

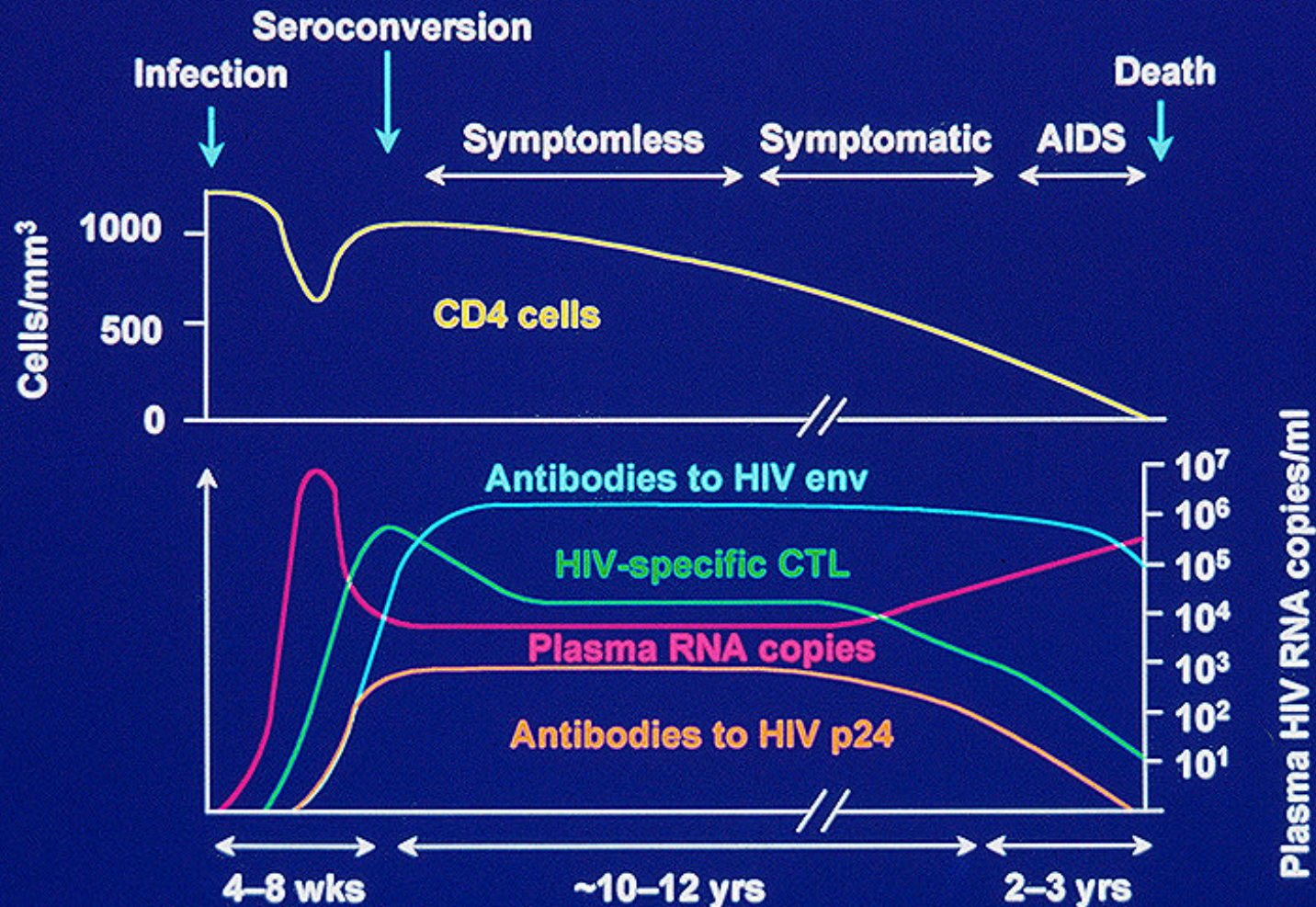
Diagnosis of HIV-1 Infection

**Estelle Piwovar-Manning
HPTN Central Laboratory
The Johns Hopkins University**

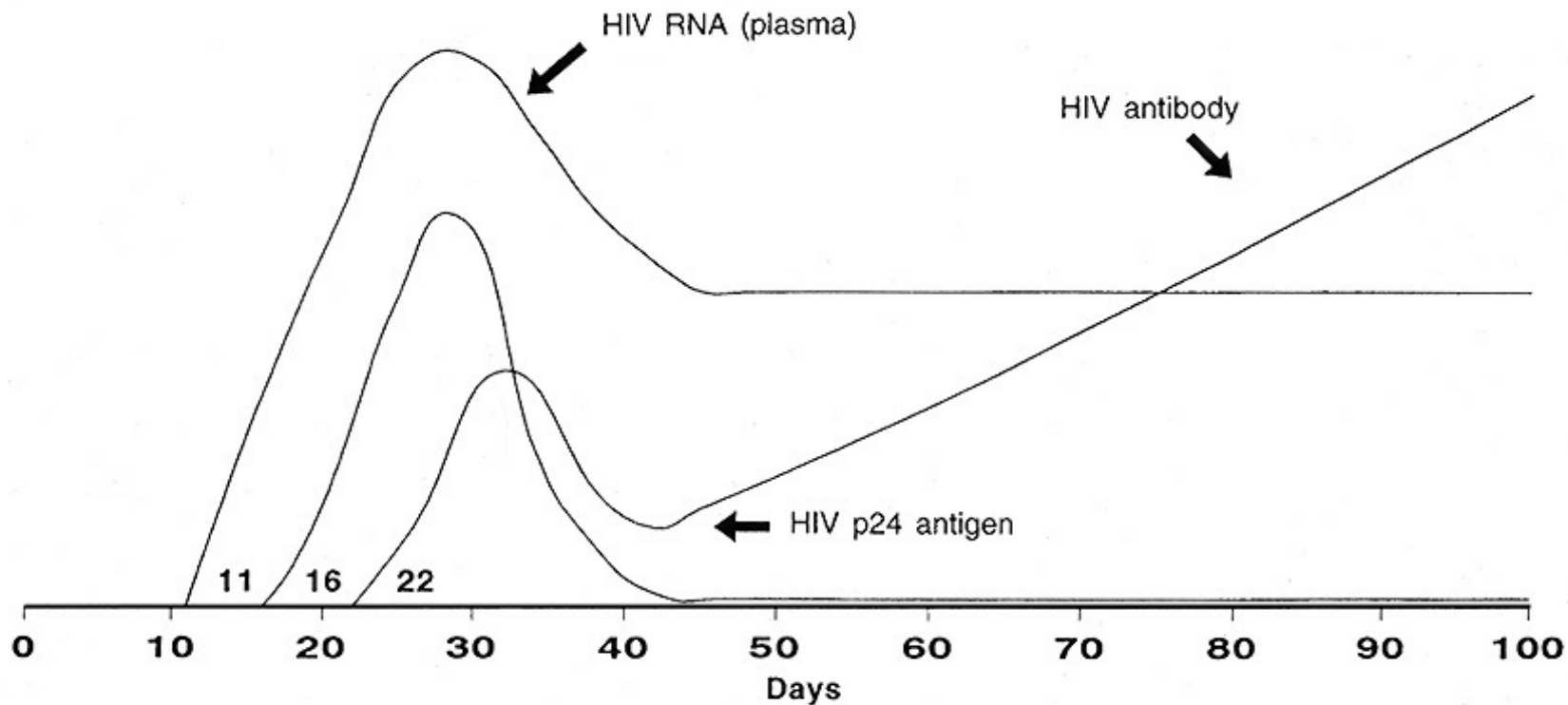
Tests Used to Diagnose HIV-1 Infection

- **HIV antibody (today's topic)**
- **HIV p24 antigen**
- **HIV DNA**
- **HIV RNA**
- **HIV Isolation (Culture)**

