Utility of rapid antibody tests to exclude HIV-1 infection among infants and children aged <18 months in a low-resource setting

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ABSTRACT

Background: Excluding HIV infection among infants and young children in resource-poor settings where nucleic acid amplification tests (NAAT) are not routinely available remains a considerable challenge.

Objectives: To assess the performance of two rapid HIV antibody tests (RT) used alone and in parallel for excluding HIV infection among acutely ill infants and children <18 months in comparison to NAAT in a region where maternal HIV prevalence was approximately 7%.

Study Design: Infants and children $\geq 2 < 18$ months admitted to hospital with an acute febrile illness had two rapid antibody tests in parallel, with single and parallel results subsequently compared against NAAT.

Results: HIV prevalence among 1602 enrolled infants was 3.4%. All 1526 infants with 2 negative RT were HIV negative by NAAT. All 46 infants with 2 positive RT were HIV positive by NAAT. The overall specificity of two rapid tests for excluding HIV infection was 99.5%. Sensitivity and specificity were $\geq 99\%$ and $>98\%$, respectively, across all age brackets $\geq 2 < 18$ months. Overall sensitivity and specificity for a single RT was 98.2% and 99%, respectively, for Determine, and 85.5% and 99.6%, respectively, for Capillus.

Conclusions: In a setting with a maternal HIV prevalence rate of <10%, a single negative RT had excellent specificity and two negative RT performed in parallel had a perfect negative predictive value for HIV infection among acutely ill patients <18 months of age. In this and similar settings, RT could assist with excluding HIV infection with much lower complexity and cost than NAAT.

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1. Background

The ability to diagnose or exclude HIV infection in infants and young children is critical for both patient management and aversion of mortality. The diagnosis of HIV in infants and young children has conventionally relied on the use of nucleic acid amplification tests (NAAT). However, NAAT is still not widely available in low-resource countries. When NAAT is available, it is unlikely to provide results rapidly enough to influence acute care decisions. HIV rapid antibody tests (RT) could provide a solution for timely infant and young child diagnosis in areas without NAAT and for areas with prolonged NAAT turnaround times.

The presence of maternal HIV antibody in HIV-exposed, uninfected infants may produce false-positive results, though the majority will serorevert prior to 15 months of age.^{1–4} The sensitivity of RT may also be influenced by seronegativity during the conversion illness associated with HIV and thus may fail to detect early acute HIV infection.⁵ The performance of a single RT compared to parallel RT has not been well described under the age of 18 months. Presently, the World Health Organization recommends RT from as early as nine months to exclude infection in non-breastfeeding infants.⁶ In practice, many providers in sub-Saharan Africa remain reluctant to use RT for children <18 months of age.

2. Objectives

The aim of this study was to evaluate the performance of two HIV-1/2 RT performed alone and in parallel to exclude HIV

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infection among children aged <18 months, who presented to a rural district hospital in Tanzania with an acute febrile illness. The clinical presentation of infected infants and young children is also described.

3. Study design

3.1. Study design and subjects

The sample subset for the present study was taken from a larger observational study aimed at measuring invasive bacterial disease in an area with intense malaria transmission. The study was conducted in Teule Hospital, Muheza, Tanga Region, in north-east Tanzania, from June 2006 to May 2007. This district hospital serves a rural population of approximately 277,000 people. HIV seroprevalence among pregnant women in Muheza District in 2007 was 7.2%.⁷ Children ages >2 months to ≤13 years with a current fever or history of fever 48 h prior to admission were eligible for enrollment into the study. Full details are described elsewhere.⁸ For the present study, we tested all available archived specimens on infants/children <18 months of age who had been tested for HIV-1 by rapid test in parallel, but without a confirmatory NAAT ($n=1546$).

