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# Current Trends in the detection of Acute HIV Infection among Blood Donors: Reliability of Pooled Nucleic Acid Amplification Technology and the Need for Population Specific Algorithms: A Systematic Review

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## Abstract

We conducted a systematic review of quantitative studies that evaluated the accuracy of nucleic acid amplification testing technologies (NAAT) compared their cost effectiveness and evaluated minipool NAAT against individual donor testing NAAT. PubMed, Cochrane and Google Scholar were used to identify relevant peer-reviewed journal articles published in English language between 1999 (when NAAT was introduced) and 2013. MeSH key words included: Human immunodeficiency virus OR HIV AND nucleic acid amplification techniques OR pooled NAAT AND blood donors. Additional filters include: minipool-NAAT and individual donor testing-NAAT. After screening for duplication and relevance, 50 out of 4,181 articles were selected.

Thirty six (36) studies which included 5 review article, 5 retrospective cohort studies, 20 cross sectional studies, 2 statistical modeling's, 2 national guidelines and 2 prospective cohort studies were further synthesized. Articles were further sub-divided into 8 groups based their focus area which includes: prevalence, clinical sensitivity of the assay, analytical sensitivity, test technology, testing algorithm, limit of detection and cost effectiveness.

Four of six studies with pool sizes of 10 to 50 donor plasmas with standard centrifugation recorded a clinical sensitivity of 100% while the remaining two whose plasma pool size of 96 and128 had sensitivities of 92.3% and 95.3%, respectively. All four studies that focused on analytical sensitivity using different samples and controls, including cadaveric samples reported 100% analytical sensitivity using various pool sizes. We recommend minipool NAAT testing after running a third generation ELISA as highly sensitive and cost effective algorithm for low income countries.

## Introduction

### Background

The prevalence of transfusion-transmissible infections (TTIs) in blood donations in developed countries is somewhat lower than in under-developed and developing nations. The prevalence of HIV in blood donations in high income countries is 0.003%, in comparison with 0.1% and 0.6% in middle and low income countries respectively [1]. This explains the difference in prevalence amongst eligible donors from these populations, the type of donors (such as voluntary non-renumurated blood donors from low risk groups) and the effectiveness of donor selection mechanisms [1]. The National prevalence of TTIs among Nigerian blood donors are 2.1%, 9.7%, and 2.5% for HIV, Hepatitis B and Hepatitis C respectively [2].

The window period (WP), a period when HIV virus cannot be detected using conventional diagnostic methods in an infected person, is very important in the diagnosis and epidemiology of this virus. The 1st generation HIV antibody ELISA was designed using viral lysate to detect anti-HIV-1 IgG antibodies in plasma or serum samples. The 2nd generation HIV antibody ELISA tests used recombinant antigens to detect anti-HIV-1 and HIV-2 IgG antibodies in plasma or serum samples. The 3rd generation HIV antibody ELISA tests increased the sensitivity of the assay by including the detection of anti-HIV IgM as well as IgG antibodies in serum or plasma samples and the 4th generation HIV antibody and p24 ELISA tests detect early or acute HIV infection by including detection of p24 antigen in plasma/serum samples. WHO recommends 4th generation ELISA (antigen/antibody combined or 3rd generation EIA, followed by p24 antigen separately) as the minimum standard for blood donor screening. Rapid test kits have less sensitivity compared to ELISAs particularly in the detection of acute HIV infection. The nucleic acid amplification tests are the most sensitive for the detection of acute HIV infection, after ruling out established HIV infections using HIV antibody detecting ELISA.

We have previously reported HIV prevalence of 3.3% among 330 HIV antibody (3rd generation Enzyme Immuno Assay (EIA)) negative blood donors in Kano-Nigeria, using P24 antigen (Dia.Pro, Milan, Italy) ELISA [3] (Table1). Nevertheless, the Nucleic acid amplification tests (NAAT) have advantage over the antigen-antibody EIAs due to their higher clinical and analytical sensitivity thereby reducing the

HIV 1 window period, from 16 days-22 days for third and fourth generation ELISAs, to an average of 8 days. The challenge of adopting this technology, particularly for resource constrained economies, is the high cost per test. Because of the cost per test of NAAT, and high sensitivity of the assay, different donor specimens are spiked and in some cases centrifuged together in one tube and tested as a single test. This also increases the efficiency and reduces the turnaround time of the assay. The master pool contains all the specimens while the sub-pools are kept, in case a positive result is found, they can be used to track the sub pools and individual donor sample(s) that are positive. There is fear of dilution of sample reducing sensitivity and chances of false negativity associated with this method.

Individual donor testing NAAT is the gold standard and in this case each individual donor specimen is tested separately. The major challenge here is the cost and probably longer turnaround time. In order to reduce the cost while maintaining high sensitivity and quality, the mini-pool (MP) NAAT has been piloted and used in different settings [4-16].

| | |
|---|---|
| P | Detection of acute HIV infection among blood donors |
| I | Minipool-NAAT Screening of blood donors and Individual donor testing nucleic acid amplification test (IDT-NAAT) |
| C | Blood donors screened by antigen-antibody ELISA |
| O | Higher sensitivity than antigen-antibody ELISA<br>Strong correlation with the gold standard (IDT-NAAT)<br>Compare cost effectiveness of mini pool NAAT with IDT-NAAT and ELISA |
| S | Systematic Review |

**Table 1:** Formulation of an answerable research hypothesis (PICOS).

## Example of pooling strategy

The aliquots obtained from each specimen will be pooled into 1 mini pool each, containing 36 equal volumes of the specimens (1 test) Westreich et al.'s table can be used for the estimation of pool size using D3 model [17-31].