Viral marker profile during course of HIV infection



INTERORGANIZATIONAL TASK FORCE



Theoretical Infectivity

HIV RNA

HIV p24 antigen

HIV antibody

Day 0

Day 11

Day 16

Day 22

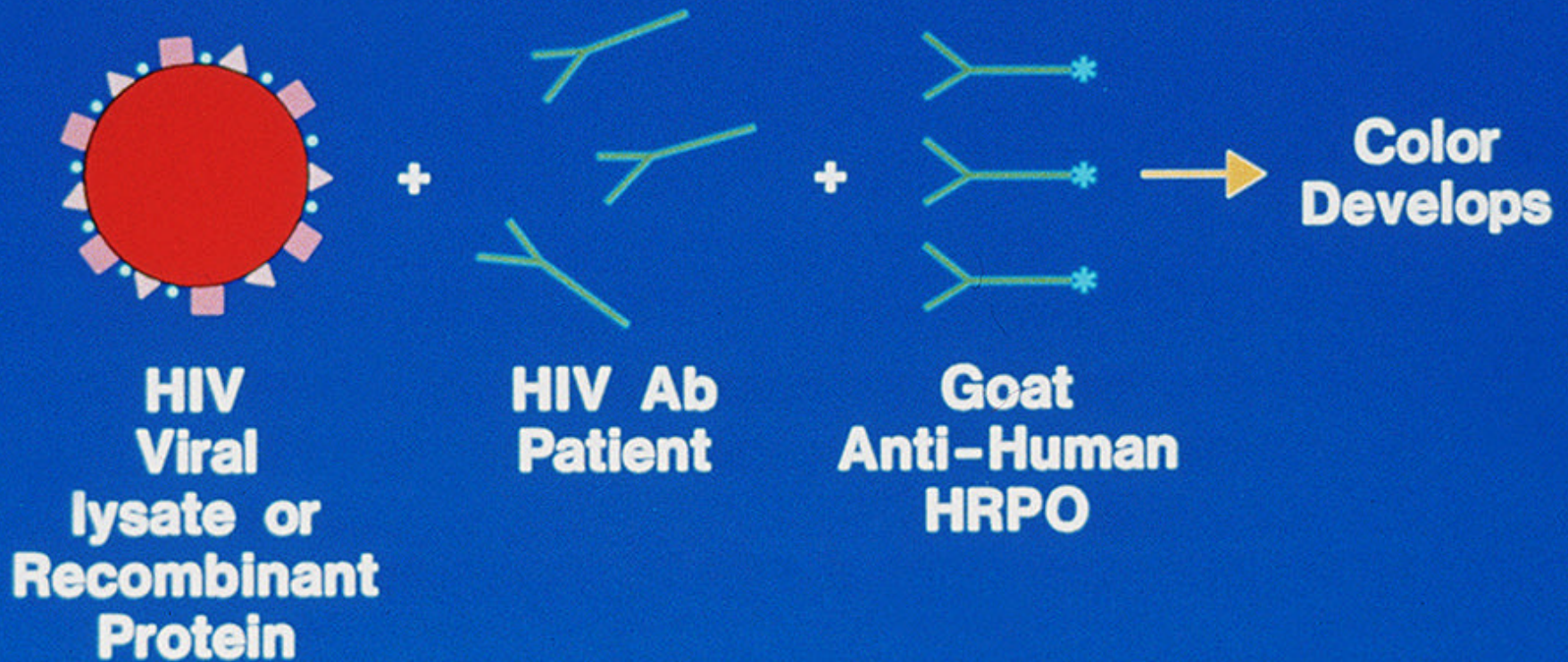
5 Days

6 Days

11 Days

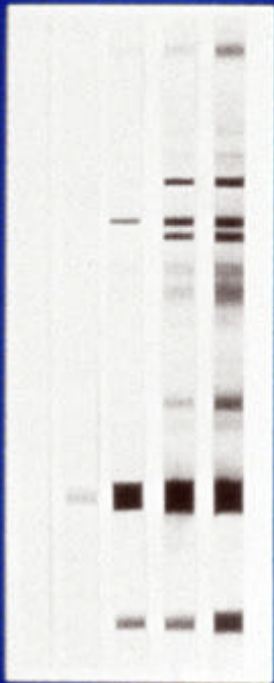
- **Detection of HIV-1 antibody is the gold standard for diagnosing HIV-1 infection in persons over 18 months of age.**
- **In the United States an enzyme immunoassay (EIA also known as ELISA) is used as the screening test. This test uses HIV antigens prepared by lysis of whole virus, recombinant and/or synthetic peptides.**
- **Typically, the test is initially performed once on serum or plasma. If reactive, then the test is repeated in duplicate. If the test is repeatedly reactive in 2 of 3 or 3 of 3 tests, then the result is confirmed by testing the sample by a licensed Western blot or Immunofluorescence assay (IFA).**

