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ORIGINAL INVESTIGATION

# Discrimination between recent and non-recent HIV infections using routine diagnostic serological assays

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## Abstract

The suitability of routine diagnostic HIV assays to accurately discriminate between recent and non-recent HIV infections has not been fully investigated. The aim of this study was to compare an established HIV recency assay, the Sedia limiting antigen HIV avidity assay (LAg), with the diagnostic assays; Abbott ARCHITECT HIV Ag/Ab Combo and INNO-LIA HIV line assays. Samples from all new HIV diagnoses in Ireland from January to December 2016 ($n=455$) were tested. An extended logistic regression model, the Spiegelhalter–Knill–Jones method, was utilised to establish a scoring system to predict recency of HIV infection. As proof of concept, 50 well-characterised samples were obtained from the CEPHIA repository whose stage of infection was blinded to the authors, which were tested and analysed. The proportion of samples that were determined as recent was 18.1% for LAg, 6.4% with the ARCHITECT, and 14.5% in the INNO-LIA assay. There was a significant correlation between the ARCHITECT S/CO values and the LAg results, $r=0.717$, $p<0.001$. ROC analysis revealed that an ARCHITECT S/CO < 250 had a sensitivity and specificity of 90.32% and 89.83%, respectively. Combining the Abbott ARCHITECT HIV Ag/Ab Combo assay and INNO-LIA HIV assays resulted in an observed risk of being recent of 100%. Analysis of the CEPHIA samples revealed a strong agreement between the LAg assay and the combination of routine assays ($\kappa=0.908$, $p<0.001$). Our findings provide evidence that assays routinely employed to diagnose and confirm HIV infection may be utilised to determine the recency of HIV infection.

**Keywords** HIV infection · Recency · Routine diagnostic assays

## Introduction

The accurate identification of recent HIV infection remains an important area of research for many reasons. First, discriminating recent from long-term established HIV infection is needed for more accurate estimates for HIV incidence which is key to monitoring and understanding the patterns of transmission in any given population and identifying any changes in pattern. This information is important to inform and measure population-based HIV prevention and treatment intervention policies, and ensures that prevention programmes are effectively targeted.

Second, at the individual patient level, accurate identification of recent HIV infection provides an opportunity for targeted contact tracing to prevent onward transmission of infection. Third, it has become more difficult to determine a patient's stage of HIV infection due to the improved antiretroviral therapies, intermittent or limited access thereto and patient migration. The availability of laboratory assays that can more accurately distinguish the different stages of HIV infection also carries a clinical benefit for the patient.

However, following HIV Ag loss and the development of the anti-HIV response, determining the timing of HIV infection becomes a challenge. Much attention is now focusing on the improvement of assay-based methods and the use of

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multi-assay algorithms (MAA) to better predict HIV infection recency [1]. One area of interest is the use of serological assays which should be less affected by factors such as HIV clade, host genetics, and routes of transmission. Furthermore, molecular-based assays are more challenging and more costly to implement. In the present study, three different assays were used to measure the relative quantity, quality, and avidity of HIV IgG simultaneously. These include the ARCHITECT HIV Ag/Ab Combo assay, a chemiluminescent microparticle immunoassay for the simultaneous qualitative detection of HIV p24 antigen and antibodies to human immunodeficiency virus type 1 and/or type 2 (HIV-1/HIV-2) in human serum or plasma. The ARCHITECT HIV Ag/Ab Combo result does not distinguish between the detection of HIV p24 antigen, HIV-1 antibody, or HIV-2 antibody. Therefore, to confirm the presence of antibody and discriminate between HIV-1 and HIV-2, the INNO-LIA HIV I/II score line assay is used. Schupbach and colleagues [2] demonstrated in 2007 that the intensity and banding pattern of a patient's antibody reaction in the INNO-Lia HIV I/II score assay also provides information on the recency of HIV infection. The INNO-LIA measures only IgG antibodies to different HIV recombinant proteins and synthetic peptides in a standardised, semi-quantitative way. As both the pattern and intensity of HIV-specific antibodies evolve during the first months after infection, it is possible to define algorithms which, with a certain diagnostic sensitivity and specificity, recognise antibody patterns characteristic of early HIV-1 infection. In the previous studies, Schupbach et al., determined the diagnostic sensitivity and specificity of more than 20 different INNO-LIA algorithms for differentiating between HIV-1 infections of less or more than 12 month duration [3–5]. Furthermore, a large study with patients who had been infected for at least 12 months and were selected to represent all clinical stages and major clades of HIV-1 was undertaken to study the specificity of the incident infection algorithms [6].

