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Maternal antibody to hepatitis B core antigen detected in dried neonatal blood spot samples

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SUMMARY

Despite Department of Health recommendations, universal antenatal testing for hepatitis B virus (HBV) is not performed throughout Scotland. We describe the evaluation of an assay to document past or present infection with HBV, by identifying maternal antibody in routine Guthrie dried neonatal blood spot samples taken when infants are 7 days old. A modified haemagglutination assay to detect antibody to hepatitis B core antigen (CORECELL, Green Cross) was validated and found to be 79% sensitive (44/56) and 100% (105/105) specific when used with dried blood spot samples made from panels of serum of known reactivity. Ninety-three percent (13/14) of HBV carriers were CORECELL positive. Sixty-six (0·5%) of 14044 routine Guthrie samples taken from babies born in Scotland from June–August 1992 were CORECELL positive indicating past or present maternal infection with HBV. A cross-sectional survey would document the maternity hospitals where universal antenatal hepatitis B screening should be urgently established.

INTRODUCTION

Eighty-five to 90 percent of infants born to mothers who are high infectivity carriers of hepatitis B (hepatitis B surface antigen and e antigen positive) will become infected with hepatitis B virus (HBV) and become chronic carriers, leading to chronic hepatitis and its sequelae [1]. Infection usually occurs during parturition, or through breastfeeding. By giving infants HBV immune globulin and vaccine, 85% of expected infections can be eliminated [1]. Therefore, knowing that a mother is an HBV carrier is very important. The Department of Health advises health authorities in the UK to screen all mothers for HBV carrier status [2]. However, only in 8 out of 15 health board areas in Scotland are all pregnant women routinely screened for HBV (personal communication, Dr D. Goldberg, Scottish Centre for Infection and Environmental Health, Ruchill Hospital, Glasgow).

The Guthrie heel-prick blood sample is taken onto filter paper cards from all infants at around 7 days postnatal age to detect inborn errors of metabolism. It has been used to help define the epidemiology of viral infection, e.g. HIV-1 [3]. Antibody to hepatitis B core antigen (anti-HBc) is present during the acute, convalescent, and chronic phases of HBV infection [4, 5]. It persists for many years after infection and may be the only marker of previous exposure [6]. Unlike hepatitis B surface antigen, anti-HBc crosses the placenta, and maternal antibody can be measured in the baby's blood [1]. Commercial vaccines contain hepatitis B surface antigen, and so anti-HBc is not induced [7]. Therefore testing Inborn Errors Screening Cards (Guthrie cards) and finding anti-HBc will indicate that the mother has had previous or has current infection with hepatitis B virus.

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We report here the validation of a haemagglutination assay for anti-HBc (CORECELL, Green Cross Laboratories), using panels of serum samples of known hepatitis B status. Field testing of 14044 routine Guthrie cards estimated the prevalence of HBV infection in women attending maternity units in Scotland during 1992.

SUBJECTS AND METHODS

The protocol was approved by the ethics committee of the Royal Hospital for Sick Children.

Patients

Panel A contained 49 dried serum spots sent by Dr I. L. Chrystie (Virology Department, St Thomas' Hospital, London SE1 7EH). Forty-one were defined by Dr Chrystie as hepatitis B surface antigen(HBsAg) positive and were assumed to be anti-HBc positive. Eight were anti-HBc positive and antibody to hepatitis B surface antigen (anti-HBs) negative. Panel B was composed of 46 anti-HBc negative sera and one positive serum sample.

Panel C contained sera sent by Ms M. Briggs (Division of Virology, Windeyer Building, 46, Cleveland St., London, W1P 6DB). These were described as 14 HBsAg-pos/anti-HBe-pos/anti-HBc-pos (low infectivity HBV carrier sera), 15 HBsAg-neg/anti-HBs-neg/anti-HBc-neg (HBV negative sera) and 15 HBsAg-neg/anti-HBs-pos/anti-HBc-pos (HBV immune sera). Two 'immune sera' when retested at the HIV Hepatitis Reference Laboratory, Ruchill Hospital, Glasgow were negative for anti-HBc. This illustrates the difficulty of finding a suitable assay for anti-HBc where serum tested in 2 regional virus laboratories gave different results [8]. These 2 sera are in the HBV negative sera in Figure 1. Panel D were 100 consecutive serum samples collected from female intravenous drug users in Glasgow. Fifty-six samples were anti-HBc positive and 44 negative.

Fourteen thousand and forty-four consecutive routine Guthrie card samples from babies born between June and August 1992 in Scotland were tested during this period.

