Hepatitis B core-related antigen rapid diagnostic test for point-of-care identification of women at high risk of hepatitis B vertical transmission: a multicountry diagnostic accuracy study

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Summary

Background Timely administration of the hepatitis B virus (HBV) birth dose vaccine, along with identifying high-risk pregnant individuals for antiviral prophylaxis, is essential for the global elimination of vertical transmission of HBV. However, in resource-limited settings, access to HBV DNA testing is scarce, and accurate rapid tests for HBeAg are lacking. We aimed to assess the diagnostic performance of a newly developed hepatitis B core-related antigen (HBcrAg) rapid diagnostic test (RDT) to identify women who are HBsAg-positive and eligible for antiviral prophylaxis.

Methods In this multicountry diagnostic accuracy study, we retrospectively validated the HBcrAg-RDT using stored plasma from pregnant women who were HBsAg-positive in cohort studies from Cambodia and Cameroon and prospectively using finger-prick capillary blood from postpartum mothers at rural health centres in Burkina Faso. We estimated the sensitivity and specificity of the HBcrAg-RDT for diagnosing high HBV DNA concentrations (≥200 000 IU/mL) using real-time PCR (rtPCR) as the reference. We compared the diagnostic performance of the HBcrAg-RDT with that of conventional HBeAg assays based on the area under the receiver operating characteristic curve (AUROC).

Findings In total, plasma samples were available for 1964 participants: 1194 stored plasma samples available for analysis from the Cambodian cohort, 501 stored samples from the Cameroonian cohort, and 269 prospectively collected samples from women in the Burkina Faso cohort. In the pooled population, the mean age was 28·1 years (SD 6·0), and 382 (20·0%) were HBeAg positive. The HBcrAg-RDT showed an overall sensitivity of 93·1% (95% CI 90·5–95·2) and specificity of 94·3% (93·0–95·4). Sensitivity and specificity were 93·4% (90·7–95·5) and 94·4% (92·9–95·6) in the retrospective laboratory-based analyses of samples from Cambodia and Cameroon, and 89·7% (75·8–97·1) and 93·9% (90·0–96·6) in the prospective real-world analysis of samples of HBsAg-positive women from Burkina Faso. The AUROC for HBcrAg-RDT (0·937 [95% CI 0·924–0·950]) in distinguishing high versus low HBV DNA concentrations at the 200 000 IU/mL threshold in the pooled data set was significantly higher than that of HBeAg rapid tests (0·822 [0·798–0·845]; p<0·0001) and similar to laboratory-based HBeAg immunoassays (ELISA and chemiluminescence assay; 0·926 [0·897–0·955]; p=0·72). In Burkina Faso, the median turnaround time for HBV DNA testing was 46 days (IQR 31–72), whereas HBcrAg-RDT provided same-day results for all participants.

Interpretation HBcrAg-RDT might offer a practical solution for integrating the prevention of vertical transmission of HBV into decentralised antenatal care in resource-limited settings, enabling timely identification and management of pregnant individuals who are at high risk of transmission.

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Introduction

Hepatitis B virus (HBV) infection is a substantial global health challenge. In 2022, it affected approximately 254 million individuals and resulted in 1·1 million deaths, making HBV the third leading cause of infectious disease-related mortality, after COVID-19 and tuberculosis.[1] The World Health Assembly endorsed the Global Health Sector Strategy on viral hepatitis in 2016, aiming to eliminate HBV as a public health threat by 2030 through a 90% reduction in new infections and a 65% reduction in deaths.[1] Given that the vast majority of individuals with chronic HBV infection live in

Research in context

Evidence before this study

We searched PubMed for studies evaluating the performance of point-of-care tests for identifying HBsAg-positive pregnant individuals with high viraemia (≥200 000 IU/mL), from database inception to Oct 17, 2024, using the terms ("rapid test" OR "point of care test") AND ("HBV DNA" OR "HBeAg" OR "e antigen") AND "pregnancy" with no language restrictions. A systematic review and meta-analysis that informed the 2020 WHO guidelines for the prevention of vertical transmission reported pooled sensitivity and specificity for HBeAg tests diagnosing high HBV DNA concentrations (≥200 000 IU/mL) of 88·2% (95% CI 83·9–91·5) and 92·6% (90·0–94·5), respectively. However, most included studies relied on laboratory-based immunoassays (ELISA and chemiluminescence assays), and a subgroup analysis of studies using rapid diagnostic tests showed lower sensitivity (70·1% [95% CI 58·2–79·9]). Additionally, one study, not included in the systematic review, assessed the use of dried blood spots for a laboratory-based HBeAg test, reporting a sensitivity of 56% and specificity of 87%. Beyond HBeAg, we found no studies evaluating point-of-care tests, including the Xpert HBV Viral Load assay, for identifying highly viraemic pregnant individuals.

Added value of this study

In this multicountry study, we assessed the performance of a newly developed hepatitis B core-related antigen (HBcrAg) rapid diagnostic test (RDT) for identifying HBsAg-positive women with high HBV DNA concentrations (≥200 000 IU/mL), using real-time PCR as the reference. Among 1194 Cambodian, 501 Cameroonian, and 269 Burkinabé women, the HBcrAg-RDT showed a sensitivity of 93·1% (95% CI 90·5–95·2) and specificity of 94·3% (93·0–95·4). The area under the receiver operating characteristic curve for HBcrAg-RDT in distinguishing high from low HBV DNA concentrations at the 200 000 IU/mL threshold was significantly greater than that of HBeAg rapid tests and similar to HBeAg laboratory immunoassays.

Implications of all the available evidence

The HBcrAg-RDT can reliably identify pregnant women eligible for antiviral prophylaxis at the point of care. Its advantages include a low production cost (<US$5), versatility with capillary blood, no need for electricity or centrifugation, operability at temperatures up to 39°C, and a rapid turnaround time of 45 min. These features facilitate its integration into routine antenatal care in decentralised health-care facilities in resource-limited settings, thereby supporting the efficient roll-out of programmes for the prevention of vertical transmission of HBV in these countries.

low-income and middle-income countries (LMICs),[2] it is crucial that interventions are tailored for practicality and effective implementation in these resource-limited settings.

