# Prevalence of the human immunodeficiency virus among patients with tuberculosis in Sierra Leone, established from dried blood spots on filter paper

F. Boillot,* M. Peeters,† A. Kosia,‡ E. Delaporte†

*formerly National Leprosy and Tuberculosis Control Programme, Freetown, Sierra Leone, currently Unité Santé UE, Port au Prince, Haiti, †Laboratoire des Retrovirus, Centre ORSTOM, France, ‡National AIDS Control Programme, Freetown, Sierra Leone

_____ S U M M A R Y

SETTING: Sierra Leone National Tuberculosis Programme.
OBJECTIVE: To evaluate serological testing in field conditions of dried blood spots (DBS) on filter paper for unlinked surveillance of human immunodeficiency virus (HIV) associated tuberculosis.
DESIGN: DBS were first evaluated against sera in 359 consenting patients on the capital city's District Tuberculosis Register (DTR). DBS eluates were tested with repeated ELISA using different antigens. Serum samples were tested with ELISA and confirmed with LIA. The cost was compared with that of rapid/simple tests on whole blood. In a second phase, DBS were applied to an unlinked countrywide serosurvey of 582 patients from the DTRs.

RESULTS: The specificity of DBS for HIV-1 and HIV-2 was 100% and sensitivity was 100% and 87.5%, respectively. The cost of the strategy was half that of rapid/simple tests on whole blood. In 1995, HIV-1 associated tuberculosis seroprevalence was 2.41%.
CONCLUSION: The proposed method for the surveillance of HIV-1 associated tuberculosis in Africa is simple, cheap and accurate. Further investigations are necessary to evaluate its sensitivity for HIV-2, and to study the epidemiology of HIV-2 in Sierra Leone.
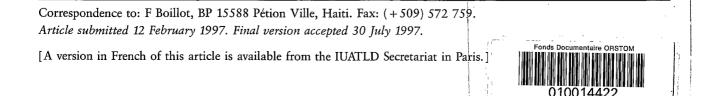KEY WORDS: tuberculosis; HIV; surveillance; filter paper; Sierra Leone.

IN SUB-SAHARAN AFRICA, tuberculosis is the most common opportunistic disease among adults infected by the human immunodeficiency virus (HIV).[1,2] An increased tuberculosis caseload for countries with limited resources poses a danger to tuberculosis control programmes.[3,4] Knowing the geographic distribution and the trend of HIV seroprevalence among tuberculosis cases would allow tuberculosis and acquired immune-deficiency syndrome (AIDS) control programmes to target interventions, forecast resource needs, and adapt control strategies, such as the use of thioacetazone, to local conditions.[5]

A survey carried out in 1993 by the Sierra Leone National AIDS Control Programme (NACP) in four hospitals located in different regions, using a repeated rapid/simple test on whole blood, found an average HIV seroprevalence of 8.4%. However, few details on the size and recruitment modalities of the sample of this survey were available. The National Leprosy and Tuberculosis Control Programme (NLTCP) wished to determine the feasibility of carrying out HIV serological surveillance of tuberculosis patients.

The detection of HIV antibodies using dried blood spots (DBS) on filter paper has been widely used for HIV seroprevalence surveys,[6–11] and has been shown to be applicable in tropical conditions.[12–15] DBS can be centralized for enzyme-linked immunosorbent assay (ELISA) testing in a single laboratory, hence limiting analytical variance, particularly the risk of error by laboratory staff with various levels of skills. The patients' anonymity is also better secured than when both sampling and testing are done in a district hospital laboratory.

The objective of the present study was to evaluate the use of DBS from patients registered in the District Tuberculosis Register with ELISA testing of the eluates, for conducting anonymous serosurveillance of HIV-associated tuberculosis.

## MATERIAL AND METHODS

In an initial validation phase, we evaluated ELISA testing of DBS on filter paper against serum samples collected from the same tuberculosis patients. In a second phase, the method was implemented countrywide for obtaining a baseline seroprevalence profile.

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## Validation phase

### Patient enrolment

From April 1994 onwards, all patients diagnosed with tuberculosis aged 18 and over, who resided permanently in Freetown district and were registered consecutively in the District Tuberculosis Register, were asked to participate. Clearance was obtained from the National Ethics and Medical Research Committee. A notice of information in English and in Krio (the lingua franca of Sierra Leone) was submitted to all patients before their consent was sought.

