WHO Prequalification of In Vitro Diagnostics Programme
PUBLIC REPORT

Product: INNO-LIA HIV I/II Score
Number: PQDx 0203-073-00

Abstract

INNO-LIA HIV I/II Score with product code 80540, manufactured by Fujirebio Europe NV, CE mark regulatory version, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 8 May 2015.

Intended use:
INNO-LIA HIV I/II Score is intended as a supplementary assay for specimens found to be reactive using an anti-HIV screening assay. INNO-LIA HIV I/II Score is a line immunoassay (LIA), to confirm the presence of antibodies against the human immunodeficiency virus type 1 (HIV-1), including group O, and type 2 (HIV-2) in human serum or plasma. The INNO-LIA HIV I/II Score also differentiates between HIV-1 and HIV-2 infections.

Test principle:
Recombinant proteins and synthetic peptides from HIV-1 and HIV-2, and a synthetic peptide from HIV-1 group O are coated as discrete lines on a nylon strip with plastic backing.

Five HIV-1 antigens are applied: sgp120 and gp41, which detect specific antibodies to HIV-1, and p31, p24, and p17, which may also cross-react with antibodies to HIV-2. HIV-1 group O peptides are present in the HIV-1 sgp120 band. The antigens gp36 and sgp105 are applied to detect antibodies to HIV-2.

In addition to these HIV antigens, four control lines are coated on each strip: anti-streptavidin line, ± cut-off line (human IgG), 1+ positive control line (human IgG) and one strong 3+ positive control line which is also the specimen addition control line (anti-human Ig). INNO-Lia HIV I/II Score is based on the enzyme immunoassay principle. The test specimen is incubated in a test trough together with the multiple antigen-coated test strip. HIV antibodies, if present in the specimen, will bind to the individual HIV antigen lines on the strip. Afterwards, a goat anti-human IgG labelled with alkaline phosphatase is added and will bind to any HIV antigen/antibody complex previously formed. Incubation with enzyme substrate (BCIP/NBT) produces a dark brown color in proportion to the amount of HIV antibody present in the specimen. Color development is stopped with sulphuric acid.

If the specimen contains no HIV-specific antibodies, the labelled antihuman antibody will not be bound to antigen/antibody complex so that only a low standard background color develops.

The test kit contains:

- Antigen-coated test strips (20 x strips), reference 57330
- Sample diluent (1 x30ml/vial), reference 57304
- Negative control (1 x 0.12ml/vial), reference 57307
- Positive control (1 x 0.12ml/vial), reference 57306
- Ready-to-use conjugate (1 x 45ml/vial), reference 57301
- Ready-to-use substrate BCIP/NBT (1 x 45ml/vial), reference 57302
- Stop solution (1 x 45ml/vial), reference 57303
- Wash solution (1 x 45ml/vial), reference 57299
- Incubation tray (2)
- Adhesive sealers (5)
- Data reporting sheet (1)
- Reading card (1)
- Instructions for use (1 copy)

Items required but not provided in the test kit:

- Distilled or deionized water
- Precision pipettes with disposable tips (10 µl, 20 - 200 µl, 200 - 1000 µl)
- Orbital mixer or rocker
- Vortex mixer or equivalent
- Graduated cylinders (10, 25, 50, 10 ml)
- Tweezers for strip handling
- Timer

Optional items:

- Vacuum aspirator (containing 5% sodium hypochlorite in the waste bottle)
- Repetitive dispenser for solutions
- Dry incubator at 37 °C

Storage:
The test kit should be stored at 2 to 8 °C.

Shelf-life:
16 months.

Summary of prequalification status for INNO-LIA HIV I/II Score

	Initial acceptance	
	Date	**Outcome**
Status on PQ list	8 May 2015	listed
Dossier assessment	N/A	MR: Abbreviated assessment
Inspection status	08 January 2015	MR
Laboratory evaluation	N/A	MR

MR: Meets Requirements
N/A: Not Applicable

INNO-LIA HIV I/II Score was accepted for the WHO list of prequalified in vitro diagnostics.

