BioscienceHorizons Volume 4 • Number 1 • March 2011 10.1093/biohorizons/hzr004

Research article

# Development of a United Kingdom National External Quality Assessment Scheme (UK NEQAS) for HIV point of care testing

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The use of HIV point of care tests (POCTs) is increasing rapidly in both laboratory and other settings. These tests are often performed by non-laboratory trained staff. At the present time there are no external quality assessment (EQA) providers in the UK offering proficiency testing schemes for HIV point of care testing. The aim of this study is to develop such an EQA scheme. Firstly, a selection of the most widely used POCTs was selected and their performance assessed using existing HIV-positive serology EQA specimens. All assays produced the correct results however intensity of results observed for the same specimen differed greatly between POCT devices. In addition the effect of various sub-groups of HIV-1 serum samples on the HIV POCT assays was investigated and no difference between the results on the POCTs was observed. Ultimately four serum specimens, two HIV-1 and one HIV-2 positive, one HIV negative, were chosen and sent to NHS laboratories and sexual health clinics for testing as part of a pilot EQA scheme for HIV POCT. Results were excellent with 97% of participants reporting correct results ($n = 20$). The study highlighted a lack of awareness of EQA particularly in non-laboratory settings, although recommendations (ISO 22870:2006) are in place for the users of such devices. In conclusion, the need for EQA for providers of point of care testing is an integral part of ensuring reliability of results and quality of care for the patient.

**Key words:** HIV, POCT, EQA, point of care.

Submitted July 2010; accepted on 20 January 2011

## Introduction

In 2009, the Health Protection Agency studied the number of people being tested for HIV at sexual health clinics and not returning for their diagnosis. The report produced[1] revealed that the number of infected individuals in the UK at the end of 2008 was estimated to be 83 000 with approximately 22 400 individuals unaware of their infection (Fig. 1).

Many patients remain anonymous at sexual health clinics and it is therefore impossible to trace them and inform them of their test result. This obviously has a severe impact on the risk of HIV transmission. It has been shown in several studies such as that by Darnell *et al.* in 2006[2] that HIV-positive individuals aware of their infection avoid practices associated with high-risk of HIV transmission.

One of the priority areas for the UNAIDS framework for 2009–2011 is to reduce the transmission of HIV. Testing for HIV at the point of care may increase the number of adults willing to take a test as the results are produced at the same time as testing. This would therefore reduce the risk of transmission and allow anti-viral treatment to start earlier.[3]

Point of care tests (POCTs) are investigations carried out near to or at the patient's side. They are rapid immunochromatographic, flow through or particle agglutination assays that provide results immediately or within minutes depending on the test device. Only a small volume of patient sample is required to perform the test, often a drop of blood from a finger prick.[4] The sample formats for use in POCTs vary between devices but can be serum, plasma, whole blood or saliva.

