WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

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

**m-PIMA HIV-1/2 Detect assay** with product codes 27011R050, 27011R010 and 27030R001, manufactured by Alere Technologies GmbH, CE marked regulatory 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**

<table>
<thead>
<tr>
<th>Date</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-Jun-2016</td>
<td>listed</td>
</tr>
<tr>
<td>18-Dec-2015</td>
<td>MR</td>
</tr>
<tr>
<td>16-18-Jul-2018</td>
<td>MR</td>
</tr>
<tr>
<td>14-Aug-2015 to 11-May-2016</td>
<td>MR</td>
</tr>
</tbody>
</table>

MR: Meets requirements

**Report amendments and/or 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 summarized in the following table, and details of each amendment are provided below.

<table>
<thead>
<tr>
<th>Version</th>
<th>Summary of amendment</th>
<th>Date of report amendment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>To reflect fulfillment of an outstanding commitment to WHO prequalification. Commitment was on the instructions for use to be clear as per WHO’s requirement.</td>
<td>2-Sept-2016</td>
</tr>
<tr>
<td>4.0</td>
<td>Rebranding of products Alere q HIV-1/2 Detect (test cartridges, and accessory products) and Alere q analyzer 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.</td>
<td>30-Jul-2019</td>
</tr>
</tbody>
</table>

\(^1\) Please note the name of product Alere q HIV-1/2 Detect and Alere q instrument products were changed to m-PIMA HIV-1/2 Detect on 3 October 2018.
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 in addition to 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 the removal of test group 3.

Intended use:

According to the claim of intended use from Alere Technologies GmbH, “m-PIMA HIV-1/2 Detect 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 RNA in human whole blood and plasma specimens. The test can be used in laboratory as well as non-laboratory environments. The m-PIMA HIV-1/2 Detect test is intended 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.

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. The test is suitable for laboratory and non-laboratory environments and for near-patient Testing.”

Assay description:

Sample Handling and Processing

According to the manufacturer “Peripheral blood may be collected from the patient either through standard finger or heel prick sampling techniques or venous blood draw. The sample volume required to run a test is 25μL. Finger or heel prick blood can be applied directly and immediately onto the m-PIMA HIV-1/2 Detect cartridge. When using EDTA anti-coagulated venous blood or plasma, the appropriate volume is transferred into the cartridge using a volumetric pipette or transfer capillary. Standard phlebotomy sample collection practices for obtaining both capillary and venous blood samples are to be followed. After applying the sample, the cartridge cap is snapped into place, eliminating the chance of sample spillage or contamination of the instrument. After closing the cap the cartridge is inserted into the m-PIMA Analyser and the test is initiated automatically. The steps described in the following subsections are performed automatically by the m-PIMA Analyser within the cartridge.”
RNA Isolation
The RNA isolation consists of following steps:

a) Complete lysis of the sample based on chaotropic salts in order 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, thus 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.

Primers are short specific oligonucleotides that 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 facilitate simultaneous detection of more than one specific nucleic acid sequence a multiplex PCR is performed. Target amplification between HIV-1 group M/N and group O, and HIV-2 is facilitated by specific primer pairs. In addition, the primer pairs allow for the amplification of internal process controls.
Detection

Detection of PCR product is based on Competitive Reporter Monitored Amplification technology utilizing an array of immobilized oligonucleotide probes and complementary fluorescently labelled reporter oligonucleotides in solution. In order 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 that 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 and 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 binds to them. In addition, the solid support to which the oligonucleotide probe is attached introduces a diffusion barrier which significantly reduces the hybridization rate. Thus, generally, solution phase reactions are kinetically favoured to solid phase reactions. Therefore the amount of reporter hybridized to the complementary probe decreases proportionally to the formation of new amplicons. This decrease is observed until a plateau in the amplification reaction is reached. Measuring the change in signal intensity of each probe can be achieved by imaging the fluorescence pattern on the array during the amplification process. Fluorescence images are collected during the annealing phase of each amplification cycle. After acquiring the hybridization pattern an algorithm is applied that is able to identify and eliminate different noise signals from the data obtained by the array assisted real-time PCR. The algorithm then calculates the cycle threshold values from the resulting amplification kinetics determining the presence of the analyte”.

Product test kit contents:

<table>
<thead>
<tr>
<th>m-PIMA HIV-1/2 Detect Cartridge Kit</th>
<th>10 x Cartridge Kit (product code 27011R010)</th>
<th>50 x Cartridge Kit (product code 27011R050)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individually pouch test cartridges</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Guide</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Instrumentation:

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equipment: m-PIMA Analyser (product code 27030R001)</td>
<td>1 unit</td>
</tr>
<tr>
<td>Alternative Equipment Kits: m-PIMA Complete (product code 27030R002) containing product codes 27030R001, 27040R007, 27040R004, 260400059, 26040R009.</td>
<td>1x per kit component</td>
</tr>
</tbody>
</table>

Consumables required but not provided:

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finger Stick Sample Collection Kit (product code 260400199)</td>
<td>100</td>
</tr>
<tr>
<td>Neonatal Sample Collection Kit (product code 270400200)</td>
<td>100</td>
</tr>
<tr>
<td>Plastic Capillaries plain (product code 270400005)</td>
<td>10 x 100</td>
</tr>
<tr>
<td>Plastic Capillaries EDTA-K2 (product code 270400006)</td>
<td>10 x 100</td>
</tr>
</tbody>
</table>

Items required but available separately:

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>USB Printer (product code 27040R007)</td>
<td>1</td>
</tr>
<tr>
<td>Power Drum (product code 27040R004)</td>
<td>1</td>
</tr>
<tr>
<td>Connectivity Pack IV (product code 260400059, compatible with software versions 0.26.01 and higher)</td>
<td>1</td>
</tr>
<tr>
<td>Printer Paper I (product code 26040R009)</td>
<td>10</td>
</tr>
</tbody>
</table>

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

Shelf-life upon manufacture:
9 months.