Among various interventions, preventing vertical transmission of HBV is vital for elimination efforts, as this route of transmission substantially increases the risk of chronic HBV infection and related liver diseases, compared with horizontal transmission later in life.[3] Over the past three decades, WHO's strategies for HBV prevention have evolved progressively, incorporating additional measures (appendix 2 p 7).[3] In 1992, WHO recommended integrating HBV vaccination into the Expanded Programme on Immunization and, in 2009, they emphasised the universal administration of the first dose of the HBV vaccine immediately after birth (birth dose) to prevent vertical transmission and early horizontal transmission.[4] In 2020, due to residual risks of vertical transmission despite infant immunoprophylaxis, particularly among women with high viral loads (≥200 000 IU/mL) or who are positive for HBeAg, WHO recommended antiviral prophylaxis for pregnant women with HBV infection and these risk factors.[5] In 2024, recognising the challenges faced by many pregnant individuals in LMICs who do not have access to HBV DNA or HBeAg testing, WHO conditionally recommended considering antiviral prophylaxis for all pregnant women with HBsAg positivity in settings where these tests are not available.[3]

HBV DNA testing remains unaffordable for many individuals in LMICs.[6] Even when accessible, the turnaround time from sample collection to receipt of HBV DNA results can be up to 2–3 months.[7] Such delays can hinder timely initiation of antiviral prophylaxis, which is crucial for effectively reducing maternal viral loads by the time of delivery.[8] Additionally, laboratory immunoassays for detecting HBeAg are not widely available in LMICs.[9] Although rapid HBeAg tests are included in the WHO Model List of Essential In Vitro Diagnostics,[10] they are not sufficiently sensitive.[11–13] Treating all pregnant individuals who are HBsAg-positive might seem advantageous, but this risks overtreatment, as only 10–30% have high viral loads (≥200 000 IU/mL) and are at elevated risk of vertical transmission.[14] Global modelling suggests that a treat-all strategy might not be cost-effective in most (64 [60%] of 106) countries with a high HBV burden.[15]

Hepatitis B core-related antigen (HBcrAg) assay quantifies, using a chemiluminescence immunoassay, HBcAg, HBeAg, and the p22 core-related protein, a phosphorylated form of HBcAg.[16,17] Serum HBcrAg concentrations have shown strong correlation with serum HBV DNA concentrations and intrahepatic covalently closed circular DNA levels in untreated individuals with chronic HBV infection.[18,19] In 2023, a rapid diagnostic test (RDT) based on immunochromatography (ESPLINE HBcrAg [RUO], Fujirebio, Tokyo,

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Japan) was developed for point-of-care detection of high HBV DNA concentrations.[20] This test (hereafter, the HBcrAg-RDT) might be particularly suited for LMICs owing to its low production cost (<US$5), no need for electricity or centrifugation, broad applicability with serum, plasma, whole blood, or dried blood spots, operability at temperatures up to 39°C, and rapid 45 min turnaround time. A preliminary study using stored sera from adults with chronic HBV infection in The Gambia found a sensitivity of 91·4% and specificity of 86·3% for identifying those with HBV DNA concentrations of 200 000 IU/mL or more.[20] Given these features, HBcrAg-RDT could potentially support not only two-step strategies (HBsAg screening followed by HBcrAg-RDT to identify individuals at high risk of vertical transmission) but also one-step strategies (HBcrAg-RDT only), similar to discussions surrounding hepatitis C core antigen testing and point-of-care nucleic acid testing for hepatitis C.[21] However, the test has not yet been formally validated in pregnant individuals.

We aimed to assess the performance of HBcrAg-RDT in identifying women with high HBV DNA concentrations (≥200 000 IU/mL), using real-time PCR (rtPCR) as the reference standard. We also aimed to compare the performance of HBcrAg-RDT with that of conventional HBeAg assays.

Methods

Study design and participants

In this multicountry diagnostic accuracy study, we retrospectively validated HBcrAg-RDT using stored plasma from HBsAg-positive pregnant women in Cambodia[8] and Cameroon,[22] and prospectively evaluated the test in Burkina Faso using finger-prick capillary blood from postpartum women attending HBsAg screening at rural health centres.[23] Because HBcrAg-RDT was tested on all women attending HBsAg screening in Burkina Faso, we assessed its performance as a one-step strategy (all women, irrespective of HBsAg status) or a two-step strategy (restricting to HBsAg-positive women). These countries were selected due to their high HBV prevalence among pregnant women,[8,24,25] the predominance of HBV genotypes B and C in Cambodia, and E and A in Cameroon and Burkina Faso,[26] and the limited availability of both HBV DNA and laboratory-based HBeAg testing.[13,22,23]

In Cambodia, the TA-PROHM study (ANRS12345) prospectively and consecutively recruited pregnant women attending antenatal care at five hospital-based maternity units across Phnom Penh, Siem Reap, Kampong Cham, and Takeo, from Oct 4, 2017, to Nov 27, 2020.[8] The study included pregnant women aged 18 years or older who tested positive for HBsAg using an RDT (SD BIOLINE HBsAg, Standard Diagnostics, Yongin-si, South Korea). Each included participant signed two informed consent forms corresponding to the screening phase and the inclusion phase of the study. Exclusion criteria included HIV or hepatitis C virus positivity, ongoing anti-HBV treatment, creatinine clearance less than 30 mL/min, severe pregnancy-related diseases, or planned delivery outside the study centre. After inclusion, plasma HBeAg presence was evaluated using an RDT (SD BIOLINE HBeAg) and HBV DNA concentrations were quantified using rtPCR (PUMA HBV kit, Omunis, Clapiers, France). Alanine aminotransferase concentrations were measured using the ABX PENTRA C400 analyser (Horiba, Kyoto, Japan). Remaining plasma samples were stored at –80°C at the Pasteur Institute of Cambodia and later used for HBcrAg-RDT testing from May to July, 2022.

