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研究報告の概要

○慢性B型肝炎患児の唾液中のB型肝炎ウイルス(HBV)DNA:唾液がHBV水平感染の伝播手段となっている可能性

目的:小児におけるHBVの水平感染の機序を検討するため、慢性B型肝炎患児46人の唾液中HBV量と血漿中HBV量を定量し、関連性を調べた。

対象および方法:デンマークにおいてB型肝炎は2000年から届出疾患となっている。2006年5月から2008年11月までに0～16歳までの慢性B型肝炎患児(HBs抗原陽性)180人に手紙を送り、両親から同意が得られた46人について、6ヶ月あるいは12ヶ月ごとに唾液と血液を得た。HBV-DNAはTaqMan Assayにて定量した(検出感度は50 IU/mL)。

結果:本研究中にHBe抗原が陽性から陰性になった2人と、HBe抗原の状態が分からない1人を調査対象外とした。25人(58%)がHBe抗原陽性で、18人(42%)がHBe抗原陰性であった。HBe抗原陽性の子供の唾液に含まれるHBV-DNA濃度は、HBe抗原陰性の子供の血漿中より39倍高かった。

考察:唾液がHBVの伝播手段になっている。子供において血漿中のHBV量と唾液への分泌量は相関する。ユニバーサルワクチン接種が、児童間のB型肝炎の唾液による感染への懸念を軽減できる可能性がある。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
小児におけるHBVの水平感染の機序を検討するため、慢性B型肝炎患児の唾液中と血漿中のHBV量の関連性を調べたところ、HBe抗原陽性患児の唾液中に高値HBV-DNAを認め、児童間での唾液によるHBV水平感染の可能性が示唆されたとの報告である。 これまで、本剤によるHBV感染の報告はない。また本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれている。さらに最終製品についてHBV-NAT陰性であることを確認しており、安全性は確保されていると考える。	これまでの使用実績やバリデーション成績に鑑み本製剤の安全性は確保されており、特別の対応を必要としないが、HBV感染に関する新たな知見等について今後も情報の収集に努める。なお、日本赤十字社では献血時のスクリーニング法としてより感度の高い化学発光酵素免疫測定法(CLEIA)および新NATシステムを導入した。

HEPATITIS B VIRUS DNA IN SALIVA FROM CHILDREN WITH CHRONIC HEPATITIS B INFECTION

IMPLICATIONS FOR SALIVA AS A POTENTIAL MODE OF HORIZONTAL TRANSMISSION

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Abstract: To explore the mechanism of horizontal transmission of hepatitis B virus (HBV) among children, we investigated the quantitative relationship between HBV in saliva and blood from 46 children with chronic hepatitis B.

We found high levels of HBV DNA in saliva of HBeAg (+) children, suggesting saliva as a vehicle for horizontal transmission of HBV among children.

Key Words: chronic hepatitis B, children, HBV DNA, saliva, horizontal transmission

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Hepatitis B virus (HBV) infection is a major global health problem and more than 350 million people worldwide are chronically infected. The course of hepatitis B infection is dependent on age at the time of infection. When infected perinatally, 90% of children become chronic carriers and 25% develop liver cirrhosis and are at risk for hepatocellular carcinoma. During childhood the HBV infected children are in a prolonged immune tolerance phase, and they constitute a silent infectious reservoir that may further maintain and spread HBV to susceptible individuals.

The most common routes of acquiring hepatitis B infection in adults are sexual contact and sharing injecting equipment. In childhood, perinatal and horizontal child-to-child transmission are the most common modes of transmission, but the mechanism of viral spread in horizontal transmission remains to be elucidated.[1–3]

Since 1992, WHO has recommended global vaccination against HBV, and by the end of 2006, 168 countries had implemented or were planning to implement a universal HBV immunization program for newborns, infants, and/or adolescents. Only 7 countries in Northern Europe have not yet implemented such a policy—Denmark, Finland, Iceland, the Netherlands, Norway, Sweden, and the United Kingdom.[4] These countries have adopted an at-risk strategy offering vaccination to individuals at high risk of infection.[5] The selective immunization strategy in Denmark includes immunization of staff and children at day-care centers before an HBsAg positive child is admitted. The Medical Officer of Health informs staff and parents before the vaccinations are given, and knowledge about the individual child with chronic hepatitis B infection is confidential. However, despite professional information, this strategy can cause social discrimination of the family and the child with chronic hepatitis B infection. The selective strategy in Denmark does not include hepatitis B vaccination before school entry, and parents are not obliged to inform the school that their child has chronic hepatitis B infection. For the parents of a child with chronic hepatitis B infection, this policy leads to fear of transmission of HBV from their child to unvaccinated children at school. The potential importance of saliva as a vehicle of spread is often a major concern, although transmission from saliva has not been documented except through percutaneous exposure (eg, a bite that breaks the skin).[6] Recent studies have shown that HBV DNA is present in saliva from infected adults and that there is a quantitative correlation between viral load in saliva and serum.[7,8]

The aim of this study was to explore the potential significance of saliva as a vehicle of transmission, and the quantitative relationship between HBV DNA in saliva and in plasma of children was determined.

MATERIALS AND METHODS

In Denmark, chronic hepatitis B infection has been a notifiable disease since the year 2000. All children nationwide, aged 0 to 16 years, notified with chronic hepatitis B ($n = 180$) were invited by letter to participate in the study during the period May 2006 to November 2008. The families of 46 children responded positively, and after written informed consent from the parents, 46 children were included in the study. Blood and saliva samples were obtained at the children's clinical visits every sixth or 12th month. The saliva samples were obtained using the saliva collection kit Oracol (Malvern Medical Developments, Worcester, United Kingdom). Blood was collected in EDTA tubes, spun, and separated into cells and plasma fractions. Purification of HBV DNA from plasma and saliva was performed using the MagNa Pure LC Instrument (Roche Applied Science, Penzberg, Germany). HBV DNA in plasma and saliva was quantitatively measured using the HBV TaqMan Assay as previously described.[9] The lower detection limit was 50 IU/mL. To monitor both loss and inhibition of the samples, a universal internal control consisting of a known number of Phocid herpesvirus type-1 particles was added to the samples, as previously described.[10] Corrections in viral load assessments were made if necessary. Data on the serological status (HBsAg, HBeAg, anti-HBeAg) were obtained from the children's clinical records. Statistical analyses were performed using mixed models with random intercepts with the statistical environment R-2.8.1 using the NLME package, taking into account repeated measurements on several of the patients. All HBV DNA values were log transformed by the natural logarithm prior to analysis, to ensure normality of standardized residuals.

RESULTS

A total of 46 HBsAg positive children were included in the study. Two children were excluded from the analyses as they converted from HBeAg (+) to HBeAg (−) during the study period, and one child was excluded due to unknown HBeAg status. Of those, 25 (58%) of the children were HBeAg (+) and 18 (42%) were HBeAg (−). Mean age at sample date was 10.2 years (SD ± 3.9 years). The number of samples collected ranged from 1 to 7 from each child. In total, we collected 117 plasma samples and 124 saliva samples from 43 children; 116 plasma and saliva samples were paired.

