WHO Prequalification of In Vitro Diagnostics Programme
PUBLIC REPORT

Product: Murex HIV Ag/Ab Combination
Number: PQDx 0144-043-00

Abstract

Murex HIV Ag/Ab Combination with product codes 7G79-09 (GE41, 96 wells) and 7G79-11 (GE42, 480 wells), manufactured by DiaSorin S.p.A - UK Branch, CE-marked regulatory version, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 30 March 2015.

Murex HIV Ag/Ab Combination is an enzyme immunoassay for the simultaneous qualitative detection of Human Immunodeficiency Virus (HIV) p24 antigen and antibodies to HIV type 1 (HIV-1 group O) and HIV type 2 (HIV-2) in human serum or plasma. This kit is intended as an aid in the diagnosis of HIV-1 and/or HIV-2 infection. Murex HIV Ag/Ab Combination is intended for manual use with an automated microplate washer and reader, and for use with fully automated microplate instrumentation using a validated protocol. Results from Murex HIV Ag/Ab Combination cannot be used to distinguish between the presence of HIV-1 p24 antigen, HIV-1 antibody, or HIV-2 antibody in a specimen.

Murex HIV Ag/Ab Combination is intended for screening individual human donors (blood or plasma) for the presence of HIV-1 p24 antigen and antibodies to HIV-1 (including subtype O) and HIV-2, and as an aid to diagnosis.

Murex HIV Ag/Ab Combination is based on microwells coated with synthetic peptide representing immunodominant regions of HIV-1 (O) and HIV-2, recombinant protein derived from the envelope regions of HIV-1 and HIV-2 and HIV pol protein, together with monoclonal antibodies raised against p24 of HIV-1. The Conjugate is a mixture of the same antigen epitopes, and different monoclonal antibodies, also raised against p24, all labelled with horseradish peroxidase.

Test and control specimens are incubated in the wells and reactive HIV-1 p24 antigen and/or antibodies to HIV-1/2 in the test or control specimens sera bind to the antibodies and/or antigens on the microwell; sample and any excess antibodies or antigen are then washed away. In a subsequent step, Conjugate is added which in turn binds to any reactive HIV-1 p24 antigen and/or specific antibodies to HIV-1/2 already bound to the reagents on the well. Specimen not containing HIV-1 p24 antigen or specific antibodies to HIV-1/2 will not cause the Conjugate to bind to the well. Unbound Conjugate is washed away and a solution containing 3,3′,5,5′- tetramethylbenzidine (TMB) and hydrogen peroxide is added.
to the wells. Wells with bound Conjugate develop a blue green colour which is converted to an orange colour which may be read at 450nm after the reaction has been stopped with sulphuric acid.

Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive in the assay (see Limitations of the Procedure).

Unless local procedures state otherwise, such specimens must be repeated in duplicate using the original source specimen. Specimens that are reactive in at least one of the duplicate repeat tests are considered repeatedly reactive in Murex HIV Ag/Ab Combination and are presumed to contain HIV-1 p24 antigen and/or antibodies to HIV-1 or HIV-2. Such specimens must be further investigated and the results of this assay considered with any other clinical and supplemental testing. Specimens that are non-reactive in both wells on repeat testing are considered non-reactive for HIV-1 p24 antigen and antibodies to HIV-1/2.

The test kit contains:

