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Next-generation point-of-care testing in pediatric human immunodeficiency virus infection facilitates diagnosis and monitoring of treatment

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Abstract

Point-of-care (PoC) testing facilitates early infant diagnosis (EID) and treatment initiation, which improves outcome. We present a field evaluation of a new PoC test (Cepheid Xpert® HIV-1 Qual XC RUO) to determine whether this test improves EID and assists the management of children living with human immunodeficiency virus (HIV) infection.

We compared 2 PoC tests with the standard-of-care (SoC) test used to detect HIV infection from dry blood spots in newborn infants at high risk of in utero infection. We also evaluated the ability of the PoC tests to detect HIV total nucleic acid (TNA) in children living with HIV infection who had maintained undetectable plasma viremia following very early combination antiretroviral therapy (cART) initiation.

Qualitative (Qual) detection of HIV using the Xpert® HIV-1 Qual XC RUO ("RUO") and Xpert® HIV-1 Qual ("Qual") PoC tests was compared in 224 infants with the SoC DBS Roche COBAS® HIV-1/HIV-2 qualitative test. The same 2 PoC tests were also evaluated in 35 older children who had initiated cART before 21 days of age and maintained undetectable plasma viremia for a mean of 25 months.

No discrepancies were observed in detection of HIV infection via the 2 PoC tests or the SoC test in the 224 neonates studied, but only 95% of the SoC test results were generated compared with 100% of the PoC test results ($P = .0009$). The cycle threshold values for the research use only (RUO) assay were the lowest of the 3 assays ($P < .0001$ in each case). In 6 of the 35 early-treated aviremic children, HIV TNA was detected by RUO but not Qual.

The RUO assay outperforms Qual in detecting HIV-1 infection. RUO would therefore potentially improve EID and assist in identifying cART-adherent early-treated children with the lowest HIV TNA levels and the highest HIV cure potential.

Abbreviations: ART = combination antiretroviral therapy, Ct = cycle threshold, DBS = dry blood spot, EID = early infant diagnosis, HIV = human immunodeficiency virus, PBMC = peripheral blood mononuclear cell, PoC = point-of-care, Qual = qualitative, RUO = research use only, SoC = standard of care, TNA = total nucleic acid.

Keywords: adherence, HIV diagnosis and monitoring, human immunodeficiency virus, pediatric HIV, pediatric infectious disease, point-of-care testing, South Africa

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The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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1. Introduction

Early infant diagnosis of human immunodeficiency virus (HIV) and initiation of combination antiretroviral therapy (cART) improve outcomes.[1] In South Africa, the standard of care (SoC) for HIV-exposed infants is the collection of a dried blood spot (DBS) sample in the delivery room, which is sent to the National Health Laboratory System for Roche COBAS® HIV-1/HIV-2 qualitative testing. The results are then sent to the local clinic for dissemination to the caregiver on the baby's first clinic visit at 1 week of age. However, many things can go wrong during this process. Point-of-care testing (PoC) is an alternative approach that has been effective in early infant diagnosis and initiating treatment within hours after birth.[1–5]

A second major challenge to the successful management of children living with HIV is cART adherence.[5] However, it can be difficult to determine levels of cART adherence for the period between clinic visits because plasma aviremia observed at clinic visits reflects recent adherence only. In the current study, undertaken at KwaZulu-Natal South Africa, we sought to evaluate a next-generation PoC test, which simultaneously detects 2 regions of the virus as opposed to 1, to determine whether this facilitated early diagnosis of intrauterine infection in babies born to HIV-infected mothers compared with current PoC and SoC tests and whether it could assist in the longer-term management of early cART-treated children living with HIV.

2. Methods

2.1. Study subjects

The study was undertaken at the Queen Nandi Regional Hospital, Empangeni, South Africa, between September 2020 and April 2021. Two hundred ten newborn babies with a high risk (defined according to South African National Guidelines[6]) of in utero HIV infection were enrolled for PoC testing within 48 hours of birth. An additional 14 infants who had previously tested positive or indeterminate for HIV infection on the SoC DBS test were enrolled for confirmatory testing within 21 days of birth. To evaluate the use of PoC tests in children living with HIV on cART, we studied 35 children in the Ucwaningo Lwabantwana cohort in whom cART had been initiated at a median of 1 day of age.[5] Participants were enrolled in the study with legal guardian-informed consent. The study was approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee.

