WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: m-PIMA HIV-1/2 Detect
WHO reference number: PQDx 0226-032-00

m-PIMA HIV-1/2 Detect assay with product code **27011R050**, manufactured by **Abbott Rapid Diagnostics Jena GmbH** [1], **CE marked version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 13 June 2016.

Summary of WHO Prequalification Assessment for m-PIMA HIV-1/2 Detect assay

	Date	Outcome
PQ listing	13 June 2016	listed
Dossier review	18 December 2015	MR
Site inspection(s) of the quality management system	27-28 April 2023	MR
Laboratory evaluation of performance and operational characteristics	14 August 2015 to 11 May 2016	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 WHO has been notified and has undertaken a review. Amendments to the report are summarised in the following table, and details of each amendment are provided below.

Version	Summary of amendment	Date of report amendment
3.0	To reflect the fulfilment of an outstanding commitment to WHO prequalification. The commitment was on the instructions for use to be clear as per WHO's requirement.	2 September 2016.
4.0	Rebranding of products Alere q HIV-1/2 Detect (test cartridges and accessory products), and Alere q analyser to m-PIMA HIV-1/2 Detect and m-PIMA Analyser, and to update the products codes of the rebranded products and to reflect the latest version of the IFU—minor editing of the performance evaluation part.	30 July 2019.

[1] Manufacturer name changed from Alere Technologies GmbH to Abbott Rapid Diagnostics Jena GmbH.

5.0	1. An additional kit insert containing further clarification (incl. pictures) on the correct interpretation of test results is to be included in all Alere q / m-PIMA HIV-1/2 Detect test kits. 2. Software and IFU change to modify the test report layout. A summary line is to be inserted indicating the overall test result and the individual results for HIV-1 M/N, HIV-1 O, and HIV-2. 3. Change in the release QC procedure comprising modified analyte concentrations, new sampling plans for sensitivity and invalid test rate, and removing test group 3.	12 March 2020.
6.0	The manufacturer changed its name and address from Alere Technologies GmbH, Löbstedter Strasse 103-105, 07749 Jena, Germany, to Abbott Rapid Diagnostics Jena GmbH, Orlaweg 1, D-07743 Jena, Germany. The EU-notified body was changed from mdc to TÜV SÜD Product Service GmbH. Revision of product labels and IFUs.	13 July 2020.
7.0	A rest-of-world version of m-PIMA HIV-1/2 Detect was created (catalogue numbers 27011-W01 and 27011-W50) and included in the WHO List of Prequalified In Vitro Diagnostic Products and the CE-marked regulatory variant.	25 November 2021.
8.0	Design change of the m-PIMA HIV-1/2 Detect cartridge and assay software to implement a second target sequence for detection of HIV-1 M as per EU requirement (EU) 2019/1244 amending the Common Technical Specifications, prolongation of the product's shelf life, and new IFU version. Addition of energy.case 05.24V (external battery, product code 5100643).	12 September 2022.
9.0	Changes to the labelling to comply with Regulation (EU) 2017/746 and label updates to include UDI information and new symbols in accordance with ISO 15223-1:2021; new IFU version. Removal of product variant 27011R010.	15 November 2023.
10.0	Minor edits to the public report. 11 December 2023	11 December 2023
11.0	Regulatory change of m-PIMA HIV-1/2 Detect and the m-PIMA Analyser to comply with the Regulation (EU) 2017/746 [IVDR]. This includes changes in product labelling & IFU for both products and an update of the Intended Use and the QC batch release process of m-PIMA HIV-1/2 Detect. Furthermore, the variant 27011R010 of m-PIMA HIV-1/2 Detect will not be manufactured anymore.	9 August 2024
12.0	Edits to the public report to clarify that the PIMA Analyser (product code 27030R001) is not prequalified since it is an instrument. The m-PIMA HIV-1/2 Detect assay with product code 27011R050 is the one that is prequalified.	22 November 2024

Intended use

According to the claim of the intended purpose from Abbott Rapid Diagnostics Jena GmbH, *"The m-PIMA HIV-1/2 Detect test is an automated, qualitative, nucleic acid amplification test for the detection of Human Immunodeficiency Virus (HIV) type 1 groups M/N and O, and type 2 RNA in human whole blood and plasma specimens using the m-PIMA Analyser for specimen processing, amplification and detection.*

The m-PIMA HIV-1/2 Detect test is intended for in vitro diagnostic use by health care professionals in a laboratory environment as well as for near patient testing.
The m-PIMA HIV-1/2 Detect test is intended to be used as an aid in the diagnosis of HIV infection in paediatric and adult individuals, or as a confirmatory assay when samples have already been tested using alternative methods (e.g. serological assays to screen for evidence of HIV infection).
The m-PIMA HIV-1/2 Detect test is not intended to be used as a donor screening test for HIV."

Assay description

Sample Handling and Processing

According to the manufacturer, "*Collect peripheral blood from the patient either through finger or heel prick sampling techniques or venous blood draw. One test run requires a sample volume of 25 µL. Finger or heel prick blood can be applied directly onto the m-PIMA HIV-1/2 Detect cartridge. When using EDTA anti-coagulated venous blood or plasma, transfer the appropriate volume onto the cartridge using a volumetric pipette or transfer capillary. Follow standard phlebotomy sample collection practices for obtaining both capillary and venous blood samples. 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, insert the cartridge into the m-PIMA Analyser. The test is initiated automatically. The m-PIMA Analyser performs the steps described in the following subsections automatically within the cartridge.*

RNA Isolation
The RNA isolation consists of the following steps:

a) *Complete lysis of the sample based on chaotropic salts to release all nucleic acids including cell-associated HIV RNA and HIV RNA from plasma-based particles.*
b) *Hybridization of oligonucleotides complementary to specific sequences of the HIV-1 and HIV-2 genome. These sequence specific capture oligonucleotides carry a 3'-terminal biotin-residue.*
c) *All biotinylated capture oligonucleotides are captured onto the surface of streptavidin-sepharose particles. As a consequence, any HIV RNA bound to a captured oligonucleotide is captured on the sepharose, too.*

d) 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

RNA which is captured onto the surface of the streptavidin-sepharose particles cannot be detected directly. Therefore, an amplification of HIV-specific nucleic acid sequences has to be performed. This is realized by PCR which allows an in vitro amplification of DNA sequences.

Since most DNA polymerases do not synthesize DNA directly from RNA a reverse transcription of RNA into cDNA is necessary. Reverse transcription is an isothermal reaction which is performed at a defined temperature. The same reverse primers as for the subsequent PCR amplification are used for RT. The DNA primers hybridize with their complementary sequence onto the RNA and form a DNA-RNA hybrid. The reverse transcriptase then transcribes the RNA 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.

Primer are short specific oligonucleotides. They hybridize readily to their complementary sequences at the appropriate annealing temperature (annealing) and form the starting point for extension by a heat stable DNA polymerase. Primer annealing and the amplification of the DNA (elongation) by DNA polymerase are carried out at defined temperatures. The three steps (denaturation, annealing and elongation) describe one PCR cycle and are repeated 45 times.

To enable simultaneous detection of more than one specific nucleic acid sequence a multiplex PCR is performed. Target amplification of HIV-1 group M/N and group O, and HIV-2 is facilitated by specific primer pairs. For HIV-1 group M two independent amplification reactions target either the gag upstream region or the pol region of the viral genome (dual target). In addition, the primer pairs allow for the amplification of internal process controls.

Detection
Detection of PCR product is based on Competitive Reporter Monitored Amplification technology. It utilizes an array of immobilized oligonucleotide probes and complementary fluorescently labeled reporter oligonucleotides in solution.

To maximize initial signal intensity, reporters used in this reaction have fluorescence labels at the 5' and the 3' ends. Under suitable conditions the reporter will specifically hybridize to the immobilized probes.

The reporter oligonucleotides are also complementary to a specific sequence of a target amplicon that is generated during PCR. These target amplicons compete 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.
Thus, the reporter is 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.

As amplicons accumulate the hybridization kinetics become more dependent on the amplicon concentration. The more amplicons are synthesized the more reporter bind to them.
In addition, the solid support to which the oligonucleotide probe is attached introduces a diffusion barrier. This significantly reduces the hybridization rate.
In general, solution phase reactions are kinetically favoured to solid phase reactions.

Therefore, the number of reporters hybridized to the complementary probe on the array 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 identifies and eliminates different noise signals from the data obtained. The algorithm then calculates the cycle threshold values from the resulting amplification kinetics determining the presence of the analyte."

Product test kit contents

m-PIMA HIV-1/2 Detect Cartridge Kit	**50 x Cartridge Kit (product code 27011R050)**
Individually pouched test cartridges	50
m-PIMA HIV-1/2 Detect cartridge guide	1

Instrumentation required but not provided:

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

Consumables required but not provided

Item	Quantity
Finger Stick Sample Collection Kit (product code 260400199) Neonatal Sample Collection Kit (product code 270400200) Plastic Capillaries plain (product code 270400005)	100 100 10 x 100

Items not required but available separately

Item	Quantity
USB Printer (product code 27040R007)	1
CONNECT Universal Gateway (product code AC-EU-01/AC-US-01)	1
Connectivity Pack IV (product code 260400059,	1
Energy.case 05.24V (product code 5100643)	1
Printer Paper I (product code 26040R009)	10
Printer Paper II (product code 26040R010)	10

Storage
The test kit should be stored at 4–30 °C.

Shelf-life upon manufacture
13 months.

Warnings/limitations
For warnings and limitations, please refer to the current version of the instructions.

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

Product dossier assessment

Abbott Rapid Diagnostics Jena GmbH[2] submitted a product dossier for m-PIMA HIV-1/2 Detect assay as per the "Instructions for compilation of a product dossier" (PQDx_018 v1). The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

[2] Manufacturer name and address changed from Alere Technologies GmbH to Abbott Rapid Diagnostics Jena GmbH, Orlaweg 1, D-07743 Jena, Germany.

Notwithstanding, certain aspects of the product dossier submitted for stringent regulatory review for CE marking were reviewed by a technical expert during the site inspection.

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

Manufacturing site inspection

An on-site inspection of Abbott Rapid Diagnostics Jena GmbH located at Orlaweg 1, Jena, 7734, Germany was conducted between 27 to 28 April 2023. At the time of considering the product application for Prequalification, the Manufacturer of the product had a well-established quality management system and manufacturing practices in place that would support the manufacture of the product of consistent quality. Routine inspections of the Manufacturer will be conducted with copies of these WHO Public Inspection Reports (WHOPIRs) published on the WHO Prequalification web page as per Resolution WHA57.14 of the World Health Assembly. To note that a WHOPIR reflects the information on the most current inspection performed at a manufacturing site for in vitro diagnostic products and gives a summary of the inspection findings.

Information on the most current inspection can be found at:

https://extranet.who.int/prequal/inspection-services/who-public-inspection-reports-whopirs-vitro-diagnostics

All published WHOPIRs are with the agreement of the manufacturer.

Product Performance Evaluation

m-PIMA HIV-1/2 Detect assay is a qualitative nucleic acid amplification test for the detection of Human Immunodeficiency Virus (HIV) type 1 groups M/N and O and type 2 in human whole blood and plasma. This evaluation was performed on venous whole blood. A volume of 25µl of whole blood/specimen is needed to perform the assay. This type of assay does require laboratory equipment and can be performed in laboratories with limited facilities.

Analytical evaluation
The assay detected the following HIV-1 subtypes: A, B, C, D, F, AE, and AG in whole blood. The total hit rate for 21 replicates (3 for each subtype) study was 100%.

The limit of detection was estimated to be 2937 IU/ml [95% Fiducial limits: 2147 – 6079]; 1758.68 copies/mL [95% Fiducial Limits 1286 – 3640] using the WHO 3rd HIV-1 International Standard. No carry-over was detected.

The repeatability assessment showed a hit rate of 100%, while the reproducibility hit rate was determined to be 100%.

Clinical evaluation

In this limited performance evaluation, on a panel of 301 infant whole blood specimens, we found, in specimens from infants aged less than 18 months, an initial sensitivity of 98.67% (95% CI: 95.27-99.84) and an initial specificity of 100.00% (95% CI: 97.59-100.00) compared to the reference results (Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0) for all specimens tested.

In specimens from individuals older than 15 years of age, we found an initial sensitivity of 90.00% (95% CI: 78.19-96.67) and an initial specificity of 100.00% (95% CI: 93.02-100.00).

The final sensitivity and specificity for infant whole blood specimens were 99.33% (95% CI: 96.34-99.98) and 100.00% (95% CI: 97.59-100.00), respectively.

