Abbott

m-PIMA™ HIV-1/2 DETECT

CARTRIDGE GUIDE

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INTRODUCTION

Virological testing using assays specifically detecting HIV DNA or RNA already plays an important role in the diagnosis of HIV infection in newborns.[1] 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.[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

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 paediatric 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

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

Store m-PIMA™ HIV-1/2 Detect cartridges at 4 - 30 °C. Refer to page 13 for further details.

Materials Required but Not Provided

m-PIMA™ Analyser (catalogue no. 27030R001) with installed software version 0.38.0 or higher.

Optional Items

Finger Stick Sample Collection Kit (100) (catalogue no. 260400199)
Neonatal Sample Collection Kit (100) (catalogue no. 270400200)
Plastic Capillaries plain (catalogue no. 270400005)

❗ 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

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 - 20).
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. 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 utilizing an array of immobilized oligonucleotide probes and complementary fluorescently labeled reporter oligonucleotides in solution.[22] 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.

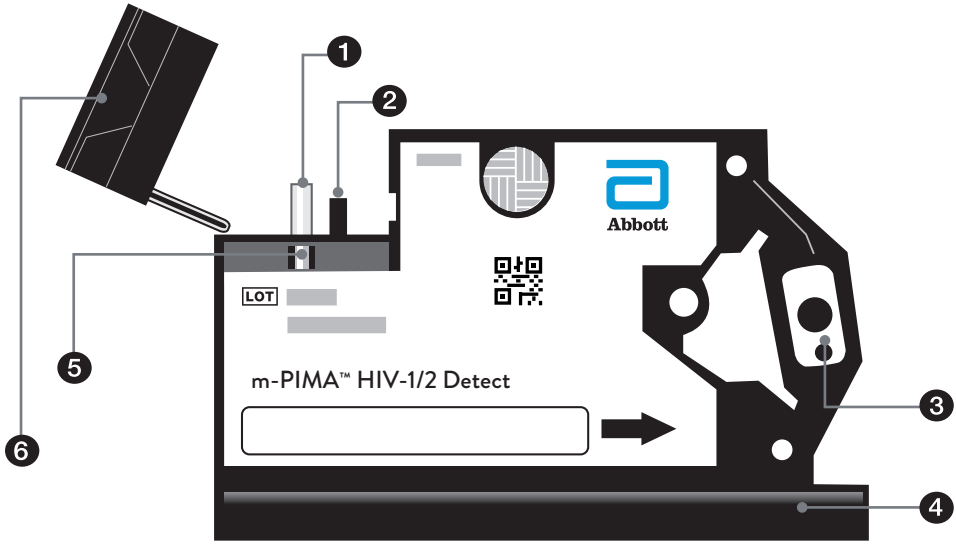
The more amplicons are synthesized the more reporter binds to them. In addition, the solid support to which the oligonucleotide probe is attached introduces a diffusion barrier which significantly reduces the hybridization rate. Thus, generally, solution phase reactions are kinetically favoured to solid phase reactions.[23] 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.



1 Sample Capillary
2 Air Channel
3 Reactor Chamber
4 Buffer Reservoir
5 Control Window
6 Cartridge Cap

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

	% (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.
- 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

- Only use m-PIMA™ HIV-1/2 Detect test cartridges at ambient temperatures of 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 result.
- 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, and change gloves after each sample collection and before handling a new cartridge (please see pages 14 and 15 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.
- Proper aseptic technique should always be used when working with RNA.
- 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

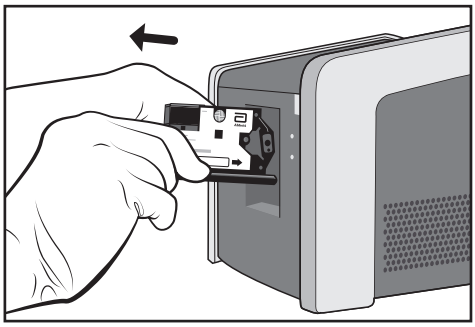
Basic Workflow

1. Switch on the m-PIMA™ Analyser and wait for the initialization to be completed. The presence of the «HOME» screen indicates the analyser 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 on page 19. 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).

