INNO-LiPA HBV Genotyping

RUO

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INNOGENETICS®
BIOTECHNOLOGY FOR HEALTHCARE

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Symbols used

Manufacturer

RUO

For research use only
Not for use in diagnostic procedures

LOT

Batch code

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REF	Catalogue number
	Use By
	Consult Instructions for Use
	Temperature limitation
	Contains sufficient for < n > tests

Amplification (AMP) reagents:

PRIMER MIX O	Outer Primer Mix
PRIMER MIX N	Nested Primer Mix

LiPA reagents:

STRIPS	Strips
DENAT SOLN	Denaturation Solution
HYBRIDIZ SOLN	Hybridization Solution
STRIN WASH SOLN	Stringent Wash Solution
RINSE SOLN 5x	Rinse Solution 5x
CONJ DIL	Conjugate Diluent
CONJ 100x	Conjugate 100x
SUBS BUF	Substrate Buffer
SUBS BCIP/NBT 100x	Substrate BCIP/NBT 100x

Intended use

The INNO-LiPA HBV Genotyping assay is a line probe assay designed to identify hepatitis B virus (HBV) genotypes A to H by detection of type-specific sequences in the HBV polymerase gene domain B to C.
For research use only. Not for use in diagnostic procedures.

Test principle

Hepatitis B virus (HBV) is a partially double stranded DNA virus that replicates through an RNA intermediate.
The first step is the isolation of the viral DNA from the sample. The purified DNA is then amplified over two rounds of PCR using biotinylated PCR primers.

The outer primers (first round of PCR) used in this test will amplify part (domain B and C) of the HBV polymerase gene. By heating, the two strands of the DNA helix are separated (denaturation) to expose the target to the outer primers. These oligonucleotide primers are complementary to very conserved regions flanking the target region. Therefor, upon cooling to a specified temperature, the primers will bind to their specific sequence (annealing at 45°C). At an elevated temperature, in the presence of dNTP's, the thermostable DNA polymerase will extend the annealed primers along the target template (extension). An exact copy of the template is produced after one cycle of denaturation, annealing, and extension. The process is repeated for 40 cycles, thus yielding a multi-fold amplified target sequence of 409 bp.

Because the amount of amplification product is generally not sufficient, a nested (second round) PCR is needed. The principle of this amplification is identical to the first with the exception that the DNA is replaced by amplified product of the first round PCR, and that the outer primers are replaced by biotinylated nested primers. The process of denaturation, annealing and extension is repeated for 35 cycli, yielding an amplified sequence of 342 bp. We advise to check on gel if outer or nested amplified product should be used.

After amplification, the biotinylated DNA material generated from the HBsAg open reading frame is hybridized with specific oligonucleotide probes immobilized as parallel lines on membrane-based strips. After hybridization, unhybridized DNA is washed from the strip, streptavidin labeled with alkaline phosphatase is added and bound to any biotinylated hybrid previously formed. Incubation with BCIP/NBT chromogen results in a purple/brown precipitate.

The INNO-LiPA HBV Genotyping strip contains one red marker line, 2 control lines, and 14 parallel probe lines. The conjugate control line is a control for the color development reaction and the amplification control line contains universal HBV probes to check the presence of amplified HBV genomic material.

Reagents

Description, preparation for use and recommended storage conditions

REMARK:

- AMP reagents and LiPA reagents should be stored immediately after arrival: AMP reagents in the pre-amplification area and LiPA reagents in the post-amplification area.

- If stored at 2 - 8°C, opened or unopened, the reagents are stable until the expiry date.
  Do not use the reagents beyond the expiry date.
- In order to avoid contamination, it is recommended to aliquot amplification reagents after first use.
- The reagents should be stored isolated from any source of contaminating DNA, especially amplified products. Disposable tubes and pipette tips (preferably cotton-plugged) should be used.
- All reagents should be brought to room temperature (20 - 25°C) approximately 30 minutes before use and should be returned to the refrigerator immediately after use. Briefly vortex and spin down all reagents before opening the vials. Close the vials immediately after use.
- Alterations in physical appearance of the kit reagents may indicate instability or deterioration.
- To minimize the possibility that strips curl before use, it is recommended to store the tube horizontally.