2.2. Sample testing

The 2 qualitative POC tests used were the Cepheid Xpert HIV-1 Qual Assay ("Qual") and the next-generation Xpert HIV-1 Qual XC RUO Assay ("RUO") using 100 µL capillary blood, both performed on the GeneXpert® Edge and the GeneXpert® 4-module Instrument systems.[7] The SoC dried blood spot sample was analyzed using the Roche COBAS® HIV-1/HIV-2 qualitative test, performed on the Cobas 6800/8800 systems.[8] Plasma RNA viral load (VL) was measured by Nuclisens EasyQ v2.0 HIV-1 RNA PCR (bioMérieux, Marcy l'Etoile, France), with a limit of detection of 20 HIV RNA copies/mL plasma. HIV DNA loads were measured by droplet digital polymerase chain reaction (BioRad, Hercules, CA) as previously described.[9]

2.3. Statistical analysis

Two-group continuous variables were compared using the *t* test (parametric) or the Mann–Whitney *U* test (nonparametric data). A 2-sided Fisher exact test was used to assess the difference in dichotomous variables.

3. Results

Evaluation of PoC and SoC tests in HIV-exposed newborns 224 infants born to HIV-infected mothers were tested for HIV infection using 2 PoC tests[7] (Fig. 1A), HIV-1 Qual ("Qual") and HIV-1 Qual XC RUO ("RUO"), in addition to the SoC birth DBS (dried blood spot) Cobas HIV-1/HIV-2 test[8] (Fig. 1). This is the first peer-reviewed evaluation of the Xpert® HIV-1 Qual XC RUO ("RUO") PoC test. The infants studied comprised 210 newborn babies tested at birth via SoC and at 20 hours of age (median; interquartile range, 13–28 hours) via PoC, and an additional 14 neonates aged 9.5 days (median; interquartile range, 7.0–12.3) who had tested positive or indeterminate on the birth DBS test.

Of the 210 newborns, 194 were negative on all 3 tests and 6 were positive on all 3. No result available from the SoC DBS test for the remaining 10 cases, due to "sample not found" (n = 4), "unsuitable specimen" (n = 2), "specimen not received" (n = 2), or "information does not match" (n = 2). In all 10 instances, these tested negative on both PoC assays. For the 14 infants whose birth SoC DBS test had given a positive (n = 10) or indeterminate result (n = 4), in confirmatory testing, 12 were positive and 2 were negative by all 3 tests, excluding 1 not tested using the SoC DBS assay because the sample had been mislaid. Thus, although there were no discrepant results, only 95% (213/224) of SoC results were generated compared with 100% of PoC test results ($P = .0009$).

Examining further the 3 assays that all detected infection in the 6 infants tested in the first 48 hours of life, the Ct values for the RUO PoC test were significantly lower than those for the other 2 tests ($P = .002$ and $P = .02$ for RUO versus Qual and versus DBS, respectively; Fig. 1B). Similarly, in the 12 infants whose confirmatory test was positive by all 3 assays, using samples obtained at exactly the same time point, Ct values for the RUO PoC test were again significantly lower than for the other 2 tests ($P < .0001$ in each case; Fig. 1C).

3.1. Evaluation of PoC testing in early ART-treated HIV-infected children

Maintaining cART adherence is a second major challenge in the management of HIV-infected children. However, measurement of plasma RNA VL at clinic visits only provides a snapshot of recent cART adherence. We hypothesized that longitudinal tracking via PoC of changes in HIV total nucleic acid (TNA) levels from 1 clinic visit to the next testing might provide additional insights into cART adherence between clinic visits. We evaluated 35 children with previously documented in utero infection whom we have followed from birth.[5] In 22 cases, HIV TNA was detected by both PoC assays, again with lower Ct values for the RUO test (Fig. 2A; $P < .0001$). In 4 cases, HIV TNA was detected by the RUO but not by the Qual PoC test, and in 9 children, HIV TNA was not detected by either PoC test.