<table>
<thead>
<tr>
<th>Coated Wells</th>
<th>96 tests (product code 7G79-09)</th>
<th>480 tests (product code 7G79-11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 microwells coated with HIV antigens and monoclonal antibodies</td>
<td>One plate</td>
<td>Five plates</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample Diluent</th>
<th>96 tests (product code 7G79-09)</th>
<th>480 tests (product code 7G79-11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green/brown buffered solution containing bovine and murine protein, detergent and saponin. Contains 0.05% ProClin® 300 preservative.</td>
<td>1 bottle of 8 ml</td>
<td>1 bottle of 18 ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>96 tests (product code 7G79-09)</th>
<th>480 tests (product code 7G79-11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV antigens and monoclonal antibodies conjugated to horseradish peroxidase and freeze dried. When reconstituted each bottle is sufficient for up to two plates.</td>
<td>1 bottle of 1.1 ml</td>
<td>3 bottles of 1.1 ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Conjugate Diluent</th>
<th>96 tests (product code 7G79-09)</th>
<th>480 tests (product code 7G79-11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow buffered solution consisting bovine protein, saponin and detergent. Sufficient to reconstitute one bottle of Conjugate</td>
<td>1 bottle of 22 ml</td>
<td>3 bottles of 22ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anti-HIV-1 Positive Control</th>
<th>96 tests (product code 7G79-09)</th>
<th>480 tests (product code 7G79-11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 bottle of 1.7 ml</td>
<td>1 bottle of 1.7 ml</td>
<td></td>
</tr>
</tbody>
</table>
Inactivated human serum in a buffer containing bovine protein.

| Anti-HIV-2 Positive Control  | 1 bottle of 1.7 ml | 1 bottle of 1.7 ml |
| HIV-1 p24 Positive Control   | 1 bottle of 1.7 ml | 1 bottle of 1.7 ml |
| Negative Control             | 2 bottles of 2.5ml | 2 bottles of 2.5ml |
| Substrate Diluent            | 1 bottle of 35 ml  | 1 bottle of 35 ml  |
| Substrate Concentrate        | 1 bottle of 35 ml  | 1 bottle of 35 ml  |
| Wash Fluid                   | 1 bottle of 125 ml | 2 bottles of 125 ml |

**Note:** a copy of the instructions for use is not part of the test kit components, and must be requested separately from the manufacturer, or the local distributor.

**Storage:**
2 to 8 °C (for all components, under which condition they will retain activity until the expiry date of the kit)

**Shelf-life:**
12 months.
Summary of prequalification status for Murex HIV Ag/Ab Combination

<table>
<thead>
<tr>
<th>Status on PQ list</th>
<th>Date</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dossier assessment</td>
<td>22 July 2014</td>
<td>MR</td>
</tr>
<tr>
<td>Inspection status</td>
<td>12 August 2014</td>
<td>MR</td>
</tr>
<tr>
<td>Laboratory evaluation</td>
<td>18 February 2014</td>
<td>MR</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Initial acceptance</th>
<th>Date</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status on PQ list</td>
<td>30 March 2015</td>
<td>listed</td>
</tr>
</tbody>
</table>

MR: Meets Requirements  
N/A: Not Applicable

Murex HIV Ag/Ab Combination was accepted for the WHO list of prequalified in vitro 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 HIV Ag/Ab Combination. Based on the established prioritization criteria, Murex HIV Ag/Ab Combination was given priority for prequalification.

Product dossier assessment

DiaSorin S.p.A UK Branch submitted a product dossier for Murex HIV Ag/Ab Combination as per the Instructions for compilation of a product dossier (PQDx_018 v3). 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 HIV Ag/Ab Combination for prequalification.

Commitments for prequalification:

1. Updated instructions for use.

Manufacturing site inspection

A comprehensive inspection was performed at the site of manufacture (Central Road, Dartford, Kent, DA1 5LR, UK) and the site of warehousing (Via Crescentino, snc, 13040 Saluggia, Italy) of Murex HIV Ag/Ab Combination in February 2014 as per the Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics (PQDx_014 v3). 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 11 August 2014.

Commitments for prequalification:
1. N/A

Laboratory evaluation
Murex HIV Ag/Ab Combination (DiaSorin S.p.A UK Branch was evaluated by WHO at the Institute of Tropical Medicine, Antwerp, Belgium – a WHO Collaborating Centre for HIV/AIDS Diagnostics and Laboratory Support. The laboratory evaluation was conducted according to the “WHO protocol for the laboratory evaluation of HIV serology assays” (PQDx_030 v1.0), and drew the following conclusions:

Murex HIV Ag/Ab Combination (DiaSorin S.p.A UK Branch) is a qualitative 4th generation sandwich enzyme immunoassay intended to screen individual human donors for the presence of HIV p24 antigen and antibodies to HIV-1, including group O, and HIV-2 or as an aid to the diagnosis of HIV infection. A volume of 100 µl of specimen is needed to perform the assay. This type of assay requires laboratory equipment and cannot be performed in laboratories with limited facilities. Reading of the results must be performed with a spectrophotometer.

