World Health Organization

# PREVENTION OF MOTHER-TO-CHILD TRANSMISSION OF HEPATITIS B VIRUS: Guidelines on antiviral prophylaxis in pregnancy

## July 2020

**Web Annex B**: Systematic review of the performance of hepatitis B e antigen test, as an alternative to HBV DNA, to assess eligibility for initiating antiviral therapy during pregnancy

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## Table of Contents

## List of Tables

## List of Figures

## List of abbreviations

| | |
|---|---|
| AASLD | American Association for the Study of Liver Diseases |
| AFR | African Region |
| AMR | Region of the Americas |
| APASL | Asian Pacific Association for the Study of the Liver |
| BCP | basal core promoter |
| CHB | chronic hepatitis B virus infection |
| CIA | counting immunoassay |
| CLIA | chemiluminescence immunoassay |
| CNKI | China National Knowledge Infrastructure |
| EASL | European Association for the Study of the Liver |
| EIA | enzyme immunoassay |
| EMR | Eastern Mediterranean Region |
| ESS | effective sample size |
| EUR | European Region |
| FIA | fluoroimmunoassay |
| FN | false negative |
| FP | false positive |
| GHSS | Global Health Sector Strategy |
| GRADE | Grading of Recommendations Assessment, Development and Evaluation |
| HBcrAg | hepatitis B core-related antigen |
| HBeAg | hepatitis B e antigen |
| HBIG | hepatitis B immunoglobulin |
| HBsAg | hepatitis B surface antigen |
| HBV | hepatitis B virus |
| HCC | hepatocellular carcinoma |
| HCV | hepatitis C virus |
| HDV | hepatitis D virus |
| lnDOR | log diagnostic odds ratio |
| MTCT | mother-to-child transmission |
| N/A | not applicable |

| | |
|---|---|
| NAT | nucleic acid test |
| NLR | negative likelihood ratio |
| N/R | not reported |
| PC | precore |
| PCR | (real-time) polymerase chain reaction |
| PLR | positive likelihood ratio |
| QUADAS-2 | Quality Assessment of Diagnostic Accuracy Studies |
| RDT | rapid diagnostic test |
| RIA | radioimmunoassay |
| Se | sensitivity |
| SEAR | South-East Asia Region |
| Sp | specificity |
| SROC | summary receiver operating characteristic curve |
| TBD | timely birth dose of hepatitis B vaccine |
| TN | true negative |
| TP | true positive |
| VL | viral load |
| WPR | Western Pacific Region |
| WHO | World Health Organization |
| 95% CI | 95% confidence interval |

# 1. BACKGROUND

Currently, the World Health Organization (WHO) estimates that chronic hepatitis B virus (HBV) infection affects close to 260 million persons and causes an estimated 900 000 deaths annually through manifestations of chronic liver disease, such as cirrhosis or hepatocellular carcinoma (HCC). The regions with the highest prevalence of chronic HBV infection (CHB) are the Western Pacific and Africa *(1)*. In 2016, the World Health Assembly endorsed the Global Health Sector Strategy (GHSS) on viral hepatitis, which calls for an elimination of HBV worldwide as a public health threat by 2030, to be accomplished through reducing the incidence of CHB by 90%, and its mortality by 65% *(2)*.

Chronic infection is more likely to develop when HBV is acquired early in life, and therefore, perinatal mother-to-child transmission (MTCT) is a major contributor to the incidence of CHB *(3)*. Moreover, the risk of developing chronic liver disease, including HCC, may be higher in those who acquired CHB through MTCT compared to those who ended up with CHB through horizontal transmission later in life *(4,5)*.

In order to achieve the WHO's global hepatitis elimination plan, it is imperative to prevent MTCT of HBV *(6)*. Since 2009, WHO makes a universal recommendation to administer timely hepatitis B vaccines to all newborn babies within 24 hours of birth to prevent MTCT and early childhood transmission *(7)*. Although the birth dose vaccines alone should be enough to prevent MTCT from mothers with CHB who have low HBV viral replication *(8,9)*, 20–30% of women with high viral load infect their newborns despite timely birth dose vaccination *(9,10)*. Therefore, in resource-rich countries, pregnant women are screened for hepatitis B surface antigen (HBsAg), and subsequently for hepatitis B e antigen (HBeAg), to identify high-risk infants who would benefit from hepatitis B immunoglobulin (HBIG) in addition to timely birth dose vaccination *(11)*. However, despite this active and passive immunoprophylaxis, a substantial proportion of infants are still infected when their mothers have a very high viral load, particularly when the serum HBV DNA level exceeds 200 000 IU/mL *(12)*. Consequently, in high-income countries, HBV DNA quantification has become a part of antenatal HBV testing to identify highly viraemic women who have a residual

risk of MTCT despite administration of both hepatitis B vaccine and HBIG to neonates at birth *(12–14)*, and who thus require antiviral therapy during pregnancy for minimizing its risk *(15)*.

However, in low- and middle-income countries, such additional measures to prevent MTCT have rarely been implemented *(16)*. Following antenatal screening for HBsAg, it is essential to quantify serum HBV DNA levels using the nucleic acid test (NAT) to decide whom to treat and not to treat during pregnancy to prevent MTCT. However, access to NAT is severely limited in these countries. The current standard NAT assay, which is real-time polymerase chain reaction (PCR), is hardly accessible due to its high cost (US$ 60–200/assay) and its need for a sophisticated laboratory with highly skilled laboratory staff *(1)*. Alternatively, detection of HBeAg using laboratory-based immunoassays, such as enzyme immunoassay (EIA) and chemiluminescence immunoassay (CLIA), or rapid diagnostic test (RDT) with lateral flow immunochromatographic assay, may largely overcome these limitations, because these tests may be more readily available and affordable (US$ 1–30/assay) than HBV DNA NAT in such settings *(17)*.

# 2. OBJECTIVES

## 2.1. Primary objective

To provide a summary estimate of the performance (sensitivity and specificity) of HBeAg tests in pregnant women with CHB with the purpose of diagnosing high HBV DNA levels for the assessment of eligibility for antiviral treatment during pregnancy to prevent HBV MTCT

The definition of high maternal HBV DNA levels that warrant antiviral therapy during pregnancy varies substantially according to the regional/national guidelines as below:

- EASL & AASLD 200 000 IU/mL *(13,14)*
- China 2 000 000 IU/mL *(18)*
- APASL 1 000 000–10 000 000 IU/mL *(19)*
- Australia & New Zealand 10 000 000 IU/mL

*Abbreviations: AASLD (American Association for the Study of Liver Diseases); APASL (Asian Pacific Association for the Study of the Liver); EASL (European Association for the Study of the Liver)*

The general eligibility criteria for initiating the immediate antiviral therapy in people with CHB set the HBV DNA thresholds much lower, but also require the presence of other disease parameters (i.e. liver inflammation and/or liver fibrosis):

- WHO 20 000 IU/mL *(20)*
- EASL, AASLD & APASL 2000 IU/mL *(13,14,19)*

Because of the variability in the definition of high HBV DNA levels as an eligibility criterion for antiviral therapy, in this systematic review we defined high HBV DNA levels to be any value greater than or equal to 20 000 IU/mL.