To investigate further factors contributing to persisting detection of HIV TNA on RUO PoC testing, we first excluded 8 children who had recurrent (>2) viral blips (plasma VL >20 copies/mL but <1000 copies/mL) or >1 viral spike (plasma VL >1000 copies/mL) since achieving an undetectable plasma RNA VL on cART. We then compared children with detectable HIV TNA (n = 18) against those with undetectable HIV TNA (n = 9) on the RUO test. There was no significant difference between the groups in age at cART initiation (mean 7.8 versus 9.6 days, $P = .52$), age at which an undetectable plasma RNA VL was first achieved (median 2 versus 3 months, $P = .09$), duration of undetectable plasma RNA VL (mean 25.4 versus 24.6 months, $P = .87$), age at PoC testing (mean 27.2 versus 26.3 months, $P = .87$), or sex ($P = .23$). However, the groups differed significantly in baseline DNA VL (median 23 versus 1185 HIV DNA copies/million peripheral blood mononuclear cells, $P = .0003$;

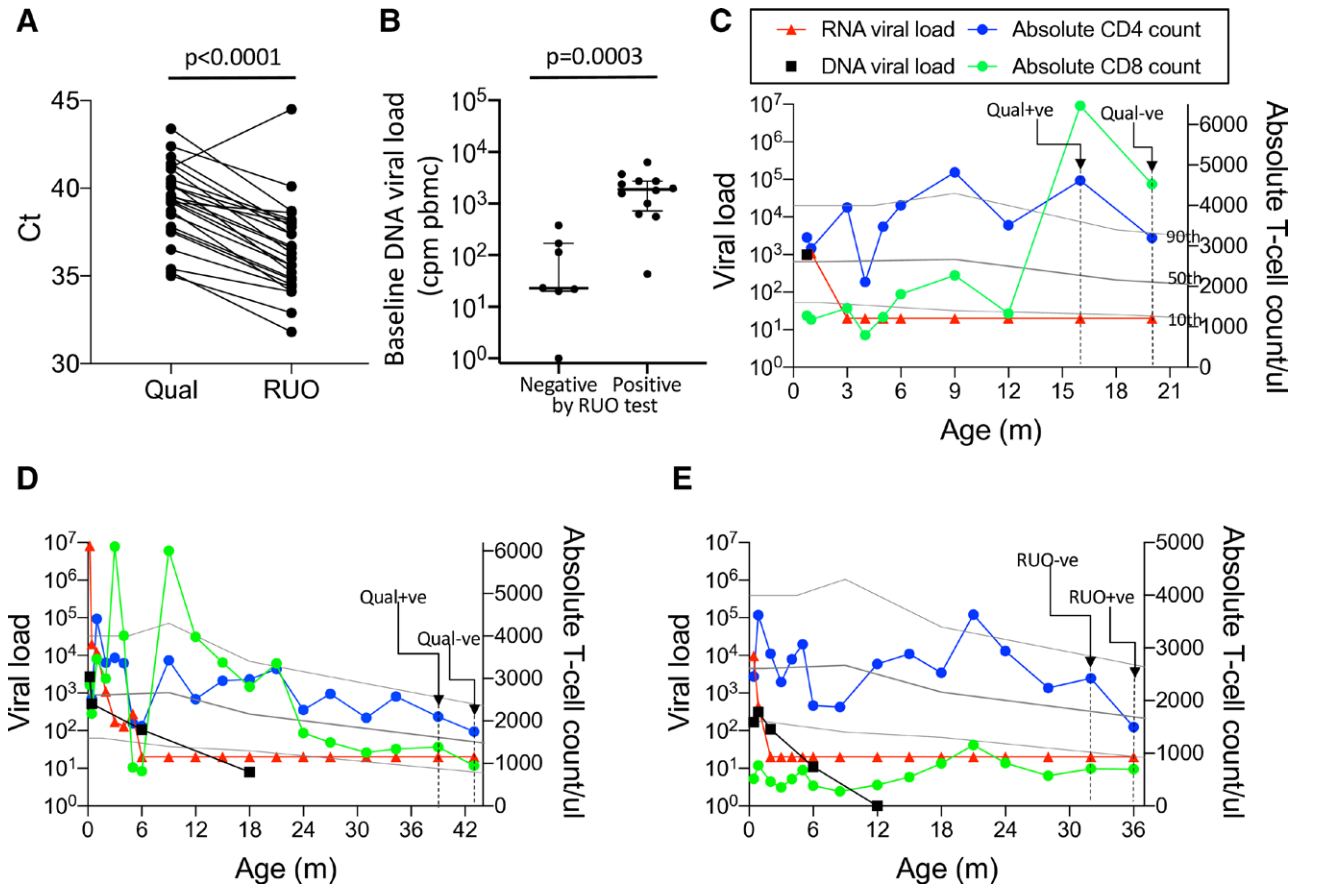

Figure 1. Comparison of 2 PoC assays with the SoC dried blood spot assay for early infant diagnosis of HIV infection. (A) Study design. Not done*: the reasons why these tests were not done are detailed in the text. (B) Ct values for the 6 infants of 210 infants tested at birth (SoC) or at a median of 20 h of age who tested positive for the 2 PoC assays, Xpert® HIV-1 Qual ("Qual"), Xpert® HIV-1 Qual XC RUO ("RUO"), and the SoC dried blood spot DBS Cobas HIV-1/HIV-2 qualitative test. (C) Ct values for the 12 children who had previously tested positive or indeterminate on the birth DBS assay and who now tested positive at a median of 9.5 days on all 3 assays. In the case of the DBS assay, only 11 of these 12 infants were tested. Ct = cycle threshold, DBS = dry blood spot, HIV = human immunodeficiency virus, PoC = point of care, RUO = research use only, SoC = standard of care.

Fig. 2B). However, contemporaneous samples were not available for measurement of DNA VL at the time points of PoC testing.