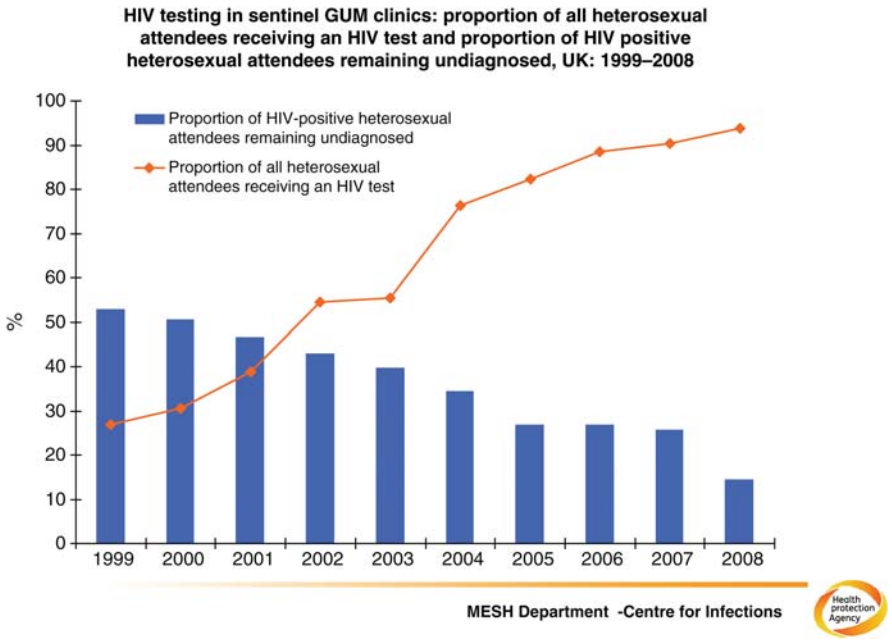


**Figure 1.** Percentage of heterosexuals positive for HIV remaining undiagnosed.

The majority of POCT devices approved for diagnostic use involve HIV-recombinant antigens and/or synthetic peptides bound to the membrane in the POCT device, the sample is applied to the membrane and if antibodies are present they form a complex with the membrane-bound antigen that induces a coloured reaction.

In addition to the detection of HIV antibodies, new fourth-generation POCTs can also detect the presence of HIV p24 antigen; antibodies to p24 antigen are membrane bound and detect p24 antigen in the sample. These tests reduce the time to diagnosis as HIV infection can be detected before seroconversion.[5]

Studies show that the use of POCTs for the detection of HIV is increasing rapidly in both laboratory and other clinical settings, especially in developing countries. For example, following the introduction of POCTs in Malawi the number of people receiving an HIV test annually rose from 5000 to 40 000 and the number of those tested who remained to receive results increased from 69% to 99.7%.[4]

HIV POCTs are often performed by non-laboratory trained individuals such as nurses.[6] The results of these tests are interpreted by the user, which is very subjective and relies heavily on user expertise and knowledge of the test principle.[4] Adequate training and quality assurance procedures should be in place, as with any other assay, to ensure reliable results.[7]

External quality assessment (EQA) has an important role in the quality assurance measures of every laboratory or clinic as participation in an EQA scheme allows the user to monitor their own quality assurance procedures and to readily identify and remedy any problems with internal quality control measures. It demonstrates to patients and other professionals a commitment to quality.[8]

Participating in an EQA scheme contributes to a laboratory's accreditation status awarded by the appropriate governing body, for instance the Clinical Pathology Accreditation. Although participation in an EQA scheme is not compulsory for sexual health clinics or laboratories, unless they wish to gain accreditation, a bulletin by the Medicine and Healthcare Products Regulatory Agency and a document by the International Organization for Standardization (ISO) relating to point of care testing (EN ISO 22870:2006) state that users of POCTs should participate in an appropriate EQA scheme.

EQA involves the analysis of simulated specimens designed to reflect the characteristics of a clinical sample. Simulated specimens of known but undisclosed content are sent at regular intervals often monthly or quarterly to participating laboratories. The participants then test the samples using their own routine procedures and report their results to the EQA provider. The results of all participants are analysed and a report produced that shows a participants own result and the anonymized results of all other participants in the scheme. The report also includes a cumulative assessment of the participant's performance allowing them to monitor the performance over a period of time.

The United Kingdom National External Quality Assessment Service (UK NEQAS) for Microbiology is the leading external quality assessment (EQA) provider in the UK. They provide EQA schemes in all disciplines related to microbiology to diagnostic laboratories across UK and worldwide.

At the time of the study there were no EQA providers in the UK offering proficiency testing schemes for HIV point of care testing. Given that the use of HIV POCTs is rising rapidly in the UK and worldwide, the study proposed that EQA of HIV POCT providers is beneficial to ensure quality, reliable results for patients especially in primary care settings where no supporting laboratory is available.

In September 2009, UK NEQAS for Microbiology published results of an HIV point of care testing questionnaire sent to 293 Genito Urinary Medicine clinics across the UK.[9] The questionnaire sought information on whether POCTs were used, frequency of use, assay type and sample format used, e.g. whole blood, serum, oral fluid, etc.

The intention of this project was to develop and distribute a pilot HIV point of care testing EQA scheme to interested participants in laboratories and other clinical settings. For this purpose, the various kits available on the market for HIV point of care testing were used to demonstrate performance on existing UK NEQAS HIV serology samples. The focus was on the detection of anti-HIV antibodies and possibly the detection of HIV p24 antigen for fourth-generation POCTs. The effect of various sub-types of HIV serum samples on the performance of HIV POCT assays was also determined.

