The screening of human sera for antibodies to infectious agents is a primary laboratory tool for the diagnosis of infectious disease. The requirements of proper collection and proper handling of sera or plasma are barriers to serologic diagnosis with samples collected at remote sites where the laboratory equipment, personnel, or infrastructure necessary for the correct handling of blood samples may not be available. Also, the requirement for venipuncture and properly trained phlebotomists is an impediment to large-scale screening and the collection of samples from pediatric patients. Finally, the long-term storage of sera requires a freezer with sufficient space to hold samples. Due to these issues, over 35 years ago Bailey et al. (4) evaluated the use of blood samples dried on filter paper as a source of antibody for the serologic diagnosis of trypanosomiasis. Subsequently, numerous publications have outlined the utility of dried blood samples (DBS) for the serologic diagnosis of infectious diseases as well as for large-scale seroprevalence studies (4–7, 9–12, 15, 16).

Herpes simplex virus type 1 (HSV-1) and HSV-2, the two types of HSV, cause both primary and recurrent infections (8). HSV-1 is commonly acquired in childhood and primarily infects the oral cavity, but it may also infect the genital tract to a lesser extent. HSV-2 generally infects the genital tract only and is acquired in late adolescence or early adulthood. Genital herpes can be associated with serious morbidity, especially when it is caused by HSV-2, because HSV-2 genital herpes has a higher rate of recurrence and entails a longer period of viral shedding than HSV-1 genital herpes. The seroprevalence of HSV-2 in the industrialized world ranges from 15 to 50%, while in the developing world the seroprevalence of HSV-2 can be as high as 65 to 70%. Recently, HSV type-specific antibody tests which allow for the detection of HSV type-specific antibodies in a routine laboratory setting have been developed. The new generation of HSV serologic tests is based on the detection of antibodies to glycoprotein G1 (gG1) and gG2, which are markers for HSV-1 and HSV-2, respectively (1–3). The gG-based HSV serology tests are now being used to determine the seroprevalence of HSV in many countries in the world. The ability to use DBS for the gG-based tests will allow for expanded studies of seroprevalence as well as expanded serodiagnosis of genital herpes in the developing world.

The present study evaluated the efficiency of eluting immunoglobulin G (IgG) from DBS on filter paper by quantitating the IgG eluted from DBS and those in corresponding serum samples. The ratio of the mean IgG concentration for all dried blood samples to the mean IgG concentration for the corresponding sera was 1:29. When the 1:29 ratio was applied to each of the 22 pairs of samples, there was a deviation of less than 15% between concentrations in the dried blood sample and in the corresponding serum sample in 19 of the pairs. No positive or negative bias was detected for the IgG eluted from dried blood. The presence of HSV-1 and HSV-2 antibodies was determined in the paired dried blood and serum samples, and no differences in the HSV serostatuses were detected for 43 of the 44 pairs. One pair's serostatus varied, with the serum sample being weakly positive for HSV-1 and the dried blood sample results being equivocal. The detection of HSV antibodies was generally consistent for dried blood samples stored for over 1 year or at room temperature for 30 days, although decreased reactivities were found in a few samples.

**Materials and Methods**

**Blood specimens.** Paired serum and whole-blood samples were collected from 22 healthy volunteers. The whole-blood samples were collected in heparinized Vacutainer tubes. Sera were collected from the clotted blood samples, aliquoted, and stored at −20°C until used. Whatman no. 3 filter paper was impregnated
with heparinized blood by the addition of 600 μl of whole blood per sample. The impregnated filter paper was allowed to dry for 2 to 4 h and stored at −20°C for 6 weeks prior to the elution steps. Some DBS were also stored for 1 year at −20°C for use in stability studies. The plasma from the heparinized blood was collected, aliquoted, and frozen at −20°C until used.

