

Causes of false positive HIV rapid diagnostic test results

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HIV rapid diagnostic tests (RDTs) have enabled widespread implementation of HIV programmes in resource-limited settings. If the tests used in the diagnostic algorithm are susceptible to the same cause for false positivity a false positive diagnosis may result with devastating consequences. In resource-limited settings, the lack of routine confirmatory testing, compounded by incorrect interpretation of weak positive test lines and use of tie-breaker algorithms, can leave a false positive diagnosis undetected. We propose that heightened CD5+ and early B-lymphocyte response polyclonal cross-reactivity are a major cause of HIV false positivity in certain settings; thus test performance may vary significantly in different geographical areas and populations. There is an urgent need for policy makers to recognize that HIV RDTs are screening tests and mandate confirmatory testing before reporting an HIV-positive result. In addition, weak positive results should not be recognised as valid except in the screening of blood donors.

Keywords

HIV, rapid diagnostic test (RDT), false positive, diagnosis, discordant, algorithms, resource-poor setting, resource-limited setting, Médecins Sans Frontières (MSF), humanitarian

Background

HIV rapid diagnostic tests (RDTs) have enabled widespread implementation of HIV programmes and surveillance in resource-limited settings. RDTs can be performed with minimum training, do not require laboratory facilities or expensive equipment and are often supplied as self-contained kits. RDTs improve uptake of test results since testing can be performed at the point of care and the result obtained during a single visit.

The reliability of HIV RDTs has been shown to be equivalent to that of laboratory-based immunoassay methods (apart from during very early seroconversion), and World Health Organization (WHO) guidelines recommend HIV diagnostic algorithms that use only RDTs [1-3]. A minimum of two positive HIV test results, or three where HIV prevalence is <5%, are needed for a positive diagnosis [1,4-6].

False positive results with HIV RDTs have been widely reported and attributed to a variety of causes [7-17]. They usually lead to discordant test results, which delay diagnosis and, if the frequency of discordant results is high, undermine confidence in testing. However, if the tests used in the diagnostic algorithm are susceptible to the same cause for false positivity this may lead to a false positive HIV *diagnosis* with potentially devastating consequences for individuals [18]. In resource-limited settings, the current common lack of routine laboratory confirmatory or follow-up testing means that a false diagnosis may not be detected; therefore specificity in these settings is of far greater importance than in resource-rich settings where a false diagnosis will be quickly discovered during the plethora of testing and review following diagnosis [19].

The WHO/UNAIDS HIV test evaluation programme was developed to provide independent, standardized assessment of HIV tests [2,20-22]. Their data, shown in Table 1, indicate that HIV RDTs do not have 100% specificity. Field trial data (Table 2) often demonstrate lower

specificity than WHO panel results [23]. Even a minor loss of specificity can significantly reduce the positive predictive value of a test when HIV prevalence is low [1,24,25]. This is particularly important if the tests used in a diagnostic algorithm are not independent and/or are susceptible to the same interference.

WHO recommends that HIV RDTs are evaluated at the national level before implementation [1] and there are comprehensive guidelines [6] for development of a diagnostic algorithm for a particular setting. However, implementation of these guidelines is generally beyond the capacity of smaller or less well-resourced programmes or not supported by funding bodies, and in many cases tests are introduced and algorithms formulated without prior local validation [26]. Even if evaluation guidelines can be followed, they assume that the target population remains serologically stable. Médecins Sans Frontières' field experience has been that individual programmes continuing to use the same RDTs can experience significant fluctuations in the frequency and nature of discordant results [27].

A 'test and treat' strategy for well individuals in settings that are hyperendemic for HIV has been much debated [28,29]. If it is widely adopted it could exacerbate the consequences of incorrect HIV diagnoses, with possible treatment toxicity and associated costs added to their already huge personal and social implications [18].

In this article we discuss some of the evidence for commonly cited causes of false positive RDT results for HIV. We hypothesize that, in some settings, false positive results may be more common than the specificities quoted by manufacturers and those determined by the WHO Independent Testing Program suggest [2,20-22], and that they might often be caused by non-specific serological interference. Serological interference may be more common in resource-limited settings, where the testing algorithm is dependent on RDTs alone and where confirmatory testing is usually not available [20-22]. An understanding of the factors

that may influence RDT results is critical to developing reliable HIV diagnostic algorithms in resource-limited settings and to the safety of rolling out wide-scale access to HIV testing and early antiretroviral therapy initiation.

Causes of false positive RDT results

False positive RDT results can be caused by user error such as misinterpretation, clerical mistakes and cross-contamination between blood samples [30-34]. Accuracy in test performance can also be negatively affected by gaps in quality assurance during the manufacturing process, or by manufacturers introducing changes in the source of the antigen/antibody without changing the name of the product. At the field level, training and supervision in association with quality control procedures are critically important in the roll out of these tests. However false positive results caused by cross-reactivity or non-specific serological reactivity/interference will not be correctable by training in correct test use. RDTs and enzyme immunoassays (EIA) share susceptibility to the common possible causes for false positive results, therefore we have included discussion of EIAs where relevant (Panel). It is important to note, however, that HIV RDTs use a restricted target antigen range and are therefore more susceptible than other immunoassays such as western blot and line immune assay (LIA) to producing a false positive test result.

