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. This public report was amended on 17 November 2016 to reflect updated labelling.

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:

	96 tests (product code 7G79-09)	**480 tests (product code 7G79-11)**
Coated Wells 96 microwells coated with HIV antigens and monoclonal antibodies	One plate	Five plates
Sample Diluent Green/brown buffered solution containing bovine and murine protein, detergent and saponin. Contains 0.05% ProClin® 300 preservative.	1 bottle of 8 ml	1 bottle of 18 ml
Conjugate HIV antigens and monoclonal antibodies conjugated to horseradish peroxidase and freeze dried. When reconstituted each bottle is sufficient for up to two plates.	1 bottle of 1.1 ml	3 bottles of 1.1 ml
Conjugate Diluent Yellow buffered solution consisting bovine protein, saponin and detergent. Sufficient to reconstitute one bottle of Conjugate	1 bottle of 22 ml	3 bottles of 22ml

Anti-HIV-1 Positive Control Inactivated human serum in a buffer containing bovine protein.	1 bottle of 1.7 ml	1 bottle of 1.7 ml
Anti-HIV-2 Positive Control Inactivated human serum in a buffer containing bovine protein.	1 bottle of 1.7 ml	1 bottle of 1.7 ml
HIV-1 p24 Positive Control HIV-1 p24 (recombinant antigen) in a buffer containing bovine protein.	1 bottle of 1.7 ml	1 bottle of 1.7 ml
Negative Control Normal human serum in a buffer containing bovine protein.	2 bottles of 2.5ml	2 bottles of 2.5ml
Substrate Diluent Colourless solution of tri-sodium citrate and hydrogen peroxide.	1 bottle of 35 ml	1 bottle of 35 ml
Substrate Concentrate 3,3',5,5'-tetramethylbenzidine (TMB) and stabilizers in an orange solution.	1 bottle of 35 ml	1 bottle of 35 ml
Wash Fluid 20 times working strength Glycine Borate 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

	Initial acceptance	
	Date	**Outcome**
PQ status amended	17 November 2016	listed
Status on PQ list	30 March 2015	listed
Dossier assessment	22 July 2014	MR
Inspection status	12 August 2014	MR
Laboratory evaluation	18 February 2014	MR

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.

Laboratory evaluation

Murex HIV Ag/Ab Combination 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 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 %.

Change notification

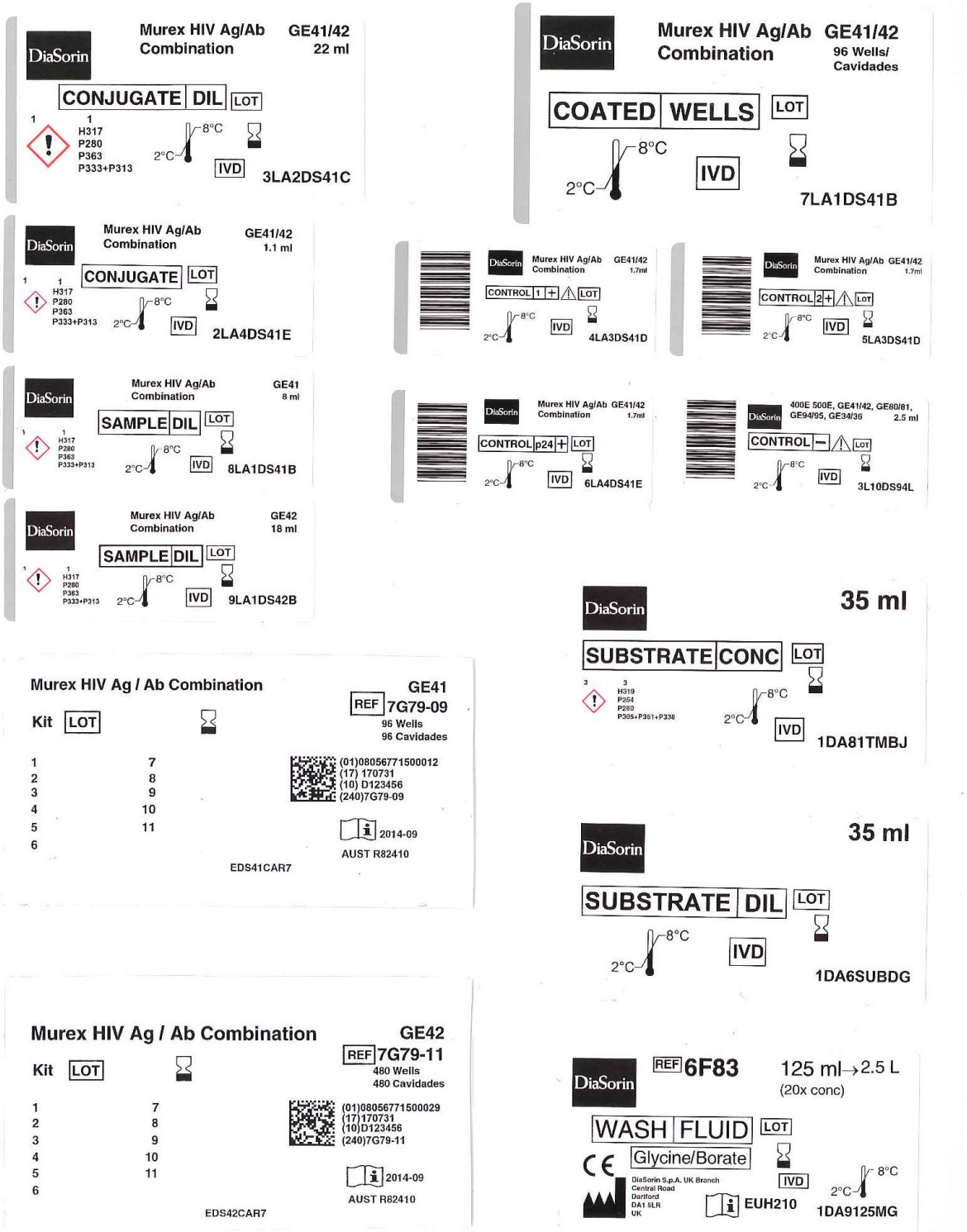
In 2016, DiaSorin S.p.A UK Branch submitted a change notification related to changes in labelling. This change notification was assessed and product was found to meet WHO prequalification requirements.