In specimens from individuals older than 15 years of age, we found a final sensitivity of 94.00% (95% CI: 83.45-98.75) and a final specificity of 100.00% (95% CI: 93.02-100.00).

In this study, the invalid rate was 5.58%.

Performance characteristics in comparison with an agreed reference standard		
	Initial (95% CI)	**Final (95% CI)**
Sensitivity %		
Infant specimens: (N=150)	98.67% (95.27-99.84)	99.33% (96.34-99.98)
Adult specimens: (N=50)	90.00% (78.19-96.67)	94.00% (83.45-98.75)
Specificity %		
Infant specimens: (N=151)	100.00% (97.59-100.00)	100% (97.59-100.0)
Adult specimens: (N=51)	100.00% (93.02-100.00)	100.00% (93.02-100.00)
Invalid rate %	5.58%	

Additional performance characteristics	
Subtype detection	21/21 were correctly classified
Limit of detection using WHO 3rd International Reference Standard	2937 IU/ml [95% Fiducial limits: 2147 – 6079]; 1758.68 copies/mL [95% Fiducial Limits 1286 – 3640]
Carry-over	0%

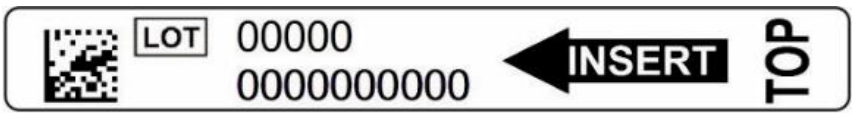
Key operational characteristics	
Validated specimen types	Venous (EDTA) and capillary whole blood. EDTA plasma.
Number of steps	4 steps
Time to result	Less than 60 minutes
Internal QC	Assay Process Controls 1- Internal process controls for HIV-1 and HIV-2 2- Positive hybridisation control 3- Negative hybridisation control
In-use stability of reagents	Reagents are all contained within the cartridge, which is single-use. Filled cartridges need to be processed immediately after sample loading.

Labelling

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

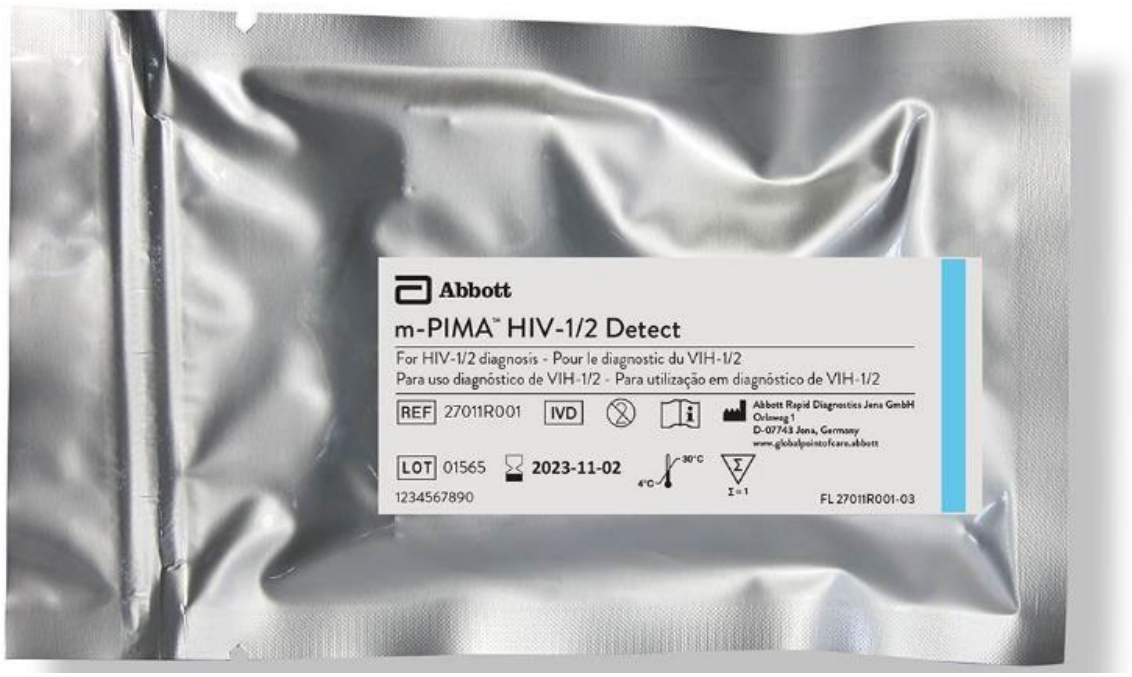
1.0 Labels

1.1. m-PIMA HIV-1/2 Detect Cartridge Top Label

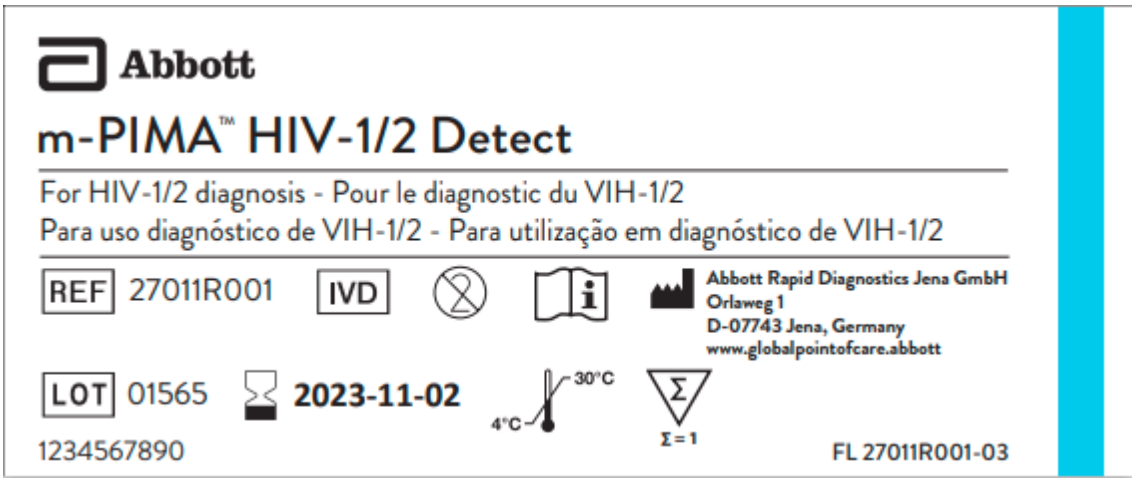


1.2. m-PIMA HIV-1/2 Detect Cartridge Label

1.3. m-PIMA HIV-1/2 Detect Foil Pouch

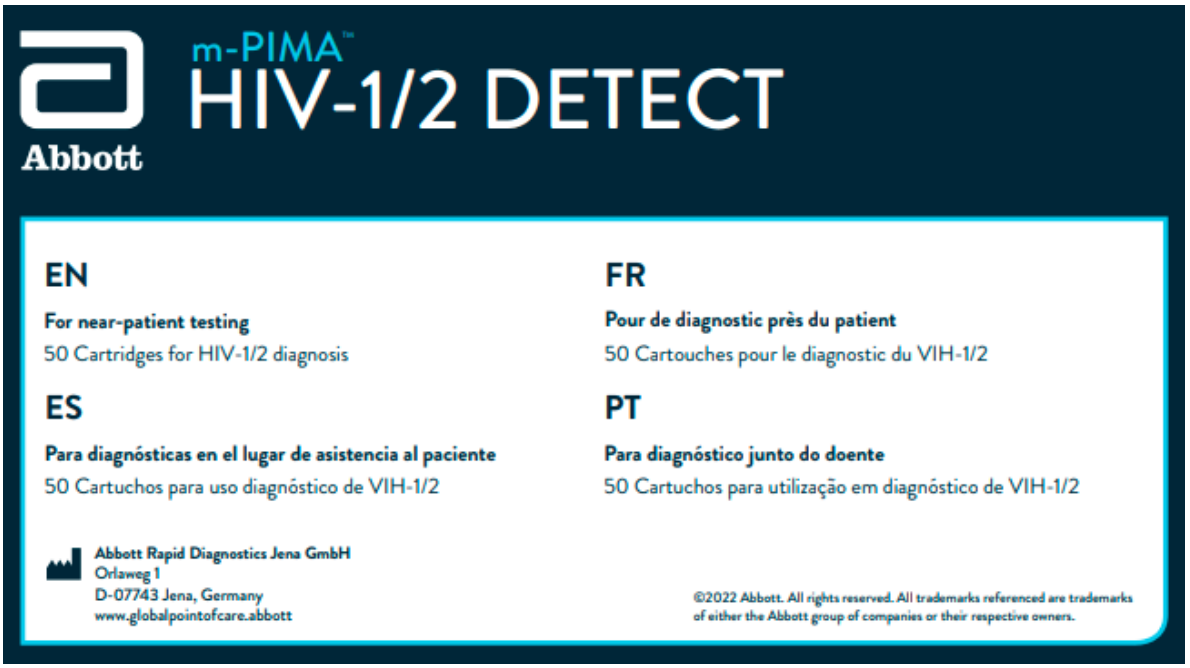


1.4. m-PIMA HIV-1/2 Detect Pouch Label

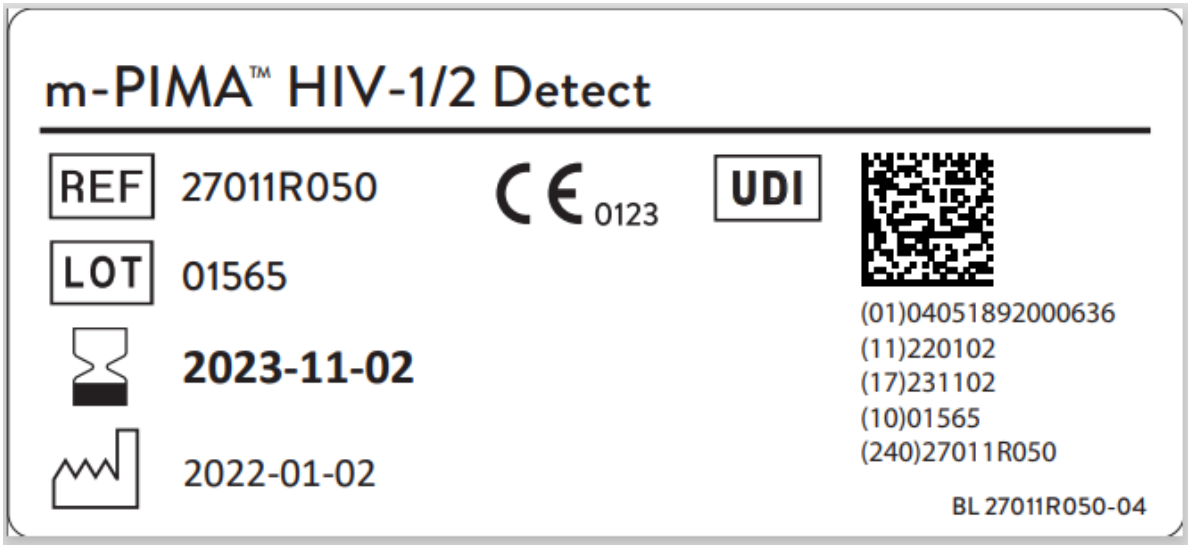


1.5. m-PIMA HIV-1/2 Detect Kit Box

1.6. Labelling details on the m-PIMA HIV-1/2 Detect Kit Box



1.7. Box Label m-PIMA HIV-1/2 Detect Kit Box



2. Instructions for use[3]

[3] 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 DETECT

CARTRIDGE GUIDE

EN

CE 0123 IVD

TABLE OF CONTENTS

INTRODUCTION

Virological testing using assays specifically detecting HIV DNA or RNA plays an important role in the diagnosis of HIV infection in newborns.[1] This is because maternal HIV antibody transmitted through the placenta may persist in the child up to 18 months of age, preventing the use of serological testing to diagnose HIV infection. Early identification of HIV infection in exposed infants and referral for antiretroviral treatment leads to improved outcomes. Virological testing can also play a role in the confirmation of HIV infection in adults who have been tested positive with alternative methods (e.g. anti-HIV antibody/antigen tests). Currently, HIV virological testing is prohibitively expensive and complex, as it requires skilled technicians and regular equipment maintenance. m-PIMA™ HIV-1/2 Detect enables virological testing at the point of care performed by non-laboratory personnel in primary health setting.[2] Besides the conventional sample type, plasma, m-PIMA™ HIV-1/2 Detect is capable of processing whole blood, requiring only small volumes (25 µL) that can be easily obtained by finger or heel prick (for neonates) sampling techniques. Using whole blood as a sample instead of plasma also takes advantage of the well documented fact that HIV-particles adhere to various types of blood cells like platelets [3-6] and monocytes,[7] but also to CD4-/CD8- T cells, probably originating from infected CD4+ T cells.[8] HIV RNA and HIV antigen were also found to be associated with erythrocytes.[9-11] In addition, intracellular HIV RNA is produced within infected lymphocytes circulating in the peripheral blood.[12-20] By using whole blood, cell-associated viral particles are included into the analysis thus providing a higher probability to detect a HIV infection.[21]

Intended Use/ Intended Purpose

The m-PIMA™ HIV-1/2 Detect test is an automated, qualitative, nucleic acid amplification test for the detection of Human Immunodeficiency Virus (HIV) type 1 groups M/N and O, and type 2 RNA in human whole blood and plasma specimens using the m-PIMA™ Analyser for specimen processing, amplification and detection.