Issues with test manufacture and interpretation

Limited and overlapping target antigens

HIV RDTs use a restricted number of HIV viral target antigens, often the envelope antigens gp160/gp120/gp41 only or these in combination with p24 (HIV1) and/or gp36 (HIV-2), with a positive result indicating the presence of antibodies to any of the included antigens [34,35]. This is in contrast to the multiple distinct bands relating to individual antigens that

are used to define a positive western blot and LIA test. The limited number of target antigens and common signal increases the susceptibility of HIV RDTs to false positive results. WHO testing guidelines recommend standardized testing strategies based on a limited number of tests, to 'maximize the accuracy of test results while minimizing cost' [36]. They also specify that HIV assays included in a testing algorithm should have different antigen preparations and test kit components. However, in practice, detailed information about the antigens in a test is often not readily available and there is likely to be significant overlap between the target antigens used in different brands of test. In addition, manufacturers are increasingly providing semi-finalized or finalized products to re-branders/re-labellers, which makes it difficult to determine an assay's provenance. In at least one case, two identical tests have been marketed under different names and by different distributors: Retroscreen HIV and ImmunoFLOW HIV1-HIV2 [37].

Over-interpretation of weak reactivity

Although weak reactivity has been demonstrated to have low specificity in some settings, manufacturers' instructions commonly direct that any reactivity, weak or strong, should be interpreted as a positive result [7-9]. For example, a study in south-western Uganda [9] reported a false positive rate of 43.9% (129/295 positive results) among 1517 patients using a 'tie-breaker' algorithm (Determine, STAT-PAK, Uni-Gold). Thirty-seven tests were found to have weak bands and exclusion of these results reduced the false positive rate to 2.3% (2/86). 123/129 false positives resulted from Determine HIV-1/2 and Uni-Gold HIV tests. The weak bands were confirmed as false positives on re-assay by an independent laboratory (Centers for Disease Control and Prevention). Similarly a false positive rate of 10.5% in

eastern Democratic Republic of Congo (DRC) was shown to be largely attributable to weak false positive RDT bands (Determine HIV-1/2 and UniGold HIV) [7]. The false positive rate fell from 10.5% to 3.3% when only strong positive results were included.

In a cohort study to determine HIV prevalence in over 15,000 people older than 2 years from south-west Tanzania, Kroidl et al also found very high rates of false positive results using Determine HIV-1/2 and HIV-1/2 STAT-PAK [38]. Most were attributable to weak bands. Only 1/50 Determine tests positive with weak bands on whole blood and 17/267 on plasma were true positives. 55/121 faintly positive on STAT-PAK were true positives. The positive predictive value of the Determine HIV-1/2 in this population was therefore 82.6% in plasma and only 32.9% in whole blood. Interpreting the faint positives as negative would decrease sensitivity for Determine RDT testing of plasma from 100% to 98.6%.

Changing the HIV testing algorithm and the manufacturer's directions to exclude the interpretation of weak bands as a positive result would increase HIV RDT specificity and positive predictive value in many settings. Weakly positive bands may be a result of non-specific serological cross-reactivity, discussed further below. However, false positive results may also present as strongly positive reactions.

Heightened CD5+ B-lymphocyte activation and polyclonal activation

Heightened CD5+ B-lymphocyte activation in the early immune response to infectious disease antigens produces broad-spectrum antibodies that can cause non-specific and unpredictable cross-reactivity in serological testing. Early broad-specificity antibodies can be expected to have low affinity. Bouillon *et al.* [39] report that 69% (299/435) of repeatedly reactive false positive third-generation immunoassay reactions in blood donors could be

abolished by treatment with thiocyanate, which acts to dissociate weak bonding. Treatment with thiocyanate did not affect the reactivity of true HIV-positive samples.

High reported rates of false positive current 3rd and 4th generation immunoassay results among African patients co-infected with a variety of parasites support the suggestion of polyclonal B-cell activation as a cause of false positive reactions.

In the large cohort study from Tanzania discussed earlier in the section on weak bands, an association with lower altitude for Determine HIV-1/2 was significant on multivariable analysis, leading the authors to postulate an association with other infections (more common with higher ambient temperature found at lower altitudes) [38]. However they did not find an association with any specific infection for which they tested, including schistosomiasis. A univariable association with *P. falciparum* infection became non-significant on multivariable analysis. It is of note that half the participants with false positive Determine RDT results were still false positive when re-tested with the Determine RDT one year later.

Human African trypanosomiasis (HAT)

Lejon *et al.* evaluated the effect of HAT infection on HIV test results using serum from patients participating in a treatment study for *Trypanosoma brucei gambiense* in the DRC [40,41]. Samples from before and 24 months after successful treatment were tested for HIV using a range of RDTs; 11/359 patients were diagnosed HIV positive (3.1%) using reference tests. Specificity was 39.1% for Determine HIV 1/2 and 85.3–92.8% for Vikia HIV1/2, ImmunoFLOW HIV1-HIV2, DoubleCheckGold HIV 1&2 and SD Bioline HIV-1/2 3.0 RDTs. A high frequency of indeterminate and false positive results was also found by the reference

tests Vironostika HIV Uni-Form II antigen/antibody (EIA) and Inno-Lia HIV I/II (LIA). After HAT cure, a significant improvement was seen in specificity for three RDTs including Determine (39.1–86.3%), UniGold (96.3–99.4%) and ImmunoFLOW (91.3–96.3%). Specificity of the Vironostika EIA also improved from 67.5% to 98.1%.

While these data could support direct cross-reactivity between HAT and HIV antigens, high levels of polyclonal B-cell activation in early HAT infection could equally be the cause.

Schistosomiasis and other helminth infection

Helminthic infections modulate [42] and stimulate immune activation [43,44] and may thereby constitute a risk for false positive reactions in diagnostic tests, including HIV tests.

