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(7)

4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial spents, when opening kild values and mucroplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken. 5. Upon receipt, store the kir at 2.8°C into a temperature controlled refrigerator or cold room.

6. Do not interchange components between different tots of the kts. It is recommended that components between two kits of the same tot should not be interchanged. Others that the resignities or aggregates. If not advice the aboratory supervisor to initiate the necessary procedures for kits.

Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.

one. 10. Do external Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each Do not

example container and internal (vials) alreis. A study conducted on an operand kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 6 months. 11. Treat all speciments as potentially infective. All human setum stechners should be handled at Blosafety Level 2, as recommended by the Center for Disease Control, Alfanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Bosafety in Microbiological and Biomedical Laboratories," ed. 1984.

12. The use of disposable plastic-ware is recommended in the use the kit after the expiration date stated on the

12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid

Costs containment of the search of the kit has to be discarded in compliance with national directives and laws concerning latoratory waste of chemical and biological substances. In particular, flight waste perevised from the hast to be concerning latoratory waste of chemical and biological substances. In particular, flight waste perevised from the hast to be trasted as potentially infective material and inactivated treatment with a 10% final concentration of household bleach for 15.18 has or heal inactivation by autocave at 121°C for 20 min.

14. Accidental spills from samples and operations have to be adsorted with apper tissues soaked with household bleach and then with water. Tissues soaked with household bleach and containers designated for latoratory/hospital waste.

15. The Surphuric Acid is an irritant, in case of spills wash the surface with planty of water.

16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used micropilates) should be handled as potentially infective and disposed decording to national directives and latoratoring aboratory wasters.

SPECIMEN: PREPARATION AND WARNINGS
 Islood is drawn asspitically. by venepuncture and nlasma or recrum-its-prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citate. EDTA and house.

and heparin.

2. Samples have to be clearly identified with codes or names in order to avoid mismaprolation of results. Bay code labeling and electronic reading is strongly recommended.

3. Heamolysed ("red") and visibly hyperilipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they

4. Sera and plasma can be stored at +2"..8"C for up to five days after collection. For longer storage periods, samples can be stored frozen at -20"C for several months. Any frozen samples Sera and plasma can be str

should not be freezedthwaved more than once as this may generate particles that could affect the test result.

5. If particles are present, centrifuge at 2.000 pm for 20 min or filter using 0.20.00 filters to clean up the sample for testing.

6. Samples whose ant-Ea 4gG antibody concentration is expected to be higher than 100 arblums hould be diuded before use, either 1:10 or 1:100 in the Calibrator 0 anblum, Diutions have to be done in clean disposable tubes by diluting 50 ut of each specimen with 450 ut of Cali 0 (1:10). Then 50 ut of the 1:10 cllution are diluted with 450 ut of the Calibrator 0 and 1:100). Mix tubes thoroughly on vortex and then proceed toward the dilution step reported in section M.

# PREPARATION OF COMPONENTS AND WARNINGS

Allow he micropiate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of storing.

In this case call Dia Pro's customer service.

Unused sings have to be placed back inside the atuminum pouch, with the desiccent supplied, firmly zipped and stored at After

ortant Note: After first opening, remaining strips are stable the humidity indicator inside the desiccant bag turns from

### Ready to

component. Mix carefully on vortex before

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

\*\*Roce: Once diluted, the wash solution is stable for 1 week at 2.8° C.

Enzyme conjugate:
Ready to use. Mix well on vortex before use,
Be careful not so contaminate the liquid with oxidizing chemicals,
air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly

4 10

Ready to use. Mix well an vortex before use. Be careful not to contaminate the liquid with oxidizing chemicals air-driven dust or microbes.

Do not expose to strong illumination, oxidizing agents and metalitic surfaces, if this component has to be transferred use only plastic, possible sterite disposable container

Mix carefully on vortex before euse

Ready to use. Mix well on vortex before use. Attention: Irritant (H315, H319; P280, P302+P352, P332+P313 P305+P351+P338, P337+P313, P362+P363).

### Legenda

Warning H statements

H319 - Causes serious eye irritation. SK:

## Precautionary P statements: P280 - Wear profective

protection/face protection;
P302 + P352 - IF ON SKIN; Wash with plenty of soap and water, gloves/protective clothing/eye

> advice/attention. P362 + P363 - Take P332 + P343 - If skin advice/attention.
> P305 + P351 + P338 - IF IN I off contaminated clothing eye initation persists: EYES: Rinse cautiously with contact lenses, if present and easy Get medical and wash it

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Microphietise have to be eathyrated to deliver the corned volume required by the assay and must be submitted to regular decontainmation (household elcohol. 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +1/2%, Decontamination of the contact of the con of spills or residues of kit components should also be carried

out regularly.

The ELISA incubator has to be set at 37°C (tolerance of +1, 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water boths are suitable for the incubations, provided that the instrument is validated for the incubations, provided that the instrument is validated for the incubation of ELISA tests.

The ELISA washer is extremely important to the overally validated and correctly optimised using the lat controls and reterence panels, before using the lat for routine laboratory tests. Usually 4-5 washing cycles (assiriation + dispensation of 350 lawled in vashing studion = 1, 2014) are obtained in order to ensure that the assay performs as expected. A soaking time set correctly their number, it is recommended to run an assay with the lat controls and well characterized negative and positive reference samples, and check to match the values reported below in the section "internal Quality Control". Regular califration of the volumes delivered by, and maintenance (decontamination and cleaning of the instructions of the manufacturer. w

4. Incubation times have a tolerance of ±5%.
5. The ELISA micropiate reader has to be equipped with a second filter (620-630nm, strongly recommended) for blanking purposes, its standard performances should be [a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability ≥ 1%. Blanking is carried out on the welf dentified in the section "Assay Procedure." The optical system of the reader to be calibrated regularly to ensure that the cortext optical density is measured. It should be regularly maintained according to the manufacturer 's

instructions.

6. When using an EUSA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully soft calibrated, controlled and regularly serviced in order to match the values reported in the section internal Quality Control. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader, in addition, the liquid handling part, of the station, dispensation, and washing) has to be validated and correctly st. Particular washing has to be validated and correctly st. Particular uses the dispensation and the vashing has to be validated and correctly st. Particular uses the dispensation and the vashing has to be validated and controlled to minimize the possibility of contamination of objected when the number of samples to be tested and controlled when the number of samples to be tested as exceed 20-30 units per run.

7. Dia Pro is customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the

requirements described. Support is also provided for the installation of new instruments to be used with the kit,

occurs

Gel

PRE ASSAY CONTROLS AND OPERATIONS
Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.

Check that the liquid components are not contaminated by wishle particles or aggregates.

Check that the Chromogen (TMB) is colourless or pale blue by assirating a small volume of it with a storile plastic

by ascirating a small volume of it with a sterile plastic phetite.
Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pount, containing the micropiate, is not purctured of dranaged.
Differ all the content of the 20x concentrated Wash Solution

Allow all the c (about 1 hr) Allow at other components to reach room temperature ) and then mix gently on vortex all liquid

7. Set the EISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the mandatures instructions. Set the right number of washing cycles as found in the validation of the instrument for the use with the kit.

8. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.

9. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.

10. Check that the micropherias are set to the required volume.

11. Check that all the other equipment is available and ready

to use.

12. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.
The kit may be used determinations as well.

ਰ੍ਹ quantitative and

### M QUANTITATIVE DETERMINATION

Dilute samples 1:101 into a property defined dilution tube (example: 1000 pt (Sample Diluent \* 10 pt sample), Do not dilute the Celibration Set as califorations are ready to use. Mix carefully, all the liquid components on vortex and then

b proceed as described below.

Place the required number of Microwells in the microwell holder, Leave the A1 and B1 empty for the operation of

100 µl of diluted samples in each properly ide Incubate the microplate for 60 min at +37°C. blanking.
Dispense 100 µl of Calibrators in duplicate. Then dispense to each properly identified well.

Important note: Strips have to be sealed with the adhesive sealing foil supplied, only when the test is carried out manually Do not cover strips when using ELISA automatic instruments.

Wash the micropiate with an automatic washer as reported

previously (section 13).

Pipetie 100 µL Enzyme Conjugate into each well, avoign A 11-81 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A 3 and 81.

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Do

Important note: Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate.

- Incubate the microplate for 60 min at +37°C. Wash microwells as in step 5. Pipette 100 µC (Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at room temperature (18-24°C) for 20 minutes.

Important note: Do not expose to strong direct illumination. High background might be generated.

- 10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of each will run the positive calibrators and the positive samples from blue to yellow.
- Measure the colour intensity of the solution in each well, as described in section 1.5, at 450nm filter (reading) and at 520-530nm (background subtraction, strongly recommended), blanking the instrument on A1 or B1 or both.

## M2. QUALITATIVE DETERMINATION

If only a qualitative determination is required, proceed described below: as

- Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrations are ready to use. Mix carefully all the liquid components on vodex and then
- Proceed as described below.

  Place the required number of Microwells in the microwell holder, Leave AI well empty for the operation of blanking.

  Dispense 100 µl of Cellboror 0 articum and Cellboror artifum in duplicate and Cellbrator 100 artifum in signers.

  Then dispense 100 µl of diluted samples in each properly identified well.
- Incubate the microplate for 60 min at +37°C.

Important note: Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually, Do not cover strips when using EUSA automatic instruments.

- Wash the microplate with an automatic washer as reported
- previously (section 1.3).
  Pipete 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the seater, Check that this red coloured component has been dispensed in all the wells,

Important nate: Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.

- Incubate the microplate for 50 mln at +37°C.
- Wash microwells as in step 5.

  Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at room temperature (18-24°C) for 20 minutes.

High background might be generated. Important note: Do not expose to strong direct illumination.

Pipette 100 µl Sulphuric Acid into all the wells using the same pipeting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from yellow to blue.

Hassure the colour intensity of the solution in each well as described in section 1.5, at 450mm filter (reading) and at 620.

630nm (background subtraction, strongly recommended), blanking the instrument on A1.

## General Important notes:

- If the second litter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450mm, Finger prints could generate false positive results on reading.
- Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self exidence of the chromogen can cocur leading to high background.

## N. ASSAY SCHEME

Reading OD	ulphune Acid	emperature	incubation	MB/H2O2	Wash step	emperature	incubation	nzyme conjugate	Yash Step	emperature	incubation	allorators pamples olluted 1:101	Method
450000	100 ul	12	20 min	100 pt	4-5 cycles	+37°C	60 min	100 H	4-5 cycles	+37°C	60 min	100 Jul	Operations

An example of dispensation scheme for Quantitative Analysis is reported below:

l	Þ	œ	>	c	0	m	m	G	Ξ
	BLX	BLX		CALT	CALT	CAL2	CALZ	CAL3	CAL3
1.3	CALA	CAL4	-	CALS	CALS	CALE	CALE	S.	S2
w	SS	00		SS	88	\$7	(1)		
A		1							
cn				ij			1	1	1
o		1				1	1	1	1
7							1	1	1
20					1	1	1	1	1
p	1				1	1	1		1
5	1				1			1	
*	1								
1	16			T	1	I		1	

An example of dispensation scheme in qualitative assays is reported below.

	1	P	ij	C	ż	¢	C	n	n		6	I
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2		S		4		00	on m		0/	8.8	88	S 10
3		11	ш	S 12		3 13	143		U.	S 16	S 17	Si
4												
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4	2	1					1	L			1	4
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	2	-		1			1			Ц	1	1
							L		1		1	1
		i		1				l				L

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O. INTERNAL QUALITY CONTROL
A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.
Control that the following data are matched:

Check	Requirements
Blank well	< 0.100 OD450nm value
CAL 1 0 arbU/ml	< 0.150 mean OD450nm value after blanking
CAL 2 5 arbU/ml	OD450nm > OD450nm CAL1 + 0.100
CAL 6	OD450nm > 1,000

If the results of the fast match the requirements stated above, proceed to the next section. If they do not, do not proceed any further and operate as follows:

Blank well  1. that the Chromogeniststrate solution  2.0.150 ODA50mm  3.0.100 missiach has been done in the passive calibrator or of meir wells has occurred use spills of positive samples or the enzyma conjugate;  5.0.150 ODA50mm  6.0.100 missiach has been done in the passive calibrator or of meir wells has occurred use spills of positive samples or the enzyma conjugate;  6.0.150 mm contrarination of the negative contaminated with positive samples or the enzyma conjugate;  6.0.150 mm contrarination of the procedure has been correctly executed;  2.0.150 mm contrarination of a worning calibrator research;  3.161 m ensistive has been done in its distribution (dispensation of a wrong calibrator research);  3.162 m executed;  4.150 arbUfml  5.150 arbUfml  5.150 arbUfml  6.150 arbufml  7.150 arbufml  7.150 arbufml  7.150 arbu	Problem	Chant
Lithal the washing procedure and washer settings are as validated in pre-publication study.  2. that the proper washing solution been used and the washer has berniere with the tone use.  3. that no mistake has been done in assay procedure (dispensation of the negative one.  4. that no contamination of the negative one.  4. that no contamination of the negative one.  5. that mistake has been done in the spills of positive samples or enzyme conjugate.  5. that mistake has been done in the state occurred the spills of positive samples or enzyme conjugate.  6. that the waster needless are blocked or partially obstructed.  1. that the procedure has been done in distribution (ex.: dispensation of wrong calibration facts are savalidated in pre qualification study.  4. that no external confirmination of a wrong calibration (dispensation of a wrong calibration facts of the waster settings are as validated in 1 pre qualification study.  3. that the wasting procedure and the pre-particular study.  4. that no external confirmination of a wrong calibration study.  4. that no external confirmination of a wrong calibration study.  5. that the wasting procedure and the pre-particular study.  6. The pre-particular confirmination of a wrong calibration study.  7. that no external confirmination of a wrong calibration study.	Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the
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m pre qualification study;  That the proper washing solution been used and he washer has been done in been used and he washer has been used and he washer has been done in assay procedure (dispensation of the negative one.  I that no installed in the social due spills of positive samples or enzyma conjugate;  I that the washer needles are blooked or partially obstructed.  I that the washing procedure and washer settings are as validated in the procedure has been done in distribution (excelled.)  I that no external confamination of a word calibrator instead).  I that the procedure has been done in distribution (as persistion of washer settings are as validated in pre qualification study.  I that the procedure has been done in distribution (dispensation of a word calibrator instead).  I that the procedure has been done in distribution (dispensation of a word calibrator instead).  I that the procedure has been done in distribution (dispensation of a word calibrator instead).  I that the vashing procedure and if the procedure and it is the procedure a	0 arbi/ml	5 G
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2 4 D \$ 00 Q N D + Q 4 D \$ 00 \$ 0 N S	coefficient of	primed with it before use;
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4, that no contamination of the negative samples or positive samples or enzyme conjugate; 5. that impropipettes haven't contaminated with positive samples or enzyme conjugate; 6. that the washer needles are blocked or partially obstructed. 1. that the procedure has been done is blocked or partially obstructed. 2. that or missive has been done is distribution (ex. dispensation of word partially obstructed. 2. that no existing procedure and washer settings are as validated in pre qualification study. 4. that no external confamination of a word partially obstructed. 5. that the procedure has been done in pre qualification instance. 6. that the procedure has been done in pre qualification study. 7. that no mistake has been done in distribution (dispensation of a word calibrator instead); 8. that the washing procedure and pre qualification study. 9. that no external confamination of a word calibrator instead); 9. that the washing procedure and pre qualification study. 9. that no external confamination of a word calibrator instead); 9. that the washing procedure and pre qualification study. 9. that no external confamination of a word calibrator instead; 9. that the washing procedure and pre qualification study. 9. that no external confamination of		1100000
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Que spils of positive samples or enzyme conjugate;  5. that metropipetes haven't contaminated with positive sample with the enzyme conjugate and the enzyme conjugate in that the procedure has been corrected.  1. that the procedure has been corrected in the procedure has been corrected in the procedure and the procedure and wrong calibrator instead).  2. that no enternal confamiliation of washer settings are as validated in pre qualification study.  4. that no external confamiliation of calibrator instead).  1. that the procedure has been done in distribution (dispensation of a wrong calibrator instead).  3. that the washing procedure and washer settings are as validated in pre qualification study.  4. that no external confamiliation of a wrong calibrator instead).  5. that the washing procedure and pre qualification study.  4. that no external confamiliation of a washer settings are as validated in the qualification study.		calibrator or of their wells has occurred
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2.5.77 - (1.5.75 - 1.5.75	ārbU/ml	executed;
2.07		2. that no mistake has been done in its
	2 ^	distribution (ex.: dispensation of a
	unm CALT	wrong calibrator instead);
7 D 77 C 11 D 7 C 11		3. Inal the washing procedure and the
74 Q 4 Q Q Q Q Q Q 4 F		ore qualification shall
Q = 9 4 2 Q & 2 4 5		4. that no external contamination of the
- 9 4 2 2 C 2 C 4 F		
94980899	AL 6	1. that the procedure has been correctly
that no mistake has been don distribution (dispensation of a calibrator instead):     that the washing procedure a washer settings are as validated pre qualification study.	uu arbu/ml	executed
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washer settings are as validated in the pre qualification study.  4 that no external contamination of the noether areas.		3. that the washing procedure and the
pre qualification study; 4. that no external contamination of the		washer settings are as validated in the
nostly control contamination of the		pre qualification study:
		4 that no external conlamination of the

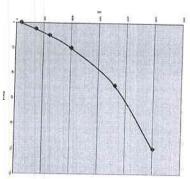
Should one of these problems have happened, after checking report to the supervisor for further actions.

### P. RESULTS

P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters Interpolation is suggested).
Then on the calibration curve calculate the concentration of anti-Ea IgG antibody in samples.

An example of Calibration curve is reported below



Important Note:

Do not use the calibration curve above to make calculations.

P.2 Qualitative method
In the qualitative method calculate the mean OD450nm values for the Calibrators 0 and 5 arbU/ml and then check that the

Example of calculation:

figures obtained by the user. Note: The following data must not be used instead or real

0.250 – 0.270 00.450am. Higher than Cal 0 + 0.100 – Accepted Calibrator 100 abbUml: riigher than 1,000 - Accepted Mean Value: Calibrator 0 arbU/ml: Lower than 0.150 - Accepted 0.022 OD450nm 0.020 - 0.024 OD450nm

off (or C) of the system.

The ratio between the OD450nm value of the sample and the OD450nm of the Calibrator 5 arbitimit (or SiCo) can provide a semi-quantitative estimation of the content of specific (gG in the The OD450nm of the Calibrator 5 arbU/ml is considered the cut-

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Q. INTERPRETATION OF RESULTS
Samples with a concentration lower than 5 arbU/mi are considered negative for article 1gG antibody.
Samples with a concentration higher than 5 arbU/mi are considered positive for article 1gG antibody.
Ea ligG results alone are not, anyway, enough to provide a clear diagnosis of EBV infection, and EBV VCA ligM results, possibly logether with EBVA ligG, are necessary in combination.
A reference andge of the minimum essential serological markers of Epstein-Barr infection, derived from Infectious Diseases Handbook, 3° edition, published by Lexi-Comp Inc., USA, is reported schematically below.

	positive	negative	POSITIVE	BATTERIAL	VCA IgM EBNA
	positive	positive	regative	negative	EBNA (or VCA) IgG
The state of the s	Reactivation	History of previous infection	Acute primary infection	No history of EBV infection	Interpretation

### Important notes:

- Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.

  When lest results are transmitted from the laboratory to another facility, effention must be paid to avoid erroneous data fransfer.

  3. Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.

R. PERFORMANCE CHARACTERISTICS
Evaluation of Performances has been conducted in an external
clinical center on negative and positive samples with reference
to a FDA approved commercial kit. U

## 1. Limit of detection

No international standard for Ea IgG Antibody detection has been defined so far by the European Community, in its absence, an Internal Goal Standard (or IGS), derived from a patient with an history of past mononucleosis infection, has been defined in order to provide the device with a constant and excellent sensitivity. j :0 :0 :7 :0

2. Diagnostic Sensitivity and Specificity.
The diagnostic performances were evaluation is performance evaluation is tudy conducted in an external centre, with excellent experience in the diagnosts of infectious diseases.

The diagnostic sensitivity was studied on samples, pre-tested positive with a different reference kit of European origin in use at the laboratory. Positive samples were collected from patients the experienced monomucleous infection.

The diagnostic specificity was determined on panels of negative samples from normal individuals and blood donors, classified negative with the reference kit, including potentially interfering

Both plasma, derived with different standard techniques of presparation (clirate, EDTA and repain), and sera have been used to determine the specificity. No faise reaching due to the method of specimen preparation has been observed. Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the tast. No interference was observed on deen and particle free

The Performance Evaluation provided the following values.

Sensitivity > 98 % Specificity > 98 %

M

Reproducibility.
 Dasa obtained from a study conducted on three samples of different Ea IgG reactivity, examined in 16 replicates in three separate rure show CV% values ranging 3-16% depending on OD450nm readings.
 The variability shown in the tables did not result in sample

S. LIMITATIONS
False positivity has been assessed as less than 2-5% of the normal population depending on the reference kit used.
Frozen samples containing fibrin particles or aggregates may generate false positive results.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with EN ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Produced by
Dia Pro Diagnostic Bioprobes Sri
Via G. Carducti n\* 27 – Sesto San Giovanni (Mi) – Italy

Epstein Barr Virus Nuclear Antigen Enzyme ImmunoAssay (ELISA) for determination of IgG antibodies to in human serum and plasma the quantitative/qualitative

for "in vitro" diagnostic use only -



## DIA.PRO

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REF EBNG CE

## EBNA IgG

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### USE

eliobay of Burkitts lymphoma and nasophanyngale carcinoma, or NPC. A member of the family Herpesvihdae, it has a worldwide distribution, such that 80 to 90% of all adults have been infected. Primary infections usually occur during the first decade of life. White elibthood infections are mostly asymptomatic. So to 70% of young adults undergoing primary EBV infections show mid to severe liness. EBV may cause a persistent, latent infection which can be reactivated under immunoscoppression or in AIDS affected patients, As humoral responses to primary EBV infections are quite rapid, the level and class of antibodies niesed in most cases allow classification as to whether the patient is still susceptible, has a current or recent primary infection. The detection of EBV specific IgG, IgM and IgA antibodies to its major immunodominant antilgens (mainly Nuclear Antigen or EBNA and Viral Capsdid Antigen or VCA) has become therefore an important and useful determination for the monitoring and follow-up of EBV infected patients.

substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti EBNA logG antibodies present washing out all the other

### D. COMPONENTS

## 2. Calibration Curve CAL Nº

Antigen in human plasma and sera. For "in vitro" diagnostic use only. enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of igG antibodies to Epstein Barr Virus Nuclear

B. INTRODUCTION

Epstein Barr Virus or EBV is the principal etiological agent of infectious monomorphisms, as well as a continuous factor in the

C. PRINCIPLE OF THE TEST in order to get rid of cossreactions with other vinuses of the same family, microplates are coated with affinity purified native EBNA artigen, capable to provide the assay with the highest In the  $\mathbf{1}^{\text{st}}$  incubation, the solid phase is treated with diluted samples and anti-EBNA IgG are captured, if present, by the

After washing out all the other components of the sample, in the production bound anti-EBNA IgG are detected by the addition of anti higG antibody, labeled with peroxidase (HRP). The enzyme captured on the solid phase, acting on the

lgG in the sample may therefore be quantitated by means of a standard curve calibrated in arbitrary units per milliliter (arbU/ml) as no international standard is available.

Each kit contains sufficient reagents to perform 96 tests

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with affinity purified native
EBNA antigen. Plates are sealed into a bag with desiccent.
Allow the microplate to reach room temperature before opening;
reseal unused strips in the bag with desiccent and store at 4°C.

Ready to use and color coded standard curve ranging:

4 mi CAL1 = 0 arbUmi

4 mi CAL2 = 3 arbUmi

2 mi CAL3 = 10 arbUmi

2 mi CAL3 = 20 arbUmi

2 mi CAL4 = 20 arbUmi

2 mi CAL5 = 20 arbUmi

4 mi CAL6 = 100 arbUmi

Mi CAL6 = 100 arbUmi

6 mi CAL6 = 100 arbUmi

7 mi CAL6 = 100 arbUmi

- a remove exidizing chemicals used as disinfectants).

  3. Triner with 80 minute range or higher.

  4. Absorbent paper tissues.

  5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at 43°C (+0.75°C tolerance).

  6. Calibrated ELISA microwal reader with 450nm (reading) and with 820-839nm (blanking) filters.

  7. Calibrated ELISA microplate washer.

  8. Vortay are 150-850.

## F. WARNINGS AND PRECAUTIONS

The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.

All the personnel involved in performing the assay have to

wear protective liaboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National

Standards are calibrated against an internal Gold Standard or IGS as no international one is defined.
Contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kalhon GC as preservatives, Standards are blue colored

## 3. Control Serum: CONTROL

1 Val. Lyophilized.
It contains feat bowine serum proteins, human IgG antibodies to EBNA at 20 arbUmit-20%, 0.2 mg/ml genlamicine sulphate and 0.1% Kalhon GC as preservatives.

## Wash buffer concentrate: WASHBUF 20X

1x80mi/bottle20x concentrated solution.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7,0+7-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

4. Enzyme conjugate: CONJ 'Xtfbm/Val. Ready to use and red colour coded. It contains furstendish peroxidase conjugated polyclonal antibodies to human igG, 5% SSA, 10 mM Tris buffer pH 6.84-0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

3.8. 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or HzOz).

Note: To be stored protected from light as sensitive to Chromogen/Substrate: SUBS TMB
 Ixlomi/vial, it contains 50 mM citrate-phosphate buffer pH 3.5.

6. Sulphuric Acid: [H2SO4\_0.3 M]
1x. Smithail contains 0.3 M H3SO, solution.
1x. Smithail contains 0.3 M H3SO, solution.
Attention: Inflant (H316, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P382+P363).

7. Specimen Diluent: DILSPE 2x00ml/val, it contains 2% casein, 10 mM Na-cifrate buffer pH 6.0 +0-0,1, 0.1% (respen 20, 0,0%) Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the sample.

## 8. Plate sealing foils n°2

9. Package insert n°1

- MATERIALS REQUIRED BUT NOT PROVIDED
   Calibrated Microphettes (1000, 100 and 10ul) and disposable plastic fips.
   EIA grade water (bidistilled or delonised, charcoal treated to

- Vortex or similar mixing tools,

Institute of Health's publication. "Biosafety in Microbiological and Biomedical Laboratories", ed. 1884.
3. All the personnel Involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available.

safe and effective 4. The laborate

when opening it was and as dust or air-born microbial agents, when opening it was and micropiates and when performing the rest. Protect the Chromogen (TMB) from strong light and avoid vitration of the bench surface where the test is undertaken, but the needed, store the ket at 2.4°C into a temperature controlled refrigerator or cold room. The laboratory environment should be controlled so as

Do not interchange components between different lots of kits. It is recommended that components between two kits

of the same for should not be interchanged.

Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervision to initiate the necessary procedures for kit

 Avoid class-contamination between serum/plasma samples by using disposable tips and changing them after each cross-contamination between

disposable Avoid cross-contamination between kit reagents by using osable tips and changing them between the use of each

external container and internal (vials) abets. A study conducted on an opened kit did not pointed out any reverant loss of activity up to six 6 uses of the device and up to 5 months.

11. Treat all specimens as potentially infective. An increase un specimens so potentially infective. one 10. Do external

11. Treat all specimens as potentially infective, All human serum specimens should be handled at Blossfely Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Blossfely in Microbiological and Biomedical phonthering." Laboratories, ed. 1954.

12. The use of disposable plastic-ware is recommended in the preparation of the figuid components or in transferring components into automated workstations, in order to avoid

discarded in compliance with referred and brooks and laws concerning laboratory waste of chemical and biological substances. In particular, flould waste generated from the wasting procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 his or neal inactivation by autociave at 121°C to 20 min. 14. Accidental spills from samples and operations have to be adsorbed with paper lissues soaked with household bleach and then with water. It issues should then be discarded in proper containers designated for laboratory/hospital waste. Waste produced during the use of the kit has to be

The Sulphuric Acid is an irritant, In case of spills, wash the

surface with plenty of water

16. Other waste materials generated from the use of the kit

16. It is a surface of the surface of the kit

16. It is a surface of the surface of the surface of the kit

16. It is a surface of the surface of the surface of the kit

16. It is a surface of the su

S. SPECIMEN: PREPARATION AND WARNINGS
 Bood is drawn assiptically. By energundure and blasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA observed in the preparation of the sample with citrate, EDTA.

and heparin,
2. Samples have to be dearly identified with codes lation of results. Bar code labeling and or names in

electronic reading is strongly recommended.

3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results

Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they

Sera and plasma can be s

after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any forzan samples should not be freezod/hawded more than once as this may generate particles that could afted the text result.

5. If particles are present, contriuge at 2,000 ipm for 20 min or filter using 0,2-0,8bt filters to clean up the sample for testing.

6. Samples whose anh-EBNA IgG antibody concentration is expected to the higher than 100 act/burn should be diffued before use, either 1:10 or 1:100 in the Calibrator 0 act/burn, Dilutions have to be done in clean disposable tubes by diffuting 50 ut of each specimen with 450 ut of Cal 0 (1:10). Then 50 ut of the 1:10 dibtion are diffued with 450 ut of the Cal 0 (1:100). Mix tubes throughly on vortex and then proceed toward the diffution step reported in section M.

# H. PREPARATION OF COMPONENTS AND WARNINGS

the microplate to reach

lurned to dark green, indicating a defect of storing, before opening the contain

## Calibration Curve

component. Mix carefully on vortex before use.

### Control Serum

vortex,
Note: The control after dissolution is not stable. Store frozen in Add the volume of ELISA grade water, reported on the label, the lyophilised powder; let fully dissolve and then gently mix 9 5

The whole content of the concentrated solution has to be dituted 20x with bidistilled water and mixed gently endower-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles. Nate: Once difuted, the wash solution is stable for 1 week at +2\_8° C.

air-driven dust or microbes.

If this component has to be transferred use only plastic, possibly

sterile disposable containers

Do not expose to strong air-driven dust or microbes

or longer storage periods, samples can be

# room lemperature (about 1 hr) Check that the desiccant is not

course surps have to be placed back inside the aluminum pouch, with the desicoant supplied, firmly zipped and stored at +2"\_8°C\_ In this case call Dia Pro's customer service Unused strips have to be placed back

Important Note: After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yeliow to green

## Ready to use

Enzyme conjugate:

Ready to use. Mix well on vortex before use.

Be careful not to contaminate the liquid with oxidizing chemicals.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be carreful not to contaminate the liquid with oxidizing chemicals If this component has to be transferred use only plastic, possible Illumination, oxidizing agents and

Ready to use component. Mix carefully on vortex before

Sulphuric Acid:

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Ready to use. Mix well on vortex before use. Attention: Irritant (H315, H319: P280, P302+P352, P332+P313, P305+ P351+P338, P337+P313, P362+P363).

using an ELISA automated work station, all critical (dispensation, incubation, washing, reading, date

H319 – Provoque une sévére irritation des yeux Mention de danger, Phrases H

P280 — Porter des gants de protection/des vêtements protection/un équipement de protection des yeux/ du visage. P302 + P352 — EN CAS DE CONTACT AVEC LA PEAU; It P280 Conseil de prudence, Phrases P Porter des

abondamment à l'eau et au savon, P332 + P313 – En cas d'irritation cutanée: consulter

: laver Ξ

de

si elles peuvent être facilement enlevées. Continue P337 + P313 - Si l'irritation oculaire persiste: YEUX: rincer avec précaution à l'eau pendant plusieurs minutes. Enlever les lentilles de contact si la viclime en porte et YEUX: P305 + P351 + P338 - EN CAS DE CONTACT AVEC LES Continuer à rincer consulter 5

P362 + P363 — Enlever les vêtements contaminés et les laver

# INSTRUMENTS AND TOOLS USED IN COMBINATION

Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol 10% southon of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly mannaned in order to show a precision of 1% and a trueness of +/-2%, Decontamination of spills or residues of kil components should also be carried

out regularly.

The ELISA incubator has to be set at +37°C (tolerance of +1-0.5°C) and regularly checked to ensure the correct temperature is meintained. Both dry incubators and water baths are suitable for the incubations, provided that the

reference panels, before using the kit for multine laboratory tests. Usually 4.5 washing cycles (aspiration + dispensation of 350µl/wall of washing solution = 1 (2yel) are sufficient to ensure that the assay performs as expected. A soaking fine of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section 'internal Quality Control'. Regular-calibration and cleaning of medies) of the washer has to be carried out according to the instructions of the manufacturer.

Includation times have a biderance of 15%.

The ELISA microplate reader has to be equipped with a strongly recommended) for banking purposes. Its standard performances should be (a) bankwidth <a href="https://doi.org/10.100/en.100.1001/en. instrument is validated for the incubation of ELISA tests. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and validated and correctly optimised using the kit controls and

sleps (dispensation, incubation, washing, reading, data franding) have to be carefully set, calibrated, controlled and regularly serviced in order for match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader, in addition, the liquid handing part of the station (dispensation and washing) has to be validated and correctly set, Perfocular altention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of confamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run, 2 bits Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

- L. PRE ASSAY CONTROLS AND OPERATIONS

  1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.

  2. Check that the liquid components are not contaminated by
- visible particles or aggregates Check that the Chromogen ( by aspirating a small ogen (TMB) is colourless or pale blue volume of it with a sterile plastic
- pipette.
  Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary spillage of liquid is present inside the box (primary pour the containing the spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged;
- Dissolve the content of the Control Serum as reported.
  Dilute all the content of the 20x concentrated Wash Solution
- as described above,
  Allow all the other components to reach room lemperature (about 1 hr) and linen mix gently on vortex all liquid
- reagents.

  Set the ELISA incubator at +37°C and prepare the ELISA waster by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.

  Check that the ELISA reader is turned on or ensure it will be

- turned on al least 20 minutes before reading.

  10, If using an automated work station, turn on, check settings and be sure to use the right assay protocol.

  11. Check that the micropipettes are set to the required volume.

  12. Check that all the other equipment is available and ready.
- lo use.

  13, in case of problems, do not proceed further with the test and

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

The -kit may be used for quantitative—and—qualitative

determinations as well.

## M1. QUANTITATIVE DETERMINATION

Dilute samples 1.101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrators are ready to use.

is is	51.0
	200

- Mix carefully all the liquid components on vortex and then proceed as described below.

  Place the required number of Microwells in the microwell holder, Leave the A1 and B1 empty for the operation of
- properly identified well, incubate the microplate for 60 min at +37°C e 100 µl of Calibrators and 100 µl Control Serum in e. Then dispense 100 µl of diluted samples in each

Important note: Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. On not cover strips when using ELISA automatic instruments.

- Wash the microplate with an automatic washer as reported
- neviously (section 1.3). Pipette 100 µ Enzyme Conjugate into each well, except A1+31 blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important note: Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur

- Incubate the microplate for 60 min at +37°C. Wash microwells as in step 5. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at room temperature (18-24°C) for 20

Important note: Do not expose to strong direct illumination. High background might be generated.

- 10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
  1. Measure the colour intensity of the solution in each well, as described in section 1.5, at 450nm filter (reading) and at 6:0. 530nm (blackground subtraction, strongly recommended) blanking the instrument on A1 or B1 or both.

## M2. QUALITATIVE DETERMINATION

only a qualitative determination is required, proceed ag

- Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibrations are ready to use, My carefully all the liquid components on vortex and then
- 2 proceed as described below.

  Place the required number of Microwells in the microwell
- holder. Leave A1 well empty for the operation of blanking.
  Disperse 100 µl of Calibrator 0 arbUrm and Calibrator 10
  arbUrm in duplicate and Calibrator 100 arbUrm in signe.
  Then dispense 100 µl of diffued samples in each properly.
- Incubate the microplate for 60 min at +37°C.

Important note: Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually, Do not cover strips when using ELISA automatic instruments.

- Wash the micropiale with an automatic washer as reported
- previously (section 1.3).

  Pipette 100 ut Enzyme Conjugate into each well, except the A1 well, and prover with the sealer. Check that this red coloured component has been dispensed in all the wells.

Important note: Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.

- Incubate the microplate for 60 min at +37°C, Wash microwells as in step 5.

  Pipette 100 ut Chromogen/Sustrate mixture into each well, the blank well included. Then incubate the microplate at room temperature (18-24°C) for 20 minutes.

Important note: Do not expose to strong direct illumination High background might be generated.

- 10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will furn the positive callingtons; the control serum and the positive samples from yellow to blue.
  11. Measure the colour intensity of the solution in each well as described in section 1.5, at 450nm (fler(reading) and at 620-650nm (fler(acid) and subtraction, strongly recommended).
- blanking the instrument on A1.

## General important notes:

- If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450mm. Finger prints could generate false positive results on reading, prints could generate false positive results on reading. Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.

## N. ASSAY SCHEME

Reading On	Sulphuric Acid	Temperature	3 <sup>rd</sup> incubation	TMB/H2O2	Wash step	Temperature	2" incubation	Enzyme conjugate	Wash step	Temperature	1 <sup>st</sup> incubation	Samples diluted 1:101	Calibrators & Control(*)	Method
45000	100 u	13	20 min	100 M	4-5 cycles	+37°C	60 min	100 µ	4-5 cycles	+37°C	60 min	100 μ	100 m	Operations

- (\*) Important Notes:

   The Cantrol Serum (CS) it does not affect the test's results calculation.

   The Control Serum (CS) used only if a laboratory intermal quality control is required by the

An example of dispensation scheme for Quantitative Analysis is reported below:

rm	O	0	to	A		
CAL2	CALT	CALI	BLK	BLK	4	
CALE	CAL5	CAL5	CALA	CAL4	2	
8 8	(3) 4	S3	S 2	S	(i)	
					4	NIC.
	Ų			Ĭ,	ch	99
					a	316
				F	7	
					00	
1					9	
					10	
					111	
					12	

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F CAL2 CAL6 S6
G CAL3 CS(7) S7
H CAL3 CS(7) S8
Legenda: BLK = Blank S = Sample CS(\*)= Control Serum - Not mandatory

reported below: An example of dispensation scheme in qualitative assays is

enda:	S2	S	CAL6	CAL3	CAL3	CAL1	CAL1	BLX	113	
w	210	Se	S8	S7	SB	SS	\$4	S3	2	
BLK = Blank	S18	\$17	818	\$15	814	S13	S12	Sii	ω	
ank	Ĭ								4	Z.
	J								(J)	Micropiate
									6	iate
S					4				7	
CAL = Calibrators					4		_		ca	
8		_		1					8	
all									10	
š									#	
ĺ						1	- 1		12	

& IOTHOOM>

S = Sample

## O. INTERNAL QUALITY CONTROL

A validation check is carried out on the calibrators any time the kit is used in order to verify whether the performances of the assay are as qualified. Control that the following data are malched:

Check	Requirements
Blank well	< 0.100 OD450nm value
CAL 1 0 arbU/ml	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
CAL 2 5 arbU/ml	OD450nm > OD450nm CAL1 + 0,100
CAL 3 10 arbU/ml	0D450nm > 0D450nm CAL1+ 0.200
CAL 6	OD450nm > 1.000

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

Problem	Check
5 0,100 00450nm	<ol> <li>that the Chromoger/Sustrate solution has not got contaminated during the assay.</li> </ol>
CAL 1 D arbulins > 0.150 OD450mm artur Usanking	in that the weathing protopours and the weather softways are as validated in the one qualification saids.  2. That the proper weathing solution has been used
10%	I that no matther has been done in the assay procedure (disponsition of a positive calibrate instead of the positive one;
	<ul> <li>Itali ne conjugate;</li> <li>Itali ne conjugate;</li> <li>Itali ne conjugate;</li> </ul>
	positive samples or with the enzyme conjugate
	is that the washer needles are not blocked or

		CAL 3 10 arbt/m 10 arbt/m CAL 1 - 0,200 CAL 1 - 0,200 CAL 6 100 arbt/m 1100 CAL 6 1,100 CA
t/mi		OD450nm < OD456nm CAL1 + 0,208
Uini ne. < OD456nm - 0.209	0.208	CAL 6 100 arbWmt
	-0.206	< 1.000 00450nm
Umi O.200 O.450m O.200	-0.208 -0.208 -0.208	

Should one of these problems have happened, after checking report to the supervisor for further actions.