Reagents supplied:

Component	Quantity	Ref.	Description
AMP reagents:			
Outer Primer Mix	1 x 0.08 ml	59058	Containing biotinylated primers and 0.05% NaN_3 as preservative.
Nested Primer Mix	1 x 0.08 ml	59055	Containing biotinylated primers and 0.05% NaN_3 as preservative.
LiPA reagents:			
Strips	1 x 20	56827	1 plastic tube containing INNO-LiPA HBV Genotyping strips marked with a red marker line.
Denaturation Solution	1 x 1 ml	56828	Alkaline solution containing EDTA. CAUTION: The vial containing the Denaturation Solution should be closed immediately after use; prolonged exposure of this solution to air leads to rapid deterioration.
Hybridization Solution	1 x 80 ml	58235	Containing SSC-buffer with detergents and preservatives. The Hybridization Solution should be pre-warmed to a temperature of at least 37°C and must not exceed 49°C (all crystals should be dissolved before use).
Stringent Wash Solution	1 x 200 ml	58237	Containing SSC-buffer with detergents and preservatives. The Stringent Wash Solution should be pre-warmed to a temperature of at least 37°C and must not exceed 49°C (all crystals should be dissolved before use).
Conjugate 100x	1 x 0.8 ml	56832	Streptavidin labeled with alkaline phosphatase in Tris-buffer containing protein stabilizers and 0.01% MIT/0.098% CAA as preservative, to be diluted 1/100 in Conjugate Diluent before use. Prepare 2 ml conjugate working solution for each test trough + 2 ml in excess. For *Auto*-LiPA, make 10 ml in excess. The conjugate working solution is stable for 24 hours at room temperature (20 - 25°C) when stored in the dark.
Conjugate Diluent	1 x 80 ml	56833	Phosphate-buffer containing NaCl, Triton®, protein stabilizers and 0.01% MIT/0.1% CAA as preservatives.
Substrate BCIP/NBT 100x	1 x 0.8 ml	56834	5-bromo-4-chloro-3-indolylphosphate and 4-nitro-blue tetrazolium in dimethylformamide, to be diluted 1/100 in Substrate Buffer before use. Prepare 2 ml substrate working solution for each test trough + 2 ml in excess. For *Auto*-LiPA, make 10 ml in excess.

			The substrate working solution is stable for 24 hours at room temperature (20 - 25°C) when stored in the dark.
Substrate Buffer	1 x 180 ml	56835	Tris buffer containing NaCl, $MgCl_2$ and 0.01% MIT/0.1% CAA as preservatives.
Rinse Solution 5x	1 x 80 ml	56831	Phosphate buffer containing NaCl, Triton® and 0.01% MIT/0.1% CAA as preservatives, to be diluted 1/5 (1 part + 4 parts) in distilled or deionized water before use. Prepare 8 ml rinse working solution for each test trough + 10 ml in excess. For *Auto*-LiPA, make 20 ml in excess. Rinse working solution is stable for 2 weeks at 2 - 8°C.
Incubation trays	3	-	Containing 8 troughs each.
Reading card	1	-	For identification of positive probes.
Interpretation chart	1	-	For interpretation of results.
Data reporting sheet	2	-	For storage of developed strips.

Materials required but not provided

- Disposable gloves.
- Disposable sterile pipette tips (preferably cotton-plugged).
- Sterile microtubes.
- Microtube racks.
- Microtube centrifuge.
- Pipettes adjustable to deliver 1 - 20 µl, 20 - 200 µl and 200 - 1000 µl.
- Autoclaved distilled water.

Specifically for amplification

For the amplification, Taq DNA polymerase and Taq amplification buffer provided by Stratagene are recommended for use.

- DNA thermal cycler and equipment.
- dNTP's (25 mM).
- 10x Taq buffer (containing 100 mM Tris-HCl pH 8.8; 500 mM KCl; 15 mM $MgCl_2$, 0.01% (w/v) gelatin, and stabilizers, Stratagene).
- Taq DNA polymerase (Taq DNA Polymerase, Stratagene).

Specifically for LiPA

- Aspiration apparatus.
- Calibrated thermometer.
- Tweezers.
- Graduated cylinders (10, 25, 50 and 100 ml).
- Timer (2 hours ± 1 minute).
- Vortex mixer or equivalent.
- Water bath with shaking platform (80 rpm; inclined lid; temperature adjustable to minimum 49°C ± 0.5°C).
- Orbital shaker (160 rpm) or rocker shaker (50 rpm).

