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Development of an avidity assay for detection of recent HIV infections

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A B S T R A C T

HIV avidity can measure the incidence of recent infections within the population. The aim of this study was to evaluate an HIV avidity assay, initially from a clinically defined group of patients and then apply the assay to a prospective study to determine the false recency rate and mean duration of recency for the assay. The assay is a commercial ELISA modified with 7 M urea. The validation of the assay used plasma from patients split into Group 1 (recently infected $N = 25$) and group 2 (established infection $N = 301$). The prospective study tested 178 newly diagnosed HIV patients for avidity. A total of 326 retrospective samples of known HIV status were collected and tested. The initial evaluation gave a sensitivity 100% (CI 86.16–100%) and specificity of 98.65% (95% CI 97.05–99.78%). The prospective study incorporating 178 newly diagnosed patients found 22 patients with low avidity. Follow-up samples obtained from low avidity patients determined the estimated mean duration of recency to be between 3 and 4 months with a false recency rate of 0.89% (CI: 0.24–2.3%). The assay described here compares well in sensitivity, specificity and false recency rate with that of other published avidity assays.

1. Introduction

HIV incidence measures the number of new infections occurring within a population during a given timeframe. Understanding the incidence of new infections allows public health campaigns to target at risk groups and promote contact tracing (Semaille et al., 2008; Fox et al., 2009; Mastro, 2013). Detecting recent HIV infection is difficult because most individuals are asymptomatic and current laboratory tests do not effectively differentiate between recent and chronic infection.

There have been several published methods for HIV recency assays including the BED-CEIA, IDE-V3 and avidity (Parekh et al., 2002; Suligoi et al., 2002; Barin et al., 2005). Antibody avidity is the binding capacity of maturing antibody to antigen, which increases over time. Various HIV avidity assays have been described including modified commercial assays (Suligoi et al., 2002, 2003; Masciotra et al., 2010; Suligoi et al., 2011) and novel avidity assays including the limiting-antigen avidity assay (Doung et al., 2012) and the Bio-Plex COOH microsphere multiplex assay (Curtis et al., 2012).

The HIV avidity assay, described in this paper, is a modified version of the Genscreen HIV 1/2 v2 ELISA from Bio-Rad. A Bio-Rad method has previously been published using diethylamine (DEA) as the chaotropic agent (Masciotra et al., 2010). Here, we describe a Bio-Rad assay where the chaotropic agent is urea. The incubation times and temperatures for the assay in this paper are different from the previously published method. Various aspects of our assay were first evaluated, including its ability to correctly classify known recent and chronic HIV infections and to determine the average duration in which the assay will recognise a newly diagnosed infection as recently acquired. This period is known as the mean duration of recent infection (MDRI). After development and validation of the assay we were, to our knowledge, in a unique position to confirm our recent infections by demonstrating a maturing antibody response using follow-up samples. Most programmes use single samples (usually anonymised) for epidemiological purposes only. The aim of this study was to evaluate an HIV avidity assay using a set of clinically defined samples and then apply the assay to a prospective study where testing follow-up samples from recent cases determined the false recency rate (FRR) and the MDRI for the assay.

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2. Materials and methods

2.1. HIV avidity assay

The avidity assay is a modification of the Genscreen HIV1/2 Version 2 (Bio-Rad). Plasma samples were initially diluted 1/400 in sample diluent and 100 μl added, in duplicate, to 2 wells of a 96 well plate. The plate was incubated at 37 °C for 30 min before being washed once with 300 μl wash buffer. The samples were then washed for 5 min at RT in 300 μl kit buffer whilst 300 μl of 7 M urea was added to the wells containing the duplicate samples (data for optimisation of urea concentration not shown). This was repeated three times for 5 min. The microtitre plate was then washed 12 times with 300 μl of wash buffer using an automated plate reader. The manufacturer's guidelines were followed for the remaining steps of the assay. The AI was calculated by taking the optical density (OD) of the urea treated sample divided by the OD of the wash buffer treated sample and expressed as a percentage. The OD was measured using the plate reader function on the Siemens BEP *III*. Samples with an OD < 1.0 were re-tested at a 1/100 dilution using the protocol described above. If the sample OD ≥ 4.0 (saturation point on the plate reader), it was re-tested at a dilution of 1/1600. The AI cut-off was based on results from a retrospective analysis of samples of known HIV status as described below. Based on the intra-assay and inter-assay variability, a zone of ±10% around this cut-off was established as an equivocal range with samples falling within this being retested in duplicate and a final AI was then calculated based on the mean of the three AI results.