Next, in a subset of 6 children, PoC testing was undertaken at 2 consecutive clinic visits, 4 months apart. In each case, plasma RNA VLs were <20 copies/mL at both time points. In 2 of the children, HIV TNA was detected by both PoC tests at the first time point but, while remaining detectable on the RUO test, had become undetectable on the Qual PoC test at the second time point. The correspondingly increased Ct values on the RUO PoC test (from 38.0 to 40.1 and from 36.7 to 38.7, respectively) were consistent with decreased viral TNA (Fig. 2C and D). In a third case, viral TNA was undetectable by both tests at the first time point and detected by the RUO test only at the second time point (Fig. 2E). Although this child remained aviremic, there was a fall in the CD4 count to between the 10th and 50th centile for age, consistent with an increase in viral TNA, suggesting imperfect cART adherence in this case.

4. Discussion

Two major challenges to managing HIV-infected children are, first, early diagnosis and treatment initiation and, second, maintaining viral suppression. We show here that, by comparing Ct values, the next-generation PoC test, "RUO", has lower Ct values than its predecessor, "Qual", and the SoC DBS test. Although these lower Ct values did not translate into any discrepant diagnoses between the 3 assays in testing 224 infants at high risk of in utero infection, we postulate that this finding will be an advantage when evaluating infants on antiretroviral prophylaxis. In only 95% of cases tested via SoC was the result generated. Although this might seem a small difference, in a country such as South Africa, where >300,000 infants are born each year to mothers living with HIV, even with an in utero transmission rate as low as 0.5%, 5% of infants not being tested corresponds to >75 infected babies being missed each year. Furthermore, the higher sensitivity of the RUO test compared to SoC DBS testing would, over a large number of HIV-exposed infants, translate into early infant diagnosis in more infants still. Finally, our evaluation of PoC testing to assist in the longer-term management of early-treated children suggested that changes in HIV TNA detection via PoC testing from 1 clinic visit to the next may provide a more faithful reflection of cART adherence over that time than a single measurement of plasma viremia. The small sample size studied is a limitation of the current study and further studies in larger numbers of infants are warranted.

