WHO Prequalification of In Vitro Diagnostics Programme
PUBLIC REPORT

Product: Enzygnost HIV Integral 4
WHO reference number: PQDx 0214-064-00

Enzygnost HIV Integral 4 with product codes **OPKR03, OPKR05, OPKR07(Q)** and **Supplementary Reagents for Enzygnost/TMB** with product code **OUVP17** manufactured by **Siemens Healthcare Diagnostics Products GmbH**, **CE-mark regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 22 March 2016. This report was amended on 04 April 2016 to correct a typographical error.

Intended use:
Enzygnost HIV Integral 4 is an enzyme immunoassay for the qualitative detection of HIV p24 antigen and specific antibodies to human immunodeficiency viruses of type 1 and 2 (HIV-1 including HIV-1 subtype O virus and HIV-2) in human serum and plasma. The enzyme immunoassay can be processed using the ELISA processors, BEP® III System, BEP® 2000 System, BEP 2000 Advance® System as well as the Quadriga® Systems. A non-automated processing of the test procedure is also possible.

Assay description:
Enzygnost HIV Integral 4 is a fourth generation enzyme immunoassay. The specific antibodies to HIV contained in the test sample bind to the antigens in the reaction wells of the HIV Integral 4 test plate and the HIV p24 antigen present in the specimen to the monoclonal anti-HIV p24 specific antibodies, accordingly. The biotinylated components (recombinant HIV proteins or synthetic peptides respectively monoclonal anti-HIV p24 antibodies) of HIV Integral 4 Conjugate 1 bind in the second step to these specific antibodies respectively to the HIV p24 antigen (antigen sandwich respectively antibody sandwich). In the third step, HIV Integral 4 Conjugate 2 (streptavidin/POD) reacts with the bound biotin conjugates. The enzyme portion of HIV Integral 4 Conjugate 2 causes the Chromogen Working Solution to turn blue. This reaction is stopped by the addition of Stopping Solution POD, which causes a color change to yellow. The color intensity is a measure of the immunochemical reactivity of the HIV-specific antibodies and the concentration of HIV p24 antigen in the specimen.

Test kit contents:

Component	**2 x 96 tests/kit Product code OPKR03**	**10 X 96 tests/kit Product code OPKR05**	**10 X 96 tests/kit Product code OPKR07(Q)**
Test Plate Recombinant proteins (E. coli) containing HIV1 gp41, HIV1 (subtype O) gp41, HIV2 gp36 as well as two monoclonal antibodies (mouse) to HIV p24 antigen coated microtitration plate	2 x 96 wells	10 x 96 wells	10 x 96 wells
Sample Buffer Phosphate buffer with BSA and TRITON X-100; coloured pink (Contains preservative phenol (≤ 1 g/L)	2 x 5 ml	6 x 5 ml	2 x 25 ml
Conjugate 1 Buffer TRIS/HCl buffer with SAPOGENAT T500 and Casein (Contains preservative phenol (≤ 1 g/L)	2 x 12.5 mL	10 x 2.5 mL	2 x 75 mL
Conjugate 1 Lyophilizate of recombinant E. coli HIV-1, HIV-2 and HIV-1 (subtype O) synthetic peptides and two monoclonal antibodies (mouse) to HIV p24, biotinylated; coloured blue (Contains Proclin 300)	2 x 12.5 ml	10 x 12.5 ml	2 x 10 mL of concentrated Conjugate 1
Conjugate 2 Streptavidin/peroxidase (POD) conjugate in TRIS/HCl buffer; coloured yellow (Contains preservative phenol (≤ 1 g/L)	2 x 12.5 mL	10 x 12.5 mL	2 x 75 mL

Component	2 x 96 tests/kit Product code OPKR03	10 X 96 tests/kit Product code OPKR05	10 X 96 tests/kit Product code OPKR07(Q)
Control, negative Stabilized human serum without HIV antigens and without antibodies to HIV-1, HIV-2 and HIV-1 (subtype O) antigens; coloured green. (Contains preservative phenol (≤ 1 g/L)	2 x 2 mL	3 x 2 mL	3 x 2 mL
Control, positive Heat-treated human serum with antibodies to HIV-1 antigens in HEPES buffer; coloured red (Contains preservative phenol (≤ 1 g/L)	2 x 2 mL	3 x 2 mL	3 x 2 mL
Instructions for use	1	1	1
Polyethylene bag	1	1	1

Items required but not provided:

Item	Product code
Supplementary reagents kit for Enzygnost®/TMB	OUVP17
Buffer/Substrate TMB	
Chromogen TMB	
Stopping Solution POD	
Washing Solution POD	
Adhesive foils	
Empty bottle for the Chromogen Working Solution	
Instructions for Use	
Non-automated processing instrumentation requirements	
Incubator	N/A
Microtitration plate washer	N/A
Spectrophotometer suitable for 96-well plates (450nm measuring and 650nm reference wavelengths)	N/A
Automated processing instrumentation requirements	

BEP® III System: for automated processing and evaluation of the test after manual dispensing of samples and controls	TBC
BEP® 2000 System/BEP® 2000 Advance System: for fully automated processing and evaluation of the test	TBC
Quadriga® System: for fully automated processing and evaluation of the test in combination with BEP® III	TBC
Precision pipettes plus tips	N/A

Storage:
The test kit should be stored unopened at 2-8 °C. Once opened, refer to IFU for storage conditions.

Shelf-life:
12 months.

Warnings/limitations:
See manufacturer's instructions for use.

WHO special warnings:
WHO reviewed the instructions for use that were current at the time of WHO prequalification, and a number of changes were suggested. Most, but not all, changes were made by the manufacturer (WHO comments relate to intended use, warnings and precautions, preparation of reagents, certain aspects of test procedure, results, nomenclature).

Furthermore, it should be clear to end-users that the product does not contain a positive quality control for HIV-1 p24 antigen. Therefore, end-users are encouraged to source external quality control material for HI-1 p24 antigen.

Summary of prequalification assessment for Enzygnost HIV Integral 4

	Date	Outcome
PQ listing	22 March 2016	listed
Dossier review	N/A	MR
Site inspection(s)	13 April 2015	MR
Laboratory evaluation	16 November 2015	MR

MR: Meets Requirements
N/A: Not Applicable

Prioritization for prequalification
Based on the established criteria, Enzygnost HIV Integral 4 was given priority for WHO prequalification.

Product dossier assessment
In accordance with the WHO procedure for abbreviated prequalification assessment, Siemens Healthcare Diagnostics Products GmbH was not required to submit a product dossier for Enzygnost HIV Integral 4 as per the *Instructions for compilation of a product dossier* (PQDx_018 v1). Notwithstanding, certain aspects of the product dossier previously submitted for stringent regulatory review were reviewed by an assessor during the site inspection.

Manufacturing site inspection
In accordance with the WHO procedure for abbreviated prequalification assessment, a shortened inspection with fewer inspectors was conducted at the site of manufacture (Emil-von-Behring Straße 76, 35041 Marburg, Germany) of Enzygnost HIV Integral 4 in 24-26 February, 2015 as per the Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics (PQDx_014 v1).

The inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality.

The inspection was concluded with the final inspection report sent 13 April 2015.

Based on the site inspection and corrective action plan review, the quality management system for Enzygnost HIV Integral 4 meets WHO prequalification requirements.

Laboratory evaluation
Enzygnost HIV Integral 4 was evaluated by WHO in the 2nd and 3rd quarter of 2015 using serum/plasma specimens. From this evaluation, we drew the following conclusions:

Enzygnost HIV Integral 4 is an enzyme immunoassay for the qualitative detection of HIV-1/2 antibodies and HIV-1 p24 antigen in human serum and plasma specimens. A volume of 100µl 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 99.2% (98.2% - 99.8%) compared to the reference results. The final sensitivity (95% CI) was 100% (99.2% - 100%) and the final specificity (95% CI) was 99.8% (99.2% - 100%) compared to the reference results. Lot to lot variation observed was within the acceptance range for most dilution series. For three series there was a difference of 2 dilutions.

For eight seroconversion panels, Enzygnost HIV Integral 4 detected on average 1 specimen earlier than the benchmark assay (Enzygnost Anti-HIV 1/2 Plus [Siemens Healthcare Diagnostics Products GmbH]) and on average 0.375 specimens earlier than Vironostika HIV Ag/Ab (bioMérieux) EIA.

For the mixed titer panel, Enzygnost HIV Integral 4 detected all specimens correctly, except one that was false reactive. For the HIV-1 p24 antigen panel, Enzygnost HIV Integral 4 correctly classified most specimens. The assay was false non-reactive for 2 specimens. For the HIV culture supernatant panel, Enzygnost HIV Integral 4 detected all HIV-1 subtypes and the HIV-2 isolate.

For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], Enzygnost HIV Integral 4 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], Enzygnost HIV Integral 4 detected to 0.39 international units. In contrast, Vironostika HIV Ag/Ab (bioMérieux) detected to 12.5 international units.

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

Labelling

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

1. Labels

1.1 Overview on kit components

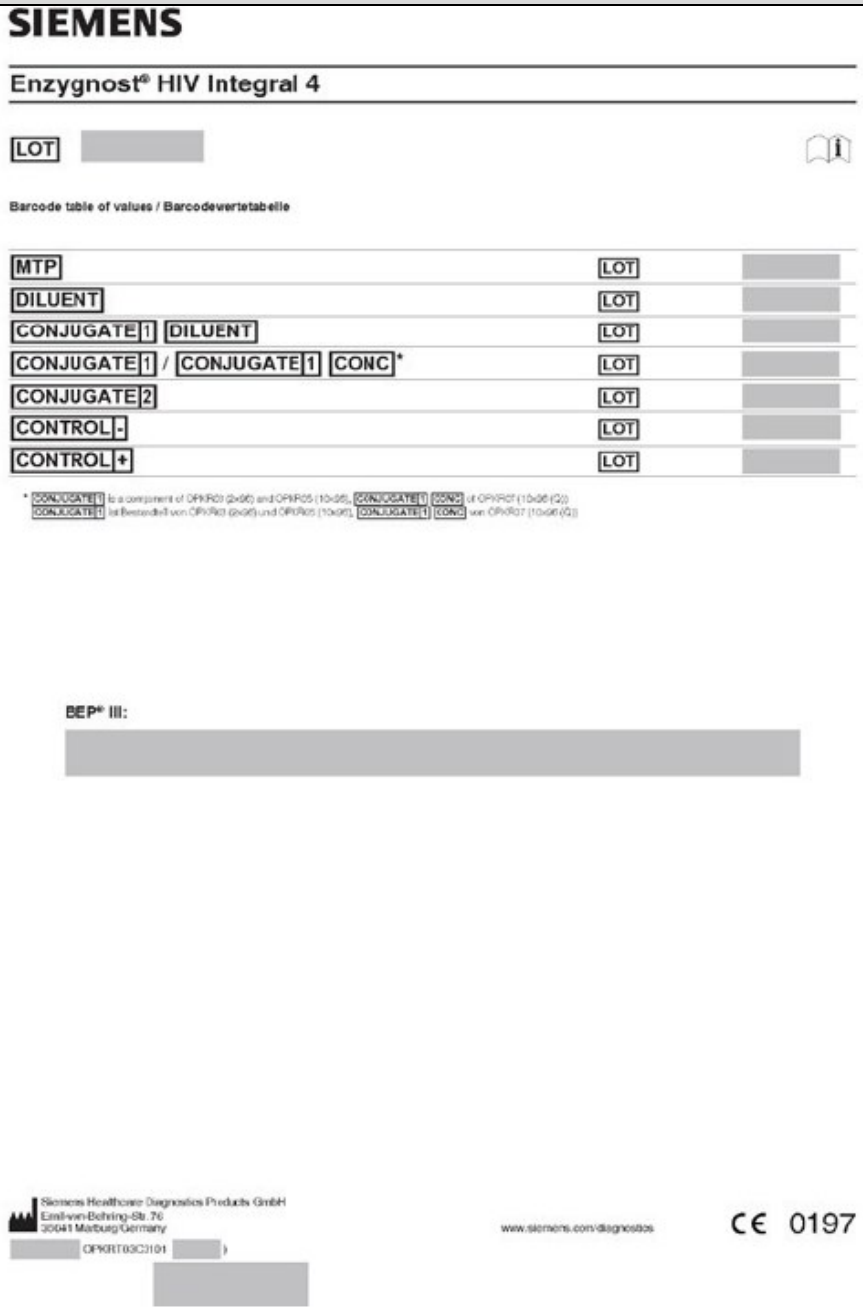
Product numbers of variants	Overview on kit components
OPKR 03 (2 x 96) OPKR 05 (10 x 96) OPKR 07 (10 x 96 (Q))	Materials Provided