**Elution and quantitation of IgG from DBS.** A 6-mm-diameter (28-mm²) disk around each DBS was cut from the filter paper and soaked overnight at 4°C for 150 μl of 0.15 M phosphate-buffered saline containing 0.05% azide. After the overnight elution step, the IgG present in each DBS and in the corresponding serum and plasma samples was quantitated with an Array nephelometer (Beckman, Brea, Calif.). All samples were handled per the manufacturer’s specifications.

**Detection of HSV type-specific antibodies.** The HerpeSelect HSV type-specific ELISA (Focus Technologies, Cypress, Calif.) was used to determine the presence of HSV-1 and HSV-2 antibodies in the paired DBS and serum samples. The serum samples were assayed per the manufacturer’s specifications. The DBS were diluted in the HerpeSelect kit specimen diluent as defined in Results, and all subsequent steps were performed as directed by the manufacturer. The results are reported as an index value, with an index of <0.9 considered to be negative, an index of 0.9 to 1.1 considered to be equivocal, and an index of >1.1 considered to be positive.

**RESULTS**

**Correlation of IgG concentrations in corresponding serum samples, plasma samples, and DBS.** The mean IgG concentrations were 1,215, 1,176, and 41.8 mg/dl for the 22 serum samples, plasma samples, and DBS, respectively (Table 1). The concentrations of IgG in plasma samples correlated closely to those in serum samples, with a correlation coefficient (r²) of 0.972. The mean IgG concentration for the DBS was 29-fold less than the mean IgG concentration for the corresponding serum samples. When the IgG concentration in each DBS was multiplied by 29 (29×DBS value) and the result was compared to the IgG concentration in the corresponding serum sample, the mean deviation of the DBS value from that for the corresponding serum sample was −0.9% (standard deviation, 11.5%), with deviations between samples ranging from −28% to −25%. However, 16 of the 22 paired IgG results had a deviation of 10% or less, and 19 of the 22 paired samples had a deviation of less than 15%. The 29×DBS values for only three samples (samples 2, 16, and 18) deviated more than 15% from the IgG concentrations in the corresponding serum samples, and all three corresponding serum samples had IgG concentrations greater than 1,000 mg/dl. The 29×DBS values were equally distributed above and below those in the corresponding serum samples, with 12 29×DBS values less than the IgG concentrations in the corresponding serum samples and 10 29×DBS values higher than the IgG concentrations in the corresponding serum samples. Figure 1 demonstrates the correlation of the 29×DBS values and the IgG concentrations in the corresponding serum samples. The correlation coefficient of the IgG concentrations in serum samples and those in DBS multiplied by 29 was 0.8055.

**Standardized dilution scheme to detect HSV antibodies in DBS.** The use of DBS to detect HSV type-specific samples was evaluated by using a standard dilution scheme for all DBS. Sera were diluted 1:101 in the standard HerpeSelect ELISAs for HSV-1 and HSV-2. As demonstrated in the IgG elution experiments, the ratio of IgG concentrations in serum samples to those in DBS elute was, on average, 1:29. Therefore, to standardize a dilution scheme for the use of DBS eluate in the ELISA, the eluate was diluted to a concentration of 1:4 in ELISA sample buffer. The 1:4 dilution of DBS eluate was then comparable to a 1:101 dilution of serum. The HSV-1 ELISA index values for the paired DBS and serum samples are shown in Table 2. Twenty-one of the 22 paired DBS and serum samples gave concordant results for the HSV-1 ELISA. Only pair 9 had different interpretations, with the serum result being positive (index = 1.3) and the DBS result being equivocal (index = 1.1). Two other serum samples (samples 6 and 10) also gave equivocal results; however, the corresponding DBS results were also equivocal for HSV-1.

The HSV-2 results are given in Table 2. Although only 4 of
the 22 pairs were positive for HSV-2 antibodies, all 22 pairs gave concordant results. Few samples were near the equivocal zone (0.9 to 1.1) for the ELISA for either HSV-1 or HSV-2. Therefore, it was difficult to assess the impact of using DBS to detect the presence of HSV type-specific antibodies in samples with reactivities near the assay’s equivocal zone. The two serum samples listed in Table 2 (samples 5 and 14) with results closest to the equivocal range for HSV-2 had indices of 1.6 and 0.6, and the corresponding DBS results were similar to the serum results. Pair 16 in Table 2 had an index of 0.3 for the serum sample but an index of 0.6 for the DBS.

