Detection of HIV-1 antibodies in blood specimens spotted on filter-paper

F. Lillo,¹ O.E. Varnier,¹ E. Mantia,² A. Terragna,² G. van der Groen,³ I. Van kerckhoven,³ P.P. Mortimer,⁴ J.V. Parry,⁴ G. Bayliss,⁴ & H. Tamashiro⁵

Described are the results of an international collaborative study to evaluate the use of whole blood samples spotted on filter-paper (BSP) for the detection of antibodies to human immunodeficiency virus type 1 (HIV-1). BSP samples were collected from 40 patients at risk for HIV-1 infection and tested blindly using commercially available HIV antibody test kits, either specifically manufactured or modified for this purpose. Parallel serum samples were also collected, and the antibody reactivity was defined and confirmed by Western blot.

The results demonstrate that recovery of antibodies from BSP samples after elution can be comparable to that from serum. Some kits can be easily adapted to test BSP samples, while others cannot. At present, detection of HIV antibodies in BSP samples should therefore be carried out using kits specifically manufactured for this purpose or by the development of a modified protocol using a panel of BSP and their corresponding serum specimens.

Introduction

The identification of human immunodeficiency virus (HIV) infection is mainly based on the detection of specific antibodies to viral antigens in samples of serum or plasma (1). Although much progress has been made in developing diagnostic reagents, antibody reactivity still requires confirmation; multiple samples are therefore often needed to complete testing (2). Epidemiological studies can be hampered by the difficulty in obtaining adequate samples of blood, particularly from infants or elderly patients, and storage of aliquots may be difficult in developing countries.

In the last 20 years newborns have been screened for metabolic disorders by testing blood spotted on filter-paper (BSP) (3). This method has also been used to diagnose several diseases, such as measles (4), viral hepatitis B (5), and, more recently, to detect antibodies to HIV (6–11).

The collection of whole blood on filter-paper for antibody assay has unique advantages over the use of serum samples. Equipment requirements are minimal — inexpensive sterile lancets and filter-papers replace the syringes, tubes, centrifuges, refrigerators, and freezers that are needed for serum collection and storage. The filter-cards used are light, hold up to eight aliquots, cannot be broken or split, can be stored at room temperature for several weeks, require minimal storage space, and can be sent by mail. The BSP technique is therefore particularly suitable for use in screening programmes in developing countries.

Use of the BSP technique to screen for HIV antibodies needs to be validated to verify whether the results obtained in different assays are comparable to those obtained by analysis of serum or plasma. The present international collaborative study therefore assessed the usefulness of different HIV antibody assays of BSP samples, using a panel of 40 paired samples of BSP and the corresponding serum samples.

Methods

Specimens

Forty blood samples (10 ml each) were collected in 1989–90 by venepuncture using heparinized syringes from patients (25 intravenous drug addicts, 11 sexual partners of HIV-1-positive subjects, and 4 homosexual males) who were attending the Clinic of Infectious Diseases, Genoa. Whole blood was spot-
the reaction was then incubated overnight at 4°C.

The only commercially available kit that is manufactured to test BSP samples is the Du Pont HIV recombinant (ENV9) enzyme-linked immunosorbent assay (ELISA), which on request is provided with a dedicated sample diluent. This test was adopted as the reference test by all the collaborating laboratories in the study. Since the other kits were not validated for testing BSP samples, partially modified protocols were used. Here only the results obtained with those kits that proved to be satisfactory for this purpose are reported. The manufacturers of the HIV assays that were unsatisfactory for testing BSP samples in our study will be informed and invited to develop specific protocols or to advise their customers not to use their kits for this purpose until specific protocols have been developed.

The following kits were satisfactory:

- the Cellular Product Inc. ELISA prepared with HIV-1 virus lysate;\(^a\)
- the Behring “Enzygnost Anti-HIV Micro” competition ELISA;\(^b\)
- the Fujirebio “Serodia-HIV” particle agglutination test;\(^c\)
- the IgG antibody-capture ELISA (GACELISA)\(^d\) developed at the Virus Reference Laboratory, London, England, in collaboration with Wellcome Diagnostics. This assay employs a recombinant-antigen directly conjugated to alkaline phosphatase; and
- the GACPAT,\(^d\) a capture assay based on a modification of the Serodia-HIV test (12).

Results

All the 40 BSP samples were tested using the Du Pont HIV recombinant (ENV9) ELISA, in parallel with the corresponding serum samples. The BSP results were concordant with those obtained for the sera, with the exception of one negative sample, which was initially reactive but negative when retested (Table 1).

The Cellular Product Inc. ELISA detected the presence of HIV-1 antibodies in all the positive BSP and serum specimens. Five HIV-1-seronegative BSP samples were initially reactive in this test and two of these samples were also repeatedly reactive when retested: these two samples were reactive only for p24 in the HIV-1 Western blot.

---

\(^a\) Cellular Product Inc., Buffalo, NY, USA.

\(^b\) Behringwerke AG, Marburg, Germany.

\(^c\) Fujirebio Inc., Tokyo, Japan.