In this limited performance evaluation on a panel of 1119 specimens, we found an initial sensitivity (95% CI) of 100% (99.2 - 100%) and an initial specificity (95% CI) of 99.4% (98.4 - 99.8%) compared to the reference results. The final sensitivity (95% CI) was 100% (99.2 - 100%) and the final specificity (95% CI) was 99.7% (98.9 - 100%) compared to the reference results. Lot to lot variation observed was within the acceptance range.

For eight seroconversion panels, Murex HIV Ag/Ab Combination detected on average 1.125 specimens earlier than the benchmark assay (Enzygnost Anti-HIV 1/2 Plus [Siemens Healthcare Diagnostics]) and on average 0.5 specimens earlier than Vironostika HIV Ag/Ab (bioMérieux) EIA.

For the mixed titer panel, Murex HIV Ag/Ab Combination correctly classified all specimens. For the HIV-1 p24 antigen panel, Murex HIV Ag/Ab Combination classified all but one of HIV-1 antigen positive/anti-HIV negative specimens. For the HIV culture supernatant panel, Murex HIV Ag/Ab Combination identified all HIV-1 and HIV-2 subtypes.

For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], Murex HIV Ag/Ab Combination detected all subtypes tested (HIV-1 A, HIV-1 B, HIV-C, HIV-1 CRF01_AE, HIV-1 O and HIV-2). For the HIV-1 p24 antigen standard [NIBSC code 90/636], Murex HIV Ag/Ab Combination detected to 1.56 international units. In contrast, Vironostika HIV Ag/Ab (bioMérieux) detected to 12.5 international units.

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

1. Labels
2. Instructions for use
1. Labels
Murex HIV Ag/Ab Combination

480 Wells / Cavidades

Enzyme immunoassay for the improved detection of anti-HIV antibody and anti-HIV p24 in plasma or serum. Detection of anti-HIV antibody is based on the principle of enzyme immunoassays (EIA). The test screening is based on the ELISA method and the detection is based on the EIA method. The test is designed for use in the diagnosis of HIV infection in adults and children, and is used for the detection of anti-HIV antibody. The test is useful for the diagnosis of HIV infection in individuals with a history of HIV exposure. The test is designed for use in the diagnosis of HIV infection in individuals with a history of HIV exposure.
2. Instructions for use

Murex HIV Ag/Ab Combination

Enzyme immunoassay for improved detection of seroconversion to human immunodeficiency virus types 1 (HIV-1, HIV-1 group O) and detection of anti-HIV-2 antibodies

The assay is intended to screen individual human donors for the presence of HIV p24 antigen and antibodies to HIV-1, including group O, and HIV-2 or as an aid to the diagnosis of HIV 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

See REAGENTS section for a full explanation of symbols used in reagent component naming.
INTENDED USE
Enzyme immunoassay for improved detection of seroconversion to human immunodeficiency virus types 1 (HIV-1, HIV-1 group O) and detection of anti-HIV-2 antibodies.
The assay is intended to screen individual human donors for the presence of HIV p24 antigen and antibodies to HIV-1, including group O, and HIV-2 or as an aid to the diagnosis of HIV infection.

SUMMARY AND EXPLANATION OF THE TEST
Two types of human immunodeficiency virus, HIV-1 and HIV-2, have been described and implicated as causative of the Acquired Immunodeficiency Syndrome (AIDS). Both are retroviruses which are transmitted by exposure to certain infected body fluids, primarily blood and genital secretions, and by transplacental passage. Infection by HIV-1 has been reported worldwide; HIV-2 infection has been reported as occurring mainly in West Africa and some European countries.
The two types of virus show substantial antigenic cross-reactivity in their gag and pol proteins, but the envelope glycoproteins are less cross-reactive.

