WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: INNOTEST HCV Ab IV
WHO reference number: PQDx 0201-073-00

INNOTEST HCV Ab IV with product codes **80068** and **80330**, manufactured by **Fujirebio Europe NV, CE-marked regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 15 February 2018.

Intended use:
The INNOTEST HCV Ab IV is an enzyme immunoassay for the detection of antibodies to human Hepatitis C virus (HCV) in human serum or plasma.

Assay description:
The wells of polystyrene microplate strips are coated with a mixture of HCV synthetic peptides/recombinant proteins derived from different immunodominant regions: the Core (2 different epitope clusters), NS3, NS4A, NS4B, as well as the NS5A region.

The antigens from these immunodominant regions are derived from different HCV genotypes (1a, 1b, 2, 3a). The test sample is incubated in such a well. Virus-specific antibodies to HCV, if present in the sample, will bind to the solid-phase antigens. Subsequently, an affinity purified rabbit anti-human IgG (H chain specific) labelled with the enzyme horseradish peroxidase (HRP) is added.

Upon a positive reaction this labelled antibody becomes bound to any solid-phase antigen/antibody complex previously formed. Incubation with enzyme substrate produces a blue color in the test well, which turns yellow when the reaction is stopped with sulfuric acid.

If the samples contain no HCV antibodies, then the labelled antibody cannot be bound specifically and only a low background color develops.

Test kit contents:

Component	**192 tests (product code 80068)**	**480 tests (product code 800330)**
Negative control: human serum containing 0.01% methylisothiazolone (MIT) and <0.1% chloroacetamide (CAA) as preservative.	1 vial (1.5ml)	1 vial (3ml)
Positive control:	1 vial (1.5ml)	1 vial (3ml=

phosphate buffer containing antibodies to HCV, protein stabilizers, Proclin 300 as presentative.		
Antigen-coated test wells (plate): containing a strip-holder with 12 x 8 HCV with a silicagel bag added as dessicant.	2 sachets	5 sachets
Sample diluent: phosphate buffer containing sodium chloride, Triton, protein stabilizers and Proclin 300 as preservative - purple colored buffer solution.	1 vial (60 ml)	1 vial (150 ml)
Concentrated wash solution: phosphate buffer containing 0.01% MIT and <0.1% CAA as preservative.	1 vial (150 ml)	1 vial (200 ml)
Conjugate diluent: phosphate buffer containing protein and enzyme stabilizers and 0.05% Proclin 300 as preservative - green colored buffer solution).	1 vial (60 ml)	1 vial (150 ml)
Concentrated conjugate 100x: rabbit anti-human IgG (H chain) labelled with horseradish peroxidase	1 vial (0.60 ml)	1 vial (1.5 ml)
Substrate buffer: phosphate citrate buffer containing 0.006% hydrogen peroxide.	1 vial (60 ml)	1 vial (150 ml)
Concentrated substrate: TMB 100x (tetramethylbenzidine (TMB) dissolved in dimethylsulfoxide (DMSO)	1 vial (1 ml)	1 vial (1.5 ml)
Stop solution: 0.9 N sulfuric acid	1 vial (30 ml)	1 vial 45 ml)
Adhesive plate sealers	8	15
Plastic minigrip bag(s).	1	2

Items required but not provided:

Item
Consumables: Distilled or deionized water Absorbent tissues
Equipment: - Precision pipette with disposable tips to deliver ranges of 20 µl to 200 µl and 200 µl to

1000 µl respectively.
- Microplate shaker
- Incubator at 37°C
- Microplate washer (alternatively, washing can be performed manually)
- Photometric reading: microplate reader, equipped with a 450 nm filter and optional 620 nm filter

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

Shelf-life upon manufacture:
16 months.

