Alere™ Knowing now matters:

Alere Determine™ HIV-1/2 Ag/Ab Combo

Product Training

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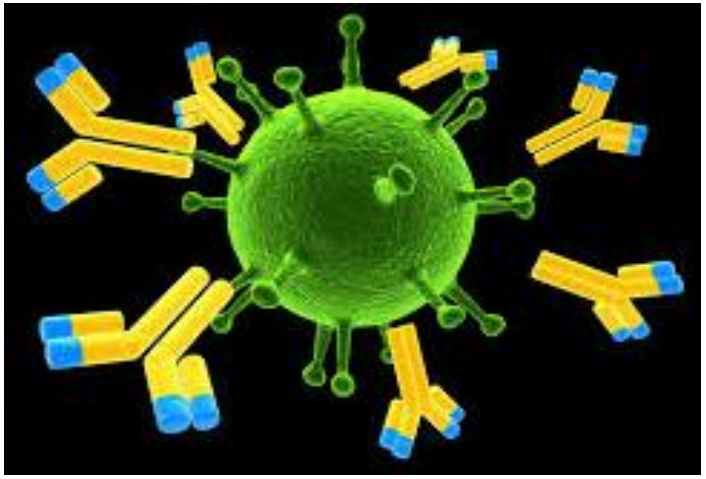
Overview

1. HIV Antigens and Antibodies
2. Product Information / Clinical Data
3. Test Procedure
4. External Controls Proficiency
5. Quiz
6. Fingerstick Demo Procedure

What Do You Know About HIV Antibodies & Antigens?

Antigen - Ag

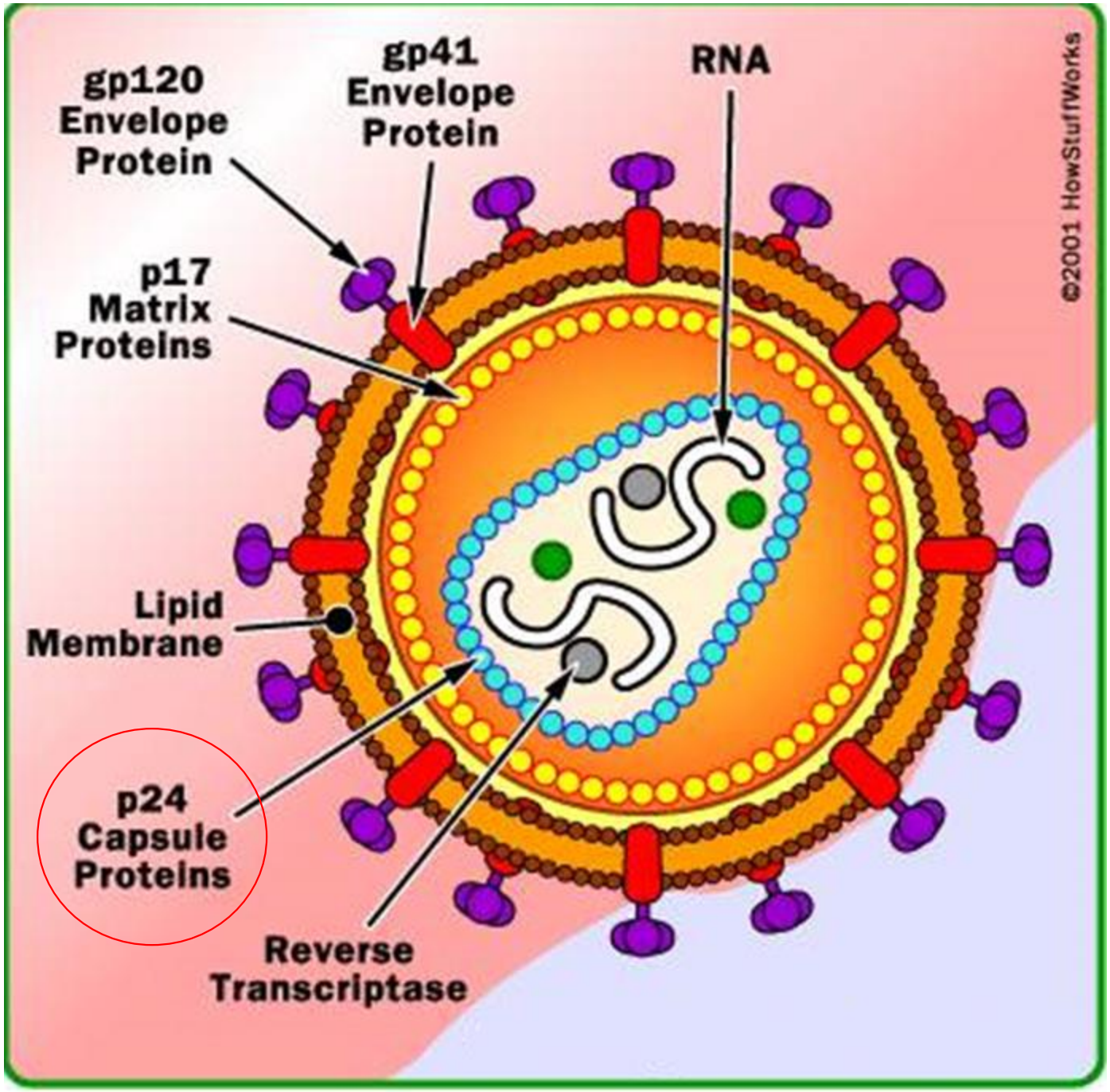
a pathogen or parts of a pathogen
(virus, bacteria, fungi and parasites) that
causes the body to produce antibodies
(detected between 2 and 4 weeks)



Antibody - Ab

a Y-shaped protein
created by your body in response to antigens
(most by 3 months, up to 6 months to be produced)

