

WHO Prequalification of In Vitro Diagnostics PUBLIC REPORT

Product: INNO-LIA HCV Score
Number: PQDx 0202-073-00

Abstract

INNO-LIA HCV Score with product codes 80538, manufactured by Fujirebio Europe NV, CE-mark regulatory version, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 24 July 2015.

INNO-LIA HCV Score is a line immunoassay (LIA) for the detection of antibodies to hepatitis C virus in human serum or plasma. It is intended for use as a supplementary test on human serum or plasma specimens found to be reactive using an anti-HCV screening procedure.

INNO-LIA HCV Score is a 3rd generation line immunoassay which incorporates HCV antigens derived from the core region, the E2 hypervariable region (HVR), the NS3 helicase region, the NS4A, NS4B, and NS5A regions. The antigens were coated as 6 discrete lines on a nylon strip with plastic backing. In addition, four control lines are coated on each strip: background control line, 3+ positive control (anti-human Ig) which is also used as sample addition control line, 1+ positive control (human IgG), and the \pm cut-off line (human IgG). INNO-LIA HCV Score is based on the principle of an enzyme immunoassay. A test sample is incubated in a trough together with the test strip. If present in the sample, HCV antibodies will bind to the HCV antigen lines on the strip.

Subsequently, an affinity-purified alkaline phosphatase-labelled goat anti-human IgG (H+L) conjugate is added and reacts with specific HCV antigen/antibody complexes, if previously formed. Incubation with the enzyme substrate produces a chesnut-like color, the intensity of which is proportionate to the amount of HCV-specific antibody captured from the sample on any given line. Color development is stopped with sulfuric acid.

The test kit contains:

20 tests (product code 80538)	
Test strips HCV antigen-coated test strips	20 tests strips
Sample Diluent Containing color-coded (green) phosphate buffer containing sodium chloride, detergent, bovine protein stabilizers and 0.3% chloroacetamide (CAA) as	1 bottle of 30 mL

preservative.	
Ready-to-use Conjugate Containing color-coded (red) goat anti-human IgG labelled with alkaline phosphatase in Tris buffer containing bovine stabilizers, detergent and 0.01% methylisothiazolone (MIT)/<0.1% CAA as preservative.	1 bottle of 45 mL
Negative Control Containing basematrix of human origin with 0.01% MIT/<0.1% CAA as preservative.	1 bottle of 0.12 mL
Positive Control Containing inactivated human serum positive for antibodies to HCV with 0.01% MIT/<0.1% CAA as preservative.	1 bottle of 0.12 mL
Ready-to-use BCIP/NBT Substrate Containing 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium in dimethyl formamide, with 0.01% MIT/<0.1% CAA as preservative.	1 bottle of 45 mL
Stop Solution Containing 0.1 mol/l sulfuric acid.	1 bottle of 45 mL
Wash Solution Containing color-coded (blue) Tris buffer containing sodium chloride, detergent and 0.02% bromo-nitro-dioxane as preservative, to be diluted 5x in distilled water.	1 bottle of 45 mL
Incubation tray	2 units with 11 troughs each
Adhesive sealers	5 units
Data reporting sheet For storage of developed strips	1 unit
Reading card For identification of reactive antigen lines.	1 unit
Instructions for use	1 unit

Storage:

The test kit should be stored at 2 - 8 °C.

Shelf-life:

15 months.

Summary of prequalification status for INNO-LIA HCV Score

	Initial acceptance	
	Date	Outcome
Status on PQ list	24 July 2015	listed
Dossier assessment	N/A	MR
Inspection status	08 January 2015	MR
Laboratory evaluation	N/A	MR

MR: Meets Requirements

NA: Not Applicable

INNO-LIA HCV Score was accepted for the WHO list of prequalified in vitro diagnostics on the basis of data submitted and publicly available information.

Background information

Fujirebio Europe NV submitted an application for prequalification of INNO-LIA HCV Score. Based on the established prioritization criteria, INNO-LIA HCV Score was given priority for prequalification.

Product dossier assessment

In accordance with the WHO procedure for abbreviated prequalification assessment, Fujirebio Europe NV was not required to submit a product dossier for INNO-LIA HCV Score as per the "Instructions for compilation of a product dossier" (PQDx_018 v1).

Commitments for prequalification:

N/A

Manufacturing site inspection

In accordance with the WHO procedure for abbreviated prequalification assessment, an abbreviated inspection was performed at the site of manufacture (Technologiepark 6, Zwijnaarde, 9052 Belgium) of INNO-LIA HCV Score in November 2014 as per the "Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics" (PQDx_014 v1).

The inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality. The manufacturer's responses to the nonconformities found at the time of the inspection were accepted 08 January 2015.

Commitments for prequalification:

N/A

Laboratory evaluation

The objective of the performance laboratory evaluation is to assess the performance and operational characteristics of commercially available in-vitro diagnostics for the purpose of advising the governments of WHO Member States on these issues. In particular, suitability for use in resource-limited settings will be assessed.

Based on the risk level associated with the use of the supplemental assays, the known general performance of supplemental assays and role of the supplemental assays in patient care in resource-limited settings, it was decided that WHO will not conduct performance evaluations of these assays as part of the prequalification assessment process.

Consequently, laboratory evaluation of INNO-LIA HCV Score was not conducted.

Labelling

- 1. Labels**
- 2. Instructions for use**

1. Labels






INNO-LIA HCV Score
Line ImmunoAssay

IVD CE 0459


Fujirebio Europe N.V.
Technologiepark 6
9052 Gent, Belgium
☎+32-9 329 13 29

289665 VO

INNO-LIA HCV Score **REF** 80538

STRIPS	1x $\frac{\pi}{20}$	REF 57329	  
SAMP DIL	1x 30 mL	REF 57304	
CONJ	1x 45 mL	REF 57301	
CONTROL -	1x 0.12 mL	REF 57307	
CONTROL +	1x 0.12 mL	REF 57308	
SUBS BCIP/NBT	1x 45 mL	REF 57302	
STOP SOLN	1x 45 mL	REF 57303	
WASH SOLN 5x	1x 45 mL	REF 57299	

DANGER



H317 H360D
P280 P201 P261 P363 P308+P313

LOT 412345
2013-12

CONTROL 0.12 mL
REF 57307 LOT 123456
2014-03
Fujirebio Europe N.V.
Belgium Q+3293291329

CONJ 45 mL
REF 57301 LOT 123456
2014-03
Fujirebio Europe N.V.
Belgium Q+3293291329

SUBS BCIP/NBT 45 mL
REF 57302 LOT 123456
2014-03
DANGER
Fujirebio Europe N.V.
Belgium Q+3293291329

STOP SOLN 45 mL
REF 57303 LOT 123456
2014-03
Fujirebio Europe N.V.
Belgium Q+3293291329

WASH SOLN 5x 45 mL
REF 57299 LOT 123456
2014-03
Fujirebio Europe N.V.
Belgium Q+3293291329

INNO-LIA HCV Score 20
STRIPS LOT 123456
REF 57329 2014-03
Fujirebio Europe N.V.
Belgium Q+3293291329

INNO-LIA HCV Score 0.12 mL
CONTROL LOT 123456
REF 57308 2014-03
Fujirebio Europe N.V.
Belgium Q+3293291329

SAMP DIL INNO-LIA HCV/HIV 30 mL
REF 57304 LOT 123456
WARNING 2014-03
Fujirebio Europe N.V.
Belgium Q+3293291329

2. Instructions for use

Special note: WHO identified some opportunities for improvement of the wording of the instructions for use submitted in the course of WHO prequalification (version number 28667 v2, 2014-03-28). Fujirebio Europe N.V. will take these into consideration in the next revision of the instructions for use.



KEY-CODE: FRI84704
 80538 INNO-LIA HCV Score
 28667 v2
 2014-03-28
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 English

INNO-LIA HCV Score



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"Note changes highlighted"

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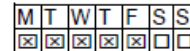
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www.e-labeling.eu/FRI84704

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









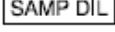
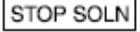

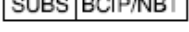
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Symbols used

	Manufacturer
	<i>In Vitro</i> Diagnostic Medical Device
	Batch code
	Catalogue number
	Use By
	Consult Instructions for Use
	Temperature limitation
	Biological risks
	Contains sufficient for <n> tests
	Conjugate
	Negative Control
	Positive Control
	Sample Diluent
	Stop Solution
	Strips
	Substrate BCIP/NBT

WASH SOLN 5x

Wash Solution 5x

Intended use

The INNO-LIA HCV Score is a Line Immuno Assay (LIA) for the detection of antibodies to human hepatitis C virus in human serum or plasma. It is intended for use as a supplementary test on human serum or plasma specimens found to be reactive using an anti-HCV screening procedure.

Test principle

The INNO-LIA HCV Score is a 3rd generation line immunoassay which incorporates HCV antigens derived from the core region, the E2 hypervariable region (HVR), the NS3 helicase region, the NS4A, NS4B, and NS5A regions.