REF	Contents			
	Number of Tests			Component
	2 × 96	10 × 96	10 × 96 (Q)	
OPKR	2	10	10	MTP
	2 × 5 mL	6 × 5 mL	2 × 25 mL	DILUENT
	2 × 12.5 mL	10 × 12.5 mL	2 × 75 mL	CONJUGATE 1 DILUENT
	2 × → 12.5 mL	10 × → 12.5 mL	--	CONJUGATE 1
	--	--	2 × → 10 mL[f]	CONJUGATE 1 CONC
	2 × 12.5 mL	10 × 12.5 mL	2 × 75 mL	CONJUGATE 2
	2 × 2 mL	3 × 2 mL	3 × 2 mL	CONTROL –
	2 × 2 mL	3 × 2 mL	3 × 2 mL	CONTROL +
	1	1	1	polyethylene bag

[f] final volume: 75 mL, refer to "Preparing Reagents"

The test plate, the conjugates, the Conjugate 1 Buffer, as well as the control, positive and the control, negative must be used in the given combination of 6-digit lot numbers printed on the package, respectively stated in the enclosed barcode table of values.

1.2 Table of assigned values (TAV)

Component	Document
TAV	

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Enzygnost® HIV Integral 4

LOT

Barcode table of values / Barcodewertetabelle

MTP	LOT	
DILUENT	LOT	
CONJUGATE 1 DILUENT	LOT	
CONJUGATE 1 / CONJUGATE 1 CONC*	LOT	
CONJUGATE 2	LOT	
CONTROL -	LOT	
CONTROL +	LOT	

* CONJUGATE 1 is a component of OPKR01 (2x96) and OPKR05 (10x96). CONJUGATE 1 CONC is in OPKR07 (10x96 (Q))
CONJUGATE 1 ist Bestandteil von OPKR01 (2x96) und OPKR05 (10x96). CONJUGATE 1 CONC von OPKR07 (10x96 (Q))

BEP® III:

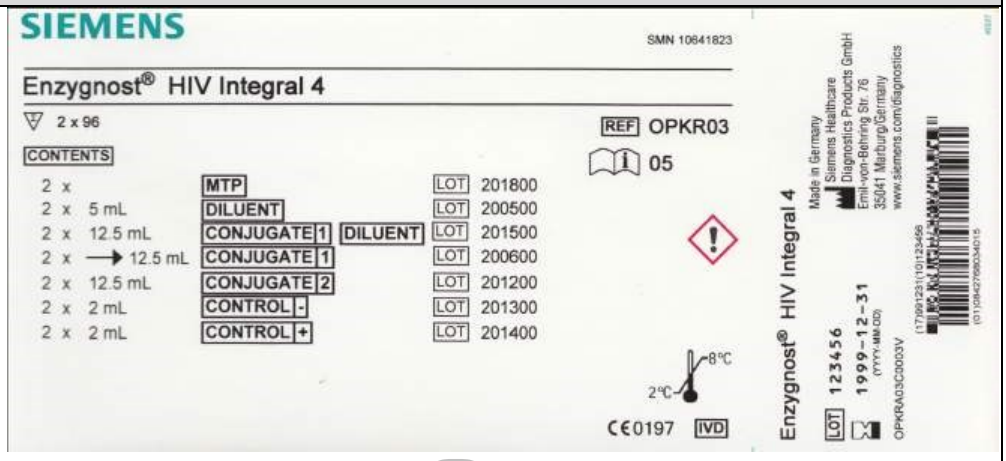
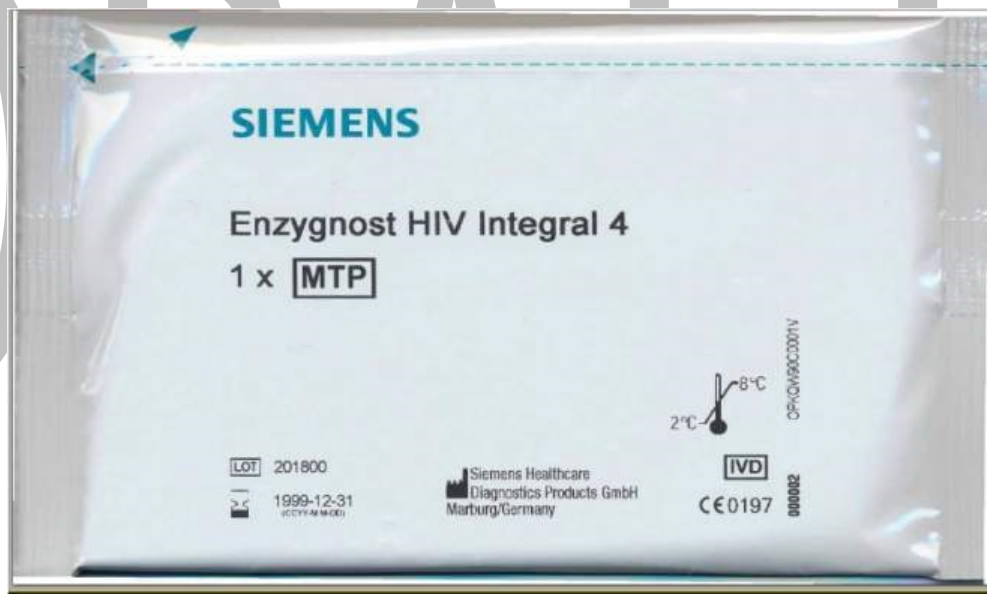
Siemens Healthcare Diagnostics Products GmbH
Emil-von-Behring-Str. 76
35041 Marburg/Germany

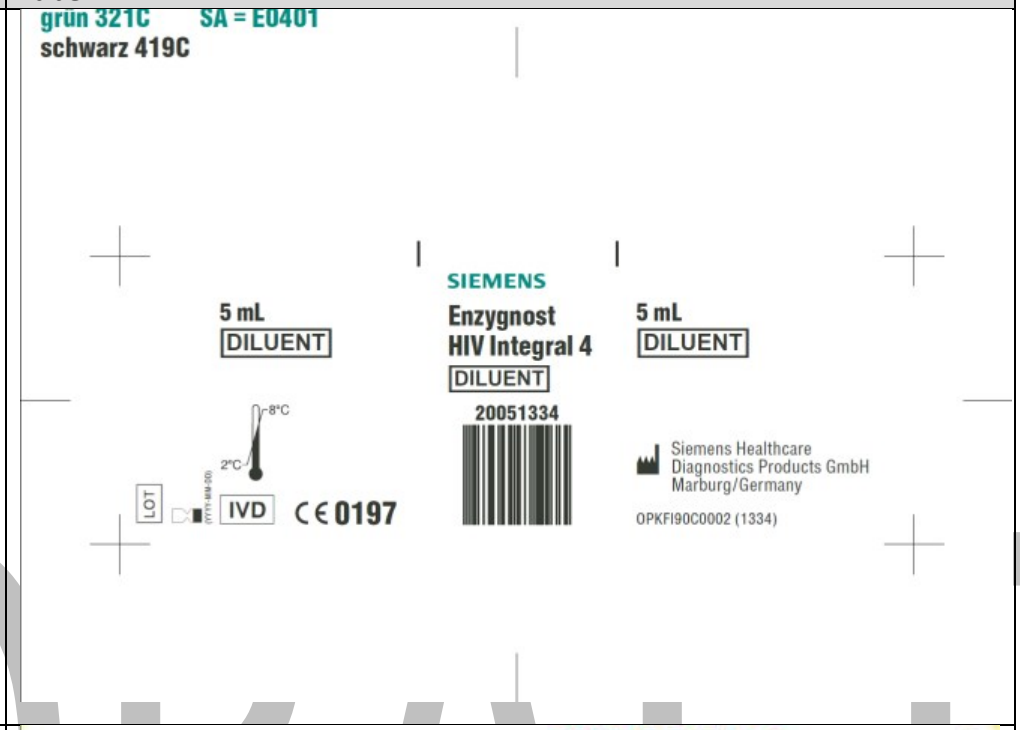
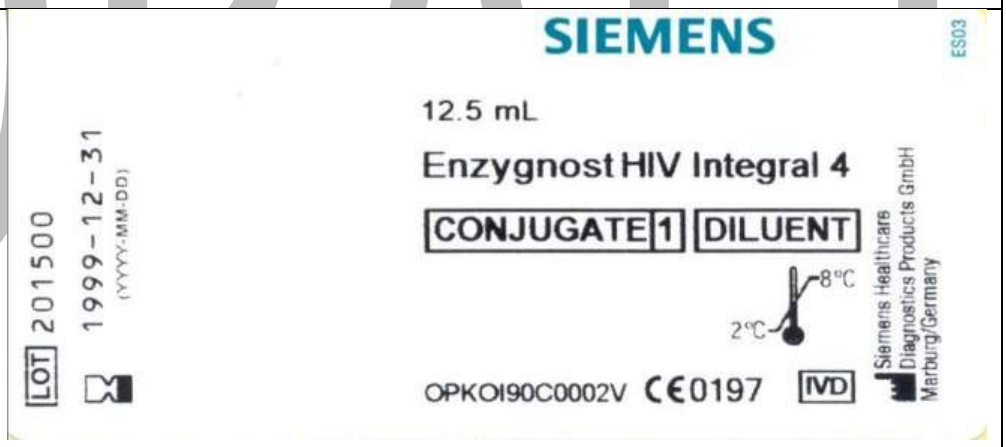
OPKRT03C3101

