WHO Prequalification of Diagnostics Programme
PUBLIC REPORT

Product: Murex HBsAg Version 3 with Murex HBsAg Confirmatory Version 3
Number: PQDx 0121-043-00

Abstract

Murex HBsAg Version 3 with Murex HBsAg Confirmatory Version 3 with product codes¹ 9F80-01, 9F80-05 and 2G27-01, manufactured by DiaSorin S.p.A UK Branch, CE marked regulatory version, was accepted for the WHO list of in vitro prequalified diagnostics and was listed on 10 October 2014.

Murex HBsAg Version 3 is an enzyme immunoassay for the detection of hepatitis B surface antigen in human serum or plasma. The assay is intended to screen individual human donors for the presence of hepatitis B surface antigen or as an aid to the diagnosis of HBV infection.

Murex HBsAg Confirmatory Version 3 functions by means of specific antibody neutralization of HBsAg in specimens that are repeatedly reactive in the Murex HBsAg Version 3.

Principle of test procedure:
In Murex HBsAg Version 3, the specimen is pre-incubated in microwells coated with a mixture of mouse monoclonal antibodies specific for different epitopes on the ‘a’ determinant of HBsAg. Affinity purified goat antibody to HBsAg conjugated to horseradish peroxidase is then added to the specimen in the well. During the two incubation steps any HBsAg present in the specimen is bound to the well in an antibody-antigen-antibody-enzyme complex. In the absence of HBsAg no conjugate will be bound. After washing to remove sample and unbound conjugate, a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide is added to the wells. Wells which contain HBsAg and hence bound conjugate will develop a purple colour which is converted to orange when the enzyme reaction is terminated with sulphuric acid.

Specimens giving an absorbance equal to or greater than the cut-off value are considered initially reactive in the assay. Such specimens should be re-tested in duplicate. Specimens that are reactive in at least one of the repeat tests are presumed to contain HBsAg and should be confirmed by testing with the Murex HBsAg Confirmatory Version 3 kit (2G27-01) and for other HBV markers.

The test kit contains:
Murex HBsAg Version 3; product code 9F80-01 (96 tests)
Each test kit contains: 1 plate of 96 coated wells, 1 (16ml) bottle of sample diluent, 1 (2.5ml) bottle of negative control, 1 (2ml) bottle of positive control, 1 (6ml) bottle of conjugate containing HRP labelled goat antibody to HBsAg, 1 bottle (35ml) of substrate diluent containing tri-sodium citrate and hydrogen peroxide, 1 (35ml) bottle of substrate concentrate containing TMB, and 1 (125ml) bottle of wash fluid. *Note: 2M sulphuric acid required as stop solution not included in this test kit configuration, although it can be provided as either code N0164 for the 15 vial pack or code N0165 for the 1 vial pack.*

**Murex HBsAg Version 3; product code 9F80-05 (480 tests)**
Each test kit contains: 5 plates of 96 coated wells, 1 (16ml) bottle of sample diluent, 1 (2.5ml) bottle of negative control, 1 (2ml) bottle of positive control, 2 (16ml) bottles of conjugate containing HRP labelled goat antibody to HBsAg, 1 bottle (35ml) of substrate diluent containing tri-sodium citrate and hydrogen peroxide, 1 (35ml) bottle of substrate concentrate containing TMB, and 1 (125ml) bottle of wash fluid. *Note: 2M sulphuric acid required as stop solution not included in this test kit configuration, although it can be provided as either code N0164 for the 15 vial pack or code N0165 for the 1 vial pack.*

**Murex HBsAg Confirmatory Version 3; product code 2G27-01 (50 tests)**
Each kit contains: 1 (1.25mL) bottle of Control Reagent, containing buffer and 1 (1.25mL) bottle of Specific Reagent, containing specific horse antibody to HBsAg. Both bottles contain Proclin®300 preservative.

Storage:
The test kit should be stored between 2°C and 8°C.

Shelf-life:
12 months for Murex HBsAg Version 3
17 months for Murex HBsAg Confirmatory Version 3.

**Summary of prequalification status for Murex HBsAg Version 3 with Murex HBsAg Confirmatory Version 3**

<table>
<thead>
<tr>
<th>Status on PQ list</th>
<th>Date</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial acceptance</td>
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<td></td>
</tr>
<tr>
<td>Dossier assessment</td>
<td>10 October 2014</td>
<td>listed</td>
</tr>
<tr>
<td>Inspection status</td>
<td>05 August 2014</td>
<td>MR</td>
</tr>
<tr>
<td>Laboratory evaluation</td>
<td>12 August 2014</td>
<td>MR</td>
</tr>
</tbody>
</table>

MR: Meets Requirements
NA: Not Applicable
Murex HBsAg Version 3 with Murex HBsAg Confirmatory Version 3 was accepted for the WHO list of prequalified diagnostics on the basis of data submitted and publicly available information.

**Background information**

DiaSorin S.p.A UK Branch submitted an application for prequalification of Murex HBsAg Version 3 with Murex HBsAg Confirmatory Version 3. Based on the established prioritization criteria, Murex HBsAg Version 3 with Murex HBsAg Confirmatory Version 3 was given priority for prequalification.