It is necessary for screening purposes to use epitopes from the envelope proteins of both viruses in addition to major cross reacting gag or pol proteins to ensure detection of antibodies against both types of virus at all stages following infection. Variants of HIV-1, classified together as group O, have been identified in samples from Cameroon and Europe. Group O is highly divergent from the originally known subtypes of HIV-1 (together classified as group M). Specific epitopes from the envelope region of this virus can be used to detect antibody to group O in infected individuals: reliance on cross reactions to the known subtypes of HIV is not satisfactory. The earliest specific antibody response following infection by HIV may be of immunoglobulin M (IgM) followed by a response in immunoglobulin G (IgG). Maximum sensitivity for detection of anti-HIV seroconversion is achieved by assays which respond to both IgM and IgG whilst HIV core antigen is typically detectable during a short period prior to antibody seroconversion.

Murex HIV AgAb Combination is designed to detect reactive HIV core antigen in addition to IgG, IgM and IgA to the envelope glycoproteins and the cross reacting pol proteins of HIV-1 and HIV-2. Consequently potentially infectious samples of serum, EDTA plasma or citrate plasma can be identified.

PRINCIPLE OF THE PROCEDURE
Murex HIV AgAb Combination is based on microwells coated with synthetic peptide representing non-antigenic regions of HIV-1 (O) and HIV-2, recombinant protein derived from the envelope regions of HIV-1 and HIV-2 and HIV pol protein, together with monoclonal antibodies raised against p24 of HIV-1. The Conjugate is a mixture of the same antigen epitopes, and different monoclonal antibodies, also raised against p24, all labelled with horse-radish peroxidase.
Test specimens and control sera are incubated in the wells and reactive HIV core and/or antibodies to HIV in the sample or control sera bind to the antibodies and/or antigens on the microwell; sample and any excess antibodies are then washed away. In a subsequent step, Conjugate is added which in turn binds to any reactive HIV core and/or specific antibody already bound to the reagents on the well. Samples not containing reactive core antigen or specific antibody will not cause the Conjugate to bind to the well.
Unbound Conjugate is washed away and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide is added to the wells. Wells with bound Conjugate develop a blue green colour which is converted to an orange colour which may be read at 450mm after the reaction has been stopped 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 conditions they will retain activity until the expiry date of the kit.

1. Coated Wells
One plate (7G78-09) or five plates (7G79-11) of 96 microwells coated with HIV antibodies and monoclonal antibodies.

2. Sample Diluant
One bottle containing 8 ml (7G79-09) or 18 ml (7G79-11) of a green/brown buffer solution, bovine and murine proteins, detergent and sorbitol. Contains 0.05% ProClin® 300 preservative.

3. Conjugate
One bottle (7G79-09) or three bottles (7G79-11) containing 1 ml of HIV antibodies and monoclonal antibodies conjugated to horseradish peroxidase and freeze dried. When reconstituted each bottle is sufficient for up to two plates.

4. Conjugate Diluent
One bottle (7G79-09) or three bottles (7G79-11) containing 25ml of a yellow solution consisting of buffer, bovine protein, sorbitol and detergents, sufficient to reconstitute one bottle of Conjugate. Contains 0.1% ProClin® 300 preservative.

Reconstitution of Conjugate
Tap the bottle of Conjugate gently on the bench to remove any material adhering to the rubber stopper. Pour the whole contents of the bottle of conjugate diluent into the bottle of conjugate, recap the latter and mix by gentle inversion. Allow to rehydrate for at least 30 minutes with occasional swirling. The reconstituted conjugate will be red in colour. Reconstituted conjugates may be returned to and pooled in the plastic conjugate diluent bottles required.

After reconstitution the Conjugate may be stored at 2 to 8°C for up to four weeks.

5. Anti-HIV Positive Control
One bottle containing 1.7 ml of inactivated human serum in a buffer containing bovine proteins. Contains 0.05% Boviban® preservative.