The Sedia limiting antigen assay, an in vitro quantitative limiting antigen (LAg) avidity enzyme immunoassay for distinguishing recent HIV-1 infections from those which are long-term, was used to simultaneously measure the quantity and avidity of HIV IgG in a specimen. Individuals with recently acquired HIV-1 infections typically have lower avidity HIV IgG than those with long-term infections [7, 8]. This assay incorporates a recombinant protein which contains the major variants of gp41 immunodominant regions among the HIV-1 group M viruses and includes a single-well avidity assay using the limiting amounts of antigen [7]. The US CDC performed testing on a number of well-characterised samples and showed that subtype bias was minimised by the use of the multi-subtype antigen. Furthermore, performance of the HIV-1 LAg-Avidity EIA in Africa has shown a false recency rate of less than 1%, suggesting improved accuracy over the previous technologies [9]. The HIV-1 LAg-Avidity EIA is based on the functional avidity of the antibodies and is, therefore, less affected by disease state than the other types of assays that have been previously used [10, 11]. The specimens classified as "recent" using the Sedia™ HIV-1 LAg-Avidity EIA have been estimated to have a mean duration of recent infection of 130 days (95% CI 118–142) [12].

Given the use of established routine serological screening and confirmatory diagnostic assays for HIV in many laboratories worldwide, in this study, we investigated whether these standard assays could be used to determine recency of infection. To confirm our investigation, a proof-of-concept analysis using samples from the CEPHIA repository (Consortium for Performance and Evaluation of HIV Incidence Assays) was performed.

## Materials and methods

### Samples

New HIV diagnoses in Ireland from January to December 2016 ($n = 455$) were included in the study. For each new HIV diagnosis, HIV INNO-LIA assay (LIA) results and risk factor data were obtained from the NVRL laboratory information management system. Patients on antiretroviral treatment or previously diagnosed (> 12 months) were excluded from the analysis ($n = 34$).

### HIV avidity testing

All samples were tested using the Sedia limiting antigen avidity assay (LAg) according to the manufacturer's protocol (Sedia Biosciences, USA). Briefly, assay controls and HIV positive specimens were diluted 1:101 in assay diluent and 100 µL of calibrator, controls, or specimens were added to the antigen-coated wells and incubated for 60 min at 37 °C. After four washes, a dissociation buffer was added to the wells to dissociate the low-avidity antibodies. After incubation for 15 min, the plate was washed. Peroxidase-conjugated goat anti-human IgG was added to the wells and the plate incubated for 30 min at 37 °C. The plates were developed and read on a spectrophotometer at a wavelength of 450 nm using a reference of 620 nm. A normalised OD (ODn) for each sample was calculated as a ratio of the OD of the sample divided by the median OD of the calibrator. The normalised cut-off in the confirmatory LAg assay for recent infection is ODn < 1.5.

### Abbott ARCHITECT® HIV Ag/Ab Combo

The automated Abbott ARCHITECT® analyser (Abbott Diagnostic Division) which uses the fourth-generation

assay for HIV Ag/Ab combo was used to test samples. Result values are given as Sample/Cut-Off (S/CO). All positive results were confirmed anti-HIV IgG positive using the INNO-LIA assay.

### INNO-LIA™ HIV I/II Score assay

All new diagnoses were subjected to confirmatory testing using the INNO-LIA HIV assay (Innogenetics N.V.). The INNO-LIA measures antibodies to different HIV antigens in a standardised, semi-quantitative way. Schupbach and colleagues [6] have shown that as both the pattern and intensity of HIV-specific antibodies evolve during the first months after infection, it is possible to define algorithms (Alg) which, with a certain diagnostic sensitivity and specificity, recognise antibody patterns' characteristic of early HIV-1 infection. The algorithm is derived from antibody reaction scores to the 7 HIV antigen bands on the LIA strip (sgp120, gp41, p31, p24, p17, sgp105, and gp36). Algorithm 15.1 [6] was applied to the INNO-LIA assay results to determine recent or non-recent HIV infection.

### Statistical analysis

Statistical analysis was conducted using SPSS 22. The Mann–Whitney *U* test for continuous variables and Fisher's exact test for categorical variables were employed. Associations between variables were determined by the non-parametric Spearman correlation test. $p \leq 0.05$ was considered significant. The Spiegelhalter–Knill–Jones approach establishes a probabilistic system for scoring information which can then assist in making a diagnosis or establishing the status of an individual patient [13, 14]. This predictive model employs the elements of logistic regression and Bayes' theorem and was used to develop a predictive scoring system which is based on the LAg assay as a 'gold standard' as this assay demonstrated 100% accuracy in identifying recency based on samples tested from the CEPHIA repository. Cut-off values for each test were LAg cut-off ODn < 1.5 and ARCHITECT S/CO cut-off < 250 to indicate recent vs long-term HIV infection (Fig. 3a). Sensitivity and specificity were calculated for each test and then used to calculate likelihood ratios for both positive and negative test results. Weights for each test results were then calculated and adjusted for dependence. The adjusted weights were then used in a logistic regression model to calculate the log (odds)—and then the probability—of an individual having a recent HIV infection. A summary of the statistical techniques used to derive Spiegelhalter–Knill–Jones weightings is outlined in the "Appendix".