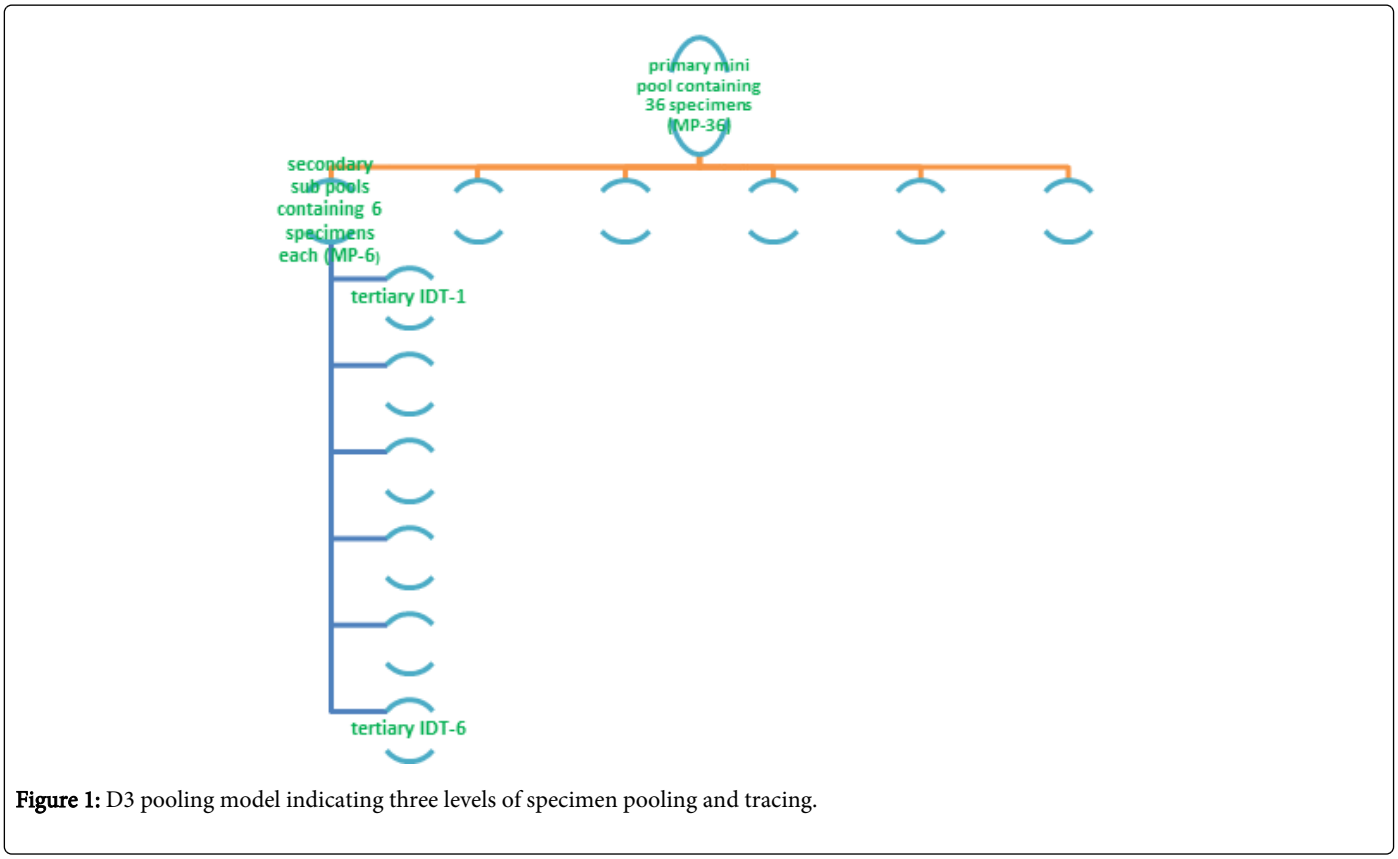


**Figure 1:** D3 pooling model indicating three levels of specimen pooling and tracing.

## Objectives

This systematic review is aimed at analyzing the validity of mini-pool NAAT in comparison to the gold standard, Individual donor NAAT in the detection of early HIV infection among blood donors. We also reviewed the cost effectiveness of mini-pool NAAT against Individual donor NAAT, third generation ELISA, and fourth generation ELISA respectively.

## Methods

### Study design

We adopted PICOS (problem, intervention, comparison, outcomes and study designs)'PRISMA (preferred reporting items for systematic reviews and meta-analyses), QUOROM (Quality of Reporting of Meta-analyses), and STROBE (strengthening the reporting of observational studies in epidemiology) guidelines of the Cochrane Collaboration for the meta-analysis and review of randomized trials and observational studies in this systematic review.

### Eligibility criteria

After getting the full text, the following inclusion criteria were applied to qualify for evaluation and data abstraction:

- Must be original research and a few expert reviews were considered (for narrative review). For quantitative review;
- Must be quantitative investigations;
- Every investigation must compare minipool and individual donor testing, clinically or analytically;
- Must have results with adequate data for calculation of confirmed reactive and non-reactive (determine sensitivity);
- The platform or reagent and pooling method must be clear.

### Search strategy

We included quantitative studies that evaluated the efficacy of nucleic acid amplification testing technology (NAAT), compared the cost effectiveness and feasibility of minipool NAAT with individual donor testing NAAT. PubMed, Cochrane and Google Scholar were used to identify relevant peer-reviewed journal articles published in English language between 1999 (when NAAT was introduced) and 2013. Initial MeSH key words included: Human immunodeficiency virus OR HIV AND nucleic acid amplification techniques OR pooled NAAT AND blood donors. Additional filters include: minipool-NAAT and individual donor testing-NAAT. For Google Scholar, Africa and validation were added to reduce the high volume of identified articles. After screening for duplication and relevance, 36 articles were selected (Figure 1). Few studies were conducted in Africa and only one (on pooled NAAT but not on blood donors) is from Nigeria. After screening for duplication and relevance, 50 articles were selected (Figure 1).

### Study population

Studies that compared pooled NAAT and IDT NAAT among subjects with acute HIV infection (blood donors and Men who have Sex with Men) were included in this review. We also included studies which compared other acute HIV infection detection techniques with the gold standard, IDT NAAT in terms of sensitivity and cost effectiveness.

### Setting

Papers that clearly described their strategy for the detection of acute HIV infection were included irrespective of the setting and studies that were set in blood bank or STI clinics were included regardless of the prevalence of HIV infection, clinical and analytical sensitivities of the technology or testing strategy.

### Intervention

The diagnostic techniques examined for detection of AHI were nucleic acid amplification techniques (Pooled and IDT), fourth generation enzyme linked immunosorbent assay (ELISA) and rapid HIV test kits. Nucleic acid amplification POCs were also examined to determine if they would serve as useful screening tools. All studies were compared with the gold standard (IDT NAAT). The sensitivity of enhancement techniques (such as ultra-centrifugation) was compared with those techniques that did not use ultracentrifugation. All sample processing methods were reviewed.

### Outcome

**Detection of acute HIV infection using pooled NAAT**

**Data extraction and management:** Data extraction logs were designed for this systematic review and were blindly validated on 4 articles by 2 reviewers (A.G and P.J.). The log validation revealed numerous ways in which the log was either ambiguous or inadequate for data collection/analysis, and it was therefore redesigned. All studies were extracted by the 2 reviewers who were masked (A.G. and P.J.) and entered into a spreadsheet. All discrepancies and discordances were discussed and fixed by the 2 reviewers.

### Data analysis

We initially planned a meta-analysis of studies on the accuracy of and pooled nucleic acid amplification versus individual nucleic acid amplification techniques in the detection of AHI among blood donors. After a careful study of the abstracted data and looking at the heterogeneity of the data, the authors resolved that a systematic review of the sub groups is more feasible, and the scope of the study was also expanded to include other blood donor screening techniques together with cost effectiveness.

## Results

Thirty six (36) studies which included 5 review articles, 5 retrospective cohort studies, 20 cross sectional studies, 2 statistical modellings, 2 national/international guidelines and 2 prospective cohort studies were further synthesized. Articles were further sub-divided into 8 groups based their focus area which includes: prevalence, clinical sensitivity of the assays, analytical sensitivity, test technology, testing algorithm, nucleic acid amplification POCs, rapid combo test kits, and cost effectiveness of test strategy (Figure 1).