**Product dossier assessment**

DiaSorin S.p.A UK Branch submitted a product dossier for Murex HBsAg Version 3 with Murex HBsAg Confirmatory Version 3 as per the “Instructions for compilation of a product dossier” (PQDx_018 v1). The information submitted in the product dossier was reviewed by WHO staff and external experts (assessors) appointed by WHO in accordance with the internal report on the screening and assessment of a product dossier (PQDx_009 v2). Based on the product dossier screening and assessment findings, a recommendation was made to accept the product dossier for Murex HBsAg Version 3 with Murex HBsAg Confirmatory Version 3 for prequalification.

Commitments for prequalification:
The manufacturer committed to amend and submit additional documentation on the following issues:

1. Additional analytical specificity studies
2. Additional evidence supporting traceability of control materials
3. Further studies in support of in use stability
4. Further studies in support of transport stability

**Manufacturing site inspection**

A comprehensive inspection was performed at the site of manufacture (Dartford, UK and Saluggia, Italy) of the Murex HBsAg Version 3 with Murex HBsAg Confirmatory Version 3 test in February 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 and successfully closed on August 11 2014.

**Laboratory evaluation**
Murex HBsAg Version 3 (DiaSorin S.p.A UK Branch) was evaluated by WHO in the 1st quarter of 2013 using serum/plasma specimens. From this evaluation, we drew the following conclusions:

Murex HBsAg Version 3 (DiaSorin S.p.A UK Branch) is an enzyme immunoassay for the detection of HBsAg in human serum or plasma. A volume of 75µl 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 515 specimens, we found an initial sensitivity (95% CI) of 100% (98.2% – 100%) and an initial specificity (95% CI) of 98.4% (96.3% – 99.5%) compared to the reference results. The final sensitivity (95% CI) was 100% (98.2% – 100.0%) and the final specificity (95% CI) was 99.0% (97.2% – 99.8%) compared to the reference results. Lot to lot variation was in the acceptance range.

For six seroconversion panels, Murex HBsAg Version 3 detected on average 0.5 specimens earlier than the benchmark assay (Monolisa Ag HBs Plus [Bio-Rad]). For the low titer panel, Murex HBsAg Version 3 correctly classified all specimens.

For the 1st International Biological Reference Preparation for Hepatitis B surface antigen NIBSC code 03/262, Murex HBsAg Version 3 detected to 0.13 IU/ml. As a comparison, Heapanostika HBsAg ULTRA detected to 0.13 IU/ml.

In this study, 0.18% of the results were recorded as indeterminate. The invalid rate was 0%.
Labelling

1. Labels
2. Instructions for use
1. Labels

Murex HBsAg version 3 (9F80-01/9F80-05)

Label 190mm x 182mm
Murex HBsAg Confirmatory version 3

Murex HBsAg Confirmatory Version 3 (2G27-01)
2. Instructions for use

Murex HBsAg Version 3

Enzyme immunoassay for the detection of hepatitis B surface antigen in human serum or plasma

The assay is intended to screen individual human donors for the presence of hepatitis B surface antigen or as an aid to the diagnosis of HBV infection.

Customer Service
For additional product information, please contact your local customer service organization.

This instructions for use must be read carefully prior to use. The instructions for use must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions for use.

Key to symbols used

<table>
<thead>
<tr>
<th>REF</th>
<th>LOT</th>
<th>Expiration Date</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>List Number</td>
<td>Lot Number</td>
<td>Expiration Date</td>
<td>Manufacturer</td>
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</tbody>
</table>

See REAGENTS section for a full explanation of symbols used in reagent component naming.
INTENDED USE

Murex HBsAg Version 3 is a rapid and sensitive enzyme immunoassay for the detection of hepatitis B surface antigen in human serum or plasma.

The assay is intended to screen individual human donors for the presence of hepatitis B surface antigen or as an aid to the diagnosis of HBV infection.

SUMMARY AND EXPLANATION OF THE TEST

The causative agent of serum hepatitis is hepatitis B virus (HBV) which is an enveloped DNA virus. During infection, HBV produces an excess of hepatitis B surface antigen (HBsAg), also known as Australian antigen, which can be detected in the blood of infected individuals. HBsAg is the first serological marker after infection with HBV appearing one to ten weeks after exposure and two to eight weeks before the onset of hepatitis. HBsAg persists during this acute phase and clears late in the convalescence period. Failure to clear HBsAg within six months indicates a chronic HBsAg carrier state. Blood from individuals in the acute or chronic state is potentially infectious to recipients and should not be transfused. Consequently, potentially infectious samples of serum, EDTA plasma or citrate plasma can be identified.

PRINCIPLE OF THE PROCEDURE

In Murex HBsAg Version 3, the sample is pre-incubated in microtitre wells coated with a mixture of mouse monoclonals specific for different epitopes on the 'a' determinant of HBsAg. Affinity purified goat antibody to HBsAg conjugated to horseradish peroxidase is then added to the sample in the well. During the two incubation steps any HBsAg present in the sample is bound to the well in an antibody-antigen-antibody-enzyme complex. In the absence of HBsAg no conjugate will be bound. After washing to remove sample and unbound Conjugate, a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide is added to the wells. Wells which contain HBsAg and hence bound Conjugate will develop a purple colour which is converted to orange when the enzyme reaction is terminated with sulphuric acid.

REAGENTS

DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also Warnings and Precautions.

All components must be stored at 2 to 8°C unless otherwise stated, under which condition they will retain activity until the expiry date of the kit.

COATED WELLS

1. Coated Wells
One plate (9FB0-01) or five plates (9FB0-05) of 96 wells coated with mouse monoclonal antibody to HBsAg.
Allow the wells to reach room temperature (18 to 30°C) before removal from the bag. Place unused wells in the sealable storage bag provided and return to 2 to 8°C.