Warnings/limitations:
For warnings and limitations, please refer to current version of instructions for use.

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

Alere Technologies GmbH 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.

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

Commitment:
Manufacturer had a commitment of revision to the instructions for use as per WHO requirements and submission was due on December 2016. Version 09 of the product IFU, which reflects amendments agreed to as a commitment to prequalification, was provided to WHO October 2018. This commitment was met and closed.

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

A comprehensive reinspection was performed at the site of manufacture (Loebstedter Str. 103-105, 07749 Jena, Germany) of m-PIMA HIV-1/2 Detect in 16 to 18 July 2018 as per the “Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics” (PQDx_014). The inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality.

The manufacturer's responses to the nonconformities found at the time of the inspection were accepted on 29 December 2018.

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

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, 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

<table>
<thead>
<tr>
<th></th>
<th>Initial (95% CI)</th>
<th>Final (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant specimens: (N=150)</td>
<td>98.67% (95.27-99.84)</td>
<td>99.33% (96.34-99.98)</td>
</tr>
<tr>
<td>Adult specimens: (N=50)</td>
<td>90.00% (78.19-96.67)</td>
<td>94.00% (83.45-98.75)</td>
</tr>
<tr>
<td><strong>Specificity %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant specimens: (N=151)</td>
<td>100.00% (97.59-100.00)</td>
<td>100% (97.59-100.0)</td>
</tr>
<tr>
<td>Adult specimens: (N=51)</td>
<td>100.00% (93.02-100.00)</td>
<td>100.00% (93.02-100.00)</td>
</tr>
<tr>
<td><strong>Invalid rate %</strong></td>
<td>5.58%</td>
<td></td>
</tr>
</tbody>
</table>

**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**: 0h:56 minutes (3 - 4 min hands-on time, 52 minutes on instrument)
- **Internal QC**: Assay Process Controls
  1. Internal process controls for HIV-1 and HIV-2
  2. Positive hybridization control
  3. Negative hybridization 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. Labels

1.1 m-PIMA HIV-1/2 Detect

Cartridge Top Label

Cartridge User Label

Primary Packaging Label
Secondary Packaging Labels

m-PIMA™ HIV-1/2 Detect

50 Cartridges for HIV-1/2 diagnosis
50 Cartouches pour le diagnostic du VIH-1/2
50 Cartuchos para uso diagnóstico de VIH-1/2
50 Cartuchos para utilização em diagnóstico de VIH-1/2

REF 27011R050
LOT 02564

2020-12-15
4°C-30°C

Alere Technologies GmbH
Loebstedter Str. 103-105
D-07749 Jena, Germany
www.alere.com

m-PIMA™ HIV-1/2 Detect

10 Cartridges for HIV-1/2 diagnosis
10 Cartouches pour le diagnostic du VIH-1/2
10 Cartuchos para uso diagnóstico de VIH-1/2
10 Cartuchos para utilização em diagnóstico de VIH-1/2

REF 27011R010
LOT 02564

2020-12-15
4°C-30°C

Alere Technologies GmbH
Loebstedter Str. 103-105
D-07749 Jena, Germany
www.alere.com
1.2 Consumables

1.2.1 Finger Stick Sample Collection Kit

Packaging Labels

![Barcode Image]

Collection of Devices as indicated on separate label

Distribution by:
Alere Technologies GmbH
Loebstedter Str. 103-105
D-07749 Jena, Germany
www.Alere-HIV.com

1.2.2 Neonatal Sample Collection Kit

Packaging Labels

![Barcode Image]

Collection of Devices as indicated on separate label

Distribution by:
Alere Technologies GmbH
Loebstedter Str. 103-105
D-07749 Jena, Germany
www.Alere-HIV.com
1.3 Instrument Kits

1.3.1 m-PIMA™ Analyser

Device Label

Packaging Label
1.3.2 m-PIMA™ Complete

Packaging Label

![Image of m-PIMA™ Complete Packaging Label]

Content / Contenu / Contenido / Conteúdo

m-PIMA™ Complete

REF 27030R002

Alere Technologies GmbH
Loebstedter Str. 103-105
D-07740 Jena, Germany
www.alere.com

2°C - 50°C

m-PIMA™ Analyser

REF 27030R001 SN NAT-04000752

PowerDrum

REF 27040R004

USB Printer

REF 27040R007

Printer Paper I

REF 26040R009

Connectivity Pack IV

REF 260400059

Falcon Wireless Communications GmbH
Gewerberei 6, D-98704 Langenwiesen, Germany
BL 27030R002-D1
1.4 Optional Items

1.4.1 PowerDrum

Device Label

---

Packaging Label

---

Contents

1x PowerDrum Case

SN APD-000266

1x PowerDrum Battery

LOT 0000008765
1.4.2 PowerDrum Component Labels

1.4.2.1 PowerDrum Battery

Battery Label

Abbott

PowerDrum Battery

Type: Lithium-Ion battery-pack 7ICR19/66-3
Nonspillable battery
Nominal voltage: 25.6 V
Nominal capacity: 6.45 Ah

Watt-hour rating per battery block:
47.1 Wh / 70.6 Wh / 47.1 Wh
Operating Temperature: 2°C - 40°C
Charging Temperature: 2°C - 40°C

Please make sure that the battery packs are recharged after each partial discharge i.e. after each use, to maintain their full capacity.

distributed by: Alere Technologies GmbH
Loebstedter Str. 103-105
D-07749 Jena, Germany
www.alere.com