The summary estimates (sensitivity and specificity) were reported at each of the different HBV DNA thresholds used in the included studies. In addition, the following subgroup analyses were considered whenever there were at least three studies:

- Type of HBeAg assay
- Type of HBV DNA assay
- Mean/median maternal age
- Maternal coinfection with HIV, HCV or HDV
- Maternal HBV genotypes
- WHO region
- Study's risk of bias (high vs low)

## 2.2. Secondary objectives

The secondary objectives were:

- Secondary Objective 1

  To provide a summary estimate of the performance (sensitivity and specificity) of HBeAg tests in pregnant women with CHB to predict the risk of HBV MTCT, by type of HBeAg assay and type of preventive measures
- Secondary Objective 2

  To provide a summary estimate of the performance (sensitivity and specificity) of different serum HBV DNA thresholds in pregnant women with CHB to predict the risk of HBV MTCT, by type of preventive measure

These secondary objectives were assessed within the studies included for the primary objective; no additional systematic review was conducted to respond specifically to these secondary objectives.

We also provided a Grading of Recommendations Assessment, Development and Evaluation (GRADE) review, and identified gaps in research.

## 2.3. Post-hoc analyses

Following the Guidelines Development Group meeting held in Geneva on 9–10 October 2019, we conducted post-hoc analyses:

- Post-hoc analysis 1:

  To assess the maternal HBV DNA threshold during pregnancy at which the risk of HBV MTCT starts to increase despite infant's immunoprophylaxis (timely birth dose vaccine with or without HBIG)
- Post-hoc analysis 2:

  To provide a summary estimate of the performance (sensitivity and specificity) of HBeAg tests in pregnant women with CHB to diagnose HBV DNA threshold specifically defined by the analysis above

# 3. METHODS

## 3.1. Narrative review question

Can HBeAg test be used instead of NAT to diagnose high HBV DNA levels in order to assess eligibility for antiviral therapy initiation in pregnant women with CHB to prevent MTCT?

## 3.2. PICO questions

We obtained evidence to answer the following questions (Table 1).

Table 1. PICO questions for this systematic review

| | **PICO2A** | **PICO2B** | **PICO2C** |
|---|---|---|---|
| | **What is the performance of HBeAg to diagnose high HBV DNA levels in pregnant women with CHB?** | **What is the performance of HBeAg in pregnant women with CHB to predict the risk of MTCT?** | **What is the performance of different HBV DNA thresholds in pregnant women with CHB to predict the risk of MTCT?** |
| **P**opulation | Pregnant women with CHB[*1] without concomitant anti-HBV therapy | Same as PICO2A | Same as PICO2A |
| **I**ntervention | Maternal HBeAg test during pregnancy[*2] | Same as PICO2A | Maternal HBV DNA levels during pregnancy[*4]<br>• Dichotomized into high and low using the following thresholds: ≥20,000, 10^5, 10^6, 10^7 and 10^8 IU/mL |
| **C**omparison | Maternal HBV DNA levels during pregnancy[*3,4]<br>• Dichotomized into high and low using the following thresholds: ≥20 000, 10^5, 10^6, 10^7, & 10^8 IU/mL | MTCT defined as:<br>• HBsAg positivity or HBV DNA positivity in infants aged 6–12 months<br>• Extended to 6–24 months in infants who received three doses of HBV vaccine | Same as PICO2B |

| | | | |
|---|---|---|---|
| **O**utcomes | Sensitivity and specificity of HBeAg tests[*5] to diagnose each of different HBV DNA thresholds | Sensitivity and specificity of HBeAg tests to predict MTCT[*6] | Sensitivity and specificity of HBV DNA test to predict MTCT[*6] |

[*1] CHB was defined as HBsAg seropositivity on two occasions at least 6 months apart. However, because new HBV infections in adults are uncommon in highly endemic areas where the vast majority of HBsAg-positive people acquired the infection perinatally or during childhood, HBsAg positivity on only one occasion (at antenatal care) in women living in highly prevalent countries was assumed to reflect CHB *(21)*.

[*2] Maternal HBeAg test performed after child delivery was not considered. The test result should be reported positive or negative; an indeterminate result was not considered for the meta-analysis. Instead, the frequency of the indeterminate result in each study was extracted and reported. The following HBeAg immunoassays were considered:

- Lateral flow immunochromatographic rapid diagnostic test (RDT)
- Enzyme immunoassay (EIA)
- Chemiluminescence immunoassay (CLIA)
- Radioimmunoassay (RIA)
- Counting immunoassay (CIA)
- Fluoroimmunoassay (FIA)

[*3] It is ideal to have both HBeAg and HBV DNA measurements from the same sample, or at least from a sampling done at the same time. However, a study was still considered whenever both markers were measured during the same period of pregnancy, even if they were not measured using samples collected on the same day.

[*4] There are two types of NAT: qualitative (undetectable or detectable) and quantitative. When NAT provided a continuous value through quantification of HBV DNA levels, the value was dichotomized into high and low according to a threshold used in each included study. In order to have a wide range of estimations, the HBV DNA threshold used in each included study to dichotomize HBV DNA levels into high and low should have been greater than or equal to 20 000 IU/mL. Similarly, when NAT provided only a qualitative binary result (detectable or

undetectable), the limit of detection of the qualitative NAT should have been greater than or equal to 20 000 IU/mL.

[*5] For example, in the case of HBV DNA levels of ≥200 000 IU/mL, the sensitivity and specificity were defined as below:

- $sensitivity = \frac{No.\ of\ women\ with\ HBV\ DNA \geq 200\ 000\ IU/mL\ who\ tested\ positive\ for\ HBeAg}{Total\ no.\ of\ women\ with\ HBV\ DNA \geq 200\ 000\ IU/mL}$
- $specificity = \frac{No.\ of\ women\ with\ HBVDNA < 200\ 000 IU/mL\ \ who\ tested\ negative\ for\ HBeAg}{Total\ no.\ of\ women\ with\ HBV\ DNA < 200\ 000\ IU/mL}$

In order to have these estimates, a study needed to provide sufficient data for us to draw a 2x2 or 2x1 table with the cross-classification of the reference test results (high vs low HBV DNA levels) and the index test results (positive vs negative HBeAg serostatus).