Labelling

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

1. Labels

Murex HIV Ag/Ab Combination (7G79-09/11)



IIID File: DS CL GE41E CE 02 02 Final

PANTONE 421 CVC PANTONE 032 CVC BLACK

GE41

REF 7G79-09

Murex HIV Ag/Ab Combination

96 Wells/Cavidades

CE 0123

Diasorin S.p.A. UK Branch
Central Road
Dartford, DA1 5LR
UK

IVD

2°C–8°C

Murex HIV Ag/Ab Combination

96 Wells /Cavidades

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

Test immunoenzymatique pour la détection améliorée de la séroconversion au virus de l'immunodéficience humaine de type 1 (VIH-1, VIH-1 groupe O) et la détection des anticorps anti-VIH-2

Enzymimmunoassay zum verbesserten Nachweis der Serokonversion nach Infektion mit dem humanen Immundefizienzvirus Typ 1 (HIV-1, HIV-1 Gruppe O) und zum Nachweis von anti-HIV-2-Antikörpern

Dosaggio immunoenzimatico per una migliore rilevazione della sieroconversione contro il virus dell'immunodeficienza umana di tipo 1 (HIV-1, HIV-1 gruppo O) e per la rilevazione degli anticorpi anti-HIV-2

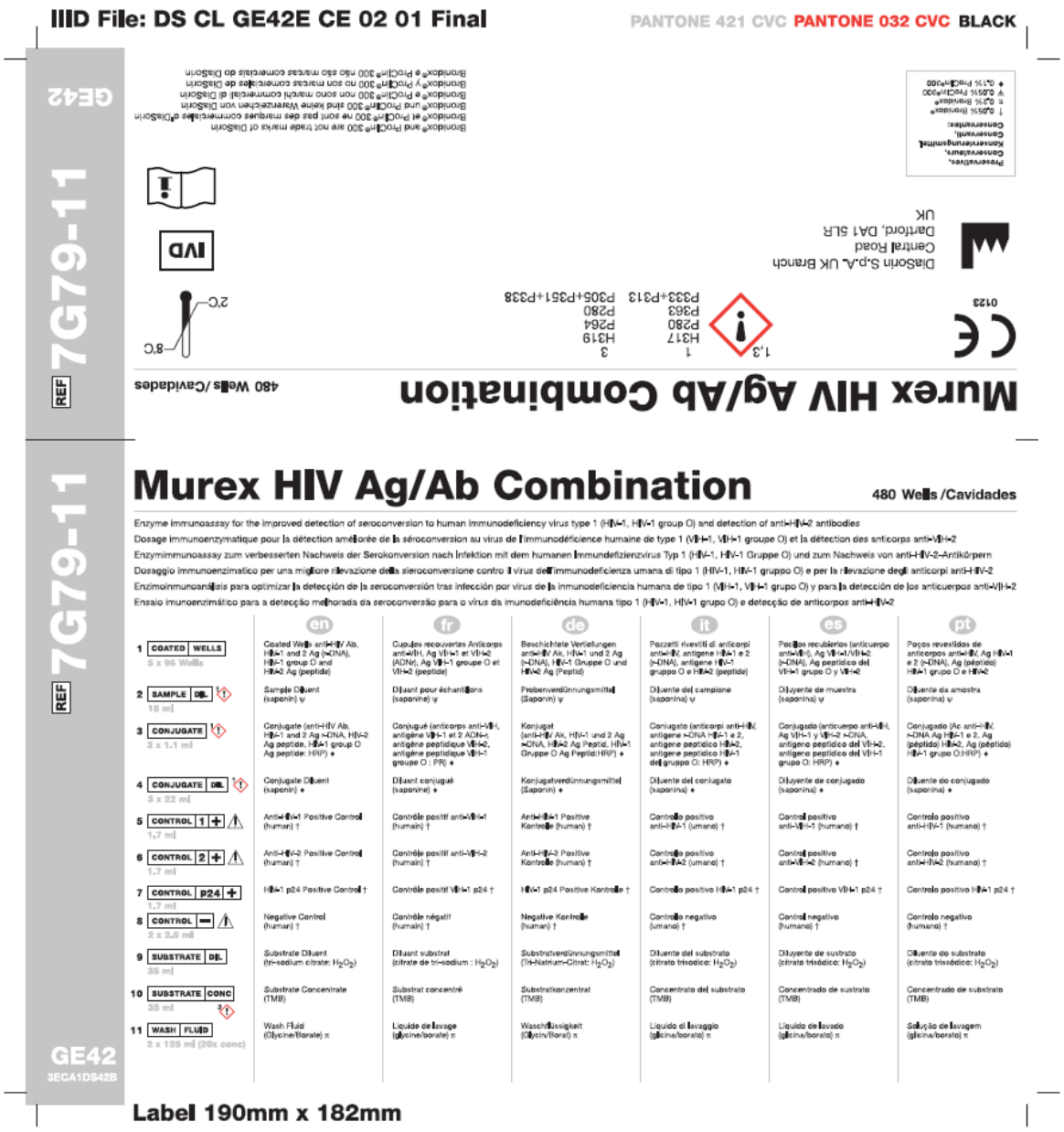
Enzimoinmunoanálisis para optimizar la detección de la seroconversión tras infección por virus de la inmunodeficiencia humana de tipo 1 (VIH-1, VIH-1 grupo O) y para la detección de los anticuerpos anti-VIH-2

Ensaio imunoenzimático para a detecção melhorada da soroconversão para o vírus da imunodeficiência humana tipo 1 (HIV-1, HIV-1 grupo O) e detecção de anticorpos anti-HIV-2