Preparation of discs (serum and blood spot)

Panel A contained 49 dried serum spots produced by absorbing 25 μl serum onto Guthrie cards (universal cotton fibre filter paper, number 903 Schleicher and Schuell) and allowing them to dry in air for 12 h. Sera from panels B, C and D were made up into dried blood spots using the technique used for HIV quality control samples at the CDC, Atlanta [9]. Routine Guthrie card dried neonatal blood spot samples were taken according to the standard protocol [10].

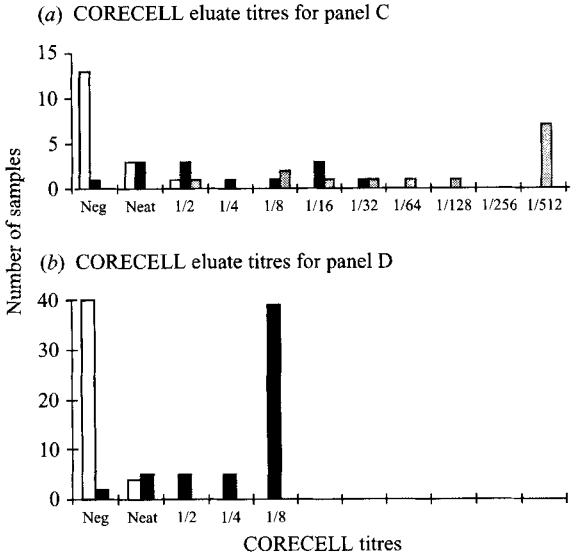

Fig. 1. (*a*) **Panel C.** Carrier sera tend to have higher CORECELL titres indicating higher levels of anti-HBc antibody than immune sera. Using a cut-off titre of 4, 13 of 14 carrier sera (93%) were positive by CORECELL on eluate samples. ⊠ = carrier, ■ = immune, □ = negative. (*b*) **Panel D.** Positive eluates were titred to a dilution of 1/8, therefore 1/8 indicates 1/8 or greater. The sensitivity of the CORECELL assay for immune sera from panel D using a cut-off titre of 4 as positive was 79% (44/56). There were no carriers in this group of patient samples.

Extraction of anti-HBc

Two, 3 mm diam disks were punched from Guthrie card serum or blood spots into flat bottomed 96-well microtitre master plates (Sterilin, Feltham, England), and eluted in 200 μl of elution buffer (phosphate-buffered saline, pH 7·2, containing 0·5% Tween 20 and 0·005% sodium azide). The plates were shaken (oscillatory shaker) at 200 cycles/min for 30 min at room temperature and left overnight at 4 °C, following which they were shaken (oscillatory shaker) for 3 min at 500 cycles/min.

Modified CORECELL screening assay

The CORECELL passive haemagglutination test (Green Cross Laboratories) is based on the ag-

glutination of sheep erythrocytes sensitised with hepatitis B core antigen (HBc) in the presence of anti-HBc. Reactive samples are titrated to a negative endpoint by serial twofold dilution. The anti-HBc titre is expressed as the maximum dilution of the original sample in which a positive agglutination pattern is macroscopically visible. The CORECELL assay has been used before as a confirmation assay for anti-HBc [11].

Samples of every eluate (20 μl) were added to 20 μl of TPHA (cell membranes from sheep and ox erythrocytes, normal rabbit testicular extract, cell components of *Treponema pallidum* [Reiter strain], gum arabic, normal rabbit serum and phosphate-buffered saline, Mast Diagnostics, Bootle, England) in 'V'-welled microtitre plates using a multichannel pipette. Twenty microlitres of a 1/4 dilution of sensitized erythrocytes (in CORECELL kit buffer) were added to each well, shaken for 5 min at 300 r.p.m. using an oscillatory shaker, and incubated at room temperature for a reaction time of 15 min. The plates were then centrifuged at 2600 r.p.m. for 2 min, and incubated at room temperature for a further 15 min before being placed on a 70° slope. Reactions were read after 10 min; sensitized red cells which were mixed with eluates containing anti-HBc agglutinated and remained as a discrete tight button. Eluates not containing antibody became an elongated 'teardrop' as the red cells fell down the slope.

Reactive eluates were titred by doubling dilutions to an endpoint. A positive sample was determined as any which remained reactive when titred to 2 wells or more, i.e. titre of 4 or greater. Controls were high positive, low positive and negative. The CORECELL kit supplied a positive control serum sample which was used at a 1/4 and 1/32 dilution.

RESULTS

Validation of the CORECELL screening test

The positive CORECELL kit control (not eluate) titred to a negative endpoint of 1/307200.

Panels A and B. Panel A: all 41 HBsAg positive and anti-HBc positive had a CORECELL titre of 32 or greater, all 8 anti-HBc positive and anti-HBs negative had a positive CORECELL titre of 8 or greater. Panel B: all 46 anti-HBc negative sera were CORECELL non-reactive, the positive sample had a CORECELL titre of 32. This initial evaluation of panels A and B indicated that the CORECELL screening test had potential when used with dried blood spot eluates.

