CDC Response to Comments from Peer Reviewers

May 16, 2014

Reviewer A.

[Reviewer Comments are in italics]

1. Provide an evaluation of the updated recommendations overall and their applicability for HIV diagnosis;

1. Comment: The updated recommendations are well-researched, and well-written. The earlier diagnosis of patients with acute infection who would have had discrepant EIA and Western blot results should benefit both the patient and public health. These recommendations will also help facilities move towards more opt-out testing.

2. Evaluate the appropriateness of the methods used to develop these recommendations and the strength of the authors' inferences;

2. Comment: Methods are appropriate for recommendations and the inferences are well-supported by the cited literature.

3. Point out any omissions or oversights in the literature cited as the evidence base for these recommendations;

3. Comment: None noted.

3. Identify any biases, oversights, omissions, or inconsistencies in the interpretations, findings, and conclusions;

3a. Comment: Line 190 states that M-class antibodies are seen 3-5 days after p24 antigen yet the x-axis on Figure 1 implies that this is longer (even if only by a few days). This Figure could be perceived as exaggerating the benefits of the 4th gen assay and therefore biased.

Response: The line drawing in Figure 1 has been revised to more closely approximate the 3-5 day interval, and the text describing the figure was amended to read: "The approximate times at which different markers appear, estimated from different data sources, are outlined schematically in Figure 1."

3b. Comment: Please see 4a. below.

4. Provide advice on the reasonableness of judgments made from the scientific evidence;

4a. Comment: Is there a reasonable judgment made from scientific evidence for the use of the phrase 'FDA-approved'? The phrase is used several times throughout the document but my main concern focuses on its use in the context of NAT. The recommendations specify that the HIV-1 NAT test should be 'FDA-approved', (line 453). I understand that there are several NAT's FDA-approved for managing HIV and not for diagnosing HIV. Nevertheless, many laboratories can conduct their own internal validation of a quantitative test for diagnostic purposes. In the absence of a good reason made from scientific evidence, the use of the phrase 'FDA-approved' could be perceived as bias towards the sole manufacturer of the NAT approved for diagnosis.

Response: CDC received conflicting comments from reviewers on this topic. (Please see comment 38 from reviewer D). As noted in the executive summary (page 4), this report provides recommendations to laboratory personnel for the use of FDA-approved assays. At each step in the algorithm, the recommended assays specify "FDA-approved," and the majority of studies reviewed in the synthesis of evidence used FDA-approved assays. No bias was intended in favor of any commercial product.

As noted by this reviewer, quantitative NATs that are FDA-approved for monitoring but not for diagnosis could be used in the algorithm if laboratories conducted the validation required by CLIA. Several studies cited in the evidence synthesis used these quantitative assays. Note, however, that all FDA-approved HIV assays also undergo evaluations of performance with non-B subtypes of HIV-1 and HIV-1 group O. Evidence indicates that some assays that are not FDA-approved, especially in the case of NAT, do not detect all HIV-1 variants, depending on the assay design and selection of targets for amplification.[1] Although subtype B predominates in the U.S., one study reported a 4% prevalence of non-B subtypes among 4,884 specimens submitted to a US commercial laboratory in 2011 for drug resistance genotyping.[2] Therefore, CDC considers the assay's ability to detect non-B subtypes (as demonstrated by the FDA-approved assays) to be important for accurate HIV diagnosis, and a justification for recommending assays that are FDA-approved.

4b. Comment: Many clinical laboratories use quantitative NAT's, not the FDA-approved qualitative NAT. This is acknowledged in lines 870 - 871. Lines 1070 - 1075 again mention the performance of NAT's but don't specify whether it was the FDA-approved qualitative NAT or the quantitative NAT's. Were they equivalent in performance?