Warnings/limitations:
- Only adequately trained personnel should be permitted to perform the test procedure.
- Specimens 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 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.
- All blood components and biological materials should be disposed of in accordance with one of the following established safety procedures.
    1. Autoclave for at least 15 minutes at 121°C.
    2. Incinerate disposable material.
    3. Mix liquid waste with sodium hypochorite 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.
- Avoid contact and inhalation of TMB substrate solution. This solution is irritating to the skin, eyes and the respiratory system, and it may also cause sensitisation by inhalation and skin contact. TMB substrate solution, containing DMSO, provokes very rapid absorption by skin.
- Negative control and wash solution contain MIT/CAA as preservative. This solution may cause sensitisation by skin contact. Wear gloves and protective goggles.
- The kit contains 0.9N sulfuric acid as stop solution.
- Use of personal protective equipment is necessary: gloves and safety goggles when manipulating dangerous or infectious agents.
- Waste should be handled according to the institutions waste disposal guidelines. Also observe all federal, state and local environmental regulations

Summary of WHO prequalification assessment for INNOTEST HCV Ab IV

	Date	Outcome
PQ listing	15 February 2018	listed
Dossier review	N/A	MR
Site inspection(s) of quality management system	25 to 27 November 2014	MR
Laboratory evaluation of performance and operational characteristics	6 September 2017	MR

MR: Meets requirements
N/A: Not applicable

Prioritization for prequalification
Based on the established criteria, INNOTEST HCV Ab IV was given priority for WHO prequalification.

Product dossier assessment
In accordance with the WHO procedure for abbreviated (abridged) prequalification assessment, Fujirebio Europe NV was not required to submit a product dossier for INNOTEST HCV Ab IV as per the "Instructions for compilation of a product dossier" (PQDx_018 v1). Notwithstanding, certain aspects of the product dossier previously submitted for stringent regulatory review were reviewed by an assessor during the site inspection.

Commitments for prequalification:

1. Changes to the reporting of performance characteristics required to fully comply with labelling as per WHO Technical Guidance Series (TGS) - 5 "Designing Instructions for Use for IVDs."

Manufacturing site inspection
In accordance with the WHO procedure for abbreviated prequalification assessment, a shortened inspection with fewer inspectors was conducted at the site(s) of manufacture (Technologiepark 6 B-9052, Ghent, Belgium) of INNOTEST HCV Ab IV in 25 to 27 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 21 January 2015.

Commitments for prequalification:

1. Control material to be routinely tested for the presence of HIV, HCV and HBV with state of the art assays (nucleic acid testing) (PQ Commitment to be executed by August 2018).

Based on the site inspection and corrective action plan review, the quality management system for INNOTEST HCV Ab IV meets WHO prequalification requirements.

Laboratory evaluation

INNOTEST HCV Ab IV (Fujirebio) was evaluated by WHO in the second quarter of 2017 using serum/plasma specimens. A volume of 20 µl of specimen is needed to perform the assay. This type of assay requires laboratory equipment and cannot be performed in laboratories with limited facilities. Reading of the results must be performed with a spectrophotometer.

In this limited performance evaluation on a panel of 483 specimens, we found the performance as shown below

Performance characteristics in comparison with an agreed reference standard		
	Initial (95% CI)	**Final (95% CI)**
Sensitivity %	100% (97.8% - 100%)	100% (97.8% - 100 %)
Specificity %	99.4% (97.8 % - 99.9 %)	100% (98.9% - 100.00 %)
Invalid rate %	0%	
Inter-reader variability %	N/A	

