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Utility of POC Xpert HIV-1 Tests for Detection-Quantification of Complex Recombinants

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BACKGROUND

○ The access to routine molecular tools for infant HIV diagnosis (EID) and viral load (VL) quantification during the clinical follow-up can reduce the HIV-associated mortality and morbidity in pediatric and adult population. Conventional molecular tools for EID and VL testing require sophisticated instruments. They are typically placed in centralized laboratories which often offer result turnaround times of several weeks or months in most resource limited countries. It can lead to long delays in clinical interventions, increasing the risk of loss of clinical follow-up in patients.

○ To improve the HIV monitoring and the linkage to care of HIV-exposed and infected patients in settings with limited laboratory infrastructure, some new, simple, fast and easy to perform molecular assays for EID and VL quantification have been developed (the point-of-care or POC assays). They can be performed directly in health centers, reducing the time for results and allowing HIV confirmation, antiretroviral treatment (ART) initiation, or treatment or adherence interventions on the same day of sampling.

○ WHO has recommended the use of dried blood spots (DBS) instead of plasma to increase access to HIV diagnosis and monitoring in resource-limited settings. Since HIV genetic variability can affect the HIV-1 detection and quantification success by molecular tools for EID and HIV quantification (POC and non-POC), more studies are needed to evaluate their performance using DBS in areas with extremely high diversity as in Kinshasa (Democratic Republic of Congo, DRC), with 1.6% HIV prevalence. Kinshasa is considered the epicenter of the HIV-1 group M epidemic, showing an extremely high genetic diversity of non-B subtypes and complex circulating (CRF) and unique (URF) recombinant strains.

OBJECTIVE

To analyze the efficacy of two POC techniques for EID and VL (Cepheid Xpert HIV-1 Qual and Xpert HIV-1 VL) *versus* non-POC Roche CAP/CTM Quantitative VL test v2.0 in DBS collected from children and adults in Kinshasa, where a large diversity of HIV-1 variants are expected.

METHODS

○ From April to November of 2016, 160 DBS were collected in Monkole Hospital (Kinshasa, DRC) from 84 children (60 HIV-, 18 HIV+, 6 HIV-exposed) and 76 HIV-infected adults (66 treated, 10 naïve).

○ DBS samples will be collected by spotting by micropipette 70µl of venous blood collected by venipuncture in EDTA-anticoagulant tubes into each dot on a Whatman 903 Protein Saver Card (Schleicher & Schuell, Dassel, Germany).

○ HIV diagnosis was firstly performed in DRC using rapid serological tests in whole blood: Alere Determine™ HIV-1/2 Ag/Ab and Abbott and Trinity Biotech Uni-Gold in children and HIV COMBI PT Roche and VIDAS HIV Duo Quick BioMerieux in adults.

○ In Madrid, Spain, HIV status in the 84 children was confirmed using BioRad Geenius™ HIV-1/2 confirmatory assay with one DBS dot. All HIV seropositive and undetermined were then tested by POC Cepheid Xpert Qual.

○ In Navarra, Spain, HIV status in adults was confirmed using two ELISAS using Roche's Cobas® e411 analyzer and Mini Vidas (BioMerieux).

○ We compared HIV-1 viremia with Cepheid Xpert HIV-1 VL (Xpert VL) and Roche CAP/CTM Quantitative VL test v2.0 (Roche VL) in all HIV+ DBS, after VL correction by plasma volume in the dot assuming an average hematocrit depending on patient´s age. We assume 39% hematocrit for children, 42% for women and 47% for men, leading to plasma volume of 42.7 µl, 40.6 µl and 37.1 µl, respectively, in 70 µl blood per dot.

○ For HIV-1 variant characterization, RNA was extracted automatically using the NucliSENS easyMAG automated platform (BioMerieux) using 2 dots and amplified using primers designed by WHO and/or ANRS in *pol* coding region. HIV-1 variant was characterized by phylogenetic analysis using MEGA6 with Tamura 3-parameters model.

RESULTS

○ We evaluated the HIV-1 quantification efficacy of different molecular assays (POC and no-POC) in dried blood carrying different HIV-1 non-B subtypes and complex recombinants.

○ Most of 160 patients with available DBS were seropositive (58.75 %) by rapid serological tests performed in Kinshasa (DRC), female (59%), and antiretroviral-experienced (54%). The mean age from the 84 children was 9.6 years (range 0-18 years old) and from the 76 adults was 46.8 years (range 24.8-73 years old).

○ HIV-1 infection was confirmed in 13 of 84 children by Xpert Qual and in 71 (93.4%) of 76 adults by both Xpert VL and Roche VL.

○ We identify false HIV+ diagnosis in DRC in 5 adults and in 5 children (range 12-158 months age), 4 of them under unnecessary ART during a mean time 35.6 months.