ANTIBODY ELISA ASSAY

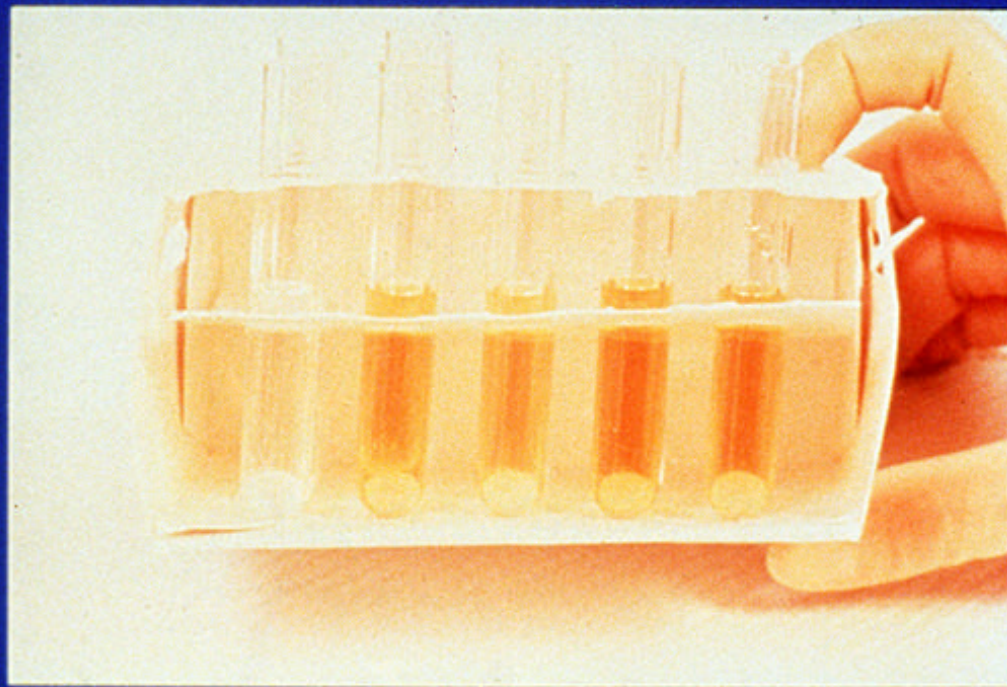


ANTIBODY ASSAYS

**Western
Blot**



EIA



- **A Western blot detects antibodies to specific HIV proteins which have been separated on nitrocellulose paper. Typically 9 viral characteristic bands can be detected on Western blot with HIV + sera, but the minimal criteria for a positive Western blot is reactivity to at least 2 of the 3 following bands (p24, gp41, and gp120/160). However, different kits use different criteria.**
- **A negative Western blot typically means the absence of no bands or no viral characteristic bands.**
- **An indeterminate Western blot result typically means the presence of any band pattern that does not meet the criteria for a positive result.**

**IMPORTANT TO STANDARDIZE
TESTING ALGORITHM AND
INTERPRETATION CRITERIA.**

**Protocols conducted under an FDA IND
should use an FDA licensed kit whenever
possible.**

- **Use of the EIA/WB testing algorithm is very sensitive and specific with less than 1 in 100,000 false positives.**
- **Most infected individuals typically have detectable antibody 3-6 weeks after exposure, but virtually all seroconvert within 6 months.**
- **In chronically HIV infected individuals false negative EIA results are very rare, and usually represents either an HIV variant such as HIV-2 or an unusual subtype (ie O, N), a technical problem, or a sample mixup. Seroreversion has been reported in older studies, but not with the more sensitive 3rd generation EIA tests. Licensed HIV-1/2 tests in the United States will detect HIV-1 and 70-80% of subtype O samples.**

Western blots should not be performed on EIA negative samples or used alone due to the high rate of indeterminate results in HIV uninfected people (roughly 15%).

PU1

- **False reactivity on EIA or Western blot assays can be due to HLA antibody, autoimmune diseases (ie lupus), cross reactivity to yeast or to other contaminating antigens used to prepare the HIV antigens.**
- **Indeterminate WB results may represent recent seroconversion or seroconversion in process. Subjects are asked to be retested in 4-6 weeks.**

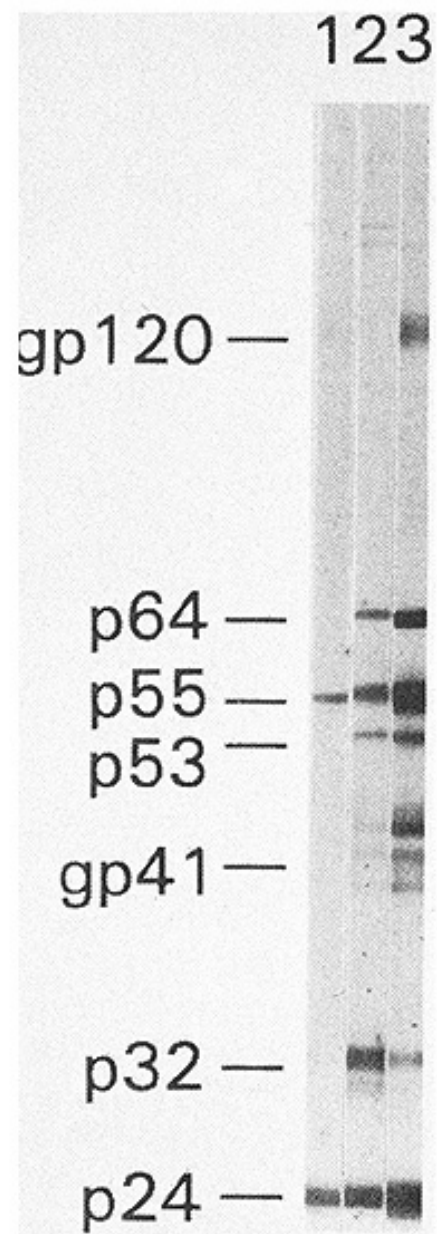


Fig 1—Western blot showing typical antibodies against core proteins 10 and 4 months (lanes 1 and 2) before ELISA-verified HIV seroconversion (lane 3).