## Results

### Patient demographics

Table 1 shows the descriptive statistics of the study cohort. The 2016 cohort of new diagnoses was predominantly male ($n = 320$, 76.0%) and MSM/bisexual (78.9%). The mean age of the cohort was 36.2 ± 9.6 years and was similar in the male (34.2 ± 4.71 years) and female (35.7 ± 9.1 years) groups. Males had higher mean HIV VL (51,745 ± 10,725 vs 17,234 ± 6,156) and mean LAg (3.72 ± 1.31 vs 3.49 ± 1.71); however, there was no statistical difference. The proportion of recent infections identified by LAg assay was similar in males and females (18.8% vs 16.7%); however, twice as many males (16.6%) compared to females (8.3%) were classified as recent using Algorithm 15.1.

### Correlation of LAg incidence assay with the INNO-LIA HIV assay

Of the 76 samples identified as recent by the LAg assay, applying the INNO-LIA HIV Algorithm 15.1 criteria further sub-divided this cohort into 39 individuals as 'recent' and 37 individuals as 'non-recent'. There was a significant difference in the mean LAg result between these two groups (mean ± SE: 0.513 ± 0.066 vs 0.785 ± 0.068, $p < 0.01$) (Fig. 1a). The number of patients with ODn < 1.0 in the groups was also significantly different ($p < 0.03$). However, HIV VL were similar between these groups (mean copies/ml ± SE: 71,930 ± 39,195 vs 66,567 ± 13,880, $p$ = NS, data not shown) and there was no significant difference in age between the groups (mean years ± SE: 32.79 ± 1.43 vs 35.05 ± 1.62, pNS) (Fig. 1b).

**Table 1** Descriptive characteristics of the study population in Ireland, 2016

| Variable | | Males ($n = 320$) | Females ($n = 96$) |
|---|---|---|---|
| Age | Mean (SD) | 34.20 (4.71) | 35.71 (9.06) |
| Avidity | Mean (SD) | 3.72 (1.31) | 3.49 (1.71) |
| ARCHITECT | Mean (SD) | 688.42 (222.13) | 608.77 (263.24) |
| Viral load | Mean (SE) | 51,745 (10,725) | 17233.78 (6,156) |
| Recent (LAg < 1.5) | *n* (%) | 60 (18.8) | 16 (16.7) |
| Recent (Alg 15.1) | *n* (%) | 53 (16.6) | 8 (8.3) |

Gender was not reported for five patients

*Alg 15.1* Algorithm 15.1 (Schupbach et al. [6])

