



INNOGENETICS®

INNO-LIA™ HCV Score

KEY-CODE: INX39257  
89539 INNO-LIA™ HCV Score  
25426 v13  
2010-12-16  
P 1242

English

**Specificity****Blood donors**

A total of 400 blood donors found to be negative for HCV antibodies were analyzed internally using this 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%; 96.4%]]. Two hundred five 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 INNO-TEST™ 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 [89.7%; 95.3%]] was obtained, while specificity after repeated testing was 93.7% [192/205; 95% CI [92.7%; 95.3%]].

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 INNO-TEST™ 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 experiments tested a panel of 13 HCV-positive samples, as well as one positive and one negative control on 3 different lots using the Angel-IA™ 24-hour sample incubation procedure while a third experimenter tested this panel on one of these lots. The use of different strips lot and performance by different experimenters resulted in the same test outcome.

**Trademarks**

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INNO-LIA™ is a Trademark of Innogenetics N.V.

Other languages see ! Autres langues voir ! Andere Sprachen siehe ! Altre lingue vedere !

Ver otros idiomas / Outras línguas ver:



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KIT VALORE INNO-LIA™ HCV Score  
80539 INNO-LIA™ HCV Score  
25426 v13  
2010-12-16  
P 1242

English



INNO-LIA™ HCV Score

IVD



“Mais changes highlighted”

04/09

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**INNOGENETICS**  
INNOVATIVE DIAGNOSTICS

KEY CODE: INX39257  
80530 INNO-LIA™ HCV Score  
25428 v1.0  
2010-12-16  
p.2/2

English

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## Symbols used

	Manufacturer
	In vitro Diagnostic Medical Device
	Batch code
	Catalogue number
	Use By
	Temperature Indicator
	Barcode
	Conc suff suff for < n > tests
	Coverage
	Negative Control
	Positive Control
	Sample Dose
	Stop Solution
	Strips
	Substrate BCP/NBT
	Wash Solution 5x

KEY CODE: INX39257  
80530 INNO-LIA™ HCV Score  
25428 v1.0  
2010-12-16  
p.3/2

English



## INNOGENETICS®

English

ENGLISH VERSION

KEY CODE: INX39257  
80538 INNO-LA™ HCV Score  
25x28 v13  
2013-12-16  
p 4/2

### Intended use

The INNO-LA™ 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 specimen source to be reactive using an anti-HCV screening procedure.

### Test principle

The INNO-LA™ HCV Score is a 3' generation line immunoassay which incorporates HCV antibodies 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: coated line 3+ positive control (anti-human IgG) which is also used as sample dilution control line, 1+ positive control (human IgG) and the ± control (anti-human IgG).

The INNO-LA™ HCV Score is based on the principle of an enzyme immunoassay. A test sample is incubated in a tray (together with other test strips). If present in the sample, HCV antibodies will bind to the HCV antigen lines on the strip. Subsequently, an affinity-purified alkaline phosphatase-conjugated goat anti-human IgG (+/-) conjugate is added and reacts with specific HCV antigenic domains, if previously formed. Incubation with the enzyme substrate produces a colorimetric-like color, the intensity of which is proportional to the amount of HCV-specific antibody capture 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 strips beyond the expiration date.  
All reagents and the plastic tube containing the test strips must be brought to room temperature 18 - 25°C approximately 30 minutes before use and 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	57429	Containing 20 INNO-LA™ HCV antigen coated test strips.
Sample Diluent	30 ml	57304	Containing color-coated (green) phosphate buffer containing sodium chloride, detergent, bovine protein stabilizers and 0.3% trichloroacetic acid (TCA) as preservative.
Ready-to-use	45 ml	57301	Containing color-coated (red) goat anti-human IgG labelled with alkaline phosphatase in its buffer containing bovine stabilizers, detergent and 0.3% methylchlorazone (MTC) 1% TCA as preservative.
Negative Control	0.12 ml	57307	Containing basematrix of human origin with 0.01% MTC/1% TCA as preservative.
Positive Control	0.12 ml	57308	Containing maculated human serum positive for antibodies to HCV with 0.01% MTC/1% TCA as preservative.
Rinse & Use	45 ml	57302	Containing 5-bromo-4-chloro-3-indolyl p-nitrophenyltetrazolium in BICIPNA substrate.
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 a clean water. Dried wash solution is stable for 2 weeks if kept at 2 - 8°C.
Incubation tray	2	-	With 11 troughs each.
Antiseptic swab	5	-	For cleaning strips.
Data reporting sheet	1	-	For storage of developed strips.
Handling card	1	-	For identification of reactive antigen lines.