Everett *et al* investigated a very high rate of false positive results with 4th-generation Murex HIV Ag/Ab Combination EIA (Abbott) in north-west Tanzania amongst a clinical trial population of young adults (16-27 years old). In addition to clinical and sociodemographic factors, a subset of samples was tested for various parasitic and autoantibodies. In the final multivariate model, independent immunological risk factors for false positive Murex EIA results were increasing levels of certain anti-schistosomal antibodies and a high rheumatoid factor titre (≥ 80), and decreasing levels of other antibodies [45,46]. Testing in older adults from the same region using the Murex assay (as well as Abbott Determine and Trinity Biotech Capillus SR tests) resulted in specificity within manufacturer range [47]. They suggest age-related differences in schistosoma-specific antibody responses or schistosomiasis prevalence may make cross-reactivity less likely with increased age [48,49].

B-lymphocyte activation is generally also more common in adolescents [46]. Furthermore, first-time exposure to schistosomiasis (and other endemic infections such as malaria) is likely to cause an acute immune response resulting in non-specific antibodies that could also

lead to false positive test results. This may also apply to displaced populations who, in a new environment, may have a less well developed immune response to the new local infectious diseases, as has been previously reported [48].

Malaria

Fonseca *et al.* [50] reported a strong correlation between malaria and HIV false positive results in one of three immunoassay tests in a sample population of migrant workers in Brazil; however, other studies have found no such association [45,51,52]. In addition, for two of the three tests, specificity was within the manufacturers' ranges, suggesting a test-specific problem (discussed later in more detail).

In their study reporting an association between malaria infection and false positive 1st and 2nd generation RDT results, Gasasira *et al.* [16] also found a strong association between younger age and false positive immunoassay and indeterminate western blot reactions, noting that younger persons with a 'less developed immune response to malaria are more likely to exhibit non-specific B-cell stimulation'.

Environmental factors

Populations in resource-limited settings are more likely to have heightened B-lymphocyte activation than those in developed countries [44,53]. Clerici *et al.* [43] reported that both Ugandans and Italians living in Uganda have a heightened immune activation that reduces to 'European' levels when these individuals take up residence in Italy. Immune activation may be directly related to environmental factors such as poor hygiene or dietary limitations or exposure to endemic infections [43,44].

Meles *et al.* [54] report a correlation between HIV RDT false positivity and low haemoglobin. However the authors note that this may also be associated with poverty, in that poverty is

likely to be associated with increased exposure to infections and hence an increased level of CD5+ B-cells.

The implication for HIV diagnostics is that patients in resource-limited settings are likely to have an augmented and broader range of cross-reacting antibodies. If this is the case then, while co-infection will likely increase the occurrence of false positive test results, the effect is indirect and not caused by cross-reactivity with a specific antigen present in the infectious agent.

Genetics

Genetic difference could be another possible factor in the higher rates of false positive RDT results observed in African settings. Hill *et al.* [55] reported extensive HLA class II DR-DQ polymorphism in The Gambia and Malawi and, citing other reports, stated that Africans have a greater HLA diversity and more class II haplotypes than Caucasians, Asians, Indians and Pacific Islanders. Extensive HLA class 1 polymorphism has also been reported in Africans [56]. The degree of similarity between a pathogen antigen and host HLA antigens will increase or decrease the level of immune response [57]. HLA polymorphism modifies the immune response to tuberculosis, leprosy, malaria, *Klebsiella*, *Bartonella henselae*, *Chlamydia*, *Shigella*, *Yersinia*, schistosomiasis, Chagas disease, dengue fever, HIV, HTLV-1, hepatitis B and hepatitis C, and may act alone or in combination with other genes conferring susceptibility to, or protection against, infectious diseases [57].

Since different populations will have different HLA polymorphisms, and therefore different responses to non-HIV infectious diseases, the nature and frequency of cross-reactive antibodies can also be expected to be population dependent. In particular the performance of HIV diagnostic tests in Caucasian populations cannot be extrapolated to non-Caucasian populations. For example Santos *et al.* [58] reported that the frequency of indeterminate

western blot reactivity varies significantly between regions and populations: 0.14%, 0.5%, 1.6%, 4.3% and 68.4% for studies in the USA, West Indies, rural Cameroon, Brazil and DRC, respectively [6]. Similarly Clark *et al.* [24] report using two tests, both with reported specificities of >99%, that gave positive predictive values of 100% and 62.8%, respectively, in a sub-population in Peru with HIV prevalence of 1.6%.

Contamination

False positive reactions may be caused by contamination by bacterial proteins (such as *Escherichia coli*) during the synthesis of recombinant HIV antigens used in HIV RDTs [24,59]. This was observed in the development stage of one recombinant HIV antigen analyzed by mass spectrophotometry (Derryck Klarkowski, personal observation). This is unlikely to be problematic when stringent procedures are used to purify the target antigens, but is a potential cause of false positive reactions if poor quality tests are used in resource-limited settings where exposure to contaminated water sources is increased.

Unlikely causes of false positive HIV RDT results

Understanding the causes of false positive results is critical to effective programme management and patient care. However, reported causes have often been based on data with limited validation or are out-dated and unlikely to apply to current HIV RDTs. These postulated causes of false positive results with limited evidence are summarized in Table 3. The reports can be categorized as historical literature relating to first-generation immunoassay testing; studies over-generalizing problems with specific test formats or brands; reports later withdrawn or corrected; insufficient evidence; and theoretical risk.

First-generation immunoassay testing

Reports of false positives with first-generation immunoassays (associated with blood transfusion, chronic hepatic disease, pregnancy, leprosy and syphilis) are not relevant to

current testing. Antigens used in manufacture of the tests were produced using viral lysate and processing commonly involved use of H9 cell lines, which resulted in some HLA class II antigens from the cells contaminating the antigen [1,60,61].