HIV ANTIGENS

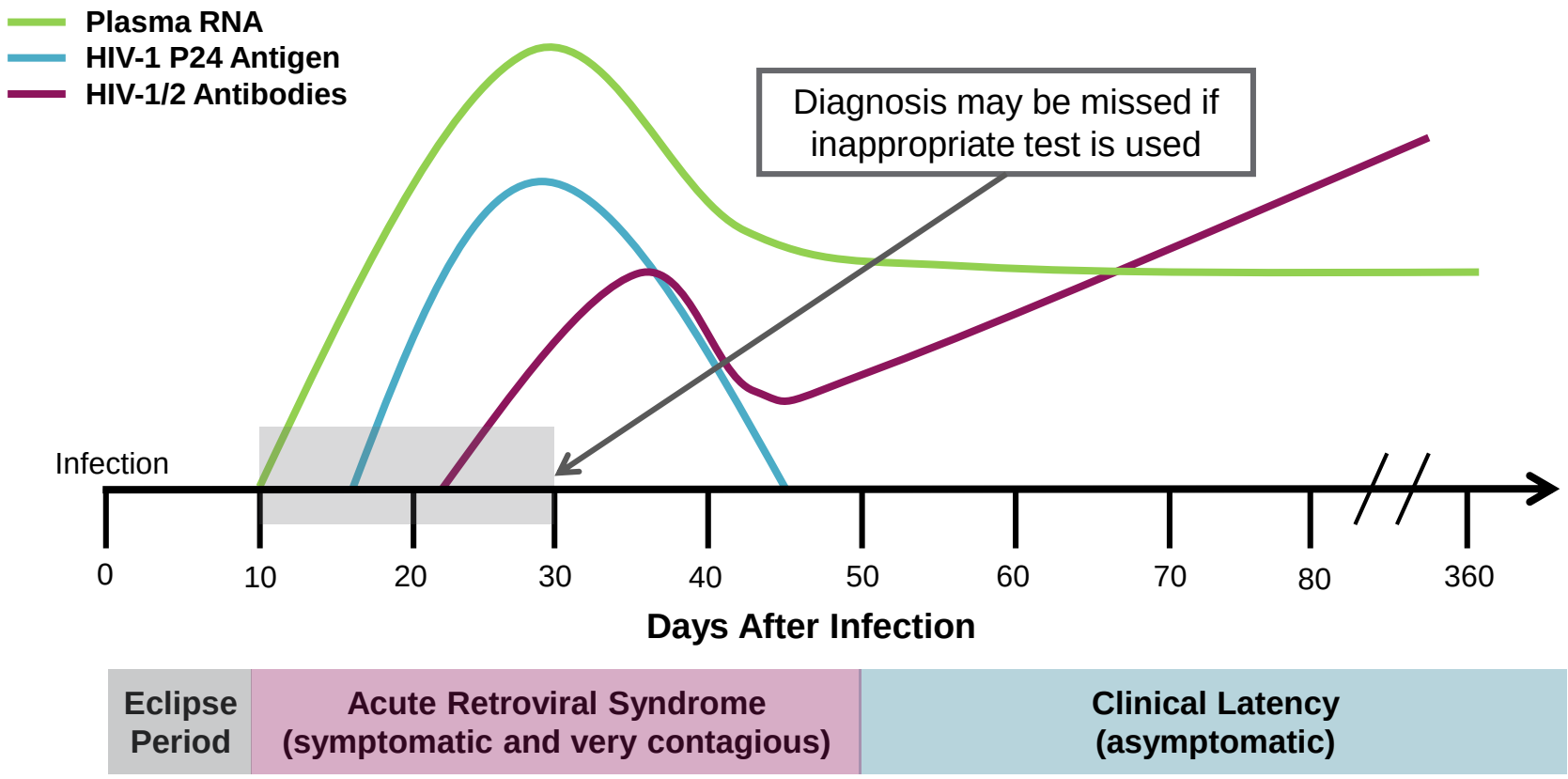


Detecting HIV earlier

- The first FDA approved rapid point-of-care test that detects both HIV-1/2 antibodies and free HIV-1 p24 antigen.
- A 4th generation test that has the ability to identify HIV earlier than 2nd and 3rd generation antibody only tests.[1]
- Detecting HIV earlier enables linkage to care sooner which may prevent onward transmission.[2]

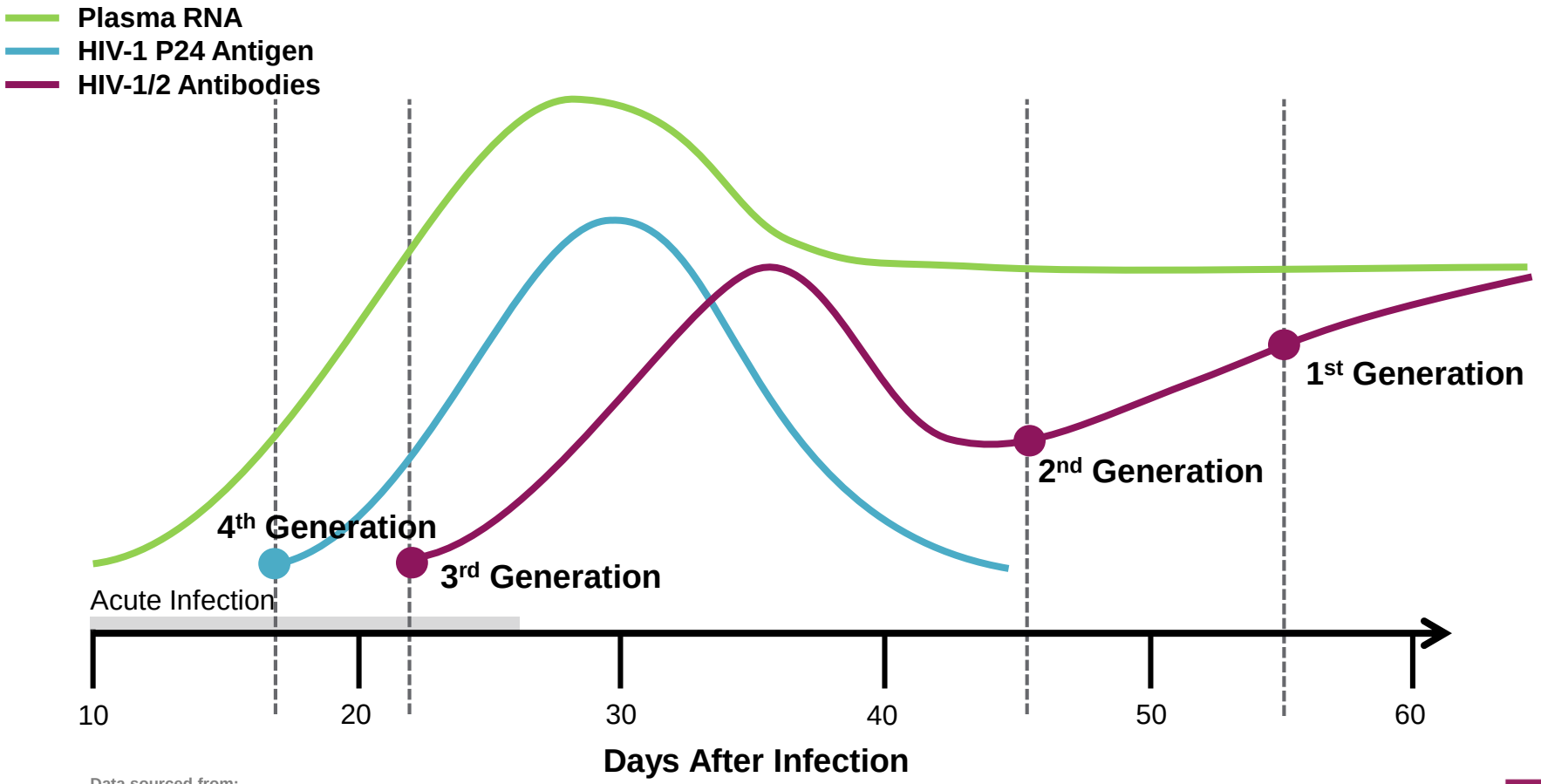
1. Masciotra S, et al. Performance of the Alere Determine™ HIV-1/2 Ag/Ab Combo Rapid Test with specimens from HIV-1 seroconverters from the US and HIV-2 infected individuals from Ivory Coast. J Clin Virol 2013. (Vol. supplement 1 pages e54-e58, DOI: 10.1016/j.jcv.2013.07.002) http://www.journalofclinicalvirology.com/article/S1386-6532(13)00277-1/fulltext.
2. Brenner BG, Roger M, Routy JP, Moisi D, Ntemgwa M, Matte C, et al. High rates of forward transmission events after acute/early HIV1 infection. J Infect Dis 2007;195:951–9.

The Progression of HIV



Adapted from: Figure 1 in Branson BM, Owen SM, Wesolowski LG, et al. Centers of Disease Control and Prevention. 2014 June 27. doi: 10.15620/cdc.23447.

