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Could HIV-1 RNA Testing be an Option as the Second Step in the HIV Diagnostic Algorithm?

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Abstract

Background: There is benefit to early HIV-1 diagnosis and treatment, but there is no Food and Drug Administration–approved quantitative assay with a diagnostic claim. We compared the performance of the Hologic Aptima HIV-1 Quant (APT-Quant) and Aptima HIV-1 Qual (APT-Qual) assays for diagnostic use and the performance of a diagnostic algorithm consisting of Bio-Rad BioPlex 2200 HIV Ag-Ab assay (BPC) followed by APT-Quant (2-test) compared with BPC followed by Geenius HIV-1/2 supplemental assay (Geenius) with reflex to APT-Qual (3-test).

Methods: Five hundred twenty-four plasma, which included 419 longitudinal specimens from HIV-1 seroconverters (78 were after initiating antiretroviral therapy [ART]) and 105 from ART-naive persons with established HIV-1 infections, were used to evaluate APT-Quant performance for diagnostic use. Specimens from 200 HIV-negative persons were used to measure specificity. For the algorithm comparison, BPC-reactive specimens were evaluated with the 2-test or 3-test algorithm. McNemar's test was used to compare performance.

Results: The APT-Quant detected more samples early in infection compared with APT-Qual. The APT-Quant specificity was 99.8%. Before ART initiation, the algorithms performed similarly among samples from different stages of infection. After ART initiation, the 3-test algorithm performed significantly better ($P = 0.0233$).

Conclusions: The APT-Quant has excellent performance for diagnostic use. The 2-test algorithm works well in ART-naive samples, but its performance decreases after the IgG response is elicited and with ART-induced suppressed viremia. Providing confirmation and viral load assay with 1 test result could be advantageous for patient care. However, additional factors and challenges associated with the implementation of this 2-test algorithm, such as cost, specimen type, and collection need further evaluation.

In 2014, the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) updated the recommendations for HIV testing by laboratories in the United States. The recommended 3-test algorithm includes initial testing

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with an Ag/Ab assay and, if reactive, an HIV-1/2 antibody differentiation immunoassay. An HIV nucleic acid test (NAT) follows when the supplemental test does not confirm infection.[1] Later, CDC released 2 technical updates to address the use of the Alere Determine Ag/Ab combo test and the Bio-Rad Geenius HIV-1/2 supplemental assay (Geenius) in the algorithm,[2,3] as well as a quick reference guide for the laboratory HIV testing algorithm using serum or plasma specimens.[4] The performance of the algorithm in individuals receiving antiretroviral therapy (ART) early in infection or when infected while taking preexposure prophylaxis has not been extensively evaluated.[5–7]

In 2015, Food and Drug Administration (FDA) approved the Bio-Rad BioPlex 2200 HIV Ag-Ab assay (BPC), which is the only screening immunoassay intended for the simultaneous qualitative detection and differentiation of HIV-1 p24 Ag, HIV-1 (groups M and O) antibodies and HIV-2 antibodies. Initial detection of p24 alone may be indicative of acute HIV-1 infection, which would ultimately require confirmation with NAT.[8,9]

The current CDC/APHL testing recommendations do not include the use of any HIV-1 viral load (VL) assay or HIV-2 NAT in the algorithm since there are no such tests approved by FDA for HIV diagnosis. However, HIV-1 VL assay is usually performed at initiation of HIV clinical care. In addition, there is only 1 HIV-1 RNA qualitative assay approved by FDA for diagnosis and it is costly compared with antibody supplemental tests, labor intensive and only needed for acute infection confirmation, which prevents its implementation in many laboratories.[10] In 2016, FDA approved the Hologic HIV-1 Quant Assay on the Panther system (APT-Quant) for monitoring HIV-1 VL assay. This is the same assay that was approved outside of the United States for HIV diagnosis and monitoring[11–13] (Aptima HIV-1 Quant Dx Assay), and it has been shown to perform similarly to FDA-approved VL assays.[14–18]

Because there is benefit in simultaneously diagnosing HIV-1 infections early and obtaining a VL measurement for patient management, we evaluated the performance of APT-Quant for diagnosis in our laboratory. In addition, we used plasma specimens from persons at different stages of HIV-1 infection to compare the performance of a 2-test diagnostic algorithm consisting of screening with BPC, a differentiation assay, followed by the APT-Quant with the currently recommended 3-test algorithm to assess the possibility of more efficiently diagnosing HIV-1 infections[19] which are more common than HIV-2 infections in the United States[20] and to examine the effects of ART on the algorithm performance.