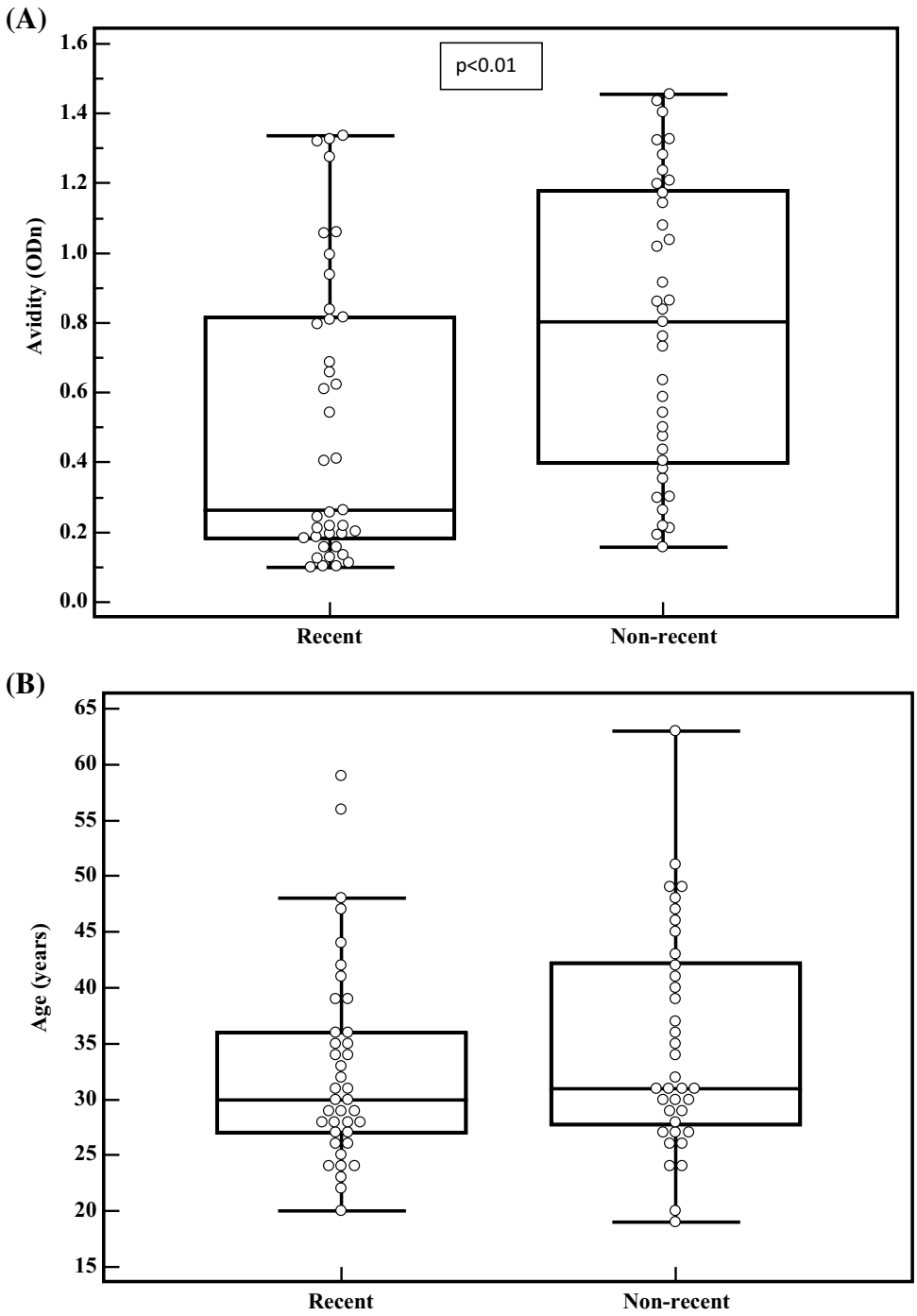



**Fig. 1** Box and whisker plot of the comparison of patients identified as recent or non-recent according to Schupbach's Algorithm 15.1 with reference to **a** avidity and **b** age

## HIV avidity correlates with ARCHITECT HIV antibody levels

There was a strong and significant correlation between the ARCHITECT S/CO values and the LAg results, $r = 0.717$, $p < 0.001$ (Fig. 2a). Receiver operator curve analysis revealed that an ARCHITECT S/CO < 250 had a sensitivity and specificity of 90.32% and 89.83% respectively, compared to LAg, as shown in Fig. 2b.

The ARCHITECT revealed high sensitivity and specificity, and performed better than the INNO-LIA HIV assay (Schupbach's Algorithm 15.1) in detecting the recent HIV. The ARCHITECT with a cut-off of S/CO < 250 denoting recent HIV correctly classified recent HIV in 70% of cases and correctly classified long-term HIV in 94.9% of cases, whereas the INNO-LIA HIV assay using Schupbach's Algorithm 15.1 correctly identified the recent HIV in 51.3% of cases and long-term HIV in 93.6% of cases.

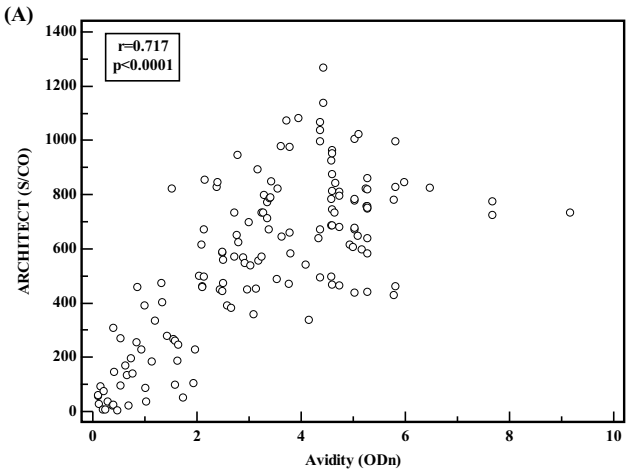



**Fig. 2** **a** Correlation of HIV avidity with HIV S/CO as determined by the ARCHITECT in 149 patient samples. **b** Receiver operator curve analysis

## Assessment of the predicted risk of recent HIV infection

Combining the results for Algorithm 15.1 and ARCHITECT assays together provided more information than any one of the markers alone. The Spiegelhalter–Knill–Jones method, which is an extension of logistic regression, was used to derive weighting for a predictive scoring system based on the available data to predict a patient's risk of having a recent HIV infection. The results of the regression model of the observed and predicted risk for each routine diagnostic test alone and in combination with the other assays are shown in Table 2.

