CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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Update to Guidelines for Diagnosing HIV Infection
- CPAL's Testing Algorithm for Diagnostic HIV Testing

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Introduction:

Since the advent of human immunodeficiency virus (HIV) testing, laboratory based methods have undergone tremendous change. The increasing availability of new assays for the diagnosis of HIV infections, such as antigen-antibody combination tests, nucleic acid tests, and rapid membrane-based tests, have offered improved detection and turn-around-times. Yet, the increasing number of options has created an increasing challenge to the clinician when asked to determine which tests to perform and how best to interpret the results.

During the evolution of this testing, adequate consensus guidelines have not been proposed to assist in the appropriate use and interpretation of these tests and testing strategies. In 2010, the CLSI, published a 'proposed' document on diagnosing HIV infections based on CDC guidelines which was followed in June 2011 with the 'approved' version of these guidelines (**CLSI. *Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus infection; Approved Guideline.* CLSI document M-53-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.[1])**

After reviewing the guidelines, holding discussions with the Medical Directors, and consulting with specialist in the field of HIV diagnosis and management, CPAL selected an algorithm that was felt to best meet the needs of our member hospitals and the patients they serve. At that time, work began to make the necessary changes for implementation of the chosen algorithm. The testing algorithm was announced in a January 11, 2013 technical bulletin. This testing algorithm included an option for Western Blot analysis to resolve testing results in some cases.

We have been informed that HIV-2 EIA and Western Blots for HIV-1 and HIV-2 resolution will no longer be available from Quest Diagnostics. The expert at Quest Diagnostics recommends replacing these tests with HIV 1 RNA, Qualitative, TMA (Quest Test Code 16185) and HIV 2 DNA/RNA Qualitative Real Time PCR (Quest Test Code 34977X). Additionally, the CDC announced DRAFT recommendations that eliminate Western Blot testing from the recommended testing algorithm and replace it with appropriate nucleic acid testing (NAT). Since NAT testing provides higher sensitivity and specificity than Western Blot, CPAL has decided to adopt this revision to the current testing algorithm.

Testing to Diagnose an HIV Infection:

Because different HIV markers, i.e., antibodies, antigens, viral nucleic acid, appear at various times after infection, detection of HIV infections requires a combination of tests. Thus, HIV diagnostic testing is carried out in a multistep process.

In general, specimens are initially tested using a highly sensitive ***HIV screening assay*** (EIA, Rapid). Specimens that are non-reactive on the initial screen are generally considered HIV negative (if a known high-risk exposure has occurred, it is recommended that follow-up testing be performed). Screening tests to detect HIV infection, although highly effective, do not always detect all individuals infected and do not always correctly classify persons who are not infected. False positive and false negative results occur with any screening assay.

If an initial (screening) assay is reactive, it is generally repeated in duplicate per manufacturer's instructions, and, if repeat results are reactive, further testing with a ***supplemental HIV test*** is performed to confirm an HIV diagnosis as is required by law.

CPAL Tests for HIV Diagnosis:

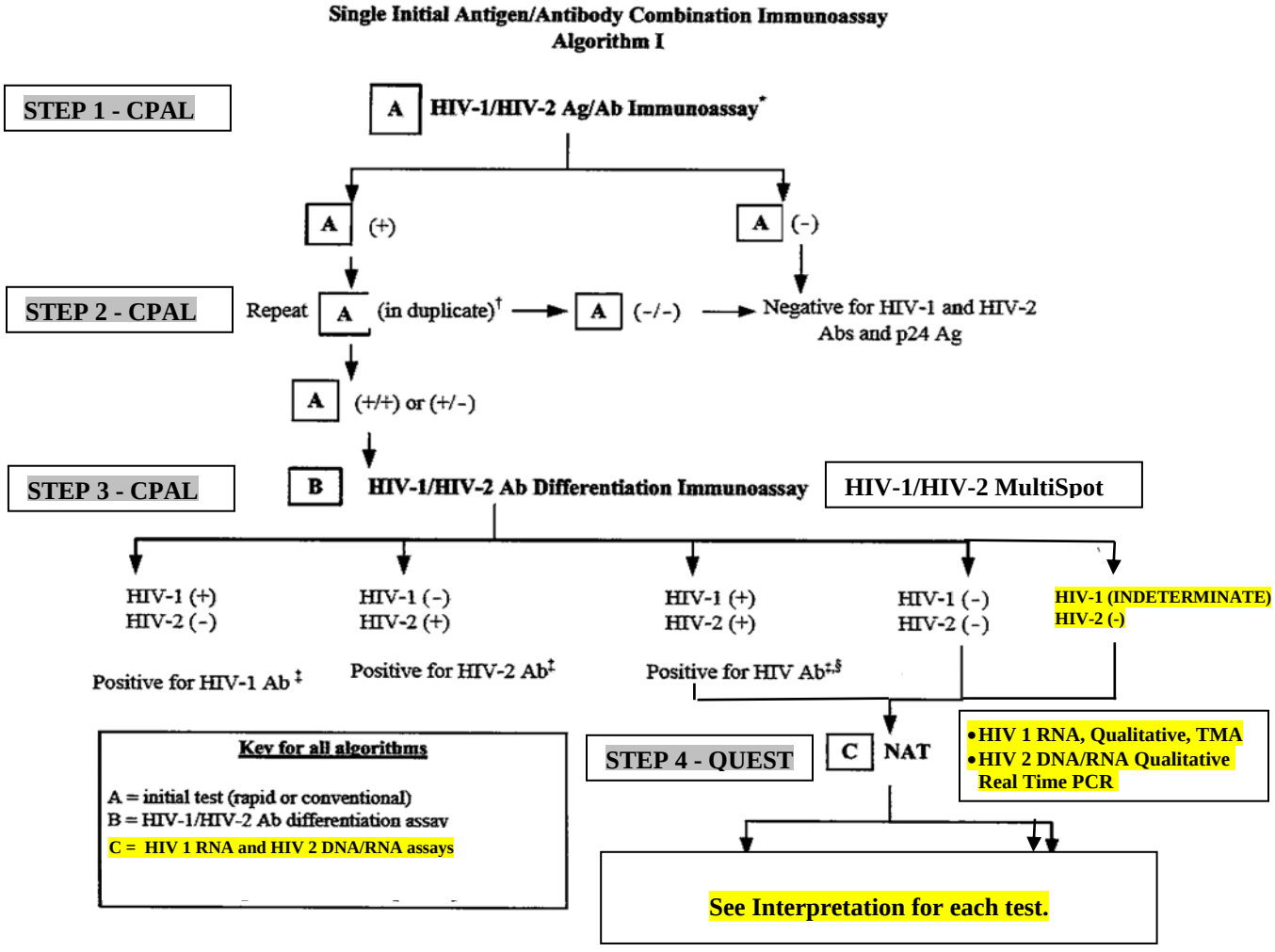
1) ***Screening HIV Tests*** – in February 2011, CPAL implemented a 4th generation HIV Combo Assay for the ***simultaneous*** detection of both HIV p24 antigen and antibodies to HIV type 1 (HIV-1 group M and group O) and/or type 2 (HIV-2) in human serum and plasma. Previous generation assays targeted antibodies OR antigen but not both. This assay is designed to shorten the time to detection post-infection.

2) ***Supplemental HIV Tests*** – in December 2012, CPAL implemented a rapid supplemental HIV test, the MultiSpot HIV Antibody Differentiation Assay. This test is

recognized as an effective replacement for the Western Blot Differentiation assay. The test is used to confirm the presence of an HIV antibody and differentiate between HIV-1 and HIV-2 infection. Additionally, it significantly decreases the time to complete the testing process for identification of an HIV infection. According to the CLSI Guidelines, the MultiSpot HIV Antibody Differentiation Assay should resolve approximately 97% of the repeatedly reactive screening tests without requiring further testing. According to new guidelines, for the 3% that remain unresolved, further testing (i.e., HIV 1 RNA, Qualitative, TMA and HIV 2 DNA/RNA Qualitative Real Time PCR) may be necessary to differentiate between a true infection and a false positive screening test.

HIV Diagnostic Algorithm:

An HIV test algorithm is a step-by-step testing plan used to determine the presence or absence of HIV infection. Following a review of the algorithms proposed in the CLSI Guidelines as well as from the recent CDC DRAFT Recommendations, the CPAL Directors selected the testing Algorithm below.



As it pertains to testing performed by CPAL:

STEP 1 - CPAL - Initial/Screen Test – CPAL routinely employs a 4th generation screening assay designed to simultaneously detect the presence of the HIV p24 antigen and antibodies to HIV-1 and HIV-2.

- **If the initial /screen test is NON-REACTIVE** – the test is resulted as NON-REACTIVE for HIV-1 and HIV-2 antibodies and p24 antigen.

- **If the initial /screen test is REACTIVE** – the screening test would automatically be repeated (per manufacturer's instructions) **in duplicate** by CPAL (***see STEP 2***).

STEP 2 - CPAL - Repeat Testing of an Initially Reactive Screening Test – an initially reactive screening test is automatically repeated in duplicate.

- **If the Repeat Screening Tests are BOTH NON-REACTIVE -** the test is resulted as NON-REACTIVE for HIV-1 and HIV-2 antibodies and p24 antigen.

- **If EITHER of the Repeat Screening Tests is REACTIVE –** a MultiSpot HIV-1/HIV-2 Antibody Differentiation Assay will automatically be performed by CPAL (***see STEP 3***).

