

p24 revisited: a landscape review of antigen detection for early HIV diagnosis

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Despite major advances in HIV testing, early detection of infection at the point of care (PoC) remains a key challenge. Although rapid antibody PoC and laboratory-based nucleic acid amplification tests dominate the diagnostics market, the viral capsid protein p24 is recognized as an alternative early virological biomarker of infection. However, the detection of ultra-low levels of p24 at the PoC has proven challenging. Here we review the landscape of p24 diagnostics to identify knowledge gaps and barriers and help shape future research agendas. Five hundred and seventy-four research articles to May 2018 that propose or evaluate diagnostic assays for p24 were identified and reviewed. We give a brief history of diagnostic development, and the utility of p24 as a biomarker in different populations such as infants, the newly infected, those on preexposure prophylaxis and self-testers. We review the performance of commercial p24 assays and consider elements such as immune complex disruption, resource-poor settings, prevalence, and assay antibodies. Emerging and ultrasensitive assays are reviewed and show a number of promising approaches but further translation has been limited. We summarize studies on the health economic benefits of using antigen testing. Finally, we speculate on the future uses of high-performance p24 assays, particularly, if available in self-test format.

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Introduction

With adoption by the United Nations General Assembly of the political declaration *On the Fast Track to Accelerating the Fight against HIV and to Ending the AIDS Epidemic by 2030* there has been a major shift in the field of HIV

medicine from optimization of palliative control, to eradication [1,2]. UNAIDS has set ambitious targets towards this end, which include goals that 90% of people living with HIV know their status by 2020, and 95% by 2030 [2,3]. Improved testing methods including detection earlier in infection, better test accuracy, increased

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self-testing, and robust linkage to care have all been highlighted as key areas for improvement [4–6].

Currently, only an estimated 46% of people living with HIV know their status [7]. Early detection has been proven to allow for better patient outcomes and lower rates of transmission [8,9]. Extending the window of detection facilitates prompt linkage to pathways of clinical care, and would also allow for accurate diagnosis of infant HIV; currently around half of HIV-exposed infants are appropriately tested with a virological assay and one-third of those who require antiretroviral therapy receive it [10–13]. Current alternative solutions include the use of dry blood spots to transfer samples to distant high-throughput laboratories, and the need for a more accessible and affordable pathogen-based point-of-care (PoC) assay for early infant diagnosis (EID), especially in resource-limited settings, remains [11,14,15].

In this review, we present outcomes of research pertaining to use of p24 as a biomarker including a short history of p24 diagnostics, use of antigen assays for early detection, characteristics of commercial antigen assays, and we discuss reasons why detection of p24 is challenging and complex. We briefly review research laboratory-stage techniques and health-economic evaluations of antigen-detection assays and present the outlook for these in the light of emerging trends in HIV research and care. This review is focussed on assays for p24 antigen as other reviews of current and emerging molecular diagnostics for HIV are existent, including articles that specifically address their suitability for resource-limited settings [16–19].

Diagnostics for HIV: a brief history

The first Food and Drug Administration (FDA)-licensed HIV test was an ELISA in 1985 [20], followed by the rapid development of immunofluorescence assays [21], agglutination and dot blot tests [22,23]. Some early tests (e.g. the HIVCHEK; Du Pont de Nemours, USA; [24]) and the Single Use Diagnostic System (Murex, Norcross, Georgia, USA [25]) could be performed in 5–20 min, though results required trained interpretation. In November 2002, the OraQuick Rapid HIV-1/2 Antibody Test was FDA-approved and was CLIA-waived shortly after in January 2003, permitting diagnosis of HIV in a nonclinical setting [26]. Detection of anti-HIV antibodies by rapid PoC test remains the mainstay of testing algorithms across many settings, with high levels of the target molecules (up to mg/ml) and generally good specificity [27,28]. Algorithms that use multiple PoC tests have been shown to be accurate, reliable and cost-effective when compared with laboratory-based tests [29,30]. These tests are unable, however, to accurately diagnose infection in infants prior to clearance of maternal antibody (transmitted prenatally and in breast

milk), in those who have not yet seroconverted [31,32] and sporadic cases where no or an atypical antibody response is mounted [33–37].

In an ideal scenario, a confirmatory test and/or viral load test follow a reactive HIV screening test. CD4⁺ cell count and sequencing can ensue to determine the individual's immune status and the drug-resistance phenotype of the virus, followed by appropriate therapy [38–41]. Nucleic acid amplification tests (NAAT; e.g. viral load tests) can be either RNA-based or DNA-based (DNA comes from integrated provirus) with purification and amplification usually required. These requirements render current NAAT unsuitable for PoC use, though emerging novel nucleic acid amplification technologies and miniaturization may bring this goal closer (reviewed in [42,43]).

