INTERPRETATION INFORMATION SHEET

Human Immunodeficiency Virus (HIV) Serology

**Anti-HIV-1/HIV-2 Plus O ChLIA:** This chemiluminescent assay (ChLIA) detects the presence of antibodies to HIV-1 and HIV-2, including HIV-1 Groups M and O. It does not discriminate between HIV-1 and HIV-2 reactivity. An HIV-1, HIV-2 or dual infection can only be confirmed serologically by more specific supplemental and/or confirmatory assays.

**Anti-HIV-1/HIV-2 Plus O EIA:** This enzyme-linked immunoassay (EIA) allows simultaneous detection of antibodies to HIV-1 and HIV-2, including HIV-1 Group O. It does not discriminate between HIV-1 and HIV-2 reactivity. An HIV-1, HIV-2 or dual infection can only be confirmed serologically by more specific supplemental and/or confirmatory assays.

**Anti-HIV-1 Western Blot (WB):** See separate sheet.

**Anti-HIV-2 EIA:** This enzyme-linked immunoassay (EIA) detects antibodies to HIV-2. However, repeatedly reactive specimens may contain specific antibodies to HIV-2, cross-reacting antibodies to HIV-1, or be non-specifically reactive. Therefore, more specific supplemental tests for antibodies to both HIV-1 and HIV-2 should be performed.

**Anti-HIV-1/2 Supplemental Assay:** See separate sheet.

Final interpretations of any serology assay should also consider the results from the nucleic acid amplification assay and additional medical history.

- It is acceptable to perform Procleix NAT HIV discriminatory testing in place of HIV antibody confirmation tests for a specimen that is reactive by both Anti-HIV-1/HIV-2 Plus O and the Procleix NAT screening test (refer to HIV NAT information below).
- A negative serology test does not exclude the possibility of exposure to or infection with HIV.
INTERPRETATION INFORMATION SHEET

Human Immunodeficiency Virus Nucleic Acid Testing (NAT)

Procleix HIV-1/HCV/HBV Assay: This assay utilizes transcription mediated amplification of nucleic acid followed by the use of specific labeled probes for detection of HIV-1 and HCV RNA and HBV DNA. The assay generates a chemiluminescent signal which is measured by a luminometer in Relative Light Units (RLU) and reported as reactive or nonreactive. The screening assay is referred to as “Ultrio Plus” and does not discriminate between HIV-1 and HCV RNA and/or HBV DNA. Specimens found to be reactive with this multiplex (or triplex) assay are then tested in HIV-1, HCV and HBV Discriminatory Assays (dHIV, dHCV, dHBV assays) to determine if they are reactive for HIV, HCV, and/or HBV. It is possible for all three discriminatory tests to be non-reactive. This may indicate a false positive NAT screening test.

Roche cobas MPX Assay: This assay is based on real time Polymerase Chain Reaction (PCR) technology using a fully automated nucleic acid extraction and purification process followed by PCR amplification of RNA and DNA using specific primers for detection of HIV and HCV RNA and HBV DNA. The assay generates a fluorescent signal produced by labeled detection probes, which is measured by an automated instrument and reported as reactive or nonreactive. The screening assay allows independent identification of HIV and HCV RNA and/or HBV DNA which eliminates the need for individual discriminatory testing. The cobas MPX test may serve as a confirmatory assay in lieu of serology confirmation in “dual-reactive” individuals when discriminated for the applicable RNA or DNA in conjunction with the corresponding antigen or antibody. It is possible for all three cobas MPX discriminatory results to be non-reactive. This may indicate a false positive nucleic acid screening test.

Discriminatory NAT Assays (Procleix dHIV, dHCV, dHBV): These assays utilize specific probe reagent directed against specific conserved regions in the viral genome to determine the presence of virus by TMA. Detection of the chemiluminescent signal is measured by a luminometer in Relative Light Units (RLU) and reported as reactive or nonreactive. Individual Procleix discriminatory assays may be performed for HIV-1, HCV or HBV and may serve as a confirmatory assay for NAT and serology (dual-reactive) samples in lieu of serology confirmation.

Limitations: Detection of HIV, HCV and HBV using nucleic acid tests is dependent on the number of viral particles present in the specimen and may be affected by stage of infection. In addition, some true positive samples may generate invalid results due to a high viral load. Final interpretations of any serology assay should also consider the results from nucleic acid amplification assays and additional medical history.

