code 03-05

CE 0123 IVD

AMPLIQUALITY HCV TYPE PLUS

Kit for the identification of Hepatitis C virus (HCV) genotypes 1 to 7 and subtypes a and b of genotype 1, by reverse transcription, PCR and Reverse Line Blot of the 5'UTR and the CORE region

USER MANUAL

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1 IDENTIFICATION OF THE DEVICE

The complete list of product codes to which these instruction for use (IFU) are intended for is reported below.

Code	Product	PKG
03-05-XX*	AMPLIQUALITY HCV TYPE PLUS	5 tests – manual format
03-05-20 M		20 tests – manual format
03-05-20 A		20 tests – automatic format

*sample code

This product complies with the Regulation (EU) 2017/746 on *in vitro* diagnostic medical device.

2 INTENDED PURPOSE

AMPLIQUALITY HCV TYPE PLUS is an *in vitro* diagnostic medical device intended to be used by professional users for the identification of Hepatitis C virus (HCV) genotypes 1 to 7 and subtypes a and b of genotype 1 in human EDTA plasma or serum from individuals with chronic HCV infection.

AMPLIQUALITY HCV TYPE PLUS is not intended for use as a screening test for the presence of HCV in blood or blood products or as a diagnostic test to confirm the presence of HCV infection. The device is intended for use in selecting individuals with chronic HCV infection for antiviral therapy and in determining the duration of therapy regimens according to the antiviral therapy prescribing information.

It is recommended to use this kit as indicated in the instructions herein.

3 KIT CONTENT

The minimum guaranteed quantities for each reagent (or reagent aliquot) are reported below

BOX RG

STORE AT -30°C/-20°C

DESCRIPTION	LABEL	CAP COLOR	03-05-XX (5 tests)	03-05-20 M (20 tests)	03-05-20 A (20 tests)
Master mix containing reagents for reverse transcription and PCR*	MASTERMIX HCV TYPE PLUS	Red	1 × 125 µL	2 x 125 µL	2 x 125 µL
Master mix containing enzymes for reverse transcription and PCR**	MULTIPLE ENZYME MIX	Violet	1 × 12,5 µL	1 × 50 µL	1 × 50 µL

*Active ingredients: primers (1.25 % v/v)
**Active ingredients : DNA polymerase (1X); RT-enzyme (1X)

BOX PC inside BOX RG

STORE AT -30°C/-20°C

DESCRIPTION	LABEL	CAP COLOR	03-05-XX (5 tests)	03-05-20 M (20 tests)	03-05-20 A (20 tests)
Positive control (DNA containing sequences corresponding to parts of the 5'UTR and the CORE region of HCV genotype 1a)	PC HCV PLUS	Blue	1 x 30 µL	1 x 30 µL	1 x 30 µL
RNA Internal control	IC RNA		1 x 125 µL	2 x 125 µL	2 x 125 µL

BOX R – manual format (03-05-20 M)

STORE AT +2°C/+8°C

DESCRIPTION	LABEL	IDENTIFICATION COLOR	03-05-20 M (20 tests)
Nitrocellulose strips with specific probes	HCV TYPE PLUS STRIP	Orange	20
Ready-to-use denaturation solution containing NaOH (< 2 %)	DEN 	White	1 x 800 µL
Ready-to-use hybridization solution	HYB-2	Red	1 x 100 mL
Conjugate diluent solution	CON-D1	Yellow	1 x 200 mL
Streptavidin - Alkaline Phosphatase conjugate #	CON	Yellow	1 x 15 µL
Ready-to-use rinse Solution	RIN	Green	1 x 200 mL
Substrate: ready-to-use NBT/BCIP solution	NBT/BCIP	Tinted bottle	1 x 50 mL
Ready-to-use stop solution	STOP	Light Blue	1 x 100 mL
Tray with 8 single-use incubation channels for hybridization and staining			3
Reading card for strip interpretation	Reading card		1
Strip collection sheet			2
CD-ROM containing			1

-HCV TYPE PLUS Strip Reader -User manual -Interpretation Table			

BOX R – automatic format (03-05-20 A)

STORE AT +2°C/+8°C

DESCRIPTION	LABEL	IDENTIFICATION COLOR	03-05-20 A (20 tests)
Nitrocellulose strips with specific probes	HCV TYPE PLUS STRIP		20
Ready-to-use denaturation solution containing NaOH (< 2 %)	DEN 	White	1 x 800 µL
Ready-to-use hybridization solution	HYB-2	Red	1 x 100 mL
Conjugate diluent solution	CON-D1	Yellow	1 x 200 mL
Streptavidin - Alkaline Phosphatase conjugate#	CON	Yellow	2 x 15 µL
Ready-to-use rinse Solution	RIN	Green	1 x 200 mL
Substrate: ready-to-use NBT/BCIP solution	NBT/BCIP	Tinted bottle	2 x 50 mL
Ready-to-use stop solution	STOP	Light Blue	1 x 100 mL
Reading card for strip interpretation	Reading card		1
Strip collection sheet			2
CD-ROM containing -HCV TYPE PLUS Strip Reader -User manual -Interpretation Table			1

BOX R – sample (03-05-XX)

STORE AT +2°C/+8°C

DESCRIPTION	LABEL	IDENTIFICATION COLOR	03-05-XX (5 tests)
Nitrocellulose strips with specific probes	HCV TYPE PLUS STRIP		5
Ready-to-use denaturation solution containing NaOH (< 2 %)	DEN 	White	1 x 800 µL
Ready-to-use hybridization solution	HYB-2	Red	1 x 100 mL
Conjugate diluent solution	CON-D1	Yellow	1 x 200 mL
Streptavidin - Alkaline Phosphatase conjugate#	CON	Yellow	1 x 15 µL
Ready-to-use rinse Solution	RIN	Green	1 x 200 mL
Substrate: ready-to-use NBT/BCIP solution	NBT/BCIP	Tinted bottle	1 x 50 mL
Ready-to-use stop solution	STOP	Light Blue	1 x 100 mL
Tray with 8 single-use incubation channels for hybridization and staining			1

Reading card for strip interpretation	Reading card		1
Strip collection sheet			1
CD-ROM containing -HCV TYPE PLUS Strip Reader -User manual -Interpretation Table			1

[#] Active ingredient: enzyme (0.025% v/v)

4 STORAGE AND STABILITY OF REAGENTS

Each component of the kit must be stored at the conditions indicated on the label of each box:

BOX RG	Store at -30°C/-20°C
BOX PC	Store at -30°C/-20°C
BOX R	Store at +2°C/+8°C

If stored at the recommended temperature, all reagents are stable until the expiration date indicated on the label.

