

The Changing Landscape of HIV Testing Q&A

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- Q. Do you know when the CDC will be coming out with its formal MMWR report with the algorithm?
- A. It was anticipated that the formal guidelines would be published in the early part of this year. I suspect that they will be published before the end of the year, but I do not have any official information on the date.
- Q. After speaking with reps from Bio-Rad regarding the use of the Multispot, it was confirmed that use of that assay for confirmation would be considered "off-label" and thus require method validation of the assay for that use. Given that such a validation could potentially mean the testing of hundreds to thousands of samples by the end-user (financially difficult for many institutions), how is that reality addressed in the CDC recommended algorithm?
- A. That is a very good question. Since I am not a CDC representative, I cannot speak on behalf of the CDC. The algorithm has many points that represent challenges for laboratories because they may require off-label use and potentially laboratory validation. We are hoping that the anticipated CDC guidelines will be published soon and provide guidance.
- Q. Would you please comment on the following: A client has a "reactive" result using a rapid test at the point of care (single test only). The lab draws venous blood and sends it to the lab for confirmatory testing. The lab uses its regular screening assay to test the sample (ARCHITECT® fourth-generation Combo). If that test is non-reactive (single test only), is it appropriate to report that sample as HIV negative? If not, what further testing would you suggest?
- A. I believe the current CDC guidelines consider a "reactive" rapid test a "preliminary positive" and recommend confirmation (Western blot, IFA). However, I would refer you to CLSI document M53-A on HIV diagnosis that was published in 2011, where several updated algorithms were presented for screening and confirmation, including specific information for point of care testing.
- Q. Since the algorithm is proposed but not accepted, should laboratories use the old guidelines and confirm with Western blot?
- A. At this point, formal guidelines from the CDC addressing the new algorithm have not been published. I would refer you to the following CLSI document: "Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection; Approved Guideline," which includes the new algorithm as one of several algorithms addressing HIV diagnosis (published June 30, 2011). ARUP is currently using the Western blot for confirmation.
- Q. Would you interpret a Western blot as positive if the following band pattern was present: p24, gp 160 with no gp 120 or p 24 with gp120 with no gp 160?
- A. Since I do not know your location or organization (different organizations have different interpretive criteria for a positive Western blot result, although the CDC is the most commonly utilized in the US) , I would refer you to the package insert instructions associated with the assay you are currently using and your institution's medical director for input.

- Q. Is the CDC algorithm for HIV testing approved by the FDA? Does ARUP offer the NAAT testing? My understanding is that the FDA does not approve algorithms per say; it clears or approves assays for a particular use, based on the data submitted with the application of the assay.
- A. ARUP currently offers a number of viral load assays with different chemistries (bDNA, real-time PCR, proviral DNA), but not the Aptima qualitative assay. In regard to the algorithm, we are waiting for the published guidelines from the CDC and evaluating if and how we can validate and offer the other components of the algorithm.
- Q. The CDC recommends a confirmation with the rapid test Multispot HIV-1 and 2 discriminatory assay. I would like to know your point of view about the line immunoassay test, called Innolia, that is not approved by the FDA but that can distinguish between HIV-1 and 2 in a more performant way?
- A. Although I am aware of the existence of the Innolia assay, I do not have any experience with this assay.
- Q. Do you have to use the new CPT Code to order 4th gen testing?
- A. The use of the new CPT code 87389 is a regulatory question.
- Q. Which instrument will you be using in August?
- A. ARUP is currently using the ADVIA Centaur automated third-generation EHIV/1/2/O assay (Siemens) and will also be performing the fourth-generation manual immunoassay from Bio-Rad beginning in August.
- Q. With the newly proposed algorithm, does this require collection of an additional specimen if the NAAT testing is required?
- A. In general, sample requirements differ and are typically more stringent, depending on the NAAT assay, compared to immunoassays (including Ag/Ab combo assays).
- Q. If the fourth-generation test is positive, Multispot is negative, and NAT is also negative, is repeat testing after a certain period recommended considering the eclipse period?
- A. According to published data, NAT testing is more sensitive than fourth-generation Ag/Ab combo assays. The eclipse is a period of time when no currently available assay can detect infection.
- Q. What is the latest information you have on the significance and prevalence of the HIV -1 P group?
- A. Group P is most closely related to SIV strains circulating in gorillas but remains extremely rare. The following reference may be useful: Vallari et al. *J Virol* 2011;85(3):1403-7.
- Q. Say we do the screening test and then do a Multispot, the patient looks to be HIV-2 positive and HIV-1 negative, would you still proceed to the NAAT testing? I'm sure you would get a negative result in this situation, but just curious if the follow-up NAAT testing is required or recommended for HIV-2 positive patients, as shown by something like the Multispot.
- A. There is no FDA-cleared method for confirmation of HIV-2 infection. HIV-2 specific EIA, or HIV-2 immunoblot, and HIV-2 DNA assays are offered by some specialty laboratories. ARUP offers an HIV-2 panel that consists of an HIV-2 specific EIA that reflexes to HIV-2 immunoblot if the HIV-2 EIA is repeatedly reactive.

