WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: m-PIMA HIV-1/2 VL
WHO reference number: PQDx 0359-032-00

The **m-PIMA HIV-1/2 VL test** with product code **27015-W50** using the **m-PIMA Analyser (product code 27030R001)**, manufactured by **Abbott Rapid Diagnostics Jena GmbH**[1], **Rest of World regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 8 April 2019.

Summary of WHO prequalification assessment for m-PIMA HIV-1/2 VL

	Date	Outcome
Prequalification listing	8 April 2019	listed
Dossier assessment	4 March 2019	MR
Site inspection(s) of the quality management system	16 to 18 July 2018	MR
Product performance evaluation	August 2018 to February 2019	MR

MR: Meets Requirements

Report amendments and product changes

This public report has since been amended. Amendments may have arisen because of changes to the prequalified product for which the WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

Version	Summary of amendments	Date of report amendment
2.0	Capillary EDTA plasma was included as a new specimen type in addition to venous EDTA plasma. The volumetric sample transfer tool was replaced by non-volumetric sample transfer tool. A finger-stick sample collection set was added as an optional accessory. Correction of the dates when the dossier review and manufacturing site inspection were conducted. Acceptance of prequalification commitment.	29 July 2019

[1] Fomerly called Alere Technologies GmbH.

3.0	1. Change the manufacturer's name and address from the manufacturer name and address from Alere Technologies GmbH, Loebstedter Str. 103-105, Jena 07749, Germany to Abbott Rapid Diagnostics Jena GmbH, Orlaweg 1, D-07743 Jena, Germany. 2. the EU notified body was changed from mdc to TÜV SÜD Product Service GmbH and revision of product labels and IFUs. 3. Issue of new assay software version for m-PIMA HIV-1/2 VL in which cycle threshold (ct) cut-off value was shifted from 40.5 ct to 41.5 ct.	8 October 2020
4.0	Modified primary packaging label (pouch label) with new single-cartridge reference number.	16 November 2022
5.0	The manufacturer removed the limitation to using the test only with patients 18 years or older from the instructions.	22 May 2023

Intended use:

According to the claim of intended use from Abbott Rapid Diagnostics GmbH, "*m-PIMA HIV-1/2 VL test is an in vitro quantitative nucleic acid amplification test designed for the quantification of Human Immunodeficiency Virus (HIV) type 1 groups M/N and O, and HIV type 2 RNA in human plasma specimens from individuals with diagnosed HIV-1 or HIV-2 infection using the Alere q instrument or m-PIMA Analyser for automated specimen processing, amplification and detection. This test is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management of HIV-1 and HIV-2 infected patients.*

This test can be used to assess patient prognosis by measuring the baseline HIV-1 and HIV-2 RNA level or to monitor the effects of antiretroviral therapy by measuring changes in EDTA plasma HIV-1 and HIV-2 RNA levels during the course of antiretroviral treatment.

This test is not intended for use as a screening test for the presence of HIV-1 and HIV-2 in blood or blood products or as a diagnostic test to confirm the presence of HIV-1 and HIV-2 infection.

The m-PIMA HIV-1/2 VL test is intended to be used by trained health care or laboratory professionals or other health care workers receiving appropriate training in the use of the device.

This test may be used in any laboratory and non-laboratory environment that meets the requirements specified in the instruction for use. This test may be used for near-patient testing".

Assay description:

According to the claim of assay description from Abbott Rapid Diagnostics Jena GmbH,

*"**Sample Handling***

Peripheral blood is collected from the patient through venous draw into an EDTA collection tube. As an alternative, capillary blood can be collected from the patient into an EDTA collection tube. Depending on the patient's individual constitution (weight, age, clinical presentation), capillary samples can be collected by finger- or heel-prick. Standard phlebotomy sample collection practices and respective guidelines for obtaining venous or capillary blood samples are to be followed. Venous whole blood samples can be stored for up to 48 hours at ambient temperature (18 - 28 °C). Capillary whole blood samples should be processed as fast as possible (latest within one hour) and tested immediately after plasma generation.
Please refer to the attached IFU for further details about sample transport and stability.

Sample Processing

To generate plasma the EDTA collection tube containing the whole blood sample is centrifuged as per legal manufacturer's instruction. Fifty microlitres (50 µL) of plasma are transferred into the m-PIMA HIV-1/2 VL cartridge. A volumetric pipette or a disposable transfer tool can be used. After applying the sample, the cartridge cap is snapped into place, eliminating the chance of sample spillage or contamination of the instrument. After closing the cap the cartridge is inserted into the m-PIMA Analyser.
The test is initiated automatically. All steps described in the following subsections are also performed automatically by the m-PIMA Analyser within the cartridge.

RNA Isolation

The isolation of RNA consists of the following steps:
1. Complete lysis of the sample based on chaotropic salts in order to release all nucleic acids.
2. Binding (Hybridization) of short nucleic acid molecules (oligonucleotides) complementary to specific sequences of the HIV-1 and HIV-2 genome (capturing). These capture oligonucleotides carry a terminal biotin molecule.
3. Capture oligonucleotides are bound onto the surface of streptavidin-sepharose particles through their biotin molecule which connects to streptavidin. As a consequence HIV RNA linked to a capture oligonucleotide is also bound on the sepharose particles.
4. Washing of the streptavidin-sepharose particles to remove all contaminants that bind non-specifically to the particles, i.e. human nucleic acids, cellular and extracellular proteins, cell membrane fragments and low molecular weight molecules.
After the washing steps, the remaining HIV RNA molecules are ready for reverse

transcription (RT) followed by polymerase chain reaction (PCR).

Reverse Transcription and Amplification

HIV RNA which is captured onto the surface of the streptavidin-sepharose particles cannot be detected directly. HIV-specific nucleic acid sequences first need to be amplified.

This is realized by PCR which allows an in vitro amplification of DNA sequences. Most DNA polymerases do not synthesize DNA directly from RNA. Therefore, a reverse transcription of the HIV RNA into cDNA is necessary. Reverse transcription is an isothermal reaction where DNA primer (short specific oligonucleotides) bind to their complementary sequence of the HIV RNA and form a RNA-DNA hybrid. With the help of the enzyme "reverse transcriptase" the HIV RNA is then transcribed into its complementary DNA by extending the oligonucleotide primer. The reverse transcription is followed by a denaturation step at a defined temperature in order to

- *deactivate the reverse transcriptase*
- *activate the DNA polymerase and*
- *separate the RNA-DNA hybrid to make the newly formed cDNA accessible for primer oligonucleotide binding and cyclic primer extension by PCR.*

Primers bind readily to their complementary sequences at an appropriate temperature (annealing). They form the starting point for extension by a heat stable enzyme called DNA polymerase. Primer annealing and the amplification of DNA (elongation) are carried out at defined temperatures. The three steps (denaturation, annealing and elongation) describe one PCR cycle and are repeated 45 times. To facilitate simultaneous detection of more than one specific nucleic acid sequence a multiplex PCR is performed.

Target amplification between HIV-1 group M/N and group O and HIV-2 is facilitated by specific primer pairs. In addition, the primer pairs allow for the amplification of internal process controls.

Detection

The detection of PCR product is based on Competitive Reporter Monitored Amplification. This technology utilizes an array of immobilized oligonucleotide probes and complementary fluorescently labeled reporter oligonucleotides in solution. In order to maximize initial signal intensity, reporters used in this reaction are labeled with fluorescence dyes on both ends. Under suitable conditions the reporters will specifically bind to the immobilized probes. The reporter oligonucleotides are also complementary to a specific sequence of a target amplicon that is generated during PCR. Amplicons are competing with the immobilized probes for binding of the reporter oligonucleotides. At the onset of the amplification reaction, none or a few target molecules are present. The reporter is therefore free to bind to its complementary probe on the array. In the presence of target template more target amplicons with a reporter specific binding site are synthesized as the amplification reaction proceeds. The more amplicons are synthesized the more reporters bind to them. In addition, the solid support to which the oligonucleotide probes are attached introduces a diffusion barrier. This significantly reduces the binding rate. In general, solution phase reactions are

kinetically favoured to solid phase reactions.
As a consequence, the amount of reporters binding to complementary probes on the solid support decreases proportionally to the formation of new amplicons. This decrease is observed until a plateau in the amplification reaction is reached. The change in signal intensity of each probe can be measured by imaging the fluorescence pattern on the array during the amplification process. Fluorescence images are collected during the annealing phase of each amplification cycle.
After acquiring the hybridization pattern an algorithm is applied to identify and eliminate different noise signals. The algorithm then calculates the cycle threshold (Ct) values from the resulting amplification kinetics determining the presence and concentration of the analyte.

Test kit contents:

m-PIMA HIV-1/2 VL	**50 x Cartridge Kit (product code 27015-W50)**
Individually pouched m-PIMA HIV-1/2 VL test cartridges	50
Disposable sample transfer tools	60
m-PIMA HIV-1/2 VL cartridge guide	1

Instrumentation:

Item	**Quantity**
Equipment: m-PIMA Analyser (product code 27030R001)	 1

Items not required but available separately:

Item	**Quantity**
USB Printer (product code 27040R007)	1
CONNECT Universal Gateway (product code AC-EU-01)	1
Connectivity Pack IV (product code 260400059)	1
Printer Paper I (product code 26040R009)	10
energy.case 05.24V (product code 5100643)	1

Optional consumables available separately:

Item	Quantity
Finger-Stick Sample Collection Set (product code 270400201)* containing the following items:	
- Safety Lancet	4 x 28
- Alcohol Pad	100
- Plastic Bandage	100
- Nonwoven Swabs	4 x (25 x 2)
- EDTA 300 µl Capillary Blood Collection System	100
- Instructions for Use	1

*Recommended for the collection of capillary whole blood samples. If other collection devices are used, please refer to the specific instructions from the legal manufacturer.

Items required but not provided:

- EDTA whole blood collection tubes
- Centrifuge

Storage:

Store cartridges at 4 - 30 °C. The surrounding temperature may lie outside this range for a limited period of time (i.e. up to 48 accumulated hours at 2 °C and up to 48 accumulated hours at 40 °C). Once removed from their protective pouch, cartridges are stable for up to 10 minutes between 10 - 40 °C and relative humidity between 30 to 85 %.

DO NOT freeze the test cartridges.

Shelf-life upon manufacture:

10.7 months.

Warnings/limitations:

Refer to current version of the manufacturer's instructions for use attached to this public report.

Prioritization for prequalification:

Based on the established eligibility criteria, the m-PIMA HIV-1/2 VL test was given priority for WHO prequalification assessment.

Dossier assessment

Alere Technologies GmbH submitted a product dossier for **m-PIMA HIV-1/2 VL** as per the "*Instructions for compilation of a product dossier*" (PQDx_018 version 3). The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

The manufacturer's responses to the nonconformities found during dossier screening and assessment findings were accepted on 04 March 2019.

Commitments for prequalification:

1. A consumer field evaluation study is to be submitted by 30 April 2019. This commitment was closed.
2. Amendments to the real-time stability report at the 12-, 15-, and 18-month periods are to be provided on 30 April 2019, 30 July 2019, and 30 October 2019, respectively. The studies are to be provided such that the reactivity of all specimens can be understood. The commitment was fulfilled. The issue was closed.
3. The Kenya study report to be submitted by 15 October 2019. The commitment was fulfilled. The issue was closed.

Based on the product dossier screening and assessment findings, the product dossier for the **m-PIMA HIV-1/2 VL** meets WHO prequalification requirements.

Manufacturing site inspection

A comprehensive inspection was performed at the site of manufacture (Alere Technologies GmbH Loebstedter Str. 103-105, Jena, Thuringia, 07749, Germany) of m-PIMA HIV-1/2 VL from 16 to 18 July 2018 as per the "*Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics*" (PQDx_014 version 4). 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 on 29 December 2018.

Based on the site inspection and corrective action plan review, the quality management system for m-PIMA HIV-1/2 VL meets WHO prequalification requirements.

Product performance evaluation

The **m-PIMA HIV-1/2 VL** (product name before rebranding: Alere q HIV-1/2 VL plasma) was evaluated by the NHLS Haematology and HIV Molecular Laboratory, Charlotte Maxeke Johannesburg Academic Hospital on behalf of WHO. The evaluation took place from August 2018 to February 2019. From this evaluation, we drew the following conclusions.

