Could HIV-1 RNA be an option as the second step in the HIV diagnostic algorithm?

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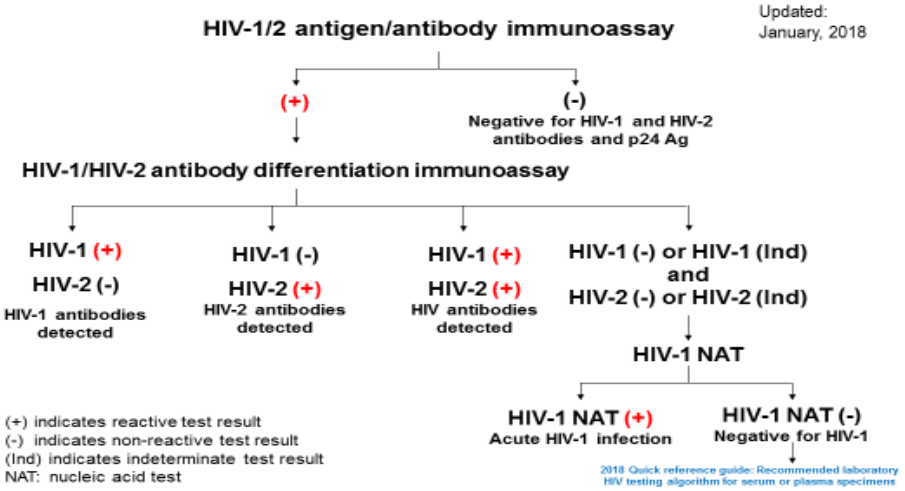
2019 HIV Diagnostics Conference

March 26, 2019

Disclaimer: This evaluation was conducted using a non-FDA approved indication for use.
Tradenames are used for informational purposes and do not constitute an endorsement by CDC.

Disclosure: No relevant financial relationships

Background



- **Implementation of the HIV diagnostic algorithm in 2014**
- **Use of an HIV-1/2 antibody differentiation assay**
  - HIV-2 infections are rare in the US
- **Use of nucleic acid testing to increase detection of acute infections**
  - Only one FDA-approved assay for diagnosis
  - Frequently, VL assays are used as third test

BioPlex® 2200 HIV Ag-Ab Assay (BPC)

- Bio-Rad Laboratories (2015)
- Multiplex flow immunoassay intended for the simultaneous qualitative detection and differentiation
  - HIV-1 p24 antigen
  - HIV-1 (group M and O) antibodies
  - HIV-2 antibodies
- An aid in the diagnosis of infection with HIV-1 and/or HIV-2, including acute HIV-1 infection in human serum or plasma
- Pediatric subjects ≥ 2 yo and pregnant women

BPC results

Index (IDX)	Retest	Retest Result	Final Interpretation
< 1.00 for all analytes	No	Not Applicable	Non-Reactive
≥ 1.00 for at least one analyte	Yes	Both retest results have an Index (IDX) <1.00 for all analytes	Non-Reactive
		Index (IDX) of at least one retest result is ≥ 1.00 for the analyte(s) that was initially reactive	**Reactive for HIV Ag-Ab** with **Reactive for HIV-1 Ag** and/or **Reactive for HIV-1 Ab** and/or **Reactive for HIV-2 Ab** or **Reactive, Undifferentiated**

- **CDC lab evaluation showed BPC performance similar to other FDA-approved lab-based Ag/Ab immunoassays in early HIV-1 infections**

Aptima HIV-1 Quant Assay on the Panther system (APT-Quant)

- Hologic (2016)
- CE-IVD marked for HIV diagnosis and monitoring (dual claim)
- High-throughput fully automated testing platform with random access
- Transcription mediated amplification (TMA) and dual target approach (LTR and integrase)
- Reported limit of detection: ~ 13 copies/ml
- Linear range of quantification: 30- 10^7 copies/ml