Response: The studies cited in lines 1070-1075 used both the qualitative and quantitative NAT on different specimens. No published studies directly compared the performance of qualitative NAT with quantitative NAT on the same specimens. Unpublished data (M. Busch, personal communication) suggest the limit of detection of the qualitative NATs is lower than that of the quantitative NAT. These unpublished data indicate that this difference in the limits of detection is unlikely to have clinical

significance when NAT is used after a reactive Ag/Ab combination assay because the limit of detection for both the qualitative and quantitative assays is much lower than the estimated threshold (30,000 – 58,000 RNA copies/ml) at which the 4th generation combination assay becomes reactive for p24 antigen.[3]

5. Ensure that scientific uncertainties are clearly identified and characterized, that the potential implications of any uncertainties for the proposed recommendations are clear,

5. Comment: The one uncertainty that needs to be addressed is the specificity of the 4th gen assays. Lines 1022 - 1039 address specificity of NAT, 3rd gen, etc., but not 4th generation. It is a concern for laboratories because the usual assumption is that as sensitivity increases, specificity decreases, and the need for additional testing increases.

Response: Data on specificities of the 4th generation assays (which are similar to those of the 3rd generation assays) were presented on lines 1027 to 1028. However, this might have been easy to overlook because it was included in the same text bullet as the specificities of the 3rd generation assays. To improve clarity, the information on specificities of 4th generation assays has been moved to a separate bullet.

6. Assess whether the authors sufficiently acknowledge limitations in the evidence used to develop the recommendations and any limitations of the recommendations themselves for the intended purpose of the accurate laboratory diagnosis of HIV infection.

6. Comment: Limitations acknowledged!

We would also welcome other comments, for example, on improving their usability or other suggestions about the use of terminology, etc.

7. Comment: A close reading of lines 725 - 726 could lead to laboratories' NOT reporting test results from initial infections. If the local practice is to run a 4th generation assay and then go straight to NAT for diagnosis (bypassing 'Multispot'), then the lab might not think they have to report the results because this practice is not part of the recommended testing algorithm. Far-fetched, but.... I'm not sure what benefit there is to using the phrase 'as part of the recommended testing algorithm' in line 726.

Response: The phrase “as part of the recommended testing algorithm” has been removed.

8. Comment: Line 1159 refers to 'single use rapid HIV tests' but table 2 in reference 46 refers to eia and chemiluminescent tests. I assumed the term 'single use' referred to the rapid single-use cartridge tests, but that is not what the table covers. I don't know why the authors are comparing the 4th gen tests to single use cartridges when the data show they are also superior to the standard automated assays used in most large laboratories. The article text says the 4th gen also performed better than the single use cartridges but

the data are not presented in detail.

Response: The paragraph in the evidence summary that includes line 1159 is intended to compare the performance of 4th generation assays and single-use rapid HIV tests during early infection. It presents the evidence in support of the recommendation for testing specimens submitted after a reactive rapid test result to the entire recommended algorithm, beginning with the 4th generation combination immunoassay. To reduce confusion about the purpose of this paragraph, each paragraph in Section 4 of the evidence synthesis has been given a subheading. The paragraph to which the reviewer refers is now labeled "d. Testing specimens submitted after reactive single-use rapid HIV test results." All data in this paragraph compare the performance of the 3rd generation and 4th generation laboratory immunoassays and the single use rapid tests, including the 4th generation combination antigen/antibody rapid test. Citations are provided for these data. Table 3 in reference 46 includes data on the performance of each of the rapid HIV antibody tests, 3rd generation antibody tests, and one 4th generation antibody test with plasma seroconversion panels. An additional citation has been added, which includes performance data on another 4th generation EIA and 4th generation rapid test with the same plasma seroconversion panels. [4]

9. Comment: These recommendations could help break the logjam about opt-out testing. One of the biggest barriers to opt-out testing is having to wait for a Western blot; this will allow inpatients, ER patients, and even clinic patients, to leave their appointment knowing their status and plugged into follow-up care.

Reviewer B.

[Reviewer comments in italics]

10. Comment: Overall, this is an extremely well written document that proposes a new and long overdue algorithm to that fundamentally changes our current strategy of diagnosing HIV infection. The rational for performing HIV-1/2 antigen/antibody combination immunoassay followed by HIV-1/HIV-2 antibody differentiation is evidence based and will make a major improvement in the diagnosis of acute HIV-1 infection and will no doubt eliminate the known problems with traditional HIV-1 western blot testing. I applaud the committee for a job well done!