The **m-PIMA HIV-1/2 VL** is an in vitro quantitative nucleic acid amplification test designed for the quantification of Human Immunodeficiency Virus (HIV) type 1 groups M/N and O, and HIV type 2 RNA in human plasma specimens from individuals with diagnosed HIV-1 or HIV-2 infection using the m-PIMA Analyser or Alere q instrument for automated specimen processing, amplification and detection.

It is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management of HIV-1 and HIV-2 infected patients.

This assay requires limited laboratory equipment and can be performed at sites with limited facilities.

A volume of 50 µl of specimen venous or capillary EDTA plasma is required to perform the assay.

Analytical specimens:

The assay's within-run and within-laboratory precision of measurement was verified. In this evaluation the precision of measurement was found to be acceptable and all %CV, calculated using the % CV formula for log-transformed data, were found to be < 35%.

The linearity of the assay was verified in HIV 1 Subtypes A, B, C, D, AG and HIV 2 groups A and B. In this evaluation the linearity for all HIV 1 subtypes and HIV 2 groups were estimated by linear regression over a range of viral loads of 10^3 to 10^6 cp/mL. All slopes were < 0.2 from an ideal value of 1, and R^2 values were all ≥ 0.98.

The limits of detection (LOD) were verified. In this evaluation, the LOD was estimated for HIV-1 to be 314 (CI 95%, 219- 617) cp/mL and for HIV-2 to be 432 (CI 95%, 305 - 867) cp/mL for HIV-2, respectively. These limits of detections are within confidence interval of the claims.

The robustness of the assay was verified, and no carry-over was observed.

Clinical specimens:

In this limited performance evaluation on a panel of 421 specimens (122 not detected, 52 detected <1000 cp/mL and 247 ≥1000 cp/mL) using the COBAS AmpliPrep/COBAS TaqMan

HIV-1 Test, v2.0 (Roche Molecular) as the reference method, we observed the following results:

The correlation was found to be within range (R^2 =0.933, P<0.0001).
Bland Altman's analysis showed a bias of -0.2109 log_{10} cp/mL and limits of agreement of -0.6029 log_{10} cp/mL to 0.1810 log_{10} cp/mL compared to the reference standard.

The sensitivity and specificity for the detection of virological failure at a threshold of 1000 cp/mL were estimated at:
Sensitivity (95% CI): 235/247 = 95.1% (95% CI: 91.7; 97.5)

Specificity (95% CI): 173/174 = 99.4% (95% CI: 96,8; 99.99)

In this study, the invalid rate (total number of errors)/ (total number of tests) *100 was 9.2%.

Labelling

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

1. Labels

m-PIMA HIV-1/2 VL primary packaging label

Abbott

m-PIMA™ HIV-1/2 VL

For HIV-1/2 viral load monitoring - Pour la surveillance de la charge virale du VIH-1/2
Para monitoreo de la carga viral de VIH 1/2 - Para monitorização da carga viral do VIH-1/2

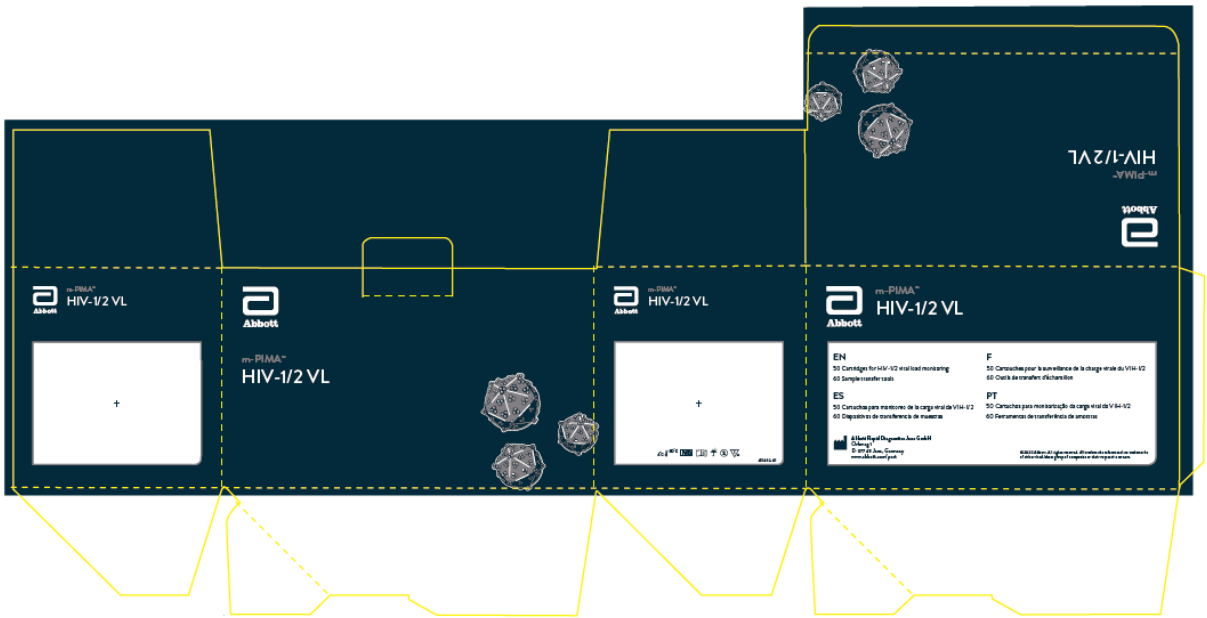
REF 270150001 IVD

Abbott Rapid Diagnostics Jena GmbH
Orlaweg 1
D-07743 Jena, Germany
www.globalpointofcare.abbott

LOT 12345 2022-12-15 4°C 30°C Σ = 1

1234567890 FL 270150001-03

m-PIMA HIV-1/2 VL secondary packaging label



m-PIMA™

HIV-1/2 VL

EN

50 Cartridges for HIV-1/2 viral load monitoring

60 Sample transfer tools

F

50 Cartouches pour la surveillance de la charge virale du VIH-1/2

60 Outils de transfert d'échantillon

ES

50 Cartuchos para monitoreo de la carga viral de VIH-1/2

60 Dispositivos de transferencia de muestras

PT

50 Cartuchos para monitorização da carga viral do VIH-1/2

60 Ferramentas de transferência de amostras

Abbott Rapid Diagnostics Jena GmbH
Orlaweg 1
D-07743 Jena, Germany
www.abbott.com/poct

m-PIMA™

HIV-1/2 VL

4°C -30°C IVD 50

411492-01

m-PIMA™ HIV-1/2 VL

4 051892 000629

REF 27015-W50

2020-12-15

LOT 02564

(241)27015W50(10)02564(17)20201215

BL 27015-W50-03

Finger-Stick Sample Collection Set (optional item) box labels

Abbott

FINGER-STICK SAMPLE COLLECTION SET

EN

Content for 100 blood collections:
Safety-Lancet Super: Lancets 4 x 28 pcs.
Alcohol Pads: Alcohol pads 1 x 100 pcs.
Plastic Bandage: Adhesive plasters 1 x 100 pcs.
Nonwoven Swabs: Nonwoven Swabs 4 x (25x2) pcs.
EDTA 300 µL Capillary Blood Collection System:
Capillary Tubes 1 x 100 pcs.

F

Contenu pour 100 prises de sang:
Safety-Lancet Super: Lancettes 4 x 28 uds
Alcohol Pads: Tampons alcoolisés 1 x 100 uds
Plastic Bandage: Pansement prédécoupés 1 x 100 uds
Nonwoven Swabs: Compresses en non-tissé 4 x (25 x 2) uds
EDTA 300 µL Capillary Blood Collection System:
Tubes capillaires 1 x 100 uds

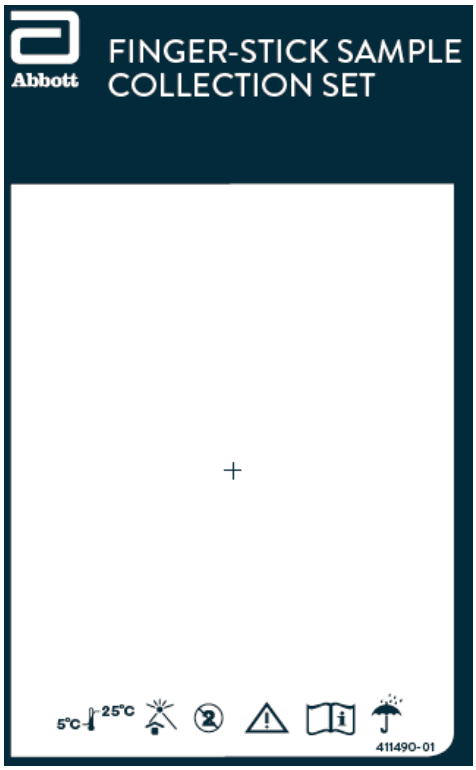
ES

Contenido para obtener 100 muestras de sangre:
Safety-Lancet Super: Lancetas 4 x 28 pièces
Alcohol Pads: Gasas con alcohol 1 x 100 pièces
Plastic Bandage: Tiras adhesivas sanitarias 1 x 100 pièces
Nonwoven Swabs: Compresas no tejidas 4 x (25x2) pièces
EDTA 300 µL Capillary Blood Collection System:
Tubos capilares 1 x 100 pièces

PT

Conteúdo para 100 recolhas de sangue:
Safety-Lancet Super: Lancetas 4 x 28 unidades
Alcohol Pads: Compressas de álcool 1 x 100 unidades
Plastic Bandage: Pensos adesivos 1 x 100 unidades
Nonwoven Swabs: Compressas não tecido 4 x (25x2) unidades
EDTA 300 µL Capillary Blood Collection System:
Tubos capilares 1 x 100 unidades

Abbott Rapid Diagnostics Jena GmbH
Orlaweg 1
D-07743 Jena, Germany
www.abbott.com/poct



Finger-Stick Sample Collection Set

4 051892 000759

REF 270400201

yyyy-mm-dd

LOT 00000xxxxx

(241)270400201(10)00000xxxxx(17)yyyymmdd

BL 270400201-02

2. Instructions for use[2]

[2] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

Abbott

m-PIMA™ HIV-1/2 VL

CARTRIDGE GUIDE

EN

IVD

TABLE OF CONTENTS

INTRODUCTION

Human Immunodeficiency Virus (HIV) is the virus that causes HIV infection. The most advanced stage of an HIV infection is the Acquired Immunodeficiency Syndrome (AIDS).[1-3] HIV is spread through contact with blood, semen, pre-seminal fluid, rectal fluids, vaginal fluids or breast milk from an HIV positive person.[4] Within three to six weeks after infection with HIV, individuals generally develop a brief acute syndrome which is accompanied by flu-like symptoms like fever, rash and chills for several weeks. This early phase is associated with high levels of viremia in the peripheral blood.[5-8] Usually four to six weeks after the onset of symptoms an HIV-specific immune response follows with a decline of plasma viremia.[9, 10] After this seroconversion, infected individuals enter a clinically stable, asymptomatic phase that can last for many years.[11-13] This period is characterized by persistent, low level plasma viremia [14] in which high rates of virus production and infection of CD4+ cells are balanced by equally high rates of virus clearance, death of infected cells and replenishment of CD4+ cells.[15-17] Though the levels of both plasma viremia and CD4+ cells are relatively stable, without antiretroviral therapy (ART) the CD4+ T lymphocytes are gradually depleted leading to severe immunodeficiency, multiple opportunistic infections, malignancies and death.[18]

HIV-2 is generally less pathogenic than HIV-1 with a longer asymptomatic stage, lower plasma viral loads, slower decline in CD4 count, lower mortality rate due to AIDS, lower rates of mother to child transmission and lower rates of genital shedding and sexual transmission.[19-28] However, a significant proportion of HIV-2-infected individuals progress to AIDS and may benefit from ART.[29, 30] In areas where HIV-2 and HIV-1 co-circulate a substantial number of patients are dually-infected with both HIV types.[31-33]

The correct differentiation between HIV-1 and HIV-2 infection is critical for diagnosis, ART and medical management of HIV-infected individuals.[34] Quantitative measurement of HIV levels in peripheral blood has greatly contributed to the understanding of the pathogenesis of HIV infection [16, 17] and has been shown to be an essential parameter in prognosis and management of HIV infected individuals.[35–40] Decisions regarding initiation or changes in antiretroviral therapy are guided by monitoring plasma HIV RNA levels (viral load), CD4+ T cell count, and the patient's clinical condition.[40, 41] The goal of antiretroviral therapy is to reduce the HIV virus in plasma to below detectable levels of available viral load tests.[40, 42]

HIV RNA levels in plasma can be quantitated by nucleic acid amplification or signal amplification technologies.[43-45]

Current methods for distinguishing between HIV-1 and HIV-2 rely on differential immunoassays with varying sensitivity and specificity.[46, 47]

The m-PIMA™ HIV-1/2 VL test is a point-of-care (POC) nucleic acid test (NAT) which directly quantifies viral RNA in human EDTA plasma samples and differentiates HIV-1 and HIV-2 using Polymerase Chain Reaction (PCR) technology with homogenous real-time fluorescence detection.