Background information

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

The manufacturer's instructions for use contained two test procedures: one manual test procedure and one automated test procedure using the Auto-LIA which is a walk-away systems with automated aspiration, pipetting and incubation. As the WHO assessment was conducted using the abbreviated prequalification assessment approach, these two test procedures were not examined in great depth.

As the regulatory version submitted for WHO prequalification assessment had previously been stringently assessed by European Community, the product was eligible for the WHO procedure for abbreviated prequalification assessment, in accordance with "Abbreviated prequalification assessment: WHO Prequalification of In Vitro Diagnostics Programme" (PQDx_173)

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 HIV I/II 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 with the WHO procedure for abbreviated prequalification assessment, an abbreviated inspection was performed at the site of manufacture (Ghent, Belgium) of INNO-LIA HIV I/II 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

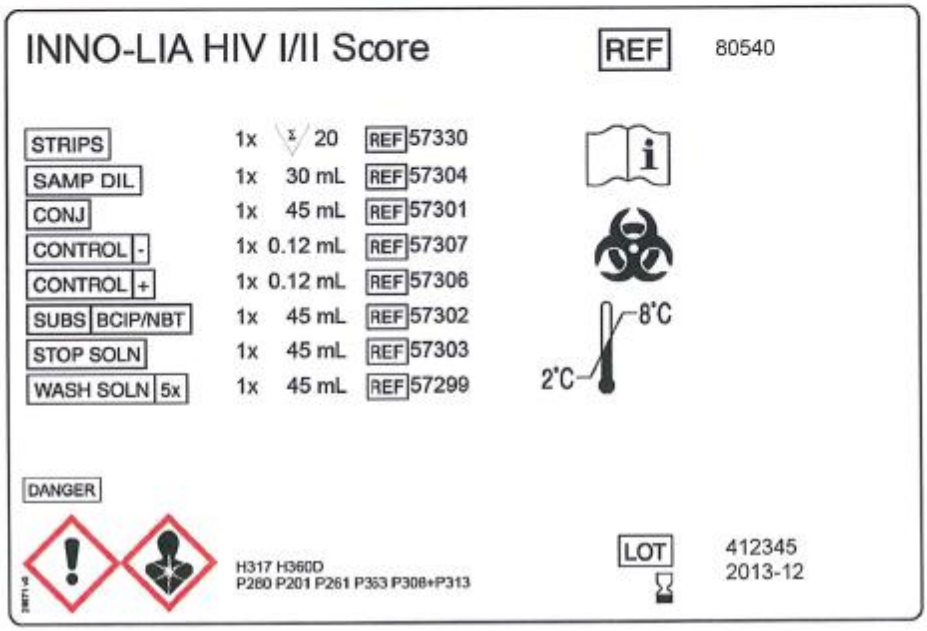
In accordance with the WHO procedure for abbreviated prequalification assessment and given the fact that INNO-LIA HIV I/II Score has been used to characterize specimens for all previous WHO test kit evaluations of HIV serology assays (including rapid diagnostic tests, enzyme immunoassays, other formats), the product was not required to undergo an WHO laboratory evaluation for its use with human serum/plasma specimens.

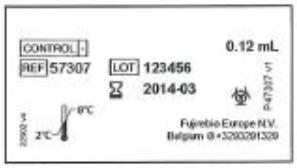
Labelling

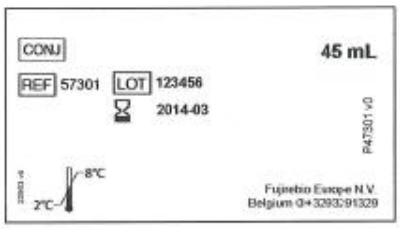
1. **Labels**
2. **Instructions for use**