Alber GmbH
Vor dem Weißen Stein 21
D-72461 Albstadt, Germany

Packaging Label

PowerDrum Battery

REF 27040R003
LOT 0000006789

Distributed by: Alere Technologies GmbH
Loebstedter Str. 103-105
D-07749 Jena, Germany
www.alere.com

Alber GmbH
Vor dem Weißen Stein 21
D-72461 Albstadt, Germany
1.4.2.2 PowerDrum Case

Device Label

---

Abbott

PowerDrum Case

SN APD-000295

Alere Technologies GmbH
Loebstedter Str. 103-105
D-07749 Jena, Germany
www.alere.com

DC Input: 24 V === 6A
DC Output: 24 V === 6A

---

Packaging Label

---

PowerDrum Case

PowerDrum Case incl. Connector Cable to m-PIMA™Analyser

REF 27040R002

SN APD-000623

2°C – 50°C

Alere Technologies GmbH
Loebstedter Str. 103-105
D-07749 Jena, Germany
www.alere.com

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1.4.3 USB Printer

Device Label

![Abbott USB Printer Label]

Packaging Label

![USB Printer Packaging Label]

1.4.4 Connectivity Packs

Connectivity Pack IV Box label:

![Connectivity Pack IV Label]
2. Instructions for use

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

CE Mark

In vitro diagnostic medical device

Catalog number

Batch code

Use by YYYY-MM-DD

Contains sufficient for < n > tests

Temperature limitation

Consult instructions for use

Do not reuse

Manufacturer

To keep dry

Attention symbol. Indicates special problems or important information. Read the accompanying text carefully.
# TABLE OF CONTENTS

4 INTRODUCTION
4 Intended Use
4 Indications for Use
4 Intended User
5 Order Information and Scope of Delivery
5 Materials Required but Not Provided
5 Optional Items

5 TEST PRINCIPLE
5 Sample Handling and Processing
6 RNA Isolation
6 Reverse Transcription and Amplification
7 Detection

8 m-PIMA™ HIV-1/2 DETECT CARTRIDGE FEATURES
8 Cartridge Components
9 Quality Control (QC) Features
10 Reagents

11 WARNINGS AND PRECAUTIONS
14 m-PIMA™ HIV-1/2 Detect Testing Procedure
14 Basic Workflow
15 How to discard used test cartridges
16 Heel Prick Sample Collection
17 Finger Prick Sample Collection
18 Venous Whole Blood Sample Collection
18 Sample Application via Transfer Capillaries
19 Test Report

21 LIMITATIONS
21 Interpretation of results
21 Matrix Effect
21 Sample Stability
24 Multiplex Testing

24 PERFORMANCE CHARACTERISTICS
25 Diagnostic sensitivity in HIV-1 fresh and frozen whole blood and plasma samples
26 Diagnostic sensitivity in HIV-2 samples
26 Diagnostic sensitivity and specificity in neonatal samples
26 Diagnostic sensitivity in seroconversion panels
27 Diagnostic specificity on frozen whole blood and plasma samples
28 Analytical sensitivity/ Limit of Detection
30 Analytical specificity
33 Genotype/subtype testing
35 Matrix effects
36 Multiplex assay
37 Carryover
37 Precision

37 TECHNICAL SUPPORT

38 REFERENCES
INTRODUCTION

Virological testing using assays specifically detecting HIV DNA or RNA already plays an important role in the diagnosis of HIV infection in newborns. 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 for the first time enables virological testing at the Point of Care performed by non-laboratory personnel in primary health setting. 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 and monocytes, but also to CD4-/CD8- T cells, probably originating from infected CD4+ T cells. HIV RNA and HIV antigen were also found to be associated with erythrocytes. In addition, intracellular HIV RNA is produced within infected lymphocytes circulating in the peripheral blood. By using whole blood, cell-associated viral particles are included into the analysis thus providing a higher probability to detect a HIV infection.

Intended Use

The m-PIMA™ HIV-1/2 Detect test 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 RNA in human whole blood and plasma samples. The test can be used in laboratory as well as non-laboratory environments. The m-PIMA™ HIV-1/2 Detect test is intended 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.

Indications for Use

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

Intended User

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. The test is suitable for laboratory and non-laboratory environments and for near-patient testing.
**Order Information and Scope of Delivery**
n-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

m-PIMA™ HIV-1/2 Detect 10x Cartridge Kit (catalogue no. 27011R010):
- 10 individually pouched test cartridges
- 1 m-PIMA™ HIV-1/2 Detect Cartridge Guide

**Materials Required but Not Provided**
m-PIMA™ Analyser (catalogue no. 27030R001) with installed software version 0.26.1 or higher.

**Optional Items**
Finger Stick Sample Collection Kit (100) (catalogue no. 260400199)
Neonatal Sample Collection Kit (100) (catalogue no. 270400200)

Before performing a 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**
Peripheral blood may be collected from the patient either through finger or heel prick sampling techniques or venous blood draw (draw as described on pages 16-18). The sample volume required to run a test is 25 μL.
Finger or heel prick blood can be applied directly and immediately onto the m-PIMA™ HIV-1/2 Detect cartridge. When using EDTA anti-coagulated venous blood or plasma, the appropriate volume is transferred into the cartridge using a volumetric pipette or transfer capillary (please refer to page 12 for allowed anti-coagulants). Standard phlebotomy sample collection practices for obtaining both capillary and venous blood samples are to be followed.
After applying the sample, the cartridge cap is snapped into place, eliminating the chance of sample spillage or contamination of the instrument. After closing the cap the cartridge is inserted into the m-PIMA™ Analyser and the test is initiated automatically. The steps described in the following subsections are performed automatically by the m-PIMA™ Analyser within the cartridge.
RNA Isolation
The RNA isolation consists of following steps:

a  Complete lysis of the sample based on chaotropic salts in order 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, thus 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 that 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 facilitate simultaneous detection of more than one specific nucleic acid sequence a multiplex PCR is performed. Target amplification between HIV-1 group M/N and group O, and HIV-2 is facilitated by specific primer pairs. In addition the primer pairs allow for the amplification of internal process controls.