Generations of HIV Tests: Days After Infection



Data sourced from:

Patel P, Mackellar D, Simmons P, et al. *Arch Intern Med.* 2010 Jan 11;170(1):66-74. DOI: 10.1001/archinternmed.2009.445

Fiebig EW, Wright DJ, Rawal BD, et al. *AIDS*. 2003 Sep 5;17(13):1871-9. DOI: 10.1097/01.aids.0000076308.76477.b8

Product Information

Product Information

Product Information

Information Type	Product Detail
Method	Lateral flow
Time to results	20 minutes
Results window	20-30 minutes after starting test
Test lines	HIV-1 p24 antigen HIV-1/2 antibodies
Storage conditions	2-30 °C (36-86 °F)
Test shelf life	18 months*
External controls shelf life	24 months*
Sample type	Whole blood/serum/plasma
Operating temperature	15 - 30°C (59 to 86°F)

CLIA-waived for Fingerstick Whole Blood
Moderate Complexity: Venipuncture Whole Blood, Serum/Plasma

*From date of manufacture

Clinical Data

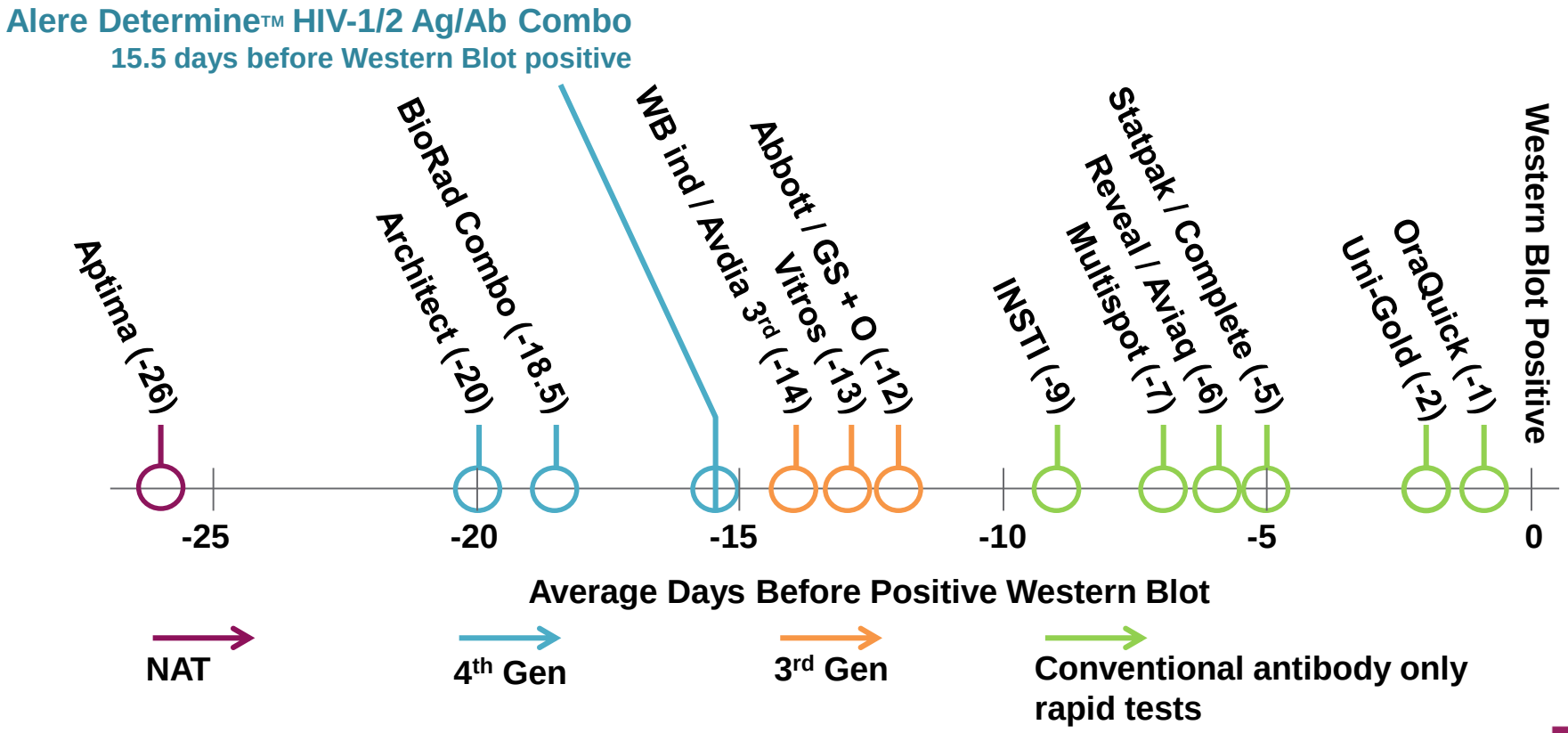
Clinical Data: Sensitivity & Specificity

Product Clinical Performance		
Sample Type	**Overall Clinical Sensitivity**	**Overall Clinical Specificity**
Fingerstick Whole Blood	99.9%	99.8%
Venous Whole Blood	99.9%	99.7%
Serum	99.9%	99.6%
Plasma	99.9%	99.7%

The sensitivity of Alere Determine™ HIV-1/2 Ag/Ab Combo for detection of antibodies to HIV-2 was estimated to be 250/250 = 100% (95% confidence interval 98.5 to 100.0%).