Optional materials:
- Dispensing multipipette (Multipipette, Eppendorf or equivalent).
- Equipment for automation of strip processing steps. For details, contact your distributor.

Safety and environment

- **Please refer to the Material Safety Data Sheet (MSDS) and product labelling for information on potentially hazardous components. The most recent MSDS version is available on the website www.innogenetics.com.**

 R20/21, R36, R61, S36/37, S45, S53

Toxic! Harmful by inhalation and in contact with skin. Irritating to eyes. May cause harm to the unborn child. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label if possible). Avoid exposure - obtain special instructions for use. Restricted to professional users.
Contains Dimethylformamide, 5-Bromo-4-chloro-3-indolyl phosphate p-Toluidine salt: Substrate BCIP/NBT 100x.

 R43, S24-37

Irritant! May cause sensitization by skin contact. Avoid contact with skin. Wear suitable gloves. **Contains 2-Chloroacetamide:** Stringent Wash Solution, Hybridization Solution, Rinse Solution, Substrate Buffer and Conjugate Diluent.


R36/38, S23-24-26
Irritant! Irritating to eyes and skin. Do not breathe aerosol. Avoid contact with skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
Contains sodium hydroxide: Denaturation Solution.

- Specimens should always be handled as potentially infectious. Therefore, all blood components and biological materials should be considered as being potentially infectious and should be handled as such. Only adequately trained personnel should be permitted to perform the test procedure.
- All blood components and biological materials should be disposed of in accordance with established safety procedures.
  - Autoclave for at least 15 minutes at 121°C.
  - Incinerate disposable material.
  - Mix liquid waste with sodium hypochlorite so that the final concentration is ± 1% sodium hypochlorite. Allow to stand overnight before disposal.
    CAUTION: Neutralize liquid waste that contains acid before adding sodium hypochlorite.
- Use of personal protective equipment is necessary: gloves and safety spectacles when manipulating dangerous or infectious agents.
- Waste should be handled according to the institution's waste disposal guidelines. All federal, state, and local environmental regulations should also be observed.

Specimen collection and handling

Use extracted DNA from HBV positive samples freshly isolated or frozen at or below -20°C, preferably never thawed. Amplified material of the HBV-pol gene domain B to C can be generated. Use then 10 µl of HBV amplified product for the LiPA process.

Remarks and precautions

- For professional use only.
- All pipette tips and tubes for the amplification process should be autoclaved. Pipette tips with cotton plugs are recommended. Use only disposable lab materials.
- Use a new sterile pipette tip for each aliquoted specimen.
- Do not mix reagents between kits, unless the components have identical lot numbers.
- To prevent PCR contamination, maximize the physical separation of the pre- and post-amplification steps. Do not return samples, equipment, or reagents to the area where you performed the previous step. If you need to return to a previous work area, first perform the appropriate decontamination safeguards.
- Use tweezers to handle strips. Do not touch strips with your hands as the oils from your hands could interfere with hybridization and color development.
- Use only **pencil** to write on the strips. The assay reagents may remove ink from the strips.
- For accurate results, use a water bath for the hybridization and wash steps and measure that the temperature is 49°C ± 0.5°C. Do not use a hot air shaker. Use a calibrated thermometer, as strict temperature control is necessary. Always close the lid of the water bath to maintain the temperature. If the temperature is too low, the assay may yield false positive results; if the temperature is too high you may observe very weak signals or false negative results. Use a shaking water bath with inclined lid for optimal temperature control.
- The motion generated by the shaking water bath during hybridization and stringent wash and by the orbital or rocker shaker during color development is critical. Adjust the speed of these instruments carefully to maximize the movement of the reagents over the strip without splashing solutions between troughs in the tray.
- Avoid splashing water from the water bath into the tray. Adjust the water level in the water bath so that it is between one-third and one-half the height of the tray. Prevent the tray from sliding by immobilizing it using weights.
- Do not allow the strips to dry between steps.

- Keep each strip in the same trough throughout the procedure.
- Aspirate liquid from a trough using a pipette that may be attached to a vacuum aspirator.

