



The Open AIDS Journal

Content list available at: www.benthamopen.com/TOAIDJ/

DOI: 10.2174/1874613601610010158



RESEARCH ARTICLE

Evaluation of the Upgraded Version 2.0 of the Roche COBAS® AmpliPrep/COBAS® TaqMan HIV-1 Qualitative Assay in Central African Children

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Received: January 10, 2016

Revised: May 03, 2016

Accepted: May 30, 2016

Abstract:

Background:

Several commercially available molecular techniques were developed based on subtype B of HIV-1, which represents only 10% of HIV strains worldwide. Indeed, in sub-Saharan Africa, non-B subtypes of HIV-1 are predominant. The aim of this study was to evaluate the performances of the COBAS® AmpliPrep/COBAS® (CAP/CTM) HIV-1 Qualitative assays to detect the broad range of HIV-1 variants circulating in Central Africa and compare to the outgoing CAP/CTM HIV-1 Quantitative test v2.0 (Roche Molecular Systems), chosen as reference gold standard molecular assay.

Methods:

The CAP/CTM HIV-1 Qualitative tests versions 1.0 and 2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA) were evaluated compared to CAP/CTM TaqMan HIV-1 Quantitative test v2.0 (Roche Molecular Systems) on 239 dried plasma spot (DPS) from 133 HIV-1-infected (with detectable plasma HIV RNA load) and 106 uninfected children, followed-up at Complexe Pédiatrique, Bangui, Central African Republic.

Results:

The version 1.0 showed low sensitivity (93.2%), with 9 (6.8%) false negative results, demonstrating under-detection of non-B HIV-1 subtypes. In contrast, the upgraded version 2.0 showed 100%-sensitivity, 100%-specificity and perfect agreement (κ coefficient, 1.0).

Conclusion:

Our evaluation in the Central African Republic demonstrates the clinical implications of the accuracy and reliability of the CAP/CTM HIV-1 Qualitative assay for early diagnosis of HIV-1 in Central African children.

Keywords: Central Africa, Early infant diagnosis, Dried plasma spot, HIV-1, Roche Molecular Systems, Subtype.

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INTRODUCTION

Early antiretroviral therapy is formally recommended to reduce HIV disease progression and mortality in children born from HIV-infected mothers [1, 2] and this necessitates early infant diagnosis of HIV infection [3]. In high HIV epidemic burden and limited resource setting such as African countries, reliable and accurate early diagnosis of HIV-1 infection of infants with a single screening PCR is therefore crucial for assessing the HIV status of a children as antibody testing cannot confirm HIV-1 infection in children less than 18 months of age [3]. High sensitivity (at least 95%) of early HIV molecular diagnosis is essential, as false negative HIV screening will cause delayed treatment of infected babies, possibly leading to death [2].

HIV genetic variability is an important factor in molecular detection and quantification of HIV [4 - 6]. Several commercially available molecular techniques were developed based on subtype B of HIV-1, which represents only 10% of HIV strains worldwide [7]. Indeed, in sub-Saharan Africa, non-B subtypes of HIV-1 are largely dominant [8]. As a consequence, certain viral variants could be missed or under quantified by HIV molecular assays [4].

The COBAS® AmpliPrep/COBAS® TaqMan (“CAP/CTM”) HIV-1 Qualitative test (Roche Molecular Systems, Inc., Branchburg, NJ, USA) is a total nucleic acid real-time polymerase chain reaction (PCR) assay that simultaneously amplifies HIV-1 proviral DNA as well as cDNA reverse-transcribed from HIV-1 RNA using a thermostable recombinant *Thermus species* Z05 DNA polymerase in a real-time format. It allows the detection of HIV-1 RNA and proviral DNA in plasma, anticoagulated fresh whole blood, dried blood spots (DBS) and dried plasma spots (DPS). The assay includes an internal control consisting of armored RNA containing binding sites identical to the HIV-1 target sequence but a unique probe-binding region. The internal control is added to each specimen prior to extraction at a defined concentration. It controls for both the extraction and amplification processes, including variable input and suboptimal amplification through inhibition. Fluorescence readings for the internal control need to conform to pre-set criteria for the specimen result to be regarded as valid. In addition to the internal control, the assay includes external controls in each run, namely a low positive control (to detect inadequate sensitivity) and a negative control (to detect contamination). The automation of CAP/CTM allows reducing technical user intervention and improving the specimen throughput.