NOTE: Procleix discriminatory HBV triplicate testing or cobas MPX testing is required to provide HBV DNA detection at approximately 2 IU/mL at >95% probability.
INTERPRETATION OF ANTI-HIV-1 WB RESULTS

The GS HIV-1 Western Blot (WB) is a qualitative test for detection of antibodies to HIV-1 and is intended to be used as an additional, more specific test in specimens found to be repeatedly reactive by screening procedures such as EIA. In this assay, purified and inactivated HIV-1 strain LAV grown in the CEM cell line is disrupted and electrophoretically resolved into protein bands. The proteins are transblotted onto nitrocellulose sheets which are then cut into strips. If specific HIV-1 antibody is present in a sample, it binds to the corresponding viral proteins resolved on the strip. Addition of a phosphatase labeled conjugate followed by incubation with enzyme substrate causes development of purple bands if HIV antibodies are present.

HIV-1 WB results are interpreted as Positive, Negative, Indeterminate or Unreadable

**POSITIVE:** A specimen is interpreted as positive when at least two of the major bands (gp160 and/or gp120, gp41 or p24) are present and are at least as intense as the Low Positive Control gp120 band. Although a positive result for antibodies to HIV-1 usually indicates infection with the virus, a diagnosis of Acquired Immunodeficiency Syndrome, AIDS, can only be established on clinical grounds, provided that an individual meets the case definition of AIDS established by the Centers for Disease Control.

**ACTION REQUIRED:** It is imperative an inquiry be made regarding blood donation history and pertinent information forwarded to the Medical Director or Technical Director of the referring blood center, regardless of where the donation may have taken place. The donor (or individual) must be assured confidentiality will be maintained.

**NEGATIVE:** A specimen is interpreted as negative when there are no bands present.

**INDETERMINATE:** A specimen is interpreted as indeterminate when one or more bands are present but it does not meet the criteria for a Positive result as described above.

**NOTE:** An Indeterminate interpretation should not be considered positive or negative but may provide useful information in the context of medical evaluation in which clinical information is available. Additional immunoblot testing and clinical evaluation including testing of a fresh specimen after six months may be utilized to evaluate an Indeterminate result.

HIV-INT REV 6
INTERPRETATION OF GEENIUS HIV 1/2 RESULTS

This immunochromatographic assay is intended for use as an additional, more specific test to confirm and differentiate antibodies to HIV-1 and HIV-2 for specimens found to be repeatedly reactive by diagnostic screening procedures. It is not approved for confirmation testing of specimens from blood, plasma, cell or tissue donors that are repeatedly reactive on HIV-1/2 donor screening assays. Due to this limitation, the assay cannot be used as a replacement for licensed HIV-1 IFA or Western blot testing of repeatedly reactive donation samples. It may, however, provide useful information for physician counseling of donors that are not confirmed by an approved HIV-1 confirmatory assay.

In this assay, highly conserved recombinant proteins and synthetic peptides representing specific HIV-1 and HIV-2 proteins are bound to a membrane solid phase which will capture HIV-1 and/or HIV-2 antibodies present in a sample. Addition of protein A conjugated to colloidal gold dye particles causes development of pink/purple bands if HIV antibodies are present.

Results for the Geenius assay are based on a combined interpretation for specific HIV-1 and HIV-2 reactivity. These results may indicate HIV-1 or HIV-2 or undifferentiated reactivity due to the high degree of cross-reactivity between HIV-1 and HIV-2 as described below.

<table>
<thead>
<tr>
<th>HIV-1 RESULT</th>
<th>HIV-2 RESULT</th>
<th>ASSAY INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab Nonreactive</td>
<td>Ab Nonreactive</td>
<td>HIV Antibody NEGATIVE</td>
</tr>
<tr>
<td>Ab Indeterminate</td>
<td>Ab Nonreactive</td>
<td>HIV-1 INDETERMINATE\textsuperscript{a}</td>
</tr>
<tr>
<td>Ab Nonreactive</td>
<td>Ab Indeterminate</td>
<td>HIV-2 INDETERMINATE\textsuperscript{b}</td>
</tr>
<tr>
<td>Ab Indeterminate</td>
<td>Ab Indeterminate</td>
<td>HIV INDETERMINATE\textsuperscript{c}</td>
</tr>
<tr>
<td>Ab Positive</td>
<td>Ab Nonreactive</td>
<td>HIV-1 POSITIVE</td>
</tr>
<tr>
<td>Ab Positive</td>
<td>Ab Indeterminate</td>
<td>HIV-1 POSITIVE</td>
</tr>
<tr>
<td>Ab Nonreactive</td>
<td>Ab Positive</td>
<td>HIV-2 POSITIVE</td>
</tr>
<tr>
<td>Ab Indeterminate</td>
<td>Ab Positive</td>
<td>HIV-2 POSITIVE</td>
</tr>
<tr>
<td>Ab Positive</td>
<td>Ab Positive</td>
<td>HIV-2 POSITIVE with HIV-1 cross-reactivity: Antibody to HIV-2 confirmed in the sample. HIV-1 positivity (with only one HIV-1 envelope band, gp160 or gp41), is due to cross-reactivity and precludes confirmation of HIV-1\textsuperscript{*}. \textsuperscript{a}Note: Differentiation features managed by proprietary algorithm.</td>
</tr>
<tr>
<td>Ab Positive</td>
<td>Ab Positive</td>
<td>HIV POSITIVE Untypable (undifferentiated): Antibodies to HIV-1 and HIV-2 confirmed in the sample. This may occur in an HIV-2 positive sample with significant cross-reactivity to HIV-1, or may be due to co-infection with both HIV-1 and HIV-2 (rare)\textsuperscript{*}. \textsuperscript{b}Note: Differentiation features managed by proprietary algorithm.</td>
</tr>
</tbody>
</table>