[*6] The outcome was stratified by the type of preventive measures: timely birth dose vaccine (yes or no); and HBIG at birth (yes or no). We only considered studies in which sensitivity and specificity estimates could be stratified by the type of preventive measure provided to the mother–child pairs. We did not consider studies that provided antiviral therapy to mothers during pregnancy, since our objective is to evaluate these HBV markers as a tool to identify pregnant women who would benefit from antiviral therapy during pregnancy.

**Other inclusion and exclusion criteria: study design, languages, dates of publication**

We included studies with any design, published in any language, which used an HBV DNA threshold to dichotomize HBV DNA levels into high and low. This threshold needed to be at least higher than 20 000 IU/mL. Moreover, studies needed to provide sufficient data to draw a 2x1 or 2x2 table with the cross-classification of the reference test results (high vs low HBV DNA levels) and the index test results (positive vs negative HBeAg serostatus) in pregnant women with CHB without concomitant anti-HBV therapy. We excluded studies that selected participants based on the index test (i.e. maternal HBeAg status) to avoid verification bias *(22)*. Studies published between 1 January 2000 and 3 April 2019 were considered.

### 3.3. Post-hoc analyses

For the post-hoc analyses, we included studies evaluating mother–child pairs, in which child outcomes could be stratified by different maternal HBV DNA levels during pregnancy with a narrow range (≤1.0 log IU/mL; such as <4.0, 4.0–4.9, 5.0–5.9, 6.0–6.9, and ≥7.0 log IU/mL). At each stratum defined by maternal viral load, there should be ≥10 infants assessed for MTCT. We excluded studies that selected participants based on maternal HBeAg status or maternal viral load to avoid verification bias *(22)*. Studies published between 1 January 2000 and 3 April 2019 were considered.

### 3.4. Search strategy

The search terms employed covered "hepatitis B infection" AND "viral load" AND "pregnancy" and their variations. The databases searched included: four English-language databases (PubMed, EMBASE, Scopus, and CENTRAL (the Cochrane Library)); and two Chinese-language databases (the China National Knowledge Infrastructure [CNKI] and the Wanfang database). The search strategies used for each of the databases are presented in **Appendix A**.

A manual search through the references of the included studies, as well as through those of relevant systematic reviews identified through the literature search, was undertaken to identify any further eligible studies. Expert opinion was also sought to include other relevant studies.

### 3.5. Conduct of the review

Titles and abstracts for all of the publications identified by the search strategy were independently screened for relevance by two reviewers (PB and KY). Following selection of potentially eligible studies, full-text reading and reviewing was independently performed. Finally, the two reviewers discussed the list of eventually eligible studies, and if discrepancies existed that could not be resolved between the two reviewers, a third reviewer (YS) was consulted in order to make the final decision. For the Chinese databases, the same procedure was followed by two independent Chinese

reviewers (YL and TZ).

For all potentially eligible studies, if information was lacking within the full-text article that limited the ability to make a final decision on whether or not the study should be included, the corresponding author of that study was contacted by mail or phone.

The final protocol for this review was registered on the international prospective register of systematic reviews (PROSPERO) with the registration number: CRD42019138227 prior to starting the data analysis.

### 3.6. Quality appraisal

The quality of included studies was assessed independently by two reviewers.
Risk of bias and applicability of population, index and reference tests to the main review questions were evaluated using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) *(23)*. A list of the signalling questions used for QUADAS2 are presented in **Appendix B**.

### 3.7. Data extraction

The data were extracted from the selected studies by the two independent reviewers for each of the English (PB and KY) and Chinese articles (YL and TZ), using a pre-piloted data extraction form (**Appendix C**). In case of disagreement in the data extracted between the two reviewers, a deliberation that involved a third person (YS) was carried out.

During data extraction, articles from the same study sites with overlapping recruitment periods, enrolment criteria, and treatment types were considered as being part of one study. The lead reviewer for both English (PB) and Chinese (YL) articles then followed up with the corresponding author(s) from each of the article groups in order to understand if there was any patient overlap. If the authors explicitly stated in their article that there is overlap, or if the authors responded to the email inquiry confirming overlap, or if the author did not respond, then only the data extracted from the most recently published article was used in data analysis. If authors denied any patient overlap between articles then data extracted from all the articles within the group were used. In the

case of a group of articles from the same study where some articles were published in Chinese and some in English, the latest English article was included in the data analysis sheet, unless a direct communication with the study authors directed the reviewers to use a different article in the group.

### 3.8. Data synthesis

All statistical analyses were performed using STATA 14.2 (Stata Corp LP, College Station, TX).

Sensitivity and specificity were estimated at each of different HBV DNA thresholds used in the included studies (≥20 000 IU/mL, ≥5 $\log_{10}$ IU/mL, ≥6 $\log_{10}$ IU/mL, ≥7 $\log_{10}$ IU/mL and ≥8 $\log_{10}$ IU/mL). In case a single study presented the results at multiple HBV DNA thresholds, all the different thresholds were used. In addition, for PICO2B and 2C, sensitivity and specificity were estimated specifically for each measure of prevention for HBV MTCT. The summary statistics were pooled only when there were at least three studies.

We performed bivariate analysis for studies allowing estimation of both sensitivity and specificity. Study-specific estimates of sensitivity and specificity along with their 95% CI were graphically presented in coupled forest plots. When there were at least three studies, the summary estimates for sensitivity and specificity along with their 95% CI were obtained using the DerSimonian–Laird bivariate random effects model. Positive and negative likelihood ratios (PLR, NLR) with 95% CIs were obtained from the pooled sensitivity and specificity. When there were <3 studies, the range of sensitivity, specificity, PLR, and NLR, were presented. Pre-test probabilities were estimated by pooling the proportion of pregnant women with CHB who had high viral loads. After the variance of the proportions was stabilized using Freeman–Tukey double arcsine transformation, these estimates were pooled using the DerSimonian–Laird random-effects model *(24)*. Post-test probabilities were computed using the pre-test probabilities and the pooled PLR and NLR.

Heterogeneity in the estimates across the studies was visually assessed using: (i) coupled forest plots displaying study-specific estimates of sensitivity and specificity, and (ii) summary scatter plots. The summary scatter plots were presented without a summary receiver operating characteristic (SROC) curve for PICO2A and with a SROC curve for PICO2B and 2C.

