Accuracy of 4th generation rapid diagnostic tests for HIV infection: a systematic review and meta-analysis

Protocol
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Background

Over 35 million people were living with HIV in 2013[1]. Rapid scale-up of delivery of antiretroviral therapy (ART) is underway in many countries with the aim of achieving universal access to treatment and prevention by 2015[1]. HIV testing and counselling (HTC) is the entry point to further HIV care and prevention and WHO recommends that individuals who are in a key population for HIV, or come from a population with a generalised HIV epidemic, test for HIV 6-12 monthly (Table 1)[2].

Table 1: World Health Organization recommendations for HIV testing[2]

Populations	Who should test for HIV?
All countries	All individuals attending health facilities with symptoms or signs of HIV infection
Countries with generalised HIV epidemics (HIV prevalence consistently above 1% in pregnant women)	All individuals attending health facilities as part of standard medical care, regardless of presenting condition All adults, 6-12 monthly
Countries with concentrated epidemics (HIV prevalence consistently above 5% in one or more key population, but below 1% in pregnant women)	Individuals from key populations, 6-12 monthly
Countries with low-level epidemics (HIV prevalence less than 5% in any defined sub-population)	Individuals from key populations, 6-12 monthly

However, rates of HIV testing for first-time testers remain suboptimal in most populations, hindering efforts to maximise access to treatment for individuals who are HIV-positive, and to HIV prevention services for those who are HIV-negative. In Saharan Africa, where two-thirds of people infected with HIV live, only half of people living with HIV are aware of their status[3]. In the UK, a country with low-level epidemic, 20% of HIV infected individuals do not know their HIV status[4]. Estimates from sub-Saharan Africa suggest that only 20% of men and women tested for HIV within the previous 12 months, substantially lower than WHO recommendations[3].

Acute HIV infection (the interval between detection of viral HIV-1 RNA and development of antibodies to HIV-1/2)[5] is increasingly associated with high transmission potential for HIV[6]. Acute infection is characterised by presence of HIV nucleic acid and/or HIV-1 p24 antigen. Individuals in the acute stage of HIV infection may experience a flu-like seroconversion illness, which may be missed or mis-classified by health professionals. Acute HIV infection is characterised by high levels of HIV RNA in the individual, which are strongly associated with risk of HIV transmission to partners[6]. Available estimates suggest that between 1% and 20% of incident HIV infections are attributable to transmission from individuals in the acute stage of infection, depending on epidemic stage, coverage of preventive means such as adult male circumcision, antiretroviral treatment as prevention (TasP), and other modelling assumptions[6]. Therefore, people with acute HIV infection are increasingly recognised as being an important population to target for HIV diagnosis and treatment.

For conventional HIV testing and diagnosis in well-resourced settings, a venous blood specimen is collected, processed and referred to a laboratory for testing to detect the presence of antibodies to HIV-1/2, usually by a combination of enzyme immunoassays (EIA) and Western blotting or line immunoassay. However, the laboratory-based nature of these diagnostics frequently results in delays of days before results can be provided and requires the individual to make up to three facility visits to complete the testing process, increasing the risk that they will not receive their result[7, 8].

Rapid diagnostic tests (RDTs) for HIV can be performed by a trained counsellor or health worker i.e. non-laboratory personnel[9]. They are quick (less than 30 minutes to result), relatively cheap, easy to use and interpret (as results are read visually) and require little equipment and no electricity beyond their storage needs in environments exceeding 30°C. RDTs can then be performed in health facilities or in community settings, improving access to HIV testing and rates of completion of HIV diagnosis[8].

HIV RDTs are either immunochromatographic (lateral flow) or use immunofiltration (flow-through) techniques, with assays to detect antibodies to HIV-1/2 infection and/or HIV-1 p24 antigen in finger-prick capillary blood and oral fluid specimens, as well as serum, plasma and venous whole blood specimens[9]. Serological RDTs for HIV-1/2 are categorised by generation, being classified on the basis of antigen and/or conjugate used. First generation assays were constructed using viral lysate as the antigen source and were sensitive, but lacked specificity and were prone to producing false positive results[10]. Second generation assays used synthetic peptides as antigen and third generation assays used recombinant proteins and labelled antigen as conjugate[10]. The first three generations of HIV RDTs detect only antibodies to HIV-1/2[10], thus individuals in the acute stage of infection, who may pose a substantial risk of HIV transmission to others may not be identified.