Intended Use

The m-PIMA™ HIV-1/2 VL test is an in vitro quantitative nucleic acid amplification test designed for the quantification of Human Immunodeficiency Virus (HIV) type 1 groups M/N and O, and HIV type 2 RNA in human plasma specimens from individuals with diagnosed HIV-1 or HIV-2 infection using the m-PIMA™ Analyser for automated specimen processing, amplification and detection. This test is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management of HIV-1 and HIV-2 infected patients.

This test can be used to assess patient prognosis by measuring the baseline HIV-1 and HIV-2 RNA level or to monitor the effects of antiretroviral therapy by measuring changes in EDTA plasma HIV-1 and HIV-2 RNA levels during the course of antiretroviral treatment. This test is not intended for use as a screening test for the presence of HIV-1 and HIV-2 in blood or blood products or as a diagnostic test to confirm the presence of HIV-1 and HIV-2 infection. The m-PIMA™ HIV-1/2 VL test is intended to be used by trained health care or laboratory professionals or other health care workers receiving appropriate training in the use of the device. This test may be used in any laboratory and non-laboratory environment that meets the requirements specified in the instruction for use.

This test may be used for near-patient testing.

Order Information and Scope of Delivery

m-PIMA™ HIV-1/2 VL 50x Cartridge Kit (catalogue no. 27015-W50):

- 50 individually pouched test cartridges
- 60 disposable sample transfer tools
- 1 m-PIMA™ HIV-1/2 VL Cartridge Guide

Store m-PIMA™ HIV-1/2 VL cartridges at 4 - 30 °C. Refer to page 15 for further details.

Materials Required but Not Provided

- m-PIMA™ Analyser (catalogue no. 27030R001) with installed m-PIMA™ software version 0.26.1 or higher.
- EDTA whole blood collection tubes
- centrifuge

Optional Items

- Finger-Stick Sample Collection Set (catalogue no. 270400201)*

  **Recommended for the collection of capillary whole blood samples. If other collection devices are used, please refer to the specific instructions from the legal manufacturer.*

TEST PRINCIPLE

Sample Handling and Storage

Peripheral blood is collected from the patient through venous draw into an EDTA collection tube. As an alternative specimen, capillary blood is collected from the patient's finger into an EDTA collection tube. Standard phlebotomy sample collection practices and respective guidelines [50, 61] for obtaining venous or capillary blood samples are to be followed. Venous whole blood samples can be stored for up to 48 hours at ambient temperature (18 - 28 °C). Capillary whole blood samples should be processed as fast as possible (latest within one hour) and tested immediately after plasma generation.
Please refer to page 25 for further details about sample transport and stability.

Sample Processing

To generate plasma the EDTA collection tube containing the whole blood sample is centrifuged as per legal manufacturer's instruction. Fifty microlitres (50 µL) of plasma are transferred into the m-PIMA™ HIV-1/2 VL cartridge. A volumetric pipette or a disposable transfer tool can be used. After applying the sample, the cartridge cap is snapped into place, eliminating the chance of sample spillage or contamination of the instrument. After closing the cap the cartridge is inserted into the m-PIMA™ Analyser.
The test is initiated automatically. All steps described in the following subsections are also performed automatically by the m-PIMA™ Analyser within the cartridge.

RNA Isolation

The isolation of RNA consists of the following steps:

1. Complete lysis of the sample based on chaotropic salts in order to release all nucleic acids.
2. Binding (Hybridization) of short nucleic acid molecules (oligonucleotides) complementary to specific sequences of the HIV-1 and HIV-2 genome (capturing). These capture oligonucleotides carry a terminal biotin molecule.
3. Capture oligonucleotides are bound onto the surface of streptavidin-sepharose particles through their biotin molecule which connects to streptavidin.
   As a consequence HIV RNA linked to a capture oligonucleotide is also bound on the sepharose particles.
4. Washing of the streptavidin-sepharose particles to remove all contaminants that bind non-specifically to the particles, i.e. human nucleic acids, cellular and extracellular proteins, cell membrane fragments and low molecular weight molecules.

After the washing steps, the remaining HIV RNA molecules are ready for reverse transcription (RT) followed by polymerase chain reaction (PCR).

Reverse Transcription and Amplification

HIV RNA which is captured onto the surface of the streptavidin-sepharose particles cannot be detected directly. HIV-specific nucleic acid sequences first need to be amplified. This is realized by PCR which allows an in vitro amplification of DNA sequences. Most DNA polymerases do not synthesize DNA directly from RNA. Therefore a reverse transcription of the HIV RNA into cDNA is necessary. Reverse transcription is an isothermal reaction where DNA primer (short specific oligonucleotides) bind to their complementary sequence of the HIV RNA and form a RNA-DNA hybrid. With the help of the enzyme "reverse transcriptase" the HIV RNA is then transcribed into its complementary cDNA by extending the oligonucleotide primer. The reverse transcription is followed by a denaturation step at a defined temperature in order to

- deactivate the reverse transcriptase
- activate the DNA polymerase and
- separate the RNA-DNA hybrid to make the newly formed cDNA accessible for primer oligonucleotide binding and cyclic primer extension by PCR.

Primers bind readily to their complementary sequences at an appropriate temperature (annealing). They form the starting point for extension by a heat stable enzyme called DNA polymerase. Primer annealing and the amplification of DNA (elongation) are carried out at defined temperatures. The three steps (denaturation, annealing and elongation) describe one PCR cycle and are repeated 45 times. To facilitate simultaneous detection of more than one specific nucleic acid sequence a multiplex PCR is performed. Target amplification between HIV-1 group M/N and group O, and HIV-2 is facilitated by specific primer pairs. In addition the primer pairs allow for the amplification of internal process controls.

Detection

The detection of PCR product is based on Competitive Reporter Monitored Amplification. This technology utilizes an array of immobilized oligonucleotide probes and complementary fluorescently labeled reporter oligonucleotides in solution.[48]
In order to maximize initial signal intensity, reporters used in this reaction are labeled with fluorescence dyes on both ends. Under suitable conditions the reporters will specifically bind to the immobilized probes. The reporter oligonucleotides are also complementary to a specific sequence of a target amplicon that is generated during PCR. Amplicons are competing with the immobilized probes for binding of the reporter oligonucleotides. At the onset of the amplification reaction, none or a few target molecules are present. The reporter is therefore free to bind to its complementary probe on the array. In the presence of target template more target amplicons with a reporter specific binding site are synthesized as the amplification reaction proceeds. The more amplicons are synthesized the more reporters bind to them. In addition, the solid support to which the oligonucleotide probes are attached introduces a diffusion barrier.
This significantly reduces the binding rate. In general, solution phase reactions are kinetically favoured to solid phase reactions.[49]
As a consequence the amount of reporters binding to complementary probes on the solid support decreases proportionally to the formation of new amplicons. This decrease is observed until a plateau in the amplification reaction is reached. The change in signal intensity of each probe can be measured by imaging the fluorescence pattern on the array during the amplification process. Fluorescence images are collected during the annealing phase of each amplification cycle.
After acquiring the hybridization pattern an algorithm is applied to identify and eliminate different noise signals. The algorithm then calculates the cycle threshold (Ct) values from the resulting amplification kinetics determining the presence and concentration of the analyte.

m-PIMA™ HIV-1/2 VL CARTRIDGE FEATURES

Cartridge Components

The m-PIMA™ HIV-1/2 VL test cartridge consists of a black solid cartridge base with an attached cartridge cap that is secured in place after sample collection is completed.
The cartridge is a completely sealed system once the cap is closed. Sample loading can be controlled by the operator via a control window.
The cartridge also consists of several internal compartments. They contain dry reagents and an on board buffer reservoir. The compartments of the cartridge are connected through a micro-fluidic network and air/liquid movement within the cartridge is regulated by the m-PIMA™ Analyser through valves within the cartridge. The RT-PCR reaction takes place within the reactor chamber of the cartridge.
All liquid waste produced during the test is sealed within the cartridge. Air pressure for moving the liquids into the different compartments is applied via a septum. The septum is pricked by a needle connected to the pneumatic module of the m-PIMA™ Analyser. Several built-in safety features prevent template contamination (filter, sealed waste container).
The m-PIMA™ HIV-1/2 VL test cartridge is shown below in figure 1.

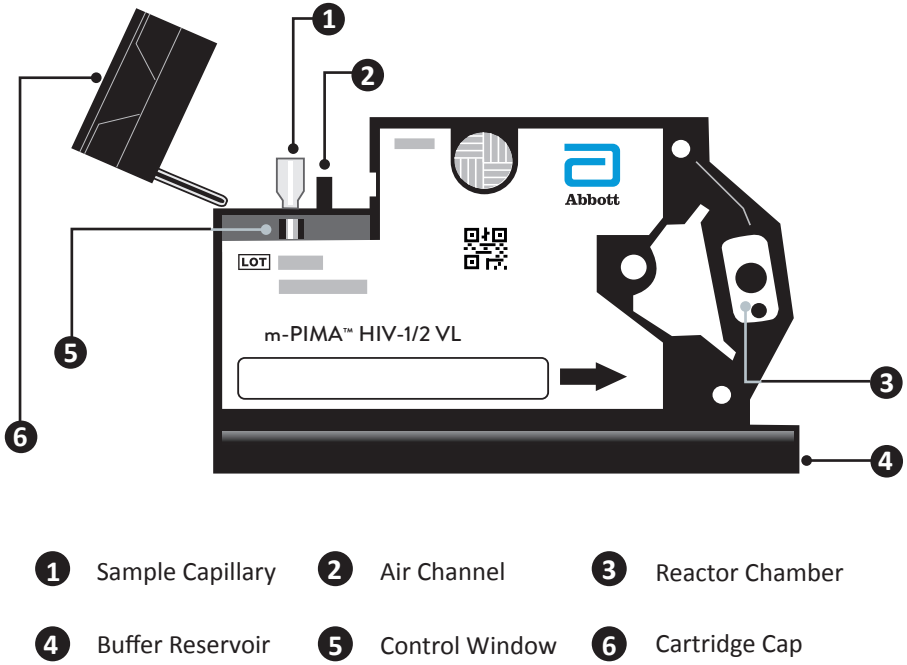


Figure 1: The m-PIMA™ HIV-1/2 VL test cartridge

Quality Control (QC) Features

Data Matrix Code (DMC)

The DMC printed on the cartridge label contains cartridge specific information including cartridge and lot identifier, the expiry date, the assay ID and calibration curve parameters. Upon insertion of the test cartridge the m-PIMA™ Analyser automatically reads the DMC. After successful reading the test will commence. In case of an expired cartridge, an illegible DMC or lack of matching software to perform the encoded assay, an error message will be displayed and the test will not start.

Sample Detection Control

The m-PIMA™ HIV-1/2 VL test requires 50 µL plasma per test run. Samples can be applied using a volumetric pipette or the disposable transfer tools provided within the cartridge kit. The test cartridge contains a sample control window, allowing the operator to control for sample loading at the lower end of the capillary. In order to detect the light-colored plasma samples a dye is placed onto the inner surface of the sample capillary. The dye mixes with the sample upon contact and allows the detection of the stained sample at the start of every test run. The m-PIMA™ Analyser checks for the stained sample via the sample control window. If no sample is detected the analysis will not start and an error message will be displayed.

Assay Process Controls

Each test cartridge has built-in process controls ensuring for proper function of the assay.

- Internal process controls for HIV-1 and HIV-2:
  These controls run together with the patient sample through all assay processing steps. They enable the detection of potential failures during lysis, RNA isolation, capturing, PCR and detection. Sequences of these positive controls are designed to hybridize to the same capture oligonucleotides and primers as the respective targets. They will be distinguished during detection by specific reporters and probes on the micro array.
- Positive hybridization control (detects if hybridization conditions are out of range):
  This control is made of probes on the array that are complementary to specific reporters in solution. It does not interfere with PCR-primers in the RT-PCR mix and therefore has to produce a valid signal above a defined threshold and not produce a Ct value.
- Negative hybridization control (detects non-specific hybridization):
  This control is made of probes on the micro array not being complementary to any reporter. The hybridization signal for this control has to be lower than a defined threshold.