	en	fr	de	it	es	pt
1 COATED WELLS 96 Wells	Coated Wells anti-HIV Ab, HIV-1 and 2 Ag (rDNA), HIV-1 group O and HIV-2 Ag (peptide)	Cupules recouvertes Anticorps anti-VIH, Ag VIH-1 et VIH-2 (ADNr), Ag VIH-1 groupe O et VIH-2 (peptide)	Beschichtete Vertiefungen anti-HIV Ak, HIV-1 und 2 Ag (rDNA), HIV-1 Gruppe O und HIV-2 Ag (Peptid)	Pozzetti rivestiti di anticorpi anti-HIV, antigene HIV-1 e 2 (rDNA), antigene HIV-1 gruppo O e HIV-2 (peptide)	Pocillos recubiertos (anticuerpo anti-VIH, Ag VIH-1/VIH-2 (rDNA), Ag peptídico del VIH-1 grupo O y VIH-2	Poços revestidos de anticorpos anti-VIH, Ag HIV-1 e 2 (rDNA), Ag (péptido) HIV-1 grupo O e HIV-2
2 SAMPLE DIL. 1 8 ml	Sample Diluent (saponin) ψ	Diluant pour échantillons (saponine) ψ	Probenverdünnungsmittel (Saponin) ψ	Diluente del campione (saponina) ψ	Diluyente de muestra (saponina) ψ	Diluente da amostra (saponina) ψ
3 CONJUGATE 1 1.1 ml	Conjugate (anti-HIV Ab, HIV-1 and 2 Ag rDNA, HIV-2 Ag peptide, HIV-1 group O Ag peptide: HRP) ♦	Conjugué (anticorps anti-VIH, antigène VIH-1 et 2 ADNr, antigène peptidique VIH-2, antigène peptidique VIH-1 groupe O : PR) ♦	Konjugat (anti-HIV Ak, HIV-1 und 2 Ag rDNA, HIV-2 Ag Peptid, HIV-1 Gruppe O Ag Peptid: HRP) ♦	Coniugato (anticorpi anti-HIV, antigene rDNA HIV-1 e 2, antigene peptidico HIV-2, antigene peptidico HIV-1 del gruppo O: HRP) ♦	Conjugado (anticuerpo anti-VIH, Ag VIH-1 y VIH-2 rDNA, antígeno peptídico del VIH-2, antígeno peptídico del VIH-1 grupo O: HRP) ♦	Conjugado (Ac anti-HIV, rDNA Ag HIV-1 e 2, Ag (péptido) HIV-2, Ag (péptido) HIV-1 grupo O:HRP) ♦
4 CONJUGATE DIL. 1 22 ml	Conjugate Diluent (saponin) ♦	Diluant conjugué (saponine) ♦	Konjugatverdünnungsmittel (Saponin) ♦	Diluente del coniugato (saponina) ♦	Diluyente de conjugado (saponina) ♦	Diluente do conjugado (saponina) ♦
5 CONTROL 1 + 1.7 ml	Anti-HIV-1 Positive Control (human) †	Contrôle positif anti-VIH-1 (humain) †	Anti-HIV-1 Positive Kontrolle (human) †	Controllo positivo anti-HIV-1 (umano) †	Control positivo anti-VIH-1 (humano) †	Controlo positivo anti-HIV-1 (humano) †
6 CONTROL 2 + 1.7 ml	Anti-HIV-2 Positive Control (human) †	Contrôle positif anti-VIH-2 (humain) †	Anti-HIV-2 Positive Kontrolle (human) †	Controllo positivo anti-HIV-2 (umano) †	Control positivo anti-VIH-2 (humano) †	Controlo positivo anti-HIV-2 (humano) †
7 CONTROL p24 + 1.7 ml	HIV-1 p24 Positive Control †	Contrôle positif VIH-1 p24 †	HIV-1 p24 Positive Kontrolle †	Controllo positivo HIV-1 p24 †	Control positivo VIH-1 p24 †	Controlo positivo HIV-1 p24 †
8 CONTROL – 2 x 2.5 ml	Negative Control (human) †	Contrôle négatif (humain) †	Negative Kontrolle (human) †	Controllo negativo (umano) †	Control negativo (humano) †	Controlo negativo (humano) †
9 SUBSTRATE DIL. 55 ml	Substrate Diluent (tri-sodium citrate: H_2O_2)	Diluant substrat (citrate de tri-sodium : H_2O_2)	Substratverdünnungsmittel (Tri-Natrium-Citrat: H_2O_2)	Diluente del substrato (citrato trisodico: H_2O_2)	Diluyente de sustrato (citrato trisódico: H_2O_2)	Diluente do substrato (citrato trissódico: H_2O_2)
10 SUBSTRATE CONC 35 ml 3	Substrate Concentrate (TMB)	Substrat concentré (TMB)	Substratkonzentrat (TMB)	Concentrato del substrato (TMB)	Concentrado de sustrato (TMB)	Concentrado de substrato (TMB)
11 WASH FLUID 125 ml (20x conc)	Wash Fluid (Glycine/Borate) π	Liquide de lavage (glycine/borate) π	Waschflüssigkeit (Glycin/Borat) π	Liquido di lavaggio (glicina/borato) π	Líquido de lavado (glicina/borato) π	Solução de lavagem (glicina/borato) π