11. Comment: Page 4, line 33. Use of the word "significant" typically has statistical implications. It might be better to delete and just say "might miss a percentage of HIV infections".

Response: To avoid any confusion about statistical significance but communicate that this percentage is non-trivial, the sentence has been changed to read "…antibody testing alone might miss a considerable percentage of HIV infections detectable by virologic tests."

12. General question: Since the Ag/Ab immunoassay will be followed by an antibody differentiation assay, will a false positive result on the first assay likely lead to a false positive result on the differentiation assay?

Response: The Reviewer refers to the possibility that shared antigens in the initial and differentiation antibody immunoassays might result in concurrent non-specific cross reactivity and a false-positive antibody test result. The available FDA-approved assays of each class (initial assays and differentiation assay) were evaluated in combination to evaluate whether false-positive results might occur. These evaluations resulted in the observation that a small number of specimens reactive on the Ag/Ab immunoassay and reactive on only one indicator of the antibody differentiation assay proved to have no detectable HIV-1 RNA.[5,6] The manufacturer revised the interpretive criteria for the differentiation assay to require a positive reaction from both HIV-1 indicators on the differentiation assay (recombinant gp41 antigen and synthetic gp41 peptide) for a positive interpretation. Thus, a positive HIV-1 antibody test result consists of 3 reactive test results: the initial Ag/Ab immunoassay and 2 indicators on the differentiation assay. Reactivity on only one indicator is considered an indeterminate result that must be further evaluated with an HIV-1 NAT.

Because this theoretical possibility of a false-positive result due to cross-reactivity still exists, the text in Section M, Additional Considerations, Medical evaluation and follow-up testing, specifies that "unexplained cross-reactivity may result in an incorrect laboratory diagnosis," and indicates that "an HIV-1 viral load test should be part of the baseline evaluation for every HIV-infected patient…" "If HIV-1 RNA is below the assay's limit of detection, repeat or additional testing is indicated to verify the diagnosis of HIV infection."

13. Comment: Acute HIV-2: These guidelines focus on the diagnosis of HIV-1 infection, HIV-2 infection and acute HIV-1 infection. Although the document clearly addresses the challenges in diagnosing acute HIV-2 infection, the end-user still does not have a clear path forward to the diagnosis of acute HIV-2. Perhaps a statement should be included that indicates that if in the rare situation, acute HIV-2 is suspected, what, if any testing or follow up testing should be performed.

Response: A new paragraph describing the prevalence and diagnosis of HIV-2 infection has been added to Section M, Additional Considerations. This paragraph is now referenced by footnotes accompanying the recommendation that describes HIV-1 NAT results and the diagnosis of acute HIV-1 infection. The new paragraph summarizes the limited evidence for and problems with diagnosis of acute HIV-2 infections, and provides guidance for follow-up testing if acute HIV-2 infection is suspected: "Because HIV-2 RNA is undetectable in at least half of HIV-2 infected patients, testing for proviral DNA may be required for definitive diagnosis.[7,8] No tests for HIV-2 RNA or DNA have received FDA approval. If additional testing for HIV-2 is requested, HIV-2 NAT for which the analytic

performance characteristics have been determined may be available from commercial laboratories, city or state public health laboratories, or CDC."

14. Comment: Page 9, line 172. I recommended changing this sentence to: " In most cases, inclusion of p24 antigen capture allows detection of HIV-1 seroconversion before seroconversion.

Response: We do not think this sentence should be revised. It might not have been apparent to the reviewer that lines 149-176 appear in a call-out box which describes the design principles of immunoassays. Lines 170-73 describe the principle of the antigen/antibody combination immunoassays which allows them to detect HIV infection before antibody seroconversion. The occurrence of p24 antigen and its clinical limitations for detecting HIV infection are described in lines 186-189, the text that accompanies this box.

15. General comment: I am not used to using the word "reactive" in the context of NAT testing. It seems to me that "NAT detected" or "NAT positive" may be better understood by the reader.