Test procedure for amplification

DNA extraction protocol: High Pure PCR Template preparation Kit (Roche Diagnostics). See protocol of mentioned kit. Other DNA extraction procedures can be used.

Amplification protocol

The following protocols (Outer and Nester amplification) were designed for optimal amplification, using
- Taq polymerase (5U/µl, Stratagene),
- 10x Taq buffer (containing 100mM Tris-HCl pH 8.8, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin, stabilizers, Stratagene),
- MicroAmp® PCR tubes (0.2 ml thin-walled tubes) and
- PE-2400 or PE-9600 (Perkin Elmer Cetus) thermal cyclers.

This protocol can be used with most commercial types of thermal cyclers, but may require some modifications provided by the manufacturer of the cycler.

Take the necessary precautions to avoid non-specific amplification, which may impair the results:
- thaw the components listed below and place them on ice
- perform all pipetting steps on ice and without delay.

Outer amplification

1. Determine the number of vials to be prepared (N) as:
   N = Number of samples + 1 Negative Control + 1 Positive Control + 1.
2. Using cotton-plugged pipette tips, prepare Master Mix in an autoclaved nuclease-free microcentrifuge tube:
3. Prepare Master Mix 1 containing:

(N x 32.4 µl)	autoclaved distilled water
+ (N x 5.0 µl)	10x Taq Amplification buffer (Stratagene)
+ (N x 0.4 µl)	dNTP mix (25 mM)
+ (N x 2.0 µl)	Outer Primer Mix
+ (N x 0.2 µl)	Taq DNA Polymerase 5U/µl (Stratagene)

Vortex briefly and aliquot 40 µl of this Master Mix 1 into (N-1) autoclaved amplification tubes.

4. Pipette 10 µl of purified DNA, or 10 µl of distilled water (no DNA to the Negative Control).
   REMARK:
   - Mix briefly and spin the mix to collect the liquid at the bottom of the tube.
5. Place the samples into the calibrated thermal block (see instructions provided by the manufacturer of the thermal cycler). Start the amplification program designed for the INNO-LiPA HBV Genotyping outer amplification.

   INNO-LiPA HBV Genotyping outer amplification profile for a PE-2400 or PE-9600 thermal cycler:

Step	Temp	Time	
1 Denature	94°C	4 min	
2 Denature	94°C	30 sec	Repeat cycle
3 Anneal primers	45°C	30 sec	steps 2 - 4,
4 Extend primers	72°C	30 sec	40 times
5 Elongate	72°C	10 min	
6 Cool to 4°C			

6. After this process, use the samples immediately for the nested amplification or store at -15/-25°C.
   NOTE:
   - Do not store the amplified DNA products together with amplification reagents or extracted DNA.

Nested amplification

1. Determine the number of vials to be prepared (N) as:
   N = Number of outer amplified samples + Outer amplified Negative Control + 1 Negative Control + 1 Positive Control + 1.
   Using a cotton-plugged pipette tip, prepare the Master Mix 2 in an autoclaved tube:

   (N x 40.4 µl) autoclaved distilled water
   + (N x 0.4 µl) dNTP mix (25 mM)
   + (N x 5.0 µl) 10x Taq Amplification buffer (Stratagene)
   + (N x 2.0 µl) Nested Primer Mix
   + (N x 0.2 µl) Taq DNA polymerase 5 U/µl (Stratagene)

Vortex briefly and aliquot 48 µl of this Master Mix into (N-1) autoclaved amplification tubes.

2. Pipette 2 µl of the outer amplified products, or 2 µl distilled water (no DNA to the Negative Control).
   REMARK:
   - Mix briefly and spin the mix to collect the liquid at the bottom of the tube.
3. Place the samples into the calibrated thermal block (see instructions provided by the manufacturer of the thermal cycler). Start the amplification program designed for the INNO-LiPA HBV Genotyping nested amplification.

   INNO-LiPA HBV Genotyping nested amplification profile for a PE-2400 or PE-9600 thermal cycler:

Step	Temp	Time	
1 Denature	94°C	4 min	
2 Denature	94°C	30 sec	Repeat cycle
3 Anneal primers	45°C	30 sec	steps 2 - 4,
4 Extend primers	72°C	30 sec	35 times
6 Elongate	72°C	10 min	
7 Cool to 4°C			

4. After this process, analyse 5 µl of both outer and nested amplified product on 2% agarose gel or store at -15/-25°C.
   It is advised based upon in-house observations, that if the outer amplified product gives a band of 409 bp, an interpretable result should be given by the LiPA assay. If no band is visible for the outer amplified product, use the nested amplified product with the INNO-LiPA HBV Genotyping.
   NOTE:
   - Do not store the amplified DNA products together with amplification reagents or extracted DNA.