If partially used, at the end of the protocol the solutions for Reverse Line Blot must be closed and stored at the correct storage temperature. Under these conditions, solutions can be used for a maximum of five (5) additional Reverse Line Blot assay sessions.

5 PRECAUTION FOR USE

- The kit must be used only as an IVD and be handled by qualified technicians who are trained in techniques of molecular biology applied to diagnostics.
- Before using the kit read the IFU carefully and completely.
- Keep the kit protected from light and heat.
- Please pay particular attention to the expiration date on the label of each box. Do not use any part of the kit past the expiration date.
- The reagents present in the kit must be considered an undividable unit. Do not use them separately or in combination with reagents from other kits or lots.
- The MASTERMIX HCV TYPE PLUS must be thawed at room temperature before use. Mix the solution by inverting the tube several times (do NOT vortex!), then centrifuge briefly.
- The MULTIPLE ENZYME MIX has to be stored at -30 °C/-20 °C. In order to avoid degradation, remove the Enzyme only before its use and return it to -30 °C/-20 °C immediately afterwards. Spin down the Enzyme before use. Do not use a vortex mixer.
- The positive and internal controls must be thawed at room temperature, mixed well and briefly centrifuged before use.
- Work quickly, particularly if preparing the reactions at room temperature. If possible, work on ice or on a cooling block.
- When using the assay make sure ambient temperature and air humidity remain in the range of +20°C to +25°C and not above 80%, respectively. A temperature and air humidity outside of these ranges may lead to invalid results. In that case the test has to be repeated.
- Avoid using instruments close to heat sources or cooling devices, since variation in temperature may impair the operation of instruments.
- The reagents, except DEN and NBT/BCIP solutions, are colorless. Any change in color may indicate degradation of the reagent.
- NBT/BCIP solution must not be exposed to direct light. It degrades quickly. The NBT/BCIP solution must have a deep yellow color and be free of precipitates.
- All reagents are odorless. Any change in odor may indicate degradation of the reagent.
- Do not cover the tray with nylon film or lids during any of the phases of the procedure.
- Store the stained and dried strips protected from light and at room temperature.

In case of any doubt concerning storage conditions or box integrity, please contact the technical support team at AB ANALITICA before use:

customersupport@abanalitica.it

Please observe the following precautions for the nucleic acid amplification:

- Use sterile filter tips and change the filter tip after every dispensation of liquid.
- Store biological samples, extracted RNA, PCR products, positive and internal controls included in the kit separate from MASTERMIX HCV TYPE PLUS and MULTIPLE ENZYME MIX.
- Do not swap the caps of reagent bottles or vials in order to avoid contamination.
- Set up pre- and post-PCR areas. Do not share instruments or consumables (pipettes, tips, tubes, etc.) between those areas.
- Change gloves frequently.
- Clean the work surfaces with 0.5% sodium hypochlorite and then with distilled water.
- For the cleaning of the GENEQUALITY X120 platform, refer to the user manual.

6 SAFETY RULES

6.1 General safety rules

- Wear disposable gloves when handling reagents and clinical samples. Wash hands after the procedure.
- Do not pipet by mouth.
- No known diagnostic method can assure the absence of infective agents. Therefore, consider every clinical sample to be potentially infectious and handle it accordingly.
- All devices that come into contact with clinical samples must be considered contaminated and disposed of as such. In case of accidental spilling of the samples, clean up with 0.5 % sodium hypochlorite. The materials you use to clean must be disposed of in special containers for contaminated products.
- Decontaminate and then dispose of clinical samples, materials and contaminated products.

For the safe disposal of the device, its accessories, and the consumables used with it, consider the following information:

- CER code: 18.01.03*
- Class: H 9
- Disposal operation: D10

6.2 Safety rules regarding the kit

Reagent of biological origin are included in MULTIPLE ENZYME MIX, CON, PC HCV PLUS, IC RNA, HYB-2, COND-1.

Risks associated with the use of this product relate to its single components.

Hazardous components:

DEN: contains NaOH < 2%

Warning:

Hazard statements: H290 May be corrosive to metals.
H315 Causes skin irritation.
H319 Causes serious eye irritation.

Precautionary and prevention statements:

P280	Wear protective gloves and clothing. Wear eye and face protection.
P264	Wash the hands thoroughly after use.
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.
P337+P313	If eye irritation persists: get medical advice.

The Safety Information Sheet (SIS) of the kit is available upon request.

7 MATERIAL REQUIRED, BUT NOT PROVIDED

7.1 Reagents

- Reagents for RNA extraction (see paragraph **Errore. L'origine riferimento non è stata trovata.**).
- DNAse- and RNAse-free sterile water.

7.2 Instruments

- Laminar flow cabinet.
- Micropipettes (range: 0.5-10 µL; 2-20 µL; 10-100 µL; 200-1000 µL).
- PCR Thermal cycler (ramp rate: 1.5°C/s and 2.7°C/s).
- Microcentrifuge (max 12-14.000 rpm).
- Electronic pipet aid (optional).
- Orbital shaker (only for manual strip development).
- Equipment for removing liquids from the hybridization trays (optional, only for manual strip development protocol).
- Equipment for automated strip development: Autoblot 3000H (*MedTec*), ProfiBlotT^{M} T48 (*Tecan Diagnostic*), DYNABLOT Heat (*DYNEX TECHNOLOGIES*).
- Equipment for manual strip development: Thermoshaker or thermal bath (Dubnoff) capable of maintaining 50°C ± 0.5°C. Recommended shaking speed: 80 rpm for the thermal bath and 250 rpm for the thermoshaker. The thermoshaker must be large enough to accommodate a tray of 8.5 cm × 12 cm × 1.7 cm.