### Materials required but not provided

Distilled or deionized water

- Protective gloves (with disposable anti-capable of delivering 10 L, 20 - 200 µl and 2000 µl, respectively)
- Orbital shaker or rocker (see Directions for Incubation)
- Graduated cylinders: 10, 25, 50 and 100 ml
- Tweezers, universal equivalent
- Timer
- Hot air fan (air dryer) or cryo incubator at 37°C
- An appropriate plastic together with disposable vials for the storage of strip solution conjugate, substrate and wash solution.
- Vacuum aspirator which contains 5% sodium hypochlorite solution in waste bottle.

### Safety and environment

Please refer to the Material Safety Data Sheet (MSDS) and product labeling for information on potentially hazardous components. The most recent MSDS version is available on the website [www.innogenetics.com](http://www.innogenetics.com).



Inert! (X) R43, S23, S43-27-60

Contains 2-Chloroacetamide, CON, SODS, BCP/PMS, CONTROL, SAMPLE Dil.

R43 May cause sensitization by skin contact

S23 Do not breathe vapour/spray

S24 Avoid contact with skin.

S37 Wear suitable gloves.

S60 This material and its container must be disposed of as hazardous waste

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-1/HIV-2, anti-HCV and HCVAg. No test method can fully rule out HIV infection. Positive controls 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.

• Autoclavable for at least 15 minutes at 121°C

• Autoclavable disposable material

• Mix liquid waste with sodium hypochlorite so that the final concentration is 1.4% sodium hypochlorite solution (hypochlorite).

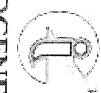
Use of personal protective equipment is necessary: gloves and gown. Specimens when manipulating dangerous or infectious agents.

• Waste should be handled according to the institutions 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-LA™ 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 mixed well by centrifugation.
- Store the specimens at 2 - 8°C. For storage longer than one week, freeze at 20°C or lower.
- Do not heat-treat samples.
- Repeatedly (more than 3 times) frozen and thawed samples may produce erroneous results.



**INNOGENETICS®**  
TEST KITS FOR CLINICAL DIAGNOSTICS

KEY-CODE: INX39257  
80638 INNO-LA™ HCIV Score  
25426 v13  
2010-12-16  
p-612

English



**INNOGENETICS®**  
TEST KITS FOR CLINICAL DIAGNOSTICS

KEY-CODE: INX39257  
80638 INNO-LA™ HCIV Score  
25426 v13  
2010-12-16  
p-712

English

## Remarks and precautions

- Do not mix reagents with different lot numbers.

- Frozen reagents, e.g. 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.

- Use a new pipette tip for each specimen.

- Make sure that the test strips are placed in the troughs with their membranes 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 sealant 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.

- Because of slips or smudges on resin interpretation of the 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. Use the required amount of test troughs taking into account that for each test run, a Positive and a Negative Control should be assayed. Identify test troughs as controls and specimens, and place them in the tray.

2. Add 1 ml of Sample Diluent to each test trough

3. Cover the troughs with an adhesive sealant (see Remarks and precautions).

4. Incubate the samples by placing the tray on a shaker or rocker (see Directions for incubation) and agitate

OVERNIGHT (18 - 24 h) at room temperature (18 - 25°C).

Note: Carefully remove the adhesive sealant from the tray, and add one strip to each of the test troughs.

The STRIP MUST BE COMPLETE (ELY SUMMERBERG).

5. Cover the troughs with an adhesive sealant (see Remarks and precautions).

6. 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

7. Add 1 ml of Sample Diluent to each test trough

8. 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)

9. Wash each test strip 3 times (5 minutes) with 1 ml Wash Solution (See Directions for washing)

10. Add 1 ml of Substrate Solution to each test trough

11. 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)

12. Aspirate liquid. Add 1 ml of Stop Solution to each trough.

13. Incubate with the stop solution by placing the test trough on the shaker or rocker and agitate for 10 minutes (10 - 30 minutes) at room temperature (18 - 25°C).