In-house evaluation of APT-Quant for diagnosis

Reported HIV-1 RNA Concentration Result[a]			
Copies /mL[b]	Log_{10} Value	HIV-1 RNA Concentration Interpretation[a]	User's Diagnostic Qualitative Interpretation[c]
Not detected	Not detected	HIV-1 RNA not detected.	Non-reactive for HIV-1 RNA.
<[LLOQ value][d] detected	<[log_{10} LLOQ value]	HIV-1 RNA is detected but at a level below the Lower Limit of Quantitation (LLOQ).	Reactive for HIV-1 RNA.
[LLOQ value] to 10,000,000	[LLOQ value] to 7.00	HIV-1 RNA concentration is within the linear range of [LLOQ value] to 10,000,000 copies/mL.	Reactive for HIV-1 RNA.
>10,000,000	>7.00	HIV-1 RNA concentration is above the Upper Limit of Quantitation (ULOQ).	Reactive for HIV-1 RNA.
Invalid	Invalid	There was an error in the generation of the result. Specimen should be retested.	Invalid.[c]

HIV-1 US Seroconverters		APT-Qual	
		Reactive	Nonreactive
APT-Quant	Reactive	328	34
	Nonreactive	7	48

(McNemar's p< 0.0001)

- In 417 samples from HIV-1 U.S. seroconverters, APT-Quant detected virus in more samples including seronegative phase than Hologic HIV-1 RNA Qualitative (APT-Qual)
- In HIV-1 established infections, both tests performed similarly
- APT-Quant non-inferior to the FDA-approved diagnostic test

Objective

To compare the performance of a two-test diagnostic algorithm consisting of screening with a Ag/Ab HIV-1/2 differentiation immunoassay, followed by HIV-1 NAT to the currently recommended three-test algorithm.

HIV samples and analysis

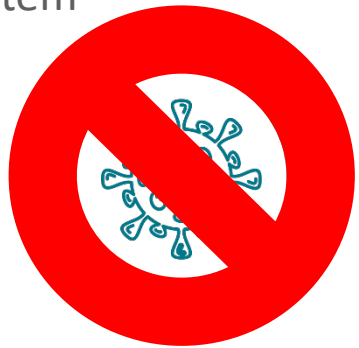
- **Specificity**
  - BPC: 596 HIV-negative samples
  - APT-Quant: 478 Hologic Aptima HIV-1 RNA Qualitative (APT-Qual) nonreactive and HIV-1 antibody negative samples
  - APT-Quant carry over contamination experiment in open platform
- **Comparison of HIV diagnostic algorithms**
  - 46 U.S. seroconverters (subtype B) with 255 longitudinal samples before and 73 after initiation of antiretroviral therapy (ART) and after BPC-seroreactivity
  - 105 Cameroonian ART-naïve established infections
    - 3 HIV-1 Group O and 102 HIV-1 Group M non-B subtypes
- **HIV testing was performed as part of studies with Bio-Rad and Hologic that provided kits**

Results

Specificity

- **Bio-Rad BioPlex 2200 Ag/Ab Combo: 99.7% [95% CI 98.8-99.9%]**

- **Hologic Aptima HIV-1 Quant: 99.8% [95% CI 98.8- 99.9]**
  - Four sequences of nine HIV-negative plasma followed by plasma with 10^7 HIV-1 RNA cop/ml tested in the Panther system
  - No carry over contamination was observed on the open system



Three-test algorithm results with early stages of HIV-1 infection before ART initiation

Three-test algorithm results with early stages of HIV-1 infection before ART initiation

BioPlex 2200 Ag/Ab assay			Geenius HIV-1/2 assay	APTIMA-Qualitative	
	HIV-1	HIV-2			
Ag	Ab	Ab	Final intrepration v1.1	NR	R
R	NR	NR	HIV-1 Ab-negative	0	42
R	NR	NR	HIV-1 indeterminate	0	2
R	R	NR	HIV Ab-negative	1	13
R	R	NR	HIV-1 indeterminate	1	6
NR	R	NR	HIV Ab-negative	0	3
NR	R	NR	HIV-1 indeterminate	1	10

NR: non reactive; R: reactive

- BPC detected p24 Ag reactive samples after HIV-1 RNA positivity

Three-test algorithm results with early stages of HIV-1 infection before ART initiation

BioPlex 2200 Ag/Ab assay			Geenius HIV-1/2 assay	APTIMA-Qualitative	
	HIV-1	HIV-2			
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R	NR	NR	HIV-1 Ab-negative	0	42
R	NR	NR	HIV-1 indeterminate	0	2
R	R	NR	HIV Ab-negative	1	13
R	R	NR	HIV-1 indeterminate	1	6
NR	R	NR	HIV Ab-negative	0	3
NR	R	NR	HIV-1 indeterminate	1	10