7.3 Disposables

- Talc free disposable gloves.
- Disposable sterile filter tips (range: 0.5 - 10 µL, 2 - 20 µL, 10 - 100 µL, 200 - 1000 µL).
- DNAse- and RNAse-free PCR tubes with safe-lock cap.
- Graduated sterile pipettes for use with pipet aid (optional).
- 50 ml Falcon® type tubes for the dilution of CON solution.
- Tweezers.

For further information, please contact the technical support team at AB ANALITICA.

8 TEST PRINCIPLE

The AMPLIQUALITY HCV TYPE PLUS is a qualitative kit based on multiplex RT-PCR (reverse transcription and PCR with biotinylated primers) coupled with Reverse Line Blot technique.

The polymerase chain reaction (PCR) method (Saiki *et al*., 1985) can be defined as an in vitro amplification reaction of a specific part of DNA by a thermostable DNA polymerase. Multiplex Polymerase Chain Reaction allows the simultaneous analysis of gene polymorphism in many different specimens. The main features of this technique are speed and specificity of the method and reduction of cross-over contamination and thus false-positive results compared with traditional PCR (Coiras *et al*., 2007).

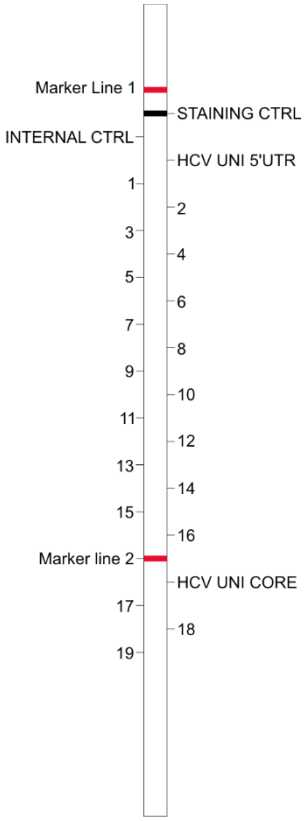
To analyze an RNA sample, the Reverse Transcription PCR (RT-PCR) is necessary. In RT-PCR, the RNA template is first converted into a complementary DNA (cDNA) using a reverse transcriptase. The cDNA is then used as a template for exponential amplification using PCR (Halford *et al*., 1998). This process can be achieved as either a one-step or a two-step reaction. In the one-step approach, the entire reaction from cDNA synthesis to PCR amplification occurs in a single tube, thus minimizing experimental variations. (Wacker & Godard 2005).

The Reverse Line Blot protocol is then performed, where the PCR products are denatured and hybridized to oligonucleotide probes that are bound to strips and that recognize specific sequences of the viral genome. Strips are then incubated with a solution that contains streptavidin-alkaline phosphatase conjugate, which binds to the biotin-marked amplicons, and subsequently with a chromogenic substrate of the enzyme. As a consequence, amplicons are stained, revealing positive bands.

9 PRODUCT DESCRIPTION

AMPLIQUALITY HCV TYPE PLUS kit enables the identification of hepatitis C (HCV) genotypes 1-7 and subtypes a, b of genotype 1; this is possible by the simultaneous analysis of the 5'UTR and CORE regions. The AMPLIQUALITY HCV TYPE PLUS kit includes a ready-to-use master mix for reverse transcription and PCR that requires the addition of the MULTIPLE ENZYME MIX and the RNA extract.

The master mix contains the dUTP/Cod UNG system that prevents carry over contaminations in the RT-PCR. The Cod UNG enzyme degrades any single or double stranded DNA containing uracil without affecting RNA. The enzyme merely requires 5 minutes at room temperature directly after addition of the RNA extract and is irreversibly deactivated by heat during the RT-PCR cycles.



The kit includes an internal control (IC RNA), which is to be added to every sample during the RNA extraction. This internal control is reverse transcribed together with the viral RNA during the RT-PCR. Emergence of the internal control band (INTERNAL CTRL) on the strip indicates that the RNA-extract is suited for analysis.

The kit also includes a positive control for PCR and genotyping. This control contains DNA sequences corresponding to the 5'UTR and the CORE region of HCV of genotype 1a.

The strips of AMPLIQUALITY HCV TYPE PLUS contain two red Marker Lines that facilitate correct positioning of the reading card on the strips (see Fig.1). Each strip has nineteen (19) bands with probes specific for the different HCV genotypes (HCV 1 to 7) and four (4) control bands.
The control bands are here described:

a) staining control band (STAINING CTRL), which allows the user to assess the staining reaction (substrate conversion).
b) internal control band (INTERNAL CTRL) recognizes the internal control (IC RNA), thus permitting assessment of the quality of the RNA extract, which provides indications concerning successful completion of the reverse transcription. Absence of this band, while other specific bands on the strip are not or only weakly visible indicates that the analysis of this sample may produce false-negative results.
c) The control band HCV UNI 5'UTR shows the presence of a PCR product from the 5'UTR.
d) The band HCV UNI CORE shows the presence of a PCR product from the CORE region. This band only needs to be considered in case that the genotype-specific bands for the 5'UTR indicate that the sample is positive for HCV of genotype 1 (refer to section 12.2).

The nineteen (19) HCV specific bands are divided in the following way:
Sixteen (16) bands (position 1 to 16) contain probes that recognize sequences of the 5'UTR and three (3) bands (position 17 to 19) contain probes that recognize sequences in the CORE region.
The band patterns are interpreted overlaying the reading card with the strip and comparing the band pattern with the included Interpretation Table. HCV TYPE PLUS Strip Reader software is available as an auxiliary tool to support the operator in the analysis (see par. 12.3).

10 SPECIMEN COLLECTION, HANDLING AND PREPARATION

The blood must be treated with EDTA. Other anticoagulation agents, like heparin, are strong inhibitors of the Taq polymerase enzymatic activity and may impair the PCR.

Extract the plasma from whole blood as soon as possible and not later than 4 hours after collection of the blood sample (Tuck *et al*., 2009).

Plasma samples are stable up to 8 hours at room temperature, up to 30 hours at +2°C/+8°C and up to 6 months if frozen at -30°C/-20°C or at -80°C/-70°C.