Response: "Reactive" is the term used in the product insert for the result of the FDA-approved qualitative NAT assay, which states "A Reactive result indicates that HIV-1 RNA was detected." However, to help with understanding when test results are reported, in Table 1, which lists the tests results to be reported to the clinical provider, the suggested language has been changed to "RNA detected."

16. Comment: There is one arm of the algorithm which may introduce a delay in diagnosis: When Ab/Ag combo test is positive and the Ab differentiation assay is HIV-1 and HIV-2 negative OR HIV-1 indeterminate/HIV-2 negative, NAT is the recommended next step. The logistical issue here is that a "second" blood draw will be required to perform NAT testing. In clinical practice, this will introduce a delay over reflex western blot testing as the WB can be performed from the initial specimen where as NAT testing will almost certainly require a second blood draw. Perhaps this is a situation where reflex WB testing might be considered?

Response: CDC has obtained differing opinions about whether a second specimen is required to perform NAT. Some laboratories request plasma specimens for HIV testing and perform NAT, if needed, on the original specimen; others require a second specimen for NAT. Although the need for NAT after a negative or indeterminate antibody differentiation assay might increase turnaround time for definitive test results, evidence indicates that reflex to Western blot before NAT would be of value in an extremely small number of cases: the HIV-1/2 differentiation assay usually becomes reactive earlier than the Western blot during HIV seroconversion, when the Western blot result is still negative or indeterminate.[5,6,9,10] Because such reflex to Western blot would be performed so rarely, costs for the test (including controls) would add to HIV

testing costs considerably,[11] while only rarely providing additional information. Finally, reflex to Western blot would have little effect on turnaround time in many laboratories that send specimens to reference laboratories for Western blot.

17. Comment: Uncommon types of HIV-1. Since NAT testing will replace Western blot. Should recommendations be made for the evaluation of patients from geographical locations where uncommon types of HIV-1 are encountered (such as N, O or P) and NAT performance may be inadequate?

Response: Evidence suggests that the recommended algorithm will be adequate to address non-B variants of HIV. Western blot will be replaced by the HIV-1/2 differentiation assay and, when necessary, HIV-1 NAT. The FDA-approved versions of these tests have been evaluated on non-B subtypes of HIV-1, group O, and in some cases, group N. The antibody differentiation assay was reactive with all 203 non-B specimens from the worldwide subtype panel and 11 of 12 group O specimens. The qualitative HIV-1 NAT was reactive with all 59 members of a non-B subtype panel and with group N and O specimens. Evaluations of sensitivity for non-B variants will be assessed as new assays are introduced and receive FDA approval.

Reviewer C.

[Reviewer comments in italics]

I have reviewed the draft recommendations for "Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations" and have the following specific comments addressing the specific review items given to reviewers.

18. Comment: The draft, updated recommendations provides a thorough description of the new HIV testing algorithm, the methods used to identify sources of data on which to base the recommendations and the specific sources. The recommended algorithm is based on a sound review of the literature and describes an approach designed to address deficiencies in the standard algorithm that has been place for many years.

19. Comment: The methods used to develop the recommendations were appropriate and based on a thorough review of the pertinent literature.

20. Comment: The literature cited was comprehensive and no omissions are noted.

21. Comment: The interpretations and conclusions were appropriate based on the literature.

22. Comment: This reviewer's only comments about uncertanties, that are appropriately addressed in the document, relates to the undifferentiated multispot results which are treated/reported as HIV antibody positive. While the data indicate that most are

associated with HIV infection, we have preliminary evidence for a "false positive" undifferentiated multispot result that is associated with a diagnosis of SLE but no apparent HIV infection. Further testing is needed to confirm this. Given the rarity of the result and limited follow up data consideration might be given to acknowledging uncertainty in interpretation.

Response: In response to this recommendation we have added, in section J, "Limitations of the recommended laboratory testing algorithm," an additional limitation to indicate that no diagnostic test or algorithm can be completely accurate and that false-positive test results have been attributed to autoimmune disorders (with citations to studies of false-positive HIV test results that include systemic lupus erythematosus and autoimmune hemolytic anemia.)[12,13] In the paragraph describing the uncertainties associated with undifferentiated results (also in section J), we have added "follow-up data are limited" to the sentence that indicates the frequency of dually reactive results is unknown.

