Dried Blood Spots on Filter Paper as an Alternative Specimen for Measles Diagnostics: Detection of Measles Immunoglobulin M Antibody by a Commercial Enzyme Immunoassay

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Background. We compared the results of a serum-based measles immunoglobulin M (IgM) test with results of tests using paired reconstituted dried filter paper blood spot (DBS) samples to assess the feasibility of using DBS samples for measles diagnostic procedures.

Methods. We collected 588 paired serum and DBS samples from 349 children aged 8 months through 12 years at Mulago Hospital in Kampala, Uganda; of these samples, 513 (87%) were collected from children with a clinical diagnosis of measles 0–33 days after rash, and 75 (13%) were collected from children hospitalized for other reasons. Eluted DBS and serum samples were tested using a commercial measles IgM enzyme immunoassay. Detection of viral RNA was attempted on a subset of 20 DBS by reverse-transcriptase polymerase chain reaction.

Results. Among the 513 sample pairs collected from children with measles, the concordances for samples collected during days 0–6 and >1 week after rash were 95.7% and 100%, respectively (P < .01). The relative sensitivity and specificity of the DBS-based assay during the first week were 98.7% and 88.9%, respectively, and the sensitivity and specificity >1 week after rash were 100% and 100%, respectively. Viral RNA was detected in 5 (26%) of 19 DBS samples tested. Among 75 sample pairs collected from children hospitalized for other reasons, concordance was 94.7%.

Conclusions. DBS samples are a feasible alternative sample for measles diagnostic procedures in high-incidence settings.

Laboratory confirmation of measles is based on detection of the measles-specific immunoglobulin M (IgM) antibodies in a single serum specimen by enzyme-linked immunoassays (EIAs). Serum-based tests usually involve collecting blood samples by venipuncture and are associated with certain requirements and precautions related to specimen handling, such as serum separation, refrigeration during transportation to the laboratory, and freezing for long-term storage. These requirements present obstacles for further strengthening of measles surveillance, particularly in developing countries with underdeveloped public health infrastructures.

Collecting a few drops of blood from a finger or heel prick onto filter paper may simplify specimen collection and obviate the other logistical barriers mentioned above. A filter paper technique for measles diagnostic testing was considered in early 1980s [1, 2]. More recently, dried filter paper blood spot (DBS) samples were shown to be suitable for the laboratory diagnosis of measles using a combination of reverse-transcriptase polymerase chain reaction (RT-PCR) analysis and measles IgM detection by a capture EIA [3], as well as for
detection of measles IgM antibody by a capture EIA after vaccination [4]. Riddel et al [5] evaluated the performance of a commercial EIA for measles IgM detection in DBS samples prepared from venous blood collected from 216 individuals with a clinical diagnosis of measles; overall, compared with paired serum samples, the relative sensitivity and specificity of the DBS-based assay were 90.2% and 98.8%, respectively. Use of DBS samples for measles surveillance was previously field-tested in Sudan, where measles IgM was detected in 200 (63%) of 312 specimens collected from patients with a clinical diagnosis of measles [6]; however, only DBS samples were collected, precluding comparative analysis with serum-based results. The rate of viral RNA detection in the DBS samples was previously documented to be much lower (28%–48%) than the rate of measles IgM detection; nevertheless, the sequence information obtained from the RT-PCR–positive samples was used to determine the genotype of measles virus responsible for the outbreak [3, 6].

To facilitate expansion of laboratory-supported surveillance in developing countries, in 2007, the World Health Organization (WHO) convened an ad hoc meeting that reviewed available data on the use of alternative samples, including DBS samples for measles and rubella diagnostic testing; attendees concluded that, although serum-based assays remain the standard approach for measles diagnostic testing, DBS samples are a viable option for measles surveillance in settings where the collection of serum samples remains challenging [7]. This study was undertaken as a part of the WHO-led effort to evaluate the use of DBS samples as an alternative to serum specimens for detection of measles-specific IgM antibody with a commercial EIA that is widely used throughout the WHO Global Measles Laboratory Network. In addition, the feasibility of detecting the measles virus RNA was to be explored on any DBS samples that remained after the completion of tests for measles IgM antibody.

**MATERIALS AND METHODS**

The project was approved by the Institutional Review Boards of the 3 collaborating institutions and by the Uganda National Council for Science and Technology. Written informed consent was obtained from a legal guardian for all study participants, and written assent was obtained from children aged ≥7 years.