The m-PIMA™ HIV-1/2 VL also considers multiple QC parameters to ensure proper function of the m-PIMA™ Analyser and consistency of raw data for data analysis.

Reagents

	% w/w
Cartridge Capillary (coated with)	
Brilliant Black	
Lysis Reagent – Lysis Chamber (solid particles, embedded in cartridge)	
Guanidin-HCl	89.82
$Na_2EDTA*2H_2O$	
$Na_4EDTA*4H_2O$	1.19
Kollidon VA64 fine	
Lactose	
Mg-Stearate	
Antifoam BC 2527	
N-Lauroylsacrosine	0.92
Proteinase K Reagent (solid particles, embedded in cartridge)	
Proteinase K	0.20
Advantose 100	
Kollidon CL-SF	
Mg-Stearate	
COST-Reagent Pellet (solid particles, embedded in cartridge)	
Tris EDTA Buffer	
Oligonucleotides	
Artificial virus containing RNA	
Cavasol	
$MgCl_2$	
BSA, acetylated	
Trizma crystals pH 7.0	
NaCl	
TRT-Reagent Pellet (solid particles, embedded in cartridge)	
Trizma Preset Crystals	
Anti-Taq-antibody	
Taq polymerase	
dNTP (dATP, dCTP, dGTP, dTTP) mix	
Reverse Transcriptase	
Cavasol	
Arginine	
Glycerol	

	% w/w
PR-Reagent Pellet (solid particles, embedded in cartridge)	
Tris EDTA Buffer	
Cavasol	
Oligonucleotides	
Streptavidin-Sepharose (solid particles, embedded in cartridge)	
Streptavidin-Sepharose	
Trehalose	
Lysis Reagent – Buffer Chamber (solid particles, embedded in cartridge)	
Guanidin-HCl	93.23
$Na_2EDTA*2H_2O$	
$Na_4EDTA*4H_2O$	1.23
Kollidon VA64 fine	
Lactose	
Mg-Stearate	
	% v/w
Washing Buffer (liquid, sealed in cartridge)	
KCl	
Trizma base	
$MgCl_2$	
Trehalose	
EDTA-solution	
Tween 20	
NaN_3	
Guanidin-HCl	0.364
$Na_4EDTA*4H_2O$	0.005
$Na_2EDTA*2H_2O$	
Triton X-100	0.018
Sulfuric acid	
Micro Array Chip (Epoxisilane functionalized glass chip, embedded in cartridge)	
Oligonucleotides	
Na_2HPO_4	
$Na_2SO_4*10H_2O$	
NaOH	

WARNINGS AND PRECAUTIONS

In Vitro Diagnostic Use

- For *in vitro* diagnostic use.
- m-PIMA™ HIV-1/2 VL cartridges are intended to be used only in connection with the m-PIMA™ Analyser.
- The use of the m-PIMA™ HIV-1/2 VL cartridge is limited to personnel trained in the use of this device.
- Only use EDTA (ethylenediaminetetraacetic acid) plasma with the m-PIMA™ HIV-1/2 VL test. The use of other sample types was not evaluated and is NOT recommended as they may result in inaccurate or invalid results.
- m-PIMA™ HIV-1/2 VL cartridges are for single use only.

Safety Precautions

- Follow proper infection control guidelines for collecting and handling all blood and plasma specimens and related items.
- Always wear powder free gloves when handling or collecting specimens or test cartridges, and change gloves after each sample collection and before handling a new cartridge.
- DO NOT pipette by mouth.
- DO NOT eat, drink, smoke, apply cosmetics or handle contact lenses in areas where samples are handled.
- Clean and disinfect spills of specimens by including the use of a disinfectant such as 1% sodium hypochlorite or other suitable disinfectant.
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state and federal regulations.
- Material Safety Data Sheet for this product is available on request through Technical Support (see page 39).

Handling Precautions

- Only use m-PIMA™ HIV-1/2 VL test cartridges at temperatures of 10 - 40 °C and relative humidity between 30 to 85 %.
- All steps to perform the test need to be compliant with the procedure described in this Package Insert. Appropriate organizational measures need to be taken to prevent patient sample mix-up and to ensure correct assignment of results to corresponding patient samples.

- Cartridges are supplied with the cap attached to the cartridge. DO NOT close the cap until the cartridge is fully loaded with sample since this can lead to an invalid test result.
- DO NOT attempt to re-open a closed cap. Damaged caps can lead to incomplete cartridge closure and an invalid test result.
- DO NOT touch the transparent foil cover of the reactor chamber (see figure 1 (3), page 9). Damaged or soiled covers can lead to an invalid test result.
- DO NOT use cartridges that are damaged, that have become wet or if the foil pouch has been damaged since reagent integrity might be compromised.
- DO NOT introduce air bubbles or blood cells when applying the plasma sample onto the cartridge capillary since this can lead to invalid test results.

Contamination and Inhibition

Amplification technologies such as PCR are sensitive to contaminations by carry-over of product from previous amplification reactions. These PCR products can lead to incorrect test results if either the clinical specimens or the cartridge capillary are contaminated during sample application. The following precautions should be considered to minimize the risks of ribonuclease (RNase) contamination, cross-contamination between samples, and inhibition. Measures to reduce the risk of contamination and inhibition include, but are not limited to the following:

- Wear appropriate personal protective equipment at all times.
- Always wear powder free gloves in the respective size when handling or collecting specimens or test cartridges, and change gloves after each sample collection and before handling a new cartridge (please see pages 19 and 22 for details).
- Always use aerosol barrier pipette tips when using a pipette or use the disposable sample transfer tools contained in the cartridge kit to prevent sample-to-sample contamination. NEVER re-use pipette tips or transfer tools.
- During collection, handling and application of samples, compliance with proper infection control guidelines and the procedures specified in this Package Insert is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of RNases into samples.
- Proper cleaning procedures should always be used when working with RNA.
- The closed cartridge design reduces the risk of amplicon contamination, though it is required to be compliant with the procedures specified in this Package Insert to avoid any contamination from HIV-1 and HIV-2 positive controls and clinical specimens.

Storage Instructions

Store cartridges at 4 - 30 °C. The surrounding temperature may lie outside this range for a limited period of time (i.e. up to 48 accumulated hours at 2 °C and up to 48 accumulated hours at 40 °C). Once removed from their protective pouch, cartridges are stable for up to 10 minutes between 10 - 40 °C and relative humidity between 30 to 85 %.
DO NOT freeze the test cartridges.

Indication of Instability or Deterioration

When a positive or negative control value is out of the expected range, it may indicate deterioration of the assay reagents. Associated test results are invalid and samples must be retested.

m-PIMA™ HIV-1/2 VL TESTING PROCEDURE

In the following Part A, collection of venous EDTA whole blood samples and plasma generation is described. Venous EDTA plasma is the standard specimen to be used with the m-PIMA™ HIV-1/2 VL test. Alternatively, capillary EDTA blood may be collected by finger-stick [61] using the Finger-Stick Sample Collection Set (see page 5) following the therein enclosed instructions. Sufficient capillary EDTA whole blood (approx. 300 µL) needs to be collected to ensure enough plasma is available after centrifugation to apply 50 µL onto the m-PIMA™ HIV-1/2 VL test cartridge.

Part A: Venous Whole Blood Sample Collection [50] and Plasma Generation

A1 Always wear a new pair of gloves in your respective size for every patient or every sample you handle!
Collect blood aseptically by venipuncture into a sterile EDTA blood collection tube suitable for at least 48 hours blood storage. Blood samples collected in plasma gel tubes should typically be processed within 6 hours. Standard phlebotomy sample collection practices for obtaining venous blood samples are to be followed.

A2 Invert collection tube 8 - 10 times to thoroughly mix blood with anticoagulant.

A3 Store at ambient temperature (18 - 28 °C) for not more than 48 hours. See also “Specimen Stability” on page 25.

A4 To generate plasma, invert the blood collection tubes gently 10 - 15 times followed by centrifugation as per EDTA blood tube manufacturer’s instruction (see figure 2 below). After generation of plasma, samples should be tested within 6 hours of storage at 18 - 28 °C or within 26 hours at 2 - 8 °C.

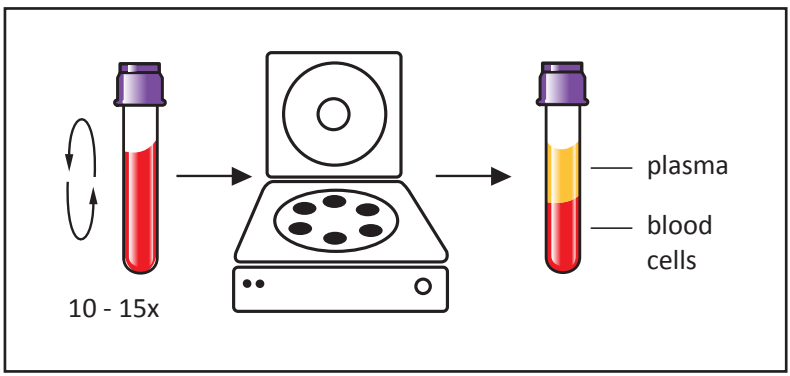


Figure 2: Generation of venous EDTA plasma

Part B: General Process

Please refer to the graphical workflow in part E on the following pages (starting page 20) on how to perform an m-PIMA™ HIV-1/2 VL test run.

B1 Switch on the m-PIMA™ Analyser and wait for the initialization to be completed.
The presence of the «HOME» screen indicates the analyzer is ready for use (image E1).
For detailed information please refer to the m-PIMA™ Analyser User Guide.
Adhere to the instructions described in the following and displayed as graphical workflow, respectively.

B2 Always wear a new pair of gloves for every cartridge!
Remove an m-PIMA™ HIV-1/2 VL cartridge from its pouch and completely flip open the cartridge cap to fully expose the sample capillary.
Only open the foil pouch when ready to load sample onto the cartridge (image E3).

B3 Transfer 50 µL of sample using a volumetric pipette and aerosol barrier pipette tips to avoid sample-to-sample contamination. Alternatively the disposable transfer tool contained in the cartridge kit can be used (see figure 3).
The sample application process is described in Part C on page 18.

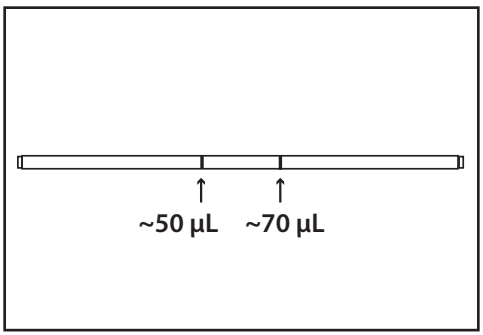


Figure 3: m-PIMA™ HIV-1/2 VL sample transfer tool

Part C: How to apply a plasma sample

C1 Prepare pipette with aerosol barrier tip or take out one transfer tool from its packaging.
To avoid contamination do not touch the far end of the pipette tip/ transfer tool that is finally getting in contact with the sample.

C2 Gently tilt the sample collection tube to support careful insertion of the pipette tip/ transfer tool into the plasma layer (image E4).
DO NOT touch the blood cell pellet.
DO NOT impurify the plasma sample with blood cells.

C3 The transfer tool will fill on its own by capillary force. It must be filled beyond the 50 µL mark (first black mark) close to the second black mark to provide sufficient plasma for correct sample volume application. The correct filling level of the transfer tool is shown in image E5.

C4 Close the upper end of the transfer tool with your finger to avoid any dripping of sample from the capillary.
Remove finger to release the plasma into the cartridge capillary.

C5 While dispensing the sample hold the pipette/ transfer tool in an approx. 45 degree angle to avoid trapping air bubbles in the cartridge capillary (image E6).

C6 The cartridge is correctly loaded when the cartridge capillary is completely full (image E7). Correct filling of the lower end of the capillary can be controlled via the sample control window (image E8).

C7 Discard the pipette tip/ transfer tool as biohazard waste after each sample application.
NEVER re-use pipette tip or transfer tool (image E8).

Part D: How to process and discard m-PIMA™ HIV-1/2 VL cartridges

D1 Once sample loading is completed, close the cartridge by capping the capillary with the cartridge cap (images E9-E11). Do not insert the cartridge into the device before ensuring that the cap is securely in place. Cartridges need to be processed immediately after sample loading.