Pregnancy

Pregnancy is one of the most commonly listed causes of HIV false positive reactions. First-generation immunoassays were susceptible to allo-immunization in pregnancy cross-reacting with contaminating cellular proteins from the cells used to culture the HIV virus [14,24,62-65]. An association between current pregnancy and false positive HIV tests or between parity and false positive results for newer tests has not been demonstrated. Recent studies suggest that the rate of false positives may be similar to that in other groups and that the relatively high number of false positive results reported among pregnant women is a function of universal screening and the low overall incidence of HIV infection in pregnant women [63,64,66].

Over-generalization

Problems can and do occur with specific test formats that are either resolved by the manufacturer or result in the test being withdrawn. Such reports should be treated with caution and not be cited as a general cause of false positive results.

For example, HIV false positive results related to an influenza vaccine in 1990 [67] were caused by a design defect, subsequently rectified, in the tests of a single manufacturer [13,68,69]. We can find no data to support direct cross-reactivity between influenza vaccination antigens as a direct cause of HIV RDT false positives, nor other HIV serological tests. However, a recent report refers to influenza vaccination as a 'known cause of indeterminate results ... for HIV antibodies' [15].

Similarly Fonseca *et al.* [50] reported a strong correlation in a Brazilian cohort between the anti-*Plasmodium falciparum* antibodies and HIV western blot *gag* reactivity. They also demonstrated that absorption with *P. falciparum* antigen removed the reactivity in seven out of nine cases. However, the false positive results occurred with only one of three immunoassay tests used, suggesting a test-specific problem. Ribeiro *et al.* [70] investigated serologic reactivity among Brazilians in Bahia diagnosed with tropical infections. A second generation immunoassay (Du Pont HIV-1) was positive in 9/100 samples from patients diagnosed with visceral leishmaniasis, all of which were negative by western blot. This test has now been discontinued.

Reports later withdrawn or corrected

Pearlman and Ballas reported a single case in which a patient presented with a false positive immunoassay result 6 weeks after receiving a rabies vaccination [71]. The patient also developed a transient indeterminate western blot. Subsequently Plotkin *et al.* tested 50 patients 2–4 weeks after rabies vaccination with no reported false positivity [72]. This finding was challenged by one of the original authors on the basis that false positivity is only likely to develop at around 6 weeks post-vaccination. To clarify the issue Henderson *et al.* [73] repeated the protocol of Pearlman and Ballas [71] with 14 volunteers and found no false positive results. Although rabies shares glycoprotein sequences with gp120 [12], the evidence appears to be against consistent false positivity being caused by the antigens used for rabies vaccination.

Evidence from the literature of other vaccinations as a cause for false positive immunoassay results is limited to a case control study from Brazil that investigated an association between increased rubella vaccination and an increase in false positive HIV tests among blood donors in São Paulo [74].

Withdrawn or corrected reports can be particularly problematic when the modification is published by a different author or in a different journal. In the case of the influenza vaccination discussed earlier (in '*Over-generalization*'), the original report was published in 1991 by MacKenzie *et al.* [67] and the correction by Buffington *et al.* [13] in 2004.

Insufficient evidence

This category includes reports with insufficient data and isolated reports that have not been corroborated by observations in other settings.

Examples include reports of false positive results caused by dengue (one publication, n=9) [75]; hepatitis B (one publication, n=20 [76]); retroviruses (one publication [77] but no evidence reported in two publications [14,78]); rabies vaccination (as described above) [73]; a recent case report describing a false positive third-generation immunoassay result and a negative western blot in a patient admitted with visceral leishmaniasis [79].

Theoretical risks

Reports in this group primarily relate to situations where there is peptide homology between HIV target antigens and infectious agents. Examples of proposed theoretical risk of interference include that from *Candida* [80]; HTLV-1 [81]; picornaviruses [59] and *Trichomonas* [82]. There appear to be no data supporting interference with HIV testing by these infectious agents in actual testing practice. Further an additional modulating factor would need to be involved to account for the absence of positive reactions among all patients with a given infection.

Cross reactivity between antibodies to *Schistosoma mansoni* and HIV-1 peptides has been reported in one publication but further studies to investigate the association have not been published [83].

Herpes simplex represents an interesting variation as Langedijk *et al.* [17] suggested homology between herpes and p24 antigen immobilized on a nitrocellulose matrix, which could have implications for some HIV RDT tests. However there appears to be no follow-up of this observation.

A further theoretical risk for sporadic or unexpected cross-reactivity is that HIV viral antigens processed by humans and bacteria have different characteristics that can potentially affect test performance, and this is relevant as the recombinant antigens used in RDTs are produced using bacteria. Craske *et al.* [84] report that differences in protein modification between eukaryotic and prokaryotic cells will produce pseudo-epitopes that are not related to the HIV antigen and that could cross-react with non HIV antibodies. A further potential problem with recombinant and peptide antigens is that, because the sequences are short, they may have a different tertiary structure to the same sequence as part of the much larger native protein and thereby form unexpected epitopes [59]. Both of these effects create the potential for unpredictable cross-reactivity.