Characteristics of the outlier studies were narratively described. A sensitivity analysis was also performed after excluding these outlier studies.

Heterogeneity was also assessed statistically, by considering the following variables as a priori potential sources of heterogeneity: type of HBeAg assay, type of reference standard (commercial PCR vs other/not reported), mean/median maternal age, maternal virological characteristics (HIV/HCV/HDV coinfection status and HBV genotypes), WHO region, and study's risk of bias (high vs low). In addition, for PICO2B and 2C, the measures of prevention for HBV MTCT were considered. Models fitted with and without the covariate were compared using likelihood ratio tests assuming equal variances. When there was good evidence ($P$<0.05) to support the heterogeneity, another model was fitted with separate variances and compared to the model with equal variances to understand if the heterogeneity observed could be due to the differences in variances between studies within a category rather than differences between categories of variables identified as potential sources of heterogeneity.

To integrate estimates from studies that provided data only for sensitivity or specificity, we performed univariate analyses using the DerSimonian–Laird univariate random effects model. When the study estimates could not be pooled (<3 studies), the range of sensitivity and specificity was presented.

Publication bias was assessed using Deeks' test, which was developed specifically for diagnostic accuracy reviews and is the method recommended in the Cochrane Handbook for Diagnostic Test Accuracy Reviews *(25)*. It tests the asymmetry of the plot of log diagnostic odds ratio (lnDOR) against 1/effective sample size $(ESS)^{1/2}$. The ESS is a function of the number of diseases ($n_1$) and non-diseased participants ($n_2$) $((4n_1{*}n_2)/(n_1 + n_2))$ and this takes into account the numbers of diseased and non-diseased participants *(26,27)*.

The post-hoc analysis 1 was conducted as below. For the studies using "IU/mL" as a unit for HBV DNA levels, MTCT risk was estimated for the following maternal HBV DNA levels during pregnancy: <4.00; 4.00–4.99; 5.00–5.99; 6.00–6.99; and ≥7.00 $\log_{10}$ IU/mL. For those using "copies/mL", the maternal viral load was transformed into "IU/mL" (by dividing by the factor of 5) and risk was estimated for the following HBV DNA levels: <4.30; 4.30–5.29; 5.30–6.29; 6.30–

7.29; and ≥7.30 $\log_{10}$ IU/mL. In case a single study presented the results at multiple HBV DNA thresholds, all different thresholds were used.

Once the HBV DNA level where the risk of MTCT was identified despite infants' immunoprophylaxis, post-hoc analysis 2 was conducted. The sensitivity and specificity of the HBeAg test to diagnose this HBV DNA threshold were estimated using the method described above.

### 3.9. GRADE review process

For each examined PICO question, the quality of the evidence was evaluated using the Grading of Recommendations Assessment, Development and Evaluation methodology (GRADE) *(28)*. We used this tool to evaluate: (i) the risk of bias; (ii) inconsistency (high heterogeneity); (iii) imprecision (confidence intervals); (iv) indirectness (use of surrogate outcomes); (v) reporting and publication bias; and (vi) other factors; for each of the outcomes. This eventually gave a score of high (further research is very unlikely to change the effect estimate), moderate, low or very low (all estimates are very uncertain). Decisions for the complex judgements within the GRADE table were made through study group consensus. The study group reviewers were supported in the process of completing this GRADE template through discussion and advice from a WHO-designated methodological expert, Professor Roger Chou (Oregon Health & Science University, USA). For this specific meta-analysis, the following rules were used to determine whether or not a group of studies had no serious, serious, or very serious issues with regard to GRADE criteria:

- GRADE scoring system:

  As cohorts and cross-sectional studies can provide reliable evidence for diagnostic accuracy, strength of evidence was initially rated as high quality *(29)*. Then, strength of evidence was lowered by one degree if there was "serious" and by two degrees if there was "very serious" risk of bias, inconsistency, indirectness or imprecision. The strength of evidence was similarly lowered by one degree if publication bias was "likely" and by two degrees if the bias was "very likely" *(30)*.
- Risk of bias:

  A study was considered as "high" overall risk of bias when multiple QUADAS-2

domains were rated as “high risk of bias”. Then, for each outcome, the number of studies with “high risk of bias” was counted. The risk of bias for the outcome was rated as “very serious”, “serious” or “not serious” when the proportion of studies rated as “high risk of bias” was >75%, >50–75% or ≤50%, respectively.

- Indirectness:

  Indirectness is linked with the level of applicability of the study population, index test or reference standard to the review question. A study was considered as “high” overall concern about applicability when at least one out of the three QUADAS-2 domains was rated as “high concern about applicability”. Then, for each outcome, the number of studies with “high concern about applicability” was counted. Indirectness for the outcome was rated as “very serious”, “serious” or “not serious” when the proportion of studies rated as “high concern about applicability” was >75%, >50–75% or ≤50%, respectively *(29)*.

- Imprecision:

  Imprecision was considered “not serious” when an absolute range in the 95% confidence intervals (95% CI) for a pooled sensitivity or specificity was ≤20%. Imprecision was “serious” or “very serious” when the range was 20–40% or >40%. Moreover, when the cumulated sample size for all included studies was <30, it was categorized as “very serious”.

- Inconsistency:

  Inconsistency was considered “not serious” when ≥75% of studies’ estimates were within +/–20% of the pooled estimate for an outcome. Inconsistency was considered “serious” or “very serious” when this proportion was 50–75% or <50%.

- Publication bias:

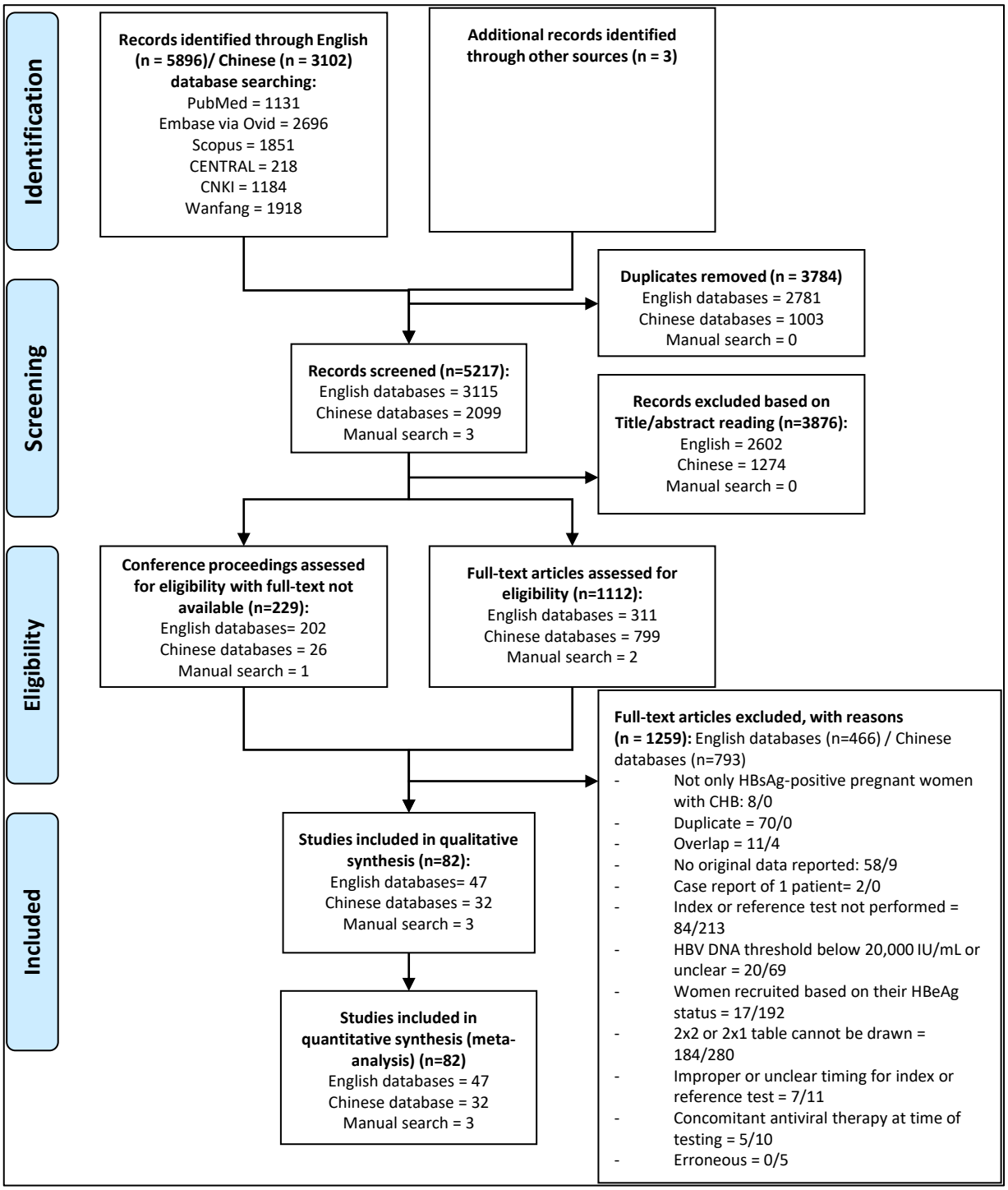
  This was not assessed as part of the GRADE, because none of the studies, except one, was designed to assess diagnostic accuracy. Therefore, the analysis based on the diagnostic odds ratio seemed irrelevant.

# 4. RESULTS

## 4.1. Flowchart for PICO questions

The search strategy identified a total of 9001 papers. After excluding 3784 articles in duplicate, 5217 were screened, and 1341 papers potentially eligible for the study were fully assessed. Finally, 82 papers were included in this review (Fig. 1). Reasons for exclusion are presented in Fig. 1.

## Fig. 1. PRISMA diagram



## 4.2. PICO2A: Performance of HBeAg to identify women with high HBV DNA viral loads

Detailed characteristics of each included study are presented in Table 2.

Table 2. Characteristics of the 82 studies included for the PICO2A

| Author, year | PICO2A | Country | Original study design | HBV DNA threshold(s) (IU/mL) | HBV DNA assay | HBeAg assay | MTCT clinical endpoint | Participants with both index and reference standard test, n | Maternal age (years) | HIV, n (%) | Children tested, n |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bai H, 2010 *(31)* | Bivariate | China | Retrospective cohort | 5 log10 | PCR Da'an Gene | FIA (Anytest SYM-BIO) | No | 120 | Mean (SD): 27.2 (3.2) | Unknown | N/A |
| Bissinger A, 2013 *(32)* | Bivariate | Germany | Cross-sectional | 5 log10 | PCR COBAS Taqman (Roche) | ELISA (ETI-EBK PLUS Diasorin) | No | 183 | Median: 31 | Unknown | N/A |
| Cai J, 2005 *(33)* | Bivariate | China | Cross-sectional | 5 log10 | Real-Time Fluorescence quantitative PCR (PG Biotech) | ELISA (WB-2496 HBeAg Wantai) | No | 709 | Unknown | Unknown | N/A |
| Cai S, 2009 *(34)* | Bivariate | China | Cross-sectional | 20,000 | Real-Time Fluorescence quantitative PCR (Kelong Bio-hightech) | ELISA (Intec HBeAg) | No | 198 | Unknown | Unknown | N/A |
| Carey I, 2017 *(35)* | Univariate (Specificity) | UK | Cross-sectional | 5 log10 | PCR COBAS Taqman (Roche) | CLIA (Architect Abbott) | No | 238 | Unknown | Unknown | N/A |
| Carey I, 2018 *(36)* | Bivariate | UK | Cross-sectional | 5 log10 | PCR COBAS Taqman (Roche) | CLIA (Architect Abbott) | No | 514 | Median: 31 | Unknown | N/A |
| Chang C, 2017 *(37)* | Univariate (Sensitivity) | USA | Retrospective cohort | 5 log10 | PCR (unknown manufacturer) | Unknown | No | 32 | Mean (SD): 29 (4) | Unknown | N/A |
| Chen T, 2018 *(38)* | Bivariate | China | Prospective cohort | 6 log10 | PCR Da'an Gene | CLIA (Architect Abbott) | Yes | 499 | Median (Range): 27 (19-42) (n=951) | Unknown | 499 |
| Chen T, 2018 *(39)* | Univariate (Sensitivity) | China | Prospective cohort | 6 log10 | PCR Da'an Gene | CLIA (Architect Abbott) | Yes | 48 | Median (Range): 24.25 (19-38) | 0/48 (0.0%) | 48 |
| Chen Z, 2017 *(40)* | Bivariate | China | Prospective cohort | 5 log10 | Real-Time Fluorescence quantitative PCR (Kehua Bio-engineering) | ELISA (AxSYM Abbott) | Yes | 211 | Mean: 26.96 | 0/211 (0.0%) | 168 |
| Cheng S, 2006 *(41)* | Bivariate | China | Prospective cohort | 20,000 | PCR (unknown manufacturer) | ELISA (Unknown | No | 110 | Unknown | Unknown | N/A |