## Proof-of-concept analysis confirms the use of routine HIV antibody assays for HIV recency determination

As further proof of concept of the trends obtained in our clinical samples, 50 highly characterised samples were obtained

**Table 2** Regression model of the observed risk of being recent and the risk to predict recency in the new scoring system using Spiegelhalter–Knill–Jones analysis

| Individual tests | Observed risk of being recent (%) | Risk predicted in new scoring system (%) |
|---|---|---|
| Algorithm 15.1 | 63.9 | 79.3 |
| ARCHITECT | 77.8 | 76.7 |
| Algorithm 15.1 & ARCHITECT | | |
| Both positive | 100.0 | 97.6 |
| Alg+, Arch− | 50.0 | 57.4 |
| Alg−, Arch+ | 62.5 | 60.1 |
| Both negative | 5.2 | 4.8 |

*Alg* Algorithm 15.1, *Arch* ARCHITECT, *Alg* + recency by applying Schupbach's Algorithm 15.1, *Arch* + a S/CO < 250

'blind' from the CEPHIA repository, and were tested and analysed. Analysis of the CEPHIA samples revealed a strong and significant correlation between the ARCHITECT assay and the LAg assay, $r = 0.891$, $p < 0.001$. There was moderate agreement between the LAg assay and Algorithm 15.1, $\kappa = 0.646$, $p < 0.001$ and there was no correlation between the HIV VL and the LAg assays, $r = -0.275$, $p = 0.058$. However, there was a strong agreement between the LAg assay and the combination of standard assays ($\kappa = 0.908$, $p < 0.001$). At ARCHITECT (S/CO) values below 250, all specimens yield a LAg < 1.5, indicating recent infection, and at values above 375, no specimens were classified as recent (Fig. 3b). There was perfect agreement between the ARCHITECT assay at an S/CO cut-off of 250 and the LAg assay at a cut-off of 1.5.

## Discussion

The present study provides insight for establishing the optimal testing strategies for the accurate and timely diagnosis of recency in HIV infected individuals using HIV serological assays which are commercially available and are used on a daily basis in many laboratories worldwide, particularly in Europe. The findings in this study show that the routine diagnostic HIV assays performed by the Abbott ARCHITECT Ag/Ab Combo assay and the INNO-LIA HIV assay can be used to provide accurate HIV staging in patients. We provide evidence of the clinical utility of these assays in new applications for HIV recency determination. The quantitative HIV antibody assays examined in this study can be completed in minutes to hours, and the cost is minimal. These assays have been extensively validated in numerous laboratories and have been found to be consistently reproducible and have been certified. These routine diagnostic assays can be quality controlled by using calibrators and quality

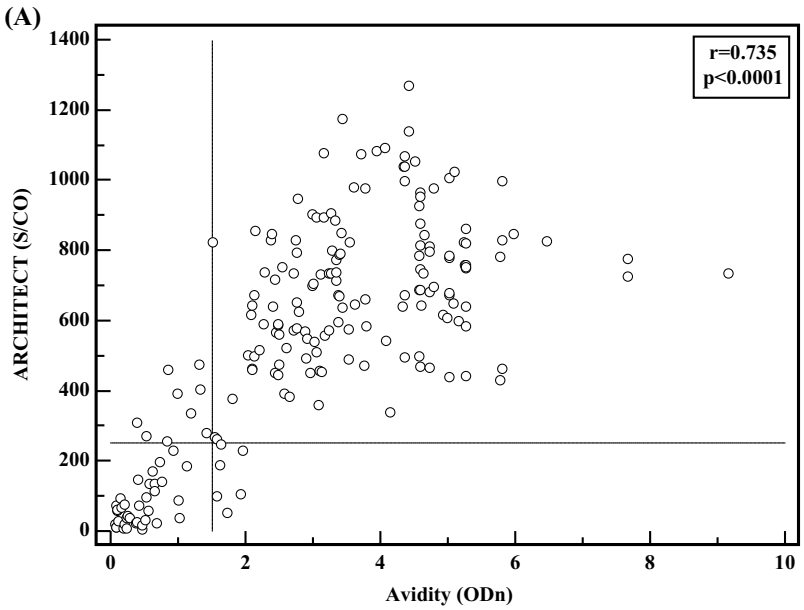
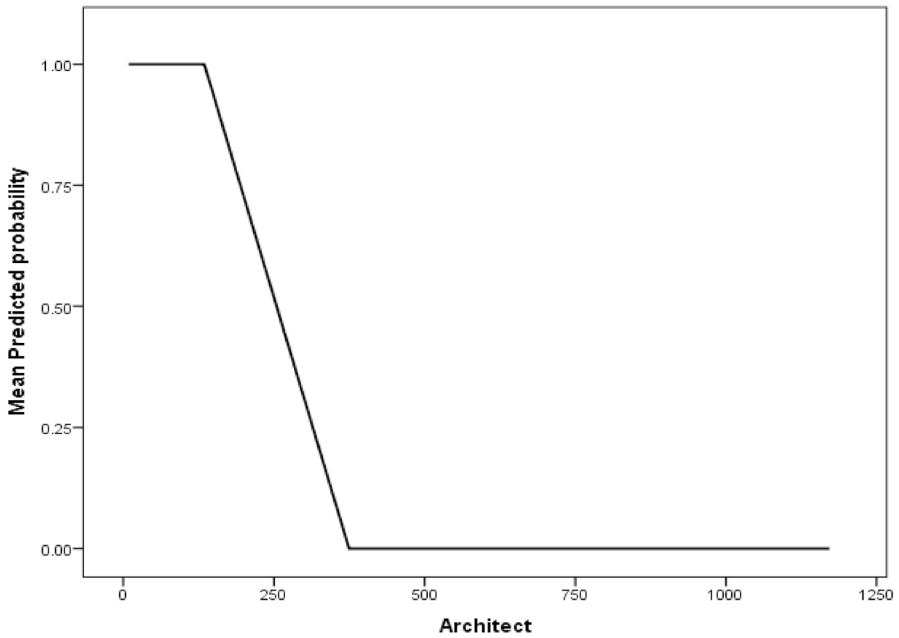