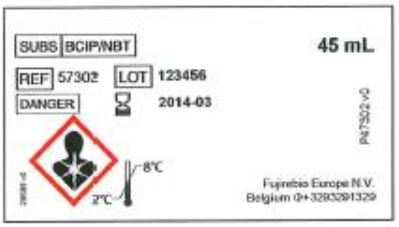
1. Labels


FUJIREBIO
INNO-LIA HIV I/II Score
Line ImmunoAssay
IVD
CE
0459
Fujirebio Europe N.V.
Technologiepark 6
9052 Gent, Belgium
Ph+32-9 329 13 29
28570 v0


INNO-LIA HIV I/II Score
REF 80540
STRIPS 1x Σ 20 REF 57330
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 57306
SUBS BCIP/NBT 1x 45 mL REF 57302
STOP SOLN 1x 45 mL REF 57303
WASH SOLN 5x 1x 45 mL REF 57299
8°C
2°C
DANGER
H317 H360D
P280 P201 P261 P353 P308+P313
LOT 412345
2013-12

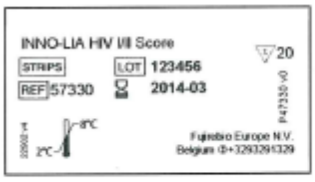

CONTROL -
0.12 mL
REF 57307
LOT 123456
2014-03
2°C
8°C
Fujirebio Europe N.V.
Belgium

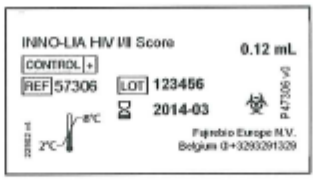

CONJ
45 mL
REF 57301
LOT 123456
2014-03
2°C
8°C
Fujirebio Europe N.V.
Belgium

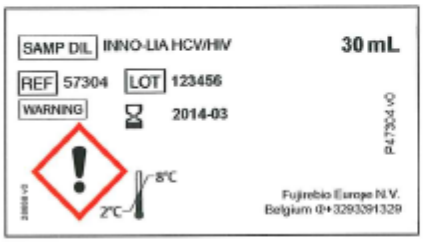

SUBS BCIP/NBT
45 mL
REF 57302
LOT 123456
DANGER
2014-03
2°C
8°C
Fujirebio Europe N.V.
Belgium


STOP SOLN
45 mL
REF 57303
LOT 123456
2014-03
2°C
8°C
Fujirebio Europe N.V.
Belgium


WASH SOLN 5x
45 mL
REF 57299
LOT 123456
2014-03
2°C
8°C
Fujirebio Europe N.V.
Belgium


INNO-LIA HIV I/II Score
20
STRIPS
LOT 123456
REF 57330
2014-03
2°C
8°C
Fujirebio Europe N.V.
Belgium


INNO-LIA HIV I/II Score
0.12 mL
CONTROL +
REF 57306
LOT 123456
2014-03
2°C
8°C
Fujirebio Europe N.V.
Belgium


SAMP DIL INNO-LIA HCV/HIV
30 mL
REF 57304
LOT 123456
WARNING
2014-03
2°C
8°C
Fujirebio Europe N.V.
Belgium

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 number2867 v2, 2014-03-28). Fujirebio Europe N.V. will take these into consideration in the next revision of the instructions for use.

FUJIREBIO

KEY-CODE: **FRI88476**
80540 INNO-LIA HIV I/II Score
28672 v2
2014-03-28
p 1/12

English

INNO-LIA HIV I/II Score

IVD CE 0459

Manufactured by:
Fujirebio Europe N.V.
Technologiepark 6
9052 Gent
Belgium
✆+32 9 329 13 29
BTW BE 0427.550.660
RPR Gent

"Note changes highlighted"

Distributed by:

Fujirebio Germany GmbH
Hans-Böckler-Allee 20
30173 Hannover
Germany
✆+49 511 857 39 31

Fujirebio Iberia S.L.
Calle Tarragona 161, Planta 14
08014 Barcelona
Spain
✆+34 93 270 53 00

Fujirebio Italia S.r.l.
Via Vaccareccia 39/A
00040 Pomezia (Roma)
Italy
✆+39 06 965 28 700