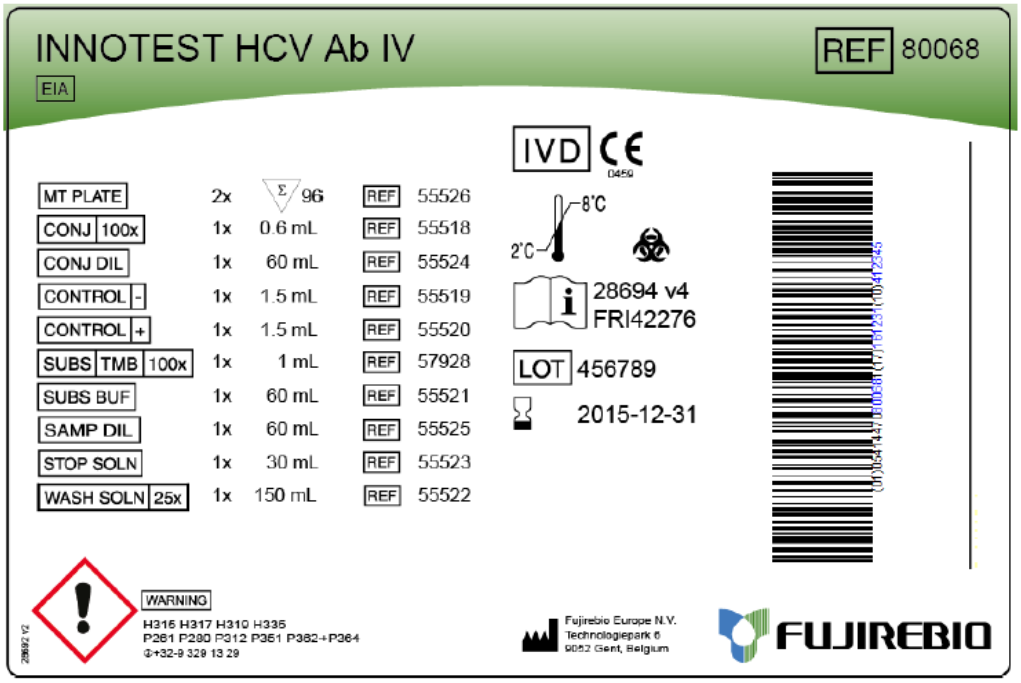
Additional performance characteristics	
Sensitivity during seroconversion on - 0.75 seroconversion panels in comparison with a benchmark assay; DiaSorin Murex Anti-HCV EIA (version 4.0)	Seroconversion sensitivity index of 0.75, therefore detection is 0.75 days earlier than the benchmark assay
Analytical sensitivity on a mixed titer panel in comparison with an agreed reference standard	15 of 15 specimens were correctly classified.
Lot to lot variation on a dilution panel in comparison with an agreed reference standard	Acceptable

Key operational characteristics	
Validated specimen types	Serum, plasma heparin, citrate or EDTA venous whole blood
Number of steps	12 with precision required
Time to result	179 minutes
Endpoint stability	15 minutes
Internal QC	Yes
In-use stability of reagents	If kept at 2-8 °C all test reagents, including the coated test wells, are stable until the expiry date given on the pack. Diluted wash solution is stable for 4 weeks if stored at 2-8 °C. Conjugate working solution is stable for 8 hours if stored at 18-30 °C. Substrate working solution is stable for 1 hour at 18-30 °C if stored in the dark.

Labelling

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

1. Labels


INNOTEST HCV Ab IV
EIA
REF 80068
IVD CE 0459
MT PLATE 2x Σ 96 REF 55526
CONJ 100x 1x 0.6 mL REF 55518
CONJ DIL 1x 60 mL REF 55524
CONTROL - 1x 1.5 mL REF 55519
CONTROL + 1x 1.5 mL REF 55520
SUBS TMB 100x 1x 1 mL REF 57928
SUBS BUF 1x 60 mL REF 55521
SAMP DIL 1x 60 mL REF 55525
STOP SOLN 1x 30 mL REF 55523
WASH SOLN 25x 1x 150 mL REF 55522
2°C 8°C
28694 v4
FRI42276
LOT 456789
2015-12-31
WARNING
H315 H317 H319 H335
P261 P280 P312 P351 P362+P364
Fujirebio Europe N.V.
Technologiepark 6
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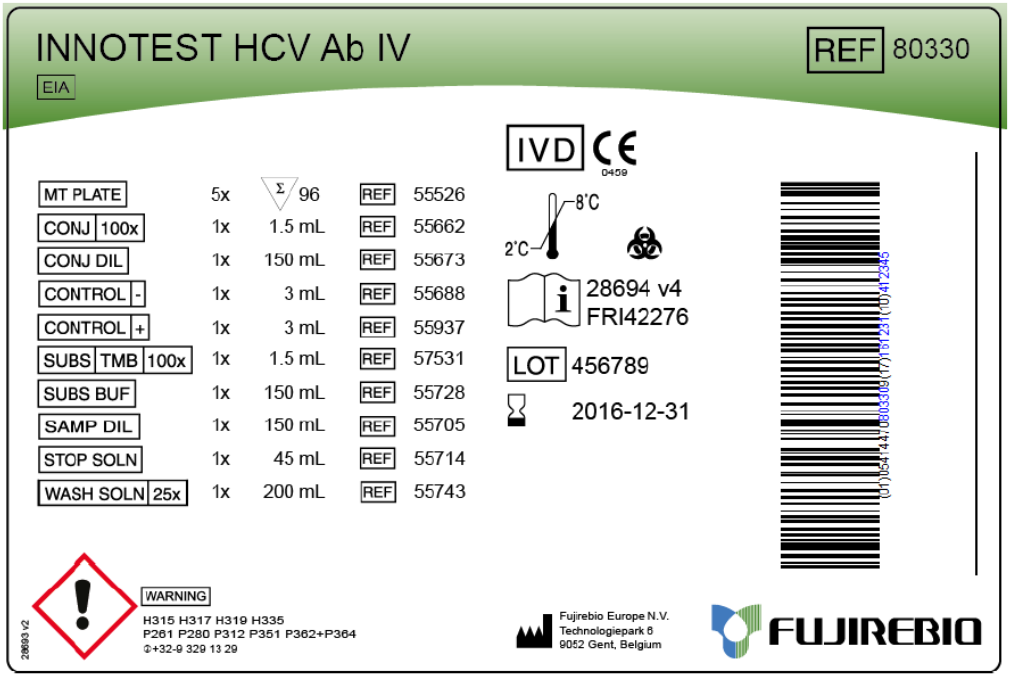
FUJIREBIO


