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Point of care and oral fluid Hepatitis B testing in remote Indigenous communities of northern Australia

Running title: Novel Hepatitis B tests in remote locations

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Abstract

Keywords: Hepatitis B; Point-of-Care Testing; Serologic Tests; Diagnosis

Background

Many Indigenous Australians in northern Australia living with chronic Hepatitis B are unaware of their diagnosis due to low screening rates. A venous blood point of care test (POCT) or oral fluid laboratory test could improve testing uptake in this region.

Objective

The purpose of this study was to assess the field performance of venous blood POCT and laboratory performance of an oral fluid Hepatitis B surface antigen (HBsAg) test in Indigenous individuals living in remote northern Australian communities.

Patients and Methods

The study was conducted with four very remote communities in the tropical north of Australia's Northern Territory. Community research workers collected venous blood and oral fluid samples. We performed the venous blood POCT for HBsAg in the field. We assessed the venous blood and oral fluid specimens for the presence of HBsAg using standard laboratory assays. We calculated the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the POCT and oral fluid test, using serum laboratory detection of HBsAg as the gold standard.

Results

From 215 enrolled participants, 155 POCT and 197 oral fluid tests had corresponding serum HBsAg results. The POCT had a sensitivity of 91.7% and specificity of 100%. Based on a population prevalence of 6%, the PPV was 100% and NPV was 99.5%. The oral fluid test had a sensitivity of 56.8%, specificity of 98.1%, PPV of 97.3% and NPV of 65.9%.

Conclusions

The venous blood POCT has excellent test characteristics and could be used to identify individuals with chronic HBV infection in high prevalence communities with limited access to healthcare. Oral fluid performance was sub-optimal.

Introduction

There are an estimated 257 million people living with Hepatitis B worldwide, causing 884 000 deaths each year, yet it is probable that only 9% know their status.[1] Indigenous Australians in the Northern Territory (NT) experience a disproportionate burden of Hepatitis B infection with an estimated 6% seroprevalence compared to 1.6% in the non-Indigenous population.[2] Many are unaware of their diagnosis as there are sub-optimal screening rates.[3] Increased rates of testing are urgently required due to the association of Hepatitis B with cirrhosis and hepatocellular carcinoma[4,5], while liver disease is among the top three diseases contributing to the reduced life expectancy of Indigenous Australians compared to non-Indigenous Australians.[6,7]

Australia's third National Strategy for Hepatitis B aims to reduce the burden of Hepatitis B in Australia, with targets of 80% for proportion diagnosed and 20% for proportion receiving antiviral treatment.[8] This strategy names Indigenous Australians as a priority population. However, achieving high screening rates is difficult in remote parts of the Northern Territory due to the mobile population, cultural and communication barriers and high staff turnover.[3]

Hepatitis B surface antigen (HBsAg) becomes detectable in blood four weeks following acquisition of the virus.[9] This is usually diagnosed using venous blood sampling, which is then analysed using laboratory equipment with enzyme immunoassay capabilities. However, this requires advanced equipment and trained technicians, controlled storage temperatures and shipping to city laboratories.[9,10]

Point of care (POCT) tests obviate the need for laboratory infrastructure at the site of testing, require minimal training, provide rapid diagnosis, and an opportunity to engage the patient in management.[11] These tests are performed on serum or whole blood and are mostly immunochromatographic tests, also called lateral flow assays.[12,13] Point of care testing for HBsAg has been shown to have variable

sensitivity (60 to 100%) and specificity (93 to 100%) in diverse populations [11,12,14-20], but has rarely been studied in very remote areas.

An alternative diagnostic strategy for HBsAg detection is the use of oral fluid, [21] a combination of saliva and gingival crevicular fluid, which is a plasma transudate.[21,22] It avoids phlebotomy, mitigates risk of needle-stick injury, is less expensive, is easier to collect than blood, can be posted to testing centres, and can be self-collected.[23-25] Oral fluid collection has also already been shown to be acceptable to a group of children for HCV testing and in adults for HIV testing. Other minimally invasive tests such as dry blood spot finger prick have also been used to identify individuals with HBsAg.[26-28] Although a simple oral sampling method that needs centralised processing may still be associated with losses to follow-up, it may prove useful for epidemiological sero-surveys. Oral fluid has variable sensitivity (78%-100%) and specificity (87%-100%) for the detection of HBsAg and depends on collection devices, population, and cut off values used in immunoassays. [9,21,24,25,29] Oral fluid tests have not been assessed in the remote northern Australian context but could have utility given the isolation of communities, ease of collection and success in some epidemiological studies.[30,31]

Materials and Methods

The study was conducted with four communities in the tropical north of Australia's Northern Territory. The Northern Territory of Australia comprises 1 337 791 square kilometres and the four communities are classified as remote or very remote by the Australian Statistical Geography Standard.[32,33] The Northern Territory Department of Health and Menzies School of Health Research Human Research Ethics Committee approved the study (HREC2014-2261 and HREC2015-2520).

Individuals living in the four remote communities and surrounding outstations aged more than 1 year were eligible for recruitment. We identified potential participants in consultation with community research workers. We also identified additional participants through recruitment in a separate study, which had identified HBsAg positive mothers and their children via the Northern Territory Pathology

Hepatitis B immunoglobulin database (unpublished). We excluded individuals unable to give consent or assent or those who were less than 1 year of age.

We consulted the community on the proposed methodology and raised awareness and shared knowledge of Hepatitis B. Two community research workers used an educational app[34] to provide education to individuals in the community. We wanted to ensure informed consent was being obtained through this education as there is a lack of shared knowledge about health, and miscommunication is pervasive in Indigenous patients.[35] We then explained the project in a culturally and linguistically appropriate manner.

We assigned a unique study number to those who provided consent and assent and collected data on age, gender, and birthplace. We then collected oral fluid using a commercial oral specimen collection device (OraSure®) that was placed between the cheek and gum for 2 minutes, then secured in the collection tube, and stored at approximately 4°C.

We then examined the medical records of enrolled participants to determine if Hepatitis B serology had been performed in the five years prior to recruitment in order to reduce unnecessary venepuncture. If serology had been taken previously, we gave individuals an opportunity to discuss these results. We placed those who had positive serology on an appropriate care pathway and referred those who were non-immune to clinic for vaccination. We offered venepuncture if individuals did not have serology from the five years prior to enrolment. The blood was collected in serum tubes. The POCT for HBsAg (Standard Diagnostics, Inc. Bioline HBsAg WB, a WHO prequalified test [36]) was then performed as recommended in the product information in the field using 0.2mL of the blood collected, and the result was read as either positive or negative by trained research staff after 15 minutes. The test strip was also photographed at the completion of the test. Early during the study, we noted some difficulties in follow up and accessing results, so the protocol was adjusted to offer all enrolled participants venepuncture.