MATERIALS AND METHODS

HIV Assays

Aptima HIV-1 Quant Assay—The APT-Quant (Hologic Inc. San Diego, CA) was performed as described in the manufacturer's package insert for plasma specimens[21] and APT-Quant results were interpreted as described in the CE-IVD APT HIV-1 Quant Dx Assay package insert since the diagnostic claim is not available in the United States.[22] The APT-Quant results that were detected either less than 1.47 $\log_{10}$ (copies/mL) or with a quantified VL were considered reactive, whereas target not detected (TND) results were considered non-reactive for diagnostic purposes. The assay targets the LTR and pol regions

of the HIV genome (HIV-1 groups M, N, and O) using transcription-mediated amplification. The Panther system is a fully automated high-throughput platform with random access able to run up to 320 samples in 8 hours. The linear range of quantification is 1.47 $\log_{10}$ copies/mL (30 copies/mL) to 7 $\log_{10}$ copies/mL (10,000,000 copies/mL) using 0.7 mL of plasma and the reported limit of detection is 1.08 $\log_{10}$ copies/mL (12 copies/ mL). The reagents for this evaluation were provided by Hologic Inc. as part of a collaboration with CDC.

Aptima HIV-1 RNA Qual Assay—The APT-Qual (Hologic, Inc.), the only FDA-approved NAT for diagnosis of acute HIV-1 infection, was performed as described in the manufacturer's package insert for plasma specimens. It also utilizes transcription-mediated amplification and detects HIV-1 RNA at greater than 98.5% for 1.47 $\log_{10}$ copies/mL (30 copies/mL) using 0.5 mL of plasma.[23]

BioPlex 2200 HIV Ag-Ab—The BPC (Bio-Rad Laboratories, Redmond, WA) is a multiplex flow immunoassay that can simultaneously detect and differentiate HIV-1 p24 antigen, HIV-1 (groups M and O) antibodies and HIV-2 antibodies in human serum or plasma.[8,9,24] The assay was performed as described in the manufacturer's package insert except for the commercial seroconversion panels that were tested in duplicate.

Geenius HIV-1/2 Supplemental Assay—The Geenius (Bio-Rad Laboratories) is a single-use immunochromatography assay designed for the confirmation and differentiation of antibodies to HIV-1 and HIV-2 in serum, plasma, and whole blood in a dual path lateral flow format. Geenius is, currently, the only supplemental antibody differentiation test approved by FDA that meets the requirements for the second step in the recommended HIV diagnostic algorithm.[1] The assay was performed as described in the manufacturer's package insert using the software version 1.1 before the release of the latest software which increased the gp140 cutoff.[25]

Sample Sets

Plasma From HIV-1 US Seroconverters—A total of 419 longitudinal plasma specimens were collected from 46 US HIV-1 seroconverters. Two hundred twenty-nine longitudinal plasma specimens were collected from 26 ART-naive donors and were obtained from Zeptometrix, Inc. (Buffalo, NY) and BBI-Seracare Diagnostics (West Bridgewater, MA). These previously characterized plasma donors were early in the process of seroconversion including samples from the eclipse phase and seroreactivity was always observed after an HIV-1 RNA-positive result.[26,27] In addition, as part of the Seroconversion Incidence Panel Project in collaboration with SeraCare Life Sciences, Inc. (Milford, MA), 190 longitudinal plasma specimens were collected from 20 donors that were HIV-1 RNA-positive at the first visit.[28] Based on records and test results, 9 subjects were ART-naive, 1 was taking ART at the time the first specimen in the series was collected and Geenius HIV-1 indeterminate, 7 started ART within 3 months of starting follow-up and after becoming Geenius HIV-1–positive, and 3 started ART >5 months after starting follow-up and after becoming Geenius HIV-1–positive. Of 190 longitudinal specimens, 78 specimens were collected after ART initiation.