D2 Press «RUN TEST» on the m-PIMA™ Analyser and insert the test cartridge in the direction indicated by the arrow on the cartridge label (image E12). Follow on-screen instruction and refer to the m-PIMA™ Analyser User Guide for further details on how to proceed with the analysis.
A test run will be completed in less than 70 minutes.

D3 Remove the cartridge when prompted by the m-PIMA™ Analyser (image E13).
Always wear gloves when removing a cartridge from the instrument!
The test result is displayed on the instrument screen.

D4 To discard of used test cartridges, preferably seal them within the gloves (see page 22).
The following example is referring to right-handed users.

- Hold the test cartridge in your left hand. With your right hand, pinch the glove of your left hand at wrist level and pull it down above the cartridge and off your fingers (images E15 and E16). Hold glove with sealed cartridge in your right hand fist.

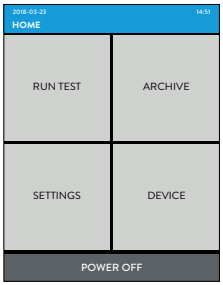
- Insert one or two fingers of your left ungloved hand under the inside rim on the palm side of the right glove; push glove inside out and down onto the fingers and over the left glove and cartridge (image E17).

- Grasp the gloves, which are now together and inside out sealing the cartridge, with your left hand and remove from your right hand (image E18).

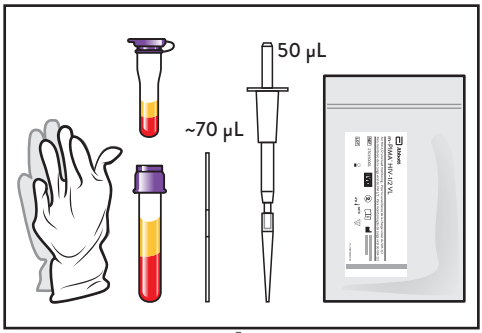
- Discard as biohazard waste (image E18).

Part E: Graphical Workflow

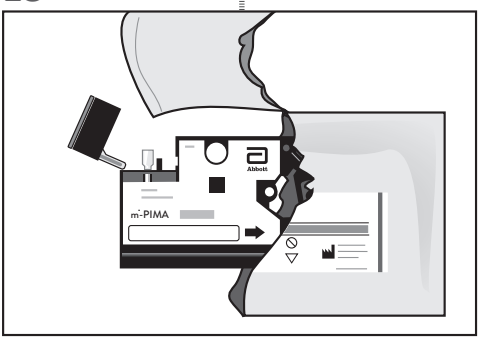
E1



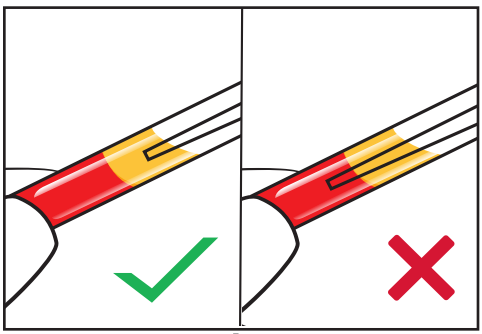
E2



E3

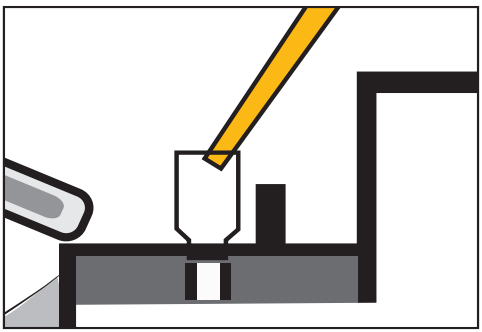


E4

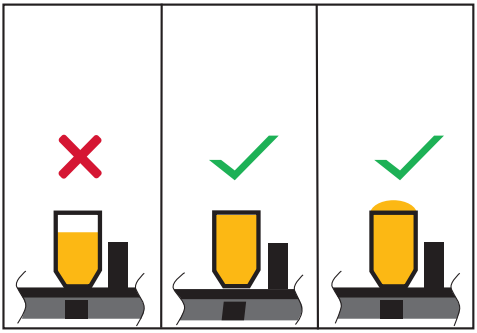


E5

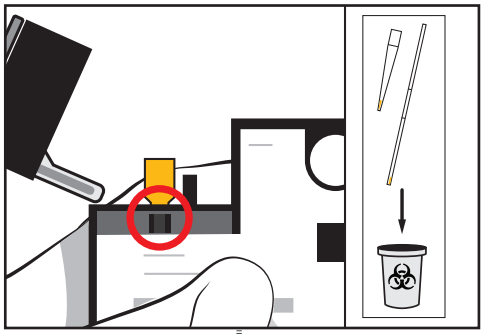
E6



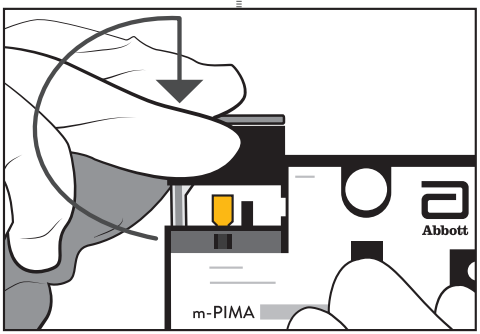
E7



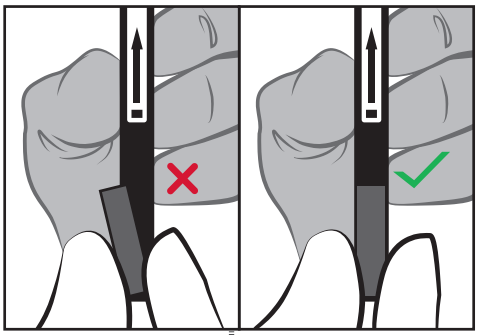
E8



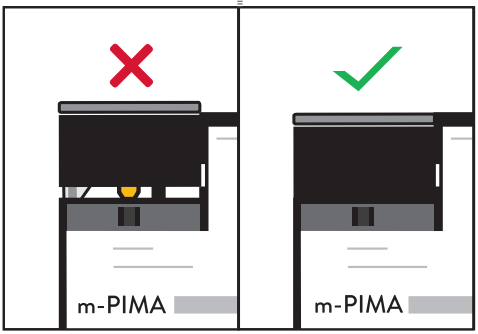
E9



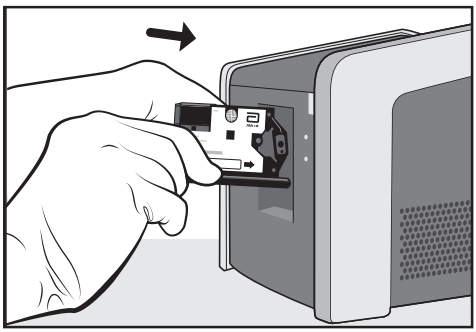
E10



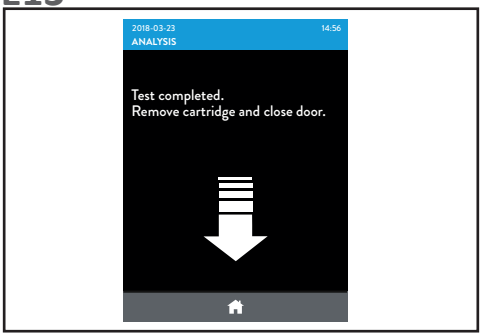
E11



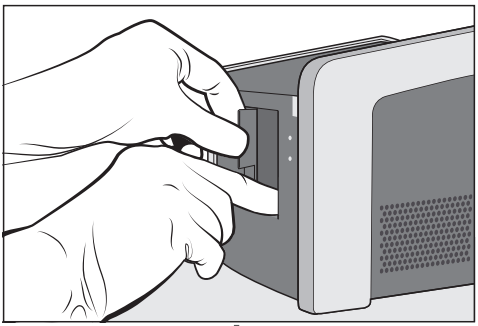
E12



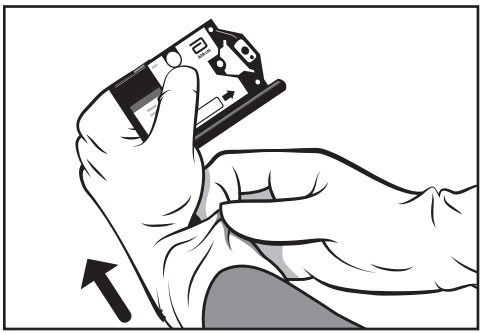
E13



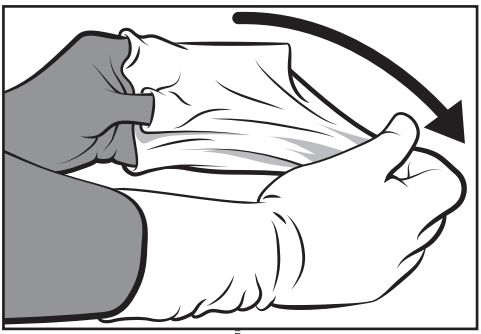
E14



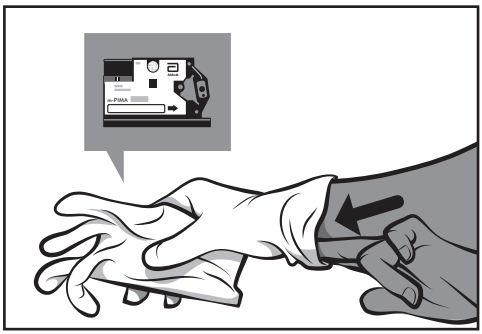
E15



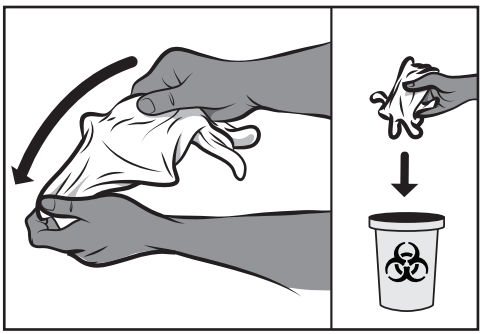
E16



E17



E18



Test Report

Test Reports are stored in the onboard archive of the m-PIMA™ Analyser. Test Reports contain the following information:
Test name, sample ID, quantitative test results for HIV-1 group M/N and group O and HIV-2, result number, date and time of test, cartridge ID (incl. lot information), operator ID, device serial number, software version as well as QC information about instrument, assay process and data analysis. Test results can be either exported onto a USB storage device, transmitted to a remote server using Connectivity Packs or printed using the USB printer available as accessories to the m-PIMA™ Analyser. For respective catalogue numbers please refer to the m-PIMA™ Analyser User Guide.

Test Result

For HIV-1 group M/N, HIV-1 group O and HIV-2 there are independent results given. Possible results are shown below:

Test Report

m-PIMA HIV-1/2 VL

Sample ID	
23-03-2018-ABC	
HIV-1 M/N	**7425 cp/mL**
HIV-1 O	**Undetected**
HIV-2	**Undetected**
Result No.	107
Date / Time	2018-03-23 15:50
Cartridge ID	0123456789
Operator	SAM MILLER
Device Serial	NAT-04000035
Software	0.26.1
QC	
Sample Detection	Pass
Device	Pass
HIV-1 Positive Control	Pass
HIV-2 Positive Control	Pass
Negative Control	Pass
Analysis	Pass

Signature

Test Report

m-PIMA HIV-1/2 VL

Sample ID	
23-03-2018-ABC	
HIV-1 M/N	**< 800 cp/mL**
HIV-1 O	**Undetected**
HIV-2	**Undetected**
Result No.	126
Date / Time	2018-04-20 10:20
Cartridge ID	0123456789
Operator	SAM MILLER
Device Serial	NAT-04000035
Software	0.26.1
QC	
Sample Detection	Pass
Device	Pass
HIV-1 Positive Control	Pass
HIV-2 Positive Control	Pass
Negative Control	Pass
Analysis	Pass

Signature

Test Report

m-PIMA HIV-1/2 VL

Sample ID	
23-03-2018-ABC	
HIV-1 M/N	**> 1000000 cp/mL**
HIV-1 O	**Undetected**
HIV-2	**Undetected**
Result No.	131
Date / Time	2018-05-11 14:40
Cartridge ID	0123456789
Operator	SAM MILLER
Device Serial	NAT-04000035
Software	0.26.1
QC	
Sample Detection	Pass
Device	Pass
HIV-1 Positive Control	Pass
HIV-2 Positive Control	Pass
Negative Control	Pass
Analysis	Pass

Signature

Interpretation of Test Results

Test Result	Interpretation
Undetected	HIV RNA is not detected.
< 800 cp/mL	HIV RNA is detected below the lower limit of quantitation of 800 cp/mL.
7425 cp/mL	HIV RNA is detected at a viral load of 7425 cp/mL (example). Calculated copies are within the measuring range between 800 cp/mL and 1000000 cp/mL.
> 1000000 cp/mL	HIV RNA is detected above the upper limit of quantitation of 1000000 cp/mL.
Error Code	Invalid test result (see m-PIMA™ Analyser User Guide Chapter 9)

If an "Undetected" viral load result is displayed on the test report for all of the three simultaneously measured analytes (HIV-1 M/N, HIV-1 O and HIV-2), then no HIV RNA is detected in the sample.
If for one or more of the simultaneously measured analytes (HIV-1 M/N, HIV-1 O and HIV-2) a quantitative result is displayed on the test report (e.g. 7425 cp/mL, < 800 cp/mL or > 1000000 cp/mL), then an HIV viral load is measured in the sample.