The geometric mean for HBV DNA in plasma from HBeAg (+) children was 41.9×10^6 IU/mL and 33.9×10^3 IU/mL in

TABLE 1. HBV DNA in Saliva and Plasma From Children With Chronic Hepatitis B Infection According to HBeAg Status

Subjects/Specimens	Log HBV DNA IU/mL	95% CI	P	Geometric Mean HBV DNA IU/mL	95% CI
HBeAg (+)					
Plasma	17.6	16.6–18.5		41.9×10^6	16.7×10^6 to 105.0×10^6
Saliva	10.4	9.5–11.4		33.9×10^3	13.0×10^3 to 86.4×10^3
HBeAg (−)					
Plasma	6.8	5.9–7.6		880	380–2038
Saliva	NA*			NA*	NA*
HBeAg (+) vs. HBeAg (−) in plasma	10.6	9.2–12.0	<0.001		
HBeAg (+) saliva vs. HBeAg (−) plasma	3.7	2.4–4.9	<0.001		

*All values below lower detection limit.

saliva, compared with 880 IU/mL in plasma from HBeAg (−) children. This showed a 39 times higher levels of HBV DNA in saliva from the HBeAg (+) children than in plasma from the HBeAg (−) children ($P < 0.001$). HBV DNA was undetectable in saliva from the HBeAg (−) children (lower detection limit 50 IU/mL). Results are shown in Table 1.

In 60% (50/84) of samples from HBeAg (+) children, HBV DNA levels in saliva were above 10^4 IU/mL, and in 33% (28/84) HBV DNA levels were above 10^5 IU/mL.

When analyzing the paired measurements of quantitative HBV DNA in plasma and saliva samples, we found a linear relationship between log HBV DNA in plasma and saliva of the HBeAg (+) children described by the equation:

$$\text{log HBV DNA in saliva} = -6.63 + 0.92 \text{ times (log HBV DNA in plasma)}$$

The relationship is presented graphically online in Figure, Supplemental Digital Content 1, http://links.lww.com/INF/A417.

DISCUSSION

Saliva has been considered a potential source of HBV transmission, and HBV DNA has been detected in saliva from adults.[7,8] We studied paired saliva and plasma samples from 43 children with chronic hepatitis B and known HBeAg status. We found a high level of HBV DNA in saliva from the HBeAg (+) children. Of note, the levels of HBV DNA were 39 times higher in saliva from the HBeAg (+) children than it was in plasma from the HBeAg (−) children.

Our findings show that saliva is a source of HBV DNA. Assuming that HBV DNA levels reflect the number of infectious particles, saliva is a potential vehicle of spread of HBV. However, studies of the infectivity of HBV DNA in saliva are limited due to lack of available animal models and cell lines that support HBV infection. It is known that HBV can survive for at least 7 days outside the body, and that infection through close interpersonal contact within households is a common mode of transmission of HBV during early childhood in high endemic countries.[2,3] It is presumed that in these settings transmission occurs from skin lesions or by sharing blood contaminated objects, although a specific pathway of transdermal exposure is rarely identified.

A significant concern for children with chronic hepatitis B infection and their parents, is the risk of infecting unvaccinated children. Older children might experience anxiousness when sharing drinks and food with friends. Because not all countries routinely vaccinate children against hepatitis B, it is a dilemma affecting families in those countries.

In samples from the HBeAg (−) children, HBV DNA was not detectable in saliva (lower detection limit 50 IU/mL) and the levels of HBV DNA were low in plasma in this group (880 IU/mL). This confirms our knowledge that HBeAg (−) children are much less infectious than HBeAg (+) children. It is shown in Figure, Supplemental Digital Content 1, http://links.lww.com/INF/A417, that HBV DNA becomes detectable in saliva at a level where log HBV DNA in plasma is around 11, corresponding to a viral load in plasma of about 60×10^3 IU/mL. It has been discussed at what levels HBV DNA of a chronic carrier should be considered to be infectious. Various guidelines are used in the European countries for when health care workers are allowed to work with exposure prone procedures, based on knowledge of HBV DNA levels at which HBV transmission has occurred. In the United Kingdom and Ireland, a cut-off limit of 10^3 HBV DNA copies/mL (=185 IU/mL) is used; in the Netherlands it is 10^5 copies/mL ($=18.5 \times 10^3$ IU/mL) and a European consensus group decided in 2003 for a cut-off level at 10^4 HBV DNA copies/mL ($=1.9 \times 10^3$ IU/mL).[11,12]

The mean viral load in saliva from HBeAg (+) children in our study was 33.9×10^3 IU/mL and 33% of these children had HBV DNA levels more than 10^5 IU/mL. Provided that the saliva is contagious, these children should be considered as highly infectious.

We found a clear association between HBV viral load in plasma and saliva. Similar results have been shown in adults.[8] As discussed, we do not know whether the HBV DNA in saliva is infectious, but it has previously been demonstrated that inoculation of chimpanzees and gibbons with saliva from hepatitis B infected individuals caused an acute infection.[13,14] Today contact tracing of the transmission of HBV using epidemiological and molecular data can identify possible sources of infection.[15]

Infection with HBV in childhood has serious consequences, as most children become chronic carriers and are at increased risk of developing liver cirrhosis and hepatocellular carcinoma. We have an ethical duty on both individual and country level to protect children from an oncogenic virus when we have the means to do so. Universal immunization can be implemented during infancy and adolescence; vaccination of adolescents provides immunization at a time of increased high-risk behavior. However, vaccination of infants is preferable because immunization of this age group is better established, and children infected at this age are at high risk of acquiring chronic infection. Universal vaccination might alleviate the fear of saliva as a potential vehicle of trans-

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mission among children, and it is the only logical strategy to protect against HBV infection.

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SEQUENCE TYPES AND ANTIMICROBIAL SUSCEPTIBILITY OF INVASIVE *STREPTOCOCCUS PNEUMONIAE* ISOLATES FROM A REGION WITH HIGH ANTIBIOTIC SELECTIVE PRESSURE AND SUBOPTIMAL VACCINE COVERAGE

Rajendra-Prasad Janapatla, PhD, Mei-Hua Hsu, MS, Jia-Fu Du, BS, Yu-Chia Hsieh, MD, Tzou-Yien Lin, MD, and Cheng-Hsun Chiu, MD, PhD

Abstract: Multilocus sequence typing was carried out on 95 invasive pneumococcal isolates belonging to the most common 7 serotypes currently circulating in Taiwan. The study confirmed continued prevalence in Taiwan of a few global clones and sequence types (STs) since the mid-1990s and identified the recent emergence of ST320 (19A) and ST902 (6A). Antimicrobial nonsusceptibility was common in the predominant STs of serotypes 14, 19A, 19F, and 23F.