**Fig. 3** **a** Correlation of Architect HIV (S/CO) and HIV avidity in all samples (149 patient samples and 49 CEPHIA samples; $n = 198$). **b** Logistic regression analysis of LAg vs Architect results of CEPHIA samples

**(B)** Logistic regression analysis of LAg vs Architect results of CEPHIA samples.




controls for accurate and precise assessments. The need for additional HIV avidity testing could, therefore, be decreased resulting in significantly reduced costs. In agreement with our findings, in a previous study, the ARCHITECT HIV Ag/Ab Combo assay was modified to include a dissociation step to determine avidity and was able to accurately distinguish recent from the established HIV infections [15].

In this study, we have also developed a predictive scoring system which was based on multi-assay parameters at diagnosis to predict the risk of having recently acquired HIV infection. This predictive scoring system combined the relative quantity and quality measures of HIV IgG simultaneously. Using the standard laboratory assays, this predictive scoring system allows an individualised assessment of risk for prediction of recency, and can be used for cross-sectional testing and biomarker information which may be an alternative to longitudinal testing. Our findings show that the combination of the ARCHITECT and INNO-LIA assays

has the potential to identify a higher proportion of recent HIV infections as, individually, the assays have a predicted risk of being recent of 76.7% and 79.3%, respectively, and, however, together, the risk is 97.6% (Table 2).

The ARCHITECT HIV Ag/Ab Combo assay is a combination assay, so S/CO values could be due to Ag only. However, as the INNO-LIA confirmatory assay which detects only IgG was positive for all ARCHITECT HIV Ag/Ab samples, the presence of IgG anti-HIV in these samples is confirmed. Experience at the NVRL indicates that p24 Ag only positivity is a rare occurrence with about 2–6 cases annually. During a recent upsurge of acute HIV infection in people who inject drugs [16], samples collected from patients who were HIV p24 Ag positive, but INNO-LIA confirmatory assay negative rarely exceeded the S/CO value of 250 in the ARCHITECT HIV Ag/Ab assay despite the possibility that IgM anti-HIV may also be present. In addition, S/CO values greater than 250 are not usually detected in the commercial seroconversion panel members which are only p24 Ag positive. It is well documented that patients on ART or elite controllers can be misclassified as recent in the incidence assays. Therefore, more research is required to fully elucidate the clinical utility of these standard diagnostic assays in the other cohorts of HIV patients who are virally suppressed such as those on ART and elite controllers.

Cross-sectional methods to determine HIV infection recency present a promising and cost-effective alternative to the repeated testing of uninfected individuals. Recently, a longitudinal study of HIV patients reported that antibody levels are stable with the higher antibody levels in viremic patients consistent with the notion that viral replication or antigen expression is responsible for the maintenance of antibody levels [17]. In the present study, however, the use of HIV VL measurement was found to be the least informative with regards to recency determination (data not shown). Indeed, HIV RNA level monitoring during treatment is mainly used to assess treatment adherence and to detect rare virologic breakthroughs related to viral resistance.

The previous reports have shown an association between the antigenic burden and antibody response in patients with HTLV-1 and HIV [18, 19]. Recent studies have also shown that declining antibody levels during ART probably reflects lower levels of antigen production and/or viral replication in the persistent HIV reservoir and reduced chronic stimulation of the humoral response [17]. Interventions to eliminate the latent reservoir of HIV to achieve 'functional cure' have been discussed widely. Further studies are required to determine if the standard routine HIV antibody diagnostic assays alone or in combination could be useful to estimate and monitor the latent reservoir during treatment.