We transported the oral fluid and blood samples in a cooled esky to the Menzies School of Health Research, Darwin, Northern Territory. We sent the oral fluid samples to the Victorian Infectious Disease Reference Laboratory (VIDRL), Melbourne, Victoria within 7 days while we stored the blood samples at –70°C at Menzies School of Health Research and these were batched to be sent to VIDRL. During one holiday period, we stored the oral fluid samples at -20°C due to laboratory and transport closures.

At VIDRL, where the saliva was not of a sufficient volume of 400 µL for the assay, specimens were suspended in up to 400 µL of 0.9% sterile saline and then vortexed. We tested for HBsAg in the serum and oral fluid, and Hepatitis B surface (HBsAb) and core antibody (HBcAb) in the serum alone using the Cobas® electrochemiluminescence immunoassay. The cut-off value used was the same for both the serum and oral fluid and was the lower limit of detection (0.05 IU/mL). The titres for HBsAb and HBsAg were tested in the serum and HBsAg in the oral fluid.

We recorded all data on a paper case report form and transferred this to a secure database at Menzies School of Health Research. We analysed the data using Stata statistical software (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC). We expressed baseline continuous variables (age) as median with interquartile range and baseline categorical variables (gender, birthplace) as frequencies. We calculated the prevalence of participants who were immune, infected, and non-immune and provided the 95% confidence interval using the exact binomial method.

We used serum HBsAg performed at VIDRL as the gold standard to calculate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the POCT. We analysed the oral fluid test using all HBsAg results available (if not performed by immunoassay during the study then we used a result from the medical record within the past 5 years) and also analysed using only contemporary HBsAg results tested at VIDRL during the study. We took population prevalence as 6%

[2]. We calculated concordance between the venous blood POCT and gold standard and the oral fluid test and gold standard with the Kappa index.

Results

254 participants met inclusion criteria between October 2015 and December 2017. 36 patients did not consent or assent and 3 participants were duplicate enrolments. We excluded this duplicate data. Baseline characteristics of the remaining 215 patients are given in Table 1 and study flow diagram is given in Figure 1. There were 155 POCT and 197 oral fluid tests, which could be analysed. For the 155 POCT, all used the laboratory serum HBsAg immunoassay result performed during the study as gold standard. For the 197 oral fluid tests, 157 used the laboratory serum HBsAg immunoassay result performed during the study as gold standard and there was an additional 40 which only had a HBsAg result from the medical record.

Serum Hepatitis B serology and HBsAg titre results, either from the immunoassay performed during the study or historical results from the medical record, are shown in Table 2. Of the 187 participants who had HBsAg, HBcAb and HBsAb results available, 34 (18.2%, 95% CI 12.9 – 24.5) were HBsAg positive, indicating active infection, 8 (4.3%, 95% CI 1.9 – 8.3) had isolated HBcAb positivity, 23 (12.3%, 95% CI 8.0 – 17.9) were immune by exposure (HBsAb and HBcAb positive, HBsAg negative), 63 (33.7%, 95% CI 27.0-40.9), were immune by vaccination, and 59 (31.6%, 95% CI 25.0 – 38.7) were not immune.

The results of the oral fluid assay as compared to the HBsAg serum result are given in Table 3. There were three false positive oral fluid results, these were “HBsAg Reactive” on the oral fluid immunoassay and titre was under the limit of detection. The median serum HBsAg titre in the 24 participants who had serum analysed by immunoassay during the study and were HBsAg positive was 1305.5 IU/mL (IQR 331.3 – 6643.1). The median HBsAg titre in the oral fluid in the 21 participants who tested positive was 0.302 IU/mL (IQR 0.17-2.54).

The oral fluid test had a sensitivity of 56.8% (95% CI 39.5 – 72.9), and specificity of 98.1% (94.6 – 99.6) when all HBsAg results were used (n=197). Based on an estimated population prevalence of 6% [2], the PPV was 97.3% (95% CI 96.1 – 98.1) and NPV was 65.9% (95% CI 37.8 – 86.0). The Cohen kappa index was 0.6345 (95% CI 0.4850-0.7840). As the HBsAg result may have changed within the past five years, we also assessed test characteristics for the subset of patients with contemporaneous serum HBsAg results. When only contemporary HBsAg results were used (n=157) the sensitivity was 54.2% (95% CI 32.8 - 74.4), and specificity 98.5% (95% CI 94.7 – 99.8). Based on an estimated population prevalence of 6% [2,] the PPV was 97.1% (95% CI 95.6 – 98.1) and NPV was 69.7% (95% CI 35.6 – 90.5). The Cohen kappa index was 0.6222 (95% CI 0.4351 - 0.8094).

The results of the venous blood POCT as compared to the HBsAg serum result are given in Table 4. The two false negatives occurred with low corresponding serum HBsAg levels of 1.3 and 9.9 IU/mL (See Figure 2). The POCT had a sensitivity of 91.7% (95% CI 73.0 – 99.0) and specificity of 100% (95% CI 97.2 – 100). Based on a population prevalence of 6%, the PPV was 100% and NPV was 99.5% (95% CI 98.0 – 99.9%). The Cohen kappa index was 0.9490 (95% CI 0.8780 – 1.000).

Discussion

Our study assessed the test characteristics of two alternative HBsAg assays in remote field settings. In comparison to a gold standard of serum HBsAg, we found the venous blood POCT to have excellent test characteristics with sensitivity of 92% and specificity of 100%, but the oral fluid assay was insufficiently sensitive for further consideration. In communities with a high prevalence (6%) of chronic hepatitis B, the venous blood POCT would provide a NPV of 99.5% and PPV of 100%. Where healthcare access is limited and engagement with care for chronic hepatitis B is poor, such a venous blood POCT may have value in identifying chronically infected individuals and facilitating ongoing clinical care.

The prevalence of HBsAg positivity was extremely high in our study and was due to the selection of some individuals who were known to be HBsAg positive as part of a mother-child study (unpublished). The population prevalence of HBsAg positivity in Indigenous individuals in the Northern Territory is estimated to be 6%.[2] We therefore used this prevalence estimate for calculations of negative and positive predictive values.