Previous studies have demonstrated improvements in outcomes from PoC testing compared to conventional testing.[1–4] The proportion of valid SoC results reaching the caregiver is reportedly as low as 53%.[2] PoC approaches have an added advantage in that the confirmatory SoC test is normally done 7 to 10 days later, when the result may be falsely negative in cases where prophylactic ART initiated at birth reduces HIV TNA levels to undetectable.[12] PoC testing avoids this issue because the confirmatory test can be undertaken immediately.

PoC testing has not so far been evaluated as an approach to assist in the management of children living with HIV. In the

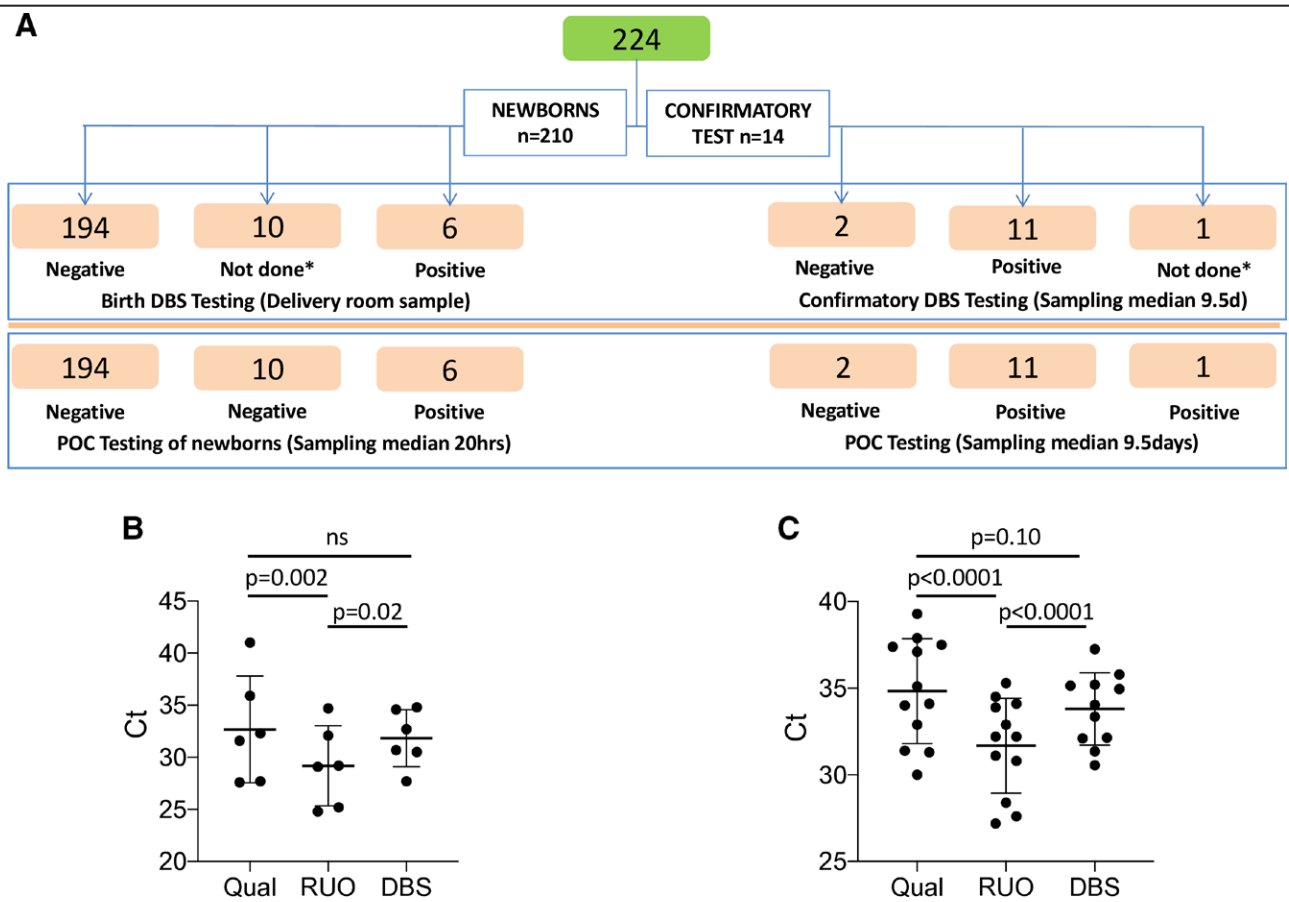

Figure 2. Evaluation of PoC testing in early ART-treated children maintaining aviremia for a mean of >24 mo. (A) Ct values for the 2 PoC assays, Xpert® HIV-1 Qual ("Qual"), Xpert® HIV-1 Qual XC RUO ("RUO"), in 22 children who tested positive for both assays. (B) Initial (baseline) DNA viral loads (cpm pbmc: copies per million peripheral blood mononuclear cells) in children who tested positive for the Xpert® HIV-1 Qual XC RUO assay ("positive by RUO test") versus those who tested negative on the Xpert® HIV-1 Qual XC RUO assay ("positive by RUO test"). (C–E) Three children tested using both PoC assays at consecutive clinic visits 4-mo apart. Gray lines indicate the normal-for-age 10th, 50th, and 90th centiles for absolute CD4 counts in HIV-uninfected children.[10,11] (C and D) Two children who tested positive on both tests at the first visit and then negative on the Qual test, but still positive on the RUO test, at the next visit. RUO Ct values increased from 38.0 to 40.1 and from 36.7 to 38.7, respectively, between the 2 clinic visits. (E) One child who tested negative on both tests on the first visit and then positive on the RUO test only at the next visit (Ct value 39.2). HIV = human immunodeficiency virus, PoC = point of care, Qual = qualitative, RUO = research use only, SoC = standard of care.