SAMPLE OIL

2. Sample Diluent
One bottle containing 16 ml of green/brown buffer containing detergents and proteins of goat and bovine origin. Mix by inversion before use. Contains 0.05% ProClin® 300 preservative.

CONTROL -

3. Negative Control
One bottle containing 2.5 ml of normal human serum. The serum is diluted in a buffer containing protein of bovine origin. Contains 0.05% Bronidox® B preservative.

CONTROL +

4. Positive Control
One bottle containing 2 ml of inactivated human serum. The serum is diluted in a buffer containing protein of bovine origin. Contains 0.05% Bronidox® B preservative.

5. Conjugate
One bottle containing 6 ml (9FB0-01) or two bottles each containing 16 ml (9FB0-05) of horseradish peroxidase labelled goat antibody to HBsAg in a red buffer containing proteins of bovine and goat origin. Mix by inversion before use. Contains 0.06% ProClin® 300 preservative.

6. Substrate Diluent
One bottle containing 35 ml of a colourless solution of tri-sodium citrate and hydrogen peroxide.

7. Substrate Concentrate
One bottle containing 35 ml of 3,3',5,5'-tetramethylbenzidine (TMB) and stabilisers in a pink solution.

Substrate Solution

To prepare the Substrate Solution add a volume of colourless Substrate Diluent to an equal volume of pink Substrate Concentrate in either a clean glass or plastic vessel. It is important that this order of addition is followed and that any pipettors and glassware used to prepare Substrate Solution are clean.

Alternatively, the Substrate Solution may be made by pouring the entire contents of the bottle of Substrate Diluent into the bottle of Substrate Concentrate. One bottle of Substrate Solution provides sufficient reagent for at least five plates - see Table 1:
Table 1

<table>
<thead>
<tr>
<th>Number of Wells</th>
<th>No. of Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td>64</td>
<td>4</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Substrate Concentrate (ml)</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
<th>2.5</th>
<th>2.85</th>
<th>3.0</th>
<th>3.5</th>
<th>4.0</th>
<th>4.5</th>
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<tr>
<td></td>
<td>6</td>
<td>12</td>
<td>18</td>
<td>22</td>
<td></td>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Substrate Diluent (ml)</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
<th>2.5</th>
<th>3.0</th>
<th>3.5</th>
<th>4.0</th>
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<tbody>
<tr>
<td></td>
<td>6</td>
<td>12</td>
<td>18</td>
<td>22</td>
<td></td>
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</tbody>
</table>

Additional reagent may be required for use with automated systems. Keep away from sunlight. The Substrate Solution should be pink; if it is purple before being used, it should be discarded and fresh Substrate Solution prepared.

The prepared Substrate Solution from this kit may be used interchangeably with that from all other Murex kits which use pink coloured Substrate Concentrate. Ensure that the Substrate Solution is prepared from Substrate Diluent and Substrate Concentrate provided together.

The prepared Substrate Solution is stable refrigerated (2 to 8°C) or at 15 to 25°C for up to two days but must be discarded if crystals have formed.

8. Wash Fluid

One bottle containing 128 ml of 20 times working strength Glycine/Borax Wash Fluid. Contains 0.2% Bromox® preservative.

Add one volume of Wash Fluid Concentrate to 19 volumes of distilled or deionised water to give the required volume or dilute the entire contents of one bottle of Wash Fluid to a final volume of 250 ml. Crystals may be observed in the Wash Fluid Concentrate but these crystals will dissolve when the Wash Fluid is diluted to working strength. When diluted, the Wash Fluid contains 0.01% Bromox® preservative.

The Wash Fluid from this kit may be used interchangeably with the Glycine/Borax Wash Fluid from any other Murex kit.

Store the working strength Wash Fluid at 18 to 32°C in a closed vessel under which conditions it will retain activity for one month.

NOTE: The Wash Fluid may develop a yellow colour on storage. This will have no effect on the performance of the assay providing the Wash Fluid is fully aspirated from the wells.

NOTE: Although the Substrate Solution and Wash Fluid are interchangeable, they must not be used beyond the expiry date printed on the component labels.

WARNINGS AND PRECAUTIONS

BVD

The reagents are for in vitro diagnostic use only.
For professional use only.
Please refer to the manufacturer’s safety data sheet and the product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

CAUTION: This kit contains components of human origin.
The human sera used for manufacture have been screened and found reactive or non-reactive for analytes as shown in Table 2 below.

<table>
<thead>
<tr>
<th>Component</th>
<th>Reactive for</th>
<th>Non-reactive for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>N/A</td>
<td>HBsAg, and antibodies to HIV1 and 2, HGV and HTLV I + II</td>
</tr>
<tr>
<td>Positive Control</td>
<td>HBsAg</td>
<td>Antibodies to HIV1 and HIV-2, and HCV</td>
</tr>
</tbody>
</table>

All reagents used in the kit have been inactivated prior to use in reagent preparation. However, all materials of human origin should be considered as potentially infectious and it is recommended that the kit and test specimens be handled using established good laboratory practice.