The performance of the venous blood POCT was comparable to other studies, which have shown sensitivities between 60 and 100% and specificities between 93 and 100%.[11,12,14-20] Performing a venous blood POCT outside of the laboratory has been associated with reduced sensitivity compared to when the venous blood POCT is performed in the laboratory.[14,37,38] Tests have also performed better in the developed compared to developing world.[10-12,37,38] For instance, sensitivity was 100% when assays were performed in the United Kingdom and 56% when performed Malawi in two different groups of individuals co-infected with HIV and Hepatitis B.[37,38] A study in the Gambia also demonstrated improved sensitivity when assays were performed in the laboratory over the field.[14] Therefore, our finding of a sensitivity of 92% in the setting of remote Indigenous communities in a tropical region is reassuring and opens the way for broader future use.

While population prevalence should not alter sensitivity of a test, a study in Brazil demonstrated that the sensitivity of a rapid diagnostic HBsAg test was 93-96% in a group attending a hepatitis clinic, 60% in the general population including underserved and remote communities and 67% in a vulnerable population consisting of beauticians and those who used crack cocaine.[11] This could reflect differences in concentration of HBsAg of the three groups and has implications for our study in that our sample population may have included more patients with higher HBsAg concentrations than our overall target population.[11] Notably, the two false negatives in our study had very low corresponding HBsAg titres and this suggests a limit of detection (Figure 2).

There was suboptimal performance of oral fluid HBsAg testing in our study population and sensitivity was lower than previous studies (78%-100%) but there was similar specificity (87%-100%).[9,21,24,25,29]

We had three samples with low-level reactivity and these were considered false positives. Low level reactivity has been reported with oral fluid in other studies.[23,29] If these samples were subsequently shown to be negative on confirmatory testing of oral fluid using another assay, specificity of the test would have increased marginally but would not alter the overall results significantly. The suboptimal results may reflect the lower levels of HBsAg in oral fluid compared to serum[9], and the lack of standard cut off absorbance values.[9,23,25,29] In addition, many samples were of insufficient volume to perform the immunoassay and required the addition of saline which may have diluted the concentration of HBsAg.

The venous blood POCT has acceptable test characteristics to enable identification of chronically infected individuals in remote Indigenous communities of northern Australia and could possibly be used in other populations with limited access to health care taking into consideration a number of factors. For instance, while HIV-HBV co-infection is rare in Indigenous Australians, there is variable performance of POCT with HIV co-infection.[37-42] In addition, the influence of HTLV-1 co-infection, endemic in Central Australia[43], is not known. False negatives have also occurred with syphilis co-infection and while there is a current outbreak of syphilis among Indigenous Australians in northern and central Australia, test performance remained acceptable.[11,42,44] Low levels of HBsAg, alanine aminotransferase level, and viral load, and differing genotypes can also hinder test performance.[10,12,41] Genotype A is typically used as the reference virus in diagnostic tests, and while it did not appear to affect results in our region, where an exclusive C4 genotype exists, it may be important in other genotypes.[41,42]

Obtaining CD4 counts at the point of care in HIV have been shown to engage individuals in care earlier, and point of care Hepatitis C RNA tests are an essential part of improving the cascade of care and reaching elimination targets for Hepatitis C.[45,46] However, there is limited data on the effect of POCTs on Hepatitis B care cascade. Multiple patients in our region express frustration at the lack of follow up to receive and discuss results for chronic Hepatitis B, and results in a feeling of disempowerment.[47]

While Hepatitis B care requires more detailed examination of bloods beyond a POCT, there could be utility in using this test as a screen to quickly identify those who are positive. This may enable targeted follow up and more effective utilisation of limited resources. Such an algorithm could also be used with other population groups who experience barriers to health care. A dry blood spot testing using finger prick at community centres and religious establishments was used to effectively identify individuals with HBsAg from the British-Chinese and South Asian population in North East England.[28] Similarly a POCT at community events for culturally and linguistically diverse populations could have utility.

The limitations of this study include that some participants did not have contemporary HBsAg serum results in the oral fluid cohort. Loss or gain of HBsAg in this group in the intervening period between serum and oral fluid testing may have altered the sensitivity and specificity of the results significantly. However, the oral fluid test sensitivity remained poor when compared to the subset of patients with a contemporaneous serum sample. The study population had a higher rate of HBsAg positivity compared to current population estimates and therefore may not be a representative sample of these communities. We tried to account for this using population prevalence estimates in calculating positive and negative predictive values. The majority of the data also comes from a single community and generalizability on the likelihood of uptake in other communities in northern Australia, as well as non – Indigenous populations is difficult. We used venepuncture, which requires more training and equipment in the remote context, and its global applicability to patients who do not ordinarily present to health care may be limited. An ideal test in this setting would provide a rapid, accurate, point-of-care result with minimal burden to individuals and staff. Finger prick sampling would therefore be more practical, however, in this study, the low rates of screening for Hepatitis B in this population meant that many patients did not have historical results and venepuncture was required to obtain a gold standard serum HBsAg result. In addition, the POCT product information recommends that only venepuncture be used. Nevertheless, the diagnostic accuracy of capillary or venous whole blood for some HBsAg rapid diagnostic tests have been shown to comparable to serum and plasma[16] and

therefore assessing rapid diagnostic tests using finger prick sampling may be an important area for future work in our region.

In conclusion, the venous blood POCT and oral fluid tests have sensitivity of 91.7% and 56.8%, and specificity of 100% and 97.8% respectively. Although saliva sampling was simple and non-invasive it was not sufficiently sensitive. The venous blood POCT has excellent test characteristics and could be used to identify and facilitate care in chronically infected individuals in communities with high prevalence and limited access to healthcare, however, requires venepuncture and associated equipment to ensure safe sampling. Future work may be to assess methods that use finger prick testing in the very remote setting to reduce the burden on individuals and staff and increase applicability.