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

Fujirebio France SARL
Les Conquérants, Bât. Le Kilimandjaro
8/10, avenue des Tropiques
91940 Les Ulis
France
✆+33 1 69 07 48 34

Other languages see / Autres langues voir / Andere Sprachen siehe / Altre lingue vedere / Ver otros idiomas / Outras línguas ver:

www.e-labeling.eu/FRI88476

✆EUROPE	+800 135 79 135
GR	00 800 161 220 577 99
IS	800 8996
LT	880 030 728
RO	0800 895 084
SK	0800 606 287
TR	0800 142 064 866
LI	+31 20 796 5692
MT	+31 20 796 5693
non-EUROPE	+31 20 794 7071
US	+1 855 236 0910
CA	+1 855 805 8539
AR, BR, CO, UY, AU, NZ, RU	+800 135 79 135

8:00 – 17:00 GMT+1

M	T	W	T	F	S	S
☒	☒	☒	☒	☒	☐	☐

TABLE OF CONTENTS

Symbols used

	Manufacturer
IVD	*In Vitro* Diagnostic Medical Device
LOT	Batch code
REF	Catalogue number
	Use By
	Consult Instructions for Use
	Temperature limitation
	Biological risks
	Contains sufficient for <n> tests
CONJ	Conjugate
CONTROL -	Negative Control
CONTROL +	Positive Control
SAMP DIL	Sample Diluent
STOP SOLN	Stop Solution

STRIPS	Strips
SUBS BCIP/NBT	Substrate BCIP/NBT
WASH SOLN 5x	Wash Solution 5x

Intended use

The INNO-LIA HIV I/II Score is a Line Immuno Assay (LIA), to confirm the presence of antibodies against the human immunodeficiency virus type 1 (HIV-1), including group O, and type 2 (HIV-2) in human serum or plasma. The INNO-LIA HIV I/II Score also differentiates between HIV-1 and HIV-2 infections. It is intended as a supplementary assay on specimens found to be reactive using an anti-HIV screening procedure.

Test principle

Recombinant proteins and synthetic peptides from HIV-1 and HIV-2, and a synthetic peptide from HIV-1 group O are coated as discrete lines on a nylon strip with plastic backing.

Five HIV-1 antigens are applied: sgp120 and gp41, which detect specific antibodies to HIV-1, and p31, p24, and p17, which may also cross-react with antibodies to HIV-2. HIV-1 group O peptides are present in the HIV-1 sgp120 band. The antigens gp36 and sgp105 are applied to detect antibodies t HIV-2.

In addition to these HIV antigens, four control lines are coated on each strip: background control line ± cut-off line (human IgG), 1+ positive control line (human IgG) and one strong 3+ positive control li which is also the sample addition control line (anti-human Ig). The INNO-LIA HIV I/II Score is based on the enzyme immunoassay principle (EIA). The test sample is incubated in a test trough together with the multiple antigen-coated test strip. HIV antibodies, if present in the sample, will bind to the individual HIV antigen lines on the strip. Afterwards, a goat anti-human IgG labelled with alkaline phosphatase is added and will bind to any HIV antigen/antibody complex previously formed. Incubation with enzyme substrate (BCIP/NBT) produces a dark brown color in proportion to the amount of HIV antibody present in the sample. Color development is stopped with sulfuric acid.

If the sample contains no HIV-specific antibodies, the labelled antihuman antibody will not be bound to antigen/antibody complex so that only a low standard background color develops.

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.

Reagents supplied:

Component	Quantity	Ref.	Description
Strips	20	57330	Containing 20 INNO-LIA HIV antigen-coated test strips.
Sample Diluent	30mL	57304	Containing color-coded (green) phosphate buffer containing sodium chloride, detergent, bovine protein stabilizers and 0.3% chloroacetamide (CAA) as preservative.
Negative Control	0.12mL	57307	Containing base matrix of human origin with 0.01% methylisothiazolone (MIT)/<0.1% CAA as preservative.
Positive Control	0.12mL	57306	Containing inactivated human serum positive for antibodies to HIV with 0.01% MIT/<0.1% CAA as preservative.
Ready-to-use Conjugate	45mL	57301	Containing color-coded (red) goat anti-human IgG labeled with alkaline phosphatase in Tris buffer containing bovine stabilizers, detergent and 0.01% MIT/<0.1% CAA as preservative.