Plasma From ART-Naive HIV-1–Positive Persons—One hundred five ART-naive antibody-positive (established infection) plasma specimens including 102 HIV-1 group M non-B subtypes and 3 HIV-1 group O were collected from discarded anonymous blood donations in Cameroon.[29]

HIV-Negative Samples—Two hundred previously characterized APT-Qual-nonreactive/ HIV-antibody negative plasma specimens were tested with APT-Quant.[30]

All specimens used in this study were unlinked from personal identifiers, and this study was determined by the CDC to be research not involving human subjects.

Analyses—To verify the performance of the APT-Quant for diagnostic use, valid APT-Quant and APT-Qual results from 417 of 419 longitudinal plasma specimens from 46 US HIV-1 seroconverters and 105 plasma specimens from HIV-1–infected persons from Cameroon were compared by the McNemar's analysis. The APT-Quant results were interpreted as described above since the diagnostic claim is not available in the United States. In addition, specificity was calculated using 200 HIV-negative plasma.

To compare the performance of the proposed 2-test algorithm to the current 3-test algorithm, 524 plasma specimens from US seroconverters and Cameroonian HIV-1–positive persons were tested with APT-Qual, APT-Quant, and BPC. If BPC-reactive, specimens were reflexed to Geenius as currently recommended in the CDC-APHL algorithm. Specimens included in this study are from previously characterized HIV-1 infections and seroreactivity was observed at or after the first HIV-1 RNA-positive result. For this analysis, ART-naive specimens were considered early infections if they had BPC-reactive and Geenius antibody-negative or indeterminate results, and established infections if they had BPC-reactive and Geenius antibody-positive results. The paired comparison of the 2-test and 3-test algorithms by McNemar's test was performed in 2 groups, BPC-reactive specimens collected before (n = 355) and after (n = 78) ART initiation to address the effects of ART on performance. Distribution of VL medians and ranges was calculated for different sample groups for further comparison.

RESULTS

Performance of the APT-Quant for Diagnostic Use

Of 419 longitudinal plasma specimens from US seroconverters, including 43 specimens collected after acquisition but negative in all HIV tests (eclipse phase), 417 had valid APT-Quant and APT-Qual results. Of the 417, 328 were reactive with APT-Qual and APT-Quant, 48 were nonreactive with both assays, 34 were APT-Quant–reactive/ APT-Qual–nonreactive and 7 were APT-Quant–nonreactive/APT-Qual–reactive. The analysis showed that APT-Quant detected significantly more HIV-1 RNA reactive samples than APT-Qual (McNemar's test, $P < 0.0001$). There was no significant difference among the 105 established HIV-1 infections from Cameroon since all specimens were reactive on both assays.

Of 200 APT-Qual-nonreactive/HIV antibody-negative plasma specimens, 199 were APT-Quant–nonreactive. The specificity using previously characterized HIV-negative specimens

was 99.5% with a 95% confidence interval of 97.2% to 99.9%. The APT-Quant performance for diagnostic use was excellent.

Performance of the Proposed 2-Test Algorithm

A total of 524 plasma specimens from US seroconverters and Cameroonian HIV-1–positive persons were tested with BPC. Of 419 plasma from HIV-1 seroconverters (subtype B), 91 were BPC-nonreactive and 328 were BPC-reactive specimens. BPC was reactive for p24 antigen and/or HIV-1 antibody only after the first positive HIV-1 RNA result. Of 105 ART-naive Cameroonian plasma (non-B subtypes), all were BPC HIV-1 antibody-reactive. Of note, no analysis of the algorithm was conducted among HIV-2 infections in this study and only HIV-1 antibody reactivity was observed among HIV-1 infections since BPC was HIV-2 antibody-nonreactive in all specimens.

For the comparison of the 2 algorithms, 433 BPC-reactive US seroconverters and Cameroonian HIV-1–positive persons were included, and the analysis was performed among 355 specimens before and 78 after ART initiation.