\textsuperscript{a}HIV-1 band(s) detected but did not meet the criteria for HIV-1 Positive  
\textsuperscript{b}HIV-2 band(s) detected but did not meet the criteria for HIV-2 Positive  
\textsuperscript{c}HIV band(s) detected but did not meet the criteria for HIV-1 Positive or HIV-2 Positive
Assay Interpretation Limitations:

- A negative or indeterminate result does not preclude the possibility of exposure to or infection with HIV. An antibody response to a recent exposure may take several months to reach detectable levels. It is recommended that testing should be completed with an FDA-approved HIV-1 nucleic acid test (NAT).
- False negative results may occur in infected individuals receiving highly active antiretroviral therapy (HAART).
- An indeterminate interpretation does not exclude the possibility of early seroconversion or a cross-reaction with other retroviruses. The homology between HIV-1 and HIV-2 can lead to cross reactivity between anti-HIV-1 and anti-HIV-2 antibodies.
- Samples that meet the HIV-1 positive criteria may, in some rare cases, show cross reactivity on one of the HIV-2 envelope bands. In most of these cases, this profile that confirms HIV-1 infection does not exclude the rare possibility of a secondary HIV-2 seroconversion (coinfection).
- Samples which meet the HIV-2 positive criteria can show cross reactivity on one or more HIV-1 bands. In most cases, this profile confirms an HIV-2 infection. However, it does not exclude the rare possibility of a secondary HIV-1 seroconversion (coinfection).
- Samples that have a Final Assay Interpretation of HIV-2 Positive with HIV-1 cross-reactivity, are both HIV-1 Ab reactive and HIV-2 Ab reactive, are generally HIV-2 positive samples with HIV-1 cross-reactivity. Such profiles do not exclude the rare possibility of HIV-1 and HIV-2 co-infection.
- Samples with reactivity to all 4 envelope bands have all been HIV-2 positive samples with HIV-1 reactivity that cannot be differentiated but these do not exclude the rare possibility of HIV-1 and HIV-2 co-infection.
- HIV-2 indeterminate results for samples from persons without any risk factors for HIV-2 infections should be confirmed by retesting before reporting final results.

REFERENCES:

Abbott Prism HIV O Plus manufacturer’s instructions
Bio-Rad GS HIV-1/HIV-2 Plus O EIA manufacturer’s instructions
Roche cobas® MPX manufacturer’s instructions
Procleix Ultro Plus Assay manufacturer’s instructions
BioRadGS HIV-1 Western Blot (manufacturer’s instructions).
Bio-Rad Geenius™ HIV 1/2 Supplemental Assay Instructions for use

Revision History

<table>
<thead>
<tr>
<th>Revision</th>
<th>Implemented</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rev 6</td>
<td>09/30/2019</td>
<td>Discontinuation of HIV-1 IFA</td>
</tr>
<tr>
<td>Rev 5</td>
<td>12/17/2018</td>
<td>CTS Algorithm Modification</td>
</tr>
<tr>
<td>Rev 4</td>
<td>05/16/2018</td>
<td>Updated for Geenius HIV 1/2 Supplemental Assay upgrade V1.3.</td>
</tr>
<tr>
<td>Rev 3</td>
<td>12/19/2016</td>
<td>Implementation of Geenius HIV 1/2 Supplemental Assay</td>
</tr>
<tr>
<td>Rev 2</td>
<td>07/01/2016</td>
<td>Implementation of Roche cobas MPX 2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Addition of limitations</td>
</tr>
<tr>
<td>Initial Release</td>
<td>05/01/2013</td>
<td>Revision History added</td>
</tr>
</tbody>
</table>

HIV-INT REV 6