Component	Quantity	Ref.	Description
Ready-to-use Substrate BCIP/NBT	45mL	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	45mL	57303	Containing 0.1 mol/l sulfuric acid.
Wash Solution	45mL	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 tips capable of delivering 10µL, 20 - 200µL, and 200 - 1000µL, respectively.
- Orbital mixer or rocker (see Directions for incubation).
- Vortex mixer or equivalent.
- Graduated cylinders: 10, 25, 50, and 100mL.
- 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 potentiall hazardous components. The most recent SDS version is available on the website www.fujirebio-europe.com.

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

Danger, Contains N,N-Dimethylformamide: SUBS BCIP/NBT
H360D P201 P281 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-HCV and HBsAg. The Negative Control has been found to be negative for anti-HIV-1/HIV-2, anti-HCV and HBsAg. No test meth can offer complete insurance that blood products will not transmit infectious agents. Therefore, a 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 perfo 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 ± 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, sta 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 HIV I/II 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 the 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. Manipulate the strips always 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 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 throughs with an adhesive sealer to avoid drying of the strips during the sample incubation.
- Unused and developed strips should be kept away from strong light and heat.
- This 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 **1mL** of **Sample Diluent** to each test trough.
6. Add 10µL of the appropriate specimen or control to their appropriately labelled troughs.
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 STRIPS 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 Directions 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 1mL **Wash Solution** (see Directions for washing).
10. Add **1mL** 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 **1mL Wash Solution** (see Directions for washing).
13. Add **1mL** 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 **1mL** of **Stop Solution** to each test 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 the 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 hair dryer for 1 minute. Developed strips will retain their color if stored in the dark.

- ***3 hours sample incubation***
- For the "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 6 - 8 - 9 and 12 have to be taken into account. Sample volume for specimens and controls will increase from 10 - 20µL (step 6) and sample incubation time changes to 3 hours (step 8). Washing after sample incubation changes for the 3 hours procedure to 3 times 6 minutes (step 9); finally the second washing is 3 times 3 minutes for the 3 hours sample incubation (step 12).

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

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

*RTU = Ready-to-use

Directions for washing

- After overnight 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 1mL of diluted wash solution to each trough and agitate on a shaker or rocker. Shaking time i 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 sampl 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 speeds generated by a 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 1000µL
  2. INC 1 min, shake speed 4
  3. PAUSE
  4. INC 960 min, shake speed 4
  5. WASH CH2 Stpos: Begin Endpos: Till end 1000µL
  6. INC 6 min, shake speed 4
  7. WASH CH2 Stpos: Begin Endpos: Till end 1000µL
  8. INC 6 min, shake speed 4
  9. WASH CH2 Stpos: Begin Endpos: Till end 1000µL
  10. INC 6 min, shake speed 4
  11. ASP

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

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

1. DISP CH1 Stpos: Begin Endpos: Till end 1000µL
2. INC 1 min, shake speed 4
3. PAUSE
4. INC 180 min, shake speed 4
5. WASH CH2 Stpos: Begin Endpos: Till end 1000µL
6. INC 6 min, shake speed 4
7. WASH CH2 Stpos: Begin Endpos: Till end 1000µL
8. INC 6 min, shake speed 4
9. WASH CH2 Stpos: Begin Endpos: Till end 1000µL
10. INC 6 min, shake speed 4
11. ASP
12. DISP CH4 Stpos: Begin Endpos: Till end 1000µL
13. INC 30 min, shake speed 4
14. WASH CH2 Stpos: Begin Endpos: Till end 1000µL
15. INC 3 min, shake speed 4
16. WASH CH2 Stpos: Begin Endpos: Till end 1000µL
17. INC 3 min, shake speed 4
18. WASH CH2 Stpos: Begin Endpos: Till end 1000µL
19. INC 3 min, shake speed 4
20. ASP
21. DISP CH6 Stpos: Begin Endpos: Till end 1000µL
22. INC 30 min; shake speed 4
23. ASP
24. DISP CH5 Stpos: Begin Endpos: Till end 1000µL
25. INC 20 min, shake speed 4
26. ASP
27. END