Demographic and clinical data were obtained from the District Tuberculosis Register and by interview, and recorded under a unique serial number in a register designed for the survey. Clinical classification was performed according to the NLTCP guidelines, based upon recommendations made by the International Union Against Tuberculosis and Lung Disease (IUATLD).[16]

Based on the 9% HIV prevalence previously observed in the selected area, a sample of 350 patients was necessary for a 3% precision with a confidence level of 95%.

### Blood sampling procedure

All patients had a finger prick for blood collection on Whatman Nr3 Chr. paper, and venous blood was sampled with disposable sterile material. Both paper and blood samples were identified by the serial number in the survey register. Papers were allowed to dry until the end of the clinic (30 min to 4 hours), put in a nylon zip-lock sheet (Sanavita essential drugs sheets) and stored in a cabinet at room temperature. The blood samples were immediately centrifuged, and the sera stored at $-20°C$.

### Laboratory methods

As the NACP laboratory could not perform ELISA testing at the time of the survey, the samples were processed by the Laboratoire Retrovirus, ORSTOM, France.

Serum was eluated from DBS in phosphate buffered saline (PBS). The samples were incubated for 90 min on a rotary shaker, followed by overnight incubation at $+4°C$. The eluates were tested by a repeated ELISA using two different antigens (Genelavia, Diagnostics Pasteur, Marnes-la-Coquette, France, and Innotest, Innogenetics, Ghent, Belgium) according to the testing strategy II recommended by the World Health Organisation (WHO).[17]

Serological testing of the same patients' sera, with ELISA (Innotest) screening and Line Immuno Assay (LIA, Innolia, Innogenetics) confirmation[18] was used as gold standard.

### Cost

We compared the cost of the proposed method with the cost of repeated rapid/simple test on whole blood.

Calculations were done using the purchase cost of the equipment and consumables used in the survey and the cost of tests as supplied in bulk by the WHO.[17] Equipment was depreciated according to the NLTCP accounting procedures.

## Implementation phase

Patients consecutively registered in the District Tuberculosis Registers throughout the country were enrolled in the implementation study. As the information obtained will constitute the baseline for further serosurveillance, the size of the sample was calculated with a precision of 1% and a confidence level of 95%. The sampling quota for each district was calculated as the proportion yielded by the district of the total number of cases registered the previous year in the country. DBS collection and testing was done as previously described.

### Analysis

Completed survey registers and laboratory results were centralized and matched at the NLTCP headquarters. Computer entry and processing of the data was done with Microsoft Excel 5.0. The reliability of computer entries was controlled by matching every tenth record with the register data. The $\chi^2$ test with a 95% confidence interval (CI) was used for comparisons.

## RESULTS

### Validation phase

#### Specimen collection

Of the 360 patients enrolled, one refused consent. Of the remaining 359 patients, 150 (41.8%) were female and 209 (58.2%) were male. The temperature recorded in the cabinet where the papers were stored ranged from 25° to 31°C and the hygrometry ranged from 68% to 92%. The mean storage duration was 36 days (STD 17).

#### Serology results

In total 6.9% (25/359) of the paper eluates and 17.8% (64/359) of the serum samples were positive with the

**Table 1** Comparison of a testing algorithm—ELISA screening, ELISA confirmation, on DBS eluates of 359 tuberculosis patients, with the algorithm—ELISA screening, LIA confirmation, on sera from the same patients serving as gold standard, for the detection of HIV-1 and HIV-2 infection

|  | Sera | | |
|---|---|---|---|
|  | Positive | Negative | Total |
| DBS eluates |  |  |  |
| Positive | 7 (HIV-1) | 0 | 7 |
| Negative | 1 (HIV-2) | 351 | 352 |
| Total | 8 | 351 | 359 |

ELISA = enzyme-linked immunosorbent assay; DBS = dried blood spots; LIA = Line Immuno Assay.

**Table 2**   Cost comparison between a repeated ELISA on DBS eluates and a repeated rapid/simple test on whole blood for a sample of 800 patients, under a high hypothesis of 18%, and a low hypothesis of 7% of tests requiring confirmation. Cost per test: ELISA equipment: US$ 0.18, DBS sampling: US$ 0.04, whole blood sampling: US$ 0.07, ELISA test: US$ 0.70, Rapid/simple test: US$ 2.00

| | Screening | | | | Confirmation | | | |
|---|---|---|---|---|---|---|---|---|
| | Sampling (US$) | Equipment (US$) | Test (US$) | Positive | Sampling (US$) | Equipment (US$) | Test (US$) | Total (US$) |
| DBS eluates | 32.48 | 141.48 | 560.00 | 7% | 0.00 | 9.90 | 39.20 | 783.07 |
| | 32.48 | 141.48 | 560.00 | 18% | 0.00 | 25.47 | 100.80 | 860.23 |
| Whole blood | 56.00 | 0.00 | 1600.00 | 7% | 0.00 | 0.00 | 112.00 | 1768.00 |
| | 56.00 | 0.00 | 1600.00 | 18% | 0.00 | 0.00 | 288.00 | 1944.00 |

