EVALUATION OF AN HIV-1/HIV-2 QUALITATIVE TEST FOR HIV EARLY INFANT DIAGNOSIS

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Shuyi Li, Guoqing Zhang, Katrina Sleeman, Shon Nguyen, Demetrius Mathis, Heather Alexander, and Clement Zeh

US Centers for Disease Control and Prevention, Center for Global Health, Division of Global HIV and TB, International Laboratory Branch, Atlanta. GA, USA. 30333

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BACKGROUND

Early Infant Diagnosis (EID) of HIV infection is key to ending the HIV pandemic. Since 2014, CDC has recommended that laboratory testing for diagnosing HIV infection should include HIV-1/HIV-2 differentiation test to confirm HIV-1/HIV-2 type(1). Currently, there is no HIV-1/HIV-2 qualitative assay for EID testing on high throughput testing systems from major manufacturers. The combination of confirmatory HIV testing and differentiation of heterotypic (HIV-1/2) and homotypic (HIV-1 or HIV-2) infections into one single test will provide clinicians with critical diagnostic data for personalized management of patients with HIV. This is particularly important in West African counties where the HIV-2 incidence is relatively high(2). Roche has developed the cobas HIV-1/HIV-2 Qualitative Test to detect and differentiate HIV-1/HIV-2 in dried blood spots (DBS). We report here the first independent analytical performance evaluation of the test conducted at a College of American Pathologists accredited laboratory at the US CDC as part of the WHO prequalification of in vitro diagnostics process.

METHODS

- The evaluation used DBS created from HIV-negative whole blood spiked with cultured virus or with HIV-1 and HIV-2 RNA International WHO Standards. Prepared DBS were stored at -80°C until day of testing(3).
- Testing was performed on a cobas 6800 system (c6800) using the HIV-1/HIV-2 DBS Qual workflow and reagent kit. The limit of detection (LOD) was calculated using PROBIT analysis.
- The reproducibility, cross-contamination, subtype detection for HIV-1 A, B, C, D, CRF02-AG and for HIV-2, and error rate were also assessed.

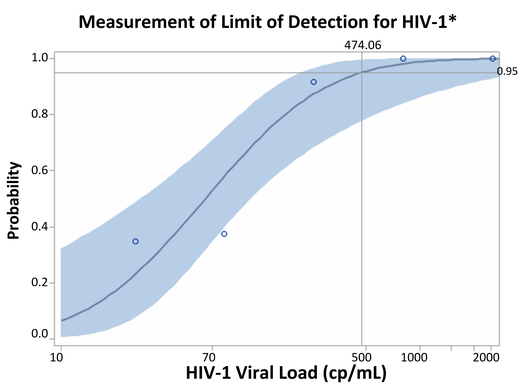
EXPERIMENT DESIGN

Analyte	Concentration (copies/mL)	Replicates
Limit of Detection (LoD)		
WHO International Standard for **HIV-1 RNA**	0.5 log_{10}-fold serial dilutions with:	24 each= 120
	• Two concentrations above the manufacturer's claimed LoD	
	• One concentration at the stated LoD	
	• Two concentrations below the LoD	
WHO International Standard for **HIV-2 RNA**	0.5 log_{10}-fold serial dilutions with:	24 each= 120
	• Two concentrations above the manufacturer's claimed LoD	
	• One concentration at the stated LoD	
	• Two concentrations below the LoD	
Reproducibility		
HIV-1 Subtype B	3x LoD	40
HIV-1 Subtype C	3x LoD	40
HIV-2	3x LoD	40
Additional subtype detection		
D	$10^{3.7}$ (5000)	3
A	$10^{3.7}$ (5000)	3
AG	$10^{3.7}$ (5000)	3
Cross-contamination		
HIV-1 Subtype B or C	$\geq 10^5$	20
Negative specimens	0	20

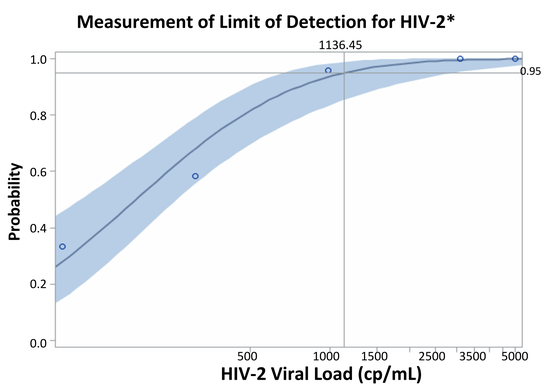
- Early Infant Diagnosis (EID) of HIV infection, proper diagnosis and HIV-1/HIV-2 differentiation are the key strategies for ending the HIV pandemic. We report here the first independent performance evaluation of the cobas HIV-1/HIV-2 Qualitative Test on the c6800 for HIV EID at a CAP accredited CDC laboratory as part of the WHO prequalification process.
- The LOD for HIV-1 was 474 copies/mL, and for HIV-2 was 1,136 copies/mL. Testing of forty replicates of three HIV-1 or HIV-2 samples over five days showed 100% reproducibility. The five major HIV-1 subtypes were all detected. No cross-contamination was detected.
- This evaluation verified the manufacturer's claims of analytical performance and provided valuable information for decision-making process related to approval for implementation in PEPFAR-supported countries.
- This is the first and only HIV-2 qualitative test commercially available on a high throughput HIV testing system. The test combines the capability of confirmatory HIV infection and HIV-1/HIV-2 differentiation, which will prove to be a useful tool in combating HIV.

RESULTS

- The LOD for HIV-1 was 474 copies/mL (95% confidence interval: 162 – 2,550 copies/mL), and for HIV-2 was 1,136 copies/mL (683 – 3,062 copies/mL).
- Testing of forty replicates of three HIV-1 or HIV-2 samples over five days, by two testers with different reagent lots, showed 100% reproducibility.
- The five major HIV-1 subtypes (A, B, C, D, CRF02-AG) and HIV-2 evaluated were all detected.
- No cross-contamination was detected and the overall error rate was 0.48% among 409 tests performed.



* Predicted Probabilities for Frequency with 95% Confidence Limits



* Predicted Probabilities for Frequency with 95% Confidence Limits

CONCLUSIONS

- **This evaluation verified the manufacturer's claims of analytical performance of the assay and provided valuable information for decision-making process related to potential approval for implementation in PEPFAR-supported countries.**
- **This is the first and only HIV-2 qualitative test commercially available on a high throughput HIV testing system. Clinical performance is currently being evaluated at PEPFAR-supported sites.**
- **The cobas HIV-1/HIV-2 Qualitative Test on the c6800 for HIV EID combines the capability of confirmatory HIV infection and HIV-1/HIV-2 differentiation, which will prove to be a useful tool in combating HIV.**

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Roche Cobas 6800

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DISCLAIMER

The findings and conclusions of this report are those of the authors and do not necessarily represent the views of US CDC

We declare no conflict of interest.

CONTACT INFO

Shuyi Li, MD, PhD
nll9@cdc.gov, CDC, Atlanta, GA