If Control Serum has used, verify the following data:

Check

If the results of th	Control Serum
If the results of the test doesn't match the requirements state	Mean OD450nm CAL4 +/-20%
o J	П

above, operate as follows

		Different from Expected value	Control Serum	The Part of the Pa
pre qualification study; 4. Ihal no external contamination of the control has occurred.	<ol> <li>that the washing procedure and the washer settings are as validated in the</li> </ol>	executed:  2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead):	1. that the procedure has been correctly	41767

Anyway, if all other parameters (Blank, CAL1, CAL2, CAL 6), match the established requirements, the lest may be considered valid

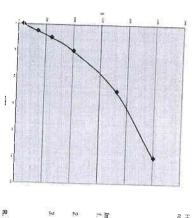
### P. RESULTS

P4 Quantitative method

If the test turns out to be valid use for the quantitative method
an approved curve titing program to draw the cathration curve
from the values obtained by reading at 450nm. (4-parameters-Then on the calibration curve calculate the concentration of ant

EBNA IgG antibody in samples

An example of Calibration curve is reported below



Important Note: Do not use the calibration curve above to make calculations,

## P.2 Qualitative method

In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 10 arbU/ml and then check that the

## Example of calculation:

Note: The following data must not be used instead or real figures obtained by the user.

Calibrator 0 arbU/ml: Mean Value: ( Lower than 0.150 - Accepted nl: 0.020 – 0.024 OD450nm 0.022 OD450nm

Calibrator 10 arbU/ml: Higher than Cal 0 + 0.200 - Accepted 0.460 OD450nm 0.450 - 0.470 OD450nm

Higher than 1,000 - Accepted Calibrator 100 arbU/mt 2.045 OD450nm

The OD450nm of the Calibrator 10 arbU/ml is considered the

cut-off (or Co) of the system.

The ratio between the OD450nm value of the sample and the OD450nm of the Calibrator 10 arbUrnl (or S/Co) can provide a semi-quantitative estimation of the content of specific gG in the

O. INTERPRETATION OF RESULTS
Samples with a concentration lower than 5 arb.l/ml are considered negative for anti EBNA (gG ambboy).

Samples with a concentration ranging 5-10 arb.l/ml are considered in the gray-zone. Samples with a concentration ranging 5-10 arb.l/ml are considered in the gray-zone. Samples with a concentration higher than 10 arb.l/ml are considered positive for anti EBNA (gG antibody.

EBNA (gG results alone are not, anyway, enough to provide a dear diagnosis of EBV infection. At least EBV VCA 1gM results.

A reference construction.

A reference range of the minimum essential serological markers of Epstein-Barr infection, derived from Infectious Diseases

Handbook, 3<sup>rd</sup> edition, published by Lexi-Comp. Inc., USA, reported schematically below:

Reactivation	positive	BOSITIVE
History of previous infer	positive	negative
Acute primary infection	negative	positive
No history of EBV infec	negative	negative
Interpretation	EBNA IgG	VCA IGM

Important notes:

1. Interpretation of results should

1. Limit of detection

No international standard for EBNA IgG Antibody detection has been defined so far by the European Community. In its absence, an internal Gold Standard (or IGS), derived from a patient with an history of past monomuoleosis infection, has been defined in order to provide the device with a constant and

2. Diagnostic Sensitivity and Specificity.

The method is based on the use of an affinity purified native EBNA antigen to provide the assay with the highest specifity to The diagnostic performances were evaluated in a performance evaluation study conducted in an extendance that the diagnostic performances were evaluated in a performance evaluation study conducted in an extendance that the diagnostic of infectious diseases and in particular in EBW infection.

The Diagnostic Sensitivity was studied on more than 50 samples, pre-tasted positive with two reference kits of European origin in use at the laboratory. Positive samples were collected from patients that experienced mononucleosis infection. The diagnostic specificity was determined on pareits of more than 50 negative samples from normal individuate and blood donors, classified negative with the reference kit, including notamishly eightfactures.

potentially interfering specimens, some standard techniques of both plasma, derived with different standard techniques of preparation (citate, EDTA and hepatin), and sera have been

Frozen specimens have also been tested to check whether samples freezing interferes with the performance of the test. No interference was observed on clean and particle free

samples.
The Performance Evaluation provided the following values:

Specificity	Sensitivity
> 98 %	> 98 %

CA IgM EBNA IgG Interpretation			
The state of the s	CA IgM	EBNA InG	Othernrefation
		-	HOMEN CANADA

Inderpresion of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations. When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous

data transfer. Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.

R. PERFORMANCE CHARACTERISTICS
Evaluation of Performances has been conducted in an external clinical center on negative and positive samples with reference to a FDA approved commercial kit.

used to determine the specificity.

No false reactivity due to the method of specimen preparation

3

## Reproducibility

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Data obtained from a study conducted on three samples of different EBNA tigG reactivity, examined in 16 replicates in three separate runs show CV% values ranging 5-20% dispending on D0450mm readings.

The variability shown in the tables did not result in sample

### S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate lates positive results.

Depending on the reference kit in use, due to some helerogeneity among different devices, the presence of 2-5% false reactivity may be seen.

### REFERENCES

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5,9,8,7,6

All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Dia Pro Diagnostic Bioprobes Srl Via G. Carducci n\* 27 – Sesto San Giovanni (MI) – Italy Produced by

qualitative/quantitative determination of Helicobacter pylori Antigen Enzyme Immunoassay for the In human stools

for "in vitro" diagnostic use only -



DIA.PRO

20099 Sesto San Giovanni Via G. Carducci nº 27 Diagnostic Bioprobes Srl

Fax -39 02 26007726 e-mail: info@diapro.it

(Milano) - Italy

REF HPAG.CE 48 Tests

HP Ag

### A. INTENDED USE

(ELISA) 약현

qualitative/quantitative determination of Helicobacter pylori Antigen (HP Ag) in human stools. The kit may be used for the the follow-up of HP-infected patients and their pharmacological freatment. For "in vitro" diagnostic use only. one-step

## B. INTRODUCTION

Helicobacter pylori (Hp) is a Gram negative bacterium, firstly isolated in gastiric mucosa by Markhall and Warren in 1983.
This bacterium is widely diffused in men, without limitations of sex and age; it has been found that infections can be transmitted directly by contact with contaminated biological fluids (saliva, sloot), body secretions) and also from contaminated food and beverages.

H.pylori, and in particular some pathogenic strains (CagA +), is the etiological agent responsible of most of active infections and

lesions of the gastric mucosa in man.

H.pylori infection moreover acts as cofactor in the development
of tumoral pathologies of the gastric apparatus and it is
suspected to be associated to some inflammatory pathologies of the genital female apparatus, evolving toward transformation. neoplastic

At the present time, the identification of Helicobacter pylori is mostly made with invasive histochemical techniques, with the determination of its unease activity on a isotopic substrate (breath lest and mass analysis), with time-consuming bacteriological culture systems and with expensive molecular

biology techniques (PCR), ELISA for HP Ag have been only recently introduced as a specific, last, non invasive (analysis of stools) and cheaper

## C. PRINCIPLE OF THE TEST

Slools from patients are used as a source of sample for the determination of HP antigen. Microplates are coated with a cockial of affinity purified mouse monoclonal antibodies directed to the most specific Helicobacter.

yplori arrigens, pylori arrigens, pylori arrigens, in the 4" incubation, the solid phase is treated with the sample, previously extracted from stoods, and simultaneously with a mixture of monodonal antibodies to Hp. conjugated with peroxidase (HPD).

After washing out all the other components of the sample, in the 2<sup>rd</sup> incubation the bound enzyme specifically present on the solid phase generates an optical signal that is proportional to the amount of H,pytori antigons present in the sample.

### D. COMPONENTS

Code HPAG CE contains reagents to perform 48 tests.

1. Microplate <u>MICROPLATE</u>
1. 1. Surps x 5 breakable microwels, coated with anti HP Ag specific affinity purified mouse monoclonal antibodies and sealed fills a bag with desicrant. Allow the microplate to reach room temperature before opening reseal unused strips in the bag with desicrant and store at 4°C.

2. Calibration Set: [AL...]

A vals - Lyophilized calibrators. To be dissolved with EIA grade water. When dissolved Calibrators have the following concentrations: 0-01-05-10 ug/mi HP Ag concentrations: 0-01-05-10 ug/mi HP Ag 10 mM Phey contain feels boyine securi, nacharated HP Ag 10 mM phosphate buffer pH 7.4+0-1, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives.

Important Note: Calibrators when dissolved are not stable.

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3. Wash buffer concentrate MASHBUF 20X 1x50m/hottle - 20X concentrated solution. Once wash solution contains 10 mM phosphate buffer and 0.05% Tween 20. ution. Once diluted, the thate buffer pH 7.0+/-0.2

4. Enzyme Conjugate CONJ

Némlívial - Ready lo use component, it contains Horseradish
Peroxidase (HRP) labeled mouse monoclonal antibodies to HP
Ag., 10 mM Tris buffer pH 6.8+4.0,1, 2% BSA, 0,1% Kathon GC
and 0,02% gentamicine sulphate as preservatives. The Enzyme Conjugate is color coded red.

5. Chromogen/Substrate SUBS TMB 1x/6ml/vial - II contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H2O2).

Note: To be stored protected from light as sensitive to

6. Specimen Diluent: DILSPE
1x60mi/viai - Suffered solution for the extraction of HP Ag from the specimen and preparation of the sample, It contains 10 mM Tris-HCI buffer pH 744-VJ.1, 2% BSA, 0.1% Kathon GC and 0.02% gerlaminier sulphale as preservatives.

The component is calor coded blue.

## 8. Plate sealing foils: n° 2

7. Sulphuric Acid <u>FBSCt. 0.3 M</u> Xt/Ont/val-. It contains 0.3 M H2SO4 solution. Attention: frilant (1415, H319, 1920, P302-P332, P332+P313, P305+P351+P338, P337+P310, P362+P363).

Package insert: n° 1

HP Ag Extraction kit n° 1
The kit contains all what is necessary to prepare n° 48 samples extracted from stools collected by patients.

# E MATERIALS REQUIRED BUT NOT PROVIDED 1. Calibrated variable volume Micropipettes ranging

Calibrated variable volume Micropipettes ranging 1000 ul

and 200 ul; disposable plastic tips.

EIA grade water (double distilled or deionised, charcoal remove oxidizing chemicals used

- in to in Timer with 60 minute range or higher.
- Absorbert paper tissues.
  Calibrated ELISA micropiate thermostatic incubator (dry or wet), set at 43°C,
  Calibrated ELISA microwell reader with 450nm (reading)

- and with 620-630nm (blanking) fillers.
  Calibrated ELISA microplate washer.
  Vortex or similar mixing tools.
  Disposable plastic mero-spoon stools collection (available upon request from Dia.Pro s.r.l.)

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.

2. All the personnel involved in performing the assay have to

2. All the personnel involved in performing the assay have to wear protective laboratory ciches, tac-free glouvs and glasses. The use of any sharp (needless) or cuffing chanes) orders should be trained in blosslely procedures as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National

Institute of Health's publication: "Biosafety in Microbiological and , ed 1984

3. The laboratory environment should be controlled so as to avoid contaminants such as dust or all-born microbial agents, when opening it vals and microplates and when performing the test. Protect the Chromogen-Substrate from strong light and avoid vibration of the bench surface where the test is

 Do not interchange components between different tots the kits. It is recommended that components between two Do not interchange compon Upon receipt, store the kit at 2.8°C into a temperature interchange components between different lots

of the same lot should not be interchanged.

6. Check that the liquid components of the kit are clear and do not contain visible heavy particles or aggregates. If not advise the laboratory supervisor to initiate the necessary procedures for kit replacement.

7. Avoid cross-contamination between samples by using

disposable tips and changing them after each sample.

8. Avoid cross-contamination between kit components by using disposable tips and changing them between the use of

9. Do not use the kit after the expiration date stated on the external container and internal (vials) jabels.
10. Treat all specimens as potentially infective, according to national regulations and laws concerning biological sample.

handling and wasting.

11. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid

12. Waste produced during the use of the kit have to be discarded in compliance with national directives and laws concerning abovetory waste of chemical and biological pushoratory waste of chemical and biological pushoratory waste of chemical form the wasting procedure, from residuals of controls and from samples above waste. Suggested procedures of inactivation are teatment with a 10% final concentration of household bleach for 16.18 his or hear thanchvation by autoclave at 121°C for 20 min. 3. Accidents spills from samples and operations have to be then with water. Tissues shaded with household bleach and containers designated for laboratory/household bleach and containers designated for laboratory/household waste. 14. The Sulphuric Acid is an irritant, in case of spills, wash the

surface with plenty of water

15. Other waste materials generated from the use of the kit example: this used for samples and controls, tools for the extraction of the sample from specimens, used microplates etc), should be handled as potentially infective and disposed according to national directives and laws concerning laboratory

G. SPECIMEN: COLLECTION, PREPARATION AND WARNINGS

I) It is recommended to collect fresh stools in the morning with the plastic-collector provided on request Together with the tat. Alternatively a conical bottomed disposable tube, provided by the taboratory to the patient, may be used.

The patient submitted to the test should not be under antibiotic or anti-bacterial readments as this pharmaceutical therapy is known to affect H-pylon up to a certain extent. depending on the antibiotic used, giving origin to false

ω interpretation.

The patient has to be asked to collect the specimen avoiding any possible contact with urine or water using the plastic spoon present in the stool collector and falling the plastic spoon present in the stool collector and falling the plastic spoon present in the stool collector and falling the plastic spoon present in the stool collector and falling the cavity of the

4 spoon.

The patient is asked to deliver the specimen the same day to the laboratory. From the time of collection, the specimen

ģ

cein he stoned in the laboratory up to 24 hr at 2,8°C or kept frozen at 20°C for longer time. 
Specimens, and then samples derived from them, have to be cleanly dentified with codes or names in order to avoid maintarpretation of results. Bar code labeling and electronic reading is recommended when the number of samples on

specimen is stored at 2.8°C. Important Note: Degradation of HP stools after 24 hrs generating false ne TP antigen heavily occurs in negative results, even if the

The next following operations are described an figures in the Instructions for Use of the Sto provided together with the kit.

Operate according to the following instructions: described and represented se of the Stool Extraction Z.

Open the stool collection device and introduce the extraction brush deeply into the specimen. Rolate the brush 2-4 times in order to collect the right amount of biological material

(about 0.2 gr).

2) Transfer the trush carefully into the test tube supplied in the kit and then add 1 ml Specimen Oblivent. Keeping the brush inside the tube, mix vigorously on vortex for 1 min 4/-10% in order to dissolve H pulyed into solition.

3) Discard the brush and insent the filtering piston, supplied with the kit, into the tube. Push gently the piston down into the tube in order to collect not more than 150-200 uld the liquid phase of the suspension, volume enough to carry out 3 N

Important Notes:

a) Be careful not to apply a too strong manual pressure on the piston. The piston could break the tube and spills could be piston. The piston could break the tube and spills could be generaled. If this should happen, use a paper towel soaked with an hospital disinfectant to clean up the contaminated

Avoid any addition of preservatives to samples, especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generaling false negative results.

H. PREPARATION OF COMPONENTS AND WARNINGS A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

Microplates:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green; indicating a defect in storing. In this case, call Dia, Po's customer service.

Unused strips have to be placed back inside the aluminum.

"An Assirvant supplied, firmly zipped and stored a Important Note pouch, with the desiccant supplied, firmly zipped and 12.8°C e: After first opening, remaining strips are s by indicator inside the desiccant bag turns

Add the volume of ELISA grade water reported in the label the lyophilized powder of each Calibrator, Let fully dissolve the property of the voles. The content and then gently mix on votes, important Note: When dissolved, Calibrators are not stable. Since Calibrators forcer, in allouds at ~20°C, century label, with the content of HP Ag present in each of them. carefully labeled

grade The concentrated solution concentrated solution has to be difuted and mixed gently end-over-end before with n ELISA : During

> preparation avoid fearing as the presence impact on the efficiency of the washing cycles, important Note: Once diuted, the wash soluweek at +2.8° C. of bubbles

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solution is stable for 1

Ready to use, Mix well on vortex before use.

Ready to use. Mix well on vortex before use.
Avoid contamination of line liquid with oxidizing chemicals, airdriven dust or microbes. Do not expose to strong light, oxidizing

possible, sterile disposable container. agents and metallic surfaces. If this component has to be transferred use only plastic, and if

Ready to use. Mix well on vortex before use

### Sulphuric Acid:

Ready to use. Mix well on vortex before use. Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363),

### Legenda

Warning H statements: skin irritation

H319 - Causes serious eye irritation

water,

water,

P332 + P313 - If skin initiation occurs: Get medical
advice-attention.

P305 + P351 + P338 - IF IN EYES; Rinse cautiously with water
for several minutes. Remove contact lenses, if present and easy
to do. Continue finsing.

P337 + P313 - If eye imitation persists: Get medical P280 — Wear protective gloves/protective dothing/ protection/face protection. P302 + P352 — IF ON SKIN: Wash with plenty of soap Precautionary P statements: P280 – Wear protective protective gloves/protective clothing/eye and

## WITH THE KIT I. INSTRUMENTS AND TOOLS USED IN COMBINATION

Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular deconfamination (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. They should also be regularly maintained in order to show a precision of 1% and a

2

trumbess of the National Market and a processon of the end of the

instructions provided by the manufacturer, Important Note: Due to the nature of the sample used and the possible presence of particles in the sample, be careful to control that the needles of the washer do not get blocked positive reference samples, and check to match the values reported below in the section "Internal Quality Control", Regular calibration of the volumes delivered an maintenance (descontamination and cleaning of needles) of the washer has to be carried our according to the instructions provided by the manufacturer. by the presence of stool bodies

4. Incubation times have a tolerance of ±5%.

The ELISA micropiete reader has to be equipped with a reading filter of 450m and with a second filter (620-630m, strongly recommended) for blanking purposes. Its standard performances should be (a) bandwidth < 10 nm, (b) absorbance ample from 0 to 2.0; (c) linearly to 2.0; repeatability ≥ 1%. Blanking is carried out on the well identified in the section "Internal Quality Control." The optical system of the reader has to be califorated regulatify to ensure that the correct optical density is measured. It should 4 10 be regularly maintained according to the manufacturer 's

6. When using an EUSA automated workstation, all critical steps (dispensation, incubation, washing reading, shaking data handling) have to be carefully set, calibrated, controlled and regularly services in order to match the values reported in the sections internal Quality Control, system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the sation (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to flase positive results. The use of EUSA automated work stations's recommendation the number of samples to be tested exceed 20-30 units per run. m units per run

Important Note: Due to the nature of the sample used and the possible presence of particles in the sample, be careful to control that the readles of the workstation do not get blocked by the presence of stool bodies. We strongly suggest to use disposable sample tips in order to avoid any block or damage of

P362 + P363 - Take off contaminated clothing and wash it

Dia/Pro's customer service offers support to the user in the setting and objecting of instruments used in combination with the kit, in order to assure full compliance with the requirements described. Support is also provided for the requirements described. Support is also provided for the substantiation of new instruments to be used with the kit.

Upon request, Dia/Pro str offers a sample preparation device able to produce a particle free sample showing excellent performances in the assay. Please inquire. Dia. Pro's customer service offers support

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Prepare the sample from stools as described in section G. and represented in the Instructions for Use of the HP Ag Extraction Kit.

2. Check the expiration date of the kit printed on the external label of the kit box, Do not use if expired.

3. Check that the fujuid components are not contaminated by naked eye visible partities or, aggregates. Check that the Chromogen/Substrate is colorless on pale the by aspirating a small volume of if with a sterile transportant plastic partial printing check that no preserve reside the box. Check that the spillage of layout is present inside the box. Check that the spillage of layout is present inside the box. Check that the or damaged.

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- Dilute all the content of the 20x concentrated Wash Solution as described above.

  Dissolve the Calibration Set as described above.

  Allow all the other components to ceach room temperature
- Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the dilute washing solution, number of washing cycles as found in the validation of the Check that the ELISA seader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on at least 20 check that the ELISA reader has been turned on the ELISA reader has the ELISA reader has been turned on the ELISA reader has the ELISA
- minutes before reading.

  9. If using an automated workstation, turn it on, check settings 03
- and be sure to use the right assay protocol.

  10. Check that the micropipettes are set to the required volume.

  11. Check that all the other equipment is evallable and ready.
- to use.

  12, In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE
The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Two procedures are available: a quantitative method able to provide a quantification of HP Ag in the specimen and a

## Quantitative Assay

Place the required number of strips in the plastic holder and carefully identify the wells for calibrators and samples. Leave A1+B1 wells empty for blanking purposes.

ω

- Pipette 100 µl Calibrators in duplicate into the calibration wells (see the example of dispensation reported below).
- up into the inner character of the piston and dispense of drops (about 100 µ) of sample into the sample well. Check on the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620mm. (samples show OD values higher than 0.100). With the Pasteur pipette supplied aspirate the liquid filtered
- Dispense then 100 µl Enzymalic Conjugate in all wells except for A1+B1, used for blanking operations.

Important note: Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur

a

Following addition of the conjugate, check that the color of the samples have turned from brown to pale reddish and incubate the microplate for 120 min at +37°C.

Important notes: Strips have to be sealed with the adhesive sealing foil only when the test is performed manually. Do not cover strips when using ELISA workstations.

- When the first incubation is over, wash the microwells
- Pipette 200 µl Chromogen/Substrate into all the wells.

  A1+81 included, incubate the micropiate protected from light at room temperature (18-24°C) for 20 min. previously described (section i.3) as

Important note: Do not expose to strong direct light as a high background-might-be-generated:

Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 6.

Measure the exter intensity of the solution in each well, as described in settlon is using a 450mm filter (reading) and a 520-550mm filter (hackground subtraction, strongly recommended), blanking the instrument on A1 or B1 or

An example of dispensation scheme is reported below:

L	Þ	7	α	7	þ	U	ì	17	ti	İ	Ø		2
	BLK	2	BLK	CALT	-	CAL:		CAL2	CALO		CALS		CAL3
2	CALA		CALA	0	-	52		83	20		25		88
u		-		-		-							
4						-			-				-
5			-	-		1			-		1		
n				l		ı			1		1		
	A 4 5 8	A BLK CAL4 3 4 5 6	A BLK CAL4 5 6	A BLK CAL4 S 6	A BLK CAL4 3: 4 5 6	A BLK CAL4  B BLK CAL4  C CAL1 S1	트로지지.	E S X X	CITIEN S		12121212	@ [5   5   5   5   5   5   5   5   5   5	

Qualitative Assay BLK = Blank CAL = Calibrator S = Sample

- Place the required number of strips in the plastic holder and carefully identify the wells for calibrators and samples, Leave A1well empty for blanking purposes.
- 2 Pipette 100 µl Calibrator 1 in duplicate, 100 µl Calibrator 2 in duplicate, 100 µl Calibrator 4 in single and then 100 µl samples. Check for the presence of samples in wells as reported before.
- Dispense 100 µl Enzymatic Conjugate in all wells, except for A1, used for blanking operations.

Important note: Be careful not to touch the inner surface of the well with the pipette tip when the conjugate is dispensed. Contamination might occur.

Following addition of the conjugate, check that the color of the samples have turned from brown to pale reddish and then incubate the microplate for 120 min at +37°C.

Important notes: Strips have to be sealed with the adhesive sealing foil only when the lest is performed manually. Do not cover strips when using EUSA workstations.

When the first incubation is over, wash the microwells as previously described (section I.3)

Pipette 200 µl Chromogen/Substrate into all the wells. At included, incubate the micropiate protected from light at room temperature (16-24°C) for 20 min.

Important note: Do not expose to strong direct light as a high background might be generated.

Pipette 100 ul Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting-sequence-as-in step 6.

Measure the color intensity of the solution in each well, as described in section 1.5 using a 450nm filer (reading) and a 820-830nm filer (background subtraction, strongly recommended), blanking the instrument on A1.

An example of dispensation scheme is reported below

1	0	œ	A		
	CALT	CAL1	BLK	1	
	SS	S4	53	2	Mic
-				ω	ropia
				4	0
١		- 1		Li)	

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Legenda: BLK = Blank CAL = Calibrator S = Sample

### important notes:

- If the second filter is not available, ensure that no fingerprints or dust are present on the external bottom of the microwell before reading at 450nm. They could generate lake positive results on reading the action results on reading should locally be performed immediately after the acid solution but definitely no longer than 20 minutes afterwards. Some self-oxidation of the chromogen can record feature in a behavior action.
- can occur leading to a higher background,

## N. ASSAY SCHEME

Operations	Washing steps n° 4-5	Chromogen/Substrate 200ul	2" incubation 20 min	Temperature room	C. de la constante de la const	In 001	Operations Calibrators & samples Enzyme Conjugate 1º incubation Mashing steps Washing steps Chromogen/Substrate 2º incubation	100 ul 100 ul 100 ul 120 mil 137°C n° 4-5 200ul 20 min roam
------------	----------------------	---------------------------	----------------------	------------------	--	--------	---	---

## O. INTERNAL QUALITY CONTROL

A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm or S/Co values have been matched in the analysis.

Requirements   Requirements	> 1.000 CD450nm value	CAL ) USANI
Blank well < 0.100 OD450nm value CAL 0 up/ml < 0.200 mean OD450nm value after blanking	UU450mm > OD450nm CAL 0 us/ml + 0.100	Car of Linding
Blank well <0.100 OD450nm value	Co.200 mean OD450nm value after blanking	Or of the same
Blank well SO 100 CDASC Requirements	SOLD CONTROLL SOLD SOLD	CAI DING
Requirement	< 0.100 ODASO	Blank well
	Requirements	101000001

If the results of the test match the requirements stated above, proceed to the next section. If they do not, do not proceed any further and perform the following checks:

	> 0.100 OD450nm CAL 0 ug/ml > 0.200 OD450nm after blanking	Problem
3. Front no mistales has them done in the assay procedure (detamentation of positive cultivations that regulative one). The restoad of the regulative one).  4. That no constantination of the cultivator or all wests where the cultivator was dissurring the task cultivation was dissurred due to spital of positive samplies of the ensyme confugate.	. That the Chromogen/Substrate southern has only become containmented utting the passay.  I that the washing procedure and the washes selfings are as validated in the pre-qualification study.  I that the proper washing southern has been used and the washer has been primed with its before use.	Check

	7 2	CAL 1 rg/ml p
<ol> <li>that micropipettes have not become contaminated with positive samples or with the enzyme conjugate</li> <li>that the washer needles are not blocked or portially obstructed.</li> </ol>	i that the procedure has been correctly performed:  2 that no missake has occurred during its distribution (ex. dispensation of negative calibrator missay).  3 that the washing procedure and the washing strongs are as validated in the pre-qualification study.  4 that no external confamination of the calibrator has occurred.	I that the procedure has been correctly performed: 2. that no mistake has occurred ouring the distribution of the collaboration of dependently of the program of the washing the washing the washing are as validated in the pre-qualification study.  3. that he wishing procedure and he washer study are as validated in the pre-qualification study.  4. that no external contamination of the

If any of the above problems have occurred, report the problem to the supervisor for further actions.

## P. CALCULATION OF RESULTS

Ouantitative Assay:
Calculate the mean Ob450nm value of the calibrators. Then
draw a calibration curve possibly using a 4 parameters fitting
curve system. Then calculate on the curve the concentration of
HP antigen in the sample.

## Cut-Off = (CAL 0 + CAL 0.1) / 2

The test results are calculated by means of a cut-off value determined from the C450nm value of the CAL 0 ug/ml (CAL 0.1) with the and the OD450nm of the CAL 0.1 ug/ml (CAL 0.1) with the

Qualitative Assay:

Important note: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.

Q. INTERPRETATION OF RESULTS
In the quantitative assay, samples showing a concentration of
Huptor antiger higher than Dis upin are considered positive.
For the qualitative assay, test results are interpreted as a ratio of
the sample OD450nm (S) and the Cut-Off value (Co),
mathematic also, Tinch according to the Astronomicals. mathematically S/Co, according to the following table:

× 1.1	1.0 - 1.1	< 1.0	S/Co
Positive	Eguivocal	Negative	Interpretation

A negative result indicates that the patient is not infected by

Any patient showing an equivocal result should be retested on a

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	Date:
ł	

A positive result is indicative of HP infection and therefore the patient should be treated accordingly.

- Intervisiation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinfact pretations.
   Any positive result should be confirmed first by repeating the result should be confirmed first by repeating the result and then, if still positive, by an alternative method before a diagnosis of HP infection is confirmed.
   When test results are transmitted from the laboratory to enother department, alterniton must be paid to avoid
- Diagnosis of HP infection has to be taken and released to the patient by a suitably qualified medical doctor. This should be done taking also into account other diagnostic erroneous data transfer.

An example of qualitative method is reported below.

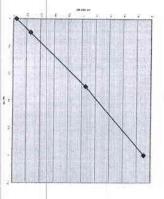
figures obtained by the Note: The following data must not be used instead or real

Cal 0.1 ug/ml: 0.210-0.230 UD450rm Mean Value: 0.220 CD450rm Higher than 6.0 u ug/ml + 0.100 – Accepted Cut-Off = (CAL 0 + CAL 0.1) / 2 = 0.135 Calibrator 1 ug/ml: 2.000 CD450rm Lower than 0,200 - Accepted 0.040 -0.060 OD450nm 0.050 OD450nm

Sample 1: 0.028 OD450nm Sample 2: 1.690 OD450nm Sample 1: \$/Co < 1.0. = nega OD450nm higher than 1,000 - Accepted

Sample 2 S/Co > 1\_1 = positive

An Example of Calibration curve is reported below:



R\_PERFORMANCE CHARACTERISTICS
Evaluation of Performances has been conducted by testing negative and positive samples in an external clinical site.

1, Limit of detection
The limit of detection of the assay has been calculated by examining serial diution of HP antigen in Sample Diluent. Results of Quality Control show that the analytical sensitivity of the assay is better than 0.05 upfird when the limit of diution is considered mean OD450nm CAL 0 upfin! + 5 SD.

extraction device supplied by Dia Pro srl.
A sensitivity of about 98% was found for n = 55.

### S. LIMITATIONS

False negative results were obtained from samples extracted from specimens stored for more than 1 day at 2, 8°C. False positive results were mostly obtained from samples still containing heavy stort bodies.

2. Diagnostic sensitivity.
The diagnostic sensitivity has been tested on parels of samples classified positive by a US FDA approved kit based on "breath test", considered by the medical literature the Gold Standard for HP Ag determination. Samples to be examined in the kit were prepared from the same specimes and extracted with the

Diagnostic sensitivity was also examined by comparison to a commercial EUSA, produced in USA. Samples to be examined in the kit were prepared from the same specimens and extracted with the extraction device supplied by Dia Pro stf...

A sensitivity of about 95% was found for n = 64

3. Diagnostic specificity:

The diagnostic specificity has been tasted on panels of samples classified negative by a US FDA approved kit based on "breath test", considered by the medical literature the Gold Standard for HP Ag determination. Samples to be examined in the kit were prepared from the same specimens and extracted with the extraction device supplied by Dia Pro sft...

A specificity of about 95% was found for n = 25.

The property of the same specimens are specimens and extracted with the extraction device supplied by Dia Pro sft...

The property of the same specimens and extracted with the extraction device supplied by Dia Pro sft...

A specificity of about 80% was found for n = 20.

No prossersation device supplied by Dia Pro sft...

No crossreaction was assessed with Campilobacter species

Precision:
 4. Precision:
 The variability shown in the lables did not result in sample misclassification. CV values ranging 4-8%, depending on OD450nm values were observed.

## ASSAY GRAPHICAL SCHEME

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Add 100 µl Calibrators, samples and conjugate to the plate and then incubate for 120 min at +37°C



Wash as described in the proper section

 $\prec$ 



Add 200 µl Chromogen/Substrate and incubate 20 min at r.t.



Add 100 µl Sulphuric Acid



Read the plate at 450nm (reading) and

at 620-630nm (blanking)



All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Dia,Pro Diagnostic Bioprobes Srl Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

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BIBLIOGRAPHY

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# IIV Ab&Ag

Fourth generation Enzyme Immunoassay Human Immunodeficiency Virus or HIV type 1&2&O and P24 HIV-1 Antigen for the determination of antibodies to in human serum and plasma

- for "in vitro" diagnostic use only -



## DIA.PRO

20099 Sesto San Giovanni Via G. Carducci nº 27 Diagnostic Bioprobes Srl

(Milano) - Italy Fax +39 02 26007726 Phone +39 02 27007161

96/192/480/960 Tests REF IVCOMB.CE

## HIV Ab&Ag

A INTENDED USE

The kit is a solid phase enzyme immunossay for the in-vitro diagnostic screening of antibodies to all subtypes of HIV-1 and HIV-2 and HIV-1 artigen (p24) in human serum or plasma. This sit is intended exclusively for the vitro diagnostic use in an authorized clinical libertatory and the test has to be carried but by specifically trained health-care professional personnel.

### B. INTRODUCTION

Epidemiological evidence indicates that an infectious agent fransmitted through intimate contact, intravenous drug use or use of infected blood or blood products leads to Acquired

Immunodeficiency Syndrome (AIDS).
This disease affects 1-cell mediated immunity, resulting in severe symphopenia and a reduced subpopulation of helper 1-lymphocytes. Destruction of this 1-lymphocyte population by the virus causes an immune deficiency, resulting in a reduced or deficient response to subsequent infections.

Consequently, infections become more severe and may cause death, AI present, there is no successful treatment for AIDS.

Immunodeficiency vitus type 1 (FWV-1).

A classify related, but distinct type of immunodeficiency vitus, designated HV-2, has also been isolated. This vitus causes a disease that is indistinguishable from AIDS.

Servlogical cross-reactivity between HV-1 and HIV-2 has been shown to be highly variable from sample to sample.

This variability requires the inclusion of artigens to both HIV-1 and HIV-2 for the screening of antibodies to HV-1 and HIV-2 and or screening of antibodies to HV-2 and consequency. The presence of anti-HIV-1 and/or anti-HIV-2 and/or HIV-1 and/or HIV-2 and consequency this blood should not be used for transitission or for manufacture of injectable products. The etiological agent has been identified as a retrovirus, human

C. PRINCIPLE OF THE TEST synthetic peptides representing immunodominant epitopes of HIV-1 and HIV-2 together with a monoclorial antibody to p24 HIV-1

The peptides and the antibody have been carefully selected to ensure the screening of antibody have been carefully selected to ensure the screening of antibody and p24 antigen to all HIV.1 subtypes, including subtype 0 and HIV.2. Serum or plasma samples are added to those wells and if antibodies specific to HIV.4 will form stable complexes with the HIV.2. Serum or plasma is an added the period of the period of the serum of the sample, they well, in case HIV.1 p24 is present in the sample, the antigen will be addition of 1) biolinylated peptides, a biolinylated in the serum of the period peptides, a biolinylated in the serum of the period peptides, a biolinylated in the serum of the period peptides and the serum of the serum of the period peptides. The involution of these artibody antigen complexes.

The involution antibody to HIV-1 p24 and; (2) horseradish peroxidase allows for the quantification of these artibody antigen complexes.

During incubation, a blue color will develop in proportion to the amount of anti-HIV-1/2 antitipoles or HIV-1.1 p24 antigen bound to the well of the service of the serv

A stop solution is added to each well and the resulting yellow color is read on a microplate reader at 450 nm.

### D. COMPONENTS

The standard format of the product code IVCOMB.CE contains reagents for 192 tests.

1. Microplate MICROPIATE
11 2 strics of 8 heakable wells coated with HIV specific gsds, gs41 and gs120 peptides and with a Monccional Antibody specific to the HIV-1 p24 Ag. Plates are scaled into a bay with discious.

2. Negative Control CONTROL 1
1x4.bml/vol. Ready to use control. It contains animal serum negative for HIV ambiocities and for p24 antigen and 0.1%. Kalhon GC as preservatives. The negative control is yellowbrown calor coded.

1x4. Omt/viel. Ready to use control. It contains inactivated HIV.1 antibody positive serum, filtered HIV. Ab&Ag negative animal serum and 0.1% Kathon GC as preservative. The Positive Control is light green color coded.

The positive control has been machinated using Enropinovalcrition. EPU.UV. This does not fully ensure the particled as potentially biohazardous, in accordance with good inhoration or machinated. 3. Positive Control HIV-1 Ab CONTROL 1+

4. Positive Control HIV-2 Ab ONTROL 24

1x4. Omivial. Ready to use control. It contains inativated HIV2
antitody positive serum, filtered HIV AbAd negative animal
serum and 0.1% Kathon GC as preservatives. The Positive
Control is daily green color coded.
Control is daily green color coded.

In produptionations BPL/UV. This does not fully ensure the
absence of violative participants, and therefore, the control stoud be
handled as potentially bichazandous, in accordance with good
lishoration markiese.

## 5. HIV-1 P24 Ag Calibrator CAL Ad

2 vials. Lyophilized. It contains not infectious recombinant p.24 antigen in a 10 mM phosphate buffer pH 7.0+4.0.2 with 0.3 mg/ml Gentlamicine. Sulphate and 0.1% Kalihon GC as stabilizers. This component is calibrated against the NIRSC 1\* International HIV-1 p.24 Ag reference sample 90/636 (diluted 1:256) as well as the EFS HIV Ag performance panel (3015the Calibrator

1) The Calibrator contains p.24 recombinant Ag with a concentration of about 410/ind. 2) The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

6. Wash buffer concentrate WASHBUF 20X 2x0 m/koutle. 20x concentrated solution. It contains 0.1% Xafhon GC, Once diluted, the wash solution contains 10 mM phosphate buffer saline pH 7.0+/0.2 and 0.05% Tween 20.

7. Conjugate # 1 CONJT|

8 vals. The vial combins (vophized blotinyates HIV18280 gp85, gp41 and gp120 peptides and a biothysteet monoclonal antibody specific for HIV 1 p24 antigen. Vials are to be resuspended with 5 nt of the Conjugate # 1 diluent.

8. Conjugate 1. Diluent CONLTEDIL 1800 milotet. Used to dissolve the typohilized powder of Conjugate # 1, it condains Tis saline Buffer supplemented with 0.05% Kalhon GC, Tween 20 and BSA.

Conjugate #2 SONJ 2
 NaSmilbottle The solution contains HRP conjugated with streptavidin in Tris saline Surfer supplemented with 0.05% Kaltinn GC. Tween 20 and BSA, This component is color coded in pink/red.

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10. Chromogen/Substrate SUBS TMB 1x45mibotite Ready-fo-use component. It contains 50 mM clirate outer; pt. 3,5–3,8,4% interphysiophoside, 0.03% tetramethy-benzidine or TMB and 0.02% hydrogen peroxide or 120.2% interphysiophoside.

: To be stored protected from light as sensitive to strong

14. Sulphuric Acid <u>PISOs O.3 M</u> 1x25m/bottle it contains 0.3 M H-SOs solution. Attention: Irriant (PI315, H319, P280, P302-P352, P305+ P351+P338, P337+P313, P362+P363) 332+P313

12. Sample Diluent: DILSPE
1x14mi/vial Contains Tre saline buffer supplemented with 0.05% Kalhon GC, anti HAMA blocker, and I ween 20; used for specimen

13. Plate sealing foils dilution. This companent is color coded in light blue.

n° 4

14. Package insert

Important note: Upon request, Dia.Pro can supply reagents 96, 480, 960 tests, as reported below: ₫

Code	Number of tests	1 Africaçãos 2 Alegaños Correo 3 Areathe Control 2 4 Pecidos e Correo 4 Areatos e Correo 5 (Vant buf conc 7 Conyugato e 1 5 Conyugato e 1 5 Conyugato e 1 5 Conyugato e 6 5 Conyugato e 6 1 Co
NCOMB.CE.96	35	n*1 12.0ml/vai 122.0ml/vai 122.0ml/vai 122.0ml/vai 122.0ml/vai 1460ml/boil 1460ml/vai 1415ml/vai 14
NCOMB.CE.480	480	n*S 1x10ml/vial 1x
NCOMB.CE.960	050	n*10 razontivial razontivial razontivial razontivial razontivial n*10 vials ax150m/bollos ax150m/bollos ax150m/bollos ax150m/bollos ax150m/bollos ax150m/bollos ax150m/bollos ax150m/bollos ax150m/bollos ax15m/bollos

- "MATERIALS REQUIRED BUT NOT PROVIDED
  Calibrated Micropipettes (200ul and 10ul) and disposable
  plastic lips.
  EA grade water (bidistilled or defonised, charcoal treated to
  remove oxidizing chemicals used as disinfectants).
  If there with 60 minute range or higher.

- Absorbent paper tissues.
   Calibrated ELISA micropiate thermostatic incursory to provide a temperature of 43°C.
   Calibrated ELISA microwell reader with 45 and with 620-630m (blanking) filters.
   Calibrated ELISA microbiate washer,
   Vortex or similar mixing bools. O1 thermostatic incubator capable
  - 450nm (reading)

- F. WARNINGS AND PRECAUTIONS

  1. The kit has to be used by skilled and properly trained to the control of a medical control of a medical doctor responsel only, under the supervision of a medical doctor responsels of the laboratory.