Q. What is the confirmatory test(s) in the CDC proposed algorithm? The Multispot test was not mentioned in this presentation as an approved confirmatory test.

A. I believe the Multispot is not specifically approved for confirmation of HIV infection.

Q. What are ARUP's plans in regards to offering a testing panel similar to the CDC's new diagnostic testing algorithm? Since the Multispot & Aptima methods are not automated, will you stick with the current algorithm until one or both tests become automated?

A. At this point, ARUP is offering the current algorithm with Western blot for confirmation. ARUP will be offering a fourth-generation HIV-1/2 plus O Ag/Ab combo assay beginning in August. The Western blot is insufficient for confirmation of a fourth generation, as it only detects antibodies and cannot confirm the p24 antigen positive samples. Additional testing will be necessary to confirm acute HIV infection. In regard to the algorithm, we are waiting for the published guidelines from the CDC and evaluating if and how we can validate and offer the other components of the algorithm.

Q. At what day of infection is NAAT testing available with good results?

A. Molecular assays that detect viral RNA directly are the most sensitive assay format and can detect HIV infection as early as day 9 to 11 post-infection, but variation exists depending on the assay chemistry and the sensitivity of the specific NAAT assay utilized.

Q. How do we confirm HIV 2 at this time? You recommended Multispot, but is there any other way?

A. ARUP does not specifically recommend the Multispot. The proposed CDC algorithm suggests an HIV-1, HIV-2 discriminatory assay at the second step in the algorithm. Practically, the only assay that has that capability is the Multispot. ARUP offers the "traditional" HIV diagnosis algorithm with Western blot for confirmation of HIV-1 infection. There is no FDA-cleared method for confirmation of HIV-2 infection. HIV-2 specific EIA, HIV-2 immunoblot, and HIV-2 DNA assays are offered by some specialty laboratories. ARUP offers an HIV-2 panel that consists of an HIV-2 specific EIA that reflexes to HIV-2 immunoblot if the EIA is repeatedly reactive.

Q. We are using the third-generation method with IFA for confirmation. Do we need to do any additional tests for confirmation?

A. ARUP is currently using a third-generation immunoassay and the Western blot for confirmation (IFA is an accepted alternative for confirmation of HIV-1 infection). Additional testing may be considered if HIV-2 or acute HIV infection is suspected.

Q. We currently use the third-generation ADVIA Centaur HIV-1/2/O platform for routine screening of HIV and confirm with the Bio-Rad Genetic Systems HIV-1 Western blot. Since all the data supports the Multispot as being more sensitive than the HIV-1 Western blot for confirmation testing (part of the suggested algorithm for fourth generation), could we replace our HIV-1 Western blot with the Multispot and still be in compliance with an accepted algorithm for HIV testing?

A. ARUP is currently screening with a third-generation immunoassay and confirming with Western blot. We are waiting for the official CDC guidelines to be published before making changes to our current algorithm. For additional information, I would refer you to the following CLSI document: "Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection; Approved Guideline," which includes the new algorithm as one of several algorithms addressing HIV diagnosis (published June 30, 2011).