## Materials and methods

Feedback from the UK NEQAS questionnaire sent to sexual health clinics across the UK identified current users of HIV POCTS and the devices employed. A selection of the most commonly used HIV POCT devices were included in this investigation namely Determine HIV-1/2 Ab (third generation), INSTI HIV-1/2 Test and the OraQuick Rapid HIV-1/2 antibody Test. In addition to the above tests, the Determine HIV-1/2 Ag/Ab Combo fourth-generation POCT was included for testing (this became available in Spring 2009). The performance of these kits was investigated using two different sets of UK NEQAS HIV serology specimens.

Plasma for EQA was provided by the NHS Blood and Transplant from living donors. All donations received by UK NEQAS were provided without patient identifiers. EQA material, from living donors, is exempt from the Human Tissue Act 2004 and ethical approval was not required.

The first set of specimens had been previously characterized by the Genscreen ULTRA HIV-1/2 EIA and INNO-LIA line assay to determine HIV group 1 or 2 and based on these results 20 specimens (16 HIV-1 and 4 HIV-2) were selected for testing. Selection was determined by HIV strain and the band profiles revealed on the line assay; a range of differing profiles were selected. Nineteen of the 20 HIV-positive specimens had been previously diluted using negative sera with dilutions varying from 1:10 to 1:117 and one specimen was not diluted. An additional specimen confirmed negative for HIV antibodies, Hepatitis B surface antigen and Hepatitis C antibodies was also included for testing. All specimens were re-tested using Genscreen ULTRA HIV-1/2 Ag/Ab EIA, these results were used as a reference for POCT results.

The second set of specimens had previously been characterized by molecular methods so the sub-group and viral load had been established; five specimens of sub-groups B, C, G and CRF02_AG were selected to test POCT performance across sub-groups.

All specimens had been heat inactivated at 56°C for 30 min and viral inactivation was fully completed by adding 1% Tween 80.

The specimens were tested according to the manufacturer's instructions for all POCT kits and reactive results were scored on the basis of colour intensity, 4+ strongest to +/− weakest.

An additional investigation was performed where the second set of specimens were re-tested as 1 in 70 dilutions, the specimens were diluted using serum confirmed negative for HIV antibodies, Hepatitis B surface antigen and Hepatitis C antibodies. The POCT signals produced by the diluted specimens were compared with the initial signals produced from the testing of the same specimen undiluted.

Current users of HIV POCTs in sexual health clinics as well as participants of the UK NEQAS HIV serology scheme that use HIV POCTs were invited to participate in the pilot EQA scheme for HIV point of care testing via letter. Those wishing to participate were asked to respond within a given time. Those who did not respond within this time limit were contacted via telephone to identify the reason for not responding and provide them with another opportunity to participate. Forty-six invitation letters were sent and 20 chose to participate in the scheme, 10 NHS laboratories and 10 sexual health clinics.

The participating sexual health clinics were given a unique identifying number (identifier) to ensure the results they reported remained confidential and anonymous. Current participants of the HIV serology scheme were already assigned with a unique identifier.

Four EQA serology specimens were distributed to each pilot scheme participant along with a reply form for the reporting of results. Instructions and safety information were also included. All returned results were analysed to determine performance with each specimen and the kits used.

## Results

Performance of POCT kits was investigated using a first set of specimens fully characterized by enzyme immunoassays (EIAs) and line assays. All specimens correctly showed reactive results for HIV-1 and HIV-2 antibody with all POCTs. However 7 of 20 specimens showed weak reactive results for HIV antibodies on both INSTI HIV 1/2 and OraQuick