GE41
3ECA2DS41C

Label 190mm x 182mm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PANTONE 421 CVC PANTONE 032 CVC BLACK
GE42
REF 7G79-11
Murex HIV Ag/Ab Combination
480 Wells /Cavidades
0123
DiaSorin S.p.A. UK Branch
Central Road
Dartford, DA1 5LR
UK
IVD
2°C
8°C
Murex HIV Ag/Ab Combination
480 Wells /Cavidades
Enzyme immunoassay for the improved detection of seroconversion to human immunodeficiency virus type 1 (HIV-1, HIV-1 group O) and detection of anti-HIV-2 antibodies
Dosage immunoenzymatique pour la détection améliorée de la séroconversion au virus de l'immunodéficience humaine de type 1 (VIH-1, VIH-1 groupe O) et la détection des anticorps anti-VIH-2
Enzymimmunoassay zum verbesserten Nachweis der Serokonversion nach Infektion mit dem humanen Immundefizienzvirus Typ 1 (HIV-1, HIV-1 Gruppe O) und zum Nachweis von anti-HIV-2-Antikörpern
Dosaggio immunoenzimatico per una migliore rilevazione della sieroconversione contro il virus dell'immunodeficienza umana di tipo 1 (HIV-1, HIV-1 gruppo O) e per la rilevazione degli anticorpi anti-HIV-2
Enzimoinmunoanálisis para optimizar la detección de la seroconversión tras infección por virus de la inmunodeficiencia humana de tipo 1 (VIH-1, VIH-1 grupo O) y para la detección de los anticuerpos anti-VIH-2
Ensaio imunoenzimático para a detecção melhorada da seroconversão para o vírus da imunodeficiência humana tipo 1 (HIV-1, HIV-1 grupo O) e detecção de anticorpos anti-HIV-2
en fr de it es pt
1 COATED WELLS 8 x 96 Wells
Coated Wells anti-HIV Ab, HIV-1 and 2 Ag (rDNA), HIV-1 group O and HIV-2 Ag (peptide)
Cupules recouvertes Anticorps anti-VIH, Ag VIH-1 et VIH-2 (ADNr), Ag VIH-1 groupe O et VIH-2 (peptide)
Beschichtete Vertiefungen anti-HIV Ak, HIV-1 und 2 Ag (rDNA), HIV-1 Gruppe O und HIV-2 Ag (Peptid)
Pozzetti rivestiti di anticorpi anti-HIV, antigene HIV-1 e 2 (rDNA), antigene HIV-1 gruppo O e HIV-2 (peptide)
Pocillos recubiertos (anticuerpo anti-VIH), Ag VIH-1/VIH-2 (rDNA), Ag peptídico del VIH-1 grupo O y VIH-2
Poços revestidos de anticorpos anti-HIV Ag HIV-1 e 2 (rDNA), Ag (péptido) HIV-1 grupo O e HIV-2
2 SAMPLE DIL 18 ml
Sample Diluent (saponin) ψ
Diluant pour échantillons (saponine) ψ
Probenverdünnungsmittel (Saponin) ψ
Diluente del campione (saponina) ψ
Diluyente de muestra (saponina) ψ
Diluente da amostra (saponina) ψ
3 CONJUGATE 3 x 1.1 ml
Conjugate (anti-HIV Ab, HIV-1 and 2 Ag rDNA, HIV-2 Ag peptide; HRP) ♦