Current guidelines recommend fourth generation antibody-antigen assays (which detect p24 and the antibody response to the virus) as the preferred method of screening for HIV [44] as they have the 'advantage of reducing the time between infection and testing HIV positive to [less than] one month, which is one to two weeks earlier than with sensitive third generation (antibody-only detection) assays' [40,45], as illustrated schematically in Fig. 1. Fourth-generation HIV antibody/antigen tests are on the list of in-vitro diagnostics that the WHO considers essential for both primary healthcare and higher level reference laboratories [46]. Several studies have investigated differences in time to first positive result for different p24-antigen assay methods including reference tests (usually serology or NAAT). These studies are summarized in Supplementary Table 1, <http://links.lww.com/QAD/B345>. Although Meier *et al.* found evidence for a second diagnostic window, a period when there is insufficient uncomplexed p24 yet too little antibody for detection, such a phenomenon has not been observed in more recent systems that can detect IgM [35,47–50].

Although antibody tests have dominated the rapid PoC diagnostics market, p24 has long been recognized as an alternative virological biomarker, especially in early attempts to close the window period of detection of HIV, and for unequivocal EID [59,60]. Polymerized capsid protein forms a protective shell around the viral RNA and its structure has been elucidated (Fig. 2) [61]. It is a ~24–25 kDa protein encoded by the *gag* gene [62], present at high copy number in HIV-1 virions; like RNA, it can be detected before seroconversion.

Beyond the standard, currently available options, there is an array of biomarkers that could potentially be used as surrogate markers for HIV diagnosis in the future, for example, micro-RNA, mRNA, and novel protein targets such as cytokines and other immune markers [66–70]. To date, we are not aware of any approaches that have progressed beyond early stage proof of concept to commercial products, or proven themselves suitable for PoC settings.

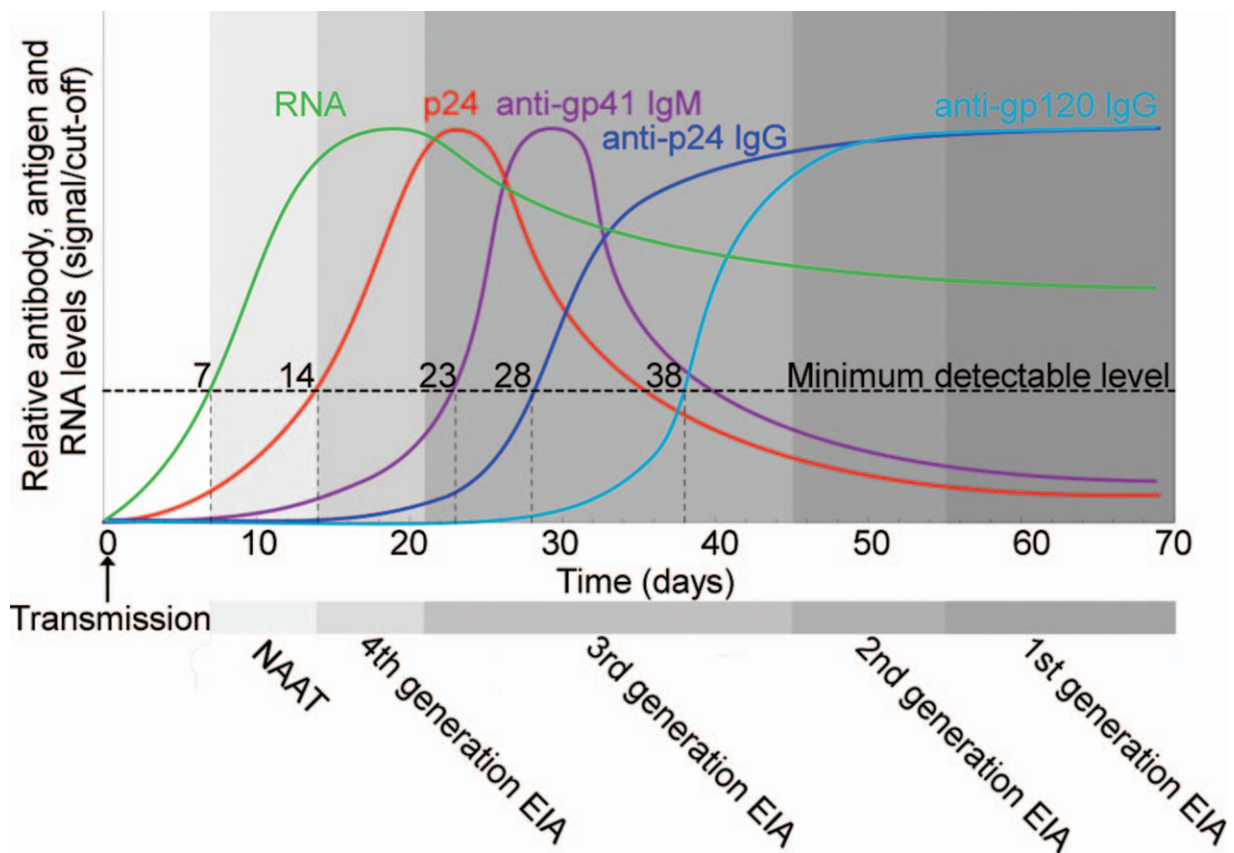


Fig. 1. Kinetics of HIV markers during acute infection to seroconversion and time-frames of detection by generations of tests. Adapted from [31,51–57]. Refer to [58] for further information about disease staging. EIA, enzyme immunoassay; NAAT, nucleic acid amplification test.