In Cameroon, the ANRS12303 study conducted prospective and consecutive enrolment of pregnant women receiving antenatal care at the Tokombéré District Hospital between Jan 31, 2009, and Dec 31, 2016.[22] After obtaining informed consent, women who tested positive for HBsAg using an RDT (VIKIA, bioMérieux, Craponne, France) were systematically invited to participate in the study. None of the participants were receiving concomitant antiviral therapy. A blood sample was collected via venepuncture and an ELISA (Monolisa, BioRad, Marnes-la-Coquette, France) was used to detect HBeAg in serum prepared from the blood sample. Frozen plasma samples were then shipped to the Angers University Hospital, France, where HBV DNA was quantified using rtPCR (Aptima HBV Quant Assay, Hologic, Marlborough, MA, USA). Alanine aminotransferase concentrations were not measured in this study. The remaining frozen samples were stored at –80°C at Angers University Hospital and later used to perform the HBcrAg-RDT from May to July, 2022.

In Burkina Faso, PREDICT-B, a prospective validation study, was conducted from May 19, 2022, to Sept 1, 2023, as part of an ancillary study within the NéoVac project.[23] The study recruited pregnant women attending 24 rural health centres in the Dô and Dafra districts of the Hauts-Bassins region. All pregnant women living in the study area who attended antenatal care and provided written informed consent were eligible for inclusion. 9 months postpartum, coinciding with the scheduled yellow fever and measles–rubella vaccinations for their children, HBV screening was done by local health-care workers at each health centre. A single finger-prick was used to obtain a capillary blood sample, with a drop applied to each of the RDTs: HBsAg (Determine HBsAg 2, Abbott, Abbott Park, IL, USA); HBcrAg-RDT; and HBeAg (Advanced Quality One Step HBeAg, INTEC Products, Xiamen, China). Women who tested positive for HBsAg had venous blood sampling, with samples transported to the Centre Muraz laboratory in Bobo-Dioulasso, Burkina Faso. Serum samples were used to quantify HBV DNA concentrations using rtPCR (Cobas AmpliPrep/Cobas TaqMan HBV assay, Roche Molecular Systems, Pleasanton, CA, USA) at the Centre Muraz laboratory and to detect HBeAg using a chemiluminescence immunoassay (ARCHITECT, Abbott, Abbott Park, IL, USA) at the Institute of Health Science Research, Bobo-Dioulasso.

This study was approved by the National Ethics Committees in Cambodia (reference 314NECHR), Cameroon (reference 2014/04/443/CE/CNERSH/SP), and Burkina Faso (reference N°2022/000082/MSHP/MESRI/CERS), as well as by the institutional review board of Kumamoto University (reference Senshin 1094) and the Institut Pasteur (reference 2018-12/IRB/9). The study is reported according to the STARD 2015 guidelines.

Procedures

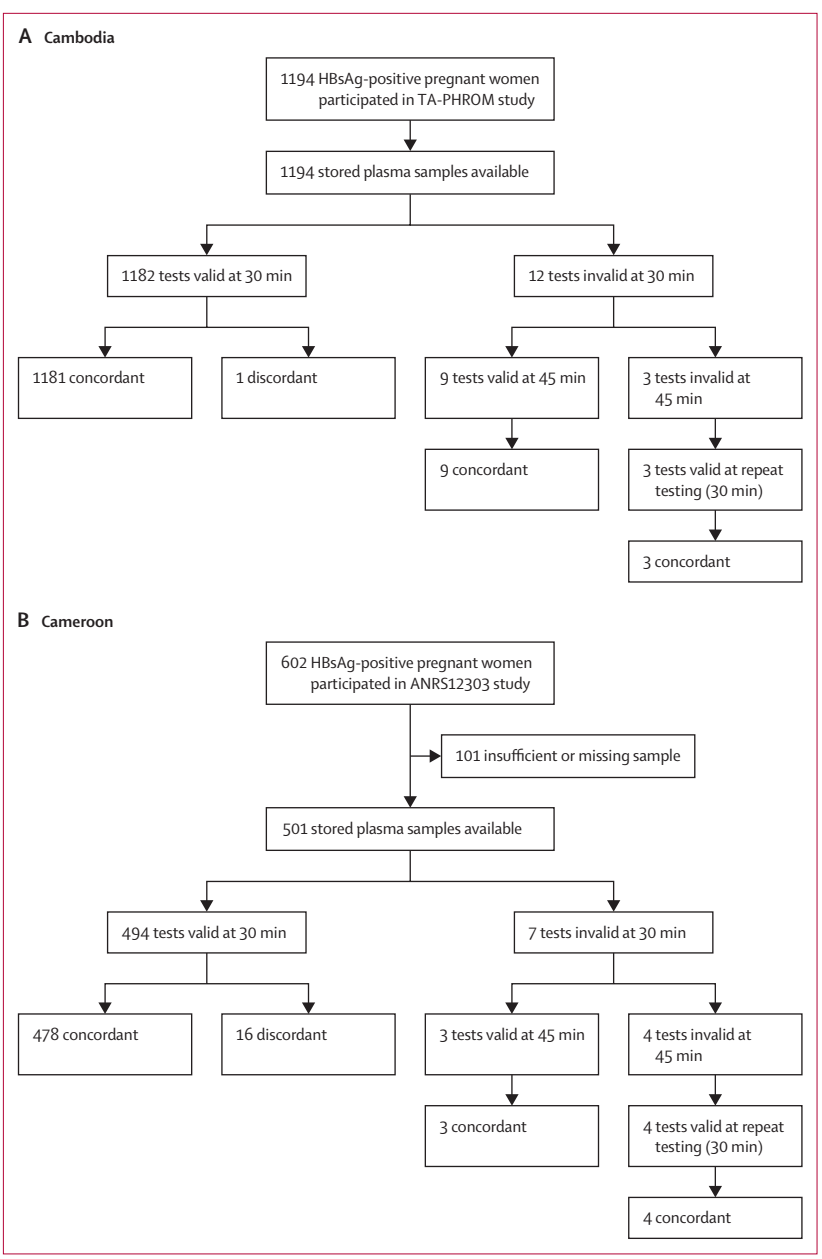
HBcrAg-RDT was done according to the manufacturer's instructions. The test kit includes a single-use plastic cassette, pretreatment solution, neutralising solution, squeeze tube, and applicator tip. No special equipment was required.