  2. When the kit is used for the soriening of blood units and plood components, it has to be used in a laboratory certified and plood components, it has to be used in a laboratory of thealth.

  3. When the kit is used for the soriening of blood units and plained by the national authority in that field (Ministry of Health or similar antify) to carry out this type of analysis.

  3. All the personnel involved in performing the assay have to the personnel involved in performing the assay have to the personnel involved in personnel involved should be trained to be used of any short of the personnel involved should be trained to be used of any short of the personnel involved should be trained to be used to any short of the personnel involved in the National Disease Control. Atlanta, U.S. and reported in the National Institute of Health's publication: "Blogaffety in Microbiological and Bornadical Laboratories", ed. 1934.

... All the personnel in vaccinated for HBV and H. safe and effective.
5. The lahr-avniring ersonnet involved in sample handling should be HBV and HAV, for which vaccines are available,

avoid containinants such as dust or air-born microbial agents opening it vals and micropiates and when performing the Protect the Chromogen/Substrate from strong light and vibration of the bench surface where the lest it The laboratory environment should be controlled so as contaminants such as dust or air-born microbial agent

Do not interchange components between different lots the kits. It is recommended that components between two in

using disposable tips and changing them after each between

Avoid cross-contamination osable tips and changing ss-contamination between kit reagents by using and changing them between the use of each

Do

discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivated before waste. Suggested procedures of inactivated are treatment with a 10% final concentration of household bleach for 15-18 has or heat inactivation by autoclave at 121°C for 20 min. 15. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with noteshold bleach and then with water. Tissues should then be discarded in proper distributions are should then be discarded in proper distributions of the proper tissues soaked with noteshold bleach and then with water. Tissues should then be discarded in proper distributions of the proper tissues to the proper tissues of the proper tissues the proper tissues the proper tissues to the proper tissues to the proper tissues to the proper tissues the proper tiss

surface with plenty of water.

12. Other waste materials generated from the use of the kit (example, tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMENDATIONS
1.Blood is drawn asspitically by venepuncture and plasma or
serum is prepared using standard techniques of preparation of
samples for clinical laboratory analysis. No influence has been
observed in the preparation of the sample with citate, EDTA

2. Avoid any addition of preservatives to samples, especially sodium actide as this chemical would affect the enzymatic activity of the confligate, penerating tisse requires testils.

3. Samples have to be clearly dendited with codes or names in order to avoid mainterpretation of results.

When the kit is electronic reading is strongly iscommended.

4. Haemotysed (red) and visibly hyperthramic [milky] samples have to be discarded as they could generate false results.

receipt, store the kit at 2,8°C into a temperature refrigerator or cold room.

of the same lot should not be interchanged.

8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit

not use the kit after the expiration date stated on the

12. Treat all specimens as potentially infective. All human serum specimens should be handled at Bissalety Level 2, as recommended by the Center for Disease Control Athania U.S. in compliance with what reported in the Institutes of Health's publication. "Biggately in Microbiological and Biomedical Laboratories," ed. 1984.

13. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid

Waste produced during the use of the kit has to

# Sample Diluent

The Lyophilized Calibrator Ag contains a non-infectious recombinant p24 antigen. The volume of EIA grade water to be used for its dissolution and to reach the appropriate p24 concentration (about 100 pg/m) is written on the vigil label. To help dissolve the lyophilized pellet, vortex a few times, at regular intervals. Complete dissolution should be achieved Note: When dissolved the Calibrator is not stable. Store in aliquods at -20°C.

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Samples containing residues of fibrin or heavy particles or microbial framents and booles should be discarded as they could give rise to lease results.

Sere and plasma can be stored at +2°.8°C for up to seven days after collection. For longer storage periods, samples can be stored frozen at -2°C for several months. Any frozen samples should not be frozen/thaved more than once as this may generate particles that could affect the test result.

If particles are present filter using 0.2-0.8u filters to dean up 7. Do not use heat inactivated samples as they could give

# H. PREPARATION OF COMPONENTS AND WARNINGS A sludy conducted on an opened kit has not shown any relevant loss of activity up to 2 months.

Microplates:

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the pouch is not broken or that some defect is present indicating a problem of storage. In this case call Dia-Pro's customer service.

Unused stings have to be placed back into the aluminum pouch, in presence of desiccant supplied, firmly zipped and storad at +2".8"C. When opened the first time, residual strips are stable

Ready to use: Mix well on vortex before

Negative Control

Positive controls are ready to use. Handle Positive Controls as potentially infective, even if HIV, if present in the control, it nas Ab

Wash buffer concentrate:

The 20x concentrates solution has to be dibited with EIA grade water up to 120 ml and mixed gently end-onex-end before use. As some self-crystals may be present into the vial, take care to dissolve all the content when preparing the solution. In the preparation avoid foaming as the presence of bubbles could give origin to a lead washing afficiency, important Note: Once diffued, the wash solution is stable for 1 week at +2.8° C.

Conjugate # 1:

The Conjugate # 1 mix solution must be prepared immediately before starting the test. Add 6 ml of Conjugate 1 diluent directly to one Conjugate # 1 vial to dissolve the lyophilized powder. This preparation (a total of 6 ml in one vial) is sufficient for 22 tests, or 4 compilete verifical strips of the micropiate. To help dissolve the lyophilized powder, vortex a few linnes, at regular intervals.

Important Note: Any unused portion of this reconstituted Conjugate # 1 Solution may be stored at +2,8° C for no more than 12 hours. After this time it has to be discarded and the empty, used container has to be washed with EIA grade water and kept dry for any following re-use.

use reagent. Mix well end-over-end before use

Chromogen/Substrate:

Ready to use. Mx well end-over-end before use.

Be cateful not to contaminate the liquid with oxidizing chemicals air-driven dust or microbes.

Do not expose to strong illumination, oxidizing agents and metallic surfaces. If this component has to be transferred use only plastic, possible sterile disposable container;

Sulphuric Acid:
Ready to use. Mix well end-over-end before use.
Aftention: Irrilant (H315, H319: P380, P302+P352;
P305+ P351+P338, P337+P313, P362+P364). 332+P313

Legenda.

Warning H statements: H315 – Causes skin irritation, H319 – Causes serious eye Irritation,

Preceutionary P statements: P280 - Wear protective glo protective gloves/protective clothing/eys protection/face

protection.

P302 + P303 - IF ON SKIN: Wash with planty of scalp and water.

P302 + P313 - If skin inhalmon occurs: Get medical advisoration in the passes of the passes o

Ready to use, Mix well end-over-end before use

# L INSTRUMENTS AND TOOLS USED IN COMBINATION

volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a truenase of 4.2%. Bocontamination of spills or residues of k1 components should also be carried calibrated to deliver the

of regularly.

The ELISA incubator has to be set at 437°C (oberance of 4-0.5°C) and regularly checked to ansure the correct temperature is maintained. Both dy incubators and water haths are suitable for the incubations, provided that the instrument is validated for the incubations, provided that the instrument is validated for the incubations, provided that the instrument is validated for the incubations, provided that the instrument is validated for the incubations. Provided that the instrument is validated for the incubation of ELISA basis.

The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit for notine laboratory reference panels, before using the kit for notine laboratory validation of 350 u/well of washing solution = 1 cycle) are sufficient to ersure that the assay performs as expected. A soading since the controls and well characterized negative of 350 u/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soading since the values reported below in the section "validation of Tasy's and passay with the kit controls and well characterized negative values reported below in the section. Validation of Tasy's and chearing of needles) of the washer has to be carried out the values reported below in the section. Passay Prochamination according to the instructions of the manufacturer.

The characterized of the washer has to be carried out a forcus of 450m and with a section of their (200.430m and the carried out of the section 14 section 14 section 15 control of the value of the section 14 section 16 control of the value of the value should be section 16 of 16 control of the value of the value of the section 16 of 16 control of the value of the v

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that the correct optical density is measured, it should be regularly maintained according to the manufacturer is

regularly services of the transfer of the sections of the section of the sections of the section of the sections of the section of th ng an ELISA automated work station, all critical

œ

# PRE ASSAY CONTROLS AND OPERATIONS Check the expiration date of the kit printed of

1. Check the expiration date of the kit printed on the external able of the kit box. Do not use if expired.
2. Check that the fluid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colories or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no bestvage occurred in transportation and no spillage of liquid is present inside the box. Check that he aluminum pouch, containing the microplate, is not punctured or demanated.

as described above.

A Dissolve the Calibrator Ag.

5. Dissolve the Calibrator Ag.

5. Dissolve the Calibrator Ag.

6. Dissolve the Calibrator Ag.

7. Dissolve the Calibrator Ag.

8. Conjugate # 1 him to Calibrator 1 Diuent (1 lyaphilized conjugate # 1 him to Experiment to the Calibrator Ag.

8. Allow all the other components to reach room temperature (about 1 hy) and then mix as described.

9. Set the ELISA incubator at 37°C and prepare the ELISA washer by priming with the diluted washing solution, number of washing sycles as found in the variedation of the instrument for its use with the kit.

8. Chack that the ELISA reader has been turned on at least 20 minutes before reading.

9. If using an automated workstation, turn it on, check settings and be sure to use the night assay protocol.

10. Check that the micropipettes are set to the required volume.

11. Check that all the other equipment is available and ready.

12. In case of problems, do not proceed further with the test and

## M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

1. Automated assay:
In case the test is carried out automatically with an ELISA system, we suggest to make the instrument dispense 50 ul sample Daluent first and then 150 ut controls and samples, before the next sample is assignated, needles have to be duly washed to avoid any cross-contamination among samples or

0

œ 7 Wash the microplate with

delivering and aspirating 350ulfwell of diluted washing solution as reported previously (socilion 1,3). Phother 150 ul Conjugate # 1 mix, propared as described before, into each well, except the 1° blanking well, and

Important note: Be careful not surface of the well with the tip Contamination might occur. filled with the Conji

10.9

Important Note: This solution must be added to the bottom of sach well-to ensure proper performance, macigually mixing of the two solutions (Conjugate 1 and Conjugate 2) may reduce the binding of streptavidin HRP (Conjugate 2) to the bindinylated respents and consequently affect the performance of the assay. Be sure to provide an adequate mixing when the Conjugate a 2 is added both in the manual and in the automated procedures.

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Incubate the microplate sealed for 30 min at +37°C.

Wash as in section 7.

Dispense 200 ul of Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at room temperature (18-25°C) for 30 minutes. Start the iming immediately after addition of this component to the first well.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

The correct number of hysphilized Conjugate # 1 must be dissolved early with 5 mt. Conjugate # 1 Diluent. Once the hysphilized powders are dissolved and mixed well, they are to be mixed together into a plastic container and the assay may

L Manual assay:

Discove the right number of lyophilized Conjugate # 1 with Conjugate # 1 Diluent before starting to dispense the samples and controls of the test.

Place the regulared number of strips in the microwell holder:
Leave the 1" well empty for the operation of blanking.
Dispense 50 ut Sample Diluent in all the wells, except A1

Important note: Strips have to be soaled with the adhesive seating this supplied, only when the test is carried out manually. On not cover strips when using ELISA automatic instruments.

with an automatic washer by 350ul/well of diluted washing

Conjugate

Incubate the microplate for 30 min at +37°C.

Pipethe 100 ul of Conjugate # 2 in all the wells, except A1, and gently agitate the microplate to mix the two conjugates.

ips have to be changed.
For the next operations follow the operative instructions reported below for the Manual Assay.

used for blanking.

4. Dispense 130 ut of Negative Control in triplicate, 150 ut HIV1 Positive Control, 150 ut HIV2 Positive Control and 150 ut of Calibrator Aq in duplicate in proper wells.

5. Dispense 150 ut of Sample in each properly identified well.

Mix. gently, the place on the work surface, evoiding overflowing and contaminating adjacent wells, in order to fully disperse the sample into the dilunct.

6. Incubate the micropiate for 60 min at +37°C. 91

## N. ASSAY SCHEME

Reading OD	Sulphuric Acid	emperature	incubation	MB/H2O2	Wash step	emperature	3 incubation	Conjugate # 2	remperature	2 incubation	Conjugate # 1	Wash step	emperature	1" incubation	Samples	Controls and calibrator	Sample Diluent	Wethod
450nm	100 cd	17	30 min	200 ul	4-5 cycles	+37°C	30 min	100 ut	+37°C	30 min	150 ul	4-5 cycles	+37°C	60 min	150 ut	150 ul	50 ul	Operations

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dispensation scher
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Ω.
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1	12	ú	4	G	61	7	90	ω	10	-	
ST.K	CALAg					ı		1			1
NC	CAL Ag		4	4	4		1	1			
NC	S1			4		1		1			
NO	S2	1	4	4	4		1	4			
POS 1 Ab	53		1	4		1		1			
POS 1 Ab	54		1	4	1	1		1			
POS 2 Ab	SE	I	Ц	4	4	4	1	4			I
POS 2 AL				4	1	1	1	4			

\* WOOME GI

Should these problems happen, after checking, residual problem to the supervisor for further actions report

### nortant note: h background Do not expose to i might be generated. strong direct illumination

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14. Pipette 100 ul Sulphuric Acid into all the wells using the same pipetting sequence as in step 13 to stop the enzymatic reaction. Addition of acid will turn the positive controls and positive samples from blue to yellowitowan.
15. Measure the color intensity of the solution in each well, as described in section 1.5, at 450m filter (reading) and at 620, 630mm (background subtraction, strongly recommended). 630nm (background subtraction, strongly blanking the instrument on A1.

Important notes:

1. If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450mm. Finger prints could generate false positive results or reading.

2. Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 30 minutes after its addition. Some self-outside on the chromogen can occur feating to high background.

3. The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.

O.INTERNAL QUALITY CONTROL A Check is carried out on the controls and the calibrator any time the kit is used in order to welfly whether I heir OD450nm values are as expected and reported in the table below.

HIV Ag Calibrator	Positive Control	Positive Control	Negative Control (NC)	Blank well	CORCA
S/Co > 1	Mean C0450mm ≥ 0.700.	Mean OD450nm ≥ 0,760;	4.0.200 mean OD450nm value after blanking Absorbance of kindhóuai negative control values must be lesso than or equal to 0.200, if one value is outside this range, discard this value and resolicable mean, if two values are outside this range the control to the propelled.	< 0,100 OD450nm value	Requirements

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and operate as follows:

	HIV Ag Calibrator S/Co < 1	Positive Controls <0.700 OD450nm	with Good In 19	Negative Control (NC) > 0.200 OD450nm	Blank well
stribution of contrigits written det Ca e negative control (size > 0.200, too. that the washing pu trings are as validation). That no atternal con- mind has cocurred. That the tyophilize arrectly with the co-	dure has been con	I that the precedure has been correctly executed:  2. Plat no mislake has been done in the distribution of contick (dispersation of register control interest of positive control, in his case, the negative control will have an OM-50mm value > 0.200 (p.o.)  3. that his washing procedure and the washing strings are as validated in the pre qualification study.  4. that no external contamination of the positive control has occurred.	and the washer has been micropials of the popular washing southing has been the saay procedure (dispiration of positive control instead of regative control instead of regative control instead of regative control in the openitor of the regative control of the regative control of their walls has occurred due to positive samples, to spills or to the enzyme conjugate. Si that indicapitations haven't got contaminated with positive samples or with the enzyme conjugate only of the washer needles are not blocked or parallely posttruies?	<ol> <li>that the washing procedure and the washer settings are as validated in the pre qualification study.</li> </ol>	1. that the Chromogen/Sustrate solution has not

any

и.
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P. CALCULATION OF THE CUT-OFF
The tests results are calculated by means of a cut-off value determined with the following formula on the mean OD450nm value of the Negative Control (NC):

## NC + 0.125 = Cut-Off (Co)

The value found for the lest is used for the interpretation of results as described in the next paragraph.

Important note. When the calculation of results is done by the operative system of an ELISA automated work silation be sure that the prosper formulation is used to calculate the cut-off value and generate the right interpretations of results.

Q. INTERPRETATION OF RESULTS
Test results are interpreted as ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following table:

~ <u>`</u>	^1	S/Co I
Positive	Negative	nterpretation

A negative result indicates that the patient has not been infected by HIV.

If the initial absorbance value is equal to or greater than the cutoff value, retest the sample in duplicate. If both relest values are
less than the cut-off, the interpretation is not reactive for HIV.

If one or both retest values are equal to or greater than the cutoff the interpretation of the test results is repeatedly reactive.

The sample should be considered reachive for HIV antibody and/or and/an according to the criteria of this HIV is a larger than the cut-

patient should be treated accordingly. A positive result is indicative of HIV infection and therefore the

### Important notes:

Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.

Prepeatedly reactive speciments stoud be submitted to a Confirmation Assay before diagnosts of HIV intection is

released.

3. When lest results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.

4. Diagnosis of HIV infection has to be done and released to the patient only by a qualified medical doctor.

## An example of calculation is reported below:

The fallowing data must not be used instead or real figures obtained by

OD450nm Mean Value: 0.115 OD450nm Lower than 0.200 - Accepted

HIV 1 Ab Positive Control: 2.000 OD450nm mean Higher than 0.700 — Accepted

HIV 2 Ab Positive-Control=2-100-00450rm nrean value Higher than 0 700 – Accepted

Calibrator Ag: 0.322 OD450nm mean valus S/Co > 1 - Accepted

-Cut-OH = 0-115+0 125 = 0.240 Sample 1: 0.070 OD450nm Sample 2: 1690 OD450nm Sample 1: S/Co <1 = negative Sample 2: S/Co > 1 = positive

NiBSC 1<sup>st</sup> International reference Reagent for HIV 1 Agroups tode 90536 - (Version 4, 12 May 2009)

Sample Lot# 1 Lot# 2 Lot# 3

IU/IIII 10045 11. Lot# 3 Lot# 3

IU

BBI Anti-HIV 1 Low Titer Performance Panel - PRB 107

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R.1 ANALYTICAL SENSITIVITY
The limit of detection (or analytical sensitivity) of the assay has been calculated by means of preparations specific for HIV.1 and HIV.2 and body and HIV.1 p.24 Ag detection, supplied by NIBSC Blanche Lane South Mimms Potters Bar Heritordshire EN6

Samples were diluted in HIV Ab&Ag negative plasma to generate limiting dilution curves and examined in duplicate. The tables below reports the mean OD450nm values and the S/Co index:

		code	code 99/674 - 8	203	1	
piq	# 10.1	9506	100.0	0706	101#	2000
tion	OD450nm	S/Co	OD450nm	S/Co	00450nm	0000
*	- Carrier	1	-			0000
ľ	19AD	214.3	Jevo	>35.0	DVE	×15.1
×	3.835	14.1	3,765	52.5		144
×	2.371	cn -4	2.268	e 7	2210	0.0
~	1.253	ži. D	1 007	-	10.0	200
	0.000		1,000	4.5	1,140	4.4
P	0.700	2.0	0.712	17.4	0.735	2.8
×	0.462	-	0.439		2840	0
×	0.281	2	0 100		4	01.0
1	102.0	1,0	0.250	1.0	0.294	-
A	681.0	0.7	6,171	0.7	0 174	7
	3 4 4 5		Contract of the last			40.00

The device sh

		code	99/750 -0	97		
Sample	Lot#	9050	2012	2706		200
District .	00000	-		41.44	1000	OBO
Suprom	MUNICIPAL	5/00	0D450nm	SICo	00450nm	500
	2000			-		4
	4.200	0.1	Ш	6,0	2.142	2.7
VE	0000					
100	0.000		0.925	3.6	1.027	i i
h	0.475		2000		1	
	2000	2.4	2000		0.486	

# NIBSC British working standard for anti HIV 1 code 99/710 – 007

Sample	Lot#	9050	Lot#	0706	Lota
Dilution	3	S/Co		250	200
10	1				1000
X		3.4		140	25
90	J				15.00
44		2.5		8.2	20
X		6.4			
9				4.5	1.2
OX	J	27		7.8	0.7
+20	1			200	0.00
100		4		1.3	0
32					
02.0		22.8		9.9	0.0
dilluent	Ц	2		2	
10000	L	0.0		0.5	0

Sample	# 101	9050	3506 Lot#	0706	101#	2000
Dilution	OD450nm	S/Co	00450nm	2000	DOMEST	2000
			-	2700	DIMAGENCE	27.00
×	gver	>14.5	Javo	0.534	DVEY	200
36	2000	-	-		0.00	-182
64	2,030	14,1	3,765	ŝ	3.774	144
4X	2.371	en -4	2268	8.7	2220	
H.			2000	5,1	61018	5.3
ex.	1.600	30	1.097	t	1 145	40
16x	0.700	2.6	0.749	2.4	25.0	3
350	2000			1	0,000	200
XXC	7000	1.1	0.439		0.483	1
64x	0.281	1 10	0.360		2000	100
1700	2400			1,00	4.534	11.1
1007	681.0	0.7	6,171	0.7	0 174	07
diluent	0.140	2.0	0.122	3.0	0 121	9

Dic	NIB
Lot#	SC Britis
8050	ih worki code s
2012	ng stand 99/750 —
9079	dard for 007
	r and HIV
2000	

	ı					
Sample	Lot#	9050	Lot	0706	Lot#	ngna
Dilution	7	S/Co		000	200	
	П	100		0/00	COASGAM	Sico
×		8.1		6.0	2 142	2.0
J.	1	-				0.4
63	256.0	1.7	0.925	3.6	1.027	u a
No.	27.70	4			1000	200
NA.	C1670	1.7	0.485	10	0.486	0
400	205.0				1000	900
000	0.630	1.1		7.2	0.289	
YEN	0.043	2	1		-	
100	0.414	C.X		0.8	0.220	0
dillent	0.470	000	ı		Charles of	0.0
Distriction of the last	0.740	Co		05	0.434	2

			4011 100	5		
Sample	Lot#	0506	Lot#	0706	100	2
Direction	2000		-	4.00	40,100	c
Commission	COSCODIN	2/00	OD450nm	S/Co	00450nm	0
44	000	200		1	tilling to the same	0
	0.000	2.2	0.611	12	0.607	2.5
24	0 204			-		Ī,
200	6.000	100	605'0	1.2	0.312	
22	0.102		3			1
1	W. 120	1,0	0.210	0,8	0.192	_
diluent	0.140	0.5	0 100	200	2	I
			4		10.73	

The devise shows a limiting dilution value at about 2x

## NIBSC HIV-1 p24 Antigen Monitor Sample code 02/146-002

				ř		
Sample	Lot#	0508	Lot # 10-1	0706	1	3000
Dilution	00450nm	S/Co	OD450nm	Sign	1	2
40	200+			3	3111	07.00
×	3.004	13.4	3.650	140		110
24	2454	,			ľ	100.00
400	101.3	2.9	2733	8,2		0.6
4X	502 c	44	1 178	2.3		
n	0.220	-				4,0
200	0.739	17.7	0.729	1.00		3.0
XOI	0.388		CVEU		I	
270	0.350			74.8		4.4
02.0	0.200	2.8	0.236	9.9		0.0
diluent	0.140	0.5	0.122	5	0.131	2

## NIBSC anti-HIV 2 monitor sample

Sample	£101	9050	3506 Lot#	0706	101#	2000
Dilution	OD450nm	S/Co	00450nm	5000	ODASON	2000
2				2000	DIMACADO	0/0
>	Jake	>14.5	Jevo	V 25.0	DVSF	4
34	1001			I	0.0	7 747-
64	2,030	14,1	3,765	32.5	3.774	14.4
X	2.377	00	2.268	8.7	2210	0.0
8×	1.253	24 10	1 007	-	21010	0.0
100	1		1000	4.5	1,140	44
103	0.700	2.0	0.712	17.7	0.735	2.8
SEX	0.462	-	0.439		7940	1
64×	0.281	7 10	0300		4.100	2,00
1700	0.400		10.000	7,50	0.289	1
VON	F. 108	0,7	6,171	0.7	0.174	0.7
tuento	0,140	0.5	0.122	0.5	0 131	0

2, 190	100	100	2004	483	700	252
0.5	1.0	100		100	3 3	
0.122	6,771	0.250	0.438	217.0	7,60.1	1
0.5	0,7	1,0	-	12	0	51.1
0.131	0,174	0.284	0.483	0.735	1.140	610.3
0.5	0.7		00	2.8	4.4	5,9

l		0000	100-001/66	10		
pic	Lot#	9320	101#	0706	# tot#	none
tion	00450nm	S/Co	-	200	COARO	2000
	2000		The state of the state of	0100	CONSTRUCTO	SICO
×	4.400	8.1	2,086	6,0	2,142	2.3
ľ	0.999	7.7	0.925	3.6	1 027	4
*	0.475	7	7,295	3		
1	2000		47.700	4,44	0,400	4.5
ľ	56775	1	0.301	1.2	0.289	
×	0.212	8.0	0.206	0 8	0.220	
P P	0.470	0.0			O'STATE OF	0.0
1	0.140	0.0	U.722	0.5	0.131	0.5

The devise shows a limiting dilution value at 8x.

		2000	2011 10 -0	37		
Sample	Lot#	0506	Lot#	0706		2000
Director	200450			4.00	40 1000	Date
Commission	COASODI	2/00	OD450nm	5700	ODAGO	200
14	0 000			0	Consolition	3700
	0.000	2.2	0.611	N	0.807	22
24	0 304					
200	0.000	100	6.308	1.2	0.312	17
X	0.103	0.7	2000			
			2,5,10	0,0	281.0	0.7
dillent	0.140	0.5	0.122	000	24	2
				5.0	2000	

		2000	P-040 140-0	100		
ample	Lot#	9050	E # 10	0706	H	2000
Hution	00450nm		O0450nm	250	1	2
4	200		1	40.00	1111	9700
X	3.654		3,650	140	1	120
2	231.0					10.0
44	101.7		2 133	2,2		3.0
4k	9 200		4 4 30		ı	200
1			11/0	4.3		4.5
OX	J		0 770	7.0	Į	
400	1			- Contract		10,32
100			0.342	1.3	Ш	4.2
32*	Ш				L	4.4
1	L		0,236	9.9		0.0
tuann		0.5	0.122	200	0.131	200

The devise shows a limiting dilution value at about 16x.

R. PERFORMANCES
Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of I/O Directive 98/79/EC).
The performance evaluation was carried out both in an external centre of excellence for I/IV diagnosis; that examined the device on a population of antibody positive and negative samples against a CE-marked kit used in the aboratory as reference, and in Diafro's laboratories as well to complete the study.

# R.2 DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The devise shows a sensitivity  $\leq 2 \text{ IU/ml}$  as required by CTS:2009.

R.2.1 Diagnostic Specificity:
In addition to the first study, where a total of more than 500 unselected blood donors, more than 200 hospitalized passents, (under examination for non-HIV pathologies) and more than 100 potentially interfacing speciments (other infectious diseases, E.coli antibody positive, patients affected by non-viral hepatic diseases, dialysis patients, pregnant women, hemoized lipemic, etc.) were tested, the diagnostic specificity was recently assessed by testing a total of 3268 pregative samples on four different iots. A value of diagnostic specificity of 100% was

No fails reactivity due to the method of syecimen preparation has been observed. Both plasma, derived with different standard facturingues of preparation (citrate, EDTA and hopain), and sera have been used to determine the value of specificity. Frozen speciments have been tested, as well, to check for metricences due to collection and storage. No interference was observed.

The diagnostic sensitivity of the test was determined on a population of HIV positive specimens.

Results are reported in the tables below.

R.2.2 Diagnostic Sensitivity

## Etablissement Français du Sang Mixed titer Performance

ō	Composition	Lot # 0505	Lot # 0706	Lot # 0906	20
L	The second secon	S/Co	SiCo	57.5	
1	THUS/ HOUSE				Total Section
ľ	(MARINE)	>14.5	0.214	×15.*	v.
-	HIV2(1/800)	0.07	10.2	40.2	
2				10000	
1	DATTER	0.4	0.4	0.4	0
	HIV1(1/700)	88	0.0	0 +	
,	LIVIATA INTERNA		41.00	3,0	u
10	DIVITION I	V13.5	>15.0	V15.4	7
0	HIV1(1/200)	100	0		1

## Performany Member

0.5 2.5 50.5	3.1 0.2 2.5 10.6	3.1 0.2 2.5 10.5 2.9	3.4 0.2 2.5 10.6 2.9 5.7	3.1 0.2 2.5 10.6 2.9 5.3 5.3 5.3	0.5 10.5 10.5 2.9 2.9 2.9 2.9 3.7 6.3	2.5 10.6 2.5 3.7 3.7 3.7 1.5	3.1 0.2 2.5 10.6 2.9 3.3 3.3 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7	3.1 0.2 2.5 10.6 3.3 3.7 3.7 3.7 3.7 3.7 3.7 2.9	3.1 0.2 10.5 10.6 2.9 3.3 3.3 3.3 3.3 3.3 3.3 3.3 3.3 3.3 3	3.9 3.1 0.5 0.2 2.3 2.5 113.4 10.5 3.3 2.9 5.9 5.3 4.0 5.3 7.5 6.3 5.3 7.7 5.3 7.2 5.3 7.2
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0.2 2.5 10.5	0.2 2.5 19.6	0.2 2.5 10.6 2.9 5.0	0.2 2.5 19.6 2.9 5.3 5.7	0.2 2.5 10.6 2.9 5.3 3.7 5.3	0.2 2.5 19.6 19.6 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0 5.0	0.2 2.5 10.6 5.3 5.3 5.3 5.3 5.3 5.3 5.3 5.3 5.3 5.3	2.5 2.5 2.6 2.0.6 2.0.6 2.0.6 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7	2.52 2.53 19.6 2.9 2.9 2.9 2.7 2.7 2.7 2.7 2.7 2.7 2.7 2.7 2.7 2.7	2.5 2.5 2.5 2.5 2.5 2.5 2.5 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7	2.52 2.53 2.93 2.93 2.93 2.93 2.93 2.93 2.93 2.9
2.5 \$0.6	10.6 2.9	2.5 10.6 2.9 5.0	2.5 10.6 2.9 5.0 3.7	25 10.6 2.9 5.7	2.5 10.6 2.9 5.3 5.7 6.3 6.3	2.5 10.6 2.9 3.7 6.3 1.2	2.5 10.6 2.9 2.7 3.7 3.7 6.3 11.5	2.5 10.6 10.6 3.0 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7	2.5 10.6 10.6 5.1 5.1 5.1 5.1 5.1 5.1 5.1 5.1 5.1 5.1	2.5 1966 2.9 2.9 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7
10.5	10.6 2.9	10.6 2.9 5.0	10.5 2.9 5.0 3.7	10.6 2.9 5.0 5.7 5.3	10.6 2.9 5.0 5.0 3.7 6.3 5.3	10.6 2.9 5.7 5.7 5.3 6.3 6.3 1.5	19.6 2.9 5.0 5.7 5.3 5.3 5.3 7.2 7.2	19.6 2.9 5.3 5.7 5.3 5.7 6.3 6.3 7.5 7.5 7.5 7.5	10.6 2.9 3.7 5.3 5.3 5.3 5.3 7.2 7.2 7.2 2.3 2.3 2.3 2.3 2.3 2.3 2.3 2.3 2.3 2	10.6 2.4 2.7 2.7 2.7 2.7 2.7 2.7 2.7 2.7 2.7 2.7
	2.9	5.3	2.9 5.0 3.7	2.9 5.0 3.7 6.3	2.9 5.0 5.7 5.3 5.3	29 5.7 3.7 6.3 3.2 1.5	2.9 5.0 5.7 6.3 1.2 1.5 7.2	5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7 3.7	29 53 3,7 2,7 1,5 1,5 7,2 2,9 6,1

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0.9	23	0.2	68.5	71.7	58.5	71.5	71.2	58.8	9.99	83.6	83.8	0.73	83.8	1,28	65,1	2.4	84.6	0.80	67.5	1.38	55.1	0.2	65.2	1.0	S/Co	Ret Kit

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Date: 2015/06

13 >14.5	12 254.5	>14.5	10 >14.5	9 11,4	V14.5	7 0.4	8 >14.5	5.6	4 >14.5	3 >14.5	2 0.4	10.2	ID# S/Co
15.7	12.0	>15.7	>15.7	10.5	-515.7	0.2	9.1	8.2	>15.7	>15.7	0.2	>16.7	S/Co

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V14.5	574.5	714.th	514.5	>14.5	>34.5	>14.5	>14.5	>14.5	>\$4.5	214.5	>14.5	>14.5	>14.5	>14.5	NCOMB.CE S/Co
17.4	17.4	17.4	17.4	19.5	17.4	17.4	17.4	18.5	17.4	15.8	17.73	17.4	17.4	18,5	Ref. Kit

20	19	18	17	6	iń	7	13	:2	1	10	(C	DC.		a	u	4	u	2	-	ID#
4.6	0.4	1.7	2.5	1.1	3.3	0.4	1,2	3.6	2.4	3,6	0,4	13,13	2,9	1.6	Nd	in.	1.6	4.5	2,3	ID# S/Co S/Co
47.5	0.5	2.4	6.3	1.5	200	6.0	2.4	i.d	33.2	12.2	0.5	1.50	5.7	123	Nd	3.0	6.0	12.2	4.3	S/Co

Moreover, in the external Performance Evaluation a total of 651 positive samples, including HIV type 1, HIV type 2, HIV type 1 mixed subtypes (including 0), HIV 1 Antigen, more than 40 early seroconversion HIV samples and cell culture supernations were evaluated and a value of 100% was found.

Finally, more than 30 panels of seroconversion containing samples of HIV 1/2/0 Antibodies and/or HIV-1 p24 Antigen positive, obtained from BBI, USA, were evaluated using IVCOMB.CE lot # 0506. In the table below results are reported.

DOUE	3019	3018	3017	3016	9013		-	Al aidure	1	-	Etabliss				100000	15	14	13	122	::	10	to.	a	7	o	· ·	44	3	2	_	- T	Memb	Pertor	
	0.0	12	1.6	4.2	8.7	SICo		IVCOMB.CE Co	HIV Ag (3015-3022) lot 2004	Performance Panel	Etablissement Français du Sang					>14.5	>14,5	>14.5	0.3	11.3	>14.5	1.1	>14.5	>18.in	>14.5	4.1	>16,5	2,524	2,454	>14.5	S/Co	Member IVCOMB.CE	Performance Panel - WWRB 303	to world
62	300	50	100	250	500	[pg/mil]	p24 Ag	Concentration HIV 1	12004	6	du Sang					V 15 G	215.9	×25×	0.4	0.3	>15.5	24	\$ 55 in	>15.9	>15.9	×15.0	>15.9	>15.9	2150	>15.9		Ref. Kit	VWRB 303	
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	The same and	PRR 956 (BE)	PRB 955 (BE)	PRB 953 (BC)	PRB 952 (BB)	PRB 950 (AZ)	PRB 949 (AY)	PRB 948 (AX)	PR8 947 (AW)	PRB 946 (AV)	PRB 944 (AT)	PRE 942 (AR)	PR8 941 (AQ)	PRS 940 (AP)	PRB 939 (AN)	PRE 938 (AM)	PHB 937 (AL)	TRB 935 (AJ)	PKE 933 (AH)	FKB 931 (AF)	TRO BOU (AE)	(The sze ov.	000 000 000	(BW) 176 BULL	(7) 976 GV.	PRB 924 (X)	PRO 922 (V)	TK0 919 (S)	(B) /(E GUL	PRESID (P)	PRE 910 (J)	Name of the last	8	Panel
	c				3	3	4	4	2	ω	i i	4	4	2	7		4	0	N	Ø	2	4	2	2	ы	5	1		2	4	2	94	First specimen detected positive in the	4 Generation HIVAbSAc
-	not detected	4	4	3			Detailed for	and decrees	Calibratan tou	not detailed	Salan	Potential for	9 1	-	000	2	not detected	7	3	on	Ca	en.	63	3	LTA.	on.	-	3	5	ts	ta	panel	scled positive in th	3 Generation

The results of the Performance with what stated by EU CTS diagnostic sensitivity of 100% R.3 PRECISION EU CTS and show on, correlate v an overall

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XCV.

Date: 2015/06

The precision of the device was assessed by determining its values in a within and between runs. In the table below results are reported for a negative sample and a low positive sample.

	·	Ţ	1
CV %	d.Deviation	JU450nm	N = 48
7.6	0.011	0.136	Sample
4.0	0.022	0,916	Low

- Aliann, M., Soniga, P., Stavis-Shousal, F., Charmann, J.-C., Tollatis, Mohamire L. and Wasis-hobson, S., 1984, Molecular Conving of Imphodrosophy-Associated Visite, Native 312: 757-768, improved March R.M., Shaw, S.M., Paposic, M., Galo, R.C. and Horty-Stadis. P. 1984, Molecular Convince and Characteristics of the Horty-Charles T., 1984, Molecular Chiming of Hospital Retirevals, 1984, 1984, Molecular Chiming of Hospital Retirevals, Native 312: 456-468, Native 312: 450-468, Native 312: 45
  - D)
- Robbiness (FTUVIII) in the Sound of Palicies with AUS, Science 22:595-508. State of Trivial in the Sound of Palicies with AUS, Science Watmedburk F. Borre-Strouser, E., Hattrient, D., Raschaum, W., Gardenburk, J.C., and Chemany, J.C., 1984. Despites of light Australia of Palicies with Australia of Palicies of Australia of Palicies of Palicies of Australia of Palicies 
value of

-	-	-	-
W A3	Sid. Deviation	OU450nm	N = 48
7.6	0.011	0.136	Sample
40	0.022	0,916	Positive

The user of this kit is advised to carefully read and understand this package insert. Sind adherence to the protocol is necessary in order to obtain reliable tast results. In particular, accurate earning and insign of insert. Sind adherence to the protocol is necessary in order to obtain reliable tast results. In particular, accurate and productible and reagent pipeting, along with careful washing and timing of incubation steps is essential for accurate and experiments of incubation steps is essential to accurate and experiments. After the EAI, tast is performed, repeatedly reactive specimens. Should be submitted for additional testing using Western Blot (WB). Assay (RIPA), tasts and DCR Rin HV nucleic acid.

The determination that a person's serum contains articodies or pack antigen to HIV has extensive medical, sociel, psychological it is recommended that contidentially, appropriate courseiling and medical availation be considered an essential sepect of the testing and their diagnosis can only be established clinically.

It is recommended that contidentially, appropriate courseiling and sequence, along and their diagnosis can only be established clinically.

A non-reactive test result al any port in the testing sequence does not preclude the possibility of exposure to or infection with HIV. The risk of an asymptomatic person, who is repeatably reactive. Testely reactive test results can be observed with a task bit of this sensitivity and specificity of the test of any on the prevalence of HIV-1 and HIV-2 architocities in the population to be screened.

Antitodies to HIV may occur due to voluntary participation in an HIV occurre study.

### BIBLIOGRAPHY L Alizan, M

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If IV vaccine study, Interpretation of this diagnostic test will depend on the type of vaccine given. Correlation with the medical history and additional setting may be necessary to accurately diagnose HIV in vaccine volunteers.

The device shows a better sensitivity than generation as it is able to detect the p24 antigen.