14. Aspirate Stop Solution.

15. 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.

Development 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 15 steps as for the test procedure "16 hours sample incubation" will be followed, but changes to steps 3 - 5, 6 and 9 have to be taken into account.

Sample volume, specimen and controls will increase from 10 to 20 µl (step 3), and sample incubation time changes to 2 and 3 hours (step 5). Washing after sample incubation changes for the 2 hours protocol to 3

times 10 minutes and for the 3 hours procedure to 3 times 6 minutes (step 6); finally the second washing is 3 times 3 minutes for the 2 and 3 hours sample incubation (step 9).

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

Incubation	16 hours sample		2 hours sample		3 hours sample	
		incubation		incubation		incubation
Specimen	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Controls	10 µl	20 µl	20 µl	20 µl	20 µl	20 µl
LIA® Test strips	16 hours + 2 hours	2 hours	3 hours			
Washing	1 ml 3 x 5 min	1 ml 3 x 10 min	1 ml 3 x 6 min			
RFLU® Conjugate	1 ml 30 min	1 ml 30 min	1 ml 30 min			
Washing	1 ml 3 x 5 min	1 ml 3 x 3 min	1 ml 3 x 3 min			
RFLU® Substrate	1 ml 30 min	1 ml 30 min	1 ml 30 min			
Stop Solution	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 tray is washed from the 3 wells of the wash 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 plate 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.

Note: Rinse strips as many times as indicated in the assay procedure.

(Do not allow the strips to dry between the washing steps.)

Make sure not to damage the surface of the test strips when agitating

Always use a clean aspiration device with filter/dish instead trap to avoid cross-contamination.

Move sure the entire strip is thoroughly washed by complete submersion in the washing solution.

Avoid the edges of the shaker or rocker when necessary

Avoid splashing of the wash solution on 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) and a 30° angle.

During incubation and washing steps, the strip/gelate should be completely submerged, with the membrane side facing upwards.

The shaker or rocker should allow a horizontal (i.e. side to side) motion of the strips in the trough, and a movement of the liquid over the strips without settling 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 small or superior to 12 mm

• recommended speed for a 13 mm circular motion is 150 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 60 mm to avoid spilling of liquid

• recommended speed is 34 rpm



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English

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80538 INNO-LIA™ HCV Score  
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2010-12-16  
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INNOGENETICS®

English

**Automated test procedure: Auto-LIA™**

The LIA™ test procedure can easily be automated using the Auto-LIA™ automate. This instrument is walk-away system with automated aspiration, pipetting, and incubation & for more information on the Auto-LIA™, please contact Innogenetics® or your local distributor.

Please read Remarks and precautions before performing the test.

**Differences Auto-LIA™ procedures:**

- 1 18 hours sample incubation Auto-LIA™
- 2 INC 1 min, shake speed 2
- 3 PAUSE
- 4 INC 900 min, shake speed 2
- 5 WASH C12 Stops, Begin Endpos, Till end 1000 µl
- 6 INC 6 min, shake speed 4
- 7 WASH C12 Stops, Begin Endpos, Till end 1500 µl
- 8 INC 6 min, shake speed 4
- 9 WASH C12 Stops, Begin Endpos, Till end 1500 µl
- 10 INC 6 min, shake speed 4
- 11 ASP
- 12 Disp CH4 Stops, Begin Endpos, Till end 1000 µl
- 13 INC 30 min, shake speed 4
- 14 WASH C12 Stops, Begin Endpos, Till end 1000 µl
- 15 INC 3 min, shake speed 4
- 16 WASH C12 Stops, Begin Endpos, Till end 1000 µl
- 17 INC 3 min, shake speed 4
- 18 WASH C12 Stops, Begin Endpos, Till end 1000 µl
- 19 INC 3 min, shake speed 4
- 20 ASP
- 21 Disp CH4 Stops, Begin Endpos, Till end 1000 µl
- 22 INC 30 min, shake speed 4
- 23 ASP
- 24 Disp CH4 Stops, Begin Endpos, Till end 1000 µl
- 25 Disp CH4 Stops, Begin Endpos, Till end 1000 µl
- 26 ASP
- 27 END

**3 hours sample incubation Auto-LIA™**

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

**23 ASP**

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 18 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).