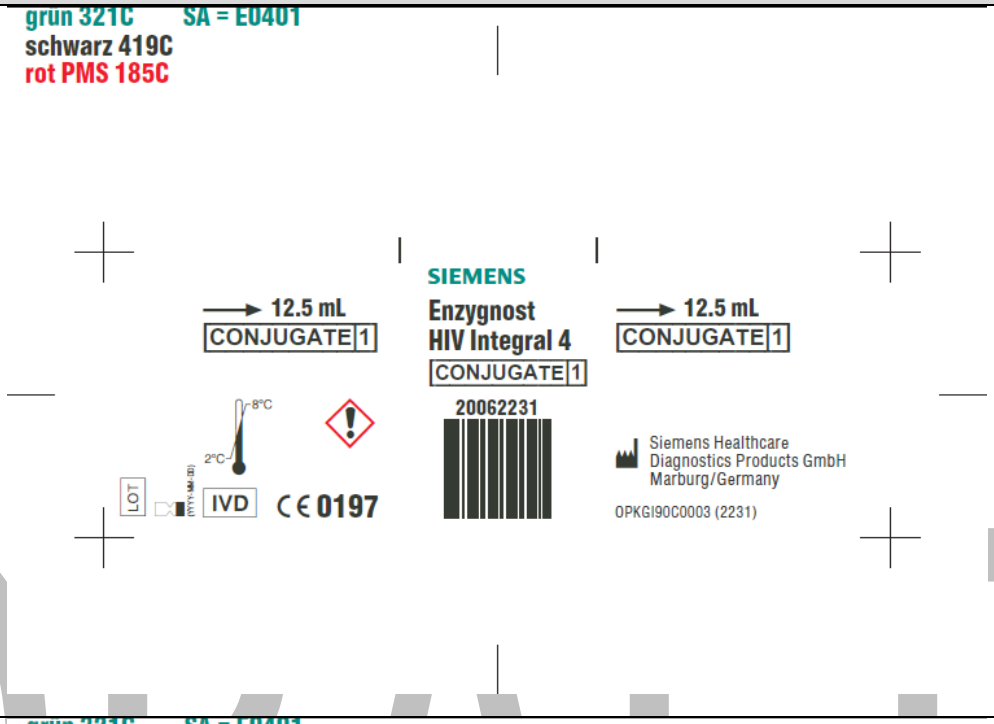
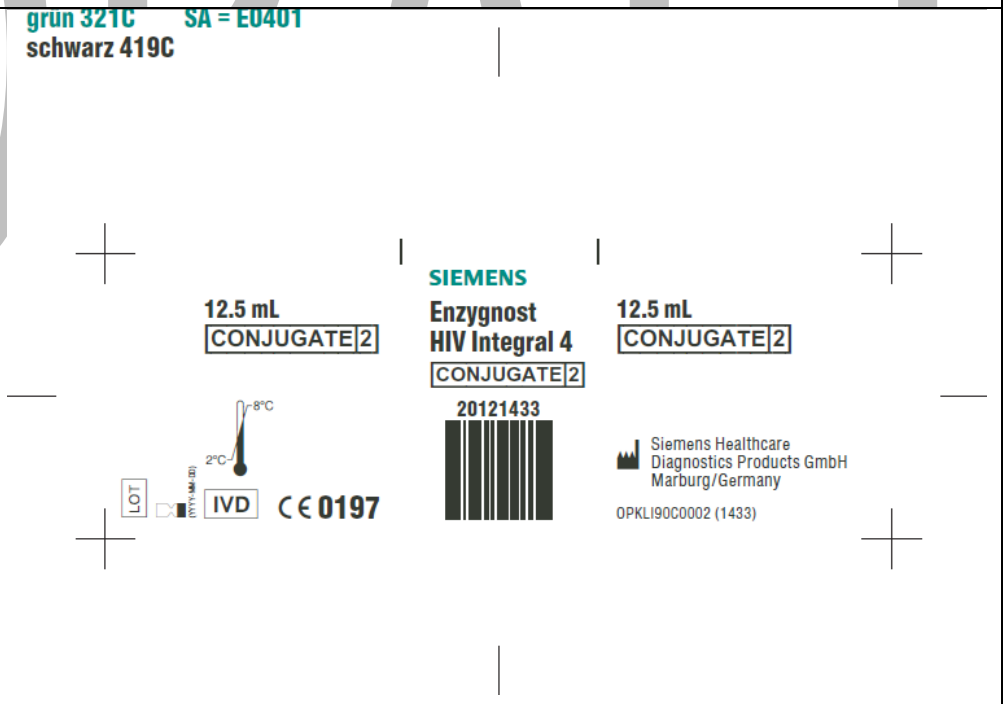
www.siemens.com/diagnostics

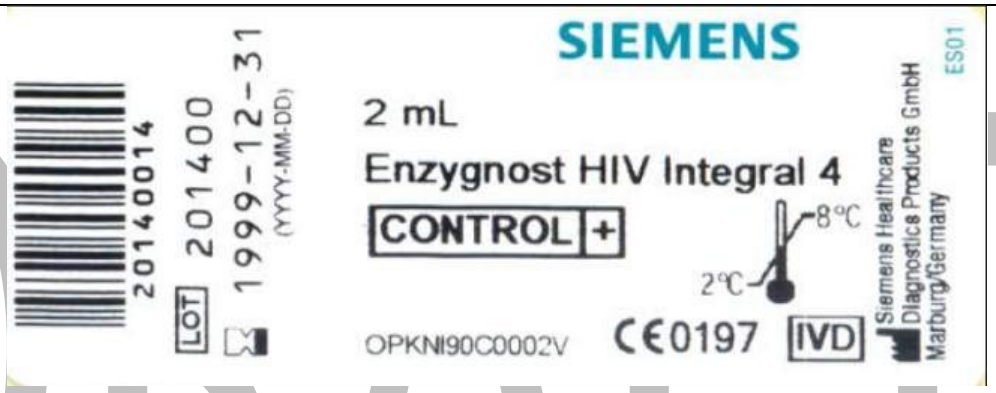
CE 0197

1.3 Labels for Product variant OPKR 03 (2x96)

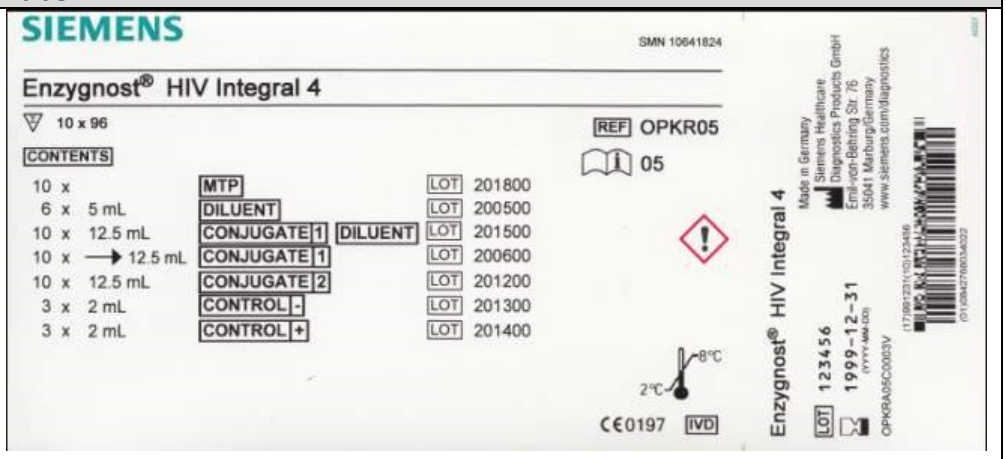
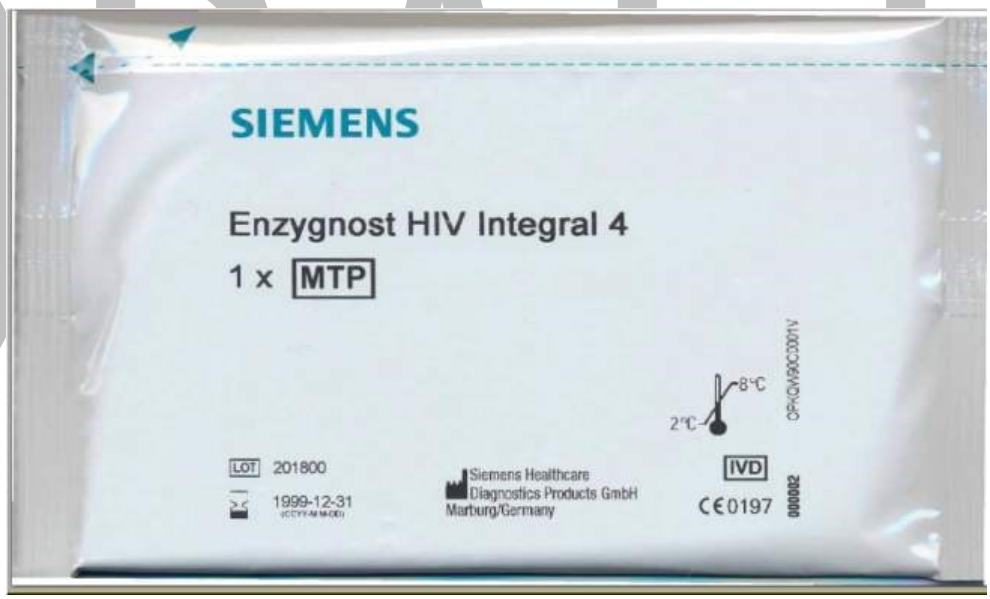
Component	Label
Outer Box	
MTP	MTP pouch with label  MTP side label 
	MTP strip label 