Serum samples are stable up to 5 days at +2°C/+8°C and up to 6 months if frozen at -30°C/-20°C or at -80°C/-70°C. It is advised to avoid storing them at room temperature.

Extracted viral RNA samples are stable up to 2 months at -80°C/-70°C. It is not recommended to store them at room temperature, at +2°C/+8°C and at -30°C/-20°C.

Repeated freeze–thaw cycles compromise nucleic acid integrity and should be avoided. For all sample types, it is recommended to aliquot into small volumes to avoid multiple freeze–thaw cycles. Plasma samples, serum samples and extracted viral RNA samples can undergo up to one freeze-thaw cycle.

As all samples are potentially infectious, make sure that they are treated according to the guidelines of good laboratory practice.

11 PROTOCOL

11.1 RNA Extraction

The AMPLIQUALITY HCV TYPE PLUS kit has been validated using the following extraction systems:

- GENEQUALITY X120 Pathogen kit (*AB ANALITICA*),
- QIAamp MinElute Virus Spin (*QIAGEN*).

For further information concerning compatible extraction methods, please contact the technical support team at AB ANALITICA.

11.2 Internal control

The AMPLIQUALITY HCV TYPE PLUS kit includes an RNA internal control (IC RNA), which allows the user to monitor extraction, reverse transcription and amplification by PCR and facilitates detection of PCR inhibition.

The internal control is to be added during the RNA extraction. The control was standardized for use of 10 µL per sample and an elution volume of 60 µL.

If the extraction system requires a different elution volume, use an accordingly adjusted volume of internal control.

For correct use of the internal control follow the instructions of the extraction system manufacturer. Please keep in mind that different extraction systems may require the internal control to be added in a different phase/step of the extraction procedure.

For further information, please contact the technical support team at AB ANALITICA.

Attention: The internal control is sensitive to physical state changes: it should NOT undergo more than three (3) freeze/thaw cycles. If performing runs with low numbers of samples, it is recommended to aliquot the reagent beforehand.

11.3 Preparing the RT-PCR

Once you have thawed the MASTERMIX HCV TYPE PLUS, homogenize it by inverting the tube(s) several times – do NOT vortex! Then centrifuge it briefly.

The MULTIPLE ENZYME MIX must be kept at its storage temperature (-20°C to -30°C) until use and returned to this temperature immediately after use. Prolonged exposure to higher temperatures may lead to degradation of the reagent.

If possible, work on ice or on a cooling block.

Prepare a pre-reaction master mix according to the following table. Make sure to prepare a volume sufficient for all samples you want to analyze as well as the positive and negative control. Also include at least one dead volume to compensate for loss of volume during pipetting.

Reagent	1 Rx
MASTERMIX HCV TYPE PLUS	12.5 µL
MULTIPLE ENZYME MIX	2.5 µL
Total volume	15.0 µL

Homogenize the pre-reaction master mix by inverting the tube several times – do NOT vortex! Then centrifuge it briefly.

Pipet 15 µL of the pre-reaction master mix into each PCR tube.

Add the extracted RNA, the positive control and the negative control to the corresponding tubes according to the following table.

Extracted RNA	10 µL of extracted RNA
Positive control	5 µL of PC HCV PLUS + 5 µL of DNase/RNase-free water
Negative control	10 µL of DNase/RNase-free water

Make sure you mix and pipet with care. Avoid the formation of bubbles in the tube and briefly centrifuge the PCR tubes before starting the reaction.

Attention:
The MASTERMIX HCV TYPE PLUS is sensitive to physical state changes: it should NOT undergo more than three (3) thaw/freeze cycles. If performing runs with low numbers of samples, it is recommended to aliquot the reagent beforehand.

The positive control is sensitive to physical state changes: It should NOT undergo more than five (5) freeze/thaw cycles. If performing runs with low numbers of samples, it is recommended to aliquot the reagent beforehand.

11.4 Activation of dUTP/Cod UNG and RT-PCR

Before starting the RT-PCR, incubate the PCR tubes with the reaction mixes at 25 °C to 28 °C for 5 min, to activate the enzyme Cod UNG.

Then start the amplification using the following thermal profile.

Number of cycles	Temperature	Time
1 cycle	48 °C	30 min
1 cycle	95 °C	10 min
45 cycles	95 °C 60 °C	30 s 90 s
-	10 °C	∞

If the visualization of the PCR products (Reverse Line Blot) is done on the same day, you can store the samples at +2 °C to +8 °C. Otherwise store the samples at -30 °C to -20 °C until use.

11.5 Visualization by Reverse Line Blot

11.5.1 Procedural notes and preliminary steps

- Before use, leave reagents and strips at room temperature for approx. 30 minutes. After use, store the reagents in the fridge.
- Heat the thermal bath/thermoshaker to 50°C and make sure that the temperature does not deviate by more than 0.5°C during the entire process.
- The room temperature should be in the range of +20°C to +25°C. Use a calibrated thermometer and scrupulously control the temperature.
- Preheat the HYB-2 and CON-D1 solutions to 50°C ± 0.5°C.
- If you use a thermal bath, make sure that the incubation tray does not slide. If necessary, fix it with weights.

- Use tweezers to manipulate the strips. Do not touch them with your hands, since the oily film on the skin can interfere with hybridization and/or staining.

11.5.2 Denaturation

- Take out the strips using tweezers, put on clean surface. Number the strips above the Marker line 1 with a pencil.
- Using the tweezers place each strip in a channel of the tray with the Marker Lines facing up.
- Add 20 µL of denaturation solution (DEN) as a single drop to one end of each channel.
- Add 10 µL of PCR product and mix by pipetting.
- Incubate for 5 min at room temperature.

11.5.3 Hybridization

- Add 2 mL of preheated HYB-2 solution to each channel with a strip. Make sure the strips are completely immersed.
- Place the tray in a thermoshaker or thermal bath preheated to 50°C ± 0.5°C. If a water bath is used, make sure the tray does not float. The water of the thermal bath MUST NOT enter the tray.
- Incubate for 60 minutes, shaking the tray gently (max. 250 rpm on a thermoshaker or max. 80 rpm in a thermal bath).