QC Parameters

QC Parameter	Underlying Control
Sample Detection	Control for the presence of sample
Device	Multiple controls for the functionality of PIMA™ Analyser
HIV-1 Positive Control	Internal process control for HIV-1
HIV-2 Positive Control	Internal process control for HIV-2
Negative Control	Control for non-specific hybridization
Analysis	Multiple controls for the analysis process, incl. positive hybridization control

LIMITATIONS

Test Results

- The performance of this test was only validated for EDTA plasma samples derived from venous whole blood and capillary whole blood after finger-stick sample collection. Other sample types were not validated and must not be used as this may lead to incorrect results.
- Specimens "Undetected" for HIV-1 or HIV-2 RNA by the m-PIMA™ HIV-1/2 VL test do not necessarily indicate the absence of an HIV infection in the respective patient. Patients receiving antiretroviral therapy (ART) or preventive therapy (e.g. PrEP, PEP, etc) may have undetectable levels of HIV RNA despite the presence of an HIV infection.
- Results from m-PIMA™ HIV-1/2 VL need to be interpreted in conjunction with other clinical and laboratory findings.
- Detection of HIV-1 and HIV-2 RNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods, transport, storage, processing procedures and patient factors (e.g. presence of symptoms, stage of the infection and viral setpoint).
- Due to inherent differences between technologies, it is recommended for operators to perform method correlation studies in their laboratory to evaluate differences in quantitation accuracy before switching from one technology to another.
- The performance of this test was validated with plasma samples derived from adults $\geq$ 18 years only. Equivalent data for infant plasma samples were not established. Therefore it is NOT recommended to use plasma specimens obtained from patients under the age of 18 for testing with m-PIMA™ HIV-1/2 VL as this may lead to incorrect results.

Specimen Transport

- Transportation of whole blood or plasma must comply with country, federal, state and local regulations for the transport of clinical, diagnostic, or biological specimens.
- Transportation of specimens has to be accomplished within the time period and under temperature conditions mentioned in the following section.

Specimen Stability

- Venous EDTA whole blood may be held at ambient temperature (18 – 28 °C) for up to 48 hours prior to preparing and testing. Venous blood samples collected in plasma gel tubes should typically be processed within 6 hours (see also instructions for use of the respective legal manufacturer).
- Venous plasma specimens may be stored at -80 °C, if storage is required. It is recommended to store specimens in aliquots, e.g. in sterile, 2.0 mL polypropylene tubes.
- Frozen venous plasma samples should be thawed at ambient temperature and, once thawed, tested immediately. It is recommended to invert the thawed sample tubes

10 - 15 times gently before pipetting. Do not vortex.
If cell debris and other solid particles are visible in the thawed venous plasma sample, it is recommended to centrifuge plasma samples in order to sediment the particulates.

Note: Storage of venous blood samples at ambient temperatures for more than 48 hours, or at temperatures exceeding 28 °C, or more than one freeze thaw cycle may negatively impact test performance, especially in samples with viral loads < 800 cp/mL.

- Capillary whole blood samples can be held at ambient temperature (18 – 28 °C) for up to 1 hour. After centrifugation the generated plasma needs to be applied directly onto the m-PIMA™ HIV-1/2 VL test cartridge and run immediately.
- To perform the test with finger-stick plasma collect approx. 300 µL initial capillary EDTA whole blood to ensure sufficient plasma is available after centrifugation. A respective Finger-Stick Sample Collection Set is available and can be ordered (see page 5).

Mutations

In rare cases mutations within the highly conserved regions of the viral genome covered by the m-PIMA™ HIV-1/2 VL primers and/or probes may result in viral under-quantitation or a failure to detect the virus.[51, 52]

Multiplex Testing

Detection and quantitation of multiple analytes has been demonstrated using a sample with equal concentrations of HIV-1 group M, HIV-1 group O, HIV-2 group A. In case of different viral load levels results of the analyte with lower concentration might be impaired.

PERFORMANCE CHARACTERISTICS

Performance characteristics of the m-PIMA™ HIV-1/2 VL test were established by testing at Alere Technologies GmbH in Jena, Germany, Abbott GmbH & Co. KG, Wiesbaden, Germany and at external sites in Germany and Uganda. Samples from African and European cohorts were tested. Due to the limited availability of samples containing HIV-1 group O and HIV-2, the majority of samples included in these studies were positive for HIV-1 group M/N only.
Plasma samples derived from venous and capillary EDTA whole blood collections were used to establish the performance characteristics of the m-PIMA™ HIV-1/2 VL test.
Both sample types showed equivalent performance.

Cohort A: Fresh venous EDTA whole blood was collected from 134 HIV infected individuals at the clinical site Wagagai Health Care Centre IV (WHC), Entebbe, Uganda. Separated plasma samples were tested at WHC and at Central Public Health Laboratories (CPHL) in Kampala, Uganda. For each plasma sample duplicate HIV-1 viral load data from Abbott RealTi*m*e HIV-1 and the m-PIMA™ HIV-1/2 VL test were available.

Cohort B: Frozen venous EDTA plasma samples from 145 European HIV-1 positive donors (Germany, France, Spain) were tested at Alere Technologies GmbH in Jena, Germany and Abbott GmbH & Co. KG, Wiesbaden, Germany. Sixteen frozen venous EDTA plasma samples were purchased from Biomex (Heidelberg, Germany), 87 from Biomnis (Sample Library, Lyon, France) and 42 from National Institute for Biological Standards and Control (Potters Bar, UK).
HIV-1 viral load data were available for each plasma sample through a certificate of the commercial supplier.

Viral load range (log_{10} cp/mL)	Sources of HIV-1 positive patient samples			Number of samples
	Biomnis	Biomex	NIBSC	
3 - 4	0	3	7	10
4 - 5	12	12	35	59
5 - 6	75	1	0	76
			in total	145

Cohort C: Frozen venous EDTA plasma samples from 107 African HIV-2 infected individuals (Ivory Coast) were provided by two commercial sources, Boca Biolistics, Pompano Beach, USA (n = 80) and BBI Solutions, Cardiff, UK (n = 27). All samples were determined to be reactive for HIV-2 Antibodies and non-reactive for Anti HIV-1, HBsAg and Anti-HCV. For all samples viral load levels were undetermined by the suppliers. Samples were tested in duplicate at Alere Technologies GmbH Jena, Germany and at an external site in Germany.

Cohort D: A total of 302 frozen venous EDTA plasma samples from presumable healthy, HIV-negative European donors (BBI Solutions, Cardiff, UK) were tested at Alere Technologies GmbH. The certificate of analysis from the supplier confirmed that the clinical samples were negative for HIV-1/2 antibodies, HIV-1 NAT, HBV NAT, Hepatitis B Surface antigen, HCV NAT, Hepatitis C virus antibodies and Syphilis.

Cohort E: Fresh venous and finger-stick EDTA whole blood was collected from 54 HIV infected individuals at the clinical site Wagagai Health Care Centre IV (WHC) and the Uganda Virus Research Institute Clinic (UVRI clinic), both in Entebbe, Uganda. Separated capillary plasma samples were tested at WHC and UVRI clinic. Separated venous plasma samples were tested at the Uganda Virus Research Institute laboratory (UVRI lab), Entebbe, Uganda. For each plasma sample HIV-1/2 viral load data from the m-PIMA™ HIV-1/2 VL test were available.

Cohort F: Fresh venous and finger-stick EDTA whole blood was collected from 83 HIV-negative individuals at the clinical sites Wagagai Health Care Centre IV (WHC) and the Uganda Virus Research Institute Clinic (UVRI clinic), both in Entebbe, Uganda. Separated finger-stick plasma samples were tested at WHC and UVRI clinic. Separated venous plasma samples were tested at the Central Public Health Laboratories (CPHL) in Kampala, Uganda.
For each plasma sample HIV-1 viral load data from Abbott RealTi*m*e HIV-1 and the m-PIMA™ HIV-1/2 VL test were available.

Limit of Detection

The Limit of Detection (LOD) is the number of copies/mL or international units/mL calculated with a Probit regression approach where the true detection rate is 95 %.
The evaluation was performed according to CLSI EP17-A2 [53] and 2009/886/EC guidelines.[54]
Pre-diluted virus preparations of HIV-1 group M (subtype B, strain IIIB), HIV-1 group O (strain MVP5180) and HIV-2 group A (strain NIHZ) of known concentrations were spiked into the EDTA plasma dilution matrix (AcroMetrix™ EDTA Plasma Dilution Matrix, Microgenics Corporation, USA) to generate spiked samples of predefined low viral load levels. To determine the LODs, each analyte was tested in 96 replicates per viral load using four different cartridge production lots and six nominal viral load levels. The results of the LOD analysis for the three analytes HIV-1 group M, HIV-1 group O and HIV-2 group A are summarized in table 1, respective detection rates for all four cartridge production lots are listed in table 2.
The m-PIMA™ HIV-1/2 VL test was designed to achieve a Limit of Detection of 800 cp/mL for each analyte. The conversion factors between virus RNA copies and International Units are 1:1.74 for HIV-1 group M and 1:0.55 for HIV-2 group A.

Note: Due to the lack of a reference material for HIV-1 group O, no conversion into IU is available.

*Table 1: LOD summary for HIV-1 group M, HIV-1 group O and HIV-2 group A**

Analyte	Limit of Detection (95 % Confidence Interval)
HIV-1 group M	342 cp/mL [95 % CI, 279 - 451]
	595 IU/mL [95 % CI, 487 - 785]
HIV-1 group O	228 cp/mL [95 % CI, 187 - 295]
	NA
HIV-2 group A	364 cp/mL [95 % CI, 292 - 484]
	200 IU/mL [95 % CI, 160 - 260]

*Representative data: results in individual laboratories may vary from these data.

Table 2: Detection Rates of the m-PIMA™ HIV-1/2 VL test

Analyte	Concentration [cp/mL]	N	$N_{detected}$	Percent detected [%]
HIV-1 group M	1000	91	91	100
	562	90	89	99
	316	87	81	93
	178	83	70	84
	100	91	49	54
	56	87	34	39
HIV-1 group O	366	94	94	100
	206	90	86	96
	116	88	68	77
	65	87	45	52
	37	92	34	37
	21	91	16	18
HIV-2 group A	506	84	80	95
	285	91	87	96
	160	91	72	79
	90	92	52	57
	51	85	29	34
	28	88	14	16

Linear Range

Linear range evaluations were performed following the CLSI EP06-A guideline [55] using samples of EDTA plasma dilution matrix (AcroMetrix™ EDTA Plasma Dilution Matrix) spiked with pre-diluted virus preparations of HIV-1 group M (subtype B, strain IIIB), HIV-1 group O (strain MVP5180) and HIV-2 group A (strain NIHZ) of known concentrations. The m-PIMA™ HIV-1/2 VL test was designed to achieve a linear range from 1000 cp/mL to 10^6 cp/mL for all three analytes. For the linearity analysis, each analyte was tested in 45 replicates of ten nominal viral load levels ranging from 1000 cp/mL to 10^7 cp/mL and using cartridges from three different cartridge production lots. For the three analytes HIV-1 group M, HIV-1 group O and HIV-2 group A, the m-PIMA™ HIV-1/2 VL test was demonstrated to be linear across the tested range from 1000 cp/mL to 10^7 cp/mL within maximum absolute bias less than or equal to 0.3 $\log_{10}$ cp/mL. Results are shown in figure 4 to figure 6.