The m-PIMA™ HIV-1/2 Detect test is intended for *in vitro* diagnostic use by health care professionals in a laboratory environment as well as for near patient testing.

The m-PIMA™ HIV-1/2 Detect test is intended to be used as an aid in the diagnosis of HIV infection in paediatric and adult individuals, or as a confirmatory assay when samples have already been tested using alternative methods (e.g. serological assays to screen for evidence of HIV infection).

The m-PIMA™ HIV-1/2 Detect test is not intended to be used as a donor screening test for HIV.

Training/ Testing Environment

The m-PIMA™ HIV-1/2 Detect 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. Suitable personnel shall be trained and experienced in related analytical techniques. This includes specimen collection and handling, test device preparation, running of tests and interpretation of results, management of all supplies, waste disposal per local regulations and generation of reports.
This test may be used in any laboratory and non-laboratory environment that meets the requirements specified in the instructions for use. Furthermore, this test may be used for near patient testing, e. g. in hospitals, Voluntary Counselling and Testing (VCT) sites or doctor´s offices.

Order Information and Scope of Delivery

m-PIMA™ HIV-1/2 Detect 50x Cartridge Kit (catalogue no. 27011R050):

- 50 individually pouched test cartridges
- 1 m-PIMA™ HIV-1/2 Detect Cartridge Guide

Store m-PIMA™ HIV-1/2 Detect 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 0.38.0 or higher.
- EDTA whole blood collection tubes for venous/ capillary samples
- centrifuge (for the generation of plasma samples)

Equipment for capillary sample collection:

- sterile safety lancet appropriate for finger stick/ heel prick
- alcohol pads
- gauze pads
- band aids

Please refer to the specific instructions from the legal manufacturer.

❗ Before performing an m-PIMA™ HIV-1/2 Detect test, please refer to these instructions for detailed information on the test procedure.

TEST PRINCIPLE

Sample Handling and Processing

Collect peripheral blood from the patient either through finger or heel prick sampling techniques or venous blood draw (draw as described on pages 18-22).
One test run requires a sample volume of 25 µL.
Finger or heel prick blood can be applied directly onto the m-PIMA™ HIV-1/2 Detect cartridge. When using EDTA anti-coagulated venous blood or plasma, transfer the appropriate volume onto the cartridge using a volumetric pipette or transfer capillary. Follow standard phlebotomy sample collection practices for obtaining both capillary and venous blood samples. 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, insert the cartridge into the m-PIMA™ Analyser. The test is initiated automatically. The m-PIMA™ Analyser performs the steps described in the following subsections automatically within the cartridge.

RNA Isolation

The RNA isolation consists of the following steps:

a Complete lysis of the sample based on chaotropic salts to release all nucleic acids including cell-associated HIV RNA and HIV RNA from plasma-based particles.

b Hybridization of oligonucleotides complementary to specific sequences of the HIV-1 and HIV-2 genome. These sequence specific capture oligonucleotides carry a 3'-terminal biotin-residue.

c All biotinylated capture oligonucleotides are captured onto the surface of streptavidin-sepharose particles. As a consequence, any HIV RNA bound to a captured oligonucleotide is captured on the sepharose, too.

d 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

RNA which is captured onto the surface of the streptavidin-sepharose particles cannot be detected directly. Therefore, an amplification of HIV-specific nucleic acid sequences has to be performed. This is realized by PCR which allows an *in vitro* amplification of DNA sequences.

Since most DNA polymerases do not synthesize DNA directly from RNA a reverse transcription of RNA into cDNA is necessary. Reverse transcription is an isothermal reaction which is performed at a defined temperature. The same reverse primers as for the subsequent PCR amplification are used for RT. The DNA primers hybridize with their complementary sequence onto the RNA and form a DNA-RNA hybrid. The reverse transcriptase then transcribes the RNA 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.

Primer are short specific oligonucleotides. They hybridize readily to their complementary sequences at the appropriate annealing temperature (annealing) and form the starting point for extension by a heat stable DNA polymerase. Primer annealing and the amplification of the DNA (elongation) by DNA polymerase are carried out at defined temperatures. The three steps (denaturation, annealing and elongation) describe one PCR cycle and are repeated 45 times.

To enable simultaneous detection of more than one specific nucleic acid sequence a multiplex PCR is performed. Target amplification of HIV-1 group M/N and group O, and HIV-2 is facilitated by specific primer pairs. For HIV-1 group M two independent amplification reactions target either the *gag* upstream region or the *pol* region of the viral genome (dual target). In addition, the primer pairs allow for the amplification of internal process controls.

Detection

Detection of PCR product is based on Competitive Reporter Monitored Amplification technology. It utilizes an array of immobilized oligonucleotide probes and complementary fluorescently labeled reporter oligonucleotides in solution.[22]

To maximize initial signal intensity, reporters used in this reaction have fluorescence labels at the 5’ and the 3’ ends. Under suitable conditions the reporter will specifically hybridize to the immobilized probes.

The reporter oligonucleotides are also complementary to a specific sequence of a target amplicon that is generated during PCR. These target amplicons compete 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. Thus, the reporter is 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.

As amplicons accumulate the hybridization kinetics become more dependent on the amplicon concentration. The more amplicons are synthesized the more reporter bind to them. In addition, the solid support to which the oligonucleotide probe is attached introduces a diffusion barrier. This significantly reduces the hybridization rate.

In general, solution phase reactions are kinetically favoured to solid phase reactions.[23] Therefore, the number of reporters hybridized to the complementary probe on the array 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 identifies and eliminates different noise signals from the data obtained. The algorithm then calculates the cycle threshold values from the resulting amplification kinetics determining the presence of the analyte.

m-PIMA™ HIV-1/2 DETECT CARTRIDGE FEATURES

Cartridge Components

The m-PIMA™ HIV-1/2 Detect test cartridge consists of a black solid cartridge base, with an attached cartridge cap that is secured in place after sample collection is completed. Sufficient sample loading can be controlled by the operator via the control window.
The cartridge also consists of several internal compartments. They contain dry reagents and an onboard buffer reservoir. The compartments of the cartridge are connected through a micro-fluidic network. Air/liquid movement within the cartridge is regulated by the m-PIMA™ Analyser through in-built valves. Inside the reactor chamber of the cartridge the RT-PCR reaction takes place. All liquid waste produced during the test is sealed within the cartridge.
The cartridge is a completely sealed system once the cap is closed. 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 Detect test cartridge is shown below in figure 1.

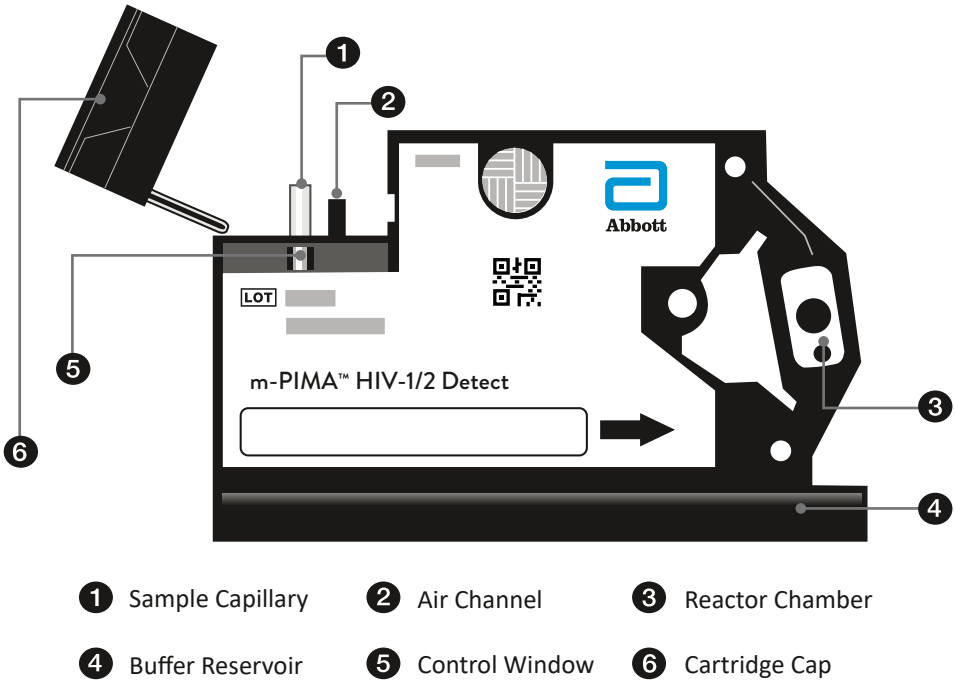


Figure 1: The m-PIMA™ HIV-1/2 Detect test cartridge

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 and the assay ID. Upon insertion of the test cartridge the m-PIMA™ Analyser automatically reads the DMC. After successful reading of the DMC, the test will commence.
In case of an expired cartridge, an illegible DMC or lack of matching software to perform the encoded assay, the m-PIMA™ Analyser displays an error message and the test will not start.

Sample Detection Control

The sample volume processed during a test is defined by the dimensions of the sample capillary. The nominal volume held by the capillary is 25 (± 2) µL. The test cartridge also contains a sample control window, allowing the operator to control for complete and correct sample loading.

At the start of every test run the m-PIMA™ Analyser checks whether sample has been loaded onto the m-PIMA™ HIV-1/2 Detect cartridge via the sample control window.
If insufficient sample has been loaded the analysis will not start, and an error message is displayed.

To also detect the presence of colourless samples such as plasma, a dye is applied to the inner surface of the sample capillary during production. Upon contact the dye stains the sample which thereafter can be detected by the m-PIMA™ Analyser.

Assay Process Controls

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

- Internal process controls for both HIV-1 and HIV-2 are deposited in the lysis chamber of the cartridge.
  These controls run together with the patient sample through all assay processing steps. They enable for 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. During detection they are distinguished by specific reporters and probes on the micro array.

- The positive hybridization control is made of probes on the array that are complementary to specific reporters in solution.
  The positive hybridization control does not interfere with PCR-primers in the RT-PCR mix. Therefore, it has to produce a valid signal above a defined threshold while it must not produce a ct value (detects if hybridization conditions are out of range).

- The negative hybridization control is made of probes on the micro array which are not complementary to any reporter.
  The hybridization signal for this control has to be lower than a defined threshold (detects non-specific hybridization).

The m-PIMA™ HIV-1/2 Detect 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)
Sample Capillary (coated with)	
$K_3EDTA*2H_2O$	65 %
Brilliant Black	
Lysis Mixture (tablet, embedded in cartridge)	
Guanidin-HCl	89.7 %
N-Lauroylsarcosine	0.92 %
$Na_4EDTA*4H_2O$	1.23 %
$Na_2EDTA*2H_2O$	
Antifoam BC 2527	
Proteinase K Tablet (tablet, embedded in cartridge)	
Proteinase K	
Capture-oligonucleotides/internal process controls (on solid support, embedded in cartridge)	
Oligonucleotides with biotin residues	
Tris-HCl	
$Na_2EDTA*2H_2O$	
Tris	
$MgCl_2$	
NaCl	
BSA, acetylated	
artificial virus RNA	
RT-PCR Mixture 1 (pellet, embedded in cartridge)	
anti-Taq-antibody	
Taq polymerase	
Reverse Transcriptase	
Tris-HCl	
dNTP (dATP, dCTP, dGTP, dTTP) mix	
RT-PCR Mixture 2 (pellet, embedded in cartridge)	
Oligonucleotides	
Oligonucleotides with Cy5 residues	
Tris-HCl	
$Na_2EDTA*2H_2O$	
Streptavidin-Sepharose (solid particles, embedded in cartridge)	
Streptavidin-Sepharose	
Washing Mixture (tablet, embedded in cartridge)	
Guanidin-HCl	93.3 %
$Na_4EDTA*4H_2O$	1.2 %
$Na_2EDTA*2H_2O$	
Buffer A (liquid, sealed in cartridge)	
NaN_3	0.04 %
Guanidin-HCl	0.32 %
$Na_4EDTA * 4H_2O$	0.005 %
$Na_2EDTA * 2H_2O$	
Triton X-100	
Tween 20	
KCl	
Tris	
$MgCl_2$	
ultrapure water	

WARNINGS AND PRECAUTIONS

In vitro Diagnostic Use

- For *in vitro* diagnostic use.
- The m-PIMA™ HIV-1/2 Detect test is not intended to be used as a donor screening test for HIV.
- m-PIMA™ HIV-1/2 Detect cartridges are intended to be used only in connection with the m-PIMA™ Analyser instrument.
- The use of the m-PIMA™ HIV-1/2 Detect cartridge is limited to professional use by personnel trained to perform the assay.
- The m-PIMA™ HIV-1/2 Detect cartridges are NOT intended for self-testing.
- m-PIMA™ HIV-1/2 Detect cartridges are for single use only.