11.5.4 Colorimetric visualization

- Shortly before the end of incubation, prepare the dilution of CON solution (streptavidine - alkaline phosphatase conjugate) as follows:
  For N samples mix in a Falcon® type tube:
  - N × 0.5 µL of CON solution
  - N × 2 mL of CON-D1 solution (preheated)
- Aspirate all the hybridization liquid from the channels and add 2 mL of preheated CON-D1 solution, place the tray in a thermoshaker or thermal bath at 50 °C ± 0.5 °C for 2 minutes, shaking gently.
- Aspirate the CON-D1 solution completely and add 2 mL of the previously diluted CON solution, place the tray in a thermoshaker or thermal bath at 50 °C ± 0.5 °C for 15 minutes, shaking gently.
- After incubation with the conjugate, aspirate all liquid and wash with 2 mL of RIN solution. Incubate at room temperature for 2 minutes, shaking the tray gently.
- Aspirate all liquid and wash again with 2 mL of RIN solution at room temperature for 2 minutes, shaking the tray gently.
- Empty the tray and add 2 mL of the ready-to-use NBT/BCIP solution.
- Incubate at room temperature in the dark for 5 minutes, shaking the tray gently.
  Please note, a prolonged incubation may increase the staining background, which may interfere with the interpretation of results.
- Empty the tray and stop the staining reaction by washing with 2 mL of STOP solution for 2 minutes.
- Remove the strips from the tray using tweezers and let them dry between two pieces of paper towel.

Make sure the strips have dried completely before proceeding to the interpretation of band patterns.

Store the developed and dried strips away from light.

NOTE: If you use an automated system for developing the strips, please contact the technical support team at AB ANALITICA to receive the appropriate preparation and assay protocol.

12 ANALYSIS OF RESULTS

Attach the strips to the strip collection sheets provided with the kit. Use the Marker Lines as reference.

To perform interpretation of the results, overlap the Reading card included in the kit on each strip by aligning the staining control band on the film with the one present on the strip.

12.1 Validation of the analytical run

The staining control band must always be visible, as it shows that the staining reaction worked correctly. The intensity of the staining control band should be comparable for all strips that were developed in the same session.

A band can be considered positive if it appears in gray/brown color at the end of the staining procedure. Intensity and color of the bands may vary, even on the same strip.

Before you begin with the analysis of the strips of your samples, check the strips of the positive and the negative control of that session:

1. The negative control should show only the staining control band and no other bands.
2. The positive control (PC HCV PLUS), which corresponds to HCV genotype 1a, should show the following bands: Staining control band; HCV UNI 5'UTR band; bands 1, 2 and 5; HCV UNI CORE band; band 17.

   Note: A faint signal for band 19 can be visible.

If one of the two controls do not show the results as described above, the session is to be considered unsuited for analysis and has to be repeated.

If the internal control (IC RNA) was added during the extraction, the INTERNAL CTRL band must be visible. Absence of the INTERNAL CTRL band indicates that the RNA extract is of suboptimal quality.

12.2 Interpretation of the results

Once attached the strips to the strip collection sheets (see par. 12), the band patterns on the strips have to be interpreted by using the Interpretation Table. HCV TYPE PLUS Strip Reader software is available as an auxiliary tool to support the operator at this stage.

When interpreting the results, take the following into consideration:

- Part A of the Interpretation Table is dedicated to the identification of genotype1, subtypes a and b, and of genotype 6 using the information from both, the 5'UTR and the CORE region.
- Part B of the interpretation Table is to be used for identification of genotypes HCV 2, 3, 4, 5 and 7 according to the information from the 5'UTR.
- If the strip shows any of the 5'UTR band patterns that correspond to the patterns described in table A (HCV genotype 1 or 6), proceed to the analysis of the CORE region band patterns and base the genotyping solely on this result.
- If there are no bands for the CORE region or the results for the CORE region are inconclusive, genotype 1 and 6 cannot be reliably distinguished and identification of HCV genotype 1 has to be based on information provided by the 5'UTR. If that is the case, make sure you note in the diagnostic report that only the 5'UTR was used for the analysis and therefore information on subtypes is not available and presence of HCV 6 cannot be excluded.
- If the 5'UTR band pattern on a strip corresponds to part B of the interpretation table (HCV genotype 2, 3, 4, 5, or 7), analyze only the pattern for the 5'UTR and ignore any bands for the CORE region.
- Each band pattern in the interpretation table was assigned a unique alphanumeric code (last row of the table). The first letter of this code corresponds to the gene region: "u" for the 5'UTR (e.g. u1, u2, u23) and "c" for the CORE region (e.g. c1, c2). The purpose of this code is to facilitate distinction of band patterns that correspond to the same genotype. Please note, the digits in the code do NOT correspond to the genotype.

If a strip shows a band pattern that is not listed in the interpretation table, this strip cannot be analyzed. In such a case, the sample may need to be genotyped using an alternative method, e.g. sequencing. Please contact the technical service at AB ANALITICA:

customersupport@abanalitica.it

12.2.1 HCV TYPE PLUS Strip Reader software

The HCV TYPE PLUS Strip Reader software is an auxiliary tool for exclusive use with the AMPLIQUALITY HCV TYPE PLUS kit.

HCV TYPE PLUS Strip Reader can produce a report containing the patient data corresponding to the sample and the information concerning the current analysis session, if available. In addition, the software allows to archive the strip in the dedicated field. The output data from the software should not be regarded as diagnostic data without operator approval.

12.2.1.1 Installation of the software

The following table lists the system requirements for installation of the software.

System requirements for HCV TYPE PLUS Strip Reader software	
Hard Disk	20 GByte free
RAM	Minimum 1 GByte
Operating system	Windows 10 or newer

The installation requires administrator access to the system.

The installation will start automatically when you insert the CD into the CD/DVD drive of the PC. Alternatively, the installation procedure can be initiated by opening/starting the file `setup.exe`, which can be found on the installation CD.

12.2.1.2 Use of the software

Start the HCV TYPE PLUS Strip Reader software.