Conjugué (anticorps anti-VIH, antigène VIH-1 et 2 ADNr, antigène peptidique VIH-2, antigène peptidique VIH-1 groupe O : PR) ♦
Konjugat (anti-HIV Ak, HIV-1 und 2 Ag rDNA, HIV-2 Ag Peptid, HIV-1 Gruppe O Ag Peptid:HRP) ♦
Coniugato (anticorpi anti-HIV, antigene rDNA HIV-1 e 2, antigene peptidico HIV-2, antigene peptidico HIV-1 del gruppo O; HRP) ♦
Conjugado (anticuerpo anti-VIH, Ag VIH-1 y VIH-2 rDNA, antígeno peptídico del VIH-2, antígeno peptídico del VIH-1 grupo O; HRP) ♦
Conjugado (Ac anti-HIV, rDNA Ag HIV-1 e 2, Ag (péptido) HIV-2, Ag (péptido) HIV-1 grupo O;HRP) ♦
4 CONJUGATE DIL 3 x 22 ml
Conjugate Diluent (saponin) ♦
Diluant conjugué (saponine) ♦
Konjugatverdünnungsmittel (Saponin) ♦
Diluente del coniugato (saponina) ♦
Diluyente de conjugado (saponina) ♦
Diluente do conjugado (saponina) ♦
5 CONTROL 1 + 1.7 ml
Anti-HIV-1 Positive Control (human) †
Contrôle positif anti-VIH-1 (humain) †
Anti-HIV-1 Positive Kontrolle (human) †
Controllo positivo anti-HIV-1 (umano) †
Control positivo anti-VIH-1 (humano) †
Controlo positivo anti-HIV-1 (humano) †
6 CONTROL 2 + 1.7 ml
Anti-HIV-2 Positive Control (human) †
Contrôle positif anti-VIH-2 (humain) †
Anti-HIV-2 Positive Kontrolle (human) †
Controllo positivo anti-HIV-2 (umano) †
Control positivo anti-VIH-2 (humano) †
Controlo positivo anti-HIV-2 (humano) †
7 CONTROL p24 + 1.7 ml
HIV-1 p24 Positive Control †
Contrôle positif VIH-1 p24 †
HIV-1 p24 Positive Kontrolle †
Controllo positivo HIV-1 p24 †
Control positivo VIH-1 p24 †
Controlo positivo HIV-1 p24 †
8 CONTROL − 2 x 2.5 ml
Negative Control (human) †
Contrôle négatif (humain) †
Negative Kontrolle (human) †
Controllo negativo (umano) †
Control negativo (humano) †
Controlo negativo (humano) †
9 SUBSTRATE DIL 35 ml
Substrate Diluent (tri-sodium citrate: H_2O_2)
Diluant substrat (citrate de tri-sodium : H_2O_2)
Substratverdünnungsmittel (Tri-Natrium-Citrat: H_2O_2)
Diluente del substrato (citrato trisodico: H_2O_2)
Diluyente de sustrato (citrato trisódico: H_2O_2)
Diluente do substrato (citrato trissódico: H_2O_2)
10 SUBSTRATE CONC 35 ml
Substrate Concentrate (TMB)
Substrat concentré (TMB)
Substratkonzentrat (TMB)
Concentrato del substrato (TMB)
Concentrado de sustrato (TMB)
Concentrado de substrato (TMB)
11 WASH FLUID 2 x 125 ml (20x conc)
Wash Fluid (Glycine/Borate) π
Liquide de lavage (glycine/borate) π
Waschflüssigkeit (Glycin/Borat) π
Liquido di lavaggio (glicina/borato) π
Líquido de lavado (glicina/borato) π
Solução de lavagem (glicina/borato) π
GE42
3ECA1DS42B
Label 190mm x 182mm