### Characteristics of the studies included in this systematic review

**Methodological quality of the included studies:** Some of the studies reviewed in this analysis were found to have limitations, leading to potential for bias in their conclusions (Table 2).

| Study | Year | Country | Specimen | Equipment | Methods Compared | Results | References |
|---|---|---|---|---|---|---|---|
| Cragin et al. | 2012 | USA | Plasma | EIA equipment | Compared 3rd generation EIA with 4th generation EIA | The use of 4th generation EIA on HIV antibody negative donor samples detects additional 266 cases with additional cost of 63,763 USD, additional 489 life years, and 395 quality adjusted life years (QALYs) and prevented 26 HIV transmissions | [24] |
| Maritz et al. | 2011 | USA | Plasma | EI equipment | Compared 3rd generation EIA and 4th generation EIA with NAAT | Cost per additional case identified is after testing a HIV antibody negative blood donor sample with a 4th generation ELISA is 5,397 | [25] |

**Table 2:** Cost effectiveness of different blood donor testing algorithms.

Group 1: Four studies focus on prevalence of HIV among different populations. Only one of these studies focused of pooled NAAT, one other study, whose limitation is small sample size, determined the prevalence of HIV antigen among HIV antibody negative blood donors using ELISA, while the fourth, a national survey, was based on antibody detection among the normal population (Tables 1 and 3).

Group 2: Six studies analyzed the clinical sensitivity of pooled NAAT. All the 6 studies had adequate sample size and compared various sizes of pooled NAAT to IDT NAAT while one of these studies used novel approaches, to estimate window period by back-extrapolation of acute viral replication dynamics.

Group 3: Four studies analysed the analytical sensitivity of different types of sample using IDT-NAAT as the gold standard. One of these authors were contacted by us in order to obtain the conversion factor for their. The authors responded that they are using a validated in-house assay and do not have a conversion factor [32].

Group 4: Ten studies looked at different performance characteristics of various NAAT HIV testing technologies with the following findings summarized in table 1. Candotti et al. [27] have in addition provided a very valuable insight on the relationship between genetic diversity and the determination the viral load conversion factor.

Group 5: Owen et al. [31] and 3 other studies have shown how different algorithms differ in sensitivity depending on the targeted population

Group 6: Two Nucleic acid amplification POC studies demonstrated good sensitivity in the detection of AHI by using heat to break antigen-antibody complex.

Group 7: One study has shown a very low sensitivity using combo rapid HIV testing. This study is limited by not employing a technique for breaking the antigen-antibody complex (using heat or PH).

Group 8: Two studies discussed the cost effectiveness of population specific algorithms in the detection of AHI.

Table 3 summarizes the prevalence of HIV in different economies and population using different technologies. Drosten et al. [4] evaluated their developed assay where they predicted an analytical sensitivity of 1,195 g eq /ml (1,195 copies/ml) from IDT-NAAT and 1,014 g eq/ml (1,014 copies/ml) to 1,470 g eq/ml (1,470 copies/ml) (The RNA input concentration at which 95% of tests are expected to be positive using probit regression analysis on replicate samples) for minipool-NAAT by combining plasma from 96 donors in one tube, centrifuged the MP-NAAT specimens (before extraction) at 48,000 g to concentrate the viral particles (Table 3).

| Group | Main findings | Authors | Year | Mothodological quality of the studies |
|---|---|---|---|---|
| 1 | HIV Prevalence | WHO | 2013 | A condensed and too generalized global over view, based on level of income the countries. Needs to provide country specific prevalence. |
| | | NACA | 2005 | More representative of Nigeria |
| | | Kwaru et al. | 2006 | Small sample size |
| | | Pilcher et al. | 2010 | Critically reviewed the effectiveness of pooled NAAT with good example from the Carolina study (that uses 90 pools) |
| 2 | Clinical sensitivity of pooled NAAT | Candotti et al. | 2004 | Adequate sample size, compared analytical and clinical sensitivity of pooled NAAT (10 pools) and IDT-NAAT with strong statistics |
| | | Drosten et al. | 2001 | Compared 96 pooled plasma with the IDT-NAAT, enough sample size, studied analytic and clinical sensitivity with strong statistical analysis. |
| | | Morandi et al. | 2012 | Compared different pool sizes ranging from 21 to 87 with IDT NAAT. |
| | | Busch et al. | 1991 | Used novel approaches, to estimate window period by back-extrapolation of acute viral replication dynamics. Incidence was derived from antibody-negative donations detected by routine MP NAAT of 37 million US donations (1999-2002) or from sensitive/Less-sensitive HIV-1 enzyme immunoassay (S/LS-EIA) results for seropositive samples from 6.5 million donations (1999). Incidences and WPs were combined to calculate risks and project yield of ID-NAAT. |

| | | | | |
|---|---|---|---|---|
| | | Sullivan et al. | 2011 | Compared MP NAAT of 128 pools with ultracentrifugation with good statistics. |
| | | Roth et al. | 2009 | International survey, good study period, compared mini pool NAAT with IDT NAAT methods, analytical, clinical sensitivities. Quite elaborate with very strong statistics. |
| 3 | Analytical sensitivity of pooled NAAT | Gubbe et al. | 2012 | Studied Cadaveric samples comparing minipool of 96 samples with IDT-NAAT with good statistics. When contacted, the authors could not provide a conversion factor on the grounds that the DRK PCR kit they used is in house not commercial. |
| | | Ethridge et al. | 2010 | Compared 16-32 pools with IDT NAT |
| | | Nugent et al. | 2009 | Determined sensitivity (analytical and clinical) of various specimens using pooled NAAT and IDT NAAT. Presented results of all dilutions including those that are less 100%. Very transparent with their findings. |
| | | Palla et al. | 2006 | Reduced size of mini pool, but did not exploit the option of ultracentrifugation with larger pool sizes. |
| 4 | Different performance characteristics of various NAAT HIV testing technologies | Patel et al. | 2010 | Pooled NAAT increased case detection by 26%. Did not try enhancement techniques like ultracentrifugation |
| | | Bush et al. | 2005 | Did not explore larger pools with enhance extraction methods such as ultracentrifugation |
| | | Allain et al. | 2004 | Focused more on occult hepatitis no other TTIs |
| | | Stramer et al. | 2004 | A good retrospective cohort study with a very good sample size. Used a pool size of 16 but did not exploit larger pool size and ultracentrifugation. |
| | | Smith et al. | 2009 | Both mini pool and matrix pool did not explore enhancement techniques |
| | | Stramer et al. | 2007 | Focused on seroconversion resulting from pooled NAT without looking at the fact that none of these reports used enhancement methods to increase sensitivity |
| | | Tang and Ou | 2012 | No detailed comparison on various approaches in NAAT blood donor screening such as IDT-NAAT, mini pool and matrix pool |
| | | Tang et al. | 2003 | Looked at analytical and clinical sensitivities and genetic diversity. Very important in determining the conversion factor |
| | | Candotti et al. | 2003 | Good sample size and strong statistical analysis. Used large pool size and got clinical sensitivity of 96.5% ,did not explore enhancement methods |
| | | Yang et al. | 2009 | Got a higher yield in IDT-NAAT compared to mini pool NAAT but did not try concentration method on the mini pools to improve sensitivity |
| | | Morris et al. | 2010 | Reduced the time for issuing NAAT results and reducing stigma among MSMs by the use of internet. Short text messages (SMS) were not tested, which is more likely to work in our setting |
| | | Weber et al. | 2002 | Very strong statistics on a highly sensitive fourth generation EIA which can serve as an alternative to NAAT for blood donor screening in low income countries |
| 5 | Testing strategies/ algorithms | Owen et al. | 2008 | provided alternative algorithms using rapid test kits for the detection of acute HIV infection |
| | | Branson et al. | 2007 | |
| | | Westreich et al. | 2008 | |
| | | Mc Mahan et al. | 2012 | |
| 6 | Nucleic acid amplification POC | Roskos et al. | 2013 | Good point of care testing for AHI. Has technique of enhancing sensitivity using heat to break antigen-antibody complex |
| | | Divena et al. | 2005 | Good point of care testing for AHI. Has technique of enhancing sensitivity using heat to break antigen-antibody complex |
| 7 | HIV rapid test combo in the detection of AHI | Rosenberg | 2012 | Should recommend antigen-antibody complex breaking technique (e.g heat and PH) to the manufacturer's to improve sensitivity |
| 8 | Cost effectiveness of different HIV testing | Cragin et al. | 2012 | |
| | | Maritz et al. | 2011 | |

