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
HIV Geenius replaces Multispot as Supplemental Antibody Test

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LOINC Code change with HIV Geenius Assay: 80203-3

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 had 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])** In 2012, the CDC published Draft Updated

Testing Recommendations. These recommendations were incorporated into the CPAL testing algorithm in January 2013. When the recommendations were finalized in June 2014, Western Blot was eliminated as a supplemental test, and replaced with nucleic acid tests.

CPAL has been notified that the BioRad HIV Multispot test, a component of the testing algorithm used to discriminate HIV-1 from HIV-2, has been discontinued. CPAL has validated the BioRad Geenius test to replace the Multispot. See below for more details of test interpretation.

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.

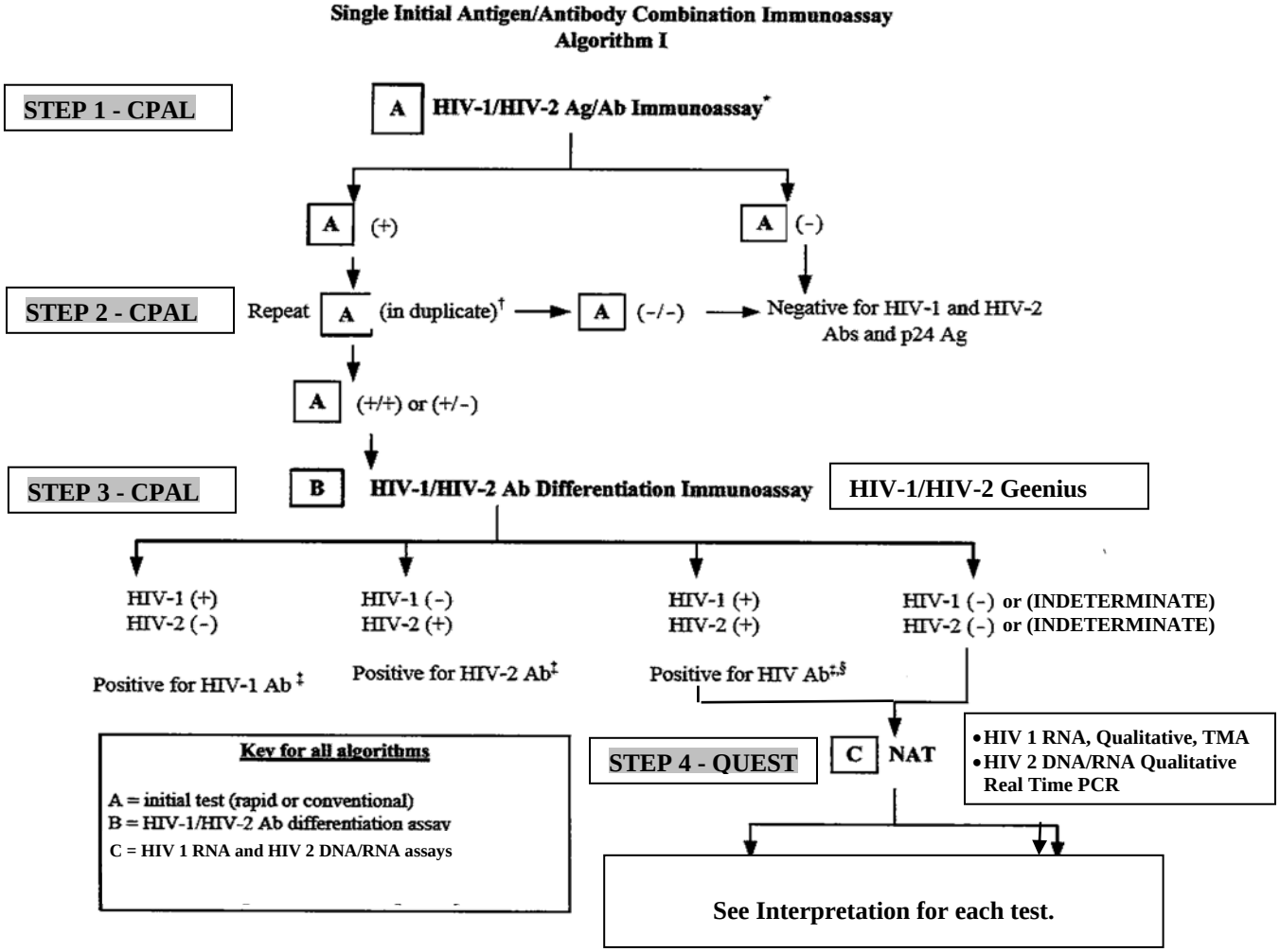
CPAL Tests for HIV Diagnosis:

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 August 2016, CPAL will replace the current BioRad Multispot test with the BioRad Geenius test. According to the CLSI Guidelines, HIV Antibody Differentiation Assay should resolve approximately 97% of the repeatedly reactive screening tests without requiring further testing. According to CDC recommendations, 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. In accordance with published guidelines and recommendations, and with consultation of Quest diagnostics for determining the appropriate nucleic acid supplemental tests, 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 Geenius HIV-1/HIV-2 Antibody Differentiation Assay will automatically be performed by CPAL (***see STEP 3***).