RDTs can be performed by trained individuals with a high degree of accuracy[10]. WHO sets minimum acceptable criteria for WHO prequalification of HIV-1/2 RDTs, including for sensitivity (≥99%) and specificity (≥98%), as well as for number of other performance (inter-reader variability, invalid rate) and operational characteristics (e.g. ease of use, degree of laboratory infrastructure require, endpoint stability, technical skill required of staff performing tests etc.)[10].

The most recent generation of HIV RDTs (fourth generation) have been developed to detect HIV-1 p24 antigen, as well as the usual HIV-1/2 antibodies tested for in previous generations of RDTs[11]. High HIV-1 viral load in acute infection should also result in high levels of target antigens, however, the duration of detectable p24 antigenemia may vary considerably. In theory, detection of the HIV-1 p24 antigen would allow earlier diagnosis of HIV when the infected individual is in the acute phase of infection and before development of antibodies to HIV-1/2. This could allow early intervention or treatment to prevent transmission of HIV to others[11]. Fourth generation HIV RDTs are currently used in clinical practice in some community and facility settings in developed and developing countries[11]. Most of these programmes use fourth generation HIV RDTs as an initial screening assay, followed by additional testing with two or three serological assays (usually RDTs), depending on the underlying disease prevalence, to confirm those the initial reactive test results. Individuals

with discrepant testing results between the first and second assays are classified as seronegative in low prevalence settings or are reflexed onto a third assay in high prevalence settings. Retesting is recommended at a defined interval e.g. 3 months for those with a non-reactive result but with on-going risk, and 14 days for those with discrepant testing results.

However, a number of studies from different settings have been published with concerning data[11-14], showing the accuracy of the single available 4th generation HIV RDT to be suboptimal for the HIV-1 p24 antigen detection component, with low sensitivity for identification of individuals in the acute phase and other individuals with low p24 antigen titers. Fourth generation HIV RDTs are more expensive than previous third and second generation RDTs and the proclaimed additional advantage of their detection of acute infection could be misplaced.

Study hypothesis

Despite increasing clinical use and expense, the diagnostic accuracy of fourth generation rapid diagnostic tests for detection of HIV-1 p24 antigen as one of the markers of acute HIV infection is suboptimal and may result in excess rates of false reassurance of no HIV infection.

Study design

Systematic review and meta-analysis

Review question

How accurate is the detection of HIV-1 p24 antigen in fourth generation HIV-1/2 rapid diagnostic tests for the diagnosis of acute HIV infection?

How accurate is the detection of HIV-1 p24 antigen and of antibodies to HIV-1/2 in fourth generation HIV-1/2 rapid diagnostic tests, when combined, for the diagnosis of non-acute HIV infection?

Objectives

1. To obtain summary estimates of the diagnostic accuracy of fourth generation HIV-1/2 rapid diagnostic tests for detection of HIV-1 p24 antigen as a marker of acute HIV infection, when compared to a reference result consisting of:
    - 3rd generation HIV-1/2 enzyme immunoassays (RDTs, EIAs or CLIAs); and
    - 4th generation HIV-1/2 enzyme immunoassays (EIAs or CLIAs); and
    - HIV-1 p24 antigen enzyme immunoassay

2. To obtain summary estimates of the diagnostic accuracy of fourth generation HIV-1/2 rapid diagnostic tests for detection of HIV-1 p24 antigen and antibodies to HIV-1/2 as a marker of non-acute infection, when compared to a reference result consisting of:

- 3rd generation HIV-1/2 enzyme immunoassays, (RDTs and/or EIAs or CLIAs), and
- 4th generation HIV-1/2 enzyme immunoassays, (RDTs and/or EIAs or CLIAs).