Pursuant to EC Regulation 72/23/EEC (CLP) hazardous reagents are classified and labelled as follows:

- **Risk Phrases**
  - H317: May cause an allergic skin reaction
- **Precautionary Statements**
  - P200: Wear protective gloves/protective clothing/eye protection/face protection
  - P603: Wash contaminated clothing before reuse
  - P233: P233: If skin irritation or rash occurs: Get medical advice/attention

- **Supplementary Information**
  - Contains: Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 5-methyl-2-isothiazolin-3-one [EC no. 220-238-5] (3:1)

- **Risk Phrases**
  - H319: Causes serious eye irritation
- **Precautionary Statements**
  - P264: Wash hands thoroughly after handling
  - P210: Wear protective gloves/protective clothing/eye protection/face protection
  - P265: P265: If exposed: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing

Pursuant to EU Reg 1223/2009 (CLP) TAKSTM FLUID is labelled as EUH305, safety data sheets available on request.

For additional information see Safety Data Sheets available on www.diaginnov.com
1. Potentially contaminated materials should be disposed of safely according to local requirement.

2. Spillage of potentially infectious material should be removed immediately with absorbent paper tissue and the contaminated area swabbed with, for example, 10% sodium hypochlorite before work is continued. Sodium hypochlorite should not be used on solid containing spills unless the spill area is first wiped dry. Materials used to clean spills, including gloves, should be disposed of as potentially biohazardous waste. Do not autoclave materials containing sodium hypochlorite.

3. Neutralised acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

4. Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.

5. The following reagents contain low concentrations of harmful substances:
   a. The Conjugate and Sample Diluent contain detergents.
   b. Subhelric acid required for the Stop Solution and hydrochloric acid used for washing glassware is corrosive and should be handled with appropriate care. If either come into contact with the skin or eyes, wash thoroughly with water.
   c. Any of the reagents come into contact with the skin or eyes wash the area extensively with water.

ANALYTICAL PRECAUTIONS
1. Do not use the reagents beyond the stated expiry date. Microbiological contamination of reagents must be avoided as this may reduce the life of the product and cause erroneous results.

2. Do not modify the Test Procedure or substitute reagents from other manufacturers or other lots unless the reagent is stipulated as interchangeable. Do not replace any of the recommended incubation times.

3. Allow all reagents and samples to come to 18 to 36°C before use. Immediately after use return all reagents to the recommended storage temperature.

4. Any glassware to be used with the reagents should be thoroughly washed with 2M hydrochloric acid and then rinsed with distilled water or high quality deionised water.

5. Avoid the use of self-defrosting freezers for the storage of reagents and samples.

6. Do not expose reagents to strong light or hypochlorite fumes during storage or during incubation steps.

7. Do not allow wells to become dry during the assay procedure.

8. Do not cross-contaminate reagents. Dedicate a pipette for use with the Substrate Solution of Muraxx assays. A pipette should also be dedicated for use with the Conjugate.

9. Do not touch or splash the rim of the well with Conjugate. Do not blow from microspoon: reverse pipetting is recommended wherever possible.

10. Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate.

11. Do not contaminate microwells with the dust from disposable gloves.

12. When using fully automated microplate processors
   i. It is not necessary to use plate lids and to tap dry the wells.
   ii. Do not allow system fluids from fully automated microplate processors to contaminate samples or reagents.

13. The possibility of cross contamination between assays needs to be excluded when validating assay protocols on fully automated processors.

14. Ensure the assay is run within the temperature limits defined in the assay protocol.

15. Do not use CO₂ incubators.

16. Do not store the Stop Solution in a shallow dish or return it to a stock bottle after use.

17. The possibility of cross contamination between assays needs to be excluded when validating assay protocols on instrumentation.
**FULLY AUTOMATED MICROPLATE PROCESSES.**

Contact your representative for details of currently available validated protocols. For instrumentation without established validated protocols, the following guidelines are recommended:

1. Do not programme times shorter than specified in the procedure.
2. For each incubation at 37°C, programmed times may be increased by up to 20%.
3. Wells containing Sample Diluent may be left for up to 60 minutes at 18 to 30°C prior to addition of sample and controls and for up to 60 minutes after the addition of sample and controls before starting step 6.
4. Ensure all ‘Analytical Precautions’ are followed.

Protocols written following these guidelines must be fully validated prior to use according to local procedures.

**RESULTS.**

**CALCULATION OF RESULTS**

Each plate must be considered separately when calculating and interpreting results of the assay.

Approved software may be used for calculation and interpretation of results.

**Negative Control**

Calculate the mean absorbance of the replicates of the Negative Control.

If one of the Negative Control wells has an absorbance more than 0.03 above the other discard the higher value.

**Cut-off Value**

Calculate the Cut-off Value by adding 0.05 to the mean of the Negative Control replicates.

**Example**

- Negative Control absorbance: well 1 = 0.071, well 2 = 0.075
- Mean Negative Control = (0.071 + 0.075)/2 = 0.073
- Cut-off Value = 0.073 + 0.05 = 0.128

**QUALITY CONTROL**

Results of an assay are valid if the following criteria for the controls are met:

- **Negative Control**
  - The mean absorbance of the Negative Control is less than 0.15 or the mean absorbance of the Negative Control is less than 0.2.

- **Positive Control**
  - The absorbance of the Positive Control is more than 0.8 above the mean absorbance of the Negative Control.

Assays which do not meet these criteria should be repeated.

In the unlikely event of the results repeatedly failing to meet either the Quality Control criteria or the expected performance of the test, please contact your representative.

### INTERPRETATION OF RESULTS

**Non Reactive Results**

Samples giving an absorbance less than the Cut-off Value are considered non-reactive in *Murine HBsAg Confirmatory Version 3*.

