HCV Ab

Confirmation

Enzyme Immunoassay for the confirmation of HCV Ab positivity in human sera or plasma

- for “in vitro” diagnostic use only -

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Code CCONF.CE
12 Tests

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A. INTRODUCTION
Hepatitis C Virus or HCV is an enveloped RNA virus recently classified in the family of Flaviviridae.
The genome encodes for structural components, a nucleocapsid protein and two envelope glycoproteins, and functional constituents involved in the virus replication and protein processing. The nucleocapsid-encoding region seems to be the most conservative among the isolates obtained all over the world.
HCV accounts for about 95% of hepatitis infections in recipients of blood transfusion and 50% of cases of sporadic NANB hepatitis. HCV commonly gives origin to asymptomatic hepatitis and chronicity develops in a high number of cases, sometime evolving in severe forms of illness, as hepato-carcinoma.
The determination of antibody to HCV has become mandatory in the screening of blood units to prevent post-transfusion hepatitis. It is also currently used to follow-up risk individuals and patients under treatment with interferon.
Confirmation of any positive result is strongly recommended in the clinical laboratory practice before considering the patient truly positive for anti HCV antibodies.

B. PRINCIPLE OF THE ASSAY
Microplates are coated by strips with HCV-specific synthetic antigens derived from “core”, “ns” and “env” regions encoding for conservative immunodominant antigenic determinants (Core, NS3, NS4, NS5 & Env).
Antigens are adsorbed to the wells composing the strips as follows:

Position	Antigen	Composition
A	None	Well for blanking operations
B	Casein	Negative internal control
C	Core	Specific synthetic antigen
D	NS3	Specific synthetic antigen
E	NS4	Specific synthetic antigens
F	NS5	Specific synthetic antigen
G	Env	Specific synthetic antigens
H	hIgG	Positive Internal Control

The strip is first treated with the sample turned out to be positive in the screening assay. Anti HCV antibodies are captured, if present, by the specific antigens.
After washing out all the other components of the sample, in the 2nd incubation bound HCV Ab are detected by the addition of anti hIgG&M antibody, labeled 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 HCV antibodies present in the sample.
Controls are included to provide an internal check of the analytical system.
The sample is confirmed positive if at least two specific reactivities are present.

C. TEST CONDITIONS AND NOTICES
1. All the reagents contained in the kit are for “in vitro” diagnostic use only.
2. Do not use the kit or reagents after the expiry date stated on labels. Do not mix reagents of different lots.
3. Procedures should be performed carefully in order to obtain reliable results and clinical interpretations.
4. Bring all the reagents to room temperature for at least 60 min, before the test is started.
5. Avoid any contamination of reagents when taking them out of vials. We recommend to use automatic pipettes and disposable tips. When dispensing reagents, do not touch the wall of microplate wells with tips, in order to avoid any cross-contamination.
6. In the washing procedure, use only the Washing Solution provided with the kit and follow carefully the indications reported in the “WASHING INSTRUCTIONS” section of this insert.
7. Ensure that the Substrate/Chromogen mixture does not come in contact with oxidizing agents or metallic surfaces; avoid any intense light exposure during the incubation step or the reagent preparation.
When preparing the Substrate/Chromogen mixture for the analysis use only plastic, disposable, clean or sterile containers.
8. Samples and materials potentially infective have to be handled with care as they could transmit infection.
All objects come in direct contact with samples and all residuals of the assay should be treated or wasted as potentially infective. Best procedures for inactivation are treatments with autoclave at 121°C for 30 min or with sodium hypochlorite at a final concentration of 2.5% for 24 hr. This last method can be used for the treatment of the liquid waste after that it has been neutralized with NaOH.
9. Avoid any contact of liquids with skin and mucosas.
Use always protective talk-free gloves, glasses and laboratory coats, according to the safety regulations.