The antigens were coated as 6 discrete lines on a nylon strip with plastic backing. In addition, four control lines are coated on each strip: **background control line**, 3+ positive control (anti-human Ig) which is also used as sample addition control line, 1+ positive control (human IgG), and the \pm cut-off line (human IgG).

The INNO-LIA HCV Score is based on the principle of an enzyme immunoassay. A test sample is incubated in a trough together with the test strip. If present in the sample, HCV antibodies will bind to the HCV antigen lines on the strip. Subsequently, an affinity-purified alkaline phosphatase-labelled goat anti-human IgG (H+L) conjugate is added and reacts with specific HCV antigen/antibody complexes, if previously formed. Incubation with the enzyme substrate produces a chesnut-like color, the intensity of which is proportionate to the amount of HCV-specific antibody captured from the sample on any given line (Fig.1). Color development is stopped with sulfuric acid.

Reagents**Description, preparation for use and recommended storage conditions**

- If kept at 2 - 8°C, opened or unopened, all reagents are stable until the expiration date. Do not freeze reagents. Do not use the kit beyond the expiration date.
- All reagents and the plastic tube containing the test strips must be taken out of the box and brought to room temperature (18 - 25°C) 60 minutes before use. All reagents and the strip tube should be returned to the refrigerator (2 - 8°C) immediately after use.
- Alterations in the physical appearance of kit reagents may indicate instability or deterioration.
- After opening the original tube containing the strips, any unused strip will be stable for 16 weeks if stored at 2 - 8°C in the closed original tube with desiccant.

Reagents supplied:

Component	Quantity	Ref.	Description
Strips	20	57329	Containing 20 INNO-LIA HCV antigen-coated test strips.
Sample Diluent	30 mL	57304	Containing color-coded (green) phosphate buffer containing sodium chloride, detergent, bovine protein stabilizers and 0.3% chloroacetamide (CAA) as preservative.
Ready-to-use Conjugate	45 mL	57301	Containing color-coded (red) goat anti-human IgG labeled with alkaline phosphatase in Tris buffer containing bovine stabilizers, detergent and 0.01% methylisothiazolone (MIT)/<0.1% CAA as preservative.
Negative Control	0.12 mL	57307	Containing basematrix of human origin with 0.01% MIT/<0.1% CAA as preservative.
Positive Control	0.12 mL	57308	Containing inactivated human serum positive for antibodies to HCV with 0.01% MIT/<0.1% CAA as preservative.
Ready-to-use BCIP/NBT Substrate	45 mL	57302	Containing 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium in dimethyl formamide, with 0.01% MIT/<0.1% CAA as preservative.
Stop Solution	45 mL	57303	Containing 0.1 mol/l sulfuric acid.
Wash Solution	45 mL	57299	Containing color-coded (blue) Tris buffer containing sodium chloride, detergent and 0.02% bromo-nitro-dioxane as preservative, to be diluted 5x in distilled water. Diluted wash solution is stable for 2 weeks if kept at 2 - 8°C.
Incubation tray	2	-	With 11 troughs each.
Adhesive sealers	5	-	
Data reporting sheet	1	-	For storage of developed strips.
Reading card	1	-	For identification of reactive antigen lines.

Materials required but not provided

- Distilled or deionized water.
- Disposable gloves.
- Precision pipettes (with disposable tip) capable of delivering 10 μ L, 20 - 200 μ L, and 200 - 1000 μ L, respectively.
- Orbital shaker or rocker (see Directions for incubation).

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- Vortex mixer or equivalent.
- Graduated cylinders: 10, 25, 50, and 100 mL.
- Tweezers for strip handling.
- Timer.
- Optional:
 - hot air fan (hair dryer) or dry incubator at 37°C.
 - a repetitive pipette together with disposable vials for the addition of stop solution, conjugate, substrate and wash solution.
 - vacuum aspirator which contains 5% sodium hypochlorite solution in a waste bottle.

Safety and environment

Please refer to the Safety Data Sheet (SDS) and product labeling for information on potentially hazardous components. The most recent SDS version is available on the website www.fujirebio-europe.com.



Warning: Contains 2-Chloroacetamide: SAMP DIL
H317 P280 P261 P363 P333+P313 P302+P352



Danger: Contains N,N-Dimethylformamide: SUBS BCIP/NBT
H360D P281 P201 P308+P313

Hazard statements

H317 May cause an allergic skin reaction.
H360D May damage the unborn child.