INNOTEST HCV Ab IV
EIA
REF 80330
IVD CE 0459
MT PLATE 5x Σ 96 REF 55526
CONJ 100x 1x 1.5 mL REF 55662
CONJ DIL 1x 150 mL REF 55673
CONTROL - 1x 3 mL REF 55688
CONTROL + 1x 3 mL REF 55937
SUBS TMB 100x 1x 1.5 mL REF 57531
SUBS BUF 1x 150 mL REF 55728
SAMP DIL 1x 150 mL REF 55705
STOP SOLN 1x 45 mL REF 55714
WASH SOLN 25x 1x 200 mL REF 55743
2°C 8°C
28694 v4
FRI42276
LOT 456789
2016-12-31
WARNING
H315 H317 H319 H335
P261 P280 P312 P351 P362+P364
Fujirebio Europe N.V.
Technologiepark 6
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FUJIREBIO

2. Instructions for use

FUJIREBIO

KEY-CODE: **FRI58541**
80068/80330 INNOTEST HCV Ab IV
28694 v6
2018-01-16
p 1/12
English

INNOTEST HCV Ab IV

IVD CE 0459

Manufactured by:

Note changes highlighted

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8:00 – 17:00 GMT+1

M	T	W	T	F	S	S
☒	☒	☒	☒	☒	☐	☐

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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 100x	Conjugate 100x
CONJ DIL	Conjugate diluent
CONTROL -	Negative control
CONTROL +	Positive control
MT PLATE	Microtiter plate
SAMP DIL	Sample diluent

STOP SOLN	Stop solution
SUBS BUF	Substrate buffer
SUBS TMB 100x	Substrate TMB 100x
WASH SOLN 25x	Wash solution 25x
WARNING	Warning
EIA	Enzyme Immunoassay

Intended use

The INNOTEST HCV Ab IV is an enzyme immunoassay (EIA) for the detection of antibodies to human Hepatitis C virus (HCV) in human serum or plasma.

Test principle

The wells of polystyrene microplate strips are coated with a mixture of HCV synthetic peptides / recombinant proteins derived from different immunodominant regions: the Core (2 different epitope clusters), NS3, NS4A, NS4B, as well as the NS5A region. The antigens from these immunodominant regions are derived from different HCV genotypes (1a, 1b, 2, 3a).
The test sample is incubated in such a well. Virus-specific antibodies to HCV, if present in the sample, will bind to the solid-phase antigens. Subsequently, an affinity-purified rabbit anti-human IgG (H chain specific) labelled with the enzyme horseradish peroxidase (HRP) is added.

Upon a positive reaction this labelled antibody becomes bound to any solid-phase antigen/antibody complex previously formed. Incubation with enzyme substrate produces a blue color in the test well, which turns yellow when the reaction is stopped with sulfuric acid.

If the samples contain no HCV antibodies, then the labelled antibody cannot be bound specifically and only a low background color develops.

Reagents

Description, preparation for use and recommended storage conditions

- If kept at 2 to 8°C, all test reagents, including the coated test wells, are stable until the expiry date given on the pack.