The promise of early detection at point of care and self-testing

Acute HIV infection is defined as the period when HIV is present, but an antibody response has not yet been mounted; recent infection covers the time when the immune response is immature, and only highly sensitive

diagnostic assays can be used [71,72]. Early detection, therefore, covers both acute and early infection, and up until a stable viral set point and immunological response is reached. In neonates or those up to 18 months old who are born to HIV-infected mothers, this period will last up until maternal antibodies have cleared [73,74]. In adults, early infection is generally between two to six months,

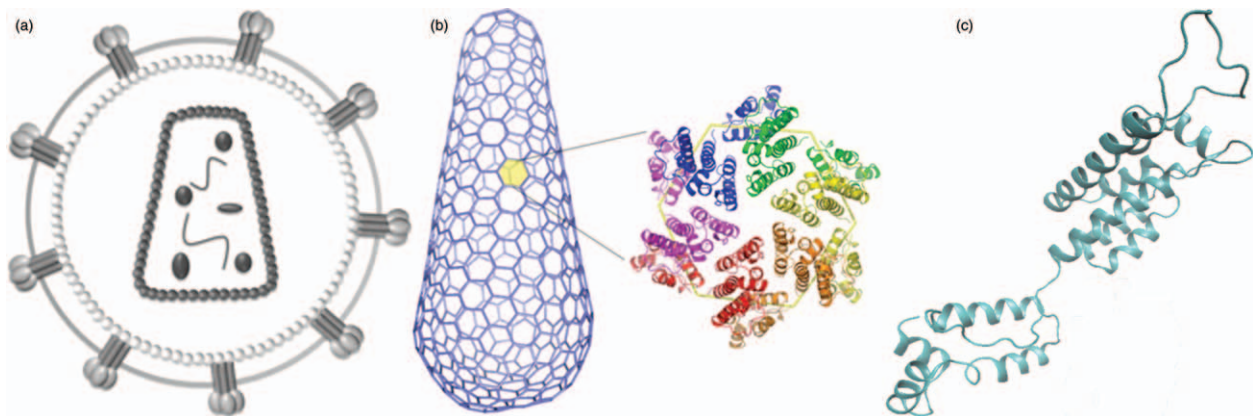


Fig. 2. Schematic of HIV and p24 structures. (a) Entire virion, (b) capsid fullerene cone superstructure made of hexamers and 12 pentamers, and (c) monomer unit. Data from [63–65].

Table 1. Summary of potential benefits of p24 testing across different population groups.

Target population or application	Current needs	Limitations of current antibody tests	Potential benefits of p24 antigen tests
Infants under 18 months old	~1.5 million births to women with HIV [80]. Half of infected neonates die before 2 if untreated [81]	Confounding maternal antibodies up to ~18 months of age [82,83]; early initiation of ART leads to undetectable antibody response [84]	Accurate EID, targeted care and treatment from birth [85,86]
Adults with acute infection (pre-seroconversion)	Increased infectiousness during acute infection [87–89]	Diagnostic window where HIV undetectable; early initiation of ART can lead to seroreversion or undetectable antibody response [31,90–92]	Earlier detection prior to seroconversion [40,89]
Adults in high-risk groups (including those on pre-exposure prophylaxis)	Recommendation of yearly testing may be insufficient for high-risk practices, and is not followed [93–95]	Diagnostic window where HIV undetectable [31]; a significant minority of attendees for testing in clinics with a high proportion of high-risk groups are acutely infected [96]	Earlier detection prior to seroconversion, no confusion over concept of diagnostic window [93]
Adult self-testers	Recent legalization of self-testing, full effects on epidemiology unknown [94,97,98]	Approved commercial self-tests rely on antibody detection (post-seroconversion) [99]	Earlier detection prior to seroconversion, no confusion over concept of diagnostic window [93]
Adults and children treated during acute/early infection	Accurate identification of HIV-positivity using antibody tests, uncertainty over true status if confirmatory assays are antibody-based tests	Early initiation of antiretroviral therapy can lead to seroreversion, or failure to develop positive serological response [90,100]	Earlier detection without need for seroconversion [40,89]
Adults and children in HIV vaccine trials	Discrimination between host response to true infection and vaccine-induced sero-reactivity; high social impact of false-positive HIV status [101]	Antibody-only tests are unable to differentiate between vaccine-induced and virally induced antibodies	Detection of virological components will unequivocally confirm infection

and fourth generation assays that can detect either antigen or antibody are recommended [27,31,40,58]. During Fiebig stage I, the earliest stage of acute infection, HIV RNA is the sole viral biomarker detectable, and therefore only NAAT can be used for virological detection [58]. Though NAAT have been extensively used in resource-rich settings to measure viral load during patient monitoring, they are generally not approved for use as qualitative diagnostic tools in most countries [40,75]. NAAT suitable for detection of HIV in acute infection are existent and reviewed in [43,76–79]; as such, NAAT will not be considered further here.