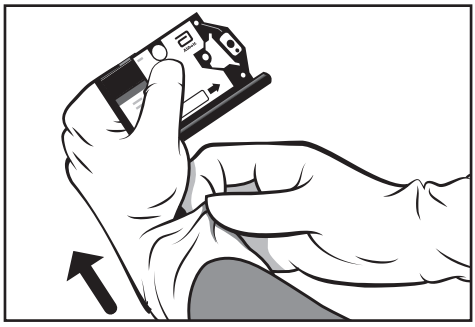
How to discard used test cartridges

(Please find a detailed description on previous page.)

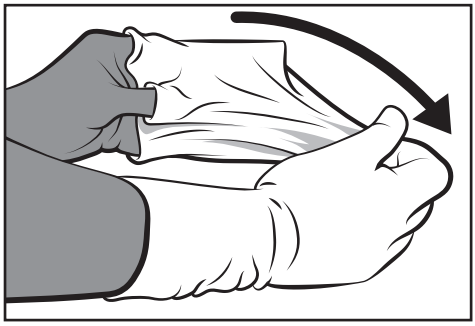
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2



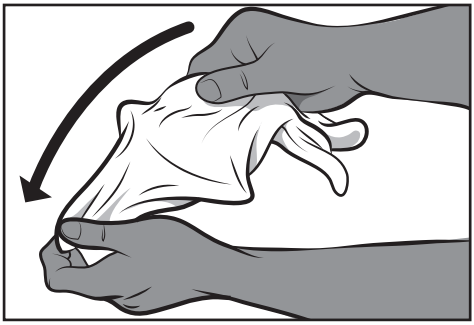
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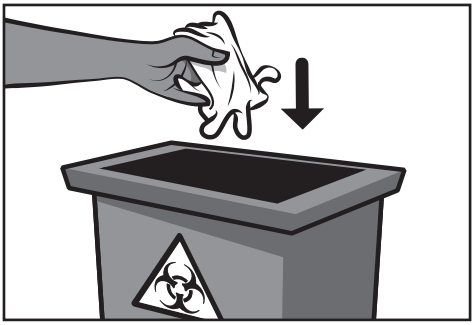
4



5



6



Finger Prick Sample Collection [26]

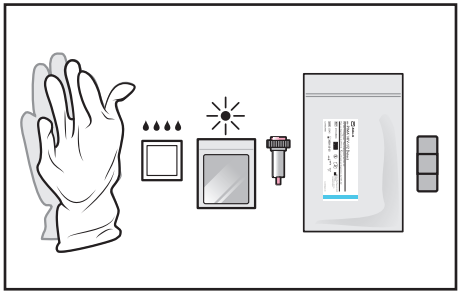
1. Always wear a new pair of gloves for every patient!
   Prepare patient for finger prick sample collection. The best locations for finger pricks are the 3rd and 4th fingers. Do not use the tip of the finger or the centre of the finger pad. Avoid the side of the finger where there is less soft tissue, 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.
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 20).
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 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, 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.

Heel Prick Sample Collection [24, 25]

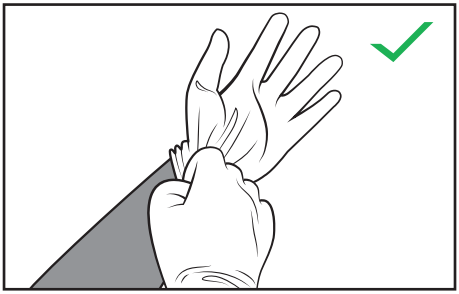
Selection of a site for capillary sampling in a paediatric patient is usually based on the age and weight of the patient. If the child is walking, the child's feet may have calluses that hinder adequate blood flow. Children older than 6 months and a body weight greater than 10 kg may be more suitable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

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 20).
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 on page 18).
   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.