Each pack contains:

1. 1 vial containing 1.5 mL (192T) or 3 mL (480T) of **negative control** (human serum containing 0.01% methylisothiazolone (MIT) and <0.1% chloroacetamide (CAA) as preservative).
2. 1 vial containing 1.5 mL (192T) or 3 mL (480T) of **positive control** (phosphate buffer containing antibodies to HCV, protein stabilizers, and Proclin 300 as preservative).
   NOTE**:** Precipitation may be seen in the positive control. This precipitation has no impact on test performance and results.
3. 2 sachets (192T) or 5 sachets (480T) containing a strip-holder with 12 x 8 HCV antigen-coated test wells (**plate**).
   A silicagel bag is added as dessicant.

After opening the aluminium foil bag containing the strips, any unused test wells will be stable for 8 weeks if stored at 2 to 8°C in the closed plastic minigrip bag with the silicagel.

4. 1 vial containing 60 mL (192T) or 150 mL (480T) **sample diluent** (phosphate buffer containing sodium chloride, Triton, protein stabilizers and Proclin 300 as preservative - purple colored buffer solution).
5. 1 vial containing 150 mL (192T) or 200 mL (480T) of concentrated **wash solution** (phosphate buffer containing 0.01% MIT and <0.1% CAA as preservative), to be diluted 1:25 with distilled or deionized water before use.

N° of tests	Wash solution(mL)		H_2O (mL)
1 x 96	40	+	960
5 x 96	200	+	4800

NOTE: Salt crystals may be formed in the concentrated wash solution after storage at 2 to 8°C. These crystals should be completely redissolved, by warming at 37°C, before dilution.

Diluted wash solution is stable for 4 weeks if stored at 2 to 8°C.

6. 1 vial containing 60 mL (192T) or 150 mL (480T) **conjugate diluent** (phosphate buffer containing protein and enzyme stabilizers and 0.05% Proclin 300 as preservative - green colored buffer solution).
7. 1 vial containing 0.60 mL (192T) or 1.5 mL (480T) of concentrated **conjugate 100x** (rabbit anti-human IgG (H chain) labelled with horseradish peroxidase), to be diluted 1:100 with conjugate diluent before use.

Number of tests	8	16	32	64	96	2 x 96	5 x 96
Conjugate (mL)	0.020	0.040	0.080	0.160	0.240	0.480	1.200
Conjugate diluent (mL)	2	4	8	16	24	48	120

Conjugate working solution is stable for 8 hours if stored at room temperature.

8. 1 vial containing 60 mL (192T) or 150 mL (480T) of **substrate buffer** (phosphate citrate buffer containing 0.006% hydrogen peroxide).
9. 1 vial containing 1 mL (192T) or 1.5 mL (480T) of concentrated **substrate TMB 100x** (tetramethylbenzidine (TMB) dissolved in dimethylsulfoxide (DMSO)), to be diluted 1:100 before use.

Number of tests	8	16	32	64	96	2 x 96	5 x 96
Substrate (mL)	0.020	0.040	0.080	0.160	0.240	0.480	1.200
Substrate buffer (mL)	2	4	8	16	24	48	120

NOTE: Concentrated Substrate TMB should be melted completely (melting point 18°C).

Substrate solution is stable at room temperature (18 to 30°C) for 1 hour if kept in the dark.

10. 1 vial containing 30 mL (192T) or 45 mL (480T) **stop solution** (0.9 N sulfuric acid).
11. 8 (192T) or 15 (480T) **adhesive plate sealers**.
12. 1 (192T) or 2 (480T) plastic **minigrip bag(s)** for storage of unused strips.

Materials required but not provided

- Distilled or deionized water.

- Precision pipettes with disposable tip to deliver in the ranges of 20 to 200 µL, and 200 to 1000 µL, respectively.
- Optionally a multichannel pipette to deliver 200 µL can be used together with disposable V-shaped troughs for addition of conjugate, substrate solution and stop solution.
- Microplate shaker.
- Incubator at 37°C.
- Microplate washer (alternatively, washing can be performed manually, e.g. by using a repeating syringe delivering 400 µL volumes and an aspirating device).
- Absorbent tissues.
- Photometric reading: microplate reader, equipped with a 450 nm filter and optional 620 nm filter.