6. Anti-HIV Positive Control
One bottle containing 1.7 ml of inactivated human serum in a buffer containing bovine proteins. Contains 0.05% Boviban® preservative.

7. HIV-1 p24 Positive Control
One bottle containing 1.7 ml of p24 (recombinant antigen) in a buffer containing bovine proteins. Contains 0.05% Boviban® preservative.

8. Negative Control
Two bottles containing 2.6 ml of normal human serum diluted in a bovine protein buffer. Contains 0.05% Boviban® preservative.
0. Substrate Diluent
One bottle containing 38 ml of a colourless solution of tri-sodium citrate and hydrogen peroxide.

10. Substrate Concentrate
One bottle containing 35 ml of 3,3’,5,5’-tetramethylbenzidine (TMB) and stabilisers in an orange solution.

Substrate Solution
To prepare the Substrate Solution add a volume of colourless Substrate Diluent to an equal volume of orange Substrate Concentrate in either a clean glass or plastic vessel.

It is important that this order of addition is followed and that any pipettes 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>
<thead>
<tr>
<th>Number of Wells</th>
<th>Number of Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>32</td>
<td>4</td>
</tr>
<tr>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td>48</td>
<td>6</td>
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<tr>
<td>56</td>
<td>7</td>
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<tr>
<td>64</td>
<td>8</td>
</tr>
<tr>
<td>72</td>
<td>9</td>
</tr>
<tr>
<td>80</td>
<td>10</td>
</tr>
<tr>
<td>96</td>
<td>11</td>
</tr>
</tbody>
</table>

Additional reagent may be required for use with automated systems. Keep away from sunlight. The Substrate Solution should be pale yellow, if it is green 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 orange coloured Substrate Concentrate. Ensure that the Substrate Solution is prepared from the 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 it must be discarded if crystals have formed.

WASH FLUID
One (709/09) or two (709/11) bottles containing 125 ml of 20 times working strength Glycine/ Borate Wash Fluid, Contains 0.2% Bromocresol 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 2500 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% Bromocresol preservative.

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

Store the working strength Wash Fluid at 18 to 20°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

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.

Low levels of fibrin precipitate may be observed in the Kit Controls and product performance is not affected by this. This is a product of certain serum batches used to manufacture the controls.

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, antibodies to HDV, HIV-1 and HIV-2</td>
</tr>
<tr>
<td>Positive Control 1</td>
<td>antibodies to HIV-1</td>
<td>HBsAg</td>
</tr>
<tr>
<td>Positive Control 2</td>
<td>antibodies to HIV-2</td>
<td>HBsAg</td>
</tr>
</tbody>
</table>

Additionally human sera used for positive controls are also tested for antibodies to HBV and may be reactive.

All reagents used has been inactivated prior to use in reagent preparation. However, all material of human origin should be considered as potentially infectious and 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 labeled as follows:

<table>
<thead>
<tr>
<th>Reactgen</th>
<th>Classificaion</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONJUGATE</td>
<td>Skin sens. 1 H377</td>
<td>Warning</td>
</tr>
<tr>
<td>SAMPLE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONJUGATE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Symbol |

HAZARD STATEMENTS
R377 May cause an allergic skin reaction.

Precautionary Statements
P280 Wear protective gloves/protective clothing/eye protection/face protection
P302 Wash contaminated clothing before reuse
P335+P333 If skin irritation or rash occurs: Get medical advice/attention

Contains
Reaction mass of: 2-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-570-7] and 2-methyl-4-isothiazolin-3-one [EC no. 220-389-5] (3:1)

The reconstituted Conjugate contains 0.1% ProClin 950 which is classified hazardous per EC Regulation 1272/2008.
11. 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.

12. Do not contaminate microtubes with dust from disposable gloves.

13. When using fully automated processors:
   i. It is not necessary to use plate lids and to dry the wells.
   ii. Do not allow system fluids to contaminate samples or reagents.
   iii. The possibility of cross-contamination between assays needs to be excluded when validating assays 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 allow 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.

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. If samples are prepared using liquid anti-coagulants e.g. citrate plasma, the dilution effect should be considered.