In conclusion, in this study, we have demonstrated that commercial serological assays used routinely to diagnose, confirm, and monitor response to treatment can also accurately classify the recent HIV infection. Our results show that the combination of the ARCHITECT and INNO-LIA assay (Schupbach's Algorithm 15.1) has the potential to correctly identify a high proportion of recent HIV infections in a cost-effective manner; however, as with most immunoassays in low prevalence settings, a definitive interpretation of recent HIV infection should include an examination of the patient's immune status and clinical history. Our findings agree with the recent findings by Grebe et al. [20] that the ARCHITECT HIV Combo assay could provide information on HIV infection staging; however, studies from the other laboratories are required to confirm our findings. The use of these assays to identify HIV recency presents a promising alternative approach to the LAg assays to estimate HIV incidence.

**Author contributions** Concept and design of the study: JH and JM; acquisition of data: JH and JM, statistical analysis: JH and OM; interpretation of data and drafting the manuscript: JH and JC; critical revision of the manuscript: CDG and GM.

## Compliance with ethical standards

**Conflict of interest** The authors declare they have no conflict of interest.

**Ethical approval** For this type of study, formal consent is not required.

## Appendix

A summary of the statistical techniques used to derive Spiegelhalter–Knill–Jones weightings is outlined by Seymour et al. [13].

To employ the Spiegelhalter–Knill–Jones method, which is an extension of logistic regression, all explanatory variables as well as the outcome variable must be binary. The LAg assay is the outcome variable and a cut-off of ODn $\leq 1.5$ is used, with those $\leq 1.5$ regarded as being recent infections. Algorithm 15.1 is presented in the data set as a binary variable (recent or non-recent), so no division or recoding is needed. A cut-off for the ARCHITECT S/CO of 250 was used in this study, with values $< 250$ regarded as recent infections.

Briefly as outlined by Seymour et al. [13], the mathematical derivation of Spiegelhalter–Knill–Jones weightings:

- The independence Bayes' equation is:

$$\text{Posterior odds} = \text{prior odds} \times \text{LR of variable 1} \times \text{LR of variable 2} \times \cdots \times \text{LR of variable } n,$$

where

(a) the posterior odds are the predicted odds of the outcome in an individual;
(b) the prior odds are the odds of the outcome in the population under study;
(c) LR is the likelihood ratio:

$$\text{LR} = (\text{sensitivity})/(1 - \text{specificity})$$

for a positive test result (in this study, recenct), OR

$$\text{LR} = (1 - \text{sensitivity})/(\text{specificity})$$ for a negative test result (in this study, non − recent).

- Taking the natural logarithms and multiplying by 100, the independence Bayes' equation becomes the following:

$$\begin{aligned}100 \times \ln(\text{posterior odds}) = {} & 100 \times \ln(\text{prior odds}) \\ & + 100 \times \ln(\text{LR of variable 1}) \\ & + 100 \times \ln(\text{LR of variable 2}) \\ & + \ldots + 100 \times \ln(\text{LR of variable } n).\end{aligned}$$

- In terms of the Spiegelhalter–Knill–Jones method, this equation becomes the following:

$$\begin{aligned}\text{Total score } (T) = {} & \text{starting score} \\ & + \text{weight of evidence of variable 1} \\ & + \text{weight of evidence of variable 2} \\ & + \cdots + \text{weight of evidence of variable } n.\end{aligned}$$

- This equation assumes that the variables are independent of one another, which is unrealistic in many cases, so the Spiegelhalter–Knill–Jones method calculates adjusted weights to account for dependence. Adjusted weights are obtained by entering the raw weights of evidence as independent variables in a logistic regression equation. This produces the final form of the predictive equation:

$$\begin{aligned}\text{Total score } (T) = {} & \text{starting score} \\ & + \text{adjusted weight of evidence for variable 1} \\ & + \text{adjusted weight of evidence for variable 2} \\ & + \cdots + \text{adjusted weight of evidence for variable } n.\end{aligned}$$

- Because $T = 100 \times \ln(\text{posterior odds})$ and because odds = probability/(1 − probability), the predicted probability can be calculated by the following:

$$\text{Predicted probability } (\%) = T = 100\ln(p/1-p).$$

Therefore, $\text{Ln}(p/1-p) = T/100$:

$$(p/p-1) = e^{(T/100)}$$
$$P = e^{(T/100)}(1-p) = e^{(T/100)} - e^{(T/100)}p$$
$$P + e^{(T/100)}p = e^{(T/100)}$$
$$P(1 + e^{(T/100)}) = e^{(T/100)}$$
$$P = e^{(T/100)}/(1 + e^{(T/100)})$$
$$\text{Predicted probability } (\%) = e^{(T/100)}/(1 + e^{(T/100)}) \times 100$$

The application of the Spiegelhalter–Knill–Jones method for the two variables studied (Algorithm 15.1 and ARCHITECT S/CO) is shown below (Table 3).

LR and Crude weights were calculated as shown below. Logistic coefficients were obtained by logistic regression analysis of the raw data using SPSS 22.