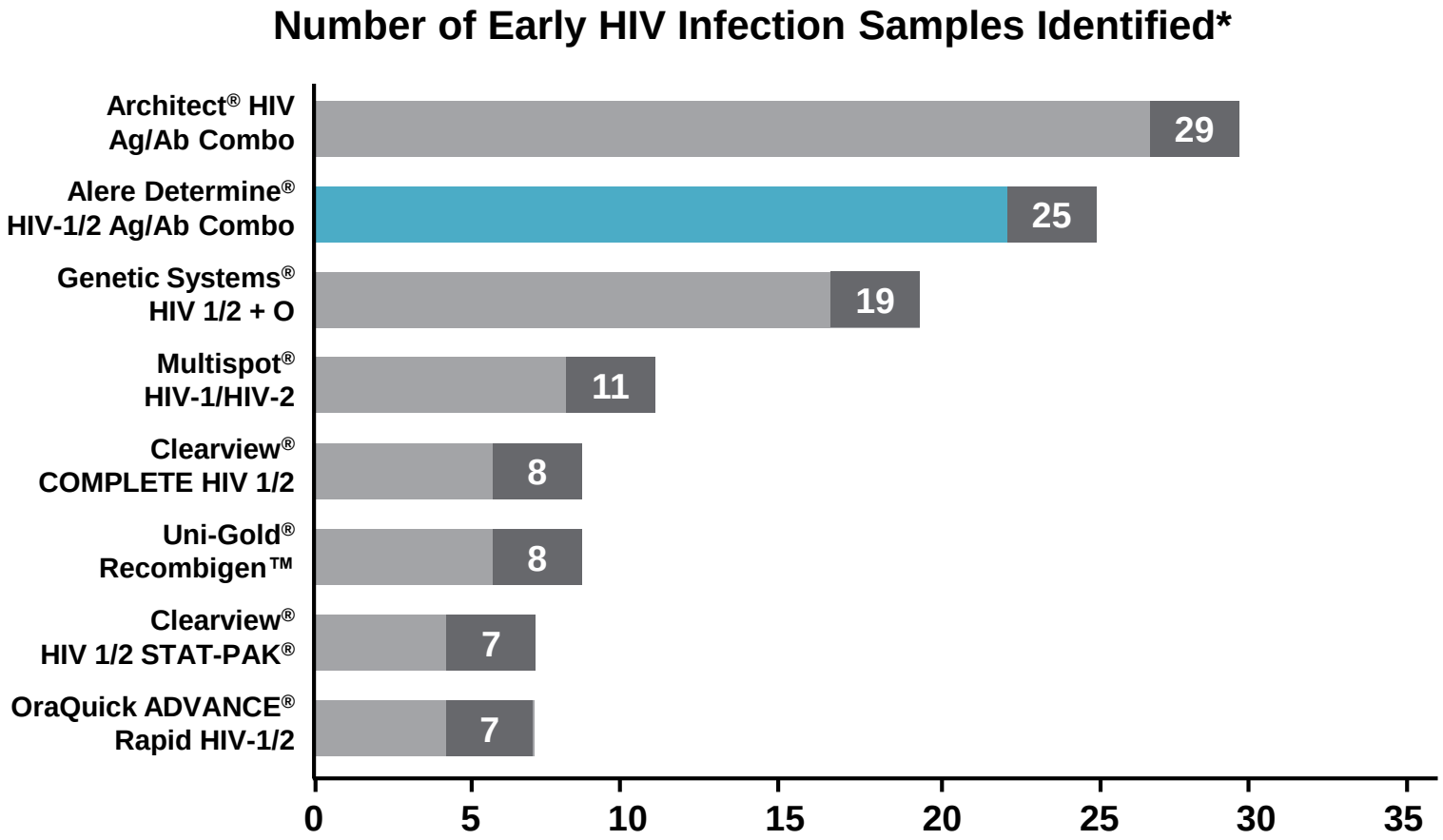
Detecting Earlier

Sensitivity of assay reactivity during early HIV-1 infections relative to number of days before first positive Western Blot



Adapted from: Masciotra S, Luo W, Youngpairoj AS, et al. *J Clin Virol*. 2013;58(Suppl 1):e54-8. doi: 10.1016/j.jcv.2013.07.002.
INSTI data sourced from: Masciotra S. HIV Diagnostics Conference, 12 December 2012, Atlanta, GA. Oral Abstract

Detecting More



*** Confirmed by NAAT**

Data sourced from: Patel P, Bennett B, Sullivan T, et al. *J Clin Virol*. 2012 May;54(1):42-7. doi: 10.1016/j.jcv.2012.01.022

Test Procedure

Materials Provided

Materials Provided

1. Alere Determine™ HIV-1/2 Ag/Ab Combo Test Cards
2. Desiccant Package
3. Chase Buffer
4. Quick Reference Card
5. Package Insert
6. Subject Information Notices
7. Customer Letter
8. Disposable Capillary Tubes
9. Disposable Workstations

1 

2 

3 

4 

5 

6 

7 

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9 

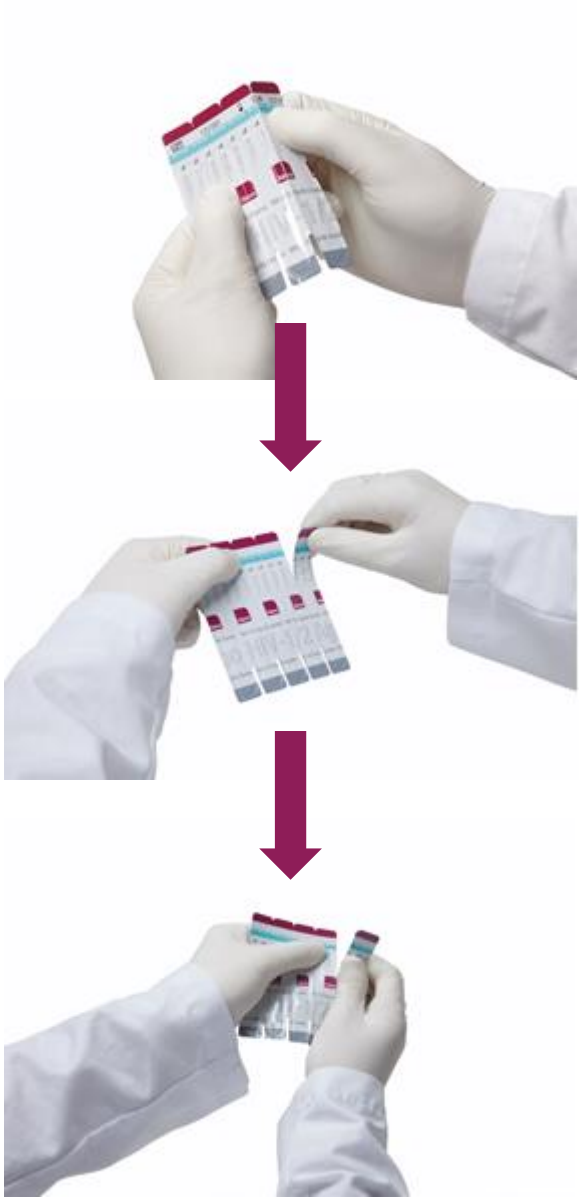
Aluminum Pouch

Test Procedure

Kit Component Preparation

1. Open the aluminum pouch containing the Alere Determine™ HIV-1/2 Ag/Ab Combo Cards.

Test Procedure



Kit Component Preparation

2. Remove the desired numbers of test units from the 5 or 10-Test Unit Card by bending and tearing at the perforation.
   NOTE: Removal of the test units should start from the right side of the Card to preserve the lot number which appears on the left side of the Card.

Test Procedure

Kit Component Preparation

3. Return the unused test units to the aluminum pouch and close the pouch with the ziplock.

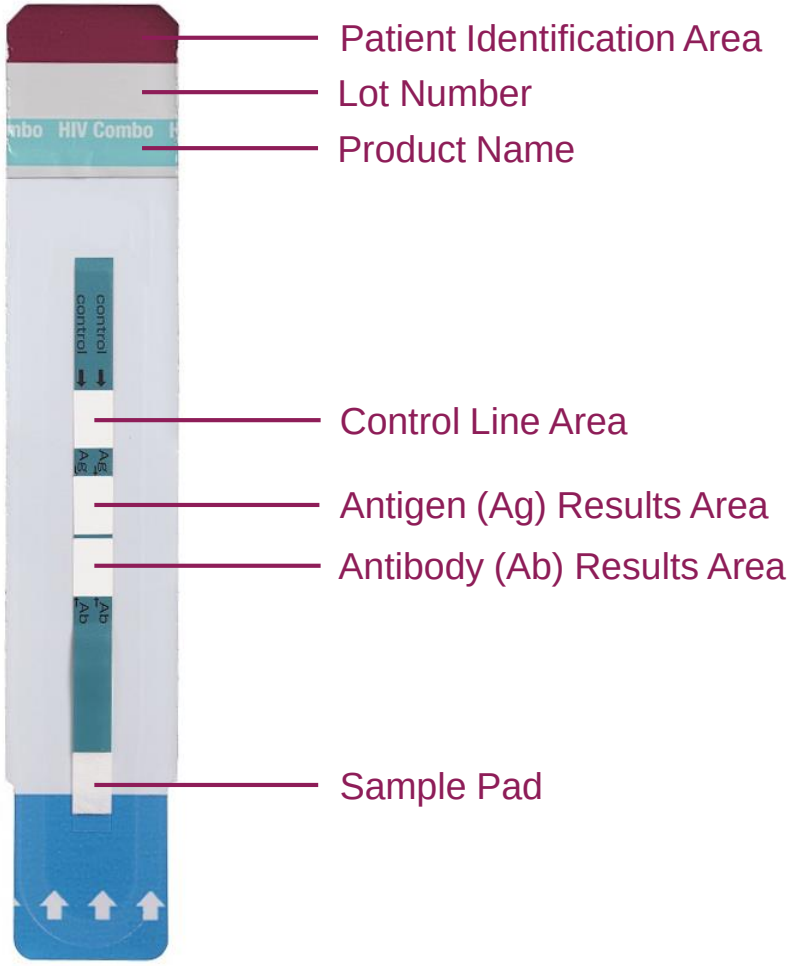
NOTE: Store the unused cards and test units only in the aluminum pouch containing the desiccant package. ***Carefully close the ziplock, so that the cards are not exposed to ambient humidity during storage.***

Test Procedure

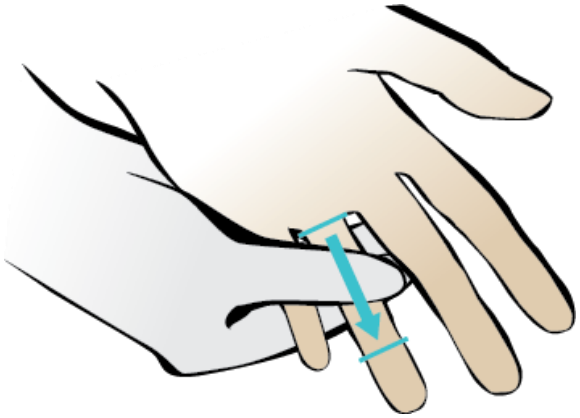
Kit Component Preparation

4. Remove the protective foil cover from each Test Unit. Lay the Test Unit flat in the workstation. The test should be initiated within 2 hours after removing the protective foil cover from each Test Unit. Do NOT touch the Sample Pad with your fingers.