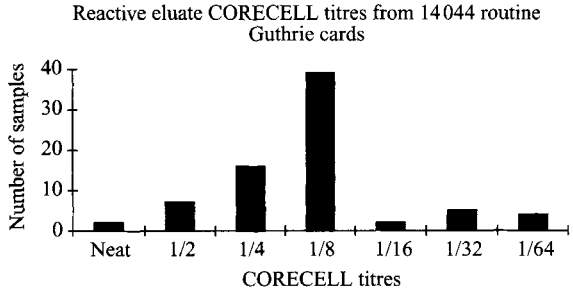

Fig. 2. CORECELL titres from 75 reactive eluates of 14044 consecutive routine Guthrie cards assayed during field testing from babies born June–August 1992.

Panel C. Figure 1*a* illustrates the results from panel C. This panel was used to establish a cut-off for a positive CORECELL screening test on Guthrie card eluate samples. The cut-off was taken as a sample which was still reactive at a dilution of 1/4. This was the cut-off chosen to help provide a high specificity, as no true negative eluate sample was found reactive at a dilution above 1/2 (Fig. 1). Testing eluate samples by CORECELL using this cut-off established a sensitivity of 93% (14/15) at detecting HBV carriers (Fig. 1*a*). It was noted that dried serum spots in panels A and B produced clearer positive results than dried blood spots made by adding red cells to test serum and then dropping the mixture onto Guthrie cards. One of the authors (SC), evaluating antibody tests for hepatitis C showed improved accuracy by using eluates from 'real' dried blood spot samples rather than spots produced after mixing known serum with red cells [9]. It may be that dried serum spots and 'real' dried blood spots provide cleaner results than made-up dried blood spots and should therefore be used in future methods to establish assay systems for use with routine Guthrie card eluates.

Panel D. Figure 1*b* illustrates CORECELL results from panel D. The sensitivity using eluate samples to identity sera from hepatitis B immune patients was 79% (44/56). The specificity of the CORECELL assay from panels A–D was 100% (105/105).

Field testing of the anti-HBc assay algorithm for dried blood eluates

Fourteen thousand and forty-four routine Guthrie card eluates were screened by CORECELL. Seventy-five eluates were reactive (Fig. 2), 66 positive to a titre of 4. Positive samples came from infants who lived in major urban centres in Scotland.

DISCUSSION

The aim of this study was to establish an assay system for anti-HBc in dried blood spot eluates. Unlike hepatitis B surface antigen, anti-HBc crosses the placenta and maternal antibody can be measured in the baby's blood [1]. Antibody to hepatitis B core antigen is not produced by vaccination, and its presence indicates maternal past or present infection with hepatitis B (HBV). Dried blood spot samples from all babies born in Scotland, arrive at one laboratory, the National Inborn Errors Screening Laboratory, at Stobhill hospital, Glasgow. It is therefore attractive to use this Guthrie card infant blood sample to help describe the epidemiology of maternal HBV infection.

We found the CORECELL assay when used on dried blood spot samples to be repeatable and simple to use. The sensitivity of 79% at detecting maternal HBV infection (past or present) would preclude its use to screen for HBV. However, it may be useful to document hospitals in Scotland where carriers of HBV may have delivered babies as the sensitivity at detecting hepatitis B carriers was 93% (13/14 samples in panel C).

The specificity of the CORECELL assay using eluate samples was 100% (105/105 anti-HBc negative samples were negative by CORECELL). The prevalence of past infection with HBV among blood donors in Scotland is 0·5% (personal communication Dr E. A. C. Follett). The prevalence of past infection or present infection with HBV from this field study was 0·5% (66/14044) using a CORECELL cut-off of 1/4. Therefore, the specificity of the CORECELL assay alone when used with dried blood spot eluates is adequate to describe the epidemiology of past infection with HBV among childbearing women. The cost of the CORECELL assay using dried blood spot eluates, with diluting procedures for erythrocytes and TPHA buffer, was £0·20 per test well. To this must be added technician time and overheads of running the laboratory.

The CORECELL anti-HBc assay is reliable and easy to use on dried blood spot eluates. The overall sensitivity in detecting past maternal infection with HBV was modest (79%), but the assay is likely to detect nearly all infants (93%) born to HBV carrier mothers. A cross-sectional study of Guthrie card eluates from all infants born in Scotland over a 12 month period, would help health boards to decide which hospitals require routine and which targeted maternal hepatitis B carrier screening, to prevent transmission of HBV to neonates.

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