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

Results

Reading

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

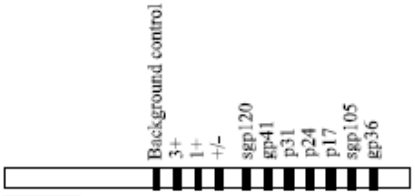


Figure 1: INNO-LIA HIV I/II Score test strip

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 < ±	-
Equal to ±	R = ±	±
Higher than ±, but lower or equal to 1+	± < R ≤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 fulfil 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 (the intensity is weaker than the ± control line).

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 fulfil the following criteria.

1. The Positive Control strip must show a reaction of at least 1+ on sgp120, gp41, p31, p24 and gp36. The p17 and sgp105 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 of the HIV 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 line is determined as being positive if a minimal rating of 1+ is observed.
ENV1 = envelope line for HIV-1: sgp120 and gp41
ENV2 = envelope line for HIV-2: sgp105 and gp36

NEG = NEGATIVE for HIV antibodies

IND = INDETERMINATE for HIV antibodies

POS = POSITIVE for HIV antibodies

No lines positive	No line ±	NEG
	1 line ±	NEG
	2 or more lines ±	IND
1 line positive (≥ 1+)		IND
2 lines positive (≥ 1+)	No ENV positive	IND
	1 ENV1 and p24	HIV-1 (*)
	2 ENV1	HIV-1
	1 ENV2 and p24	HIV-2 (**)
	2 ENV2	HIV-2
	Other combinations	IND
3 or more lines positive (≥ 1+)	No ENV positive	IND
	1 ENV1 and 1 ENV2	HIV
	1 or 2 ENV1	
	1 ENV1	HIV-1 (*)
	2 ENV1	HIV-1
	1 or 2 ENV2	
	1 ENV2	HIV-2 (**)
	2 ENV2	HIV-2
	2 ENV1 and 1 ENV2	HIV-1
	1 ENV1 and 2 ENV2	HIV-2
	2 ENV1 and 2 ENV2	HIV (***)

(*) If a rating of ± is obtained on both ENV2 antigen lines, the sample is HIV positive but not typable.
(**) If a rating of ± is obtained on both ENV1 antigen lines, the sample is HIV positive but not typable.
(***) Evaluation of a follow-up sample with alternative testing methods (eg. PCR) is required to confirm an HIV-1 and/or HIV-2 infection.

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 limitation of the procedure as mentioned below.

Limitations of the procedure

- The protocol provided must be strictly followed to obtain optimal performance of the assay.
- A sample giving a positive reaction on the background control line may give cross-reactions with other HIV antigens lines and can not be determined as positive for HIV antibodies.
- If an indeterminate or untypable result is obtained, it is recommended to test an additional patient sample after a few weeks.
- Analysis of a follow-up sample is required, if designation of HIV positivity is based on the positive score of only 2 HIV-antigen bands. In case reactivity is seen on the sgp120 and gp41 lines (regardless of reactivity on the background control line), it is possible that there was aspecific reactivity with some type of anti-streptavidin antibodies. Additional testing with other test methods is recommended.
- A negative result does not preclude the possibility of exposure to HIV or infection with the virus.