Product Information



Test Procedure



For <u>whole blood (fingerstick)</u> samples using the disposable Capillary Tube provided with the kit:

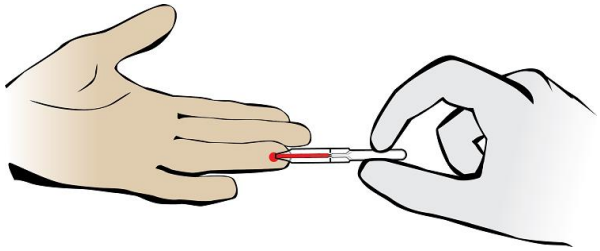
Caution: The Capillary Tube must be used to collect the fingerstick sample.

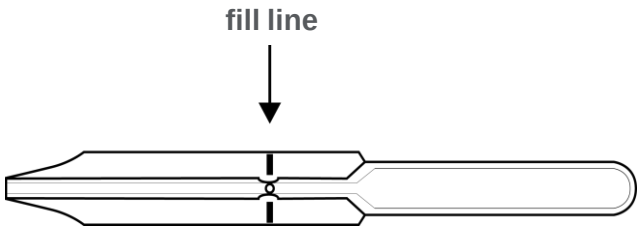
To optimize whole blood circulation:

- Warm the hand by washing in warm water (or holding it in a heating pad or hand warmer).
- Massage the finger with a downward motion several times before performing the fingerstick.

Clean the finger of the person being tested with an antiseptic wipe. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad. Using a sterile lancet capable of producing 50 µL of blood, puncture the skin just off the center of the finger pad and wipe away the first drop with sterile gauze.

Test Procedure





For <u>whole blood (fingerstick)</u> samples using the disposable Capillary Tube provided with the kit:

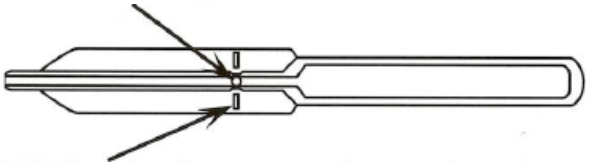
To collect an adequate sample volume:

- Quickly express blood down the fingertip by gently squeezing across the entire finger, to the last joint (not to the end of the fingertip).
- Do not squeeze or “milk” the fingertip to accelerate bleeding.

Collect the second drop of blood by holding the capillary tube **HORIZONTALLY**, and touch the tip of the capillary tube to the blood sample.

NOTE: Filling of the capillary is automatic – do NOT squeeze the bulb while sampling. Maintain this position until the flow of the sample has reached the fill line and stopped.

Test Procedure

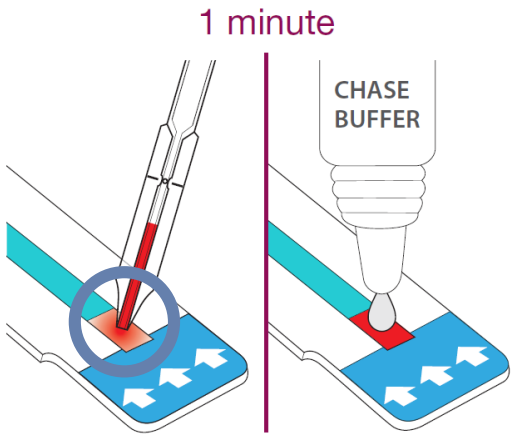


For <u>whole blood (fingerstick)</u> samples using the disposable Capillary Tube provided with the kit:

Additional information for using the MICROSAFE® Tube:

- The air vent regulates volume, and the fill line indicates total sample collected.
- Filling is automatic. Never squeeze the tube while sampling.
- Hold the tube horizontally, and touch the tip of the MICROSAFE Tube to the blood sample.

Test Procedure



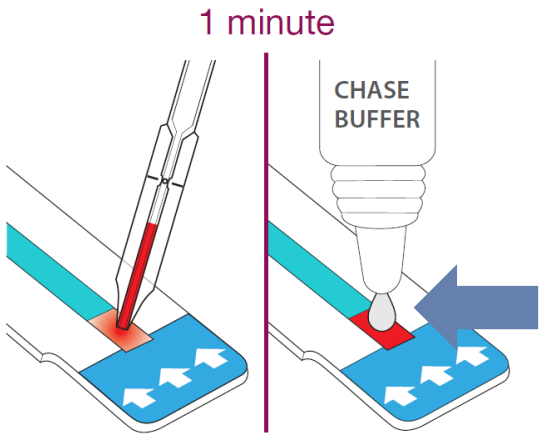
For <u>whole blood (fingerstick)</u> samples using the disposable Capillary Tube provided with the kit:

To add sample to the test strip:

1. Touch the tip of the Capillary Tube containing the blood sample to the Sample Pad (marked by the arrow symbol) and gently squeeze the bulb. Avoid air bubbles. Wait until all the blood is transferred from the Capillary Tube to the Sample Pad.

   Caution: Do not lift the Capillary Tube from the Sample Pad before all the blood has been transferred – a bubble may form which will prevent the complete transfer of sample. If a sample won't expel, cover the small opening at the mark on the capillary with a gloved finger. Then squeeze the bulb until the sample is fully dispensed onto the Sample Pad.

Test Procedure



For <u>whole blood (fingerstick)</u> samples using the disposable Capillary Tube provided with the kit:

2. When all of the blood is transferred to the Sample Pad, wait one minute to ensure the Chase Buffer does not overflow the Sample Pad.
3. Add one drop of Chase Buffer to the Sample Pad.
4. Read the test result between **20 and 30 minutes** after the addition of the Chase Buffer. Do not read Test Results after 30 minutes.

NOTE: Discard the used pipette tips, Capillary Tube, Test Units and any other test materials into a biohazard waste container.

Interpretation of Results

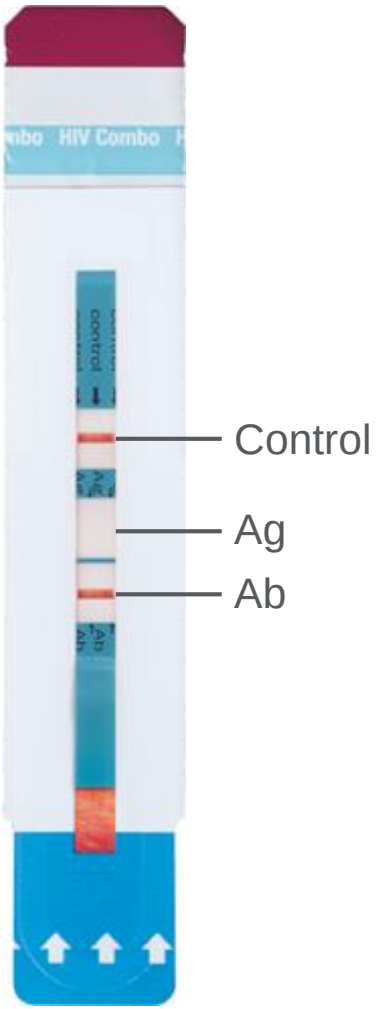
NOTE: When testing whole blood samples, a faint pink background may be visible on the test membrane.