Key Words: sequence type, serotype, *Streptococcus pneumoniae*, antimicrobial susceptibility, pneumococcal conjugate vaccine, Taiwan

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Dissemination of multiple antibiotic resistant clones of *Streptococcus pneumoniae* across regions and countries is well documented.[1–5] Global clones and their variants, which have spread in Taiwan, include Spain[6B]-2, England[14]-9, Taiwan[19F]-14, Colombia[23F]-26, Spain[23F]-1, and Taiwan[23F]-15.[5,6] Recently, Hsieh et al, reported the emergence of invasive serotype 19A isolates in Taiwan among the 2007 invasive pneumococcal isolates.[3] To prevent pneumococcal infections, 7-valent pneumococcal conjugate vaccine (PCV7) is being widely used.[1,7] PCV7 has significantly reduced invasive pneumococcal diseases (IPD) caused by vaccine serotypes, but serotype 19A has dramatically increased in some countries, but not in others.[1,8] In Taiwan PCV7 was not available until October 2005.[3] The vaccine is now being used in the private sector, with a low penetration in the pediatric population.[3] The aim of this study was to determine the sequence types (STs) of common serotypes that caused IPD in Taiwan after the introduction of PCV7. We also analyzed antimicrobial susceptibility patterns of these isolates.

METHODS

The 95 IPD isolates were selected for sequence typing and antimicrobial susceptibility testing because their serotype distribution covered the most common 7 serotypes currently circulating in Taiwan.[3] All these pneumococcal isolates were identified as described previously by Hsieh et al.[6] An IPD isolate was from a positive blood culture or pleural fluid culture from a child with a consolidation pattern upon chest x-ray. IPD also included primary bacteremia without focus defined as a positive blood culture from a child with fever but without a focal lesion. These isolates were collected from Chang Gung Children's Hospital (CGCH) during 2005–2007. The age range of children was from 1 to 9 years, with a median of 4.5 years. Prior to all experiments, the *S. pneumoniae* isolates were cultivated in trypticase soy agar with 5% sheep blood (in CO_2 incubator). Antimicrobial susceptibility to penicillin, ceftriaxone, erythromycin and imipenem was assayed by E-test (AB Biodisk, Solna, Sweden) and interpretation was based on Clinical and Laboratory Standards Institute standards.[9] The susceptible, intermediately resistant, and resistant MIC interpretative breakpoints for penicillin (nonmeningitis criteria) were ≤ 2 µg/mL, 4 µg/mL, and ≥ 8 µg/mL, respectively.[9] Serotyping was performed by latex agglutination and confirmed by Quellung reaction (Statens Serum Institute, Copenhagen, Denmark). All the serotypes were double checked by a PCR method described earlier.[10] The nucleotide sequences of 450-bp internal regions

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販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)			台湾	

研究報告の概要

○台湾における微量のB型肝炎ウイルス(HBV)DNAを検出するための個別検査とミニプール検査の有効性

背景:財政的な制約は、現在も台湾の、ルーチンの血液スクリーニングとしてのNAT実施において、主要な問題となっている。実現可能な解決策を講じるため、TIGRISシステム(Novartis Diagnostics)のPROCLEIX ULTRIO(Ultrio)分析を用いて、個別供血検査(IDT)と4本のミニプール法(MP4)双方の実施成績を評価した。

試験デザインおよび方法:分析感度はWHO国際標準品により決定した。供血者10,290名(IDT 4210名、MP4 6080名)に検査を行った。潜在的HBV陽性供血者(HBs抗原陰性/NAT陽性)を最高9ヵ月間、追跡調査した。Ultrio分析とHBs抗原検査結果が不一致の場合、さらにHBV抗体血清検査、代替NAT、HBV DNA定量検査ならびに塩基配列決定の解析を行った。

結果:検出の95%検出限界(IU/mL)(95%信頼区間)は以下のとおり:
ヒト免疫不全ウイルスType 1(HIV-1)18(12～34)、C型肝炎ウイルス(HCV)4.4(2.8～8.9)、HBV6.3(4.4～11)。再検査率は、IDT 0.55%とMP4 0.33%であった。HIVまたはHCV陽性症例は認められなかったが、潜在的HBV陽性例は12名(IDT 9名、MP4 3名)であった。そのうちの11名は、genotypeがB2であることが判明した。そのうちの10名は、追跡調査のために再来院し、ほとんどがオカルトHBV感染症(OBI)であると判明した。IDTの陽性率 9/4210(0.21%)はMP4の3/6080(0.05%)と比べ4倍高かった($p < 0.05$)。

結論:MP4と比較したIDTの高い陽性率は、OBIキャリアが顕著である台湾のような地域で、高感度NAT法を実施する有益性を示している。

使用上の注意記載状況・その他参考事項等

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血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
微量のB型肝炎ウイルス(HBV)DNAを検出するための個別NATとミニプールNATの有効性の評価を行い、オカルトHBVキャリアが多い台湾で、高感度NATの有益性が示されたとの報告である。 これまで、本剤によるHBV感染の報告はない。また本剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれている。さらに最終製品についてHBV-NAT陰性であることを確認しており、安全性は確保されていると考える。	これまでの使用実績やバリデーション成績に鑑み本製剤の安全性は確保されており、特別の対応を必要としないが、HBV感染に関する新たな知見等について今後も情報の収集に努める。なお、日本赤十字社では献血時のスクリーニング法としてより感度の高い化学発光酵素免疫測定法(CLEIA)および新NATシステムを導入した。

The efficacy of individual-donation and minipool testing to detect low-level hepatitis B virus DNA in Taiwan

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BACKGROUND: Financial constraints are the main concern in implementing nucleic acid testing (NAT) as routine blood screening in Taiwan. The PROCLEIX ULTRIO assay (Ultrio) on the TIGRIS System (Novartis Diagnostics) was evaluated for its operational performance both for individual-donation testing (IDT) and in minipools of 4 (MP4) to develop a feasible solution.

STUDY DESIGN AND METHODS: Analytical sensitivity was determined by testing WHO international standards. We tested 10,290 blood donors, 4210 in IDT and 6080 in MP4. Potential hepatitis B virus (HBV) yield donors (hepatitis B surface antigen [HBsAg] negative/NAT reactive) were evaluated for up to 9 months' follow-up. Discordant results between the Ultrio assay and the HBsAg tests were further analyzed by HBV antibody serology, alternative NATs, HBV DNA quantification, and sequencing.