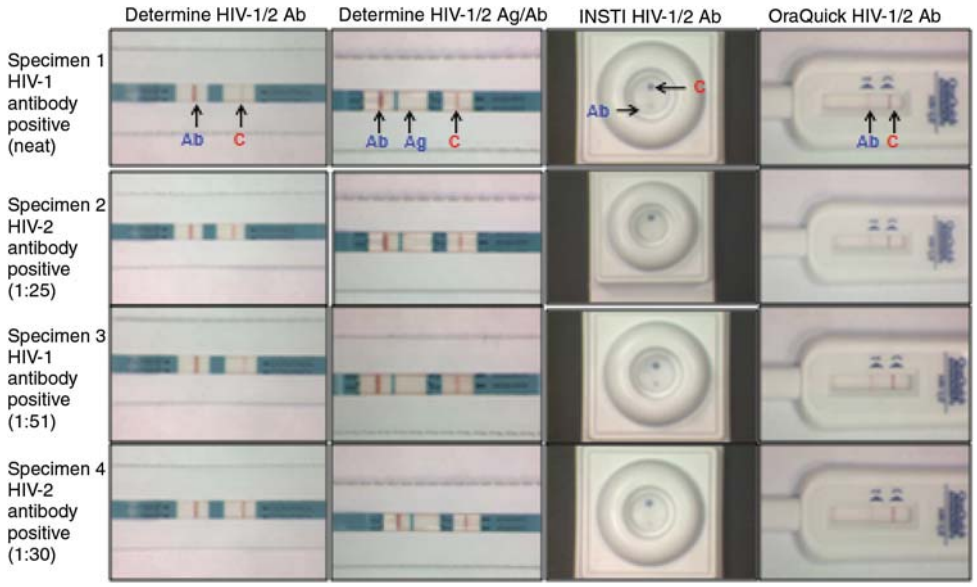


**Figure 2.** Comparison of reactive results on POCT kits. The antibody test area is indicated 'Ab', the antigen test area by 'Ag' and the control area by 'C'. Colour development in these test areas indicates a reactive result.

Rapid HIV-1/2 antibody kits, including 3 of 16 HIV-1 and all four HIV-2 specimens. All specimens tested negative for HIV p24 antigen. All results were valid as control lines were clearly observed in all tests. Accuracy of the results obtained were assured as re-testing of the specimens using the Genscreen EIA matched those from the previous characterization and the results obtained from an outside reference laboratory.

The variation in signal strength of the POCTs depicted in Fig. 2 was typical of the variation observed in this study for all specimens on the different POCTs. Both Determine POCTs produced very clear reactive results with every specimen. All signals for the Determine tests were graded at 3+ or above (Fig. 2). For the INSTI HIV-1/2 test device an example of a 2+ signal can be seen for specimen 3, a 1+ signal for specimen 4 and a +/− signal for specimen 2. All OraQuick Rapid HIV-1/2 antibody devices in this example show signal strength of 2+ apart from specimen 2 where a signal of 1+ is displayed.

An investigation into OraQuick Rapid HIV-1/2 antibody and the INSTI HIV-1/2 test results in comparison with the INNO-LIA line assay results showed a correlation between line assay gp41 band intensity and reactive signal strength on the OraQuick Rapid HIV-1/2 antibody devices for HIV-1. A similar finding was reported in a study by O'Connell *et al.* in 2003[10] where individuals with low levels of gp41 antibodies yielded false-negative results on OraQuick Rapid HIV-1/2 antibody devices. No correlation was made between INSTI HIV-1/2 test results and the INNO-LIA results.

An investigation into the effect of HIV-1 subtypes on POCT results was performed using a second set of specimens fully characterized by molecular methods. All HIV-1 sub-type specimens produced clear reactive signals on the POCTs. No effect was observed due to differences in sub-type. Interestingly, the reactive signals observed were overall much stronger than the signals observed with the first set of specimens (Fig. 3).

It was anticipated that the differences in signal strength observed may be due to dilution of the specimens. An additional investigation was performed where the second set of specimens were re-tested as 1 in 70 dilutions. The results were compared against the initial signals produced from the testing of the same specimen undiluted (Table 1).

There was little difference observed with Determine tests as signals were all very clear whether the specimen was neat or diluted, this was expected due to the performance of the tests throughout the study. An effect on signal strength was observed with some of the INSTI HIV-1/2 and OraQuick Rapid HIV-1/2 antibody test results, a 4-fold decrease in some cases (Table 1). However, a clear correlation between signal strength and dilution factor could not be made.

In order to select the most suitable specimens for distribution in the pilot scheme, the results of the POCT investigations were evaluated and the serology specimens selected were those from the first set that produced the strongest reactive signals across all POCT devices. Based on these evaluations three serology specimens, two HIV-1 and one HIV-2-positive were selected. Additionally, a serology specimen confirmed negative for HIV selected from UK NEQAS stocks was also included for distribution in the pilot scheme.