- **The sensitivity/specificity of licensed tests in the United States varies by manufacturer and kit, but generally is greater than 99.5% if performed correctly.**
- **However, the predictive value of a positive or negative result is highly dependent on the prevalence of HIV infection in the population tested, the quality of the laboratory, and drawing and reporting procedures. Transcription and labeling errors are probably the most common reason for false negative/positive results.**
- **Ideally, a positive Western blot result should be confirmed by testing an additional blood sample before rendering a diagnosis of HIV infection or treating a subject with antiretroviral therapy.**

LIMITATIONS OF CURRENT VIRAL SCREENING/DIAGNOSTIC TESTS

- A. SENSITIVITY MAY BE LESS THAN 100% DUE TO
 - 1) LOW LEVEL OF ANTIBODY TO SPECIFIC ANTIGEN
 - 2) NO ANTIBODY
 - 3) VARIANT STRAIN OF VIRUS
- B. SPECIFICITY MAY BE POOR DUE TO CELLULAR (OR E. COLI. YEAST) CONTAINMENTS
- C. PREDICTIVE VALUE DEPENDENT ON PREVALENCE
- D. PRESENCE OF ANTIBODY MAY NOT INDICATE CURRENT INFECTION, ONLY PAST EXPOSURE

Rapid tests for detection of HIV Antibody

Rapid HIV Diagnostics

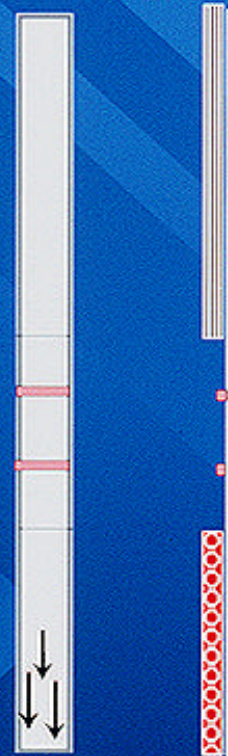
Testing Formats



**flow-through
device**



agglutination



dipstick

Advantages of Rapid HIV Antibody Tests

- **Fast (10-20 min) and simple**
- **Typically Inexpensive (\$1-2/test)**
- **Immediate result for subject**
- **Whole blood can be used**
- **No equipment, refrigeration required**
- **No electricity required**
- **No multiple timing steps**
- **Built in controls**

Commonly Used Rapid HIV Antibody Tests

- | | |
|-------------|----------------------------|
| ➤ Determine | Abbott Laboratories |
| ➤ Oraquick* | OraSure |
| ➤ Unigold* | Trinity Biotech |
| ➤ Multispot | Trinity Biotech |
| ➤ Stat-Pak | Chembio Diagnostic Systems |
| ➤ Hemastrip | SDS |
| ➤ SUDS* | Abbott Laboratories |
| ➤ Reveal* | MedMira Laboratories |

* FDA approved

Types of Rapid Tests

- One class of rapid tests is the "dot blot" or "immunoblot"; they produce a well-circumscribed colored dot on the solid phase surface if the test is positive. Most of these rapid assays now incorporate a built-in control that indicates that the test was performed correctly. This control is an anti-human immunoglobulin that binds any immunoglobulin in the sample and produces a separate indicator when all reagents are added appropriately. In addition, several varieties are available that include two "dots", which allow the differentiation of HIV-1 and HIV-2 infection

Types of Rapid Tests

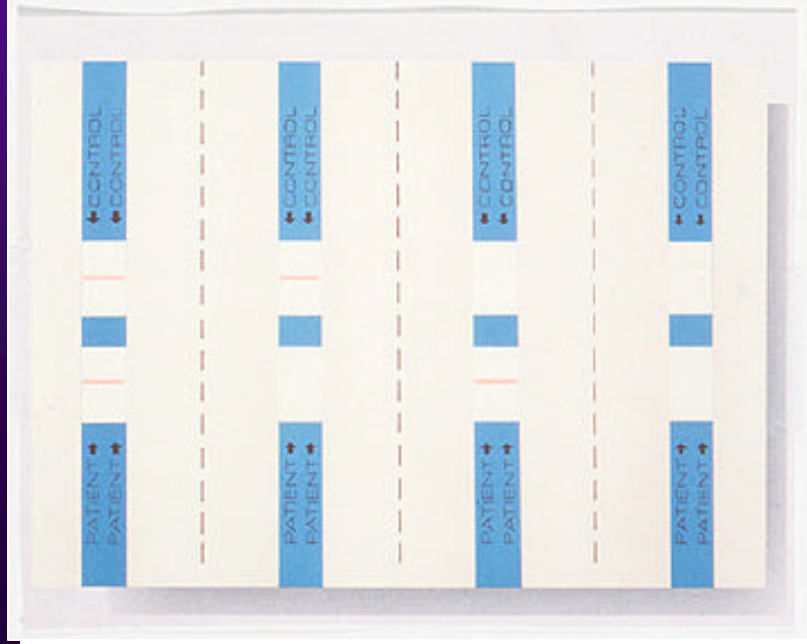
- **The procedures for the dot-blot assays are similar regardless of the exact format of the test. Most require drop-wise additions of reagents in the following sequence: buffer, sample, wash buffer, conjugate, wash buffer, substrate, and stop solution. Some assays substitute an IgG binding dye (protein A gold reagent) for the anti-immunoglobulin conjugate, thereby decreasing the procedure by one step**