The first version (v1.0) of CAP/CTM HIV-1 Qualitative test used a single-target strategy with *gag* primers and FAM-labeled *gag* probe (package insert). Maritz *et al.*, have reported in South Africa, where the majority of HIV-1 infections are subtype C, that the CAP/CTM HIV-1 qualitative assay v1.0 had a sensitivity and positive predictive value of 98.8% and 99.9%, respectively, but also pointed the possibility of discrepant results with gold standard reference molecular assays [9, 10]. In 2014, the second version (v2.0) of CAP/CTM HIV-1 Qualitative test was updated by Roche Molecular Systems, by using a dual-target strategy with *gag* and *LTR* primers and FAM-labeled *gag* and *LTR* probes, similarly as the CAP/CTM HIV-1 Quantitative test, version 2.0, used for HIV-1 RNA quantitation [11].

Recently, the performance of the CAP/CTM HIV-1 Qualitative test v2.0 was evaluated using DPS of infants from Senegal and Guinea Bissau, in a context with a genetic diversity of West Africa where HIV-1 group M CRF02_AG is highly prevalent [12]. The aim of this study was to evaluate the performances of the CAP/CTM HIV-1 Qualitative assays to detect the broad HIV-1 variants circulating in Central Africa against the outgoing CAP/CTM HIV-1 Quantitative test v2.0 (Roche Molecular Systems), chosen as reference gold standard molecular assay.

MATERIALS AND METHODS

Study Population

Blood samples were prospectively collected during a three-year period (2007-2010) from 133 children (age range, 8 weeks-17 months) born to HIV-1-infected mothers, followed up and treated with antiretroviral drugs at the Complexe Pédiatrique, the principal HIV pediatric clinic in Bangui, Central African Republic. HIV-1 RNA viral load in these children was detectable using a generic G2 real-time PCR assay for HIV1 RNA quantification assay (Biocentric, Bandol, France) on an Applied Biosystems 7500 real-time PCR system (threshold of detection > 300 copies/ml) [13]. Plasma was separated, aliquoted and kept frozen at -80°C. 106 additional HIV-1-negative plasma samples were also collected from children (age range, 10 weeks-24 months) followed up at the Complexe Pédiatrique, and aliquots were kept frozen at -80°C. One frozen plasma aliquot from each HIV-infected and HIV-negative child was transported to the virology laboratory of the Hôpital Européen Georges Pompidou, Paris, France, for further analyses.

Ethics Statement

This study was formally approved by the Scientific Committee of the Faculté des Sciences de la Santé (“FACSS”) of Bangui (so-called “Comité Scientifique Chargé de la Validation des Protocoles d’Etudes et des Résultats”/“CSCVPER”), reference #2UB/FACSS/CSCVPER/05, constituting the National Ethical Committee. Informed written consent was obtained from all mothers for themselves and on behalf of their respective children participating in the study.

HIV-1 RNA Quantification

Plasma HIV-1 viral loads were quantified by CAP/CTM HIV-1 Quantitative test v2.0 (Roche Molecular Systems), chosen as reference molecular assay for HIV-1 RNA load. We checked that all HIV-positive plasma showed detectable HIV RNA load by the CAP/CTM HIV-1 Quantitative test v2.0 (median, 2,501 copies/ml; range, 351-876,456).