NR: non reactive; R: reactive

- BPC and Geenius agreed on HIV-1 antibody reactivity
- HIV-2 antibody reactivity was not observed with either test

Three-test algorithm results with early stages of HIV-1 infection before ART initiation

BioPlex 2200 Ag/Ab assay			Geenius HIV-1/2 assay	APTIMA-Qualitative	
	HIV-1	HIV-2			
Ag	Ab	Ab	Final intrepration v1.1	NR	R
R	NR	NR	HIV-1 Ab-negative	0	42
R	NR	NR	HIV-1 indeterminate	0	2
R	R	NR	HIV Ab-negative	1	13
R	R	NR	HIV-1 indeterminate	1	6
NR	R	NR	HIV Ab-negative	0	3
NR	R	NR	HIV-1 indeterminate	1	10

NR: non reactive; R: reactive

- 79 samples from seroconverters were from early stages of HIV-1 infection
- The three-test algorithm detected 76/79 (96.2%)

Comparison of the algorithms on early stages of HIV-1 infection before ART initiation

BioPlex 2200 Ag/Ab assay			Geenius HIV-1/2 assay	APTIMA-Qualitative		Panther HIV-1 RNA Quant-diagnostic				
	HIV-1	HIV-2						Detected		
Ag	Ab	Ab	Final intrepration v1.1	NR	R	NR	R	Total <1.47 log(cop/ml)	Total quantified	Median VL log (cop/ml)¥	Range VL log (cop/ml)
R	NR	NR	HIV-1 Ab-negative	0	42	0	42	0	42	5.09	2.45- >7
R	NR	NR	HIV-1 indeterminate	0	2	0	2	0	2	7	6.45- >7
R	R	NR	HIV Ab-negative	1*	13	0	14	0	14	5.77	3.6 - >7
R	R	NR	HIV-1 indeterminate	1	6	1	6	0	6	3.87	1.81- 6.65
NR	R	NR	HIV Ab-negative	0	3	0	3	1	2	3.29	<1.47- 3.3
NR	R	NR	HIV-1 indeterminate	1*	10	0	11	1	10	2.89	<1.47- 4.89

NR: non reactive; R: reactive; ¥ >7 log(cop/ml) was considered as 7 for the median

- The two-test algorithm detected 78/79 (98.7%)
  - VL range: <1.47 to >7 log(cop/ml)
  - *2 samples APT-Qual NR were APT-Quant R with VL < 1.47 and 4.89 log (cop/ml)
- Similar performance of both algorithms (McNemar's *p=0.4795)*

Three-test algorithm results with late stages of HIV-1 infection before ART initiation

BioPlex 2200 Ag/Ab assay			Geenius HIV-1/2 assay	HIV-1 positive
Ag	HIV-1 Ab	HIV-2 Ab	Final intrepration v1.1	
R	R	NR	HIV-1 Positive	20
NRDHAL	R	NR	HIV-1 Positive	19
NR	R	NR	HIV-1 Positive	241
NR	R	NR	HIV untypable	1

NR: non reactive; R: reactive; NRDHA: not reportable due to high antibody titer

- 176 samples from seroconverters were Geenius HIV-1 positive
- All Cameroonian established HIV-1 infections were Geenius HIV-1 positive
  - One sample was also Geenius HIV-2 reactive (untypable), but further testing showed no evidence of HIV-2 infection

Comparison of the algorithms on established HIV-1 infection before ART initiation

BioPlex 2200 Ag/Ab assay			Geenius HIV-1/2 assay	HIV-1 positive	Panther HIV-1 RNA Quant-diagnostic					
	HIV-1	HIV-2					Detected			
Ag	Ab	Ab	Final intrepration v1.1		NR	R	Total <1.47 log(cop/ml)	Total quantified	Median VL log (cop/ml)*	Range VL log (cop/ml)
R	R	NR	HIV-1 Positive	20	0	20	2	18	4.8	<1.47- >7
NRDHAL	R	NR	HIV-1 Positive	19	0	19	0	19	5.59	2.11- 6.45
NR	R	NR	HIV-1 Positive	241	2*	239	5	234	4.23	<1.47- 6.16
NR	R	NR	HIV untypable	1	0	1	0	1	4.44	4.44