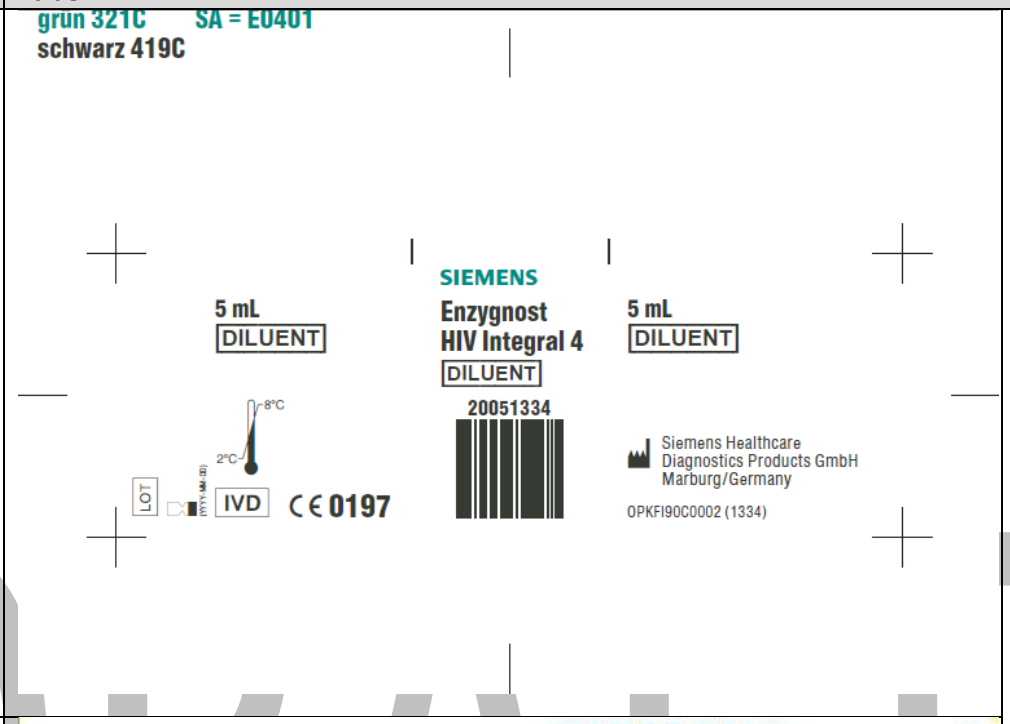
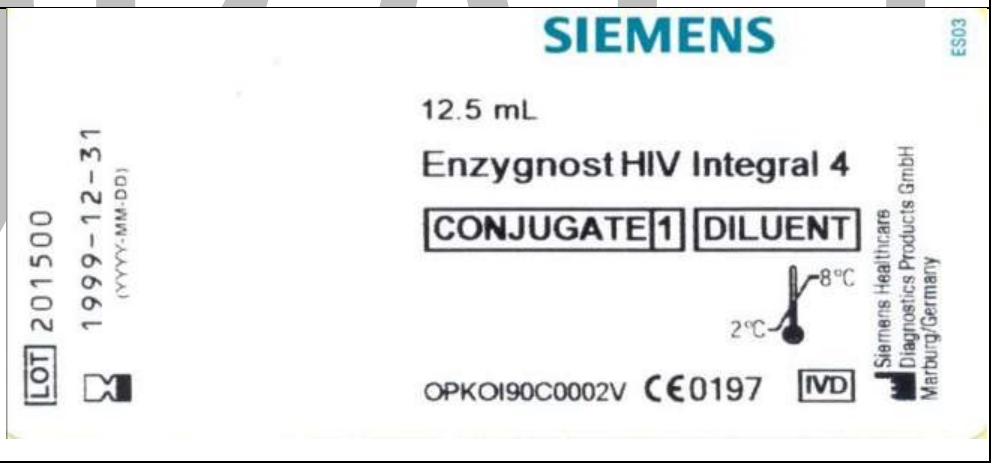
Component	Label
Diluent (5 mL)	
Conjugate 1 Diluent	

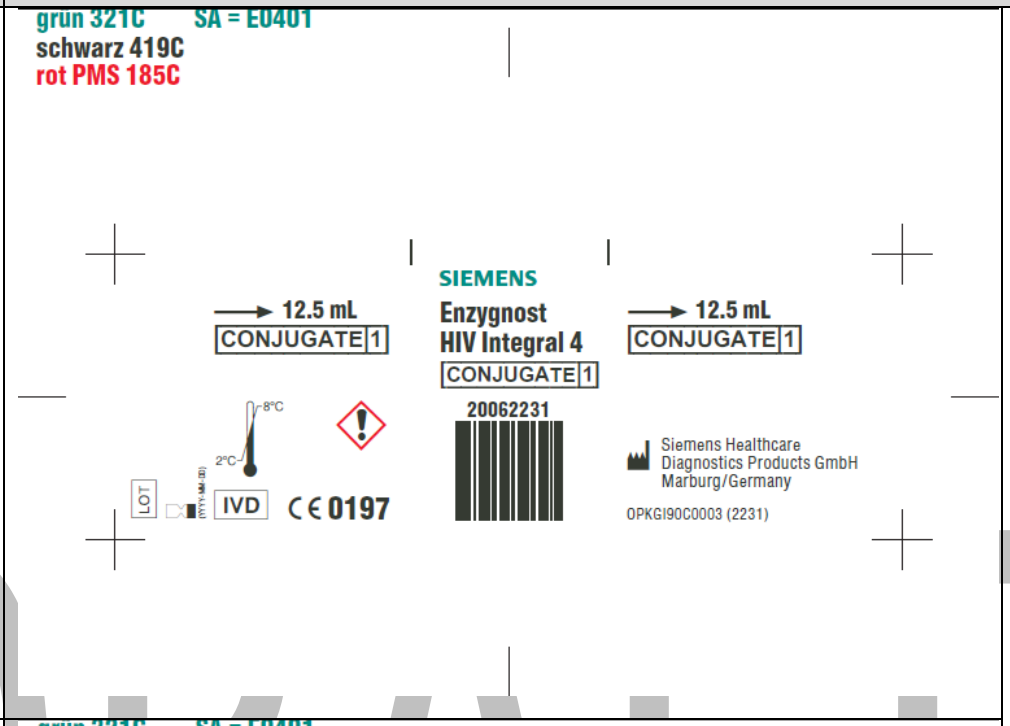
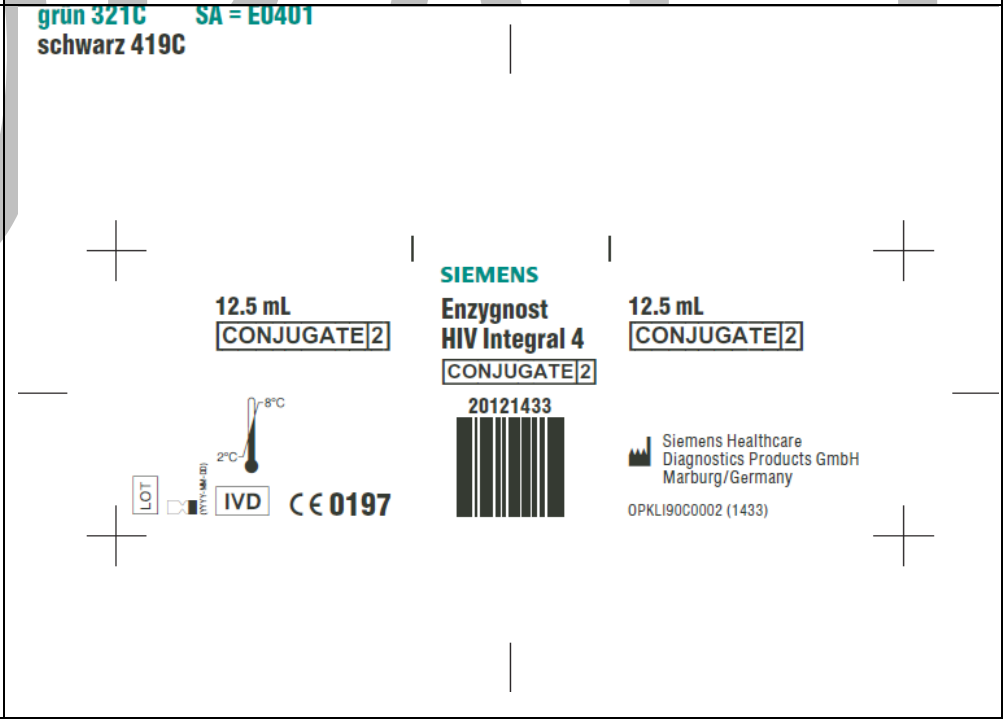
Component	Label
Conjugate 1 (12.5 mL)	
Conjugate 2 (12.5 mL)	

Component	Label
Control -	
Control +	

1.4 Labels for product variant OPKR 05 (10x96)

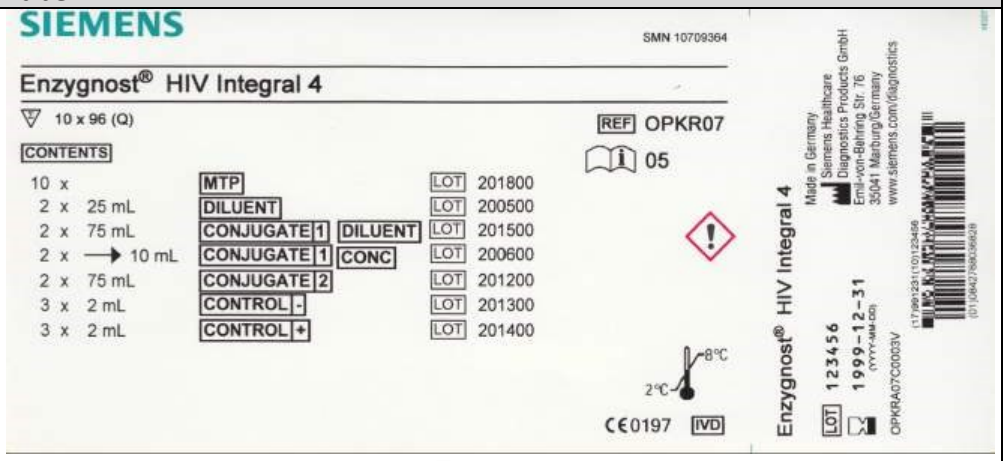
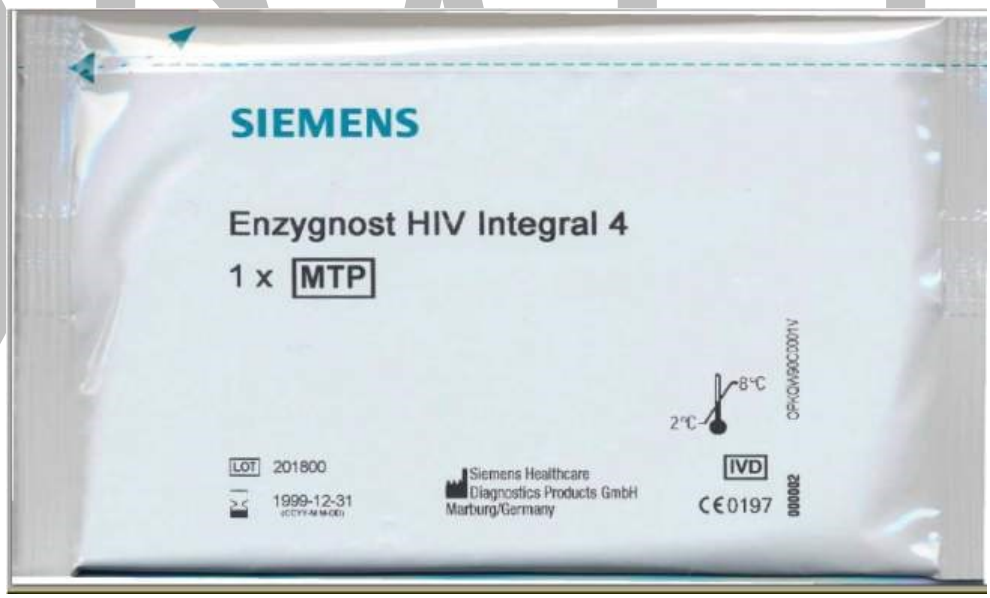
Component	Label
Outer Box	
MTP	MTP pouch with label  MTP side label 
	MTP strip label 