To approach the field situation in sub-Saharan Africa, where the use of DBS has become a good alternative to venous blood samples [14, 15], DPS were constituted on Whatman 903 filter paper (Schleicher & Schuell, Whatman, Versailles, France), as previously described [14, 15]. Each pre-printed circle was completely filled by 50 µl of plasma. After air-drying for 3 hours at room temperature, the Whatman filter papers were stored in plastic bags with silica desiccants and humidity indicator cards. The bags were kept at -20°C until the testing. Two DPS cards were prepared for each patient. The test was performed according to the manufacturer's instructions. Briefly, a 12 mm punch of each DPS card was incubated with specimen extraction reagent (Specimen Pre-Extraction Reagent, SPEX), as lysis buffer, at 56°C and at 1000 rpm during 10 minutes on a thermomixer; then samples were loaded into the CAP for automated HIV-1 DNA extraction, with further step of real-time amplification-detection carried out on the CTM analyser 48. The same DPS cards were tested in two successive periods: in 2013 first by CAP/CTM HIV-1 Qualitative test v1.0 (Roche Molecular Systems) and in 2014 by the CAP/CTM HIV-1 Qualitative test v2.0 (Roche Molecular Systems).

Frozen plasma specimens for each of the children were separately used to generate protease and reverse transcriptase HIV-1 *pol* sequences with the commercial assay ViroSeq HIV-1 Genotyping System v2.0 (Abbott Park, Illinois, USA) using the Genotyping software from National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/projects/genotyping/>).

Statistical Analysis

Sensitivity (Se) was calculated as the number of real positives divided by the sum of real positives plus false negatives. Specificity (Sp) was calculated as the number of real negatives divided by the sum of real negatives plus false positives. HIV-1 prevalence in children less than 18 months in the Central African Republic may be estimated between 0.7 to 1.1%, as previously reported [16 - 18], and the lower and higher estimations of HIV-1 prevalence in children less than 18 months were used in calculating positive predictive value (PPV) and negative predictive value (NPV) according to Bayes' formulae, as follows: $PPV = \text{sensitivity} \times \text{prevalence} / [\text{sensitivity} \times \text{prevalence} + (1 - \text{specificity}) \times (1 - \text{prevalence})]$, and $NPV = \text{specificity} \times (1 - \text{prevalence}) / [(1 - \text{sensitivity}) \times \text{prevalence} + \text{specificity} \times (1 - \text{prevalence})]$.

The confidence interval for each variable was calculated at 95% (95%CI) using a normal distribution. The 95%CI of the estimated sensitivities, specificities, PPV, and NPV were calculated using the formula: $f \pm 1.96 [f(1-f)/n]^{1/2}$, where *f* is the sensitivity, the specificity, PPV, or NPV and *n* is the number of specimens tested.

The Cohen's coefficient κ was interpreted according to the Landis and Koch scale (< 0 as indicating no agreement and 0-0.20 as slight, 0.21-0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1 as near perfect agreement) [19].

RESULTS

The Roche CAP/CTM HIV-1 Quantitative test v2.0 was used as the reference to evaluate the performances of both Roche CAP/CTM HIV-1 Qualitative assays v1.0 and v2.0, including their Se, Sp, PPV and NPV. For clinical significance of usage the Roche CAP/CTM HIV-1 Qualitative assays results on children diagnosis, the efficiency of these assays to accurately discriminate between positive and negative DPS was estimated by the Cohen's κ coefficient. The Cohen's κ corresponds to the number of negative and positive DPS correctly detected by the Roche CAP/CTM HIV-1 qualitative assay, divided by the total number of positive and negative samples detected plus the false results, multiplied by 100 [20].

Among 133 HIV-1-positive DPS, all samples except 9 (93.2%) were positive by Roche CAP/CTM HIV-1 Qualitative Test v1.0, and all (100%) by CAP/CTM HIV-1 Qualitative test v2.0. Among HIV-1-negative DPS, all samples were negative by both Roche CAP/CTM HIV-1 Qualitative test versions v1.0 and v2.0. The calculations of the Se, Sp, PPV and NPV between the Roche CAP/CTM HIV-1 Qualitative assays v1.0 and v2.0 by reference to the Roche CAP/CTM HIV-1 Quantitative test v2.0 are depicted in Table 1.