RESULTS: The 95% limits of detection in IU/mL (95% confidence interval) were as follows: human immunodeficiency virus Type 1 (HIV-1), 18 (12-34); hepatitis C virus (HCV), 4.4 (2.8-8.9); and HBV, 6.3 (4.4-11). The retest rates were 0.55% for IDT and 0.33% for MP4. No HIV or HCV yield cases were found, while there were 12 potential HBV yield cases, nine from IDT and three from MP4 testing. Eleven of them were successfully genotyped as B2. Ten of them returned for follow-up and mostly were determined as occult HBV infection (OBI). The IDT yield rate of 9 in 4210 (0.21%) was fourfold greater than the MP4 yield rate of 3 in 6080 (0.05%; $p < 0.05$).

CONCLUSION: The higher yield rate for IDT versus MP4 demonstrates the benefit to implement a more sensitive NAT strategy in regions having significant OBI carriers such as Taiwan.

Introduction of nucleic acid amplification testing (NAT) has been shown to result in the improvement of blood safety in many countries around the world.[1] NAT markedly reduces the window period (WP) defined as the time between infection and first detectable viral marker, compared to serologic assays. NAT can detect not only WP infections for human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV), but also occult HBV infection (OBI), which are missed by even the most sensitive hepatitis B surface antigen (HBsAg) tests. NAT has been introduced in North America, many European countries, Australia, New Zealand, and parts of Asia including Japan, Hong Kong, and Singapore. However, at the time of this study, it has not been implemented in Taiwan. While NAT screening for HIV-1 and HCV is more widespread than for HBV, the recent advancement of automated or semiautomated systems with multiplex tests has facilitated the

ABBREVIATIONS: d = discriminatory (HBV, HCV, HIV-1 assay); IDT = individual donor testing; LOD(s) = limit(s) of detection; MP4 = minipool of 4; OBI(s) = occult hepatitis B virus infection(s); qPCR = quantitative polymerase chain reaction; S/CO = signal-to-cutoff; TTHBV = transfusion-transmitted HBV; WP = window period.

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simultaneous screening of all three viruses. The two assays currently commercially available are the Chiron PROCLEIX ULTRIO assay (Novartis Diagnostics, Emeryville, CA) and the Roche cobas MPX assay (Roche Molecular Systems, Pleasanton, CA).

Taiwan is an endemic area for HBV infection, with an HBsAg seroprevalence of 17.3% compared to 4.4% for HCV[2] and 0.012% for HIV.[3] Adoption of anti-hepatitis B core antigen (HBc) screening that correlates with HBV exposure, in many low-prevalence countries, resulted in the deferral of only a small number of donors. However, adding this safety measure in Taiwan, where anti-HBc seropositivity is reported to be 16% to 90% in the general population,[4,5] would defer far too many otherwise acceptable donors.

Taiwan has implemented widespread HBV vaccination since 1985 and adopted third-generation HBsAg blood screening tests to limit HBV infections. Nonetheless, one study[6] reported that at least 3% of the population carried occult HBV and hence transfusion-transmitted HBV (TTHBV) infections still occur underscoring the need for additional blood safety measures. Wang and coworkers[7] estimated that approximately 0.02% of donated blood in Taiwan could transmit HBV and predicted the HBV NAT yield to be 20-fold higher in Taiwan than in low-prevalent regions such as the United States. A more recent study[6] showed the rate of transfusion transmission of HBV in Taiwan to be 7- to 40-fold higher than that observed in low-prevalence countries with approximately 0.1% of the transfused recipients acquiring TTHBV. The same study showed that even some vaccinated children with low levels of anti-HBs developed HBV viremia posttransfusion, highlighting the continued threat of TTHBV despite the use of sensitive HBsAg blood screening and more than 20 years of HBV vaccination.[6]

While many recent evaluations of NAT systems in Asian populations have demonstrated their clinical utility, especially for HBV,[8-13] each country undertook evaluations of NAT, given the complexity and cost of NAT, in its own setting and determined which multiplex test is best suited to their circumstances. A recent pilot study[13] of minipool NAT screening of Taiwanese blood donors with an alternative technology showed yield rates 0.10 and 0.01% for HBV and HCV, respectively, that were higher than those observed in Hong Kong.[8]

The objective of this study was to evaluate both the performance of the Ultrio assay on the automated TIGRIS System under standard operational conditions and its ability to identify infectious units in seronegative Taiwanese blood donations (yield). A secondary objective was to determine which configuration of the Ultrio assay, individual donor testing (IDT) or minipool of 4 (MP4) testing, would provide the optimal combination of operational efficiency and blood safety in Taiwan.

MATERIALS AND METHODS

PROCLEIX ULTRIO assay

The Ultrio assay is an in vitro NAT utilizing transcription-mediated amplification for the qualitative detection of HIV-1 RNA, HCV RNA, and HBV DNA simultaneously in human plasma. The technology has been previously described.[14-16]

Analytical sensitivity

To verify the analytical sensitivity for detecting HIV-1, HCV, and HBV, diluted panels of World Health Organization (WHO) international standards (HIV-1 RNA International Standard 97/656, HCV RNA International Standard 96/798, and HBV DNA International Standard 97/746) were tested. The WHO international standards were obtained from the National Institute for Biological Standards and Control (NIBSC, Hertfordshire, UK) and panels were prepared at Acrometrix (Benicia, CA), by serially diluting the respective standard with nonreactive human plasma and storing aliquots at −80°C. Four sets of each WHO standard panel were prepared and tested, each set consisting of eight concentrations, with eight replicates of 1.5-mL aliquots for each concentration. The analytical ranges for each WHO standard were as follows: 0.23 to 30 IU/mL for HCV, 0.78 to 100 IU/mL for HIV-1, and 0.31 to 40 IU/mL for HBV. Aliquots were stored frozen at −20°C until testing. Eight replicates of each concentration were tested on each of three different days, to give a total of 24 replicates for each dilution of each virus. An additional eight replicates of all concentrations were tested with the PROCLEIX HIV-1, HCV, and HBV discriminatory assays (dHIV-1, dHCV, dHBV) on a fourth day. A Probit statistical model[17] was applied to the analytical sensitivity data and the 95% limit of detection (LOD) was calculated for the Ultrio assay and the discriminatory assays.

Operational performance

System reliability was assessed by computing the total sample invalid rate, the failed run rate for both IDT and MP4 testing, and the non-repeatable-reactive rate. A total of two reagent master lots were used in 64 test runs over 11 weeks by three operators.

Assay reproducibility

Signal-to-cutoff (S/CO) ratio results, including the means, standard deviations, and coefficients of variation (CV), from both assay controls and viral calibrators, were used to assess assay reproducibility. Data were taken from the routine testing runs only and did not include proficiency runs or runs of the WHO standards. Data were separately collected for the two master lots used in the study and the

three operators who performed the assays. Results from the operator who performed the fewest tests were combined with those of another operator for purposes of analysis.

Blood donor testing

A total of 10,290 different and consecutive blood donor specimens were collected at the Taipei Blood Center from August 13 to October 4, 2007. These blood donors had met the routine blood donation criteria established by Taiwan Health Authority and had consented to NAT screening of their blood. The study was conducted according to the regulatory guidelines in Taiwan and followed the Good Clinical Practice and Good Laboratory Practice Guidelines consistent with the principles originating in the Declaration of Helsinki. A separate BD VACUTAINER PPT plasma preparation tube (Becton Dickinson and Company, Franklin Lakes, NJ) was collected exclusively for NAT assay.