| | | | | | | manufacturer) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cheung K, 2019 *(42)* | Bivariate | China | Cross-sectional | 6, 7 & 8 log10 | PCR COBAS Taqman (Roche) | CLIA (Elecsys Roche) | No | 325 | Median (IQR): 32 (26-38) | Unknown | N/A |
| Chotun N, 2017 *(43)* | Bivariate | South Africa | Prospective cohort | 20,000 & 5 log10 | PCR COBAS Taqman (Roche) | CLIA (Architect Abbott) | Yes | 6 | Mean (Range): 27 (21-34) | 0/6 (0.0%) | 4 |
| Dervisevic S, 2007 *(44)* | Bivariate | UK | Prospective cohort | 5 log10 | PCR COBAS Taqman (Roche) | Unknown | No | 114 | Unknown | Unknown | N/A |
| Ding Y, 2013 *(45)* | Bivariate | China | Prospective cohort | 7 log10 | PCR COBAS Taqman (Roche) | CLIA (Architect Abbott) | Yes | 249 | Unknown (Range: 16-45) (n=4536) | Unknown | 249 |
| Dolman G, 2018 *(46)* | Bivariate | UK | Cross-sectional | 5 log10 | Unknown | CLIA (Architect Abbott) | No | 423 | Median (IQR): 30 (28-35) | Unknown | N/A |
| Dopico E, 2013 *(47)* | Bivariate | Spain | Cross-sectional | 7 log10 | PCR Abbott | CLIA (Ortho Vitros) | No | 4 | Unknown | Unknown | N/A |
| Dyson J, 2014 *(48)* | Bivariate | UK | Retrospective cohort | 20,000 & 7 log10 | Unknown | Unknown | No | 56 | Median (Range): 28 (18-40) (n=81) | 2/81 (2.5%) | N/A |
| Eilard A, 2019 *(49)* | Bivariate | Sweden | Prospective cohort | 7 log10 | PCR COBAS Taqman (Roche) | CLIA (Architect Abbott) | Yes | 42 | Mean: 32.3 | 0/42 (0.0%) | 44 |
| Elefsiniotis I, 2007 *(50)* | Bivariate | Greece | Cross-sectional | 7 log10 | PCR Amplicor HBV Monitor test (Roche) | ELISA (AxSYM Abbott) | No | 63 | Unknown | Unknown | N/A |
| Foaud HM, 2019 *(51)* | Bivariate | Egypt | Prospective cohort | 5 log10 | Real-time PCR MX 400 | ELISA (ETI-EBK PLUS Diasorin) | Yes | 41 | Mean: 27.3 | 0/41 (0.0%) | 39 |
| Fujiko M, 2015 *(52)* | Bivariate | Indonesia | Cross-sectional | 6 log10 | PCR COBAS Taqman (Roche) | ELISA (Monolisa HBeAg-Ab PLUS Bio-Rad) | No | 64 | Median (Range): 29 (Range: 18-42) | 0/64 (0.0%) | N/A |
| Godbole G, 2013 *(53)* | Bivariate | UK | Cross-sectional | 6 & 7 log10 | PCR Abbott | CLIA (Elecsys Roche or Architect Abbott) | No | 293 | Median (Range): 29 (15-46) | Unknown | N/A |
| Guo F, 2007 *(54)* | Bivariate | China | Cross-sectional | 6 log10 | Real-Time Fluorescence quantitative PCR (PG Biotech) | ELISA (Intec HBeAg) | No | 319 | Unknown | Unknown | N/A |
| Han Y, 2007 *(55)* | Bivariate | China | Prospective cohort | 20,000 | PCR (Haoyuan Biotech) | CLIA (Elecsys Roche) | No | 69 | Mean (Range): 26 (22-38) | Unknown | N/A |
| Hao X, 2015 *(56)* | Bivariate | China | Prospective cohort | 5 log10 | Real-Time Fluorescence quantitative PCR (Shanghai Fosun | ELISA (Kehua HBeAg test) | No | 154 | Mean (SD): 27.4 (4.3) | Unknown | N/A |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Pharmaceutical Group) | | | | | | |
| Hu Q, 2007 *(57)* | Bivariate | China | Cross-sectional | 20,000 & 7 log10 | Real-Time Fluorescence quantitative PCR (PG Biotech) | ELISA (Robio RongSheng) | No | 420 | Mean (Range): 25 (18-37) (n=3658) | 0/420 (0.0%) | N/A |
| Huang L, 2014 *(58)* | Bivariate | China | Retrospective cohort | 5 log10 | Unknown | ELISA (Dayou HBeAg Dakewe) | No | 324 | Unknown (Range: 20-36) | Unknown | N/A |
| Jackson V, 2015 *(59)* | Univariate (Sensitivity) | Ireland | Retrospective cohort | 6 log10 | Real-Time Fluorescence quantitative PCR (Kehua Bio-engineering) | CLIA (Architect Abbott) | Yes | 36 | Median (Range): 26 (16-40) | Unknown | 21 |
| Jin C, 2007 *(60)* | Bivariate | China | Cross-sectional | 6 log10 | PCR Da'an Gene | Unknown | No | 204 | Unknown | Unknown | N/A |
| Johannessen A, 2017 *(61)* | Bivariate | Ethiopia | Prospective cohort | 6 log10 | PCR Abbott | ELISA (Vidas HBeAg/anti-Hbe Biomérieux) | No | 63 | Median (Range): 28 (18-41) (n=131) | Unknown | N/A |
| Köse S, 2011 *(62)* | Bivariate | Turkey | Retrospective cohort | 20,000 & 5,6,7 log10 | PCR COBAS Taqman (Roche) | ELISA (Liaison Diasorin) | No | 7 | Mean 26.5 (4.0) | 0/7 (0.0%) | N/A |
| Kubo A, 2014 *(63)* | Bivariate | USA | Retrospective cohort | 5 &7 log10 | Real-Time Fluorescence quantitative PCR (Kehua Bio-engineering) | ELISA (Unknown manufacturer) | Yes | 835 | Mean (SD): 31 (5.3) (n=4446) | Unknown | 835 |
| Lao PDR T, 2012 *(64)* | Bivariate | China | Cross-sectional | 8 log10 | PCR COBAS Taqman (Roche) | Unknown | No | 56 | Mean: 31.95 | Unknown | N/A |
| Latthaphasavang V, 2019 *(65)* | Bivariate | Lao PDR | Prospective cohort | 5 log10 | Real-time PCR (Fast-track Diagnostics) | ELISA (Monolisa HBeAg-Ab PLUS Bio-Rad) | Yes | 153 | Median (IQR): 28 (24-30) | 1/153 (0.7%) | 120 |