same way that, in diabetics, a blood glucose measurement gives a less valuable reflection of glycemic control over a 3-month period than hemoglobin A1c, plasma viremia in children living with HIV only gives a snapshot of cART adherence. CD4 counts give a hint of ongoing viral replication but are influenced by many other factors than HIV replication. HIV antibody status similarly reflects long-term adherence in early-treated children since HIV-infected children who have maintained suppression of viremia on cART do not develop antibodies against HIV and therefore become antibody negative once maternal antibody is lost.[13,14] An additional advantage of a PoC test here is that the implications of the test can be discussed with the mother at the clinic visit, and any issues surrounding cART adherence can be addressed immediately in the light of the PoC test results.

In future studies, it would be useful to compare directly PoC test Ct values with HIV DNA loads because the PoC assay is more convenient than the methods available to quantify total HIV DNA from peripheral blood mononuclear cells. The finding here that only children with low initial HIV DNA loads had undetectable total HIV DNA on the RUO PoC test undertaken after a mean of 24 to 25 months of aviremia is consistent with previous studies showing time to undetectability is longer if the starting HIV DNA load is higher.[9,14] However, an alternative hypothesis for future studies would be that PoC testing at 2 years, for example, might distinguish children with high potential for cure/remission via additional interventions such as broadly neutralizing antibody versus those whose viral reservoir is more refractory to cART.[15,16]

5. Conclusion

This study shows that the next-generation RUO PoC test outperforms the "Qual" and SoC assays in detecting HIV infection. The RUO test would likely improve health outcomes and may also assist in the management of early ART-treated children living with HIV.

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Author contributions

NB coordinated the clinical aspects of the study, analysed the data and wrote the manuscript. NM, SM, RF, GC, JvL, YG, CK, KC, RB, MA undertook the clinical management of the subjects, provided the clinical samples and contributed to writing the manuscript. EA, MCP, TN, and JMP undertook data

analysis and helped in writing the manuscript. PG conceptualised and led the study, analysed the data and assisted in writing the manuscript.

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