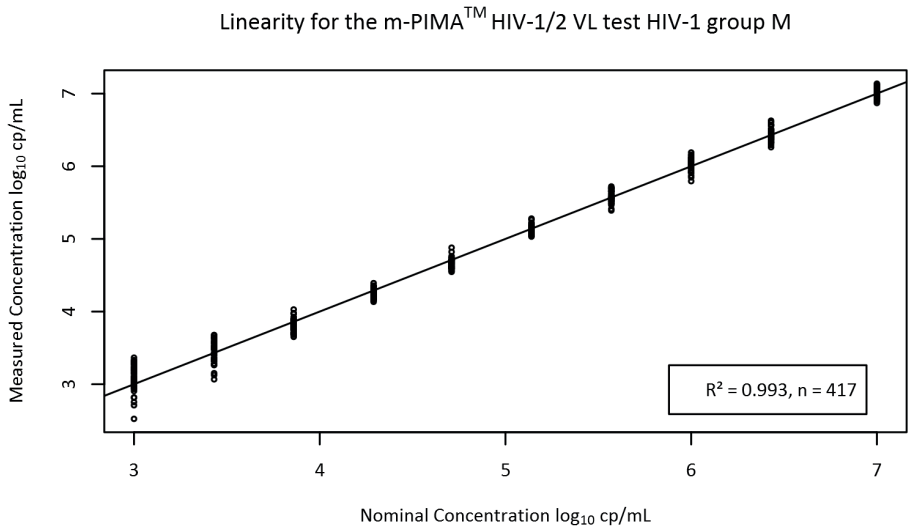


Figure 4: Linearity for HIV-1 group M Data

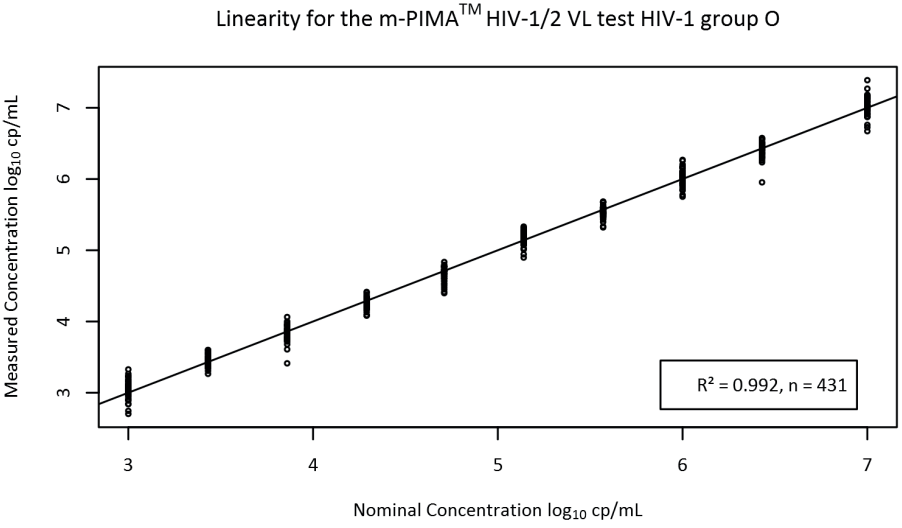


Figure 5: Linearity for HIV-1 group O Data

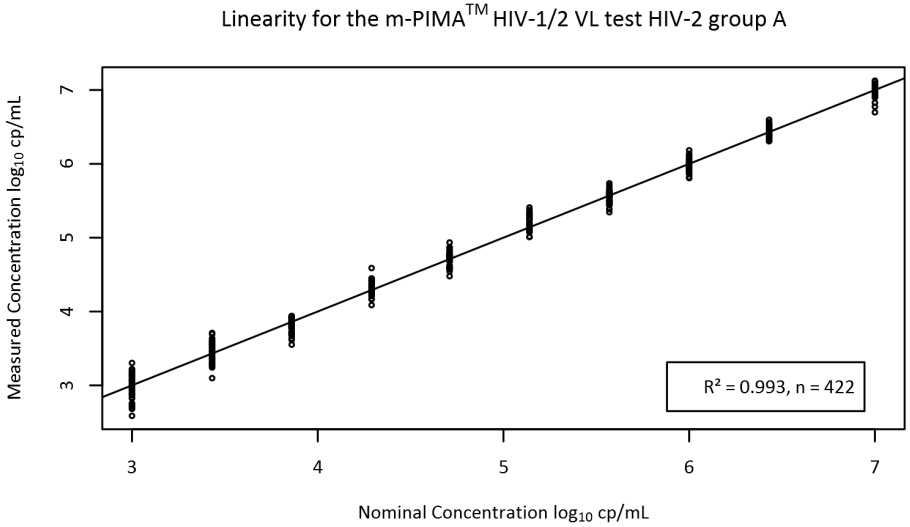


Figure 6: Linearity for HIV-2 group A Data

Measuring Range

For the determination of the measuring range, data from the linearity study were extended by testing replicates at virus concentrations of 800 cp/mL and 1000 cp/mL. Evaluations were performed following CLSI guidelines EP17-A2 [53] and MM06-A2.[56]

Tested samples were prepared by spiking pre-diluted virus preparations of HIV-1 group M (subtype B, strain IIIB), HIV-1 group O (strain MVP5180) and HIV-2 group A (strain NIHZ) of known concentrations into EDTA plasma dilution matrix (AcroMetrix™ EDTA Plasma Dilution Matrix, Microgenics Corporation, USA).

The m-PIMA™ HIV-1/2 VL test was designed to achieve a lower limit of quantitation of 800 cp/mL, and an upper limit of quantitation of 10^6 cp/mL for the three analytes.

The lower limit of quantitation was determined by testing 96 replicates for each analyte at virus concentrations of 800 cp/mL and 1000 cp/mL using cartridges from four different cartridge production lots. The upper limit of quantitation was determined by testing 45 replicates for each analyte at virus concentrations up to 10^7 cp/mL using cartridges from three cartridge production lots. For the three analytes HIV-1 group M, HIV-1 group O and HIV-2 group A, the m-PIMA™ HIV-1/2 VL test was demonstrated to have a measuring range from 800 cp/mL to 10^7 cp/mL within a maximum total error (Westgard model) less than or equal to 0.8 $\log_{10}$ cp/mL.

Precision

Reproducibility, within-laboratory precision and repeatability of the m-PIMA™ HIV-1/2 VL test were evaluated according to CLSI EP05-A3 guideline [57] and 2009/886/EC [54] on Common Technical Specifications for In Vitro Diagnostic Medical Devices.

Pre-diluted virus preparations of HIV-1 group M (subtype B, strain IIIB), HIV-1 group O (strain MVP5180) and HIV-2 group A (strain NIHZ) of known concentrations were spiked into EDTA plasma dilution matrix (AcroMetrix™ EDTA Plasma Dilution Matrix, Microgenics Corporation, USA) to generate spiked samples with virus concentrations of 5000 cp/mL (3.7 $\log_{10}$ cp/mL) and 10^6 cp/mL (6.0 $\log_{10}$ cp/mL).

The m-PIMA™ HIV-1/2 VL test was designed to achieve a reproducibility of less than or equal to 0.25 $\log_{10}$ cp/mL. At each of two sites two laboratory operators performed three days of testing in duplicate with each of three cartridge production lots using six m-PIMA™ Analysers per analyte at 3.7 $\log_{10}$ cp/mL target concentration. Tests with 6.0 $\log_{10}$ cp/mL were conducted at a single site only. Precision was evaluated by using a random effects model with terms for site, cartridge production lot, m-PIMA™ Analyser, operator and day with $\log_{10}$ transformed results. The standard deviation (SD) due to each variance component (site, cartridge production lot, m-PIMA™ Analyser, operator, day, repeatability) and the total variance (reproducibility/within-laboratory) was calculated on the $\log_{10}$ scale along with the lognormal percent coefficient of variation (CV).

Table 3: Precision Estimates at Site 1

Analyte	Concentration [$\log_{10}$ cp/mL]			Variability attributable to Variance Components [SD / lognormal %CV]					Within-Laboratory Precision [SD / lognormal %CV]
	Expected	Observed (Average)	N	Lot	Device	Operator	Day	Repeatability	
HIV-1 group M	3.70	3.65	67	0 / 0	0.03 / 7.3	0.02 / 5.0	0 / 0	0.09 / 21.2	0.10 / 23.0
	6.00	5.98	63	0.01 / 3.4	0.02 / 4.4	0 / 0	0.01 / 3.3	0.05 / 11.9	0.06 / 13.6
HIV-1 group O	3.70	3.61	70	0.05 / 11.5	0.06 / 14.6	0 / 0	0.06 / 14.9	0.08 / 18.6	0.13 / 30.7
	6.00	5.93	68	0.05 / 10.9	0 / 0	0.01 / 1.2	0.01 / 2.7	0.09 / 22.0	0.11 / 24.9
HIV-2 group A	3.70	3.63	68	0.05 / 10.8	0 / 0	0 / 0	0.07 / 15.5	0.10 / 23.5	0.13 / 30.5
	6.00	5.97	69	0.04 / 10.1	0.03 / 7.4	0 / 0	0.02 / 5.0	0.06 / 15.0	0.09 / 20.3

Table 4: Precision Estimates at Site 2

Analyte	Concentration [$\log_{10}$ cp/mL]			Variability attributable to Variance Components [SD / lognormal %CV]					Within-Laboratory Precision [SD / lognormal %CV]
	Expected	Observed (Average)	N	Lot	Device	Operator	Day	Repeatability	
HIV-1 group M	3.70	3.62	56	0.09 / 20.2	0.03 / 6.9	0 / 0	0.01 / 3.2	0.07 / 16.4	0.12 / 27.4
HIV-1 group O	3.70	3.50	65	0.13 / 29.5	0.05 / 10.6	0.02 / 5.2	0 / 0	0.16 / 38.5	0.21 / 51.5
HIV-2 group A	3.70	3.56	65	0.06 / 13.9	0.02 / 5.3	0.02 / 5.6	0 / 0	0.14 / 32.1	0.15 / 36.2

Table 5: Precision Estimates of Multi-Site Analysis

Analyte	Concentration [log_{10} cp/mL]			Variability attributable to Variance Components [SD / lognormal %CV]						Reproducibility [SD / lognormal %CV]
	Expected	Observed (Average)	N	Site	Lot	Device	Operator	Day	Repeatability	
HIV-1 group M	3.70	3.63	123	0.02 / 4.3	0.04 / 10.0	0.02 / 5.3	0.01 / 1.8	0 / 0	0.09 / 20.9	0.10 / 24.4
HIV-1 group O	3.70	3.56	135	0.07 / 15.4	0.09 / 21.3	0.05 / 12.7	0.01 / 3.0	0.04 / 8.1	0.13 / 30.8	0.19 / 44.6
HIV-2 group A	3.70	3.60	133	0.04 / 9.2	0.06 / 13.2	0.01 / 3.3	0.01 / 2.2	0.05 / 10.7	0.12 / 28.0	0.15 / 34.8

Reproducibility and repeatability of the m-PIMA™ HIV-1/2 VL test were furthermore evaluated with non-laboratory operators. Within six days three non-laboratory operators performed duplicate testing using each of two cartridge production lots on three m-PIMA™ Analyser per analyte at 3.7 log_{10} cp/mL and 6.0 log_{10} cp/mL target concentration. Results generated by non-laboratory operators showed similar results as outlined in table 3 and 4.

Precision testing by analysis of 54 different finger-stick plasma samples from cohort E included effects from twelve different m-PIMA™ Analyser. Reproducibility of the m-PIMA™ HIV-1/2 VL test using capillary plasma was 0.06 log_{10} cp/mL.

Genotype/Subtype

The performance of the m-PIMA™ HIV-1/2 VL test with HIV-1 and HIV-2 genotypes/subtypes was evaluated by analysis of cell culture supernatants representing HIV-1 genotypes/subtypes (Group M subtypes A, B, C, D, AE, F, G, AG-GH; Group N; Group O) and HIV-2 genotypes (Group A, Group B).

Nominal concentrations were assigned to dilutions of each cell culture stock material of HIV-1 and HIV-2 genotypes/subtypes in EDTA plasma dilution matrix (AcroMetrix™ EDTA Plasma Dilution Matrix, Microgenics Corporation, USA) using the Abbott RealTi*m*e HIV-1 assay for HIV-1 and a non-approved comparative assay for HIV-2.