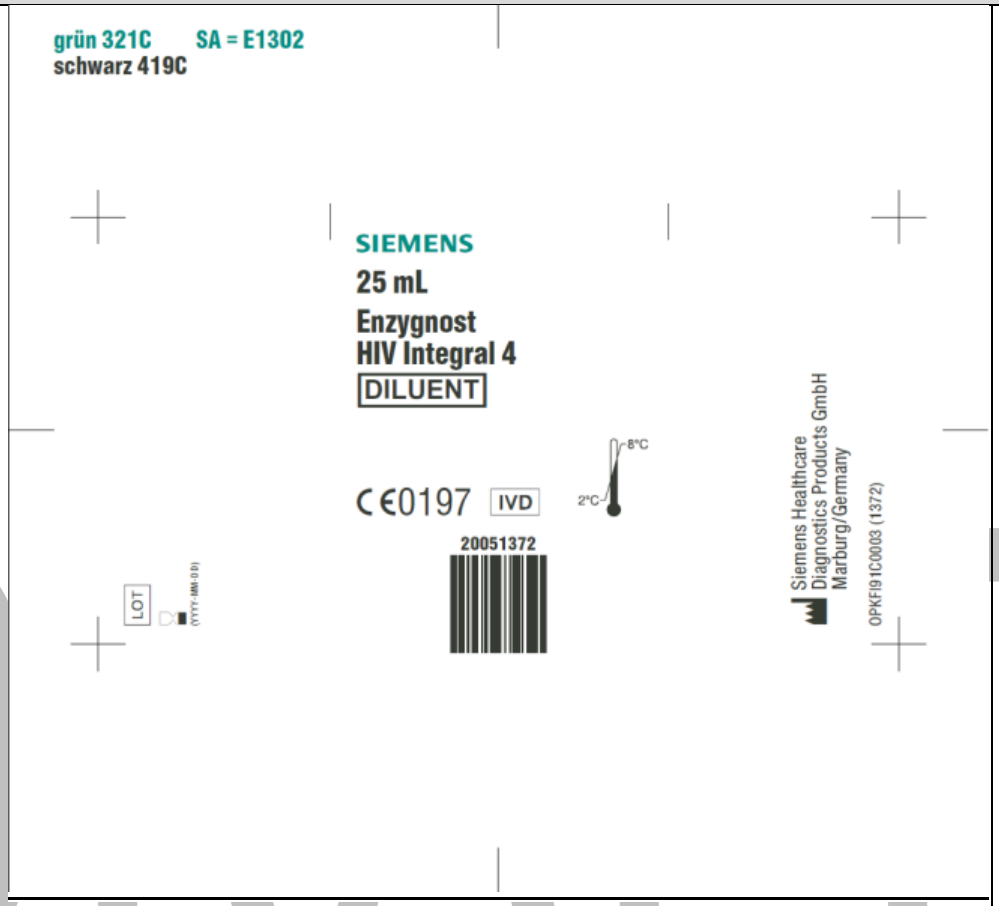
Component	Label
Diluent (5 mL)	
Conjugate 1 Diluent	

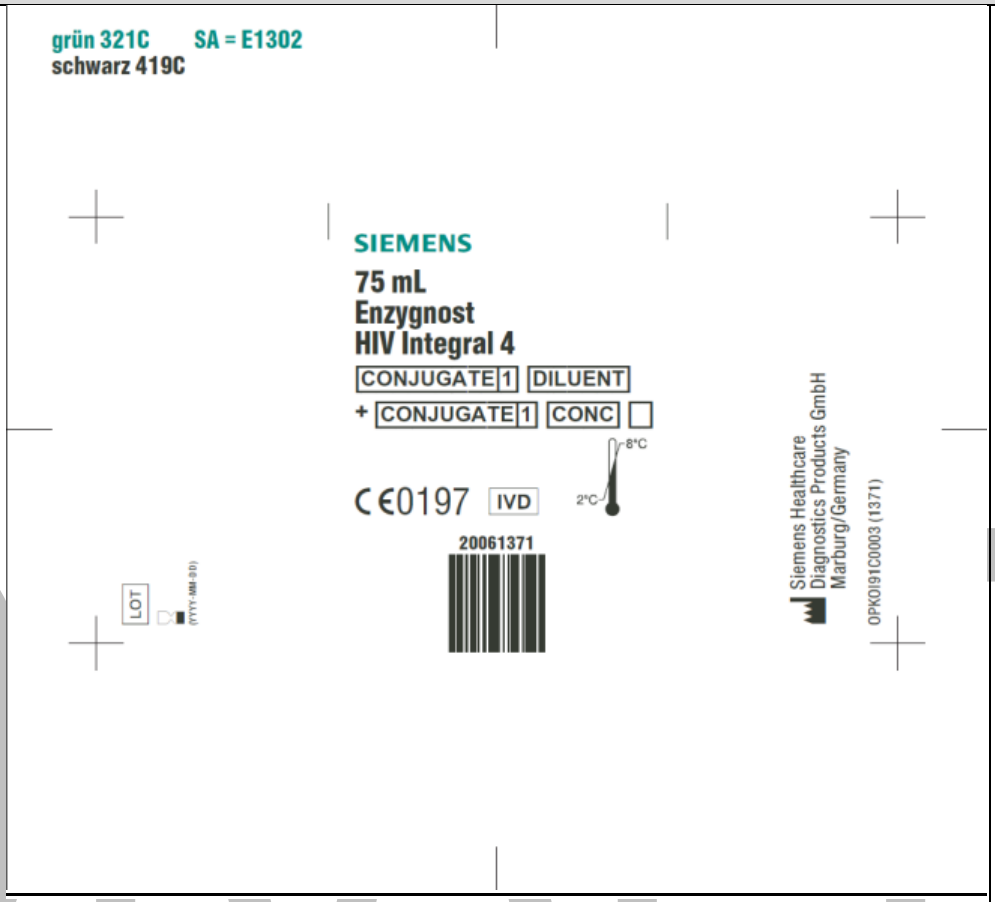
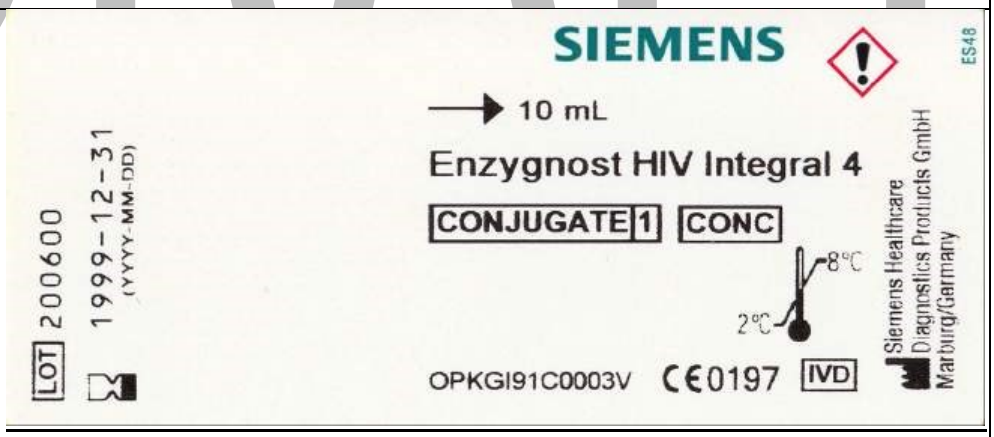
Component	Label
Conjugate 1 (12.5 mL)	
Conjugate 2 (12.5 mL)	

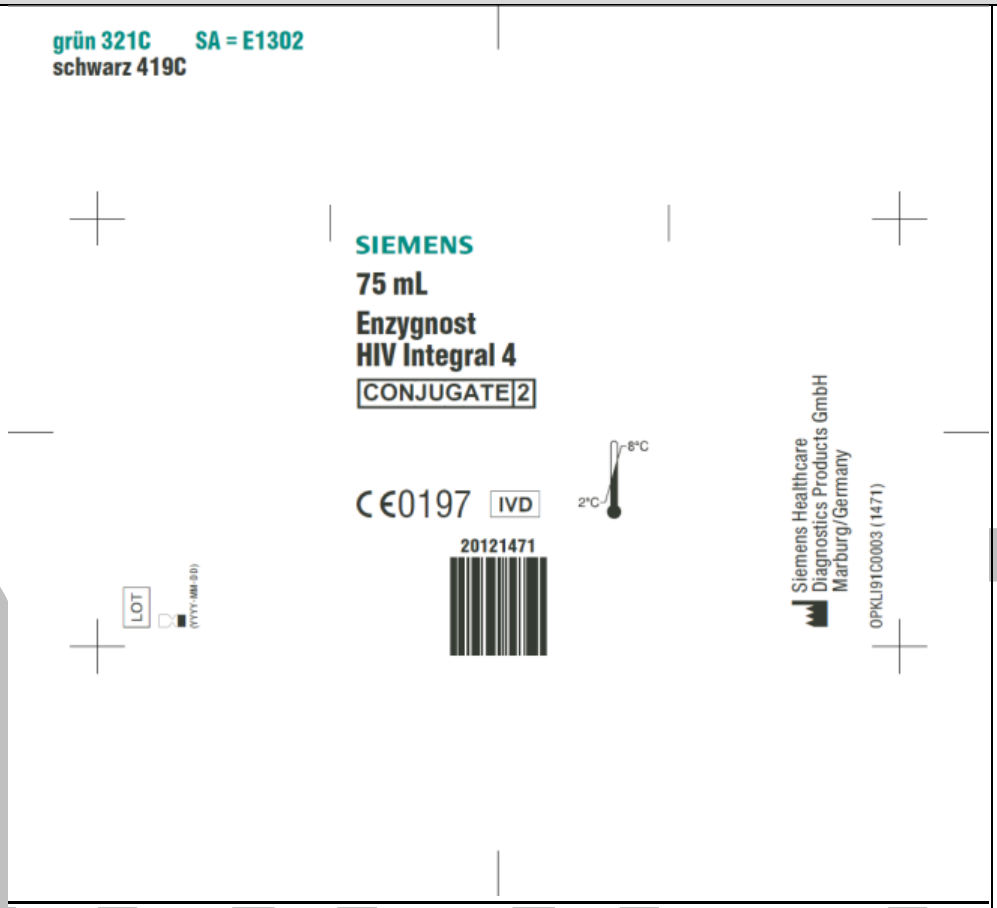
Component	Label
Control -	
Control +	

1.5 Labels for product variant OPKR 7 (10x96 Q)

Component	Label
Outer Box	
MTP	MTP pouch with label  MTP side label 
	MTP strip label 

Component	Label
Diluent (25 mL)	

Component	Label
Conjugate 1 Diluent (75 mL)	
Conjugate 1 Conc (10 mL)	

Component	Label
Conjugate 2 (75 mL)	
Control -	

Component	Label
Control +	

2. Instructions for use (excerpt containing only the English version)

SIEMENS

Enzygnost® HIV Integral 4

Enzyme immunoassay for the qualitative detection of HIV p24 antigen and specific antibodies to human immunodeficiency viruses of type 1 and 2 (HIV1 including HIV1 subtype O virus and HIV2) in human serum and plasma.