Key potential advantages of p24 antigen-based testing in specific populations are listed in Table 1, including early detection for specific target groups and EID.

EID and those in acute stages of infection have repeatedly been identified as key demographics who would benefit enormously from virological tests suitable for PoC use in resource-limited settings. The confounding effect of maternal antibodies means that a positive result from an antibody-based test administered to neonates cannot be accurately interpreted, and these antibodies can persist until 12–18 months [74]. If no virological-based alternative is available at an earlier stage, the child must be recalled for testing after weaning and many do not return. Without treatment, around half of infected

children will die by two years old [102]. Current options include testing via dry blood spots for RNA, DNA or p24 antigen; here, sensitivity is limited because of small sample volume, cross-reactions because of release of intracellular contents and high cross-contamination rates [103–106]. Sending samples away for testing in centralized laboratories additionally entails a longer turnaround time with the potential for samples or results to get lost, and extra effort and cost required to transmit results and recall patients for repeat testing or to begin therapy [12].

In the last few years, two molecular technologies described as PoC have progressed through WHO prequalification stages (the Alere Q HIV-1/2 Detect and the Cepheid Xpert HIV-1 Qual) and have been tested in a limited number of field trials, which reflect routine clinical workflows to varying degrees [107–110]. Although game-changers in terms of turn-around time and with fewer infants lost to follow-up and a higher proportion begun on antiretroviral medication [14,109], these technologies are intended for ‘trained health or laboratory professionals’ and come at a cost that remains prohibitive for scale-up [11].

For adults, low-cost and PoC p24 testing would be transformative in high-risk populations to detect those acutely infected, before antibody-based tests can be used, and to initiate treatment as early as possible. Those acutely

infected are key drivers of the epidemic, with the highest rates of transmission during this time [88]. Adult HIV incidence has remained stubbornly high, largely because of failure to routinely test for and detect acute infection at scale [89]. As the number of people taking preexposure prophylaxis steadily rises, the need to test for acute infections also increases to reduce the risk of drug-resistant strains emerging during monotherapy [111,112], and self-testing with rapid PoC tests alongside pre-exposure prophylaxis for high-risk groups are being discussed [113,114].

The maximum benefit of p24-based testing will only be realized when the diagnostic is available in rapid, PoC format compatible with the ASSURED criteria and suitable for use in resource-limited settings and self-testing [115]. The key challenge of detection by rapid PoC tests is the clinical range of p24 in the blood, which spans at least four orders of magnitude from under 0.1–10³ pg/ml; though levels above 10 pg/ml are only briefly reached during acute infection [116–134]. In particular, differences in population groups that present for testing mean that rapid antibody tests are less sensitive in high-income settings, as a higher proportion of infections in those presenting for testing are at the acute stage [135]. The ratio of detected infections to true infections is therefore lower.

Sensitivity and specificity of commercial p24 assays

As p24 detection is challenging, while there are a number of laboratory-based automated systems on the market, currently only one PoC assay in rapid-test format exists (Supplementary Table 2, <http://links.lww.com/QAD/B345>). The laboratory tests are complex, automated equipment-intensive ELISA-type assays. The Alere Determine HIV-1/2Ag/Ab until recently was the sole fourth generation lateral flow PoC assay and has now been replaced by the Alere HIV Combo. All the current laboratory-based assays listed show sensitivity and specificity levels approaching 100%. The performance of the Alere Determine HIV-1/2Ag/Ab has been much more variable depending on the trial and population groups tested (Supplementary Tables 2 and 3, <http://links.lww.com/QAD/B345>). The range of sensitivities of 0–99.8% obscures the mostly low results from the antigen-detection portion of the test in acute infection, with sensitivity at 0% in six of 11 primary studies and under 52% in 10 of 11 of these. Specificity is insufficiently high for this test to be useful for screening with too many false positives generated, particularly in low-prevalence settings (Supplementary Tables 2 and 3, [136–147], summarized in [148], <http://links.lww.com/QAD/B345>). Follow-up studies of the Alere HIV Combo in the literature are

currently insufficient in number to assess whether performance has significantly improved [149,150].