A mask of a strip appears that shows the position of all probes/bands on the strip.

Cover the strip with the reading card aligning the Marker Lines on the reading card with those of the strip and check which bands are present.

In the mask on the screen select the bands that are visible in the strip. The selected bands on the screen should turn blue. When the pattern on the screen corresponds to the band pattern on the strip, click `EVALUATE`

The word ANALYSIS and the analysis result (genotype and possibly subtype) appear in the upper text field to the right of the strip mask.

The text field below contains the words EVALUATION DETAILS and information regarding the analyzed viral region (5'UTR and/or CORE) and the ID (alphanumeric code) of the identified band pattern.

The software does also check the presence or absence of the control bands and evaluate the quality of the RT-PCR.

Via the menu `NOTES` you can access explanations of the error and warning messages (see Tab. 1) of the software that appear when the software does not recognize a band pattern or detects contradicting results.

Verify and validate the results comparing the bands on the strip with the band pattern identified by the software.

If you want to print a report of the results, select PRINT from the menu FILE . This report will contain the analysis result, the patient data corresponding to this sample and the information concerning the current analysis session you may have entered. In addition, the software allows you to archive the strip in the dedicated field.

In order to analyze the next sample, click RESET . This will clear the strip mask and the text fields.

Tab 1. Explanation of software messages

Software message	Interpretation	Action
ATTENTION: SIGNAL OF INTERNAL CONTROL ABSENT	The software reports that the band of the Internal Control is absent. **CASE 1:** The band is absent because no IC RNA was added to the sample during the RNA extraction. **CASE 2:** The IC RNA was added to the sample during extraction, but the corresponding band on the strip is missing.	Take the appropriate action according to the problem/case. **CASE 1**: It is not possible to determine whether the sample is suitable or the efficacy of the RT-PCR. However, if the band pattern on the strip can be interpreted, it is possible to proceed with the analysis. **CASE 2:** Absence of the IC RNA band is an indication for suboptimal quality of the RNA extract or inhibition of the RT-PCR. It is advisable to repeat extraction and analysis of this sample.
SUBTYPE CANNOT BE DETERMINED	In absence of bands for the CORE region HCV subtypes 1a and 1b cannot be discriminated. Genotyping is based solely on analysis of the 5'UTR. In addition, presence of the HCV 6 subtypes c-v cannot be excluded.	Report presence of HCV genotype 1, adding a note that genotyping was based solely on analysis of the 5'UTR and presence of HCV 6 subtypes c to v cannot be excluded. Possibly, try to repeat the analysis using a fresh extract that has been eluted in a smaller volume (in order to facilitate amplification on the CORE region). If the signal is still absent, use an alternative method (e.g. sequencing) to determine the subtype. Please note, the signals for probes 17 and 18 (CORE region) may be absent in patterns u12 and u22. In this case, these patterns may correspond to genotype 1 (subtype different from subtype 1a and 1b). In this case an alternative method (e.g. sequencing) is required to determine the subtype.

Software message	Interpretation	Action
PATTERN OF 5'UTR REGION NOT RECOGNIZED	The strip presents an unknown pattern for the 5'UTR (pattern not included in the interpretation table of AMPLIQUALITY HCV TYPE PLUS).	Check if the pattern on the strip was correctly entered into the software (make sure the bands on the strip and in the software correspond). If the problem persists, contact the technical support at AB ANALITICA.
PATTERN OF CORE REGION NOT RECOGNIZED	The strip presents an unknown pattern for the CORE region (pattern not included in the interpretation table of AMPLIQUALITY HCV TYPE PLUS).	Check if the pattern on the strip was correctly entered into the software (make sure the bands on the strip and in the software correspond). If the problem persists, contact the technical support at AB ANALITICA.
ATTENTION! BAND PATTERNS DO NOT MATCH	**CASE 1:** The number and type of bands on the strip indicate a possible cross-contamination (e.g. all CORE region bands are present).	Repeat the analysis starting from a new RT-PCR. If the problem persists, repeat the analysis starting with a new RNA extraction. If the problem persists, contact the technical support at AB ANALITICA.
	CASE 2: The number and type of bands on the strip could be compatible with a possible mixed infection.	We suggest to repeat the analysis starting from a new RT-PCR, in order to exclude the possibility of a cross-contamination event. If the result is confirmed, there is the possibility of a mixed infection. The software HCV TYPE PLUS Strip reader is not able to identify mixed infections and the performance of the assay for the detection of coinfections has not been assessed. Further investigations with other molecular techniques are required: DNA sequencing may provide additional information.

13 TROUBLESHOOTING

Bands weak or absent (including staining control band)

- *No or not enough conjugate or substrate used.*

► Repeat the visualization run by using the correct volumes of solutions as required by the protocol.

► If you use an automated system for developing the strips, verify the accuracy of the protocol and to have prepared the suitable quantity of solutions.

- *The substrate has degraded due to prolonged exposure to light and heat.*

► Store correctly the substrate solution, protected from light and heat.

Staining not homogenous

- *The strips were not completely immersed during the incubation phases.*

► Repeat the visualization run. Make sure that strips are completely immersed during each phase of protocol.

► Use the suitable volume of each solution required by the protocol.

► If you use an automated system for developing the strips, verify the accuracy of the protocol and to have prepared the suitable quantity of solutions.

- *The tray with the strips was not sufficiently agitated during hybridization.*

► Repeat the visualization run by using the recommended shaking speed, as indicated in the paragraph 11.5.

► If you use an automated system for developing the strips, verify the accuracy of the protocol.

Bands very weak or absent in presence of the staining control band

- *Extracted RNA is not* suitable for reverse transcription and/or amplification and the reaction is inhibited.

► Make sure to perform the nucleic acid extraction correctly.

► If an extraction method uses wash steps with solutions containing ethanol, make sure no ethanol residue remains in the extracted RNA.

► If present, be sure to remove the magnetic beads present in the eluate.

► Use one of the extraction systems listed in Appendix 1.

► Make sure to correctly store extracted RNA prior to using it.

- *The clinical sample is not suited for analysis*

► Make sure the clinical samples are correctly stored before performing the assay.