The retrospective validation was done by laboratory technicians at the Pasteur Institute of Cambodia for samples from Cambodia and at the Angers University Hospital for samples from Cameroon. Plasma samples were first thawed at room temperature, then 50 µL were added to a tube containing six drops (150 µL) of pretreatment solution. After mixing and allowing the solution to stand for 10 min at room temperature, two drops (50 µL) of neutralising solution were added, followed by a second mixing. Two drops of the prepared sample were then placed on the sample window of the cassette, which was left on a flat surface to allow migration for 30 min. Test results were interpreted by two readers who were masked to each other's assessments. A positive result was indicated by blue lines appearing on both the control and test areas, a negative result by a blue control line with no test line, and an invalid result by a pink or absent control line. If the result was invalid, the test was re-read after 15 min; if still invalid, the test was repeated using the remaining specimen, which had already been treated with both the pretreatment and neutralising solutions. Photographs were taken at the time of interpretation. In cases of disagreement between the two readers, a third reader's interpretation based on the photograph was used.

The prospective evaluation in Burkina Faso closely followed the procedure used in the retrospective evaluation, with a few differences: a drop of finger-prick capillary blood was used, the tests were done by health-care workers at rural health centres, interpretation was done by a single examiner, and no further readings were conducted in cases of invalid results at 30 min. At all three sites, test interpreters were blinded to the HBV DNA concentration results.

Statistical analysis

We estimated the sensitivity, specificity, and positive and negative predictive values with their exact binomial 95% CIs for HBcrAg-RDT to identify women with high HBV DNA concentrations (≥200 000 IU/mL), using rtPCR as the reference standard. To compare the diagnostic performance of HBcrAg-RDT for detecting high viraemia with that of conventional HBeAg assays, we calculated the area under the receiver operating characteristic curve (AUROC) for each test and compared them using the DeLong method. For the retrospective validation, in which two masked readers interpreted the results, we assessed inter-rater agreement using the κ statistic. In the Burkina Faso cohort data, we evaluated the test's sensitivity and specificity in HBsAg-positive women (two-step strategy) and in all women regardless of HBsAg status (one-step strategy). We also recorded the turnaround time, defined as the interval between HBsAg screening and the completion of the second test, for both



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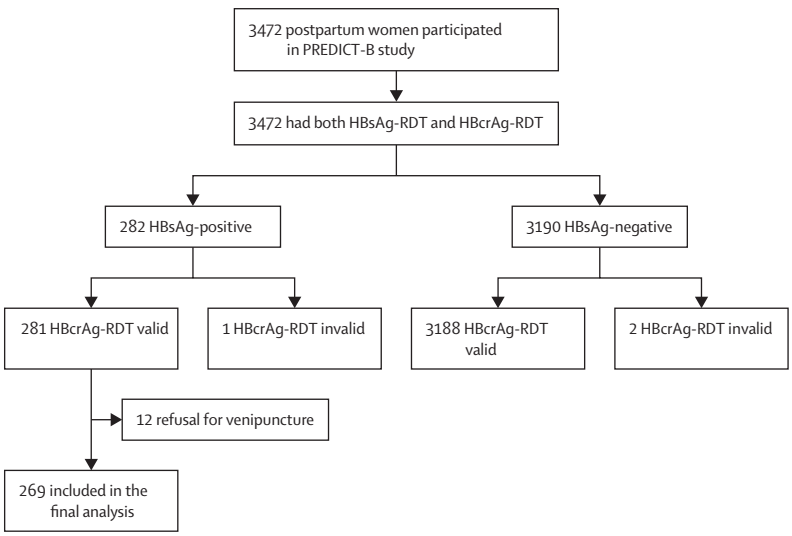

Figure 1: **Flow chart of study participants and their test results**
(A) Cambodia (serum sample). (B) Cameroon. (C) Burkina Faso. HBcrAg-RDT=hepatitis B core-related antigen-rapid diagnostic test. HBsAg-RDT=HBsAg-rapid diagnostic test. HBV=hepatitis B virus.

	Cambodia (n=1194)	Cameroon (n=501)	Burkina Faso (n=269)	Overall (n=1964)
Age, years	29·5 (5·3)	25·2 (6·2)	26·9 (6·1)	28·1 (6·0)
Alanine aminotransferase, U/L	20 (15–28)	NA	21 (17–30)	21 (16–29)
Conventional HBeAg*				
Positive	253/1194 (21·2%)	94/501 (18·8%)	35/217 (16·1%)	382/1912 (20·0%)
Negative	941/1194 (78·8%)	407/501 (81·2%)	182/217 (83·9%)	1530/1912 (80·0%)
HBcrAg-RDT				
Positive	385 (32·2%)	110 (22·0%)	49 (18·2%)	544 (27·7%)
Negative	809 (67·8%)	391 (78·0%)	220 (81·8%)	1420 (72·3%)
Hepatitis B virus DNA, IU/mL				
<2000	635 (53·2%)	365 (72·9%)	192 (71·4%)	1192 (60·7%)
2000–19 999	141 (11·8%)	36 (7·2%)	25 (9·3%)	202 (10·3%)
20 000–199 999	51 (4·3%)	12 (2·4%)	13 (4·8%)	76 (3·9%)
≥200 000	367 (30·7%)	88 (17·6%)	39 (14·5%)	494 (25·2%)

Data are mean (SD), median (IQR), n/N (%), or n (%). Some percentages may not total 100% due to rounding. HBcrAg-RDT=hepatitis B core-related antigen-rapid diagnostic test. NA=not available. *HBeAg results are based on rapid diagnostic test (SD BIOLINE HBeAg) in Cambodia, enzyme-linked immunoassay (Monolisa) in Cameroon, and chemiluminescence immunoassay (ARCHITECT) in Burkina Faso.

