# Use of a Dry-Plasma Collection Device to Overcome Problems with Storage and Transportation of Blood Samples for Epidemiology Studies in Developing Countries

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**Studies are difficult in areas lacking modern facilities due to the inability to reliably collect, store, and ship samples. Thus, we sought to evaluate the use of a dry plasma collection device for seroepidemiology studies. Plasma was obtained by fingerstick using a commercial dry plasma collection device (Chemcard Plasma Collection Device) and serum (venipuncture) from individuals in Kazakhstan. Plasma samples were air dried for 15 min and then stored desiccated in foil zip-lock pouches at 4 to 6°C and subsequently shipped to the United States by air at ambient temperature. Serum samples remained frozen at −20°C until assayed. *Helicobacter pylori* status was determined by enzyme-linked immunosorbent assay (HM-CAP EIA) for the dry plasma and the serum samples. The results were concordant in 250 of the 289 cases (86.5%). In 25 cases (8.6%), the dry plasma samples gave indeterminate results and could not be retested because only one sample was collected. Five serum samples were positive, and the corresponding dry plasma samples were negative; one serum sample was negative, and the corresponding plasma sample was positive. The relative sensitivity and specificity of the Chemcard samples to serum were 97.6 and 97.9%, respectively, excluding those with indeterminate results. Repeated freeze-thawing had no adverse effect on the accuracy of the test. We found the dry plasma collection device to provide an accurate and practical alternative to serum when venipuncture may be difficult or inconvenient and sample storage and handling present difficulties, especially for seroepidemiologic studies in rural areas or developing countries and where freeze-thawing may be unavoidable.**

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*Helicobacter pylori* is a major human pathogen that is etiologically linked to gastritis, peptic ulcer disease, gastric adenocarcinoma, and primary gastric B-cell lymphoma (1, 3). There is continued interest in the epidemiology of *H. pylori* infection in order to better characterize the prevalence of infection, as well as the natural history and mode of transmission of the infection (1, 3). One problem with obtaining specimens from developing countries, where the infection is most common, has been the ability to reliably collect, store, and assay serum or plasma samples (N. Broutet, G. Duperrex, B. Bergery, and F. Megraud, Letter, Lancet 354:1529–1530, 1999). The problems relating to storage and transport of these samples have often been the limiting factor in determining which questions can be addressed in seroepidemiologic studies, especially in areas lacking modern medical facilities. Shipment of frozen serum samples is expensive, requiring the use of dry ice and expedited shipping schedules, and requires compliance with national and international regulations governing the shipment of biohazardous materials. Delays are not uncommon and can result in compromised samples. A method that eliminated the need to store and ship frozen serum would therefore be welcome. We evaluated the feasibility of using a simple device that allows collection of dry plasma from a fingerstick that does not require freezing for storage or shipment and does not require

biohazardous clearance for shipping or handling for obtaining blood samples in Kazakhstan.

## MATERIALS AND METHODS

We evaluated the utility of the dried plasma device using the commercially available HM-CAP EIA (Enteric Products, Inc., Stony Brook, N.Y.). We compared results obtained when the dry plasma sample was rehydrated and assayed via the HM-CAP EIA to results obtained using a conventional serum sample collected from the same patient in order to determine if use of the dry plasma device could be an accurate, reproducible alternative to the use of serum.

**Blood sample collection.** Serum and plasma samples were collected by local physicians in Kazakhstan using a patented dry plasma collection device (Chemcard; Chematics, Inc., North Webster, Ind.) which consists of a laminate of a semipermeable membrane, through which blood cellular and particulate matter cannot pass. This membrane is over a second membrane designed to absorb a measured amount of plasma. A hanging drop of blood obtained via fingerstick was touched to the test area of the commercial dry plasma collection device. The correct amount of blood applied was signified by a change in the color from white to red of the integrated control, indicating when an adequate volume of blood had been applied to the card. The top filter was removed after 3 min, and the card was air dried for 15 min. The resultant dried plasma sample was then stored in a desiccated zip-lock pouch at between 4 and 6°C for up to 2 months before being shipped to the United States by air at ambient temperature.

A venous blood sample was also obtained from each individual at the same time that the plasma sample was obtained. The blood was allowed to clot, and the serum was separated by centrifugation. Sera were stored at −20°C and then shipped to the United States for analysis.