Types of Rapid Tests

- **Other rapid test formats include dipsticks, in which antigen is attached on the "teeth" of comb-like devices; several of these rapid tests have the ability to differentiate HIV-1 and HIV-2. Disadvantages include a subjective interpretation, difficulty in reading if the laboratorian is color-blind, and a higher cost than that of the ELISA.**

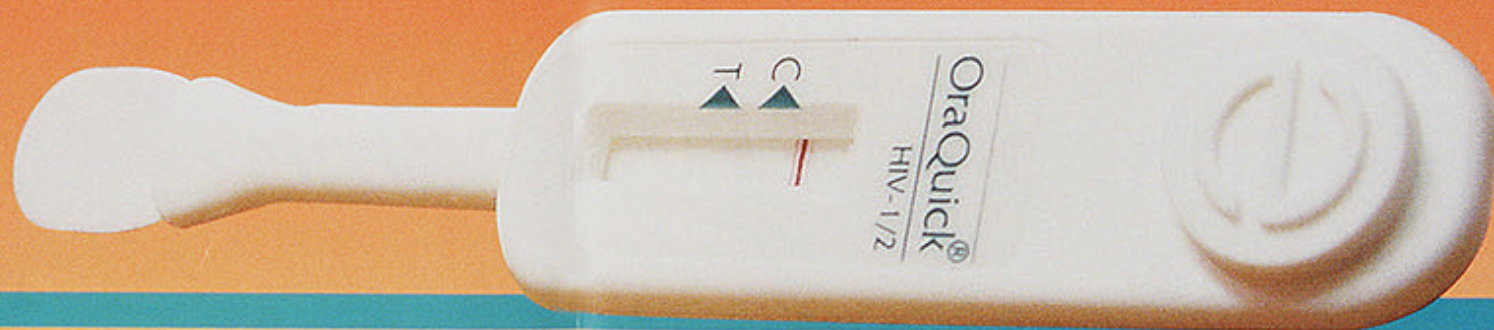
ABBOTT DETERMINE™

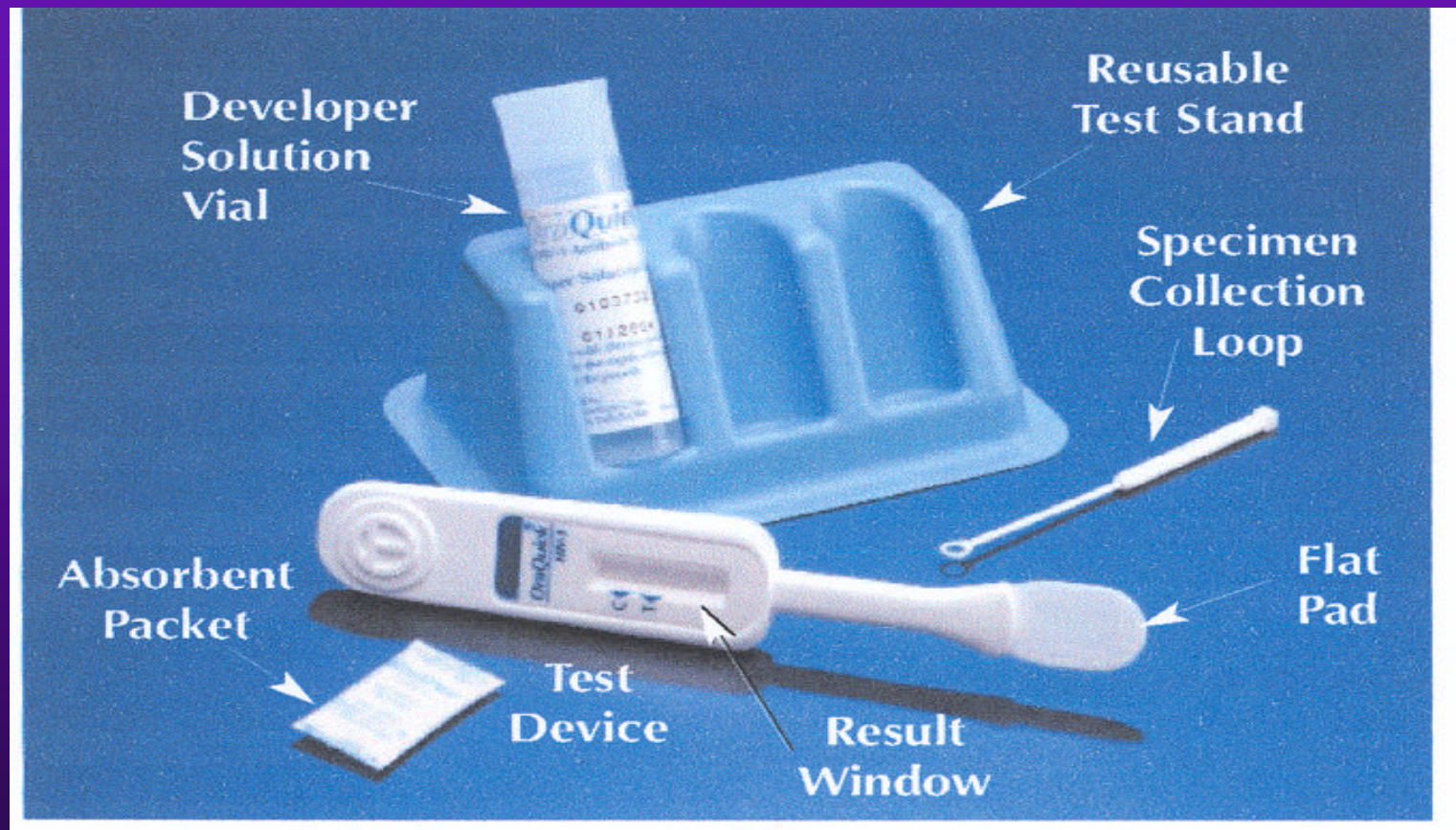
RAPID HIV 1/2 ANTIBODY TEST



OraQuick[®]
HIV-1/2

THE
RAPID ORAL HIV
TEST





ADEQUATE
LIGHTING
REQUIRED



Reading Test Result

Look at the Result Window of the Test Device



Read results after 20 minutes but not more than 60 minutes.

Non-Reactive



Test is **NON-REACTIVE** if:

- A reddish-purple line appears next to the triangle labeled "C" and **NO** line appears next to the triangle labeled "T".

Reactive



Test is **REACTIVE** if:

- A reddish-purple line appears next to the triangle labeled "C" and a reddish-purple line appears next to the triangle labeled "T". One of these lines may be darker than the other.

NOTE: The test is **Reactive** if **any** reddish-purple line appears next to the "T" triangle and next to the "C" triangle, no matter how faint these lines are.

Invalid



Test is **INVALID** if:

- NO** reddish-purple line appears next to the triangle labeled "C" (see Diagram a and b), or
- A red background in the Results Window makes it difficult to read the result **after 20 minutes** (Diagram c), or
- If any of the lines are **NOT** inside the "C" or "T" triangle areas (see Diagram d1 and d2).

Interpretation of Test Result

