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Short communication

Commercial enzyme immunoassay adapted for the detection of antibodies to hepatitis C virus in dried blood spots

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Abstract

Background: Dried blood spots (DBS) provide a convenient method for blood sample collection in many settings where the prevalence of infection with hepatitis C virus (HCV) is increasing. Consequently, HCV assays are required that produce reliable results using samples derived from DBS.
Objectives and study design: The optimum buffer for the elution of samples from DBS was selected and the performance of a commercial enzyme immunoassay (EIA) was evaluated using these DBS eluates and paired plasma samples.
Results: DBS with paired plasma samples were compared using this modified commercial EIA, which was found to have an estimated sensitivity and specificity of approximately 100% for detecting anti-HCV antibodies in DBS.
Conclusion: A DBS-based assay for the detection of antibodies to HCV will prove valuable for collecting epidemiological data in the field or in under resourced settings.

Keywords: Hepatitis C virus; Immunoglobulin; Immunologic tests; Immunological diagnosis; Routine diagnostic tests

1. Introduction

It is estimated that 170 million people worldwide are infected with hepatitis C virus (HCV). HCV is the second most common aetiological agent of chronic liver disease and hepatocellular carcinoma. The highest HCV prevalence rates are found in west central and northern Africa, the eastern Mediterranean, and central Asia (WHO, 2003). The global incidence of HCV is predicted to be 3–4 million people per annum (Brown and Gaglio, 2003), with highest rates of infection in injection drug users (IDU). Accurate sero-surveillance of populations is necessary to identify areas where public education and health services need to be improved.

Collection of plasma or sera for anti-HCV screening can be problematic; in IDU cohorts where venous access may be poor, in pediatric studies or in under resourced settings. The use of dried blood spots (DBS) offers many advantages over conventionally acquired venous samples. DBS are stable at room temperature for several weeks (Behets et al., 1992; Solomon et al., 2002), and enable transport without refrigeration. Collection of DBS by a single-use retractable lancet is simple and inexpensive (Biggar et al., 1997; Parker and Cubitt, 1999). DBS samples have been used successfully in antibody screening studies for a number of pathogens (Ades et al., 2000; Hutchinson et al., 2004; McDade et al., 2000; Parker and Cubitt, 1992, 1999; Parker et al., 1999; Tappin et al., 1991).

We performed a comparison of different DBS elution buffers, using a commercial enzyme immunoassay (EIA) and a suitable buffer was selected. Matched plasma and DBS samples were compared in the assay in order to evaluate the performance of the assay using the two sample types. The sensitivity and specificity of the modified assay was calculated using DBS eluates as samples.

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2. Materials and methods

2.1. Origin of the samples

Anti-HCV reactive whole blood samples were collected from high risk groups including 14 individuals attending St. Vincent's Outpatients Liver Clinic, Melbourne in December 2001 and a cohort of 89 Melbourne IDU (Melbourne IDU cohort) recruited by the Macfarlane Burnet Institute for Medical Research and Public Health, Melbourne between February and September 2003. Negative samples were obtained from 94 individuals undergoing testing at St. Vincent's Haematology Laboratory in February 2004. Venous blood was collected into EDTA or heparin tubes, while serum was also collected from participants in the Melbourne IDU cohort. Ethics approval was granted for this study, under St. Vincent's Health HREC project no. 021/01 and the Alfred Hospital HREC project no.178/02.

All plasma samples were tested for anti-HCV antibodies using the Monolisa anti-HCV PLUS Version 2 EIA (Bio-Rad, France) and confirmed using the Murex anti-HCV (version 4.0) EIA (Murex Biotech, Kyalami, South Africa). Samples reactive in both assays were considered positive, and those non-reactive by Monolisa EIA were considered negative. From a total of 197 plasma samples, 80 were anti-HCV positive, and 117 were negative.

2.2. Preparation of dried blood spots

Approximately 80 μl of each whole blood sample was spotted onto Schleicher and Schuell cards (Grade 903) and plasma was immediately recovered from the remaining whole blood sample after centrifugation. DBS were air-dried at room temperature, then placed in envelopes, inside zip lock bags containing a silica desiccant, prior to storage at −20 °C. Serum and plasma samples were stored at −20 °C. The time of storage for both sample types before testing ranged from 1 week to 11 months.

2.3. Elution of dried blood spots

A 6 mm disc was punched from each DBS and placed in a 96-well plate in 200 μl of elution buffer. The plate was incubated on a shaking platform at room temperature for 30 min, and then incubated at 4 °C overnight before shaking at room temperature for 30 min prior to testing.

Positive and negative samples were chosen based on the results obtained from previous testing using PBS/T (150 mM sodium chloride, 50 mM phosphate, pH 7.4 containing 0.05% Tween-20 and 0.005% sodium azide) as the eluant. Samples were selected that had demonstrated either a DBS sample absorbance/cut-off (S/Co) ratio that was similar to the plasma S/Co ratio or where the matched DBS and plasma S/Co ratios had differed when tested previously. The effect of different elution buffers on the S/Co ratios was investigated using the Monolisa EIA. DBS from three positive and three negative samples were eluted in duplicate in 200 μl of either, PBS/T, 0.3% skim milk powder in 10 mM tris pH 7.4 containing 150 mM sodium chloride, Monolisa EIA specimen diluent or specimen diluent from the Genetic Systems rLAV EIA (Bio-Rad, France). Each DBS eluate was tested on the Monolisa EIA by a modified protocol described below. Duplicates of matched plasma samples were tested in parallel by the standard protocol.

2.4. Commercial anti-HCV EIAs

DBS eluates were analysed on the Monolisa EIA using a modified protocol in which 80 μl of eluate was added to 20 μl of specimen diluent (McCarron et al., 1999). In the standard assay protocol, 20 μl of serum or plasma is added to 80 μl of specimen diluent. In both the standard and the modified DBS protocol, the total volume in the test well is 100 μl. The four-fold increase in sample volume in the modified protocol partially compensates for the dilution of plasma that occurs as a result of elution of whole dried blood. The final dilution of plasma in the test wells was 1/50 when 80 μl of DBS eluate was used, which was more dilute by 10-fold than the corresponding plasma samples. All other steps were performed according to the manufacturer's instructions. A S/Co ratio was calculated to compare results from different runs of the same assay. Samples with S/Co ratios $\geq$1.00 were considered reactive, and <1.00 were considered non-reactive.

2.5. Statistical analysis

SPSS© (SPSS Inc., Chicago, Ill) was used to perform *t*-tests for independent samples to compare DBS and serum or plasma S/Co ratios. The delta values (Crofts et al., 1988) for plasma and DBS positive and negative sample populations were calculated using Microsoft Excel 2000 (Microsoft Corporation).

3. Results

3.1. Selection of DBS elution buffer

DBS samples eluted using the Genetic Systems elution buffer had the lowest levels of non-specific reactivity when compared with DBS eluted using other elution buffers (Fig. 1(A)). Levels of non-specific reactivity in DBS eluate were comparable to those of the matched plasma samples when the Genetic Systems elution buffer was used. All of the elution buffers analysed demonstrated an equivalent capacity to elute antibody from anti-HCV positive DBS (Fig. 1(B)). The decreased reactivity observed for eluted DBS from the HCV positive sample Pos 3 (Fig. 1(B)) resulted from the low antibody titre present in this sample (data not shown). Even though the sample volume for DBS eluates is increased in the modified Monolisa EIA used for this analysis, the elution of samples from DBS still results in 10-fold less sample added