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Short communication

Diagnosis of HIV-1 infection: Performance of Xpert Qual and Geenius supplemental assays in fourth generation ELISA-reactive samples

Aviya Rakovsky[a,1], Yael Gozlan[a,1], Ravit Bassal[b], Marina Wax[a], Rachel Shirazi[a], Mary Bakhanashvili[c], Zipi Kra-Oz[d], Sara Radian-Sade[e], Haim Ben-Zvi[f], Licita Schreiber[g], Dana G. Wolf[h,i], Yonat Shemer-Avni[j], Daniel Chemtob[k,l], Ella Mendelson[a,m], Orna Mor[a,m,*]

[a] National HIV Reference Laboratory, Central Virology Laboratory, Ministry of Health, Tel-Hashomer, Ramat-Gan, Israel
[b] Israel Center for Disease Control, Ministry of Health, Tel-Hashomer, Ramat-Gan, Israel
[c] HIV Laboratory, Infectious Diseases Unit, Sheba Medical Center, Ramat-Gan, Israel
[d] Virology Laboratory, Rambam Medical Center, Haifa, Israel
[e] HIV Laboratory, Kaplan Medical Center, Rehovot, Israel
[f] Virology Laboratory, Rabin Medical Center, Petach Tikva, Israel
[g] Maccabi Health Services, Mega Laboratory, Rehovot, Israel
[h] Virology Laboratory, Hadassah Medical Center, Jerusalem, Israel
[i] Hadassah University Medical School, Jerusalem, Israel
[j] Virology Laboratory, Soroka Medical Center, Beer-Sheva, Israel
[k] Department of Tuberculosis and AIDS, Ministry of Health, Jerusalem, Israel
[l] Faculty of Medicine, Braun School of Public Health & Community Medicine, Hebrew University-Hadassah Medical School, Jerusalem, Israel
[m] School of Public Health, Sackler Faculty of Medicine, Tel-Aviv University, Ramat-Aviv, Israel

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ABSTRACT

Background: Architect (AR) and Vidas (VD) fourth generation HIV screening immunoassays, which identify early stages of HIV infections, could have false positive results especially at low signal/cutoff (S/C) AR values. Geenius HIV1/2 (GS) is a specific confirmation line immunoassay that is not highly sensitive to early HIV infections. An HIV-1 RNA assay may better detect such infections.

Objectives: To evaluate all AR-VD reactive samples with GS results, and to assess Xpert Qual HIV-1 RNA assay (XQ) as an alternative to GS, in the first low S/C AR-VD-reactive samples from a tested individual.

Study design: First AR-VD-reactive-GS-tested results from all individuals with resolved HIV status, collected between March 2015 and March 2017 (n = 749), were retrospectively assessed. Samples with AR-VD-reactive-GS-discordant results and those with low S/C AR-VD-reactive results, were tested by XQ. Receiver operating characteristic (ROC) analysis of GS and XQ sensitivity/specificity was performed.

Results: Overall, 94.1% (705/749) of AR-VD-reactive results were true HIV-1 positive. All samples with < 3 S/C AR values were false positive. XQ resolved all first samples with AR-VD-reactive-GS-discordant results. The diagnostic accuracy of XQ in low (≤33 S/C) AR-VD-reactive samples was better than that of GS (97.6%, 81/83 versus 73.5%, 61/83, $p < 0.01$). ROC analysis for low S/C AR samples was optimal for pooled XQ and GS results.

Conclusions: Incorporating XQ in the current screening algorithm for the first AR-VD-reactive-GS-discordant samples may significantly reduce overall turn-around time of HIV-1 diagnosis.