Lessons learnt

In addition to low viral loads, research has suggested that failure to detect p24 may be associated with:

1. Insufficient immune complex disruption (ICD). Sequestering of p24 by host anti-p24 antibody may lower the sensitivity of assays by several orders of magnitude. ICD dissociates host antibodies, allowing the assay antibodies to bind p24, and sensitivity levels may be considerably enhanced. Dissociation is conventionally via heat or acid-based techniques. Publications on these methods peaked in the early 1990s, later petering out as efforts to mainstream and consolidate advances in nucleic acid testing gained traction. A summary of methods from studies using ICD is presented in Supplementary Table 4, <http://links.lww.com/QAD/B345>.
2. Early infant diagnosis. Studies have reported widely variable sensitivities of p24 antigen tests for EID (summarized in [151]). For example, Quinn *et al.* [152] found good sensitivity for infants older than one month, but all nine specimens under one month were negative; Lewis *et al.* [153] found very poor performance in all infants under three months; Parpia *et al.* [154] found very high sensitivity and specificity in infants despite using a test with a limit of detection of 20 pg/ml. In part, this variability may relate to lack of stratification between infants infected in utero, during birth or breast-feeding, which significantly alter the timing of the window period. False negatives may also result if neonates received antiretrovirals as part of 'prevention of mother-to-child transmission' programs [155]. As a neonate born to an infected mother will have high levels of anti-HIV antibodies, use of ICD techniques have been highlighted [156,157]. Supplementary Table 3, <http://links.lww.com/QAD/B345> contains details of studies for which ICD was used or compared, including for EID.
3. Viral subtypes (Fig. 3). In assessments of non-B subtypes, Spacek *et al.* found poor and variable performance especially for RNA loads below 400 and above 500 000 copies/mL in Uganda [131]. Variable sensitivity depending on genotype has been found using combined assays in a French study that concluded: 'many HIV Ag/Ab assays could fail to detect HIV primary infection due to HIV-1 non-B, non-M and HIV-2 strains' [158]. Beelaert *et al.* [145] found p24 assays could not detect HIV-2, and some failed to detect outlier subtypes (one group O, one subtype F and two subtype H out of 50 tested). However, others found good performance with multiple subtypes. For example, Pascual *et al.* [125] found performance of a modified ELISA to be good compared with NAAT (the Roche Monitor RNA) including for subtypes A to F. Ribas *et al.* [130] demonstrated the ability of a modified

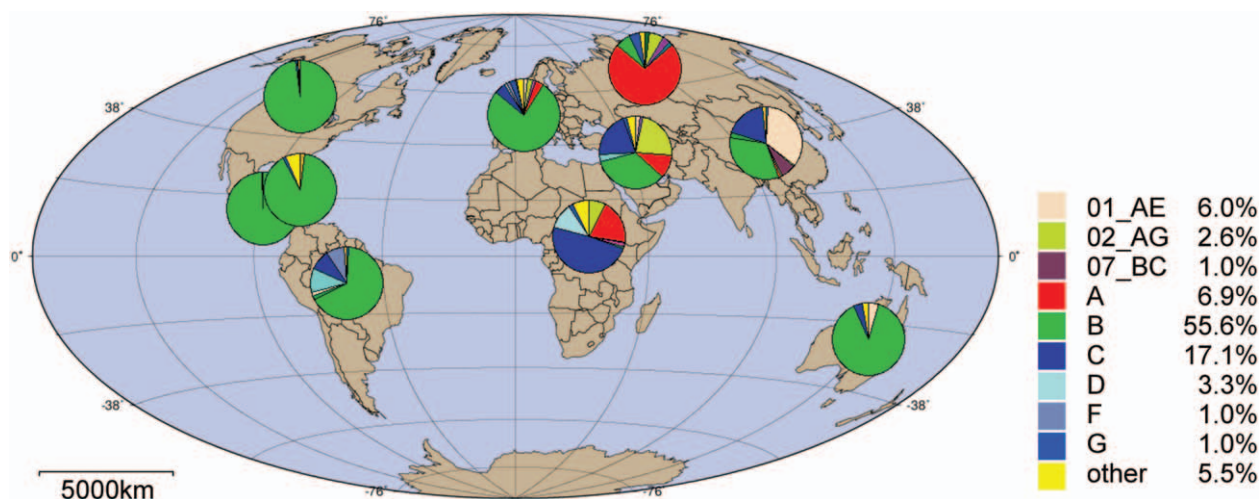


Fig. 3. Prevalence of HIV subtypes worldwide. Source: Los Alamos HIV Sequence Database (<http://www.hiv.lanl.gov/>). Proportions are calculated from total available sequence data available from each region and may reflect localized biases in sampling.

ELISA to detect various subtypes and recombinant forms of p24. Subtype diversity panels (e.g. <https://eqapol.dhvi.duke.edu/viral-diversity>) enable researchers to ensure that their reagents are validated against a wide range of subtypes found worldwide (for example, [65]).

HIV-2 is relatively uncommon outside West Africa (~one to two million infected [159]). Suppliers of antibodies often claim cross-detection, though not all demonstrate sufficient cross-reactivity to be useful, as shown by a report that found no activity for many AIDS Reagent Program antibodies against HIV-2 in a laboratory ELISA (www.aidsreagent.org) [160].