**Stability of HSV antibodies in DBS stored frozen and at room temperature.** DBS samples were stored at −20°C for 1 year to determine the effects of long-term storage on the HSV-1 and HSV-2 reactivities. An additional DBS for each set of samples was stored at room temperature for 30 days beyond the 1 year at −20°C. Table 3 presents the original HSV-1 and HSV-2 index values for these DBS as well as the values for the same samples after storage for 1 year at −20°C or after storage for 1 year at −20°C plus an additional 30 days at room temperature. Since the HSV index values for the samples were obtained 1 year apart, the effect on HSV index values of different ELISA runs as opposed to that from degradation of samples over 1 year is difficult to interpret.

All 14 DBS that originally tested positive for HSV-1 remained positive after being stored at −20°C for 1 year; however, one of the three equivocal samples (sample 9) was negative for HSV-1 after being stored at −20°C, while the other two equivocal samples (samples 6 and 10) remained equivocal. Three samples stored at room temperature for an additional 30 days showed different results for HSV-1 antibody interpretation, with indices of samples 6 and 9 decreasing from the equivocal to the negative range and the index for sample 11 decreasing from the weakly positive to the equivocal range. The other 14 HSV-1-positive samples remained positive after the additional storage at room temperature. The mean index values for the samples stored frozen for 1 year showed slightly higher reactivities than the original index values; however, the mean index values for samples stored at room temperature dropped 15% compared to those for the samples kept frozen.

The limited number of HSV-2-positive samples obtained in the random serum panel did not allow for extensive studies on the effect of storage on HSV-2 reactivity. Sample 5 was originally weakly positive for HSV-2 but was negative for HSV-2 after being stored frozen and at room temperature. The other three samples that were originally positive for HSV-2 remained positive after being stored frozen and at room temperature; however, the index values of these samples decreased. The DBS kept at room temperature for an additional 30 days after 1 year at −20°C gave HSV-2 index values similar to those of the DBS kept only at −20°C; thus, room temperature storage had no apparent additional detrimental effects on antibody reactivity. The mean index values for the two sets of stored DBS were 42 to 43% lower than the original index values for HSV-2 obtained 1 year earlier.

**DISCUSSION**

The utility of DBS for infectious disease testing has been known for many years (4–7, 9–12, 15, 16). As the need to

| Sample | Original DBS | Frozen DBSa | FR/RT DBSb | Index values from HSV-1 assay on: | Frozen DBS | FR/RT DBS |
|--------|--------------|-------------|------------|----------------------------------|------------|------------|
| 1      | 0.5          | 0.4         | 0.4        | 0.2                               | 0.1        | 0.2        |
| 2      | 4.8          | 5.5         | 4.4        | 3.2                               | 1.3        | 1.5        |
| 3      | 4.8          | 5.4         | 5.0        | 3.2                               | 1.3        | 1.5        |
| 4      | 0.4          | 0.2         | 0.2        | 0.4                               | 0.2        | 0.2        |
| 5      | 5.3          | 5.3         | 4.5        | 1.5                               | 0.9        | 0.6        |
| 6      | 1.1          | 1.0         | 0.6        | 0.4                               | 0.1        | 0.1        |
| 7      | 5.2          | 5.3         | 5.4        | 0.5                               | 0.1        | 0.1        |
| 8      | 0.2          | 0.2         | 0.2        | 0.5                               | 0.0        | 0.0        |
| 9      | 1.1          | 0.8         | 0.6        | 0.4                               | 0.1        | 0.1        |
| 10     | 1.0          | 1.1         | 1.1        | 0.3                               | 0.1        | 0.2        |
| 11     | 1.5          | 1.2         | 1.0        | 11.8                              | 8.6        | 8.7        |
| 12     | 0.2          | 0.1         | 0.2        | 0.4                               | 0.1        | 0.1        |
| 13     | 0.2          | 0.1         | 0.2        | 0.4                               | 0.1        | 0.1        |
| 14     | 4.4          | 4.8         | 4.4        | 0.6                               | 0.1        | 0.2        |
| 15     | 4.6          | 6.7         | 5.1        | 0.3                               | 0.1        | 0.1        |
| 16     | 4.8          | 6.2         | 3.6        | 0.6                               | 0.1        | 0.1        |
| 17     | 4.8          | 3.6         | 3.1        | 0.1                               | 0.1        | 0.1        |
| 18     | 5.1          | 5.6         | 4.0        | 0.5                               | 0.2        | 0.2        |
| 19     | 4.8          | 4.5         | 3.8        | 0.4                               | 0.1        | 0.1        |
| 20     | 5.2          | 6.3         | 6.2        | 0.3                               | 0.1        | 0.1        |
| 21     | 5.0          | 6.3         | 6.0        | 0.3                               | 0.0        | 0.0        |
| 22     | 4.3          | 3.3         | 2.4        | 9.4                               | 6.6        | 5.4        |