The BPC, Geenius, APT-Qual, and APT-Quant (median VLs and ranges) reactivities in plasma collected before ART initiation from HIV-1 seroconverters and ART-naive HIV-1–positive persons are described in Table 1. Of 355 BPC-reactive with valid Geenius results, Geenius identified 75 samples as HIV antibody-negative or HIV-1 indeterminate (early infections) only among seroconversion specimens and 280 as HIV-1–positive or untypable (established infections) among specimens from seroconverters and Cameroonian HIV-1–positive persons. Of 75 specimens from early HIV-1 infections, the 3-test algorithm was positive for 72 (96.0%) using APT-Qual, whereas the 2-test algorithm was positive for 74 (98.7%) using APT-Quant. Furthermore, APT-Quant quantified 72 (96.0%) of the 74 reactive samples; 2 were detected <1.47 $\log_{10}$ (cop/mL) and 1 was TND and APT-Qual-nonreactive. The paired comparison analysis showed no significant difference ($P = 0.4795$) between the 2-test (BPC/APT-Quant) and 3-test (BPC/Geenius/APT-Qual) algorithms in early HIV-1 infections. Of the remaining 280 specimens from the United Sates and Cameroon described in Table 1, 279 were Geenius HIV-1–positive and 1 from Cameroon was HIV-positive untypable. The APT-Qual was nonreactive in 3 and of those APT-Quant was nonreactive in 2 specimens and the third was detected less than 1.47 $\log_{10}$ (copies/mL). One Geenius HIV-1–positive specimen produced an invalid APT-Quant result, and thus was excluded from the statistical analysis. Of note, all plasma from Cameroon were reactive on both HIV-1 RNA tests. The 2-test (BPC/APT-Quant) algorithm was positive for 277 (99.6%) of the 279 established HIV-1 infections with valid results. Among 355 plasma from ART-naive persons infected with HIV-1 the overall paired comparison of the proposed 2-test algorithm and the current 3-test algorithm showed no significant difference ($P = 0.2482$).

The BPC, Geenius, APT-Qual, and APT-Quant (median VLs and ranges) reactivities in 78 plasma collected after ART initiation from HIV-1 seroconverters in the context of the 3-test and 2-test diagnostic algorithm are described in Table 2. Of those, 77 were BPC HIV-1 antibody-reactive and 1 was BPC antigen and HIV-1 antibody-reactive (early ART initiation). Among specimens from persons receiving ART, the current 3-test algorithm performed significantly better ($P = 0.0233$); 71 specimens were positive using both

algorithms whereas 7 were only positive using the current 3-test algorithm. Of those 7 BPC-reactive/APT-Quant-nonreactive, 3 were Geenius HIV antibody-negative or indeterminate and APT-Qual reactive and 4 were Geenius HIV-1–positive.

Geenius results indicated seroreversion occurred in 3 of 8 seroconverters who initiated ART early in the infection (see Appendix Table 1), but seroreversion was not observed in any of the specimens from persons who initiated ART more than 2 months after follow-up was initiated (time of infection is unknown). Of the 3 seroconverters with seroreversion 1 was Geenius HIV-1 indeterminate with APT-Quant detected <1.47 $\log_{10}$ (copies/mL) at the first time point and presented a delayed seroconversion to Geenius HIV-1–positive and later seroreversion to Geenius HIV-1 indeterminate then to HIV-antibody negative within 481 days of viral suppression. In this seroconverter, the 3-test algorithm was positive for 10 of 10 specimens whereas the 2-test algorithm was positive for 8 of 10 specimens. The second seroconverter was Geenius HIV-1–positive at first time point with a VL of 5.64 $\log_{10}$ (copies/mL) while on ART and within 2 months became virally suppressed for 147 days and Geenius became HIV-1 indeterminate and APT-Qual-reactive but, APT-Quant-nonreactive. The 3-test algorithm was positive for 10 of 10 specimens whereas the 2-test algorithm was positive for 8 of 10 specimens. The last seroconverter was Geenius HIV-1 indeterminate and had a VL greater than 7.00 $\log_{10}$ (copies/mL) at the first time point and initiated ART within 2 months of seroconversion to Geenius HIV-1–positive but became and remained Geenius HIV-1 indeterminate for 149 days and then became Geenius HIV-1–positive again. Despite the low viremia, both algorithms were positive for 10/10 specimens.