Results for amplification

Visualization

- The presence of the amplified product can be checked on a 2% agarose gel.
- Load 5 µl of amplified product per slot.

- The nested amplicon should appear as a band with a length of 342 bp.
- The outer amplicon should appear as a band with a length of 409 bp.

Quality control

- Include at least one blank for extraction.
- Include at least one positive and one negative control each time amplification is performed. As with any new laboratory procedure, the inclusion of additional positive and negative controls should be considered until a high degree of confidence is reached in the ability to correctly perform the procedure. If the addition of a positive control is desirable, use a known positive sample.
- If a positive band is obtained in the gel for the negative control, the entire run should be discarded and the complete procedure should be repeated.

NOTE:

- A single-round PCR protocol is also available, as an alternative to the outer and nested amplification. This protocol was tested on a limited amount of samples. For this single-round PCR protocol, AmpliTaq Gold and buffer II provided by Applied Biosystems are recommended for use.
  - DNA thermal cycler and equipment
  - BSA (10mg/ml)
  - $MgCl_2$ 25mM (Applied Biosystems)
  - dU/dN (5mM/2.5mM)
  - Amperase (1U/µl)
  - AmpliTaq Gold 5U/µl (Applied Biosystems)
  - 10x PCR buffer II (Applied Biosystems)

1. Determine the number of vials to be prepared (N) as: N = Number of samples + 1 Negative Control + 1 Positive Control + 1.
2. Using cotton-plugged pipette tips, prepare Master Mix in an autoclaved nuclease-free microcentrifuge tube.

3. Prepare Master Mix containing:
   (N x 7.5 µl) autoclaved distilled water
   + (N x 0.5 µl) BSA
   + (N x 5.0 µl) 10x PCR buffer II
   + (N x 5.0 µl) $MgCl_2$
   + (N x 2.0 µl) Nested Primer Mix
   + (N x 4.0 µl) dU/dN
   + (N x 0.5 µl) Amperase
   + (N x 0.5 µl) AmpliTaq Gold
   Vortex briefly and aliquot 25 µl of this Master Mix into (N-1) autoclaved amplification tubes.
4. Pipette 25 µl of purified DNA, or 25 µl of distilled water (no DNA to the Negative Control).
   REMARK:
   - Mix briefly and spin the mix to collect the liquid at the bottom of the tube.
5. Place the samples into the calibrated thermal block (see instructions provided by the manufacturer of the thermal cycler).

   INNO-LiPA HBV Genotyping single-round amplification profile:

Step	Temp	Time	
1 Activation	50°C	2 min	
2 Denature	95°C	10 min	
3 Denature	95°C	20 sec	Repeat cycle
4 Anneal primers	50°C	20 sec	steps 3 - 5,
5 Extend primers	72°C	1 min	44 times
6 Elongate	72°C	5 min	
7 Cool to 4°C			

6. After this process, use the samples immediately with the INNO-LiPA HBV Genotyping test strip or store at -15/-25°C.
   NOTE:
   - Do not store the amplified DNA products together with amplification reagents or extracted DNA.

Test procedure for LiPA

Denaturing the samples

CAUTION: Using a calibrated thermometer, ensure that the temperature of the shaking water bath is 49°C ± 0.5°C, and adjust the temperature if necessary before adding a sample tray.

1. Equilibrate a shaking water bath to 49°C ± 0.5°C.
2. Place the Hybridization Solution and Stringent Wash Solution in a water bath at 37 to 49°C to dissolve all crystals. Ensure that the water bath does not exceed 49°C. Mix by shaking the bottle before use.
3. Using tweezers, remove one strip for each sample. With a pencil, write an identification number above the red marker line on the strip. Always include a strip for the negative control (amplified product of an HBV negative sample).
   NOTE:
   - Do not place the strip in the trough until step 2 in Hybridizing the samples.
4. Place one trough for each sample/strip in the tray.
5. Add 10 µl Denaturation Solution to the upper corner of each trough.
   NOTE:
   - Immediately close the vial containing the Denaturation Solution after each use. Prolonged exposure to air causes the solution to deteriorate.
6. Add 10 µl sample or negative control to the Denaturation Solution in each trough. Carefully mix by pipetting up and down.
7. Allow denaturation to proceed for 5 minutes at room temperature.