3.2. Laboratory procedures

As part of the initial study, infants and children had two rapid HIV-1/2 serum antibody tests performed at the time of study entry: Capillus (Trinity Biotech, County Wicklow, Ireland) and Determine (Abbott Laboratories, Abbott Park, IL, USA).⁹ RT were performed at the bedside by nurses, trained and supervised by the study senior medical team. Medical history, physical examination, and a blood culture were also obtained.⁸ Children under the age of 18 months with two positive RT had CD4+ T-lymphocyte count and percentage along with serum or plasma HIV-1 RNA measured by NAAT (Abbott Real-Time m2000 System, Abbott Molecular, Illinois, USA).^{10,11} Children with discordant RT had HIV-1 enzyme linked immunosorbent assay (ELISA) (Vironistika Uniform II Plus-O Test, bio-Mérieux, NC, USA), but no further testing if negative on ELISA. Children under the age of 18 months with two negative RT also had no further testing at the time of the initial study. The present study tested samples without prior NAAT from the original 2006 to 2007 study that had been in storage at -80°C for 4–5 years, which included all infants/children <18 months with concordant negative RT or discordant RT with negative ELISA. These samples ($n=1546$) were tested with HIV-1 RNA PCR by a pooled method, using 10 samples per well and 24–96 wells per run. Two positive and one negative control were used for each run. The assay platform participated successfully in the US National Institutes of Health Viral Quality Assessment program (VQA, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL, USA). If HIV-1 RNA was detected in a pool, all contributing samples were re-tested individually. HIV-1 RNA PCR testing was also repeated on a subset of the originally positive samples to confirm positivity after 4–5 years in storage. Finally, HIV antibody testing was repeated for samples with initially discordant RT results and undetectable HIV-1 RNA PCR. In March 2008, after the main study and before the present study, the Tanzania Ministry of Health HIV testing guidelines required replacement of the Capillus HIV-1/HIV-2 with the SD Bioline HIV-1/HIV-2 test (Standard Diagnostics Inc, South Korea). Consequently repeat rapid antibody testing on stored samples was done with the Determine and SD Bioline rapid diagnostic tests. HIV-infected was defined as a single positive HIV-1 RNA PCR >400 copies/ml; HIV uninfected was defined as a single negative undetectable HIV-1 RNA PCR.

Table 1

HIV rapid test (RT) results among children <18 months using Determine and Capillus RT, by age group, Teule Hospital, Tanzania.

	NAAT positive	NAAT negative
≥2 <6 months		
RT concordant positive	6	0
RT discordant	4	2
RT concordant neg	0	229
≥6 <9 months		
RT concordant positive	17	0
RT discordant	0	8
RT concordant negative	0	331
≥9 <12 months		
RT concordant positive	7	0
RT discordant	3	5
RT concordant negative	0	362
≥12 <18 months		
RT concordant positive	16	0
RT discordant	2	6
RT concordant negative	0	604

3.3. Data analysis

Data were first scanned into a Microsoft Access database using the Cardiff Teleform system (Cardiff, Vista, California, USA), and then initially analyzed in Stata 10.0 (StataCorp LP, College Station, TX, USA). The present analysis was done using Stata 11.0. Sensitivity and specificity of a single RT, concordant negative RT, discordant RT, and concordant positive RT was determined and compared against HIV-1 RNA PCR results. The test of medians was used to compare median HIV-1 RNA levels.

3.4. Research ethics

The study was approved by ethics committees of the Tanzania National Institute for Medical Research and the London School of Hygiene and Tropical Medicine, UK.

4. Results

A total of 1602 samples from children aged <18 months who had previously undergone RT with both Capillus and Determine were available. Results were grouped into the following age categories: ≥2 <6 months ($n=241$); ≥6 <9 months ($n=356$); ≥9 <12 months ($n=377$); ≥12 <18 months ($n=628$). Of the 1602 samples, 1526 (95.3%) had concordant negative RT results, and 46 (2.9%) had concordant positive RT results (Fig. 1). The 1526 concordant negative specimens were all negative on testing by NAAT (HIV-1 RNA PCR) performed after 4–5 years in storage. The 20 specimens with discordant RT and negative ELISA were also all NAAT-negative when tested for the present study. The 46 specimens with concordant positive RT were positive on NAAT confirmatory testing performed during the original study period. Twelve of the samples that were originally NAAT positive were retested in 2011, and all were still positive on retest.