- A **Non-Reactive** test result means that anti-HIV-1 antibodies **were not detected** in the specimen. The test result is interpreted as **NEGATIVE for HIV-1 antibodies**. Follow CDC Guidelines to inform the test subject of the test result and its interpretation.

- A **Reactive** test result means that anti-HIV-1 antibodies **have been detected** in the specimen. The test result is interpreted as **PRELIMINARY POSITIVE for HIV-1 antibodies**. Follow CDC Guidelines to inform the test subject of the test result and its interpretation.

- An **Invalid** test result means that there was a problem running the test, either related to the specimen or to the Device. An **invalid result cannot be interpreted**. Repeat the test with a new Pouch and fingerstick blood sample. Contact OraSure Technologies' Customer Service if you are unable to get a valid test result upon repeat testing.



Bethlehem, PA 18015 USA • Ph. 800-ORASURE (800-672-7873) • Fax 610.882.3572 • www.orasure.com

©2001, 2003 OraSure Technologies, Inc.
U.S. Patent # 6,203,081 and various international and U.S. patents pending.
OraQuick® is a registered trademark of OraSure Technologies, Inc.
L3061 0306 (rev. 04/03)



Uni-Gold™ Recombigen® HIV was designed as a rapid immunoassay based on the immunochromatographic sandwich principle and is intended to detect antibodies to HIV-1 in human serum, plasma and whole blood (venipuncture).

Uni-Gold™ Recombigen® HIV Test employs genetically engineered recombinant proteins representing the immunodominant regions of the envelope proteins of HIV-1. The recombinant proteins are immobilised at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitized as a control region.