ANTIBODY REACTIVE

(Two Lines - Control and Ab Line)

A **PINK/RED** Control line appears in the Control Area AND a **PINK/RED** Ab line **must** appear in the Lower Test Area of the Test Unit. The intensity of the Ab and Control lines may vary. Any visible **PINK/RED** color in both the Control and Lower Test Areas, regardless of intensity, is considered REACTIVE.

A Reactive test result means that HIV-1 and/or HIV-2 antibodies have been detected in the specimen. The test result is interpreted as PRELIMINARY POSITIVE for HIV-1 and/or HIV-2 antibodies.



Interpretation of Results

NOTE: When testing whole blood samples, a faint pink background may be visible on the test membrane.

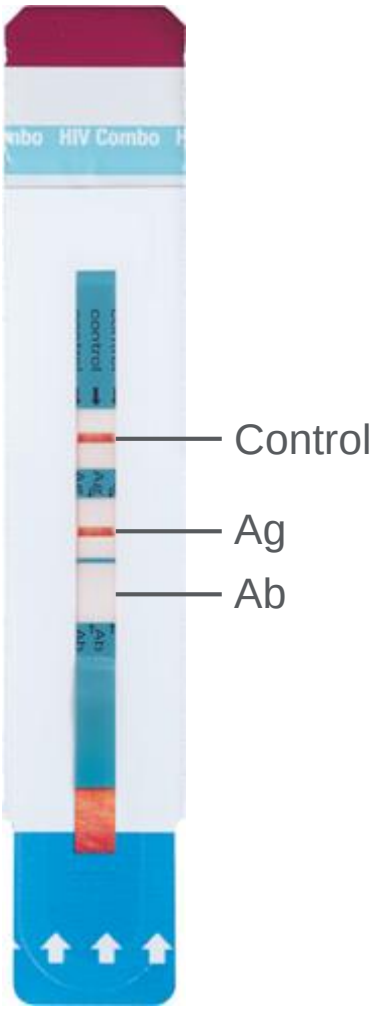
ANTIGEN (HIV-1 p24) REACTIVE

(Two Lines - Control Line and Ag Line)

A **PINK/RED** Control line appears in the Control Area AND a **PINK/RED** Ag line must appear in the Upper Test Area of the Test Unit. The intensity of the Ag and Control lines may vary. Any visible **PINK/RED** color in both the Control and Upper Test Areas, regardless of intensity, is considered REACTIVE.

A Reactive test result means that HIV-1 p24 antigen has been detected in the specimen. The test result is interpreted as PRELIMINARY POSITIVE for HIV-1 p24 antigen.

NOTE: A test result that is PRELIMINARY POSITIVE for HIV-1 p24 antigen in the absence of reactivity for HIV-1 or HIV-2 antibodies may indicate an acute HIV-1 infection in the test subject. In this case the acute HIV-1 infection is distinguished from an established HIV-1 infection in which antibodies to HIV-1 are present.



Interpretation of Results

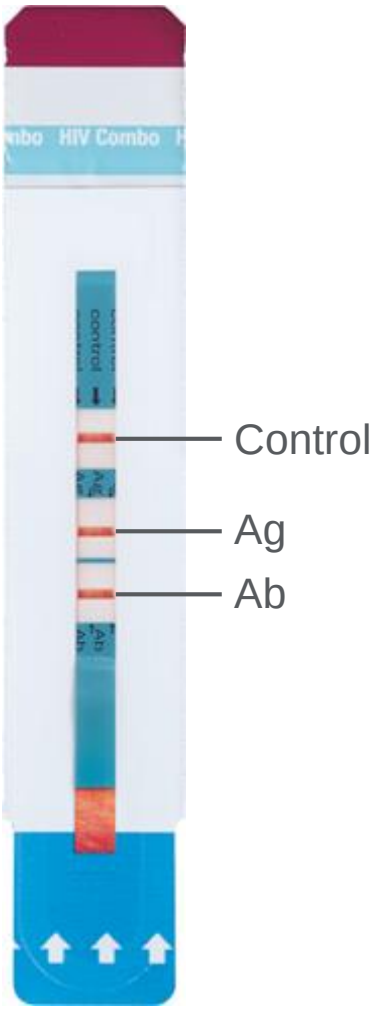
NOTE: When testing whole blood samples, a faint pink background may be visible on the test membrane.

ANTIBODY REACTIVE AND ANTIGEN (HIV-1 p24) REACTIVE

(Three Lines - Control, Ab and Ag Lines)

A **PINK/RED** Control line appears in the Control Area AND a **PINK/RED** Ab line must appear in the Lower Test Area AND a **PINK/RED** Ag line appears in the Upper Test Area of the Test Unit. The intensity of the Ab, Ag and Control lines may vary. Any visible **PINK/RED** color in the Control Area, the Lower Test Area and the Upper Test Area, regardless of intensity, is considered REACTIVE.

The test result is interpreted as PRELIMINARY POSITIVE for HIV-1 and/or HIV-2 antibodies and HIV-1 p24 antigen.



Interpretation of Results

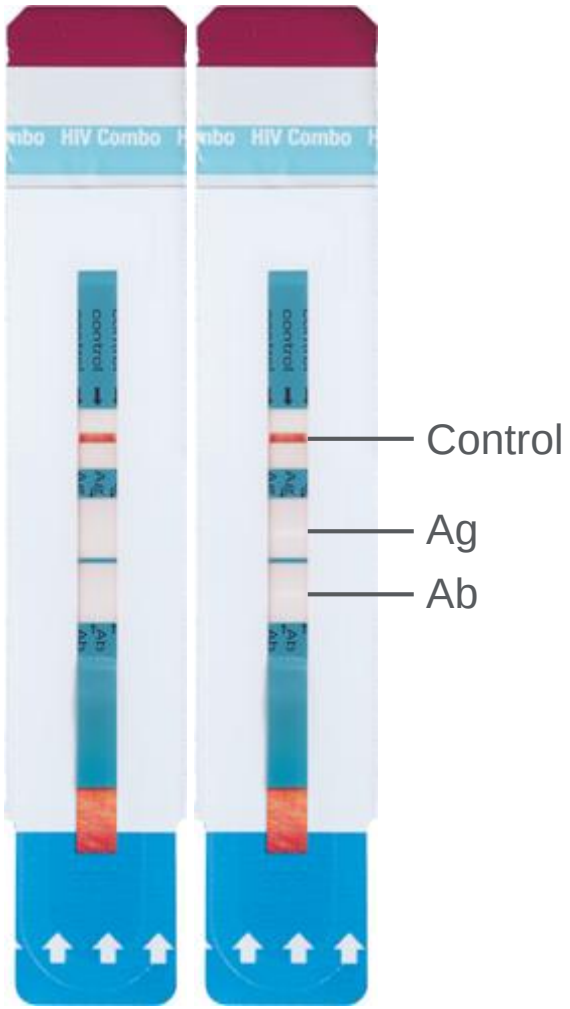
NOTE: When testing whole blood samples, a faint pink background may be visible on the test membrane.