There were 30 RT discordant samples, of which 9 (30%) were NAAT-positive and 21 (70%) were NAAT-negative. Of these 30, 23 (76.7%) were Determine positive and Capillus negative, and 7 (23.3%) were Determine negative and Capillus positive (Fig. 1). Among infants ≥2 <6 months, 6 had discordant results, 4 of whom were NAAT-positive. There were 8 discordant results among infants ≥6 <9 months, all of which were negative by NAAT. For infants ≥9 <12 months, there were also 8 discordant results, with 3 NAAT-positive. Finally, for children ≥12 <18 months, there were also 8 discordant results, with only 2 NAAT-positive (Table 1). On repeat RT of the 21 samples with discordant RT and negative NAAT, 7 (33.3%) were discordant using SD Bioline and Determine (Table 2). Thus, of 1547 patients aged <18 months with negative NAAT, 7

Fig. 1. Flowchart of study population included in the analysis: children <18 months with two rapid antibody tests (RT) performed in parallel followed by confirmatory nucleic acid amplification test (NAAT).

Table 2

HIV Rapid test (RT) results among children < 18 months after repeat RT with SD Bio-line on discordant negative samples ($n = 21$), by age group, Teule Hospital, Tanzania.

	NAAT positive	NAAT negative
$\geq 2 < 6$ months		
RT concordant positive	6	0
RT discordant	4	0
RT concordant neg	0	231
$\geq 6 < 9$ months		
RT concordant positive	17	0
RT discordant	0	4
RT concordant negative	0	335
$\geq 9 < 12$ months		
RT concordant positive	7	0
RT discordant	3	2
RT concordant negative	0	365
$\geq 12 < 18$ months		
RT concordant positive	16	0
RT discordant	2	1
RT concordant negative	0	609

(0.5%) had discordant RT (Table 2). Following repeat testing, discordant RT among infants/young children with negative NAAT decreased with increasing age group from 6 months onward (Fig. 2).

The negative predictive value (95% confidence intervals) for excluding HIV-infection using two RT in parallel was 100% (99.7–100%) for all patients $\geq 2 < 18$ months. The positive predictive value of one or more positive RT for correctly detecting HIV infection was 88.7% (78.5–94.4%) for all patients $\geq 2 < 18$ months. Specificity by age bracket ranged from 98.8 to 100%, and sensitivity,

from 99 to 100% (Table 3). Sensitivity and specificity for a single RT was 98.2% (90.4–99.7%) and 99% (98.4–99.4%), respectively, for Determine, and 85.5% (73.8–92.4%) and 99.6% (99.2–99.8%), respectively, for Capillus (Table 4).

Finally, there was a range of clinical presentations and laboratory values among the 55 infants/children with a positive NAAT in the study cohort (Table 5). The median (range) HIV-1 RNA level was 399,995 (684, 14,674,839) copies/mL. Eight (14.5%) had HIV-1 RNA levels $> 5 \times 10^6$ copies/mL. The median HIV-1 RNA level for those with concordant positive RT was 348,818 (684, 14,674,839) copies/mL, compared to 1,391,712 (15,495, 3,354,803) copies/mL for those with discordant RT, though this difference was not statistically significant ($p = 0.253$). Median (range) CD4+ T-lymphocyte absolute count for the 25 who had results was 618 (44, 3616) cells/mm³, and median (range) CD4+ T-lymphocyte percentage was 14 (3–30). Nine (16.4%) of the 55 were diagnosed with a bloodstream infection during their hospitalization. Thirty (54.5%) had a diagnosis of very severe or severe pneumonia as defined by the WHO.¹² Twelve (22%) died during hospitalization, 7 (58%) of whom died within 48 h of admission.

5. Discussion

In a cohort of pediatric patients < 18 months presenting to hospital with an acute febrile illness in Tanzania, a country with a generalized HIV epidemic, two negative RT performed in parallel had a negative predictive value for HIV infection of 100%, and a single negative RT had a negative predictive value of 99.9% (Determine) and 99.5% (Capillus). No infected infant had two negative RT; no uninfected infant had two positive RT; and discordant RT results occurred in <2% of those tested. Our analysis demonstrates the utility of RT for excluding HIV infection in the acutely ill infant or young child in this low-resource setting where rapid turnaround NAAT is unavailable.