Safety Precautions

- Follow proper infection control guidelines for handling all blood specimens and related items.
- Always wear powder free gloves when handling or collecting specimens or test cartridges. Change gloves after each sample collection and before handling a new cartridge (please see pages 16 and 17 for details).
- 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 1.0 % 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.

Handling Precautions

- Only use m-PIMA™ HIV-1/2 Detect test cartridges at 10 - 40 °C and relative humidity below 90 %.
- Only use venous EDTA whole blood or plasma and capillary whole blood (from the finger or heel) with the m-PIMA™ HIV-1/2 Detect test. The use of other sample types was not evaluated and is NOT recommended as they may result in inaccurate or invalid results.
- DO NOT use clotted blood samples since they may lead to invalid or inaccurate test results.
- When using heel prick samples make sure that the pricking site is clean and not contaminated with maternal blood.
- 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. 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.

Contamination and Inhibition

The following precautions should be observed to minimize the risks of RNase contamination, cross-contamination between samples, and inhibition:

- Always wear powder free gloves when handling or collecting specimens or test cartridges. Change gloves after each sample collection and before handling a new cartridge (please see pages 16 and 17 for details).
- When using a volumetric pipette, always use aerosol barrier pipette tips to prevent sample-to-sample contamination. If no aerosol barrier pipette tips are available use single use transfer capillaries. NEVER re-use pipette tips or transfer capillaries.
- During collection, handling and application of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of ribonucleases (RNases) into samples.
- Always use proper aseptic techniques when working with RNA.

- Amplification technologies such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results can occur if either the clinical specimen or sample capillary of the cartridge become contaminated by accidental introduction of even a few molecules of amplification product.

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 93 % relative humidity.
  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 DETECT TESTING PROCEDURE

Part A: Basic Workflow

A1. Switch on the m-PIMA™ Analyser and wait for the initialization to be completed. The presence of the «HOME» screen indicates the analyser is ready for use.

A2. Always wear a new pair of gloves for every cartridge!
Remove a m-PIMA™ HIV-1/2 Detect 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 on the cartridge.

A3. Apply sample onto the m-PIMA™ HIV-1/2 Detect cartridge. Different sample collection and application methods are described in Part C to Part G. Enough sample is applied when the sample capillary is completely filled, and the sample is visible in the control window.

A4. Once sample loading is completed, close the cartridge by capping the capillary with the cartridge cap as shown in the images E11 - E14 on page 21. Do not insert the cartridge into the m-PIMA™ Analyser before ensuring that the cap is securely in place.

A5. Filled cartridges need to be processed directly after sample loading.

A6. Press «RUN TEST» on the m-PIMA™ Analyser. Insert the test cartridge in the direction indicated by the arrow on the cartridge label. Follow on-screen instruction or refer to the m-PIMA™ Analyser User Guide for details on how to proceed with the analysis.
A test run is completed in less than one hour.
Refer to the following Part B on how to discard used test cartridges.

A7. Remove the cartridge when prompted by the m-PIMA™ Analyser (step B1). The test result is displayed on the screen. Always wear gloves when removing a cartridge from the m-PIMA™ Analyser!

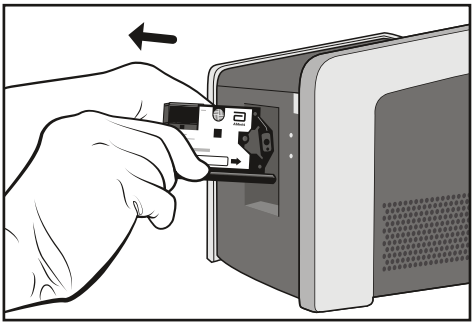
A8. To discard of used test cartridges, preferably seal them within the gloves (the following example is referring to right-handed users):

- 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 over the cartridge and off your fingers (step B2 and B3). Hold glove with wrapped cartridge in your right-hand fist.
- Insert one or two fingers of your left un-gloved hand under the inside rim on the palm side of the right glove. Push glove inside out and down over the fingers and the left-hand glove wrapping the cartridge (step B4).
- Grasp the glove, which is now inside out, with your left hand and remove from your right hand (step B5).
- Discard as biohazard waste (step B6).

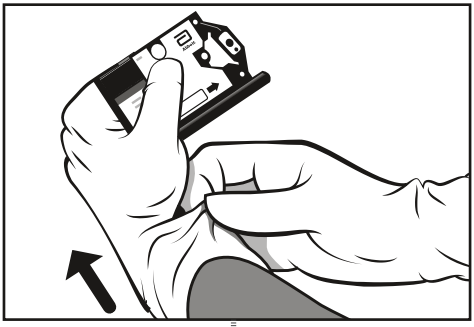
Part B: How to Discard used Test Cartridges

(Please find a detailed description on previous page.)

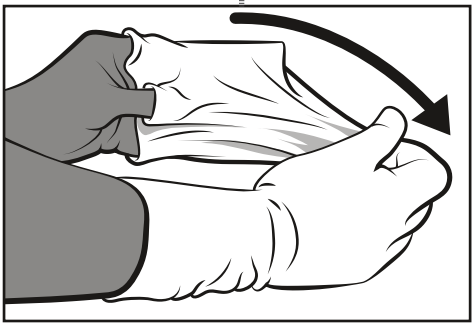
B1



B2

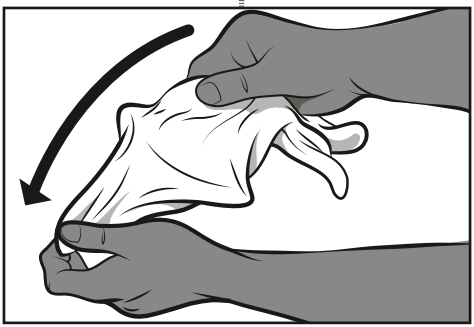


B3

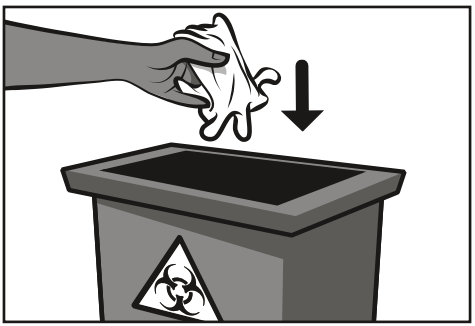


B4

B5



B6



Part C: Finger Prick Sample Collection [26]

C1. Always wear a new pair of gloves for every patient!
Prepare patient for finger prick sample collection. The best locations for finger pricks are the 3rd and 4th fingers. Do not use the tip of the finger or the centre of the finger pad. Avoid the side of the finger where there is less soft tissue, where vessels and nerves are located, and where the bone is closer to the surface. The 2nd (index) finger tends to have thicker, callused skin. The 5th finger tends to have less soft tissue overlying the bone. Avoid puncturing a finger that is cold, cyanotic, swollen, scarred, or covered with a rash. Avoid fingers with rings on.

C2. Warm fingers if needed. Have the patient hold their hand downwards to increase the blood flow to the finger.
Note: The patient should always sit higher than the person performing the finger prick.

C3. Wipe the tip of the appropriately selected finger with an alcohol swab and let the alcohol air dry.

C4. If the cartridge is filled directly from the pricked finger, continue with C5.
If a transfer capillary is used, please refer to the Part G on sample application via transfer capillary (page 22).

C5. Remove one m-PIMA™ HIV-1/2 Detect cartridge from its foil pouch and open the plastic cap to fully expose the sample capillary.

C6. Use a sterile lancet to make a skin puncture just off the centre of the finger pad.
To obtain a representative blood sample constant blood flow is of utmost importance. When using an automated lancet, it is essential to press the lancet firmly onto the finger and maintain contact while ejecting the lancet. Do not squeeze or apply strong repetitive pressure (milking) to the site; this may result in tissue-fluid contamination of the specimen. If necessary, gently massage the finger to ensure a steady blood flow.

C7. Wipe off the first drop of blood with a dry gauze swab. Ensure steady blood flow that generates large enough drops of blood. If necessary, wipe off another drop, until the blood flows freely.

C8. Allow blood to flow freely from the pricked finger directly into the sample capillary by holding the cartridge at a 45-degree angle for sample loading. Wait until the sample capillary is completely filled with blood. Avoid trapping air bubbles in the sample capillary. Enough sample is applied when the sample control window is filled with blood. Then remove the cartridge from the finger and let the patient apply direct pressure to the wound side with a clean dry swab.

C9. Continue with step A4 of the Basic Workflow description in Part A (page 16).

C10. Apply a band aid to the patient's finger.

Part D: Heel Prick Sample Collection [24, 25]

Selection of a site for capillary sampling in a paediatric patient is usually based on the age and weight of the patient. If the child is walking, the child's feet may have calluses that hinder adequate blood flow. Children older than 6 months and a body weight greater than 10 kg may be more suitable for finger prick sample collection.
Please refer to your institutional standard operating procedures in this regard.

D1. It is recommended to comfort the baby. Ask the parent/ caregiver to assist.

D2. Ensure the baby is warm, comfortable and is held in a secure position for taking the sample. Additional warming of the foot is not required.

D3. Always wear a new pair of gloves for every patient! Clean the pricking site of the heel. The heel should be completely dry before taking the sample.

D4. If the cartridge is filled directly from the pricked heel, continue with step D5.
If a transfer capillary is used, please refer to the Part G on sample application via transfer capillary (page 22).

D5. Remove one m-PIMA™ HIV-1/2 Detect cartridge from its foil pouch and open the plastic cap to fully expose the sample capillary.

D6. Use a sterile lancet appropriate for neonates to make a skin puncture.
The external and internal limits of the calcaneus are the preferred pricking sites (hatched areas in image E3 on page 20).
To obtain a representative blood sample constant blood flow is of utmost importance. When using an automated lancet, it is essential to press the lancet firmly onto the heel and maintain contact while ejecting the lancet. Do not squeeze or apply strong repetitive pressure (milking) to the site; this may result in tissue-fluid contamination of the specimen. If necessary, gently massage the heel to ensure a steady blood flow.

D7. Wipe off the first drop of blood with a dry gauze swab. Ensure steady blood flow that generates large enough drops of blood. If necessary, wipe off another drop, until the blood flows freely.

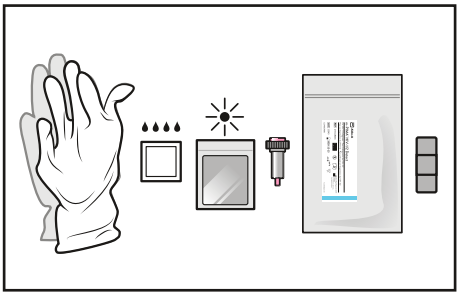
D8. Allow blood to flow freely from the pricked heel directly into the sample capillary by holding the cartridge at a 45-degree angle for sample loading. Wait until the sample capillary is completely filled with blood. Avoid trapping air bubbles in the sample capillary. Enough sample is applied when the sample control window is filled with blood.
Then remove the cartridge from the heel and let the parent/ caregiver apply direct pressure to the wound side with a clean dry swab.

D9. Continue with step A4 of the Basic Workflow description in Part A (page 16).

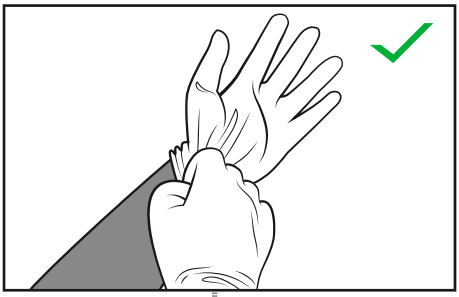
D10. Apply a band aid to the infant's heel.