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23 22 Microbiologica, 1989 Jan (12)(1)(1).
Goustini J. Lange JM, Knore WJ, Tourissen MB, Epstein LG,
Damme SA, van den Sego H, Benedinveld C, Shirt L, Bakker M, and
Palinogenosis of HIV and its implications for serodiagnosis and
monitoring of emistral fluerapy. J Viral Methods. 1987. Aug;17(1):

21. Ljammyo E. Brudstenje-Baden U. Ratasanev A. Utassa E. Konno G. Galvatim G. Kadman G. Galvatim 
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All the IVO Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Dia.Pro Diagnostic-Bioprobes-Sri Via G. Carducci n\* 27 – Sesto San Giovanni (Mi) – Italy

M 0318

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## M61 LASH

"Capture" Enzyme Immuno Assay (ELISA) for the determination of IgM antibodies to Herpes Simplex Virus type 1 in human plasma and sera

- for "in vitro" diagnostic use only -

### DIA.PRO

Diagnostic Bioprobes Srl Via G. Carducci n° 27 20099 Sesto San Giovanni (Milano) - Italy Phone +39 02 27007161 Fax +39 02 26007726



REF HSVIM, CE

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### MBI IV2H

preservatives, Tween 20, 0.09% sodium azide and 0.1% Kathon GC as

3. Positive Control: CONTROL + The negative control is cpale yellow color coded..

as preservatives. 0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC positive for HSV1 IgM, 2% casein, 10 mM tris buffer pH 6.0+/-1x4.0 ml/vial. Ready to use control. It contains 1% human serum

The positive control is green colour coded.

preservatives. serum, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as reported in the label. It contains anti HSV1 IgM, fetal bovine 4. Calibrator: CAL ...mi
N° 1 lyophilized vial. To be dissolved with EIA grade water as

vial may vary from lot to lot. Please use the right volume reported on the label. Note: The volume necessary to dissolve the content of the

with 1.9 ml of Antigen Diluent as reported in the specific section. N° 6 lyophilized vials. The vials contain gamma-ray inactivated HSV1 in protein buffer. The solution contains 2% bovine proteins, 10 mM Tris HCl buffer pH 6.8+/-0.1, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC. To be dissolved 5. Lyophilized HSV1 Ag: AG HSV1

1x60ml/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC. 6. Wash buffer concentrate: WASHBUF 20X

preservatives. Kathon GC and 0.2 mg/ml gentamidine sulphate as containing 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.1% 1x0.8 ml/visl. 20x concentrated solution of a HS/v1-specific antibody, labeled with HRP and diluted in a protein buffer 7. Enzyme conjugate: CONJ 20X

coloured with 0.01% red alimentary dye gentamicine sulphate as preservatives. The reagent is code pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.2 mg/ml the Immunocomplex. The solution contains 10 mM Tris buffer onoilereparetion for the preparation of 16 ml. Protein buffer solution of 16 ml. Protein buffer solution for the preparation of 8. Antigen Diluent: AG DIL

0.1% Tween 20, 0.09% sodium axide and 0.1% Kathon GC as samples. It contains 2% casein, 10 mM ths buffer pH 6.0+/-0.1, 2x60.0 ml/vial. Proteic buffered solution for the dilution of 9. Specimen Diluent: DILSPE

The reagent is color coded with 0.0°% blue alimentary dye.

10. Chromogen/Substrate: SUBS TMB 1x16ml/vial. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine (TMB), solution at pH 3.5-3.8, Use of the solution at perceivide (Lincol

11. Sulphuric Acid: H2SO4 0.3 M strong illumination. Note: To be stored protected from light as sensitive to .0.02% hydrogen peroxide (HsOs) and 4% dimethylsulphoxide.

Attention: Initiant (H315, H319; P280, P302+P352, 332+P313, P365+P363) 1x15ml/vial. It contains 0.3 M H2SO4 solution.

12. Plate sealing foils n° 2

13. Package insert n° 1

A. INTENDED USE

monitoring of risk of neonatal defects due to HSV infection plasms and sera with the "capture" system.

The device is intended for the follow-up of HSV1 infected patients and for the Enzyme ImmunoAssay (ELISA) for the determination of IgM antibodies to Herpes Simplex Virus types 1 in human

For "in vitro" diagnostic use only. during pregnancy.

в. ІИТRODUCTION

just a few of type-specific sequences. possessing an high number of cross-reactive determinants and to induce the synthesis of several proteins during infection, large complex DNA-containing viruses which have been shown Herpes Simplex Virus type 1 (HSV1) and type 2 (HSV2) are

The majority of primary and recurrent genital herpetic infections are caused by HSV2; while non genital infections, such as

reactivations of a latent one, in the absence of evident clinical mportant in the diagnosis of acute/primary virus infections or The detection of virus specific IgG and IgM antibodies are common cold sores, are caused primarily by HSV1.

A-symptomic infections may happen for HSV in apparently healthy individuals and during pregnancy. Severe herpetic infections may happen in immuno-compromised and suppressed patients in which the disease may evolve toward suppressed patients in which the disease may evolve toward suppressed patients.

important in the monitoring of "risk" patients and in the follow up he determination of HSV specific antibodies has then become ¢ritical pathologies.

of acute and severe infections.

C. PRINCIPLE OF THE TEST

phase coated with anti high antibody. class antibodies in the sample are first captured by the solid The assay is based on the principle of "IgM capture" where IgM

phase are detected by the addition of a preparation of insclivated HSV1, labeled with a HSV1 specific antibody conjugated with peroxidase (HRP). particular IgG antibodies, the specific IgM captured on the solid After washing out all the other components of the sample and in

conjugate and then the chromogen/substrate is added. After incubation, microwells are washed to remove unbound

to HSV1 present in the sample. be detected and is proportional to the amount of IgM antibodies hydrolyzed to a colored end-product, whose optical density may In the presence of bound conjugate the colonless substrate is

the clinician to make a correct interpretation of results. shown by a sample is true or not (Confirmation Test), helpful for A system is described how to control whether the positivity

D. COMPONENTS

The kit contains reagents for 96 tests.

12 strips x 8 microwells coated with anti human IgM affinity 1. Microplate: MICROPLATE

unused strips in the bag with desiccant and store at 2..8°C. purified goat antibody, in presence of bovine proteins.
Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal

proteins, 2% casein, 10 mM tris buffer pH 6.0+/-0.1, 0.1% 1x4.0 ml/vial. Ready to use control. It contains 1% human serum 2. Negative Control: CONTROL

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then with water. Tissues should then be discarded in proper adsorbed with paper tissues soaked with household bleach and 14. Accidental spills from samples and operations have to be

15. The Sulphunc Acid is an irritant. In case of spills, wash the surface with plenty of water containers designated for laboratory/hospital waste.

according to national directives and laws doncerning laboratory should be handled as potentially infective and disposed (example: tips used for samples and controls, used microplates) 16. Other waste materials generated from the use of the kit

### G. SPECIMEN: PREPARATION AND WARNINGS

observed in the preparation of the sample with citrate, EDTA samples for clinical laboratory analysis. No influence has been serum is prepared using standard techniques of preparation of 1. Blood is drawn aseptically by venepuncture and plasma or

and heparin.

2. Samples have to be clearly identified with codes or names in

electronic reading is strongly recommended. order to avoid misinterpretation of results. Bar code labeling and

could give rise to false results. microbial filaments and bodies should be discarded as they Samples containing residues of fibrin or heavy particles or have to be discarded as they could generate false results. 3. Haemolysed ("red") and visibly hyperliperiic ("milky") samples

generate particles that could affect the test result. should not be frozen/thawed more than once as this may after collection. For longer storage periods, samples can be stored trozen at -20°C for several months. Any trozen samples 4. Sera and plasma can be stored at +2°..8°C for up to five days

filter using 0.2-0.8u filters to clean up the sample for testing. 5. If particles are present, centrifuge at 2,000 rpm for 20 min or

### H. PREPARATION OF COMPONENTS AND WARNINGS

"syluow E of qu bris solves of the device and up to 8 A study conducted on an opened kit has not pointed out any

### Microplate:

before opening the container. Check that the deign not turned dark green, indicating a defect in storing. In this case, call Dia, Pro's customer service. Check that the desiccant has Allow the microplate to reach room temperature (about 1 hr)

with the desiccent supplied, firmly zipped and stored at +2°.8°C. After first opening, remaining strips are stable until Unused strips have to be placed back into the aluminum pouch,

to dieen. the humidity indicator inside the desiccant bag turns from yellow

Ready to use. Mix well on vortex before use. Negative Control:

Ready to use. Mix well on vortex before use. Positive Control:

Calibrator:

the lyophilized powder. Let fully dissolve and then gently mix on Add the volume of ELISA grade water reported on the label to

Trozen in aliquots at -20°C. Important Note: The solution is not stable. Store the Calibrator

### Wash buffer concentrate:

+5.8° C. Note: Once diluted, the wash solution is stable for 1 week at bubbles could impact on the efficiency of the washing cycles. use. During preparation avoid foaming as the presence of 20x with bidistilled water and mixed gently end-over-end before The whole content of the concentrated solution has to be diluted

### Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and E. MATERIALS REQUIRED BUT NOT PROVIDED

disposable plastic tips.

EIA grade water (double distilled or deionised, charcoal

disinfectants). oxidizing chemicals remove

Timer with 60 minute range or higher. 3°

.3

Absorbent paper tissues.
Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
Calibrated ELISA microwell reader with 450nm (reading) .9

Calibrated ELISA microplate washer. ١, and with 620-630nm (blanking) filters.

,8

Vortex or similar mixing tools.

### F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical dather respectively.

in biosafety procedures, as recommended by the Center for The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained wear protective laboratory clothes, talc-free gloves and glasses. All the personnel involved in performing the assay have to doctor responsible of the laboratory.

Biomedical Laboratories", ed. 1984. Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and

safe and effective.  $3.~\rm All$  the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available,

vibration of the bench surface where the test is undertaken. test. Protect the Chromogen (TMB) from strong light and avoid when opening kit vials and microplates and when performing the avoid contaminants such as dust or air-born microbial agents, The laboratory environment should be controlled so as to

controlled refrigerator or cold room. Upon receipt, store the kit at 2..8°C into a temperature

the kits. It is recommended that components between two kits Do not interchange components between different lots of

repracement. Isboratory supervisor to initiate the necessary procedures for kit visible heavy particles or aggregates, If not, advise the Check that the reagents are clear and do not contain of the same lot should not be interchanged.

samples by using disposable tips and changing them after each serum/plasma petween cross-contamination DIOVA

disposable tips and changing them between the use of each Avoid cross-contamination between kit reagents by using

up to six 6 uses of the device and up to 3 months. on an opened kit did not pointed out any relevant loss of activity external container and internal (vials) labels. A study conducted 10. Do not use the kit after the expiration date stated on the

publication: "Biosafety in Microbiological and Biomedical aboratorles", ed., 1984. recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's 11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as

cross contamination. components into automated workstations, in order to avoid preparation of the liquid components or in transferring The use of disposable plastic-ware is recommended in the

before waste. has to be treated as potentially infective material and inactivated discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as particular, interpretation of the procedure. 13. Waste produced during the use of the kit has to be

16-18 hrs or heat inactivation by autoclave at 121°C for 20 min... treatment with a 10% final concentration of household bleach for Suggested procedures of inactivation are

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and maintenance (decontamination and cleaning to ne carried out according to and positive reference samples, and check to match the values reported below in the section "Internal Quality Control". Regular calibration of the volumes delivered by, and maintenance (decompanies from the section of the volumes delivered by. set correctly their number, it is recommended to run an assasy with the kit controls and well characterized negative of 20-30 seconds between cycles is suggested, in order to ensure that the assay performs as expected, A soaking time of 350ul/well of washing solution = 1 cycle) are sufficient to

strongly recommended) for blanking purposes. Its standard reading filter of 450nm and with a second filter (620-630nm, The ELISA microplate reader has to be equipped with a Incubation times have a tolerance of +5%. the instructions of the manufacturer.

When using an EUSA automated work station, all critical that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's should be commissionally in parameters and market before a solution to be 2.0; (c) linearly to 2.0; (c) linearly to 2.0; (d) linearly to 2.0; (d) linearly to 2.0; (d) linearly to 2.0; (d) linearly to on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure system of the reader has to be calibrated regularly to ensure a system of the reader has to be confined to the control of the pould have the correct optical densured.

exceed 20-30 units per run. is recommended when the number of samples to be tested adjacent wells. The use of ELISA automated work stations and controlled to minimize the possibility of contamination of attention must be paid to avoid carry over by the needles attention must be paid to washing. This must be studied liquid handling part of the station (dispensation and washing) has to be validated and correctly set Particular validated as for the washer and the reader. In addition, the has to be installed in the operating system of the unit and the section "Internal Quality Control". The assay protocol regularly serviced in order to match the values reported in steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and

installation of new instruments to be used with the kit. requirements described. Support is also provided for the with the kit, in order to assure compliance with the settling and checking of instruments used in combination 7. Dia. Pro's customer service offers support to the user in the

### L. PRE ASSAY CONTROLS AND OPERATIONS

Check that the liquid components are not contaminated by label (primary container). Do not use the device if expired. Check the expiration date of the kit printed on the external

that the aluminum pouch, containing the microplate, is not no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check a small volume of it with a sterile plastic pipette. Check that visible particles or aggregates. Check that the Chromogen/Substrate is colonless or pale blue by aspirating

as described above. Dilute all the content of the 20x concentrated Wash Solution punctured or damaged

(about 1 hr) and then mix gently on vortex all liquid Allow all the other components to reach foom temperature Dissolve the Calibrator as described above and gently mix.

number of washing cycles as found in the validation of the according to the manufacturers instructions. Set the right Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution,

7. Check that the ELISA reader is turned on or ensure it will be instrument for its use with the kit.

Check that the micropipettes are set to the required volume. and be sure to use the right assay protocol. If using an automated work station, turn on, check settings turned on at least 20 minutes before reading.

Ag/Ab Immunocomplex:

Proceed carefully as follows:

1. Dissolve the content of a lyophilized vial with 1.9 ml of Conjugate/Antigen Diluent. Let fully dissolved the lyophilized

and mix gently on vortex. Then add 0.1 ml of it to the vial of the dissolved HSV1 Ag Gently mix the concentrated Enzyme Conjugate on vortex. 5 content and then gently mix on vortex.

Dissolve and prepare only the number of vials necessary to important Notes:

before the dispensation of samples and controls into the The preparation of the immucomplex has to be done right any residual solution frozen in aliquots at -20°C. the test. The Immunocomplex obtained is not stable. Store

plate. Mix again on vortex gently just before its use.

Ready to use. Mix well on vortex before use Specimen Diluent:

Chromogen/Substrate:

air-driven dust or microbes. Be careful not to contaminate the liquid with oxidizing chemicals, Ready to use. Mix well on vortex before use.

metallic surfaces. Do not expose to strong illumination, oxidizing agents and

sterile disposable container If this component has to be transferred use only plastic, possible

Sulphuric Acid:

P305+ P351+P338, P337+P313, P362+P363). Ready to use. Mix well on vortex before use. Attention: Irritant (H315, H319; P280, P302+P352, 332+P313,

:epue6e-

H315 – Causes serious eye irritation. H319 – Causes serious eye irritation. Warning H statements:

protection, Precautionary P statements:
P280 – Wear protective gloves/protective clothing/eye protection/face

P332 + P313 – If skin irritation occurs: Get medical advice/attention.

P396 + P361 + P398 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. b305 + b325 - IE ON SKIN: Mesh with plenty of sosp and water.

P362 + P363 - Take off contaminated clothing and wash it before reuse. Continue rinsing. P337 + P313 - If eye irritation persists: Get medical advice/attention.

### WITH THE KIT I. INSTRUMENTS AND TOOLS USED IN COMBINATION

ont regularly. of spills or residues of kit components should also be carried should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination could accidentally come in contact with the sample. They of bleach, hospital grade disinfectants) of those parts that volume required by the assay and must be submitted to regular deconfamination (household alcohol, 10% solution Micropipettes have to be calibrated to deliver the correct

baths are suitable for the incubations, provided that the 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water The ELISA incubator has to be set at +37°C (tolerance of +/-

tests. Usually 4-5 washing cycles (aspiration + dispensation reference panels, before using the kit for routine laboratory validated and correctly optimised using the kit controls and performances of the assay. The washer must be carefully The ELISA washer is extremely important to the overall instrument is validated for the incubation of ELISA tests.

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- Incubate the microplate for 60 min at +37°C.
- Wash microwells as in step 6.
- at room temperature (18-24°C) for 20 minutes. well, the blank well included. Then incubate the microplate Pipette 100 µl Chromogen/Substrate mixture into each
- High background might be generated. Important note: Do not expose to strong direct illumination.
- turn the positive control and positive samples from blue to same pipetling sequence as in step 10. Addition of acid will 11, Pipette 100 µl Sulphuric Acid Into all the wells using the
- blanking the instrument on A1. 630nm (background subtraction, strongly recommeded). described in section I.5, at 450nm filter (reading) and at 620-12. Measure the color intensity of the solution in each well, as yellow.
- If the second filter is not available ensure that no finger Important notes:
- Stop Solution and anyway not any longer than 20 minutes Reading has to be carried out just after the addition of the positive results on reading. reading at 450nm. Finger prints could generate false

prints are present on the bottom of the microwell before

occur leading to high background. after its addition. Some self oxidation of the chromogen can

### N. ASSAY SCHEME

mn024	Reading OD
In 001	Sulphuric Acid
,1,7	Temperature
nim 02	3 <sup>rd</sup> incubation
In 001	TMB/H2O2 mix
4-5 cycles	gnidasW
+37°C	Temperature
nim 09	2nd incubation
lu 001	Immunocomplex
4-5 cycles	gnirlssW
+37°C	Temperature
nim 09	1st incubation
In 001	Samples diluted 1:101
ID 001	Controls&calibrator(*)

### (\*) Important Notes:

- calculation, therefore it does not affect the test's The Calibrator (CAL) does not affect the Cut Off
- internal quality control is required by the The Calibrator (CAL) used only if a laboratory results calculation.
- пивтвретем.

An example of dispensation scheme is reported below:

Microplate
A Company

	ajuure	5 = 5	Ic			egativi G = P <sub>v</sub>				LK = Big or-Not n	d :ebo f) = Calibrat	CVF	
										018	25	Н	
										68	ıs	9	
										88	bC	Э	
										LS	CAL(*)	Ξ	i
									į - T	98	CAL(*)	D	
4										98	NC	0	1
							j .			t/S	NC	8	
										23	BLK	$\forall$	]
12	11	10	6	8	L	9	9	Þ	3	5	land b		

10. Check that all the other equipment is available and ready

11. In case of problems, do not proceed further with the test and

advise the supervisor.

M¦ASSAY PROCEDURE

below, taking care to maintain the same incubation time for all The assay has to be carried out according to what reported

:Yssss bətsmotuA f.M

the samples in testing.

wells of the microplate. instrument dispense 100 ll diluted samples into the proper samples. When all the samples have been diluted make the to be duly washed to avoid any cross-contamination among dilution tube. Before the next sample is aspirated, needles have The whole content is then dispensed into a properly defined Specimen Diluent and then 10 µl sample (1:101 dilution factor). system, we suggest to make the instrument aspirate 1000 µl In case the test is carried out automatically with an ELISA

in the platform and finally dispense the whole content in the 100 pl Specimen Diluent, then 10 pl liquid from the first dilution second dilution platform. Make then the instrument aspirate first of 1:10 each (90 µl Specimen Diluent + 10 µl sample) into a This procedure may be carried out also in two steps of dilutions

Do not dilute controls/calibrator as they are ready to use. Dispense 100 µl calibrators/controls in the appropriate calibration/control wells. proper well of the assay microplate.

For the next operations follow the operative instructions reported

delaying the first washing operation accordingly. calculated by the instrument and taken into consideration by the dispensation of the first and the last sample will be It is strongly recommended to check that the time lap between below for the Manual Assay.

Dilute samples 1:101 by dispensing first 10 th sample and :Yesse launeM 2 .M

- Place the required number of Microwells in the microwell 5. then 1 ml Specimen Diluent into a dilution tube; mix gently
- Dispense 100 µl of Negative Control and 100 µl of Calibrator in the proper wells in duplicate. Dispense 100 µl 3, operation of blanking. holder. Leave the well in position A1 empty for the
- i əsn ol of Positive Control in single into the proper well. Do not dilute controls and the calibrator as they are ready
- colored and that controls and calibrator have been wells and then check that all the samples wells are blue Dispense 100 µl diluted samples in the proper sample
- Incubate the microplate for 60 min at +37°C ,beansqsib

Do not cover strips when using ELISA automatic instruments. sealiing foil, supplied, only when the test is carried out manually. Important note: Strips have to be sealed with the adhesive

- previously (section 1.3). Wash the microplate with an automatic washer as reported '9
- sealer. Check that all wells are red colored, except A1, well, except the blanking well A1, and cover with the Pipette 100 µl of the Ag/Ab Immunocomplex into each

Immunocomplex. Contamination might occur. dAlgA et it with the tip filled with the AglAb Important note: Be careful not to touch the plastic inner

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1. that the procedure has been correctly	Calibrator
performed;  2. That no mistake has occurred during its distribution (e.g.: dispensation of negative control instead);  3. That the washing procedure and the washer settings are as validated in the pre qualification study;  4. That no external contamination of the	z'\ > °O)/S

may be considered valid. Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test

### P. CALCULATION OF THE CUT-OFF

The test results are calculated by means of the mean OD450nm value of the Negative Control (NC) and a mathematical calculation, in order to define the following cut-off formulation:

### Cut-Off = NC + 0.250

results as described in the next paragraph. The value found for the test is used for the interpretation of

off value and generate the correct interpretation of results. ensure that the proper formulation is used to calculate the cutthe operating system of an ELISA automated work station, Important note: When the calculation of results is performed by

### Q. INTERPRETATION OF RESULTS

shd the Cut-Off value (or S/Co) according to the following table: Test results are interpreted as a ratio of the sample OD450nm

Positive	2.1 <
Equivodal	2.1 - 0.1
9vitsp9M	0.1 >
Interpretation	00/S

examining a second sample taken from the patient after 1-2 Any patient showing an equivocal result, should be re-tested by adute infection of Herpes Simplex Virus type 1. A negative result indicates that the patient is not undergoing an

Positive result is indicative of a Herpes Simplex Virus type 1 weeks from first testing.

An example of calculation is reported below:

real figures obtained by the user. Important Note: The following data must not be used instead or

Higher than 1.000 – Accepted 1.850 OD450nm Positive Control: Lower than 0.150 - Accepted 0.100 OD450nm :enleV neeM Negative Control: 0.100 - 0.120 - 0.080 OD450nm

Cut-Off = 0.110+0.250 = 0.360

2/C0 = 5'8

S/Co higher than 1.2 - Accepted mn02400 026.0 :ənjex ueəjy MN02440 006.0 - 000.1 Calibrator:

Sample 2 S/Co > 1.2 = positive Sample 1 S/Co < 1 = negative 1.580 OD450nm Sample 2: Mn024QO 270.0 : Laldines

### O. INTERNAL QUALITY CONTROL

are as qualified. is used in order to verify whether the performances of the assay A validation check is carried out on the controls any time the kit

Control that the following data are matched:

-WAY-JEJ	Sil	Requiremen		SIN	Parameter
		9ulsv mn02t	20 SO.	0 >	Blank well
after	eulev	Mn02400	0.200	>	Negative
			gniAr	plai	Control mean
	%08	> noitainev to			value (NC)
		mn034			lostioo Control

proceed to the next section. If the results of the test match the requirements stated above,

following checks: If they do not, do not proceed any further and perform the

The lines oppositely	
1. Insit the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of the distribution of the control (dispensation of 3. that the washing procedure and the washing procedure.	Politive Control
partially obstructed.	Jostao D evitien
enzyme conjugate 6. that the washer needles are not blocked or	
procedure (dispensation of positive control instead of negative control 4. That no contamination of the negative control was dispensed that occurred due to positive samples, to spills or to the enzyme conjugate; that micropipettes have not become to the enzyme with the contaminated with positive samples or with the contaminated with positive samples or with the	
3. that no mistake has been done in the assay	%ariation > 30%
perore use;	to Insicition
2. that the proper washing solution has been used and the washer has been primed with it	after blanking
sindy:	> 0.200 OD450nm
settings are as validated in the pre qualification	9 0 500 ODVE00E
I that the washing procedure and the washer	Negative Control
1, that the Chromogen/Substrate solution has not become contaminated during the assay	Blank well > 0.05 OD450nm
Сивск	Problem

to the supervisor for further actions. It sny of the above problems have occurred, report the problem

### \*\* Important Note:

Calibrator

Requirements Среск If the Calibrator has used, verify the following data:

2/C0 > 1.2

above, operate as follows: If the results of the test doesn't match the requirements stated

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False positive reactions may be anyway pointed out and the procedure reported in section T, able to verify whether or not a positive result is real.

4. Precision:

Results are reported as follows:

19)	= M) eviteneM
raR # 1이	HSV1M.CE:

	11.00			(at = N) evitege
egerevA eulsv	unı <sub>n</sub> e	unu puz	unu jaj	saulev neaM
201.0	911.0	701.0	580.0	mn024 GO
110.0	£10.0	710.0	400.0	Std.Deviation
48.01	69.11	15.82	51.2	% ΛO

			(91	ow reactive (N =
egerevA eulsv	un ac	Zud run	nui let	Mean values
714.0	124.0	964.0	£6£.0	mn024 GO
610.0	700.0	610.0	150.0	Std.Deviation
99.4	88.1	85.4	£6,7	% AO

Average	uru <sub>p/</sub> g	nun bns	nun tet	Mean values
sulsv	E STARTED	0.2.14		In a large state
1.613	1.541	1,530	697.1	mn02+ do
S40.0	750.0	990.0	0.034	noilsiveQ.bl2
2.77	2.39	09.6	2.31	CΛ %

660'0	260'0	660.0	101.0	mn021 do
Average	3 <sub>th</sub> run	Zug tun	unı jet	Mean values
	10			(ar = N) evitegel
			7.UN #	18A1M.CE: lot

Average	ยนา เกย	Zug run	unı ısı	Mean values
660'0	260'0	660.0	101.0	mn021 do
110.0	610.0	110.0	600.0	notisived.bt2
pt.ff	04.61	11,11	16.8	% ΛO

Average	g <sub>ed</sub> run	nun bas	nun tef	seulsv nseM
604.0	0.420	965.0	0.412	Mn024 GO
\$10.0	\$10.0	600.0	910.0	noteived,bts
2,92	98.S	2.27	3.64	% AO

	CA %
0 350.0 S40.0 noils	Sid.Devis
	097 00
	sv nsəM

lot # RD3	HSV1M.CE:
-----------	-----------

% ΛO

7700	7,00	1 3100	1 6100	Inoitsive O.bl2
904.0	0412	868,0	304.0	mn024 QO
Average Suley	um <sub>as</sub> g	Zuq tun	nun 181	Mean values
			(9	r = M) svitassy wo
10.50	98.01	40.8	12.6	- % ΛO
110.0	0.00	600.0	0.012	Sid Deviation
001.0	Z60'0	211.0	960'0	mu03+ do
Average value	ary <sup>57</sup> 8	nuı bnS	ոսո նշ ի	Mean values
				(gr = N) avnega

3,77

samples has not revealed any interference in the system. We cross reaction were observed.

The Performance Evaluation has provided a value > 98%,

Indignment errors and misinterpretations.

Indignment errors and misinterpretations.

Important notes:

Important notes:

Judgment errors and misinterpretations.

2. Particular attention in the interpretation of results has to be VSH to noise an infection of pregnancy for an infection of pregnancy and consistent little of or over the control of the co

due to the rack of severe neonatal malformations.

In pregnancy monitoring, it is strongly recommended that any positive result is confirmed first with the procedure described below and secondly with a different device for HSV IgM detection, before taking any preventive medical

action.

Any positive sample should be submitted to the Confirmation Test reported in section T before giving a result of positivity. By carrying out this test, false reactions, leading to a misinterpretation of the analytical result, can be

leading to a misinterpretation of the analytical result, can be revealed and then ruled out.

When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous another facility, attention must be paid to avoid erroneous

another facility, attention must be paid to avoid erroneous data transfer.

Diagnosis of infection has to be taken and released to the patient by a suitably qualified medical doctor.

### R. PERFORMANCE CHARACTERISTICS

1. Limit of detection
No international standard for HSV1&2 IgM Antibody detection
has been defined so far by the European Community.

has been defined so far by the European Community. In its absence, an Internal Gold Standard (or IGS), calibrated on the preparation named "Accurun – Anti HSV2 IgM plasma" produced by Boston Blomedica Inc., USA, code 9106072, has produced by Boston Blomedica Inc., USA, code 9406072, has produced by Boston Blomedica Inc., USA, code 9406072, has produced in order to provide the device with a constant and

been defined in order to provide the device with a constant and excellent sensitivity.

The limit of detection of the assay has been therefore calculated on the IGS. A limiting dilution curve was prepared in the

on the IGS. A limiting dilution curve was prepared in Megative Control (NC).
Results of Quality Control are given in the following table:

### OD450nm values

HSV1M.CE	Lot # RD2 HSV1M, CE	Lot # RD1	SOI
334.0	094.0	0.450	X١
882.0	008.0	772.0	2X
381.0	861.0	912.0	ΧÞ
980.0	380.0	0.115	NC

< SPW SISYBUR ON HIGH BOURSES
CE marked kit. The value obtained from the analysis was >
Ad avuised pauleonia solduna at ta attach
yaluation study on panels of 40 samples classified positive by
courselled a the cores was
The diagnostic sensitivity has been tested in a performance
rougduoziic seusitivity:

3. Diagnostic specificity:
The diagnostic specificity has been determined in the performance evaluation on panels of more than 300 specimens, negative with the reference kit, derived from normal individuals of European origin.
Soft bissma, derived with different standard separates.

or European origin.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity. No false reactivity due to the method of specimen preparations.

method of specimen preparation has been observed.

Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was interferes with the performance of the test. No interference was abserved on clean and particle free samples.

A study conducted on more than 60 potentially cross-reactive.

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alluser to noitsterpretain ett of betroger zi eldst gniwollot eft

l rue positive	esis 4	Problem of contam.	Honard Idionii
0.1 >	2.1 <	0.r >	r =     Interpretation
2,1 <	2.1 <	0, r >	ia
	2\C		Mell

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specifications and acceptance criteria. market only if conforming with the EC technical submitted to a quality control and released into the System in compliance with ISO 13485 rule, Each lot is under the control of a certified Quality Management All the IVD Products manufactured by the company are

Dia. Pro Diagnostic Bioprobes Srl Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy Manufacturer:



### S. LIMITATIONS

may affect the absorbance values of the samples with Bacterial contamination or heat inactivation of the specimen generate false positive results. Frozen samples containing fibrin particles or aggregates may

booled ones. Ton bns seldmss elignis gnitset for testing single sandten and consequent alteration of the level of the analyte.

the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be Diagnosis of an infectious disease should not be established on

### T. CONFIRMATION TEST

released to the doctor. sample before a diagnosis of primary infection of HSV is The confirmation test has to be carried out on any positive lead to an operation of abortion, a confirmation test is reported. the follow-up of pregnancy, where a false positive result could In order to provide the medical doctor with the best accuracy in

Prepare the Antigen/Conjugate Complex as described in Proceed for confirmation as follows:

not use any lyophilized antigen vial for this procedure ! This in 500 ul Antigen Diluent and mixed gently on vortex. Do Then 25 ul concentrated Enzymatic Conjugate are diluted the proper section. This reagent is called Solution A.

The Negative Control is dispensed in the strip in positions B1+C1. This is used for the calculation of the cut-off and The well A1 of the strip is left empty for blanking. 3 solution is called Solution B.

dispensed in the strip in position D1+E1. The positive sample to be confirmed, diluted 1:101, is 9

The strip is incubated for 60 min at +37°C, 9

,8 After washing, the blank well A1 is left empty.

100 µl of Solution A are dispensed in wells B1+C1+D1.

The strip is incubated for 60 min at +37°C, 10. Then 100 µl of Solution B are added to well E1.

and at 620-630nm (background subtraction,), blanking the their color intensity is measured at 450nm (reading filter) all the wells and the strip is incubated for 20 min at r.t. of the shall shall are added to all the wells and then then the shall are solded to the shall be 15 After washing, 100 µl Chromogen/Substrate are added to

1.0 a problem of dispensation or contamination in the first If the sample in position D1 shows a S/Co value lower than Interpretation of results is carried out as follows:

of the sample is in fact not dependent on the specific 1.2 and in position E1 shows a S/Co value still higher than 1.2 the sample is considered a **false positive**. The reactivity If the sample in position D1 shows a S/Co value higher than 7. M has to be repeated to double check the analysis. test is likely to be occurred. The Assay Procedure in Section

the sample is considered a true positive. The reactivity of 0.1 and in position E1 shows a S/Co value lower than 1.0 If the sample in position D1 shows a S/Co value higher than conjugate has occurred.

HSV and not due to any crossreaction. the sample is in fact dependent on the specific presence of

I'A no Insmuntani

presence of HSV1 and a crossreaction with enzymatic

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination in human serum and plasma Herpes Simplex Virus type 1 of IgG antibodies to

for "in vitro" diagnostic use only -



DIA.PRO

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(Milano) - Italy

REFIISVIGCE

## HSV1 lgG

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### USE

1 in human plasma and sera. For "in vitro" diagnostic use only. Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Herpes Simplex Virus type

Hetpes Simplex Virus type 1 (HSV1) and type 2 (HSV2) are arge complex DNA-containing viruses which have been shown to induce the synthesis of several proteins during infection, possessing an high number of crossreactive determinants and just a few of type-specific sequences. The majority of primary and recurrent genital herpetic infections are caused by HSV2, while non genital intentions, such as

common cold sores, are caused primarily by HSV1.

The detection of virus specific (gC and (gM anthodies are important in the diagnosis of authoritymany virus infections or reactivations of a latent one, in the absence of evident clinical

Asymptomatic infections may happen for HSV in apparently healthy individuals and during pregnancy. Severe herpetic infections may happen in immunocompromised and suppression patients in which the disease may evolve toward critical

The determination of HSV specific antibodies has then become important in the monitoring of 'risk' patients and in the follow up of acute and severe infections.

## C. PRINCIPLE OF THE TEST

Microplates are coated with native inactivated HSV1.

The solid phase is first treated with the diluted sample and IgG to HSV are captured, if present, by the antigens, safety, in the control of the sample, in the 2<sup>th</sup> incubation bound and HSV1 tigG are detected by the addition of polycional specific anti higG anotherised by the addition of polycional specific anti higG anotherised with

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of ant HSV1/1g6 antibodes present in the sample. A Calibration Curve, calibrated against an internal Gold Standard, making possible a quantitative determination of the fig6 antibody in the patient.

## D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE

n° 1.1 2 strips x 8 microvells coated with native UV inactivated HSVI in presence of bowne proteins.

Pales are sealed into a bag with desicant. Allow the microplate to reach room temperature before opening; rescal unused strips in the bag with desicant and store at 2.8°C.

### Ready to use and color coded standard or human plasma positive for HSV1 IgG ranging: 2. Calibration Curve: CAL N'... standard curve derived from

4ml CAL 1 = 0.arbU/ml
4ml CAL2 = 5 arbU/ml
2ml CAL3 = 10 arbU/ml
2ml CAL4 = 20 arbU/ml
2ml CAL4 = 20 arbU/ml
2ml CAL4 = 50 arbU/ml
4ml CAL6 = 100 arbU/ml

Slandards are calibrated in arbitrary units against an internal Gold Standard (or IGS). It contrains human serum proteins, 2% casein, 10 mM Na-clirate buffer pH 6.0 +/-0.1,0.1% Tween 20,0.08%, Na-azzide and 0.1% Kathon GC as preservalives. Standards are blue colored.

Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

4. Wash buffer concentrate: <u>WASHBUF 20X</u> 1x60m/houtle20x concentrated solution, Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0,05%. Tween 20 and 0,1% Kathon GC.

5. Enzyme conjugate: [CON.]

2dmit/val, Ready to use and red colour coded, it contains forecastish prevoluties conjugated polycional antibodies to human igG, 5% BSA, 10 mM Tris buffer ph 6,8-7-0,1, 0,1% Adhton GC, 0,02% gentamicine sulphate as preservatives and n 142. MM allianoscopies. 0.01% red alimentary dye.

6. Chromogen/Substrate: SUBS TMB
1x16m/wal. It contains 50 mM citrate-phosphate buffer pH 3.53.8, 4%, dimethylsubphoxide, 0.03%, forta-neghyl-benzidine (or
TMB) and 0.02% hydrogen peroxide (or H-O<sub>1</sub>).
Note: To be stored protected from light as sensitive to
strong illumination.

7. Sulphuric Acid [<u>HSS0: 0.3 M</u> 1x15m/val; It contains 0.3 M HSS0: solution, 1x15m/val; It contains 0.3 M HSS0: solution, Attention: Irriant (H315; H319: p-180; p-1802-p-1852, 332+p-1313, p-1805+p-1851+p-1838, p-1837+p-113, p-1862+p-1863)

8. Specimen Dissent: DILSPE 2x60m/vial. It contains 2% casely, 10 m/M Na-cifrate buffer pH 6.0 +4-0.1, 0.1% Tween 20, 0.09% Na-azide 0.1% and Kathon GC as preservatives. The reagent is blue colour coded.

## Plate sealing foils n°2

10. Package insert n°1

- E. MATERIALS REQUIRED BUT NOT PROVIDED
   Calibrated Microprettes (1000 ul, 100 ul and 10 ul) and disposable plastic lips.
   EIA grade water (doubte distilled or delonised, charcoal treated to remove codizing chemicals used as
- Timer with 60 minute range or higher,

- Absorbent paper tissues
  Calibrated ELISA microplate thermostatic incubator (dry or with, set at +37°C (+4.5°C tolerance).
  Calibrated ELISA microwell reader with 450nm (reading) and with 520-630nm (blanking) filters.

gn on 4 to

- Calibrated ELISA microplate washer. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.

2. All the personnel involved in performing the assay have to wear protecher laboratory oldries, tall-free gloves and glasses. The use of any sharp (needles) or cutting (blasses) devices, should be avoided. All the personnel involved should be trained in blosafely procedures, as recommended by the Center for Disease Control, Allanda, U.S. and reported in the National Institute of theith's publication: "Biosafety in Microbiological and Bonnedical Indianal Control and tooks."

safe and 4. The Biomedical Latoratories', ed. 1994.

3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available.

4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening bit vials and micropiales and when performing the itest. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.

5. Upon receipt, store the kit at 2.5°C into a temperature controlled refrigerator or cold room.

6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.

7. Check that the reagents are clear and do not contain visible heavy particles or aggregates.

Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each

cross-contamination between kit reagents by using them between the use of each

one. On our use the kill after the expansion date stated on the external container and internal (vials) labels. A study conducted on an opened kill did not pointed out any relevant loss of activity in the set of uses of the opicie and up to annotate.

11. Treat all specimens as potentially infective, All human serum specimens should be handled at Blossfely, Level 2, as recommended by the Center for Dissase Control, Allana U.S. In compliance with what reported in the Institutes of Haath's Laboratories, ed. 1994.

12. The use of disposable plastic-ware is recommended in the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.

13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, flauld waste generated from the wasting procedure, from residuals or controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10½ final concentration of household blasch for 16-18 has or heat inactivation by autobave at 121°C for 20 min. 14. Accidental spills from samples and conformation have to be adsorbed with paper tissues soaked with household blasch had been with water. Tissues should then the discarded in proper tissues soaked with household beach and then with water. Tissues should then the discarded in proper tissues to should have been and then with water. Tissues should then the discarded in proper tissues to should have been and then with water. Tissues should then the discarded in proper tissues to should have been and then with water. Tissues should have the discarded in proper tissues to should have the discarded in proper tissues. Waste produced during

(example: fits used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS
 Reload is drawn asspirately by venepurature and plasma or serum is prepared using standard sehon-judges of prepared ton of samples for clinical laboratory analysis. No influence has been asserved in the preparation of the sample with citrate, EDTA and happain.
 Samples have to be clearly identified with codes or names in order to avoid misinfarmetation of results. Bar code labeling and electronic reading is strongly recommended.

H. PREPARATION OF COMPONENTS AND WARNINGS
A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3

Microplate:

Allow the microplate to reach room temperature before opening the container. Check that the not turned dark green, indicating a defect in storium this case, call Dis. Pro's customer service.

Unused strips have to be placed back into the all with the desiccant supplied, firmly zipped a Atter first opening, remaining strips are stable un It opening, I remaining strips are stable until the humidity desiccant bag turns from yellow to green. o reach room temperature (about 1 hr)
mainer. Check that the desiccant has
indicating a defect in storing. and stored ω

Ready to use component . Mix carefully on

reported on the label, to e and then gently mix on

after dissolution is not stable.

## Wash buffer concentrate

a Determine reason is a surregy recommendate.

A Harmolysed ("red") and visibly hyperdepientic ("milky") samples of have to be discarded as they could generate false results. Samples containing residues of florin or heavy particles or enuicobial filaments and bodies should be discarded as they at a could give rise to false results.

A. Sera and plasma can be stored at +2". 8"C for up to five days after collection. For longer storage periods, samples can be stored frozen at -20"C for several months. Any frozen samples to should not be frozen/thaved more than once as this may 5. If particles are present, contribuge at 2.000 from for 20 min or filter using 0.2-0.80 filters to clean up the sample for testing.

vortex before use

Add the volume of ELISA grade water, rep the tyophilised powder, let fully dissolve ar vortex.

Note: The control after dissolution is not a aliquots at -20°C. Add the volume

Store frozen

The whole content of the concentrated solution has to be diluted 20x with idestilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbies could impact on the efficiency of the washing cycles.

\*\*Note: Once diluted, the wash solution is stable for 1 week at +2.8° C.

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly

Ready to use. Mix well on vortex before use, be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.

Do not expose to strong illumination, oxidizing agents and matalic surfaces.

If this component has to be transferred use only plastic, possible seried disposable contamer.

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Doc.

