Parvovirus B19 IgG

Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Parvovirus B19 in human serum and plasma

- for “in vitro” diagnostic use only -

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REF PARVOG.CE
96 Tests

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A. INTENDED USE
Enzyme ImmunoAssay (ELISA) for the quantitative/qualitative determination of IgG antibodies to Parvovirus B19 in human plasma and sera.
For "in vitro" diagnostic use only.

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 erythrovirus. Parvovirus B19 is a non-enveloped, icosahedral virus that contains a single-stranded linear DNA genome. It is classified as erythrovirus because of its capability to invade red blood cell precursors in the bone marrow. Three genotypes (with subtypes) have been recognized. The viral capsid is composed of two structural proteins , namely VP1 (83kD) and VP2 (53 kD). Infection by Parvovirus B19 spreads through respiratory secretions but also through blood or blood products. The infection causes a mild illness characterized by an erythematous maculopapular facial rash called fifth disease or erythema infectious. It is tipical in childhood and is also seen in adults. A person usually gets sick within 4 to 14 days after getting infected with parvovirus B19 but about 20% of children and adults who get infected with this virus will not have any symptoms. Infection during pregnancy presents the risk of transmission to the fetus that may result in hydrops fetalis. People with weakened immune systems caused by leukemia, cancer, organ transplants, or HIV infection are at risk for serious complications from fifth disease. It can cause chronic anemia that requires medical treatment.
Therefore the detection of Parvovirus B19-specific antibodies becomes very important.

C. PRINCIPLE OF THE TEST
Microplates are coated 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 2nd incubation bound anti Parvovirus IgG are detected by the addition of polyclonal specific anti hIgG antibodies, labelled with peroxidase (HRP).
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 Parvovirus IgG antibodies present in the sample. A Calibration Curve, calibrated against the 2nd W.H.O international standard for Anti-Parvovirus 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.

1. Microplate: MICROPLATE
12 strips x 8 microwells coated with Parvovirus B19 antigens.
Plates are sealed into a bag with desiccant. Allow the microplate 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 to use and color coded standard curve derived from human plasma positive for Parvovirus B19 IgG and titrated on WHO standard ranging:
4ml/vial CAL1 = 0 WHO IU/ml
4ml/vial CAL2 = 3 WHO IU/ml
2ml/vial CAL3 = 6 WHO IU/ml
2ml/vial CAL4 = 12 WHO IU/ml
2ml/vial CAL5 = 20 WHO IU/ml
4ml/vial CAL6 = 40 WHO IU/ml.
Standards are calibrated against the 2nd W.H.O international standard for Anti-Parvovirus B19 code 01/602.
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 0.045% ProClin 300 as preservatives. Standards are blue colored.

3. Control Serum: CONTROL …ml
1 vial. Lyophilized.
It contains bovine serum proteins, human plasma positive to Parvovirus B19 calibrated at 12 WHO IU/ml ± 10%, 0.2 mg/ml gentamicine sulphate and 0.045% ProClin 300 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.

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 0.045% ProClin 300.

5. Enzyme conjugate : CONJ
2x8ml/vial.Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.045% ProClin 300, 0.02 mg/ml gentamicine sulphate as preservatives and 0.01% red alimentary dye.

6. Chromogen/Substrate: SUBS TMB
1x16ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2) and 4% dimethylsulphoxide.
Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H_2SO_4 0.3 M
1x15ml/vial. It contains 0.3 M H_2SO_4 solution.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

8. Specimen Diluent: DILSPE
2x60ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. The reagent is blue colour coded.

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.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet), set at +37°C (+/-0.5°C tolerance)..
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
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 doctor responsible of the laboratory.
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 in biosafety 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 Biomedical Laboratories", ed. 1984.
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 laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials 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.
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. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. 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 up to six 6 uses of the device and up to 3 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety 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: "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 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 inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills 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/hospital waste.
15. 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 (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 WARNINGS

1. Blood is drawn aseptically 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 citrate, EDTA and heparin.
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 ("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 could give rise to false results.
4. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.

Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.

5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.
6. Samples whose anti-Parvovirus B19 IgG antibody concentration is expected to be higher than 40 IU/ml should be diluted before use, either 1:10 in the Specimen Diluent. Dilutions have to be done in clean disposable tubes by diluting 50 ul of each specimen with 450 ul of Specimen Diluent (1:10). Mix tubes thoroughly on vortex and then proceed toward the dilution step reported in section M.

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.

Microplate:

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 manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C.
After first opening, remaining strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

Calibration Curve

Ready to use component. Mix carefully on vortex before use.

Control Serum

Add the volume of ELISA grade water, reported on the label, to the lyophilised powder; let fully dissolve and then gently mix on vortex.
Note: *The control after dissolution is not stable. Store frozen in aliquots at –20°C. Do not thaw and freeze the aliquot again.*

Wash buffer concentrate:

The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. 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 foaming as the presence of bubbles could give origin to a bad washing efficiency.
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.
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 sterile disposable containers.