STEP 3 - CPAL – Geenius 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. In addition to the interpretive comments shown below, all HIV Geenius results will include the following comment: '**Note: The HIV Ab/Ag screen detects antibodies to HIV-1 and HIV-2, as well as HIV p24 Antigen. The HIV Geenius confirms only the presence of antibodies against HIV-1 and/or 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. Note: Co-infection with HIV-2 cannot be ruled out. If clinically relevant, additional testing should be performed including HIV-2 DNA/RNA Qualitative Real Time PCR (Quest code 34977X).'**

- **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. Note: Co-infection with HIV-1 cannot be ruled out. If clinically relevant, additional testing should be performed including HIV-1 RNA Qualitative, TMA (Quest code 16185).'**

- **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 (***see STEP 4***).
    - This will be reported as **'HIV Positive Untypable – 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 Positive against 1 envelope protein/HIV-2 Positive –** This confirms the presence of HIV-2 antibody. However, HIV-1 cross reactivity is detected, and additional testing will be required to rule out dual infection (***see STEP 4***).

- This will be reported as **'HIV-2 Positive with HIV-1 cross reactivity. HIV-1 positivity (with only one HIV-1 envelope band, gp160 or gp41) is due to cross reactivity and precludes confirmation of HIV-1. Additional testing should be performed on a new sample including HIV-1 RNA Qualitative, TMA (Quest code 16185).**

- **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***).
  - This will be reported as **'HIV 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).**

- **If result is INVALID –** Rarely, due to interfering factors, the Geenius test may generate an 'INVALID' result. Further testing should be performed to determine if this is a true HIV infection or false positive screening assay (***see STEP 4***).
  - 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).'**

Occasionally, due to interfering factors, the Geenius test may generate an 'INDETERMINATE' result. The Geenius test is able to generate an 'INDETERMINATE' individually for HIV-1 and 2. If only one of the results is 'INDETERMINATE,' the test can generate a partial result. Therefore, there are multiple interpretations and supplementary testing recommendations that could be generated. Further testing should be performed to determine if this is a true HIV infection or false positive screening assay (***see STEP 4***).

- **HIV-1 Positive/HIV-2 Indeterminate –** This confirms the presence of an HIV-1 antibody; however, further testing will be required to rule out dual infection (***see STEP 4***).
  - This will be reported as **'HIV-1 Positive – Positive for HIV-1 Antibody. Note: Co-infection with HIV-2 cannot be ruled out. If clinically relevant, additional testing should be performed with a new sample including HIV-2 DNA/RNA Qualitative Real Time PCR (Quest Test Code 34977X).'**

- **HIV-1 Indeterminate/HIV-2 Positive** – This confirms the presence of HIV-2 antibody; however, further testing will be required to rule out dual infection (***see STEP 4***).
  - This will be reported as **'HIV-2 Positive – Positive for HIV-2 Antibody. Note: Co-infection with HIV-1 cannot be ruled out. Additional testing should be**

performed with a new sample including HIV-1 RNA, Qualitative, TMA (Quest Test Code 16185).'

- **HIV-1 Indeterminate/HIV-2 Negative** – HIV-2 antibodies were not detected. While some bands were detected for HIV-1, they did not meet the criteria for a positive test result (***see STEP 4***).
    - This will be reported as **'HIV-1 Indeterminate – Indeterminate for HIV-1 Antibody. HIV-1 band(s) detected but did not meet criteria for HIV-1 Positive. 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).'**

- **HIV-1 Negative/HIV-2 Indeterminate –** HIV-1 antibodies were not detected. While some bands were detected for HIV-2, they did not meet the criteria for a positive test result (***see STEP 4***).
    - This will be reported as **'HIV 2 Indeterminate – Indeterminate for HIV-2 Antibody. HIV-2 band(s) detected but did not meet the criteria for HIV-2 Positive. 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).'**

- **HIV-1 Indeterminate/HIV-2 Indeterminate –** While some bands were detected for HIV-1 and HIV-2, they did not meet the criteria for positive test results (***see STEP 4***).
    - This will be reported as **'HIV Indeterminate – Indeterminate for HIV Antibodies. HIV band(s) detected but did not meet criteria for HIV-1 or HIV-2 Positive. 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).'**

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.

3. Centers for Disease Control and Prevention and Association of Public Health Laboratories. *Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations.* Available at http://dx.doi.org/10.15620/cdc.23447. Published June 27, 2014. Accessed June 13, 2016.

4. Bio-Rad Geenius HIV-1/2 Supplemental Assay Test Package Insert (Ref #72461) 12/2014.