NONREACTIVE

(One Line – Control Line)

A **PINK/RED** Control line appears in the Control Area of the Test Unit, and no **PINK/RED** Ab or Ag line appears in the Lower Test Area and the Upper Test Area of the Test Unit, respectively.

A NONREACTIVE test result means that HIV-1 or HIV-2 antibodies and HIV-1 p24 antigen were not detected in the specimen.



Interpretation of Results

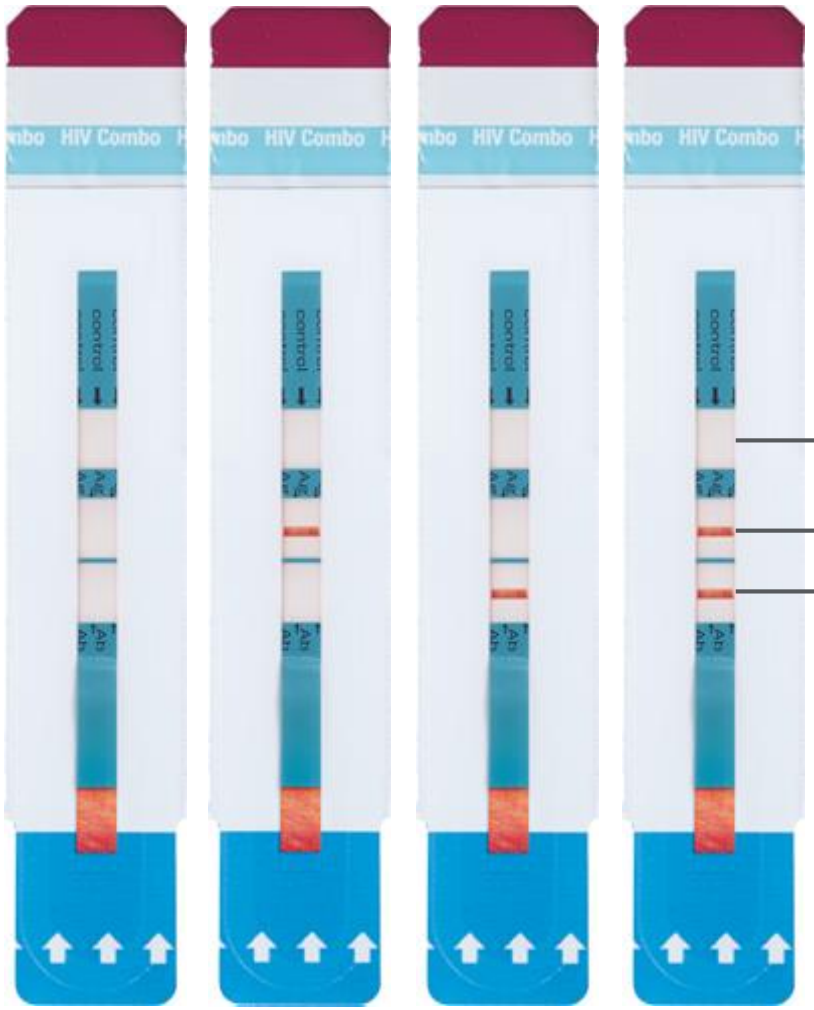
NOTE: When testing whole blood samples, a faint pink background may be visible on the test membrane.

INVALID

(No Control Line)

If there is no **PINK/RED** Control line in the Control Area of the Test Unit, even if a **PINK/RED** line appears in the Lower Test Area or the Upper Test Area of the Test Unit, the result is INVALID and the test should be repeated.

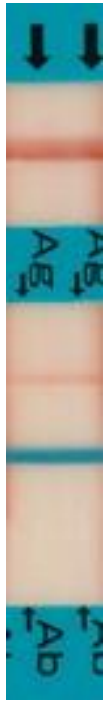
If the problem persists, contact Alere™ Technical Support.



Alere™

A

B



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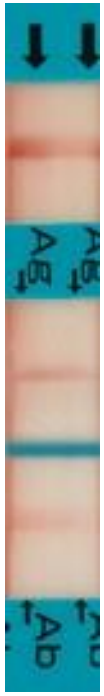

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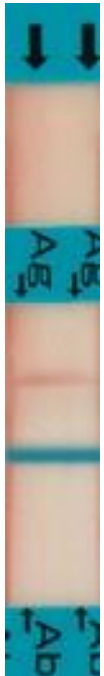
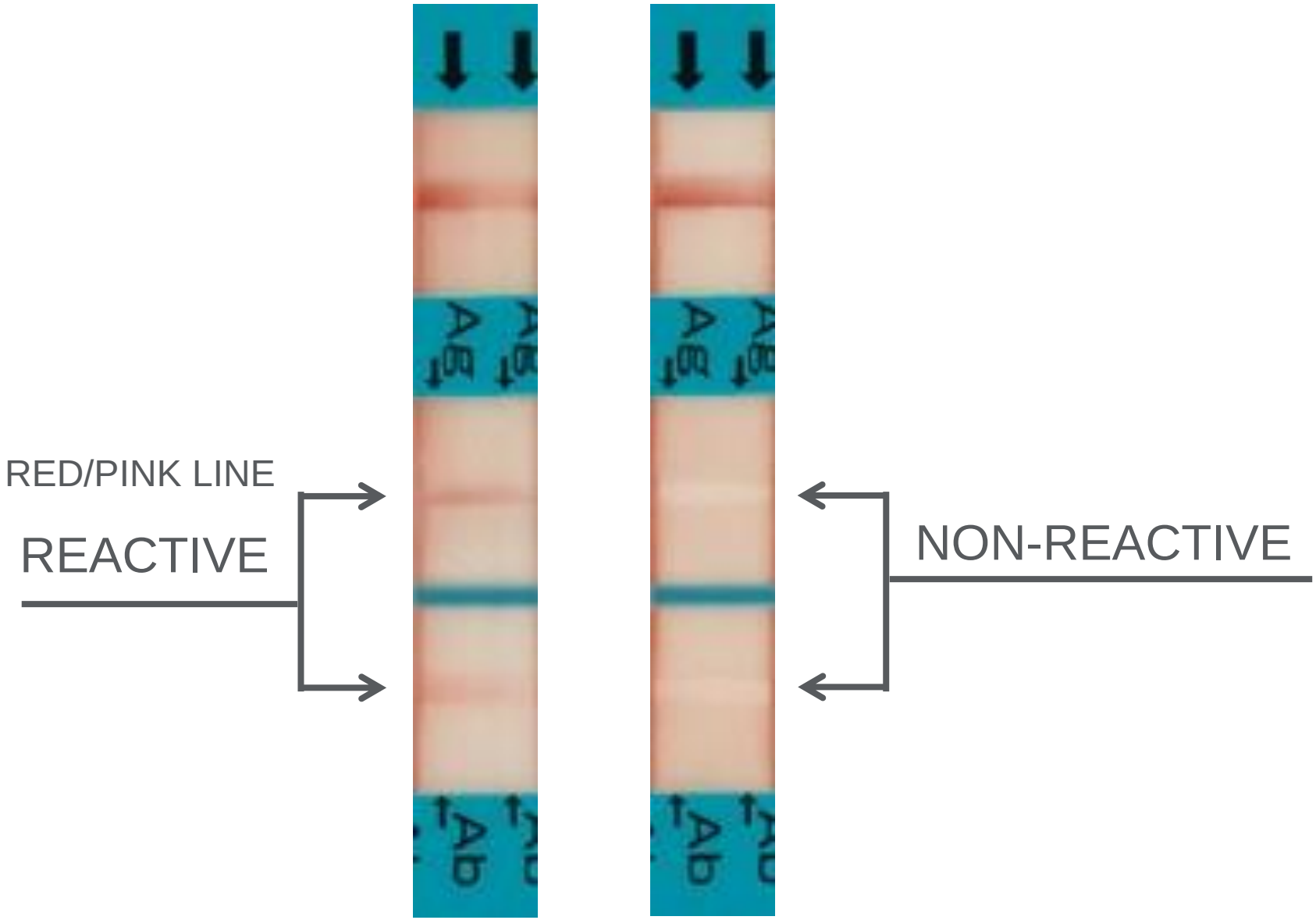
G



H

Test lines



External Controls

Determine™ HIV-1/2 Ag/Ab Combo Controls

Catalog #: 7D2628

Storage and Stability

Store at 2 to 8°C (36 to 46°F) Do not use beyond the indicated expiration date. Open the Control Vials only when you are performing tests. Recap and store the Control Vials in their original container at 2 to 8°C (36 to 46°F) after use.