STEP 3 - CPAL – MultiSpot HIV-1/HIV-2 Antibody Differentiation Assay – this test is designed to confirm the presence of an HIV antibody and differentiate between HIV-1 and HIV-2.

- **If HIV-1 Positive/HIV-2 Negative –** this confirms the presence of an HIV-1 antibody and of HIV-1 infection.
    - **This will be reported as 'Reactive – Positive for HIV-1 Antibody'.**

- **If HIV-1 Negative/HIV-2 Positive** – this confirms the presence of an HIV-2 antibody and of HIV-2 infection.
    - **This will be reported as 'Reactive – Positive for HIV-2 Antibody'.**

- **If HIV-1 Positive/HIV-2 Positive** – this confirms the presence of an HIV antibody; however, further testing will be required to rule out dual infection.
    - **This will be reported as 'Positive for HIV antibody – Type cannot be Differentiated. Additional testing should be performed on a new sample including HIV 1 RNA, Qualitative, TMA (Quest Test Code 16185) and HIV 2 DNA/RNA Qualitative Real Time PCR (Quest Test Code 34977X).'**

- **If HIV-1 Negative/HIV-2 Negative –** the presence of an HIV antibody could not be confirmed; initial reactive screen result may be due to the presence of p24 antigen which may be seen in early infections prior to the development of antibodies. Further testing should be performed to determine if this is a true HIV infection or false positive screening assay (***see STEP 4***).
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    - **This will be reported as 'HIV 1 and 2 Antibody Negative – additional testing should be performed with a new sample including HIV 1 RNA, Qualitative, TMA (Quest Test Code 16185) and HIV 2 DNA/RNA Qualitative Real Time PCR (Quest Test Code 34977X). Note: The HIV Antibody/Antigen Screen detects antibodies to HIV-1 and HIV-2 as well as HIV p24 antigen. The HIV Multispot confirms only the presence of antibodies against HIV-1 and/or HIV-2.'**

- **If result is INVALID -** occasionally, due to interfering factors, the MultiSpot test may generate an 'INVALID' result.
    - **This will be reported as 'INVALID – No Results could be Obtained. Additional testing should be performed with a new sample including HIV 1 RNA, Qualitative, TMA (Quest Test Code 16185) and HIV 2 DNA/RNA Qualitative Real Time PCR (Quest Test Code 34977X).'**

- **If result is INDETERMINATE -** occasionally, due to interfering factors, the MultiSpot test may generate an 'INDETERMINATE' result.
    - **This will be reported as 'INDETERMINATE for HIV-1 Antibody – Additional testing should be performed with a new sample including HIV 1 RNA, Qualitative, TMA (Quest Test Code 16185). Note: The HIV Antibody/Antigen Screen detects antibodies to HIV-1 and HIV-2 as well as HIV p24 antigen. The HIV Multispot confirms only the presence of antibodies against HIV-1 and/or HIV-2.'**

STEP 4 - Quest – Supplemental HIV Testing – additional testing will be required to determine if this is a true HIV infection or false positive screening assay. ***This testing is NOT performed by CPAL.*** It is recommended that the following be ordered by the physician and that the ordering laboratory send these directly to a qualified reference laboratory, i.e. Quest Diagnostics:

1. **HIV 1 RNA, Qualitative, TMA (Quest Test Code 16185)** – this will require a new specimen be collected for this testing.

    Note – <u>DO NOT</u> order a Quantitative HIV Assay (HIV Viral Load) – these assays are not to be used for diagnosing an HIV infection; they are to be used for monitoring the HIV viral load in an already confirmed HIV positive patient while managing HIV viral therapy.

- **If HIV 1 RNA, Qualitative, TMA is NON-REACTIVE/NEGATIVE** – target was not detected; no detectable HIV-1 RNA.

- **If HIV 1 RNA, Qualitative, TMA is REACTIVE/POSITIVE** – target was detected; indicates HIV-1 RNA was detected supporting a diagnosis of HIV-1 infection.

2. **HIV 2 DNA/RNA Qualitative Real Time PCR (Quest Test Code 34977X)** – this will require a new specimen be collected for this testing.

   - **If HIV 2 DNA/RNA Qualitative Real Time PCR is NEGATIVE**– no HIV-2 DNA/RNA detected; HIV-2 infection unlikely.

   - **If HIV 2 DNA/RNA Qualitative Real Time PCR is POSITIVE**– target was detected; indicates HIV-2 DNA/RNA was detected supporting a diagnosis of HIV-2 infection.

If, at any time during the HIV testing process, you should have any questions, please feel free to contact CPAL at 851-1416.

References:

1. **CLSI.** ***Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus infection; Approved Guideline.*** **CLSI document M-53-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.**

2. **CDC.** ***DRAFT Recommendations: Diagnostic Laboratory Testing for HIV Infection in the United States.*** **Presented at the 2012 HIV Diagnostics Conference Feedback Session; December 14, 2012.**