**ELISA test procedure.** The HM-CAP EIA for immunoglobulin G antibodies for *H. pylori* was performed according to the manufacturer's instructions. The plasma collection pad was removed from the card and placed in the bottom of a test tube. A portion (170 μl) of EIA wash buffer was added to each tube so that the collection pad was totally immersed in the wash buffer, and this was hydrated for 10 min. The collection pad and wash buffer were vortexed three times for 10 s each time. The wash buffer used for the extraction of the plasma was assayed in the HM-CAP EIA as if it were a diluted serum sample (i.e., 100 μl of the wash buffer containing the plasma was added to each test well of the microwell plate,

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and the EIA was performed in accordance with the manufacturer's instructions). All samples were assayed within 30 min to 1 h of rehydration and extraction. Serum and card samples were evaluated on separate assays on separate days. For the HM-CAP EIA, quantitative enzyme-linked immunosorbent assay (ELISA) values (EV) were extrapolated and interpreted for each sample, for either serum or dried plasma, according to the manufacturer's instructions.

**Reproducibility.** Six plasma card samples were obtained from each of three patients. These patients had previously been demonstrated to have *H. pylori* antibody titers which were negative, low positive, and mid-range positive as judged by HM-CAP analysis of serum samples obtained by venipuncture. All six samples were obtained on the same day. The dried plasma samples were stored desiccated at 4 and 6°C until rehydration, extraction, and analysis on the HM-CAP EIA. The plasma samples were assayed as follows: samples 1 and 2 were evaluated in tandem on the same assay at 2 weeks, samples 3 and 4 were evaluated in tandem on the same assay at 1 month, and samples 5 and 6 were analyzed in tandem on the same assay at 3 months.

**Effect of freeze-thawing.** Three EDTA whole-blood samples giving plasma card results in the range of high-negative-indeterminate, low-positive, and high-positive EV on EIA were used. For each sample, a total of 10 plasma cards were spotted. The dried plasma samples thus obtained were stored in a desiccated zip-lock pouch. One sample at each EV was stored at 2 to 8°C for a total of 7 days. The remaining seven were frozen at −20°C overnight. The following day, all seven cards for each were removed to 2 to 8°C for a minimum of 4 h to thaw. One card was left at 2 to 8°C for the remainder of the experiment. The remaining six were refrozen at −20°C overnight. This pattern was continued until all the plasma cards were at 2 to 8°C, each having undergone 1, 2, 3, 4, 5, 6, or 7 freeze-thaw cycles. On the final day, all 10 pouched plasma cards for each EV level were removed to room temperature, rehydrated, and assayed in tandem on the HM-CAP EIA assay. The reproducibility of results for the three which were never frozen and thawed versus the remaining seven which had undergone freeze-thaw cycles 1 through 7 was evaluated.

## RESULTS

*H. pylori* infection was defined as a positive HM-CAP EIA from the frozen serum sample. Values of <1.8 were scored as negative, and those that were >2.2 were scored as positive. Values of 1.8 to 2.2 were considered indeterminate and repeated as directed by the manufacturer. If the repeat result was positive, the sample was scored as positive, and if negative, the sample was scored as negative. If the repeat result was indeterminate, the sample was scored as indeterminate.

A total of 289 simultaneously obtained plasma and serum samples were collected. Of these, 204 simultaneously obtained pairs of plasma and serum samples were positive and 46 were negative on the HM-CAP EIA. Five serum samples were positive, whereas the corresponding plasma samples were negative. One serum sample was negative, whereas the corresponding plasma sample was positive. Insufficient material remained to retest the plasma samples that yielded indeterminate results, and so the 25 indeterminate plasma results (8.6% of the samples) were excluded from the comparative analysis. The relative sensitivity and relative specificity of the dried plasma samples compared to the serum samples were 97.6 and 97.9%, respectively, excluding the indeterminate samples.

Of the 25 indeterminate plasma sample results, 17 had corresponding serum EV results in the low-positive range of <3.0. One of the twenty-five had a corresponding serum result in the high-negative range of 1.4 EV, and two had corresponding serum results which were also indeterminate. Of eight sera yielding indeterminate results, two had corresponding plasma card results in the high-negative range of ≥1.0 EV.

**Reproducibility.** Results obtained using the dried plasma card were very reproducible, as shown in Table 1. Duplicate samples assayed in the HM-CAP EIA in tandem were reproducible within 0.1 EV or 6.0%. All six samples gave a standard deviation of 0.2 EV or less. These results are consistent with the HM-CAP assay reproducibility, as stated in the manufacturer's package insert.