23. Comment: The second comment relates to data from Coomb's et al. (2013) who have provided some evidence for false negative 4th generation tests specifically in samples with S/CO near the 1.0 cutoff for the Architect assay. Given assay imprecision, some note of this data may be warranted – advising users that follow up testing of samples with results near the cutoff may be warranted to assess antigen conversion to positive or seroconversion in the case of a potential window period.

Response: Currently, the evidence for the yield and effect on specificity is insufficient to justify a recommendation to conduct follow-up testing for specimens with S/CO values below but near the 1.0 cutoff for the Architect assay. In the product insert, the manufacturer defines no intermediate or "grey zone" for values near the cutoff of 1.0 with the Architect assay. The study by Coombs et al,[14] based on comparisons of detectable HIV-1 RNA in specimens from a high-risk population with previously observed high prevalence of acute HIV infection, found suggestive evidence for defining an intermediate or "gray zone" of S/CO values between 0.5 and 1.0 for identifying acute HIV-1 infections. However, the authors attributed this to the broader dynamic range of the chemiluminescent immunoassay (compared with enzyme immunoassays), and concluded that a more precise definition warrants further study. CDC will continue to monitor the potential utility of using S/CO values, but we are unable to make an assay-specific recommendation for a specific range of S/CO values that differs from that recommended by the manufacturer.

24. Comment: The limitations of the evidence used for development of the recommendations are acknowledged sufficiently.

25. Comment: Although it is clearly stated (line 36) that the recommendations were based on tests approved by the FDA as of December 2012, the approval of the Determine 4th generation rapid test less than 1 year later is problematic. It would be reasonable to

consider an addendum to address this and clarify is this assay should be used as described in lines 102 – 104 and 425 – 433.

Response*:* This comment was raised by several reviewers. A footnote has been added to the relevant recommendation statements indicating that data are insufficient to recommend use of the Determine HIV-1/2 Ag/Ab Combo rapid test as the initial assay in the algorithm. The recommendation specifying use of the recommended algorithm on specimens after reactive rapid HIV test results was revised to clarify that it applies to reactive results from any rapid HIV test, including the Determine Combo 4th generation test. The summary of evidence now specifies that available data indicate that 4th generation laboratory-based antigen/antibody immunoassays are more sensitive during seroconversion than any of the rapid HIV tests available in the U.S. as of May 2014, including the Determine Combo 4th generation rapid test.

26. Comment: Line 173: There appears to be an extra quotation mark after combo in this line.

Response: Extra quotation mark was removed.

27. Comment: Line 477 – 478: Determine Ab/Ag combo was approved August 9, 2013.

Response: The sentence in the rationale for Recommendation 4 was revised to indicate that the laboratory-based antigen/antibody immunoassays detect HIV infection earlier during seroconversion than any of the rapid tests available as of May 2014, including the rapid HIV-1/HIV-2 antigen/antibody combination test.

28. Comment: Lines 572 – 576: Lines 183 – 184 identifies the eclipse phase as lasting approximately 10 days. This statement seems somewhat inconsistent with lines 572 – 576 in which the eclipse phase is described as not well defined.

Response: The estimate of approximately 10 days for the duration of the eclipse phase in Lines 183-184 t was derived from a mathematical model based on evolution of viral sequences that used the diversity in sequences obtained early in infection and compared the estimated time of infection with the time interval (predicted by the model) to the most recent common ancestor (i.e., the transmitted strain). Lines 574 to 576 have been revised to specify that the duration of the eclipse period between infection and the appearance of RNA is not well-defined "from clinical studies."

29. Comment: Lines 593 – 599: shouldn't consideration be given to the possibility of indeterminate differentiation immunoassay results being falsely positive, even if rarely so?