Table 1: **Characteristics of study participants**

HBcrAg-RDT and HBV DNA testing. Factors associated with false positive and false negative results were identified using the χ^2 test, Fisher's exact test, or Kruskal–Wallis rank-sum test. Data analysis was done using STATA 16.

Sample size was calculated for the prospective study in Burkina Faso (PREDICT-B). Assuming a true sensitivity of 90%,[20] 54 cases of high viral load were required to ensure that the lower boundary of the 95% CI would exceed 75%, with a two-sided significance level of 5% and a power of 80%. Assuming that approximately 10% of women would test positive for HBsAg[25] and 15% of these would have high viral loads,[14] we aimed to recruit 3600 women for HBsAg screening.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

In Cambodia, plasma samples were available for all women who were HBsAg-positive in the TA-PROHM study (n=1194; figure 1A). The median age was 29 years (IQR 26–33); 253 (21·2%) participants were HBeAg-positive, and 367 (30·7%) had high HBV DNA concentrations (≥200 000 IU/mL; table 1). In Cameroon, plasma samples were insufficient or missing for 101 (17%) of 602 women analysed in the original study (figure 1B). Among the 501 women with available samples, the median age was 24 years (IQR 20–30); 94 (18·8%) were HBeAg-positive, and 88 (17·6%) had high HBV DNA concentrations (≥200 000 IU/mL; table 1).

In Burkina Faso, 3472 women were prospectively included. HBsAg-RDT, HBcrAg-RDT, and HBeAg-RDT were simultaneously performed on samples from all participants at rural health centres (figure 1C). HBsAg was positive in 282 (8·1%) women, and 270 (95·7%) of these women consented to venipuncture, one of whom had an invalid HBcrAg-RDT and was excluded from analysis. The median age of women who had valid HBcrAg-RDT results was 26 years (IQR 22–31); 35 (16·1%) of 217 women who had HBeAg chemiluminescence assay testing were positive, and 39 (14·5%) of 269 women had HBV DNA concentrations greater than or equal to 200 000 IU/mL (table 1). The median turnaround time from sample collection to receipt of HBV DNA result was 46 days (IQR 31–72), whereas the HBcrAg-RDT was conducted on the same day as sample collection for all participants.

At 30 min, valid results were obtained from 1182 (99·0%) of 1194 samples in Cambodia, 494 (98·6%) of 501 samples in Cameroon, and 3469 (99·9%) of 3472 in Burkina Faso (figure 1). In Cambodia and Cameroon, an additional reading was conducted at 45 min for samples with initially invalid results; this yielded valid results in nine of 12 samples in Cambodia and three of seven samples in Cameroon. For samples that remained invalid after 45 minutes, the leftover pretreated samples were tested with a second cassette, and valid results were achieved in all such cases in both countries.

In Cambodia and Cameroon, two readers independently interpreted the results. In Cambodia, the test results were concordant except for one (0·1%) of 1194 samples (κ 0·9981 [95% CI 0·9891–0·9997]). In Cameroon, 16 (3·2%) of 501 samples had discordant results (κ 0·9088 [0·8545–0·9435]). Disagreements were resolved by a third

	Sensitivity (n/N [95% CI])	Specificity (n/N [95% CI])	Positive predictive value (n/N [95% CI])	Negative predictive value (n/N [95% CI])	AUROC (95% CI)	Difference in AUROC between HBcrAg-RDT and HBeAg (95% CI)	p value *vs* the AUROC of HBcrAg-RDT
HBcrAg-RDT							
Cambodia, plasma (n=1194)	94·0% (345/367 [91·1–96·2])	95·2% (787/827 [93·5–96·5])	89·6% (345/385 [86·1–92·5])	97·3% (787/809 [95·9–98·3])	0·946 (0·932–0·960)	..	..
Cameroon, plasma (n=501)	90·9% (80/88 [82·9–96·0])	92·7% (383/413 [89·8–95·0])	72·7% (80/110 [63·4–80·8])	98·0% (383/391 [96·0–99·1])	0·918 (0·886–0·951)	..	..
Burkina Faso, capillary blood (n=269)	89·7% (35/39 [75·8–97·1])	93·9% (216/230 [90·0–96·6])	71·4% (35/49 [56·7–83·4])	98·2% (216/220 [95·4–99·5])	0·918 (0·868–0·969)	..	..
Overall (n=1964)	93·1% (460/494 [90·5–95·2])	94·3% (1386/1470 [93·0–95·4])	84·6% (460/544 [81·2–87·5])	97·6% (1386/1420 [96·7–98·3])	0·937 (0·924–0·950)	..	..
HBeAg							
Cambodia, plasma RDT (n=1194)	65·4% (240/367 [60·3–70·3])	98·4% (814/827 [97·3–99·2])	94·9% (240/253 [91·4–97·2])	86·5% (814/941 [84·2–88·6])	0·819 (0·794–0·844)	0·127 (0·101 to 0·152)	<0·0001
Cameroon, serum ELISA (n=501)	88·6% (78/88 [80·1–94·4])	96·1% (397/413 [93·8–97·8])	83·0% (78/94 [73·8–89·9])	97·5% (397/407 [95·5–98·8])	0·924 (0·889–0·958)	–0·006 (–0·035 to 0·046)	0·78
Burkina Faso							
Serum, chemiluminescence assay (n=217)	90·3% (28/31 [74·3–98·0])	96·2% (179/186 [92·4–98·5])	80·0% (28/35 [63·1–91·6])	98·4% (179/182 [95·3–99·7])	0·933 (0·878–0·987)	–0·008 (–0·536 to 0·552)	0·73
Capillary blood, RDT (n=229)	71·9% (23/32 [53·3–86·3])	98·5% (194/197 [95·6–99·7])	88·5% (23/26 [69·9–97·6])	95·6% (194/203 [91·8–98·0])	0·852 (0·772–0·931)	0·071 (–0·023 to 0·165)	0·049
Overall							
All (n=1912)*	71·2% (346/486 [66·9–75·2])	97·5% (1390/1426 [96·5–98·2])	90·6% (346/382 [87·2–93·3])	90·8% (1390/1530 [89·3–92·3])	0·843 (0·823–0·864)	0·095 (0·073 to 0·116)	<0·0001
RDT only (n=1423)	65·9% (263/399 [61·0–70·6])	98·4% (1008/1024 [97·5–99·1])	94·3% (263/279 [90·9–96·7])	88·1% (1008/1144 [86·1–89·9])	0·822 (0·798–0·845)	0·122 (0·103 to 0·140)	<0·0001
ELISA or chemiluminescence assay only (n=718)	89·1% (106/119 [82·0–94·1])	96·2% (576/599 [94·3–97·6])	82·2% (106/129 [74·5–88·4])	97·8% (576/589 [96·3–98·8])	0·926 (0·897–0·955)	–0·006 (–0·522 to 0·534)	0·72