**Reactive Result**

Samples giving an absorbance equal to or greater than the Cut-off Value are considered initially reactive in the assay (see Limitations of the Procedure). Such samples should be retested in duplicate using the original sample source. Samples that are reactive in at least one of the re-tests are presumed to contain HBsAg and should be confirmed by using the Murine HBsAg Confirmatory Version 3 kit (ECID-01) and tests for other HBV markers. Samples that are non-reactive in both wells on re-test should be considered non-reactive.

**WASH PROCEDURES.**

Protocol for recommended washers and procedures for verifying washers and analysers can be obtained from your representative. The following protocol is recommended:

- **Protocol for automated microplate strip washer**
  - Perform 5 wash cycles using working strength Wash Fluid. Ensure, where possible, that:
    - Flow-through washing with a fill volume of 600 µl/well is used with instrumentation supplied by DeSerco. When using other instrumentation for which this is not possible, ensure that the wash is fully completed.
    - The dispense height is set to completely fill the well with a slight positive meniscus, without causing an overflow.
    - The time taken to complete one aspirate/wash cycle is approximately 30 seconds.
  - Ensure that no liquid is left in the well (by use of a double aspirate step in the final cycle where possible).
  - After washing is completed, invert the plate and tap out any residual Wash Fluid onto absorbent paper.

**NOTE:** Do not allow the wells to become dry during the assay procedure.

Washers must be rinsed with distilled water at the end of the test to avoid blockage and corrosion.

### SEMI-AUTOMATED PROCESSING

**Step 1** Prepare Substrate Solution and Wash Fluid.
- Use only the number of wells required for the test.

**Step 2** Add 25 µl of Sample Diluent to each well.

**Step 3** Add 75 µl of Samples or Controls to the wells.

**Step 4** To each plate add 75 µl of the Negative Control to wells A1 and B1 and 75 µl of Positive Control into well C1.
- Add the Controls to the designated wells after dispensing the samples.

**Step 5** Cover the plate with a lid and incubate for 60 minutes at 37°C ± 1°C.

**Step 6** Add 50 µl of Conjugate to each well.
- Incubate at 10 sec intervals up to 10 minutes.

**Step 7** Cover the plate with the lid and incubate for 30 minutes at 37°C ± 1°C.

**Step 8** At the end of the incubation time wash the plate 5 times as described under Wash Procedures.
- After washing is completed, invert the plate and tap out any residual Wash Fluid onto absorbent paper.

**Step 9** Immediately after washing plate, add 100 µl Substrate Solution to each well.

**Step 10** Cover the plate with a lid and incubate for 30 minutes at 37°C ± 1°C while colour develops.
- A purple colour should develop in wells containing reactive samples.

**Step 11** Within 15 minutes read the absorbance of each well at 450 nm using 620 nm to 800 nm as the reference wavelength if available.
- Blank the instrument on air (no plate in the carriage).
SPECIFIC PERFORMANCE CHARACTERISTICS

The performance of Murex HBsAg Version 3 has been determined by testing samples from random blood donors, patients with acute and chronic hepatitis B infection, patients with mutant forms of hepatitis B infection and patients with diseases unrelated to hepatitis B infection.

In addition, its performance against the French A.F.S.S.A.R.S. panels and other commercially available seroconversion samples has been evaluated.

1. Donor Samples

The Murex HBsAg assay demonstrated a specificity of 99.6% in a study where a total of 12,000 routine donor samples were screened with Murex HBsAg Version 3. In the study, 0.18% (22/12,000) of samples were initially reactive and 0.03% (47/12,000) were repeatedly reactive. None of the repeatedly reactive samples with Murex HBsAg version 3 and the alternative assays were confirmed as positive for the presence of hepatitis B surface antigen.

The specificity of Murex HBsAg Version 3 on presumed negative samples from donors is estimated to be 99.97% (12,000/12,100) to 99.99% (12,000/12,030) by the binomial distribution.*

2. Clinical Samples

Samples from patients at various stages of hepatitis B infection and patients with conditions unrelated to hepatitis B were tested in three regional virus reference laboratories and at DiaSorin.

A total of 630 samples from patients suffering from acute and chronic hepatitis B infection were tested with Murex HBsAg version 3. All 630 samples were confirmed with an alternative immunoassay for HBsAg and found to be positive in both assays.

A further six samples from patients infected with mutant forms of hepatitis B infection, confirmed by DNA sequencing, were also tested with Murex HBsAg version 3 and were all detected successfully.

In addition 908 potentially cross-reactive samples from patients with conditions unrelated to hepatitis B infection, including other acute viral infections, anicteric,icteric and haemorrhagic samples, were tested with Murex HBsAg Version 3. A total of 466 of these samples were non-reactive with Murex HBsAg Version 3. Of the 466 non-reactive samples, one was false reactive and showed no other hepatitis markers; the remaining sample was anti-HBs positive.

3. Seroconversion Panels

A total of 22 commercially available HBV seroconversion panels were tested with Murex HBsAg Version 3. Comparison with two other commercially available microplate based immunoassays for the detection of hepatitis B surface antigen showed that Murex HBsAg Version 3 detected HBsAg six days earlier in one panel, four days earlier in another panel, two days earlier in one panel, one day earlier in four panels and at the same day in the remaining 15 panels.

4. Assay Reproducibility

Ten replicates of each of five samples were tested on ten separate test occasions with two separate batches to assess the reproducibility of Murex HBsAg Version 3. The results of the study are summarised in Table 3 and Table 4.