Concentrations of 10^4 cp/mL and 10^6 cp/mL were analyzed with 10 replicates per concentration level using one cartridge production lot each. Additional concentrations targeted near the limit of quantitation of 800 cp/mL (2.90 log_{10} cp/mL) were analyzed with 25 replicates using two cartridge production lots.

For all genotypes/subtypes except for HIV-1 group N and HIV-2 group B, the m-PIMA™ HIV-1/2 VL test was evaluated to have a maximum total error (Westgard model) less than or equal to 0.8 log_{10} cp/mL within the tested measuring range from 800 cp/mL to 10^6 cp/mL. Analysis showed a detection rate higher than 95 % for all genotypes/subtypes at 800 cp/mL except for HIV-2 group B as shown in table 6.

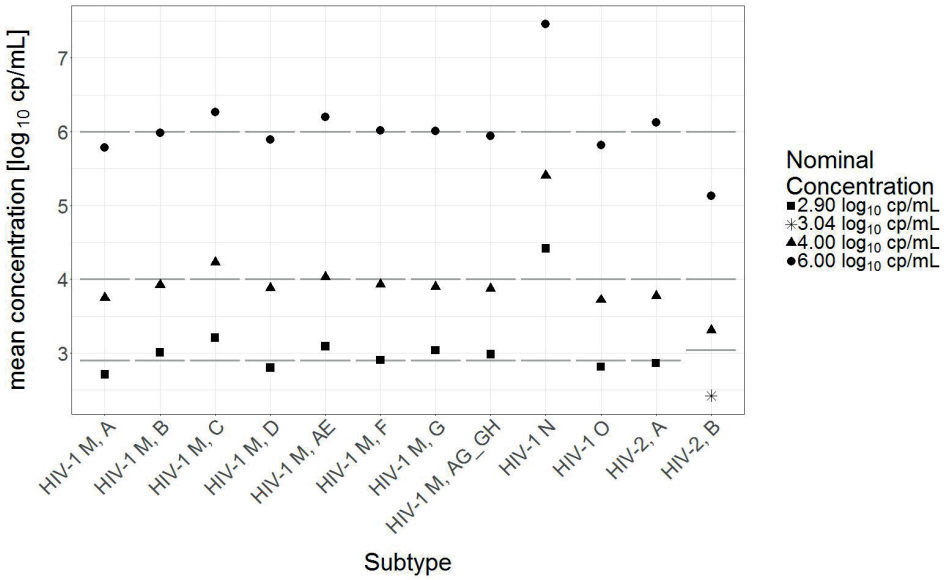


Figure 7: Quantitation of HIV-1 and HIV-2 Genotypes/Subtypes

Table 6: Sensitivity of HIV-1 and HIV-2 Genotypes/Subtypes

Type	Group	Subtype	Isolate	Lowest Concentration [cp/mL] > 95 % Detection Rate	Detection Rate [%]
HIV-1	M	A	92UG037	800	100
		B	92TH014	800	100
		C	98TZ017	800	100
		D	94UG114	800	100
		AE	92TH001	800	100
		F	93BR020	800	100
		G	RU570	800	100
		AG_GH	VI525	800	96
	N	N/A	YBF30	800	100
	O	N/A	MVP5180	800	100
HIV-2	A	N/A	CBL20	800	100
	B	N/A	Seattle	1100	100

Matrix Effects

To evaluate potential effects of sample matrix on the performance of the m-PIMA™ HIV-1/2 VL test samples from HIV infected individuals were tested. The matrix effect was determined using a total of 54 matched pairs of venous plasma and capillary plasma samples from cohort E. For matched pairs of venous plasma and capillary plasma samples Pearson's coefficient of correlation was 0.994, the slope was 1.00 [95 % CI, 0.97 - 1.03] and the offset was -0.035 $\log_{10}$ cp/mL [95 % CI, -0.19 - 0.12].

While only HIV-1 M/N was detected in the samples from this cohort, the results are considered to be representative for all analytes of the m-PIMA™ HIV-1/2 VL test (HIV-1 group M/N, HIV-1 group O and HIV-2).

Diagnostic Specificity

The specificity of the m-PIMA™ HIV-1/2 VL test was determined by analysis of 302 venous plasma samples from HIV-negative blood donors from Cohort D and 83 capillary plasma samples from HIV-negative blood donors from Cohort F using one cartridge production lot each. The presence of HIV-1 group M/N, HIV-1 group O and HIV-2 was analyzed simultaneously.

HIV was not detected in all 302 venous specimens and all 83 capillary specimens, respectively. This equates to 100 % diagnostic specificity for all three analytes and both sample types (one-sided lower 95 % confidence limit is ≥ 99.0 % for venous plasma and ≥ 96.5 % for capillary plasma).

Cross-Reactivity

The following viruses, bacteria and fungi were evaluated for potential cross-reactivity in the m-PIMA™ HIV-1/2 VL test. Patient samples, cultured virus material (Human T-lymphotropic virus type 2) or purified DNA (S. aureus, S. epidermidis), all diluted in EDTA plasma dilution matrix (AcroMetrix™ EDTA Plasma Dilution Matrix, Microgenics Corporation, USA) were tested in HIV-negative as well as HIV-positive samples by adding 10000 cp/mL virus from HIV-1 group M (subtype B, strain IIIB), HIV-1 group O (strain MVP5180) and HIV-2 group A (strain NIHZ).

	Specimen
Viruses	Human T-lymphotropic virus type 1
	Human T-lymphotropic virus type 2
	Herpes simplex virus type 1
	Herpes simplex virus type 2
	Hepatitis B virus
	Hepatitis C virus
Bacteria	*Staphylococcus epidermidis*
	Staphylococcus aureus
Fungi	Candida *albicans*

No interference in the performance of the m-PIMA™ HIV-1/2 VL test was observed in the presence of the potential cross-reactants for all HIV-positive and HIV-negative samples tested. HIV-positive specimen results were within ± 0.3 $\log_{10}$ cp/mL from a HIV-positive control (without cross-reactants).

Interfering Substances

Elevated concentrations of endogenous (table 7) and exogenous substances (table 8) were evaluated for potential interference in the m-PIMA™ HIV-1/2 VL test. Targeted concentrations according to CLSI guideline EP07-A2 [58] of interfering substances were added into EDTA plasma dilution matrix (AcroMetrix™ EDTA Plasma Dilution Matrix, Microgenics Corporation, USA). An additional cohort of 10 plasma samples from pregnant donors (3rd trimester) was also tested. Substances were tested as HIV-negative and as HIV-positive samples by adding 10000 cp/mL virus from HIV-1 group M (subtype B, strain IIIB), HIV-1 group O (strain MVP5180) and HIV-2 group A (strain NIHZ).

Table 7: Endogenous Substances

Endogenous Substance	Concentration tested
Hemoglobin	200 mg/dL
Triglycerides	3300 mg/dL
Bilirubin	20 mg/dL
Albumin	6 g/dL

Table 8: Exogenous Substances

Pool	Exogenous Substance	Concentration tested
1	Tenofovir, Lamivudine, Efavirenz	three times the peak plasma level (c_{max})
2	Tenofovir, Lamivudine, Efavirenz, Emtricitabin, Nevirapine, Zidovudine, Raltegravir, Enfuvirtide	
3	Tenofovir, Lamivudine, Efavirenz, Co-trimoxazole	
4	Zidovudine, Lamivudine, Lopinavir, Ritonavir, Atazanavir	
5	Isoniazid, Rifampicin, Pyrazinamide, Ethambutol, Streptomycin	
6	Peginterferon alfa-2a, Peginterferon alfa-2b, Ribavirin	
7	Ciprofloxacin, Flucytosine, Acyclovir	
8	Fluconazole	
9	Azithromycin	
10	Epirubicin, Idarubicin, Doxorubicin, Daunorubicin, Mitomycin	
N/A	Biotin	1500 ng/mL

No interference in the performance of the m-PIMA™ HIV-1/2 VL test was observed in the presence of the potential interfering substances for all HIV-positive and HIV-negative samples tested. HIV-positive specimen results were within ± 0.3 $\log_{10}$ cp/mL from a HIV-positive control (without interfering substances).

Whole System Failure Rate

The whole system failure rate for the m-PIMA™ HIV-1/2 VL test was determined by testing 111 replicates of HIV-1 group M (subtype B, strain IIIB), 115 replicates of HIV-1 group O (strain MVP5180) and 108 replicates of HIV-2 group A (strain NIHZ).
The analytes were spiked into HIV negative EDTA plasma matrix (AcroMetrix™ EDTA Plasma Dilution Matrix, Microgenics Corporation, USA) to a virus concentration of three times the respective limit of detection. Tests were conducted using two cartridge production lots. There were no false negative tests, resulting in a whole system failure rate of 0 % for all three analytes.

Method Correlation

HIV-1 RNA quantitation

HIV-1 RNA quantitation was compared between the m-PIMA™ HIV-1/2 VL test and the Abbott RealTi*m*e HIV-1 assay according to CLSI EP09-A3 guideline [60] and 2009/886/EC [54] on Common Technical Specifications for In Vitro Diagnostic Medical Devices. In total, 134 samples of Cohort A were tested in Uganda and 145 samples of Cohort B were tested in Germany, each with both assays in duplicates. Of the 279 samples, 161 samples had two replicates within the measuring range of both assays. Applying extreme studentized deviation algorithm three samples were identified as outliers. The data from the remaining 158 samples were analyzed using the Passing-Bablok linear regression method (figure 8). Pearson's coefficient of correlation was 0.933, the slope was 0.96 [95 % CI , 0.91 - 1.02] and the offset was -0.06 $\log_{10}$ cp/mL [95 % CI, -0.39 - 0.21].

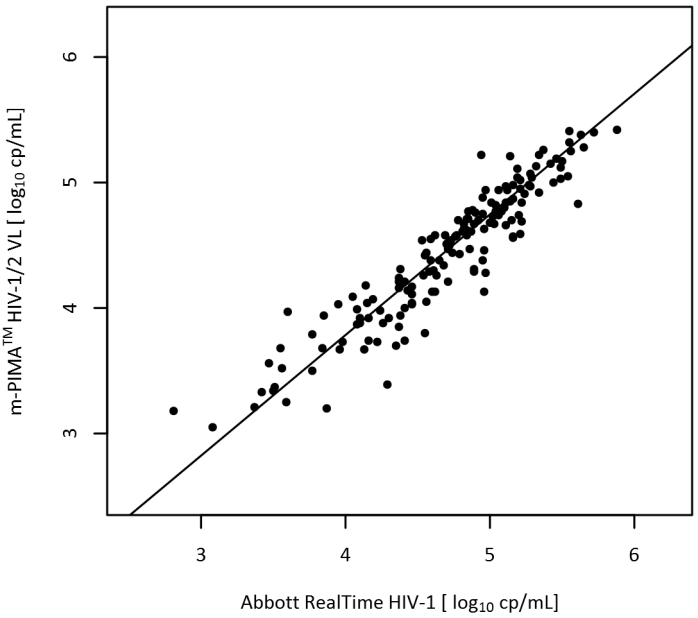


Figure 8: Method Correlation HIV-1

HIV-2 RNA quantitation

HIV-2 RNA quantitation was compared between the m-PIMA™ HIV-1/2 VL test and a quantitative, non-commercial, non-approved HIV-2 assay of the Institute of Clinical and Molecular Virology, Erlangen, Germany.[59]

In total, 107 patient samples of Cohort C and 11 diluted cell culture supernatants were tested in duplicate with both assays. Of the 118 samples, 22 samples had two replicates within the measuring range of both assays. This data were analyzed using the Passing-Bablok linear regression method. Pearson's coefficient of correlation was 0.925, the slope was 1.02 [95 % CI, 0.82 - 1.31] and the offset was -0.58 $\log_{10}$ cp/mL [95 % CI, -2.02 - 0.41].

TECHNICAL SUPPORT

For Technical Support please contact your local distributor or call the respective number for your region:

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EXPLANATION OF SYMBOLS

In vitro diagnostic medical device

Catalog Number

Batch Code

Use by YYYY-MM-DD

Contains Sufficient For < n > Tests

Temperature Limitation

Consult Instructions For Use

Do Not Reuse

Manufacturer

To Keep Dry

Attention Symbol.
Indicates special problems or important information.
Read the accompanying text carefully.

Abbott

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Orlaweg 1
D-07743 Jena, Germany
www.abbott.com/poct

Printed on 100% recycled paper.

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