Detection
Detection of PCR product is based on Competitive Reporter Monitored Amplification technology utilizing an array of immobilized oligonucleotide probes and complementary fluorescently labeled reporter oligonucleotides in solution. In order to maximize initial signal intensity, reporters used in this reaction 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 that 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 and 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 binds to them. In addition, the solid support to which the oligonucleotide probe is attached introduces a diffusion barrier which significantly reduces the hybridization rate. Thus, generally, solution phase reactions are kinetically favoured to solid phase reactions. Therefore the amount of reporter hybridized to the complementary probe decreases proportionally to the formation of new amplicons. This decrease is observed until a plateau in the amplification reaction is reached. Measuring the change in signal intensity of each probe can be achieved by imaging the fluorescence pattern on the array during the amplification process. Fluorescence images are collected during the annealing phase of each amplification cycle. After acquiring the hybridization pattern an algorithm is applied that is able to identify and eliminate different noise signals from the data obtained by the array assisted real-time PCR. 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 a control window. The cartridge also consists of several internal compartments containing dry reagents and an onboard buffer reservoir. The compartments of the cartridge are connected through a micro-fluidic network and air/liquid movement within the cartridge is regulated by the m-PIMA™ Analyser through valves within the cartridge. The RT-PCR reaction takes place within the reactor chamber of the cartridge. All liquid waste produced during the test is sealed within the cartridge.

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).

Risks regarding PCR template contamination of samples, instrument, cartridge or the environment are minimized as the test is designed to capture un-spliced RNA whereas amplicons generated in the test process represent short targets downstream of the capture binding sites.
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, an error message will be displayed by the m-PIMA™ Analyser 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 sample loading.

At the start of every test run the m-PIMA™ Analyser checks whether sample has been loaded into the m-PIMA™ HIV-1/2 Detect cartridge via the sample control window. If insufficient sample has been loaded into the test cartridge the analysis will not start and an error message is displayed. In order to allow sample presence detection of colorless sample such as plasma, a dye is applied onto the inner surface of the sample capillary. The dye mixes with the sample upon contact and allows sample detection.

Assay Process Controls
Each test cartridge has built in process controls ensuring for 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 and 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 and will be distinguished during detection 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 and therefore 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 not being 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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>% (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Capillary (coated with)</td>
<td></td>
</tr>
<tr>
<td>K$_2$EDTA*2H$_2$O</td>
<td>65 %</td>
</tr>
<tr>
<td>Brilliant Black</td>
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<tr>
<td>Lysis Mixture (tablet, embedded in cartridge)</td>
<td></td>
</tr>
<tr>
<td>Guanidin-HCl</td>
<td>89.7 %</td>
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<tr>
<td>N-Lauroylsarcosine</td>
<td>0.92 %</td>
</tr>
<tr>
<td>Na$_2$EDTA*4H$_2$O</td>
<td>1.23 %</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Antifoam BC 2527</td>
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</tr>
<tr>
<td>Proteinase K Tablet (tablet, embedded in cartridge)</td>
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</tr>
<tr>
<td>Proteinase K</td>
<td></td>
</tr>
<tr>
<td>Capture-oligonucleotides/internal process controls (on solid support,</td>
<td></td>
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<tr>
<td>embedded in cartridge)</td>
<td></td>
</tr>
<tr>
<td>Oligonucleotides with biotin residues</td>
<td></td>
</tr>
<tr>
<td>Tris-HCl</td>
<td></td>
</tr>
<tr>
<td>Na$_2$EDTA*2H$_2$O</td>
<td></td>
</tr>
<tr>
<td>Tris</td>
<td></td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td></td>
</tr>
<tr>
<td>BSA, acetylated</td>
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</tr>
<tr>
<td>artificial virus RNA</td>
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<td>RT-PCR Mixture 1 (pellet, embedded in cartridge)</td>
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<tr>
<td>anti-Taq-antibody</td>
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<td>Taq polymerase</td>
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<td>Reverse Transcriptase</td>
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<td>Tris-HCl</td>
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<tr>
<td>dNTP (dATP, dCTP, dGTP, dTTP) mix</td>
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<tr>
<td>RNase Inhibitor</td>
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<tr>
<td>RT-PCR Mixture 2 (pellet, embedded in cartridge)</td>
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<tr>
<td>Oligonucleotides</td>
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<tr>
<td>Oligonucleotides with Cy5 residues</td>
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</tr>
<tr>
<td>Tris-HCl</td>
<td></td>
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<tr>
<td>Na$_2$EDTA*2H$_2$O</td>
<td></td>
</tr>
<tr>
<td>Streptavidin-Sepharose (solid particles, embedded in cartridge)</td>
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<tr>
<td>Streptavidin-Sepharose</td>
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<tr>
<td>Washing Mixture (tablet, embedded in cartridge)</td>
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<tr>
<td>Guanidin-HCl</td>
<td>93.3 %</td>
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<td>Na$_2$EDTA*4H$_2$O</td>
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<td>0.005 %</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Triton X-100</td>
<td></td>
</tr>
<tr>
<td>Tween 20</td>
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</tr>
<tr>
<td>KCl</td>
<td></td>
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<td>Tris</td>
<td></td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td></td>
</tr>
<tr>
<td>ultrapure water</td>
<td></td>
</tr>
</tbody>
</table>
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.

The use of the m-PIMA™ HIV-1/2 Detect cartridge is limited to personnel trained to perform the assay.