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. Stop Solution (0.5 mol/l sulphuric acid): 20 ml (for 0.5M) and 11 ml (for 2.0M) of analytical grade concentrated sulphuric acid (18M) to about 80 ml of distilled or deionized water and then make up to 100 ml with more water. Alternatively, the following reagent can be used: 1N Sulphuric Acid (Code N0164 - 50 ml pack and N0165 - 1 litre pack).

2. Freshly distilled or high quality deionised water is required for dilution of Wash Fluid, for preparation of the Stop Solution and for use in conjunction with automated washers.

3. Micropipettes and Multichannel micropipettes of appropriate volume.

4. Incubator capable of maintaining the temperature limits defined in the assay protocol.

5. Moulded Heating Block (Code 0F09-02). For use in laboratory incubators. The moulded heating block should ideally be kept in the incubator used. If this is not possible it must be placed in the incubator at least four hours before beginning the assay.

6. Instrumentation
   a. Automated microplate stripwashes.
   b. Microplate reader.
   c. Fully automated microplate processors.

All instruments must be validated before use. Please consult your representative for details of recommended systems, software protocols for instrumentation and validation procedures.

7. Disposable Reagent Troughs. (Code 0F24-01).

8. Sodium hypochlorite for decontamination. (Refer to Health and Safety Information)

9. Sodium hydroxide solution (0.1M). (Refer to Analytical Precautions)
TEST PROCEDURE
Please read Analytical Precautions carefully before performing the test.
Addition of the various components of the assay to the wells may be confirmed visually by examining the plate for the following colours:
Sample Diluent is green/brown in colour. On addition of Sample or Control the colour will change to blue/green. The colour change will vary from sample to sample but some change should always be visible. The addition of sample or control may be confirmed using a microplate reader at 700 nm or 520 nm with a reference of 630 nm.
Reconstituted Conjugate is red in colour. The addition of Conjugate may be confirmed using a microplate reader at 490 nm with a reference of 680 nm.
Substrate Solution is initially pale yellow with any reactive wells becoming blue green. On addition of Stop Solution the blue green colour of the reactive will change to orange, whilst the negatives will change to pink. The addition of Substrate Solutions may be confirmed using a microplate reader at 450 nm (no reference).

SEMI AUTOMATED PROCESSING

Step 1
Reconstitute and mix the Conjugate, prepare the Substrate Solution and Wash Fluid.

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 Sample Diluent to each well.

Step 4
Add 100 µl of Samples or 100 µl Controls to the wells.

Step 5
Cover the wells with the lid and incubate for 60 minutes at 37°C ± 2°C.

Step 6
At the end of the incubation time wash the plate as described under Wash Procedures.

Step 7
Immediately after washing add 100 µl of Conjugate to each well.

Step 8
Cover the wells with the lid and incubate for 30 minutes at 37°C ± 2°C.

Step 9
At the end of the incubation time wash the plate as described under Wash Procedures.

Step 10
Immediately after washing add 100 µl of Substrate Solution to each well.

Step 11
Cover the wells with the lid and incubate for 30 minutes at 37°C ± 2°C.

Step 12
Add 60 µl of Stop Solution (0.5% to 2M sulphuric acid) to each well.

Step 13
Within 15 minutes read the absorbance at 490 nm using 520 nm to 650 nm as the reference wavelength if available. Blank the instrument on air (no plate in the carriage).

FULLY AUTOMATED PROCESSORS
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 5 minutes.
3. Wells containing Sample Diluent may be left for up to 50 minutes at 18-30°C prior to the addition of Sample and for up to 60 minutes after the addition of samples or Controls before starting step 6 in the assay protocol.
4. Ensure all Analytical Precautions are followed.