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Transparency declaration

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Tables and Figure Legends

Table 1: Baseline demographics of study participants

	All participants (n = 215)	Point of Care (n = 155)	Oral fluid test (n =197)
Age (median age, IQR)	27 (12-39) (n=207)	27 (13-39)	27 (13-39)
Gender (female)	143 (67%)	107 (69%)	131 (67%)
Community	Community A: 170 (79%) Community B: 14 (7%) Community C: 17 (8%) Community D: 14 (7%)	Community A: 124 (80%) Community B: 13 (8%) Community C: 14 (9%) Community D: 4 (3%)	Community A: 155 (79%) Community B: 13 (7%) Community C: 16 (8%) Community D: 13 (7%)

Table 2: Hepatitis B serology of participants based on serum tested during this study or pathology results within the past 5 years

	All participants (n = 215)	Point of Care (n = 155)	Oral fluid test (n =197)
HBsAg positive	37 (19%, n=198)	24 (16%, n=155)	37 (19%, n=197)
HBsAb positive (>10IU/ml)	87 (46%, n=188)	72 (47%, n=155)	87 (46%, n=188)
Anti-HBc positive	70 (36%, n=197)	51 (33%, n=155)	70 (36%) (n=194)

Table 3: Serum and Oral Fluid HBsAg

	HBsAg Oral Fluid (all results)			**HBsAg Oral Fluid (contemporary serum HBsAg only)**		
HBsAg Serum	*Positive*	*Negative*	**Total**	*Positive*	*Negative*	**Total**
Positive	21	16 (FN)	37	13	11 (FN)	24
Negative	3 (FP)	157	160	2 (FP)	131	133
Total	24	173	197	15	142	157
Sensitivity (95% CI)	56.8% (39.5-72.9)			54.2% (32.8-74.4)		
Specificity (95% CI)	98.1% (94.6-99.6)			98.5% (94.7-99.8)		
PPV (95% CI)*	65.9% (37.8-86)			69.7% (35.6-90.5)		
NPV (95% CI)*	97.3% (96.1-98.1)			97.1% (95.6-98.1)		
Cohen kappa index	0.6345 (0.4850-0.7840)			0.6222 (0.4351-0.8094)		

FN = False negative, FP = False positive
*Based on a population prevalence of 6%

Table 4: Serum and Point of Care HBsAg

	HBsAg Point of Care		
HBsAg Serum (all contemporary)	*Positive*	*Negative*	**Total**
Positive	22	2 (FN)	24
Negative	0 (FP)	131	131
Total	22	133	155
Sensitivity (95% CI)	91.7% (73.0-99.0)		
Specificity (95% CI)	100% (97.2-100)		
PPV (95% CI)	100%		
NPV (95% CI)	99.5% (98.0-99.9)		
Cohen kappa index	0.9490 (0.8780-1.000)		

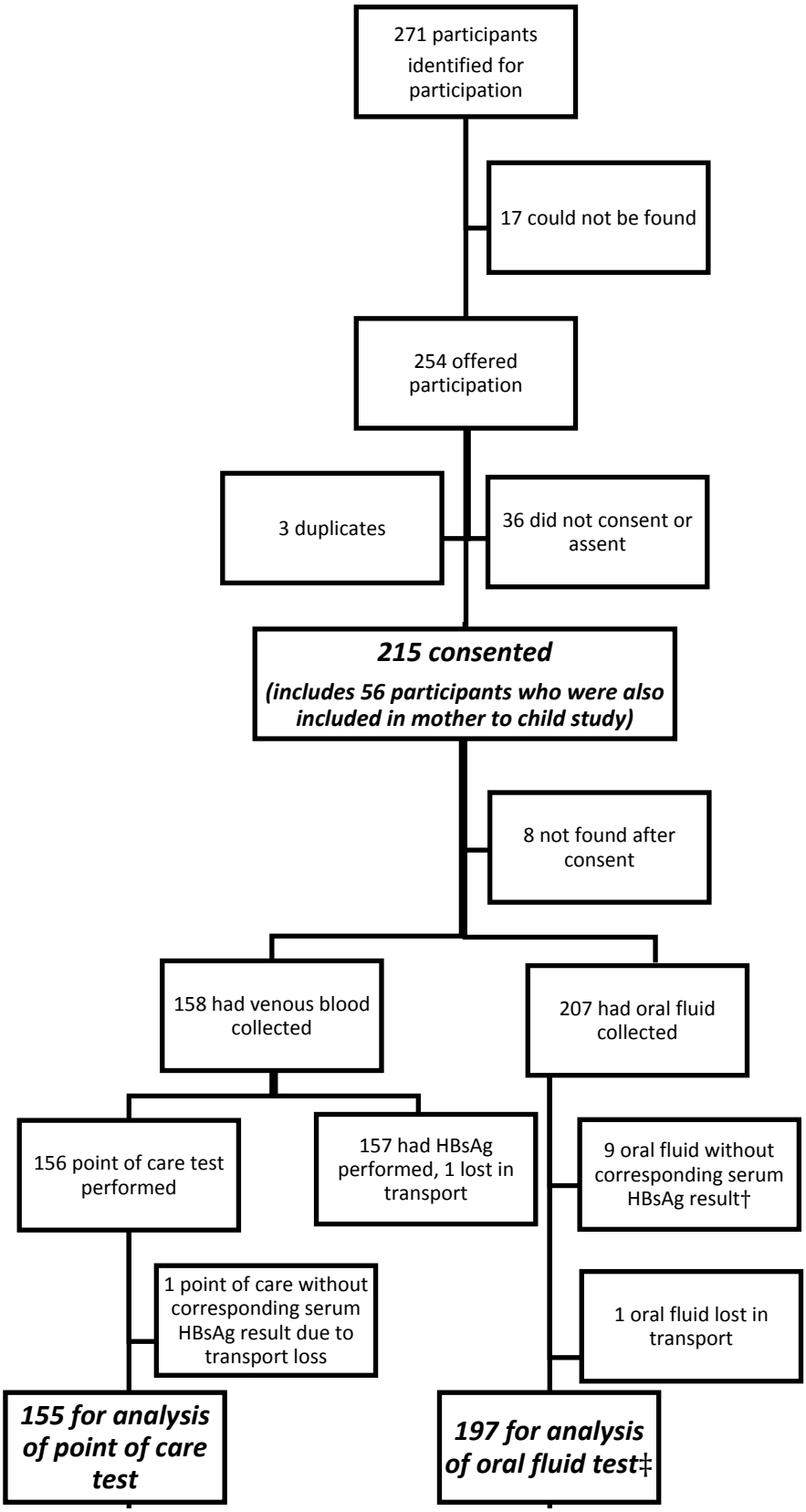
*FN = False negative, FP = False positive
*Based on a population prevalence of 6%

Figure 1: Study flow diagram

†1 incorrectly identified as not requiring HBsAg bloods, 6 phlebotomy unsuccessful, 2 lost to follow up

‡ 157 used serum HBsAg from VIDRL as gold standard, 40 used HBsAg taken from the medical record (3 of which were collected after oral fluid test but within study period)

Figure 2: Serum Hepatitis B Surface Antigen Titre from lab testing during the study and Point of Care Result