| | | | | |
|---|---|---|---|---|
| | algorithms in the detection of AHI | | | |

**Table 3:** Summary of studies included in evaluating the efficacy of methods of detection of acute HIV infection among blood donors.

Table 4 summarizes the sensitivities (100%) of different platforms (clearly defined) using different controls, specimens and standards. Analytical sensitivities of 10 to 2,130 viral copies/ml were observed in plasmas of pool sizes of 10 to 96 irrespective of the pool size. A study group [10] estimated that HIV-1 risks with current MP-NAAT screening in the US are approximately 1 per 2 million units and that these risks could be reduced to 1 in 3 to 4 million units by ID-NAAT screening. The assumptions of this study are based on estimates of B clade which may not apply to non-B clades. Allain [11] reported that Pooled NAAT (pool of 10) may reduce sensitivity of occult hepatitis B by 50% while increase in extraction plasma volume from 200 µl to 500 µl or 1,000 µl will increase the sensitivity. An evaluation of three years (March 1999 to January 2002) of implementation of minipool NAAT using Gen Probe (Gen Probe San Diego, CA USA) and Cobas AmpliScreen (Roche Molecular Systems, Mannheim, Germany) revealed a consistent sensitivity and specificity (1 in 3.1 million donations) of the technique, irrespective of the platform (p=0.74) [12]. From Table 4 (below) four out of six studies of pool sizes 10 to 50 donor plasmas used standard centrifugation and have recorded sensitivity of 100%. The remaining two whose plasma pool sizes were 96 and128 got sensitivities of 92.3 and 95.3 respectively. While ultracentrifugation of a pool size of 45 samples by Morandi et al. recorded a sensitivity of 100% and that of 96 samples by Drosten et al. was quite less (92.3%) under the same condition. Interestingly, Sullivan et al. evaluated 128 plasma pools, without ultracentrifugation and got a higher sensitivity than Drosten et al. Detection threshold of these studies range between 400 and $10^5$ viral copies/ml. All the 6 studies (Table 1) have compared the pooled NAAT with the gold standard (IDT-NAAT).

| Study | Year | Location | Specimen/ Standard | Controls | Test/Methods | Gold standard | Platform(s) | Pool size | Targeted population | Prevalence | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WHO | 2013 | International | Blood donor plasma | Positive and Negative controls | EIA, rapid tests, -Low income countries, EIA, NAAT- Middle income countries, EIA, NAAT- High income countries | NAAT | various | 10-128 | Blood donors | Low income countries: 0.6%; Middle income countries: 0.1%; High income countries: 0.03% | [1] |
| NACA | 2005 | Nigeria | Blood donor plasma | Positive and Negative controls | Antigen/antibody EIA | | EIA equipment | NA | Blood donors | 2.10% | [2] |
| Kwaru et al. | 2006 | Nigeria | Blood donor plasma | Positive and Negative controls | EIA antibody/antigen | | EIA equipment | NA | Antibody negative blood donors | 3.30% | [3] |
| Pilcher | 2010 | USA (review) | Plasma DBS | NA | NA | NA | NA | 9000.00% | high risk group | Antibody positive 4%; NAAT positive 0.02%; Piloting DBS for EQA | [22] |

**Table4:** Prevalence of HIV among different groups and populations using different technologies.

Table 5 (below) summarizes the analytical sensitivity of pooled NAAT and IDT-NAAT on different specimens and standards by different assays at various settings. The German study used in-house PCR with 96 donors in one master pool recorded a sensitivity(100%) that is comparable with pool sizes between 10 and 32 at even much lower detection threshold (9.0 copies/ml) [33-35].

| Platform/Assay | Pool size | No. of pools tested | Centrifugation/ Enrichment speed | Minipool NAAT reactive | Individual NAAT reactive | Viral load range/ threshold | Confirmed reactive | Sensitivity of minipool (%) | Authors/Year/ Location | References |
|---|---|---|---|---|---|---|---|---|---|---|
| A multiplex real-time quantitative reverse transcription (RT)-PCR assay | 10 | 267 | Standard method (at least 12,500 Xg for 15 min) | 1 (0.4%) | 1 (10%) | $10^5$ copies/ml | 1 (100%) | 100 | Candotti et al. 2004, Ghana | [21] |
| 5'-nuclease based reverse transcription (RT)-PCR | 96 | 1791 | 48,000 Xg for 60 min | 36 | 39 | 400 copies/ml | 39 | 92.3 | Drosten et al. 2001, Germany | [4] |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| assay/Roche Amplicor monitor | | | | | | | | | | |
| PEG Amplicor | 45 | 234 | 50,000 Xg for 80 min | 5 | 5 | 500 copies/ml | 5 | 100 | Morandi et al. 1998, Switzerland | [5] |
| Roche Amplicor v1.5 | 25 | 993 | Standard method ( at least 12,500 Xg for 15 min) | 16 | 16 | 400-$10^5$ copies/ml | 16 | 100 | Man Charurat et al. 2012, Nigeria | [9] |
| In-house (Modified Kellog and Kwok's method) on Perkin Elmer automated thermocycler | 50 | 873 | Standard method ( at least 12,500 Xg for 15 min) | Initial: 55; Repeatedly (testing with second primer): 21 | 1 | NA | 1 | 100 | Busch et al. 1991, USA | [33] |
| Aptima HIV-1 RNA qualitative assay, Gen-Probe Inc) | 128 | 21 | Standard method ( at least 12,500 Xg for 15 min) | 20 | 21 | 1,520-500,000 copies/ml | 20 | 95.23 | Sullivan et al. 2011, USA | [35] |
| Dynamic Range: range within which measurements can be made<br>Covers LOQ to LOL<br>Detection threshold/ LOQ: (limit of quantitation): lowest level at which quantification can reliably be made<br>Equal to 10 × Average Signal for blank i.e. 10Sbl<br>LOL: Limit of Linearity: point where signal is no longer corresponding to the level of analyte | | | | | | | | | | |