Feedback from the UK NEQAS HIV POCT questionnaire identified users of HIV POCTs in sexual health clinics. These

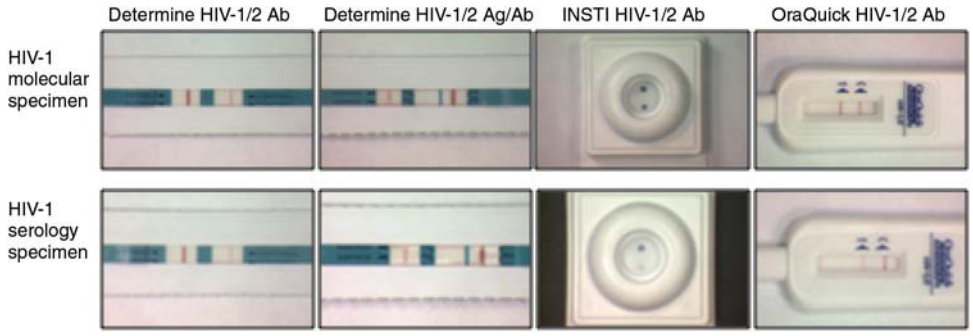


**Figure 3.** Comparison of reactive results on POCTs between HIV serology specimens (first set) and HIV molecular specimens (second set). Both specimens had not been previously diluted.

**Table 1.** Comparison of the reactive signals observed on the POCTs between neat and diluted specimens

| Specimen | Dilution used | Determine HIV-1/2 Ab | IN5TI HIV-1/2 Ab | OraQuick HIV-1/2 Ab |
|---|---|---|---|---|
| Q2659 | Neat | 4+ | 4+ | 4+ |
| Q2659 | 1/70 | 2+ | 1+ | +/− |
| Q3873 | Neat | 3+ | 4+ | 4+ |
| Q3873 | 1/70 | 4+ | 3+ | 3+ |
| Q3874 | Neat | 3+ | 4+ | 4+ |
| Q3874 | 1/70 | 4+ | 3+ | 1+ |
| Q3877 | Neat | 3+ | 3+ | 3+ |
| Q3877 | 1/70 | 3+ | 2+ | 1+ |
| Q3882 | Neat | 3+ | 4+ | 4+ |
| Q3882 | 1/70 | 2+ | 3+ | +/− |

clinics as well as current participants of the UK NEQAS HIV serology scheme that use HIV POCTs were invited to participate in the pilot scheme. Overall 20 responses were received and 18 sets of results were returned. Two sets of results were pending as the participants were awaiting test kits (Fig. 4).

The four EQA serology specimens were distributed to each pilot scheme participant along with a reply form for the reporting of their results. All returned results were analysed to determine performance with each specimen and the kits used.

The most popular kits used were Determine HIV-1/2 Ag/Ab, Determine HIV-1/2 Ab and INSTI HIV-1/2 Ab (Fig. 5). Three participants chose to test the samples using a combination of kits, namely Determine HIV 1/2 Ab and Unigold ($n = 1$), Determine HIV 1/2 and Immunocomb II HIV 1/2 Bispot ($n = 1$) and HIV Determine and INSTI HIV-1/2 ($n = 1$).

Overall performance was excellent in this pilot scheme with 97% of participants reporting the correct results. One participant reported an indeterminate result for specimen 0216 using the INSTI HIV-1/2 Ab test and one participant reported HIV p24 antigen positive for the negative specimen 0217 using Determine HIV-1/2 Ag/Ab Combo test (Fig. 6).

## Discussion

The investigation into the performance of the POCTs using the first set of HIV serology specimens revealed that all POCTs showed correct results. The reactive results with the INSTI HIV-1/2 and OraQuick Rapid HIV-1/2 antibody kits were very weak in some cases and could lead to difficulties in interpretation whilst the Determine kits produced clear reactive results. It seems that in some cases dilution of the specimens did have an effect on the intensity of reactive signals observed with the INSTI HIV-1/2 and OraQuick Rapid HIV-1/2 antibody results. Although a clear correlation between the dilution factor and signal intensity could not be made, the effect dilution of the sera may have on POCT results should be taken into account when selecting specimens for future distributions.

The investigation into the effects of various sub-types of HIV-1 on the POCTs showed very strong reactive results on all devices regardless of sub-type. However, a limited panel of sub-types were tested and only from within HIV-1 group M. Increasing immigration has introduced greater incidence of HIV-1 circulating recombinant forms (CRF), non-B sub-types, HIV-1 group O and HIV-2 to other parts of world where they are not usually encountered.[11] A study at King's College and St. Thomas Hospitals showed that the prevalence of newly acquired infections with HIV-1 non-B subtypes is increasing in the UK and Europe, 42 out of 183 individuals included in the study infected with HIV-1 non-B sub-type had acquired their infection in the UK.[12] This poses a challenge to the POCT devices largely based on HIV-1 sub-group B antigens and peptides. UK NEQAS for Microbiology provides EQA schemes worldwide including parts of Africa where the prevalence of non-B sub-groups and HIV-2 is higher. Further studies should assess all subtypes with the aim of incorporating current circulating strains into future distributions.