**Results****Reading**

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

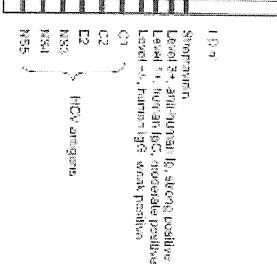


Figure 1. INNO-LIA™ HCV Score test strip

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
Lowest (not visible)	R < 1
Equal to 1	R = 1
Higher than 1, but lower or equal to 1+	1+ < R < 1+
Higher than 1+, but lower than 3+	1+ < R < 3+
Equal to 3+	R = 3+
Higher than 3+	R > 3+

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.

- 15 WASH C12 Stops, Begin Endpos, Till end 1000 µl
- 16 WASH C12 Stops, Begin Endpos, Till end 1000 µl
- 17 INC 3 min, shake speed 4
- 18 WASH C12 Stops, Begin Endpos, Till end 1000 µl
- 19 INC 3 min, shake speed 4
- 20 ASP
- 21 Disp CH4 Stops, Begin Endpos, Till end 1000 µl



INNOGENETICS

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### Validation

Check the validity of the positive and the negative control strips and the validity of the control levels on each strip.

- Validation of the test strip.**

  1. The positive control strip MUST show a reaction of at least 1+ or C1. C2, H2G2 and NS1 antigen line. The E2 and B1G2 antigen line may show a negative result.
  2. The negative control strip must show a negative (nothing/no reaction) at a level of at least less than control line ± 10%.
  3. The control levels 1+ and 2+ 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+

Fast Performance

360

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Manual 16-hour sample incubation procedures were obtained internally on 13 BBL seroconversion panels (PHM 904-III-916), on 2 BBL flow User panels (PHM 1003 and 2014), and on the SFT-994 panel. The t<sub>1/2</sub> seroconversion panels started with a negative blood and had narrow bleeding intervals. These results were compared with CHIRONE® RIBA® and HIV 3.0 SIA results (Table 1 and Table 2).

Table 1: Overview results ABF setconversion periods

Detection sensitivity patterns towards Chiron RIBA HCV 3.0 SIA				
Assay	Earlier	Equal	Later	Stayed negative
INNO-LIA HCV Score (2 hours Auto-LIA procedure)	7	4	1	1
INNO-LIA HCV Score (16 hours manual procedure)	7	4	1	1

Table 2: Overview results BBI for INNO samples and SFTS94 panel

Assay	Number of depicted positive samples/panel <sup>a</sup>	PHV 103	PHV 204	SFTS 94
INNO-LIA HCV Score (2 hours Auto-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 256 samples, originating from HCV-infected patients and were analyzed internally on the INNO LiPA™ HCV Score using the All samples scored positive, with the exception of a single sample. In addition, the INNO LiPA™ HCV Score using the AutoLiPA™ 2-HD internally on 98 HCV-confirmed samples. All major HCV genotypes were covered in this sample set (Table 3).

were loaded on 2x3 sample strips, originating from non-HCV-infected patients and found to be positive on 2 screening assays. All samples screened positive, with the exception of a single sample which scored indeterminate. In addition, the INNO-*LiPA*-HCV Score using the Auto-LiPA™ 2-hour sample incubation procedure, internally on 99 HCV-demonstrated samples.

#### **Limitations of the procedure**

- The protocol provided must be strictly followed to obtain optimal performance of the assay. Samples with a single HCV antibody or IgM can be indicative for HCV seropositivity. They are therefore stored as indeterminate if an IND-TERMINATE result is obtained it is recommended to test an additional patient sample after 8 weeks. As with testing a positive reaction on the sheep serum control line may give cross-reactions with other HCV antigens lines and can not be determined as positive for HCV antibodies. Anti-HCV antibodies may be undetectable in early infection. In a therapeutic 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.

Note: no genotype & sample was available for testing

All 99 positive samples scored positive.

In total, this is resulting in a sensitivity upon institutions

[S90, S91, S92, S93, S94, S95, S96, S97, S98]