Conclusions

HIV RDT results may vary significantly in different geographical areas, among different populations and over time [27]. Evaluation of tests with the use of a national serobank to guide algorithm development (as per current WHO guidelines) would go some way towards addressing this problem, however, the evaluation programme required is beyond the capacity of some countries and will not pick up variation between populations within a

country. Validation at programme level may quickly become invalid with population changes, in transient populations or during outbreaks of infectious disease [45]. In addition to population changes, the rapid rate of development of new tests and discontinuation of older ones may mean that algorithms become quickly outdated, necessitating repeats of the validation process. Furthermore, the shift towards increased sensitivity in new tests in response to the focus on early detection has led to inclusion of IgM detection and p24 antigen, which may increase the potential for non-specific reactivity [19,40,85,86]. While this is likely to be of minimal significance in settings in which confirmatory testing is done routinely, it is likely to have major consequences in resource-limited settings where confirmation is rare.

Our analysis of the literature suggests that HIV false positive results with current tests are more likely caused by polyspecific antibodies resulting from an independent infection than by direct antibody cross-reactivity with an independent infectious agent. We propose that early B-lymphocyte response/polyspecific cross-reactivity can be a significant cause of HIV false positive results, with the implication that test characteristics may vary significantly in different geographical areas and among different populations. Factors that may also be involved include differences in HLA polymorphism modulating the nature and frequency of cross-reactive antibodies in different populations, and pseudo-epitopes created in HIV RDT manufacture.

The current widespread use of a tie-breaker algorithm, where two positive tests and one negative test are interpreted as an overall positive result, is highly susceptible to false results caused by cross-reactivity. The finding of both positive and negative results for the same blood sample is an alert to potential cross-reactivity and should not be followed by a single, deciding 'tie-breaker' test which may also be subject to the same cross-reactivity.

Any potential advantage of the use of tie breaker algorithms by reducing loss to follow-up is lost when balanced against the consequences of false positive HIV test results.

We also propose that many of the commonly cited causes of false positive HIV tests, in particular blood transfusion, hepatitis, malaria, pregnancy and vaccination, are unlikely to cause direct interference with current HIV RDTs. Other sources of confusion include over-generalization of a problem related to a specific brand of test; reports later withdrawn or corrected; insufficient evidence, particularly insufficient sample size; and theoretical cross-reactivity not reported as problematic in actual case studies.

Although the social and personal consequences of false positive HIV tests and diagnosis are widely recognised, RDT false positive results are often dismissed as insignificant in studies from resource-rich settings. This stems from the perspective that these tests should be considered 'preliminary positives', needing confirmation with more specific laboratory based testing, or that false positive results will be quickly detected as part of subsequent viral load tests. However, in most resource-limited settings, confirmatory laboratory-based tests are not readily available, and it may be in these settings that the consequences of a false positive diagnosis are most serious.

HIV RDTs, despite their name, are screening rather than diagnostic tests and are clearly indicated as such by their manufacturers. We propose that the specificity of HIV RDT algorithms would be significantly improved by the universal implementation of confirmation testing. Confirmatory tests will not resolve all situations. However they do provide a safeguard for cross-reactivity against a single antigen (such as gp41) and are therefore a significant improvement on no confirmatory testing [7,18].

In order for this to be possible, there is an urgent need for the development of simpler and cheaper confirmatory tests. Where confirmatory testing is not yet implemented, immediate

measures to reduce the risk should be introduced: diagnostic algorithms and manufacturer instructions should be changed to state that weak positive results are indeterminate and require further testing; and the use of tie-breaker algorithms should be discontinued.

Expert commentary

We propose that early B-lymphocyte response/polyspecific cross-reactivity can be a significant cause of HIV false positive results, with the implication that test characteristics may vary significantly in different geographical areas and among different populations. Therefore current algorithms that rely on evaluation of HIV RDTs with the use of a national serobank to guide their development will be inadequate. Furthermore, the shift towards increased sensitivity in new RDTs in response to the focus on early detection in 'treatment as prevention' strategies may increase the potential for non-specific reactivity which may have major consequences in resource-limited settings where confirmation testing is uncommon. The strengthening of HIV testing algorithms, including the implementation of field serological confirmatory testing, is important, particularly in settings where heightened CD5+ and polyclonal B-lymphocyte activation is likely such as where population changes occur, in transient populations or during outbreaks of infectious disease. This dictates the universal implementation of HIV confirmation testing and the exclusion of interpreting weak positive reactions as positive during screening.

Five-year view

Over the next 5 years the use of HIV RDTs will increase in resource-limited settings as the focus of HIV programmes moves to 'treatment as prevention' with widespread community HIV testing. Especially as lower HIV prevalence populations become routinely tested, this will increase the number of people falsely diagnosed with HIV using current RDTs and HIV

testing algorithms. Recognition of this problem will hopefully lead to greater implementation of cheap, easy-to-use, highly specific point-of-care HIV confirmation tests in improved diagnostic algorithms that will minimise this risk but still allow access to HIV testing for the millions of people who need it.

Key issues

- HIV rapid diagnostic tests (RDTs) have enabled widespread implementation of HIV programmes and surveillance in resource-limited settings, but false positive results from HIV RDTs can go undetected in these settings because of the lack of routine confirmatory testing
- Interpretation of weak positive test lines as positives instead of indeterminate increases the risk of falsely diagnosing HIV on RDTs. HIV RDTs use a restricted number of viral target antigens, increasing susceptibility to false positive results
- The tie-breaker algorithm is highly susceptible to error when false positives are caused by cross-reactive antibodies and should be abandoned
- The shift towards increased sensitivity in new tests in response to the focus on early detection (in 'treatment as prevention' strategies) has led to inclusion of IgM detection and p24 antigen, which may increase the potential for non-specific reactivity
- Many repeatedly cited causes of false positive results are based on data with limited validation or are out-dated and unlikely to apply to current HIV RDTs. These include false positive results caused by influenza vaccination, pregnancy and blood transfusion