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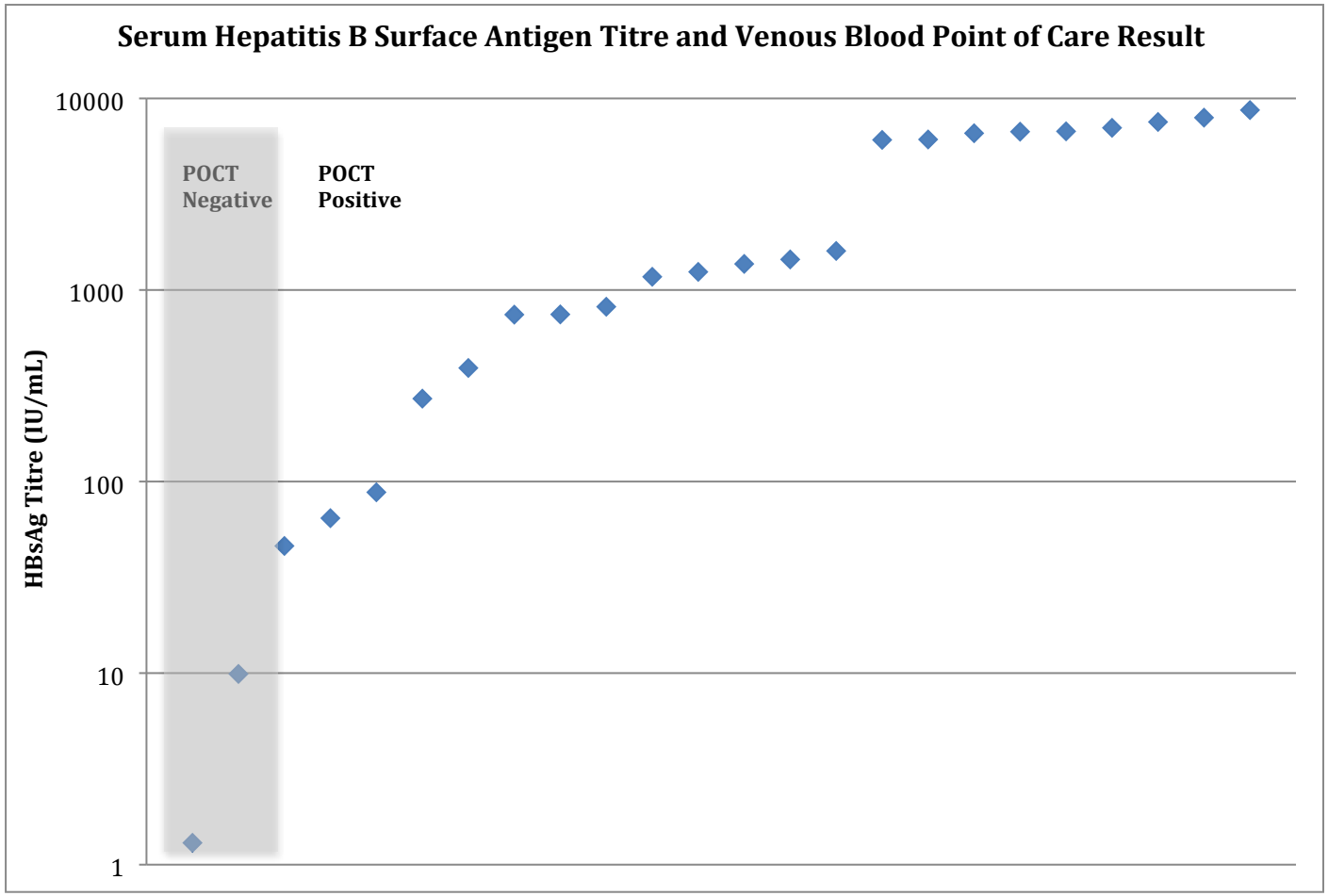

Figure 2: Serum Hepatitis B Surface Antigen Titre from lab testing during the study and venous blood Point of Care Test (POCT) Result. Participants are ordered along the x-axis in ascending HBsAg titre. The participants with a negative POCT are in the grey region and those with positive POCT in the white region of the plot.

Point of care and oral fluid Hepatitis B testing in remote Indigenous communities of northern Australia

Running title: Novel Hepatitis B tests in remote locations

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Abstract

Keywords: Hepatitis B; Point-of-Care Testing; Serologic Tests; Diagnosis

Background

Many Indigenous Australians in northern Australia living with chronic Hepatitis B are unaware of their diagnosis due to low screening rates. A venous blood point of care test (POCT) or oral fluid laboratory test could improve testing uptake in this region.

Objective

The purpose of this study was to assess the field performance of venous blood POCT and laboratory performance of an oral fluid Hepatitis B surface antigen (HBsAg) test in Indigenous individuals living in remote northern Australian communities.

Patients and Methods

The study was conducted with four very remote communities in the tropical north of Australia's Northern Territory. Community research workers collected venous blood and oral fluid samples. We performed the venous blood POCT for HBsAg in the field. We assessed the venous blood and oral fluid specimens for the presence of HBsAg using standard laboratory assays. We calculated the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the POCT and oral fluid test, using serum laboratory detection of HBsAg as the gold standard.

Results

From 215 enrolled participants, 155 POCT and 197 oral fluid tests had corresponding serum HBsAg results. The POCT had a sensitivity of 91.7% and specificity of 100%. Based on a population prevalence of 6%, the PPV was 100% and NPV was 99.5%. The oral fluid test had a sensitivity of 56.8%, specificity of 98.1%, PPV of 97.3% and NPV of 65.9%.

Conclusions

The venous blood POCT has excellent test characteristics and could be used to identify individuals with chronic HBV infection in high prevalence communities with limited access to healthcare. Oral fluid performance was sub-optimal.

Introduction

There are an estimated 257 million people living with Hepatitis B worldwide, causing 884 000 deaths each year, yet it is probable that only 9% know their status.[1] Indigenous Australians in the Northern Territory (NT) experience a disproportionate burden of Hepatitis B infection with an estimated 6% seroprevalence compared to 1.6% in the non-Indigenous population.[2] Many are unaware of their diagnosis as there are sub-optimal screening rates.[3] Increased rates of testing are urgently required due to the association of Hepatitis B with cirrhosis and hepatocellular carcinoma[4,5], while liver disease is among the top three diseases contributing to the reduced life expectancy of Indigenous Australians compared to non-Indigenous Australians.[6,7]

Australia's third National Strategy for Hepatitis B aims to reduce the burden of Hepatitis B in Australia, with targets of 80% for proportion diagnosed and 20% for proportion receiving antiviral treatment.[8] This strategy names Indigenous Australians as a priority population. However, achieving high screening rates is difficult in remote parts of the Northern Territory due to the mobile population, cultural and communication barriers and high staff turnover.[3]

Hepatitis B surface antigen (HBsAg) becomes detectable in blood four weeks following acquisition of the virus.[9] This is usually diagnosed using venous blood sampling, which is then analysed using laboratory equipment with enzyme immunoassay capabilities. However, this requires advanced equipment and trained technicians, controlled storage temperatures and shipping to city laboratories.[9,10]

Point of care (POCT) tests obviate the need for laboratory infrastructure at the site of testing, require minimal training, provide rapid diagnosis, and an opportunity to engage the patient in management.[11] These tests are performed on serum or whole blood and are mostly immunochromatographic tests, also called lateral flow assays.[12,13] Point of care testing for HBsAg has been shown to have variable

sensitivity (60 to 100%) and specificity (93 to 100%) in diverse populations [11,12,14-20], but has rarely been studied in very remote areas.