The study group included 349 children hospitalized at the Mulago Hospital in Kampala, Uganda, from May 2002 through March 2003. Of these, 274 (78%) of the children received a clinical diagnosis of measles by a qualified health care worker prior to being offered participation in the study (the measles group), and 75 (22%) were hospitalized for reasons other than measles (the nonmeasles group). Patients were not included in the study if they met any one of the following exclusion criteria: (1) age ≤8 months, (2) history of measles vaccination ≤8 weeks from the rash onset date, (3) known severe preexisting illness, such as those requiring treatment with immunosuppressive therapy (eg, corticosteroids, cancer chemotherapy, and renal failure requiring dialysis) and (4) permanent residence outside of the Kampala District.

Patient information, including demographic characteristics, vaccination history, history of present disease, and a family history of fever and rash during the year preceding the enrollment, was obtained from the primary caretaker using a standardized questionnaire. At enrollment, all patients underwent a standardized physical examination by a study physician, including measurement of axillary temperature and ascertainment of clinical signs of measles. The clinical diagnosis of measles was established according to the WHO case definition (any case with fever, maculopapular [nonvesicular] rash, and at least 1 of the following: cough, coryza, or conjunctivitis) [8].

All participants were asked to provide a set of clinical samples (serum and DBS) at enrollment. In addition, participants in the measles group were randomized for a follow-up visit during the second, third, or fourth week after rash onset and were asked to provide a second set of samples at that time. Capillary blood was collected by a finger or heel prick for both the DBS sample and the serum sample. Using universal precautions, a finger or heel was pricked by a sterile single-use self-launching pediatric lancet. For the DBS sample, ~100 μL of blood was placed on each of the two 13-mm diameter circles on Schleicher & Schuell #903 filter paper. The rest of the blood from the prick (~300 μL) was collected in a microtiter tube, allowed to clot, and separated by centrifugation; the serum was stored at −20°C.

The DBS samples were dried at room temperature for a minimum of 4 hours and, thereafter, were individually wrapped in clean wax paper and placed in a plastic zip-lock bag. Up to 30 wrapped filter paper specimens were placed in a single bag, along with sachet of 1g of silica gel desiccant (Minipax Absorbent Packets; Aldrich Chemical). The DBS and serum samples were stored at −20°C for 3–15 months after collection until shipment on dry ice to the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, where the laboratory testing was performed. DBS controls were prepared at the CDC as previously described [9] and were treated as test samples.

The CDC method for elution of serum from DBS has been described previously [10]. The Dade Behring Enzygnost Anti-Measles Virus IgM enzyme immunoassay was used to detect measles-specific IgM antibody in serum and eluted DBS samples per manufacturer’s instructions, with one modification; the IgM-positive Behring control serum Anti-Measles Virus Reference P/N were dispensed only on the first pair of wells of the first plate and on the last pair of wells of the last plate of each run, rather than on each individual plate. Test controls were treated in accordance with the kit’s instructions. Runs were validated with the qualitative evaluation criteria, and results were calculated in accordance with the kit’s instructions. Samples giving an equivocal result were retested. We also retested samples with
discordant results between the serum and DBS samples and samples from runs with high background. An error was detected on one 46–serum sample plate, and testing was repeated twice with consistent results; the repeat test results were considered in the analysis. To evaluate the accuracy of the remaining results, a systematic sample of /C2420% of all other serum samples was retested, yielding results that were consistent with the initial test results. Subsequently, the initial results were retained in the final analyses for all other serum samples except for the above-described group of 46 samples.

Viral RNA Detection
A subset of 20 DBS samples was tested for the presence of measles virus RNA by RT-PCR. Selection of this subset was based on the availability of a sufficient amount of specimen after completion of the serologic testing. Two 6-mm punches from each DBS were placed in 200 µL of RNase-free water and were incubated for 30 min. Samples were centrifuged at 11500 rpm for 1 min at room temperature, and RNA was extracted from the supernatant as directed by the manufacturer (Qiagen). Real-time RT-PCR assays to detect measles RNA and messenger RNA from a cellular gene, RNaseP, were performed as previously described [11]. Standard RT-PCR and sequence analysis were performed as previously described [12].

Data Analysis
The laboratory results were entered in a Microsoft Excel file and were analyzed using SAS, version 9.1 (SAS); Microsoft Office Excel 2003; and Epi Info, version 6.04d (CDC) [13–15]. The assay results obtained from the paired DBS and serum samples were analyzed cross-sectionally by week of sample collection following the rash onset for the measles group and separately for the nonmeasles group. In each analysis, the relative sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated by comparing the results of the assay performed using the DBS samples with the results of the assay performed using the corresponding serum samples. Sample pairs in which one or both samples had an equivocal final assay result were disregarded from these calculations. Concordance between the DBS-based and corresponding serum-based assay results was evaluated by considering all sample pairs in the study. The weighed kappa and R² statistics were calculated to measure the level of agreement between the DBS and serum samples. Differences between the measles group and the nonmeasles group study participants were examined with the χ² or Kruskal-Wallis H test, as appropriate.