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

a) Protocol for automated stripwasher
Perform 5 wash cycles using working strength Wash Fluid. Ensure, where possible, that:
(i) Flow-through washing with a volume of 600 µl/well is used with instrumentation supplied by DiaSorin. When using other instrumentation for which this is not possible, ensure that the well is completely filled.
(ii) The dispense height is set to completely fill the well, with a slight positive meniscus, without causing an overflow.
(iii) The time taken to complete one aspirate/wash/soak cycle is approximately 30 seconds.
(iv) Ensure that no liquid is left in the well (by use of a double aspirate step in the final cycle where possible).
(v) 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 or deionised water at the end of the test to avoid blockage and corrosion.

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 Negative Controls.

Example:

Well 1 = 0.084, Well 2 = 0.086, Well 3 = 0.070
Total = 0.240

Mean Negative Control = 0.240/3 = 0.080

If one of the Negative Control Wells has an absorbance more than 0.15 O.D. above the mean of all three, discard that value and calculate the new Negative Control mean from two remaining replicates.

Cut-off value
Calculate the Cut-off value by adding 0.160 to the mean of the Negative Control replicates (see above).

Mean Negative Control = 0.080
Cut-off value = 0.080 + 0.160 = 0.230
INTERPRETATION OF RESULTS

Non-reactive Results
Samples giving an absorbance less than the Cut-off value are considered negative in the assay.

Reactive Results
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).

Unless local procedures state otherwise, all samples must be retested in duplicate using the original source. Samples that are reactive in at least one of the duplicate retests are considered repeatedly reactive in Murex HIV Ag/Ab Combination and are presumed to contain reactive HIV core antigen and/or antibodies to HIV-1 or HIV-2. Such samples must be further investigated and the results of this assay considered with any other clinical and/or assay information. Samples that are non-reactive in both wells on retest are considered non-reactive for HIV core antigen and HIV antibodies.

No sample addition
Absorbance values significantly higher than the Negative Control may be obtained in wells where the sample has been omitted but all the reagents have been added.

SPECIFIC PERFORMANCE CHARACTERISTICS

The performance of Murex HIV Ag/Ab Combination has been determined by testing samples from random blood donors, patients with AIDS diagnosed according to CDC criteria, patients with AIDS Related Complex (ARC), other patients with known antibody to HIV-1 (including group O), patients with confirmed HIV-2 infection and patients at risk of HIV infection or in other clinical categories. In addition, its performance on commercially available seroconversion panels has been evaluated.

Diagnostic Sensitivity
A total of 497 specimens from patients with confirmed HIV-1 infection were tested and found to be reactive with Murex HIV Ag/Ab Combination. The specimens were taken from patients at various stages of HIV infection and included 24 specimens from patients with HIV-1 subtype C infection and a further 159 specimens from patients infected with HIV-1 subtypes other than subtype B.

In addition, a total of 100 specimens from patients with confirmed HIV-2 infection were also tested with Murex HIV Ag/Ab Combination and found to be reactive.

The diagnostic sensitivity of Murex HIV Ag/Ab Combination on this population of specimens is therefore estimated to be 100% (67/67) with a lower 95% confidence limit of 99.38% (63/63) by the binomial distribution.

A total of 26 commercial HIV-1 seroconversion panels were tested with Murex HIV Ag/Ab Combination. Using the presence of both core (p24) and an envelope (gp120/160) band on Western blot as the reference criteria, Murex HIV Ag/Ab Combination detected antibody to HIV as early or in the same sample as Western blot in all the panels.

Diagnostic Specificity
The Murex HIV Ag/Ab Combination assay demonstrated a specificity of 99.5% in a study where specimens from European blood donor population were tested. A total of 9,290 routine donor plasma specimens were screened with Murex HIV Ag/Ab Combination at three European blood transfusion centres. The results are summarised in Table 3. In the study, 99.75% (9299/9299) of specimens were non-reactive and 0.25% (23/9299) were repeatedly reactive. One of the repeatedly reactive specimens was weakly positive with the Murex HIV Antigen mAb (‘B77’). None of the remaining 20 specimens were confirmed as positive for the presence of HIV-1 antigen or antibody to HIV-1 or HIV-2.