CONCLUSIONS

This study evaluates an HIV diagnostic algorithm that differentiates HIV-1 from HIV-2 antibodies with p24 detection at screening, followed by an HIV-1 RNA assay that provides detection and quantification when diagnosing HIV-1 infections. The Hologic Aptima HIV-1 Quant, approved and labeled outside of the United States as Aptima HIV-1 Quant Dx Assay has primarily been evaluated to compare its performance to other VL assays.[11,14–18] Our study of the evaluation of APT-Quant for diagnostic use showed excellent agreement with the reference FDA-approved assay (APT-Qual) for detection of HIV-1 RNA including for different HIV-1 subtypes. Specificity of Aptima HIV-1 Quant was also high. Of note, the 2 HIV-1 RNA assays were not run in parallel for the set of commercial seroconversion panels and plasma specimens were tested in singlet and 2 specimens with invalid results were not repeated. However, our results show that the assay performance is as good as the diagnostic assay, therefore, it is acceptable as a supplemental diagnostic test. The APT-Quant not only performs well in early and established infections with different HIV-1 subtypes but also offers an automated high throughput alternative that provides fast and accurate results for diagnosing and monitoring HIV-1 infections from the same sample.

Differentiating HIV-1 from HIV-2 infections may be of limited utility compared to detection of acute infection in the United States.[19,20] Therefore, the use of 2 automated high-throughput platforms for a differentiation screening assay and rapid NAT would facilitate HIV-1 diagnosis and likely decrease turnaround time for results which could lead to faster initiation of care in clinical settings compared with the current 3-test diagnostic algorithm

using standard screening assays and a labor-intensive and manual NAT. One limitation of this study was that Geenius results were interpreted with software version v1.1, before Bio-Rad adjusted the gp140 cutoff to address HIV-2 indeterminate results, but a recent evaluation showed that identification of HIV-1 infection is not affected with the new software version.[25] The agreement between BPC and Geenius was excellent for identifying HIV-1 infections in the current study population. However, we did not evaluate the performance of the algorithm among HIV-2–positive specimens that would likely require the use of an HIV-1/2 antibody differentiation assay for confirmation if the HIV-1 RNA result is undetectable in the second step. The other advantage of using BPC is the identification of p24 antigen-reactive specimens to identify acute HIV-1 infection at screening. The results show that BPC was p24-only reactive in specimens from early stages of HIV-1 seroconversion, right after HIV-1 RNA becomes detectable. This may be beneficial for determining acute infection in the absence of a second antibody test. High VLs may indicate acute infection; however, our results show that very early in HIV-1 infection, when the supplemental assay is either negative or indeterminate, VLs can range from less than 1.47 to greater than 7.00 $\log_{10}$ (copies/mL). A similar range was also observed when p24 antigen was nonreactive, and IgG was already present in the sample (Geenius HIV-1–positive). The downside of the evaluated 2-step algorithm is that BPC is the only FDA-approved differentiation screening assay that runs in a large-sized automated high-throughput platform, thus, nationwide implementation of this 2-test algorithm may be challenging for small- and medium-sized laboratory settings.

The APT-Quant as a second step in the algorithm not only detects HIV-1 but also provides a VL needed for initial care and treatment. Before ART initiation, the 2 algorithms performed similarly among samples from different stages of HIV-1 infection, although the 2-test algorithm may reduce the turn-around time for results and initiation of ART. In the evaluated sample sets, in ART-naive samples, the VL results among BPC p24 only-reactive samples ranged from 2.45 to greater than 7.00 log (copies/mL). Thus, a 2-test algorithm using standard Ag/Ab combo assays that do not differentiate Ag from Ab reactivity, followed by VL may require further evaluation to establish a VL threshold to identify acute infections. In contrast, in a small number of specimens (n = 78) collected after ART initiation the current 3-test algorithm performed significantly better and the use of a supplemental antibody assay was shown to be advantageous because the VL assay alone could not confirm the observed BPC antibody reactivity.