Part E: Graphical Workflow (example for heel prick sample collection)

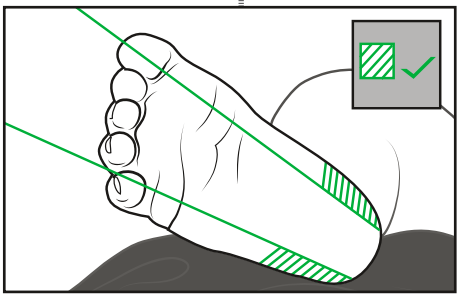
E1



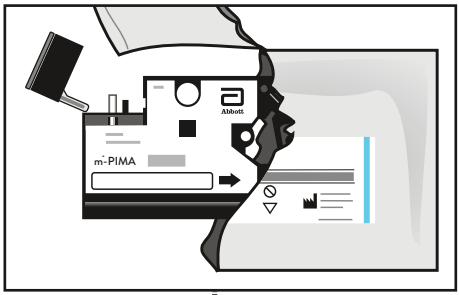
E2



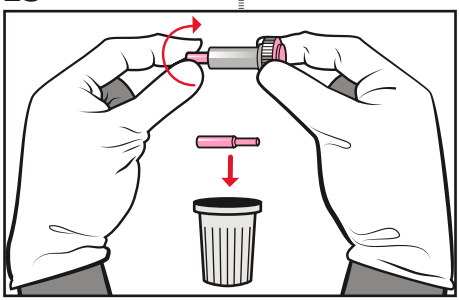
E3



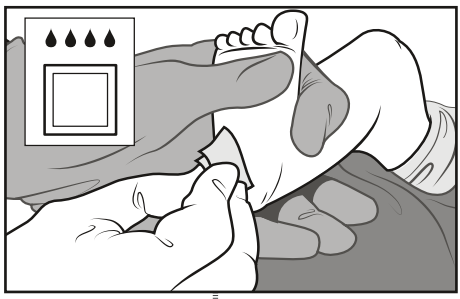
E4



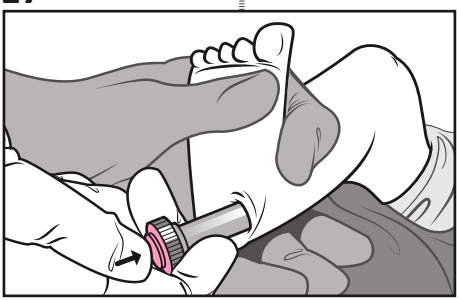
E5



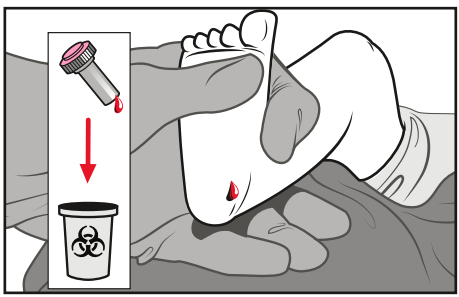
E6



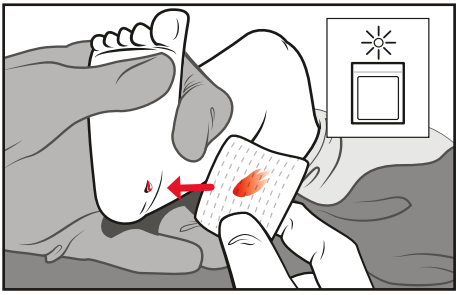
E7



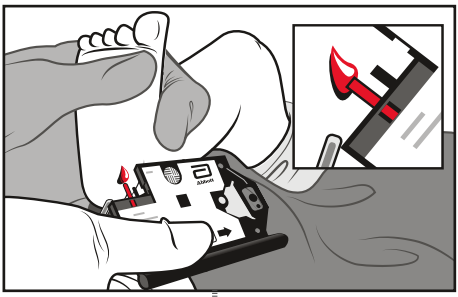
E8



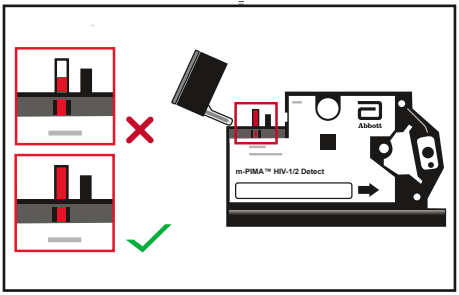
E9



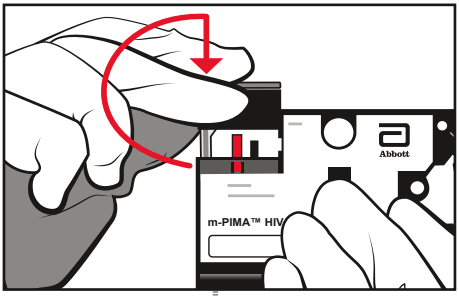
E10



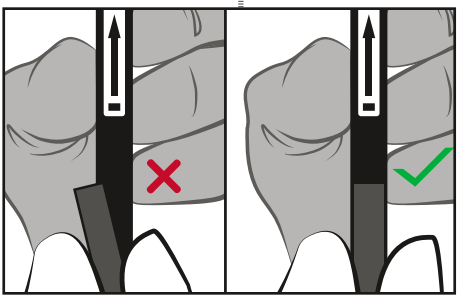
E11



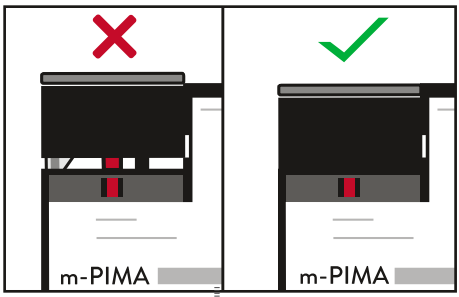
E12



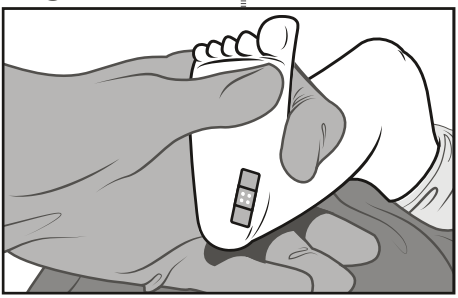
E13



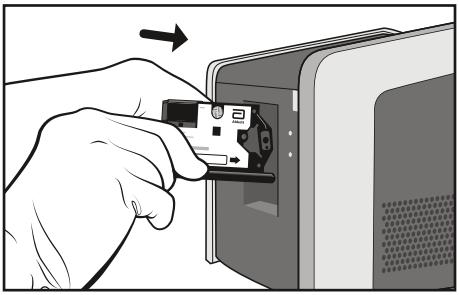
E14



E15



E16



Part F: Venous Whole Blood Sample Collection [27]

F1. Always wear a new pair of gloves for every patient or every sample you handle! Collect blood aseptically by venipuncture into a sterile EDTA (ethylenediaminetetraacetic acid) blood collection tube.

F2. Invert collection tube 8 - 10 times.

F3. Store at ambient temperature (18 - 28 °C). The sample must be analysed within 24 hours of draw. If samples need to be stored for longer periods, please refer to the section on Sample Stability (page 26) for detailed information.

F4. Before using sample for testing, invert collection tube 10 - 15 times to ensure proper sample mixing.

F5. If plasma needs to be tested, collection tubes should be centrifuged according to the tube manufacturer's instructions.

F6. If the cartridge is filled with a volumetric pipette, continue with step F7. If a transfer capillary is used, please refer to Part G on sample application via transfer capillary (see below).

F7. When using a volumetric pipette, always use aerosol barrier pipette tips to avoid sample-to-sample contamination. If no aerosol barrier pipette tips are available use single use transfer capillaries. NEVER re-use pipette tips.

F8. Apply 25 µL into the sample capillary of the m-PIMA™ HIV-1/2 Detect cartridge and continue with step A4 of the Basic Workflow description in Part A (page 16).

Part G: Sample Application via Transfer Capillaries

Transfer Capillaries can be used to apply all sample types compatible with m-PIMA™ HIV-1/2 Detect. For samples containing EDTA, use transfer capillaries without additional anticoagulant. For blood collected directly from a pricked finger or heel, always use transfer capillaries containing EDTA.
Always use a new transfer capillary for every patient!

G1. When collecting whole blood directly from a pricked finger or heel, put one end of the EDTA coated transfer capillary in contact with the blood drop. Hold the transfer capillary almost horizontal. When collecting venous whole blood/plasma, insert a plain transfer capillary into the collection tube and hold both tube and transfer capillary almost horizontal, without spilling the sample.

G2. Allow the transfer capillary to fill without trapping air bubbles.
Stop filling when enough sample is drawn in to apply 25 µL securely and correctly onto the m-PIMA™ HIV-1/2 Detect cartridge.

G3. Close the opposite end of the transfer capillary with your finger to prevent unwanted release of the sample.

G4. Put the open end of the transfer capillary in contact with the sample capillary of the test cartridge and hold the transfer capillary almost vertical. Remove the finger from the other end. The sample capillary will automatically draw blood from the transfer capillary. Observe the descent in fill level of the transfer capillary. It will stop when enough sample is transferred to the cartridge capillary.

G5. Dispose of the transfer capillary as potentially infectious materials in accordance with local, state, and federal regulations and continue with step A4 of the Basic Workflow description in Part A (page 16).

Test Report

Test Reports are stored in the onboard archive of the m-PIMA™ Analyser.
Reports contain the following information:
Test name, sample ID, qualitative test results for HIV-1 M/N and 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 device, assay process and data analysis.
Test results can be either exported onto a USB storage device, transmitted to a remote server using Connectivity Pack IV (Cat. no. 260400059) or the CONNECT Universal Gateway (Cat. no. AC-EU-01/AC-US-01) or printed using the USB Printer (Cat. No. 27040R007) available as additional equipment for the m-PIMA™ Analyser.

Test Result

A qualitative result (detected/ not detected) is provided for analytes HIV-1 (groups M/N and O) and HIV-2.
If for one or more of the simultaneously measured analytes (HIV-1 M/N, HIV-1 O and HIV-2) an “HIV detected (positive)“ result is displayed then HIV RNA is detected and the sample is HIV positive.
If an “HIV not detected” 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 (see examples for printed test reports on page 24).

For displayed Error Codes please refer to the m-PIMA™ Analyser User Guide (Chapter 9).

Test Result Reporting (examples)

Test Report for HIV positive results

Test Report

m-PIMA HIV-1/2 Detect

Sample ID
23-07-2019-ABC

HIV detected (positive)

HIV-1 M/N	Detected
HIV-1 O	Not detected
HIV-2	Not detected
Result No.	107
Date / Time	2022-07-23 15:50
Cartridge ID	0123456789
Operator	SAM MILLER
Device Serial	NAT-04000935
Software	0.38.0

QC

Sample Detection	Pass
Device	Pass
HIV-1 Positive Control	Pass
HIV-2 Positive Control	Pass
Negative Control	Pass
Analysis	Pass

Signature

Test Report readout if no HIV is detected

Test Report

m-PIMA HIV-1/2 Detect

Sample ID
2347-SRT-2019

HIV not detected

HIV-1 M/N	Not detected
HIV-1 O	Not detected
HIV-2	Not detected
Result No.	34
Date / Time	2022-08-12 11:50
Cartridge ID	0123456554
Operator	OP-23
Device Serial	NAT-040001098
Software	0.38.0

QC

Sample Detection	Pass
Device	Pass
HIV-1 Positive Control	Pass
HIV-2 Positive Control	Pass
Negative Control	Pass
Analysis	Pass

Signature

QC Parameters

Sample Detection:	control for presence of sample
Device:	multiple QC parameters for the functionality of the m-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 QC parameters for the analysis process, incl. positive hybridization control

LIMITATIONS

Interpretation of Results

Specimens “Not detected” for HIV-1 or HIV-2 RNA by m-PIMA™ HIV-1/2 Detect do not necessarily indicate the absence of an HIV infection in the respective patient. As with any diagnostic test, results from m-PIMA™ HIV-1/2 Detect need 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 and patient related factors (e.g. age, presence of symptoms, stage of the infection and viral setpoint).

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. m-PIMA™ HIV-1/2 Detect is not intended for confirmation of an HIV infection in patients receiving ART or preventive therapy.

Genetic Variants/ Mutations

The m-PIMA™ HIV-1/2 Detect assay targets conserved parts of the 5’-UTR (untranslated region) in the HIV-1 genome and of the 5’-LTR (long terminal repeat) region in the HIV-2 genome, respectively.

While the detection of HIV-1 group M analytes is based on two amplification reactions targeting the *gag* upstream region (5’-UTR) and the *pol* region of the viral genome (dual target), the detection of HIV-1 group N and HIV-1 group O is based solely on the *gag* upstream region (single target).