Response: Lines 593 to 599 have been revised to indicate that "11% to 15% of specimens with indeterminate [differentiation immunoassay] results proved to be HIV-negative.[5,6]"

30. Comment: Effective April 7, 2014, patients will have the right to receive laboratory results directly from laboratories. While there are built in "delays" which should allow providers time to receive, review and communicate results to their patient's, laboratories may still be faced with providing results to individuals with uncertain ability to appropriately interpret the results. Should recommendations be included to provide guidance on appropriate approach(s) to communicate results directly to patients by laboratories?

Response: CDC appreciates this suggestion and will begin to work with the Association of Public Health Laboratories, other laboratory partners, stakeholders, and consumer representatives to develop approaches to communicate test results directly to patients. Because the process for developing appropriate language and assessing comprehension in audiences with different levels of health literacy may be time-consuming, we anticipate such guidance would be issued as a companion document so as not to delay release of the updated recommendations for testing.

Reviewer D.

[Reviewer comments in italics]

I want to thank the committee for the years of persistent hard work that went into this much needed new guideline. Your efforts are greatly appreciated by those of us that deal daily with diagnosing HIV and the difficulties we have faced in the past with suboptimal tests and algorithms. I congratulate you all on the final publication of this guidance document. It has been my pleasure to have assisted you.

31. Comment: I have attached a document with my comments added.

Response: The Reviewer's comments in the document have been transcribed and are included in this document with responses to reviewers.

32. Comment: I only see 1 major point that I think needs clarification. This relates to the new 4th generation rapid tests. People are assuming that they can replace the 4th generation immunoassay in this algorithm and if positive can go directly to the Multispot assay without running a 4th generation immunoassay. I interpret that this is not correct since there was no evaluation of this tests for the algorithm this would not be acceptable. The Alere test would have to follow the same testing algorithm as the other rapid tests. This needs to be clearly stated in the document.

Line 38 – Should a comment be made here specifically stating that 4th generation rapid tests and HIV-2 viral load testing were not included in the decision tree as FDA approved tests were not available at the time to be evaluated in this algorithm. It is my understanding that if Determine is positive you still must do an EIA then go to Multispot. This needs to be clarified. They are equating the 4th generation EIAs with the 4th generation rapid.

Line 84 -- Should you clarify here that this does not include the 4th generation rapid tests?

Line 176 - In the mention of commercially available tests, section on generation classification: Again should a note go in about 4th generation rapids but not evaluated for this algorithm.

Line 438 – In recommendation statement for 4th gen initial assay: This needs to be clarified that this does not include rapid 4th generation. There is a lot of confusion about this.

Line 637 –Section on specimens submitted after reactive rapid HIV test results - add " including 4th generation rapid tests ." Since 4th generation were not evaluated a positive should be repeated with a 4th generation immunoassay, if the immunoassay is negative, can they stop also as with the other rapid tests?

You do nicely state below, but since Alere was approved before this document was finalized many will assume it meets the primary screening criteria as with the 4th generation immunoassays.

Anticipating continued improvements in laboratory diagnostic techniques, CDC and APHL will monitor the introduction and FDA approval of diagnostic assays for HIV infection and update these recommendations when necessary. CDC and APHL will continue to monitor the performance of the laboratory testing algorithm and review the performance of the recommended algorithm at least every five years.

If I am incorrect please advise. Thanks

<u>Response</u>: These comments were raised by several reviewers. These updated recommendations were based on tests that were FDA-approved as of December 2012 for which sufficient evidence was available for their performance as part of the algorithm. A clarification was added in the Executive Summary to indicate these recommendations do not include the rapid HIV-1/HIV-2 antigen/antibody combination test approved by the FDA in August 2013, for which evidence of performance in the algorithm was insufficient. A footnote has been added to the relevant recommendation statements indicating that data are insufficient to recommend use of the Determine HIV-1/2 Ag/Ab Combo rapid test as the initial assay in the algorithm. The statement

specifying use of the recommended algorithm, beginning with an antigen/antibody combination assay, on specimens after reactive rapid HIV test results was revised to clarify that it applies to reactive results from any rapid HIV test. The summary of evidence now specifies that available data indicate that 4th generation laboratory-based antigen/antibody immunoassays are more sensitive during seroconversion than any of the rapid HIV tests available in the U.S. as of May 2014, including the 4th generation rapid test.