4. Low prevalence settings. In low prevalence settings, false positives necessitate uncertainty and further testing. Currently, third generation tests have lower false-positive rates compared with fourth generation tests, suggesting that laboratory-based screening should instead be used for those at high risk of infection [137]. Tamhane *et al.* [161] suggested adjusting positive readout thresholds in order to optimize a modified ELISA for a given prevalence (using receiver-operator curves), but this approach would be difficult to implement in simple PoC tests.
5. Use in resource-poor settings. A potential use of antigen detection tests is their easy adaptation for resource-poor settings compared with NAAT. A number of studies in low-income countries or nonclinical, community settings found very poor performance of the Alere Determine rapid test, inconsistent with manufacturer evaluations, but largely consistent with each other. For example, studies led by Conway, Duong, Jones, Rosenberg, Chetty, and Taegtmeier all reported that the antigen-detection portion of the test failed to detect any cases of acute infection and Kilembe, Faraoni, and Brauer found limited detection levels at one of 34, three of 17 and three of 30, respectively [137–144,146] (Supplementary Table 2, <http://links.lww.com/QAD/B345>). In one study, the

specificity was sufficiently poor at 86.1% for the test to be usable as a screening tool, but in another study, the antigen portion successfully highlighted 32/39 antigen-positive cases of acute infection [136,145]. Bulterys *et al.* [162] further found limited sensitivity of a modified ELISA for p24 in African children compared with reports in developed countries, though this study could also be affected by patient age and HIV subtype (see point 2).

6. Choice of antibodies. Lack of adequate antibodies may, in part, explain poor performance observed in earlier studies, particularly efficacy of monoclonal antibodies for binding certain subtypes [163], and variable binding affinity [65,164,165]. These issues can potentially be overcome by careful selection from wide screening of antibodies, or potentially using multiple antibodies concurrently [160,166].
7. Stability of test components and target. For both research studies and clinics, samples may be stored and processed in batches. For studies, in particular, analysis can occur years after collection. Storage at 4 °C even for brief periods permits immune complexes to form, though freezing does not [167]. Cold-chain transportation is excluded from the criteria for ASSURED rapid tests though required for reagents of many laboratory-based assays [4,115]. Even for those that do not require refrigeration, conditions during transport or storage in resource-limited settings regularly exceed guidance on maxima for temperature (usually 30 °C), sometimes by 15° or more, and humidity (65%), leading to invalid results in some studies [168,169], but not others [170].

Emerging and ultrasensitive approaches

The limited sensitivity of most p24-detection assays has led to the widely-held belief that p24 tests 'are relatively insensitive and therefore have a limited utility in clinical