Routine serologic testing of donor specimens for HBsAg (Murex HBsAg v3.0, Abbott Diagnostics, Dartford, UK), anti-HCV (Murex anti-HCV v4.0, Abbott Diagnostics, Kyalami, South Africa), and anti-HIV-1 and -2 (Murex HIV 1.2.O, Abbott Diagnostics) was performed according to Taipei Blood Center's established standard operating procedures. Study specimens were linked to donors to permit follow-up evaluations.

Of the 10,290 specimens, 4210 were tested in IDT format and 6080 were tested in 1520 pools of MP4 format. MP4 testing was performed by pooling equal aliquots of plasma from four donation specimens. If a pool was reactive in the Ultrio assay, each specimen from the reactive pool was individually tested to identify the reactive specimen(s).

All Ultrio assay–reactive specimens, whether identified through IDT or MP4, were further tested with the discriminatory assays to determine specific viral activity. When the Ultrio assay was nonreactive and the donor specimen was seronegative, the testing was considered complete.

Supplemental serologic and alternative NAT

Donor specimens with discordant results between the Ultrio assay and the serologic tests of record were retested using specimens taken directly from the plasma unit. Supplemental serologic tests for HBV, HCV, and HIV were the HBsAg neutralization test (Quest Diagnostics, San Juan Capistrano, CA), anti-HCV recombinant immunoblot assay (Novartis Diagnostics, Emeryville, CA), and anti-HIV-1/2 Western blot (MP Diagnostics, Singapore), respectively. Additional supplemental serologic tests included anti-HBs (AxSYM, Abbott Diagnostics, Wiesbaden, Germany), anti-HBc Total and IgM (Quest Diagnostics), and anti-HCV (AxSYM, Abbott Diagnostics). Alternative NAT comprised two assays: the NGI HBV UltraQual assay (NGI, Los Angeles, CA), a polymerase chain reaction (PCR) assay with a 95% LOD of 0.9 IU/mL, and Cambridge University Laboratories quantitative (q)PCR assay (Cambridge, UK), with a 95% LOD of 20 IU/mL.[18]

For HIV, HCV, and HBV, the confirmed presence of viral genome without detectable viral antigen or specific antibody was identified as WP infection when follow-up samples confirmed seroconversion. For HBV, samples with the presence of DNA associated with anti-HBc and/or anti-HBs were defined as OBI.[19]

HBV nucleic acid sequencing and genotyping

Viral DNA was quantified from 500 µL of plasma.[20,21] In addition, after ultracentrifugation of 5 to 8 mL of plasma depending on the volume available, full-length HBV genome minus 50 bp in the precore region (approx. 3150 bp), pre-S/S region (approx. 1190 bp), and 300 bp in the basic core promoter/precore region were amplified using nested PCR. Amplified products were directly sequenced and those with sequences of greater than 1000 bp were phylogenetically analyzed.[20,21] Deduced amino acid sequences were compared to sequences of HBV strains of Genotypes B and C published in the GenBank database.

RESULTS

Analytical sensitivity

The 95% LOD for HIV-1, HCV, and HBV of the Ultrio assay and the corresponding discriminatory assays, as determined by Probit analysis, are shown in Table 1.

Assay reproducibility

For both reagent master lots used in the study the percent CVs for the reactive calibrators was less than 5%. There was 100% agreement between the expected and observed S/CO ratio results for the Ultrio assay controls. The three

TABLE 1. 95% LODs for Ultrio and discriminatory assays as determined by WHO panel tested by IDT

WHO panel	Assay tested	Estimated 95% LOD, IU/mL (95% CI)
HIV RNA 97/656	Ultrio*	18 (12-34)
	dHIV†	14 (8.1-48)
HCV RNA 96/798	Ultrio	4.4 (2.8-8.9)
	dHCV	8.5 (3.8-63)
HBV DNA 97/746	Ultrio	6.3 (4.4-11)
	dHBV	12 (5.6-69.1)

* Performed on 3 separate days with eight replicates per day, for a total of 24 replicates.
† Performed on 1 day with a total of eight replicates.

operators gave consistent and reproducible results (with no significant differences) for the reactive control specimens (data not shown).

Operational performance

A total of 4210 donations in IDT and 6080 donations in 1520 pools of MP4 were tested with the Ultrio assay on the TIGRIS platform. A summary of the testing data is shown in Tables 2 and 3. The non–repeat-reactive rates were 0.07% for IDT and 0.13% for MP4. There were 23 invalid results among 4210 specimens tested IDT (0.55%) and 5 invalid results among the 1520 pools tested (0.33%). All invalid results were valid when the tests were repeated. The retest specimen rate of 0.27% was mostly a result of assay processing errors.

Seronegative donor specimens tested in IDT and MP4

Testing results for 10,290 donor specimens by serology and by Ultrio assay in IDT (4210) and in MP4 (6080) are shown in Fig. 1A. None of the NAT-only-reactive samples were discriminated as either HIV or HCV. Among the 4179 seronegative specimens tested in IDT, 10 were Ultrio assay reactive. Six of these were discriminated as HBV, while four were nonreactive in discriminatory testing. These 10 specimens were further analyzed; 9 of 10 were found to be positive for HBV by alternative PCR, viral load, or genotyping and were regarded as potential yield cases. The results are summarized in Table 4. For donor IDT-A9, it was considered an indeterminate result. IDT-A9 was initially Ultrio assay reactive but no HBV, HCV, or HIV nucleic acid detectable (data not shown). Among the 6044 seronegative specimens in MP4, three were reactive in the Ultrio assay and were all discriminated as HBV and were also reactive in the NGI HBV UltraQual assay. These three specimens were further studied as potential yield cases as summarized in Table 4. In total, there were 12 potential yields cases, nine from IDT and three from MP4. They were between ages of 30 and 63, with equal male-to-female ratio.

Follow-up study of potential yield cases

Among the 12 potential yield cases, 10 donors joined in the follow-up study; results of the samples are listed in Table 4. All index samples and follow-up samples were anti-HBc positive, except the index sample of donor MP4-A3. Donors IDT-A1 and IDT-A3 became HBV DNA negative a few months after the index donations.

The combination of molecular and serologic marker data allows further definition of the diagnostic phase of HBV infection (Table 4). The presence of anti-HBc in all but one index sample excluded preseroconversion WP infection and in four cases anti-HBs were also detected indicating resolved infection. In IDT-A9 where molecular confirmation was doubtful, the presence of anti-HBc did not particularly help the diagnostic process because 16% to 90% in the Taiwanese general population[4,5] carry this marker. The potential yield cases were genotyped as B2, except donor IDT-A10. This sample could not be amplified in any of the four different regions targeted although the viral load tested by qPCR provided a positive result.