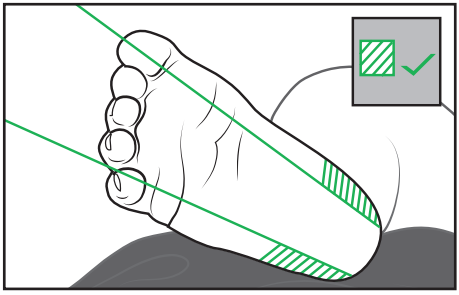
1



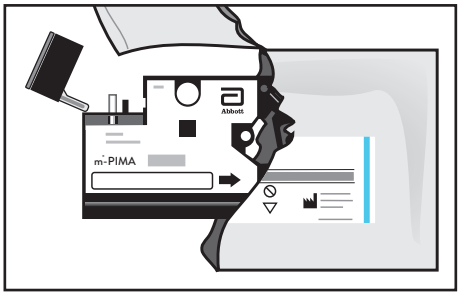
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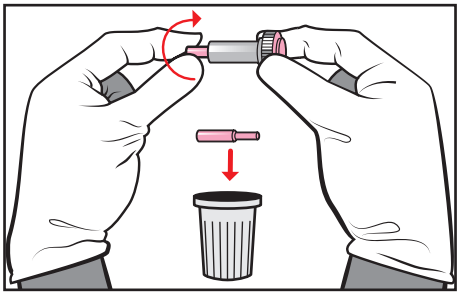
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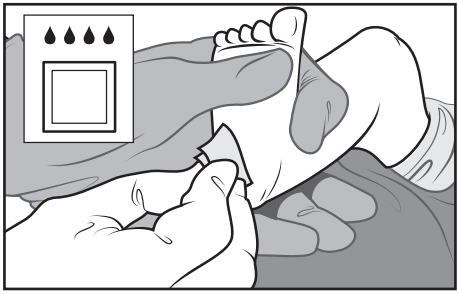
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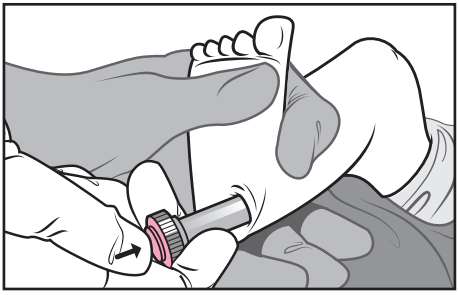
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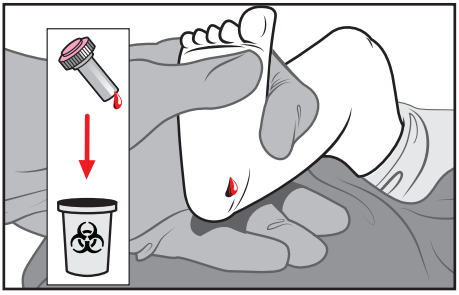
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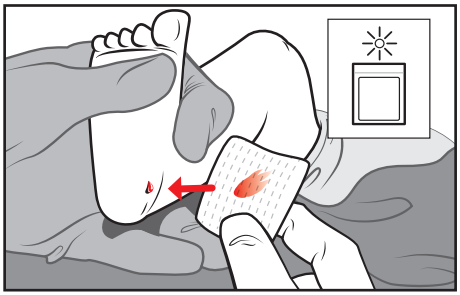
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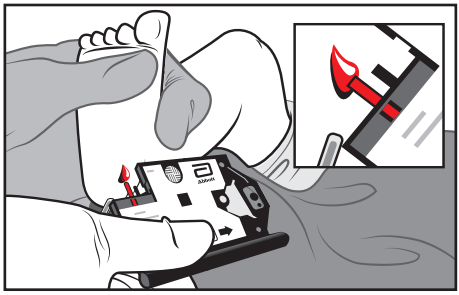