| Lee L, 2015 *(66)* | Bivariate | Singapore | Prospective cohort | 20,000 | PCR artus HBV PCR Kits, QIAGEN | CLIA (Architect Abbott) | Yes | 161 | Mean (SD): 32.4 (4.7) | Unknown | 154 |
| Li X, 2018 *(67)* | Bivariate | China | Retrospective cohort | 20,000 & 5, 6, 7 log10 | Real-Time Fluorescence quantitative PCR (Shanghai Fosun Pharmaceutical Group) | CLIA (Architect Abbott) | No | 716 | Unknown | 0/716 (0.0%) | N/A |
| Li Y, 2013 *(68)* | Bivariate | China | Prospective cohort | 20,000 & 7 log10 | Real-Time Fluorescence quantitative PCR (Toyobo) | ELISA (Unknown manufacturer) | No | 140 | Unknown | 0/140 (0.0%) | N/A |
| Liang P, 2017 *(69)* | Bivariate | China | Cross-sectional | 5 log10 | ABI 7500 real-time PCR system (Applied Biosystems) | CLIA (Architect Abbott) | No | 391 | Unknown | 0/391 (0.0%) | N/A |
| Lin J, 2002 *(70)* | Bivariate | China | Retrospective cohort | 20,000 & 5, 6 log10 | Real-Time Fluorescence quantitative | ELISA (WB-2496 | No | 69 | Unknown | Unknown | N/A |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | PCR (Biotromics technologies) | HBeAg Wantai) | | | | | |
| Liu CP, 2015 *(71)* | Univariate (Sensitivity) | China | Retrospective cohort | 6 log10 | PCR Da'an Gene | ELISA (HBeAg Santa-Cruz) | Yes | 256 | Mean: 25.7 | 0/256 (0.0%) | 256 |
| Liu H, 2013 *(72)* | Bivariate | China | Case-control | 5 log10 | PCR Da'an Gene | CLIA (Elecsys Roche) | No | 259 | Mean (SD): 28 (4) | Unknown | N/A |
| Liu Y, 2016 *(73)* | Univariate (Sensitivity) | China | Prospective cohort | 6 log10 | ABI 7500 real-time PCR system (Applied Biosystems) | CLIA (Architect Abbott) | Yes | 110 | Mean 27.7 | 0/110 (0.0%) | 78 |
| Lu H, 2009 *(74)* | Bivariate | China | Prospective cohort | 5 & 8 log10 | Real-Time Fluorescence quantitative PCR (PG Biotech) | FIA (Anytest SYM-BIO) | No | 33 | Mean (Range): 26.2 (21-34) | Unknown | N/A |
| Lunel-Fabiani F, 2018 *(75)* | Bivariate | Cameroun | Prospective cohort | 20,000 & 5, 6, 7, 8 log10 | HBV Aptima, Hologic | Unknown | Yes | 594 | Median (IQR): 24 (20-30) | 1/592 tested (0.2%) | 133 |
| Ma Y, 2014 *(76)* | Bivariate | China | Prospective cohort | 20,000 | PCR (unknown manufacturer) | ELISA (Unknown manufacturer) | No | 675 | Mean (SD): 26.5 (2.8) | Unknown | N/A |
| Michitaka K, 2012 *(77)* | Bivariate | Japan | Prospective cohort | 6 log10 | Real-Time Fluorescence quantitative PCR (Kehua Bio-engineering) | CLIA (Unknown manufacturer) | No | 21 | Median (IQR): 34 (IQR: 25-40) | Unknown | N/A |
| Peng N, 2012 *(78)* | Bivariate | China | Cross-sectional | 5 log10 | Real-Time Fluorescence quantitative PCR (Kehua Bio-engineering) | CLIA (Architect Abbott) | No | 410 | Unknown | Unknown | N/A |
| Peng S, 2019 *(79)* | Bivariate | China | Prospective cohort | 6 log10 | PCR Da'an Gene | ELISA (Kehua HBeAg) | Yes | 1219 | Mean: 29.17 | 0/1219 (0.0%) | 93 |
| Pirillo M, 2007 *(80)* | Bivariate | Uganda and Rwanda | Cross-sectional | 6 log10 | In-house PCR | ELISA (Murex HBeAg/anti-Hbe) | No | 7 | Unknown | 7/7 (100.0%) | N/A |
| Pirillo M, 2015 *(81)* | Bivariate | Malawi | Prospective cohort | 20,000 & 5, 6, 7, 8 log10 | Versant kPCR HBV DNA 1.0 (Siemens) | CLIA (Architect Abbott) | Yes | 21 | Median (IQR): 27 (23-30) (n=27) | 27/27 (100.0%) | 17 |
| Punzalan C, 2012 *(82)* | Bivariate | USA | Retrospective cohort | 7 log10 | Unknown | Unknown | Yes | 200 | Unknown | Unknown | 200 |
| Rouet F, 2004 *(83)* | Bivariate | Ivory Coast | Cross-sectional | 5 log10 | Non-PCR (Quantiplex HBV DNA, Bayer) | ELISA (Murex HBeAg/anti-Hbe) | No | 77 | Mean: 25 (n=501) | 45/77 (58.4%) | N/A |
| Samadi Kochaksaraei G, 2016 *(84)* | Bivariate | Canada | Prospective cohort | 7 log10 | PCR COBAS Taqman (Roche) | CLIA (Architect Abbott) | No | 99 | Median (IQR): 32 (29-35) | 0/99 (0.0%) | N/A |
| Ségéral O, 2018 *(85)* | Bivariate | Cambodia | Prospective cohort | 5 & 6 & 7 log10 | PCR targeting the S gene of HBV (PUMA HBV kit, Omunis) | RDT (SD Bioline HBeAg test) | No | 128 | Median (IQR): 29 (25-32) | Unknown | N/A |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | (n=250) | | |
| Sellier P, 2017 *(86)* | Univariate (Sensitivity) | France | Prospective cohort | 5 log10 | PCR COBAS Taqman (Roche) | CLIA (Architect Abbott) | No | 27 | Mean (SD): 28.2 (4.7) | 0/27 (0.0%) | N/A |
| Seo K, 2018 *(87)* | Univariate (Sensitivity) | DR Korea | Retrospective cohort | 6 log10 | PCR COBAS Taqman (Roche) | RIA (Abbott-Hbe Abbott) | No | 41 | Median (Range): 32 (22-40) | 0/41 (0.0%) | N/A |