Mean % REACd 3.15 3.36 2.84 1.48 0.86 0.84 107 91 58 57

**Table 3. Index values obtained from DBS before and after storage**

**Table 2. Correlation of HSV-1 and HSV-2 index values for corresponding serum samples and DBS**

| Pair no. | Index | Result interpretation | Index | Result interpretation |
|----------|-------|-----------------------|-------|-----------------------|
| Serum    | DBS   | Serum DBS             | Serum | DBS DBS               |
| 1        | 0.4   | 0.5                   | 0.2   | 0.2                   |
| 2        | 6.1   | 4.8                   | 0.2   | 0.3                   |
| 3        | 5.7   | 4.8                   | 2.4   | 3.1                   |
| 4        | 0.3   | 0.4                   | 0.1   | 0.3                   |
| 5        | 5.4   | 5.3                   | 1.6   | 1.5                   |
| 6        | 1.1   | 1.1                   | Eq    | Eq                    |
| 7        | 6.6   | 5.2                   | 0.2   | 0.4                   |
| 8        | 0.2   | 0.2                   | 0.2   | 0.5                   |
| 9        | 1.3   | 1.1                   | 0.2   | 0.3                   |
| 10       | 1.1   | 1.0                   | 0.2   | 0.3                   |
| 11       | 1.4   | 1.5                   | 11.9  | 11.7                  |
| 12       | 0.3   | 0.2                   | 0.3   | 0.4                   |
| 13       | 0.2   | 0.2                   | 0.2   | 0.4                   |
| 14       | 4.8   | 4.4                   | 0.6   | 0.6                   |
| 15       | 6.2   | 4.6                   | 0.4   | 0.2                   |
| 16       | 5.6   | 4.8                   | 0.3   | 0.6                   |
| 17       | 5.6   | 4.8                   | 0.2   | 0.1                   |
| 18       | 6.0   | 5.1                   | 0.5   | 0.5                   |
| 19       | 5.8   | 4.8                   | 0.3   | 0.4                   |
| 20       | 7.0   | 5.2                   | 0.3   | 0.2                   |
| 21       | 6.5   | 5.0                   | 0.2   | 0.2                   |
| 22       | 3.8   | 4.3                   | 9.7   | 9.4                   |

* Eq, equivocal.
investigate new or emerging diseases continues, especially in the developing world, the need to collect, store, and transport blood samples will also continue. In this study we determined the consistency between IgG eluted from blood dried on filter paper and IgG present in corresponding serum samples. There is a slight variation in the concentration of IgG obtained from DBS and that obtained from sera; however, the majority of sample pairs give very consistent values, with a 1:29 ratio, on average, between IgG concentrations in DBS eluate and those in serum samples. When the concentrations of IgG in the DBS were multiplied by 29 for comparison to concentrations in serum samples, 16 of the 22 pairs had less than a 10% deviation between concentrations, and only 4 samples had deviations of 15% or greater. No positive or negative bias resulting from elution was noted for this panel of sera. Finally, the four pairs with the greatest differences between the 29 × DBS value and the serum IgG result did not have any significant differences between their HSV-1 and HSV-2 results.