### use con

Mix carefully on vortex before

use

to be installed in the oberating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needless used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to the tested expressed only an existence of the provision 
Diluent

Sulphuric Acid: Ready to use. Mix well on waterition: Irritant (H315, H319305+ P351+P338, P337+F x well an variex before use. (H315, H319, P280, P302+P352, 332+P313, 38, P337+P313, P382+P363).

Warning H staten H315 – Causes s H319 – Causes s

tements: s skin irritation s serious eye irritation,

Precautionary P statements: P280 - West protective gi gloves/protective clothing/eye protection/:

#302 + #352 = IF ON SKIN: Wash with plenty of soap and water, #312 + #313 = If skin initiation occurs; Get medical advocationation, #935 + #951 = #938 = IF IN EVEST. Rives caudiously with water to several minutes. Remove contact lenses; if present and easy to of Conduce rissing. #937 + #9312 = if yet initiation persists: Get medical advice/attention, #937 + #9313 = if yet initiation persists: Get medical advice/attention, #932 + #933 - Take off contaminated clothing and wash it before reuse.

## I. INSTRUMENTS WITH THE KIT AND TOOLS USED IN COMBINATION

Nicorpipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol. 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +0.2%. Decontamination of shills or residues of kit components should also be carried

out regularly.

The ELISA incubator has to be set at -37°C (beteance of +20°C) and regularly chacked to ensure the correct temperature is maintained. Both dry incubators and water hattis are suitable for the incubations. Provided that the temperature is maintained. Both dry incubators and water hattis are suitable for the incubation of ELISA tests.

The ELISA wester is cortemely important to the overall performances of the assay. The waster must be carefully validated and contectly optimised using the kit controls and reference panels, before using the kit control and characterized negative and positive reference samples, and characterized by needles) of the washer has be carried out according to the instructions of the manufacturer.

5. The ELISA microplate reader has to be captipped with a second filter (620-630nm, performances should be (a) benefits equally to resure that the correct optical density is measured, it should be regularly to resure that the correct optical density is measured. It should be regularly manufacturer in manufacturer.

5. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, controlled and regularly to record to natch the values reported in the section in necklation and characterized controlled and regularly serviced in order to natch the values reported in the section in action and characterized the section and characterize

exceed 20-30 units per run.

Dia Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

PRE ASSAY CONTROLS AND OPERATIONS
Check the expiration date of the kit printed on the external about (primary container). Do not use if expired,
Check that the liquid components are not contaminated by

visible porticles of aggregates. See the concurrence of the concurrence of concurrence o

7

leagents.

8. Set the ELISA incubator at 43°C and prepare the ELISA washer by priming with the diduted washing solution, according to the manufactures instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.

9. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.

10. It using an automated work station, turn on, check settings an automated work station, turn on, check settings on architecture to use the right assay protocol.

10. Check that the mining-plettes are set to the required volume, to check that all the other equipment is available and ready to read.

to use,
3. In case of problems, do
advise the supervisor. , do not proceed further with the test and

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all. The samples in testing.

The kit may be used for quantitative and qualitative determinations as well.

## QUANTITATIVE DETERMINATION

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Sample Diluent and then 10 µl sample (1:101 dilution fractor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl samples into the proper wells of the

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This procedure may be carried out also in two steps of dilutions of 1.10 each (90; µl Sample Diluent + 10 µl sample) into a second offuline plotform. Make then the instrument segirate first 100 µl Sample Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper well of the assay micropiate.

Do not dilute Calibrators and the dissolved Control Serum as they are ready to use.

Dispense 100 µl calibrators/control in the appropriate calibrations/control in the appropriate

For the next operations follow the operative instructions reported below for the Manual Assay. calibration/control wells

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

### Manual assay: 1. Dilute sample

- Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample), Do not dilute the Califoration Set as califorators are ready to use. Mix carefully all the liquid components on vortex and then
- proceed as described below.

  Place the required number of microwells in the microwell holder. Leave the A1 and B1 emply for the operation of
- Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each
- properly identified well.
  Incubate the microplate for 60 min at +37°C.

Important note: Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. On not cover strips when using EUSA automatic instruments.

- Wash the microplate with an automatic washer as reported
- previously (section 1.3).
  Pretter 100 µl Enzyme Conjugate into each well, expert
  A1+31 blanking wells, and cover with the sealer. Check
  that this red coloured component has been dispensed in all
  the wells, except A1 and B1.

Important nate: Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.

- Incubate the microplate for 60 min at +37°C. Wash microwells as in step 5. Pleete 100 µl. Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at room temperature (18-24°C) for 20

Important note: Do not expose to strong direct illumination. High background might be generated.

- Pipette 100 µl Sulphurto Acid to stop the enzymatic reaction into all the walls using the same pipetting-sequence as in step 9, Addition of sacid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
   Measure the colour intensity of the solution in each well, as described in section 15, at 450nm filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1 or B1 or both.

M2\_QUALITATIVE DETERMINATION....
If only a qualitative determination is required, proceed described below:

Automated assay:
Proceed as described in section M1.

- Manual assay: Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent +10 µl sample). Do not dilute the Calibration Set as calibrations are ready to use Microscattly all the flightd components on vortex and then proceed as described below.

  Place the required number of Microwells in the microwell place the required number of Microwells in the microwell.
- holder. Leave A1 well empty for the operation of branking. Dispense 100 µ of Calibrator 0 artiUm1 and Calibrator 5 and Um1 in objecte and Calibrator 100 and DUM1 in single. Then dispense 100 µ of diluted samples in each properly.
- Incubate the microplate for 60 min at +37°C;

Important note: Strips have to be sealed with the adhesive sealing foil supplied, only when the test is carried out manually. Do not cover strips when using EUSA automatic instruments.

- Wash the microplate with an automatic washer as reported
- previously (section 1.3).

  Phoetic 100 µi Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.

Important note: Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur

- Incubate the microplate for 60 min at +37°C
- Wash microwells as in step 5.

  Wash microwells as in step 5.

  Pipette 100 µi Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at room temperature (18-24°C) for 20 minutes.

High background might be generated. Important note: Do not expose to strong direct illumination

- 2. Pleate 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will um the positive calibrators, the control serum and the positive samples from blue to yellow.

  1. Measure the colour intensity of the solution in each well, as described in section 1.5, at 450mm filter (reading) and at 620-
- 630nm (background subtraction, strongly recommended), blanking the instrument on A1,

- General Important notes:

  If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.
- Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.

## N, ASSAY SCHEME

Method	Operations
Calibrators & Control (*)	100 µi
Samples diluted 1:101	100 11
1 <sup>st</sup> incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µI
2 <sup>rd</sup> incubation	60 min
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 1
3 <sup>™</sup> incubation	20 min
Temperature	F
Sulphuric Acid	100 u
Reading OD	450nm

- (\*) Important Notes:

  The Control Serum (CS) it does not affect the test's
- results calculation,
  The Control Serum (CS) used only if a laboratory
  the Control is required by the Management

An example of dispensation scheme for Quantitative Analysis reported below:

-	2	ω	4	O	9	7	ĆO	60	0 -4	14:114	
BLK	CAL4	S	j						-		
BLK	CAL4	\$2									
CALI	CALS							I			
CALT	CALS	n) 4.									
CALZ	CAL6	S S									
CAL2	CALE										
CAL3	CS(*)										
CAL3	CS(")	88									

10 mm00m>

Legenda: BLK = Blank CAL = Calibrator CS(") = Control Serum - Not mandatory S = Sample BLK = Blank

An example of dispensation scheme in qualitative assays reported below:

### Microplate

OB>	CAL E	0 0 0 0 4 0	S S S									
0	CAL2	88			1	4	1		1	1		1
m	CAL2	S 7			1		1					
73	CAL6	8 8				4	4				1	
0	S			П		1	Ц	Ц	Ц			Ш
I.	\$2	S 10		1	4	1	1	1	1		1	

BLK = Blank S = Sample CAL = Calibrators

Legenda:

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O. INTERNAL QUALITY CONTROL

A validation check is carried out on the calibrators any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

Check	Requirements
Blank well	< 0.050 OD450nm value
CAL 1 0 arbU/ml	an 00
CAL 2 5 arbU/ml	OD450nm > OD450nm CAL1 + 0.100
CAL 6 100 arbU/ml	OD450nm > 1,000

If the results of the test match the requirements stated above proceed to the next section.

they do not, do not proceed any further and operate

exemial containingtion of the	
washer seuritys are as validated in the pre	
3. that the washing procedure and the	
librator instead) :	
distribution (dispensation of a wrong	< 1,000 OD450nm
executed;	and arbutmi
1. that the procedure has been correctly	CALB
Calibrator has occurred.	
4. that no external contamination of the	
qualification study:	
3. That the washing procedure and the	0.100
	0D450nm CAL1+
distribution (ex.: dispensation of a wrong	OD450nm <
2. that no mistake has been done in its	
executed:	5 arbU/ml
* That the boundary	CAL 7
b. that the washer needles are not blocked or	
the enzyme conjugate	
contaminated with positive samples or with	
5, that micropipettes haven't got	
conjugate	
spile of positive samples or the enzyme	
calibrator or of their wells has occurred due	
4. that no contamination of the securive	
calibrator instead of the negative one:	
assay procedure (dispensation of a northwe	
3. that no mistake has been done in the	> 30%
used and the washer has been primed with it	coefficient of variation
2 that the proper washing solution has been	ager planking
qualification study;	> U.15U QD450nm
washer settings are as validated in the pre-	C arbC/ml
f. that the washing procedure and the	CAL 1
not got contaminated during the assay	> 0.050 OD450pm
1. that the Chromogen/Sustrate solution has	Blank well
CONTR	

Should one of these problems have happened, after checking, report to the supervisor for further actions.

\*\* Note:

If Control Serum has used, verify the following data

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If the results of the test doesn't match the requirements stated above, operate as follows:

	Different from expected value	Control Serum	Problem
washer settings are as validated in the pre- qualification study: 4. That no external contamination of the control serum has occurred.	2. that no mistake has been done in its distribution (dispensation of a wrong calibrator instead)  3. that the washing procedure and the	ne proced	Check

Anyway, if all other parameters (Blank, CAL1, CAL2, CAL6), match the established requirements, the test may be considered valid.

### P. RESULTS

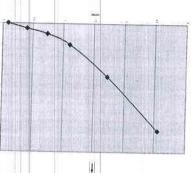
## P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters

Interpolation is suggested).
Then on the calibration curve calculate the concentration of anti-Herpes Simplex Virus type 1 lgG antibody in samples.

An example of Calibration curve is reported in the next page.

## Example of Calibration Curve:



R. PERFORMANCES

It Limit of detection

The limit of detection of the assay has been calculated by means of an internal Gold Standard in absence of an international preparation to refer to.

The limit of detection has been calculated as mean OD450nm Calibrator 0 arbUm+ 5 S.D.

Tine table below reports the mean OD450nm values of this

the assay for three lots. standard when diluted in negative plasma and then examined in

P.2 Qualitative method in the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 5 arbUrml and then check that the

Example of calculation

The following data must obtained by the user.

Calibrator 0 arbU/ml: 0.020 - 0.024 OD450nm 0.022 OD450nm

Particular attention in the interpretation of results has to be in the follow-up of pregnancy for a primary infection of HSV to the risk of neonatal malformations.

- interpretation of results should be done under the supervision of the laboratory supervision to reduce the risk of judgment errors and misriterpretations.

  When test results are transmitted from the laboratory to
- another facility, attention must be paid to avoid erraneous data transfer.

  In the follow-up of pregnancy for HSV infection a positive result (presence of igG antibody > 5 archVm) should be confirmed to ruled out the risk of a false positive result and a false definition of protection.

Important Note:

Do not use the calibration curve above to make calculations

not be used instead or real figures

Lower than 0.150 - Accepted

Higher than Cal 0 + 0,100 - Accepted Calibrator 5 arbU/ml: 0.350 – 0.370 OD450nm 0.360 OD450nm

Calibrator 100 arbU/ml: 2 Higher than 1.000 — Accepted 2,245 OD450nm

Q. INTERPRETATION OF RESULTS Samples with a concentration lower than 5 arbU/ml are considered negative for ant HSV1 IgG antibody.

Samples with a concentration higher than 5 arbU/ml are considered positive for ant HSV1 IgG antibody.

V due

### Important notes:

Diagnostic sensitivity;
 The diagnostic sensitivity as been tested in a performance evaluation study on panels of samples classified positive by a kit US FDA approved. Positive samples from different stage of HSV infection were tested. The value, obtained from the analysis of more than 300 specimens, has been > 98%.

## Diagnostic specificity:

The diagnostic specificity has been determined on panels of negative samples from not infected individuals, classified negative with a tit US FDA approved.

Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been

used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for interferences due to collection and storage.

Potentially interfering samples derived from patients with different pathologies (mostly ANA, AMA and RF positive) and No interference was observed rom pregnant women were tested.

on more than 100 specimens. No crossreaction was observed.

An overall value > 88% of specificity was found when examined

3. Precision.
It has been calculated on the Calibrator 5 arbU/ml, considered the cut-off of the assay, examined in 16 replicates in three separate runs for these lots.

## HSV1G.CE Lot # 1804

0.024	UD 450nm 0.292 0.290 0.285	Mean values 1st run 2nd run 3 <sup>rd</sup> run	1000
0.025	0,289	Average	

## Mean OD450nm values (n = 2)

Mean values 1st run

2nd run ).382 ).629 7.59

3" run

OD 450nm

0.365 0: 0.022 0: 6.02 7

Dor:

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lgG arbU/ml	HSV1G.PU Lot # 0703	HSV1G.PU Lot # 1263	
0	0.077	0.034	
5	0.355	0,404	
10	0.742	0.713	
20	1.254	1.216	
50	1.952	1.928	
001	2,623	2.261	

# The assay shows a limit of detection far better than 5 arbU/ml.

In addition the preparation code Accurum n° 150, produced by Boston Biomedica Inc., BBI, USA, was tested in dilutions to determine the limit of its detection and provide a further value of analytical sensitivity.

Mean values	tstrun	2nd run	3" Pan	Average
OD 450nm	0,322	0.298	0.304	805.0
Std Deviation	0.019	2010	0000	0.000
The state of the s	0.010	810.0	0.010	0.018
CV %	5.59	6.38	80.3	574

The variability shown in the tables above did not result in sample misclassification.

## Mean OD450nm values (n = 2)

S. LIMITATIONS OF THE PROCEDURE

ution	HSV1G,CE Lot # 1004	HSV1G.PU Lot # 1203	HSV1G.PU Lot # 0204/2
×	1.248	1.218	1 3000
2×	0.860	0.848	0.876
×	0.545	0.526	0.583
×	0.315	0.300	0.329
6 X	0,164	0.152	0.148
2×	0.082	0.064	0.072
IM/DO	0.057	0.050	0.047
1m/Ud	0.288	0.355	0.318

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each bit is submitted to a quality control and repleased into the market only if conforming with the EC technical specifications and acceptance criteria.

## Manufacturer:

Dia:Pro Diagnostic Bioprobes Srl Via:G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy



# HSV2 IgM

"Capture" Enzyme Immuno Assay (ELISA) for the determination Herpes Simplex Virus type 2 in human plasma and sera of IgM antibodies to

for "in vitro" diagnostic use only -



## DIA.PRO

20099 Sesto San Giovanni Via G. Carducci nº 27 Diagnostic Bioprobes Srl

(Milano) - Italy e-mail: into(wdiapro.it Fax +39 02 26007726 Phone +39 02 27007161

REF HSV2M.CE 96 tests

## HSV2 IgM

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### A INTENDED

during pregnancy.
For "in vitro" diagnostic use only, Enzyme ImmunoAssay (ELISA) for the determination of IgM antibodies to Herpas Simplax Virus types 2 in human plasma and sets with the "capture" system. The devise is intended for the follow-up of HSV2 intended palients and for the monitoring of risk of neonatal defects due to HSV intection

### B. INTRODUCTION

Helipes Simplex Virus type 1 (HSV1) and type 2 (HSV2) are large complex DNx-containing viruses which have been shown to induce the synthesis of several proteins during infection, possessing an high number of cross-reactive determinants and

just a few of type-specific sequences. The majority of primary and recurrent genital herpetic infections are caused by HSV2, while non genital infections, such as common cold sores, are caused primarily by HSV1.

The detection of virus specific igG and igM antibodies are important in the diagnosis of acutelythinary virus infections or reactivations of a latent one, in the absence of evident clinical

Asymptomatic infections may happen for HSV in apparently healthy addividuals and during pregnancy. Severe hepetic infections may happen in immuno-compromised and suppressed patients in which the disease may evolve toward

critical pathologies.

The determination of HSV specific antibodies has then become important in the monitoring of "risk" patients and in the follow up of acute and severe infections.

## C. PRINCIPLE OF THE TEST

The assay is based on the principle of "gM capture" where IgM class antibodies in the sample are first captured by the solid phase coaled with anti high ambibody.

After washing out all the other components of the sample and in particular tigG ambibodies, the specific IgM explured on the solid phase are interested by the addition of a preparation of inactivated ISVZ, Taibeded with a HSVZ specific antibody conjugated with percondase (HRP).

After Incubation, microwells are washed to remove unbound conjugate and then the chromogenisubstrate is added. In the presence of bound conjugate the coloriess substrate is hydrolyzed to a colored end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies.

to HSV2 present in the sample.

A system is described how to control whether the positivity shown by a sample is true or not (Confirmation Test), helpful for the clinician to make a correct interpretation of results.

D. COMPONENTS
The kit contains reagents for 96 tests

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with anti human IgM affinity
purified goat antihody, in presence of bovine proteins.
Plates are sealed into a bag with desicoant. Allow the
microplate to reach-norm temperature before opening reseal
unused strips in the bag with desicoant and store at 2,8°C.

Negative Control: CONTROL |
 1x4.0 ml/vial. Ready to use control. It contains 1% human serum proteins, 2% casein, 10 mM fris buffer pH 6.0+/-0.1, 0.1%

The negative control is pale yellow color coded Tween 20, 0.09% sodium azide and 0.1% Kathon preservatives. GC.

a)

3. Positive Control: CONTROL 3 1x4.0 m/wal. Ready to use control. Il contains 1% human serum positive for HSV2 19th, 2% casein, 10 mM iris buffer pH 6.04-0.1.0.1% Tween 20. 0.09% sodium azide and 0.1% Kathon GC

The positive control is green colour coded.

4. Calibrator: CAL \_\_mi
W 1 yaphilized wal, To be dissolved with EIA grade water as reported in the label, It contains and HSV2 IgM, fetal bovine serum, 0.2 mg/mi gentamicine sulphale and 0.1% Kalhon GC as

Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

S. Lyophilized HSV2 Ag: [AG HSV2]
N° 6. lyophilized visis. The visis contain lyophilized gamma-ray inactivated HSV2 in protein whiter. The solution contains 2% bovine proteins, 10 mM Tris HCl buffer pH 5.8+4.0,1,0,2 mg/ml gerlamicine sulphale and 0,1% Kathon GC. To be dissolved with 1.9 ml of Antigen Diluent as reported in the specific section.

6. Wash buffer concentrate: MASHBUF 20XI 1x60m3bottle, 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2 0.05% Tween 20 and 0.1% Kathon GC.

8. Antigen Dituent: AG DIL 11 val of 16 mL Problem Buffer solution for the preparation of the immunocomplex. The solution contains 10 mM Tris buffer pH 6.84-4.0.1, 2% BSA, 0.1% Kathon GC and 0.2 mg/ml gertlamnicine subhabite as preservatives. The reagent is code coloured with 0.01% red alimentary dye 7. Enzyme conjugate: CONJ 2000

1x0.8 milvial. 20x concentrated solution of a HSV2-specific antibody, labeled with HRP and diluted in a protein buffer containing 10 mM Tris buffer pH 6.84-0.1, 2% 85A, 0.1% Kathon GC and 0.2 mg/ml gentamicine sulphate as

ακου.υ ml/vial. Proleic buffered solution for the dilution of samples. It contains 2% casein, 10 mM this buffer.pH 6.04-0.1, 0.1% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives. 9. Specimen Diluent : DILSPE

The reagent is color coded with 0.01% blue alimentary dye.

Chromogen/Substrate: SUBS TNIS
 X15m/Wal. It contains a 50 mM citrale-phosphate buffered solidion at pH 35-38. 0.33% retra-methyl-penzidine (TMB).
 0.02% hydrogen peroxide (THC) and 4% dimethylsulphoxide.
 Note: To be stored protected from light as sensitive to

10. Sulphuric Acid. <u>FESO. 0.3 M</u> "Exf5m/Val. It contains 0.3 M HSOs solution. Attention: Infant, [4715, H.319, p.280, P.302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P353)

11. Plate sealing foils n° 2

12, Package insert nº 1

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- Ν MATERIALS REQUIRED BUT NOT PROVIDED Calibrated Micropipettes (1000 ut, 100 ut and 10 E ) and
- disposable plastic tips.

  EIA grade water (double distilled or treated to remove oxidizing chadisinfectants).

  Timer with 60 minute range or higher. tips. (double distilled or chemicals deionised, used , charc gg
- மையம்
- Absorben paper tissues.
   Calibrated ELISA microplate thermostatic incubator (dry or well) set at 4770 (#4.570 bideance).
   Calibrated ELISA microwell reader with 450nm (reading) and with 520-650nm (blanking) filters.
   Calibrated ELISA microplate washer.
   Vortex or similar mixing tools.

## The kit has to be used by sk

- The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
- 2. All the personnel involved in performing the assay have to war protective laboratory clothes, tale-fee gloves and glasses. The use of any sharp (needles) or curring (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Alfanta, U.S. and reported in the National Institute of Health's publication. "Biosafety in Microbiological and Biomedical Laboratories," ed. 1984, and the personnel involved in sample handling should be vecchated for HBV and HAV, for which vaccines are available,
- sale and effective.

  The laboratory environment should be controlled so as to avoid conteminants such as dust or air-born microbial agents, when opening kit visits and micropistics and when performing the test. Protect the Chromogen (TMB) from strong light and avoid when the best of the performance where the test is undertaken.

  Lypon receipt, store the kit at 2.8°C into a temperature controlled refrigerator or cold from.
- Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same fol should not be interchanged.
   Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit
- Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each
- Avoid cross-contamination between kit re posable tips and changing them between kit reagents by using ween the use of each

- one.

  10. Do not use the kit after the expiration date stated on the external container and internal (viais) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six is uses of the device and up to 3 months.

  11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosefety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the institutes of Health's publication. Biosefety in Microbiological and Biomedical
- Laboratories", ed. 1984.

  12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations. In order to avoid
- 13. Waste produced during the use of the kit has to be decarated in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the wasting procedure, from residuals of controls and from samples has to be treated as potentially infective metarial and inactivation before waste. Suggested procedures of inactivation are

- treatment with a 10% final concentration of household bleach for 6-18 the or heat inactivation by autoclave at 121°C for 20 min.

  14. Accidental spalls from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hostplat waste.

  15. The Sulphuric Acid is an initiant, in case of spills, wash the
- surface with plenty of water

  5. Other waste materials, generated from the use of the kit (example; tigs used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory

## . SPECIMEN: PREPARATION AND WARNINGS

Ready to use. Negative Control: Ready to use. Mix well on:

Add the volume of ELISA grade water reported on the label the lyophilized powder. Let fully dissolve and then gently mix.

Important The e solution is not -20°C. stable. Store the Calibrator

The whole content of the concentrated solution has to be diluted 20x with bidsilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

- Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for cinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA

- after collection. For longer storage periods, samples can be stored flozen at 20°C for several months. Any frozen samples should not be frozen/thewed more than once as this may generate particles that could affect the test result.

  5. If periodes are present, centrifuge at 2,000 pm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS
A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desicoant has not turned day green, indicating a defect in storing, in this case, call Dia, Pros customer service. Unused strips have to be placed back into the aluminum pouch, with the desicoant supplied, firmly zipped and stored at 12°.8°C. After first opening, remaining strips are stable until the humidity indicator inside the desicoant bag turns from yellow

vortex before use

Ready to use. Mix well on

vorlex before use.

Positive Control

## Wash buffer concentrate

Note: Onco +2.8° C.

diluted, the wash

soution is stable

fgr

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- 2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results, Bar code labeling and electronic reading is strongly recommended.

  3. Haemolysed (Ted') and visibly hyperlipemic (Tmiky') samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they microbial filaments and bodies should be discarded as they

Ready to use. Mix well on vortex before use. Be careful not to contaminate the liquid with oxidizing chemicals, Do not expose to strong illumination, oxidizing agents and metallic surfaces.

Ready to use; Mix well on vortex before

Specimen Diluent

air-driven dust or microbes. Chromogen/Substrate:

If this component has to be transferred use only plastic, possible

- ould give rise to false results.

  4. Sera and plasma can be stored at +2"...8"C for up to five days.

  For Immer storage periods, samples can be

Sulphuric Acid:
Sulphuric Acid:
Ready to use. Mix well on vortax before use.
Attention: Irritant (H315, H319; P280, P802,+P362, 332+P313,
P305+ P351+P338, P337-P313, P362+P363).

Warning H statements: H315 – Causes skin imitation, H319 – Causes scrious eye imitation,

- Precautionary P statements P280 Wear protective gl protective glaves/protective clothing/eye protection/face
- P302 + P352 IF ON SKIN: Wash with plenty of soap and water, P332 + P313 If skin initiation occurs; Get medical advise/attention, P305 + P351 P333 IF is IF P4755. Rises caudiously with valer for several minutes. Remove contact lenses; if present and easy to do. Continue finding. P332 If skin provided the passes of the passe

# I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

- Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to egular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +1-2%. Decontamination of spills or residues of kit components should also be carried out membrane.
- out regularly.

  2. The ELISA incubator has to be set at +37-0 (tolerance of +1-0.57-0) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubations provided that the instrument is validated for the incubation of ELISA tests.

  3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be paretify.

Cal

validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests, Usually 4-5 washing podiest (septimion - dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking strate of 20-30 seconds between cycles is suggested, in order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to make the values reported below in the section "Internal Quality Control". Regular calibration of the volumes delivered by and maintenance (decondamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

Proceed carefully as follows:

1. Dissolve the content of a lyophilized vial with 1.9 ml of Conjugate/Antigen Dissolve Let fully dissolved the lyophilized content and then gently mix on vortex.

2. Gently mix the concentrated Enzyme Conjugate on vortex. Then add 0.1 ml of it to the vial of the dissolved HSV2 Ag

Important Notes

and mix gently

s necessary to stable. Store a ni

Dissolve and prepare only the number of wals necessary the test. The Inmunocomplex obtained is not stable. SI any residual solution force in aliquots at 20°C. The preparation of the Immunocomplex has to be done in before the dispensation of samples and conflost into plate. Mix again on vortex gently just before its use

right to the

- 4. Incobation limits have a tolerance of £5%.

  The ELISA micropiate reader has to be equipped with a reading filter of 450m and with a second filter (520-530m, strongly recommended) for blanking purposes. Its standard parformances should be (a) banking purposes. Its standard parformances should be (a) banking is carried out on the well identified in the section "Assay Procedure". The optical system of the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical detaily is measured. It should be instructions:

  System of the reader has to be calibrated regularly to ensure that the correct optical detaily is measured. It should be instructions:

  System of the reader has to be carefully set, earling, data handling) have to be carefully set, eating, data handling) have to be carefully set, eating, data handling) have to be carefully set, eating, and the reader, in addition, the section "internal Quality Corrections of the unit and regularly services in order to match the values reported in the section standard and correctly set. Particular validated as for the washer and the reader. In addition, the disease of the controlled to mismize the possibility of contamination of all entered 20.30 until set run.

  The section "internal controlled to mismize the possibility of contamination of all glacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20.30 until set run. 7
- acceed 20-30 units per run.

  Das Pros customat service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

- L PRE ASSAY CONTROLS AND OPERATIONS

  1. Check the expiration date of the kit printed not a charge at latel (primary container). Do not use the device if expired, alter (primary container). Do not use the device if expired, containinated by visible particles or aggregates. Check that the Chromogenet/Substrate is colories or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of fliquid is present inside the box (primary container). Check that the aluminum pouch, containing the micropiate, is not purchased or dramanum.
- punctured or damaged.

  Dilute all the content of the 20x concentrated Wash Solution
- as described above.

  Dissolve the Calibrator as described above and gently mix.

  Allow all the other components to reach norm temperatur.

  (about 1 hr) and then mix gently on vortex all liqui liquid
- reagents.
  Set the ELISA incubator at \*37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
- Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.

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- If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
   Check that the microphothes are set to the required volume, to the check that all the other equipment is available and ready the case of problems, do not proceed further with the test and advise the supervisor.

## M. ASSAY PROCEDURE The assay has to be car

the samples in testing. M.1 Automated assay: The assey has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

#

In case the test is carried out automatically with an EUSA system, we suggest to make the instrument aspirate 1000 µl Specimen Dilibent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube Before the next sample is aspirated, needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispenses 100 µl diluted samples into the proper wells of the microplate.

This procedure may be carried out also in two steps of dilutions of 1:10 each (90 µl Specimen Diluent + 10 µl sample) into a second dilution platform. Make then the instrument aspirate first 100 µl Specimen Diluent, then 10 µl liquid from the first dilution in the platform and finally dispense the whole content in the proper wall of the assay interoplate.

Do not dilute controls/calibrator as they are ready to use. Dispense 100 µl calibrators/control using the second of the

calibration/control wells.

For the next operations follow the operative instructions reported below for the Manual Assay.

It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

## M. 2 Manual assay: Dilute samples

- Dilute samples 1:101 by dispensing first 10 µl sample and then 1 ml Specimen Diluent into a dilution tube; mix gently
- on vortex.

  Place the required number of Microwells in the microwell holder. Leave the well in position A1 empty for the
- operation of blanking.

  Disperse 100 µi of Negative Contris and 100 µi of Calibrator in the proper wells in duplicate. Disperse 100 µi of Positive Control in single into the proper well. Do not didude controls and the calibration as they are ready to use!
- Dispense 100 µl difuted samples in the proper sample wells and then check that all the samples wells are blue colored and that controls and calibrator have been
- dispensed. Incubate the microplate for 80 min at +37°C.

Important note: Strips have to be sealed with the adhesive sealing foil supplied only when the test is carried out manually. Do not cover strips when using EUSA automatic instruments.

- Wash the microplate with previously (section 1.3) an reported
- Pipette 100 µl of the Ag/Ab immunocomplex into each well, except the blanking well A1, and cover with the sealer. Check that all wells are red colored, except A1.

Important note: Be careful not to touch the plastic surface of the well with the tip filled with the Ammunocomptex Contamination might occur. Ag/Ab

- œ incubate the microplate
- ထ Wash microwells as in step 6
- 10 Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at room temperature (18-24°C) for 20 minutes.

High background might be generated. Important note: Do not expose to strong direct illumination

- Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10. Addition of acid will turn the positive control and positive samples from blue to
- yellow:

  Measure the color intensity of the solution in each well, as described in section 1.5, at 450nm filter (reading) and at 620blanking the instrument on A1. (background subtraction, strongly recomm

### N. ASSA

Reading OD .	huric Acid	emperature	ncubation	MB/H2O2 mix		emperature	incubation	mmunocomplex	Washing 4	Temperature	Incubation	Samples diluted 1:101	Cunitals@callbrator (*)
450nm	100 ul	1.1	20 min	100 u	4-5 cycles	+37°C	60 min	100 ul	4-5 cycles	+37°C	60 min	100 ul	100 ul

- (\*) Important Notes:
  The Calibrator (CAL) does not affect the Cut Off calculation, therefore il does not affect the test's results calculation
- The Calibrator (CAL) used only if a laboratory internal quality control is required by the

An example of dispensation scheme is reported below

	į	_						
	Þ	œ	n	O	m	m	0	Ξ
1	BLK	NC	NC	CAL(*)	CAL(*)	PC	S)	S2
2	\$3	22	SS	SS	\$7	SS	98	S10
w								
4								
4   5   6								
5 6 7								
7							1	
30							1	
D	1		I					
3	1							
1								1
	ı	Т	П					П

PC = Positive Control

3	
g	
H.	
릐	
+37°C	
200	

	Ņ		7-4
over leading to high background.	Reading has to be carried out just after the addition of the	positive results on reading.	<ol> <li>If the second filter is not available ensure that no linger prints are present on the bottom of the microwell before</li> </ol>

2	
20	
-	
賣	
a	

Positive Centrol < 1.000 OD450nm	Megative Control (NC)  > 0,200 OD450nm  after blanking  coefficient of  variation > 30%	Blank well
1. that the procedure has been correctly performed.  2. that no missake has occurred during the destribution of the control (dispensation organities control instead of positive control).  3. that the vashing procedure and the washer seruly; as the washer or positive control, acting are as weldered in the pre-qualification study;	I. that the washing procedure and the washer study, are as validated in the pre qualification study, and the washer study. It has been primed with it whose washing solution has been used and the washer has been primed with it whose washer has been primed with it whose washer has been done in the assay procedure (dispensation of positive control of the wash where the control was dispensed that accurred due to positive another, to split or in the excurred due to positive another, to split or in the excurred out to positive another, to split it in the properties have not become contaminated with positive samples or with the enzyme conjugate.	Check  I that the Chromogen/Substrate solution has

If any of the above problems have occurred, report the problem to the supervisor for further actions:

\*\* Note:

If the Calibrator has used, verify the following data

If the results of the test doesn't match the requirements stated above, operate as follows:

c	)
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ZNA	
ODAL	
7	
ğ	

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A validation check is carried out on the controls any time the kill is used in order to verify whether the performances of the assay are as qualified. Control that the following data are matched:

Probler Calibrator S/Co < 1.2

performed;
2. that no mistako has occurred during its distribution (ex. dispensation of negative control instead)

control instead

control instead

control instead

washer settings are as validated in the pre
qualification study

that no external contamination of the

Check
The procedure has been correctly

Parameter	Requirements
Blank well	< 0.05 OD450nm value
Negative	< 0.200 OD450nm value after
Control mean	blanking
value (NC)	coefficient of variation < 30%
Positive Control	Positive Control   > 1,000 OD450nm

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and perform the following checks:

P. CALCULATION OF THE CUT-OFF
The test results are calculated by means of the mean ODASDInm
value of the Negative Counted (NC) and a mathematical
calculation, in order to define the following cut-off formulation:

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

aidr has occurre

Problem	Check
ank well	1 that the Chromogen/Substrate solution has
05 OD450nm	
gative Control	1. that the washing procedure and the washer
0)	settings are as validated in the pre qualification
,200 OD450nm	sludy;
er blanking	2 that the proper washing solution has been
	used and the washer has been primed with it
efficient of	before use:
iation > 30%	3, that no mislake has been done in the assay
	procedure (dispensation of positive control
	egative control;
	4, that no contamination of the negative control
	or of the wells where the control was dispensed
	has occurred due to positive samples, to spills
	or to the enzyme conjugate:
	content illustrates have not become
	enzyme continuate
	6 that the washer needles are not blocked or
	partially obstructed.
sitive Control	1. that the procedure has been correctly
,000 OD450nm	
	2. that no mistake has occurred during the
	of the control (dispensation
	negative control instead of positive control).
	J. Inal the washing procedure and the washer
	durings are as validated in the pre-qualification
	4. that no external contamination of the positive
	control has occurred

Test results are interpreted as a ratio of the sample OD450nm and the Cut-Off value (or S/Co) according to the following table

Interpretation

Q INTERPRETATION OF RESULTS

Important note: When the calculation of results is performed by the operating system of an CLISA automated work station, ensure that the proper formulation is used to adducte the core of value and generate the correct interpretation of results.

The value found for the test is used for the interpretation of results as described in the next paragraph.

Cut-Off = NC + 0.250

1.0 - 1.2 > 1.2 < 1.0 S/Co

Equivocal Positive Negative

weeks from first teeling.
A positive result is indicative of a Herpes Simplex Virus type 2 A negative result indicates that the patient is not undergoing an acute infection of Herpes Simplex Virus type 2.

Any patient showing an equivocal result, should be re-tested by examining a second seance from the patient after 1-2.

An example of calculation is reported below

real figures obtained by the user. Important Note: The following data must not be used instead or

Mean Value: 0.100 OD450nm Lower than 0.200 – Accepted Positive Control: 1.850 OD450nm Higher than 1.000 – Accepted Negative Control: 0.090 - 0.110 - 0.070 OD450nm Mean Value: 0.100 OD450nm

Cut-Off = 0.100+0.250 = 0.350

S/Co = 2.8

Sample 1: Sample 2: Sample 1 S/Co < 1 = negativeSample 2 S/Co > 1.2 = positive0.070 OD450nп 1.690 OD450nп

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- therpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of Julyment errors and misreterpretations.

  Producular attention in the interpretation of results has to be used in the follow-up of pregnancy for an infection of HSV due to the risk of severe neonatal malformations.

  In pregnancy monitoring, it is strongly recommended that any positive result is conditioned first with the procedure described below and secondly with a different device for HSV lyM detection, before taking any preventive medical
- Any positive sample should be submitted to the Confirmation Test reported in section T before giving a result of positivity. By carrying out this test, false reactions, invading to a misinterpretation of the analytical result, can be revealed and then ruled out.

  When lest results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous
- deta transfer.

  Diagnosis of infection has to be taken and released to the patient by a suitably qualified medical doctor

## False positive rea ruled out in the reported in section result is real

Results are reported as follows:

Negative (N = 16)				
Mean values	ist run :	2nd run	379 1110	Average
OD 450nm	0.092	0.113	0.097	101.0
064 5			Van de la ca	20101
DID DEVIBUOR	0.011	0.019	0.010	0.013
CV%	12.25	16.83	10.24	1211

			-	200 / 000
0.027	D.006	0.052	0.023	CHARACTER
100		-	0.000	City Therein
1 543	1.527	1.574	1.530	COC 45Unm
value				200
Average	37 AM	2nd run	Cut-155	Mean values
0.0				
20	7.48	0.00	3.92	CV%
0.017	0.033	0.000	0,018	OIG DEVISION
0,452	0.435	0.471	0.451	CO LOCATION
antes				00 450
MOETHWA	3"745	2nd run	Tst run	Sanjes ustray

reactive (N = 16)	16)			
dan values	18175	Znd run	37700	Average
DD 450mm	1.530	1.574	1.527	1543
d Deviation	0.023	0.052	D 006	7500
CV %	1.40	3.33	0.37	272

## R. PERFORMANCE CHARACTERISTICS

1. Limit of detection

1. Limit of detection

1. Limit of detection

1. In the mational standard for HSV182 IgM Antibody detection

1. In the been defined so far by the European Community

1. In its absence, an internal Gold Standard (or (GS), palbinated on

1. In the preparation named "Accurum" Anti HSV2 IgM plasm,

1. In the preparation named "Accurum" Anti HSV2 IgM plasm,

1. In the preparation named "Accurum" Anti HSV2 IgM plasm,

1. In the preparation of the p

The limit of detection of the assay has been therefore calculated on the IGS. A limiting dilution curve was prepared in Negative Control (NC). (NC)

Results of Quality Control are given in the following table:

### OD450nm values

1	2X 0.343	0.56C	IGS HSV2M,
0000	0.324	0.572	CE HSV2M.CE D1 Lot#RD2
0	0.348	0.590	HSV2M.CE Lot # RD3

0.132

0.139

Diagnostic sensitivity.
 The diagnostic sensitivity has been tested in a clinical trial on panels of 40 samples classified positive by a kit US FDA approved. The value obtained from the analysis was > 98%.

The diagnostic specificity has been determined in a performance evaluation study on panels of more than 300 specimens, negative with the reference kit, derived from normal individuals of European origin.

reparation (chief. EDIA and hepain), and seria have been used to determine the specificity. No false reactivity due to the method of specimen preparation has been observed. Frozen specimens have also been tested to check whether this observed on clean and particle three samples. In a study conducted on more than 60 potentially consu-reactive. Both plasma derived with different standard techniques of ittate. EOTA and heparin), and sera have been

Diagnostic specificity
 The diagnostic specific

samples has not revealed any interference in the system. No cross reaction were observed.

The Performance Evaluation has provided a value > 98%.