**Results of repeated freeze-thawing.** The EV of each sample having undergone freeze-thaw cycles 1 through 7 were comparable to the mean of the three samples that were never frozen

TABLE 1. Reproducibility of Chemcard EV results on HM-CAP EIA[a]

| Sample no. | EV for: | | | Assay date |
|---|---|---|---|---|
| | Patient A | Patient B | Patient C | |
| 1 | 0.4 | 2.7 | 3.6 | 2 wk |
| 2 | 0.4 | 2.7 | 3.7 | 2 wk |
| 3 | 0.4 | 2.9 | 3.7 | 1 mo |
| 4 | 0.4 | 3.0 | 3.7 | 1 mo |
| 5 | 0.5 | 2.5 | 3.4 | 3 mo |
| 6 | 0.4 | 2.5 | 3.2 | 3 mo |

[a] For patients A, B, and C, the mean EV ± the standard deviations and the coefficients of variation were 0.4 ± 0.04 and 9.7%, 2.7 ± 0.2 and 7.5%, and 3.6 ± 0.2 and 5.8%, respectively.

(Table 2). Results were reproducible within a standard deviation of 0.2 EV and/or coefficient of variation of <6% for each EV level.

## DISCUSSION

The results of the two methods of blood collection were comparable, with a specificity and sensitivity of >97%. The comparative analysis showed that the two collection methods were highly reliable and reproducible, with 250 of 256 determinations having essentially identical HM-CAP EIA results. There were only six instances (2.3%) in which the results differed between the collection methods. The only disadvantage to the Chemcard (compared to serum) is that samples yielding indeterminate results cannot be retested. This can be overcome by collection of two or more samples per patient, and this is recommended for future studies. In this study we collected only one sample and those with indeterminate values could not be retested. The results of this comparative study in Kazakhstan are similar to those obtained in the carefully controlled conditions in the United States in which 84 patients were tested and the results were compared to the HM-CAP (2), despite the potential problems associated with collection and storage in an underdeveloped country.

Problems related to blood collection, storage, and shipping are the major impediments to conducting seroepidemiologic studies in areas where modern facilities are lacking. In rural areas, storage and transportation are often the critical elements responsible for the failure or success of a study, and the failure of any support system (refrigerator, freezer) or a delay

TABLE 2. Effect of repeated freeze-thaw cycles of Chemcard EV for three sera as determined by HM-CAP EIA[a]

| Freeze-thaw cycle | EV level in serum: | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| 0 | 1.8[b] | 3.1[b] | 5.0[b] |
| 1 | 1.6 | 2.6 | 5.1 |
| 2 | 1.8 | 2.9 | 5.2 |
| 3 | 1.7 | 2.7 | 4.9 |
| 4 | 1.8 | 3.0 | 5.0 |
| 5 | 1.7 | 2.7 | 4.9 |
| 6 | 1.6 | 2.6 | 5.3 |
| 7 | 1.6 | 2.7 | 4.8 |

[a] The mean EV levels ± the standard deviations and the coefficients of variation for sera 1, 2, and 3 were 1.7 ± 0.1 and 5.4%, 2.8 ± 0.2 and 6.8%, and 5.0 ± 0.2 and 3.3%, respectively.

[b] Mean of three Chemcard determinations for unfrozen samples.

in transit may result in the loss of valuable specimens and necessitate the recollection of samples (Broutet et al., Letter).

Dried-blood collection has been previously recognized as a rapid, convenient, and inexpensive means of collecting samples for later quantitative laboratory analysis and is especially useful where collection of a blood sample is more difficult and/or less desirable, for example, in infants (4–8; G. F. Batstone, E. J. Coombes, and T. R. Gamlen, Letter, Lancet **2:**99, 1984; J. V. Kenny, Letter, Trop. Doct. **23:**128, 1993; S. O'Shea, J. Mullen, K. Corbett, I. Chrystie, M. L. Newell, and J. E. Banatvala, Letter, AIDS **13:**630–631, 1999; G. T. Werner, G. G. Frosner, and C. Epp, Letter, Trans. R. Soc. Trop. Med. Hyg. **79:**135–136, 1985). Typically, a standardized area of the dried-blood sample is punched out and rehydrated in an attempt to obtain a relatively standardized sample. The Chemcard collects a standardized amount of plasma without contaminating red blood. Plasma, unlike serum, is stable at ambient temperature and thus may allow for the archiving of samples for longitudinal studies and possibly for comparison of acute and convalescent or quantitative laboratory analysis, which is not possible in rapid test devices.

The Chemcard allowed collection of plasma samples with little extraneous equipment (e.g., a fingerstick apparatus such as disposable lancets) and could be performed with minimal training or discomfort. The dried sample was also simple to handle, store, and transport, and shipping costs were minimal because the samples were compact and could be shipped at ambient temperature, thus producing a saving both in convenience and in shipping costs. In addition, the results of the freeze-thaw experiment showed that this method is very robust. Together, these features of the Chemcard make it highly desirable for seroepidemiologic studies in both developed and developing countries.

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