Finally, in 10 donors, at least one follow-up sample was obtained and this

TABLE 2. Summary of IDT and MP4 run results on Ultrio TIGRIS

Variable	IDT	MP4	Total
Number of individual donor samples	4,210	6,080	10,290
Total number of pools tested	4,210	1,520	5,730
Number of initially reactive pools	32	23	55
Initial reactive rate	0.76	1.51	0.96
Number of resolved pools	NA	21	21
Number of reactive donation(s) on discrimination assay	28	21	49
Non-repeat-reactive IDT/pools (%)	4 (0.09)	2 (0.13)	6 (0.1)
Total number of batches	21	24	45
Total invalid batch (%)	1* (4.76)	0 (0)	1 (2.22)
Total retested donor samples† (%)	23 (0.55)	5 (0.33)	28 (0.27)
Assay processing error	22	5	27
Internal control invalid	1	0	1

* Caused by negative control and HIV-1–positive control volume error.
† The retested donor samples resulted from invalid tests or invalid batch.
NA = not available.

TABLE 3. Summary of serology and Ultrio results observed

Result	IDT HIV-1	IDT HCV	IDT HBV	IDT Total	MP4 HIV-1	MP4 HCV	MP4 HBV	MP4 Total
Seropositive/Ultrio nonreactive	4	1	4	9	7	0	9	16
Seropositive/Ultrio reactive and discriminated	0	2	20	22	0	4	16	20
Seronegative/Ultrio reactive	0	0	6	6	0	0	3	3
Seronegative/Ultrio reactive and nondiscriminated				4*				0
Seronegative/Ultrio nonreactive				4169				6041

* Three confirmed reactive; one indeterminate result by alternative NAT (see Table 4).

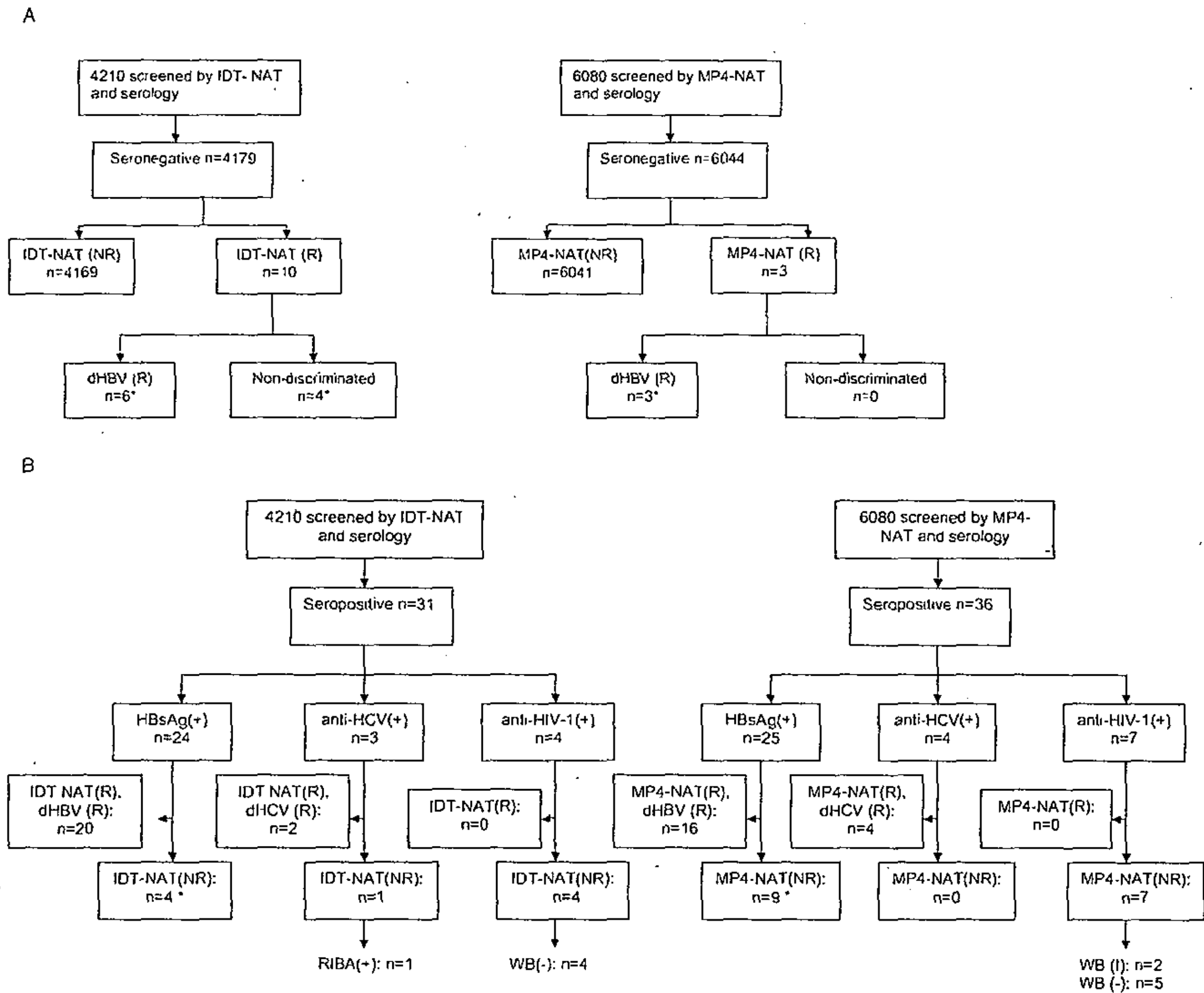

Fig. 1. (A) Results of seronegative donations screened by IDT and MP4 and serology. NR = nonreactive; R = reactive. *All these samples are discussed in Table 4. (B) Results of seropositive donations screened by IDT and MP4 and serology. NR = nonreactive; R = reactive. I = indeterminate; RIBA = recombinant immunoblot assay; WB = Western blot. *All these samples are discussed in Table 5.

refined the preliminary classification obtained on the basis of molecular and serologic results. In eight cases, the results obtained in the index samples were reproduced in the follow-up samples available confirming the diagnosis of OBI. In two cases, IDT-A3 and IDT-A6, a low level of anti-HBs was found in follow-up samples indicating cases of resolved infections with fluctuating levels of anti-HBs. In IDT-A5 HBsAg was detected in the follow-up sample together with the persistence of anti-HBc already present in the index sample. This profile suggested a chronic HBV infection with fluctuating, low-level, HBsAg. In MP4-A3, the follow-up sample became anti-HBc positive, while it was negative in the index donation. And the presence of HBV DNA in index donation of MP4-A3 suggested that this donor was a window case during that time.