Hybridizing the samples

1. Carefully add 2 ml Hybridization Solution to each trough, taking care not to contaminate other troughs. Gently shake the trough to mix reagents.
2. Immediately place a strip with the marker line facing up into a trough.
   NOTE:
   - Wear disposable gloves and use tweezers when handling the strips.
3. Ensure that the strip is completely submerged in the solution. Do not cover the troughs with microplate sealers so as to avoid cross-contamination.
4. Place the tray into the 49°C ± 0.5°C shaking water bath and immobilize the tray between two heavy weights. Set the water bath to approximately 80 rpm, close the lid, and incubate the

tray for 60 minutes. Ensure that each strip remains completely submerged and floats freely.

5. When hybridization incubation is complete, remove the tray from the water bath.

Washing the strips

CAUTION:

- Hold the tray at a low angle so the liquid accumulates at one end of a trough, above the marker line on the strip, for easy removal. Do not damage the surface of the strip. Avoid splashing or transferring solutions between troughs.

1. Aspirate the solution from the trough using a pipette, preferably attached to a vacuum aspirator.
2. Add 2 ml Stringent Wash Solution to each trough and rinse the strip by rocking the tray 1 minute at 20 - 25°C. Aspirate the solution from each trough.
   NOTE:
   - A multipipette is useful for dispensing the Stringent Wash Solution.
3. Repeat the wash by adding 2 ml Stringent Wash Solution to each trough and rinsing the strip by rocking the tray for 1 minute at 20 - 25°C. Aspirate the solution from each trough.
4. Add 2 ml Stringent Wash Solution to each trough, place tray into the 49°C ± 0.5°C shaking water bath and immobilize the tray between two heavy weights. Set the water bath to approximately 80 rpm, close the lid and incubate the tray for 30 ± 1 minutes.
5. Prepare the rinse working solution and conjugate working solution (See Reagents).

Developing the color

CAUTION:

- Hold the tray at a low angle so the liquid accumulates at one end of a trough, above the marker line on the strip, for easy removal. Do not damage the surface of the strip. Avoid splashing or transferring solutions between troughs.

1. Aspirate the solution from the trough using a pipette.
2. Add 2 ml rinse working solution to each trough and wash the strip by rocking the tray for 1 minute at room temperature. Aspirate the solution from each trough. Repeat this step once.
3. Add 2 ml conjugate working solution to each trough, place the tray on a shaker (orbital at 160 rpm or rocker at 50 rpm) at 20 - 25°C, and incubate for 30 ± 1 minutes.
   NOTE:
   - Prepare the substrate working solution about 10 minutes before the end of the conjugate incubation (See Reagents).
4. When the incubation is complete, remove the tray from the shaker and aspirate the solution from each trough using a pipette.
5. Add 2 ml rinse working solution to each trough and wash the strip by rocking the tray for 1 minute at 20 - 25°C. Aspirate the solution from each trough. Repeat this step once.
6. Add 2 ml Substrate Buffer to each trough and wash the strip by rocking the tray for 1 minute at 20 - 25°C. Aspirate the solution from each trough.
7. Add 2 ml substrate working solution to each trough, place on shaker at 20 - 25°C, and incubate for 30 ± 1 minutes.
8. When the incubation is complete, stop the color development by washing the strips: remove the tray from the shaker, aspirate the solution from each trough, and then add 2 ml distilled water to each trough and place the tray on the shaker for at least 3 minutes. Repeat this step once.
9. Using tweezers, remove each strip from its trough and place the strip with the marker line facing up on absorbent paper.
10. Dry the strips completely before reading the results. Store the developed and dried strips in the dark.

Automated test procedure: *Auto*-LiPA and *Auto*-LiPA 48

The LiPA test procedure is extremely well suited for automation. Therefore, the *Auto*-LiPA and *Auto*-LiPA 48 are designed to fully handle hybridization, stringent wash and color development steps. The *Auto*-LiPA and *Auto*-LiPA 48 are featured as a walk-away system with automated heating and cooling, and with automated aspiration and pipetting.
For more information and specific protocols on *Auto*-LiPA and *Auto*-LiPA 48, please contact your local distributor.

