THE USE OF DRIED BLOOD SPOTS AND A MODIFIED PARTICLE AGGLUTINATION TEST FOR COST-EFFECTIVE SERO-EPIDEMIOLOGICAL HIV SURVEYS

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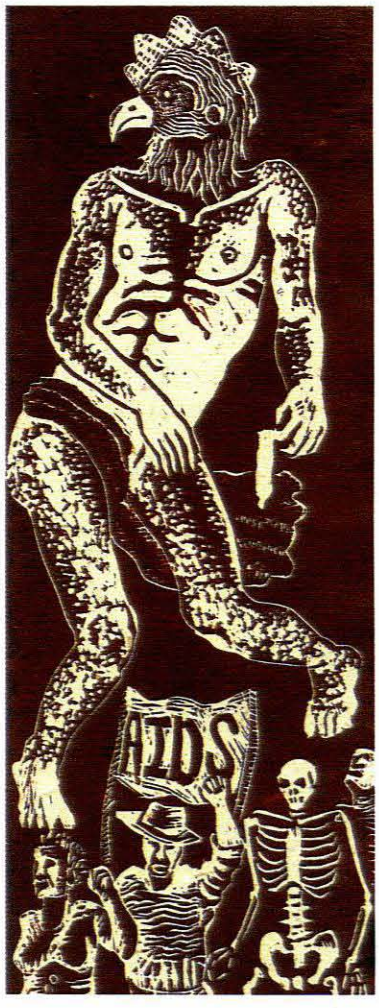
The AIDS epidemic in South Africa has now reached the alarming level at which almost 1 in every 10 South Africans is infected. This figure is extrapolated from the 10th National Antenatal HIV Survey, which was conducted during October 1999,[1] and translates into an absolute figure of around 4.2 million people infected with HIV in South Africa, one of the highest in the world. It is therefore important that educational and awareness programmes reduce the rate of new infections. The importance of finding the right programme for the right targeted population lies in the fact that an estimated 1 600 new infections occur daily in South Africa. New 'incidence' enzyme-linked immunosorbent assays (ELISAs) can be a great help in evaluating the success of any intervention programme,[2] but their performance has not yet been reported on in South Africa. The alternative of using figures obtained from the Department of Health's national HIV seroprevalence surveys of women attending antenatal clinics is not a satisfactory solution as these are too crude for true representation or comparison of intervention studies. The 1999 national survey used 487 sites as representative of the country as a whole, and information gained from these clearly showed that the epidemic differs in different geographical regions in South Africa. Calculating the total estimated HIV- positive males, children and elderly from these figures would be grossly inaccurate owing to different infection rates and other co-factors in individuals other than pregnant women attending antenatal clinics.



Additional sero-epidemiological surveys are frequently performed in order to guide programme management, as they help to formulate prevention strategies and monitor intervention efforts in certain populations. High costs and fear of breaches in confidentiality reduce the statistical power of these results in many cases.

The use of dried blood spots (DBS) for the testing of HIV-1 antibodies has been described since 1988.[3] Numerous studies have proved that this method of prevalence testing has high sensitivity and specificity and is easy to use, as well as having the advantage of being less expensive than conventional tests on whole blood.[4-6] The elution from the spots can be tested for HIV RNA, DNA or antibodies to HIV, as they all seem to be stable for long periods of time even when stored at room temperature.[7,8] A modified particle agglutination anti-HIV-1 testing method[9] is particularly useful as it reduces the cost of a normal ELISA kit to almost one-tenth so the cost per test is approximately R2.50.