We have assumed absence of knowledge regarding the HIV status of the mother as this was not documented in the study and in routine care, its presence or reliability cannot be assumed at the time of admission of a child with a febrile illness. However, it is a conservative assumption since the information on maternal status is now often available from testing in the antenatal or intrapartum period, and in addition, certain illnesses such as pneumonia or malnutrition increase the probability of HIV infection in

Fig. 2. Percentage of uninfected children with discordant rapid test (RT) by age, test.

Table 3

Sensitivity, specificity, positive predictive value (+PV), negative predictive value (–PV) with 95% confidence intervals (using ≥ 1 positive rapid test (RT) and 2 negative RT^a), Teule Hospital, Tanzania.

	Sensitivity (%)	Specificity (%)	+PV (%)	–PV (%)
All, $\geq 2 < 18$ months	100 [0.931–1]	99.5 [0.991–0.998]	88.7 [0.785–0.944]	100 [0.997–1]
$\geq 2 < 6$ months	99 [0.71–1]	100 [0.983–1]	99 [0.71–1]	100 [0.983–1]
$\geq 6 < 9$ months	99.4 [0.807–1]	98.8 [0.97–0.995]	81 [0.6–0.923]	100 [0.988–1]
$\geq 9 < 12$ months	99 [0.71–1]	99.5 [0.98–0.999]	83.3 [0.552–0.953]	100 [0.989–1]
$\geq 12 < 18$ months	99.4 [0.816–1]	99.8 [0.991–1]	94.7 [0.754–0.991]	100 [0.993–1]

^a 21 discordant samples with negative NAAT retested with SD Bioline/Determine; all others, Capillus/Determine.

infants. While our estimate of sensitivity and specificity are unaffected by pre-test probability, our estimates of positive predictive value or negative predictive value are likely to be unrealistically low and high, respectively, if the mother is known to be HIV-positive.

Our results are relevant to a number of different scenarios. First, for any high risk infant – whether born to an HIV-infected mother or with signs and symptoms suggestive of immunosuppression – a single test is obviously insufficient. Two RT with complementary qualities – either highly specific or sensitive – is recommended. For infected infants and children < 18 months in our study, we had no concordant negative RT results, and among those with two negative RT, not a single patient was HIV-infected. Discordant tests require NAAT, but in the acute care setting we are looking for reassurance for the mother and provider, or, confirmation of infection. In either scenario, a correct diagnostic test will avert illness and relieve anxiety.

A single negative test in a low risk infant, which also includes a setting of low population prevalence, is highly predictive for excluding HIV infection and is an acceptable screening option. Finally, a single positive test, even one with no suspicion of HIV infection or very low risk, should have a second test with a different RT. Close follow-up and NAAT will be needed in time.

While HIV NAAT is the preferred diagnostic method for infants and children aged < 18 months, its use is still not widespread. It is estimated that only 8% of HIV-exposed infants in resource-poor settings had access to a virological testing method in 2008.^{13,14} Even when available, turnaround times for NAAT may average 4 weeks or more, meaning the value of NAAT for acute care decision-making is low. An acutely ill child with two negative or even a single negative RT would allow clinicians to focus on diseases other than HIV; alternatively, a positive RT could inform the differential diagnosis to include conditions typically associated with immune suppression. The majority of HIV-infected children will die before their second birthday without access to antiretroviral therapy,¹³ and in this study most children that died with HIV did so within 48 h of admission, making timely identification of the HIV-infected infant or young child critical. HIV antibody testing using RT is simpler to perform, faster, and much cheaper than NAAT.¹⁵