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, and change gloves after each sample collection and before handling a new cartridge (please see pages 14 and 15 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

1. Only use m-PIMA™ HIV-1/2 Detect test cartridges at ambient temperatures of 10 - 40 °C and relative humidity below 90 %.

2. 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 result.

3. When using heel prick samples make sure that the pricking site is clean and not contaminated with maternal blood.

4. 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.

5. DO NOT attempt to re-open a closed cap. Damaged caps can lead to incomplete cartridge closure and an invalid test result.

6. DO NOT touch the transparent foil cover of the reactor chamber. Damaged or soiled covers can lead to an invalid test result.

7. 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:

1. Always wear powder free gloves when handling or collecting specimens or test cartridges, and change gloves after each sample collection and before handling a new cartridge (please see pages 14 and 15 for details).

2. 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.

3. 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.

4. Proper aseptic technique should always be used when working with RNA.

5. Amplification technologies such as PCR are sensitive to accidental introduction of product from previous amplification reactions.
Incorrect results could 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 ambient temperature (4-30 °C). The surrounding temperature may lie outside this range for a limited period of time (i.e. up to 48 hours at 2 °C and up to 72 hours at 40 °C). Once removed from their protective pouch, cartridges are stable for up to 10 minutes at 40 °C and 90 % 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**

**Basic Workflow**

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

2. 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.

3. Apply sample onto the m-PIMA™ HIV-1/2 Detect cartridge. Enough sample is applied when the sample control window is filled with blood.

4. Once sample loading is completed, close the cartridge by capping the capillary with the cartridge cap as shown in the images 11-14 of the center fold. Do not insert the cartridge into the device before ensuring that the cap is securely in place.

5. Filled cartridges need to be processed immediately after sample loading.

6. Press «Run Test» on the m-PIMA™ Analyser and 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.

7. Remove the cartridge when prompted by the m-PIMA™ Analyser (step 1). Always wear gloves when removing a cartridge from the instrument! The test result is displayed on the instrument screen.

8. 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 above the cartridge and off your fingers (step 2 and 3). Hold glove with sealed cartridge in your right hand fist.
   - Insert one or two fingers of your left ungloved hand under the inside rim on the palm side of the right glove; push glove inside out and down onto the fingers and over the left glove and cartridge (step 4).
   - Grasp the gloves, which are now together and inside out sealing the cartridge, with your left hand and remove from your right hand (step 5).
   - Discard as biohazard waste (step 6).
How to discard used test cartridges
(Please find a detailed description on previous page.)

1

2

3

4

5

6
Heel Prick Sample Collection \(^{(24, 25)}\)

Selection of a site for capillary sampling in a pediatric 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.

1. It is recommended to comfort the baby. Ask the parent to assist. Ensure the baby is cuddled and in a secure position for taking the sample.
2. Ensure the baby is warm and comfortable. Additional warming of the foot is not required.
3. 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.
4. If the cartridge is filled directly from the pricked heel, continue with step 5. If a transfer capillary is used, please refer to the section on sample application via transfer capillary (page 18).
5. Remove one m-PIMA™ HIV-1/2 Detect cartridge from its foil pouch and open the plastic cap to fully expose the sample capillary.
6. 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 3 of the center fold). 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, gentle massaging of the heel may be conducted in order to ensure a steady blood flow.
7. Wipe off the first drops 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.
8. 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. Enough sample is applied when the sample control window is filled with blood. Then remove the cartridge from the heel and let the parent apply direct pressure to the wound side with a clean dry swab.
9. Continue with step 4 of the Basic Workflow description (page 14).
10. Apply a band aid to the infant’s heel.
**Finger Prick Sample Collection** *(26)*

1. 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 center 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 fifth 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.

2. 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.

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

4. If the cartridge is filled directly from the pricked finger, continue with step 5. If a transfer capillary is used, please refer to the section on sample application via transfer capillary (page 18).

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

6. Use a sterile lancet to make a skin puncture just off the center 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, gentle massaging of the finger may be conducted in order to ensure a steady blood flow.

7. Wipe off the first drops 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.

8. 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. 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.

9. Continue with step 4 of the Basic Workflow description (page 14).

10. Apply a band aid to the patient’s finger.
Venous Whole Blood Sample Collection \(^{(27)}\)

1. 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.

2. Invert collection tube 8 – 10 times.

3. Store at ambient temperature (18 – 28 °C). The sample must be analyzed within 24 hours of draw. If samples need to be stored for longer periods, please refer to the section on Sample Stability (page 21) for detailed information.

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

5. If plasma needs to be tested, collection tubes should be centrifuged at 800-1600 x g for 20 minutes at ambient temperature.

6. If the cartridge is filled with a volumetric pipette, continue with step 7. If a transfer capillary is used, please refer to the section on sample application via transfer capillary (see below).

7. 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.

8. Apply 25 µL into the sample capillary of the m-PIMA™ HIV-1/2 Detect cartridge and continue with step 4 of the Basic Workflow description (page 14).

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 capillaries without additional anticoagulant. For blood collected directly from a pricked finger or heel, always use capillaries containing EDTA (please refer to page 3 for information regarding available capillary options).

Always use a new transfer capillary for every patient!

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

2. Allow the capillary to fill approximately half way without trapping air bubbles.

3. Close the opposite end of the transfer capillary with your finger to prevent unwanted release of the sample.
4. Put the open end of the transfer capillary in contact with the sample capillary of the test cartridge and hold the transfer capillary almost vertically. 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.

5. Dispose of the transfer capillary as potentially infectious materials in accordance with local, state and federal regulations and continue with step 4 of the Basic Workflow description (page 14).