ELISA = enzyme-linked immunosorbent assay; DBS = dried blood spots.

first ELISA and required confirmation. The categorical serologic results are presented in Table 1. One serum was found positive with the Innotest ELISA and confirmed by LIA as being HIV-2 positive. The Genelavia ELISA on the eluate failed to detect this serum, but a control with the Innotest ELISA was positive.

The sensitivity of the DBS strategy was 100% for HIV-1 and 87.5% for HIV-1 and -2. The specificity was 100% for both HIV-1 and -2. The negative predictive value was 99.7%. The seroprevalence observed using DBS in the capital city district during the validation phase was 1.9% (95% CI 0.5%–3.4%).

### Cost

We estimated the cost of the rapid/simple test on whole blood strategy under a high hypothesis of 18% (ELISA on serum) and a low hypothesis of 7% (ELISA on eluates) of samples positive on screening. The cost comparison is presented in Table 2. Using rapid/simple tests on whole blood is 2.3 times more expensive than the proposed method.

### Implementation phase

#### Patient enrolment

With a 2% estimated seroprevalence and a 1% precision at a confidence level of 95%, 753 patients were calculated to be necessary for the implementation phase. Unfortunately during the study, civil war resulted in massive population displacement and interruption of activities in several districts. The survey was, however, completed in 9 of the 14 districts by the

end of March 1995: 601 patients were registered and 19 foreign patients were excluded; the remaining 582 give a 77% achievement of the enrolment target. The population of patients enrolled was representative of the recruitment of the NLTCP.

### Laboratory results

The seroprevalence results are presented in Table 3: 9/242 (3.7%) patients residing in the capital city district were HIV positive. These results are higher than in the validation phase, but the difference is not significant ($\chi^2 = 2.09$, $P > 0.05$). Overall, 14/582 patients had confirmed HIV-1 infection. Five of the 9 HIV positive patients of urban origin and all five HIV-positive patients of rural origin were new cases of tuberculosis. Despite a higher seroprevalence rate in the capital city, residence in that area was not a risk factor (odds ratio [OR] = 2.58, 95% confidence interval 0.88–7.53).

## DISCUSSION

This study shows the feasibility of using DBS to perform HIV serosurveillance in a national tuberculosis control programme. Collection of DBS is simple and inexpensive, making it attractive for countries with limited resources. The need for a clinical laboratory

**Table 3**   Distribution of the 582 tuberculosis patients surveyed in the implementation phase, according to the clinical type and the type of case

| Type of tuberculosis | New cases | Previously treated | Failure | Relapse | Total |
|---|---|---|---|---|---|
| Smear positive pulmonary | 381 | 124 | 16 | 23 | 544 |
| Smear negative pulmonary | 18 | 1 | 0 | 4 | 23 |
| Extra-pulmonary | 15 | 0 | 0 | 0 | 15 |
| Total | 414 | 125 | 16 | 27 | 582 |

**Table 4**   Prevalence and prevalence rates with their 95% confidence intervals (CI), obtained from the filter paper eluates in the implementation phase ($n$ = 582), according to the type of tuberculosis and the type of case. No HIV-positive failure or relapse case was found

| Type of tuberculosis | New cases n (%) | Previously treated n (%) | Total n (%) |
|---|---|---|---|
| Smear positive pulmonary | 8/381 (2.10) | 4/124 (3.23) | 12/544 (2.21) |
| CI | 0.66%–3.54% | 0.12%–6.34% | 0.98%–3.44% |
| Smear negative pulmonary | 2/18 (11.11) | 0 | 2/23 (8.70) |
| CI | 0%–25.63% | | 0%–20.22% |
| All types of tuberculosis | 10/414 (2.42) | 4/125 (3.23) | 14/582 (2.41) |
| CI | 0.94%–3.90% | 0.12%–6.34% | 1.17%–3.65% |

to perform serological tests is a constraint on HIV surveillance. However in this study we were required to use an overseas laboratory to conduct these assays.