There are important differences in RT with respect to sensitivity for early diagnosis of acute HIV infection in adults.^{16–18} Differences in the performance of RT among infants have also

been described.^{19,20} In our study, there were nine infected infants/children with discordant RT, and all but one was Capillus negative/Determine positive. False negative Capillus results among infected infants have been reported elsewhere, and have been shown to be associated with low HIV-1 viral load and concurrent antiretroviral therapy.²⁰ Furthermore, the Capillus assay is difficult to perform, using three different reagents and six different steps in the process which typically require a high level of laboratory skill.¹⁶ The Capillus assay is no longer part of Tanzania's national algorithm for HIV testing.²¹ The Determine assay has been shown to be a more sensitive assay compared to other RT for the detection of HIV exposure and seroreversion during infancy.¹⁹

The higher median HIV-1 RNA PCR levels among infected infants with discordant RT in our study compared to those infected with concordant RT is interesting. Whether this represents discordance due to acute HIV infection cannot be determined. Acute HIV infection, while well-described in adults,^{5,22,23} is less well-described among infants and young children. Adults with acute HIV infection have a burst of plasma viremia typically in the first several weeks after infection.^{5,24} In contrast to adults, vertically infected infants maintain high levels of virus (typically $> 10^5$ HIV-1 RNA copies/mL) for several months while typically preserving a normal CD4+ lymphocyte percentage for age.^{24–26} A child with acute HIV infection may have a false negative antibody test because the child has not yet mounted an antibody response to the virus²⁷; or, a child's primary production of antibody may be suppressed by the presence of maternal IgG antibody.²⁸ Given that the majority of infants in our study were breastfed, acute postnatally acquired HIV infection may explain some of the discordant results among infected infants.

Our study was limited by the fact that samples were in storage for up to five years before performing the majority of the NAAT testing, which could affect assay sensitivity. However, 12 previously NAAT-positive samples were retested after 4–5 years in storage and all were still positive. Furthermore, no test required repeating to show concordance. Another limitation is the relatively low prevalence of HIV infection in our population of 3.4%. Thus, while sensitivity was high across all age groups, confidence intervals are wide and positive predictive value is clearly affected by the low prevalence. We also did not include infants less than 2 months of age in the study and thus our results cannot be applied to this age group. Furthermore, we did not collect information regarding maternal or infant use of highly active antiretroviral therapy (HAART), though

Table 4

Sensitivity, specificity, positive predictive value (+PV), negative predictive value (–PV) with 95% confidence intervals for a single Capillus or Determine rapid test for infants and children $\geq 2 < 18$ months of age, Teule Hospital, Tanzania.

	Sensitivity (%)	Specificity (%)	+PV (%)	–PV (%)
Capillus	85.5 [0.738–0.924]	99.6 [0.992–0.998]	88.7 [0.774–0.947]	99.5 [0.99–0.997]
Determine	98.2 [0.904–0.997]	99 [0.984–0.994]	78.3 [0.672–0.864]	99.9 [0.996–1]

Table 5Select demographic characteristics of HIV-infected infants and children $\geq 2 < 18$ months of age, Teule Hospital, Tanzania.