Seroreactive donor specimens tested in IDT and MP4

Testing results for the 31 seroreactive donor specimens identified among the 4210 tested in IDT and for the 36 seroreactive specimens within the 6080 samples screened in MP4 are shown in Fig. 1B. Among the 31 specimens tested in IDT, 24 were HBsAg reactive, three were anti-HCV reactive, and three anti-HIV reactive. Twenty of the 24 HBV-seroreactive specimens were also dHBV reactive.

TABLE 4. Confirmation and possible status of HBV yield cases in Taiwan

		HBV DNA				HBV serological markers			Possible
Donor ID	Time (days)	dHBV	Alt PCR*	Viral load (IU/mL)†	Genotype‡	HBsAg (PRISM)	Anti-HBc§	Anti-HBs (mIU/L)¶	HBV status of donors
IDT-A1	Index	R[5]	P	7	B2	N	P	N	OBI
	81		P			N	P	N	
	199		P			N	P	N	
	261		N			N	P	N	
IDT-A2	Index	R	P	15	B2	N	P	488	OBI
	85		P			N	P	367	
	276		P			N	P	434	
IDT-A3	Index	R	P	N	B2	N	P	N	OBI
	82		P			N	P	13	
	144		N			N	P	8	
	215		N			N	P	6	
IDT-A4	Index	R	P	48	B2	N	P	N	OBI
	77		P			N	P	N	
IDT-A5	Index	R	P	<5	B2	P**	P	N	CHBV††
	215		P			P	P	N	
IDT-A6	Index	R	P	<5	B2	N	P	N	OBI
	160		P			N	P	11	
IDT-A7	Index	NR	P	N	B2	N	P	N	OBI
	189		P			N	P	N	
IDT-A8	Index	NR	N	N	B2	N	P	86	OBI
	185		N			N	P	65	
	256		N			N	P	66	
IDT-A9	Index‡‡	NR	N	N	NA	N	P	P	Ind§§
IDT-A10	Index‡‡	NR	N	6.4	NA	N	P	P	OBI
MP4-A1	Index	R	P	9	B2	N	P	N	OBI
	175		P			N	P	N	
MP4-A2	Index‡‡	R	P	N	B2	N	P	N	OBI
MP4-A3	Index	R	P	N	B2	N	N	N	WP
	253		P			N	P	N	New infection

* Alternative PCR by NGI HBV UltraQual.
† Results of Cambridge qPCR with numbers indicating viral load in IU/mL. <5 indicates a signal too low to allow reliable quantification.
‡ HBV genotyped by sequencing.
§ The results correspond to IgG anti-HBc. All the anti-HBc IgM determinations were N.
¶ Anti-HBs is given either qualitative (P or N) or quantitative in mIU/L.
** HBsAg N by Abbott Murex (S/CO = 0.9) and Ortho Assays; P by PRISM in subsequent analysis.
†† Chronic HBV infection with low and fluctuating HBsAg level.
‡‡ Donor was lost to follow-up.
§§ Indeterminate result, possibly contamination or OBI.
N = negative; NA = not available; NR = not reactive; P = positive; R = reactive.

while four were nondiscriminated and were further investigated (Table 5).

Thirty-six seroreactive specimens (25 HBsAg, four anti-HCV, and seven anti-HIV) were involved in NAT MP4 testing. Sixteen of the 25 HBsAg-reactive specimens were dHBV reactive and were considered true positive while nine were not and were further investigated as shown in Table 5.

Of the seven anti-HCV–reactive specimens (three IDT and four MP4), six were HCV RNA reactive. One of the three IDT-reactive specimens was found to be dHCV nonreactive. Of the 11 anti-HIV–reactive specimens (four IDT and seven MP4), none were HIV RNA reactive and none were confirmed antibody positive by Western blot (see Fig. 1B).

DISCUSSION

In a region where up to 90% of the population has evidence of past exposure or ongoing infection for HBV,[5] undetected OBIs pose a great threat to blood safety. While NAT only yield cases may occur under a number of circumstances—1) acute infection in the WP, 2) tail end of a chronic HBV infection, 3) persistence of low-level HBV replication in the presence of anti-HBs, and 4) escape mutant not detected by current HBsAg assays[22,23]—for this discussion we restrict the term of OBI to refer to HBV infection with the presence of anti-HBc and/or anti-HBs with no other detectable HBV markers except for HBV DNA.[24] While the transfusion transmission risk is lower for OBIs than for WP infections,[25] OBIs numerically pose a more significant threat to the blood supply, especially in HBV-endemic countries.[1,26]

In Asia, Taiwan in particular, many reports indicated that HBV DNA could be present, generally at a low level, in HBsAg-negative but anti-HBc–positive blood donations.[1,7,27] The proportion of this type of blood donation (1%-7%) was considerably higher than in low-prevalence Western countries (0%-3.5%).[26,28-31] Identifying and

TABLE 5. Profile of the 13 Ultrio-nonreactive initially HBsAg-positive (Abbott Murex) specimens

	HBV serologic markers					HBV DNA		
	HBsAg			HBV antibodies				
Donor ID	Murex (S/CO[3])	PRISM	Neutralization	Anti-HBc	Anti-HBs*	Ultrio IDT (Reactive/Total)	Ultrio dHBV	Alt PCR†
IDT-B1	28.56	P	P	P	N	NR (0/3)	NA	R
IDT-B2	10.89	P	P	P	N	NR (0/3)	NA	NR
IDT-B3	6.68	P	P	P	N	NR (0/3)	NA	NR
IDT-B4	3.56	P	P	P	N	R (1/3)	NR (0/3)	R
MP4-B1	50.66	P	P	P	N	R (3/3)	HBV	R
MP4-B2	3.47	P	P	P	N	R (1/3)	HBV	R
MP4-B3	1.59	P	P	P	N	R (2/3)	NR (0/3)	NR
MP4-B4	1.45	P	P	P	N	NR (0/3)	NA	R
MP4-B5	10.72	P	P	P	N	NR (0/3)	NA	NR
MP4-B6	1.32	N	Not confirmed	P	N	NR (0/3)	NA	NR
MP4-B7	1.12	N	Not confirmed	N	>1000	NR (0/3)	NA	NR
MP4-B8	2.22	N	Not confirmed	N	>1000	NR (0/3)	NA	NR
MP4-B9	1.00	N	Not confirmed	N	N	NR (0/3)	NA	NR

* Anti-HBs is given either qualitative (P or N) or quantitative in mIU/L.
† Alternative NAT: NGI HBV UltraQual assay used LOD = 0.9 IU/mL.
NA = not available; NR = nonreactive; R = reactive.

excluding such donations from the blood supply in Taiwan is important since it was demonstrated that this type of blood can be infectious by transfusion.[6,7,25] The risk of HBV transmission with the anti-HBc–"alone" blood has been reported to cover a wide range (0.4%-90%). In contrast, in a Japanese study, no donations containing both HBV DNA and anti-HBs were found infectious through transfusion.[25] However, a recent report from Slovenia presented two cases of HBV transmission by transfusion of an OBI unit containing low levels of anti-HBs.[32] Furthermore, vaccinated children with low levels of anti-HBs but relatively immunocompromised appeared to be susceptible to HBV infection after transfusion with HBsAg-negative blood products.[6] Therefore, on the basis of these studies, it appears important for blood safety in Taiwan that routine HBV NAT be implemented in addition to the current HBsAg screening.