An alternative diagnostic strategy for HBsAg detection is the use of oral fluid, [21] a combination of saliva and gingival crevicular fluid, which is a plasma transudate.[21,22] It avoids phlebotomy, mitigates risk of needle-stick injury, is less expensive, is easier to collect than blood, can be posted to testing centres, and can be self-collected.[23-25] Oral fluid collection has also already been shown to be acceptable to a group of children for HCV testing and in adults for HIV testing. Other minimally invasive tests such as dry blood spot finger prick have also been used to identify individuals with HBsAg.[26-28] Although a simple oral sampling method that needs centralised processing may still be associated with losses to follow-up, it may prove useful for epidemiological sero-surveys. Oral fluid has variable sensitivity (78%-100%) and specificity (87%-100%) for the detection of HBsAg and depends on collection devices, population, and cut off values used in immunoassays. [9,21,24,25,29] Oral fluid tests have not been assessed in the remote northern Australian context but could have utility given the isolation of communities, ease of collection and success in some epidemiological studies.[30,31]

Materials and Methods

The study was conducted with four communities in the tropical north of Australia's Northern Territory. The Northern Territory of Australia comprises 1 337 791 square kilometres and the four communities are classified as remote or very remote by the Australian Statistical Geography Standard.[32,33] The Northern Territory Department of Health and Menzies School of Health Research Human Research Ethics Committee approved the study (HREC2014-2261 and HREC2015-2520).

Individuals living in the four remote communities and surrounding outstations aged more than 1 year were eligible for recruitment. We identified potential participants in consultation with community research workers. We also identified additional participants through recruitment in a separate study, which had identified HBsAg positive mothers and their children via the Northern Territory Pathology

Hepatitis B immunoglobulin database (unpublished). We excluded individuals unable to give consent or assent or those who were less than 1 year of age.

We consulted the community on the proposed methodology and raised awareness and shared knowledge of Hepatitis B. Two community research workers used an educational app[34] to provide education to individuals in the community. We wanted to ensure informed consent was being obtained through this education as there is a lack of shared knowledge about health, and miscommunication is pervasive in Indigenous patients.[35] We then explained the project in a culturally and linguistically appropriate manner.

We assigned a unique study number to those who provided consent and assent and collected data on age, gender, and birthplace. We then collected oral fluid using a commercial oral specimen collection device (OraSure®) that was placed between the cheek and gum for 2 minutes, then secured in the collection tube, and stored at approximately 4°C.

We then examined the medical records of enrolled participants to determine if Hepatitis B serology had been performed in the five years prior to recruitment in order to reduce unnecessary venepuncture. If serology had been taken previously, we gave individuals an opportunity to discuss these results. We placed those who had positive serology on an appropriate care pathway and referred those who were non-immune to clinic for vaccination. We offered venepuncture if individuals did not have serology from the five years prior to enrolment. The blood was collected in serum tubes. The POCT for HBsAg (Standard Diagnostics, Inc. Bioline HBsAg WB, a WHO prequalified test [36]) was then performed as recommended in the product information in the field using 0.2mL of the blood collected, and the result was read as either positive or negative by trained research staff after 15 minutes. The test strip was also photographed at the completion of the test. Early during the study, we noted some difficulties in follow up and accessing results, so the protocol was adjusted to offer all enrolled participants venepuncture.

We transported the oral fluid and blood samples in a cooled esky to the Menzies School of Health Research, Darwin, Northern Territory. We sent the oral fluid samples to the Victorian Infectious Disease Reference Laboratory (VIDRL), Melbourne, Victoria within 7 days while we stored the blood samples at –70°C at Menzies School of Health Research and these were batched to be sent to VIDRL. During one holiday period, we stored the oral fluid samples at -20°C due to laboratory and transport closures.

At VIDRL, where the saliva was not of a sufficient volume of 400 µL for the assay, specimens were suspended in up to 400 µL of 0.9% sterile saline and then vortexed. We tested for HBsAg in the serum and oral fluid, and Hepatitis B surface (HBsAb) and core antibody (HBcAb) in the serum alone using the Cobas® electrochemiluminescence immunoassay. The cut-off value used was the same for both the serum and oral fluid and was the lower limit of detection (0.05 IU/mL). The titres for HBsAb and HBsAg were tested in the serum and HBsAg in the oral fluid.

We recorded all data on a paper case report form and transferred this to a secure database at Menzies School of Health Research. We analysed the data using Stata statistical software (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC). We expressed baseline continuous variables (age) as median with interquartile range and baseline categorical variables (gender, birthplace) as frequencies. We calculated the prevalence of participants who were immune, infected, and non-immune and provided the 95% confidence interval using the exact binomial method.

We used serum HBsAg performed at VIDRL as the gold standard to calculate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the POCT. We analysed the oral fluid test using all HBsAg results available (if not performed by immunoassay during the study then we used a result from the medical record within the past 5 years) and also analysed using only contemporary HBsAg results tested at VIDRL during the study. We took population prevalence as 6%

[2]. We calculated concordance between the venous blood POCT and gold standard and the oral fluid test and gold standard with the Kappa index.

Results

254 participants met inclusion criteria between October 2015 and December 2017. 36 patients did not consent or assent and 3 participants were duplicate enrolments. We excluded this duplicate data. Baseline characteristics of the remaining 215 patients are given in Table 1 and study flow diagram is given in Figure 1. There were 155 POCT and 197 oral fluid tests, which could be analysed. For the 155 POCT, all used the laboratory serum HBsAg immunoassay result performed during the study as gold standard. For the 197 oral fluid tests, 157 used the laboratory serum HBsAg immunoassay result performed during the study as gold standard and there was an additional 40 which only had a HBsAg result from the medical record.