Chromogen/Substrate:
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 metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container
Sample Diluent
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, P362+P363).

Legenda:

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

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation 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 rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. **Micropipettes** 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 +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The **ELISA incubator** has to be set at +37°C (tolerance of +/-0.5°C) 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 incubation of ELISA tests and the right temperature of +37°C is assured to the microplate.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The **ELISA microplate reader** has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm mandatory for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 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 that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. 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 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 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.
7. 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.

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.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. 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 microplate, is not punctured or damaged.
5. Dissolve the content of the lyophilised Control Serum as reported in the proper section.
6. Dilute all the content of the 20x concentrated Wash Solution as described above.
7. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
8. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturer instructions. Set the right number of washing cycles as reported in the specific section.
9. Check that the ELISA reader is turned on or ensure it will be turned 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.
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.
The kit may be used for quantitative and qualitative determinations as well.

M1. QUANTITATIVE DETERMINATION:

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 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 the A1 and B1 empty for the operation of blanking.
3. Dispense 100 µl of Calibrators in duplicate. Then dispense 100 µl 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 whenever an internal quality control is required by the management to check the overall performances of the laboratory itself. In case, dispense 100 ul of the Control Serum, prepared according to instructions, in duplicate into a proper 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 ELISA automatic instruments.*

5. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section I.3).
6. Pipette 100 µl Enzyme Conjugate into each well, except A1+B1 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.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. 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 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 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 I.5, at 450nm filter (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 or B1 or both (mandatory).

M2. QUALITATIVE DETERMINATION

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

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µl Sample Diluent + 10 µl sample). Do not dilute the Calibrator 1 (0IU/ml) and Calibrator 5 (20IU/ml) 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 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 operation of blanking.
3. Dispense 100 µl of Calibrator 1 (0 IU/ml) and 100 µl Calibrator 5 (20 IU/ml) in duplicate, and 100 µl Control Serum, prepared according to instructions, in single. Then dispense 100 µl of diluted samples in each 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 ELISA automatic instruments.*

5. Wash the microplate with an automatic washer by delivering and aspirating 350 µl/well of diluted washing solution as reported previously (section I.3).
6. 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, 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.*

7. Incubate the microplate for **60 min at +37°C**.
8. Wash microwells as in step 5.
9. 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.*

10. 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 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 I.5, at 450nm filter (reading) and at 620-630nm (background subtraction), blanking the instrument on A1 or B1 or both (mandatory).

General Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive 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 occur leading to high background.*

N. ASSAY SCHEME

Method	Operations
Calibrators & Control(*) Samples diluted 1:101	100 µl 100 µl
1st incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme conjugate	100 µl
2nd incubation	60 min
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2	100 µl
3rd incubation	20 min
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

(*) Important Notes:

- *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.*

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

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL4	S 1									
B	BLK	CAL4	S 2									
C	CAL1	CAL5	S 3									
D	CAL1	CAL5	S 4									
E	CAL2	CAL6	S 5									
F	CAL2	CAL6	S 6									
G	CAL3	CS(*)	S 7									
H	CAL3	CS(*)	S 8									

Legenda: BLK = Blank CAL = Calibrator CS(*) = Control Serum - Not mandatory
S = Sample

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

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 2	S 10									
B	CAL1	S 3	S 11									
C	CAL1	S 4	S 12									
D	CAL5	S 5	S 13									
E	CAL5	S 6	S 14									
F	CAL5	S 7	S 15									
G	CS(*)	S 8	S 16									
H	S 1	S 9	S 17									

Legenda: BLK = Blank CAL = Calibrator CS(*) = Control Serum - Not mandatory
S = Sample

O. ASSAY 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 expected and required by the IVDD directive 98/79/EC.
The Control Serum is used only when required by the management for an internal verification of the performances of the laboratory itself.

Control that the following data are matched:

Check	Requirements
Blank well	< 0.050 OD450nm value
CAL 1 0 IU/ml	< 0.150 mean OD450nm value after blanking coefficient of variation < 30%
CAL 2 3 IU/ml	OD450nm > OD450nm CAL1 + 0.100
CAL 5 20 IU/ml	OD450nm > 0.750
CAL 6 40 IU/ml	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
Blank well > 0.050 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
CAL 1 **0 IU/ml** > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of a positive calibrator instead of the negative one; 4. that no contamination of the negative calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate; 5. that micropipettes haven't got contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.

CAL 2 **3 IU/ml** OD450nm < OD450nm CAL1 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (ex.: dispensation of a wrong calibrator 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 calibrator has occurred.
CAL 5 **20 IU/ml** < 0.750 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator 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 positive control has occurred.
CAL 6 **40 IU/ml** < 1.000 OD450nm	1. that the procedure has been correctly executed; 2. that no mistake has been done in its distribution (dispensation of a wrong calibrator 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 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:

Check	Requirements
Control Serum	Mean OD450nm CAL4 ± 20%

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

Problem	Check
Control Serum Different from expected value	First verify that: 1. the procedure has been correctly performed; 2. no mistake has occurred during its distribution (e.g.: dispensation of a wrong sample); 3. the washing procedure and the washer settings are correct; 4. no external contamination of the control has occurred. 5. the Control Serum has been dissolved with the right volume reported on the label. If a mistake has been pointed out, the assay has to be repeated after eliminating the reason of this error.