**Table 5:** Summary of clinical sensitivity of different strategies pooled NAAT plasma testing at different settings.

Table 6 summarizes different performance characteristics of different platforms and methods in early detection of acute HIV infection (AHI).

| Platform/Assay | Pool size/ Dilution factor | Specimen/ Standard | Viral load range/ Threshold | Analytical sensitivity of minipool | Authors | References |
|---|---|---|---|---|---|---|
| DRK Baden-Würtemberg-Hesse NAAT system. (In-house NAAT, German Red Cross) | 96 | Control 40 | 9.0 copies/ml* (WHO/ NIBSC NAAT standard 97/650) | 100% | Gubbe et al. 2012, Germany | [32] |
| | | Cadaveric 32 | | | | |
| Aptima HIV-1 RNA qualitative assay, Gen-Probe Inc) | 16 | WHO HIV RNA standard | 1,070 copies/ml of infected donor plasma/pool) | 100% | Ethridge et al. 2010 USA | [34] |
| | 32 | | 2,130 copies/ml of infected donor plasma/pool | 100% | | |
| Aptima HIV-1 RNA qualitative assay, Gen-Probe Inc) | 10 | Semen | 50 copies/ml | 100% | Nugent et al. 2009 USA | [6] |
| | | Plasma | 10 copies/ml | 100% | | |
| Ampliscreen | 16 | Plasma | 515 copies/ml | 100% | Palla et al. 2006 Italy | [26] |
| *a conversion factor of 1 IU/ml=1.039 copies/ml was used (i.e 5.56 log10 IU/ml=5.35 log10 copies/ml for WHO 2nd standard 95/650) | | | | | | |

**Table 6:** Summary of analytical sensitivity of different assays, testing different pooled NAATs specimens/standards.

The result of 267(rapid test HIV, HBsAg, HCV negative) mini pools of 10 plasmas from a Ghanaian teaching hospital using a multiplex (NAAT) assay revealed one pool (0.4%) as HIV-1 positive which contained one positive donation with a viral load of 105 IU/ml (109 copies/ml) [21] (Table 7).

| Study | Year | Location | Specimen/ Standard | Controls | Test/Methods | Gold standard | Platform(s) | Pool size | Variable/ Performance characteristics | LOD | Estimated window period | Case Detection rate | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patel et al. | 2010 | USA | Plasma/ Serum | | HIV antibody/antigen EIA | | APTIMA Gen-probe | 16 | LOD: pooled NAAT after negative third-generation test results detected | 30 copies/ml | | Increased HIV case detection by 2.2% | [8] |
| | | | | | OraQuick | | | | | | | | |
| | | | | | HIV antibody | | | | | | | | |
| | | | | | NAAT (IDT and Pooled) | | | | | | | | [10] |
| Busch et al. | 2005 | USA | Plasma | | MP-NAAT; IDT- NAAT; sensitive/Less-sensitive HIV-1 enzyme immunoassay (S/LS-EIA) | IDT-NAAT | Gen probe | 16, 24 | IDT Window period | | 5.6 days | | |
| | | | | | IDT- NAAT | | Cobas Amplicreen | | MP-NAAT to IDT NAAT detection | | 3.4 days | | |
| | | | | | sensitive/Less-sensitive HIV-1 enzyme immunoassay (S/LS-EIA) | | | | MP NAAT to p24 antigen NAAT detection | | 6.0 days | | |
| Allain | 2004 (review) | USA | Plasma | | Rapid test NAAT | | | | NAT sensitivity | 50 copies/ml | | | [11] |
| | | | | | HBsAg NAAT | | | | | | | | |
| Stramer et al. | 2004 | USA | Plasma | | IDT NAAT | IDT-NAAT | Cobas Amplicreen | 24 | NAAT Detection rate | 100% | | 10 of 37,164 | [13] |
| | | | | | Pooled NAAT | | Gen-probe | 16 | P24 detection rate | | | 2 of 37,164 | |
| | | | | | | | | | Sensitivity of MP-NAAT | | | | |
| Smith et al. | 2009 | | Plasma | | Minipool NAAT | IDT-NAAT | Ultrasensitive Amplicor | 10 | Pooled NAAT sensitivity | | | | [14] |
| | | | | | Matrix NAAT | | HIV-1 monitor viral load assay | | Pooled NAAT NPV | | | | |
| | | | | | IDT-NAAT | | | | LOD | 50 copies/ml | | | |
| Stramer | 2007 (review 1999-2006) | | Plasma | | Minipool NAAT | | Various platforms | | Difference between IDT NAAT and MP NAAT in detection rate | | | 1 to 2 in 5 million units | [15] |
| | | | | | IDT NAAT | | | | Risk reduction of MP NAAT compared to serological testing | | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tang and Ou | 2012 | NA (review) | Whole blood, Plasma, DBS | | DNA/RNA Qualitative assays | | APTIMA | | Applicability | Pooled NAAT | | | [18] |
| | | | | | | | Proleix | | | Early infant diagnosis (EID) | | | |
| Tang et al. | 2007 | USA | Plasma | HIV-1 internal control | Abbott RealTime HIV-1 assay for quantification of HIV-1 group M | | | | Dynamic range | 40 copies to 106 copies of HIV-1 RNA/ml | | | [23] |
| | | | | | Subtypes A–H, group O, and group N isolates | | | | HIV-1 RNA detected at different concentrations (LOD)with 95% probability | 25 copies/ml for 1.0ml of plasma | | | |
| | | | | | | | | | | 39 copies/ml for 0.6mL of plasma | | | |
| | | | | | | | | | | 65 copies/ml for 0.5ml of plasma, and | | | |
| | | | | | | | | | | 119 copies/ml for 0.2ml of plasma indicating higher sensitivity with increasing volume | | | |
| Candotti et al. | 2003 | UK | Plasma | Positive and Negative controls | EIA | IDT-NAAT | EIA equipment | 96 | Compared Pooled NAAT with IDT-NAAT after 3rd generation EIA | 23 to 56 copies/ml | | | [27] |
| | | | | | Pooled NAAT | | Cobas Amplicor 2.0 | | Clinical Sensitivity | | | | |
| | | | | | IDT-NAAT | | | | Estimated 95% analytical sensitivity using replicate standards | | | | |
| Yang et al. | 2009 | Taiwan | Plasma | Positive and Negative controls | EIA | IDT-NAAT | Proleix Ultrio/ TRIGIS | 4 | Estimated 95% LoD on WHO standards (replicates): | 18(12-34) copies/ml | | | [28] |
| | | | | | Pooled NAAT | | | | | Clinical sensitivity | | | |
| | | | | | IDT-NAAT | | | | | | | | |
| Morris et al. | 2010 | USA | Plasma | Positive and Negative controls | NAAT | NA | Rapid test (Oraquick | NA | NAAT Increase yield | | | 23% | [29] |
| | | | | | | | NAAT( Cobas Amplicor, Proleix) | | Use of internet and voice mail to deliver NAAT result | | | | |
| Weber et al. | 2002 | Germany | Plasma | Positive and Negative controls | EIA | NAAT | New Cobas core HIV combi | NA | Sensitivity | | | 100% | [30] |
| | | | | | NAAT | | | | | | | | |

*conversion factor for HIV IU/ml to Copies/ml=1.039

**Table 7:** Methodology, performance characteristics, major findings and locations of different studies on blood donor screening.