Data are shown for the two-step strategy (women who were screened as HBsAg-positive). AUROC=area under the receiver operating characteristic curve. HBcrAg-RDT=hepatitis B core-related antigen-rapid diagnostic test. RDT=rapid diagnostic test. *HBeAg results are based on rapid diagnostic test (SD BIOLINE HBeAg) in Cambodia, enzyme-linked immunoassay (Monolisa) in Cameroon, and chemiluminescence immunoassay (ARCHITECT) in Burkina Faso.

Table 2: **Performance of HBcrAg-RDT and HBeAg tests in identifying HBsAg-positive women with HBV DNA concentrations ≥200 000 IU/mL**

reader using photographs, and these final results were used to estimate HBcrAg-RDT sensitivity and specificity.

In the pooled data from all three countries, the overall sensitivity for identifying HBsAg-positive women with high HBV DNA concentrations (≥200 000 IU/mL) was 93·1% (460/494 [95% CI 90·5–95·2]) and the specificity was 94·3% (1386/1470 [91·1–96·2]; table 2). In Cambodia, the sensitivity was 94·0% (345/367 [91·1–96·2]) and the specificity was 95·2% (787/827 [93·5–96·5]). In Cameroon, the sensitivity was 90·9% (80/88 [82·9–96·0]) and the specificity was 92·7% (383/413 [89·8–95·0]). The pooled sensitivity was 93·4% (95% CI 90·7–95·5) and pooled specificity was 94·4% (92·9–95·6) in the retrospective laboratory-based analyses of samples from Cambodia and Cameroon.

In Burkina Faso, HBcrAg-RDT was used for all participants, regardless of their HBsAg status. Among HBsAg-negative women (n=3190), the HBcrAg-RDT returned negative results for 3179 (99·7%) women, false-positive results for nine (0·3%), and invalid results for two (0·1%). When considering a one-step strategy—in which the HBcrAg-RDT is administered to all women regardless of HBsAg status—the sensitivity for detecting women with HBsAg positivity with high HBV DNA concentrations was 89·7% (35/39 [95% CI 75·8–97·1]), with a specificity of 99·3% (3395/3418

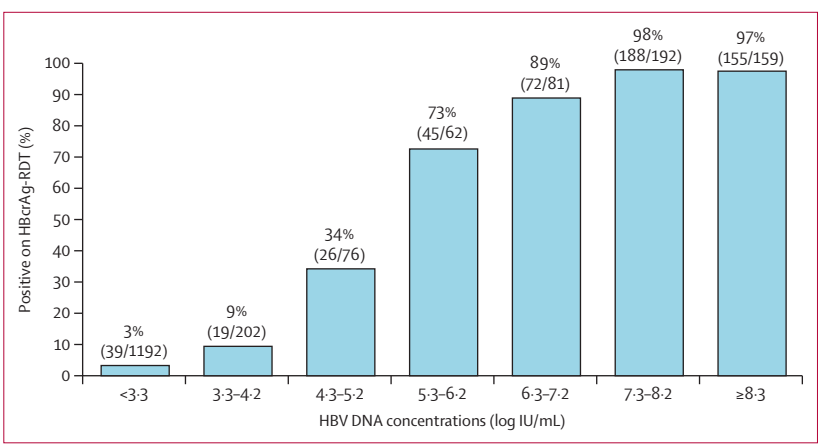

Figure 2: **Proportion of HBcrAg-RDT positivity across different HBV DNA concentrations**
HBcrAg-RDT=hepatitis B core-related antigen-rapid diagnostic test. HBV=hepatitis B virus.

[99·0–99·6]; table 2). For the two-step strategy, in which the HBcrAg-RDT was used only for HBsAg-positive women, the sensitivity was 89·7% (35/39 [75·8–97·1]) and specificity was 93·9% (216/230 [90·0–96·6]).

Factors associated with false-negative and false-positive results are shown in appendix 2 (p 4). Among samples with high HBV DNA concentrations (≥200 000 IU/mL), false negatives were more likely when viral loads were close to the threshold (200 000–1 999 999 IU/mL) and when HBeAg was negative. For samples with low viraemia (<200 000 IU/mL), false positives were more frequent with HBV DNA concentrations near the threshold (20 000–199 999 IU/mL), HBeAg positivity, and alanine aminotransferase concentrations greater than or equal to