The detection of polymorphisms is enabled by using a combination of several primers. Though rare, genetic variants/ mutations, e.g. due to base changes, deletions or insertions within the genomic regions targeted by the m-PIMA™ HIV-1/2 Detect assay may affect the primer and/or probe binding sites resulting in decreasing assay detection efficiency and/or increasing error rates.[28, 29] These effects need to be considered when interpreting the m-PIMA™ HIV-1/2 Detect test results. Negative test results may require further testing using technologies with different genomic targets if the clinical and laboratory findings indicate an infection with HIV.

False positive HIV-1 M/N test results may occur in cases where HIV-1 O is present in the sample at a concentration of 100000 cp/mL or higher.

Matrix Effect

Due to the inclusion of cell-associated HIV RNA into the analysis, the number of virus particles per given sample volume is higher in whole blood samples than in plasma samples.
Samples of 186 patients from cohort B (see page 27) with corresponding plasma viral loads between 0 and 2491 cp/mL (Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 version 2.0) were analysed. While the diagnostic sensitivity (95 % confidence interval) with Roche COBAS® using 1 mL plasma was 48.4 % [41.0 %, 55.8 %], the sensitivities with m-PIMA™ HIV-1/2 Detect using a sample volume of 25 µL was 51.4 % [43.8 %, 59.0 %] for capillary blood, 56.6 % [49.1 %, 63.9 %] for venous whole blood, but only 2.7 % [0.9 %, 6.2 %] for the corresponding plasma samples, respectively, indicating a loss of sensitivity for low viral load samples when using plasma instead of whole blood.

Sample Stability

Venous whole blood, collected into EDTA tubes, can be stored at ambient temperature (18 – 28 °C) for up to 24 hours after draw before testing with m-PIMA™ HIV-1/2 Detect.
If testing is not possible within 24 hours, whole blood or plasma samples should be aliquoted and frozen at least at -80 °C. Frozen samples are stable for up to 11 days.
Frozen samples should be thawed at ambient temperature and, once thawed, tested immediately.
It is recommended to invert the thawed sample tubes 10 - 15 times before pipetting.

Note: Sample storage at ambient temperatures for more than 24 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 < 4000 cp/mL.

Multiplex Testing

Multiplex testing refers to the simultaneous presence of HIV-1 group M/N, HIV-1 group O and/or HIV-2 in the same patient sample. In case of larger viral load differences, the ability to detect the analyte present in lower concentration may be reduced (see also section “Multiplex Assay” on page 40).

PERFORMANCE CHARACTERISTICS

Performance characteristics of the m-PIMA™ HIV-1/2 Detect test were established by testing at Abbott Rapid Diagnostics Jena GmbH in Jena, Germany, and at external sites in Mozambique, Uganda, the United States of America and Germany.
Performance characteristics established with the m-PIMA™ HIV-1/2 Detect product including only a single target for HIV-1 group M detection are indicated as "single target" in the following sections.
Samples from several 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.

Cohort A: A total of 254 matched pairs of frozen venous EDTA whole blood and plasma samples from treatment naïve HIV-1 positive donors after seroconversion were collected at clinical sites in Germany and tested at Abbott Rapid Diagnostics Jena GmbH (formerly Alere Technologies GmbH). For each plasma sample HIV-1 viral load data from Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 version 2.0 was available.

Cohort B: Fresh venous and finger prick whole blood from 200 HIV-1 positive donors after seroconversion (91.5 % on ART) tested at clinical sites in Germany. Matching plasma samples were tested at Abbott Rapid Diagnostics Jena GmbH. For each plasma sample HIV-1 viral load data from Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 version 2.0 was available.

Cohort C: Fresh finger prick whole blood from 200 HIV-1 positive donors after seroconversion (73.5 % on ART) tested at a clinical site in Uganda. Matching venous whole blood samples were tested at the Uganda Virus Research Institute. For each sample HIV-1 plasma viral load data from Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 version 2.0 was available.

Cohort D: Frozen EDTA plasma samples from 101 HIV-2 positive donors after seroconversion (72 % on ART) collected at clinical sites in several African countries and tested at the University of Washington (UW), USA.
For each plasma sample HIV-2 viral load data from a HIV-2 laboratory defined assay, developed at UW, utilizing the Abbott m2000 platform, was available.[30]

Cohort E: Fresh heel prick whole blood samples from 223 children (median age 1 month; range: 1 - 11) born to HIV infected mothers tested at clinical sites in Mozambique. For each whole blood sample positivity for HIV-1 was determined using the Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative test.

Cohort F: Venous EDTA Plasma samples obtained from individuals during HIV-1 antibody seroconversion. Ten commercially available panels including 56 samples of early seroconversion were purchased from SeraCare, Mulford, USA and HISS Diagnostics GmbH, Freiburg, Germany and tested at Abbott Rapid Diagnostics Jena GmbH. For each plasma sample positivity for HIV-1 was determined using the Abbott Determine™ HIV Early Detect (4th generation, HIV-1 p24 antigen and HIV-1/2 antibodies).

Cohort G: A total of 1203 frozen venous EDTA whole blood and plasma samples from presumably healthy European donors (commercially available through BBI Solutions, Cardiff, UK and through the Institut für Transfusionsmedizin der Friedrich Schiller Universität Jena, Germany) were tested at Abbott Rapid Diagnostics Jena GmbH. The clinical samples have been tested negative for HIV-1/2 antibodies, HIV-1 NAT, Hepatitis B Surface antigen, HCV NAT, Hepatitis C virus antibodies and Syphilis.

Cohort H: Fresh venous EDTA whole blood and plasma samples from presumably healthy European donors (commercially available through the Institut für Transfusionsmedizin der Friedrich Schiller Universität Jena, Germany) were tested negative for HIV-1/2 antibodies, HCV antibodies, Hepatitis B Surface Antigen, Hepatitis B Core-Protein antibodies, Syphilis, irregular antibodies, HCV NAT and HIV-1 NAT (Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 version 2.0).

Cohort I: Fresh venous EDTA whole blood samples from 113 presumably healthy European donors (commercially available from Biomex GmbH, Heidelberg, Germany) were tested negative for HIV-1/2 antibodies, HCV antibodies, Hepatitis B Surface Antigen.

Diagnostic Sensitivity in HIV-1 Fresh and Frozen Whole Blood and Plasma Samples (single target)

Diagnostic sensitivity of a test is defined as the proportion of subjects with the clinical condition of interest who have a positive test result and is expressed as a proportion or percentage. Diagnostic sensitivity was determined using a total of 295 venous whole blood samples, 235 plasma samples and 74 capillary blood samples from cohorts A, B and C with corresponding plasma viral loads above the limit of detection of m-PIMA™ HIV-1/2 Detect (≥ 2491 cp/mL determined with Roche COBAS®).
The observed diagnostic sensitivity [95 % confidence intervals] of m-PIMA™ HIV-1/2 Detect for HIV-1 in venous whole blood, plasma and capillary whole blood samples was 98.98 % (292/295) [97.06 %, 99.79 %], 99.57 % (234/235) [97.65 %, 99.99 %] and 98.65 % (73/74) [92.70 %, 99.97 %], respectively. No samples reactive for HIV-2 were found in these cohorts.

Diagnostic Sensitivity in HIV-2 Samples (single target)

Diagnostic sensitivity was determined using a total of 37 plasma samples from cohort D with corresponding plasma viral loads above the limit of detection of m-PIMA™ HIV-1/2 Detect (≥ 952 cp/mL, equivalent to ≥ 98 cp/mL determined with Abbott m2000).
The observed diagnostic sensitivity [95 % confidence intervals] of m-PIMA™ HIV-1/2 Detect for HIV-2 in plasma samples was 97.30 % (36/37) [85.84 %, 99.93 %]. Of the 101 patients in cohort D, 8 were co-infected with HIV-1 and HIV-2 according to historical serological test data. Of these, 4 patients showed detectable viral load for one or both virus types on m-PIMA™ HIV-1/2 Detect. No discrepant results were observed for HIV-2.
One of the patients reactive for HIV-1 on Abbott m2000 was undetected on the m-PIMA™ HIV-1/2 Detect, but the viral load for this sample was below the limit of detection of the m-PIMA™ HIV-1/2 Detect. The other 4 patients had undetectable virus with both m-PIMA™ HIV-1/2 Detect and the reference method.

Diagnostic Sensitivity and Specificity in Neonatal Samples (single target)

Diagnostic sensitivity and specificity for neonatal specimens was determined using a total of 223 heel prick whole blood samples from cohort E.
The observed diagnostic sensitivity and specificity [95 % confidence intervals] of m-PIMA™ HIV-1/2 Detect for HIV-1 in heel prick samples was 100 % (18/18) [84.7 %, 100 %] and 100 % (205/205) [98.5 %, 100 %], respectively. No samples reactive for HIV-2 were found in this cohort.

Diagnostic Sensitivity in Seroconversion Panels

Diagnostic sensitivity in seroconversion panels was determined using the 10 panels from cohort F.
A total of 56 panel members were tested. One member of panel 0600-0251 resulted in an invalid test run for m-PIMA HIV 1/2 Detect and was therefore excluded from data analysis. m-PIMA™ HIV-1/2 Detect detected HIV-1 in 39 out of the valid 55 panel members compared to 22 out of 55 detected by Abbott Determine™ HIV Early Detect used as reference method. In 10 out of 10 panels m-PIMA™ HIV-1/2 Detect detected HIV-1 M/N earlier than the reference (mean difference 6.8 days).
For detailed results see table 1.

Table 1: Seroconversion sensitivity

Panel 0600-	Number of panel members tested	Number of panel members detected		Days to first HIV detected		Difference in days to first HIV detected (m-PIMA™ minus Abbott)
		m-PIMA™ HIV-1/2 Detect	Abbott Determine™ HIV Early Detect	m-PIMA™ HIV-1/2 Detect	Abbott Determine™ HIV Early Detect	
0227	4	3	1	4	7	-3
0230	4	3	2	6	18	-12
0237	4	4	4	0	7	-7
0240	5	4	3	40	47	-7
0245	7	3	2	14	17	-3
0250	10	6	2	15	26	-11
0251	10*	5	2	61	70	-9
0253	4	4	2	0	7	-7
0260	4	3	2	7	9	-2
0261	4	4	2	0	7	-7
					Mean Difference	-6.8

** Due to an invalid test result for m-PIMA™ HIV-1/2 Detect only 9 panel members were analysed.*

Diagnostic Specificity on Frozen Whole Blood and Plasma Samples (single target)

Diagnostic specificity of a test is defined as the proportion of disease-free subjects who have a negative test result and is expressed as a proportion or percentage.
Diagnostic specificity was determined using a total of 600 venous whole blood samples and 603 plasma samples from cohort G. The observed diagnostic specificity [95 % confidence intervals] of m-PIMA™ HIV-1/2 Detect for venous whole blood and plasma samples from presumably healthy donors was 100 % (600/600) [99.50 %, 100 %] and 100 % (603/603) [99.50 %, 100 %], respectively.

Diagnostic Specificity on Fresh Whole Blood Samples

Diagnostic specificity was determined using 204 fresh venous whole blood specimens from healthy donors from cohort H (n = 91) and I (n = 113). The observed diagnostic specificity [95 % confidence intervals] of m-PIMA™ HIV-1/2 Detect for venous whole blood was 100 % (204/204) [98.21 %, 100 %].

Analytical Sensitivity/ Limit of Detection

m-PIMA™ HIV-1/2 Detect was designed to achieve analytical sensitivities for all three analytes of 4000 cp/mL. During an in-house study at Abbott Rapid Diagnostics Jena GmbH the analytical sensitivity of m-PIMA™ HIV-1/2 Detect was determined by analysing the lower limit of detection (LOD) for each analyte (HIV-1 group M/N, HIV-1 group O and HIV-2). The limit of detection is the number of copies/mL or international units/mL, where the true detection rate is 95 % and was calculated with a Probit regression.
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 HIV negative venous whole blood samples from cohort H to generate spiked samples of predefined low viral load levels. To determine 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 2. Respective detection rates are listed in table 3. The conversion factors between virus RNA copies and International Units (IU) are 1:1.74 for HIV-1 group M/N based on the 3rd HIV-1 WHO International Standard and 1:0.55 for HIV-2 group A based on the 1st HIV-2 WHO International Standard.

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

*Table 2: 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	636 copies/mL [95 % CI, 517 - 840] **
	1107 IU/mL [95 % CI, 900 - 1462]
HIV-1 group O	826 copies/mL [95 % CI, 673 - 1080]
	NA
HIV-2 group A	1037 cp/mL [95 % CI, 843 - 1359] ***
	570 IU/mL [95 % CI, 464 - 747]

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

** One copy of HIV-1 RNA is equivalent to 1.74 International Units (IU) based on the 3rd HIV-1 WHO International Standard.