*Representative performance data are shown: results obtained at individual laboratories and with different populations may vary.

Table 3

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Number of Assays</th>
<th>Mean Absorbance/Cut-Off Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intra-assay %CV</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>3.96</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>1.29</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>4.44</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>0.98</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Table 4

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Number of Assays</th>
<th>Mean Absorbance/Cut-Off Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intra-assay %CV</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>3.19</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>1.19</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>3.73</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>0.55</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.18</td>
</tr>
</tbody>
</table>

LIMITATIONS OF THE PROCEDURE

1. The Test Procedure and Interpretation of Results must be followed.
2. The test has only been evaluated for use with individual (unpooled) serum, EDTA plasma, or citrate plasma samples.
3. A negative result with an antigen detection test does not preclude the possibility of infection.
4. Non-repeatable reactive results may be obtained with any EIA procedure.
5. The most common sources of error are:
   a. Improper delivery of Sample, Conjugate or Substrate into the wells.
   b. Contamination of Substrate or Conjugate.
   c. Contamination with conjugates from other assays.
   d. Blocked or partially blocked washer probes.
   e. Insufficient aspiration leaving a small volume of Wash Fluid in the wells.
   f. Failure to ensure that the bottom surface of the wells is clean and dry, and that no air bubbles are present on the surface of the liquid in the wells before a plate is read.
   g. Failure to read at the correct wavelength or use of an incorrect reference wavelength.
6. The use of highly haemolysed samples, incompletely clotted sera, plasma samples containing fibrin or samples with microbial contamination may give rise to erroneous results.
7. This test has not been evaluated for use with samples from cordovirus.

BIBLIOGRAPHY


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UK

D010334GB
September, 2014
Instruction for use for Murex HBsAg Confirmatory version 3

Murex HBsAg Confirmatory Version 3

For the confirmation of the presence of hepatitis B surface antigen in serum and plasma samples.

Customer Service
For additional product information, please contact your local customer service organization.

This instructions for use must be read carefully prior to use. The instructions for use must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions for use.

Key to symbols used

See REAGENTS section for a full explanation of symbols used in reagent component naming.
INTENDED USE
Murix® HBsAg Confirmatory Version 3 (2G27) is used to confirm the presence of hepatitis B surface antigen (HBsAg) in serum or plasma samples found to be reactive in the Murix® HBsAg Version 3 (9F80) assay.

SUMMARY AND EXPLANATION OF THE TEST
Murix® HBsAg Confirmatory functions by means of specific antibody neutralisation of HBsAg in samples that are repeatedly reactive in the Murix® HBsAg (9F80) assay. In contrast to the goat and mouse antibodies used in the HBsAg (9F80) assay, the specific antibody used in the confirmatory reagent is derived from horse, this precaution minimizes the risk of confirming false positive samples containing anti-species antibodies.

PRINCIPLE OF THE PROCEDURE
For each test sample two assay wells are assigned. The Murix® HBsAg assay is run according to the usual procedure except that the Sample Diluent is substituted with Control Reagent in the control well and Specific Reagent in the specific well. During the first incubation the horse anti-HBs in the Specific Reagent will compete with the mouse antibodies immobilised on the well for any HBsAg present in the sample and will reduce the amount of HBsAg binding to the well. In the control well there is no competition and the HBsAg will bind normally. Conjugate is then added and the assay completed in the normal way. In samples containing HBsAg there will be a significant difference between the A405 generated in control and specific wells, if the inhibition in the specific well exceeds 50%, the sample is considered to be confirmed reactive.

REAGENTS
DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS.
See also Warnings and Precautions.

1. Control Reagent
One bottle containing 125 ml of a yellow coloured buffer containing detergents and material of horse, goat, human and bovine origin. Contains 0.038% ProClin® 300 preservative.

2. Specific Reagent
One bottle containing 125 ml of specific horse antibody to HBsAg in a red coloured buffer containing detergents and material of horse, goat, human and bovine origin. Contains 0.038% ProClin® 300 preservative.

WARNINGS AND PRECAUTIONS

IVD
The reagents are for in vitro diagnostic use only.
For professional use only.
Please refer to the manufacturer’s safety data sheet and the product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

CAUTION: This kit contains components of human origin.
The human sera used for manufacture have been screened and found reactive or non-reactive for analytes as shown in Table 1 below:

<table>
<thead>
<tr>
<th>Component</th>
<th>Reactive for</th>
<th>Non-reactive for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Reagent</td>
<td>N/A</td>
<td>HBsAg, anti-HCV and anti-HIV-1/HIV-2</td>
</tr>
<tr>
<td>Specific Reagent</td>
<td>N/A</td>
<td>HBsAg, anti-HCV and anti-HIV-1/HIV-2</td>
</tr>
</tbody>
</table>

All material of human origin should be considered as potentially infectious and it is recommended that this kit and test specimens be handled using established good laboratory practice.

Pursuant to EC Regulation 1272/2008 (CLP) hazardous reagents are classified and labelled as follows.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>CONTROL REAGENT</th>
<th>SPECIFIC REAGENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classification</td>
<td>Skin sens. 1 H317</td>
<td></td>
</tr>
<tr>
<td>Signal Word</td>
<td>Warning</td>
<td></td>
</tr>
<tr>
<td>Symbols / Photoimage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazard Statements</td>
<td>P317 May cause an allergic skin reaction</td>
<td></td>
</tr>
<tr>
<td>Precautionary Statements</td>
<td>P284 Wear protective gloves/ protective clothing/ eye protection/ face protection</td>
<td>P363 Wash contaminated clothing before reuse. P304+P341 If skin irritation or rash occur: Get medical advice/attention</td>
</tr>
<tr>
<td>Contain</td>
<td>Reaction mass of: 8-chloro-2-methyl-4-isothiazole-3-one [EC no. 247-600-7] and 2-methyl-2H-isothiazole-3-one [EC no. 220-229-3] (3:1)</td>
<td></td>
</tr>
</tbody>
</table>

See also Murix® HBsAg Version 3 (9F80) Instructions for Use.