○ Among the 84 HIV+ total samples, 80 (95.2%) could be detected by Xpert VL and 82 (97.6%) by Roche VL, providing quantified viremia values (≥40 *vs.* ≥20 c/dot or ≥936-1078 *vs.* ≥468-539 c/ml plasma depending on hematocrit) in 65 (77.4%) *vs.* 66 (78.6%) DBS, respectively (TABLE 1).

○ Considering age, HIV-1 could be quantified by both assays in most HIV+ children (92.3%, 12/13) and in 53 (74.6%) or 52 (73.2%) of 71 HIV+ adults by Xpert VL or Roche VL, respectively.

○ Two specimens not detected by Roche were detected by Xpert (<40c/ml) and 4 DBS below limit of detection of Xpert could be only detected by Roche (<20c/ml).

○ The three tests were able to detect and/or quantify HIV-1 in DBS collected in an area with multiple and highly diverse different variants circulating, including complex recombinants (Kinshasa, DRC).

○ Most (91.7%) of 24 obtained HIV-1 sequences (7 children/17 adults) were unique recombinant forms (URF), with >1000 c/ml by both VL tests in all but one sample.

○ A high correlation (R^2=0.91) was observed among viremia values (FIGURE 1) in the 78 HIV+ DBS quantified by both VL assays. However, we observed significant VL differences (≥0.5log, range from -0.55 to 1.07) in 12 (15.4%) of 78 cases with VL above detection limit by both assays (FIGURES 2 and 3), differing across samples.

TABLE 1. HIV-1 VL quantification in 84 HIV+ DBS from 13 HIV+ children and 71 HIV+ adults using two molecular assays.

		Xpert VL (POC assay)			
		Not detected	Detected not quantified <40c/dot	Quantified ≥40c/dot	Nº DBS
Roche VL (Non-POC assay)	Not detected	-	2	0	2
	Detected not quantified <20c/dot	4	9	3	16
	Quantified (≥20c/dot) ≥20-39c/dot	0	3	7	10
	Quantified (≥20c/dot) 40-1000c/dot	0	1	27	28
	Quantified (≥20c/dot) >1000c/dot	0	0	28	28
	Nº DBS	4	15	65	84

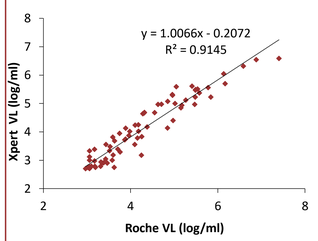


FIGURE 1. Correlation between Xpert VL and Roche VL assays. Scatter plot with a simple linear regression analysis of 78 samples (13 children + 65 adults) which were quantified (VL of ≥40 or ≥ 20copies/ml) by both assays.

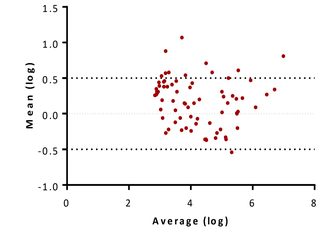


FIGURE 2. Bland-Altman plot: difference vs average Xpert VL vs. Roche VL of of 78 samples (13 children + 65 adults) quantified by both assays.

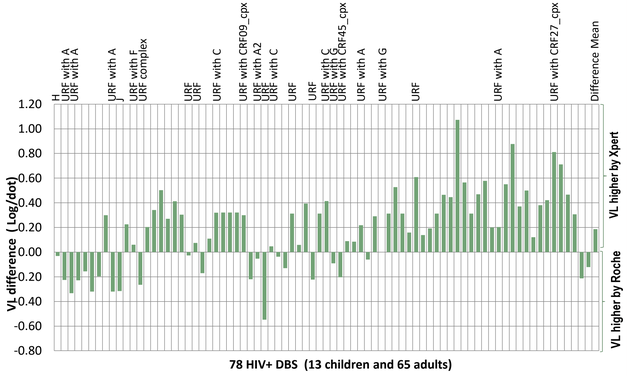


FIGURE 3. HIV-1 viremia differences by Xpert VL *vs.* Roche VL in 78 HIV+ DBS quantified by both assays and HIV-1 variants in 24 samples with available sequence.

CONCLUSION

○ POC Xpert VL and Roche VL can successfully detect and quantify complex recombinants URF. However, due to its simplicity and rapid results, POC use can improve the early diagnosis in HIV-exposed infants and the early detection of ART failures in countries with complex recombinants HIV-1 strains and limited infrastructures, as in Democratic Republic of Congo.

○ We provide new data related to the HIV molecular epidemiology in Kinshasa, reporting an extremely high rate of unique inter-subtype recombinants in recently infected population.

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