- Heightened CD5+ B-lymphocyte activation in the early immune response to infectious disease antigens produces broad-spectrum antibodies that can cause non-specific and unpredictable cross-reactivity. High rates of false positive immunoassay results among African patients co-infected with a variety of parasites support polyclonal B-cell activation as a cause of false positivity
- Populations in resource-limited settings are more likely to have heightened B-lymphocyte activation than those in developed countries due to environmental factors; we propose that early B-lymphocyte response/polyspecific cross-reactivity can be a significant cause of HIV false positive results in some settings
- Genetic difference (higher rates of HLA polymorphism) could be another factor in some settings
- HIV RDT results may thus vary significantly in different geographical areas and among different populations
- Strengthening of HIV algorithms and the implementation of confirmatory testing that are feasible for use in resource-limited settings are urgent priorities
- There is an urgent need for the development of simpler and cheaper confirmatory tests

Conflicts of interest

The authors declare they have no conflicts of interest.

Author contributions

DK was the originator of the article, did the first draft and literature search.

KS analysed and synthesised information and completed the second draft of the manuscript.

DOB worked on subsequent drafts.

LS worked on subsequent drafts.

All authors approved the final version of the article.

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Table 1. Reported WHO parameters for a selection of commonly used HIV rapid diagnostic tests (RDTs) [2]

| Simple/rapid assay | Manufacturer | Sensitivity (%) (95% CI) | *Specificity (%) (95% CI) |
|--------------------------------|---|--------------------------|---------------------------|
| Determine HIV-1/2 | Abbott Laboratories, Wiesbaden-Delkeheim, Germany; Dainabot, Osaka, Japan | 100 (95.5–100) | 99.4 (96.7–100) |
| Uni-Gold HIV | Trinity Biotech, Bray, Ireland | 100 (95.5–100) | 100 (97.9–100) |
| Capillus HIV-1/HIV-2 | Trinity Biotech | 100 (95.5–100) | 100 (97.9–100) |
| SD Bioline HIV-1/2 3.0 | Standard Diagnostics, Hyderabad, India | 100 (97.7–100) | 99.3 (97.6–99.9) |
| Genie II HIV-1/HIV-2 | Bio-Rad, Hercules, CA, USA | 100 (97.7–100) | 99.7 (98.1–100) |
| First Response HIV-1/HIV-2 WB | PMC Medical Pty., Daman, India | 100 (95.5–100) | 98.8 (95.8–99.9) |
| HIV-1/2 STAT-PAK | Chembio Diagnostic Systems, Medford, NY | 98.2 (96.6–99.2) | 99.3 (98.1–99.9) |
| OraQuick HIV-1/2 | OraSure Technologies, Bethlehem, PA, USA | 98.1 (94.5–99.6) | 100.0 (98.8–100) |
| Retrocheck HIV WB/Core HIV 1&2 | Qualpro Diagnostics, Goa, India; Core Diagnostics, Birmingham, UK | 100 (98.8–100) | 99.1 (97.8–99.8) |

*Note the lower bound of the confidence interval, which is frequently overlooked.

Table 2. Field reports on commonly used HIV RDTs

| Test (sample) | Location | No. of tests Total (pos) | Specificity (%) (95% CI) | Reference |
|--|-------------------------|--------------------------|--------------------------|---|
| Capillus HIV-1/HIV-2 (serum) | Kisumu, W Kenya | 754 (409) | 99.4 (97.9–99.8) | Zeh <i>et al.</i> 2011 [87] |
| Capillus (whole blood) | NW Tanzania | 789 (145) | 99.7% (98.9–100%) | Everett <i>et al.</i> 2009 [47] |
| Stat-Pak HIV 1/2 (serum/plasma, whole blood) | USA | 439 pos, 5789 (280) | 99.9 (99.6–100) | Delaney <i>et al.</i> 2011 [88] |
| Stat-Pak HIV 1/2 (plasma) | Mbeya, Tanzania | 13,139 (1170) | 99.3% (99.1–99.4) | Kroidl <i>et al.</i> 2012 [38] |
| Stat-Pak HIV 1/2 (serum) | Rakai, Uganda | 150 (99*) | 99.1 (95.3–99.9) | Kagulire <i>et al.</i> 2011[8] |
| Stat-Pak HIV 1/2 (serum) | East Kasai, DRC | 359 (11) | 98.3 (96.9–100) | Lejon <i>et al.</i> 2010 |
| Determine HIV 1/2 (serum) | Kisumu, W Kenya | 753 (409) | 99.1 (97.5–99.7) | Zeh <i>et al.</i> 2011 [87] |
| Determine HIV 1/2 (serum) | Rakai, Uganda | 150 (99*) | 85.2 (77.4–91.1) | Kagulire <i>et al.</i> 2011 [8] |
| Determine HIV 1/2 (whole blood) | Northern Malawi | 2099 (815) | 97.2 (96.7–98.1) | Molesworth <i>et al.</i> 2010 [89] |
| Determine HIV 1/2 (NS) | Blantyre, Malawi | 200 (100) | 100 (96.4–100) | Piwowar-Manning <i>et al.</i> 2011 [90] |
| Determine HIV 1/2 (NS) | Lilongwe, Malawi | 200 (100) | 100 (96.4–100) | Piwowar-Manning <i>et al.</i> 2011 |
| Determine HIV 1/2 (NS) | South Africa | 203 (102) | 100 (96.4–100) | Piwowar-Manning <i>et al.</i> 2011 |
| Determine HIV 1/2 (NS) | Zambia | 200 (100) | 98.1 (93.1–99.8) | Piwowar-Manning <i>et al.</i> 2011 |
| Determine HIV 1/2 (NS) | Zimbabwe | 200 (100) | 99 (94.6–100) | Piwowar-Manning <i>et al.</i> 2011 |
| Determine HIV 1/2 (whole blood) | Dar Es Salaam, Tanzania | 2433 (390) | 99.6 (99–99.9) | Lyamuya <i>et al.</i> 2009 [91] |
| Determine HIV 1/2 (whole blood) | Kampala, Uganda | 940 (45) | 96.2 (94.7–97.3) | Eller <i>et al.</i> 2007 [92] |
| Determine HIV 1/2 (serum) | East Kasai, DRC | 359 (11) | 39.1 (33.9–44.2) | Lejon <i>et al.</i> 2010 [40] |
| Determine HIV 1/2 (serum) | East Kasai, DRC | 162 (1) | 86.3 (81–91.7) | Lejon <i>et al.</i> 2010 [40] |
| Determine HIV 1/2 (whole blood) | NW Tanzania | 789 (145) | 99.7 (98.8 to 100%) | Everett <i>et al.</i> 2009 [47] |