## Individual markers

### ARCHITECT S/CO versus LAg as the gold standard

Sensitivity = 21/30 = 70%.

Specificity = 112/118 = 94.9%.

The observed risk of being recent when ARCHITECT < 250 (suggests recency) is 21/27 = 77.8% (as shown in Table 2 in the manuscript).

Likelihood ratios for recency:

Sensitivity/100 − Specificity = 70.0/100 − 94.9 = 70.0/5.1 = 13.73.

Ln13.73 = 2.62.

2.62 × 100 = 262 = weighting to be applied to a recent test.

Likelihood ratios for non- recency:

100 − Sensitivity/Specificity = 100 − 70.0/94.9 = 30.0/94.9 = 0.316.

Ln0.316 = − 1.152.

− 1.152 × 100 = − 115.2 = weighting to be applied to a non-recent test.

### Algorithm 15.1 versus LAg as the gold standard

Sensitivity = 39/76 = 51.3%.

Specificity = 323/345 = 93.6%.

The observed risk of being recent when Algorithm 15.1 suggests that recency is 39/61 = 63.9% (as shown in Table 2 in the manuscript).

Likelihood ratios for recency:

Sensitivity/100 − Specificity = 51.3/100 − 93.6 = 51.3/6.4 = 8.016.

**Table 3** Derivation of scoring system based on Spiegelhalter–Knill–Jones method

| | Recent (%)) | Non-recent ($n=345$) | LR | Crude weights | Logistic coefficients (SE) | Adjusted weights | Adj. weights × 100 |
|---|---|---|---|---|---|---|---|
| Prior probability | 0.18 | 0.82 | | 22 | − 1.171 (0.324) | − 1.17 | − 117 |
| Algorithm 15.1 | | | | | | | |
| Recent | 39 | 22 | 8.05 | 208 | 0.012 (0.003) | 2.5 | 251 |
| Non-recent | 37 | 323 | 0.52 | − 65 | | − 0.78 | − 78 |
| Architect (S/CO) | | | | | | | |
| < 250, recent | 21 | 6 | 13.77 | 262 | 0.009 (0.002) | 2.36 | 236 |
| ≥ 250, non-recent | 9 | 112 | 0.32 | − 115 | | − 1.04 | − 104 |

Ln8.016= 2.081.

2.081 × 100 = 208 = weighting to be applied to a recent test.

Likelihood ratios for non-recency:

100 − sensitivity/specificity = 100 − 51.3/93.6 = 48.7/93.6 = 0.520.

Ln0.520= − 0.654.

− 0.654 × 100 = − 65.4 = weighting to be applied to a non-recent test.

## Predictive analysis for the two markers—ARCHITECT S/CO and Algorithm 15.1

### ARCHITECT S/CO (recent) and Algorithm 15.1 (recent) versus LAg as the gold standard

See Table 4.

$$\text{Predicted probability (\%)} = 100\ln(p/1-p)$$
$$= -117 + (0.009 \times 100)(262) + (0.012 \times 100)(208)$$
$$= -117 + 235.8 + 249.6 = 368.4$$
$$\ln(p/1-p) = 3.684$$
$$(p/1-p) = 39.815.$$

Predicted risk of being recent (as measured by the LAg as gold standard) if both tests suggest the recent infection = 39.815/1 + 39.805 = 39.815/40.815 = 97.6% (as shown in Table 2 in the manuscript).

### ARCHITECT S/CO (non-recent) and Algorithm 15.1 (non-recent) versus LAg as the gold standard

See Table 5.

$$\text{Predicted probability (\%)} = 100\ln(p/1-p)$$
$$= -117 + (0.009 \times 100)(-115) + (0.012 \times 100)(-65)$$
$$= -117 + (-103.5) + (-78) = -298.5$$
$$\ln(p/1-p) = -2.985$$
$$(p/1-p) = 0.0505.$$

Predicted risk of being non-recent (as measured by the LAg as gold standard) if both tests suggest non-recent infection = 0.0505/1 + 0.0505 = 0.0505/1.0505 = 4.8% (as shown in Table 2 in the manuscript).

**Table 4** Crosstabulation of LAg with ARCHITECT (S/CO < 250)

| | Gold standard (LAg) | | |
|---|---|---|---|
| | Recent | Non-recent | Total |
| ARCHITECT | | | |
| Recent | 21 | 6 | 27 |
| Non-recent | 9 | 112 | 121 |
| Total | 30 | 118 | 148 |

**Table 5** Crosstabulation of LAg with Algorithm 15.1

| | Gold standard (LAg) | | |
|---|---|---|---|
| | Recent | Non-recent | Total |
| Algorithm 15.1 | | | |
| Recent | 39 | 22 | 61 |
| Non-recent | 37 | 323 | 360 |
| Total | 76 | 345 | 421 |

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