The specificity of Murex HIV Ag/Ab Combination on presumed negative European blood donors is estimated to be 99.75% (9299/9299) with 95% confidence limits of 99.67% (9295/9299) to 99.83% (9299/9299) by the binomial distribution. A total of 267 specimens from patients with conditions unlinked to HIV infection were also tested with Murex HIV Ag/Ab Combination. These included specimens from pregnant women and patients suffering from autoimmune disease and other acute viral infections. A total of five specimens were reactive with Murex HIV Ag/Ab, four were reactive with two other commercially available screening assays. In Western blot studies four produced indeterminate results and one was negative.

In addition, 38 true-positive, true-negative and haemolyzed specimens were also tested and found to be non-reactive.

The overall diagnostic specificity of Murex HIV Ag/Ab Combination on confirmed negative specimens during this performance evaluation is estimated to be 99.75% (969/970), with 95% confidence limits of 99.67% (959/969) to 99.83% (970/970) by the binomial distribution.

The reproducibility of Murex HIV Ag/Ab Combination was assessed by testing two of the assay controls and four quality assurance panel members for 20 replicates on four separate occasions. The results from the testing are summarised in Table 4.

Table 3

<table>
<thead>
<tr>
<th>Centre</th>
<th>Number of presumed negative specimens tested</th>
<th>Number of repeatedly reactive specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>509</td>
<td>6 (0.12%)</td>
</tr>
<tr>
<td>B</td>
<td>2893</td>
<td>9 (0.32%)</td>
</tr>
<tr>
<td>C</td>
<td>3992</td>
<td>6 (0.18%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>9290</td>
<td>21 (0.23%)</td>
</tr>
</tbody>
</table>

*Represents performance data are shown. Results obtained at individual laboratories and with different populations may vary.

Table 4

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Number of Assays</th>
<th>Number of Replicates</th>
<th>Mean Absorbance/ Cut-off ratio</th>
<th>Intra-assay %CV</th>
<th>Inter-assay %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>4</td>
<td>10</td>
<td>0.266</td>
<td>8.7</td>
<td>11.3</td>
</tr>
<tr>
<td>HIV-1 Positive Control</td>
<td>4</td>
<td>10</td>
<td>8.287</td>
<td>4.3</td>
<td>4.7</td>
</tr>
<tr>
<td>QA01</td>
<td>4</td>
<td>10</td>
<td>3.672</td>
<td>4.6</td>
<td>7.3</td>
</tr>
<tr>
<td>QA02</td>
<td>4</td>
<td>10</td>
<td>4.696</td>
<td>5.6</td>
<td>12.9</td>
</tr>
<tr>
<td>QA03</td>
<td>4</td>
<td>10</td>
<td>3.006</td>
<td>3.9</td>
<td>4.2</td>
</tr>
<tr>
<td>QA04</td>
<td>4</td>
<td>10</td>
<td>1.663</td>
<td>6.8</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Sensitivity on AFSSAPS HIV Ag standard
The sensitivity of Murex HIV Ag/Ab Combination on the AFSSAPS HIV Ag standard was determined at three testing centres.

Table 5

<table>
<thead>
<tr>
<th>Centre</th>
<th>Sensitivity HIV Ag pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
</tr>
<tr>
<td>Mean</td>
<td>28</td>
</tr>
</tbody>
</table>

The data shown in Table 5 was obtained during this testing but may not be exactly reproducible on other testing occasions.
LIMITATIONS OF THE PROCEDURE
1. The Test Procedure and Interpretation of Results must be followed.
2. This test has only been evaluated for use with individual (unpooled) serum, EDTA plasma or citrate plasma samples.
3. A negative result with an antigen/antibody detection test does not preclude the possibility of infection with HIV.
4. A positive result with Murex HIV Ag/Ab Combination should be confirmed by at least one other test.
5. Non-reproducible reactive results may be obtained with any EIA procedure.
   The most common sources of error are:
   a) Improper delivery of Sample, Conjugate or Substrate into the wells.
   b) Contamination of Substrate with 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 (450 nm) or use of an incorrect reference wavelength (not 520 nm to 690 nm).
6. The use of highly heamolyzed 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 cadavers.

BIBLIOGRAPHY

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