Precautionary statements

P201 Obtain special instructions before use.
P261 Avoid breathing mist/vapours/spray.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P281 Use personal protective equipment as required.
P308+P313 If exposed or concerned: Get medical advice/attention.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P363 Wash contaminated clothing before reuse.
P302+P352 IF ON SKIN: Wash with plenty of soap and water.

- Specimens, Positive Control and Negative Control should always be handled as potentially infectious.
- The Positive Control has been found to be negative for anti-HIV and HbsAg. The Negative Control has been found to be negative for anti-HIV-1/HIV-2, anti-HCV and HbsAg. No test method can offer complete insurance that blood products will not transmit infectious agents. Therefore, all blood components and biological materials should be considered as being potentially infectious and should be handled as such. Only adequately trained personnel should be permitted to perform the test procedure. All blood components and biological materials should be disposed of in accordance with established safety procedures.
 - Autoclave for at least 15 minutes at 121°C.
 - Incinerate disposable material.
 - Mix liquid waste with sodium hypochlorite so that the final concentration is \pm 1% sodium hypochlorite. Allow to stand overnight before disposal. CAUTION: Neutralize liquid waste that contains acid before adding sodium hypochlorite.
- Use of personal protective equipment is necessary: gloves and safety spectacles when manipulating dangerous or infectious agents.
- Waste should be handled according to the institution's waste disposal guidelines. All federal, state, and local environmental regulations should also be observed.
- Do not aspirate the stop solution in a waste bottle, which contains sodium hypochlorite.

Specimen (collection and handling)

- The INNO-LIA HCV Score may be performed on human serum or plasma collected in tubes containing citrate, heparin or EDTA as anticoagulants.
- Before storage, serum or plasma should be separated from blood clot or blood cells by centrifugation.
- Store the specimens at 2 - 8°C. For storage longer than one week, freeze at -20°C or lower.
- Do not use heat-treated specimens.
- Repeatedly (more than 3 times) frozen and thawed samples may produce erroneous results.

Remarks and precautions

- Do not mix reagents with different lot numbers.
- Frozen reagents, eg. stored too close to cooling element, can cause erroneous results!
- Make sure correct sample volume and washing times are used for the test procedure needed.

- Avoid microbial contamination of reagents.
- Ensure that the samples and controls are homogeneous before use.
- Do not touch the membrane of the strip. Always manipulate the strips with the plastic backing.
- Use a new pipette tip for each specimen.
- Make sure that the test strips are placed in the troughs with their membrane side facing upwards.
- All incubation steps should be performed using an orbital shaker or rocker (use rocker only for overnight sample incubation).
The shaking of the solutions over the strips is important in achieving even line staining and maximum sensitivity. During shaking, the strip surface should be completely submerged.
- Cover the troughs with an adhesive sealer to avoid drying of the strips during the overnight sample incubation.
- Unused and developed strips should be kept away from strong light and heat.
- The kit should only be used by personnel trained in clinical laboratory practices.
- Re-use of strips or troughs will result in erroneous results.
- Cutting strips will result in erroneous interpretation of the results.

Manual test procedure

Please read Remarks and precautions before performing the test.

- 16 hours sample incubation

1. Take the required amount of test troughs.
2. For each test run, a Positive and a Negative Control can be assayed for internal control purposes.
3. Identify the test troughs as specimen (and controls) and place them in the tray.
4. Make sure that patient or control specimen does not spill over into other wells. Carefully add patient or control specimen and reagents during the entire manual test procedure to avoid cross-contamination.
5. Add 1 mL of Sample Diluent to each test trough.
6. Add 10 µL of the appropriate specimen or control to the appropriately labelled trough.
7. Remove the required amount of test strips from their container, and add one strip to each of the test troughs. The test strip is placed membrane side upwards into the trough using tweezers.
THE STRIP MUST BE COMPLETELY SUBMERGED.
8. Cover the troughs with an adhesive sealer (see Remarks and precautions).
Incubate the samples by placing the tray on a shaker or rocker (see Direction for incubation) and agitate OVERNIGHT (16 ± 2 h) at room temperature (18 - 25°C).
NOTE: Carefully remove the adhesive sealers to avoid cross-contamination.
9. Wash each test strip 3 times (5 minutes) with 1 mL Wash Solution (see Directions for washing).
10. Add 1 mL of Conjugate Solution to each test trough.
11. Incubate with the conjugate by placing the test tray on the shaker or rocker and agitate for 30 minutes at room temperature (18 - 25°C).
12. Wash each test strip 3 times (5 minutes) with 1 mL Wash Solution (See Directions for washing).
13. Add 1 mL of Substrate Solution to each test trough.
14. Incubate with the substrate by placing the test tray on the shaker or rocker, and agitate for 30 minutes at room temperature (18 - 25°C).
15. Aspirate liquid. Add 1 mL of Stop Solution to each trough.
16. Incubate with the stop solution by placing the test trough on the shaker or rocker, and agitate for 10 - 30 minutes at room temperature (18 - 25°C).
17. Aspirate Stop Solution.
18. Remove the strips from the test troughs and place them, membrane side upwards, on absorbent paper using tweezers. As soon as the strips have dried completely, results can be interpreted. To accelerate the drying process, place strips in a dry incubator at 37°C for 30 minutes or use a hot air fan for 1 minute. Developed strips will retain their color if stored in the dark.