Sensitivity of the CAP/CTM HIV-1 Qualitative test v1.0 was only 93.2%, compared with 100% for the CAP/CTM HIV-1 Qualitative test v2.0. The 9 DPS from HIV-1-infected children that were negative by the CAP/CTM HIV-1 Qualitative Test v1.0 had plasma HIV-1 RNA loads that ranged from 1,433 to 61,326 copies/ml (Table 2). All children with discordant results between CAP/CTM HIV-1 Qualitative test v1.0 and CAP/CTM HIV-1 Qualitative test v2.0 showed HIV-1 RNA load above 1,000 copies/ml (the threshold of therapeutic failure according to the 2013-revised WHO recommendations). HIV-1 subtypes were determined from genotyping results from protease and reverse transcriptase *pol* genes and indicated that the children in this study showed broad genetic diversity of HIV-1 group M [CRF11 (35%), CRF01_AE (19%), A1 (15%), G (8%), CRF02_AG (8%), D (3%), H (3%), CRF13 (3%), F1 (2%), CRF15 (2%), B (1%), C (1%)] (data not shown). HIV-1 subtypes of the 9 discordant samples are also shown in Table 2, with no dominant subtype among the group. We also examined the fluorescent measurement and the cycle threshold (Ct) values (as reported by the AmpliLink® version 3.3.1 software) of CAP/CTM results between samples with concordant positive results between the qualitative and quantitative assays, to those with discordant results. Samples with negative results in the CAP/CTM HIV-1 Qualitative test v1.0 had similar values for absolute fluorescent index and Ct value to true negative sample.

Table 1. Sensitivities, specificities, positive predictive values, negative predictive values and agreements between Qualitative and Quantitative CAP/CTM HIV tests (Roche Molecular Systems).

	N	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV	PPV	NPV	k
								at estimated prevalence=0.7%		at estimated prevalence=1.1%		
Qualitative test v1.0	239	124	106	0	9	93.2% [88.9-97.0]*	100.0% [97.3-100.0]*	100.0% [97.3-100.0]*	100.0% [99.5-100.0]*	100.0% [97.3-100.0]*	99.9% [99.4-100.0]*	0.92
Qualitative test v2.0	239	133	106	0	0	100.0% [97.3-100.0]	100.0% [97.3-100.0]	100.0% [97.3-100.0]	100.0% [97.3-100.0]	100.0% [97.3-100.0]	100.0% [97.3-100.0]	1.00

TP: True positive according to CAP/CTM HIV-1 Quantitative test v2.0; TN: True negative according to CAP/CTM HIV-1 Quantitative test v2.0; FP: False positive; FN: False negative; PPV: Positive predictive values; NPV: Negative predictive values.

*Confidence interval.

The concordances between the results on DPS of the CAP/CTM HIV-1 Qualitative test v1.0 and v2.0 and those of the gold standard HIV-1 detection by molecular testing using Roche CAP/CTM HIV-1 Quantitative test v2.0 was assessed by calculating the Cohen's κ coefficient. For both CAP/CTM HIV-1 Qualitative assays the concordance between qualitative and reference assays was almost perfect, that of the v2.0 of the CAP/CTM HIV-1 Qualitative test being the highest, demonstrating perfect agreement.

Table 2. Characteristics of the 9 HIV-1-infected children undetectable by the CAP/CTM HIV-1 Qualitative test v1.0 (Roche Molecular Systems).