Determine™ HIV-1/2 Ag/Ab Combo Controls

Catalog #: 7D2628

Intended Use

The Alere Determine™ HIV-1/2 Ag/Ab Combo Controls are quality control reagents for use with the Alere Determine™ HIV-1/2 Ag/Ab Combo Assay only.

Determine™ HIV-1/2 Ag/Ab Combo Controls

Materials Provided

- 1 Package Insert
- 40 Disposable Pipettes*
- Four (4) Controls Vials:
  - One (1) HIV-1 Reactive Control
  - One (1) HIV-2 Reactive Control
  - One (1) HIV-1 p24 Antigen Control
  - One (1) Nonreactive Control


Determine™
HIV-1/2 Ag/Ab Combo Controls

NOTE: The disposable pipettes provided with the Alere Determine™ HIV-1/2 Ag/Ab Combo Control Kit are for use with the external controls only and are NOT to be used for testing patient samples.

**Additional Disposable Pipettes are available upon request*

When to perform controls

- Each new untrained operator prior to performing tests on patient specimens
- When opening a new test kit lot
- Whenever a new shipment of test kits is received
- If the temperature of the test storage area falls outside of 2 to 30°C (36 to 86°F)
- If the temperature of the testing area falls outside of 15 to 30°C (59 to 86°F)
- At periodic intervals as indicated by the user facility

It is the responsibility of each laboratory using Alere Determine™ HIV-1/2 Ag/Ab Combo to establish an adequate quality assurance program to ensure the performance of the device under its specific locations and conditions of use.

Test Procedure

Procedure for Using Controls with the Alere Determine™ HIV-1/2 Ag/Ab Combo Assay

External control material stored at refrigerated temperatures must be brought to room temperature (15 to 30°C; 59 to 86°F) prior to testing.

The desired number of test units from the 5- or 10-test card can be removed by bending and tearing at the perforation. The test(s) should be initiated within 2 hours after removing the protective foil cover from each test.

Test Procedure

Procedure for Using Controls with the Alere Determine™ HIV-1/2 Ag/Ab Combo Assay

1. Removal of the test units should start from the right side of the test card to preserve the lot number which appears on the left side of the test card.

Test Procedure

Procedure for Using Controls with the Alere Determine™ HIV-1/2 Ag/Ab Combo Assay

2. Remove the protective foil cover from each test and place it in the workstation.
3. Label the Test Device with Control Reagent name or identification number

CONTROLS SET UP / LABELING!!!

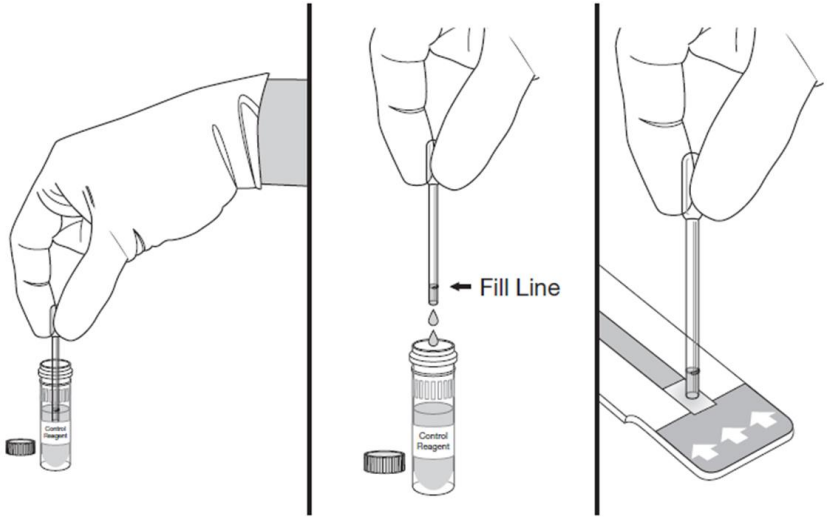
Test Procedure

Procedure for Using Controls with the Alere Determine™ HIV-1/2 Ag/Ab Combo Assay

4. Open a Control Vial containing the Control Reagent.
   - **If using the disposable pipettes provided** with the Alere Determine™ HIV-1/2 Ag/Ab Combo Control Kit
     a. Squeeze the pipette bulb and place the pipette tip into the Control Reagent.
     b. Gently release the bulb to bring the liquid above the fill line on the pipette.
     c. Raise the pipette and gently squeeze the bulb to bring the liquid down to the fill line.
     d. Touch the pipette tip to the Sample Pad and squeeze the pipette bulb to release all of the liquid.

NOTE:

- *Use a disposable pipette with each new Control Reagent.*
- ***Do not add Chase Buffer** when running External Controls.*



Test Procedure

Procedure for Using Controls with the Alere Determine™ HIV-1/2 Ag/Ab Combo Assay

5. Read the Test Result between 20 and 30 minutes after the addition of the Control Reagent in a well-lit area.

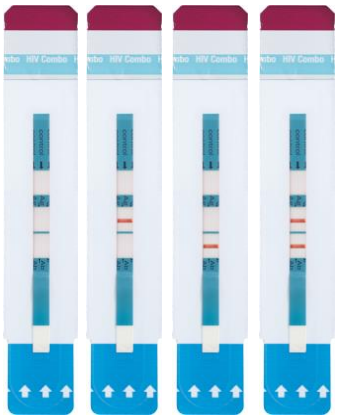
   Do not read Test Results after 30 minutes.

Nonreactive

HIV-1 / HIV-2 Ab Reactive

HIV-1 p24 Ag Reactive

INVALID



Test Procedure

Procedure for Using Controls with the Alere Determine™ HIV-1/2 Ag/Ab Combo Assay

6. Discard the used Test Device and any other test materials into a biohazard waste container.

Test Procedure

Procedure for Using Controls with the Alere Determine™ HIV-1/2 Ag/Ab Combo Assay

7. Reseal the Control Reagent Vials and store them in their original container at 2 to 8°C (36 to 46°F).

Store at:

2 - 8°C or **36 - 46°F**

VERY IMPORTANT STEPS!!!

1. Clean the finger with an antiseptic wipe and make sure the fingertip is completely dry before puncturing with lancet.
2. Use a sterile 2.0mm x 1.5mm blade lancet capable of producing 50ul of blood.
3. Wipe away the first drop of blood. Do not squeeze the fingertip.
4. After adding the blood to the sample pad, wait 1 minute before applying the chase buffer.
5. Hold the chase buffer vertically right above the sample pad and apply one (1) free flowing drop in the center of the sample pad.
6. If moving the test, make sure the workstation/tray is flat/level so that the buffer is not shaken off the sample pad.
7. Read the results between 20-30 minutes. Only read pink/red lines.

