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Letter to the Editor

Stability of Human Immunodeficiency Virus Type 1 Antibodies in Whole Blood-Impregnated Filter Papers under Various Tropical Conditions

The deliberate exposure of whole blood specimens with high and low human immunodeficiency virus type 1 (HIV-1) antibody titers to an adverse tropical environment by Behets et al. (2) was associated with a progressive decline in enzyme immunoassay optical density ratios. This, along with the simultaneous exposure of quality control sera with high and low HIV-1 titers to 14 different standardized environmental conditions during the rainy season in Kinshasa, Zaire, was a remarkable attempt to simulate the harsh environmental rigors prevailing in developing countries. The exercise lasted 20 weeks and involved regular monitoring of the microenvironment of the blood samples for humidity changes by employing innovative microenvironment cards. Storage in gas-impermeable bags with a desiccant produced maximum stability for HIV-1 antibody detection and was recommended for sample storage in harsh environments.

Before universal acceptance of the recommendations of this outstanding study (2), it would be essential to carry out identical investigations in other seasons at Kinshasa itself and also to carry the metal cargo container in peripheral areas in Zaire. The metal cargo containers could be transported by the routine modes of transportation and stored in remote areas in shaded or even unshaded locations in non-air-conditioned houses. The retrieved whole blood preparations and quality control sera could be tested in Kinshasa to ascertain whether there were any remarkable differences in declines in enzyme immunoassay optical density values or whether there were any repeatedly false-positive results attributable to storage in peripheral areas in different seasons. Furthermore, it might be worthwhile to carry out similar experiments in regions where ambient temperatures would be less than 10°C. Such situations are not that infrequent in developing countries, where facilities in which room temperature is maintained around 20°C are not available.

Future investigations on the field stability of HIV-1 antibodies in which warehouse temperature, humidity, and air velocity are monitored would provide invaluable guidance to ensure field stability of vaccines and to eliminate any decline in the potency of meticulously calibrated antibody reference preparations (1). The therapeutic failures of antivenom preparations against Russell viper venom (Viper russelli pulchella) to clear antigenemia in 19 of the 20 patients in the interior region of Sri Lanka bitten by Russell vipers are alarming (5). The performance of a freeze-dried measles vaccine in immunization centers during the 1990s has been dismal. The inadvertent use of vaccine batches with potencies below 10^2 50% tissue culture infectious doses was associated with a mere 26% seroconversion (4). A search is on for a live polio virus vaccine that is stable at 45°C that would withstand field rigors (3). Extended investigations are essential to define the prevailing harsh environmental hazards in tropical countries. Development of environmentally stable immunodiagnostic tests for HIV (6) and therapeutic or prophylactic vaccines, as well as determination of ideal conditions for carriage of specimens from the field, should be undertaken, and the outstanding studies of Behets et al. (2) should be extended in other geographic locations in the near future.

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Author's Reply

We thank Dr. Arya for his interest in our study regarding the stability of blood specimens stored on filter papers under various tropical conditions. While it would have been interesting to have extended our studies for a longer period of time and at different temperatures in Zaire, these experiments are no longer possible with the unfortunate closure of Projet SIDA due to recent political difficulties. Fortunately, extensive stability studies have been performed by the Centers for Disease Control (CDC) as part of a quality assurance program for the use of dried blood spots (DBS) on filter papers for a variety of tests used in newborn screening. These tests have measured the stability of protein and nonprotein analytes, thyroid-stimulating hormones, thyroxin, phenylalanine, galactose, progesterone, hemoglobins, and HIV-specific antibodies. In these studies, blood spots on sheets of filter paper are dried routinely for 24 h at ambient temperature and then placed in plastic zip-closure bags with low gas permeability containing desiccant packages and humidity indicator cards (2). The DBS materials are stored at −70°C, and the humidity is maintained below 30%. Bags with low permeability to gas are important for maintaining active desiccant. The source of filter paper is important, and only paper certified for whole-blood collection must be used. We have found that these conditions are ideal for long-term storage of DBS for all analytes that have been studied.

Humidity is the primary contributor to instability of analytes in DBS.

Results from stability studies indicate that under a variety of heat and humidity conditions, HIV antibodies in DBS are reasonably stable. Under a variety of climate conditions and seasons around the world (45 states and 17 countries), we have not detected any DBS stability problems with DBS in plastic bags containing desiccant during these "round-robin" tests of transportation conditions. These round-robin tests involved double-package distribution to a given location in the world and return of one package to the CDC. All return samples were tested in the same assays to minimize analytical variance. We do not recommend use of metal cargo containers for transport of DBS. Wood or heavy cardboard would retain and conduct less heat and result in less potential for compromise of DBS. If airtight containers with desiccant cannot be used, the best shipping storage conditions are paper containers that acclimate quickly. Containers that sweat with change in temperature must be avoided unless desiccant is used. Finally, storage containers with DBS (with or without desiccant) brought from low to ambient temperatures must be allowed to acclimate before being opened.

Environmental control stability studies are carried out both as accelerated and as longitudinal studies at the CDC. DBS are stored at multiple temperatures, either in zip-closure bags with desiccant or unprotected. A reduction in HIV antibody titer of approximately 15% is seen within 30 days of storage in ambient laboratory conditions (unprotected); at higher temperatures this loss in titer is enhanced (2). Samples stored in controlled humidity at the same ambient conditions were stable for up to 190 days. Ongoing longitudinal stability studies have demonstrated that HIV antibodies in DBS stored under our controlled conditions of humidity and at a low temperature are stable for at least 56 months. DBS samples with significant temperature-induced loss in titer (55°C for 160 days) were examined by quantitative assessment of HIV-specific bands on Western immunoblots, and it was determined that the loss in titer appeared to be proportional across all HIV-specific bands.

For stability testing, a dilution series that extends from an optical density of 2.0 to 0.1 and that contains all Western blot HIV-specific bands is preferred. This aspect is important in testing the stability of HIV antibody, because HIV-positive samples frequently have very high OD values and cannot be tested because of loss in titer. For 4 years the CDC has monitored a DBS dilution series prepared and stored 5 years ago for stability by enzyme immunoassay and Western blot testing and has found no significant change in response after storage under the above-described conditions and after transportation to laboratories across the United States.

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