Recently, a cohort study from Nigeria on the detection of acute HIV infection among high risk groups adopted an algorithm using multistage, pooled reverse transcriptase polymerase chain reaction (PCR) to screen for the presence of HIV RNA using Roche Amplicor version 1.5 (Roche Diagnostics, Mannheim, Germany) using a master pool in a 1:25 dilution [9]. Minipool of 25 specimens of different high risk populations were tested and repeated by individual NAAT from 993 subjects with a detection limit of 400 to $10^5$ copies /ml and analytical and clinical sensitivity of 100% respectively (determined by the number of MP-NAAT reactive/IDT-NAAT reactive(at LoD) × 100).

An international survey involving 59 countries has reported that as of 2008, majority of countries still practice pooled NAAT discriminating this data by pool size was inconclusive. Although larger pool sizes may seem to be associated with low sensitivity, Germany recorded comparable results with countries that use lower pool size. Ultracentrifugation of pooled plasma before extraction and volume of plasma used for extraction are some of the factors to consider in addition to pool size and analytical sensitivity of the assay [13]. Another study found minipool (from 10 patients) to give comparable result with individual donor testing for monitoring of patients on ARVs [14].

## Need for population specific screening algorithms

In South Africa, a 53-year-old donor who had donated 53 times previously testing negative by IDT-NAAT; 3 months following the transmitting donation, returned for a another donation and tested HIV antibody confirmed positive. The transmitting donation tested nonreactive by p24 antigen and antibody; the fresh frozen plasma tested RNA positive with a viral load of 12 copies/ml. The infected donor and recipient were linked by phylogenetic analysis. An algorithm that combines HIV-1 NAAT results with the Western blot indeterminate and negative results reveals that less than 0.04% of these donors are true HIV 1 positive, 91.6% of the anti-HIV confirmed positive samples that tested NAAT reactive had a signal-cutoff ratio of 15 or greater ELISA (Abbott Laboratories, Abbott Park, Ill). In contrast, of those anti-HIV repeat reactive samples that tested NAAT nonreactive and Western blot negative or indeterminate, 98.5% had an Abbott ELISA signal-cutoff ratio less than 15 [15]. The need for an optimized and realistic algorithm and pool size that is cost effective, for different settings and incidences via-as-vis methodology (such as hard spinning), taking into account HIV 1 genetic diversity, primer-probe mismatch and platforms as summarized in Table 6 below, cannot be over emphasized [16-23,26,27,36-45]. Cannillo et al. recently, reported that CAD as the most frequent cardiac complication in HIV patients (particularly those with low CD4 and high viral load) treated with HAART. They however, opined that the development of heart failure and atrial fibrillation in these patients is frequent and deserves both clinical care in diagnosis, prevention, and treatment. This also necessitates the need for further studies focusing on the efficacy and safety of both prevention and treatment of heart failure and atrial fibrillation in these patients [46-48]. We hypothesize a careful formulation of an algorithm, targeting patients on long term HAART to include more frequent of CD4 count, viral load (both peripheral and intra-monocytic), drug resistance monitoring, blood film(in search of activated monocytes), lipid/coagulation profile and other risk factors of heart failure.

## Application of dipstick NAAT technology

Simple and low cost plastic cartridge systems have been designed as a platform that can be easily modified to accommodate different specimens during sample preparation, and different uniform heating stages of DNA amplification assays coupled to nucleic acid amplification lateral flow and isothermal-mesofluidic systems. One of these universal platforms has tested 19 pools of 10 plasma samples from West Africa and recorded a high sensitivity. This simple, rapid, and robust test format at reduced cost is valuable for the screening of blood samples by laboratory workers in blood banks or diagnostic laboratories in developing countries [20,36]. These assays are aimed at increasing analytical sensitivity breaking the antigen-antibody complex and reducing the window period at low cost.

## Rapid HIV antigen antibody (combo) assays

Table 8 shows the results of a field evaluation in Malawi which reported low sensitivity and specificity of the combo rapid HIV test kit in the detection of acute HIV infection [16].

| Study | Year | Location | Specimen/ Standard | Controls | Test/methods | Gold standard | Platform(s) | Pool size | Variable/ Performance characteristics | Findings | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Owen et al. | 2008 | USA | Plasma | Positive control | Rapid tests | - | | | Sensitivity in | Established HIV infection | [31] |
| | | | | | | | GS HIV-1/2_O Third-generation EIA: | | | 99.80% | |
| | | | | | | | Abbott Third-generation EIA: | | | 99.40% | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Negative control | EIAs | | Vir HIV-1_O Second-generation EIA: | | | 99.70% | |
| | | | | | | | GS HIV-1/2 peptide Second-generation EIA: | | | 98.70% | |
| | | | | | | | GS rLAV Second-generation EIA: | | | 97.40% | |
| | | | | | | | Vir HIV-1 First-generation EIA: | | | 99.00% | |
| | | | | | | | Oraquick Rapid test: | | | 98.60% | |
| | | | | | | | Reveal Rapid test: | | | 99.00% | |
| | | | | | | | Multispot Rapid test: | | | ND | |
| | | | | | | | Uni-Gold Rapid test: | | | 98.40% | |
| | | | | | | | Procleix NAAT: | | | 97.4 | |
| | | | | | NAATs | | CDC RNA NAAT: | | | 95.80% | |
| | | | | | | | AmpliScreen NAAT: | | | 92.60% | |
| | | | | | | | Genetic Systems HIV-1 Western blot) | | | - | |
| | | | | | | | and Cambridge Biotech HIV-1 Western blot. (used interchangeably) | | | | |
| Branson et al. | Reviewed data from CDC, 2007. | USA | Whole blood | | OraQuick Advance | - | NA | | Sensitivity | 99.6% (98.5–99.9) | [7] |
| | | | Plasma | | Rapid HIV-1/2 Antibody | | | | | | |
| | | | | | Uni-Gold Recombigen | | | | | | |
| | | | | | | | NA | | | 100% (99.5–100) | |
| | | | | | Clear view HIV 1/2 Stat-Pak | | | | | | |
| | | | | | | | NA | | | 99.7% (98.9–100) | |
| | | | | | Clear view Complete HIV 1/ | | | | | | |
| | | | | | Reveal G3 Rapid | | | | | 99.7% (98.9–100) | |
| | | | | | HIV-1 Antibody Test | | NA | | | | |
| | | | | | | | NA | | | 99.8% (99.2–100 | |
| | | | | | MultispoHIV-1/ HIV-2 Rapid Test | | | | | 100% (99.9–100) | |
| Westreich et al. | 2008 | | Plasma | | Minipool NAAT | IDT-NAAT | | | Pooling algorithms and optimization: | All the three pooling strategies postulated by this simulation | [17] |
| | | | | | Three postulated algorithms: | | | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | two-stage minipool (D2), three-stage hierarchical pools (D3), and Square arrays with master pools (A2m). | | | | | have demonstrated improved efficiency and PPV for AHI case detection compared to individual NAAT. | |
| Mc Mahan | 2012 | USA (modelling and simulation) | Urine, Swabs, Blood, Plasma | | Various-including pooled NAAT with example from Pilcher et al. 2005 North Carolina studies | | | 90 | Simulated a formula for decoding and retesting positive master pools: | Simple and efficient modelling applicable in so many pooling strategies | [19] |