Patient	Age (months)	Sex	Antiretroviral treatment	HIV-1 subtype	Viral load (copies/ml)	CD4 count (/mm ³)
#A	9	M	AZT+d4T+NVP	CFR01	1,641	854
#B	8	M	AZT+3TC+EFV	CRF15	14,609	376
#C	13	M	AZT+3TC+EFV	CFR11	61,326	763
#D	17	F	AZT+d4T+NVP	CRF01	20,451	379
#E	16	F	AZT+d4T+NVP	D	18,934	63
#F	9	F	d4T+3TC+EFV	CFR01	11,091	1,118
#G	14	F	AZT+d4T+NVP	CRF11	1,433	821
#H	7	M	AZT+d4T+NVP	CRF11	3,860	621
#I	10	M	AZT+3TC+NVP	CRF11	19,542	442

AZT: Zidovudine; EFV: Efavirenz; d4T: Stavudine; NVP: Nevirapine; 3TC: Lamivudine M: Male; F: Female

DISCUSSION

In the present study, the CAP/CTM HIV-1 Qualitative assays versions 1.0 and 2.0 were evaluated compared to CAP/CTM HIV-1 Quantitative test v2.0 on 239 DPS samples from 133 HIV-1-infected (with detectable plasma HIV RNA load) and 106 non-infected Central African children. The CAP/CTM HIV-1 Qualitative test v2.0 is the upgraded version of the CAP/CTM HIV-1 test for HIV-1 load quantification, using a dual-target strategy with *gag* primers and FAM-labeled *gag* probe, in addition with *LTR* primers and FAM-labeled *LTR* probe. This quantitative assay has been previously fully validated [11, 21], and was chosen as reference gold standard molecular assay.

The CAP/CTM HIV-1 Qualitative test v1.0 showed lower sensitivity (93.2%), with 9 (6.8%) false negatives within HIV-positive DPS and multiple circulating recombinant forms and one subtype were under-detected by the v1.0 assay. These findings clearly demonstrate that the CAP/CTM HIV-1 Qualitative assay v1.0 under-detects non-B subtypes of HIV-1 strains circulating in Central Africa. It is well documented that HIV genetic diversity can influence plasma HIV-1 detection and quantification by nucleic acid amplification tests in patients infected by non-B subtypes [4, 6]. Several authors have reported the failure of commercial assays for viral load monitoring in patients infected by non-B subtypes [22 - 24]. The HIV epidemic in the Central African Republic is characterized by broad genetic diversity included mainly non-B subtypes or circulating recombinant forms of HIV-1 group M [4]. The under-detection of non-B subtypes of HIV-1 likely occurs because the target of the CAP/CTM HIV-1 Qualitative test v1.0 is limited to *gag* gene of subtype B HIV-1. In contrast, the upgraded CAP/CTM HIV-1 Qualitative test v2.0 showed 100%-sensitivity, 100% specificity, and perfect agreement (κ coefficient, 1.0). This observation demonstrates that the upgraded version of the CAP/CTM HIV-1 Qualitative test, which now uses a dual-target strategy with *gag* and *LTR* primers and probes, performs as well as the CAP/CTM HIV-1 Quantitative test v2.0 [11], whose initial version underquantified several non-B subtype of v2.0 HIV-1 group M [11, 25]. Our results are in keeping with the performance of the CAP/CTM HIV-1 Qualitative test v2.0 on DBS of infants from Senegal and Guinea Bissau [12].

CONCLUSION

Taken together, our evaluation in the Central African Republic, a country of broad HIV-1 genetic diversity, demonstrates that the upgraded version of the CAP/CTM HIV-1 Qualitative assay is appropriate for routine use for early diagnosis of HIV in children by molecular tool. The upgraded assay may be recommended for early children diagnosis in Central Africa, especially in reference laboratory using automation and with high throughput activity.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The study was supported by GIP-ESTHER (Ensemble pour une Solidarité Thérapeutique Hospitalière En Réseau) France – Central African Republic. We are thankful to Miss Rosine Feissona for excellent technical assistance. Dr MA Jenabian is the holder of the Canada Research Chair in Immuno-virology Tier 2.

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