Performance of the Geenius™ HIV 1/2 Supplemental Assay in the CDC HIV testing algorithm

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Background

In June 2014, CDC published updated recommendations for laboratory diagnosis of HIV infection in the United States (Figure 1, the "CDC algorithm"), which recommend the use of an assay that differentiates HIV-1 from HIV-2 after a reactive HIV-1/HIV-2 screening test.

- Previously, only the Multispot HIV-1/HIV-2 Rapid Test (Multispot) was FDA-approved for this use.
  - Multispot is also approved as a screening assay
- A new test, the Geenius™ HIV 1/2 Supplemental assay (Geenius), received FDA approval in October 2014 and is an additional test that could be used as the differentiation assay in the CDC algorithm
- Geenius uses an automated reader and a proprietary software algorithm to distinguish HIV-1 from HIV-2 reactivity

Multispot HIV-1/HIV-2 **Geenius HIV-1/HIV-2**

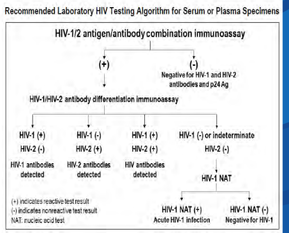
Methods

We evaluated Geenius with a panel of 873 specimens previously tested with FDA-approved screening tests, Multispot and the Aptima RNA assay (NAT) and classified by the CDC algorithm. This panel consisted of:

- 658 classified as HIV-1 by the CDC algorithm (HIV-1+)
- 8 classified as acute HIV-1 infections (acute), and
- 207 challenge specimens with reactive results on at least one HIV-1/HIV-2 screening test selected from 5415 specimens classified as negative based on negative 4th generation screening and Aptima tests:
  - 181 with a reactive HIV screening test result other than Multispot,
  - 26 with only a reactive Multispot screening test result.

- We compared Geenius results to those from the CDC Algorithm and Multispot. The 26 samples that only screened reactive using Multispot were excluded from the Multispot/Geenius comparison because Multispot can not be used as a supplemental test after a reactive Multispot screening test result.

Figure 1: CDC's recommended* laboratory HIV testing algorithm



* http://www.cdc.gov/hiv/pdf/hivtestingalgorithmRecommendation-final.pdf

Results

Geenius results compared with CDC Algorithm

- Geenius classified 656 of 658 (99.7%) HIV-1+ specimens as HIV Positive
  - 654 (99.4%) were classified HIV-1 positive, 2 (0.3%) HIV Positive untypable
- For 207 challenge specimens with reactive screening test results that were algorithm negative
  - 1 (0.02% of 5415 algorithm negative specimens) was HIV-1 positive by Geenius (weakly reactive for p31 and gp41 only)
  - 21 specimens were initially Geenius indeterminate; (17/21, 81%) were HIV-2 indeterminate with only a weak gp140 band (Figure 3)
  - 10/17(59%) HIV-2 Indeterminate specimens were HIV negative when retested as specified in the package insert
  - After retesting a total of 11 indeterminate results remained (see Table 1)

Figure 2: Geenius assay interpretation

Result Category	Geenius Interpretation Criteria
HIV Negative	Absence of HIV-1 and HIV-2 bands
HIV-1 Positive	Presence of at least 2 HIV-1 bands including at least one ENV (gp160 or gp41)
HIV-2 Positive	Presence of both gp140 and gp36 HIV-2 bands
HIV-2 Positive with HIV-1 cross-reactivity	Antibody to HIV-2 confirmed. Antibody to HIV-1 due to cross-creativity
HIV Positive Untypable (undifferentiated)	Antibodies to HIV-1 and HIV-2 confirmed. 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)
Indeterminate samples (HIV-1 IND, HIV-2 IND and HIV IND)	Presence of HIV-1 or HIV-2 band(s) which does not meet the HIV-1 or HIV-2 positivity criteria

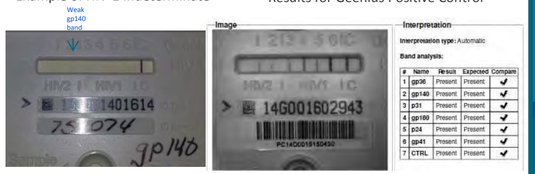
Table 1: Comparison of Geenius results with CDC Algorithm results

	Geenius interpretation						
	HIV Negative	HIV Indeterminate			HIV Positive		
CDC Algorithm interpretation		HIV Ind	HIV-2 Ind	HIV-1 Ind	HIV Positive Untypable	HIV-1 Positive	Row Total
HIV-1 Positive	1			1	2	654	658
Acutely Infected	7		1†				8
HIV-1 Negative	195	2	7*	2		1	207