**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, instrument, assay process and data analysis QC information.
- Test results can be either exported, transmitted to a remote server using Connectivity Pack IV or printed using the USB Printer available as accessories to 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 20).
Test Result Reporting (examples)

Test Report for HIV positive results  
Test Report readout if no HIV is detected

**Note:** The indicated test result summary lines “HIV detected (positive)” and “HIV not detected” have been introduced with m-PIMA™ Analyser software version 0.26.3. Previous software versions display individual analyte test results only.

**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 “Undetected” 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. Though rare, mutations within the highly conserved region of the viral genome covered by the primers and/or probes used for m-PIMA™ HIV-1/2 Detect may result in the failure to detect the virus.\cite{28, 29}

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 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.

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 below) with corresponding plasma viral loads between 0 and 2491 cp/mL (Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 version 2.0) were analyzed. 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 and 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, samples should be aliquoted and frozen at least at -80 °C either as whole blood or plasma within 24 hours after draw. 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 M/N, HIV-1 O and/or HIV-2 in the same patient sample. The capability of multiplex testing is potentially affected by viral load ratios that exceed the ones which were tested by Alere Technologies GmbH. In case of larger viral load differences, the ability to detect the analyte present in lower concentration may be reduced.

**PERFORMANCE CHARACTERISTICS**

Performance characteristics of the m-PIMA™ HIV-1/2 Detect test were established by testing at Alere Technologies GmbH in Jena, Germany, and at external sites in Mozambique, Uganda, the United States of America and Germany. 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 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 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 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 128 samples of early seroconversion were purchased from Helvetica Health Care, Geneva, Switzerland and tested at Alere Technologies GmbH. For each plasma sample positivity for HIV-1 was determined using the Abbott ARCHITECT® HIV Ag/Ab Combo.

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) were tested at Alere Technologies GmbH. The clinical samples have been tested negative for HIV-1/2 antibodies, HIV-1 NAT, HBV 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).

Diagnostic sensitivity in HIV-1 fresh and frozen whole blood and plasma samples
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
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 of the 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
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. m-PIMA™ HIV-1/2 Detect detected HIV-1 in 46 out of the total 128 panel members compared to 42 out of 128 detected by Abbott ARCHITECT® HIV Ag/Ab Combo. In 4 out of 10 panels m-PIMA™ HIV-1/2 Detect detected HIV-1 M/N earlier, up to 4 days, than the reference. For detailed results see table 1.
Table 1: Seroconversion Sensitivity

<table>
<thead>
<tr>
<th>Panel</th>
<th>Number of panel members tested</th>
<th>Number of panel members detected</th>
<th>Days to first HIV detected</th>
<th>Difference in days to first HIV detected (m-PIMA™ minus Abbott)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>m-PIMA™ HIV-1/2 Detect</td>
<td>Abbott ARCHITECT® HIV Ag/Ab Combo</td>
<td>m-PIMA™ HIV-1/2 Detect</td>
</tr>
<tr>
<td>12007</td>
<td>9</td>
<td>6</td>
<td>6</td>
<td>117</td>
</tr>
<tr>
<td>6247</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>9016</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>9018</td>
<td>11</td>
<td>3</td>
<td>3</td>
<td>28</td>
</tr>
<tr>
<td>9020</td>
<td>22</td>
<td>4</td>
<td>3</td>
<td>87</td>
</tr>
<tr>
<td>9022*</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td>9024</td>
<td>12</td>
<td>2</td>
<td>1</td>
<td>49</td>
</tr>
<tr>
<td>9025</td>
<td>12</td>
<td>2</td>
<td>2</td>
<td>85</td>
</tr>
<tr>
<td>9076</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>66</td>
</tr>
<tr>
<td>9079</td>
<td>25</td>
<td>17</td>
<td>17</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>128</td>
<td>46</td>
<td>42</td>
<td>543</td>
</tr>
</tbody>
</table>

*No Abbott ARCHITECT® HIV Ag/Ab Combo results were provided for the samples 9022-01 and 9022-07, but both Abbott PRISM HIV Ag/Ab Combo and Abbott Murex HIV-1 Ab/Ag Combo were non-reactive.

Diagnostic specificity on frozen whole blood and plasma samples

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 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) [99.51 %, 100 %] and 100 % (603/603) [99.50 %, 100 %], respectively.
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 Alere Technologies the analytical sensitivity of m-PIMA™ q HIV-1/2 Detect was determined by analyzing the lower limit of detection 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. Cartridges from three different lots were used. Commercially obtained HIV-1 group M and HIV-2 group A was used. For HIV-1 group O, virus was purified from cell culture supernatant at Alere Technologies GmbH. These analytes were spiked at defined concentration into HIV negative venous whole blood samples from cohort H at concentrations outlined in tables 2, 3 and 4.

During the WHO evaluation of m-PIMA™ HIV-1/2 Detect for listing on the WHO list of prequalified in vitro diagnostic products the limit of detection was determined using the WHO 3rd HIV-1 International Standard spiked in whole blood.[31] The conversion factors between virus RNA copies and International Units (IU) are 1:1.67 for HIV-1 group M/N based on the 1st 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.