| | | | | |
|--|-----------------|---------------|---------------------|----------------------------------|
| Determine HIV 1/2 | Rakai, Uganda | 1000 (93) | 91.7% (90-93.4) | Singer <i>et al.</i> 2005 [93] |
| Determine HIV 1/2 (plasma) | Mbeya, Tanzania | 12916 (1184) | 97.87 (97.59-98.12) | Kroidl <i>et al.</i> 2012 [38] |
| Determine HIV 1/2 (whole blood) | Mbeya, Tanzania | 1696 (26) | 96.83 (95.87-97.61) | Kroidl <i>et al.</i> 2012 [38] |
| First Response (serum) | Rakai, Uganda | 150 (99*) | 97.4 (92.6-99.5) | Kagulire <i>et al.</i> 2011 [8] |
| HIV (1+2) Rapid Test Strip (plasma) | Cameroon | 446 (187) | 98.8 (96.6-99.6) | Aghokeng <i>et al.</i> 2004 [92] |
| HIV-SPOT (plasma) | Ethiopia | 12 124 (1437) | 99.5 (99-99.8) | Meless <i>et al.</i> 2002 [94] |
| ImmunoComb II HIV 1 & 2 (plasma) | Cameroon | 446 (187) | 89.6 (85.3-92.7) | Aghokeng <i>et al.</i> 2004 [92] |
| OraQuick Advance HIV-1/2 (oral fluid) | SE Zimbabwe | 584 (174) | 100% | Pascoe <i>et al.</i> 2009 [95] |
| OraQuick Advance HIV-1/2 (whole blood) | Kampala, Uganda | 940 (45) | 99.8 (99.1-99.9) | Eller <i>et al.</i> 2007 [90] |
| OraQuick Advance HIV-1/2 (serum) | East Kasai, DRC | 359 (11) | 98 (96.5-99.5) | Lejon <i>et al.</i> 2010 [40] |
| OraQuick Advance HIV-1/2 (serum) | East Kasai, DRC | 162 (1) | 99.4 (98.2-100) | Lejon <i>et al.</i> 2010 [40] |
| Retrocheck HIV (plasma) | Cameroon | 446 (187) | 98.5 (96.1-99.4) | Aghokeng <i>et al.</i> 2004 [92] |
| SD Bioline HIV 1/2 (Standard Diagnostics, Hyderabad, India) (plasma) | Cameroon | 446 (187) | 92.7 (88.8-95.3) | Aghokeng <i>et al.</i> 2004 [92] |
| Uni-Gold (serum) | Rakai, Uganda | 150 (99*) | 97.4 (92.6-99.5) | Kagulire <i>et al.</i> 2011 |
| Uni-Gold (serum) | East Kasai, DRC | 359 (11) | 96.6 (94.6-98.5) | Lejon <i>et al.</i> 2010 |

*Extrapolated (not given in paper); NS: sample not specified. DRC=Democratic Republic of Congo.

Panel: Evolution of HIV testing

| Generation | 1 st (1985) | 2 nd (1987) | 3 rd (1991) | 4 th (1997) |
|----------------------|---|---|---------------------------|--|
| Antigen | Viral lysate | Viral lysate | Recombinant & synthetic | Recombinant & synthetic |
| Detects | Antibody (immunoassay)* | Antibody (immunoassay) | Antibody (immunoassay) | Antibody/antigen (combined) |
| Conjugate | Antibody | Antibody | Antigen (‘sandwich’) | Antigen/antibody (2 different assay formats combined) |
| Window period | 8-10 weeks | 4-6 weeks | 2-3 weeks | 2 weeks |
| Rapid tests** | No | Yes | Yes | Yes |
| Problems | Antigen preparation e.g. Contamination (with proteins from cells used to culture virus causing false positives) | Contamination (bacteria derived antigen preparations) | Non-specific reactivity | Combination of assay formats to maximise sensitivity. Reduces specificity due to non-specific reactivity especially if common signal |

***Immunoassay** – an antigen is used to react with antibodies raised against the infecting HIV of an infected person, which is detected in various ways. Most common in HIV testing is an enzyme immunoassay (EIA) in which an enzymatic reaction is used to create a signal on the attaching antibody. Other types include chemiluminescent microparticle immunoassay (CMIA). A western blot is another type of immunoassay in which proteins are separated, resulting in distinct bands rather than a common signal.

Four generations of immunoassay have been used for screening and diagnosis since 1985 when commercial immunoassays for HIV detection first became available.