NR: non reactive; R: reactive; NRDHA: not reportable due to high antibody titer; ¥ >7 log(cop/ml) was considered as 7 for the median

- The two-test algorithm detected 279/281 (99.3%)
  - VL range: <1.47 to >7 log(cop/ml)
  - 7 samples were APT-Quant R with VL < 1.47 log (cop/ml)
  - *2 samples were APT-Quant and APT-Qual NR
- Similar performance of both algorithms (McNemar's *p=0.4795)*

Three-test algorithm results with HIV-1 infections after ART initiation

BioPlex 2200 Ag/Ab assay			Geenius HIV-1/2 assay	APTIMA-Qualitative	
	HIV-1	HIV-2			
Ag	Ab	Ab	Final intrepration v1.1	NR	R
R	R	NR	HIV-1 Positive	0	1
NRDHAL	R	NR	HIV-1 Positive	0	4
NR	R	NR	HIV Ab-negative	0	1
NR	R	NR	HIV-1 indeterminate	0	8
NR	R	NR	HIV-1 Positive	10	49

NR: non reactive; R: reactive; NRDHA: not reportable due to high antibody titer

- 9 samples from seroconverters showed seroreversion
- Three-test algorithm detected 73/73 (100%)

Comparison of the algorithms on HIV-1 infections after ART initiation

BioPlex 2200 Ag/Ab assay			Geenius HIV-1/2 assay	APTIMA-Qualitative		Panther HIV-1 RNA Quant-diagnostic					
	HIV-1	HIV-2						Detected			
Ag	Ab	Ab	Final intrepration v1.1	NR	R	NR	R	Total <1.47 log(cop/ml)	Total quantified	Median VL log (cop/ml)	Range VL log (cop/ml)
R	R	NR	HIV-1 Positive	0	1	0	1	0	1	6.9	-
NRDHAL	R	NR	HIV-1 Positive	0	4	0	4	0	4	3.18	1.57- 5.64
NR	R	NR	HIV Ab-negative	0	1	1	0	0	0	TND	
NR	R	NR	HIV-1 indeterminate	0	8	2	6	3	3	2	1.53- 2.63
NR	R	NR	HIV-1 Positive	10	49	6	53	18	35	2.84	1.51- 5.58

NR: non reactive; R: reactive; NRDHA: not reportable due to high antibody titer

- The two-test algorithm detected 64/73 (87.7%)
  - VL range: Target not detected (TND)- 6.9 log (cop/ml)
  - 3/9 that seroreverted and 6/64 Geenius HIV-1 positive samples were APT-Quant TND
- Three-test algorithm performed better after ART initiation
  - McNemar's *p=0.0077*

Limitations of the study

- NAT was done in singlet
- APT-Qual and APT-Quant were not performed in parallel for a set of ART-naïve seroconversion panels
- Geenius HIV-1/2 differentiation assay was performed using software v1.1 prior the update to address HIV-2 indeterminate results
- Small number of samples from ART-treated persons

Summary results

- The BPC/APT-Quant algorithm performed similar to the BPC/Geenius/APT-Qual in ART-naïve samples at different stages of HIV-1 infection
- The three-test algorithm performed better than the two-test algorithm in samples with lower viremia due to ART
- BPC accurately identified early and established HIV-1 infections
- Despite the limitations, BPC and Geenius v1.1 showed great concordance for HIV-1 antibody differentiation

Conclusions

- **APT-Quant, an automated HIV-1 RNA assay, works well for diagnosis and quantification as a second step in the proposed algorithm in different stages of HIV-1 infection**
  - No FDA-approved dual claim assay
- **APT-Quant performance decreases after the IgG response is elicited and with suppressed viremia due to ART**
  - Use of HIV antibody test after undetectable viral load results
- **Confirmation with a dual claim RNA assay is advantageous for patient care**
- **However, additional factors such as the implications of off-label use and cost associated with the implementation of a second-step quantitative NAT algorithm need to be explored**

Acknowledgements

Wei Luo

Vickie Sullivan

Tara Smith

Rebecca Rossetti

William Fowler

Krystin A. Price

S. Michele Owen

Laura Wesolowski

Kevin Delaney

Steven Ethridge

Bill Switzer

Jeff Johnson

Disclaimer

The findings and conclusions in this presentation are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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