Enzymimmunoassay zum qualitativen Nachweis von HIV p24-Antigen und spezifischen Antikörpern gegen humane Immundefizienz-Viren der Klasse 1 und 2 (HIV1 inklusive HIV1-Subtyp O-Virus und HIV2) in Human- Serum und -Plasma.

Dosage immunoenzymatique destiné à la détection qualitative de l'antigène p24 du VIH et des anticorps spécifiques dirigés contre les virus d'immunodéficience humaine de type 1 et 2 (VIH-1 et VIH-2), y compris le sous-type O du VIH-1 dans le sérum ou le plasma humain.

Metodo immunoenzimatico per l'identificazione qualitativa dell'antigene e degli anticorpi HIV p24 specifci per i virus dell'immunodeficienza umana di tipo 1 e 2 (virus HIV1 incluso il l'HIV1 sottotipo O e HIV2) nel siero umano o nel plasma.

Enzimoinmunoanálisis para la detección cualitativa del antígeno p24 del VIH y anticuerpos específicos contra los virus de inmunodeficiencia humana tipo 1 y 2 (VIH1 incluido el subtipo O del VIH1 y el VIH2) en suero sanguíneo y plasma humanos.

Ensaio imunoenzimático para a detecção qualitativa do antigénio p24 do VIH e de anticorpos específicos contra o vírus da imunodeficiência humana tipo 1 e 2 (VIH1, incluindo o subtipo O do VIH1, e VIH2) em soro e plasma humanos.

OPKRG03C0505(1372) 2015-04

Enzygnost® HIV Integral 4

Revision bar indicates update to previous version.

Intended Use

Enzyme immunoassay for the qualitative detection of HIV p24 antigen and specific antibodies to human immunodeficiency viruses of type 1 and 2 (HIV1 including HIV1 subtype O virus and HIV2) in human serum and plasma.

The enzyme immunoassay can be processed using the ELISA processors, BEP® III System, BEP® 2000 System, BEP 2000 *Advance*® System as well as the Quadriga® Systems. A non-automated processing of the test is also possible. For *in vitro* diagnostic use.

Summary and Explanation

Acquired immunodeficiency syndrome (AIDS) was first recognized in 1981 as a clinical picture in its own right. Two different human immunodeficiency viruses, HIV1 (synonym: LAV/HTLV-III) and HIV2 are considered as causal pathogens[1,2,3]. The serological determination of antibodies to HIV plays an essential role, especially in the field of transfusion medicine in order to prevent the further spread of the disease. To exclude a transmission via blood transfusions or blood-derived products to the greatest possible extent, blood banks and manufacturers of plasma products introduced testing of blood donors for anti-HIV1 and anti-HIV2 antibodies on a routine basis. In 1990 a new HIV subtype was described, HIV1 subtype O[4,5,6], which is detectable specifically with today's assays, together with the established HIV1 and HIV2 isolates. To narrow the diagnostic window between the occurrence of HIV infection and its first serological detection, it was sensible to supplement the antibody test with a test for HIV p24 antigen as HIV p24 antigen was detected in some HIV seroconversion samples before HIV antibodies were detectable[7]. Although the detection of HIV antibodies and/or antigen does not allow a definite conclusion on whether infectious HIV1 or HIV2 is present in the blood at the time of blood collection, and also a negative result does not exclude the presence of HIV1 or HIV2 with certainty, the combined antigen/ antibody test provides currently the best serological way of detecting and eliminating blood donations from HIV-infected donors with a high probability[7,8].

Principles of the Procedure

The specific antibodies to HIV contained in the test sample bind to the antigens in the reaction wells of the HIV Integral 4 test plate and the HIV p24 antigen present in the test sample to the monoclonal anti-HIV p24 specific antibodies, accordingly. The biotinylated components (recombinant HIV proteins or synthetic peptides respectively monoclonal anti-HIV p24 antibodies) of HIV Integral 4 Conjugate 1 bind in the second step to these specific antibodies respectively to the HIV p24 antigen (antigen sandwich respectively antibody sandwich). In the third step, HIV Integral 4 Conjugate 2 (streptavidin/POD) reacts with the bound biotin conjugates. The enzyme portion of HIV Integral 4 Conjugate 2 causes the Chromogen Working Solution to turn blue. This reaction is stopped by the addition of Stopping Solution POD, which causes a color change to yellow. The color intensity is a measure of the immunochemical reactivity of the HIV-specific antibodies and the concentration of HIV p24 antigen in the sample.

Reagents

Reagent	Description	Storage	Stability once opened[c]	Stability after reconstitution
Enzygnost® HIV Integral 4 test plate MTP 96 wells	with a mixture of recombinant proteins (*Escherichia coli*) containing HIV1 gp41, HIV1 (subtype O) gp41, HIV2 gp36 as well as two monoclonal antibodies (mouse) to HIV p24 antigen coated microtitration plate	2–8 °C 15–25 °C in the bag with desiccant	28 days 6x8 hours[d]	n.a. n.a.
Enzygnost® HIV Integral 4 Sample Buffer DILUENT 5 mL or 25 mL	phosphate buffer with BSA and TRITON X-100; colored pink[a]	2–8 °C 15–25 °C	28 days 6x8 hours[d]	n.a.
Enzygnost® HIV Integral 4 Conjugate 1 Buffer CONJUGATE 1 DILUENT 12.5 mL or 75 mL	TRIS/HCl buffer with SAPOGENAT T500 and Casein[a]	2–8 °C	use immediately once opened	n.a.
Enzygnost® HIV Integral 4 Conjugate 1 CONJUGATE 1 → 12.5 mL or CONJUGATE 1 CONC → 10 mL (final volume 75 mL)	lyophilizate of recombinant (E. coli) HIV1-, HIV2- and HIV1- and HIV1 (subtype O) synthetic peptides and two monoclonal antibodies (mouse) to HIV p24, biotinylated; colored blue[b]	2–8 °C 15–25 °C	n.a.	28 days 6x8 hours[d] or 24 hours[e]
Enzygnost® HIV Integral 4 Conjugate 2 CONJUGATE 2 12.5 mL or 75 mL	streptavidin/peroxidase (POD) conjugate in TRIS/HCl buffer; colored yellow[a]	2–8 °C 15–25 °C	28 days 6x8 hours[d] or 24 hours[e]	n.a.
Enzygnost® HIV Integral 4 Control, negative CONTROL – 2 mL	stabilized human serum without HIV-antigens and without antibodies to HIV1, HIV2 and HIV1 (subtype O) antigens; colored green[a]	2–8 °C 15–25 °C ≤ –20 °C	28 days 6x8 hours[d] 12 weeks	n.a.
Enzygnost® HIV Integral 4 Control, positive CONTROL + 2 mL	heat-treated human serum with antibodies to HIV1 antigens in HEPES buffer; colored red[a]	2–8 °C 15–25 °C ≤ –20 °C	28 days 6x8 hours[d] 12 weeks	n.a.

a Preservative: phenol (≤ 1 g/L)
b Preservative: PROCLIN 300
c use each component by the expiry date at the latest
d number of cycles of standing time open in the laboratory or on board the systems when used within 28 days after first opening and closed storage between cycles at 2–8 °C
e on board the BEP® 2000 System

Stored unopened at 2 to 8 °C, all components of the test kit may be used up to the expiry dates given on the labels.

Warnings and Precautions

For *in vitro* diagnostics use.

The test was developed for testing individual samples, not for pooled samples.

Warning! CONJUGATE 1, CONJUGATE 1 CONC
H317: May cause an allergic skin reaction.
P261, P272, P280, P363, P302 + P352, P333 + P313, P501: Avoid breathing dust/fume/gas/mist/vapours/spray. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/protective clothing/eye protection/face protection. Wash contaminated clothing before reuse. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents and container in accordance with all local, regional, and national regulations.

CAUTION! POTENTIAL BIOHAZARD
Each donor or donor unit was tested and found to be negative for human immunodeficiency virus (HIV) 1 and 2 (with the exception of HIV Integral 4 Control, positive), hepatitis B virus (HBV) and hepatitis C virus (HCV) using either tests found to be in conformance with the In Vitro Diagnostic Directive in the EU or FDA approved tests. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with appropriate caution.

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

Caution: This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

It is advisable to wear protective gloves throughout the entire test procedure. Please follow the recommendations of the manufacturer concerning the compatibility between gloves and exposed materials.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all government requirements. It is recommended that solid infectious materials should be autoclaved for at least 1 hour at 121 °C. All aspirated liquids should be collected in two receptacles connected in series. Both should contain a disinfectant suitable for inactivating human pathogens. The concentrations and times specified by the manufacturer must be observed.

Buffer/Substrate TMB, Chromogen Working Solution and Stopping Solution POD must not be allowed to come into contact with heavy metal ions or oxidizing substances (do not use pipettes with metal parts which are in direct contact with the liquid). The substrate reaction steps must not be performed in the vicinity of disinfectants containing hypochlorite. If the Chromogen Working Solution has spontaneously developed a blue color before being transferred into the test plate, this indicates that the solution is contaminated; in such cases, prepare a fresh solution in a clean container. Skin contact with the above mentioned solutions is to be avoided.

Preparing Reagents

Bring all reagents and test samples to 15 to 25 °C before starting with the test. Do not remove the foil pouch from the test plates during this step. If reagents or reagent working solutions need to be mixed, avoid foam formation.

To avoid a frequent change of syringes when processing large series of samples on the BEP® III System, the kit 10 x 96 (Q) is recommended.

HIV Integral 4 test plate: Before starting the test processing, remove not required strips from the holder and store these in the enclosed polyethylene bag for later use.

HIV Integral 4 Conjugate 1 Buffer: ready to use

A slight whitely precipipate at the bottom of the vial can be dissolved by short agitation of the buffer before use. The precipitate does not affect the performance of the test and is not caused by microbial contamination.

HIV Integral 4 Conjugate 1 (2x96 and 10x96 Kit): Transfer the entire contents of one vial Conjugate 1 Buffer into a vial Conjugate 1. Dissolve the lyophilizate completely by slight agitation and equilibrate at 15 to 25 °C for at least 15 minutes.

HIV Integral 4 Conjugate 1 Concentrate (10x96 (Q) Kit): Reconstitute the lyophilizate with 10 mL Conjugate 1 Buffer by slight agitation. Retransfer the complete contents to the Conjugate 1 Buffer vial. Rinse the emptied Conjugate 1 Concentrate vial with 10 mL of the now blue colored solution and transfer it after

slight agitation to the Conjugate 1 Buffer vial. Mix thoroughly. Before use, equilibrate the Conjugate 1 Working Solution for at least 15 minutes at 15 to 25 °C. Document the addition of reconstituted Conjugate 1 Concentrate to Conjugate 1 Buffer by using the check box on the buffer vial label.