*** One copy of HIV-2 RNA is equivalent to 0.55 International Units (IU) based on the 1st HIV-2 WHO International Standard.

Table 3: Detection Rates of the m-PIMA™ HIV-1/2 Detect test

Analyte	Concentration (cp/mL)	N_{valid}	$N_{detected}$	Percent detected
HIV-1 group M	1605	95	95	100
	903	95	95	100
	508	93	85	91
	285	92	69	75
	161	91	46	51
	91	95	40	42
HIV-1 group O	1514	93	93	100
	851	93	90	97
	479	91	76	84
	269	94	58	62
	151	89	39	44
	85	95	23	24
HIV-2 group A	1778	88	88	100
	1000	93	90	97
	562	94	78	83
	316	96	54	56
	178	93	38	41
	100	91	23	25

Analytical Specificity (single target)

Analytical specificity of a test is defined as the ability to detect only the intended target and that the detection of that target is not affected by cross-reactivity or interfering substances.

Cross-reactivity refers to potentially interfering organisms, i.e. other pathogens. Interfering substances refer to endogenous substances that may occur under sample related conditions like non-infectious diseases, medical conditions, or exogenous substances such as drugs. These substances were either spiked into the samples or were already detectable in commercially available sample panels. HIV negative samples from cohort H were spiked with defined amounts of HIV-1 group M (subtype B strain IIIB), HIV-1 group O (strain MVP5180), and HIV-2 group A (strain NIHZ) purified virus (one analyte per sample) to reach a concentration of 12000 cp/mL. Drug interference was tested by adding three times Peak Plasma Level into whole blood.

Cross-Reactivity

The susceptibility of m-PIMA™ HIV-1/2 Detect to cross reactivity with relevant pathogenic organisms was evaluated for whole blood, plasma and serum samples. Cross-reactants included pathogens that frequently occur in co-infections with HIV, but also in normal human flora and include viruses, fungi, protozoa, and bacteria (see tables 4 and 5).

Table 4: List of pathogens: non-clinical samples

Viruses	**Bacteria**
HTLV-1 HTLV-2 HBV HCV HAdV HSV-1 (HHV-1) HSV-2 (HHV-2) VZV (HHV-3) EBV (HHV-4) HCMV (HHV-5) HHV-6	Salmonella Enteritidis Salmonella Typhimurium Salmonella Paratyphi Staphylococcus epidermidis Streptococcus pneumoniae Streptococcus mutans MSSA MRSA Chlamydia pneumoniae Escherichia coli Propionibacterium acnes
Fungi	**Protozoa**
Candida albicans Cryptococcus neoformans Pneumocystis jirovecii	Toxoplasma gondii

Table 5: List of pathogens: clinical samples

Organism/ Agent	Number of Patient Samples
Treponema pallidum (mixed antibody titer panels)*	10
HCV (serological and NAT pos)**	10
HBV (serological and NAT pos)**	10
HCMV EBV HSV-1 HSV-2 Rubella Virus Toxoplasma gondii (IgG pos)*	12

** confirmed antibody positive samples*
*** confirmed nucleic acid and antibody positive samples*

A total of 26 pathogens provided as non-clinical samples (purified pathogen or purified genomic DNA) have been tested with at least 10 replicates with m-PIMA™ HIV-1/2 Detect. In a total of 304 test runs with HIV-negative and 306 test runs with spiked HIV-positive samples no cross-reactivity with the tested organisms was observed.
No false positive HIV results were obtained for any HIV negative samples, and no false negative for HIV positive samples (for all three analytes).
In addition, 42 clinical samples (9 different pathogens) have been tested for cross-reactivity with the m-PIMA™ HIV-1/2 Detect test. No false positive HIV results were obtained for any HIV negative samples, and no false negative for HIV positive samples (for all three analytes). None of the pathogens revealed cross-reaction with the m-PIMA™ HIV-1/2 Detect test.

Endogenous Interfering Substances and Medical Conditions

The susceptibility of m-PIMA™ HIV-1/2 Detect to interference by elevated levels of endogenous substances and several medical conditions was evaluated for plasma and serum samples (see table 6).

Table 6: List of endogenous substances or samples from donors with medical conditions

Clinical Sample	Number of Patients
Double-stranded DNA	10
Anti-nuclear antibodies (1:320-1:10000 titer)	10
Bilirubin (5.1-13.4 mg/dL)	10
Cholesterol (99-220 mg/dL)	10
Contraceptive pill	10
Ovarian cancer	10
Renal failure	10
Rheumatoid factor (295-7900 IU/mL)	10
Third trimester pregnancy	10
Total T3	10
Type II Diabetes	10
IV drug abuser	10
Non-viral liver disease	10

A total of 130 different clinical samples (representing 13 different clinical conditions) have been used for interference testing with m-PIMA™ HIV-1/2 Detect. Of 130 test runs with HIV-negative samples, no false positive results for all three analytes were generated. Of 130 test runs with spiked HIV-positive samples, no false negative results for all three analytes were generated. No interference from endogenous substances or medical conditions was observed.

Drug interference

The susceptibility of m-PIMA™ HIV-1/2 Detect to interference by drugs commonly prescribed to HIV infected individuals was evaluated for plasma samples (see table 7).

Table 7: List of drugs

HIV drugs	
Protease Inhibitors Lopinavir, LPV Ritonavir	**Nucleoside/Nucleotide Analogue Inhibitors of Reverse Transcriptase** Abacavir sulfate, ABC Emtricitabine, FTC Stavudine, d4T Tenofovir disoproxil fumarate, TDF Lamivudine, 3TC Zidovudine, AZT
Non-Nucleoside/Nucleotide Analogue Inhibitors of Reverse Transcriptase Efavirenz, EFV Nevirapine, NVP	**Integrase Inhibitors** Raltegravir, RAL
HCV/HBV drugs	**Compounds for treatment of Herpes Viruses**
Immune Modulator Ribavirin Peginterferon alfa-2a Peginterferon alfa-2b	**Nucleoside Analogues** Acyclovir Ganciclovir
Compounds for treatment/ prevention of opportunistic infections in HIV disease	
Anti Fungal Fluconazole	**Anti Fungal/ Bacterial** Co-trimoxazole
Anti Mycobacterial Isoniazid Rifampicin Pyrazinamide Ethambutol Streptomycin	

Of 284 test runs with HIV-negative samples, no false positive results for all three analytes were generated. Of 280 test runs with spiked HIV-positive samples, no false negative results for all three analytes were generated. No interference by drugs was observed.

Genotype/ Subtype Testing

The analytical performance of m-PIMA™ HIV-1/2 Detect with HIV-1 and HIV-2 subtypes was evaluated by testing 20 HIV-1 isolates (HIV-1 group M subtypes A through H, HIV-1 group N, HIV-1 group O and Circulating Recombinant Forms) and 5 HIV-2 isolates (HIV-2 group A, B, A/B). All isolates used in this study were members of subtype panels provided by the German National Reference Center for Retroviruses (GNRCR), Munich, Germany, by the former German National Reference Center for Retroviruses, Erlangen, Germany, and by the National Institute for Biological Standards and Control (NIBSC), UK.
Viruses were cultivated on cells and supernatants were diluted in HIV-negative plasma and quantified using different real time PCR assays at GNRCR, Munich and Erlangen. Viruses provided by the NIBSC were cultivated on cells, supernatants were diluted in HIV-negative plasma and freeze-dried.
The specimens were used at Abbott Rapid Diagnostics Jena GmbH to generate different dilution levels. Subtypes were tested at the 0.5-fold, 1-fold and 3-fold limit of detection.

All subtypes were successfully detected by m-PIMA™ HIV-1/2 Detect. Neither false positive results at all concentrations nor false negative results at 3-fold LOD for HIV-1 group M/N, HIV-1 group O or HIV-2 were observed.
For details on the tested isolates see tables 8 and 9:

Table 8: HIV-2 group detection of the m-PIMA™ HIV-1/2 Detect test

		m-PIMA™ HIV-1/2 Detect ($N_{detected}$ / N_{valid})		
Groups	Isolate	0.5x LOD	1x LOD	3x LOD
Group A	CDC77618	10/10	10/10	10/10
Group A	7924A	10/10	10/10	10/10
Group A	60415K	10/10	10/10	10/10
Group A/B	7312A	10/10	10/10	10/10
Group B	CDC310319	10/10	10/10	10/10

Table 9: HIV-1 group and subtype detection of the m-PIMA™ HIV-1/2 Detect test

		m-PIMA™ HIV-1/2 Detect ($N_{detected}/N_{valid}$)		
Groups/Subtypes	Isolate	0.5x LOD	1x LOD	3x LOD
Group M/Subtype A	92UG029	8/10	10/10	10/10
Group M/Subtype B	92TH026	10/10	10/10	10/10
Group M/Subtype C	92BR025	10/10	9/10	10/10
Group M/Subtype D	92UG021	8/10	10/10	10/10
Group M/Subtype F	93BR029	7/10	8/10	10/10
Group M/Subtype F	93BR020	9/10	10/10	10/10
Group M/Subtype G	RU570	6/10	10/10	10/10
Group M/Subtype G	P962	8/10	10/10	10/10
Group M/Subtype H	VI557	10/10	10/10	10/10
Group M/Subtype J	SE9173	8/10	10/10	10/10
Group M/Subtype CRF01 (AE)	92TH022	8/10	10/10	10/10
Group M/Subtype CRF02 (AG)	01CM.0005BBY	6/10	10/10	10/10
Group M/Subtype CRF02 (AG)	01CM.0008BBY	9/10	10/10	10/10
Group M/ Subtype AG-GH	VI525	9/10	10/10	10/10
Group M/ Subtype CRF01 A, G, J, U	96CM1849	8/10	10/10	10/10
Group N	YBF30	10/10	10/10	10/10
Group O	MVP5180	8/10	10/10	10/10
Group O	CA-9	4/10	10/10	10/10
Group O	13740	8/10	10/10	10/10
Group O	2549-95	5/10	10/10	10/10

Sample Matrix Effects (single target)

To evaluate potential effects of the sample matrix on the performance of m-PIMA™ HIV-1/2 Detect, spiked HIV negative samples and samples from HIV positive cohorts were tested.

Matrix effect in spiked samples

Fresh matched venous whole blood and plasma samples from cohort H were spiked with virus preparations of HIV-1 group M (subtype B, strain IIIB) at a concentration of 12000 cp/mL. The samples were analysed with m-PIMA™ HIV-1/2 Detect at 3 days with two runs per day on 20 analysers. Valid tests (venous whole blood: n=56; plasma: n=59) were used to analyse the matrix effect. For all tests on venous whole blood and matching plasma samples spiked with 12000 cp/mL HIV-1 M (subtype B, strain IIIB), HIV-1 M/N was 100 % successfully detected with m-PIMA™ HIV-1/2 Detect. No influence of blood cells and blood cell components on the detection rate was observed.
There were no false positive results for HIV-1 O and HIV-2. The results are considered to be representative for all analytes of the m-PIMA™ HIV-1/2 Detect test (HIV-1 group M/N, HIV-1 group O and HIV-2).

Matrix effect in patient samples with viral loads ≥ 2491 cp/mL

Matrix effect was determined using a total of 235 matched pairs of venous whole blood and plasma samples, and 74 matched pairs of venous whole blood and capillary blood samples from cohorts A, B and C with corresponding plasma viral loads above the limit of detection of m-PIMA™ HIV-1/2 Detect (≥ 2491 cp/mL determined with Roche COBAS®).
For matched pairs of venous whole blood and plasma samples there was 100 % agreement (CI for difference in sensitivity -1.8 %, +1.8 %) between the m-PIMA™ HIV-1/2 Detect test results.
For matched pairs of venous whole blood and capillary blood samples there was 98.65 % agreement (CI for difference in sensitivity -7.7 %, +4.2 %) between the m-PIMA™ HIV-1/2 Detect test results (please also refer to the limitation section on page 25).
While only HIV-1 M/N was detected in the samples from these cohorts, the results are considered to be representative for all analytes of the m-PIMA™ HIV-1/2 Detect test (HIV-1 group M/N, HIV-1 group O and HIV-2).

Multiplex Assay

To evaluate sensitivity within HIV-1/ HIV-2 dual-infections, the two virus types were tested at different concentrations (high/ low) in the same sample on the m-PIMA™ HIV-1/2 Detect test. Defined concentrations of the HI virus preparations were spiked into HIV negative whole blood samples of presumably healthy European donors from cohort H according to the table below.