- *The thermal profile used is different from that reported in this user manual.*

► Check the instrument programming and then repeat the PCR session. Pay particular attention to the thermal profile.

- *The reaction mix did not work correctly.*

► Make sure the MASTERMIX HCV TYPE PLUS has been stored at -30°C/-20°C. Avoid unnecessary thaw/freeze cycles.

► Make sure the MULTIPLE ENZYME MIX has been stored at -30°C/-20°C and returned it to -30°C/-20°C immediately after use, in order to avoid degradation.

► Do not use the kit past the expiration date reported on the label.

- *Hybridization and incubation temperatures were not set correctly.*

► Repeat the visualization run by setting the temperature, as reported in the paragraph 11.5.

► Verify that the instrument used for the reverse line blot protocol could reach and maintain the temperature reported in the paragraph 11.5, within a range of ± 0.5°C.

Very weak bands pattern for the positive control

- *The positive control was not stored correctly and is degraded*

► Make sure to store the positive control at -30°C to -20°C.

► Do not use the product past the expiration date.

Unexpected results

If all strips of the genotyping run show abnormal staining (e.g. high background staining, non-specific results, etc.), one of the following problems may have occurred:

- *The incubation temperature was not correct.*

► Repeat the visualization run by setting the temperature as reported in the paragraph 11.5.

► Verify that the instrument used for the reverse line blot protocol could reach and maintain the temperature reported in the paragraph 11.5, within a range of ± 0.5°C.

- *Solution HYB-2 and/or solution CON-D1 were not properly preheated.*

► Repeat the visualization run by accurately preheating the HYB-2 and CON-D1 solutions, as indicated in the paragraph 11.5.

- *Contamination from neighboring incubation channels due to spilling during the initial washing steps.*

► Carefully dispense and aspirate the solutions during the first steps of incubation and washing. Change filter tip at each step of dispensation/aspiration.

In case of further problems or questions, please contact the technical support team at AB ANALITICA:

customersupport@abanalitica.it

fax (+39) 049-8709510

tel. (+39) 049-761698

14 DEVICE LIMITATIONS

The kit can have reduced performance if:

- the clinical sample is not suitable for this analysis (blood treated with anticoagulant other than EDTA, for example heparin);
- the extracted RNA is not suitable for RT-PCR (due to the presence of PCR inhibitors or to the use of an inappropriate extraction method);
- the kit was not stored correctly.

As for any other diagnostic device, even if it has been reduced to the minimum, there is a residual risk of obtaining invalid/false positive/false negative results; this risk cannot be eliminated or further reduced.
This genotyping test functions only within the limits of the genomic region in which the probes were selected. Thus, sequencing may complete the analysis in the uncertain cases.
Moreover, the presence of possible mutations/polymorphisms in the region of pairing of the probe and/or of the primers could compromise the genotyping of pathogenic RNA. The product is an auxiliary tool for the genotyping of HCV and therefore the obtained results have to be interpreted taking into account all clinical data and other laboratory tests related to the patient. In addition, please consider that verification and validation by qualified personnel of the output data from the provided software HCV TYPE PLUS Strip Reader have to be performed for all sample results.

If information from the HCV CORE region on the strip is not available, it is not possible to identify the subtypes 1a or 1b of genotype 1.

The assay is not able to identify the subtype d of the genotype HCV 6.

The assay is not able to identify the recombinant HCV 2k/1b, which is typed as genotype HCV 2

In rare cases, uninterpretable patterns may appear. This may be due to sequence heterogeneity of the HCV genome, mixed infections or cross contaminations, recombinant HCV isolates (Kalinina *et al*., 2002).

15 DEVICE PERFORMANCE

The Summary of Safety and Performance (SSP) is stored as part of the technical documentation of the device and, until EUDAMED is fully functional, is made available to the public upon request.

15.1 ANALYTICAL PERFORMANCE

15.2 ACCURACY

15.2.1 Precision of measurement

Precision is commonly evaluated by performing a replication experiment to observe the variability in results generated under the normal operating conditions in the laboratory. For qualitative genotyping assays, precision can be expressed as a percentage by dividing the number of repeated results in agreement by the total number of results.
Precision of the AMPLIQUALITY HCV TYPE PLUS kit was tested using 6 RNA samples of INSTAND-EQAS positive for HCV 1a, HCV 1b, HCV 2, HCV 3a, HCV 4a, HCV 5a. Each sample was tested in 2 replicates for each run, for a total of 240 replicates. Testing was planned in a between-day format, with a total of 20 operating days to perform all the runs, one run per day. Other significant sources of variation, such as different operators and instruments were introduced. The obtained results allowed to calculate the total precision for the AMPLIQUALITY HCV TYPE PLUS kit overall for genotypes 1 – 5, as shown below:

HCV Genotype	Total number of replicates	Total number of replicates in agreement	Percent results in agreement %
1a	40	40	100 (40/40)
1b	40	40	100 (40/40)
2	40	40	100 (40/40)
3a	40	40	100 (40/40)
4a	40	40	100 (40/40)
5a	40	40	100 (40/40)
Overall	**240**	**240**	**100 (240/240)**

On a total of 240 tested samples, the percentage of results in agreement is 100%. No differences in the data were observed between genotypes, operators or instruments.

15.3 ANALYTICAL SPECIFICITY

15.3.1 Cross reactivity

Cross-reactivity of AMPLIQUALITY HCV TYPE PLUS was assessed by a primer alignment tool (i) in GenBank and by an experimental study (ii).

(i) The tool “Nucleotide BLAST” (Basic Local Alignment Search Tool) available on the website http://blast.ncbi.nlm.nih.gov/Blast.cgi, revealed the absence of non-specific pairing for Dengue virus, Zika virus, Human Immunodeficiency virus (HIV) and Hepatitis B virus (HBV).

(ii) The experimental study was performed in singlicate with human samples containing following pathogens, infecting plasma district: Epstein-Barr virus (EBV), Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2), Cytomegalovirus (CMV), Varicella-zoster virus (VZV), Parvovirus B19, Adenovirus (ADV), BK virus (BKV), Enterovirus, Human herpes virus type 6 (HHV 6), Human herpes virus type 8 (HHV 8), JC virus (JCV).

