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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 additional 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 additional more specific supplemental and/or confirmatory assays.

Anti-HIV-1 IFA: 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, additional 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.

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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.

cobas TaqScreen HIV/HCV/HBV Assay: This assay utilizes reverse transcription of RNA followed by automated Polymerase Chain Reaction (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 is referred to as “MPX 2.0” and it allows independent identification of HIV and HCV RNA and/or HBV DNA which eliminates the need for individual discriminatory (AmpliScreen) testing historically used for discrimination of multiplex (triplex) reactive samples. At this time, the individual discriminatory results are not currently approved as a confirmatory assay for NAT and serology (dual-reactive) samples so serology confirmation is required for all serology reactive samples regardless of NAT reactivity. It is possible for all three discriminatory tests 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 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: cobas TaqScreen MPX 2.0 duplicate testing is required to provide HBV DNA detection at approximately 2 IU/mL at >95% probability.

INTERPRETATION OF ANTI-HIV-1 IFA RESULTS

The HIV-1 immunofluorescence assay (IFA) 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, specific HIV-1 antibodies present in a specimen bind to HIV-1 infected human T cells which are fixed to a microscope slide. Uninfected cells are included on the microscope slide for comparison purposes. Bound HIV-1 antibodies are detected with anti-human immunoglobulin conjugated to fluorescein isothiocyanate, which fluoresces when exposed to UV light. Interpretation of the degree and pattern of fluorescence of the infected cells compared to uninfected cells determines the HIV-1 status of a sample.

HIV-1 IFA results are interpreted as Positive, Negative or Indeterminate.

POSITIVE: A specimen is interpreted as positive when there is a specific cytoplasmic staining pattern in the HIV-1 infected cells and there is a significant difference in the intensity of fluorescent staining and the pattern of fluorescence between the HIV-1 infected and uninfected cells. Although a positive IFA 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 local blood bank, regardless of where the donation may have taken place. The patient must be assured confidentiality will be maintained.

NEGATIVE: A specimen is interpreted as negative when there is no specific fluorescent staining of the infected cells and there is no significant difference between the HIV-1 infected and uninfected cells.

INDETERMINATE: A specimen is interpreted as indeterminate when there is fluorescent staining present in both the HIV-1 infected and uninfected cells OR when it is not possible to clearly differentiate the intensity of fluorescent staining and the pattern of fluorescence between the HIV-1 infected and uninfected cells OR when duplicate retests are discordant.

NOTE: A sample with an initial indeterminate result is retested in duplicate before a final interpretation is made.

Indeterminate IFA interpretation does not imply that HIV-1 antibodies are, or are not, present in the specimen. It means that the HIV-1 status cannot be resolved and results must not be considered positive or negative. In most cases, indeterminate IFA results are due to the presence of non-specific staining. Non-specific staining can occur as a result of a variety of conditions and may mask the presence of specific HIV-1 staining. The correct evaluation in such situations must be based on subsequent testing and clinical evaluation.

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

HIV-1 RESULT	HIV-2 RESULT	ASSAY INTERPRETATION
Negative	Negative	HIV NEGATIVE
Indeterminate	Negative	HIV-1 INDETERMINATE ^a
Negative	Indeterminate	HIV-2 INDETERMINATE ^b
Indeterminate	Indeterminate	HIV INDETERMINATE ^c
Positive	Negative	HIV-1 POSITIVE
Positive	Indeterminate	HIV-1 POSITIVE
Negative	Positive	HIV-2 POSITIVE
Indeterminate	Positive	HIV-2 POSITIVE
Positive	Positive	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*. *Note: Differentiation features managed by proprietary algorithm.
Positive	Positive	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)*. *Note: Differentiation features managed by proprietary algorithm.

^aHIV-1 band(s) detected but did not meet the criteria for HIV-1 Positive

^bHIV-2 band(s) detected but did not meet the criteria for HIV-2 Positive

^cHIV 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 be repeated on a freshly collected specimen after 2 to 4 weeks.
- 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. It is recommended that testing be repeated on a freshly collected specimen after 2 to 4 weeks.
- 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 (co-infection).
- Samples which meet the HIV-2 positive criteria can show cross reactivity on one or more HIV-1 bands. In most cases, an HIV-1 indeterminate profile associated with an HIV-2 positive profile is a true HIV-2 only infection but this does not exclude the possibility of a secondary HIV-1 seroconversion (co-infection).
- Samples that meet both HIV-1 and HIV-2 positive criteria but are reactive with only one envelope band (gp160 or gp41) are generally HIV-2 positive samples with HIV-1 cross-reactivity but this does 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
cobas™ TaqScreen MPX Test, Version 2.0 manufacturer's instructions
Procleix Ultrio Plus Assay manufacturer's instructions
Sanochemia Pharmazeutika AG Fluorognost™ HIV-1 IFA (manufacturer's instructions).
Bio-Rad Geenius™ HIV 1/2 Supplemental Assay Instructions for use

Revision History

Revision	Implemented	Reason
Rev 3	12/19/2016	<ul style="list-style-type: none">▪ Implementation of Geenius HIV 1/2 Supplemental Assay
Rev 2	07/01/2016	<ul style="list-style-type: none">▪ Implementation of Roche cobas MPX 2.0▪ Addition of limitations
Initial Release	05/01/2013	<ul style="list-style-type: none">▪ Revision History added