Results for LiPA

Reading

Figure 1 illustrates the position of the different oligonucleotide probes on the INNO-LiPA HBV Genotyping strip. A line is considered positive when a clear purple/brown band appears at the end of the test procedure.

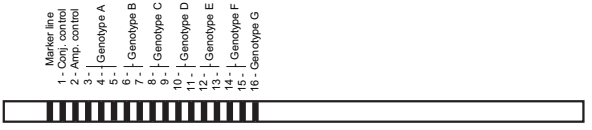

Fig. 1: Location of the red colored marker line, the conjugate control line (Conj. control), the amplification control line (Amp. control) and the 14 probe lines on the INNO-LiPA HBV Genotyping strip.

Quality control

- The uppermost red line is the marker line. This line allows correct orientation of the strip.
- The first positive line should be lined up with the "Conj. control" line on the plastic reading card. This line controls for the addition of reactive Conjugate and Substrate Solution during the detection procedure. It should always be positive and should have approximately the same intensity on each strip in the same test run.
- The second positive line ("Amp. control" on reading card) controls for the addition of amplified material for hybridization. If HBV is present in the sample and correct sample processing and amplification has occurred, then the target amplicon hybridizes to this "Amp. control" line.
- The Negative Control strip should be blank except for the conjugate control line. This demonstrates no contamination during the assay performance.

Interpretation of the results

Use the interpretation chart AT ALL TIMES for correct interpretation of the HBV genotype.

NOTE:

- Isolates from different genotypes can cross react with the same probe line. Please, check carefully on the interpretation chart which probe line patterns apply to each individual genotype.

REMARK: (patterns not present on the interpretation chart):

- Interpretation of mixed infections:
  The existence of mixed genotype infections in chronic HBV carriers has been documented.
  When multiple genotype infections are detected by using the INNO-LiPA HBV Genotyping assay, the results should be interpreted as follows:
  - If multiple genotype specific lines show for different genotypes, a mixed genotype should be reported.
    For example, positive lines 6 - 7 together with positive lines 8 - 9 is reported as a B/C co-infected sample.
  - For HBV genotype G, it has been shown that this genotype is co-infected with HBV genotype A in a majority of cases.
    For example, a reactivity pattern where the genotype G specific line 16 is present together with at least 2 positive genotype A lines should be reported as an A/G co-infected sample.
  - If multiple genotype specific lines show for one genotype, together with a single positive line for a second genotype, this should be reported as a single genotype.
    The genotype reported should be the one with the multiple lines positive. Please note that a single line 16 is indicative for the presence of a genotype G.

- Indeterminate results:
  Indeterminate (IND) results should be reported in the following cases:
  - Single reactive lines for more than one genotype in conjunction with a positive amplification control line (line 2). Please note that a single line 11 and a single line 15 reactivity should be interpreted as genotype H.
  - Absence of positive genotype-specific lines in conjunction with a positive amplification control line (line 2).
  - All lines positive.

For any support on the interpretation, contact your distributor.

Limitations of the procedures

- Use of this kit should be limited to personnel who are trained in the techniques of HBV nucleic acid extraction and amplification.
- Good laboratory practice and careful performance of the procedure previously specified allow specific amplification.
- Because of the occasional very high load of HBV infected samples, extreme caution should be taken to avoid contamination.
- Polymerase inhibition (e.g. by heparin haemoglobin) might be the reason for complete failure of the assay.
- Powder from disposable gloves and sodium hypochlorite have an inhibiting effect on amplification.
- HBV infections with mixed viral genotype can occur. The primers amplify all genotypes simultaneously. In a sample with mixed infection and low viral load, it is possible that not all genotypes will be detected due to PCR competition.
- This assay is based on the sequence variability in the HBV polymerase gene domains B and C, allowing differentiation between HBV genotypes A to H. Due to some sequence heterogeneity of the HBV genome, indeterminate patterns or false-reacting probe lines may occasionally be produced.
- Results showing weak reactivity on all lines are occasionally observed and yield an indeterminate result. Samples showing this pattern should be re-amplified or re-extracted.