HIV Integral 4 Conjugate 2: ready to use

HIV Integral 4 Sample Buffer: ready to use

HIV Integral 4 Control, negative: ready to use

HIV Integral 4 Control, positive: ready to use

Specimen Collection and Handling

Collecting the Specimen

Suitable specimens are individual samples (human sera or CPDA/EDTA/heparinized/citrated plasma) obtained by standard laboratory techniques.

Storing the Specimen

The samples should be stored for no more than 3 days at 18 to 25 °C or 8 days at 2 to 8 °C. If samples are frozen within this period, they can be stored at below –20 °C for up to 2 years and 6 months if repeated freeze-thaw cycles are avoided.

Procedure

Materials Provided

REF	Contents			
	Number of Tests			Component
	2 × 96	10 × 96	10 × 96 (Q)	
OPKR	2	10	10	MTP
	2 × 5 mL	6 × 5 mL	2 × 25 mL	DILUENT
	2 × 12.5 mL	10 × 12.5 mL	2 × 75 mL	CONJUGATE 1 DILUENT
	2 × → 12.5 mL	10 × → 12.5 mL	--	CONJUGATE 1
	--	--	2 × → 10 mL[f]	CONJUGATE 1 CONC
	2 × 12.5 mL	10 × 12.5 mL	2 × 75 mL	CONJUGATE 2
	2 × 2 mL	3 × 2 mL	3 × 2 mL	CONTROL –
	2 × 2 mL	3 × 2 mL	3 × 2 mL	CONTROL +
	1	1	1	polyethylene bag

[f] final volume: 75 mL, refer to "Preparing Reagents"

The test plate, the conjugates, the Conjugate 1 Buffer, as well as the control, positive and the control, negative must be used in the given combination of 6-digit lot numbers printed on the package, respectively stated in the enclosed barcode table of values.

Materials Required but not Provided

Item	Description
Supplementary Reagents for Enzygnost®/TMB REF OUVP	Buffer/Substrate TMB Chromogen TMB Stopping Solution POD Washing Solution POD adhesive foils empty bottle for the Chromogen Working Solution For details on kit size and components refer to the respective Instructions for Use.
BEP® III System	for automated processing and evaluation of the test after manual dispensing of samples and controls
BEP® 2000 / BEP 2000 *Advance*® System	for fully automated processing and evaluation of the test

Item	Description
Quadriga® Systems	for fully automated processing and evaluation of the test in combination with BEP® III
Pipettes	piston-type pipettes with fixed or variable volumes, or single- and multichannel pipettes with adjustable volumes

The following items are required additionally if the test is not processed automatically:

Incubator	covered water bath (37 ±1 °C) or similar incubation method
Washing device	microtitration plate washer
Photometer	photometer suitable for microtitration plates, measuring wavelength of 450 nm, reference wavelength of 650 nm (between 615 nm and 690 nm as appropriate). For SURE measurements, wavelength 405 nm is also required.

All the equipment used in the test must have been validated.

Test Procedure

Non-automated Test Procedure

1. **Preparing Reagents:** Refer to "Preparing Reagents".
2. **Assay scheme:** The necessary number of test plate wells is given by the number of test samples plus the number of determinations (n = 5) for HIV Integral 4 Control, positive and negative.
3. **Pre-dispense buffer:** Dispense 25 µL of Sample Buffer into each required well of the test plate.
4. **Dispense samples:** Dispense 100 µL Control, negative into each of the first 3 wells (A1-C1), 100 µL Control, positive into the next well (D1) and 100 µL of undiluted sample into each of the subsequent wells. At the end of the series, respectively test plate, fill the last well with 100 µL Control, positive. Do not mix well content!

   Important:

   It is not permitted to first pipette Control, positive into the wells at the start and end of the sample series, and then put the samples in-between.

   Alternative pipetting scheme: Dispense 100 µL Control, negative into each of the first 3 wells (A1-C1), 100 µL Control, positive into each of the next 2 wells (D1-E1), and 100 µL of undiluted sample into each of the subsequent wells. Do not mix well content!

   Each sample must be pipetted with its own pipette tip. The pipetting steps for Sample Buffer and sample must be completed within 30 minutes per test plate. After completing the pipetting steps, seal the test plate with foil and place immediately into the incubator.

   Pipetting control (optional):

   The correct pipetting of the controls and samples can be checked visually (HIV Integral 4 Control, negative (green), HIV Integral 4 Control, positive (red), sample and empty wells (clear)) or qualitatively by photometric measurement at 405 nm against 650 nm (the so-called SURE function). For details refer to the document "BEP® III System/ BEP® 2000 System/ BEP 2000 *Advance*® System SURE Specifications".
5. **Incubate samples:** Incubate for 30 ±2 minutes at 37 ±1 °C, then proceed immediately to the wash step.
6. **Wash:** Remove foil and aspirate all wells. Fill each well with approximately 300 µL diluted Washing Solution POD, aspirate the plate, and repeat the wash cycle two times. After completing the wash cycles, proceed immediately to the next reagent dispensing step (otherwise the wells may dry out).
7. **Dispense Conjugate 1:** Pipette 100 µL Conjugate 1 Working Solution into each well. Then seal the test plate with fresh foil and place immediately into the incubator.
8. **Incubate Conjugate 1:** Incubate for 30 ±2 minutes at 37 ±1 °C, then proceed immediately to the wash step.
9. **Wash:** As described in step 6.
10. **Dispense Conjugate 2:** Pipette 100 µL Conjugate 2 into each well. Then seal the test plate with fresh foil and place immediately into the incubator.
11. **Incubate Conjugate 2:** Incubate for 30 ±2 minutes at 37 ±1 °C, then proceed immediately to the wash step.
12. **Wash:** Remove foil and aspirate all wells. Fill each well with approximately 300 µL diluted Washing Solution POD, aspirate the plate, and repeat the wash cycle three times. After completing the wash cycles, proceed immediately to the next reagent dispensing step (otherwise the wells may dry out).

13. **Dispense substrate:** Pipette 75 µL of Chromogen Working Solution into each well, then seal the microtitration plate with fresh foil.
14. **Incubate substrate:** Immediately after the substrate dispensing step, incubate at 18 to 25 °C for 30 ±2 minutes, protected from light.
15. **Stop reaction:** Remove the foil. Add 75 µL Stopping Solution POD to each well, keeping to the same timing as during the substrate dispensing step.
16. **Measure:** Read the test plate at 450 nm within one hour. The recommended reference wavelength is 650 nm (or where appropriate between 615 and 690 nm).

Procedure for the BEP® III System

When using the BEP® III, the test plates must be prepared up to the sample dispensing step (steps 1 to 4 in the section "Non-automated Test Procedure"). Ensure that partially loaded test plates are supplemented with "water-filled strip" to at least half plates (6 test strips). Immediately afterwards place the uncovered test plates, i.e. not covered with foil, into the BEP® III. All subsequent processing steps are performed fully automatically by the instrument (see BEP® III Instruction Manual).

The settings for the incubation times in the BEP® III software may differ from the times in the section "Non-automated Test Procedure" for technical reasons (system speed) but have been validated for Enzygnost® on the BEP® III.

Procedure for fully automated Systems (BEP® 2000 and Quadriga®)

The sample dispensing steps and subsequent processing of the test are performed fully automatically by the analyzer (see respective Instruction Manual). Ensure that partially loaded test plates are supplemented with "water-filled strips" to at least half plates (6 test strips).

Sample processing with the BEP® 2000 and Quadriga® System may differ from the information given under "Non-automated Test Procedure", but has been validated for Enzygnost® on the respective system.

Internal Quality Control

To evaluate the test the following criteria must be fulfilled:

1. HIV Integral 4 Control, negative: $-0.010 \leq A \leq 0.130$
2. HIV Integral 4 Control, positive: $0.900 \leq A \leq 2.700$

If one of the three absorbance values of HIV Integral 4 Control, negative is outside the specification, this value can be neglected.

Both absorbance values for HIV Integral 4 Control, positive must comply with the respective specification.

If these conditions are not met, the test is not valid for evaluation. In this case, the software of BEP® III, BEP® 2000 and Quadriga® will give the notice of an invalid test result. The test must be repeated after investigating the cause.

Results

The evaluations are performed automatically with the BEP® III, the BEP® 2000 and the Quadriga® Systems. Please consult the relevant Instruction Manual. The following sections must be taken into account when performing measurements without software support.

Evaluation using the Cut-off

To calculate the cut-off, use the mean of the valid absorbance values of HIV Integral 4 Control, negative and add a value of 0.180:

$\bar{A}_{neg} + 0.180 = \text{cut-off}$

Based on the cut-off, the samples are classified as follows:

HIV	**negative**	$A <$ cut-off
HIV	**reactive**	$A \geq$ cut-off

Evaluation using the Ratio

An interpretation of the test results is also possible by calculating the quotient of A_{sample} and cut-off:

$$\text{ratio} = \frac{A_{sample}}{\text{cut-off}}$$

The ratio is calculated automatically by the BEP® III, BEP® 2000 and Quadriga® Systems. With this method results from different runs can be standardized and made comparable with each other.

Based on the ratio, the samples are classified as follows:

HIV	**negative**	ratio	< 1.0
HIV	**reactive**	ratio	≥ 1.0

Assessment of the Results

Reactive test samples (absorbance ≥ cut-off, respectively a ratio ≥ 1) have to be tested again in duplicate. A sample is considered repeatedly reactive if at least one repeat measurement has an absorbance value ≥ cutoff, respectively a ratio ≥ 1.0. If the absorbance value of both repeat measurement is < cut-off respectively ratio < 1.0, the sample is considered HIV negative according to the test criteria. All reactive samples must be clarified according to a recognized confirmation method (e.g., immunoblot, nucleic acid amplification assay). It is recommended to analyze a follow-up sample about two weeks later.

Results should always be interpreted in conjunction with the patient´s medical history, clinical presentation, and other findings.

Limitations

1. Anticoagulants (citrate, CPDA, EDTA, heparin) do not interfere with the test result.
2. Samples from pregnant woman and samples containing the following potentially interfering substances were investigated: HBsAg, antibodies to *E. coli*, HBc, HCV, CMV, HTLV-I, HTLV-II, HAV, HHV-8, HSV-2, PCP, as well as Syphilis and Toxoplasmosis positive samples. With these samples no interference with the test results has been observed.
3. Heat treated samples should not be used.
4. Incompletely coagulated sera and microbially contaminated samples should not be used. Any particulate components in the sample (e.g. fibrin clots, erythrocytes) should be removed before the test.
5. Previously frozen samples and samples stored on the clot may show increased unspecific reactivity.
6. If thawed samples are used, ensure that the material is thoroughly homogenized.
7. Highly reactive samples may cause a precipitation of the dye during the stopping reaction. This does not interfere with the photometric evaluation.
8. The control sera were produced using native human sera. Therefore, turbidity may occur but does not impair the test result.
9. This product is not intended for use with samples drawn post mortem.
10. Patient samples may contain heterophilic antibodies that could react in immunoassays to give a falsely elevated or depressed result. This assay has been designed to minimize interference from heterophilic antibodies. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed.
11. As it is not possible to differentiate between maternal IgG (from HIV infected mothers) and antibodies of an active infection, Enzygnost® HIV Integral 4 is not recommended for testing infants younger than 2 years.
12. Siemens Healthcare Diagnostics has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Application Sheets or these Instructions for Use.