33. Comment: Overall the recommendations are clearly written. There are just a few suggestions for minor clarifications that are aimed at the local lab and situations/questions that I think might arise.

34. Comment: The methods are appropriate and sufficient.

35. Comment: I am in agreement with the advice

36. Comment: Clarification of the status of HIV-2 testing in the summary and clarification of the use of the new 4th generation rapid tests.

Response: A statement was added to the executive summary: "The recommendations do not include the rapid HIV-1/HIV-2 antigen/antibody combination assay approved by the FDA in August 2013 (for which evidence of performance in the algorithm was insufficient) or HIV-2 nucleic acid tests (NAT) none of which are FDA-approved. A new subsection devoted to HIV-2 infection was added to Section M, Additional Considerations, which is referenced by footnotes with each of the relevant recommendations.

37. Comment: Lines 88-89 -- Do some assays not require repeating a reactive sample in duplicate? Do some states (ex NY still; require repeating in duplicate? If so maybe we should add a statement that not only deals with manufacturer recommendations but also regulatory (State, CLIA, CAP) guidelines?

Response: The recommendation was amended to say "(or repeatedly reactive, if repeat testing is recommended by the manufacturer or required by regulatory authorities)"

38. Comment: Lines 94:95: Specify a NAT FDA-approved for diagnosis. The viral load assays are all FDA approved but not for this indication.
Line 453: Specify "FDA NAT approved for the diagnosis of HIV-1 infection"

Response: CDC received conflicting comments from reviewers on this topic. (See comment 4a from Reviewer A.) Quantitative NATs that are FDA-approved for monitoring but not for diagnosis could be used in the algorithm if laboratories conducted the validation required by CLIA. Several studies cited in the evidence synthesis used these quantitative NAT. The ability of NAT to detect non-B subtypes,

which must be demonstrated for FDA approval, is a justification for recommending assays that are FDA-approved. However, published studies demonstrated adequate performance of FDA-approved quantitative NAT as part of the algorithm,[6,15] and unpublished data indicate that the limit of detection of the quantitative assays is much lower than the estimated threshold (30,000 – 58,000 RNA copies/ml) at which the 4th generation combination assay becomes reactive for p24 antigen.[3] CDC makes no recommendation for off-label use of an FDA-approved assay. However, as noted by Reviewer A, laboratories can conduct their own internal validation of a quantitative test for diagnostic purposes, and sufficient evidence demonstrates adequate performance in the algorithm. In addition, the qualitative NAT FDA-approved for HIV diagnosis is available in far fewer laboratories than quantitative HIV-1 (viral load) RNA assays. To facilitate prompt diagnosis of acute HIV infection when faced with discordant screening and supplemental antibody test results, clinicians could order a viral load test to differentiate acute HIV-1 infection from false-positive IA results.

39. Comment: Lines 100-101 A reactive 4th gen, negative Multispot, negative HIV-1 NAT does not always indicate false-positive 4th gen assay. Acute HIV-2 infection is a possibility. Should we put a precautionary note for follow up depending on risk factors for HIV-2? A short statement referring to what you mention on page 18 section 1.

Response: Although a possibility, based on epidemiologic evidence and expert opinion, the probability of acute HIV-2 infection in this circumstance is extremely low (compared with a false-positive immunoassay result), and mentioning it as part of the recommendation might encourage unnecessary additional testing. A footnote was added, "See Section M, Special Considerations, for a discussion of issues related to acute HIV-2 infection," and in section M, additional information was added about (1) the theoretical possibility of acute HIV-2 infection in this circumstance and (2) guidance for HIV-2 testing, if further investigation is clinically indicated. The statement "Follow-up testing for HIV-2 should be performed if clinically indicated" was added to the suggested language for reporting the results from this sequence of test results.

40. Comment: Line 204 – (regarding laboratory stages) Should a comment be made that in rare circumstances in late stage HIV antibodies can be undetectable.