	×	S HSV2
242	1.560	/2M.CE # RD1
0 224	0.572	HSV2M.CE Lot # RD2
0000	0.590	HSV2M.CE Lot # RD3

## HSV2M,CE: lot # RD3

G: - M SAMPRO				
Mean values	1st run	2nd run	3 <sup>rd</sup> run	Average
9D-450mm	0.104	0 108	0,000	SPIER
E		W. 100	660.0	0.104
DOI DEVIATION	0.015	0.010	0.011	0.012
CV%	14.4	9.2	17.11	11.57

300.Deviation 0.040 0.004	l		wear values 1st run 2nd run	High reactive (N = 15)	5.0 1.4	2.0		0.435	200	unitary seniors senior and senior	VALUE CONTROL OF THE PROPERTY
0.024	1,558		3" tun		2.0	1	0.009	0.440		3" (40)	
2000	1,564	value	Average		1.7	0,000	0.000	0.434	BUSBA	Average	

interpretation in T, able to	۲
oretati able t	
- 2.2	
of re-	
yw es	
way pointer esults with whether or	
S w	
ME WIT	
9 7 6	
ed out and the proc	
0 9 0	
d the	
666	

### Precision

Mean values	(strun	2nd run	370 1110	Average
OD 450nm	0.092	0.173	7.097	1010
Std Powerster	000		10000	20101
DODGE AND DIS	0.011	0.019	0.010	0.013
CV %	12.25	16.83	10.24	1211

an values	1st run	2nd run	3""141	Operany
				walter.
C #300m	0.451	0.471	0.435	0.452
Deviation	0,018	0.000	0.023	0000
70.00	-	-	0.000	0.011
CV 79	3.92	0,00	7.48	00
reactive (N = 16)	16)			
an values	ist rus	Znd run	37700	Average
				valle

## HSV2M.CE: jot # RD2

Mean values	1st run	Zod run	3rd nun	Ауенад
00.000				White.
CC 4000m	0.095	0.101	0.097	0.098
Std. Deviation	0.006	0.008	0.005	2000
CV %	6.30	7.92	5	545

STILL FOR THE STATE	UTZ 2351	2nd run	3" nun	Average
QD: 450nm	0.431	0.428	0.453	0.427
Std.Deviation	0.023	8:00	2000	0000
CV %	5.3			1,000,0
04. An	5.3	A	5.10	4.9

FOR IGALIAN IN II	lat			
Mean values	1st zun	2nd run	3 min	Avers
CC 45Unm	0.431	0.428	0.453	0.40
Std.Deviation	0.023	0.059	2000	2
2/6/		20.00	0.050	20,02
04. An	5.3	i.		
High reactive (N =	16)	,	0.10	4
High reactive (N =		1,5	08.0	A
Mean values		2nd run	3" വന	Awera
Mean values		2nd ryn	3" 740	Averag Averag
Mean values OD 450nm		2nd run 1.552	3 <sup>10</sup> n/n	Avera valu
Mean values  OD 450nm  Std Deviation		2nd run 1.552 0.025	3 <sup>m</sup> n/n 1.541	Averag value 1,550

	2.53	1.01	1,50	0.75
0	0.039	0.025	0.031	CANADIOU
	1.541	1,552	1.556	*DODES
*				100
4	3" กมก	2nd run	UNITES	Samen

G: = N SAMPRO				
Mean values	fstrun	2nd run	Jan na	Averag
201.00				Anthe
WILDES AC	0.704	0.108	0.099	0.104
Sid Deviation	0.015	0.010	0.031	0013
CV %	12.4	000		1
	20,000	44		17.57

Mean values	tential	2nd run	Sile (M)	Average
				BUSBA
OU 45Umm	0.425	0.436	0.440	0.434
Sid Deviation	0.000	2000	2000	
200	-	0,000	Chana	0,000
-			2	
igh reactive (N =	4	100	2.0	1.7
gh reactive (N	16)		2.0	1.7
igh reactive (N =	600	2nd run	3 <sup>10</sup> run	Average Average
igh reactive (N =		2nd run	3 mn	Average Average
igh reactive (N = Mean values		2nd run 1,562	3 <sup>st</sup> run	Average value
igh reactive (N - Mean values OD 450nm Std.Deviation		2nd n.n 1.562 0.034	3.0 run 1,558 0,024	Average value 1.564

generale false positive results. Frozen samples containing fibrin particles or aggregates may Doc.: INS HSV2M.CE | Page | 8 of 8 | Rev.: 3 | Date: 2015/10

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent attention of the layed of the analyte.

This test is suitable only for testing single samples and not

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be ocled anes

## T, CONFIRMATION TEST

In order to provide the medical doctor with the best accuracy in the follow-up of pregnancy, where a false positive result could lead to an operation of abortion, a confirmation test is reported. The confirmation test has to be carried out on any positive sample before a diagnosis of primary infection of HSV is Proceed for confirmation as follows released to the doctor,

Prepare the Antiger/Conjugate Complex as described in the proper section. This reagent is called Solution A. Then 25 ut concentrated Enzymatic Conjugate are dated in 500 ut Antigen Diluent and mixed gently on vortex. Do not use any lyophilized antigen vial for this procedure if This solution is called Solution B.

The well A1 of the strip is left empty for blanking.

The Negative Control is dispensed in the strip in positions B1+C1. This is used for the calculation of the cut-off and

110.98.76

M

SICo values.

SICo values.

The positive sample to be confirmed, diluted 1:101, is dispetised in the strip in position D1+E1.

The strip is incubated for 60 min at +37°C.

After washing, the blank well A1 is left emply.

Then 100 µl of Solution A are dispetised in wells B1+C1+D1.

The strip is incubated for 60 min at +37°C.

The strip is incubated for 60 min at +37°C.

The strip is incubated for 60 min at +37°C.

After washing, 100 µl Chromogen/Substrate are added to all the wells and the strip is incubated for 20 min at +1.

The properties of the strip is incubated to 20 min at +1.

Their color intensity is measured at 450mm (teading filter) and at 620-630mm (background subtraction, strongly recommended), blanking the instrument on A1. 12

Interpretation of results is carried out as follows:

If the sample in position D1 shows a S/Co value lower than
1.0 a problem of dispensation or confirmation in the first
test is likely to be occurred. The Assay Procedure in Section
M has to be repeated to double check the analysis.

If the sample in position D1 shows a S/Co value higher than
1.2 and in position E1 shows a S/Co value higher than
1.1 the sample is considered a false positive, The reaching
of the sample is in fact not dependent on the specific presence\_of\_HSV2\_and\_a\_crossreaction\_with\_enzymatic

conjugate has occurred.

If the sample in position D1 shows a S/Co value higher han 1,2 and in position E1 shows a S/Co value lower than 1,0 the sample is considered a true positive. The reactivity of the sample is considered on the specific presence of the sample is in fact dependent on the specific presence of HSV and not due to any construction.

The following table is reported for the interpretation of results

interpretation	interpretation Problem of	False	True
E1	<1.0	> 1.2	^
2	< 1.U	> 1.2	> 1
etation	< 1.0	V 12	4 A V

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Dia-Pro Diagnostic Bioprobes Srl Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination Herpes Simplex Virus type 2 in human serum and plasma of IgG antibodies to

for "in vitro" diagnostic use only



## DIA.PRO

20099 Sesto San Giovanni Via G. Carducci nº 27 Diagnostic Bioprobes Srl

e-mail: info(diapro.it Fax -39 02 26007726 Phone -39 02 27007161 (Milano) - Italy

REF HSV2G.CE 96 Tests

### HSV2 lgG

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infections may happen in immunocompromised and suppressed patients in which the disease may evolve toward critical Asymptomatic infections may happen for HSV in apparently healthy individuals and during pregnancy. Severe herpetic

pathologies.

The determination of HSV specific antibodies has then become important in the monitoring of "risk" patients and in the follow up of acute and severe infections.

## C. PRINCIPLE OF THE TEST

are with synthetic HSV2 specific

peroxidase (HRP).

The enzyme captured on the solid phase, acting on the

### D. COMPONENTS

n° 1. 12 strips x 8 microwells coated with synthetic HSV2-

## A INTENDED USE

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Herpes Simplex Virus type 2 in human plasma and sera. For "in vitro" diagnostic use only.

Herpes Simplex Virus type 1 (HSV1) and type 2 (HSV2) are large complex DNA-containing viruses which have been shown to induce the synthesis of several poleris during infection, possessing an high number of crossreactive determinants and just a few of type-specific sequences.

The majority of primary and recurrent genital infections, such as common cold sortes, are caused by HSV2, while non genital infections, such as common cold sortes, are caused primarily by HSV1.

The detection of virus specific 19G and 1gM antibodies are important in the diagnosis of acute/primary virus infections or reactivations of a latent one, in the absence of evident clinical executions.

## coated

glycoprotein G or gG.
The solid phase is first treated with the diluted sample and IgG.
The solid phase is first treated with the diluted sample and IgG.
To HSV2 are captured, if present, by the antigents.
After washing out all the other components of the sample, in the Carl incuthation bound and HSV2 IgG are detected by the addition of polycional specific anti hlgG antibodies, labelled with

substrate/chromogen mixture, generales an optical signal that is proportional to the amount of anti HSV2 IgG antibodies present in the sample. A Calibration Curve, colibrated against an internal Gold Standard, makes possible a quantitative determination of the IgG antibody in the patient.

Each kit contains sufficient reagents to perform 96 tests.

## 1. Microplate: MICROPLATE

specific gG in presence of bovine proteins.

Plates are sealed into a bag with desiccant. Allow the microbale to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2.8°C,

2. Calibration Curve: CAL N°.

Ready lo use and color coded standard curve derived human plasma positive for HSV2 lgG ranging: from

4ml CAL1 = 0 arbU/ml
4ml CAL2 = 5 arbU/ml
2ml CAL3 = 10 arbU/ml
2ml CAL4 = 20 arbU/ml
2ml CAL4 = 20 arbU/ml
2ml CAL5 = 50 arbU/ml
4ml CAL6 = 100 arbU/ml

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical-presonal-confu-undocture supervision-of a medical-decided property of the laboratory.

2. All the personnel involved in performing the assay have to wear protective laboratory dothes, talc-free gloves and plasses. The use of any sharp (needles) or cutting (blades) devices

Standards are calibrated in arbitrary units against an internal Gold Standard (or IGS),

It contains human serum proteins, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. Standards are blue colored.

3. Control Serum: [CONTROL ...m]

1 vial. Lyophilized, It confains felal bovine serum proleins, human lgG antibodies to HSV2 at about 20 arbUm ± 20%, 0.2 mg/m1 geniamicine sulphale and 0.1% Kathon GC as

Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

## 4. Wash buffer concentrate: WASHBUF 20X

1x60ml/bottle20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

5. Enzyme conjugate: CONJ
2x6mt/val. Ready to use and red colour coded. It comains transcratish peroxidase conjugated polyclonal antibodies to human 1g6, 5% BSA, 10 mM. Tis buffer pH 6.8+/0,1, 0,1% Yathon GC, 0,02% gen

6. Chromogen/Substrate: SUBS TMB

1x16ml/vial. It contains 50 mM citrale-phosphate buffer pH 3.5-38. 4% dimeth/stulphoxide. 0.03% tetra-methyl-benzicine (or TMB) and 0.02% thydrogen persode (or H.0-). Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: [H:SO: 0.3 M] Ixf5mWal, It contains 0.3 M H:SO: solution. Ixf5mWoal, It contains 0.4 M H:SO: solution. Altention: Irinari (H:315, H:319: P280, P302+P352, 332+P313, P305+ P351+P338, P337+P313, P362+P363)

## 2x60ml/vial, It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide 0.1% and Kathon GC as preservatives. The reagent is blue colour coded. 8. Specimen Diluent: DILSPE

9. Plate sealing foils n°2

10. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED 1. Calibrated Micropipettes (1000 ul. 100 ul and 10 ul) and

disposable plastic tips.

EIA grade water (double distilled or deionised, charcoal remove oxidizing chemicals pesn

as

Timer with 60 minute range or higher.

Absorbent paper tissues

Calibrated ELISA microplate thermostatic incubator (dry or

wet), set at +37°C (+1-0,5°C tolerance),.
Calibrated ELISA microwell reader with 450nm (reading)

Vortex or similar mixing tools. and with 620-630nm (blanking) filters, Calibrated ELISA microplate washer.

Blomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handing should should be avoided. All the personnal involved should be trained in bloasety procedures, as recommended by the Center for Disease Control. Attanta, U.S. and reported in the Astonal Institute of Health's publication: "Biossfery in Microbiological and

vaccinated for HBV and HAV, for which vaccines are available

when opening kit was and microbial agents, lest. Protect the Chromogen (TMS) from strong light and detection of the bench surface where the tests undertaken of the bench surface where the tests undertaken, controlled refrigateator or cold from.

5. Upon receipt, store the kit at 2.8°C into a temperature controlled refrigateator or cold from.

6. Do not interchance The laboratory environment should be controlled so as to contaminants such as dust or air-born, microbial agents,

 Do not interchange components between different lots of the kits, It is recommended that components between two kits of the same fot should not be interchanged.
 Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not advise the laboratory supervisor to intitate the necessary procedures for kit replacement 8. Avoid

samples by using disposable tips and changing them after each sample.

Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each cross-contamination between

100

10. Do not use the kit after the expiration date stated on the external container and internal (vais) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially inflighter. All human setum specimens should be handled at Blossfety Levet 2, as recommended by the Center for Disease Contral, Atlanta, U.S. in compliance with what reported in the institutes of Health's publication. "Biosafety in Microbiological and Biomedical to be presented in the contral cont

Laboratories", 12. The use 12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid

13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substainces. In particular, fluud waste generated from the wasting procedure, from residuals of controls and from samples before waste. Suggested procedures or inactivated treatment with a 10% final compeniation of household bleach or 16-18 his or heat mactivation by autoclave at 121°C for 20 min. 14. Accidental spills from samples and operations have to be then with water. Tissues should then be discarded in proper listures soaked with household bleach and comitaines designated for laboratory/hospital waste.

The Sulphurc Acid is an intriant. In case of spills, was the surface with penny of water.

15. The Sulphurc Acid is an intriant. In case of spills, was the 16. Other waste materials periorated from the use of the kit (crampler its used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laboratory wastes. Waste produced during discarded in compliance will

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysts. No influence has been observed in the preparation of the sample with citate, EDTA

and heparin.

2. Samples have to be clearly identified with codes or names in order to evold misinterpretation of results. Ber code labeling and reading is strongly recommended

3. Haemolysed ("red") and visibly hyperlipemia ("nilky") samples have to be discarded as they could generale false results. Samples containing residues of florin or heavy particles or microbial flaments and bodies should be discarded as they could give rise to false results, and the stored at +2". 8"C for up to five days after collection. For longer storage periods, samples can be stored flozen at -20"C for several months. Any flozen samples of should not be flozen/thaved more than once as this may seperate particles hat could affect the last result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or differ using 0.2-0.50 if flors to clean up the sample for lesting.

H. PREPARATION OF COMPONENTS AND WARNINGS
A sludy conducted on an opened kit has not pointed out any relevant loss of activity up to 5 re-uses of the device and up to 3 months

Allow the microplate to reach from temperature (about 1 hr) before opening the container. Check that the desicoant has not furned dark green, indicating a defect in storage. In this case, call Dia-Pro's customer service, the container of the duminum pouch, with the desicoant supplied, firmly zipped and stored at 2°.8°C. desicoant supplied, firmly zipped until the humidity indicator incide the advantage of the containing strips are stable until the humidity indicator incide the advantage of the containing strips are stable until the humidity indicator incide the advantage.

indicator inside the desiccant bag lurns from yellow to green.

## Calibration Curve

Ready to use component. Mix carefully on vorlex before use,

Add the volume of ELISA grade water, reported on the label, to the lyophilised powder, let fully dissolve and then gently mix on Note: The Control Serum after dissolution is not stable. Store frozen

Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted. The whole content of the concentrated solution has to be diluted. 20x with bidsilled water and mixed garily end-over-end before use.

During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

Note: Once diluted, the wash solution is stable for 1 week at

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with
air-driven dust or microbes. sterile disposable containen If this component has to be transferred use only plastic, possibly oxidizing chemicals

## Chromogen/Substrate:

IF this air-driven dust or microbes.
Do not expose to strong illumination, Be careful not to contaminate the liquid with oxidizing chemicals sterile disposable container Ready to use. Wix-well-on-vortex before use component has to be transferred use only plastic, possible oxidizing agents and

### Diluent

. Mix carefully on vortex before use

"Sulphuric Acid."

Ready to use, Mix well on vortex before use,
Attention Irritant (H315, H319, P280, P302+P352, 332+P313,
P305+ P351+P338, P337+P313, P362+P363),

Dec.:

Warning H statements: H315 – Causes skin imitation, H319 – Causes serious eye imitation,

Precautionary P statements:
P280 — Wear protective gloves/protective clothing/eye protection/face

protection. P332 – IF ON Sight: Wash with plenty of scap and water. P332 + P333 – If skin irritation occurs: Get medical authorization to 2305 + P353 – If Skin irritation occurs: Ruse caudiously with water to several intuities. Reference contact irritation previous continue irritation persists: Get medical advice/attention. P352 + P353 – Take off contaminated atching and wash it before reuse.

# I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

ired by the assay and must be submitted to intamination (household alcohol, 10% solution to be calibrated to deliver the

regular deconsimilation (household action). (10% solution of bleach, hospital grade disfribetants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a threness of +1.2%. Decontamination of spills or residues of kit components should also be earlied out regularly.

10°C) and regularly checked to ensure the correct temperature is maintained Both dry incubators and water batts are suitable for the incubations, provided that the instrument is validated for the incubations provided that the instrument is validated and retiremently important to the overall validated and correctly optimised using the kit combis and reference pariets, before using the kit for routine laboratory tests. Usually 4.5 washing cycles (spsination + dispensation of 350-lukwell of washing solution = 1 cycle) are sufficient to ensure that the assay performs are expected. A spaking time set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative reported below in the section. Thermal Quality Control. Regular calification of the volumes delivered below in the section. Thermal Quality Control. Regular califications of the earlied out according to the instructions of the manufacturer.

10°C and maintenance (accordamination and cleaning of the instructions of the manufacture.

20°C incubation limes have a follarinor of ±5%.

20°C interactive that the correct of 45%.

20°C interactive that is a carried out according to the instructions of the section 10°C 20°C (interactly to 20°C) repeatability ≥ 1%. Blanking is carried on the well be septiability = 6°C and the correct optical density is measured. It should be instructions.

instructions.

When using an ELISA automated work station, all critical steps (dispersation, incubation, washing, reading, data handling) have to be cerefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section 'internal Quality Control'. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part, of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid earry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations

is recommended when the number of samples to be tested exceed 20-30 units per run.

Dia Pro's customer service offers support to the user in the

setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

g é

PRE ASSAY CONTROLS AND OPERATIONS
Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
Check that the liquid components are not contaminated by

Check that the Chromogen (TMB) is colourless or paie blue Check that the Chromogen (TMB) is colourless or paie blue check that the Chromogen of it with a sterile plastic

pipette.

4. Check that no breakage occurred in transportation and no spillage of figurd is present inside the box (primary container). Check that the alluminium pouch, containing the micropiate, is not punctured or damaged.

5. Dissolve the content of the yophilised Control Serum as reported in the proper section.

6. Dilute all the content of the 20x concentrated Wash Solution Ċ

as described above. Allow all the other Allow all the other (about 1 hr) and

0

inagents.

Set the ELISA mubblor at +37°C and prepare the ELISA washer: by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing opides as found in the validation of the instrument for its use with the kit.

Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading. It was an accordance work station, turn on, check settings and be sure to use the right assay protocol.

These has the microphysics are set to the required volume, to case of problems, do not proceed further with the test and advise the supervisor. components to reach room temperature then mix gently on vortex all liquid

10. 90

12.1

## M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking case to maintain the same incubation time for a the samples in tasting.

The kit may be used for quantitative and qualitative delerminations as well.

## . QUANTITATIVE DETERMINATION

Automated assay:

In case the test is carried out automatically with an ELISA system; we suggest to make the instrument aspirate 1000 µs Sample Diluent and then 10 µs sample (1:101 dilution factor).

The whole content is then dispensed into a property defined dilution tube. Before the next sample is saspirated, needles have be duly washed to avoid any cross-contamination among a samples. When all the samples have been diluted make the instrument dispenses 100 µs samples into the proper wells of the morrowners.

n microplate

In This procedure may be carried out also in two steps of dilutions
of 1:10 each (90 µl Sample Diluent + 10 µl sample) into a
second dilution platform. Make their the instrument aspirate first
of 100 µl Sample Diluent, then 10 µl liquid from the first dilution in
the platform and finally dispense the whole content in the proper
seel of the assay microplate.

Do not dilute Calibrators and the dissolved Control Serum as
they are ready to use.

100 pi calibrators/control control wells. ₽. the appropriate

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For the next operations follow the operative instructions reported below for the Manual Assay. It is stongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and laken into consideration by detailed the first theoretic and laken into consideration by delaying the first washing operation accordingly

- Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl. Sample Diluent : 10 µl sample). Do not dilute the Calibration Set as calibration set ready to use. Mix. carefully all the liquid components on vortex and then
- proceed as described below.

  Place the required number of microwells in the microwell holder. Leave the A1 and B1 empty for the operation of

'n

ω Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each properly identified well.

Incubate the microplate for 60 min at +37°C.

Important note: Sirps have to be sealed with the adhesive sealing fail, supplied, only when the test is carried out manually. Do not cover strips when using EUSA automatic instruments.

- Wash the microplate with an automatic washer as reported
- previously (section 1.3).

  Pipette 100 µ Enzyme Conjugate into each well, except A1+61; blanking wells, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1 and B1.

Important note: Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur,

- hocubate the microplate for 60 min at +37°C. Wash microwells as in step 5.

  Pipete 100 µ I Chromogen/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the microplate at room temperature (18-24°C) for 20

Important note: Do not expose to strong direct illumination, High background might be generated.

Pipette 190 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipeting sequence as in Step 9. Addition of acid will turn the positive calibrations; the control serum and the positive samples from blue to yellow.
 Measure the colcul intensity of the soution in each well, as described in section 1.5 at 450m filter (reading) and at 620-630nm (background subtraction, strongly recommended), blanking the instrument on A1 or B1 or both.

M2 QUALITATIVE DETERMINATION If only a qualitative determination i described below: is required, proceed Se

### Automated assay Proceed as described in section M1.

### Manual assay:

- Diluis samples 1:101 into a properly defined dilution table (example: 1000 µ Sample Diluent +10 µ sample). Do not dilute the Calibration Series as calibratis are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.

  Place the required number of Microwells in the microwell holder, Leave A1 well empty for the operation of blanking.

N

- identified well.
  4. Incubate the microplate for 60 min at ±37°C. Dispense 1i arbU/ml in a Then disper

Do not cover strips when using EUSA automatic instruments Important note: Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually.

- except A1

Important note: Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.

Important note: Do not expose to strong direct illumination. High background might be generated.

- General Important notes:

  1. If the second filter is not available ensure that no linger prints are present on the bottom of the microwell before reading at 450mm. Finger prints could generate false positive results on reading.
- occur leading to high background.

## N. ASSAY SCHEME

Reading OD	Sulphuric Acid	Temperature	3" incubation	IMB/H2O2	Wash step	remperature	2 incubation	Enzyme conjugate	wash step	lemperature	1 incubation	Samples diluted 1:101	Calibrators & Control (*)	Method
450nm	100 ul	T.1.	20 min	100 山	4-5 cycles	+37°C	60 min	100 µI	4-5 cycles	+37°C	60 min	100 µl	100 µl	Operations

of Calibrator 0 arbU/ml and Calibrator 5 cate and Calibrator 100 arbU/ml in single.  100 µl of diluted samples in each properly	ell.	dupli dupli
Calibrator 0 arbU/ml and Calibra and Calibrator 100 arbU/ml in s of diluted samples in each pro	8	icate
brator 0 arbU/ml and Calibra Calibrator 100 arbU/ml in s diluted samples in each pro		학문
or 0 arbU/ml and Calibn librator 100 arbU/ml in s led samples in each pro	g	
arbU/ml and Calibra ator 100 arbU/ml in s samples in each pro	utec	alibra
I/ml and Calibra 30 arbU/ml in solies in each pro	Sa	at of
ni and Calibra arbU/mi in s as in each pro	量	-
nd Calibrator 5 bU/ml in single. In each properly	88	95
Calibrator 5 rd in single. sch properly	標	E 3
brator 5 single. properly	9	五色
or 5	Sold	l Si
56.6591	en)	다 다
	~	e-sun

- Wash the microplate with an automatic washer as reported
- previously (section (.3).

  Plotte 100 µ Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells.

- 10 00 7
- Incubate the microplate for 60 min at +37°C. Wash microwalts as in step 5. Papets 100 µC homonogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at room temperature (18-24°C) for 20 minutes.

10. Pipette 100 µl Sulphuric Acid into all the wells using the same pipeting sequence as in step 9. Addition of acid will turn the positive calibrators, the control serum and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section L5 at 450nm filter (reading) and at 520-530nm (background subtraction, strongly recommended), blanking the instrument on A1.

Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can

Reading OD	Sulphuric Acid	Temperature	3" incubation	TMB/H2O2	Wash step	remperature	2 incubation	Enzyme conjugate	Wash step	lemperature	1 incubation	camples diluted 1:101	Calibrators & Control (*)	Method
450nm	100 ul		20 min	100 µ	4-5 cycles	+37°C	60 min	100 μ	4-5 cycles	+37°C	60 min	100 µ	100 µl	Operations

(\*) Important Notes:
The Control Serum (CS) it does not affect the test's

results calculation

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The Control Serum (CS) used only if a laboratory Management quality control is require

reported below: An example of dispensation scheme for Quantitath

### Microplate

	-			t		-		Ų	1	
1	BLK	BLK	4	100	CALT	CAL2	CAL2	2	CAL3	CAL3
2.	CAL4	CAL4	200	CARO	CAL5	CAL6	CALE		CS(*)	CS(*)
co	S	52		000	52	S	200		57	8.8
+		1					1	l		
S										
æ						1	1	L		
7						1		L		
203	-					1	1			
D										
á	1	I								1
1	1	T								
	1	T	Γ			1	ſ		T	

Legenda CS(\*) = Control Serum - Not mandatory BLK = Blank CAL = Calib dalory S

An example of dispensation scheme in qualitative reported below:

### wiicropiate

	2	3	-4	Un)	(T)	7	œ	9	'n	*	3
BLK	SS							1	1		
CALI	SS			4	1	4	1		1	1	
CALT	0		1	1	1	1	1	1			1
CALZ	co				4			1	1		I
CAL2				1	4	1	1	1	1		
CAL6			1	1	1	1	1	1	1		1
ST	S	8 16		4		1	1	1	1	1	
S2				4		1	1	1	1	1	

I O M M O O M >

BLK = Blank S = Sample CAL = Calibrators

Legenda

O.INTERNAL QUALITY CONTROL

A validation check is carried out on the calibrators any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched

1 1111	100 arbU/ml
OD450nm > 1,000	CAL 6
0.100 OD450nm CAL1 +	5 arbU/ml
coefficient of variation < 30%	
< 0.150 mean OD450nm value after	CAL 1
< 0.050 OD450nm value	Diank well
Requirements	Check

If the results of the test match the requirements stated above, proceed to the next section.

follows: do not, do not proceed any further and operate

ed by the are Analysis is	Problem Blank wall  0.056 00450nm CAL 1  0 abbum  0.150 00450nm site blanking coefficient of variation  30%	
	coefficient of variation	
	CAL 2 5 arbUml	1. that the procedure has executed:
bralor S = Sample	00450nm <u>&lt;</u> 00450nm CAL1+ 0.100	<ol> <li>Inal no mistal distribution (ex.: calibrator instead);</li> <li>that the was washer settings as qualification study;</li> </ol>
e assays is		4. that no external contamination
	CAL 6 100 arbU/m! ≤ 1.000 OD450cm	that the procedure has executed.     that no mistake has be distribution.
0 11 12	≤ 1,000 CD450nm	distribution (dispensation of a wrong caltrapor instead);  3. that the washing procedure and the washer settings are as validated in the pre-qualification study.  4. In all no external contamination of the positive reports her present the pre-present the procession of the procession.
		a. Inal no external contar positive control has occurred

Should one of these problems have happened, after checking, report to the supervisor for further actions.

### \*\* Note:

If Control Serum has used, verify the following data:

If the results of the lest doesn't match the requirements stated above, operate as follows:

		Different from expected value	Control Serum	Problem
4. that no external contamination of the control serum has occurred.	3. that the washing procedure and the washier settings are as validated in the pre-	2 that no mistake has been done in its distribution (dispensation of a wrong	1, that the procedure has been correctly	Check

Anyway, if all other parameters (Blank, CAL1, CAL2, CAL5), match the established requirements, the test may be considered valid.

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### P. RESULTS

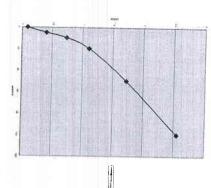
## P.1 Quantitative method

If the lest turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters interpolation is suggested).

Then on the calibration curve calculate the concentration of anti-Herpes Simplex Virus type 2 tgG antibody in samples.

An example of Catibration curve is reported in the next page,

## Example of Calibration Curve :



P.2 Qualitative method
In the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 5 artiUlml and then check that the

## Example of calculation

figures

Calibrator 0 arbUlmi: 0.020 - 0.024 OD450nm

Lower than 0,150 - Accepted

Calibrator 5 arbUrni:

Calibrator 100 arbUfml: 2 Higher than 1 000 — Accepted 2,245 OD450nm

Important Note:

Do not use the calibration curve above to make calculations.

The following data must not be used instead or real obtained by the user.

0.022 OD450nm

Calibrator 5 arbUrni: 0.350 = 0.370 OD450nm Mean Value 0.350 OD450nm Higher than Cal 0 + 0.100 - Accepted

Q. INTERPRETATION OF RESULTS
Samples with a concentration lower than 5 arbul/ml
considered negative for anti HSV2 IgG antibody,
Samples with a concentration higher than 5 arbul/ml
considered positive for anti HSV2 IgG antibody. аге 976

## Mean OD450nm values (n = 2)

nc vic	non.	200	30	10	5		7	c	0	arbU/ml
2.030	0.000	1.169		0,550	3	0.353	0.000	220.0	0000	Lot # 1203
2.276		1 471	2000	0.606		0.384		0.030		HSV2G,PU
1.776	0.000	208 0	0,000	0 227	Cinco	0360	010.13	0.014	The Contract	HSV2G

The assay shows a limit of detection far better than 5 arbU/ml,

 Diagnostic sensitivity.
 Diagnostic sensitivity, has been lested in a performance evaluation study on parels of samples classified positive by a kill US FDA approved. Positive samples from different stage of HSV infection were tested. The value, obtained from the analysis of infection were tested. more than 300 specimens, has been > 98%

Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for a primary infection of HSV due to the risk of neonatal malformations.

### Important notes: Interpretation

- data transfer.

  In the follow-up of pregnancy for HSV infection a positive result (presence of lig6 artiblody > 5 arbUrm) should be confirmed to ruled out the risk of a false positive result and a

## R. PERFORMANCES

I. Limit of detection
 The limit of detection of the assay has been calculated by means of an internal Gold Standard in absence of an international preparation to refer to.
 The limit of detection has been calculated as mean OD450nm Calibrator 0 arbU/ml + 5 SD.

the assay for three lots. The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined in

	20	8 6	5	nic	arbU/ml Lo
2 030	1.169	0.580.0	0.353	220.0	HSV2G.PU Lot # 1203
3770	1,471	0.606	0.384	0.030	HSV2G.PU Lot # 1103
-	0.895	0.557	0,269	0.014	HSV2G Lot # 0304/2

100 3.102 3.353 2,893

The diagnostic specificity has been determined on panels of negative samples from and infected individuals, classified regative with a tit US FDA approved.

Both plasma, derived with different standard techniques of preparation (citrate, EDPA and heporn), and sera have been used to determine the value of recognition. Diagnostic specificity has been
The diagnostic specificity has been used to determine the value of specificity.
Frozen specimens have been tested, as well, to check for

interferences due to collection and storage.

No interference was observed.

Potentially interfering samples derived from patients with different pathologies (mostly ANA, ANA and RE positive) and from pregnant women were tested.

No crossreaction was observed, An overall value > 88% of specificity was found when examined on more than 100 specimens.

- Interpretation of results should be done under the supervision of the laboratory supervisor to the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.

  When test results are transmitted from the laboratory to another facility, attention must be paid to avoid errorsous

# The variability shown in the tables above did not result sample misclassification.

	odiron,

## S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with crossequent attention of the level of the analyte. Finzers examples containing from particles or aggregates after thewing may generate some false presults. This test is suitable only for testing single samples and not nonled ness.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symplomation of a single test result as other diagnostic data should be

paoted ones

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It has been calculated on the Calibrator 5 arbly/ml, considered the cut-off of the assay, examined in 16 replicates in three separate runs for three lots.

Results are reported as follows:

All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISQ 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical

specifications and acceptance criteria

Mean values 1st run

2nd run

310 run

0.286 0.022

0.303 0.037 12.4

un 3<sup>th</sup>nin Average value 3 0.256 0.282 7 0.026 0.026 7.74 9.28

## Manufacturer

Dia Pro Diagnostic Bioprobes Srl Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy



Std.Deviation OV W

0.375

0.384 0.022 5.73

0.394 0.015 3.81

Average value 0.384 0.019 4.87

HSV2G.PU: lot 1103
Mean values Istrun

2nd run

3" 745

Mean values 1:

ist run

2nd run

3 run

0.352 0.017

0.345 0.332 0.020 0.024 5.78 7.23

Average value 0.343 0.020 5.95

quantitative/qualitative determination of Enzyme ImmunoAssay (ELISA) for the lgG antibodies to Rubella Virus in human serum and plasma

for "in vitro" diagnostic use only -



## DIA.PRO

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96/192/480 Tests REFRUBGICE

## Rub IgG

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### A, INTENDED USE

plasma and sera. For "in vitro" diagnostic use only. Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Rubella Virus in human

B.INTRODUCTION

Rubella is a small spherical enveloped virus, 55-80nm in Rubella is a small spherory member of the genus Rubivirus of the

lamily Togaviridiae.

The virus contains single positive stranded 42s RNA molecule and only one serotype is known. The virus encodes for at least associated protein, C, and two ponstitutural proteins.

The defection of Ruelia-specific (gG and IgM antibodies is very important for the serotogical diagnosis of both congenital and primary postnatal rubelia infections as they can lead to severe

birth defects,

The absence of Rubella-specific IgG antibodies in sera, Characteristically of long-term duration after primary infections, in presence of virus-specific IgM, is indicative for the risk of

defects in newborn infants. """ """ in invasive for the IRSK of Highly specific Rubeita IgG assays provide the clinician with helpful and reliable test for the monitoring of these risks in pregnancy and for the monitoring of the immunological response upon vaccination."

## C. PRINCIPLE OF THE TEST

Microplates are coaled with native Rubella Virus, highly purified by sucrose gradient contribugation and inactivated. The solid phase is first freated with the diluted sample and IgG to Rubella Virus are captured, if present, by the antigens, After washing out all the other components of the sample, in the "incutation bound and Rubella IgG are detected by the autition of polycional specific anti higG antibodies, labelled with addition of polyclonal specific anti htgG peroxidase (HRP).

The enzyme captured on the solid phase, acting on the substrateletromogen mixture, generates an optical signal that is proportional to the amount of anti Rubella Virus IG antitodies present in the sample. A Catination Curve, calibrated against the 1st W.H.O. International standard for anti-Rubella immunoglobulin code RUBI-1-4, makes possible a quantitative determination of the IgG antibody in the patient.

## D. COMPONENTS

The standard kit contains reagents to perform 96 tests.

1. Micropiate: MICROPLATE
12 strips x 8 microwells coated with highly purified and U
12 strips x 8 microwells coated with highly purified and U
12 strips x 8 microwells coated with desiccant. Allow to
Plates are sealed into a bag with desiccant. Allow to
micropiate to reach room temperature before opening; rese
unused strips in the bag with desiccant and store at 2.36 °C.

Calibration Curve CAL IV...

Ready to use and color coded standard curve derived frollumen plasma positive for Rubella JgG and illitated on WH

standard ranging
4 mi CAL1 = 0 WHO IU/ml
4 mi CAL3 = 00 WHO IU/ml
2 mi CAL4 = 20 WHO IU/ml
2 mi CAL4 = 50 WHO IU/ml
2 mi CAL4 = 50 WHO IU/ml
2 mi CAL5 = 250 WHO III/ml
4 mi CAL6 = 250 WHO III/ml

Standards are calibrated against the 1" W.H.O. international standard for anti-Rubella immunogobatin code RUB1-1.94, If constants human securin proteins, 2% caselm, 10 mM Na-ditrate buffer pH 6.0 +4.0.1, 0.1% fives n 20, 0.05%, Na-azibe and 0.1% Kathon GC as preservatives. Standards are blue colored.

## 3, Control Serum: CONTROL ...mi 1 vial. Lyophilized.

It contains fetal bowine serum proteins, human IgG antibodies to Rubelle. Virus calibrated at 20 WHO IU/m! + 10%, 0.2 mg/ml gertamiche suphate and 0,1% 48/non GCas preservatives. Mote: The volume necessary to dissolve the content of the valumay vary from lot to lot. Please use the right volume reported on the date!

4. Wash buffer concentrate: WASHBUF 20X 1x60ml/bottle20x concentrated solution.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

5. Enzyme conjugate: CONJ
2-8milylar, Ready to use aind red colour coded, it contains
introduced to the control provided to the contains
introduced to the control provided to the control of the control

6. Chromogen/Substrate: SUBS TMB
1x/fint/vial. If contains 50 mM clirate-phosphate buffer pH 3.53.6. 0.03% left-amothyl-braziline (or TMB) and 0.02%,
hydrogen peroxide (or HoOs) and 4% dimethylssiphocoide.
Note: To be stored protected from light as sensitive to
strong illumination.

7. Sulphurie Acid: [<u>HSS0...03M</u> 7. Sulphurie Acid: [<u>HSS0...03 M</u> HSG0.solution. 7. Sulphurie: <u>P380</u>, P302-P332. P332+P313. P305-P351+P338, P337+P313, P362+P363).

8. Specimen Diluent: DILSPE 2x50mWal. Inches 25 casein, 10 mM Na-citrate buffer pH 6.0 +4.01, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. The reagent is blue colour coded.

## 9. Plate sealing foils n°2

10. Package insert n°1

Important note: Only upon specific request, Dia.Pro can supply reagents for 192 and 480 tests, as reported below:

## -- m S REQUIRED BUT Micropipelles (1000

- NOT PROVIDED 0 ul. 100 ul and 10 ul) and
- disposable plastic tips.

  EIA grade water (double distilled or defonised, charcoal treated to remove oxidizing chemicals used as
- 60 minute range or higher.
- paper Ussues.
- Calibrated ELISA microplate thermostatic incubator (dry or wert), set at \*37°C (+4.0.5°C oberance).

  Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.

  Calibrated ELISA microplate washer.

  Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained ischnical personnel only, under the supervision of a medical doctor responsible of the laboratory.

2. All the personnel involved in performing the assay have to wear protective laboratory oddhes table-free gloves and glosses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in bineafeth unconditions as terrormanded by the Contraction. in blosalety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of health's publication: Blosafety in Microbiological and Biomedical Laboratories', ed. 1984.

3. All the personnel involved in sample handling should be vacchasted for HBV and HAV, for which vaccines are available.

safe and effective 4. The laborate

4. The laboratory environment should be controlled so as to avoid confaminants such as dust or alribom microbial agents, when opening kit vitals and microplates and when performing the vibration of the bench surface where the test is undertaken.

5. Upon receipt, store the kit at 2.8°C into a temperature controlled refrigerator or cold room.

6. Do not interchange components between different lots of the kits. It is recommended that components between two kits. Check that the reagents are clear and do not contain visible heavy particles or aggregates: If not, advise the rendamment.

Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each

disposable tips Avoid cross-contamination between kit reagents by using losable tips and changing them between the use of each

10. Do not use the kit after the expiration date stated on the external container and internal (valis) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 3 months.

11. Treat all specimens are potentially infective. All human serum specimens should be handled at Biosafety Level 2, as in compliance with what reported in the Institutes, of Health's publication. Biosafety in Microbiological and Biomedical

Laboratories ed. 1984.

12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.

13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning aboration waste of chemical and biological substances. In particular, library disconting and from samples washing procedure, from residuals of controls and from samples has to be reasted as potentially infective material and flactivates before waste. Suggested procedures of inactivation are treatmen with a 10% final concentration of household bleach for 16-18 his or heal inactivation by autoclave at 121°C for 20 min.