Serum Hepatitis B serology and HBsAg titre results, either from the immunoassay performed during the study or historical results from the medical record, are shown in Table 2. Of the 187 participants who had HBsAg, HBcAb and HBsAb results available, 34 (18.2%, 95% CI 12.9 – 24.5) were HBsAg positive, indicating active infection, 8 (4.3%, 95% CI 1.9 – 8.3) had isolated HBcAb positivity, 23 (12.3%, 95% CI 8.0 – 17.9) were immune by exposure (HBsAb and HBcAb positive, HBsAg negative), 63 (33.7%, 95% CI 27.0-40.9), were immune by vaccination, and 59 (31.6%, 95% CI 25.0 – 38.7) were not immune.

The results of the oral fluid assay as compared to the HBsAg serum result are given in Table 3. There were three false positive oral fluid results, these were “HBsAg Reactive” on the oral fluid immunoassay and titre was under the limit of detection. The median serum HBsAg titre in the 24 participants who had serum analysed by immunoassay during the study and were HBsAg positive was 1305.5 IU/mL (IQR 331.3 – 6643.1). The median HBsAg titre in the oral fluid in the 21 participants who tested positive was 0.302 IU/mL (IQR 0.17-2.54).

The oral fluid test had a sensitivity of 56.8% (95% CI 39.5 – 72.9), and specificity of 98.1% (94.6 – 99.6) when all HBsAg results were used (n=197). Based on an estimated population prevalence of 6% [2], the PPV was 97.3% (95% CI 96.1 – 98.1) and NPV was 65.9% (95% CI 37.8 – 86.0). The Cohen kappa index was 0.6345 (95% CI 0.4850-0.7840). As the HBsAg result may have changed within the past five years, we also assessed test characteristics for the subset of patients with contemporaneous serum HBsAg results. When only contemporary HBsAg results were used (n=157) the sensitivity was 54.2% (95% CI 32.8 - 74.4), and specificity 98.5% (95% CI 94.7 – 99.8). Based on an estimated population prevalence of 6% [2,] the PPV was 97.1% (95% CI 95.6 – 98.1) and NPV was 69.7% (95% CI 35.6 – 90.5). The Cohen kappa index was 0.6222 (95% CI 0.4351 - 0.8094).

The results of the venous blood POCT as compared to the HBsAg serum result are given in Table 4. The two false negatives occurred with low corresponding serum HBsAg levels of 1.3 and 9.9 IU/mL (See Figure 2). The POCT had a sensitivity of 91.7% (95% CI 73.0 – 99.0) and specificity of 100% (95% CI 97.2 – 100). Based on a population prevalence of 6%, the PPV was 100% and NPV was 99.5% (95% CI 98.0 – 99.9%). The Cohen kappa index was 0.9490 (95% CI 0.8780 – 1.000).

Discussion

Our study assessed the test characteristics of two alternative HBsAg assays in remote field settings. In comparison to a gold standard of serum HBsAg, we found the venous blood POCT to have excellent test characteristics with sensitivity of 92% and specificity of 100%, but the oral fluid assay was insufficiently sensitive for further consideration. In communities with a high prevalence (6%) of chronic hepatitis B, the venous blood POCT would provide a NPV of 99.5% and PPV of 100%. Where healthcare access is limited and engagement with care for chronic hepatitis B is poor, such a venous blood POCT may have value in identifying chronically infected individuals and facilitating ongoing clinical care.

The prevalence of HBsAg positivity was extremely high in our study and was due to the selection of some individuals who were known to be HBsAg positive as part of a mother-child study (unpublished). The population prevalence of HBsAg positivity in Indigenous individuals in the Northern Territory is estimated to be 6%.[2] We therefore used this prevalence estimate for calculations of negative and positive predictive values.

The performance of the venous blood POCT was comparable to other studies, which have shown sensitivities between 60 and 100% and specificities between 93 and 100%.[11,12,14-20] Performing a venous blood POCT outside of the laboratory has been associated with reduced sensitivity compared to when the venous blood POCT is performed in the laboratory.[14,37,38] Tests have also performed better in the developed compared to developing world.[10-12,37,38] For instance, sensitivity was 100% when assays were performed in the United Kingdom and 56% when performed Malawi in two different groups of individuals co-infected with HIV and Hepatitis B.[37,38] A study in the Gambia also demonstrated improved sensitivity when assays were performed in the laboratory over the field.[14] Therefore, our finding of a sensitivity of 92% in the setting of remote Indigenous communities in a tropical region is reassuring and opens the way for broader future use.

While population prevalence should not alter sensitivity of a test, a study in Brazil demonstrated that the sensitivity of a rapid diagnostic HBsAg test was 93-96% in a group attending a hepatitis clinic, 60% in the general population including underserved and remote communities and 67% in a vulnerable population consisting of beauticians and those who used crack cocaine.[11] This could reflect differences in concentration of HBsAg of the three groups and has implications for our study in that our sample population may have included more patients with higher HBsAg concentrations than our overall target population.[11] Notably, the two false negatives in our study had very low corresponding HBsAg titres and this suggests a limit of detection (Figure 2).

There was suboptimal performance of oral fluid HBsAg testing in our study population and sensitivity was lower than previous studies (78%-100%) but there was similar specificity (87%-100%).[9,21,24,25,29]

We had three samples with low-level reactivity and these were considered false positives. Low level reactivity has been reported with oral fluid in other studies.[23,29] If these samples were subsequently shown to be negative on confirmatory testing of oral fluid using another assay, specificity of the test would have increased marginally but would not alter the overall results significantly. The suboptimal results may reflect the lower levels of HBsAg in oral fluid compared to serum[9], and the lack of standard cut off absorbance values.[9,23,25,29] In addition, many samples were of insufficient volume to perform the immunoassay and required the addition of saline which may have diluted the concentration of HBsAg.