1. Background

The laboratory testing algorithm for the diagnosis of HIV infection, updated and issued in 2014 by the Centers for Disease Control and Prevention (CDC), recommends addition of an HIV-1 RNA-specific molecular assay to confirm or deny HIV infection for samples reactive in fourth generation screening assays and negative or indeterminate in the confirmation immunoassay [1]. Both the Aptima HIV-1 RNA (Gen-

* Corresponding author at: National HIV Reference Laboratory, Laboratory of Clinical Virology, Ministry of Health, Sheba Medical Center, Ramat-Gan, Israel; Sackler Faculty of Medicine, Tel-Aviv University, Ramat-Aviv, Israel.
E-mail address: orna.mor@gmail.com (O. Mor).
[1] AR and YG contributed equally to this publication.

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Probe Incorporated, San Diego, CA, USA) and the Xpert QUAL HIV-1 (XQ, Cepheid, CA, USA) qualitative HIV-1 RNA assays qualify for such cases [2,3].

In Israel, screening for HIV infection is performed with the Architect HIV Ag/Ab Combo assay (AR, Abbott Diagnostics, Abbott Park, IL, USA), that detects HIV-1 p24 antigen and anti-HIV-1/HIV-2 antibodies. Reactive samples are then tested by Vidas HIV DUO ULTRA (VD, Biomérieux, Marcy-l'Etoile, France), that separates p24 antigen and anti-HIV-1/HIV-2 antibodies. All AR-VD-reactive samples are transferred to the national HIV reference laboratory (NHRL) for confirmation with the Geenius HIV-1/2 (GS, Bio-Rad Laboratories, 68 Hercules, California) differentiation assay [4], which separates anti-HIV-1 and anti HIV-2 antibodies. To date, a molecular RNA assay has not been introduced. Both AR and VD fourth generation ELISA assays facilitate early detection of new infections [5,6]. Moreover, for AR, a positive association between higher signal-to-cutoff (S/C) values and confirmation of HIV infection was suggested [7], while low S/C AR values were shown to characterize acute HIV-1 infection [6]. Despite the high sensitivity of AR and VD, false-positive results mandate the use of supplementary assays like GS, to confirm HIV infection [8]. Excellent specificity was reported for GS [9]. However, discordance between the screening assays and GS results, may occur [10] and testing of a new sample, taken at least two weeks after the first one, is required to confirm HIV-1 infection [4]. Such circumstances delay referral to a medical center and initiation of anti-HIV-1 therapy.

2. Objectives

To retrospectively assess the performance of AR-VD and of GS for all first AR-VD-reactive-GS-tested samples collected between March 2015 and March 2017 in Israel. To compare XQ to GS in all samples with AR-VD-reactive-GS-discordant results and in all VD reactive samples with low S/C AR values.

3. Study design

All AR-VD-reactive samples (> 0.75 S/C AR, > 0.25 units VD), tested by GS between March 2015 and March 2017, were retrospectively evaluated. True HIV status was defined HIV-1 positive when the sample or any of the following samples was GS HIV-1 positive, HIV-1 negative when all samples were GS-indeterminate or GS-negative. Individuals for whom the true HIV status could not be determined, were excluded.

Only the first AR-VD-reactive-GS-tested samples from a tested individual were considered. The S/C AR value above which all samples were retrospectively defined true-positive was recorded. All samples unresolved by GS were tested by XQ. Also, all samples with a S/C AR value below the defined threshold of true positives, were tested by XQ. The XQ assay was performed with 100 microL serum and according to the manufacturer instructions [11].

The false-positive rate of AR-VD reactive results, as well as the overall sensitivity, specificity and diagnostic accuracy of GS and XQ in identifying true HIV status was determined. When evaluating GS performance, all indeterminate interpretations were considered incorrect as they failed to provide final verdict and required another round of testing with a later time point sample.

The Receiver Operator Characteristics (ROC) analysis was used to assess the optimal sensitivity and specificity of the GS and the XQ assays. SAS Enterprise Guide 7.1® (SAS Institute Inc., Cary, NC, USA) was used for this analysis.