No specimens included in the study were positive for HIV p24 antigen. It was anticipated that the use of Determine HIV-1/2 Ag/Ab Combo will increase as at the time of the

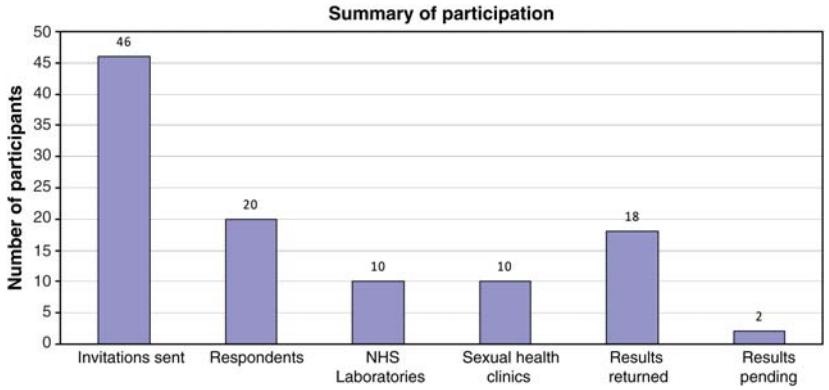


**Figure 4.** Summary of participation in the pilot EQA scheme.

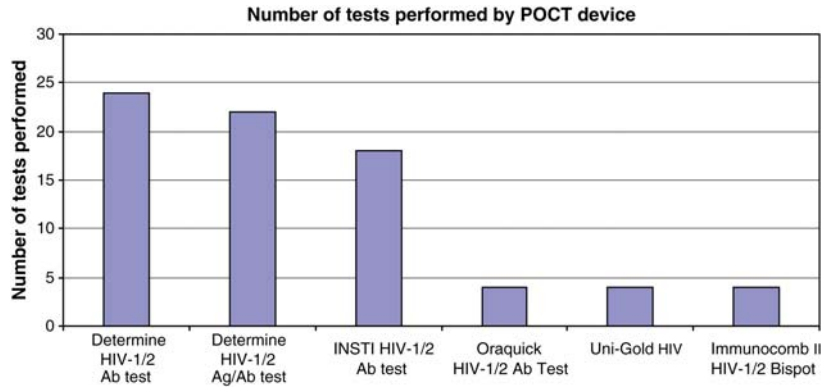


**Figure 5.** Number of POCTs performed by participants.

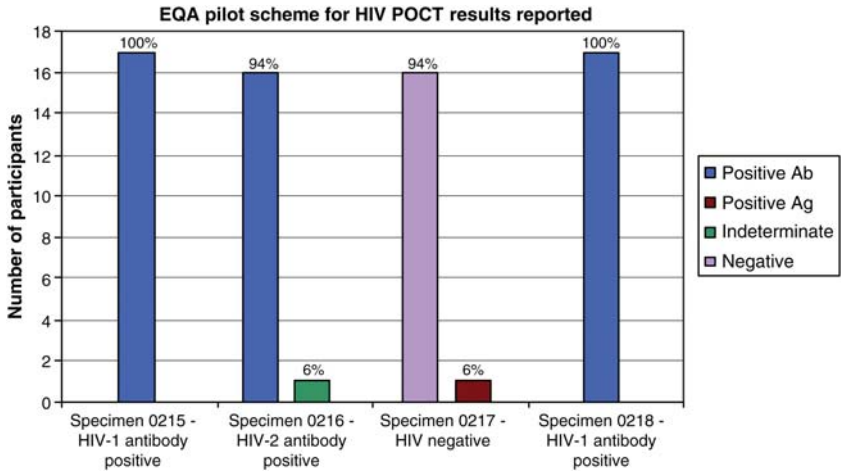


**Figure 6.** Results reported by the participants of the pilot. The percentage shown refers to the number of correct results reported out of the test results received. Two participants have not examined the specimens to date as kits were not available.

study it was already employed by 24% of the participants. It was recommended that p24-antigen-positive material is sourced for future investigations. It may prove difficult to source antigen-positive material as blood would need to be donated from recently infected individuals before seroconversion has occurred or from patients who have progressed to late stage AIDS when levels of p24 antigen increase as antibody levels diminish. It may also be possible to spike existing UK NEQAS specimens with p24 antigen as recombinant p24 antigen is commercially available.