The venous blood POCT has acceptable test characteristics to enable identification of chronically infected individuals in remote Indigenous communities of northern Australia and could possibly be used in other populations with limited access to health care taking into consideration a number of factors. For instance, while HIV-HBV co-infection is rare in Indigenous Australians, there is variable performance of POCT with HIV co-infection.[37-42] In addition, the influence of HTLV-1 co-infection, endemic in Central Australia[43], is not known. False negatives have also occurred with syphilis co-infection and while there is a current outbreak of syphilis among Indigenous Australians in northern and central Australia, test performance remained acceptable.[11,42,44] Low levels of HBsAg, alanine aminotransferase level, and viral load, and differing genotypes can also hinder test performance.[10,12,41] Genotype A is typically used as the reference virus in diagnostic tests, and while it did not appear to affect results in our region, where an exclusive C4 genotype exists, it may be important in other genotypes.[41,42]

Obtaining CD4 counts at the point of care in HIV have been shown to engage individuals in care earlier, and point of care Hepatitis C RNA tests are an essential part of improving the cascade of care and reaching elimination targets for Hepatitis C.[45,46] However, there is limited data on the effect of POCTs on Hepatitis B care cascade. Multiple patients in our region express frustration at the lack of follow up to receive and discuss results for chronic Hepatitis B, and results in a feeling of disempowerment.[47]

While Hepatitis B care requires more detailed examination of bloods beyond a POCT, there could be utility in using this test as a screen to quickly identify those who are positive. This may enable targeted follow up and more effective utilisation of limited resources. Such an algorithm could also be used with other population groups who experience barriers to health care. A dry blood spot testing using finger prick at community centres and religious establishments was used to effectively identify individuals with HBsAg from the British-Chinese and South Asian population in North East England.[28] Similarly a POCT at community events for culturally and linguistically diverse populations could have utility.

The limitations of this study include that some participants did not have contemporary HBsAg serum results in the oral fluid cohort. Loss or gain of HBsAg in this group in the intervening period between serum and oral fluid testing may have altered the sensitivity and specificity of the results significantly. However, the oral fluid test sensitivity remained poor when compared to the subset of patients with a contemporaneous serum sample. The study population had a higher rate of HBsAg positivity compared to current population estimates and therefore may not be a representative sample of these communities. We tried to account for this using population prevalence estimates in calculating positive and negative predictive values. The majority of the data also comes from a single community and generalizability on the likelihood of uptake in other communities in northern Australia, as well as non – Indigenous populations is difficult. We used venepuncture, which requires more training and equipment in the remote context, and its global applicability to patients who do not ordinarily present to health care may be limited. An ideal test in this setting would provide a rapid, accurate, point-of-care result with minimal burden to individuals and staff. Finger prick sampling would therefore be more practical, however, in this study, ~~Finger prick would be more practical in this setting, but~~ the low rates of screening for Hepatitis B in this population meant that many patients did not have historical results and venepuncture was required to obtain a gold standard serum HBsAg result. In addition, the POCT product information recommends that only venepuncture be used ~~for this particular test~~. Nevertheless, the diagnostic accuracy of capillary or venous whole blood for some HBsAg rapid

diagnostic tests have been shown to comparable to serum and plasma[16] and therefore assessing rapid diagnostic tests using finger prick sampling may be an important area for future work in our region.

In conclusion, the venous blood POCT and oral fluid tests have sensitivity of 91.7% and 56.8%, and specificity of 100% and 97.8% respectively. Although saliva sampling was simple and non-invasive it was not sufficiently sensitive~~Oral fluid performance was sub-optimal and not sufficient to recommend its use~~. The venous blood POCT has excellent test characteristics and could be used to identify and facilitate care in chronically infected individuals in communities with high prevalence and limited access to healthcare, however, requires venepuncture and associated equipment to ensure safe sampling. Future work may be to assess methods that use finger prick testing in the very remote setting to reduce the burden on individuals and staff and increase applicability.~~.~~

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Transparency declaration

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Tables and Figure Legends

Table 1: Baseline demographics of study participants

	All participants (n = 215)	Point of Care (n = 155)	Oral fluid test (n =197)
Age (median age, IQR)	27 (12-39) (n=207)	27 (13-39)	27 (13-39)
Gender (female)	143 (67%)	107 (69%)	131 (67%)
Community	Community A: 170 (79%) Community B: 14 (7%) Community C: 17 (8%) Community D: 14 (7%)	Community A: 124 (80%) Community B: 13 (8%) Community C: 14 (9%) Community D: 4 (3%)	Community A: 155 (79%) Community B: 13 (7%) Community C: 16 (8%) Community D: 13 (7%)

Table 2: Hepatitis B serology of participants based on serum tested during this study or pathology results within the past 5 years

	All participants (n = 215)	Point of Care (n = 155)	Oral fluid test (n =197)
HBsAg positive	37 (19%, n=198)	24 (16%, n=155)	37 (19%, n=197)
HBsAb positive (>10IU/ml)	87 (46%, n=188)	72 (47%, n=155)	87 (46%, n=188)
Anti-HBc positive	70 (36%, n=197)	51 (33%, n=155)	70 (36%) (n=194)

Table 3: Serum and Oral Fluid HBsAg

	HBsAg Oral Fluid (all results)			**HBsAg Oral Fluid (contemporary serum HBsAg only)**		
HBsAg Serum	*Positive*	*Negative*	**Total**	*Positive*	*Negative*	**Total**
Positive	21	16 (FN)	37	13	11 (FN)	24
Negative	3 (FP)	157	160	2 (FP)	131	133
Total	24	173	197	15	142	157
Sensitivity (95% CI)	56.8% (39.5-72.9)			54.2% (32.8-74.4)		
Specificity (95% CI)	98.1% (94.6-99.6)			98.5% (94.7-99.8)		
PPV (95% CI)*	65.9% (37.8-86)			69.7% (35.6-90.5)		
NPV (95% CI)*	97.3% (96.1-98.1)			97.1% (95.6-98.1)		
Cohen kappa index	0.6345 (0.4850-0.7840)			0.6222 (0.4351-0.8094)		

FN = False negative, FP = False positive
*Based on a population prevalence of 6%

Table 4: Serum and Point of Care HBsAg

	HBsAg Point of Care		
HBsAg Serum (all contemporary)	*Positive*	*Negative*	**Total**
Positive	22	2 (FN)	24
Negative	0 (FP)	131	131
Total	22	133	155
Sensitivity (95% CI)	91.7% (73.0-99.0)		
Specificity (95% CI)	100% (97.2-100)		
PPV (95% CI)	100%		
NPV (95% CI)	99.5% (98.0-99.9)		
Cohen kappa index	0.9490 (0.8780-1.000)		

*FN = False negative, FP = False positive
*Based on a population prevalence of 6%

Figure 1: Study flow diagram

†1 incorrectly identified as not requiring HBsAg bloods, 6 phlebotomy unsuccessful, 2 lost to follow up

‡ 157 used serum HBsAg from VIDRL as gold standard, 40 used HBsAg taken from the medical record (3 of which were collected after oral fluid test but within study period)

Figure 2: Serum Hepatitis B Surface Antigen Titre from lab testing during the study and Point of Care Result