Fewer eluates than sera were found positive with the first ELISA. This is compatible with a decrease in the antibody titre of filter papers stored under tropical conditions observed by other authors.[12,14] The specificity of the approach is good, but the low seroprevalence observed makes it difficult to determine the sensitivity with precision. ELISA testing of the eluates failed to identify the only patient infected with HIV-2. Other studies using DBS found either only HIV-1 antibodies, or the HIV type was not specified.[6-14] Further investigations to determine the reasons for the low sensitivity for HIV-2 are needed.

The HIV seroprevalence observed in the two phases is much lower than in the previous survey performed in selected hospitals. Recruiting patients from sentinel institutions may result in a substantial recruitment bias. The observed seroprevalence of 2.4% among all types of tuberculosis and 2.1% among new cases of smear positive pulmonary tuberculosis are closer to the 4.9% observed in Senegal[20] than to the 47% observed in Cote d'Ivoire.[20] This study illustrates the challenges and advantages of conducting HIV seroprevalence surveys using the District Tuberculosis Register as a basis for enrolling patients.

## Acknowledgements

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_____ **RÉSUMÉ**

CADRE : Programme National de la Tuberculose, Sierra Leone.
OBJECTIF : Evaluation de l'utilisation sur le terrain de la technique des taches de sang séchées sur papier filtre pour la surveillance de la tuberculose associée au virus de l'immunodéficience humaine (VIH).
SCHÉMA : La technique a été évaluée par comparaison

avec le sérum chez 359 patients consentants enregistrés dans le Registre de district de la tuberculose dans la capitale. Des tests répétés par ELISA utilisant des antigènes différents ont été effectués sur les éluats des papiers-filtres. Les serums ont été testés par ELISA avec confirmation par LIA (étalon). Les coûts ont été comparés à ceux de tests rapides/simples sur du sang complet. Au cours

d'une deuxième phase, la technique a été utilisée dans une enquête de séro-surveillance nationale anonyme chez 582 patients enrégistrés dans les Registres de la tuberculose des districts.

RÉSULTATS : La spécificité pour VIH-1 et VIH-2 de la méthode des taches de sang séchées est de 100%, et la sensibilité est respectivement de 100% et de 87,5%. Cette stratégie ne coûte que la moitié de celle utilisant les tests rapides/simples sur sang complet. En 1995, la séroprévalence de VIH-1 associé à la tuberculose était de 2,41%.

CONCLUSION : La méthode proposée pour la surveillance en Afrique de l'infection à VIH-1 associée à la tuberculose est simple, peu coûteuse et précise. Des investigations complémentaires s'imposent pour évaluer sa sensibilité en matière de VIH-2, ainsi que l'épidémiologie de l'infection à VIH-2 en Sierra Leone.

_____ RESUMEN

MARCO DE REFERENCIA : Programa Nacional de Control de la Tuberculosis de Sierra Leona.

OBJETIVOS : Evaluar la utilización en terreno de la técnica de manchas de sangre secas (DBS) sobre papel filtro, con test ELISA de los eluatos, para la serovigilancia independiente de la tuberculosis asociada a VIH.

MÉTODO : En 359 pacientes que habían consentido, inscritos en el Registro Distrital de Tuberculosis en la capital, se aplicó la técnica DBS en comparación con las pruebas séricas. Los eluatos de DBS fueron sometidos al test ELISA a repetición usando diferentes antígenos. Los sueros fueron sometidos al test ELISA con confirmación con LIA. Los costos fueron comparados a aquéllos de los tests rápidos/simples en sangre completa. En una segunda fase, la técnica DBS se aplicó en un estudio independiente de serovigilancia a nivel nacional en 582 pacientes inscritos en los Registros Distritales de Tuberculosis.

RESULTADOS : La especificidad es de 100% para VIH-1 y VIH-2. La sensibilidad es de 100% para VIH-1 y de 87,5% para VIH-2. El costo de esta estrategia representa la mitad de aquélla que utiliza los tests rápidos/simples en sangre completa. En 1995 la seroprevalencia de la tuberculosis asociada a VIH-1 era de 2,41%.

CONCLUSIÓN : El método propuesto para la vigilancia de la tuberculosis asociada a VIH-1 en Africa es simple, barato y seguro. Se necesitan nuevas investigaciones para evaluar su sensibilidad para el VIH-2 y sobre la epidemiología del VIH-2 en Sierra Leona.