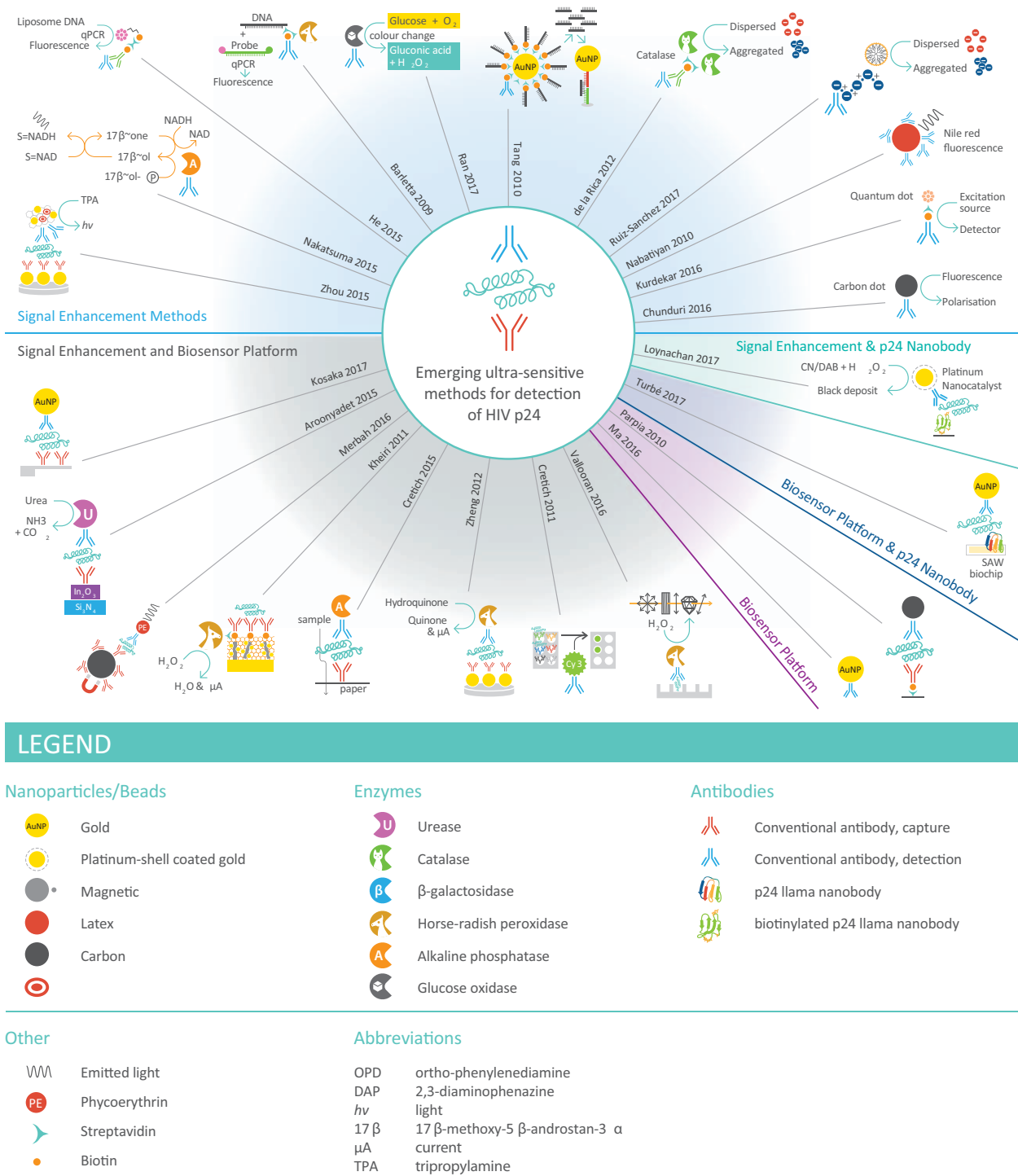


Fig. 4. Schematics of the biosensors in Supplementary Table 5, <http://links.lww.com/QAD/B345>, grouped according to whether novelty is in the structural component of the biosensor, the signal enhancement mechanism, or another/ multiple components. For further details and references, refer to Supplementary Table 5, <http://links.lww.com/QAD/B345>.

practice' [171] or that the practical limit of detection for p24 is of the order of 3 to 4 pg/ml [172]. Emerging, proof of concept assays from research laboratories have exceeded this by several orders of magnitude, but have not progressed through the product development pathway (see [79] for current or imminently launching

products). Figure 4 illustrates methods for achieving sensitive detection of p24 for which quantitative limits have been reported, with details in Supplementary Table 5, <http://links.lww.com/QAD/B345>. A number of these tests approach single molecule limits of detection; sensitivity is, therefore, limited primarily by the volume

of sample analyzed. Further development of these technologies into PoC format would greatly facilitate translation into the clinic.

Cost-effectiveness of using p24 assays for HIV detection

Health-economic analyses of using p24 assays as screening tests for HIV include [173–179] (Table 2). Models are dependent on a wide range of variables, such as prevalence, source population (e.g. use in blood banks, use in sexual health clinics, use in accident and emergency), gross domestic product per capita, and the current cost, sensitivity and specificity of the p24 antigen test under consideration (including false-positive rate) meaning that a model may only be applicable to one country or type of clinic. Antigen tests considered were laboratory-based fourth generation antigen/antibody immunoassays, not PoC tests. Prevalence strongly affects measures of cost-effectiveness; in blood banks this depends on donor population, for example, unpaid donor vs. paid donor vs. familial donors [178]. Other factors that affect cost-effectiveness include rates of linkage and retention in care, as well as improvements in partner testing [6,180]. As cheaper p24 antigen tests become available, particularly those in self-test format, parameters for these studies will shift considerably requiring cost-benefit recalculation for each target population. Currently no target product profile exists that could guide development towards key goals such as sensitivity, cost or simplicity of use.

Monitoring of HIV infection using p24?

To date, most applications of p24 assays have focused on diagnosis during acute infection and EID. Other utilities,

however, have been investigated such as prognosis and treatment monitoring. Established (correlated, but independent) predictors of disease progression and treatment failure are CD4⁺ cell count and HIV viral load [182]. Regular monitoring of patients on antiretroviral therapy is critical to prevent drug-resistant strains arising through treatment failure or poor adherence, and viral load assays are universally recommended for this purpose [183,184]. Though p24 detection for diagnosis of HIV is standard, it is not clear that p24 quantification can provide clinically meaningful data on treatment monitoring. According to a review of literature between 1997 and 2010 [66], relatively few studies investigate the relationship between p24 levels and progression of disease; just two were identified [121,124] and stated that: ‘In both studies higher [p24] levels were associated with an increased risk of progression.’ Although these might suggest that using p24 levels may add value, this could only be used in situations where optimal monitoring methods (i.e. quantitative NAAT) were not routinely available, and in this era of the recommendation to ‘treat all,’ it is unclear that predominantly untreated patients, as found in these studies, would be often encountered.

Several additional studies of correlation of p24 levels to markers of disease progression (CD4⁺ lymphocyte counts, RNA and quantitative viral load measurements) or health outcomes were identified for this review; the main findings from these studies are detailed in Supplementary Table 6, <http://links.lww.com/QAD/B345>. In general, the correlation of p24 levels with RNA viral load is not strong and varies between settings for several reasons; Erythrocytes clear immune complexes [128] and one study found limited correlation between the amount of erythrocyte-associated p24 antigen and p24 antigen in plasma [185].