Due to the consistency of IgG eluted from the DBS with IgG in serum, it is possible to use a single dilution of DBS eluate (1:4) in the HSV type-specific ELISA method tested. Pairs of samples with clearly negative or positive results gave similar results with both serum and DBS. Samples with ELISA results near the cutoff between positive and negative would be expected to be most problematic, and one serum sample that was weakly positive for HSV-1 antibody had a corresponding DBS result that was equivocal. All other pairs with HSV-1 and/or HSV-2 results near the equivocal range for serum gave the same results with the DBS. The HSV antibodies captured on DBS are stable when stored for over 1 year at −20°C or for an additional 30 days at room temperature. All 16 DBS with original HSV-1 or HSV-2 index values above 1.5 remained positive after extended storage. Five DBS had index values between 1.0 and 1.5; the interpretation of results for three of these five specimens changed after they were stored frozen for 1 year, and the interpretation of results for one additional DBS changed after additional storage at room temperature. The present study included a limited number of HSV-2-positive samples, so the conclusions apply primarily to HSV-1 antibodies. It should be noted, however, that the stability and reactivity of antibody stored and recovered from a solid phase are the parameters that were investigated in this study. It would be expected that HSV-1 and HSV-2 antibodies would have similar stabilities and recoveries when eluted from a solid matrix. The subsequent reactivities to recombinant glycoproteins would be expected to be similar. Although the mean reactivity of the serum panel to HSV-1 did not appear to degrade over 1 year, the HSV-2 indices did decrease. It was not possible to determine the contribution of either the degradation of IgG over 1 year’s storage or differences in ELISAs run 1 year apart to the differences in HSV index values obtained originally and 1 year later. No DBS were stored at temperatures below −20°C to determine stability at lower temperatures. The type of filter paper used, the diameter of the filter paper used to elute the IgG, and the elution parameters were kept constant in the study and allowed for the application of the 1:29 serum-to-DBS ratio in this study. Since other elution parameters were not investigated, changes in the parameters provided may yield different results.

In summary, blood samples dried on filter paper and stored for short periods of time give results concordant with those of corresponding blood samples tested by the standard HSV ELISA procedure. The elution of proteins, in this case IgG, appears to be quite consistent with the method used in this investigation. Some DBS stored at −20°C for over 1 year demonstrated decreased HSV reactivities; however, the decreased reactivities are evident only in samples with low antibody levels or equivocal results. The storage of DBS at room temperature for an additional month caused a slightly higher loss of reactivity. Although experiments to investigate the effects of short-term storage at room temperature were not performed, it would appear that short-term room temperature storage, perhaps 1 to 2 weeks, would have a minimal effect on the reactivity of IgG eluted from DBS.

The need for blood samples that can be easily obtained, stored, and transported continues. The ease of using dried blood samples allows for blood samples to be collected and stored under minimal conditions worldwide and without the need for sophisticated equipment or training. As new organisms become known and new laboratory tools become available, it will be necessary to ensure the validity of older methods such as those using DBS. Although genital herpes is not a new disease, the incidence of HSV infection continues to increase and has been implicated as a possible cofactor in the acquisition of human immunodeficiency virus (13, 14). Both HSV-1 and HSV-2 cause genital herpes; however, HSV-2 genital herpes has a higher incidence of recurrence, prolonged viral shedding, and thus an increased risk of viral transmission than HSV-1 genital herpes does (8). The development of type-specific HSV serologic tools utilizing gG1 and gG2 has allowed for the accurate determination of HSV-1 and HSV-2 infection. The ability to use DBS together with HSV type-specific serologic tools will aid in the evaluation of the incidence of HSV-2 worldwide.

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