4. Results

Between March 2015–March 2017, AR-VD-GS screening results for 749 individuals with a definitive HIV diagnosis, were available for retrospective assessment (Fig. 1). 5.9% (44/749) of the first AR-VD-reactive samples, were false-positives. All samples with > 33 S/C AR-VD-reactive results (88.9%, 666/749) were true positives, while those with ≤ 33 S/C values (11.1%, 83/749) were either true or false positives. All samples with ≤ 3 S/C AR values (n = 35) were false-positives. Eighty one (10.8%) of the 749 tested AR-VD-reactive samples were GS negative or indeterminate; 77.1% (64/83) of which had ≤ 33 S/C values and 2.6% (17/666) had > 33 S/C AR values. XQ correctly resolved HIV-1 status in all the GS negative or indeterminate first AR-VD-reactive samples.

In samples with ≤ 33 S/C AR values, XQ diagnostic accuracy was significantly better than GS (97.6%, 81/83 versus 73.5%, 61/83, $P < 0.01$). XQ sensitivity (94.9%, 83.1%–98.6%, 95% CI) and specificity (100%, 92.0%–100.0%, 95% CI) were also greater than GS (48.7%, 33.9%–63.8% at 95% CI and 95.4%, 84.9%–98.7%, 95% CI, respectively). However, in two 31 S/C AR-VD-GS-positive samples, XQ was negative. Retrospective analysis revealed that these samples were from two antiretroviral-treated, HIV-1-positive individuals. In both cases, HIV-1 plasma viral load was also not detected.

ROC analysis for ≤ 33 S/C samples (n = 83) tested by GS and XQ, revealed that the largest area under the curve (AUC) was obtained when results of both assays were pooled (AUC 0.77 for GS alone, 0.89 for XQ alone and 0.934 for pooled XQ and GS results, Fig. 2). The maximum sum of sensitivity and specificity (97.4% and 84.1%, respectively) obtained for these samples was only reached when XQ and GS results were pooled together.

5. Conclusions

This study shows that XQ was superior to GS in correctly defining HIV status in the first taken, low AR-VD-reactive samples, and resolved all first samples from individuals found to be negative or indeterminate by GS. In these cases, HIV-1 infection was confirmed by GS at later time points, when individuals were fully seroconverted. Being an RNA-based assay, with a detection limit of > 350 copies/ml [11], XQ was expected to identify HIV-1 in first AR-VD-reactive blood samples. However, it failed to detect HIV-1 in samples with undetectable HIV-1 plasma viral load derived from patients already under antiretroviral treatment. Such cases, as well as elite controllers, which are free of HIV-1 RNA, still prevents the general use of an RNA assay, instead of a serological assay, for confirmation of all screening results.

This study further demonstrated that low S/C AR-VD-reactive samples could be false-positives, as has been shown by others as well [12]. It has therefore been suggested that HIV confirmatory screening be performed on samples with low S/C only. Here, all samples with < 3 S/C AR-VD-reactive results were false-positives and all those with > 33 S/C AR-VD-reactive results were true-positives, similar to the 32.7 cutoff value already reported by others [8]. However, these S/C values were not tested rigorously, and could be influenced by the study parameters. Therefore, unless thoroughly studied, they should not be used in a clinical setting as concrete cutoff values.

One of the limitations of this study lay in the total number of samples screened in Israel for identification of HIV infection as well as the number of HIV negative samples found to be AR- positive-VD-negative was not available, therefore, the overall false positive rate of the AR alone could not be calculated. Full assessment of the screening algorithm, including AR-negative, AR-positive-VD-negative and the added value of VD results on the final diagnosis, is under investigation.

Israel is characterized by low HIV prevalence [13] and low infection rate, where only a minor proportion (4.1%, 236/5830) of individuals between 2000 and 2014 were identified during acute infection [14]. With this low incidence and with the higher cost of XQ compared to GS, use of the XQ assay as the major supplementary assay for all AR-VD-reactive samples, may not be cost-effective. However, early diagnosis of discordant screening and GS results could be accurately, effectively and rapidly resolved by XQ. Both GS and XQ assays can be completed in a single day, with a single sample [15,16]. With the AR-VD-GS-negative/