**Table 8:** Performance characteristics of different HIV testing strategies.

## Cost effectiveness of NAAT and EIAs algorithms in the detection of AHI in blood donors

HIV Screening with the 4th-generation assay may serve as a cost-effective means for acute HIV infection (AHI) detection in settings with high incidence who could not afford NAAT [24]. However, studies have shown that pooled NAAT is most cost effective after third-generation EIA (or rapid tests) among populations with high incidence, (as indicated in Table 9) [25]. A study on the cost effectiveness of pooled NAAT for instance, concluded that pooled NAAT is not cost effective in MSM with low incidence if the antibody testing is done frequently as recommended. In this group, pooled NAAT screening is only cost effective when antibody testing frequency is 5 years or greater, however, at that time, most of the benefits achieved occur long after the acute phase and have already been achieved with antibody testing alone [43]. This strategy is more cost effective among blood donors. A cost-effectiveness study from a high income country proved the use of triplex MP-6 NAT to be more cost effective for prevention of HBV transmission than triplex ID-NAT. Incremental cost effectiveness ratios (ICER) of averting HBV transmission by MP-6-NAT or ID-NAT were €303,218 and €518, 995 per quality adjusted life year (QALY) successively [44]. A similar study from a low income country reported ICER of p24 antigen testing, MP-NAT and ID –NAT in addition to HIV antibody testing was less favourable for ID-NAT, followed MP-NAT and p24-antigen screening respectively. Therefore, in analyzing the cost effectiveness of pooled NAAT algorithms, the target population, the laboratory analysis, reporting of result, and counseling management and level of income must also be considered [27]. More evaluations of pooled NAAT are needed from Sub-Saharan Africa to enable generalizability of this cost effectiveness in our settings.

| Population | True Positive | False Negative | True Negative | False Positive | Sensitivity | Specificity | Inference |
|---|---|---|---|---|---|---|---|
| Acute HIV infection (AHI) | 0 | 8 | 824 | 14 | 0% | 98.30% | Very poor sensitivity in the detection of AHI |
| | | | | | | (97.2-95.1) | |
| Established HIV infection | 162 | 1 | 643 | 5 | 99.40% | 99.20% | High sensitivity in the detection of established HIV infection |
| | | | | | (96.6-100) | (98.2-99.7) | |

**Table 9:** Sensitivity and specificity of a rapid HIV antigen/antibody (combo) rapid HIV test kit in the detection of acute HIV infection.

The need for population specific NAAT algorithm and optimization of assays and pool sizes including modelling and simulation cannot be over emphasized [15,17,19,31]. Tang and Ou have summarized various molecular diagnostic techniques, their application, turn-around time, including blood donor screening in a review [18]. Tang et al. [23] in a study of genetic diversity on quantification of HIV 1, determined sensitivity of HIV assays by testing different dilutions (from 5 to 250 copies/ml) of a viral standard from the virology quality assurance (VQA) Laboratory of the AIDS Clinical Trial Group. 57 replicates of each panel member were shared between 12 assay runs, three amplification lots, and three set of instruments. The level of replicates detected at each concentration were: 25 copies/ml (95% CI 20–33) for 1.0 ml; 39 copies/ml (95% CI 33-49) for 0.6 ml, 65 copies/ml (95% CI 51-88) for 0.5 ml, and 119 copies/ml (95% CI 102-150) for 0.2 ml. An evaluation of 50,000 donor sample using a modified Genesis 200 method yielded a failure rate of 8.6% which was reduced to 4.7 when the results from a critical equipment failure period were removed [27].

Analytical sensitivities (95%CI) of 18 (12-34) [97(65-184 copies/ml)for hepatitis B and 22 IU-54 IU (23 to 56 copies/ml)for HIV, were observed with an IDT-NAAT yield rate(number of positive samples/

total number of samples tested) of 9 in 4210 (0.21%), four times higher than the minipool-NAAT yield rate of 3 in 6080 (0.05%; $p<0.05$) for HBV and 26/27 (96.2%) for HIV respectively. The one HIV specimen missed by minipool NAAT of the latter was traced to an identification error during specimen preparation [27,28]. Turnaround time was reduced and delivery was increased among high risk groups in a survey which piloted delivery of NAAT results through the internet [29]. Short messages may be more feasible for our setting, considering our low internet coverage.

The major public health implication of early detection of HIV infection is reduced viral transmission [22]. A study which compared HIV antibody negative donor samples with a P24 antigen has recorded a prevalence of 3.3 % among some Nigerian blood donors [3]. An estimated transmission of 1 in 2,857 through blood transfusion in Nigeria could be reduced to about1 transmission in 4,286 to 5,714 units by the introduction of pooled NAAT. Patel et al. [8] and other studies tested a pooled NAAT of different sizes among different groups using different algorithms and found that pooled NAAT after a third generation ELISA increased the overall sensitivity by 2.2%. This is said to be the most cost effective algorithm, particularly when targeting population with high prevalence [24,25]. The use of rapid HIV antigen/antibody (combo) screening test in the detection of acute HIV infection has yielded a very low sensitivity [16]. A study has however reported that the new Cobas Core HIV combi ELISA (Roche Diagnostics, GmbH, Penzberg, Germany) to have an improved sensitivity compared with 3rd and 4th generation assays and RT PCR. This new assay has sensitivity comparable to that of Abott HIV1 AG monoclonal A (Abbott, North Chicago, USA) for early detection of HIV in seroconversion panels. The diagnostic window period is reduced by this assay by 3.6-5.7 days compared to 3rd generation ELISAs [30]. This study has compared the new assay with a variety of single ELISAs and gold standard which adds to its validity. Studies of isothermal nucleic acid amplification system coupled to a lateral flow device have been presented by a group and will be useful in the acute detection various pathogen. The heating block will aid in antigen-antibody complex dissociation [20,36].