RESULTS

Study Participants
All 274 children in the measles group met the clinical case definition of measles; no one in the nonmeasles group met that definition. Compared with the nonmeasles group, children in the measles group were younger, were more evenly distributed by sex, and were less likely to have reported receiving at least 1 dose of measles vaccine or having prior measles-like illness (Table 1).

Laboratory Results
A total of 588 paired DBS and serum samples were collected, including 513 sample pairs collected from 274 children in the measles group and 75 sample pairs collected from 75 children in the nonmeasles group. In the measles group, sample pairs were collected during the first week after rash onset from 253 (93%) of the children enrolled on days 0 (16 children), 1 (56), 2 (66), 3 (51), 4 (26), 5 (25), and 6 (13) after rash onset. A total of 260
sample pairs collected during weeks 2–5 after rash onset included those collected at enrollment from 19 (7%) and those collected at the follow-up visit from 241 (69%) of the children in the measles group; these 260 sample pairs were collected during weeks 2 (89 sample pairs), 3 (87), 4 (78), and 5 (6) from onset. Very good agreement between measles-specific IgM antibodies detected in eluates from DBS, compared with the paired serum specimens, was observed in sample pairs collected from children who received a clinical diagnosis of measles (Table 2). Among 253 sample pairs collected during the first week after rash onset, 242 (95.6%) had concordant DBS- and serum-based results; relative sensitivity and specificity of DBS samples, compared with paired serum samples, were estimated at 98.7% and 88.9%, respectively (Table 2). Concordance between the results of measles IgM detection in DBS and in paired serum samples during the first 3 days after rash onset (days 0–2) was 94.9% (weighted kappa, 0.74; 95% confidence interval [CI], 0.58–0.90), which was very similar to the concordance among sample pairs collected at 3–6 days after rash onset of 96.5% (weighted kappa, 0.59; 95% CI, 0.25–0.94). Therefore, the data for all sample pairs collected during the first week after rash onset are presented together (Table 2).

An analysis of the 11 (4.4%) discordant sample pairs found that 7 (64%) were collected on days 0–2 after rash onset (ie, during the time when detection of measles IgM by EIA in a conventional serum sample is known to be less sensitive) [13]. For 9 of the 11 children, including 6 of those who had their initial sample pair collected on days 0–2, 2 with the initial sample collected on day 3 and 1 whose initial sample was obtained on day 6 after rash onset, a second (follow-up) sample pair collected 7–25 days after rash onset was available. All of these 9 follow-up sample pairs had concordant results, with both the DBS and serum samples testing positive for measles IgM antibody. A 100% agreement between DBS-based and serum-based results was observed for 260 samples collected during weeks 2–5 (Table 2).

The nonmeasles group had the lowest concordance between the DBS-based and serum-based results (94.7%; Table 2), although this finding was not statistically significant, compared with the measles group (Yates-corrected $\chi^2 = 1.55; P = .2$). Of the 4 discordant nonmeasles sample pairs, 3 pairs had an equivocal DBS-based result, whereas the paired serum-based result was negative for measles IgM; 1 pair had a DBS-based result that was negative and a serum-based result that was positive for measles IgM. In addition, 1 sample pair collected in the nonmeasles group tested positive for measles IgM in both the serum and paired DBS samples. Both of the 2 children in the nonmeasles group whose serum samples had test results that were positive for measles IgM were hospitalized due to malaria, and both patients had a history of measles vaccination; neither had a record of a measles-like rash either before or after the recruitment into this study. The 1 child in the nonmeasles group

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<th>Table 2. Detection of Measles-specific Immunoglobulin M (IgM) Antibodies in Dried Blood Spots (DBS), Compared With Corresponding Serum Samples</th>
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**NOTE.** NPV, negative predictive value; PPV, positive predictive value.
with a serum test result positive for measles IgM but a discordant (measles IgM-negative) DBS result had a record of blood transfusions due to anemia.

The optical density values for DBS samples and corresponding serum samples yielded an R² of .4765 in the measles group and .2852 in the nonmeasles group (data not shown).

RNA from the cellular gene, RNaseP, was detected in 19 (95%) of 20 DBS samples, indicating a successful RNA extraction; all of these 19 participants had a positive measles IgM antibody result both in the DBS samples and in the paired serum samples. Measles virus RNA was detected in 5 (26%) of the 19 samples with an adequate RNA sample, including 1 (50%) of 2 samples collected 1 day after rash, 3 (50%) of 6 samples collected on day 2 after rash, and 1 (12.5%) of 8 samples collected on day 3 after the rash. Sequence analysis of PCR products from the positive samples indicated the presence of wild-type measles viruses in genotype D10, which was the predominant genotype in Uganda at the time of this study (data not shown) [14].