Seroreversion was only observed in seroconverters that initiated ART early in infection, but not in absence of ART or when ART was initiated later in the infection. The 3 seroconverters with documented initiation of ART early in the infection, specifically the one that was on ART at the first time point with Geenius HIV-1 indeterminate and VL detected less than 1.47 $\log_{10}$ (copies/mL) could be similar to serological data observed among patients who become infected while taking HIV pre-exposure prophylaxis, where delayed seroconversion has been observed due to the presence of suboptimal drugs and low-level viral replication.[7]

The availability of an HIV-1 RNA test with a dual claim for both diagnosis and monitoring would be advantageous for patient care and management due to decreased turn-around time for results and a potential decrease in cost in situations where a 3-step algorithm is needed to identify infection. However, there is no FDA-approved dual claim assay and regulatory

burden may delay future approvals. Laboratories may run into challenges validating the off-label use in clinical laboratories, such as access to plasma specimens instead of serum that is commonly used for HIV serology. Furthermore, there may be instances when a sample with an Ag/Ab-reactive result followed by an undetectable VL, would benefit from a supplemental antibody test because our results show that APT-Quant performance decreases after the IgG response is elicited and with suppressed viremia. Additional factors and challenges associated with the implementation of this 2-test algorithm, such as the implications of off-label use, sample type, cost of platforms and tests, and efficient and accurate identification in the rare cases of HIV-2 need to be considered and further evaluated.

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Disclaimer:

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Appendix

APPENDIX TABLE 1.

Test Results in Plasma Specimens From Three HIV-1 Seroconverters With Seroreversion

Sample ID	Sample Date	ART	Ag-Ab	Ag	HIV-1 Ab	HIV-2 Ab	Geenius Final Interpretation v1.1	APT-Qual Result	APT-Quant log_{10} (Copies/ml)
SC1–1	1/23/2009	YES	R	NR	R	NR	HIV-1 INDETERMINATE	R	<1.47
SC1–2	2/24/2009	YES	R	NR	R	NR	HIV-1 INDETERMINATE	R	2
SC1–3	4/1/2009	YES	R	NR	R	NR	HIV-1 INDETERMINATE	R	1.53
SC1–4	4/24/2009	YES	R	NR	R	NR	HIV-1 INDETERMINATE	R	2.64
SC1–5	6/1/2009	YES	R	NR	R	NR	HIV-1 INDETERMINATE	R	<1.47
SC1–6	71712009	YES	R	NR	R	NR	HIV-1 POSITIVE	R	1.98
SC1–7	8/6/2009	YES	R	NR	R	NR	HIV-1 POSITIVE	R	1.67
SC1–8	10/27/2009	YES	R	NR	R	NR	HIV-1 INDETERMINATE	R	<1.47
SC1–9	2/2/2010	YES	R	NR	R	NR	HIV-1 INDETERMINATE	R	TND

Sample ID	Sample Date	ART	Ag-Ab	Ag	HIV-1 Ab	HIV-2 Ab	Geenius Final Interpretation v1.1	APT-Qual Result	APT-Quant log_{10} (Copies/ml)
SC1–10	5/19/2010	YES	R	NR	R	NR	HIV NEGATIVE	R	TND
SC2–1	12/3/2008	YES	R	n/r	R	NR	HIV-1 POSITIVE	R	5.64
SC2–2	1/7/2009	YES	R	NR	R	NR	HIV-1 POSITIVE	R	2.84
SC2–3	2/4/2009	YES	R	NR	R	NR	HIV-1 POSITIVE	R	<1.47
SC2–4	3/4/2009	YES	R	NR	R	NR	HIV-1 POSITIVE	R	<1.47
SC2–5	3/31/2009	YES	R	NR	R	NR	HIV-1 POSITIVE	NR	<1.47
SC2–6	5/6/2009	YES	R	NR	R	NR	HIV-1 POSITIVE	R	TND
SC2–7	6/3/2009	YES	R	NR	R	NR	HIV-1 INDETERMINATE	R	TND
SC2–8	9/3/2009	YES	R	NR	R	NR	HIV-1 POSITIVE	R	<1.47
SC2–9	12/4/2009	YES	R	NR	R	NR	HIV-1 POSITIVE	R	5.34
SC2–10	3/10/2010	YES	R	NR	R	NR	HIV-1 POSITIVE	R	<1.47
SC3–1	2/18/2009	NO	R	R	R	NR	HIV-1 INDETERMINATE	R	>7.00
SC3–2	3/16/2009	NO	R	NR	R	NR	HIV-1 POSITIVE	R	4.7
SC3–3	4/13/2009	YES	R	NR	R	NR	HIV-1 POSITIVE	R	3.05
SC3–4	5/19/2009	YES	R	NR	R	NR	HIV-1 POSITIVE	R	3.11
SC3–5	6/23/2009	YES	R	NR	R	NR	HIV-1 INDETERMINATE	R	1.94
SC3–6	7/21/2009	YES	R	NR	R	NR	HIV-1 INDETERMINATE	R	2.82
SC3–7	8/13/2009	YES	R	NR	R	NR	HIV-1 INDETERMINATE	R	2.65
SC3–8	11/19/2009	YES	R	NR	R	NR	HIV-1 INDETERMINATE	R	1.94
SC3–9	2/26/2010	YES	R	NR	R	NR	HIV-1 POSITIVE	R	1.83
SC3–10	5/26/2010	YES	R	NR	R	NR	HIV-1 POSITIVE	R	2.08