IND= Indeterminate; HIV IND includes specimens with indeterminate Geenius results for both HIV-1 and HIV-2, HIV-positive untypable includes specimens with positive Geenius results for both HIV-1 and HIV-2 (See Figure 2), †There was insufficient volume to repeat this specimen on a new Geenius device as specified in the package insert,*10 of 17 specimens initially classified as HIV-2 Indeterminate were HIV negative when repeated

Figure 3: Reactivity of HIV-2 indeterminate results compared to the positive control

Example of HIV-2 Indeterminate Results for Geenius Positive Control



Geenius results compared with Multispot results

- Multispot provides 5 possible interpretations when used as a supplemental test; Geenius provides 8 possible interpretations (listed in Figure 2 and Table 2)
- Neither Geenius nor Multispot was HIV-1 positive with any of the 8 acute infection specimens
- Two specimens classified as HIV positive (untypable) by the Geenius assay were initially reactive for both HIV-1 and HIV-2 using Multispot, but were classified as HIV-1 positive only when that test was repeated with the 1:10 dilution protocol, a procedure not used with Geenius
- 9 specimens classified as HIV-1 only positive by Geenius were initially HIV-1/HIV-2 positive (undifferentiated) using Multispot but resolved as HIV-1 only after the dilution protocol
- Of 181 challenge specimens:
  - 14 specimens with HIV-2 Indeterminate Geenius results required repeat testing, compared to 2 with undifferentiated Multispot results that required the dilution protocol
    - Both HIV-1/HIV-2 undifferentiated specimens were HIV-negative at 1:10 dilution and would have been categorized as HIV Positive (untypable)
    - 9/14 (64%) specimens with an HIV-2 Indeterminate Geenius result were HIV negative when repeated
  - 1 specimen gave an HIV-1 false-positive Geenius result and 2 were false-positive HIV undifferentiated using Multispot

Table 2: Comparison of **initial** Geenius and Multispot results for 181‡ specimens classified as HIV-negative by the CDC algorithm but reactive on at least one screening test

		Geenius interpretation								
		HIV Negative	HIV Indeterminate			HIV Positive				
		HIV Negative	HIV Indeterminate	HIV-2 Indeterminate	HIV-1 Indeterminate	HIV Positive, Untypeable	HIV-2 Positive with HIV-1 cross-reactivity	HIV-2 Positive	HIV-1 Positive	Row Total
Multispot differentiation test interpretation	HIV-1 Only (both spots)	0	0	0	0	0	0	0	0	0
	HIV-2 Only	0	0	0	0	0	0	0	0	0
	HIV Positive Undifferentiated HIV-1 Indeterminate (only 1 of 2 spots)	1	0	1†*	0	0	0	0	0	2
	HIV Negative	161	0	13*	1	0	0	0	1	176
		3	0	0	0	0	0	0	0	3

‡ Includes 5 specimens reactive on Multispot and another test and 176 specimens reactive on 1 or more other screening tests; does not include 26 specimens which were only false-positive on Multispot
† This specimen became HIV negative after the 1:10 dilution protocol with Multispot and remained HIV-2 indeterminate after being repeated on Geenius
* Overall, 9 of 14 (64%) specimens with HIV-2 Indeterminate results resolved as HIV-negative when repeated according to the package insert instructions

Discussion

- The Geenius test demonstrated 99.7% (656/658) agreement with HIV-1+ samples identified by the CDC algorithm
  - Neither Geenius nor Multispot detected acute HIV-1 infection specimens reactive with a 4th generation assay

- HIV-2 cross-reactivity is a well known problem
  - Fewer of the 658 HIV-1+ samples required additional testing to resolve HIV-2 cross-reactivity with Geenius than with Multispot
  - 11 HIV-1+ specimens were initially reactive for HIV-1 and HIV-2 by Multispot
    - 9 of these were classified HIV-1 only by Geenius
    - 2 were classified HIV-1 by Multispot after the package insert dilution protocol but were HIV positive untypable by Geenius
  - 2 HIV negative specimens were HIV positive undifferentiated after the Multispot dilution protocol
    - 1 was HIV-1 negative, and 1 was HIV-2 Indeterminate by Geenius

- In challenge specimens with false-positive results on one or more (3rd generation, 4th generation or rapid) HIV-1/HIV-2 screening tests
  - 14 specimens with HIV-2 Indeterminate Geenius results required repeat testing, compared to 2 with undifferentiated Multispot results that required the dilution protocol
  - Repeat Geenius testing for specimens classified as HIV-2 indeterminate resolved many, but not all, of these specimens as HIV negative
  - 1 specimen gave a false-positive Geenius result and 2 were false-positive using Multispot
  - Specimens classified as HIV negative or indeterminate would go on to HIV-1 NAT in the current algorithm

- Excluding specimens that screened reactive only with Multispot, 5 specimens with a false-positive screening test had an HIV-2 indeterminate Geenius result with a weak gp140 band. Additional studies are needed to determine whether additional testing or follow-up would resolve specimens with a final result of HIV-2 Indeterminate.

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Questions? Contact:

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