On balance, p24 could be used in the future to monitor for early infection in patients belonging to high-risk

Table 2. Cost-effectiveness studies comparing p24 antigen testing to a baseline.

Conclusion	Reference	Study location	Study population	Reference scenario
p24 antigen tests are cost-effective	[174]	US	3 030 303 outpatients	No testing
	[173]	US	1 500 000 people eligible for screening: 13–64 years, no risk criteria	Third generation EIA
	[175]	US	10 000 MSM and 10 000 IDU; testing biannually/quarterly ^a	Testing annually
	[176]	US	Entire population aged 15–64 (>186 000 000) or sub-groups	Status quo for testing frequency and method [181]
p24 antigen tests are not cost-effective	[177]	US	16 000 000 donor units	Third generation antibody testing
	[178]	Ghana	Blood bank donors to 193 transfused patients	Third generation antibody testing and no testing considered
	[179]	US	2744 from San Diego early test program	Third generation antibody testing

The assessment is made in comparison to third-generation antibody tests, to the status quo, or to no testing. EIA, enzyme immunoassay; IDU, injecting drug user; MSM, men who have sex with men; US, United States.

^aCost-effectiveness was validated solely for MSM, not IDU.

groups and using preexposure prophylaxis, but the evidence for monitoring therapy using p24 is not as unequivocal as suggested by early enthusiasts [186,187]. As those involved in HIV care increasingly begin to look towards HIV cure, assays for p24 could yet come into focus again, especially if adequately sensitive tests can be made sufficiently simple to be used for self-testing.

Conclusion and outlook

The future of HIV diagnostics and monitoring will include PoC testing of an increasing number and variety of target population subsets (outlined in Table 1), each with specific and diverse requirements. Though testing of large numbers of vaccine trial recipients is currently in the pipeline, the need is immediate and ongoing for EID when mothers are known to be HIV positive, and home testing on a regular basis for adults at an elevated risk of becoming infected to catch those in the window period. With the rollout of preexposure prophylaxis in many countries, diagnostic tests that would facilitate cheap and routine testing for those in the window period, potentially by self-testing, will become highly desirable. In multiple scenarios, PoC testing for p24 antigen offers an attractive alternative to nucleic acid detection for diagnostics that are targeted at the pathogen, rather than the host response, and a simple p24 test for the presence of the virus could be transformative. In places where the facilities and technology available do not permit adherence to recommended methods for monitoring treatment, a simplified quantitative or semi-quantitative p24 test to detect resurgence of virus could permit investigations into treatment compliance or the need to switch from a failing therapy regimen.

These advantages will only be realized when PoC tests are sufficiently sensitive so as to enable detection significantly before antibody tests, and as easy to use as current PoC rapid tests. Ideally, the test would detect p24 at fg/ml (10^{-15} g/ml) to ng/ml (10^{-9} g/ml) levels, with a minimum detection limit of the order of 10^{-10} g/ml. Many approaches currently at the research stage of development from the last decade can detect p24 at these levels. However, many of these are early-stage experimental studies and have not yet made a successful transition out of the research laboratory, and through clinical trials. The challenge to develop these into simple low-cost diagnostics in the field is substantial.

Many of the studies that assessed the properties of p24 antigen during infection, particularly the correlation of p24 antigen with other biomarkers, were conducted over a decade ago. More investment into fundamental clinical research of this kind, as well as for translating cutting-edge technology for p24 detection out of the laboratory and into the field is needed. Further clinical studies with the

most up-to-date technology are merited, in addition to updated health-economic analyses for fourth generation rapid tests in different population groups.

The requirements of HIV detection technologies for diagnosis could shortly be radically altered by the introduction of cure therapies and vaccination programs. As the next generation of technologies comes through, with many intended for use in resource-limited settings, HIV p24 could yet see a fresh role as a virological target.

Methods

This is a scoping review, though a systematic search was initially used to identify articles of interest. PubMed was searched for articles from the outset of the HIV epidemic to January 2018 using the following keywords (HIV or AIDS or autoimmune diseases syndrome or human immunodeficiency virus) and (p24) and (test OR diagnostic). Identified studies were further screened. Studies were excluded if they met any of the following criteria:

1. Not in English
2. Not about HIV
3. Not about use of p24 as a clinical and/or diagnostic marker
4. Reports on chemical modification or inhibition of p24 (drug-type studies)
5. Purely immunological studies
6. Unable to obtain full-text
7. Conference abstracts alone

Five hundred and seventy-four papers were scrutinized further for potential inclusion in the review, and associated citations pursued if not identified in the original search. In addition, WHO and UNAIDS reports were sourced as appropriate.

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Royal Society Wolfson Merit Award.

Conflicts of interest

There are no conflicts of interest.

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