2. Instructions for use

DiaSorin The Diagnostic Specialist

en

REF 7G79-09 / 11
GE41/42

Revised September, 2014

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.

IVD

Key to symbols used

REF	List Number	IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number	2°C–8°C	Store at 2-8°C
	Expiration Date		CAUTION: Consult accompanying documents
	Manufacturer		Consult instructions for use

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

1

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[1].

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[2,3]. Variants of HIV-1, classified together as group O, have been identified in samples from Cameroon and Europe[4,5]. 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[6]. The earliest specific antibody response following infection by HIV may be of immunoglobulin M (IgM) followed by a response in immunoglobulin G (IgG)[7]. 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 Ag/Ab 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 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 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 450nm 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 condition they will retain activity until the expiry date of the kit.

COATED | WELLS

1. Coated Wells

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

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 | DIL

2. Sample Diluent

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

CONJUGATE

3. Conjugate

One bottle (7G79-09) or three bottles (7G79-11) containing 1.1 ml of HIV antigens and monoclonal antibodies conjugated to horseradish peroxidase and freeze dried. When reconstituted each bottle is sufficient for up to two plates.

CONJUGATE | DIL

4. Conjugate Diluent

One bottle (7G79-09) or three bottles (7G79-11) containing 22ml of a yellow solution consisting of buffer, bovine protein, saponin and detergent, 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 if required.

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

CONTROL | 1 | + ⚠

5. Anti-HIV-1 Positive Control

One bottle containing 1.7 ml of inactivated human serum in a buffer containing bovine protein. Contains 0.05% Bronidox® preservative.

CONTROL | 2 | + ⚠

6. Anti-HIV-2 Positive Control

One bottle containing 1.7 ml of inactivated human serum in a buffer containing bovine protein. Contains 0.05% Bronidox® preservative.

CONTROL | p24 | +

7. HIV-1 p24 Positive Control

One bottle containing 1.7 ml of p24 (recombinant antigen) in a buffer containing bovine protein. Contains 0.05% Bronidox® preservative.