2.2. Retrospective evaluation

Group 1 consisted of 25 patients diagnosed with a recently acquired HIV infection, defined as having one or more of the following: (a) an anti-HIV negative result ≤ 12 months ago, or (b) a clinically defined seroconversion illness, or (c) a negative/indeterminate western blot that later became positive (Table 1). Each sample used within group 1 was either the first HIV positive sample or a sample taken within 1 month of diagnosis. All viral loads were >1000 copies/ml indicating recent infection. The majority of group 1 were subtype B (64.0%) with the remainder subtype C (12%) or subtype unknown (24%). The full laboratory and clinical details for group 1 is shown in Table 1. Group 2 consisted of 301 patients known to have an established HIV infection, i.e. diagnosed as being HIV positive for >1 year. A monitoring viral load sample from each established patient was used in this panel. The subtype profile for group 2 was: B (40.9%), C (19.9%), A (6.6%), G (3.0%), CRF-02 AG (3.0%), CRF-01-AE (2.0%), D (0.3%) and complex recombinants (0.7%). The subtype profile was not known for 23.6% of group 2. Information on the number of group 2 on treatment was unknown. The viral load for 238/301 (79.1%) of group 2 was <50 copies/ml which suggested some of these patients may have been on treatment. The remainder of group 2 had an average viral load 53,880 copies/ml (range 50 copies/ml–1,740,538 copies/ml). The samples from both groups were derived from patients who attended clinics between January 2006 and January 2012, and had been stored at −80 °C.

We used follow-up samples, where available, from group 1 patients to estimate the MDRI of the assay. The number of follow-up samples and the time taken between these samples were dependent on the timing of out-patient clinic attendance(s). The follow-up samples had to show an increase in avidity over the cut-off. An estimate of the MDRI avidity assay was calculated based on the clinical/laboratory information available: (a) time from a clinically defined seroconversion illness, or (b) time from a negative/indeterminate western blot that became positive on follow-up samples. As HIV antibodies can take ~1–2 months to develop following exposure, a period of 1 month was taken as the time for antibody development in this study. A seroconverter panel HIV9079 (ZeptoMAtrix Corporation) was also used.

2.3. Intra-assay and inter-assay variability

Intra-assay variability was assessed by means of the coefficient of variation (CoV) of a low avidity and a high avidity sample tested 10 times on one avidity run. The inter-assay variability, again expressed as a CoV, was assessed using data from one low and one high avidity control sample tested on 14 different runs of the assay.

2.4. Prospective evaluation

Between April 2012 and September 2013, all new HIV positive patients detected at the West of Scotland Specialist Virology Centre in Glasgow and the Specialist Virology Centre in Edinburgh were tested for HIV avidity. Laboratory data (ImmunoCombII HIV1 & 2 Biospot, viral load, CD4 count, last known HIV negative test) and clinical information (evidence of seroconversion, markers of advanced disease) were available. Further assessment of the assay's ability to detect recent HIV was obtained from testing follow-up samples from patients with an initial low avidity result. The timing and availability of follow-up samples was dependent on out-patient clinic attendance.

2.5. Calculation of sensitivity, specificity and FRR

Sensitivity was calculated as the proportion of recently infected individuals who had an avidity index of <40%, whilst specificity was determined as the proportion of individuals with a longstanding infection with an AI ≥ 40%. The FRR was determined as the proportion of persons with longstanding infections misclassified as recent infections, having an AI < 40%.

3. Results

3.1. Assay evaluation

3.1.1. Retrospective evaluation

A total of 326 retrospective samples of known HIV status were collected and tested for avidity. The group consisted of subtype B (42.6%), subtype C (19.3%), subtype A (6.1%), other subtypes and recombinant forms (8.3%) and subtype unknown (23.6%). An avidity result was obtained for 317 of the samples; nine patients were excluded due to having either a continuing low OD ($n = 8$) or high OD ($n = 1$) following the second round dilution at 1/100 and 1/1600 respectively. All 25 samples from group 1 (recent) had an AI ≤ 34%, all but one being <30% (Tables 1 and 2). The majority (99.0%) of group 2 (established) had an AI > 40%. However, there were 3 samples that had an AI < 40% but ≥31% (Table 2). As per the protocol, these samples were re-tested in duplicate but the mean AI of the three samples remained below 40%. The sample from group 1 with an AI of 34% was also repeated in duplicate and the mean AI for this sample was 21%. This individual had a negative confirmatory antibody result 15 days prior to this sample which is in keeping with a recent infection. Based on this data, an AI cut-off of <40% was determined to maximise the sensitivity of the assay for detecting recent infections whilst minimising any compromising effect on specificity. Additionally, the results of the inter-assay variability assessment led to a ±10% grey zone being incorporated into the assay, with samples falling within this range (AI of between 30 and 50%) to be repeat tested in duplicate to validate the avidity result. Testing of 326 retrospective samples using this protocol gave a sensitivity of 100% (95% CI 86.16–100%) for identification of recent