However, this may also prove to be difficult in practice as antibodies present in the serum sample would form complexes with p24 antigen upon addition rendering it undetectable by the POCTs.

Participant performance in this pilot scheme was excellent. Constructive feedback was received from those who participated, although overall there was a lack of awareness of EQA schemes within sexual health clinics and the role the schemes had in this setting. It became apparent that, in most cases, the clinics had never encountered, or even heard of EQA before and were unaware how this type of assessment would benefit them. Upon explaining the purpose and benefits of EQA, more interest was shown and participating clinic numbers rose from 3 to 10. Personally contacting the clinics was a very time-consuming process as it proved difficult to make contact with the appropriate people especially when some clinics were only open once or twice a week. In some cases those contacted were unaware of who would give consent for participation and were unwilling to help any further. Often telephone messages and follow-up emails were left unanswered. It was accepted that a lack of awareness of EQA was the main reason for the low number of participating sexual health clinics however correspondence with those who chose not to participate also highlighted other reasons for not participating. Cost implications were a concern for those clinics operating as charities, even though the pilot was free of charge they had to take into account the costs of kits involved in testing. Some clinics were interested in the scheme but did not participate due to lack of staff and time as certain clinics only operate 1 or 2 days a week they felt EQA was another burden on their heavy workload. In a minority of cases, a general lack of interest in EQA was encountered where the clinics in question felt that their own internal quality assurance measures were sufficient. For future pilot studies, it is recommended that further promotion of the scheme be carried out at the local and management level. It would be important to target higher management levels of primary care trusts, charities and organizations involved in sexual health such as the Terrence Higgins Trust or the British Association for Sexual Health and HIV to further promote the benefits of EQA and make them aware of the recommendations from the Medicines and Healthcare products Regulatory Agency and from the International Organization for Standardization. If needed, an EQA scheme may be developed that also provided quality and technical training.

The term point of care infers that the test is carried out at the patient's side to action care immediately. However, it does have a wider use and so it is important that an EQA provider identifies the users of POCTs and how they are used in all types of settings. For example, it is not necessary to perform these tests in a laboratory and so testing can be encountered in settings such as sexual health clinics, in developing countries where charitable organizations visit remote villages and test the whole population or in hospitals or healthcare settings in cases of accidental exposure. It is possible that these tests are performed due to ease of use rather than as a point of care or rapid test and it is often the case that the patients sample is referred to a laboratory for confirmatory testing, which delays the result for the patient.

In the UK there are now recommendations for HIV POCTs to be used for screening in many settings. Sir Liam Donaldson, the UKs chief medical officer, and Professor Christine Beasley, the UKs chief nursing officer, have requested that HIV testing be carried out in all healthcare settings where appropriate.[13] This includes general practices, hospital emergency departments and other departments within hospitals where HIV-positive patients unaware of their infection status may present with diseases common to AIDS and HIV, for example thoracic departments where cases of pneumonia are encountered. It also recommends HIV screening using rapid assays for all new patients taken on by GP surgeries. Labour wards are also recommended users of HIV POCTs in order to screen women in labour who have not yet been tested during routine ante-natal appointments. Further research should ascertain the extent to which HIV POCTs are used in these settings in order to identify possible EQA participants.

At this current time where there is no cure or vaccine for HIV, preventing its transmission is the only way to reduce the number of new infections. HIV remains a significant threat to public health in the UK and worldwide however early diagnosis, that can be improved with the use of point of care testing, together with effective treatment means that those affected are living longer and have a decreased risk of transmitting the infection. This study concludes that the need for EQA for providers of this testing is an integral part of ensuring reliability of results and quality of care for the patient.

## Author biography

C.A. gained a BSc (Hons) in Biomedical Science in 2010 and hopes to embark on a successful and rewarding career in diagnostic microbiology. Her more immediate aspirations are to complete the IBMS specialist portfolio and gain an MSc in Medical Microbiology.

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