Evaluation of a fourth-generation avidity assay for recent HIV infections among men who have sex with men in Amsterdam

Serological assays to distinguish recent (<4–6 months) and established (≥4–6 months) infections using a single specimen from individuals newly diagnosed with human immunodeficiency virus (HIV) have become increasingly popular as part of HIV surveillance (Murphy & Parry, 2008). These RITA (Recent Infection Testing Algorithm) assays enable more in-depth understanding of HIV transmission, and allow better estimations of HIV incidence and the identification of risk factors for recent infections (RIs). Current assays are based on the evolving immunological response over time. An example is an assay that determines the avidity index (AI) for anti-HIV antibodies by adding a denaturing agent that disturbs the binding of low-avidity antibodies (Suligoi et al., 2011). The increasing affinity of antibodies after an infection results in a higher AI over time.

We used a fourth-generation assay (Architect HIV Ag/Ab Combo; Abbott) to measure proportions of RIs in newly diagnosed HIV patients in the Netherlands by comparing the performance of this assay in two sample panels from newly HIV-diagnosed men who have sex with men (MSM) at an Amsterdam sexually transmitted infections clinic. In the first panel, the performance of the Architect instrument was compared in plasma and serum. In the second panel, this assay was compared to previously obtained results (Murphy et al., 2009) from four other RITA assays: a less-sensitive (detuned) enzyme immunoassay (EIA) (Vironostika), a proportional assay (BED-capture EIA), the third-generation AxSYM EIA and an immunodominant assay (IDE-V3-EIA).

The first panel included plasma samples (n=200) collected between 2009 and 2012 as part of the ‘Delay’ study (Van Veen et al., 2013) and recently used for incidence estimation (Sane et al., 2014). Parallel serum samples were available from 185 MSM, of which 90% of samples were collected ≤2 weeks before plasma sample collection. AI measurements were successfully established in both samples for 183 individuals.

The median age of the HIV-positive MSM was 35 years (range 17–71 years). Overall, 76% of the men originated from the Netherlands or other European countries, 15% from South America and 9% from other regions.

The second panel included serum samples (n=172) from diagnostic testing (2007 or earlier) collected from MSM at the same clinic for a European RITA validation study by Public Health England and the European Centre for Disease Prevention and Control (Murphy et al., 2009). For this validation, the samples were tested with the four RITA assays, as described above, to provide insight into the comparability among assays. We compared results from the fourth-generation Architect assay to these results (n=170; two samples were excluded). The median age of MSM in this panel was 35 years (range 19–70 years).

In both panels, the anti-HIV-AI was measured using the Architect assay (AI cut-off: 0.80, corresponding mean RITA ‘window period’: 180 days) (Suligoi et al., 2011). The protocol was validated for serum samples from HIV-positive individuals. Since our largest sample set included plasma, we first compared the assay performance for both types of specimens. The AI was determined by testing two aliquots of thawed sample diluted with either 1 M guanidine hydrochloride or PBS, using 50 μl serum and 450 μl diluent. After 5–10 min incubation at room temperature, the samples were assayed using the Architect HIV Ag/Ab Combo test. The AI was calculated by dividing the sample cut-off ratio for the measurement in the presence of guanidine by the signal to cut-off ratio of the sample diluted in PBS. Samples were defined as ‘RI’ (<6 months from seroconversion) if the AI was ≤0.80, and as ‘established infection’ (>6 months from seroconversion) if the AI was >0.80.

Cut-off values for RI with the other assays were: 1.0, 170 days (detuned); 0.8, 153 days (BED); 0.5, 178 days (IDE-V3); and 0.8, 142 days (AxSYM). Data were analysed using SPSS version 19 (IBM).

Fig. 1 shows a scatter plot of the quantitative AI for HIV antibodies for serum–plasma comparison (n=183 pairs) in the first panel. The median ±SD AI in serum specimens (0.92±0.21) was similar to that of plasma specimens (0.92±0.22). Pearson correlation was 0.84.

In 165 of 183 sample pairs (90%), the conclusion (RI or established infection) was concordant. In 56 of these 165 cases (34%) the conclusion was RI, while 109 sample pairs (66%) were from patients with established infections. The median ±SD AI in serum was 0.64±0.12 and 0.99±0.11 for RIs and established infections, respectively. In plasma, the values were 0.63±0.12 and 0.99±0.12, respectively.

The comparative evaluation of the Architect assay using the second panel and four other assays showed large variability in the results. In 64% of the samples, all five assays showed concordant RIs or established infections; in 36%, however, one or more assays showed discordant results.

The highest proportion of RIs was detected using the BED (73/170, 43%), followed by the detuned (63/170, 37%), AxSYM (50/170, 29%), Architect (48/170, 28%) and IDE-V3 (20/170, 12%). Relative comparison with the Architect as reference (=1.0) showed that this assay sits in the results. In 64% of the samples, all five assays showed concordant RIs or established infections; in 36%, however, one or more assays showed discordant results.

In conclusion, we used the fourth-generation Architect avidity assay, for the
first time in the Netherlands, to distinguish RIs from established HIV infections in MSM attending an Amsterdam sexually transmitted infections clinic. We showed that both serum and plasma samples are suitable for this assay. Proportions of RIs were similar for the third-generation AxSYM and fourth-generation Architect AI assays, but differed from other RITA assays. This variability in test performance likely results from differences in test cut-off points and ‘false recent’ rates. The Architect assay is a useful tool for distinguishing RIs from established HIV infections, but requires (as do other assays) serious consideration of sample and data collection if used for national surveillance purposes (European Centre for Disease Prevention and Control, 2013). Furthermore, acute infections could be missed since p24-positive, antibody-negative samples will result in a false-high AI, caused by high avidity of monoclonal antibodies contained in the assay reagents for p24 detection (Suligoi et al., 2011). Additional measurement for p24 could differentiate between true and false-high AIs.

Acknowledgements

We thank B. Suligoi from the Istituto Superiore di Sanita in Rome for the laboratory protocol. We thank J. Parry, A. Charlett and F. Barin from EuroHIV Work Package 7 for testing Dutch samples. We thank B. van Benthen and M. van der Sande (RIVM) for providing comments. J. van der Helm, M. van Rooijen and A. Speksnijder (PHS Amsterdam) are thanked for providing data and samples. This study was funded by the National Institute for Public Health and Environment (RIVM). The authors declare no conflicts of interest. The ‘Delay’ study was approved by the Medical Ethics Committee of the Free University Amsterdam. Patients gave consent for the anonymous use of leftover samples for scientific research purposes.

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Abbreviations: AI, avidity index; EIA, enzyme immunoassay; HIV, human immunodeficiency virus; MSM, men who have sex with men; RI, recent infection; RITA, Recent Infection Testing Algorithm.

Fig. 1. Scatter plot of AI results from 183 paired serum and plasma specimens (cut-off value: 0.80).