14. Accidental spills from samples and operations have to be adsorbed with paper tissues socied with household bleach and then with water. Inssues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an infant. In case of spills, wash the

surface with plenty of water 16. Other waste materials

(example: tps used for samples and controls, used micropiales) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by conspuncture and plasma or seturn is prepared using standard techniques of preparation or samples for clinical aboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA

Samples have to be clearly identified with codes or names in der to avoid misinterpretation of results. Bar code labeling and

H. PREPARATION OF COMPONENTS AND WARNINGS
A Study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3

\$ ¥ Unused strips have to be placed back into the aluminum pouch with the desicoant supplied, firmly zipped and stored at 8°C firmly zipped and stored

Ready to use

Control Serum the lyophilised powders volume of ELISA grade water illised powder; let fully dissolv reported on the label

Note: The control after dissolution is not stable. Store frozen in aliquots at  $-20^{\circ}\mathrm{C}$ 

Wash buffer concentrate
The 20x concentrate solution has to be dituted with EIA grade water up to 1200 ml and mixed gently end-over-end before use,

generated from the use of the

selectricitic processing processing part contended.

5. Hearnobysed ("red") and visibly hyperlipemic ("milly") samples

5. Hearnobysed ("red") and visibly hyperlipemic ("milly") samples

5. Shame to be discarded as they could generate falste results.

5. Samples containing residues of fibrin or heavy patricles or

5. Shame and phasma can to bodies should be discarded as they

6. Shame and phasma can be stored at +2". 8"C for up to five days

after collection. For longer storage periods, samples can be

5. Should not be frozenthaved more than once as this may

5. It particles are present, contribuge at 2.000 upm for 20 million

6. Samples whose and-Rubella ligG antibody concentration is

5. It particles that could affect the tast result.

6. Samples whose and-Rubella ligG antibody concentration is

5. It particles and the Calibrator 0 IU/lm. Dictions have

6. Samples whose and-Rubella ligG antibody concentration is

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6. Samples whose and Rubella ligG. antibody concentration is

6

### Microplate

Allow the micropiate to reach room temperature (about 1 before opening the container. Check that the desicoant not turned data green, indicating a defect in manufacturing in this case, call Dia Pro's oustomer service. Check that the desiccant has

After first opening, indicator-inside the remaining strips are stable until the humidity destucant bag furns from yellow to green.

## Calibration Curve

component. Mix carefully on vortex before use.

dissolve and then gently mix on

As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution. In the preparation avoid foeming as the presence of bubbles could give origin to a back washing efficiency. York: Once diluted, the wash solution is stable for 1 week at +2.8° C.

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Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, and diven dust or microbes.
If this component has to be transferred use only plastic, possibly

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals

air-driven dust ar microbes. Do not expose to strong illumination, oxidizing agents meiailic surfaces and

If this component has to be transferred use only plastic, possible sterite disposable container

Ready to use component. Mix carefully on vortex before use

## Sulphuric Acid

Ready to use, Mix well on vortex before use. Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P382+P363).

Warning H statements: H315 – Causes skin irritation, H319 – Causes serious eye irritation.

P280 – Wear protection/face protection/face protection.
P302 + P352 – IF ON S Precautionary P statements: P280 – Wear protective protective ON SKIN, Wash with plenty of soap and gloves/protective clothing/eye

to do. Continue rinsing. P337 + P313 - If eye odvice/attention. P305 + P351 + P38 = IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy P332 + P313 - If skin irritation irritation persists: occurs: Get Get medica medical

advice/attention, P362 + P363 - Take off contaminated clothing and wash

I. INSTRUMENTS AND TOOLS USED IN COMBINATION
WITH THE KIT

1. Microplettes have to be calibrated to deliver the correct

1. Violentes fave to be calibrated to deliver the correct

1. Violentes fave to be calibrated to deliver the correct

1. Violentes fave to be calibrated to deliver the solution of the contamination (household allow of the contamination follows parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a of spills of residues of kit components should also be carried to show a feet and a functions.

out regularly.

The ELISA incubator has to be set at +37°C (tolerance of +/2,3°C) and regularly checked to ensure the connect
temperature is maintained. Both dry incubators and water.

Safts are suitable for the incubations, provided that the
instrument is validated for the incubation of ELISA, heats.

The ELISA, washer is extremely important to the overall
performances of the assay. The washer must be earefully
validated and correctly optimised using the kit controls and
reference panels, before using the kit for routine laboratory.

tests. Usually 4.5 washing cycles (aspiration + dispensation of 350/ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A seaking time of 20-30 seconds between cycles is suggested, in order to set connectly their number, it is recommended to run an ansay with the kil controls exemples, and check to match the values reported below in the sections "Validation of 1 test and positive reference samples, and check to match the values reported below in the sections "Validation of 1 test and "Assay Performances". Regular calibration of the volumes delivered by, and maintenance (etcontamination and cleaning of needles) of the washer has to be exampled with the control of the volumes delivered by, and with a second filter (625-630nm and cleaning of needles) of the washer has to be exupped with a scood filter (625-630nm as according to the instructions of the manufacture. It is standard performances should be (a) bandwidth < 10 mm, b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability > 1%. Blanking is carried out on the well dentified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure the optical of system of the reader has to be calibrated regularly to ensure the structions. 4 0

B. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated controlled and regularly serviced in order to match the values reported in the sections. Validation of Test and "Assay Performances". The assay protocol has to be installed in the operating reader. In audition, the liquid handling part of the station system of the unit and volidated as for the vasitated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended where the number of samples to be tested exceed 20-30 units per run, instrument does not fit with the vials supplied in the kit them with the same label peeled out from the original value them with the same label peeled out from the original value. This operation is important in order to avoid mismatching contents of visits, when transferring them, When the set is firmly cannet.

setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit. firmly capped.

Dia Pro's customer service offers support to the user

- L. PRE ASSAY CONTROLS AND OPERATIONS

  I. Check the expiration date of the kit printed on the external label (primary contamp). Do not use of expired on 2. Check that the liquid components are not contaminated by visible particles or eggregates.

  Check that the Chromogen (TMB) is colourless or pale blive by asbriding a small volume of it with a sterile priestic by the contamination of the with a sterile priestic and contamination of the with a sterile priestic and contamination of the with a sterile priestic and definition of the contamination of the
- Divte all the content of the 20x concentrated Wash Solution
- as described above.
  Allow all the other or
  (about 1 hr) and It mix gently on vortex n temperature dex all liquid

- 8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diffused washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kill.

  9. Check that the ELISA reader is turned on or ensure a will be furned on at least 20 minutes before reading.

  10. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.

  11. Check that the micropipettes are set to the required volume.

  12. Check that all the other equipment is available and ready to use.

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

The left may be used for quantitative and qualitative. determinations as well

## M1, QUANTITATIVE DETERMINATION:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument aspirate 1000 µl Sample Diluent and then 10 µl sample (1:101 dilution factor). The whole content is then dispensed into a properly defined dilution tube. Before the next sample is aspirated needles have to be duly washed to avoid any cross-contamination among samples. When all the samples have been diluted make the instrument dispense 100 µl samples into the proper wells of the

This procedure may be carried out also in two steps of disclores of 1:10 sech (90 µl Sample Diluent + 10 µl sample) into a second disclore platform. Make then the instrument septrate first the platform and finally discense the whole content in the proper to which the platform and finally discense the whole content in the proper Do not dilute Calibrations and the dissolved Control Serum as they are ready to use.

Dispense 100 µl calibrators/control in the appropriate

It is strongly recommended to check that the time lap between the dispersation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly. For the next operations follow the operative instructions reported below for the Manual Assay.

- Manual assay:

  1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibration Set as calibraters are ready to use.

  Mix carefully all the liquid components on vortex and then
- proceed as described below.

  Place the required number of microwells in the microwell holder. Leave the A1 and B1 empty for the operation of Dispense 100 µl of Calibrators and 100 µl Control Serum in duplicate. Then dispense 100 µl of diluted samples in each blanking.
- properly identified well.

  4. Incubate the microplate for 60 min at +37°C.

Important note: Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using EUSA automatic instruments.

5 Wash the microplate with

Wash the microplate with an automatic washer delivering and aspirating 350 µl/well of cliuted wash solution as reported previously (section (3)) atic washer by diluted washing

> Pipette 100 µl Enzyme Conjugate into each well, except A1+81 blanking wells, and cover with the sealor. Check that this red coloured component has been dispersed in all the wells, except A1 and B1.

Contamination might occur. Important note: Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate.

- Incubate the microplate for 60 min at +37°C.

Important note: Do not expose to strong direct illumination High background might be generated.

- 10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive califrators, the control serum and the positive samples from blue to yellow. It wessure the colour intensity of the solution in each well, as described in section 15, at 450m filter (reading) and at 620, 530nm (background subtraction, strongly recommended), blanking the instrument on A1 or B1 or both.

## M2. QUALITATIVE DETERMINATION

described below: only a qualitative determination is required, proceed

Proceed as described in section M1

Automated assay:

- Manual assay:

  1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 100 µl sample) Do not dilute the Calibration Set as calibrators are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.

  2. Place the required number of Microwells in the microwell holder Leave A1 well empty for the peacin of blanking.

  3. Disperses 100 µl of Calibrator 1 (0 lUmi) and 100 µl calibrator 2 (10 lUmi) in adjuncte, and 100 µl Calibrator 6 (250 lUmi) in single. Then disperses 100 µl of diluted samples in each properly identified well.

Important note: Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using EUSA automatic instruments.

- Wash the microplate with an automatic washer by delivering and aspirating 350 µ/well of diluted washing selution as

Contamination might occur, Important note: Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate

- Wash microwells as in step 5.

  Pipette 100 µl Chromogan/Substrate mixture into each weil, the blank well included. Then incubate the micropiate at room temperature (18-24°C) for 20 minutes.

- Wash microwells as in slep 5.
  Pipeta: 100 µl Chromogan/Substrata mixture into each well, the blank wells A1 and B1 included. Then incubate the micropiate al room temperature (18-24°C) for 20
- **.**

reported previously (section I.3).

Pipette 100 µi Endyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that his rod coloured component has been dispensed in all the wells.

- Incubate the microplate for 60 min at +37°C

Important note: High background

Do not expose to strong direct illumination might be generated.

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 Pipette 100 µ Suphuric Acid into all the wells using the same pipeting sequence as in sep 9. Addition of acid will furn the positive calibrators, the control serum and the positive samples from thus to yellow.
 Measure the colour intensity of the solution in each well, as described in section 1.5, at 450mm filler (reading) and at 650-630mm (background subtraction, strongly recommended). blanking the instrument on A1.

| No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No. | No.

## General Important notes:

If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false

A validation check is carried out on the controls any time the kill is used in order to verify whether the performances of the assay are as qualified. Control that the following data are matched:

O. INTERNAL QUALITY CONTROL

positive results on reading.

Reading has to be earned out just after the addition of the Stop Solution and suryway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can after its addition. Some self oxidation of the chromogen can

į,

N

occur leading to high background.

The Control Serum (CS) does not affect the test results calculation. The Control Serum may be used only when a laboratory internal quality control a laboratory internal quality control is required by the management.

### N. ASSAY SCHEME

Sulphuric Acid	emperature	3" incubation			emperature	incubation	gate	Wash step 4	emperature	"incubation	Samples diluted 1:101	Calibrators & Control	
100 LI	r.t.	20 min	100 µl	4-5 cycles	+37°C	60 min	100 µI	4-5 cycles	+37°C	60 mIn	100 山	100 µ	Operations

An example of dispensation scheme for Quantitative Analysis is reported below:

further and operate as follows:	proceed any in the next section. If they do not, do not proceed any	and to the sent makes the requirements stated above	If the results of the test match the requirements along a

20 IU/ml ±10% OD450nm > 1.000 coefficient of variation < 30% OD450nm > OD450nm CAL1 + 0,100

150 mean OD450nm value after

		coefficient of variation > 30%	0 IU/ml > 0.150 OD450/m after blanking	> 0.050 OD450nm	Problem
5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugato 6. that the washer needles are not blocked or partially obstructed.	assay procedure (disperination of a positive calibrator instead of the negative one; 4, that no contamination of the negative calibrator or of their wells has occurred que spills of positive samples or the enzyme conjugate;	used and the washer has been primed with it before use;  3. That no mistake has been done in the	that the washing procedure and the washer settings are as validated in the pre-qualification study.  I that the proper washing solution has been	<ol> <li>that the Chromogen/Sustrate solution has not got contaminated during the assay</li> </ol>	Check

Legenda	M	G	T	m	c	6	co	2	
BLK	CALI	CALS	CALZ	CAL2	CALI	CALT	BLK	SCX	
= Black	SS	cs	CALE	CALG	CALS	CAL5	CAL4	CAL4	
P = 5	8.8	87	Sa	85	tri 4-	83	82	S	
old to								Į.	
305				4					3
0	4		4	4					¢
è		1		4			1		100
1		4	1	1	1		1		0
	1	4	1	1	1	1	1	1	q
									ë
							Ī		-
1		I			T		T		12

reported below: An example of dispensation scheme in qualitative assays

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b) a value higher than +1-20%, is obtained in this case the last at invalid and the DiaPro's customer fervice has to be called.	Precision of the laboratory might not enable this less to match the expected value +/-10%. Report the problem to the Supervisor for acceptance or relucator this result.	by reported after alminishing the reason of this error, if no mistake has feen found, proceed as fellows:  a) a value up to +1-20% is obtained: the owner.	o, are country system has been dissolved with the right volume popured on the label. If a mistake has been pointed out, the assay has to	d. no external contemination		expected units  (ex. dispersation of a wrong sample)	1. The procedure has been correctly performed		dualification study;	re as	2	calibrator instead):	-	250 IU/ml executed:	S DCCLIMBO	4, that no external c	washer settings are as	calibrator ins	OD450nm CAL1+ distribution (av. dispersion	
*/-20% is obtained in this and the DiaPro's customer	ory might not enable the test of value +/-10%. Report the for for acceptance or refusal	ating the reason of this error, bund, proceed as follows:  O'N is obtained the owerall	has been dissolved with the high the label.	no external contemination of the standard has	<ol> <li>I've washing procedure and the washer settings are correct.</li> </ol>	<ol> <li>no mistake has occurred during its distribution (ex. dispensation of a wrong sample)</li> </ol>	en correcty gerforment	occurred.		e as validated in the pre	Drocedure and	of a	e has been done in its	dure has been correctly		al contamination of the	u. That the washing procedure and the washer settings are as validated in the pre-	(ex. dispensation of a wrong itead).	mistake has been done in its	

Should one of these problems have happened, after checking, report to the supervisor for further actions.

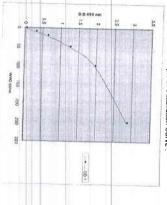
### P. RESULTS

## P.1 Quantitative method

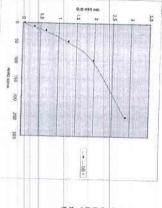
If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450nm (4-parameters

An example of Calibration curve is reported in the next page

## Example of Calibration Curve



interpolation is suggested).
Then on the calibration curve calculate the concentration of anti-Rubella Virus IgG antibody in samples.



1. Limit of detection

		70 0.402					
2000	0.241	0.451	0.742	1.007		Lot. 0503	RUBG CE
	0.231	0.425	0.724	1,354	20000	Lot 0603	RUBG,CE

mportant Note: To not use the calibration curve above to make calculations,

2.2 Qualitative method in the qualitative method, calculate the mean OD450nm values for the Calibrators 0 and 10 IU/ml and then check that the assay

## xample of calculation:

he following data must not be used instead or real figures blained by the user.

ean Value: 0.260 OD450nm igher than Cat 0 + 0.100 — Accepted alibrator 250 IU/mi: 2.845 OD450nm ower than 0,150 – Accepted alibrator 10 IU/ml: 0,250 – 0,270 OD450nm gher than 1.000 - Accepted ean Value: alibrator 0 IU/ml: 0.022 OD450nm 0.020 - 0.024 OD450nm

onsidered negative for anti Rubella Virus IgG antibody by most the international medical literature. interpretation of results amples with a concentration lower

Samples with a concentration higher than 10 WHO IUImi are considered positive for anti Rubella Virus IgG antibody. This titler is considered the Invest concentration of IgG to provide an effective immunological protection against a second inflation of Rubella Virus by NCCLS, USA.

Particular attention in the interpretation of results has to be used in the follow-up of pregnancy for an inflation of Rubella Virus due to the risk of severe neonatal mailtormations.

### Important notes:

- Interpretation of results straud be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misrinerpretations.

  When test results are transmitted from the laboratory to another featility, attention must be paid to avoid error account of the feating and the results are transmitted. The supervision is positive result (presence of log antibody > 10 (Ulmi) should be confirmed to ruled out the risk of a false positive result and a false definition of protection.

## R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what suggested in NCCLS's approved guideline for Rubella IgG testing (I/LA6.A)

The limit of detection of the assay has been calculated by means of the 1"W.H.O international standard for anti-Rubella immunoglobulin code RUBI-194. The limit of detection has been calculated as mean OD450mm Calibrator 0 IU/ml + 5 St. The table below reports the mean OD450mm values of this standard when diluted in negative plasma and then examined in the asse for three lots,

10 IU/ml	1.292 0.701	Lot. 0503 1.301 0.742	1000000000
0	0.701		
10	0.402		
S	0.211		1
Std 0	0.024		

2. Diagnostic sensitivity.
The diagnostic sensitivity has been tested in an external study of performance evaluation (University Hospital, Microbiology Department, Salamanca, Span) on panels of samples classified positive by a lat US FDA approved. Positive samples from different stage of Rubella Virus infection were tested.

The value, obtained from the analysis of more than 300 specimens, has been > 98%.

Mean values

0.195

Celibrator 250 IU/ml (N = 16)

0.020

from pregnant women were tested.

If has been calculated on three samples, a negative, a low positive and a positive, examined in 16 replicates in three separate runs for three lots. Results are reported as follows:

### RUBG,CE: lot 0303

	8.9	8.6	9.3	CV %
0.005	0.005	0.005	0,004	UONEABCT DIS
0	0.052	0.054	0.048	OD NOVIEM
value	The State of the S	- Camming		200
×	3" пип	2nd run	una 181	MICHIEL MAILES

Ob 45/mm   O.530   O.553   O.694   O.595					
ues 1st run 2nd run 3 <sup>10</sup> run 1 run 0.530 0.500 0.484 run 0.0034 0.022 0.019 run 5.4 4.4 4.0		15	250 JU/ml (N	Calibrator	
ues 1st run 2nd run 3 nun 1 nn 0.530 0.503 0.484 uan 0.034 0.022 0.019	0 4.9		4.4	0.4	0.5
ues 1strun 2nd nun 3 <sup>11</sup> nun 4 1m 0.530 0.503 0.484 1un 0.034 0.022 0.019			-		2000
ues 1st run 2nd run 3 <sup>tt</sup> run 4		0.0	0.022	0,034	UDITAL OF
es 1st nun 2nd nun 3 <sup>11</sup> nun 4			- Constitution	2	Die Description
es fisteur 2ndrum 3 <sup>th</sup> rum J		0.4	0.503	DEC.D	OC AUGUST
fist run 2nd run	value	1	The second		200
100	run Average	4	מנה מחב	uma ne	Charles A section
				***	Mean universe

### RUBG\_CE: lot 0503

3 299 0.228

Mean values 1		26.89	OCCUPATION OF	CHIDDEN ON THE		Mean values 1	
SE STATE	Calibrato	8.5	0.004	0.046		nu St	Calibrati
2nd run	r 10 IU/ml (N	8.9	0.005	0.052		200 000	Of 0 (LE/m) (N
3 <sup>th</sup> fun	= 16)	9	0.005	0.051	0 1901	340	= 161
Average		9.2	0.005	0.049	Agenda		

0,504

0.508

The assay shows a limit of detection for better than 10 IU/ml

Mean values

Calibrator 250 IU/ml (N = 15)
1st run 2nd run 3<sup>rd</sup> run

3.281

3.281 0.155

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3. Diagnostic specificity.

The diagnostic specificity has been determined in the same centre on panels of negative samples from not infected individuals, classified negative with a kit US FDA approved. Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the value of specificity. Those in specimens have been stated, as well, to check for interferences due to collection and storage.

Mean values

Calibrator 10 IU/ml (N = 16)
1st run 2nd run 3<sup>rd</sup> run

0.052

0.052

Calibrator 0 (Um) (N = 16)

No interference was observed.

Potentially interfering samples derived from patients different pathologies (mostly ANA, AMA and RF positive) ) and

No crossreaction was observed.
An overall value > 88% of specificity was found when examined on more than 100 specimens.

The variability shown sample misclassification

in the tables above did

not result in

S. Accuracy
The assyriation of the dependent of the service of the

## S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte. Frozen samples containing from particles or aggregates after thaving may generate some false results. This test is suitable only for testing single samples and not conclud conse.

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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   Volk W.A. (1982) in "essential of Medical Microbiology", 2"
   S.Jose, Torono, 100
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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

0318

Manufacturer:
Dia Pro. Diagnostic Bioprobes Srl.
Via G. Carducci n° 27 — Sesto San Giovanni (Mi) - Italy

# Parvovirus B19 9

Enzyme ImmuncAssay (ELISA) for the IgM antibodies to Parvovirus B19 in human serum and plasma qualitative determination of

for "in vitro" diagnostic use only



## DIA.PRO

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REF PARVOM.CE

## Parvovirus B19 IgM

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### A. INTENDED USE

Enzyme immunoAssay (ELISA) for the qualitative determination of light antibodies to Parvovirus B19 in human plasma and For "in vitro" diagnostic use only

The B19 virus, generally referred to as parvovirus B19 was the first (and until 2005 the only) known human virus in the family of paroviruses, genus exphrovirus, Parvovirus B19 is a non-enveloped, icosahedral virus. that contains a single-standad linear DNA, genus exphrovirus, parvovirus B19 is a non-enveloped, icosahedral virus. that contains a single-standad linear DNA genus explored virus that contains a single-standad linear DNA genus explored virus the bone marrow. These genotypes (with subtypes) have been recognized. The viral capital is composed of two structural proteins, namely VP1 (83kD), and VP2 (53 kD), infection by Parvovirus B19 streads through respiratory secretions ou also through blood or blood poducts. The infection causes a mild liness characterized by an enythermaticus maculopopular locial rash called fifth disease or exythema infections. It is tipical in within 4 to 14 days after getting infected with parvovirus B19 but about 20% of children and adults who get infected with his virus will not have any symptoms, Infection during pregnancy presents the risk of transmission to the fetus that may react in hydrops fetalis. In particular the presence of 1gM antibodies is reported to be correlated to the aute phase of ilineas, while 1gG antibodies become present a different thers shortly after pople with weakened immune systems caused by leukemia carost, organ transplants, or HVI infection are at 15k for serious that the first province and the first province and the first province and the first province and the province of the province of the province of the province on the first shortly after pople with weakened immune systems caused by leukemia carost, organ transplants, or HVI infection are at 15k for serious that the first province medical treatment.

that requires medical treatment.
Therefore the detection of Parvovirus B19-specific antibodies becomes very important.

The arrayme captured on the solid phase, acting on the substrate/shoringen mature, generates arroptical signatifiatis proportional to the arrount of anti-Parvoirus (gM antibodies present in the sample. The presence of IgM in the sample may therefore be determined by means of a cut-off value able to discriminate between negative and positive samples. Neuralization of IgG anti-Parvoirus, carried out discrimination of IgG anti-Parvoirus, carried out discript in the well-is performed in the assay in order to block interferences due to this class of antibodies in the determination of IgM.

## C. PRINCIPLE OF THE TEST

Microplates are coated with Parvovirus B19 antigens. The solid phase is first treated with the diluted sample and IgM. The solid phase is first treated with the diluted sample and IgM to Parvovirus B19 are captured. If present, by the antigens. After washing out all the other components of the sample, in the 2" incubation bound anti-Parvovirus IgM are detected by the addition of polydonal specific anti-hgM antibodies, labelled with peroxidase (HRP).

5. Wash buffer concentrate WASHBUF 20X

1x80ml/hottle20x concentrated solution.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+4.0.2, 0.05% (tween 20 and 0.05% Kathon GC.

Enzyme conjugate (CON)
 Ix familyal, Ready to use and red colour coded. It contains for the control antibodies to human IgM, 5% BSA, 10 mM Tits buffer pH 6.84–0.7, 0.1% Kathon GC and 0.02% gentamicine suphate as preservatives.

Specimen Diluent: DILSPE
 2x60mWial, It contains 2% casein, 10 mM Na-ctrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.5% NP40, 0.05% Na-azide and 0.1% Kathon GC as preservatives, 10 be used to dilute the

## D. COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with Parvovirus B19 antigens.

Plates are sealed into a bag with desiccant. Allow microplate to reach room temperature before opening; re unused strips in the bag with desiccant and store at 2..8°C. Allow the ning; reseal

2. Negative Control: CONTROL 1 K4.0 mWnb, Ready to use. If contains, hunan plasma negative to Parvovirus 819, 2% seasen, 10 mM Na-dirate buffer pH 6.0 +0.1, 0.1% Tween 20, 0.09% Na-axide and 0.1% Kalton GC

The Negative Control is pale yellow color coded,

3. Positive Controt: CONTROL 1 1x4.0 m/wsi. Ready to use. It contains human plasma positive to Parvovirus 819, 2% tasten, 10 mM Na-citrate buffer pH 6.0 +0-1, 0.1% Tween 20, 0.08% Na-azide and 0.1% Kathon GC

as preservatives. The Positive Control is green yellow color caded.

## 4. Callbrator: CA

n\* 1 vial. Lyophilized reagent to be dissolved with EIA grade water as reported in the latel. It contains bovine serum proleins, human plasma positive to Parvovirus. 0.2 might gentamicine sulphate and 0.1% Kathon GC as preservatives. Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

7. Chromogen/Substrate: SUBS\_TMB X15ml/Wal. It contains 50 mM circate-phosphate buffer pH 3.5-3.8, 4% dimethysubhoxide, D 0.0% tetra-methy-benzidine (or TMB) and 0.02% hydrogen peroxide (or HxOz). Note: To be stored protected from light as sensitive to

8. Sulphuric Acid: [<u>H2SO4\_0.3 M</u> \*Xf5m/wallt contains 0.3 M H5QL solution, \*Xf5m/wallt contains 0.3 M H5QL solution, \*Attention: infrant-(H315, H319; <u>P380</u>, <u>P902+P382</u>, <u>P332+P313</u>, P305+P351+P338, P337+P313, P382+P363).

10. Neutriking Reagent: SOLN NEUT 14. Neutriking Reagent: SOLN NEUT 14. Neutrikins goat and higG, 2% casein, 10 mM Nachrate buffer pH 6.0 +4-0, 1.0, 1% Tween 20. 0.09% Na-azide and 0.1% Kathon GC as preservatives.

## 11. Plate sealing foils n°2

12. Package insert n°1

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Ν MATERALS REQUIRED BUT NOT PROVIDED Calibrated Microphettes (1000 til, 100 til and 10 til) and spossable plastic tips.

LIA grade water (double distilled or delonised, charcos) resided to termove oxidizing chemicals used a famove oxidizing distilled or deionis axidizing chemicals used as

disinfectan Timer with

υ 4 rQ

3 Timer with 50 minute range or higher.
Absorbent paper issues.
Calibrated ELISA micropalar themostate incubator (of web) set at +37°C (+4-0.5°C tolerance).
Calibrated ELISA microwell reader with 450nm (reader with 520-630nm (blanking) fillers.
Calibrated ELISA micropiate washer. 6, 450nm (reading) (dŋ Q

Vortex or similar mixing tools

## WARNINGS AND PRECAUTIONS

responsible of the laboratory. kit has to be used by skilled and properly trained personnel only, under the supervision of a medical

Institute of Health's purificances, ed. 1984.

Biomedical Laboratories\*, ed. 1984.

All the personnel involved in sample handling should be in biosafety procedures, as recommended by the Center for Disease Control, Atlanta U.S. and reported in the National 2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained

avoid contaminants such as dust or air-born microbial agents, when opening sit visits and micropiates and when performing the set. Protect the Chromogen (TMB) from strong light and avoid unation of the bench surface where the test is undertaken, Lipon receipt, store the ket at 2.8°C into a temperature controlled refrigerator or cold noon. sale and effective.

4. The laboratory environment should be controlled so as to 4. The laboratory environment such as dust or all-born microbial agents. avoid contaminants such as a dust or all-born microbial agents.

Do not interchange components between different lots of the kits. It is recommended that components between two kits

of the same of should not be interchanged.

7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not advise the laboratory supervisor to initiate the necessary procedures for kit

8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each

disposable Avoid cross-contamination between kil reagents by using posable tips and changing them between the use of each

10. Do not use the kit after the expiration date stated on the external container and internal (viais) labels. A study conducted on an opened kit did not pointed out any rolement loss of activity in Treat all a specimens as protentially infective. All human serum specimens should be handled at Blossifety, Level 2, as in compliance with what reported in the Institutes of Health's publication. Blossety in Microbiological and Biomedical Somethins

publication: Biosafety in Microbiological and Biomedical aborationles; ed. 1984.

12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or, in transferring components into automated workstations, in order to avoid

3. Weste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as operating infective majerated and inactivation are before waste. Suggested procedures of inactivation are cross conlamination.

13. Waste produced during the discarded in compliance with na

treatment with a 10% final contentration of household bleach for 16-18 his or heat inactivation by autoclave at 121°C for 20 min.

14. Accidental squills from samples and operations have to be adsorbed with paper tissues sooked with household bleach and adsorbed with paper issues should then be discarded in proper containers designated for laboratory/hospital waste.

15. The Sulphuric Add is an initiant, in case of spills, wash the

surface with plenty of water

16. Other waste matchials generated from the use of the kit (example: tigs used incorplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory

order to avoid misinterp Samples have to be clearly identified with codes or names in wider to avoid misinterpretation of results. Bar code labeling and

electronic reading is strongly recommended.

3. Hearnolysed ("red") and visibly hypertipemic ("milky") samples have to be discarded as they could generate state results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give irse to false results.

4. Seria and plasma can be stored at \*2"...8"C for up to five days after collection. For longer storage periods, samples can be stored frozen at \*20". Or several months. Any frozen samples should not be frozen/thawad more than once as this may be full procedured to the controlled single the test result.

5. If particles are present, centringe at 2,000 fpm for 20 min or filter using 0.2-0.5u filters to clean up the sample for resting.

# H. PREPARATION OF COMPONENTS AND WARNINGS

Allow the microplate to reach temperature (about 1 hr) ck that the desiccant is not

### Ready to a

Ready to use components. Mix carefully on vortex before use

calibrator r is not stable and then gently mix Store it frazen in

Wash buffer concentrate:

The whole content of the concentrated solution has to be disused. 20x with hotistiled water and mixed gamily end-over the customer of the presence of buttons could impact on the efficiency of the washing cycles.

Value, Conce diluted, the wash solution is stable for 1 week at +2 8°C.

S. SPECIMEN: PREPARATION AND WARNINGS
 I. Blood is drawn assiptitally. By venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citate, EDTA.

before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of manufacturing in this case call Dia Pro's ousbrowner service. Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly appeal and stored at 22, 8°C. When opened the first lime, residual strips are stable till the indicator of humidity inside the desiccant bag turns from

### Control

use components. Mix carefully on vortex before use

## Positive Control

Calibrator

Add the volume of ELISA grade water, rethe tyophilized powder; let fully dissolve vortex. reported on the label

Note: The dissolved aiiquots at -20°C.

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Enzyme conjugate
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals,
air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly

Chromogen/Substrate:
Ready to use. Mix well on untax before use.
Recardy to use. Mix well on untax before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metalfic surfaces. If this component has to be transferred use only plastic, possible

### Sample Diluent

Ready to use component. Mix carefully on vortex before use

Neutralizing Reagent
Ready to use component. Mix carefully on vortex before use,

Ready to use. Mix well on vortex before use. Attention: Irritant (H315, H319: P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Warning H statements: H315 – Causes skin irrilation. serious

### Precautionary P statements: P280 – Wear protective eye irritation

P280 – Wear protective gloves/prot protection/face protection, P302 + P352 – IF ON SKIN: Wash with water: gloves/protective plenty of soap clathing/eye

7

and

P332 + advice/atte P305 + P351 + P338 - IF IN EYES: P313 - If skin . EYES: Rinse cautiously with water contact lenses, if present and easy irritation occurs: Get medical

for several minutes. F to do. Continue rinsin P337 + P313 advice/attention. P362 + P363 - Take off contaminated clothing and wash it us rinsing. 113 – If eye irritation persists Get medical

## I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

Micropipeties have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decondamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in conflact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried

of repulsivi recovers of in composition satisful about the garrier of the ELISA incubator has be set at +37°C foreignee of +1. CyC) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water hatts are suitable for the incubations, provided that the instrument is validated for the incubations, provided that the instrument is validated for the incubation of ELISA lessis.

The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routhe laboratory reference panels, before using the kit for routhe laboratory of 350ut/wail of washing southor = 1 cycle) are sufficient to ensure that the assay proferoms as expected. A capacity of the course that the assay proferoms as expected. A capacity time of 20-30 seconds between cycles is suggested, in order to

set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section "internal quality Control". Regular calibration of the volumes delivered by and maintenance (decontainmation and cleaning of needles) of the washer has to be carried out according to and maintenance (decontamination an needles) of the washer has to be carried the instructions of the manufacturer.

di 4. Inclubation there have a tolerance of ±5%.

The ELISA micropiale reader has to be exclupped with a reading filter of 450m and with a second filter (820-530nm, strongly recommended) for blanking purposes, its standard performances should be (a) bandwidth < 10 nm, (b) absorbance range from (b to ≥ 20; (c) linearity to ≥ 10; especiability ≥ 1%. Blanking is carried out on the well dentified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.

When using an EUSA automated work station, all critical staps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated confrolled and regularly serviced in order to matich the values reported in the sections. The memal Quality Control. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Farticular attention must be paid to avoid carry over by the needles ared controlled or mishize the possibility of contamination or adjacent wells. The use of EUSA automated work stations is recommended when the number of samples to be tested exceed 20:30 units her run.

is 'ecommended when the number of samples to be tested exceed 20-30 units per un. Da-Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit. In order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

- L PRE ASSAY CONTROLS AND OPERATIONS

  1. Check the expiration date of the kit printed on the external lanet (nitmary container). Do not use if expired.

  2. Check that the liquid components are not contaminated by
- visible particles or eggregates.
  Check that the Chromogen (TMB) is colourless or pale by aspirating a small volume of it with a sterile pi nale blue plastic
- pleet.
  4. Check to.
  5. Check that no breakage occurred in transportation and no spillage of inquid is present inside the box (primary container). Check that the aluminium pouch, containing the micropiate is not punctured or damaged.
  5. Dissolve the content of the Solibration as reported.
  6. Distance the content of the 20x concentrated Wash Solution ф.
- as described above.

  Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid
- setyents.

  Retail of the ELISA incubation at +37°C and prepare the ELISA washer by priming with the diulted washing solution, according to the manufacturers instructions. Sat the right number of washing openes as found in the validation of the instrument for its use with the kit.

  Check that the ELISA reader is furned on or ensure it will be under on at least 20 minutes before reading.

  To, if using an automated work station, turn on, check settings and be sure to use the right assay protocol.

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. Check that all the other equipment is available and ready

13. In case of problems, do not proceed further with the test and advise the supervisor

M. ASSAY PROCEDURE
The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

- Dilute samples 1,101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Control/Califoration as they are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
- N Place the required number of Microwells in the microwell holder, Leave A1 well empty for the operation of blanking.
- Not dispense Neutralizing Reagent in A1 used for blanking operations and in the wells used for the Controls and the Calibrator
- 4. Dispense 50 μl. Neutralizing Reagent in all the samples

Dispense 100 µl of Negative Control in duplicate, 100 µl of Positive Control in single, 100 µl of Calibrator in duplicate and 100 ul of diluted samples in each properly identified well.

Incubate the microplate for 60 min at +37°C.

Important note: Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using EUSA automatic instruments.

- Wash the microplate previously (section 1.3). with an automatic as reported
- Pipette 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells.

Important note: Be careful not to touch the plastic inner surface of the well with the fip filled with the Enzyme Conjugate. Contamination might occur.

- 9. Incubate the microplate for 60 min at +37°C.
- Wash microwells as in step 6.
- 11. Pipette 100 µl Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at room temperature (18-24°C) for 20 minutes.

Important note: Do not expose to strong direct illumination. High-background might be generated

- 12. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn, the positive catibrators, the control serum and the positive samples from blue to yellow.
- Measure the colour intensity of the solution in each well, as described in section 1.5, at 450nm filler (reading) and at 820n-630nm (background subtraction, strongly recommended), blanking the instrument on A1.

General Important notes:

1. If the second filter is not available ensure that no linger prints are present on the bottom of the microwell before

N

reading at 450mm. Finger prints could generate false pastive results on reading. Peeding has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self existence of the chromogen can occur leading to high background.

## N. ASSAY SCHEME

Reading OD	Sulphuric Acid	emperature	3 incubation	MB/H2O2	Wash step	emperature	Incubation	Enzyme conjugate	Wash step	emperature	incubation	Samples diluted 1:101	(only for samples)	Neutralizing Reagent	Controls & Calibrator (*)	Method
450nm	100 ul	H	20 min	100 µ	4-5 cycles	+37°C	60 min	100 Jul	4-5 cycles	+37°C	60 min	100 μ		50 H	100 µ	Operations

- (") Important Notes:

  The Calibrator (CAL) does not affect the Cut calculation, therefore it does not affect the (e. test's
- results calculation.
  The Calibrator (CAL) used only if a laboratory internal quality control is required by the Management.

An example of dispensation scheme is reported in the table below:

	Þ	œ	C	O	m	т	0	T
		1	- 1	-	CAL(*)			S2
2	S3	\$4	SS	Si	S7	SB	89	S10
W		ij			j			
b.								
OI.								
21								
1								
0	1					1		1
٥						1	1	
5	1							
4								
3			T	T		T	T	T

Legenda: BLK = Blank NC = Negative Control
CAL = Calibrator - Not mandatory PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as expected and required by the VDD directive 99/79/EC. Control that the following data are matched:

Positive Control	Negative Control		Blank well	Check
OD450nm > 1.000	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%	A STATE OF TOWNERS AND ASSESSMENT	< 0.100 ODASOS (10)	Requirements

I sulls of the test match the to the next section. requirements stated above

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If they do not, do not proceed any further and follows:

operate ag Important note: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to generate the correct interpretation of results.

< 1,000 GD450nm	m after atton >	> 0.100 00450nm	Problem
I, that the proordure that seem commity executery.  2, that in a missible that bean come in its distribution (disprimestation of a warrag condit);  3, that if the washing procedure and the washing softings are as validated in the pre qualification study;  4, that no external contamination of the positive control that occurred.	If that the contrary procedure and the wester softings are as validated in the pro- qualification.  I had he proper weaking golden has been used and the weaking solden has been used and the weaking solden has been solded in the pagative and the properties of a passing corner instead of the regative area. The sold procedure of the propasition of a passing corner instead of the pagative area. The sold procedure of the pagative area and the sold procedure of the sold pagative area and the sold was contrary to the sold procedure of the sold the pagative area of the sold procedure and the sold the pagative area of the sold the sold procedure and the sold the sold pagative area of the sold procedure and the sold the sold procedure area of the sold procedure and the sold procedure area of the sold procedure and the sold procedure area of the sold procedure and the sold procedure area of the sold procedure and the sold procedure area of the sold procedure and the sold procedure area of the sold procedure and the sold procedure area of the sold procedure and the sold procedure area of the sold procedure and the sold procedure area of the sold procedure and the sold procedure area of the sold procedure and the sold procedure area of the sold procedure and the sold procedure area of the sold procedure and the sold procedure area.	It let the Chromoger/Sustrate solution has not got containmaind during the assault.	Check

Should one of these problems have happened, after checking, report to the supervisor for further actions.

### \*\* Note:

If Calibrator has used, verify the following data:

if the resuits of the test doesn't match the requirements stated above, operate as follows:

Problem Calibrator	Check  1. that the procedure has been or
S/Co < 1.0	<ol> <li>that the procedure has been correctly executed;</li> </ol>
	2. that no mistake has been done in its distribution (dispensation of a wrong
	control instead);
	washing procedure and the
	pre qualification study:
	<ol> <li>that no external contamination of the</li> </ol>
	calibrator has occurred.

Anyway, if all other parameters (Blank, Negative Control, Positive Control), match the established requirements, the test may be considered valid.

P. RESULTS.

If the lest turns out to be valid, results are calculated from the mean OD450rm value of the Negative Control (NC) by means of a cut-off value (Co) determined with the following formula:

Cut-Off = NC + 0.250

Q. INTERPRETATION OF RESULTS
 Test results are interpreted as a ratio of the sample QQ450nm value (S) and the cut-off value (Co), or SICo, according to the following table:

×1.1	0.9-1.	< 0.9	5/60
Positive	Equivocal	Negative	Interpretation

A negative result indicates that the patient has not developed IgM entitlodies to Parcevirus.