D. CONTENT OF THE KIT
a – Microplate MICROPLATE
n°1. 12 strips x 8 wells coated by strip with synthetic HCV antigens. Strips are contained in a sealed bag with a desiccant and a frame. Bring the strips necessary to the test to room temperature before use, and close firmly the bag to prevent any moisture formation inside.

b – Enzyme Conjugate CONJ
n°1x16ml. Proteic buffer solution containing a specific anti-hIgG&M antibody, labeled with HRP, ready to use.
It contains proteic stabilizers, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC.

c – Washing Solution WASHBUF 20X
n°1x60ml. 20x concentrated solution to be diluted up to 1200 ml with EIA grade water. It contains phosphate buffer, Tween 20 and Kathon GC as preservative.
Once diluted, the wash solution is stable for 1 week at 2-8°C.

d – Chromogen/Substrate SUBS TMB
1x16ml. The solution contains tetramethyl-benzidine (TMB) and hydrogen peroxide (H2O2) with activators and stabilizers, diluted in a phosphate/citrate buffer. The solution is ready to use.
***Warning** : Store protected from light.*

e – Stop Solution H_2SO_4 O.3 M
n°1x15ml It contains a solution of 0.3 M H2SO4
***Warning** : Irritant ! (Xi. R36/38 S2,26,30)*

f – Negative Control CONTROL -
n°1x3ml Human serum base not reactive for anti-HCV antibodies. It contains 0.09% sodium azide and 0.1% Kathon GC as preservatives.
The Negative Control is pale yellow color coded.

g – Positive Control CONTROL +
n°1x3ml Human serum base highly reactive for HCV Ab. It contains 0.09% sodium azide and 0.1% Kathon GC as preservatives. The Positive Control is green color coded.

h – Sample Diluent DILSPE
n°1x20ml. Proteic solution for the preparation of samples. It contains a detergent, proteic stabilizers, 0.1% sodium azide and 0.1% Kathon GC as preservatives.

***Note**: All human serum derived materials have been tested as negative for HBsAg, and HIV antibodies with FDA approved kits. The Positive Control has been inactivated for HCV. Anyway, handle these components as potentially infective.*

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes of variable volume and disposable plastic tips.
2. EIA grade water (bidistilled 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 capable to provide a temperature of +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and possibly 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. When the kit is used for the confirmation of positive results obtained from screening blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. 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.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. 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/Substrate from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2..8°C into a tem perature controlled refrigerator or cold room.
7. 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.
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 replacement.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
12. 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.
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 cross contamination.
14. 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..
15. 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.
16. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
17. 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 RECOMMANDATIONS

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. Avoid any addition of preservatives to samples; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labelling and electronic reading is strongly recommended.
4. 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.
5. 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 should not be frozen/thawed more than once as this may generate particles that could affect the test result.

6 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.

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-use of the device and up to 6 months.

Microplates:

Allow the microplate 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 storage.
In this case call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2°.8°C. When opened the first time, res idual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

Negative Control:

Ready to use. Mix well on vortex before use.

Positive Control:
Ready to use. Mix well on vortex before use. Handle this component as potentially infective, even if HCV, if present in the control, has been chemically inactivated.

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.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (Xi R36/38; S2/26/30)
Legenda: R 36/38 = Irritating to eyes and skin.
S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

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

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 (tole rance of +/-0.5°C) and regularly checked to ensure the corre ct 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.
3. 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 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 soaking 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 O "Internal Quality Control" . Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
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 ideally with a second filter (620-630nm) 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. 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 of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue by aspirating a small volume of it with a sterile transparent plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
5. 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.
6. Check that the ELISA reader has been turned on at least 20 minutes before reading.
7. Check that the micropipettes are set to the required volume.
8. Check that all the other equipment is available and ready to use.
9. 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.

1 – Leave the A1 well empty for blanking operations. Dilute 20 ul sample to confirm with 1 ml diluent (1:50 dilution).
Do not dilute controls (if tested) as they are prediluted and ready-to-use.
Then dispense the controls and/or the diluted sample to confirm, each one into one strip module according to the following table:

position	sample
A	blanking well
B	**100 ul** control or diluted sample to confirm
C	**100 ul** control or diluted sample to confirm
D	**100 ul** control or diluted sample to confirm
E	**100 ul** control or diluted sample to confirm
F	**100 ul** control or diluted sample to confirm
G	**100 ul** control or diluted sample to confirm
H	**100 ul** control or diluted sample to confirm

Cover the strip with the sealer and incubate the strip module for **60 min at +37°C** .