- 2 and 3 hours sample incubation

For the "2 hours and 3 hours sample incubation" protocol the same 18 steps as for the test procedure "16 hours sample incubation" will be followed, but changes to steps 6 - 8 - 9 and 12 have to be taken into account. Sample volume for specimens and controls will increase from 10 to 20 µL (step 6) and sample incubation time changes to 2 and 3 hours (step 8). Washing after sample incubation changes for the 2 hours procedure to 3 times 10 minutes and for the 3 hours procedure to 3 times 6 minutes (step 9); finally the second washing is 3 times 3 minutes for the 2 and 3 hours sample incubation (step 12).

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Summary test procedures with highlighted differences (bold), given in following table:

	16 hours sample incubation	2 hours sample incubation	3 hours sample incubation
<i>Sample Diluent</i>	1 mL	1 mL	1 mL
<i>Specimen</i>	10 µL	20 µL	20 µL
	16 hours sample incubation	2 hours sample incubation	3 hours sample incubation
<i>Controls</i>	10 µL	20 µL	20 µL
<i>LIA test strips</i>	16 hours ± 2 hours	2 hours	3 hours
<i>Washing</i>	1 mL/3 x 5 min	1 mL/3 x 10 min	1 mL/3 x 6 min
<i>RTU* Conjugate</i>	1 mL/30 min	1 mL/30 min	1 mL/30 min
<i>Washing</i>	1 mL/3 x 5 min	1 mL/3 x 3 min	1 mL/3 x 3 min
<i>RTU* Substrate</i>	1 mL/30 min	1 mL/30 min	1 mL/30 min
<i>Stop Solution</i>	1 mL/10 - 30 min	1 mL/10 - 30 min	1 mL/10 - 30 min

*RTU = Ready-to-use

Directions for washing

- After overnight, 2 hours and 3 hours incubation, carefully remove the adhesive plate sealer.
- The liquid is aspirated from the trough with a pipette, preferentially attached to a vacuum aspirator, which contains 5% sodium hypochlorite solution in the waste bottle.
The tray is held at an angle to allow all liquid to flow to one side of the trough (to the uncoated plastic backing part of each strip).
- Add 1 mL of diluted wash solution to each trough and agitate on a shaker or rocker. Shaking time is indicated in the assay procedure.
- Repeat these steps as many times as indicated in the assay procedure.
- NOTE:
 - Do not allow the strips to dry between the washing steps.
 - Make sure not to damage the surface of the test strips when aspirating.
 - Always use a clean aspiration device with disinfectant trap to avoid cross-contamination.
 - Make sure the entire strip is thoroughly washed by complete submersion in the washing solution.
 - Adapt the speed of the shaker or rocker when necessary.
 - Avoid splashing of the Wash Solution over the edges of the troughs.

Directions for incubation

- All the incubation steps (sample, conjugate, substrate, and stop solution incubation) and also the washing steps should be performed on a shaker or rocker (use rocker only for overnight sample incubation).
- During incubation and washing steps, the strip surface should be completely submerged, with the membrane side facing upwards.
- The shaker or rocker should allow a reciprocal (to- and- fro) motion of the strips in the trough, and a movement of the liquid over the strips without spilling over the trough.
- The speed generated by an orbital or longitudinal shaker or rocker is critical in achieving even line staining and maximum sensitivity.

Recommendations for an orbital shaker:

- diameter of the circular motion should be equal or superior to 13 mm
- recommended speed for a 13 mm circular motion is 160 rpm
- recommended speed for a 24 mm circular motion is 90 rpm.

Recommendations for a rocker:

- the difference between highest and lowest point should not exceed 80 mm to avoid spilling of liquid
- recommended speed is 34 rpm.

Automated test procedure: Auto-LIA

The LIA test procedure can easily be automated using the *Auto-LIA* automate. This instrument is a walk-away system with automated aspiration, pipetting, and incubation. For more information on the *Auto-LIA*, please contact Fujirebio Europe N.V. or your local distributor.