Definitions

HIV NAT yield	Interval between detectable HIV NAT and detection of antibodies to HIV-1/2
HIV eclipse period	Interval between HIV acquisition and detectable HIV NAT and/or detectable HIV-1 p24 antigen
HIV acute infection	Interval between detectable HIV NAT and detection of antibodies to HIV-1/2
HIV window period	Interval between HIV acquisition and detectable antibodies to HIV-1/2.
Recent HIV infection (incidence)	Diagnosis of recent infection is recommended through an algorithm approach including assays measuring different biological properties of antibody maturation (e.g., antibody proportion, antibody avidity, etc.) in combination with additional information (e.g., p24 antigen, HIV viral load, antiretroviral therapy, CD4 cell count).
Fourth generation HIV-1/2 rapid diagnostic test **(INDEX TEST)**	An immunochromatographic (lateral flow) or immunofiltration (flow-through) rapid diagnostic test for HIV-1/2 infection that detects p24 antigen and antibodies to HIV-1/2 on separate test lines/bands/spots. A reactive test occurs when the test is interpreted as the presence of a visual line/band/spot to either the p24 antigen component, or to the HIV antibody component or to both, in addition to the control line/band/spot.
HIV confirmatory testing algorithm **(Reference standard)**	A series of assays performed for confirmation of HIV infection on specimens also tested with a fourth generation HIV-1/2 rapid diagnostic test. No single standard algorithm for diagnosis of acute HIV infection is internationally accepted, therefore, algorithms that include at least a fourth generation HIV-1/2 antigen/antibody immunoassay and HIV-1 p24 antigen enzyme immunoassay
Fourth generation HIV-1/2	A enzyme immunoassay or chemiluminescent

antigen/antibody immunoassay	immuno assay with capacity to detect both HIV-1 p24 antigen and HIV-1/2 antibodies, usually combined together.
HIV-1 nucleic acid test	A nucleic acid amplification or signal amplification test that detects presence of HIV-1 TNA

† Other tests (such as Western blot) may have been included in confirmatory testing algorithms for acute HIV infection in some studies. However, there is increasing evidence that these confirmatory algorithms may have suboptimal sensitivity as Western blotting is relatively insensitive. Studies including such testing algorithms will be included in the review and will have their reference standard assessed for risk of bias. Where reference standard does not include tests listed above, data will be excluded from analysis.

Searches

Scoping searches have been undertaken to inform the development of the draft search strategy. We will systematically search the MEDLINE and Embase databases using major subject headings and keywords to identify published studies meeting the inclusion criteria that have evaluated the diagnostic accuracy of fourth generation Ag/Ab HIV-1/2 rapid diagnostic tests. The draft search strategy (which may be refined before the final search is performed) is shown in Appendix 1. Additionally, published abstracts of the Conferences on Retroviruses and Opportunistic Infection and International AIDS Society Conferences will be systematically searched.

Types of studies to be included

Cross-sectional studies in which the diagnostic accuracy of fourth generation Ag/Ab HIV-1/2 rapid diagnostic tests were evaluated against the reference standard for HIV-1 p24 antigen, for anti-HIV 1/2 and for HIV infection. Prospective cohort studies and diagnostic case control studies with controls sampled from the same patient population will also be included. We shall also include test accuracy studies performed at the baseline of randomised control trials.

Inclusion criteria

- Studies published between 2005 and 2014 (4th generation Ag/Ab HIV-1/2 rapid diagnostic tests were introduced into clinical practice in 2008)
- Studies that evaluate the diagnostic accuracy of fourth generation Ag/Ab HIV-1/2 rapid diagnostic tests for detection of HIV p24 antigen, for anti-HIV1/2, for HIV infection
- Studies including adult (>15 years old) participants only (alternative HIV diagnosis strategies are recommended for children)

No restrictions will be placed on inclusion by study country, clinical or laboratory setting or the language in which the study is published.

Exclusion criteria

- Studies that do not provide sufficient data to allow estimation of either sensitivity and or specificity of fourth generation Ag/Ab HIV-1/2 rapid diagnostic tests for diagnosis of HIV-1/2 infection
- Studies that are reviews or commentaries, case-studies or case series, editorials, economic analyses, qualitative studies or case control studies with healthy controls.

Methods

Selection of studies

Studies identified by the final search strategy will be imported into a study database (Endnote, Thompsons Reuters).

Two reviewers (PM and EA) will independently review the titles and abstracts of all studies identified by the final search strategy against inclusion and exclusion criteria (phase 1 selection). Studies not meeting inclusion criteria will be discarded. Discrepancies between reviewers will be resolved by group decision with a third reviewer (MT).