2 – Peel out the plate sealer and wash the strip module according to instructions.

3 – Add 100 ul of Conjugate to all the wells, except A1. Incubate the module sealed for **60 min at +37°C** .

4 – Peel out the plate sealer and wash the strip according to the instructions. Then add 100 ul of the Chromogen/Substrate mixture to all the wells, A1 included.

5- Incubate the strip module for **20 min at room temperature**, protected from light.

6 – Stop the enzymatic reaction by adding 100 ul of the Stop Solution to all the wells, A1 included.
Read the strip module at 450nm and 620-630nm blanking the instrument on A1 well.

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 450nm. 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
Controls Samples diluted 1:50	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm

O. INTERNAL QUALITY CONTROL

A check is carried out on the controls any time the kit is used in order to verify whether their OD450nm values are as expected and reported in the table below.

Check	Requirements
Blank well	< 0.100 OD450nm value
H well	>0.750 OD450nm value after blanking
Negative Control (NC)	<0.200 OD450nm value in wells from B to G After blanking
Positive Control (PC)	<0.200 OD450nm value in well B after blanking >B+0.350 OD450nm in all wells from C to G after blanking

If the results of the test match the requirements stated above, proceed to the next section.
In case data above do not match the correct values, before repeating the test check carefully the expiration date of the kit, the performances of the instruments used for the assay , the procedure of distribution of controls and samples and operate as follows:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Sustrate solution has not got contaminated during the assay
Negative Control (NC) >0.200 OD450nm value in wells from B to G After blanking	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 positive control instead of negative control; 4. that no contamination of the negative control or of their wells has occurred due to positive samples, to spills or to 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.
Positive Control >0.200 OD450nm value in well B after blanking <B+0.350 OD450nm in any of the wells from C to G after blanking	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of controls (dispensation of negative control instead of positive control. 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.
H well < 0.750	1. that the procedure has been correctly executed; 2. that no mistake has been done in the distribution of the Enzyme Conjugate 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the Enzyme Conjugate has occurred.

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

P. INTERPRETATION OF RESULTS
If the validity of the assay is confirmed, examine the following table for the interpretation of results.

Classification	Results
Negative	Wells from C to G with OD450nm < B + 0.350
False Positive	B well with OD450nm > 0.350
Indeterminate	One well from C to G with OD450nm > B + 0.350. B well must have OD450nm < 0.350
Positive	At least 2 wells from C to G with OD450nm > B + 0.350. B well must have OD450nm < 0.350

Important notes:
1. *Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.*

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

3. *Diagnosis of HCV infection has to be done and released to the patient only by a qualified medical doctor.*

Q. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC) for Confirmatory/Supplementary assays for anti HCV determination.

1. LIMIT OF DETECTION

In absence of a defined international standard (none is indicated in the product-specific CTS) Dia.Pro Diagnostic BioProbes s.r.l. has used working standard supplied by NIBSC, UK.

The S/Co ratio obtained on the British Working Standard, NIBSC, code 99/588-003-WI, for three lots of CCONF.CE, are reported in the following table:

NIBSC working standard

CCONF.CE Lot ID	S / Co				
	Core	NS3	NS4	NS5	Env
0904	**2.6**	**2.8**	0.8	0.3	0.1
1104	**2.9**	**2.6**	0.6	0.2	0.1
0105	**3.1**	**2.7**	0.7	0.4	0.2

2. DIAGNOSTIC SPECIFICITY AND SENSITIVITY

The diagnostic sensitivity and specificity of the device was evaluated in the clinical trials conducted at the Hospital Universitario "La Fè" – Servicio de Microbiologia, Valencia, Spain, on more than total 800 specimens.

2.1 Diagnostic specificity:

It is defined as the probability of the assay of scoring negative in the absence of specific analyte.

A total of more than 450 samples were tested, including blood donations, clinical samples and potentially interfering specimens.

No false positive results were observed from the study of these specimens, matching what required by CTS.

2.2 Diagnostic Sensitivity

It is defined as the probability of the assay of scoring positive in the presence of specific analyte.