\(^d\) Wellcome Diagnostics, Dartford, England.
Table 1: Results obtained for the detection of HIV-1 antibodies in blood spotted on filter-paper (BSP) and serum samples using various commercially available test kits

<table>
<thead>
<tr>
<th>Kit</th>
<th>Serum samples</th>
<th>BSP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. +ve No. –ve</td>
<td></td>
</tr>
<tr>
<td>Du Pont HIV-1 recombinant ELISA</td>
<td>34 6</td>
<td></td>
</tr>
<tr>
<td>Cellular Product Inc. ELISA</td>
<td>34 6</td>
<td></td>
</tr>
<tr>
<td>Enzygnost Anti-HIV Micro</td>
<td>34 6</td>
<td></td>
</tr>
<tr>
<td>GACELISA</td>
<td>34 6</td>
<td></td>
</tr>
<tr>
<td>GACPAT</td>
<td>35 5</td>
<td></td>
</tr>
</tbody>
</table>

The Behring Enzygnost Anti-HIV Micro ELISA detected HIV-1 antibodies in all the positive serum samples, except one sample that was reactive (p24 reactive in the Western blot). The same results were also obtained with the GACELISA and the GACPAT.

With the Serodia-HIV particle-agglutination test only one HIV-1-negative serum sample was reported here as positive. Western blot analysis of the serum revealed the presence of p24 reactivity. The Serodia-HIV was also used at the Virus Reference Laboratory to test the BSP panel: the results obtained were concordant with the exception of one HIV-1-negative sample, which was reactive.

The Behring Enzygnost Anti-HIV Micro ELISA detected HIV-1 antibodies in all the positive serum samples, except one sample that was reactive (p24 reactive in the Western blot). The same results were also obtained with the GACELISA and the GACPAT.

With the Serodia-HIV particle-agglutination test only one HIV-1-negative serum sample was reported here as positive. Western blot analysis of the serum revealed the presence of p24 reactivity. The Serodia-HIV was also used at the Virus Reference Laboratory to test the BSP panel: the results obtained were concordant with the exception of one HIV-1-negative sample, which was reactive.

Conclusions

The results that we have reported confirm and extend previous observations that whole blood collected on filter-paper can be effectively used instead of serum samples for HIV-antibody testing. The method can easily be carried out by individuals who have received only a little training, taking the following storage precautions: the BSP need to be carefully dried after collection and stored wrapped in sealed plastic bags in a dry, dust-free place.

Other HIV assays should be investigated to determine whether they are suitable for use with BSP specimens. Some commercial kits may easily be adapted for this purpose as shown by our results, while others may not be suitable for use in their original form or may require to be modified, e.g., by the provision of adequate elution diluents by the manufacturers as accessory reagents. At present, however, the detection of HIV antibodies in BSP samples should be performed using only specifically manufactured kits. Whenever there is the need to test BSP samples and such kits are not available, either the kits reported here to be satisfactory should be used or a modified protocol should be developed using a panel of BSP and their corresponding serum specimens.

Acknowledgements

The HIV-antibody kits evaluated in this study were kindly provided by the WHO Global Programme on AIDS, Geneva, Switzerland.

Résumé

Détection des anticorps anti-VIH-1 dans des prélèvements sanguins recueillis sur papier filtre

Le diagnostic de l'infection par le virus de l'immunodéficience humaine type 1 (VIH-1) s'appuie principalement sur la détection d'anticorps spécifiques dans le sérum des sujets infectés. Bien que de grands progrès aient été faits dans la préparation des réactifs d'identification de ces anticorps, les résultats positifs doivent toujours être confirmés et plusieurs échantillons de sérum sont souvent nécessaires. Les ponctions veineuses sont parfois difficiles à réaliser chez les nourrissons ou les personnes âgées et la conservation des prélèvements sanguins peut présenter des difficultés dans les pays en développement.
L'étude collective présentée ici visait à vérifier si des échantillons de sang total recueillis sur papier filtre pouvaient effectivement être utilisés pour diagnostiquer l'infection à VIH-1 et pour obtenir des données épidémiologiques dans les pays en développement. Des échantillons de sérum prélevés de façon classique et des échantillons de sang recueillis sur papier filtre ont été obtenus auprès de 40 patients de l'Institut des maladies infectieuses de Gênes et envoyés aux laboratoires participants. Ces échantillons ont été testés à l'aide de trusses commerciales d'anticorps anti-VIH conçues spécialement pour les essais sur papier filtre ou adaptées à cette fin. Les résultats des essais qui se sont révélés satisfaisants sont résumés ci-après. La trousse Du Pont ENV9 (spéciale pour essai sur papier filtre), l'essai immuno-enzymatique par compétition de Behring, le GACELISA avec capture d'IgG et le GACPAT (modification de l'épreuve d'agglutination de particules de Serodia) ont donné des résultats concordants pour les sérum et les échantillons de sang sur papier filtre. L'essai immuno-enzymatique sur lysat de virus de Cellular Product Inc. et l'épreuve d'agglutination de Serodia ont donné des taux de concordance de 95% et 97,5% respectivement, en raison des faux positifs.

Les résultats de cette étude confirment et complètent les observations antérieures selon lesquelles des échantillons de sang recueillis sur papier filtre pouvaient effectivement remplacer les échantillons de sérum pour la recherche des anticorps anti-VIH. Toutefois, les trusses utilisées aux fins de diagnostic doivent être spécialement conçues en fonction de cette technique ou vérifiées avec une série d'échantillons de sang sur papier filtre bien caractérisés et avec les sérum correspondants.

References