No cross-reactivity was observed in this study.

15.3.2 Endogenous and exogenous substances

Potential endogenous and exogenous interference was assessed by an experimental study.

Interference was assessed through a study comparing the test sample containing the tested substance with a control sample. Plasma samples were spiked with AcroMetrix® HCV high Control (Thermo Scientific™). The AcroMetrix™ EDTA Plasma Dilution Matrix (Thermo Scientific ™) was used as a negative sample. For each sample, 10 extracted replicates were tested. A substance will be considered “not interfering” if the genotype expected is correctly detected in at least the 95% of the results.
The following substances were evaluated:

Substances	Tested Concentration
EDTA	30 mM
Heparin	40 USP/mL
Lipids	1420 mg/dL
Bilirubin	32 mg/dL
Peg-Interferon	30.9 ng/mL and 51.5 ng/mL
Ribvirin	8244 ng/mL and 13740 ng/mL
Hemoglobin	2 g/L

Interference on the performance of the AMPLIQUALITY HCV TYPE PLUS kit was observed only in the presence of heparin, at a concentration of 40 USP/mL.
No interference was observed in the presence of the other substances at the concentration reported.

15.4 MEASURING RANGE OF THE ASSAY

15.4.1 Limit of Detection (LoD)

To assess the sensitivity limit, the WHO International Standard HCV-RNA was used. Serial dilutions of the quantified reference standard, ranging from 13000 to 812 IU/mL of viral genome, were tested in 3 separate analyses in order to determine the analytical sensitivity. After extraction six replicates of each RNA were amplified in 3 consecutive RT-PCR runs. The analytical sensitivity limit as calculated by a Probit analysis with a confidence interval of 95 % (p=0.05) is1904 IU/mL of viral genome.

15.5 CLINICAL PERFORMANCE

15.5.1 DIAGNOSTIC SENSITIVITY AND DIAGNOSTIC SPECIFICITY

The analysis was conducted at the genotype level and at the subtype level considering subtypes a and b of genotype 1 separately.
All the 235 positive HCV samples were interpretable and consistent in terms of genotype with the reference method. The diagnostic sensitivity at the genotype level resulted of 100% (235/235).
The accuracy of the device at the subtype level was analyzed in order to assess the subtypes a and b of genotype 1. A total of 148 genotypes 1 was analyzed in the study: 54 of them were subtyped as 1a and 90 as 1b by the AMPLIQUALITY HCV TYPE PLUS kit.
For four samples the device was not able to assign the subtype because of the absence of signals in the CORE region of the strip.
A summary of the results obtained by genotyping with the AMPLIQUALITY HCV TYPE PLUS kit in comparison to the results obtained with the reference method is shown below.

		Genotype obtained with AMPLIQUALITY HCV TYPE PLUS							
Expected genotype		**1**	**2**	**3**	**4**	**5**	**6**	**1+3**	**Total**
	1	148							148
	2		42						42
	3			20					20
	4				14				14
	5					6			6
	6						4		4
	1+3							1	1
	Total	**148**	**42**	**20**	**14**	**6**	**4**	**1**	**235**

Tab.2: Summary of the genotyping results obtained with the AMPLIQUALITY HCV TYPE PLUS kit in comparison with the reference method.

	AMPLIQUALITY HCV TYPE PLUS		Total
	Positive	Negative	
Positive	235	0	235
Negative	0	111	111
Diagnostic sensitivity	100%		
Diagnostic specificity	100%		
PPV	100%		
NPV	100%		
PLR	∞		
NLR	0		

Table 3: Diagnostic sensitivity, diagnostic specificity, PPV, NPV, PLR and NLR

The diagnostic specificity and sensitivity at genotype level of the AMPLIQUALITY HCV TYPE PLUS are 100%, the PPV and NPV are 100%, the PLR is ∞ and the NLR is 0.

16 NOTICE TO THE USER

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
“Serious incident” means any incident that directly or indirectly led, might have led or might lead to any of the following:
(a) the death of a patient, user or other person
(b) the temporary or permanent serious deterioration of a patient's, user's or other person's state of health
(c) a serious public health threat.

17 REFERENCES

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18 SYMBOLS

SYMBOL	TITLE OF SYMBOL
CE 0123	CE mark
IVD	Medical device for *in vitro* diagnostics
	Refer to instructions for use
	Protect from direct light
	Temperature range
	Expiration date
	Manufacturer
LOT	Lot number
REF	Product code
	Sufficient for “N” tests
	Warning; Corrosive substance
UDI	Unique Device Identification

	STEP	REQUIRED REAGENTS	CONDITIONS OF INCUBATION	INCUBATION TIME
1	**Denaturation of PCR product**	**10 µL PCR product + 20 µL DEN**	**Room temperature**	**5 min**
2	**Hybridization**	**HYB-2** (preheated to 50 °C) **+ denatured PCR product**	**Shaking at 50 °C**	**60 min**
3	**Stringent washing**	**CON-D1** (preheated to 50 °C)	**Shaking at 50 °C**	**2 min**
4	**Incubation with Streptavidine-AP conjugate**	**Diluted CON** (0.5 µL **CON** + 2 ml preheated **CON-D1** [50 °C])	**Shaking at 50 °C**	**15 min**
5	**Rinse**	**RIN**	**Shaking at Room temperature**	**2 min**
6	**Rinse**	**RIN**	**Shaking at Room temperature**	**2 min**
7	**Staining**	**STAINING SOLUTION** (ready-to-use NBT/BCIP solution)	**Shaking IN DARK at Room temperature**	**5 min**
8	**Stopping the staining reaction**	**STOP**	**Shaking at Room temperature**	**2 min**

ATTENTION! This is only a short protocol. For complete instructions, refer to section 11.5

20 DOCUMENT REVISION CONTROL

Revision	Date of issue	Reason of issue
Rev. 1	27/05/2024	First issue under IVDR
Rev. 2	10/03/2025	Update with: - Information that the kit is qualitative - Cross reference for extraction systems in par. “7.1 Reagents” - Availability of the SSP
Rev. 3	18/07/2025	Update with more details regarding HCV Strip Reader software