DISCUSSION

The findings indicate that DBS are a feasible alternative to serum for serologic confirmation of measles infection. In samples collected from persons with a clinical diagnosis of measles, detection of measles-specific IgM antibody in DBS compared favorably to the results of a serum-based assay, with an overall concordance of 97.9%. Even though the results from DBS samples indicated a lower relative sensitivity and, in particular, a reduced relative specificity during the first week after rash onset, the concordance with the results from the serum samples was still 95.6%. Furthermore, our study documented virtually identical performance characteristics when DBS and serum samples were collected >7 days after rash onset. Thus, in settings in which venous blood collection, serum separation, or refrigeration are challenging, a repeat DBS sample collection >7 days after rash onset may help to confirm the diagnosis for sporadic, clinically suspected measles cases in which measles IgM-negative result from a DBS sample that is collected during the first week of disease, during which the measles IgM detection may be less reliable.

The rate of viral RNA detection by RT-PCR in DBS samples in our study was comparable to previously published results [6], which suggested that RT-PCR alone would not have adequate sensitivity for case confirmation. However, the few positive samples were used to obtain genetic information about the circulating strains of measles virus, which demonstrates the usefulness of DBS as a means to expand virological surveillance to remote areas that lack infrastructure to collect, store, and transport the standard samples for virus detection [7, 15].

The strengths of this study include a large sample size, the availability of reliably documented presence or absence of clinical signs of measles at enrollment for all participants, and the availability of an acute phase and a convalescent phase sample pair for most participants with a clinical diagnosis of measles. Because >90% of study participants with a clinical diagnosis of measles presented during the first week after rash onset, it was possible to minimize bias in ascertaining rash onset. Nonetheless, several limitations must be considered when interpreting the results. None of the measles IgM-negative DBS samples collected from individuals who met the clinical case definition for measles were further evaluated for a different febrile rash disease with clinical features similar to those of measles (eg, rubella). However, 9 of 10 of these study participants had an available follow-up DBS sample obtained beyond the first week after rash onset, and all of these participants had test results that were positive for measles IgM. It should also be noted that this study was implemented in a high-incidence setting; in low-incidence settings, the comparative characteristics of the DBS-based assay may be different. Additionally, the number of nonmeasles participants was not sufficient to further explore the potential difference in the performance of the DBS-based assay and the serum-based assay between that group and the measles group. We could not further evaluate 2 individuals with serum-based assay results positive for measles IgM among children who were hospitalized for reasons other than measles, because follow-up blood samples were not collected for nonmeasles study participants. Finally, the study results may not be representative of conditions usually encountered in routine surveillance systems in the developing countries where DBS specimens are most likely to be of wider use, because all clinical samples for this study were collected by trained study staff at a university hospital, were immediately processed for long-term storage by highly trained laboratory personnel, and were subsequently shipped to the CDC measles laboratory, where the testing was done in batches.

In our study, the correlation between the optical densities in DBS and companion serum samples was lower than that previously reported in a study that used the same commercial assay [5]. However, the percentage agreement between DBS samples and companion serum samples collected from children with a clinical diagnosis of measles in this study, as well as the estimated relative sensitivity and specificity of the DBS-based assay, compared with the serum-based assay, are consistent with those reported by previously published studies that evaluated the detection of measles IgM by EIA in paired DBS samples among naturally infected [3, 5] or recently vaccinated individuals [4].

In most countries, serum samples collected from persons with a clinical diagnosis of measles or rubella are tested for both measles IgM and rubella IgM, either in parallel or in sequence, as a means of differential diagnosis of acute fever and rash or within the framework of an integrated measles and rubella surveillance system. In a recent study, DBS samples have been proven as a reliable means for rubella diagnostic testing in a setting with high rubella incidence [16], but the performance of a DBS-based assay for differential diagnosis of measles and rubella has not yet been evaluated.
In conclusion, although the comparative characteristics of the DBS-based assay during the first week after rash onset has limitations for routine application in the diagnosis of sporadic or individual cases, these samples are acceptable for use in confirmation of measles outbreaks and for measles diagnostic testing in other high-incidence settings or where collection of conventional serum samples remains challenging. Additional studies should be conducted in settings with a lower measles incidence, where the positive predictive values of the clinical diagnosis of measles would be less reliable, and in areas where samples are routinely tested for both measles and rubella.

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**References**