

# AN IMMUNOASSAY FOR THE IDENTIFICATION OF RECENT HIV-1 INFECTIONS: DEVELOPMENT AND VALIDATION FOR USE ON DRIED BLOOD SPOTS

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## Aim

To develop and to validate an immunoassay able to identify recent HIV-1 infections that can be used on dried blood spots (EIA-RI, for *Elisa for recent infection*).

## Preliminary developments

A single, indirect ELISA was developed to quantify antibodies towards four HIV-1 antigens: consensus peptides of the immunodominant epitope of gp41 (IDE), consensus V3 peptides, recombinant integrase, and recombinant p24. Serum samples were diluted 1:100, and incubation times were 30' (serum dilution), 30' (conjugate), 30' (substrate). Results indicated that the IDE and V3 peptides were the best able to discriminate between the antibodies present in serum from recently ( $\leq 6$  months) infected individuals and those with long-lasting infection. Combined quantification of antibody binding to these two synthetic antigens allowed us to identify recent infections with a sensitivity of 88.3%. The specificity was 97.6% in patients with long-term infection (but not AIDS) and 86.0% in patients suffering from AIDS. Therefore the EIA-RI format includes quantification of antibody binding to IDE and V3 on two separate wells for each serum sample. (F Barin et al., AIDS submitted)

## Samples and methods

The serum samples were divided into a training sample (n = 260) and a validation sample (n = 500) to allow an external validation of the parameters estimated in the training sample (Table). Serial specimens (n = 213) came from 81 seroconverters from either a local panel or the ANRS PRIMO cohort. The remaining 547 samples were from either a local panel or the ANRS SEROCO/HEMOCO cohorts.

**Table:** Description of the serum samples used to evaluate the EIA-RI on dried serum spot.

Population	Training sample		Validation sample	
	Sera	patients	Sera	patients
Seroconverters $\leq 180$ days	60	24	92	57
Seroconverters > 180 days	46	22	15	10
Chronic patients without AIDS	154	154	250	250
AIDS patients	/	/	143	143
<b>TOTAL</b>	<b>260</b>	<b>178</b>	<b>500</b>	<b>450</b>

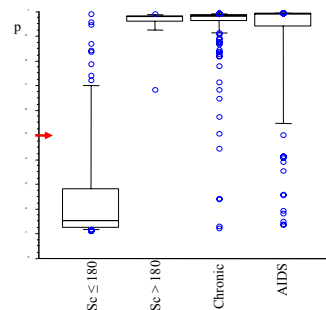
Filter paper was spotted with 20  $\mu$ l of serum and allowed to dry (DDS). Disks were punched out of the dried spot (diameter = 6 mm), placed in 500  $\mu$ l of PBS-TW-NBCS buffer and then incubated at RT for one hour in an ultrasonic cleaner. The eluted serum was directly placed in the wells of the microplates (100  $\mu$ l per well) and subjected to the EIA-RI.

The aim of the statistical analysis was to classify the samples in two groups: those corresponding to a recent infection ( $\leq 6$  months) and those not corresponding to a recent infection. A logistic regression, which gives the probability P of being classified as a non-recent seroconverter, was used for this purpose with two techniques: a bootstrap resampling procedure and generalized estimating equation (GEE) logistic models. Thresholds were explored using ROC curves.

## Results

The logistic regression equation was as follows:  $p = \exp(-2.11234 + 0.15367*ide.r - 0.01242*V3.r) / [1 + \exp(-2.11234 + 0.15367*ide.r + 0.01242*V3.r)]$ , where *ide.r* and *V3.r* are the ratios absorbance sample / absorbance negative control in the IDE and V3 wells, respectively. The final threshold, 0.50, was chosen on the basis of the sensitivity and specificity levels obtained on the validation sample in order to get the best sensitivity given an overall specificity higher than 95%.

The distribution of the probability of being classified as a non recent seroconverter in the different population groups of the validation sample showed a clear discrimination between sera collected within the first six months of infection and those collected later (Figure). Twelve of the 92 DSS collected within 180 days of primary infection were misclassified as non-recent seroconverters, giving a sensitivity of 87.0%. None of the 15 late serum samples from seroconverters was misclassified (100% specificity), whereas five of the 250 chronic patients and 13 of the 143 AIDS patients were misclassified as recent seroconverters giving specificities of 98.0% and 90.9%, respectively.



**Figure:** Distribution of the probability P of being classified as a non-recent seroconverter (arrow indicates cut-off : 0.5)

## Conclusion

This simple immunoassay allows the identification of recently HIV-1 infected patients with performances compatible with its use in the determination of the HIV-1 incidence in population-based studies. This is the first time that an assay able to identify recent infection has been validated on samples collected on filter papers. This is particularly relevant for large field studies and in settings where collection, centrifugation, storage and shipment are difficult.

## Acknowledgements

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