****Rapid test (RDT)** use rapid or short incubation times allowing rapid results and point of care diagnosis. HIV rapid tests discussed here are all immunoassays and are therefore subject to the same problems and potential sources of error as other immunoassays.

Table 3. Postulated causes of false positive results in HIV RDTs with limited supporting evidence

| Cause | | 1 st G | OG | W | ID |
|---------------------------|--|-------------------|----|---|----|
| Anaemia | Correlation reported but more likely associated with poverty [94] | | | | ✓ |
| Blood transfusion | Historic; publications related to first-generation immunoassay only [96,97] | ✓ | | | |
| <i>Candida</i> infections | Evidence of antigen homology with gp120 [80]. No supporting data of actual problems with HIV RDTs | | | | ✓ |
| Chagas | Very limited data. No correlation between Chagas and 118 indeterminate WB cases [58] | | | | ✓ |
| Dengue fever | Very limited data. Six unidentified HIV RDTs against nine patients with dengue fever. Two tests gave false positive results (4/9, 2/9 patients) [75] | | | | ✓ |
| Leprosy | Historic; publications related to first-generation immunoassay only [10] | ✓ | | | |
| Helminths | Very limited data [42-44]. No association shown with indeterminate WB in one publication [54] | | | | ✓ |
| Chronic hepatic disease | Very limited data restricted to 1988 related to first-generation immunoassay [98]. A possibility that increased globulins could cause interference but no reported data for HIV RDTs [99] | ✓ | | | |
| Hepatitis A | One study showed no false positive tests with 10 hepatitis A samples [51] | | | | ✓ |
| Hepatitis B | No false positives reported from 10 serum samples with antibodies to hepatitis B tested with Determine and Capillus tests [51]; 191 immunoassay positives including 118 indeterminate WB reactions [58] and 206 samples from persons with repeatedly reactive immunoassay and a control population [14] | | | | ✓ |
| | One paper reported hepatitis B as the cause of two false positive HIV tests [76] but provides no evidence; other causes may equally have caused the false positivity | | | | ✓ |
| Herpes | Celum <i>et al.</i> [14] reported no correlation between herpes simplex virus type 2 and HIV in 206 cases with repeatedly reactive immunoassay and a control population. Suggested homology between herpes and p24 antigen that is immobilized on a nitrocellulose matrix, which could have implications for some HIV RDT tests [17] | | | | ✓ |

| Cause | | 1 st G | OG | W | ID |
|--|--|-------------------|----|---|----|
| HTLV 1/2 | HTLV-1 and HIV share a closely related gp24 antigen [81] and come from the same viral family. Early reports suggested possible cross-reactivity with HTLV-1/2 [11,58] (and the related animal lentiviruses) [77], but consensus now is that cross-reactivity between HTLV-1/2 and HIV is at best very uncommon | | | | ✓ |
| Leishmaniasis | Insufficient evidence with a single immunoassay false positive [39,79]; report of problems with an EIA test later withdrawn [70] | | | | ✓ |
| Myeloma | Very limited data related to first-generation immunoassay [99]. Possibly related, Melles <i>et al.</i> [54] report a correlation between HIVSPOT and heavy smoking, and speculate that the higher plasma viscosity in smokers may cause interference with RDTs | ✓ | | | |
| Picornaviruses | Very limited data. Picornaviruses are reported to be widespread and cause annual occurrences of gastrointestinal and respiratory influenza [59]. Some studies suggest a possible homology between HIV and picornaviral proteins [100] but there appears to be no evidence this is a cause of HIV false positives | | | | ✓ |
| Pregnancy | Historic; publications related to first-generation immunoassay only [66] | ✓ | | | |
| Retroviruses: bovine, caprine, feline | Other retroviruses within the lentivirus group have been suggested as a source of cross reactive antibodies; supportive evidence includes the existence of analogous glycoprotein sequences and observation of crossed antigenic reactivity summarized by Tesoro-Cruz <i>et al.</i> [77]. However cross-reactivity, even it occurred, is likely to be in the <i>gag</i> region and therefore unlikely to affect HIV RDT testing. No association found between indeterminate HTLV-1, bovine immunodeficiency virus and bovine and feline leukemia virus WBs and false positive immunoassay results in two reports [14,101]. | | | | ✓ |
| Syphilis | No HIV false positives were reported in studies by Manca <i>et al.</i> (n=318) [102]; Celum <i>et al.</i> (n=206, STIs); Lien <i>et al.</i> [51] (10 syphilis and 30 high-risk STI panels). | ✓ | | | |
| <i>Trichomonas</i> | Fiori <i>et al.</i> [82] reported that although anti-gp41 can cross-react with the human form of alpha-actin only 3/140 sera containing anti- <i>Trichomonas</i> alpha-actin antibodies reacted with two immunoassay tests (2.8% false positive rate) and conclude that this data do not support cross-reactivity between <i>Trichomonas</i> and HIV testing | | | | ✓ |
| Tuberculosis (<i>Mycobacterium tuberculosis</i>) | Despite the high incidence of tuberculosis in populations being tested for HIV, cross-reactivity has not been reported. Meles <i>et al.</i> [54] reported no association with tuberculosis in an Ethiopian study of indeterminate WB cases (n=91) | | | | ✓ |

| Cause | | 1 st G | OG | W | ID |
|-------|--|-------------------|----|---|----|
| | | | | | |

1stG=restricted to first-generation immunoassay testing. OG=over-generalization of specific test or brand false positives. W=reports corrected or withdrawn. ID=insufficient data to establish validity. WB=western blot.