Any patient showing an equivocal result should be released on a second sample taken 1-2 weeks after the initial sample.

A positive result is indicative of an ongoing Parvovirus infection and therefore the patient should be treated accordingly.

- interpretation of results should be done under the supervision of the laboratory supervision to reduce the risk of judgment errors and mainterpretations.

  When lest results are transmitted from the laboratory to
- another facility, attention must be paid to avoid erroneous
- deta transfer.

  Diagnosis has to be done and released to the patient by a suitably qualified medical doctor.

## An example of calculation is reported below.

The following data must not be used instead or rea/ figures

Lower than 0, 150 - Accepted Negative Control: 0.100 ~ 0.120 ~ 0.080 OD450nm Mean Value: 0.100 OD450nm

Higher than 0, 1000 - Accepted Positive Control: 1,500 OD450nm

Cut-Off = 0.100+0.250 = 0.350

S/Co higher than 1.0 - Accepted 0.500 - 0.540 OD450nm 0.520 OD450nm

Sample 1: 0.080 OD450nm Sample 2: 1.800 OD450nm Sample 1 S/Co < 1.0 = negative Sample 2 S/Co > 1.2 = positive

Evaluation of Performances has been conducted in accordance to what suggested in NCCLS's approved guideline G24-A2. R. PERFORMANCE CHARACTERISTICS Evaluation of Performances has been con-

## Limit of detection

No international standard for Parvovirus B19 IgM. Antibody detection has been defined so fair by the European Community. In its absence, an Internal Gold Standard (or IGS), has been defined in order to provide the device with a constant and

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7 of 7	7 of 7 Rov.: 2
	Rev. 2

Negative Control	8x	4x	XX	×	LG.S. Dilution
0.065	0.225	0.407	0.804	1,418	PARVOM.CE Lot P1
0.070	0.212	0.383	0.885	1,219	PARVOM.CE Lot P2

# Diagnostic Sensitivity and Specificity:

The Diagnosic Sensitivity was calculated on a panel of 50 samples classified positive for the IgM anti Parvovirus B19 by a reference kit CE marked.

A value of 2 88% was observed when referring to the reference device.

The Diagnostic Specificity was calculated on a panel of more than 100 samples classified negative with the reference device.

A value 2 98% was observed.

These findings are summarized in the following table.

Specifi	Sensiti
city >	vity
98 %	98 %

4. Precision: It has been calculated on three samples, a negative, a low positive and a positive, examined in 16 replicates in three separate runs for two lots, Results are reported as follows:

Masonalina	-	-	
SERVICE CORPORA	Tall Turn	2nd run	3" 700
OD 450nm	3.350	2 937	7 170
OX DIVISION	-	2,000	2,170
OD DEVISION	0.174	0.199	0.197
CV %	5.2	7.0	7.1

The variability shown in the tables above did not result in sample misclassification.

Accuracy
 The assay accuracy has been checked by the dilution and recovery lests.

S. LIMITATIONS OF THE PROCEDURE
Berterial confarmation or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.

Frozen samples containing thim particles or aggregates after thewing may generate some false results.

This test is suitable only for testing single samples and not pooled ones.

Diagnosis of an intectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

## PARVOM.CE: lot P1

5115	13.0	13.0	0.0	000
0.010	00.00		1	200
0010	0018	0.018	ELD'A	THE DESIGNATION OF THE PERSON
0.140	2000	-	0000	Std Danistics
0 440	0 126	0.138	0,140	Sec. accounts
Dritte			-	OD 4500m
BEIRAN	d (880)	1000		
	210	2nd 117	(F) 182	Christian Agenda

atd Deviation	UN 45GNM	00 450	Schwick reference	Mean	The state of the s	C 8 70	Contraction of	Std Deviation	OD 4500m	The second second	Ween value
0 224	3.032		una 151	Positive	1	3.2	1	0.000	Ī	United	
0.000	2.671		2nd run	e Control (N = 16		58	0.057	118'0		2nd run	Table of the last
	3.352		3" (10)	= 16)	200	50	0.056	0.950	The second	3" run	10,00
4.000	3,000	value	Average		6.0	200	0.048	0.974	Value	Average	

5.5

7.0

## PARVOM.CE: lot P2

		11.3	12.5	64.30
013	0.0	0.010	0,013	HOUSE AND AND
0.094	0	0.091	0.101	
				00 450
UNIT	30	2nd rum	uni tet	Sarten A contract

Company of the Compan				
STRUMP IND	ist run	2nd run	35 1111	Average
D.450nm	200		The second	COURA
The state of the s	1221	1.251	0.970	1,153
Contract	0.085	0.085	0.090	5.047
CV %	6.9	5.7	9.3	70

3

Produced by
Dia Pro Diagnostic Bioprobes Srl
Via G. Carducci n° 27 – Sesto San Giovanni (Mt) – Italy

(g) \_ =

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

# Parvovirus B19 OG

quantitative/qualitative determination of Enzyme ImmunoAssay (ELISA) for the lgG antibodies to Parvovirus B19 in human serum and plasma

for "in vitro" diagnostic use only



## DIA.PRO

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REF PAR VOG.CE 96 Tests

## Parvovirus B19 lgG

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Date: 2016/07

### A INTENDED USE

plasma and sera. For "in vitro" diagnostic use only. Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Parvovirus B19 in human

B.INTRODUCTION

The B19 virus, generally referred to as parvovirus B19 was the first (and until 2005 the only) known human virus in the family of parvoviruses, genus enythrovirus. Parvovirus B19 is a temperature of the first (and until 2005 the only) known human virus in the family of parvoviruses, genus enythrovirus that contains a single-stranded inteat DNA genome. It is classified as epythrovirus because of its capability to invede red blood cell precursors in the bone memory. Three genoplyses with subtypes) have been recognized. The viral capabil is composed of two structural proteins, remely VP1 (83kD) and VP2 (83 ND), infection by Parvovirus B19 spreads through respiratory secretions but also through blood or blood products. The infection causes a mild liness characterized by an exythematory secretions but also through blood or blood products. The infections it is tipical in within 4 to 14 days after getting infected with parvovirus B19 blood within 4 to 14 days after getting infected with parvovirus B19 blood about 20% of children and adults who get infected with this virus will not have any symptoms. Infection during preparency presents the risk of transmission to the feats that may result in bydrops festals. People with weakered immune systems are at risk for serious complications from fifth disease. It can cause chronic anemia that requires medical tratament. Therefore the descrition of Parvovirus B19-specific antibodies becomes very important. becomes very important

C PRINCIPLE OF THE TEST

Microplates are coaled with Parvovirus B19 antigen.
The solid phase is first treated with the diluted sample and IgG
to Parvovirus B19 are captured, if present, by the antigens.
After washing out all the other components of the sample, in the
2<sup>th</sup> incubation bound ant Parvovirus IgG are detected by the
addition of polydonal specific anti hIgG antibodies, labelled with
personaines (AED).

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti-Parvovinus IgG antibodies the semination of anti-Parvovinus IgG antibodies the 2<sup>rd</sup> W.H.O. international standard for Anti-Parvovinus B19 code 01/602, makes possible a quantitative determination of the IgG antibody in the patient.

## D. COMPONENTS

The kit contains reagents to perform 96 tests

Microplate: MICROPLATE
 12 stips x 8 microwells coaled with Panovirus 819 antigens,
 12 stips x 8 microwells coaled with Panovirus 819 antigens,
 Pales - are - seeled - into a bag with desicoant. Allow the
 microplate to reach room temperature before opening, reseal
 unused strips in the bag with desicoant and store at 2.8°C.

2 Calibration Curve: CAL N°...

Ready-to-use and color coded standard curve derived from human plasma positive for Parovirus B19 IgG and litrated on WHO standard ranging:

4mi/vial CAL1 = 0 WHO IU/mi
2mi/vial CAL2 = 3 WHO IU/mi
2mi/vial CAL2 = 5 WHO IU/mi
2mi/vial CAL4 = 12 WHO IU/mi

2ml/vial CAL5 = 20 WHO IU/ml 4ml/vial CAL6 = 40 WHO IU/ml

Standards are calibrated against the 2<sup>rd</sup> W.H.O international standard for Ami-Parovirus B19 code 01/602.
I contains human serum potents, 2<sup>rd</sup> casein, 10 mM Na-citrate order pH 6.0 +4.0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. Standards are blue colored.

## 3. Control Serum: CONTROL ...ml

vial. Lyophilized.

It contains bovine serum proteins, human plasma positive to Parvovirus B19 asilibrated at 12 WHO IU/mi ± 10%, 0.2 mg/mi gentamicine sulphate and 0.1% Kathon GC as preservatives, Koter. The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

## 4. Wash buffer concentrate: WASHBUF 20X 1x60ml/bottle20x concentrated solution.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

3. Enzyme conjugate: CONJ 2x8mi/wai.Reary to use and red colour coded, it contains thoseradish peroxidese conjugated polyclonal ambiodies to human igG, 5% SSA, 10 mM Tris buffer pH 6,84\*-0,1, 0.1%, Kathon GC, 0,02 mg/mi gentamicine sulphate as preservatives and 0,01% red alimentary dye.

6. Chromogen/Substrate: SUBS\_TMB
1x15m/val. It contains 50 mM ottrate-phosphate buffer pH 3.53.6. 0.03% letter-mettly-benzione (or TMB) and 0.02% hydrogen peroxide (or HoOs) and 4% dimethylsulphoxide.
Note: To be stored protected from light as sensitive to

7. Sulphurit Acid: [HSQ: 0.3 M It Sfamilyal: It contains 0.3 M HSQ: solution, 1x15ml/val: It contains 0.3 M HSQ: solution, Attention, Irland; (1415), 5431; P302; P302; P332; P332; P313, P305; P351; P338, P337; P313, P362; P363).

8. Specimen Diluent: DILSPE 2x6omWall. It contains 2% casein, 10 mM Na-citrale buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.05% Na-azide and 0.1% Kathon GC as preservatives. The reagent is blue colour coded.

## Plate sealing foils n°2

10. Package insert n°1

- E. MATERIALS REQUIRED BUT NOT PROVIDED

  1. Calibrated Micropipettes (1000 ul, 100 ul and 10 ul) and
- disposable playlic tips.

  EA grade water (double distilled or delionised, charcoal treated to remove oxidizing chemicals used as
- disinfectants). Timer with 60 minute range or higher.

- Absorbent popular tissues.
   Calibrated ELISA micropiate themostatic incubator (dry or well) set at -27°C (+4.5°C tolerance).
   Calibrated ELISA microwell reader with 450mm (reading) and with 520-520nm (blanking) filess.
   Collivated ELISA micropiate weather.
   Collivated ELISA micropiate weather.
   Collivated ELISA micropiate weather.

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## WARNINGS AND PRECAUTIONS

doctor responsible of the laboratory.

2. All the personnel involved in performing the assay have to The kit has to be used by skilled and protectinical personnal only, under the supervision. sion of a y trained medical

wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in blosafety procedures, as recommended by the Center for Disease Control, Allaria, U.S. and reported in the National Institute of Health's publication: "Blosafety in Microbiological and

vaccinated for safe and effecti inedical Laboratories, ed. 1884.
All the personnel involved in sample handling should be contacted for HBV and HAV, for which vaccines are available,

4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vals and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.

5. Upon receipt, store the kit at 2.8°C into a temperature

controlled refrigerator or cold room.

5. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.

7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the aboratory supervisor to initiate the necessary procedures for kit

8. Avoid cross-conlamination between serum/plasma samples by using disposable tips and changing them after each

Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each 5 9

Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity.
 Treat all specimens as potentially interlive. All human serum specimens should be handled at Blosafety Level 2 as recommended by the Centur for Disease Control. All alman, U.S. no compliance with what reported in the Institutes of Health's publication. "Blosafety in Microbiological and Sciencedical Laboratories", ed. 1984.
 The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to aucid cross containmation.

discarded in compliance with national directives and laws substances. In particular, liquid waste generated from the washing procedure, liquid waste generated from the washing procedure, increasing the procedure of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household beach for 14. Accidental synile from samples and operations have to be interested with parely tissues soaked with household beach and then with water. Itssues should then be discarded in proper containers designated for lateral to the second of the proper states with planty of water.

15. The Sulphuric Acid is an infrant, in case of spills, wash the state with planty of water.

16. Other waste materials generated from the use of the kit (example; its used for samples and controls, used increpiates) should be handed as potentially infective and disposed according to national directives and increasing laboratory wasters. Waste produced during discarded in compliance with the use of the kit has to

# G. SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn assphically by venepuncture and plasma serum is prepared using standard techniques of preparation samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, ED. and heparin. Blood is drawn aseptically EDT/

2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading its strongly recommended.

3. Haemolysed (fred\*) and visibly hyperlipemic ('milky') samples have to be discarded as they could generate false results. Samples combining residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could generate the processor of the process could give rise to false results

before opening the container. Check that the desicoant has not turned dark green, indicating a defect in manufacturing. In this case, call Dia Pros customer service. Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at 2",gC.

Alter first opening, remaining strips are stable until the humidity indicators. microplate to reach room temperature (about 1 hr) ainer. Check that the desiccant has

inside the

### Calibration Curve

Ready to use comp

Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on Add the volume of ELISA grade water,

vortex.

Note: The control after dissolution is not stable. Store trozen aliquots at -20°C. Do not thaw and freeze the aliquot again.

The 20x-concentrated solution has to be difficiently life. A grade water up to 1200 ml and mixed genily end-over-end before use. As some salt crystals may be present into the validation of the validation.

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful-not to contaminate the liquid with oxidizing chemicals

H. PREPARATION OF COMPONENTS AND WARNINGS
A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3

### Allow the n

onent. Mix carefully on vortex before use

### Serum

dissolve all the content when preparing the solution.

In the preparation avoid feating as the presence of bubbles could give origin to a bad wasting efficiency.

Note: Once diluted, the wash solution is stable for 1 week at

aindriven dust or microbes,
If this component has to be transferred use only plastic, possibly
sterile disposable containers.

a Seria and processors of the stored at +2".8"C for up to five days after collection. For longer strage periods, samples can be stored frozen at +20"C for several months. Any frozen samples e should not be frozen/thawed more than conce as this may 5. If particles that could affect the test result.

5. If particles are present, centifuge at 2.000 rpm for 20 min or filter using 0.2-0.5 this files to clean up the sample for testing.

6. Samples whose anti-Parvoyung B19 jgg antibody concentration is expected to be higher than 40 lulmi should be falled before use, either 1:10 in the Specimen Dileent. Districtions have to be done in clean disposable tubes by diluting 50 ut on each specimen with 450 ut of Specimen Dileent. (1:10). Mix tubes throughly on vorkex and then proceed toward the dilution it step reported in section M.

P332 + advice/atte

+ P313 - If skin

irritation

occurs: Get

medica

P280 – Wear protective gloves/protective dothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and

Precautionary P statements: P280 - Wear protective

Warning H statements: H315 – Causes skin irritation H319 - Causes serious

eye irritation

Legenda:

Ready to use. Mix well on vortex before use. Attention: Irrilant (H315, H319; P280, P302+P352, P332+P313

P337+P313,

P362+P363).

Sulphuric Acid

Ready to use component. Mix carefully on vortex before If this companent has to be transferred use only plastic, possible

sterile disposable container

remaining strips are stable unit the humidity desiccant bag turns from yellow to green,

# L INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohal, 10% solution of bleach, hospital goade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a truenass of 41.2%. Decontamination of spills or residues of kit components should also be carried

of regularly.

The ELISA incubator has to be set at 43°C (tolerance of 440.5°C) and regularly checked to ensure the correct temperature is maintained, 80th dry incubators and water hatts are suitable for the incubations, provided that the instrument is validated for the incubations, provided that the regular temperature of 43°C to assured to the micropaids.

The ELISA washer is extremely important to the ownership performances of the assay. The washer must be carefully validated and correctly optimised using the lift controls and tests, ubusilly 45 washing cycles (aspiration + dispursation of 350 ulwell of washing solution = 1 overlal are sufficient to ensure that the assay performs as expected. A soaking time, set ornectly their number, it is recommended on un an assay with the kit controls and well characterized negative and "Assay Performances". Regular calibration of their controls and well characterized negative and ceaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals,
Be careful not to contaminate the liquid with oxidizing chemicals,
Be not expose to strong illumination, oxidizing agents and
metallic surfaces.

4 01 8

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5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and with a second filter (620-630nm, strongly recommended) for blanking purposes, its standard performances should be (a) beardwidth < 10 nm, (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability < 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure regularly mantiamed according to the manufacturer 's instructions.

instructions.

B. When using an ELISA automated work station, all critical steps (dispersation, incubation, washing, reading, data handling) have to be carefully set, calibrated controlled and regularly serviced in order to match the values sported in the sections "Validation of Test" and "Assay Performances. The assay protocol has to be installed in the operating system of the fruit and validated as for the washer and the reader. In addition, the liquid handling part of the station dispersation and washing; has to be validated as for over by the needles used to dispensing and for washing. This must be studied and controlled to minimize the passibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be fested exceed 20-30 units per run, setting and checking of instruments used in contiliation to the user in the with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit. 6

7

advice/attention, P362 + P363 - Take off contaminated clothing and wash it before reuse.

eye irritation

persists:

Get

medical

to do. Continue rinsing. P337 + P313 - If

P305 + P351 + P338 – IF IN EYES: Rinse cauliously with water for several minutes. Remove contact tenses, if present and easy

- 1 PRE ASSAY CONTROLS AND OPERATIONS
  Check the expiration date of the kill printed on the external label (gramary container). Do not use if expired.
  Check that the liquid components are not contaminated by
- ω visible particles or aggregates.
  Check that the Chomogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic
- picette.

  Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the
- O micropiate, is not punctured or damaged.
  Dissolve the content of the lyophilised Control
  reported in the proper section. Serum as
- o Dilute all the content of the 20x concentrated Wash Solution
- as described above.

  Allow all the other components to reach room (about 1 hr) and then mix gently on vorte vortex lemperature a liquid
- reagents.

  8. Set the ELISA incubator at +37°C an prepare the ELISA washer by priming with the diluted washing solution, according to the meantleaturer instructions. Set the right number of washing opties as found in the validation of the instrument for its use with the kit.

  9. Check that the ELISA reader is turned on or ensure it will be turned on an iteast 20 minutes before reading.

  10. If using an automated work station, turn on check settings and be sure to use the right assay protects.

  11. Check that the microphettes are set-to-the required volume of 12. Check that all the other equipment is available and ready
- to use.

  13. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE
The assay has to the carried out according to whal below, laking care, to maintain the same incubation to the samples in testing. me for all

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The I ₹. may as well bе used ō quantitative and qualitative

## ... ₹

- N Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Dilutent + 10 µl sample). Do not dilute the Calibration Set as calibrations are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.

  2. Place the required number of microwells in the microwell holder, Leave the A1 and B1 empty for the operation of
- blacking

  5. Dispense 100 µ of Calibrators in duplicate. Then dispense 100 µ of diluted samples in each properly identified well. The Control Serum doesn't have to be used in every single analysis: it may be used wherever an internal quality control is required by the management to check the overall performances of the laboratory itself. In case, dispense 100 µ of the Control Serum, prepared according to instructions, in duplicate into a proper well. ça

Important note: Strips have to be sealed with the adhesive sealing foil supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic naturanents.

Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diuted washing solution as reported previously (section 1.3).

E. Pipeter 100 µ Enzyme Conjugate into each well, except A1+B1 thanking wells, and cover with the sealer, Check that this red coloured component has been dispensed in all the sealers. the wells, except A1 and B1

Important note: Be careful not to touch the plastic inner surface of the well with the fip filled with this Enzyme Conjugate. Contamination might occur.

- Incubate the micropilate for 60 min at +37°C. Wash microwells as in step 5.

  Pipetre 100 µ Orbromogon/Substrate mixture into each well, the blank wells A1 and B1 included. Then incubate the micropilate at room temperature (18-24°C) for 20

Important note: Do not expose to strong direct illumination. High background might be generated.

- 10. Pipette 100 µl Sulphuric Acid to stop the enzymatic reaction into all the wells using the same pipeting sequence as in step 9. Acidition of ead will turn the positive calibrations: the control serum and the positive samples from blue to yellow, the section 1. Measure the colour intensity of the solution in each well, as described in section 1.5, at 450nm filter (carding) and at 202, 530nm (tackground subtraction, strongly recommended). blanking the instrument on A1 or B1 or both

## MZ QUALITATIVE DETERMINATION

described below: is required, proceed as

- 1. Dilute samples 1:101 into a properly defined dilution table (example: 1000 µl Sample Diluent \* 10 µl sample). Do not dilute the Calibration 1 (Quilum) and Calibration 5 (Quilum) as they are ready to use. The Control Serum doesn't have to be used in every single analysis, it may be used whenever an internal quality control is required by the management to check the overall performances of the laboratory itself. Mix concludy, all the liquid components on vortex, and then received the description of the laboratory itself.
- proceed as described below.

  Place the required number of Microwells in the microwell holder, Leave A1 well empty for the operation of blanking.

- w Dispense 100 µi of Calibrator 1 (0 IU/ml) and 100 µi Calibrator 5 (20 IU/ml) in duplicate, and 100 µi Control Serum. Prepared according to instructions, in single, Then dispense 100 µi of diuted samples in each property identified well.

Important note: Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. On not cover strips when using EUSA automatic instruments.

- and aspirating 350 pl/well of diluted washin Wash the microplate with an automatic washe
- reported previously (section 1.3).

  Pipetts 100 µl Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells,

except A1

Important note: Be careful not to louch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate.

- Incubate the micropiate for 60 min at +37°C.
   Wash microwells as in step 5.
   Pipette 100 Li Chromoger/Substrate mixture into each well, the blank well included. Then incubate the micropiate at room temperature (18-24°C) for 20 minutes.
   Important note: Do not expose to strong direct illumination. High background might be generated.

10. Pipette 100 µl Sulphunic Acid into all the wells using the same pipeting sequence as in step 9, Addition of acid will furn the positive calmiplots, the control sorum and the positive samples from blue to yellow.

11. Measure the colour intensity of the solution in each well as described in section 1.5, at 450nm filter (reading) and possible of 10.

## General Important notes:

- If the second filter is not available ensure that no finger parties are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false possive results on reading.

  2. Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self-oxidation of the chromogen can
- leading to high background.

## N, ASSAY SCHEME

Reading OD	Sulphuric Acid	Temperature	3 incubation	TMB/H2O2	Wash step	lemperature	2 incubation	Enzyme conjugate	Wash step	anuberature	1" incubation	Samples diluted 1:101	Calibrators & Control(*)	Method
450nm	100 ul	1.1	20 min	100 pl	4-5 cycles	+37°C	60 min	100 pl	4-5 cycles	+37°C	60 min	100 H	100 µl	Operations

Incubate the microplate for 60 min at +37°C

# An example of dispensation scheme for Quantit reported below:

	# 11 TO THE POST OF THE POST O
	o solution as
	T by delivering
	Thu dollars
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	,	۵	S P	n s		4	ø	9			
-	2	C.	a	U	σ	7	Of	9		10	10 11
BLK	CAL4	S		-							
BLK	CALA	52						T			
CAL1	CAL5	S3						Т		I	
CAL1	CALS	4						Т	-		
CAL2	CAL6	co Co						П	-		
CAL2	CALE	60						1	-		
CAL3	CS?	\$7							-+-		
CAL3	CS(*)	SS &S						ı,	4	1	-

A B COUL OT \$

An example of dispensation scheme in quality reported below:

	The state of	2	ω	4	5	50	×	84	n	n D	6	p
Þ	BLK	co G	12				***			-1		
œ	CAL1	54	\$ 12		1	- 1	4					
O	CALI	to to				- 1	-					
O	CALS	os en	S 14			- 1	-					
m	CAL5	87	S 15			- 1	4					
m	CS(*)	88	S 16			- 1	-					
G	S	88	S 17			- 1	4					
I	(O	S 10	co co			- 1	-					

O. ASSAY QUALITY CONTROL

A validation check is carried out on the Calibrator kit is used in order to verify whether the performassay are as expected and required by the 198/79/EC.

management for an the laboratory itself. The Control Serum n is used only when req internal verification of the pe

Control that the following data are matched:

Reading OD	Iphuric Acid	mperature	incubation	//B/H2O2	/ash step	emperature	incubation	izyme conjugate	ash step	emperature	incubation	amples diluted 1:101	alibrators & Control(*)	ethod
450nm	100 ut	1.1	20 min	100 pl	4-5 cycles	+37°C	60 min	100 pl	4-5 cycles	+37°C	60 min	100 11	100 pt	Operations

(\*) Important Notes:

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The Control Serum (CS) it does not affect the test's results calculation.

The Control Serum (CS) used only if a laboratory internal quality control is required by the Management.

proceed to the next if the results of the test match the requirements stated above tsection.

If they do not, do not proceed any follows: further and operate

0 100 × 0.05		CHECO
3		1. Inst the Chromogen/Sustrate solution has
10 94 65		1 that the washing procedure and the washer settings are as validated in the pre
	dnm	qualification study;  2. that the proper washing solution has been
coefficient > 30%	coefficient of variation > 30%	used and the washer has been primed with it before use;  3. that no mistake has been done in the
		calibrator instead of the negative one.  4. that no contamination of the negative calibrator at of their webs has occurred due spits of positive samples or the enzyme conjugate;
erum - Not mandatory		<ol> <li>Inat micropipettes haven't got contaminated with positive samples or with the enzyme conjugate</li> <li>Inat the washer needles are not blacked or partially obstructed.</li> </ol>
3 IU/ml OD450nm < OD450nm CAL1 alive assays is 0.100	+	
9 16 11 12	8 to 0 c //	<ol> <li>Inat the washing procedure and the washer settings are as validated in the pre qualification study;</li> <li>Inat no external contamination of the cultivator has occurred.</li> </ol>
CAL 5 22 NJ/m 20 NJ/m		i, that the procedure has been cornectly exercised. 2. Index no mistake has been done in its distribution (dispensation of a wrong cathration instead):  3. Index the weathing procedure and the washer settings are as validated in the pro- qualification study;  4. Index no external contamination of the
CAL 6 40 IU/ml 41,000 OD450nm vs any time the rmances of the IVDD directive		to the procedure has been correctly sercuted:  I that the procedure has been correctly sercuted:  I that no mistake has been done in its distribution (dispensation of a wrong calibrator instead).  I that the washing procedure and the washer settings are as validated in the pre-washer settings are as validated in the pre-washer settings are as validated in the pre-
quired by the erformances of	U A U	4. that no external contamination of the backing portrol has occurred.

Should one of these problems have happened, after checking, report to the supervisor for further actions.

Check	Requirements
Blank well	< 0.050 OD450nm value
CAL 1 0 IU/mil	
	coefficient of variation < 30%
CAL 2	OD450nm > OD450nm CAI 1 +
3 IU/ml	0.100
CAL 5	OD450nm > 0.750
20-IU/ml-	
CAL 6	OD450nm > 1,000
40 iU/ml	

\*\* Note:

if-Control Serum has used, verify the following data

Control Serum I	Check
/lean OD450nm CAL4 ± 20%	Requirements

If the results of the test doesn't match the requirements stated above, operate as follows:

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tigator a	Check
ontrof Serum	First verify that:
ifferent from	<ol> <li>the procedure has been correctly performed:</li> </ol>
xpected value	<ol> <li>no mistake has occurred during its distribution (e.g.: dispensation of a wrong sample):</li> </ol>
	<ol><li>the washing procedure and the washer settings are correct;</li></ol>
	<ol> <li>no external contamination of the control has occurred.</li> </ol>
	<ol><li>the Control Serum has been dissolved with the right volume reported on the label.</li></ol>
	if a mistake has been pointed out, the assay has to be repeated after eliminating the
	reason of this error.

Anyway, if all other parameters (Blank, CAL1, CAL2, CAL5, CAL6), match the established requirements, the lest may be considered valid.

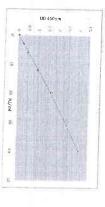
## P.1 Quantitative method

If the test turns out to be valid, use for the quantitative method an approved curve fitting program to draw the calibration curve from the values obtained by reading at 450mm (4-parameters therepolation is suggested).

Then on the calibration curve calculate the concentration of anti-

Parvovirus B19 lgG antibody in samples

## Example of Calibration Curve :



important Note: Do not use the calibration curve above to make calculations.

P.2 Qualitative method: national representative method: adouble the mean OD450nm values for Calibrator 1(0 IU/mt), and for Calibrator 5( 20IU/mt) and then check that the assay is valid.

In this case the results are calculated by means of a cut-off value determined with the following formula:

## Cut-Off(Co) = CAL5/5

Important note: When the calculation of results is performed by the operating system of an ELISA automated work station, existing that the proper formulation is used to calculate the out-off value and generate the correct interpretation of results.

### Q. INTERPRETATION OF R

0

## Q.1 Quantitative method

Samples with a concentration between 3 and 5 WHO IU/ml are considered equivocal for anti Parvovirus 819 IgG antibody. Samples with a concentration higher than 5 WHO IU/ml are considered positive for anti Parvovirus 819 IgG antibody. This titler is considered the lowest concentration of IgG to considered negative for anti Parvovirus B19 IgG antibody most of the international medical literature. provide an effective immunological protection mples with a concentration y by

## Q.2 Qualitative method

Results are interpreted as ralio between the sample OD450nm and the cul-off value or S/Co.

>1.2	0.8 - 1.2 E	< 0.8	S/Co Inte
Positive	quivocal	vegative	nterpretation

### Important notes: 1. Interpretation

## An example of calculation is reported below

Calibrator 20 IU/ml: 1.489 - 1.545 OD450nm Mean Value: 1.517 OD450nm Lower than 0\_150 - Accepted

Sample 1: 0.028 OD450nm Sample 2: 1.890 OD450nm Sample 1 S/Co < 0.9 Sample 2 S/Co > 1.0

## R. PERFORMANCES

## Limit of detection

The limit of detection of the assay has been calculated by means of the 2<sup>rd</sup> WHO international standard for Anti-Parvoirus 819 code 01/602. The limit of detection has been calculated as mean 00450mm Calbibrator 01/Jml + 5 SD. The liable below reports the mean 00450mm values of this standard when difficied in negative plasma and then examined in the assa for two lots.

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Results are interpreted according to the following table:

>1.2	0.8 - 1.2	< 0.8	S/Co ii
Positive	Equivocal	Negative	nterpretation

Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample.

Interpretation of results should be done under the supervision of the laboratory supervision to reduce the risk of judgment errors and misinterpretations.

When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous

Diagnosis has to be done and released to the patient by a

The following data must not be used instead or real figures obtained by the user.

Higher than 0.750 - Accepted Calibrator 0 (U/m): Mean Value: 0.020 - 0.024 OD450nm 0.022 OD450nm

## Cut-Off = 1.517 / 5 = 0.303

## negative positive

Evaluation of Performances has been conducted in accordance to what suggested in NCCLS's approved guideline C24.4.2.

ω	on	12	20	40	Im/UI OHW
0.266	0.473	0.954	1.686	3.041	PARVOG.CE Lot P1
U 222	0.549	0.925	1.570	3.110	PARVOG.CE Lot P2

The assay shows a limit of detection far better than 3 IU/ml.

2. Diagnostic sensitivity & Specificity:

The Diagnostic Sensitivity was calculated on a panel of 50 samples classified positive for the IgG anti parvovirus B19 by a reference kit CE marked.

A value of 2 98% was observed when referring to the reference

The Diagnostic Specificity was calculated on a panel of more than 100 samples classified negative with the reference device. A value 2 98% was observed.

These findings are summarized in the following table.:

Sensitivity > 98 % Specificity > 98 %

4. Procysion: It has been calculated on three samples, a negative, a low positive and a positive, examined in 16 replicates in three separate runs for two lots. Results are reported as follows:

### PARVOG.CE: lot P1

117	15.2	15.3	10.6	CV %
0.00	0.011	0.010	0,007	ubitesan or
0.066	0.069	0.065	0.054	CO SOUTH
Avera	3 745	200 nun	umina	Capital value

CV %	CION	1280		Santa range			CV %	- Company
9.9	0.031	0.313		Unit 351	Calibrat		10.6	2000
11.7	0.036	0.305		2nd tun	Z		15.3	0,010
6.9	0.024	0.352	1000	371 041	= 16)		16.2	0.011
9.5	0,030	0.323	British.	Senary		-	13.7	0,009

	39	4,6	4.7	CV%
0.00	0.082	0.084	2.085	old Deviation
1.33	2,077	1,799	1,780	CD 4300m
Aven	3 (4)	aler min		OF SEC.

### PARVOG.CE: lot P2

Samen uespe

0.089 0.012 13.8

20 (U/m) (N = 16)	3. 0	0.084 0.082	17117
Average	4.4	0.084	4 000
Manufacturer:	market only if conforming with the EC specifications and acceptance criteria.	System in compliance with ISO 13485 rule.	of the first of the same of th

Mean values

Calibrator 20 IU/mi (N = 16)
1st nun 2nd nun 3 nun

Callbrator 3 IU/mt (N = 16)
1st run 2nd run 3" run

2.085 0.081

2,040 0,099

The variability sample misclass		
shown in sification.		
the		
tables		
above		
di		
not		
result		
3		

5. Accuracy

The assay accuracy has been checked by the dilution and recovery tests. Any "hook effect", underestimation likely to happen at high doses of analyte, was ruled out up to 77 IU/ml.

## S. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the nayle. Prozen samples containing from particles or aggregates after mawing may generate some false results.

This test is suitable only for testing single samples and not

Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be

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All the IVD Products manufactured by the company are under the control of a certified Quality Management e. Each lot is ased into the EC technical

Via G. Carducci n	Dia, Pro	
"27 - Sesto San Gio	Diagnostic Bioprobes S.r.I.	Manufacturer:

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## 1BSAgone Version ULTRA

Hepatitis B surface Antigen or HBsAg in human serum and plasma Fourth generation Enzyme for the determination of Immunoassay (ELISA)

for "in vitro" diagnostic use only -



DIA.PRO

20099 Sesto San Giovanni Via G. Carducci nº 27 Diagnostic Bioprobes Srl (Milano) - Italy

e-mail: info/aidiapro.it Fax +39 02 26007726

Phone +39 02 27007161

# HBsAg One version ULTRA

Hepatitis B surface Antigen or HBsAg is the most importa-protein of the envelope of Hepatitis B Virus, responsible acute and chronic viral hepatitis.

The surface antigen contains the determinant "a", common to all the known viral subtypes, immunologically distinguished by two

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one-step determination of Hepatitis B surface Antigen or HBsAg in human plasma and sera. The kit is intended for the screening of blood units, is able to detect HBsAg mutants and finds application in the follow-up of A INTENDED USE Fourth generation En Enzyme Immunoassay (ELISA) for the ation of Hepatitis B surface Antigen or

distinct subgroups (ay and ad).
The ability to detect HSSAy with high sensitive immunoassays in the last years has led to an understanding of its distribution and epidemiology worldwide and to radically decrease the risk of infection in transfusion.

HBV-infected patients.
For "in vitro" diagnostic use only.

## B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B Virus infection as follows:

"Republis B is one of the major diseases of markind and it a serious global public health publieri. Hippatilis means inflammation of the liver, and the most common cause is infection with one of 5 vinces, called hopatific A.B.C.D, and E. All of these vinces can cause an acute disease with symptoms stating several weeks including yellowing of the skin and systs (purnice); dark urner, extreme talique, nausea, vomiting and abformatia pain. It can take several months to a year to feat it again, reported B virus can cause chorunic infortion in which the patient never yellow of the virus and cause chorunic infortion in which the patient never leading and the virus and cause chorunic infortion in which the patient reverse leading to the virus and many years later develops crimbiss of the liver or liver center.

(HBV is the most serious type of viral repairts and the only type causing chonic hepptilis for which a variches is available. Hepatilis B vitus is reasonabled by contact with shoot or body fullidar of an interest person in the same way as human ammunodeficiency virus (HV), the virus that causes AIDS. However, HBV is 50 to 100 times now interesting that HV. The main ways of gailing indeeds with HBV are: (s) perinals if from moriner to bady at the british; (b) Aids: loc-hind transmission; (c) unsafe injections and iransissions; (d) sexual contact.

Woodwarde, most inhections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-serialized needles and sylongs. In many developing countries, among tall children become intected with the virus. In many industrialized countries (ap. Western Eurose and North America), the patient of transmission is different. In these countries, imother-co-triant, and child-localize transmission accumulated by up to me that of children before childrend household repaids a virus of the programmes, were implemented. However, the napoliny of infections in these countries are acquired during young adulthood by sexual activity, and impeting drug use, to addition, helpatitis is the major infectious occurrationed house of health workers, and most health care workers have necessed teppatitis if which is the major infectious occurrationed house of health workers, and most health care workers have necessed teppatitis if which is the major infectious occurrationed households of health workers, and most health care workers have necessed teppatitis in the major infectious occurrations.

Hopatilis is wrus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chooler HV infection are also found in the southern paris of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chancrally infected. Infection is less common in Western Europe and North America, where less than 1% are chanically infected.

Young children who become infected with HBV are the most likely to develop chronic intection, About 90% of infants infected during the first year-of-like and 19th to 50% of centilities infected of his part of the service of centilities infected plants interested between 1 to 4 years of age develop chronic infection. The rick of death from HBV-statistal liker-develop of the centilities is approximately 25% to persons who become chronically infected during childrend. Chronic hepatitis 8 in some patients a transfer with chronic phagatitis 8 in some patients. Posterits with chronics are sometimes given liker transplants, with awaying suppose, this professional to previously his design with vaccine than to by and cure it.

Hepatitis 8 vaccine has an outstanding record of safety and effectiveness. Since 1982, over one pilion doses of Impatitis 8 vaccine have been faced worklowdu. The vaccine is present as a soviety of those informacional obsess. Studies have inhorn that the vaccine is permitted to the safety of the properties of they have only of been refused in many countries where 8% to 15% of children used to be now enhanced to the safety that and of chinne milection have been refused to less than 1% in immunitated groups of children. Since 1981, VNO has called to all countries to add hopatitis 8 vaccine not interior analysis and immunitation programs.

C.PRINOPLE OF THE TEST

A mix of mause manadonal antibodies specific to the determinants "a," of and "y of HBSAq is fixed to the surface of microwells. Patient's serum/plasma is added to the microwell logother with a second mix of mouse manadonal antibodies, conjugated with Horseradish Peroxidase (HRP) and directed against a different epitope of the determinant "e" and against "pes".

The specific immunocomplex, formed in the presence of HBsAg

in the sample, is captured by the solid phase.

At the end of the one-step incubation, microwells are washed to remove ulharind serum potents and HRP conjugate.

The chromogen/substrate is then added and, in the presence of captured HRRA immunoromplex, the coordiese substrate is hydrolyzed by the thound HRP conjugate to a colored end-product. After blocking the enzymatic reaction, its optical density the product. After blocking the enzymatic reaction, its optical density.

The color intensity is proportional to the amount of HBsAg

present in the sample.

The version ULTRA is particularly suitable for automated screenings and is able to detect 's' mutants.

### D. COMPONENTS

The standard configuration contains reagents to perform tests and is made of the following components: 192

1. Microplate MICROPLATE

\*\*2. 12 strips of 8 breakable wells coated with anti HBsAg, affirity purified mouse monodonal antibodies, specific to "s", "y and "d" determinants, and sealed into a bag with desiccant.

2. Negative Control CONTROL.

14. Imivial. Ready to use control. It contains goat serum, 10 mM phosphate buffer pH 12+4-0,1, 0,09% Ma-azde and 0,1% Kathon GC as preservatives. The negative control is pale yellow color coded.

3. Positive Control SONTROL 1

74. Onflyial. Ready to use control. It contains goat serum, non infectious recombinant HBs-4g, 10 mM phosphate buffer pH 7.4+1-0.1, 0.02% gentamicine sulphate and 0.1% Kathnon GC as preservatives. The positive control is color coded green.

4. Calibrator A. Calibrator. To be dissolved with EIA or 2 visits. Lyophilized calibrator, To be dissolved with EIA grade water as reported in the label. Contains letal boring serum, non infectious recombinant. Hashq, at 0.5 Lifant (2" WHO international standard for HBshq. NIBSC code 00/588). The properties of the HBshq. NIBSC code 00/588). The properties and 0.1% Kathon GC as preservatives.

Subnate and 0.1% Kathon GC as preservatives.

And the properties of the content of the vial may vary from for to lot. Please use the right volume reported on the label.

5. Wash buffer concentrate WASHBUF 200/ 2.46m/bottle. 201x concentrated solution. Once distred the wesh solution contains 10 mm phosphase buffer pH 7.0+-02. 0.05% Tween 20 and 0.1% Kathon SC.