Dried blood spots (DBS) have been used worldwide for the neonatal screening of congenital disorders (2, 8). Seroepidemiological studies have been conducted on DBS residual to neonatal screening to assess human immunodeficiency virus (HIV) prevalence among childbearing women (9, 10). Recently, several studies were focused on DBS for detecting drug resistance mutations (6) and for tracking global spreading of HIV type 1 subtypes (4) in proviral HIV DNA. However, RNA is notoriously less stable, and standardization of DBS for viral RNA detection would be of great benefit for application to large field studies, particularly in settings where collection, preparation, storage, and shipment of samples at controlled temperature can be difficult.

For hepatitis C virus (HCV) RNA detection on dried spot samples, the available data are much less exhaustive. A complete match between frozen serum and dried plasma spots, though with a loss of titers after room temperature storage, has been observed (1). This study was aimed at developing a simple, sensitive, and reproducible method for using whole blood collected onto filter paper (dried blood spots) for detection and genotyping of hepatitis C virus RNA that can be useful in large field studies, particularly in settings where collection, preparation, storage, and shipment of samples at controlled temperature can be difficult.

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99%; specificity > 95%). No genotype restriction was observed.

The detection limit was established by limiting dilution analysis. Specifically, blood samples from two viremic patients (viral burdens, 604,000 and 807,000 IU/ml) were serially diluted with blood from an HCV-seronegative individual. Seven-fivefold serial blood dilutions were spotted to form multiple replicate DBS strips. Four replicate pairs of each dilution (24 tests per run) were tested with TMA (after 1 and 5 weeks), giving a total of 48 tests performed, and with RT-PCR. The results, shown in Table 1, indicate that TMA was positive in 100, 85.7, and 75% of DBS containing, respectively, 1,328, 265, and 53 IU of HCV RNA. Similar sensitivity was observed with RT-PCR (not shown).

To evaluate the cross-contamination risk, a panel of DBS strips was prepared by spotting in close vicinity and, in alternate order, 15 blood samples from HCV RNA-positive patients and 15 blood samples from HCV Ab-negative individuals. Representative RT-PCR results are shown in Fig. 1, indicating a complete correspondence between expected and observed results. Similar results were obtained with TMA (not shown).

To test the stability of HCV RNA in DBS over time, a set of replicate DBS from 16 HCV RNA-positive patients was stored at room temperature and assayed at intervals of 2 to 4 weeks over an 11-month period. The results indicate that 100% positivity was preserved along the whole observation period.

To test the suitability of DBS for HCV genotyping, a replicate set of DBS from the eight patients with known genotype, stored at room temperature for 11 months, was extracted as for RT-PCR and genotyped by using the Line Probe assay (Bayer

![FIG. 1. Detection of HCV RNA in DBS from HCV-positive and -negative individuals. To control the risk of cross-contamination in using DBS for HCV RNA detection, DBS strips were prepared with blood from 30 different patients that was expected to be positive (uneven lines) or negative (even lines). The strips were spotted to realize a lattice of alternately HCV RNA-positive and -negative samples. DBS strips were wrapped in their individual paper covers and stored together in the same plastic bag for 1 week at room temperature before the assay. Representative results obtained with RT-PCR are shown.](http://jcm.asm.org/)

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**TABLE 1. Establishment of detection limit of HCV qualitative RNA assay (TMA) applied to DBS**

<table>
<thead>
<tr>
<th>HCV RNA serum concn (in IU/ml)</th>
<th>Estimated input (in IU) of HCV RNAa</th>
<th>No. positive/no. tested</th>
<th>% Positive results</th>
</tr>
</thead>
<tbody>
<tr>
<td>604,000</td>
<td>33,250</td>
<td>8/8</td>
<td>100</td>
</tr>
<tr>
<td>120,800</td>
<td>6,640</td>
<td>8/8</td>
<td>100</td>
</tr>
<tr>
<td>24,160</td>
<td>1,328</td>
<td>8/8</td>
<td>100</td>
</tr>
<tr>
<td>4,830</td>
<td>265</td>
<td>6/7</td>
<td>85.7</td>
</tr>
<tr>
<td>960</td>
<td>53</td>
<td>6/8</td>
<td>75</td>
</tr>
<tr>
<td>190</td>
<td>10.6</td>
<td>0/8</td>
<td>0</td>
</tr>
<tr>
<td>38</td>
<td>2.1</td>
<td>0/8</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0/8</td>
<td>0</td>
</tr>
</tbody>
</table>

a Uncoagulated blood from a viremic patient (viral burden, 604,000 IU/ml) was diluted with fresh blood from an HCV-seronegative individual. Eight fivefold serial blood dilutions were spotted to form multiple replicate DBS that were extracted and assayed as described in the text.

b Estimated HCV RNA input in the DBS was determined by adjusting values for viremia in serum for the volume of blood spotted and for the relative hematocrit value.
The genotype, determined in all tested DBS, was in agreement with that obtained on frozen serum samples. Since HCV genotype may influence the response to antiviral treatment, severity of infection, and performance of diagnostic assays (7, 16), these aspects are particularly relevant in view of the possibility of using the method described here in large field studies and for application in settings where collection, centrifugation, storage, and shipment can be difficult, as is often the case in developing countries.

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REFERENCES