CONTROL | − ⚠

8. Negative Control

Two bottles containing 2.5 ml of normal human serum diluted in a bovine protein buffer. Contains 0.05% Bronidox® preservative.

SUBSTRATE | DIL

9. Substrate Diluent

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

SUBSTRATE | CONC

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 1
Volume of Substrate Concentrate and Substrate Diluent Required

Number of Wells											Number of Plates			
8	16	24	32	40	48	56	64	72	80	96	1	2	3	4
Substrate Concentrate (ml)														
0.5	1.0	2.0	2.5	2.5	3.0	3.5	4.0	4.5	4.5	6.0	6	12	18	22
Substrate Diluent (ml)														
0.5	1.0	2.0	2.5	2.5	3.0	3.5	4.0	4.5	4.5	6.0	6	12	18	22

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

11. Wash Fluid

One (7G79-09) or two (7G79-11) bottles containing 125 ml of 20 times working strength Glycine/Borate Wash Fluid. Contains 0.2% Bronidox® 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% Bronidox® 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 30°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 2

Component	Reactive for	Non-reactive for
Negative Control	N/A	HBsAg, antibodies to HCV, HIV-1 and HIV-2
Positive Control 1	antibodies to HIV-1	HBsAg
Positive Control 2	antibodies to HIV-2	HBsAg

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

All reactive serum used has been inactivated prior to use in reagent preparation. However, 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 labeled as follows:

Reagents:	CONJUGATE | DIL	SAMPLE | DIL	CONJUGATE *
Classification:	Skin sens. 1 H317		
Signal Word:	Warning		
Symbols / Pictograms:			
Hazard Statements:	H317 May cause an allergic skin reaction.		
Precautionary Statements:	P280 Wear protective gloves/protective clothing/ eye protection/face protection. P363 Wash contaminated clothing before reuse. P333+P313 If skin irritation or rash occurs: Get medical advice / attention.		
Contains:	Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1).		

* The reconstituted Conjugate contains 0.1% ProClin® 300 which is classified hazardous per EC Regulation 1272/2008.

Reagents:	SUBSTRATE | CONC
Classification:	Eye Irrit. 2 H319
Signal Word:	Warning
Symbols / Pictograms:	
Hazard Statements:	H319 Causes serious eye irritation
Precautionary Statements:	P264 Wash hands thoroughly after handling P280 Wear protective gloves/protective clothing/ eye protection/face protection. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Pursuant to EC Regulation 1272/2008 (CLP), WASH | FLUID is labeled as EUH210, safety data sheets available on request.

For additional information see Safety Data Sheets available on www.diasorin.com

1. Potentially contaminated materials should be disposed of safely according to local requirements.
2. Spillage of potentially infectious material should be removed immediately with absorbent paper tissue and the contaminated area swabbed with, for example, 1.0% sodium hypochlorite before work is continued[8]. Sodium hypochlorite should not be used on acid 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. If any of the reagents come into contact with the skin or eyes wash the area extensively with water.
6. Sulphuric acid required for the Stop Solution and hydrochloric acid used for washing glassware are corrosive and should be handled with appropriate care. If either come into contact with the skin or eyes, wash thoroughly 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 reduce any of the recommended incubation times.
3. Allow all reagents and samples to come to 18 to 30°C before use. Immediately after use return 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 Murex assays. A pipette should also be dedicated for use with the Conjugate.
9. The Sample Diluent in this assay has the potential to cause false positive results in anti hepatitis B surface antigen (anti-HBs) assays if reagent cross contamination occurs.

   If running Murex HIV Ag/Ab Combination in conjunction with an anti-HBs assay on a fixed tip instrument ensure that the possibility of cross contamination is excluded during the validation process.
10. Do not touch or splash the rim of the well with Conjugate. Do not blow out from micropipettes; reverse pipetting is recommended whenever possible.
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 microwells with dust from disposable gloves.
13. When using fully automated processors
    i) It is not necessary to use plate lids and tap 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 CO_2 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.