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.](http://www.fujirebio-europe.com)

Warning

SAMP DIL | CONJ DIL | CONTROL +

Contains mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one

H317 P261 P280 P362+P364 P333+P313 P302+P352

SUBS | TMB | 100x

Contains dimethyl sulfoxide

H315 H319 H335 P280 P261 P305+P351+P338 P362+P364 P312 P302+P352

Hazard statements

H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.

Precautionary statements

P261	Avoid breathing mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352	IF ON SKIN: Wash with plenty of water/...
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P312	Call a POISON CENTER/doctor/…/ if you feel unwell.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash it before reuse

- Only adequately trained personnel should be permitted to perform the test procedure.
- Specimens 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 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.
  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 hypochorite 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.
- Avoid contact and inhalation of TMB substrate solution. This solution is irritating to the skin, eyes and the respiratory system, and it may also cause sensitisation by inhalation and skin contact. TMB substrate solution, containing DMSO, provokes very rapid absorption by skin.
- Negative control and wash solution contain MIT/CAA as preservative. This solution may cause sensitisation by skin contact. Wear gloves and protective goggles.
- The kit contains 0.9N sulfuric acid as stop solution.
- 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.

Specimens

- Serum or plasma, containing heparin, citrate and EDTA as anticoagulant, can be used. Please note there will be no SAM (Sample Addition Monitoring) color change of the sample diluent when using EDTA-plasma specimens.
- Specimens should be free of microbial contamination when tested.
- Additives may give erroneous results.
- Insoluble material should be removed from all samples by centrifugation before testing.
- Before storage, serum or plasma should be separated from blood clot or blood cells by centrifugation.
- Store the samples at 2 to 8°C. For storage longer than one week, freeze in aliquots at -20°C.
- Do not use any heat-treated specimens.

Remarks and precautions

- Do not use the kit beyond the expiry date.
- Do not mix reagents between kits, unless the components have identical lot numbers.
- Frozen reagents, eg. stored too close to cooling element, can cause erroneous results!
- All vessels used to prepare conjugate working solutions and substrate solutions must be cleaned thoroughly and finally rinsed with distilled water.

- Do not touch the top of the plates with your fingers to avoid contamination.
- Avoid microbial contamination of reagents.
- Ensure that the samples and controls are homogeneous before use.
- Use a new pipette tip for each specimen aliquoted.
- Ensure that specimen is added to the microwell. Failure to add specimen may produce an erroneous nonreactive result. Addition of specimens and controls to the microwells should be verified visually or by photometric reading.
- There will be no SAM (Sample Addition Monitoring) color change of the sample diluent when using EDTA-plasma specimens.
- To avoid contamination, do not touch the edges of the wells with the pipette tips when adding sample or conjugate.
- Do not expose substrate solution to strong light during incubation or storage. **Place the plate in the dark during the incubation of the substrate.** Substrate solution must be colorless when used. If the solution turns blue, it must be replaced.
- Re-use of the coated test wells results in erroneous results.
- Precipitation may be seen in the positive control. This precipitation has no impact on test performance and results.
- The kit should only be used by personnel trained in clinical laboratory practices.
- Do not use blood collection tubes for the preparation of the reagent working solutions.

Test procedure

Please read 'Remarks and precautions' before performing the test.

All test materials must be brought to room temperature (18 to 30°C) approximately 30 minutes before use and returned to the refrigerator (2 to 8°C) immediately after use. To avoid water condensation into the wells, the aluminum foil bag must be kept closed until the device is stabilized at room temperature.
Before starting the assay, adjust the temperature of the incubator to 37 ± 1°C.

1. Take the strip-holder with the required number of strips, ensuring that for one strip, one SAM control well, one negative and one positive control should be included; for more strips, at least one SAM control well, two negative and two positive controls should be included in each strip holder. During the test run, strips stay in the strip-holder and can be marked on one edge.
2. Add **200 µL** of **sample diluent** to each test well including the SAM control well.
3. Add **20 µL** of **specimen or control** to each appropriate test well, except to the SAM control well. A color change from purple to dark blue indicates that the specimen or control has been added to the microwell.
   The SAM color change can also be read photometrically at a wavelength of 620 nm:
   - Blank the reader on the SAM control well according to the instrument manufacturer's instructions.
   - Each control or specimen should exhibit a value of greater than or equal to 0.100.