The concentrations in cp/mL and IU/mL [95 % confidence intervals] of virus RNA that can be detected with a positivity rate greater than 95 % as determined by Probit analysis are as follows:*

- HIV-1 group M (IIIB Strain): 2491 cp/mL [2046 cp/mL, 3319 cp/mL] and 4160 IU/mL [3417 IU/mL, 5543 IU/mL]
- HIV-1 group M (WHO 3rd Int. Standard): 1759 cp/mL [1286 cp/mL, 3640 cp/mL] and 2937 IU/mL [2147 IU/mL, 6079 IU/mL]
- HIV-1 group O (MVP5180 Strain): 943 cp/mL [790 cp/mL, 1262 cp/mL]
- HIV-2 group A (NIHZ Strain): 952 cp/mL [794 cp/mL, 1239 cp/mL] and 524 IU/mL [437 IU/mL, 681 IU/mL]

*Representative data: results in individual laboratories may vary from these data.
### Table 2: HIV-1 group M (IIIB Strain)

<table>
<thead>
<tr>
<th>Concentration (cp/mL)</th>
<th>N_{valid}</th>
<th>N_{detected}</th>
<th>Percent detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>640</td>
<td>83</td>
<td>44</td>
<td>53%</td>
</tr>
<tr>
<td>1080</td>
<td>79</td>
<td>52</td>
<td>66%</td>
</tr>
<tr>
<td>1920</td>
<td>82</td>
<td>74</td>
<td>90%</td>
</tr>
<tr>
<td>3320</td>
<td>80</td>
<td>79</td>
<td>99%</td>
</tr>
<tr>
<td>5760</td>
<td>82</td>
<td>82</td>
<td>100%</td>
</tr>
<tr>
<td>10000</td>
<td>85</td>
<td>85</td>
<td>100%</td>
</tr>
</tbody>
</table>

### Table 3: HIV-1 group O (MVP5180 Strain)

<table>
<thead>
<tr>
<th>Concentration (cp/mL)</th>
<th>N_{valid}</th>
<th>N_{detected}</th>
<th>Percent detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>74</td>
<td>48</td>
<td>65%</td>
</tr>
<tr>
<td>720</td>
<td>80</td>
<td>67</td>
<td>84%</td>
</tr>
<tr>
<td>1240</td>
<td>72</td>
<td>72</td>
<td>100%</td>
</tr>
<tr>
<td>2120</td>
<td>81</td>
<td>81</td>
<td>100%</td>
</tr>
<tr>
<td>3720</td>
<td>85</td>
<td>85</td>
<td>100%</td>
</tr>
<tr>
<td>6440</td>
<td>80</td>
<td>80</td>
<td>100%</td>
</tr>
</tbody>
</table>

### Table 4: HIV-2 group A (NIHZ Strain)

<table>
<thead>
<tr>
<th>Concentration (cp/mL)</th>
<th>N_{valid}</th>
<th>N_{detected}</th>
<th>Percent detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>240</td>
<td>78</td>
<td>30</td>
<td>38%</td>
</tr>
<tr>
<td>400</td>
<td>83</td>
<td>56</td>
<td>67%</td>
</tr>
<tr>
<td>720</td>
<td>81</td>
<td>70</td>
<td>86%</td>
</tr>
<tr>
<td>1240</td>
<td>78</td>
<td>77</td>
<td>99%</td>
</tr>
<tr>
<td>2200</td>
<td>81</td>
<td>81</td>
<td>100%</td>
</tr>
<tr>
<td>3800</td>
<td>78</td>
<td>78</td>
<td>100%</td>
</tr>
</tbody>
</table>
Analytical specificity

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 specimen-related conditions like noninfectious diseases, medical conditions or exogenous substances such as drugs. These substances were either spiked into the samples or 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 5 and 6).

Table 5: List of pathogens: non-clinical samples

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTLV-1</td>
<td>Salmonella Enteritidis</td>
</tr>
<tr>
<td>HTLV-2</td>
<td>Salmonella Typhimurium</td>
</tr>
<tr>
<td>HBV</td>
<td>Salmonella Paratyphi</td>
</tr>
<tr>
<td>HCV</td>
<td>Staphylococcus epidermidis</td>
</tr>
<tr>
<td>HAdV</td>
<td>Streptococcus pneumoniae</td>
</tr>
<tr>
<td>HSV-1 (HHV-1)</td>
<td>Streptococcus mutans</td>
</tr>
<tr>
<td>HSV-2 (HHV-2)</td>
<td>MSSA</td>
</tr>
<tr>
<td>VZV (HHV-3)</td>
<td>MRSA</td>
</tr>
<tr>
<td>EBV (HHV-4)</td>
<td>Chlamydia pneumoniae</td>
</tr>
<tr>
<td>HCMV (HHV-5)</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>HHV-6</td>
<td>Propionibacterium acnes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Protozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>Toxoplasma gondii</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td></td>
</tr>
<tr>
<td>Pneumocystis jirovecii</td>
<td></td>
</tr>
</tbody>
</table>
Table 6: List of pathogens: clinical samples

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

* 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 7).
Table 7: List of endogenous substances or samples from donors with medical conditions

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

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 8).
Table 8: List of drugs

<table>
<thead>
<tr>
<th>HIV drugs</th>
<th>Nucleoside/Nucleotide Analogue Inhibitors of Reverse Transcriptase</th>
<th>Integrase Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protease Inhibitors</strong></td>
<td>Lopinavir, LPV</td>
<td>Ritonavir</td>
</tr>
<tr>
<td>Lopinavir, LPV</td>
<td>Abacavir sulfate, ABC</td>
<td></td>
</tr>
<tr>
<td>Ritonavir</td>
<td>Emtricitabine, FTC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stavudine, d4T</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tenofovir disoproxil fumarate, TDF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lamivudine, 3TC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zidovudine, AZT</td>
<td></td>
</tr>
<tr>
<td><strong>Non-Nucleoside/Nucleotide Analogue Inhibitors of Reverse Transcriptase</strong></td>
<td>Efavirenz, EFV</td>
<td></td>
</tr>
<tr>
<td>Efavirenz, EFV</td>
<td>Nevirapine, NVP</td>
<td></td>
</tr>
<tr>
<td><strong>HCV/HBV drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Immune Modulator</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribavirin</td>
<td>Acyclovir</td>
<td></td>
</tr>
<tr>
<td>Peginterferon alfa-2a</td>
<td>Ganciclovir</td>
<td></td>
</tr>
<tr>
<td>Peginterferon alfa-2b</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Compounds for treatment of Herpes Viruses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Anti Fungal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Anti Fungal/ Bacterial</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Anti Mycobacterial</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoniazid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifampicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethambutol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 (group M subtypes A through H, group N, group O and Circulating Recombinant Forms) and 8 HIV-2 isolates (HIV-2 group A, B, A/B and an indeterminate subtype). All isolates used in this study were members of subtype panels provided by the German National Reference Center for Retroviruses (GNRCR), Erlangen, Germany.
Viruses were cultivated on cells and supernatants were diluted in HIV-negative plasma and quantified using different real time PCR assays at GNRCR. HIV-samples were originally obtained from virus isolates contributed by the NIH AIDS Research and Reference Reagent Program, USA or from the Program EVA Centre for AIDS Reagents, NIBSC, UK. The specimens were used at Alere Technologies GmbH without further dilution. All subtypes were successfully detected by m-PIMA™ HIV-1/2 Detect. Neither false positive nor false negative results for HIV-1 group M/N, HIV-1 group O or HIV-2 were observed. 