ANALYTICAL PRECAUTIONS
See also Murix® HBsAg Version 3 (9F80) Instructions for Use.

1. Avoid cross-contamination of controls, samples or other reagents with the Specific Reagent.
2. Do not use the reagents beyond the stated expiry date. Microbiological contamination of reagents must be avoided as this may reduce the life of the product and cause erroneous results.
3. Do not modify the Test Procedure or substitute reagents from other manufacturers or other lots. Do not reduce any of the recommended incubation times.
4. Allow all reagents and samples to come to 18 to 30°C before use. Immediately after use return all reagents to the recommended storage temperature.
5. Any glassware to be used with the reagents should be thoroughly washed with 2M hydrochloric acid and then rinsed with distilled water or high quality deionized water.
6. Avoid the use of self-defrosting freezers for the storage of reagents and samples.
7. Do not expose reagents to strong light or hypochlorite fumes during storage or during incubation steps.
8. Do not allow wells to become dry during the assay procedure.
9. The possibility of cross contamination between assays needs to be excluded when validating assay protocols on instrumentation.
SPECIMEN COLLECTION, TRANSPORT AND STORAGE

SPECIMEN COLLECTION
Serum, EDTA plasma or citrate plasma samples may be used. Ensure that the serum samples are fully clotted. Remove any visible particulate matter from the sample by centrifugation.

SPECIMEN TRANSPORT AND STORAGE
Store samples at 2 to 8°C. Samples not required for assay within 72 hours should be removed from the clot or cell pellet and stored frozen (-18°C or colder). Avoid multiple freeze-thaw cycles. After thawing, ensure samples are thoroughly mixed before testing.

PROCEDURE
MATERIALS REQUIRED BUT NOT PROVIDED
1. Murex HbsAg Version 3 (IF80).
2. Glass or plastic tubes or microplates for diluting samples.
3. Micropipettes and Multichannel micropipettes of appropriate volume.
4. Physiological Saline (0.86 g NaCl in 100 ml distilled water) for diluting samples.
5. Other materials and equipment as for Murex HbsAg Version 3 (IF80).

TEST PROCEDURE
Please read "Analytical Precautions" carefully before performing the test.

Notes:
1. Include a minimum of one Negative Control and one Positive Control in each assay run.
2. Mix the Control and Specific Reagent thoroughly by inversion prior to use.
3. Addition of the various components of the assay to the wells may be confirmed visually by examining the plate for the following colours.
   Control Reagent: is yellow and changes to green on addition of the controls or sample, the Positive Control and some plasma samples will produce only a slight change but some difference should always be visible.
   Specific Reagent: is red and changes to purple on addition of the controls or sample.
   Samples diluted in saline will give little or no colour change with either reagent.
4. Samples giving a maximum absorbance reading in Murex HbsAg Version 3 should be diluted 1/100 in 0.86% Saline before use. All other samples can be tested undiluted.
5. Two assay wells are used for each sample and control (see Figure 1).

| Step 1 | Prepare the Substrate Solution and Wash Fluid. See Murex HbsAg Version 3 (IF80) Instructions for Use. |
| Step 2 | Use only the number of wells required for the test. Avoid touching the tops or bottoms of the wells. |
| Step 3 | Add 25 µl of Control Reagent into appropriate wells (see Figure 1). |
| Step 4 | Add 25 µl of Specific Reagent into appropriate wells (see Figure 1). |
| Step 5 | Add 75 µl of Murex HbsAg Controls and Test Samples to the wells (see Figure 1). |
| Step 6 | Shake the plate using a plate shaker for 10 seconds. Alternatively, the plate may be manually agitated by gently tapping the sides for 10 seconds. |
| Step 7 | Proceed from and including Step 5 of the Murex HbsAg Version 3 (IF80) Test Procedure as described in the Instructions for Use. |

RESULTS
CALCULATION OF RESULTS
When reading the absorbance of each well at 450 nm, a reference wavelength in the range 620 to 690 nm may be used if available (Auxy, eg). Blank the instrument on air (no plate in carriage).

Each plate must be considered separately when calculating and interpreting results of the assay. Approved software may be used for calculation and interpretation of results.

1. Negative Control
   Calculate the mean absorbance of the Negative Control incubated with Specific and Control Reagent.