Table 10: Test results for spiked HIV-1/HIV-2 duplex samples

Sample	Tested concentration ($N_{detected}/N_{valid}$)		
	HIV-1 group M/N	HIV-1 group O	HIV-2 group A
1	3x LOD (58/59)	–* (0/59)	100000 IU/mL (59/59)
2	–* (0/58)	3x LOD (53/58)	100000 IU/mL (58/58)
3	1000000 IU/mL (59/59)	–* (0/59)	3x LOD (59/59)
4	–* (1/57)	1000000 IU/mL (57/57)	3x LOD (57/57)

* *Negative for the analyte*

For all samples, the higher concentrated analytes were always detected with m-PIMA™ HIV-1/2 Detect. As described in “Limitations”, section “Genetic Variants/ Mutations” on page 25 high concentrations of HIV-1 group O may lead to false positive results for HIV-1 group M/N.

In this study one false positive result was observed for HIV-1 group M/N in the presence of high HIV-1 group O concentrations.

Low concentrated analytes were not detected for HIV-1 group M and HIV-1 group O in one case and five cases, respectively. However, the limit of detection (including the 95 % confidence interval) in multiplex samples with high concentrations of HIV-2 was proven to be less than 4000 cp/mL for both HIV-1 group M and HIV-1 group O.

Negative and Positive Predictive Value, Likelihood Ratio (single target)

The negative and positive predictive value as well as the negative and positive likelihood ratio of the m-PIMA™ HIV-1/2 Detect test were determined by analysis of 292 HIV-positive EDTA venous whole blood and 234 plasma samples (cohort A, B, C) as well as 600 HIV negative EDTA venous whole blood and 603 plasma samples (cohort G) in comparison to the COBAS® AmpliPrep/COBAS ® TaqMan HIV-1 test v2.0.
The evaluation was performed according to CLSI EP12-A2.[32]
The m-PIMA™ HIV-1/2 Detect test shows a positive predictive value of 100 % for both sample types and a negative predictive value of 99.5 % and 99.8 % for EDTA venous whole blood and plasma samples, respectively.
The positive likelihood ratio (LR+) could not be calculated as no false positive test result was observed during analysis. This provides strong evidence that a positive test result is more likely to be a true positive than a false positive test result.
The negative likelihood ratio (LR-) of 0.010 and 0.004 for EDTA venous whole blood and plasma samples, respectively, is smaller than 0.1 which provides strong evidence that a negative test result is more likely to be a true negative than a false negative test result.

Carryover (single target)

Potential sample carryover in the automated m-PIMA™ Analyser when used with the m-PIMA™ HIV-1/2 Detect test was evaluated by testing high titer HIV-1 group M (subtype B, strain IIIB) samples (at a target concentration of $3x10^7$ cp/mL) interspersed with HIV negative venous whole blood samples from cohort H (n = 144).
m-PIMA™ HIV-1/2 Detect test did not exhibit detectable carryover from high positive samples to negative samples.

Precision

To evaluate the precision of the m-PIMA™ HIV-1/2 Detect test three operators performed duplicate testing using three cartridge production lots on three m-PIMA™ Analysers on six days. All experiments were performed with HIV negative venous whole blood samples (cohort H) spiked with virus preparations of HIV-1 group M (subtype B, strain IIIB), HIV-1 group O (strain MVP5180) and HIV-2 group A (strain NIHZ) at two different concentrations, representing the 3-fold and 7-fold limit of detection.
For all tests each analyte was 100 % successfully detected with m-PIMA™ HIV-1/2 Detect.

TECHNICAL SUPPORT

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

Europe	+44 161 483 9032	EME.techsupport@abbott.com
Middle East	+965 2202 2828	EME.techsupport@abbott.com
Africa	+27 10 500 9700	ARCIS.techsupport@abbott.com
Asia Pacific	+61 7 3363 7711	AP.techsupport@abbott.com
Latin America	+57 601 482 4033	LA.techsupport@abbott.com
Russia & CIS	+7 499 403 9512	ARCIS.techsupport@abbott.com

For users and/or patients belonging to EU/EEA:
Any serious incident that has occurred in relation to an m-PIMA™ HIV-1/2 Detect cartridge shall be reported to the manufacturer and the competent authority of the Member State in which the user or patient is established.

REVISION HISTORY

This revision: version 14, 30-May-2023
Previous revision: version 13, 21-Dec-2021

Updates to previous revision:
- Established conformity to Regulation (EU) 2017/746 on in vitro diagnostic medical devices throughout this document
- Updated format for testing procedure starting page 16
- Updated Sample Stability on page 26 regarding frozen whole blood and plasma samples
- Updated cohorts on page 28
- Updated Performance Characteristics (and added “single target” indication where applicable) starting page 27:
    - Diagnostic Sensitivity in Seroconversion Panels
    - Diagnostic Specificity for Fresh Whole Blood Samples
    - Analytical Sensitivity/LOD
- Added Negative and Positive Predictive Value, Likelihood Ratio on page 41
- Updated Technical Support telephone numbers on page 42
- Added instructions on incident reporting, page 42
- Added reference to Summary of Safety and Performance, page 43

- Updated reference 1 (page 43) and 26 (page 44)
- Minor edits to the text for improved readability

SUMMARY OF SAFETY AND PERFORMANCE

Summary of Safety and Performance for m-PIMA™ HIV-1/2 Detect is available in the European database on medical devices (Eudamed) at https://ec.europa.eu/tools/eudamed

REFERENCES

(1) WHO. Consolidated guidelines on HIV prevention, testing, treatment, service delivery and monitoring: recommendations for a public health approach. Geneva: World Health Organization; 2021.

(2) Jani I.V., Meggi B., Mabunda N., et al. 2014. Accurate early infant HIV diagnosis in primary health clinics using a point-of-care nucleic acid test. J Acquir Immune Defic Syndr 67(1):e1-4

(3) Bruisten S., van Gemen B., Koppelman M., et al. 1993. Detection of HIV-1 distribution in different blood fractions by two nucleic acid amplification assays. AIDS Res Hum Retroviruses 9:259-65.

(4) Lee T.H., Stromberg R.R., Heitman J.W., et al. 1998. Distribution of HIV type 1 (HIV-1) in blood components: detection and significance of high levels of HIV-1 associated with platelets. Transfusion 38:580-8.

(5) Torre D. and Pugliese A. 2008. Platelets and HIV-1 infection: old and new aspects. Curr HIV Res 6:411-8.

(6) Beck Z., Jagodzinski L.L., Eller M.A., et al. 2013. Platelets and erythrocyte-bound platelets bind infectious HIV-1 in plasma of chronically infected patients. PLoS One 8:e81002.

(7) Zhu T., Muthui D., Holte S., et al. 2002. Evidence for human immunodeficiency virus type 1 replication in vivo in CD14 (+) monocytes and its potential role as a source of virus in patients on highly active antiretroviral therapy. J Virol 76:707-16.

(8) Kaiser P., Joos B., Niederost B., et al. 2007. Productive human immunodeficiency virus type 1 infection in peripheral blood predominantly takes place in CD4/CD8 double-negative T lymphocytes. J Virol 81:9693-706.

(9) Cena M., Schwachsa N., Garcia M.N.,et al. 2004. Determination of HIV-1 p24 antigen associated with erythrocytes: potential uses. Int Conf AIDS. 2004 Jul 11-16. 15:abstract no. B11460.

(10) Garcia M.N., dos Ramos Farías M.S., Schvachsa N.,et al. 2006. Detection of HIV-1 antigen associated to erythrocytes in patients with undetectable viral load in plasma for more than one year. Poster presentation from 2006 International Meeting of The Institute of Human Virology Baltimore, USA. 17-21 November, 2006. Retrovirology 3 (Suppl 1):P19.

(11) Hess C., Klimkait T., Schlapbach L., et al. 2002. Association of a pool of HIV-1 with erythrocytes in vivo: a cohort study. Lancet 359:2230-4.

(12) Arens M., Joseph T., Nag S. et al. 1993. Alterations in spliced and unspliced HIV-1-specific RNA detection in peripheral blood mononuclear cells of individuals with varying CD4-positive lymphocyte counts. AIDS Res Hum Retroviruses 9:1257-63.

(13) Bagnarelli P., Menzo S., Valenza A., et al. 1992. Molecular profile of human immunodeficiency virus type 1 infection in symptomless patients and in patients with AIDS. J Virol 66:7328-35.

(14) Furtado M.R., Murphy R. and Wolinsky S.M. 1993. Quantification of human immunodeficiency virus type 1 tat mRNA as a marker for assessing the efficacy of antiretroviral therapy. Infect Dis 167:213-6.

(15) Graziosi C., Pantaleo G., Butini L., et al. 1993. Kinetics of human immunodeficiency virus type 1 (HIV-1) DNA and RNA synthesis during primary HIV-1 infection. Proc Natl Acad Sci U S A 90:6405-9.

(16) Gupta P., Kingsley L., Armstrong J., et al. 1993. Enhanced expression of human immunodeficiency virus type 1 correlates with development of AIDS. Virology 196:586-95.

(17) Michael N.L., Vahey M., Burke D.S., et al. 1992. Viral DNA and mRNA expression correlate with the stage of human immunodeficiency virus (HIV) type 1 infection in humans: evidence for viral replication in all stages of HIV disease. J Virol 66:310-6.

(18) Patterson B.K., Till M., Otto P., et al. 1993. Detection of HIV-1 DNA and messenger RNA in individual cells by PCR-driven in situ hybridization and flow cytometry. Science 260:976-9.

(19) Schnittman S.M., Greenhouse J.J., Lane H.C., et al. 1991. Frequent detection of HIV-1-specific mRNAs in infected individuals suggests ongoing active viral expression in all stages of disease. AIDS Res Hum Retroviruses 7:361-7.

(20) Seshamma T., Bagasra O., Trono D., et al. 1992. Blocked early-stage latency in the peripheral blood cells of certain individuals infected with human immunodeficiency virus type 1. Proc Natl Acad Sci U S A 89:10663-7.

(21) Steinmetzer K., Seidel T., Stallmach A., et al. 2010. HIV load testing with small samples of whole blood. J Clin Microbiol 48:2786-92.

(22) Ullrich T., Ermantraut E., Schulz T., et al. 2012. Competitive Reporter Monitored Amplification (CMA) - Quantification of Molecular Targets by Real Time Monitoring of Competitive Reporter Hybridization. PLoS ONE 7(4): e35438. doi:10.1371/journal.pone.0035438

(23) Soderlund, H. 1990. DNA hybridization: comparison of liquid and solid phase formats. Ann Biol Clin (Paris) 48:489-91.

(24) UK NHS. Guidelines for Newborn Blood Spot Sampling. February 2012. Available at: http://newbornbloodspot.screening.nhs.uk/bloodspotsampling#fileid11952

(25) WHO. WHO guidelines on drawing blood: best practices in phlebotomy. Geneva: WHO; 2010. Available at: http://whqlibdoc.who.int/publications/2010/9789241599221_eng.pdf

(26) CLSI GP42 Collection of Capillary Blood Specimens; Approved Standard-7th Edition. Clinical and Laboratory Standards Institute; USA, 2020.

(27) CLSI GP41.Collection of Diagnostic Venous Blood Specimens; Approved Standard-7th Edition, Clinical and Laboratory Standards Institute, 2017.

(28) Chudy M, Kress J, Halbauer J, et al. 2014. Risk Minimization Measures for Blood Screening HIV-1 Nucleic Acid Amplification Technique Assays in Germany. Transfus Med Hemother 41:45-51.

(29) Korn K, Weissbrich B, Henke-Gendo C, et al. 2009. Single-point mutations causing more than 100-fold underestimation of human immunodeficiency virus type 1 (HIV-1) load with the Cobas TaqMan HIV-1 real-time PCR assay. J Clin Microbiol 47:1238-40.

(30) Chang M., Gottlieb G.S., Dragavon J.A., et al. 2012. Validation for clinical use of a novel HIV-2 plasma RNA viral load assay using the Abbott m2000 platform. J Clin Virol 55:128-133.

EXPLANATION OF SYMBOLS

IVD	*In vitro* diagnostic medical device
REF	Catalogue number
LOT	Batch code
	Use-by date
Σ = n	Contains sufficient for < n > tests
4°C 30°C	Temperature limit
	Consult instructions for use Electronic instructions for use (eIFU) are available at www.globalpointofcare.eifu.abbott
	Do not re-use
	Manufacturer
	Date of manufacture
	Keep dry
	Attention Symbol. Indicates special problems or important information. Read the accompanying text carefully.
CE	CE Mark
UDI	Unique Device Identifier

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