The diagnostic sensitivity has been assessed in the Performance Evaluation on a total number of 300 specimens reflecting different stages of antibody pattern and genotypes. 298 samples were detected positive and 2 samples were detected indeterminate.

The device showed correct identification of samples as positive or indeterminate; none turned out to be negative, matching in full what required by CTS.

Seroconversion panels were studied as well with reference to a licensed device produced in US (whose data are extracted from BBI's data sheets).

The results of three of them are reported below.

BBI PHV 901 (genotype 1a)

Sample ID	CCONF result	RIBA 3 result
01	NEG	NEG
02	NEG	NEG
03	**POS**	***IND***
04	**POS**	**POS**
05	**POS**	**POS**
06	**POS**	**POS**
07	**POS**	**POS**
08	**POS**	**POS**
09	**POS**	**POS**
10	**POS**	**POS**
11	**POS**	**POS**

BBI PHV 904 (genotype 1a)

Sample ID	CCONF result	RIBA 3 result
01	NEG	NEG
02	NEG	NEG
03	NEG	NEG
04	NEG	NEG
05	***IND***	***IND***
06	***IND***	***IND***
07	***IND***	***IND***

BBI PHV 905 (genotype 1a)

Sample ID	CCONF result	RIBA 3 result
01	NEG	NEG
02	NEG	NEG
03	NEG	NEG
04	***IND***	***IND***
05	***IND***	***IND***
06	***IND***	***IND***
07	**POS**	**POS**
08	**POS**	**POS**
09	**POS**	**POS**

Moreover, the diagnostic sensitivity of the Product was assessed on the panel EFS Ac HCV, lot n°01/08.03.2 2C/01/A, supplied by the Etablissement Francais Du Sang (EFS), France, and composed of samples derived from patients of European origin, with the following results:

EFS: ac HCV Panel

Sample	Lot # 0904	Lot # 1104	Lot # 0105	EFS Result
HCV 1	**pos**	**pos**	**pos**	**positive**
HCV 2	***ind***	***ind***	***ind***	***indeterminate***
HCV 3	**pos**	**pos**	**pos**	**positive**
HCV 4	**pos**	**pos**	**pos**	**positive**
HCV 5	**pos**	**pos**	**pos**	**positive**
HCV 6	neg	neg	neg	negative

In addition, the device was studied on the BBI's Panel of Performances PHV 205.

Results are reported in the following table.

BBI PHV 205

Sample ID	CCONF result	RIBA 3 result
01	**POS**	**POS**
02	NEG	NEG
03	**POS**	**POS**
04	**POS**	**POS**
05	***IND***	***IND***
06	**POS**	***IND***
07	**POS**	**POS**
08	**POS**	***IND***
09	**POS**	**POS**
10	**POS**	**POS**
11	**POS**	**POS**
12	**POS**	**POS**
13	**POS**	**POS**
14	**POS**	**POS**
15	**POS**	**POS**
16	**POS**	**POS**
17	**POS**	**POS**
18	**POS**	**POS**
19	**POS**	**POS**
20	**POS**	**POS**
21	**POS**	**POS**
22	**POS**	**POS**
23	**POS**	**POS**
24	**POS**	**POS**
25	NEG	NEG

2.3 Precision

The negative control and the positive control were used to verify this parameter, by testing 12 replicates of the same sample on three lots of product.
Results of one lot are reported below.

Negative Control

Values	O.D mean	CV%
None (blank well)	0,001	0,0
Casein	0,012	15,7
CORE	0,031	18,8
NS3	0,036	16,3
NS4	0,146	11,3
NS5	0,039	11,8
ENV	0,039	12,4

Positive Control

Values	O.D mean	SD	CV%
None (blank well)	0,001	0,000	0,0
Casein	0,041	0,005	11,4
CORE	3,973	0,024	0,6
NS3	3,981	0,000	0,0
NS4	3,981	0,000	0,0
NS5	2,646	0,095	3,6
ENV	1,067	0,086	8,0

R. LIMITATIONS

No limitation has been observed from the Performance Evaluation.
Please refer anyway to the Section G for samples and sampling methods.

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