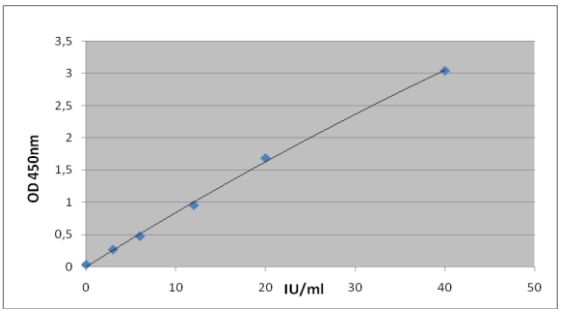
Anyway, if all other parameters (Blank, CAL1, CAL2, CAL5, CAL6), match the established requirements, the test may be considered valid.

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 Parvovirus B19 IgG antibody in samples.

Example of Calibration Curve :



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 Calibrator 1(0 IU/ml), and for Calibrator 5(20IU/ml) 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, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS

Q.1 Quantitative method

Samples with a concentration lower than 3 WHO IU/ml are considered negative for anti Parvovirus B19 IgG antibody by most of the international medical literature.
Samples with a concentration between 3 and 5 WHO IU/ml are considered equivocal for anti Parvovirus B19 IgG antibody.
Samples with a concentration higher than 5 WHO IU/ml are considered positive for anti Parvovirus B19 IgG antibody.
This titer is considered the lowest concentration of IgG to provide an effective immunological protection.

Q.2 Qualitative method

Results are interpreted as ratio between the sample OD450nm and the cut-off value or S/Co.

Results are interpreted according to the following table:

S/Co	Interpretation
< 0.8	Negative
0.8 – 1.2	Equivocal
> 1.2	Positive

Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample.

Important notes:

1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
3. *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 real figures obtained by the user.

Calibrator 0 IU/ml: 0.020 – 0.024 OD450nm
Mean Value: 0.022 OD450nm
Lower than 0.150 – Accepted
Calibrator 20 IU/ml: 1.489 - 1.545 OD450nm
Mean Value: 1.517 OD450nm
Higher than 0.750 – Accepted

Cut-Off = 1.517 / 5 = 0.303

Sample 1: 0.028 OD450nm
Sample 2: 1.890 OD450nm
Sample 1 S/Co < 0.9 negative
Sample 2 S/Co > 1.0 positive

R. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what suggested in NCCLS's approved guideline C24-A2.

1. Limit of detection

The limit of detection of the assay has been calculated by means of the 2nd W.H.O international standard for Anti-Parvovirus B19 code 01/602. The limit of detection has been calculated as mean OD450nm Calibrator 0 IU/ml + 5 SD.
The table below reports the mean OD450nm values of this standard when diluted in negative plasma and then examined in the assay for two lots.

WHO IU/ml	PARVOG.CE Lot P1	PARVOG.CE Lot P2
40	3.041	3.110
20	1.686	1.570
12	0.954	0.925
6	0.473	0.549
3	0.266	0.233
1.5	0.112	0.087
Std 0	0.030	0.055

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 ≥ 98% 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 ≥ 98% was observed.
These findings are summarized in the following table.

Sensitivity	≥ 98 %
Specificity	≥ 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:

PARVOG.CE: lot P1

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.064	0.065	0.069	0.066
Std.Deviation	0.007	0.010	0.011	0.009
CV %	10.6	15.3	15.2	13.7

Calibrator 3 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.313	0.305	0.352	0.323
Std.Deviation	0.031	0.036	0.024	0.030
CV %	9.9	11.7	6.9	9.5

Calibrator 20 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	1.790	1.799	2.077	1.888
Std.Deviation	0.085	0.084	0.082	0.084
CV %	4.7	4.6	3.9	4.4

PARVOG.CE: lot P2

Calibrator 0 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.099	0.089	0.087	0.092
Std.Deviation	0.017	0.012	0.009	0.013
CV %	17.4	13.8	10.3	13.8

Calibrator 3 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.334	0.362	0.380	0.358
Std.Deviation	0.021	0.035	0.029	0.028
CV %	6.4	9.7	7.7	7.9

Calibrator 20 IU/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.085	2.040	2.591	2.239
Std.Deviation	0.081	0.099	0.125	0.101
CV %	3.9	4.9	4.8	4.5

The variability shown in the tables above did not result in sample misclassification.

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 analyte.
Frozen samples containing fibrin particles or aggregates after thawing may generate some false results.
This test is suitable only for testing single samples and not pooled ones.
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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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.

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