Age Group (months)	Parallel RT ^a	ELISA	HIV-1 RNA PCR	Confirmatory HIV-1 RNA PCR ^b	CD4	CD4%	Death
0 < 6	CP	–	>10,000,000	–	338	13	Y
0 < 6	CP	–	473,074	–	562	7	N
0 < 6	CP	–	>10,000,000	>10,000,000	–	–	N
0 < 6	D	Pos	3,354,803	–	–	–	Y
0 < 6	D	–	1,364,430	–	–	–	Y
0 < 6	D	Pos	3,296,500	–	182	3	N
0 < 6	CP	–	5,087,096	–	–	–	N
0 < 6	CP	–	36,002	–	–	–	N
0 < 6	D	IND	1,440,593	–	375	14	N
0 < 6	CP	–	349,311	1,522,687	–	–	N
$\geq 6 < 9$	CP	–	9,937,669	–	470	9	N
$\geq 6 < 9$	CP	–	109,350	–	597	11	Y
$\geq 6 < 9$	CP	–	154,867	–	357	25	N
$\geq 6 < 9$	CP	–	795,686	–	702	8	N
$\geq 6 < 9$	CP	–	50,988	17,043	–	–	Y
$\geq 6 < 9$	CP	–	684	–	–	–	N
$\geq 6 < 9$	CP	–	633,117	–	–	–	N
$\geq 6 < 9$	CP	–	711,240	376,795	–	–	N
$\geq 6 < 9$	CP	–	14,674,839	8,089,390	–	–	N
$\geq 6 < 9$	CP	–	314,776	–	–	–	N
$\geq 6 < 9$	CP	–	>10,000,000	>10,000,000	–	–	Y
$\geq 6 < 9$	CP	–	8,309,771	–	1923	12	Y
$\geq 6 < 9$	CP	–	348,325	143,608	526	16	N
$\geq 6 < 9$	CP	–	8,102	–	–	–	N
$\geq 6 < 9$	CP	–	4,076,489	–	–	–	Y
$\geq 6 < 9$	CP	–	133,548	–	217	–	N
$\geq 6 < 9$	CP	–	63,360	–	–	–	Y
$\geq 9 < 12$	D	Pos	332,346	–	–	–	N
$\geq 9 < 12$	CP	–	78,250	–	723	9	Y
$\geq 9 < 12$	CP	–	>10,000,000	–	44	–	N
$\geq 9 < 12$	D	Pos	15,495	2643	–	–	N
$\geq 9 < 12$	D	Pos	56,486	–	–	–	N
$\geq 9 < 12$	CP	–	3710	–	–	–	N
$\geq 9 < 12$	CP	–	2,064,573	–	577	9	N
$\geq 9 < 12$	CP	–	4281	–	940	18	N
$\geq 9 < 12$	CP	–	146,091	–	1129	11	N
$\geq 9 < 12$	CP	–	341,303	–	–	–	N
$\geq 12 < 18$	CP	–	231,890	–	618	15	N
$\geq 12 < 18$	D	Pos	2,764,591	–	–	–	N
$\geq 12 < 18$	CP	–	206,501	–	1190	25	N
$\geq 12 < 18$	CP	–	4,695,058	–	1161	30	Y
$\geq 12 < 18$	D	Pos	1,391,712	–	352	7	N
$\geq 12 < 18$	CP	–	4,505,055	–	–	–	N
$\geq 12 < 18$	CP	–	819,483	–	1645	16	N
$\geq 12 < 18$	CP	–	213,027	–	908	17	N
$\geq 12 < 18$	CP	–	325,460	–	–	–	N
$\geq 12 < 18$	CP	–	217,347	61,415	–	–	N
$\geq 12 < 18$	CP	–	61,625	–	–	–	N
$\geq 12 < 18$	CP	–	650,468	244,639	–	–	N
$\geq 12 < 18$	CP	–	135,878	–	3616	30	Y
$\geq 12 < 18$	CP	–	289,509	–	940	18	N
$\geq 12 < 18$	CP	–	399,995	665,402	–	–	N
$\geq 12 < 18$	CP	–	4,484,289	3,966,528	–	–	N
$\geq 12 < 18$	CP	–	4,392,507	–	1806	28	N
$\geq 12 < 18$	CP	–	602,017	–	–	–	N

^a CP, concordant positive; D, discordant.^b Performed after 4–5 years in storage.

given the time period was five years ago, it is unlikely that many infants/children in this age range would have been diagnosed and on HAART. Finally, our study was conducted only in Tanzania, and the results may not be generalizable to other resource-poor settings, particularly where different HIV-1 subtypes may exist.

In summary, our findings suggest that a single RT may provide highly predictive results on the true HIV status of a low risk infant whose maternal status is unknown and that two RT used in parallel can provide highly predictive results in infants born to HIV positive mothers. While the utility of RT may not be surprising for children > 12 months of age, that RT performed well among younger age groups has important implications. In this and similar settings, RT can be used to reasonably exclude infection in the

acutely ill infant or young child until NAAT is widely and rapidly available.

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Competing interests

No conflicts of interest to declare.

Ethical approval

The study was approved by ethics committees of the Tanzania National Institute for Medical Research and the London School of Hygiene and Tropical Medicine, UK.

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