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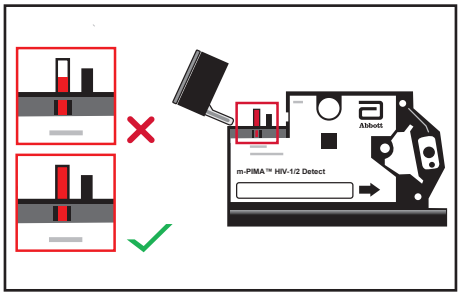
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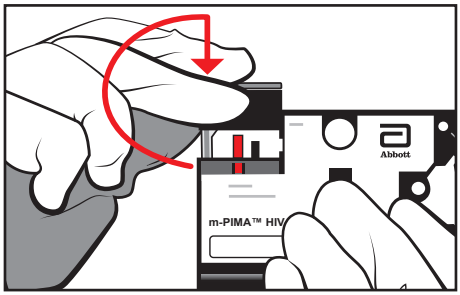
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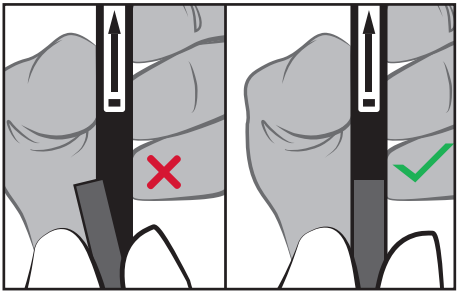
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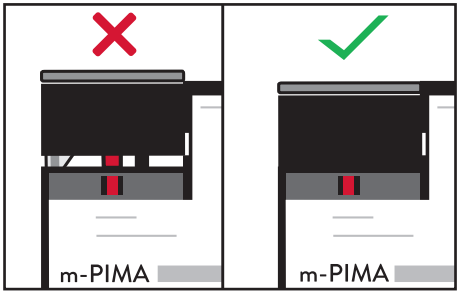
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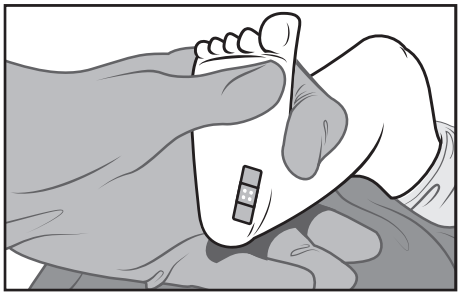
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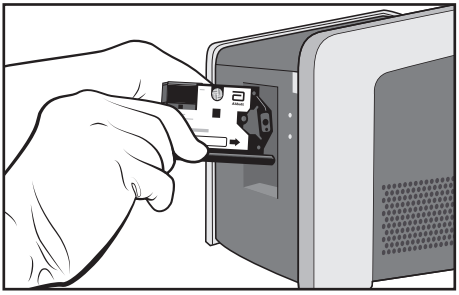
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15