Please read Remarks and precautions before performing the test.

Detailed Auto-LIA procedures

- 16 hours sample incubation *Auto-LIA*
 1. DISP CH1 Stpos: Begin Endpos: Till end 1000 µL
 2. INC 1 min, shake speed 4
 3. PAUSE
 4. INC 960 min, shake speed 4
 5. WASH CH2 Stpos: Begin Endpos: Till end 1000 µL

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6. INC 6 min, shake speed 4
7. WASH CH2 Spos: Begin Endpos: Till end 1000 µL
8. INC 6 min, shake speed 4
9. WASH CH2 Spos: Begin Endpos: Till end 1000 µL
10. INC 6 min, shake speed 4
11. ASP
12. DISP CH4 Spos: Begin Endpos: Till end 1000 µL
13. INC 30 min, shake speed 4
14. WASH CH2 Spos: Begin Endpos: Till end 1000 µL
15. INC 3 min, shake speed 4
16. WASH CH2 Spos: Begin Endpos: Till end 1000 µL
17. INC 3 min, shake speed 4
18. WASH CH2 Spos: Begin Endpos: Till end 1000 µL
19. INC 3 min, shake speed 4
20. ASP
21. DISP CH6 Spos: Begin Endpos: Till end 1000 µL
22. INC 30 min; shake speed 4
23. ASP
24. DISP CH5 Spos: Begin Endpos: Till end 1000 µL
25. INC 20 min, shake speed 4
26. ASP
27. END

- 2 hours sample incubation *Auto-LIA*

1. DISP CH1 Spos: Begin Endpos: Till end 1000 µL
2. INC 1 min, shake speed 4
3. PAUSE
4. INC 120 min, shake speed 4
5. WASH CH2 Spos: Begin Endpos: Till end 1000 µL
6. INC 10 min, shake speed 4
7. WASH CH2 Spos: Begin Endpos: Till end 1000 µL
8. INC 10 min, shake speed 4
9. WASH CH2 Spos: Begin Endpos: Till end 1000 µL
10. INC 10 min, shake speed 4
11. ASP
12. DISP CH4 Spos: Begin Endpos: Till end 1000 µL
13. INC 30 min, shake speed 4
14. WASH CH2 Spos: Begin Endpos: Till end 1000 µL
15. INC 3 min, shake speed 4
16. WASH CH2 Spos: Begin Endpos: Till end 1000 µL
17. INC 3 min, shake speed 4
18. WASH CH2 Spos: Begin Endpos: Till end 1000 µL
19. INC 3 min, shake speed 4
20. ASP
21. DISP CH6 Spos: Begin Endpos: Till end 1000 µL
22. INC 30 min; shake speed 4
23. ASP
24. DISP CH5 Spos: Begin Endpos: Till end 1000 µL
25. INC 20 min, shake speed 4
26. ASP
27. END

CH1 = Sample Diluent
 CH2 = Wash Solution
 CH4 = Conjugate
 CH5 = Stop Solution
 CH6 = Substrate

- 3 hours sample incubation *Auto-LIA*

For the "3 hours sample incubation" protocol the same 27 steps as for the test procedure "2 hours sample incubation" will be followed, but changes to steps 4 - 6 - 8 and 10 have to be taken into account. Sample incubation time changes to 180 minutes for the 3 hours procedure (step 4) and washing after 3 hours sample incubation changes to 3 times 6 minutes (steps 6 - 8 - 10).

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Results

Reading

The identity and location of the antigens and controls coated on the strip are as follows:

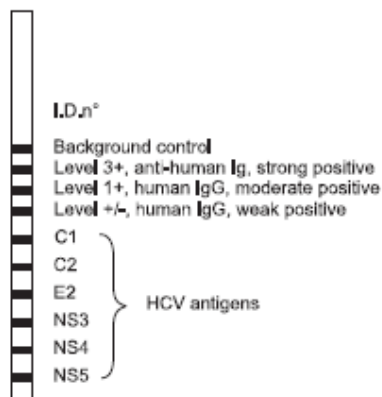


Figure 1: INNO-LIA HCV Score test strip

The intensity of the reaction on the control lines on each strip is used to assign the reactivity ratings for each antigen on that strip:

Intensity of antigen line reaction (R)		Rating
Lower than ±	$R < \pm$	-
Equal to ±	$R = \pm$	±
Higher than ±, but lower or equal to 1+	$\pm < R \leq 1+$	1+
Higher than 1+ but lower than 3+	$1+ < R < 3+$	2+
Equal to 3+	$R = 3+$	3+
Higher than 3+	$R > 3+$	4+

A reactivity rating must be made separately for each strip. Use the reading card for correct interpretation. Identification of the lines is obtained by alignment of the 3+ control line on the developed strip with the corresponding 3+ control line on the reading card.