     Make sure specimens and controls are adequately mixed with the sample diluent by pipetting up and down 5 times or by using a plate shaker at 1000 rpm for 1 minute.

4. **Cover** the strips with an adhesive sealer. **Incubate** for 60 ± 3 minutes at 37 ± 1°C.
   NOTE: Prepare conjugate working solution during incubation, see "Reagents".
5. **Wash** each well **6 times** (see "Directions for washing").
6. Add **200 µL** prepared **conjugate working solution** to each well including the SAM control well.
   A photometric read at a wavelength of 450 nm to document conjugate addition can be performed after addition of conjugate working solution to the microwell strips:
   - Do NOT blank the reader on the SAM control well.
   - Each control or specimen should exhibit a value of greater than or equal to 0.950.
7. **Cover** the strips with a new adhesive sealer. **Incubate** for 60 ± 3 minutes at 37 ± 1°C.
   NOTE: Prepare substrate solution during incubation, see "Reagents".
8. **Wash** each well **6 times** (see "Directions for washing").
9. Add **200 µL** prepared **substrate solution** to each well.
10. **Incubate** for 30 ± 1 minutes at room temperature in the dark.
11. To stop the reaction, add **50 µL stop solution** to each well in the same sequence and at the same time intervals as the substrate solution. Tap the strip holder carefully to ensure thorough mixing.
12. **Read** the absorbance of the solution in the wells within 15 minutes after step 11 at 450 nm with a microplate reader. **Do NOT blank the reader on the SAM control well.**

Directions for washing

Pre-rinse the washer with diluted wash solution.

Perform **manual wash** as follows:
- Aspirate completely the liquid from all wells by lowering an aspiration tip gently to the bottom of each well.
- Take care not to scratch the inside of the well surface.
- After each aspiration invert the plate and tap it dry on absorbent tissue.
- Fill the wells with 400 µL wash solution.
- Leave to soak for a minimum of 30 seconds, then aspirate the liquid.
- Perform the step 6 times.

In the absence of recommended washer or protocol, carry out **automatic washing** as follows: Perform 6 wash cycles ensuring that:
- The fill volume is 400 µL/well.
- The dispensing height is set to completely fill the well.
- The time taken to complete one aspiration/wash/soak cycle is approximately 30 seconds.

Incomplete washing will adversely affect the test outcome. Contamination of wash solution and washer can cause extensive problems. In case problems occur, desinfect the wash bottles and washer overnight with a 4% formaldehyde solution.

Results

Abbreviations:
P = mean of the absorbance of positive controls
S = mean absorbance of the test sample

Validation

Check the validity of individual negative and positive controls (absorbances at 450 nm).
- Each of the negative controls should be lower than 0.100.
- Each of the positive controls should be higher than 0.800.
- Calculate P eliminating controls under 0.800.

If more than half the number of controls have to be eliminated, the test run should be repeated after careful investigation into the source of errors.

Test result

Calculate the cut-off value as: (P/2.75).
A sample is NON-REACTIVE if S < (P/2.75).
A sample is REACTIVE if S ≥ (P/2.75).

IMPORTANT REMARK:
It is advised not to make a correction for a blank. This is because samples which are borderline positive before correction can become borderline negative afterwards. In this case, all OD values are lowered with the OD-value of the blank; the value of the cut-off is only lowered with the blank value divided by 2.75.

A sample reactive upon initial testing must be retested in duplicate before results interpretation.
A repeatedly reactive sample must be confirmed with an additional confirmatory test.

Limitations of the procedure

The INNOTEST HCV Ab IV assay procedure was designed to detect antibodies to HCV in human plasma and serum. Insufficient data are available to interpret tests performed on other body fluids. Therefore, testing of such specimens is not recommended.

A negative result with the INNOTEST HCV Ab IV does not preclude the possibility of exposure to, or infection by, the hepatitis C virus. Levels of antibodies to HCV may be undetectable in early infection. The presence of antibodies to HCV does not constitute a diagnosis of hepatitis C but may be indicative of recent and/or past infection by hepatitis C virus.

Test performance

Six external studies were performed in France (one study, in 5 sites, coordinated by The Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS) Paris), Germany (2 studies at Blood Bank Ulm and Max von Pettenkofer-Institut München) and Belgium (3 studies at the Blood Transfusion Center Antwerp, AZ-VUB Brussels, and CRI Ghent), respectively. Additional tests were performed internally. All tests were performed on human sera. The sensitivities and specificities given below were calculated against a reference test.