For details on the tested isolates see tables 9 and 10:

**Table 9: HIV-1 Group and Subtype Detection of the m-PIMA™ HIV-1/2 Detect test**

<table>
<thead>
<tr>
<th>Groups/Subtypes</th>
<th>Isolate</th>
<th>HIV-1 M/N</th>
<th>HIV-1 O</th>
<th>HIV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group M/Subtype A</td>
<td>92UG029</td>
<td>10/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Group M/Subtype A</td>
<td>00KE_KER2018</td>
<td>10/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Group M/Subtype B</td>
<td>92TH026</td>
<td>10/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Group M/Subtype B</td>
<td>90TH_BK132</td>
<td>10/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Group M/Subtype C</td>
<td>92BR025</td>
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<td>Group O</td>
<td>MVP5180</td>
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<td>10/10</td>
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<td>Group O</td>
<td>CA-9</td>
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### Table 10: HIV-2 Group Detection of the m-PIMA™ HIV-1/2 Detect test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Isolate</th>
<th>HIV-1 M/N</th>
<th>HIV-1 O</th>
<th>HIV-2</th>
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<tr>
<td>Group A</td>
<td>HIV-2 CDC77618</td>
<td>0/10</td>
<td>0/10</td>
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<td>Group A</td>
<td>HIV-2 CDC310072</td>
<td>0/10</td>
<td>0/10</td>
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<td>Group A</td>
<td>HIV-2 7924A</td>
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<td>0/10</td>
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<td>0/10</td>
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<td>0/10</td>
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<td>Group A/B</td>
<td>HIV-2 7312A</td>
<td>0/10</td>
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<td>Group B</td>
<td>HIV-2 CDC310319</td>
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<td>0/10</td>
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<td>indeterminate</td>
<td>HIV-2 MIR</td>
<td>0/10</td>
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</tr>
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</table>

### Matrix effects

To evaluate potential effects of sample matrix on m-PIMA™ HIV-1/2 Detect performance, 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 analyzed with m-PIMA™ HIV-1/2 Detect at 3 days with two runs per day on 20 analyzers. Valid tests (venous whole blood: n=56; plasma: n=59) were used to analyze the matrix effect. For all tests on venous whole blood and matching plasma 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 21). 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 potential effects of the presence of multiple analytes at different concentrations in the same sample on m-PIMA™ HIV-1/2 Detect performance, defined concentrations of HIV-1 group M/N and O and HIV-2 virus preparations were spiked into HIV negative whole blood samples of presumably healthy European donors from cohort H according to the table below.

**Table 11: HIV-concentrations used to prepare multiplex samples**

<table>
<thead>
<tr>
<th>HIV Concentration</th>
<th>Spiked sample</th>
<th>Log cp/mL HIV-1 M/N</th>
<th>Log cp/mL HIV-1 O</th>
<th>Log cp/mL HIV-2</th>
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<tbody>
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<td>low</td>
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<td>5.08</td>
<td>4.08</td>
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</tr>
<tr>
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<tr>
<td></td>
<td>6</td>
<td>6.00</td>
<td>6.00</td>
<td>7.00</td>
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</table>

For all 203 tests on multiplex samples at different concentrations, HIV-1 M/N, HIV-1 O and HIV-2 were 100% successfully detected with m-PIMA™ HIV-1/2 Detect. No interference of the different analytes on each other’s detectability (at the spiked concentrations) was observed.
**Carryover**
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 (strain IIIB) samples (at a target concentration of 3x10^7 cp/mL) interspersed with negative samples (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, 6 HIV negative whole blood samples from cohort H were spiked with virus preparations of HIV-1 group M subtype B (strain IIIB) at a concentration of 8000 cp/mL. For all 348 tests on spiked venous whole blood samples performed on 8 different m-PIMA™ Analyser over the course of 6 days HIV-1 M/N was 100 % successfully detected with m-PIMA™ HIV-1/2 Detect. 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).

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

<table>
<thead>
<tr>
<th>Region</th>
<th>Number</th>
<th>Email</th>
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</thead>
<tbody>
<tr>
<td>Europe</td>
<td>+44 161 483 5884</td>
<td><a href="mailto:EME.techsupport@alere.com">EME.techsupport@alere.com</a></td>
</tr>
<tr>
<td>Russia &amp; CIS</td>
<td>+27 21 5315 999</td>
<td><a href="mailto:Afrisupport@alere.com">Afrisupport@alere.com</a></td>
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<tr>
<td>Africa</td>
<td>+61 7 3363 7166</td>
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<tr>
<td>Asia Pacific</td>
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<td><a href="mailto:technical.service@alere.com">technical.service@alere.com</a></td>
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<tr>
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<td>Latin America</td>
<td>+57 2 6618797</td>
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REFERENCES


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