Validation

Before reading the test results, the validity of the control levels on each strip should be checked and should fulfill the following criteria.

Validation of a single strip:

1. The control levels 1+ and ± as well as the strong positive control level 3+ should be visible.
2. The intensity of the control level 3+ should be greater than that of level 1+ and the intensity of the level 1+ should be greater than that of level ±.
3. The background control line should have a negative rating.

In case the Positive and Negative Control were assayed, the validity of the Positive and Negative Control strips should be checked before reading the test results and should fulfill the following criteria.

1. The Positive Control strip must show a reaction of at least 1+ on C1, C2, NS3 and NS4 antigen line. The E2 and NS5 antigen line may show a negative rating.
2. The Negative Control strip must show a negative rating (no reaction at all or at least less than control level ± for all the HCV antigen lines).

NOTE:

- The strip must be completely dried to avoid any misinterpretation due to faintly visible bands appearing after addition of stop solution.
- Do not place paper on top of the strips as long as they are wet.
- Weak control bands can be observed for samples containing high IgG levels (above the normal IgG range).
- In case of unexpected results or when a test procedure error is suspected, the test should be repeated and Positive and Negative Control should be included in a new test run.

Interpretation of the results

Extensive evaluations have shown that results may be interpreted as follows:

A sample is **NEGATIVE** for HCV antibodies:

- if all HCV antigen lines have a negative reactivity rating
- if only one HCV antigen line has a reactivity of ±, except when the reactivity is observed for NS3.

A sample is **POSITIVE** for HCV antibodies:

- if at least two HCV antigen lines have a reactivity of ± minimum or higher.

A sample is considered **INDETERMINATE** for HCV antibodies:

- if one HCV antigen line has a reactivity rating of 1+ or higher
- if the NS3 line reacts with a reactivity of ± or higher and all other antigen lines are negative.

Interpretation software: LiRAS for Infectious diseases

The LiRAS for infectious diseases software is designed to assist with the interpretation of the LIA results. Please contact your local distributor to obtain the latest updated version.

WARNING: Do not use the automated interpretation without taking into account the limitations of the procedure mentioned below.

Limitations of the procedure

- The protocol provided must be strictly followed to obtain optimal performance of the assay.
- Samples with a single ± reactivity or higher on NS3 can be indicative for HCV seroconversion. They are therefore scored as indeterminate.
- If an INDETERMINATE result is obtained, it is recommended to test an additional patient sample after a few weeks.
- A sample giving a positive reaction on the **background control line** may give cross-reactions with other HCV antigens lines and can not be determined as positive for HCV antibodies.
- In case reactivity is seen on following antigen lines (regardless of reactivity on the background control line): C1, C2, E2, NS4 and NS5, it is possible that there was aspecific reactivity with some type of anti-streptavidin antibodies. Therefore the strip should be interpreted as **INVALID**. Additional testing with other test methods is recommended.
- Anti-HCV antibodies may be undetectable in early infection.
- In a hemodialysis setting antibodies may be undetectable.
- The use of diluted samples may give erroneous results.
- Parameters for assessing liver damage and HCV RNA positivity should be further investigated in HCV antibody-positive subjects before initiating treatment or invasive procedures.
- Some patient samples can produce an equal reactivity on all antigen lines (in some cases, in combination with the background control line) across the strip. When these reactivities have the same intensity around the cut-off level (± rating), results should be interpreted as indicated below:
 - Equal reactivity on all antigen lines (in some cases in combination with the background control line) between cut-off level (± rating) and 1+ rating is considered as **INVALID** and additional testing with other test methods is recommended.
 - Equal reactivity on all antigen lines below cut-off level (± rating) is considered as **NEGATIVE** on the condition that the reactivity of the background control line is also below cut-off level.
 - Equal reactivity on all antigen lines higher than 1+ level is considered as **POSITIVE** on the condition that the reactivity of the background control line is below cut-off level.

Test performance

Sensitivity

Seroconversion panels/low-titer panels

The results of the INNO-LIA HCV Score using the *Auto-LIA* 2-hour sample incubation procedure and the manual 16-hour sample incubation procedure were obtained internally on 13 BBI seroconversion panels (PHV 904 till 916), on 2 BBI low titer panels (PHV103 and 204), and on the SFTS94 panel. The 13 seroconversion panels started with a negative bleed and had narrow bleeding intervals. These results were compared with CHIRON RIBA HCV 3.0 SIA results (Table 1 and Table 2).

