

## Dried Blood Spot Specimens for use in the Determination of Recent HIV-1 Infection and HIV-1 Incidence Surveillance

a report by

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The HIV pandemic continues to spread worldwide, with over 43,000,000 people infected with HIV or suffering with AIDS. The majority of those infected are in sub-Saharan Africa, but increases in HIV infection in Asia, India and Eastern Europe are alarming. In the absence of a vaccine, efforts at diagnosis and therapy appear to be the only viable alternatives for containing the spread of HIV infection. Despite millions of dollars allocated to global health programmes, progress is slow in the identification of those who have been infected with HIV and is even slower in providing therapeutic drugs.

Surveillance of the HIV pandemic originally evaluated the prevalence of infection. Prevalence was easily measured in a cross-sectional population by the blood HIV antibody tests in common use. The measurement of HIV incidence and the extent of recent infection was difficult to estimate, generally requiring long-term cohorts and prospective follow-up in specific populations. HIV-1 incidence testing serves to locate recent infections in HIV-1 positive populations and where the risk of infection is high. The ability to discriminate between recent and long-term infections leads to improved targeting and evaluation of HIV prevention and therapeutic programmes and research, such as vaccine and microbicide trials and studies of early therapeutic intervention.

After autumn 1999, the Centers for Disease Control and Prevention (CDC), introduced a laboratory tool – serological testing algorithm for recent HIV seroconversion (STARHS) – to measure HIV incidence. The first step in the testing algorithm for recent infection is the identification of HIV-1 positive specimens. The second step is subjecting HIV-1 positive specimens to incidence testing that identifies recently infected people.

The STARHS serological testing depends on the differences between the high HIV-1 antibody titre in specimens from subjects with long-term infection and the lower titres from recent infection. Blood specimens from subjects with long-term infection and those with a recent infection are detected at the blood dilutions used in commercial HIV antibody assays, for instance the Abbott 3A11 or the Vironostika®

HIV-1 Microelisa assay. When the assay is detuned, the procedure is carried out at a higher dilution designed to detect only the high antibody titres found in long-term infection. The sensitive/less sensitive (LS) assays such as the 3A11-LS (no longer commercially available) and the Vironostika LS-enzyme immunoassay (EIA) still in use have made incidence surveillance an easy and practical laboratory procedure. Implementation of STARHS by public health departments has proven to be successful in estimating recent infection in populations at risk from HIV infection worldwide. It is important that the determination of HIV infection trends are accessible and implementable at a local level, enabling agencies to plan, implement and evaluate surveillance and prevention programmes that target the areas where HIV infection is spreading.

A simple, quantitative assay called the immunoglobulin G (IgG) Capture BED EIA has been introduced for the detection of early infection, thereby estimating incidence. Originally developed by the CDC, this test is now commercially available as the Calypte HIV-1 BED Incidence EIA. This assay supplants earlier commercially available tests (detuned assays) such as the Vironostika-LS and extends the possibilities worldwide for determining HIV incidence. Detuned assays based on more extensive dilution of the blood specimen used commercial assays designed to detect HIV-1 sub-type B, the major sub-type in the US. Worldwide, the predominant sub-type is C, predominating in southern and eastern Africa, India and Nepal. The following sub-types are also found – sub-type B and E in Thailand, C in China and other countries in Asia and A, D and F in Africa. The IgG Capture BED EIA was designed with a target antigen, with the HIV-1 gp41 branched chain synthetic peptide containing amino acid sequences for the immunodominant regions of the sub-types B, E and D. However, the assay has been successfully used in non-sub-type B countries such as Cambodia and Rwanda.

The advantages of the Calypte HIV-1 BED Incidence EIA are a 1:100 dilution step for the blood specimen, instead of the high dilutions required in the detuned assays, a typical EIA test

procedure and the use of controls and calibrators to ensure quantitative results within the appropriate optical density (OD) parameters expected for recent infections.

The Calypte HIV-1 BED Incidence EIA has been tested and used by the CDC in national HIV surveillance efforts as well as international studies in Asia including Thailand, Cambodia, China and India and in Africa in Ethiopia, Zimbabwe, Kenya and South Africa. Other public health researchers in Australia, The Netherlands, the UK and Switzerland have also used this assay.

The BED Incidence EIA was validated with serum or plasma as the test specimen. With an increasing use of dried blood spots (DBS) as the collection procedure for HIV testing, the CDC and Calypte Biomedical have embarked on the validation of DBS specimens for use with the Calypte HIV-1 BED Incidence EIA. The testing of blood dried on filter paper is a technique that has been used for nearly 40 years in the collection of heel stick blood for measuring phenylketone levels in newborn babies. In developing countries with low-resource settings there may not be a phlebotomist available for blood collection. The use of a finger stick and inexpensive filter paper for blood collection is usually possible and can bring blood collection to remote sites with non-medical personnel. DBS has therefore been adopted for HIV testing, including HIV antibody, HIV-1 DNA and viral RNA in countries such as South Africa and The Gambia, West Africa. In the UK, HIV prevalence in pregnant women in an ethnically diverse population was estimated using 490,879 DBS collected from 1998 to 2002.

The fact that the DBS was stable for over four years when kept dry is another useful attribute of this collection procedure. A CDC study presented at the HIV Diagnostics Meeting in February 2005 described the use of DBS or dried serum spots (DSS) to detect recent seroconversion by the BED Incidence EIA. DBS and DSS were prepared by spotting 100µl of blood or 50µl of serum onto Schleicher and Schuell (SS)#903 filter paper, being dried overnight. A 6mm punch from the DBS and the DSS were eluted overnight using 200µl of specimen diluent (provided with the BED Incidence EIA kit reagents) in a 96-well non-binding microwell blank plate (not currently) provided. The next day, 50µl of eluted material was transferred to a well of the BED assay plate already containing 50µl of specimen diluent. ■

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