16



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 analysed within 24 hours of draw. If samples need to be stored for longer periods, please refer to the section on Sample Stability (page 24) 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 (please refer to page 5 for further information). For blood collected directly from a pricked finger or heel, always use capillaries containing EDTA.
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 halfway 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 Packs or printed using the USB Printer available as accessories to the m-PIMA™ Analyser.

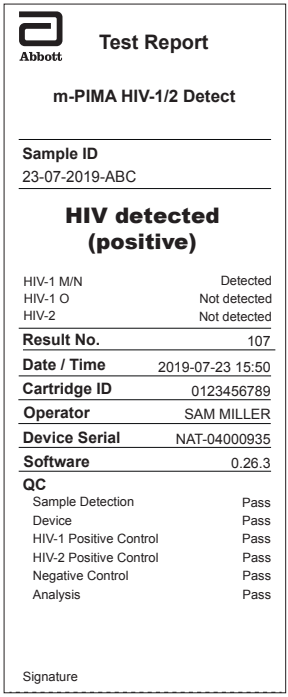
Test Result

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 22).

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



Abbott

Test Report

m-PIMA HIV-1/2 Detect

Sample ID
23-07-2019-ABC

HIV detected (positive)

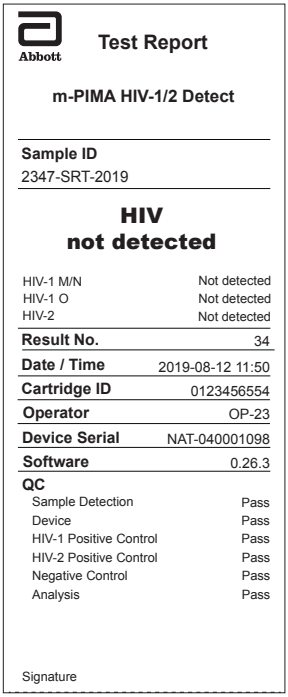
HIV-1 M/N	Detected
HIV-1 O	Not detected
HIV-2	Not detected
Result No.	107
Date / Time	2019-07-23 15:50
Cartridge ID	0123456789
Operator	SAM MILLER
Device Serial	NAT-04000935
Software	0.26.3

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



Abbott

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	2019-08-12 11:50
Cartridge ID	0123456554
Operator	OP-23
Device Serial	NAT-040001098
Software	0.26.3

QC

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

Signature

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 “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 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), 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 the 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 an increasing error rate.[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 100,000 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 below) 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 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 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 38).

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.
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 128 samples of early seroconversion were purchased from Helvetica Health Care, Geneva, Switzerland and tested at Abbott Rapid Diagnostics Jena 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 Abbott Rapid Diagnostics Jena 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

Panel	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 ARCHITECT® HIV Ag/Ab Combo	m-PIMA™ HIV-1/2 Detect	Abbott ARCHITECT® HIV Ag/Ab Combo	
12007	9	6	6	117	117	0
6247	9	3	3	21	21	0
9016	10	3	2	27	30	-3
9018	11	3	3	28	28	0
9020	22	4	3	87	90	-3
9022*	9	3	2	23	25	-2
9024	12	2	1	49	53	-4
9025	12	2	2	85	85	0
9076	9	3	3	66	66	0
9079	25	17	17	40	40	0
Total	128	46	42	543	555	

**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 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. 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 Abbott Rapid Diagnostics Jena 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)

Concentration (cp/mL)	N_{valid}	$N_{detected}$	Percent detected
640	83	44	53%
1080	79	52	66%
1920	82	74	90%
3320	80	79	99%
5760	82	82	100%
10000	85	85	100%

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

Concentration (cp/mL)	N_{valid}	$N_{detected}$	Percent detected
400	74	48	65%
720	80	67	84%
1240	72	72	100%
2120	81	81	100%
3720	85	85	100%
6440	80	80	100%

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

Concentration (cp/mL)	N_{valid}	$N_{detected}$	Percent detected
240	78	30	38%
400	83	56	67%
720	81	70	86%
1240	78	77	99%
2200	81	81	100%
3800	78	78	100%

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

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 6: 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 7).

Table 7: 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 8).

Table 8: 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 (group M subtypes A through H, group N, 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 9 and 10:

Table 9: 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 10: 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

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 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 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 23).
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 11: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)	100,000 IU/mL (59/59)
2	–* (0/58)	3x LOD (53/58)	100,000 IU/mL (58/58)
3	1,000,000 IU/mL (59/59)	–* (0/59)	3x LOD (59/59)
4	–* (1/57)	1,000,000 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 23 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 4,000 cp/mL for both HIV-1 group M and HIV-1 group O.

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 three operators performed duplicate testing using three cartridge production lots on three m-PIMA™ Analysers on six days. All experiments were performed with EDTA venous whole blood samples spiked with virus preparations of HIV-1 group M (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 % 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:

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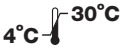
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