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 (-15°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 (0.5M to 2M Sulphuric Acid).** e.g. add between 3.0 ml (for 0.5M) and 11 ml (for 2.0M) of analytical grade concentrated sulphuric acid (18M) to about 80 ml of distilled or deionised water and then make up to 100 ml with more water. Alternatively, the following reagent can be used: 1N Sulphuric Acid (Code N0164 - 15 vial pack and N0165 - 1 vial 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 5F09-02). 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 stripwasher.
   b) Microplate reader.
   or
   c) Fully automated microplate processor.
   All instruments must be validated before use.
   Please contact your representative for details of recommended systems, software protocols for instrumentation and validation procedures.
7. **Disposable Reagent Troughs.** (Code 5F24-01).
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 570 nm or 620 nm with a reference of 690 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 690 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 reactives 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.	**25 µl**
Step 4	**Add** 100 µl of **Samples** or 100 µl **Controls** to the wells. For each plate use the first column of wells for the assay Controls. Add the Controls to the designated wells after dispensing the samples. Pipette 100 µl of the Negative Control into each of three wells A1 to C1 and 100 µl of the p24, anti-HIV-1 and HIV-2 Positive Controls into wells D1 to F1 respectively. Use of a white background will aid visualisation of sample addition.	**100 µl**
Step 5	Cover the wells with the lid and **incubate** for 60 minutes at 37˚C ±1˚C.	**60 mins**
Step 6	At the end of the incubation time **wash** the **plate** as described under **Wash Procedures**.	
Step 7	Immediately after washing the plate, **add** 100 µl of **Conjugate** to each well.	**100 µl**
Step 8	Cover the wells with the lid and **incubate** for 30 minutes at 37˚C ±1˚C.	**30 mins**
Step 9	At the end of the incubation time **wash** the **plate** as described under **Wash Procedures**.	
Step 10	Immediately after washing the plate, **add** 100 µl of **Substrate Solution** to each well.	**100 µl**
Step 11	Cover the wells with a lid and **incubate** for 30 minutes at 37˚C ±1˚C. Keep away from direct sunlight. A blue green colour should develop in wells containing reactive samples.	**30 mins**
Step 12	**Add** 50 µl of **Stop Solution** (0.5M to 2M sulphuric acid) to each well.	**50 µl**
Step 13	Within 15 minutes **read** the absorbance at 450 nm using 620 nm to 690 nm as the reference wavelength if available. Blank the instrument on air (no plate in the carriage).	**A_{450}**

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 500 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 absorbant 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.

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 60 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 5 in the assay protocol.
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 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 0.150 to the mean of the Negative Control replicates (see above).

Mean Negative Control	–	0.080				
Cut-Off value	–	0.080	+	0.150	–	0.230

QUALITY CONTROL

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

Negative Control

The mean absorbance is less than 0.15

Positive Controls

The absorbance of each of the Positive Controls 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 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, such 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 O infection and a further 139 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% (597/597) with a lower 95% confidence limit of 99.38% (593/597) 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 earlier or in the same sample as Western blot in all of the panels.

Diagnostic Specificity

The Murex HIV Ag/Ab Combination assay demonstrated a specificity of ≥99.5% in a study where specimens from a 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, 99.77% (9269/9290) of specimens were non-reactive and 0.23% (21/9290) were repeatedly reactive. One of the repeatedly reactive specimens was weakly positive with the Murex HIV Antigen mAb (8E77). 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 99.78% (9269/9289) with 95% confidence limits of 99.67% (9258/9289) to 99.87% (9277/9289) by the binomial distribution.*

A total of 267 specimens from patients with conditions unrelated to HIV infection were also tested with Murex HIV Ag/Ab Combination. These included specimens from pregnant women and patients suffering with 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 lipaemic, icteric and haemolysed 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 99.78% (9569/9590) with 95% confidence limits of 99.67% (9558/9590) to 99.86% (9577/9590) by the binomial distribution.*

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

Assay Reproducibility

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

Table 3
Reactivity of Murex HIV Ag/Ab Combination with presumed negative specimens from routine European blood donors

Centre	Number of presumed negative specimens tested	Number of repeatedly reactive specimens
A	3095	6[a] (0.19%)
B	2803	9 (0.32%)
C	3392	6 (0.18%)
TOTAL	9290	21 (0.23%)

[a] includes one specimen which was weakly positive in Murex HIV Antigen mAb (8E77)

Table 4
Murex HIV Ag/Ab Combination - Assay Reproducibility

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

Sensitivity on AFSSAPS HIV Ag Standard

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

Table 5
Sensitivity on AFSSAPS HIV Ag standard

Centre	Sensitivity HIV Ag pg/ml
1	31
2	28
3	25
Mean	28

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-repeatable reactive results may be obtained with any EIA procedure.

   The most common sources of error are:

   a) Imprecise 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 620 nm to 690 nm).
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 cadavers.

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