Performance Characteristics

Specificity

For the determination of specificity, 8419 HIV negative sera were investigated at two evaluation sites and a specificity of 99.90 % (initial testing) and 99.93 % after retesting was obtained. For the determination of specificity in plasma, 8306 EDTA plasmas were investigated at two sites and a specificity of 99.95 % (initial testing) and 99.96 % after retesting was obtained. The results of the specificity studies are summarized in the following table.

Site		Specimen	Number of samples	Initial reactive samples (specificity in %)	Retest reactive samples (specificity in %)
A	Blood donors	serum	5648	99.91	99.91
B	Blood donors	serum	2771	99.89	99.96
B	Blood donors	EDTA plasma	2772	99.89	99.93
C	Blood donors	EDTA plasma	5534	99.98	99.98

Site		Specimen	Number of samples	Initial reactive samples (specificity in %)	Retest reactive samples (specificity in %)
-	**Hospitalized persons**	serum and EDTA plasma	262	99.62	99.62
-	**Samples with potentially interfering substances**	serum and various types of plasma	551	99.82	99.82

In relation to sample population, test procedure and other factors different values may be obtained, which however have to be in accordance with the Common Technical Specifications for in vitro diagnostic medical devices (CTS).

Sensitivity

The diagnostic sensitivity was determined using 1504 HIV positive samples. All samples were tested as reactive.

Detailed information on the subtypes is available for 690 of the 1504 HIV positive samples and is summarized in the following table.

Sample population		Number of samples	Number of reactive samples
Group	**Subtype**		
HIV-1 M	A	49	49
	B	248	248
	C	43	43
	D	24	24
	F	14	14
	G	26	26
	H	8	8
	J	2	2
	K	3	3
	CRF01_AE	23	23
	CRF02_AG	22	22
	CRF03_AB	3	3
	CRF06_cpx	2	2
	CRF07_BC	2	2
	CRF09_cpx	1	1
	CRF13_cpx	1	1
	CRF14_BG	1	1
	CRF01_AE/CRF15_01B	1	1
	G/CRF02_AG	1	1
	K/CRF09_cpx	1	1
	A/AD (recombinant)	1	1
	A2C (recombinant)	1	1
	B/F (recombinant)	1	1
HIV-1 O	-	21	21
HIV-2	-	191	191

The reactivity of the test with seroconversion samples was investigated using 59 seroconversion panels. It was found that Enzygnost® HIV Integral 4 exhibits a sensitivity in detecting seroconversions which is comparable to or better than similar tests. Nevertheless, it cannot be ruled out that individual samples may escape detection when the test is used on a large scale.

During a study, a mean analytical sensitivity of 0.29 IU/mL was determined for Enzygnost® HIV Integral 4 using HIV-1 p24 Antigen ("1st International Reference Reagent" of the WHO, 1992[9]). Typically, the analytical sensitivity of Enzygnost® HIV Integral 4 is < 0.50 IU/mL.

Based on HIV-1 Antigen Standard (BioRad) the mean analytical sensitivity for Enzygnost® HIV Integral 4 was 6.10 pg/mL. Typically the analytical sensitivity of Enzygnost® HIV Integral 4 is < 12 pg/mL.

Precision

10 samples with different HIV specific reactivities were tested to determine the repeatability and the within-device variation coefficients (CV) (8-fold replicates in 5 runs). The calculation was performed using analysis of variance.

Exemplary results obtained from an internal study on the BEP® III are summarized below.

Sample	Status	Mean Absorbance (A)	Repeatability CV (%)	Within-device CV (%)
FP03	low reactive (near cut-off)	0.329	5.9	14.4
FP04	low reactive (near cut-off)	0.392	5.2	5.2
FP05	low reactive (near cut-off)	0.327	6.8	8.4
FP06	low reactive (near cut-off)	0.385	6.8	13.4
FP07	low reactive (near cut-off)	0.389	4.6	4.8
FP08	reactive	1.387	4.3	7.2
FP09	reactive	1.757	5.6	5.7
FP10	reactive	1.458	4.0	4.0
FP11	reactive	1.685	5.5	8.5
FP12	reactive	1.487	2.3	3.6

Interferences

The following substances do not interfere with the test results (false-positive reactivity of HIV negative samples, respectively signal reduction of HIV positive samples) when present in samples at the concentrations indicated.

Interferent	no interference up to....
Bilirubin	400 mg/L
Hemoglobin	10 g/L
Triglycerides	8 g/L
Rheumatoid factors	2300 IU/mL
Biotin	32 µg/L
HAMA	90 µg/L

Information on Sample Preparation

Data presented in the chapter "Performance Characteristics" was established with samples which were centrifuged as follows:

Time (minutes)	Centrifugation speed (x g)
15	2600
5	2323
5	1500
10	3000
8	3500

Note

The values cited for specific performance characteristics of the assay represent typical results and are not to be regarded as specifications for Enzygnost® HIV Integral 4.

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CE 0197

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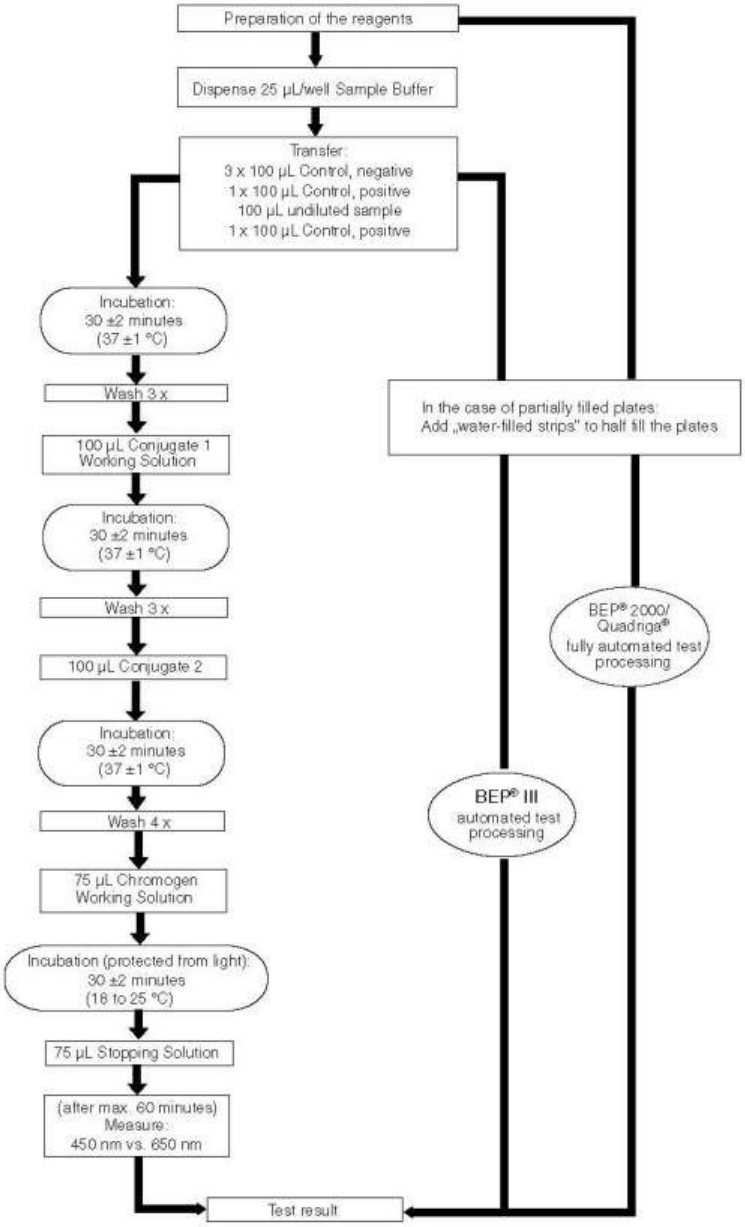
Table 2 Test Procedure



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Definition of Symbols / Bedeutung der Symbole / Définition des symboles / Definizione dei simboli / Definición de símbolos / Definição de símbolos

	Do not reuse / Nicht zur Wiederverwendung / Ne pas réutiliser / Non riutilizzare / No reutilizar / Não reutilizar
	Use By / Verwendbar bis / Utiliser jusque / Utilizzare entro / Fecha de caducidad / Prazo de validade
	Batch Code / Chargenbezeichnung / Code du lot / Codice del lotto / Código de lote / Código do lote
	Catalogue Number / Bestellnummer / Référence du catalogue / Numero di catalogo / Número de catálogo / Referência de catálogo
	Caution, consult accompanying documents / Achtung, Begleitdokumente beachten / Attention voir notice d'instructions / Attenzione, vedere le istruzioni per l'uso / Atención, ver instrucciones de uso / Atenção, consulte a documentação incluída
	Manufacturer / Hersteller / Fabricant / Fabbricante / Fabricante / Fabricante
	Authorized representative in the European Community / Bevollmächtigter in der Europäischen Gemeinschaft / Mandataire dans la Communauté européenne / Mandatario nella Comunità Europea / Representante autorizado en la Comunidad Europea / Representante autorizado na Comunidade Europeia
	Contains sufficient for <n> tests / Inhalt ausreichend für <n> Tests / Contenu suffisant pour "n" tests / Contenuto sufficiente per "n" saggi / Contenido suficiente para <n> ensayos / Conteúdo suficiente para "n" ensaios
	Biological Risks / Biogefährdung / Risques biologiques / Rischio biologico / Riesgos biológicos / Riscos biológicos
	*In Vitro* Diagnostic Medical Device / *In Vitro* Diagnostikum / Dispositif médical de diagnostic *in vitro* / Dispositivo medico-diagnostico *in vitro* / Producto sanitario para diagnóstico *in vitro* / Dispositivo médico para diagnóstico *in vitro*
	Temperature Limitation / Temperaturbegrenzung / Limites de température / Limiti di temperatura / Límite de temperatura / Limites de temperatura
	Consult instruction for Use / Gebrauchsanweisung beachten / Consulter les instructions d'utilisation / Consultare le istruzioni per l'uso / Consulte las instrucciones de uso / Consulte as instruções de utilização
	Non-sterile / Nicht steril / Non stérile / Non sterile / No estéril / Não estéril
	CE mark / CE Zeichen / Marquage CE / Marchio CE / Marca CE / Marca CE
CONTENTS	Contents / Inhalt / Contenu / Contenuto / Contenido / Conteúdo
	Reconstitution volume / Rekonstitutionsvolumen / Volume de reconstitution / Volume di ricostituzione / Volumen de reconstitución / Volume de reconstituição
LEVEL	Level / Konzentration / Niveau / Livello / Nivel / Nível
	Keep away from sunlight and heat / Vor Sonneneinstrahlung und Hitze schützen / Maintenir hors de portée de la lumière du soleil et de la chaleur / Non esporre alla luce del sole e al calore / Mantener protegido de la luz solar y del calor / Manter protegido da luz solar e do calor