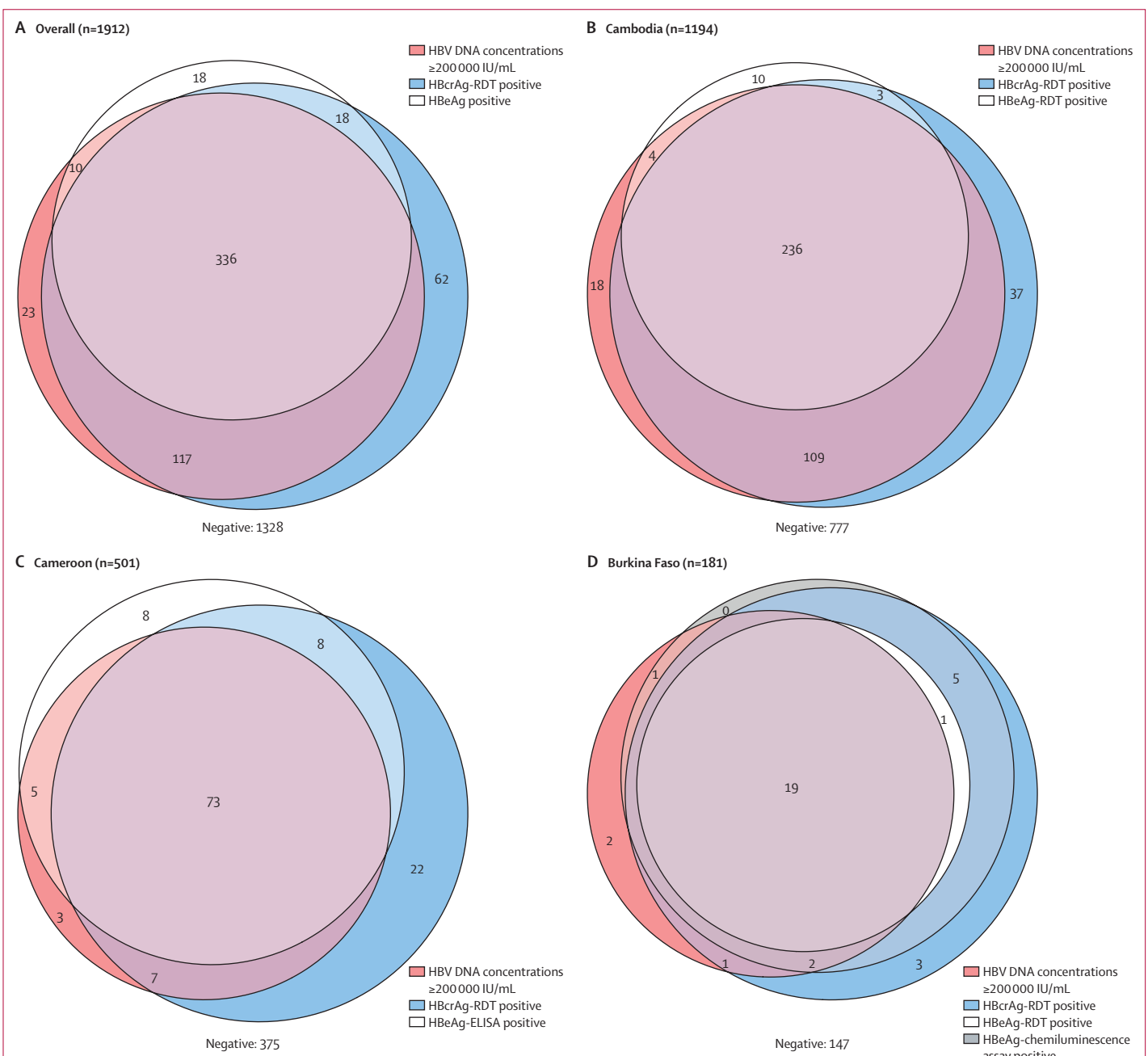

Figure 3: **Euler diagrams illustrating the overlaps among participants with HBV DNA concentrations ≥200 000 IU/mL, HBeAg positivity, or HBcrAg-RDT positivity**
(A) Overall. (B) Cambodia. (C) Cameroon. (D) Burkina Faso. HBeAg results are based on rapid diagnostic test (SD BIOLINE HBeAg) in Cambodia, enzymel-inked immunoassay (Monolisa) in Cameroon, and chemiluminescence immunoassay (ARCHITECT) in Burkina Faso. HBcrAg-RDT=hepatitis B core-related antigen-rapid diagnostic test. HBeAg-RDT=HBeAg-rapid diagnostic test. HBV=hepatitis B virus. RDT=rapid diagnostic test.

40 U/L. The proportion of HBcrAg-RDT positivity across different HBV DNA concentrations is shown in figure 2.

The performance of conventional HBeAg assays in identifying highly viraemic women is shown in table 2. The AUROC for HBcrAg-RDT was significantly higher than that of HBeAg-RDT in both Cambodia (SD BIOLINE HBeAg) and Burkina Faso (Advanced Quality). By contrast, the AUROCs for HBcrAg-RDT and laboratory-based HBeAg immunoassays were similar in both Cameroon and Burkina Faso (table 2). Euler diagrams illustrating the overlaps among participants testing positive for each of these tests are shown in figure 3.

Discussion

In this multicountry validation study, the HBcrAg-RDT accurately identified 93·1% (95% CI 90·5–95·2) of women who were HBsAg-positive and had high HBV DNA concentrations (≥200 000 IU/mL) and 94·3% (93·0–95·4) of those with lower concentrations. Its performance was significantly better than that of HBeAg-RDTs and similar to laboratory-based HBeAg immunoassays, with immediate test results available on the same day as the HBsAg screening. These findings suggest that the use of HBcrAg-RDT might represent a valid option for identifying highly viraemic individuals in resource-limited, decentralised antenatal care settings, where access to HBV DNA testing and laboratory-based HBeAg immunoassays is limited.

In 2020, WHO recommended HBeAg as an alternative in the absence of HBV DNA quantification, a decision supported by findings from a WHO-commissioned systematic review.[5,27] This meta-analysis, which included 41 studies, reported a pooled sensitivity of 88·2% (95% CI 83·9–91·5] and specificity of 92·6% (90·0–94·5] for HBeAg testing—primarily using laboratory-based immunoassays—in identifying pregnant women with high HBV DNA concentrations (>200 000 IU/mL).[5,27] This diagnostic performance is similar to that of HBcrAg-RDT in our study. Furthermore, a modelling study based on a survey of 555 health-care workers from 41 African countries suggested that the majority (≥70%) would prefer to use RDTs over HBV DNA testing if the RDT showed a sensitivity of 90%, specificity of 90%, a cost of US$5, and a turnaround time of 60 min.[28] The HBcrAg-RDT meets all these criteria, making it a suitable choice for these settings.