The method employed for DBS testing using a modified Serodia particle agglutination assay (Fujirebio Inc., Japan) involves the following procedure. A drop of blood sample is collected onto No. 3 Whatman blotting paper and left to

dry. DBS samples can be stored in sealed plastic bags at room temperature, although if a prolonged period of storage is envisaged storage at –20°C is advisable. At the time of testing a 5 mm punch of each DBS is placed in a flat-bottom microtitre plate. The sample is eluted by adding 100 µl TPHA buffer (Fujirebio) to each well, and the plate is incubated at 4°C overnight. A 1:5 dilution (5 µl + 20 µl) of the eluted sample is made in a V-shaped microtitre plate in TPHA buffer. Gelatin particles sensitised with inactivated HIV-1 antigens are reconstituted according to the manufacturer's instructions (Fujirebo) and 25 µl of a 1:10 dilution of the reconstituted particles is added to each well. A positive and a negative control blood spot, treated in the same way as the test samples, are included in each plate. The plate is covered, shaken and left for 3 hours on a vibration free surface at room temperature. Results are read visually. A positive result is recorded on observation of a tight discrete agglutination pattern. Non-reactivity is identified by the presence of a small definite pellet at the bottom of the well (Fig. 1).

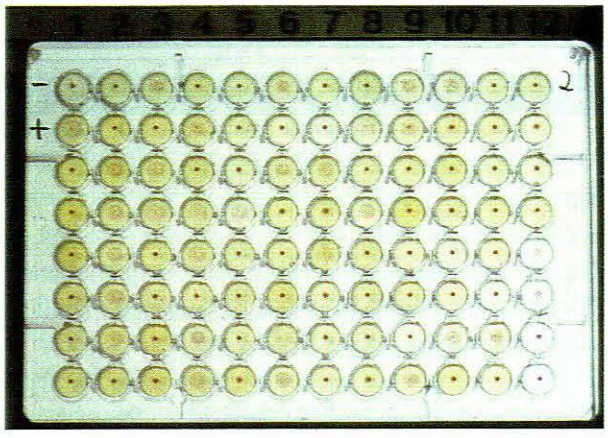


Fig. 1. A microtitre plate with positive (fuzzy centre) and negative (tight red button) results.

We have extensively used and tested this modified particle agglutination in various epidemiological studies. The patient populations tested included infants in the neonatal unit of Ga-Rankuwa Hospital over a 3-year period. The drawing of blood from neonates requires a specially trained health care worker (HCW), while a DBS from a heel prick can be obtained easily. The second group of patients tested (unlinked anonymous testing) were those who attended the antenatal clinic (ANC) and sexually transmitted disease clinic (STD) at two different locations served by the Centurion Municipal Department of Health during the period January - October 1999. The third group of patients participated in an anonymous unlinked seroprevalence survey. These were inpatients at Ga-Rankuwa Hospital, a large 1 400-bed referral hospital on the border of three provinces. In the course of 1 day a team of volunteer hospital HCWs briefed hospital patients in groups of ± 15 patients at a time about HIV (ways of transmission and prevention). The patients were asked to consent to a finger prick, which was done by a health care worker. Thereafter the patients had to blot their blood on blotting paper, let it dry, and deposit it into a collection box. The high participation percentage of 90.8% (912/1 004) in this 1-day point prevalence study was largely due to the facts that a small amount of blood was asked for and that it was obvious to most patients that the method employed conferred total confidentiality.

One hundred consecutive HIV antibody requests to our diagnostic laboratory were analysed as a quality control procedure using DBS and the modified Serodia particle agglutination assay (Fujirebio). All 38 blood samples that tested positive with our routine diagnostic HIV 1/2/0 Axsym assay (Abbott) also tested positive with the modified Serodia particle agglutination assay, i.e.100% sensitivity. Of the 62 negative results obtained by the HIV 1/2/0 Axsym assay, 1 was falsely detected as positive by the modified Serodia particle agglutination assay, i.e. 98.4% specificity. We therefore recommend that this test be used for unlinked sero-epidemiological surveys only and not for diagnostic purposes.

In conclusion, the use of DBS as an alternative method for sero-epidemiological surveys is not only relatively cheap, but can be performed by minimally trained field workers and is perceived by patients to be more confidential than other methods if performed in the right way. Samples can be stored at room temperature and mailed in the standard mail service in a sturdy envelope.

DBS methods have proved to be safe, easy to perform and reliable and provide a useful method for large-scale sero-epidemiological surveys especially in developing countries and isolated rural regions where resources are limited.

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