## Discussion

The currently estimated transmission of TTIs of 1 per 2 million transfused units of blood, in the United States could be reduced to 1 in 3 to 4 million units by ID-NAAT screening [10]. The majority of blood donors from high income countries are aged between 45 years and 64 years old, while those from low income countries are aged between 18 years to 24 years old [43]. The fact that voluntary non-renumurated blood donation is the safest, and family replacement and paid donations are likely to be unsafe and the fact that the latter accounts for 36%, 26%, 0.3% for low income countries, middle income countries and high income countries respectively make comparison between the risk rate of these countries difficult.

The strong correlation in sensitivity was observed by Drosten et al. [4] despite the pooling of plasma from large number of donors, may be due to the high concentration of HIV-1 particles recovered by hard spinning [4]. (Table 3) (1 genome equivalent (eq)/ml=1 RNA copy/ml). The % clinical sensitivity=number of samples reactive by pooled NAAT/number of samples reactive by IDT-NAAT at the lowest limit of detection × 100. A strong agreement, in analytical sensitivity and specificity was also reported using different pool sizes, specimen types (including DBS) and assays, compared to individual donor testing ruling out the speculated serious negative impact of dilution (optimum at 10 to 45 different donor samples) [4-6,8,9,21,26,27,32,34,35]. The sensitivity obviously negatively correlates with the pool size, particularly from 96 to 128. Bush et al. [33] used in house reagent which might not have been properly validated. Initially they got 54 false positives, after repeating with a second primer, they still got 20 false positive results. Primer mismatch, critical equipment malfunction and contamination may be responsible. Proper quality control measures and method validation are quite desirable. The number of assays ran over a period of time in a molecular diagnostic laboratory may also account for error rate. The WHO fact sheet and the National HIV response review have clearly stated the prevalence of HIV among blood donors both globally and internationally [1,2]. Branson et al. [7] reviewed the Carolina studies where they emphasized the efficacy of pooled NAAT of 100 different subjects in the detection of acute HIV infection with good sensitivity, among the high risk group. The study by Owen et al. has not provided data on the sensitivity of 2 EIAs and 2 NAATs (Ampliscreen (Roche Molecular Systems, Mannheim, Germany) and CDC RNA (ref 29 CDC, Atlanta, USA) in the detection of AHI. This is a very important missing piece of information, particularly the NAATs. It is however interesting to note that some of the third generation EIAs that are licensed in the United States, (GS HIV-1/2_O Third-generation EIA (Bio-Rad Laboratories Redmond WA USA) and Abbott Third-generation EIA)(Abbott Laboratories, IL,USA)have a moderately high sensitivity in the detection of AHI, 14 and 12 days reactivity before Western Blot respectively, while Proleix (Gen-Probe Diego LA, USA) NAAT was very sensitive (reactive 26 days before Western Blot positive result.)

A study from Nigeria has shown the feasibility of pooled NAAT, Amplicor 1.5 Monitor in the detection of acute HIV infection among high prevalence population in a resource limited setting [9]. The German Red Cross could not provide a conversion factor when contacted by e-mail, on the basis that their assay is in-house not standard assay. For this reason, the conversion factor of 1IU/ml=1.039 copies/ml was used (1.e 5.56log10 IU/ml=5.35log10 copies/ml for WHO 2nd standard 95/650 for NAAT assays calibration was maintained. The conversion factor can change as the sensitivity of the assay increases and it is genotype dependent. For instance, 95/650 is applicable for genotype B and will not give accurate values for non-B clades. In some industrialized countries, NAAT is still applied to minipool of plasma of various sizes in order to reduce the cost of screening donated blood for the three major transfusion-transmitted viruses. However, from some reported cases of minipool negative transfusion transmission of HIV, it was discovered that these donors' sero-conversions were recent, which explains the low viral load. Most importantly, the involved donors later revealed high risk factors, such as recent male to male unprotected sexual insertion or reception undisclosed in the original deferral interview. Retesting of archived donor samples with ID-NAAT proved reactive [11].

Applying WHO adjusted age direct standardization [43], the prevalence of HIV in Nigerian blood donors is 700 times higher than that of high income countries. Therefore, a projected risk of 700 transmissions in 2 million units (1 transmission in 2,857 units) through blood transfusion in Nigeria could be reduced to 1 transmission in 4,286 to 5,714 units by the introduction of pooled NAAT. Overall, this would reduce the number of transmissions by approximately 346,804 transmissions per year in Nigeria (assuming donations of 10% of the population of 167million a year, as recommended by WHO and HIV antibody prevalence of 2.1%). About 25-30% of more than 2,000 low viral load minipool NAAT tested infected blood donors identified require individual donation NAAT

for detection [11]. The obvious impact of NAAT screening is definitely greater than that of serological screening, despite the rare occurrence of sero-positive but NAAT-negative donations which may be due to extraction failure, personnel identification error, and primer-probe mismatch which indicates that serological screening must be maintained even with the most sensitive NAAT testing performed on individual donations. Therefore, the additional cost-effectiveness of NAAT is marginal since the safety benefits used by most health analysts are restricted to the prevention of transmission of NAAT-only yields and the cost of NAAT testing is still expensive.

## Conclusion

The size of the pool, the reagents, platform, methodology (e.g. enrichment by hard spinning) and the incidence of HIV in the targeted population are quite critical to the success and cost effectiveness of MP-NAAT. Low viral load sero-conversion panels are prone to viral RNA dilution of the mini-pools, below the detection threshold of the NAAT, though various studies have confirmed its reliability [4-6,8,9,17,21,26,27,32,34,35]. An average clinical sensitivity of 98% was recorded for pooled NAAT using IDT NAAT as v from the studies included in this review. From this review, Pooled NAAT and IDT-NAAT will reduce the window period by detecting those antibody negative donors who were not detectable by the third and fourth generation ELISAs. This means additional HIV positive blood donors will be captured by complimenting the ELISAs with NAAT. It is however important to also replicate the evaluation of the new Cobas core HIV Combi ELISA and compare its cost effectiveness with that of pooled NAAT. From the studies presented here, the difference in sensitivity between the pooled NAAT and the gold standard, IDT-NAAT are not statistically significant (p=1.00) using Probit regression analysis. It is evident from all the studies in this review that the pooled NAAT will reduce the window period of HIV among blood donors and other targeted populations for the detection of AHI. An evaluation of the new Cobas core HIV combi [30] with improved sensitivity for the detection of AHI and the isothermal DNA amplification/ lateral flow point of care (POC) assay may give an alternative algorithm for secondary and primary health care levels in low income countries [35]. This underscores the need for studies from low income countries to test the feasibility of NAAT implementation to determine if NAAT is affordable and effective for safe blood supply [34]. From this review, minipool NAAT testing after running a third generation assay can be a recommended as highly sensitive and cost effective algorithm for blood donor screening in low and middle income countries. The benefit of 3rd generation assays is to exclude all antibody negative donors, which will save cost. However, very few validations studies were done in Sub Saharan Africa, where water, electricity and access road pose a serious challenge.

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