The HBcrAg-RDT is currently under evaluation for registration as a CE-marked in-vitro diagnostic, under the name Highly Sensitive Rapid Test for the Detection of HBeAg (ESPLINE HBeAg-hs, Fujirebio, Tokyo, Japan), owing to its enhanced sensitivity for HBeAg detection compared with conventional RDTs. This will be the first CE-marked RDT for HBeAg in compliance with the In Vitro Diagnostic Regulation, with WHO prequalification anticipated in the future. This HBcrAg-RDT has shown its applicability to the target population and broadly meets the ASSURED criteria (appendix 2 pp 5–6),[29] offering substantial potential to simplify and decentralise care for the prevention of vertical transmission of HBV. Notably, the HBcrAg-RDT complements WHO's 2024 strategy to prevent vertical transmission by providing a practical and scalable alternative for identifying pregnant individuals with high viral loads in settings where HBV DNA or HBeAg testing is unavailable. This targeted approach reduces the risk of overtreatment associated with treating all HBsAg-positive women and aligns with WHO's emphasis on optimising resources in LMICs. Following HBsAg screening, reflex testing can be done at antenatal care services by lay health workers without the need for additional blood sampling or transport to central laboratories. For samples obtained via venepuncture, leftover plasma or serum can be used for the HBcrAg-RDT. Where finger-prick capillary blood is used for HBsAg detection, the second drop can be placed into a pretreatment solution tube. If HBsAg is positive, the HBcrAg-RDT can be done immediately, whereas the pretreated sample is discarded if HBsAg is negative. This test and treat approach facilitates the immediate initiation of antiviral prophylaxis on the same day as HBV screening. In Burkina Faso, the median turnaround time for HBV DNA testing was 46 days (IQR 31–72), whereas HBcrAg-RDT results were available on the same day for all participants. Depending on the test's cost and local HBV prevalence, a one-step strategy, bypassing previous HBsAg screening, might also be viable, as false-positive results for HBcrAg-RDT were rare (nine [0·3%] of 3188) among HBsAg-negative women.

An important question not addressed in this study is how to identify HBsAg-positive individuals who would benefit from long-term antiviral therapy for their own health. Although this topic is beyond the scope of this Article, a recent analysis in Cambodia, using the same sample set, suggested that combining HBcrAg-RDT with alanine aminotransferase testing might effectively identify those eligible for long-term antiviral therapy based on the 2018 American Association for the Study of Liver Diseases criteria, with a sensitivity of 87·5% (95% CI 75·9–94·8) and specificity of 75·3% (71·6–78·7).[30] These findings, together with the present analysis, suggest the potential for a streamlined and simplified care model, extending from prevention of vertical transmission to post-delivery maternal treatment, in resource-limited settings. Individuals identified as being HBsAg-positive during antenatal care should undergo both HBcrAg-RDT and alanine aminotransferase measurement. Irrespective of alanine aminotransferase concentrations, those testing positive on HBcrAg-RDT should initiate antiviral prophylaxis immediately. After the peripartum period, those with low alanine aminotransferase concentrations could discontinue antiviral therapy, whereas those with elevated alanine aminotransferase concentrations should continue treatment. The effectiveness of this approach requires further evaluation.

This study has limitations. First, the sample size in Burkina Faso did not meet our initial target of 3600 participants, indicating the need for additional data to validate the HBcrAg-RDT in real-world field settings. Second, the samples in Burkina Faso were collected 9 months postpartum rather than during pregnancy; however, previous studies suggest HBV DNA concentrations remain stable before and after childbirth, except in those receiving antiviral prophylaxis.[31] Third, although rare, discrepancies were observed between readers in interpreting results. To mitigate this, we plan to provide a colour chart to assist readers in accurately interpreting results. Fourth, differences in sample types, reference assays, and study designs (retrospective or prospective) across countries might have collectively introduced variability in HBcrAg-RDT performance, making it difficult to isolate the effect of each factor. Nevertheless, this reflects real-world conditions, where diverse samples and assays are used in various settings. Fifth, a recent study suggested variations in HBcrAg components across genotypes,[17] raising the question of whether HBcrAg-RDT performance varies by genotype. Although we could not assess this directly in our study, we believe such variation is unlikely, given the consistently good performance observed in Cambodia (predominantly genotypes B and C) and Cameroon and Burkina Faso (genotypes A and E). This is further supported by a meta-analysis showing a strong correlation between serum HBV DNA concentrations and HBcrAg concentrations, irrespective of genotype.[19] Sixth, although the turnaround time observed in the prospective study in Burkina Faso reflects a research context rather than real-life settings coordinated by national programmes, it aligns with findings from an implementation pilot programme in Uganda.[7] Finally, this study focused solely on estimating the diagnostic performance of the test. Further research is planned to evaluate the effect of integrating the test into routine antenatal care on outcomes related to the prevention of vertical transmission of HBV, including child infection.

In conclusion, this study found a high performance of the HBcrAg-RDT in identifying women with elevated HBV DNA concentrations who should be prioritised for antiviral prophylaxis. HBcrAg-RDT shows promise as a viable alternative for identifying pregnant individuals eligible for peripartum antiviral prophylaxis, particularly in decentralised, resource-limited settings.

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Contributors
YS and YT conceived the study. All authors participated in data collection and investigation. JPV and YS analysed the data. JPV and YS wrote the first draft of the manuscript. All authors reviewed, edited, and approved the final version of the manuscript. JPV, YT, and YS accessed and verified the data. All authors had access to the data and had final responsibility for the decision to submit for publication.

Declaration of interests
YS has received a research grant and honoraria for lectures from Gilead Sciences and research materials from Abbott Laboratories and Fujirebio. YT has received funding and honoraria for lectures from AbbVie, Gilead Sciences, OTSUKA Pharmaceutical and GlaxoSmithKline; has received funding from Fujirebio, Sysmex Corporation, and Janssen Pharmaceutical; and has received honoraria for lectures from Chugai Pharmaceutical, ASKA Pharmaceutical Holdings, Takeda Pharmaceutical, AstraZeneca, Eisai, and HU frontier. GC is an employee and stock holder in Abbott Laboratories. All other authors declare no competing interests.

Data sharing
De-identified participant data will be shared on reasonable request after approval by the scientific committee of each study site. Requests should be made to the corresponding author.

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