If antibodies to HIV-1 are present in the sample, they combine with an HIV-1 antigen/colloidal gold reagent and this complex binds to the immobilized antigens in the test region of the device forming a visible pink/red band. The control line should always appear as a visible pink/red band in the control region of the device to indicate that the test device is functioning correctly. A positive result is visualized by a pink/red band in the test region of the device. A negative reaction occurs in the absence of detectable levels of human immunoglobulin antibodies to HIV-1 in the specimen; consequently no visually detectable band develops in the test region of the device.

- **Rapid testing algorithms vary considerably, but generally two different rapid tests are used simultaneously or sequentially in testing serum, plasma, or whole blood (fingerstick) if the first test is reactive.**
- **If two different rapid tests are discrepant, then a third different rapid test or a Western Blot is performed. For protocols performed under an IND involving HIV infected subjects, one of the rapid tests used should be the FDA approved OraQuick and should be confirmed by a licensed Western blot.**

**No matter which tests are used,
it is essential in performing testing that:**

- **Controls be used to validate test results**
- **SOPS be developed for pre-analytical, testing, and post analytical steps**
- **Training and competency of technicians performing the tests be documented**
- **Testing procedures and algorithms be consistently followed.**

Conclusion

- **There are a variety of rapid sensitive and specific HIV antibody screening assays and available for use.**
- **For clinical protocols, a diagnosis of HIV infection should be confirmed by western blot in enrolled subjects.**
- **FDA approved tests MUST be used if protocol done under an IND if at all possible.**
- **Testing algorithm must be validated.**
- **Testing should follow Good Laboratory Practices.**

035 specific requirements

- **Anticoagulated blood will be tested for evidence of HIV infection using tests that have been validated at the study site. These same tests will be used to test plasma specimens archived at enrollment for participants who test HIV-positive at their first HIV testing timepoint during follow-up.**
- **At all sites, any of the following rapid HIV tests may be used, provided the tests have been validated at the site:**
 - **Abbott Determine**
 - **OraSure OraQuick**
 - **Uni-Gold Recombigen**

035 specific requirements

- **At sites choosing to use the OraSure OraQuick and Uni-Gold Recombigen tests, FDA-approved test kits must be used.**
- **Perform all tests according to their site SOPs and package inserts .**
- **Site staff should be aware of the different testing timeframes for each rapid test, and place appropriate timekeeping devices in all testing settings to ensure that each test is read at the appropriate timepoint.**

Test	Testing Location	Specimen Type	Tube/Container	Kit	Kit Supplier
HIV antibody tests	In clinic	Anticoagulated blood	Lavender top tube	Abbott Determine	Local
	In clinic	Anticoagulated blood	Lavender top tube	OraSure OraQuick FDA Approved version	US-based or local
	In clinic	Anticoagulated blood	Lavender top tube	Uni-Gold Recombigen FDA approved Version	US-based or local
	Local lab	Anticoagulated blood	Lavender top tube	FDA-approved HIV EIA (US site only)	Local
	Local lab	Anticoagulated blood	Lavender top tube	Genetic Systems WB FDA approved Version	US-based or local

035 specific requirements

- **When tests are performed in clinic settings, the same documentation and quality control (QC) practices required in the laboratory must be undertaken in the clinic.**
- **In-clinic testing and QC procedures must be documented on log sheets that are maintained in the clinic and reviewed by the study site Laboratory Manager (or designee) at least once per month.**
- **Once the log sheets are reviewed by the Laboratory Manager (or designee) they may then be stored in the local laboratory, if desired.**

035 specific requirements

- In the event that proper QC procedures are not followed in the clinic, or that adequate QC is not maintained, the Laboratory Manager is responsible for ensuring that corrective action is taken and documented.
- Sample log sheets are available from the HPTN Central Lab.

035 specific requirements

- **At all sites, when Western blot (WB) testing is required, the FDA-approved Genetic Systems WB, manufactured by Bio-Rad, must be used. Perform this test according to the site SOPs and package insert .**
- **All tests, and associated QC procedures, must be documented on local laboratory log sheets or other laboratory source documents.**

035 specific requirements

- **HIV infection status is the primary endpoint for HPTN 035.**
- **Use of the HIV test kits listed above has been negotiated with the US Food and Drug Administration, and no other tests may be used without approval from the HTPN Central Lab.**

035 specific requirements

- **All sites must maintain an adequate inventory of the HIV test kits they have selected and validated for use in HPTN 035.**
- **Kit inventories should be monitored closely and re-supply orders placed at 8 to 12 weeks in advance (or longer depending on their sites procurement policies) of actual need.**

035 specific requirements

- All sites will be required to report their kit inventories as well as Kit Lot numbers to the HPTN Central Lab on a monthly basis.
- Notify the Central Lab immediately if any kit inventory or quality control problems are identified, so that appropriate action can be taken.