Assay performance characteristics are critical to the interdiction of potentially infectious donations. A UK model, adjusted for test and processing errors, revealed that 22% of the risk of transfusion-transmitted infections (including HBV, HCV, and HIV) was the result of test failures and operational errors,[33] underscoring the need for a robust, reliable screening assay. The Ultrio assay in our hands had both a low invalid test rate of 0.27% and a low overall non–repeatable-reactive rate of 0.07% for IDT and 0.13% for MP4. These characteristics, along with its high assay sensitivity and specificity, provide a suitable system for routine screening of the blood supply in Taiwan.

The most critical assay attribute for detection of low-level viremia is analytical sensitivity. Our evaluation showed the Ultrio assay to be highly sensitive with 95% LODs of 18.41, 4.38, and 6.28 IU/mL for HIV-1, HCV, and HBV, respectively, and 13.97, 8.54, and 12.04 IU/mL for the respective discriminatory assays (Table 1). These results are consistent with the claims stated in the package insert (PROCLEIX ULTRIO assay, Package Insert IN0167EN rev. 2, 2004, Gen-Probe Inc., San Diego, CA) and with the findings of other investigators.[6,11,31,35]

While it was demonstrated that testing in plasma pools of small sizes was essentially as efficient as IDT for HIV-1 and HCV, pooling had a substantial impact on the efficacy of detecting low-level HBV DNA. Results presented in Tables 3 and 4 show that proportionally more HBV DNA–positive samples were identified among HBV-containing donations in IDT (87.9%) than in MP4 (67.9%). Our study provides an opportunity to determine the distribution of concordant and discordant blood donor samples between the two main HBV tests: HBsAg and HBV DNA. Among the HBV-containing donations, IDT identified 60.6% positive for both HBsAg and HBV DNA, 12.1% HBsAg only, and 27.3% HBV DNA only, whereas MP4 identified 57.2% positive for both HBsAg and HBV DNA, 32.1% HBsAg only, and 10.7% HBV DNA only. This distribution is similar to the data in our previous study[13] (58.6, 26.8, and 14.6%, respectively). Although the two testing populations in this study show different HBsAg-reactive rates (0.57% for IDT and 0.41% for MP4), they are not much different compared to the 0.48% reactive rate of Taiwanese donor population in 2007 (from Taiwan Blood Services Foundation annual report 2007). The distribution observed in an area like Taiwan, where HBV Genotypes B and C are prevalent, considerably differs from data generated in Ghana, West Africa, where Genotype E is prevalent and, tested with the Cambridge qPCR used in this study, 84% of samples were HBsAg and HBV DNA positive, 6% HBsAg only, and 10% DNA only.[20]

Additionally, the data presented in Table 5 suggest that some HBsAg-positive samples may carry an extremely low level of HBV DNA, below the LOD of most assays currently available for blood testing. This lack of sensitivity would be further compounded by any level of

pooling. Several options can be offered to address this issue in addition to IDT-NAT, such as extraction from larger plasma volume or concentration of viral particles by high-speed centrifugation.[36] Nevertheless, data of our study demonstrate that, at least for the time being, HBsAg and HBV DNA screening are complementary and that both are beneficial for the blood safety.

One important issue for NAT is the confirmation and characterization of yield cases to appropriately inform the implicated donors. As shown in Table 4, there are three successive levels of supplementary testing that can help to achieve this goal: 1) alternative NAT assays for HBV DNA, 2) detection of other HBV serologic markers to refine the HBV infection profile, and 3) testing follow-up samples to reach a suitable diagnosis. To verify potential (HBsAg-negative, NAT repeat-reactive) and probable (HBsAg-negative, NAT-reactive, and alternative NAT-reactive on an alternate specimen) yield cases, we subjected index samples to molecular analysis and genotyping and we tested follow-up specimens from these donors with six different serologic tests and three alternative NAT assays. Parts of the HBV genome (pre-core, pre-S, and S) along with the full genome were amplified in most index cases. All yield cases were Genotype B2, which is the predominant genotype in Taiwan.[37] The qualitative NAT (NGI HBV UltraQual) with a 95% LOD of 0.9 IU/mL detected HBV DNA in follow-up specimens from 9 of 11 potential yield donors, whereas a quantitative NAT with a LOD of 100 IU/mL (Quest Diagnostics) was not able to quantify DNA in any of the follow-up specimens, although it detected an HBV signal in six donors (data not shown). A third highly sensitive quantitative NAT with a LOD of 20 IU/mL (in-house PCR, Cambridge University Laboratories), only being used for testing the index donations, found HBV DNA levels ranging from less than 5 to 48 IU/mL, underscoring the assay sensitivity as a defining factor for the detection of DNA in these low-level specimens. In addition, nested amplification of multiple regions of the HBV genome after concentration by ultracentrifugation proved to be the most reliable and sensitive method of confirmation (Table 4). These data illustrate the need for alternative NATs with high assay sensitivity in confirming the presence of HBV DNA in donation samples.

The seroconversion of HBsAg and/or other HBV markers in a donor with a totally seronegative index donation distinguishes between WP infection and other diagnoses. In Case MP4-A3, anti-HBc is detected after 8 months (Table 4). Both HBV DNA and anti-HBs levels are known to fluctuate in some cases. Here, examples of such fluctuations are seen in Cases IDT-A1 and IDT-A3.

The HBV yield rate for the IDT Ultrio assay (0.21%) in Taiwan was about five times higher than was observed in Hong Kong[8] and was 10- to 100-fold higher than reported in countries with low HBV prevalence. The 12 yield cases, 10 of which were verified by NAT reactivity in follow-up specimens, are consistent with the finding of our previous study[13] on a different cohort of our donor population and with a different NAT system.

The results of this study could be used to estimate the impact of adding NAT for the whole blood donor population in Taiwan. HBV DNA screening by IDT together with HBsAg testing would initially identify 3919 confirmed donations per 500,000 donors tested. Comparing to current HBsAg screening alone, it will interdict 1069 additional infectious donations potentially transfused to more than 1000 recipients.

In summary, our study demonstrated that the great majority of our yield cases were of OBI and that these yield samples had very low viral load, necessitating the use of a highly sensitive NAT for detection. The yield rate observed with the IDT approach was higher than that observed with MP4 approaches in this study which confirmed the higher clinical utility of the more sensitive IDT approach. Implementation of HBV NAT screening, especially with the IDT format, shows promise in enhancing the safety of the blood supply in Taiwan.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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