- The use of diluted samples may give erroneous results.
- 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

A total of 12 BBI seroconversion panels (PRB 903, 904, 908, 910, 912, 916, 919, 922, 923, 924, 925, 927, including 25 early seroconversion samples) and 3 BBI low-titer panels (PRB 103 till 105, including 17 early seroconversion samples) were analyzed internally on INNO-LIA HIV I/II Score using the *Auto*-LIA II 3-hour sample incubation procedure and the manual 16-hour procedure. These results were compared with Western blot (Table 1 and Table 2). All seroconversion panels started with a negative bleed and had narrow bleeding intervals.

Table 1: Overview results BBI seroconversion panels

	Detection seroconversion panels towards Western Blot		
Assay	*Earlier*	*Equal*	*Later*
INNO-LIA HIV I/II Score (3 hours *Auto*-LIA procedure)*	2	9	0
INNO-LIA HIV I/II Score (16 hours manual procedure)*	2	9	0

Remark*: One panel (PRB924) did not become positive for either test, so not included in this overview

Table 2: Overview results BBI low titer panels

	Number of detected positive samples/panel		
Assay	***PRB103***	***PRB104***	***PRB105***
INNO-LIA HIV I/II Score (3 hours *Auto*-LIA procedure)*	13	9	14
INNO-LIA HIV I/II Score (16 hours manual procedure)*	13	11	14
Western Blot	14	9	12

HIV-positive samples

A total of 273 HIV-1-positive and 120 HIV-2-positive samples that were found positive on Vironostika HIV Uni-Form II Ag/Ab and on INNO-LIA HIV Confirmation, were analyzed internally using the *Auto*-LIA II 3-hour sample incubation procedure.

Of the 273 HIV-1-positive samples, all 273 samples were identified as positive for HIV-1 antibodies, resulting in 100% sensitivity and differentiation capacity (273/273; 95% CI [98.6%;100.0%]).

Of the 120 HIV-2-positive samples, 108 samples were correctly identified as positive for HIV-2 antibodies, 10 samples were scored positive for HIV antibodies but untypable, and 2 samples were indeterminate. For this HIV-2 sample population, including the 2 indeterminate results, a sensitivity of 100% (120/120; 95% CI [96.9%; 100.0%]) was observed, and a differentiation capacity of 91.5% (108/118; 95% CI [85.1%; 95.3%]).

Specificity

Blood donors

A total of 300 blood-donor samples found negative for HIV antibodies were analyzed internally using the manual 16-hour sample incubation procedure. After initial testing, 290 samples were scored negative, 9 samples were indeterminate, and 1 sample scored positive for HIV-2 antibodies. Upon

repeated testing in duplicate, this initial positive blood sample scored positive for HIV-2 antibodies once, then indeterminate a second time, and was positive on INNO-LIA HIV Confirmation. This sample was found to be negative on Vironostika HIV Uni-Form II Ag/Ab and on Genelabs Diagnostics HIV Blot 2.2. Specificity calculated on this sample set was 96.7% (290/300; 95% CI [94.0%-98.2%]).

Clinical samples

Two hundred six clinical samples were tested internally using the manual 16-hour sample incubation procedure. One hundred ninety-eight samples scored negative, 7 were indeterminate, and 1 scored positive. This latter sample was found to be positive upon repeated testing in duplicate and upon testing on the INNO-LIA HIV Confirmation, while a negative result was obtained on Vironostika HIV Uni-Form II Ag/Ab and on Genelabs Diagnostics HIV Blot 2.2. For this sample set, a specificity of 96.1% (198/206; 95% CI [92.5% -98.0%]) was observed.

Potentially interfering samples

One hundred twenty-four potentially interfering samples were tested internally using the manual 16-hour sample incubation procedure. Of these, 117 were negative and 7 indeterminate. The specificity for this set of samples was 94.4% (117/124; 95% CI [88.8%-97.2%]).

Reproducibility

A panel of 5 HIV-positive samples, as well as one positive and one negative control were tested on 2 different lots by 4 experimenters using the *Auto*-LIA II 3-hour sample incubation procedure. The use of different strip lots and performance by different experimenters resulted in the same test outcome, except for one sample for which an indeterminate result instead of an HIV-1-positive result was obtained in 1 of the 5 observations.

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.