Response: Although data indicate antibody responses may wane in late stages of infection or after prolonged effective treatment, leading in some cases to an indeterminate Western blot result, we were unable to find evidence that antibodies become completely undetectable by the currently available immunoassays.

41. Comment: Need to add limitation regarding a lack of current data to support the use of the new 4th generation rapids as the primary screen with a direct reflex to multispot.

Response: Addressed in response to Comment 32.

42. Comment: Terminology: clarify that in the algorithm 4th generation immunoassay does not include 4th generation rapid tests.

Response: A footnote was added to each statement that recommends initial testing with an FDA-approved antigen/antibody combination immunoassay. The footnote states "Exception: As of May 2014, data are insufficient to recommend use of the FDA-approved single-use rapid HIV-1/HIV-2 antigen/antibody combination immunoassay as the initial assay in the algorithm." In the Summary of Evidence, a notation was made to Table 2, which lists by brand name the FDA-approved assays included in the evidence synthesis, that "The Determine HIV 1-2 Ag/Ab Combo rapid test, FDA approved in August 2013, was not included in the evidence synthesis because there were no studies evaluating its performance as part of the algorithm." Although the 4th generation rapid test is not included in the algorithm, in the description of 4th generation assays in "Box 2, Evolution of HIV Immunoassay Technology," the last sentence was amended to indicate that "Examples commercially available in the U.S. as of May 2014 include one HIV-1/HIV-2 enzyme immunoassay and one HIV-1/HIV-2 chemiluminescent immunoassay, and one HIV-1/HIV-2 rapid test that uses separate indicators for antigen and antibody reactivity."

Reviewer E.

[Reviewer comments in italics]

I have reviewed the draft of the Updated Recommendations for Laboratory Testing for the Diagnosis of HIV Infection. In general the recommendations are well written and will be a valuable resource for laboratories performing HIV testing. The only corrections I have are minor and are as follows:

43. Comment: Page 8, after line 196. It might be useful to add another bullet for the "Eclipse Period" before the bullet for "Seroconversion Window Period."

Response: The following bullet was added: " The eclipse period is the initial interval after infection with HIV when no laboratory markers are consistently detectable."

44. Comment: Move headings for Table 1 on page 23between lines 725 and 726 to Table 1 on page 24.

Response: The formatting error introduced by the addition of line numbers has been corrected.

45. Comment: Page 27 lines 841-843. If 1,858 abstracts were reviewed and 1,751 were excluded, there would be 107 remaining articles, not 39.

Response: The transcription error has been corrected as follows:

The literature search identified 1,858 abstracts of potentially relevant articles. Of these, 1,778 were excluded because they were background articles, did not contain assay performance data, or evaluated assays that were not FDA-approved. Of the remaining 80, 39 articles contained data relevant to the key questions for evaluating individual assays or diagnostic algorithms for HIV, 4 studies related to costs or cost-effectiveness, and 2 studies related to potential harms from indeterminate HIV test results; 14 studies described viral dynamics of HIV and generic laboratory markers, without identifying specific assays; 6 studies described HIV-2 distribution and diagnosis with assays that are not FDA-approved, 3 studies evaluated HIV-1 diagnosis in infants, 7 studies modeled transmission attributable to acute HIV-1 infection and 5 studies evaluated the potential benefits of antiretroviral therapy for acute HIV-1 infection.

46. Comment: Page 54, line 1458. Correct spelling on "indirect."
47. Comment: Page 58, line 1654. Skip space between "with" and "repeatedly"
48. Comment: Page 60, line 1708. Correct spelling on "antigen"

Response: We thank the reviewer for the very careful review of the references. All typographical errors have been corrected.

References:

1. Mouinga-Ondeme A, Mabika-Mabika A, Alalade P, et al. Significant impact of non-B HIV-1 variants genetic diversity in Gabon on plasma HIV-1 RNA quantitation. *J Med Virol.* 2014;86:52-7.
2. Pyne MT, Hackett J, Jr., Holzmayer V, Hillyard DR. Large-scale analysis of the prevalence and geographic distribution of HIV-1 non-B variants in the United States. *J Clin Microbiol.* 2013;51:2662-9.
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