Two reviewers (PM and MT) will subsequently independently review the full text of all selected studies (phase 2 selection) using a piloted questionnaire form to determine final selection status for qualitative and quantitative analysis (Appendix 2). The piloted questionnaire will record reasons for exclusion. Discrepancies between reviewers will be resolved by group decision with a third reviewer (EA).

Data extraction

Details of the study will be extracted from the final set of included studies into a piloted data extraction form by two independent reviewers (PM and MT), including:

- Author
- Year study conducted
- Year study published
- Country of study
- Epidemic setting (generalised, concentrated or low-level)
- Study design
- Site of study procedures (laboratory, clinical facility, community)
- Study population characteristics
- Index test characteristics (name of test)
- Conduct of index test
    - Where test was performed and read?
    - Who performed and read test?
    - Were independent test readers used?
    - Did testers and readers receive formal training in test use?
- Conduct of reference standard

- What was the reference standard algorithm used?
- Was reference standard performed in an accredited laboratory with formal external quality assurance?
- Who performed reference standard?
- Did individuals performing reference standard receive formal training in reference standard?

For each study, 2x2 contingency tables will be completed by cross-tabulating test results separately for acute and non-acute HIV infection, where data is available. For each test, all summary parameters of interest (see the list of outcomes) with corresponding measures of variability (95% CIs) will be ascertained or calculated, if reported data permits. Calculated parameters will be marked as 'calculated'.

Data extraction forms will be compared between receivers for each study. Discrepancies will be resolved by reviewing the source data and data extraction sheets as a group with a third reviewer (MT).

Risk of bias and quality assessment

The QUADAS-2 tool will be used to assess risk of bias in included studies. Two reviewers (PM, EA) will independently complete a piloted questionnaire, which includes signalling questions across four domains (selection; index test conduct; reference standard conduct; and participant flow and timing). Completed questionnaires will be compared between reviewers and discrepancies resolved by group discussion with a third reviewer (EA).

A study will be judged to be at overall low risk of bias if responses to all signalling questions give low concerns about bias. If a study has one or more signalling questions where the response indicated likely bias it will be judged to be at high risk of bias. A judgement of 'unclear' will be given where inadequate information to assess risk of bias is available.

Outcome measures

PRISMA guidelines for reporting will be followed.

For both objective 1 and objective 2, the following outcomes measures (and 95% confidence intervals) will be ascertained from the manuscript or calculated:

- Sensitivity
- Specificity
- Positive predictive value
- Negative predictive value

Summary outcome measures and subgroup analysis

We shall first perform descriptive analyses by graphing paired forest plots of sensitivity and specificity and by plotting estimates of sensitivity and specificity in receiver operating characteristic space (ROC space).

If sufficient data is available, meta-analyses will be done to estimate the diagnostic performance of the tests. For both objective 1 and 2, separate summary estimates (and 95% confidence intervals) for sensitivity and specificity will be calculated using bivariate models. The data for the 4th gen tests are categorical; yes (positive) or no (negative); the results of the test depend on the presence or absence of the line. Because we expect the positivity thresholds of the included studies to be similar, we shall use the bivariate model to pool estimates of sensitivity and specificity.

Heterogeneity between studies for both objective 1 and objective 2 will be estimated. For both objectives 1 and 2, stratified summary estimates will be calculate for:

- Study site (laboratory, facility, community)
- Risk of bias (low, or high/uncertain) – see below
- Epidemic setting (generalised, or concentrated/low-level)

If there is sufficient data, evidence of publication bias will be assessed using the Deeks test.

We shall use software, Review manager (RevMan 5.2) for descriptive analyses (to generate forest and summary ROC plots) and to perform the meta-analysis, Analysis will be done using Stata (Statacorp, USA).

Dissemination plan

Study results will be submitted for publication to a high-impact journal with a focus on HIV testing, public health and laboratory science. Additionally, results will be disseminated by presentation at international conferences.

Timeline

	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
Protocol writing and publication									
Systematic literature search									
Review of study abstracts									
Review of full text and data extraction									
Data analysis									
Report writing and									

results dissemination									

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