Table 1: Overview results BBI seroconversion panels

Assay	Detection seroconversion panels towards Chiron RIBA HCV 3.0 SIA			
	Earlier	Equal	Later	Stayed negative
INNO-LIA HCV Score (2 hours <i>Auto-LIA</i> procedure)	7	4	1	1
INNO-LIA HCV Score (16 hours manual procedure)	7	4	1	1

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Table 2: Overview results BBI low titer panels and SFTS94 panel

Assay	Number of detected positive samples/panel		
	PHV 103	PHV 204	SFTS 94
INNO-LIA HCV Score (2 hours <i>Auto</i> -LIA procedure)	10	18	38
INNO-LIA HCV Score (16 hours manual procedure)	12	20	41
CHIRON RIBA HCV 3.0 SIA	12	23	31

HCV-positive samples

A total of 257 samples, originating from HCV-infected patients and found to be positive on 2 screening assays, were analyzed internally on the INNO-LIA HCV Score using the *Auto*-LIA 2-hour sample incubation procedure. All samples scored positive, with the exception of a single sample which scored indeterminate.

In addition, the INNO-LIA HCV Score using the *Auto*-LIA 2-hour sample incubation procedure was performed internally on 99 HCV genotyped samples.

All major HCV genotypes were covered in the sample set (Table 3).

Table 3. Genotype distribution of the tested HCV- positive samples

Genotype of tested samples	No. samples tested
1	30
1a	15
1b	15
2	21
3a	21
4	21
4	1
4a	15
4 non-a	5
5	6
Total	99

Note: no genotype 6 sample was available for testing.

All 99 positive samples scored positive.

In total, this is resulting in a sensitivity, upon inclusion of the single indeterminate result, of 100% (356/356; 95% CI [98.9%; 100.0%]).

Specificity**Blood donors**

A total of 400 blood donors found to be negative for HCV antibodies were analyzed internally using the manual 16-hour sample incubation procedure.

After initial testing, 377 samples were scored negative, 22 samples scored indeterminate, and one sample scored positive, resulting in an initial specificity of 94.3% (377/400; 95% CI [91.5%; 96.1%]). The initial positive blood sample scored negative after repeated testing in duplicate, resulting in a specificity after re-testing of 94.5% (378/400; 95% CI [91.8%; 96.3%]).

Clinical samples

Two hundred five clinical samples were tested internally using the manual 16-hour sample incubation procedure. One hundred eighty-nine of them scored negative, 11 were indeterminate, and 5 scored positive. Three of these 5 samples scored negative after repeated testing in duplicate.

One of the other 2 samples scored positive after repeated testing, while the other sample scored indeterminate. Both samples were negative on Ortho HCV 3.0 ELISA with Enhanced SAve and on INNOTEST HCV Ab IV. One of these 2 samples was tested on CHIRON RIBA HCV 3.0 SIA as well, and was found to be negative. For this sample set, an initial specificity of 92.2% (189/205; 95% CI [87.7%; 95.1%]) was obtained, while specificity after repeated testing was 93.7% (192/205; 95% CI [89.5%; 96.3%]).

Potentially interfering samples

One hundred thirty-seven potentially interfering samples were tested internally using the manual 16-hour sample incubation procedure. One hundred twenty-seven samples turned out to be negative, 9 were indeterminate, and one scored positive. Upon repeated testing in duplicate, this sample scored positive, and indeterminate, respectively. This sample was found to be negative on Ortho HCV 3.0 ELISA with Enhanced SAve, on INNOTEST HCV Ab IV, and on CHIRON RIBA HCV 3.0 SIA. On this set of samples, a specificity of 92.7% (127/137; 95% CI [87.1%; 96.0%]) was obtained.

Reproducibility

Two experimenters tested a panel of 13 HCV-positive samples, as well as one positive and one negative control on 3 different lots using the *Auto-LIA* 2-hour sample incubation procedure while a third experimenter tested this panel on one of these lots. The use of different strip lots and performance by different experimenters resulted in the same test outcome.

Trademarks

- LIA is a worldwide registered trademark® such as in Europe, USA, China.
- INNO-LIA is a registered trademark® in the USA and Japan and is a trademark™ in the rest of the world.
- LIRAS is a registered trademark® in Europe and is a trademark™ in the rest of the world (including USA).
- Auto-LIA is a registered trademark® in the USA and is a trademark™ in the rest of the world.