Specificity

A. A total of 8076 unselected donor samples were screened with INNOTEST HCV Ab IV at four blood transfusion centers and at Fujirebio Europe N.V. The data are presented in Table 1.

Table 1. Detection of antibody to HCV in serum samples from unselected blood donors

Study	N° samples tested	Repeatedly reactive on IT HCV Ab IV, negative on other screening and confirmation test	Repeatedly reactive on IT HCV Ab IV, reactive on other screening and confirmation test	Repeatedly reactive on IT HCV Ab IV, indeterminate on other confirmation test	Specificity (%)
France (2 sites)	2011	4	1	1	99.80% (2005/2009)
Belgium	2039	4	0	0	99.80% (2035/2039)
Germany	2024	4	0	0	99.80% (2020/2024)
Fujirebio Europe N.V	2002	4	0	0	99.80% (1998/2002)
Total	8076	16	1	1	99.80% (8058/8074)

The specificity of this test on samples from unselected blood donors is 99.80% (8058/8074).

B. A total of 280 potentially reactive samples were tested at two sites (AZ-VUB and Fujirebio Europe N.V), including HBV-, RF-, HTLV-, EBV-, syphilis-, and Toxo IgG-positive samples, samples from pregnant women, autoimmune patients and some with non-viral cirrhosis, and some hemolytic and lipemic samples. All samples were negative on INNOTEST HCV Ab IV. In addition, 793 out of the 794 clinical samples (CRI Ghent, AZ-VUB Brussels) which tested negative on other antibody-assays, were also negative on INNOTEST HCV Ab IV.

The overall **specificity** of INNOTEST HCV Ab IV is **99.80%** (9131/9148).

Sensitivity

A. At 4 independent centers (3 French and 1 German) and at Fujirebio Europe N.V., a total of 551 samples from patients infected with HCV were tested. All major HCV genotypes were covered in the sample set (Table 2).

Table 2. Genotype distribution of the tested HCV- positive samples

Genotype of tested samples	No. samples tested
1	142
2	35
3	37
4	31
4	17
4a	3
4 non-a	11
5	9
6	10
Mixed	1
Not determined	286
Total	**551**

All samples tested (551/551) were screening-positive for antibodies to HCV on INNOTEST HCV Ab IV.
A total of 365 samples, including 72 'same day' fresh samples, from patients in a clinical setting which were found to be HCV-positive with other antibody-tests, were tested. They all (365/365) tested positive using INNOTEST HCV Ab IV.
In total, 916 samples found to be positive for HCV antibodies , were typed positive using the INNOTEST HCV Ab IV (916 / 916), resulting in a sensitivity of 100% (with 95% CI [99.6,100%]).

B. The performance of the assay regarding sensitivity on seroconversion – and performance panels was assessed at different centers. A total of 33 seroconversion panels (20 commercial, 13 in-house panels) was tested. Of these 33 seroconversion panels, 22 started with a negative bleed and had narrow bleeding intervals. In addition, follow-up samples from 70 patients infected with HCV (locally recruited at 4 French centers) were evaluated on the assay. The INNOTEST HCV Ab IV showed on the panels evaluated a comparable sensitivity to other registered assays.
In conclusion, INNOTEST HCV Ab IV is a very sensitive screening assay for the detection of antibodies to HCV in serum.

The effect of different anticoagulants

In total, 50 samples from 25 paired HCV positive serum/EDTA-plasma couples, and 101 samples from 24 paired HCV negative serum/EDTA-plasma/citrate-plasma/heparin-plasma couples, 1 paired HCV negative serum/citrate-plasma couple and 1 paired HCV negative serum/EDTA-plasma/heparin-plasma couple, all spiked with strong HCV positive samples, were tested internally on INNOTEST HCV Ab IV. All 151 samples gave a positive test result. No differences were observed in results obtained from any set of samples or any type of plasma anticoagulant.

Precision

Inter-assay, intra-assay, and inter-lot variations were evaluated on a certain number of positive samples.

No significant variations (in OD-values or in S/N values) were observed between the different tests.

Trademarks

INNOTEST is a trademark of Fujirebio Europe N.V., registered in US and other countries.