ART: antiretroviral therapy; NR: non-reactive; R: reactive; n/r: Not reportable due to high antibody levels; TND: target not detected. For APT-Quant, all samples with detected virus were considered reactive for diagnosis and 1.47 log10 (cop/ml) is the lower limit of quantification of APT-Quant.

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TABLE 1.

Test Results in Plasma Specimens From Persons With HIV-1 Infection Before ART Initiation

BioPlex Ag/Ab Assay			Aptima Qual		Aptima HIV-1 Quant Log$_{10}$ (copies/mL)					
							Detected			
Ag	**HIV-1 Ab**	**Geenius HIV-1/2 Assay**	**NR**	**R**	**NR**	**R**	**Total <1.47**	**Total S1.47**	**Median VL**	**Range VL**
R	NR	HIV Ab-neg	0	41	0	41	0	41	5.05	2.45->7.00
R	**R**	HIV Ab-neg	1	14	0	15	0	15	5.81	3.60->7.00
NR	**R**	HIV Ab-neg	0	3	0	3	1	2	3.29	<1.47–3.30
R	**R**	HIV-1 ind	1	8	1	8	0	8	5.42	1.81->7.00
NR	**R**	HIV-1 ind	1	6	0	7	1	6	4.10	<1.47–4.89
R	**R**	HIV-1 pos	0	20*	0	21	2	19	4.87	<1.47->7.00
n/r	**R**	HIV-1 pos	0	19	0	19	0	19	5.59	2.11–6.45
NR	**R**	HIV-1 pos	3	236	2	236*	2	234	4.23	<1.47–6.16
NR	**R**	HIV untyp	0	1	0	1	0	1	4.44	4.44
Total			6	348*	3	351*	6	345		

*One additional invalid result; values >7 log$_{10}$ (copies/mL) was considered as 7 for the median VL. For APT-Quant, all samples with detected virus were considered reactive for diagnosis and 1.47 log$_{10}$ (copies/mL) is the lower limit of quantification of APT-Quant.

NR, nonreactive; R, reactive; n/r, not reportable due to high antibody levels; Ab-neg, antibody negative; ind, indeterminate; pos, positive; untyp, untypable.

TABLE 2.

Test Results in Plasma Specimens From HIV-1 Seroconverters After ART Initiation

BioPlex Ag/Ab Assay			Aptima Qual		Aptima HIV-1 Quant Log_{10} (copies/mL)					
							Detected			
Ag	HIV-1 Ab	Geenius HIV-1/2 Assay	NR	R	NR	R	Total <1.47	Total >1.47	Median VL	Range VL
R	**R**	HIV-1 pos	0	1	0	1	0	1	6.90	—
n/r	**R**	HIV-1 pos	0	4	0	4	0	4	3.18	1.57–5.64
NR	**R**	HIV Ab-neg	0	1	1	0	0	0	TND	—
NR	**R**	HIV-1 ind	0	12	2	10	3	7	2.00	1.53–2.63
NR	**R**	HIV-1 pos	10*	50	4	56	18	38	2.84	1.51–5.58
Total			10*	68	7	71	21	43		

Values >7 log_{10} (copies/mL) was considered as 7 for the median VL.

*2 samples were APT-Qual and APT-Quant nonreactive. For APT-Quant, all samples with detected virus were considered reactive for diagnosis and 1.47 Log_{10} (copies/mL) is the lower limit of quantification of APT-Quant.