   Example:

   \[
   \begin{align*}
   A_{\text{mean}} \text{ of Negative Control with Specific Reagent (NS)} &= 0.080 \\
   A_{\text{mean}} \text{ of Negative Control with Control Reagent (NC)} &= 0.085 \\
   \text{Total} &= 0.165 \\
   \text{Mean Negative Control} &= 0.165/2 = 0.083
   \end{align*}
   \]
2. Cut-off value  
This is calculated as the mean absorbance of the Negative Controls + 0.05

3. Positive Control  
Calculate the percentage inhibition of the Positive Control with Specific Reagent.  
Example:  
\[ \frac{A_{\text{PC,inc}} - A_{\text{SS,inc}}}{\text{PO - NC}} \times 100 \]  
where:  
- \( A_{\text{PC,inc}} \) is the absorbance of Positive Control incubated with Specific Reagent  
- \( A_{\text{SS,inc}} \) is the absorbance of Specific Reagent incubated with Control Reagent  
- \( \text{PO} \) is the absorbance of Positive Control  
- \( \text{NC} \) is the absorbance of Specific Reagent

4. Reactive Samples  
Calculate the inhibition of reactive samples.  
Example:  
\[ \frac{(A_{\text{PC,inc}} - A_{\text{SS,inc}})}{(\text{PO} - \text{NC})} \times 100 \]  
where:  
- \( A_{\text{PC,inc}} \) is the absorbance of Positive Control incubated with Specific Reagent  
- \( A_{\text{SS,inc}} \) is the absorbance of Specific Reagent incubated with Control Reagent  
- \( \text{PO} \) is the absorbance of Positive Control  
- \( \text{NC} \) is the absorbance of Specific Reagent

For samples giving maximum absorbance readings or absorbance readings greater than 2.0 with the Control Reagent, an absorbance value of 2.0 should be used for the calculations.

QUALITY CONTROL  
Results of an assay run are valid if the following criteria for the controls are met:  

Negative Control  
The mean \( A_{\text{SS,inc}} \) is less than 0.15 or the mean \( A_{\text{PC,inc}} \) is less than 0.2.  

HbsAg Positive Control  
The mean \( A_{\text{PC,inc}} \) or \( A_{\text{SS,inc}} \) of the Positive Control incubated with Control Reagent is more than 0.8 above the mean absorbance of the Negative Control.  
The absorbance of the Positive Control incubated with Specific Reagent is reduced by at least 80% of the Positive Control incubated with Control Reagent.  
Assays which do not meet these criteria should be repeated.  
In the unlikely event of the results repeatedly failing to meet either the Quality Control criteria or the expected performance of the test, please contact your representative.

INTERPRETATION OF RESULTS  
Confirmed reactive result  
For a sample to be considered as confirmed reactive in the Murex HbsAg Version 3 assay (9750), the following criteria must be met:  
(i) The absorbance with the Control Reagent must be equal to or greater than the Cut-off value. If the sample has been tested undiluted and does not meet this criterion it should be considered as indeterminate. If the sample is diluted and fails this criterion it should be tested again but undiluted.  
(ii) Inhibition by the Specific Reagent must be equal to or greater than 60%. For strongly reactive samples, if the inhibition is less than 60% and the absorbance with the Control Reagent is greater than 2.0 the sample should be repeated at a dilution of 1/100 or 1/10,000 as appropriate. Percentages may occasionally exceed 100%.  

Nonreactive result  
Providing the absorbance in the control well is less than 2.0 then samples giving less than 60% inhibition by the Specific Reagent are considered negative and therefore false reactive in Murex HbsAg Version 3 (9750). A negative percentage inhibition may be encountered and should also be considered as a negative result.

SPECIFIC PERFORMANCE CHARACTERISTICS  
A total of 49 samples, which were reactive with at least two immunosassays for the detection of hepatitis B surface antigen, have been tested with Murex HbsAg Confirmatory Version 3 (2012). The samples included specimens from patients at various stages of hepatitis B infection.  
All of the samples were confirmed when tested without dilution in Murex HbsAg Confirmatory Version 3 (2012).  
In addition a total of 506 samples, which were reactive with Murex HbsAg Version 3 (9750) and at least one other immunosassay for the detection of hepatitis B surface antigen, have been tested with Murex HbsAg Confirmatory Version 3 (2012). The samples included specimens from patients infected with known subtypes or mutant forms of hepatitis B.  
All of the 100 samples which gave a below maximum absorbance reading with Murex HbsAg Version 3 (9750) were confirmed when tested without further dilution in Murex HbsAg Confirmatory Version 3 (2012).  
Of the remaining 506 samples, which gave maximum absorbance reading with Murex HbsAg Version 3 (9750), 94 were confirmed with no further dilution and 22 were confirmed with a dilution factor of greater than 100 with Murex HbsAg Confirmatory Version 3 (2012).

LIMITATIONS OF THE PROCEDURE  
See also the Murex HbsAg Version 3 (9750) Instructions for Use.  

1. The Test Procedure and Interpretation of Results must be followed.  
2. This confirmatory assay discriminates between true and false reactivity in Murex HbsAg Version 3 (9750). This assay has been shown to be effective with mutated forms of HbsAg, however it is possible (as with any neutralisation assay) that some forms of HbsAg may fail to be inhibited. To establish the HBV status of an individual this assay should be used in conjunction with other HBsAg assays and tests for HBV markers, e.g. anti-Hbc IgM, anti-Hbc, anti-Hbe, anti-HBs, HBV DNA.  
3. This test has only been evaluated for use with individual (un pooled) serum, EDTA plasma or citrate plasma samples.  
4. Some false positive results occur due to reactivity with animal proteins. The absorbance of these samples will be reduced by both the Control and Specific Reagent. Rarely, the Specific Reagent may have a greater effect than the Control Reagent, which, upon calculation, will appear as confirmation. In this situation, if the absorbance is also markedly decreased by the Control Antibody, the sample is unlikely to be positive and must be tested by another method.  
5. A negative result with an antigen detection or confirmatory test does not preclude the possibility of infection with HBV.

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