| Shao Z, 2011 *(88)* | Bivariate | China | Prospective cohort | 5 & 8 log10 | PCR Amplicor HBV Monitor test (Roche) | ELISA (AxSYM Abbott) | Yes | 212 | Unknown | Unknown | 158 |
| Sheng Q, 2018 *(89)* | Bivariate | China | Prospective cohort | 6 log10 | PCR COBAS Taqman (Roche) | CLIA (Architect Abbott) | No | 441 | Mean (SD): 31.1 (4.5) (n=14314) | Unknown | N/A |
| Shi Y, 2012 *(90)* | Bivariate | China | Cross-sectional | 20,000 & 7 log10 | Real-Time Fluorescence quantitative PCR (Kehua Bio-engineering) | ELISA (Suzhou Sym-Bio) | No | 452 | Unknown | Unknown | N/A |
| Söderström A, 2003 *(91)* | Bivariate | Sweden | Retrospective cohort | 5,6 & 7 log10 | PCR Amplicor HBV Monitor test (Roche) | ELISA (AxSYM Abbott) | Yes | 46 | Unknown | Unknown | 264 |
| Tang A, 2018 *(92)* | Univariate (Sensitivity) | USA | Cross-sectional | 5 log10 | Real-Time Fluorescence quantitative PCR (Kehua Bio-engineering) | CLIA or ELISA (ELISA: Quest and BioReference; CLIA: LabCorp) | No | 203 | Mean (Range): 29.2 (18-55) (n=978) | Unknown | N/A |
| Tang Y, 2013 *(93)* | Bivariate | China | Cross-sectional | 5 &7 log10 | Real-Time Fluorescence quantitative PCR (Kehua Bio-engineering) | ELISA (Kehua HBeAg test) | No | 500 | Mean (Range): 27.8 (18-40) | Unknown | N/A |
| Thilakanathan C, 2018 *(94)* | Bivariate | Australia | Retrospective cohort | 5 & 6 log10 | PCR COBAS Taqman (Roche) | CLIA (Architect Abbott) | Yes | 642 | Median (Range): 30 (15-44) | Unknown | 469 |
| Van Zonneveld M, 2003 *(95)* | Univariate (Sensitivity) | The Netherlands | Prospective cohort | 6 log10 | Non-PCR (Digene Hybrid Capture I/II assay) | Unknown | Yes | 8 | Median (Range): 20 (17-25) | 0/8 (0.0%) | 8 |
| Wang F, 2009 *(96)* | Bivariate | China | Retrospective cohort | 5 log10 | Real-Time Fluorescence quantitative PCR (Sangon) | ELISA (Kehua HBeAg test) | No | 1237 | Unknown | Unknown | N/A |
| Wang J, 2015 *(97)* | Univariate (Sensitivity) | China | Prospective cohort | 6 log10 | PCR Da'an Gene | CLIA (Architect Abbott) | Yes | 48 | Mean (SD): 29.31 (3.82) | 0/48 (0.0%) | 48 |
| Wang J, 2018 *(98)* | Bivariate | China | Cross-sectional | 6 log10 | Real-Time Fluorescence quantitative PCR (Kehua Bio-engineering) | Unknown | No | 567 | Mean (SD): 27.27 (3.35) (n=14132) | Unknown | N/A |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| Wang L, 2016 *(99)* | Bivariate | China | Randomized controlled trial | 20,000 & 5,6, 7 log10 | PCR Abbott | FIA (Anytest SYM-BIO) | No | 31 | Median (Range): 28.52 (19-39) | 31/31 (100.0%) | N/A |
| Wang M, 2018 *(100)* | Bivariate | China | Retrospective cohort | 5 log10 | PCR Abbott | Unknown | No | 76 | Unknown (Range: 20-40) | 0/76 (0.0%) | N/A |
| Wang X, 2015 *(101)* | Bivariate | China | Prospective cohort | 5 log10 | Real-Time Fluorescence quantitative PCR (Shanghai Fosun Pharmaceutical Group) | CLIA (Architect Abbott) | No | 409 | Unknown | 0/409 (0.0%) | N/A |
| Wang X, 2015 *(102)* | Bivariate | China | Case-control | 5 log10 | PCR COBAS Taqman (Roche) | ELISA (Unknown manufacturer) | No | 120 | Unknown | Unknown | N/A |
| Wang Z, 2018 *(103)* | Bivariate | China | Cross-sectional | 6 log10 | Unknown | Unknown | No | 253 | Mean (SD): 29.38 (4.77) | Unknown | N/A |
| Wei K, 2017 *(104)* | Bivariate | China | Cross-sectional | 5, 6, 7 & 8 log10 | PCR Abbott | CLIA (Architect Abbott) | No | 1741 | Median (Range): 26 (15-43) (n=1741) | 0/1741 (0.0%) | N/A |
| Wiseman, E, 2009 *(105)* | Bivariate | Australia | Prospective cohort | 5 & 8 log10 | PCR Amplicor HBV Monitor test (Roche) | CLIA or ELISA (AxSYM Abbott or Architect Abbott) | Yes | 313 | Unknown | 0/313 (0.0%) | 138 |
| Xu C, 2018 *(106)* | Bivariate | China | Cross-sectional | 6 log10 | Real-time fluorescence quantitative PCR reagents (Shenyou Biotechnology) | CLIA or ELISA (Kehua HBeAg or Architect Abbott) | No | 214 | Median (IQR): 25.3 (22.7-30.2) | 0/214 (0.0%) | N/A |
| Xu H, 2008 *(107)* | Bivariate | China | Cross-sectional | 20,000 & 6 log10 | Real-Time Fluorescence quantitative PCR (PG Biotech) | ELISA (Intec HBeAg) | No | 198 | Unknown | Unknown | N/A |
| Yin Yu-zhu, 2013 *(108)* | Bivariate | China | Retrospective cohort | 5 &7 log10 | PCR Da'an Gene | ELISA (Intec HBeAg) | Yes | 1355 | Mean (SD): 28.83 (3.92) | Unknown | 1360 |
| Zhang L, 2017 *(109)* | Bivariate | China | Cross-sectional | 20,000 & 5, 6, 7, 8 log10 | Real-Time Fluorescence quantitative PCR (Sansure Biotech) | ELISA (Unknown manufacturer) | No | 491 | Unknown | Unknown | N/A |
| Zhang Q, 2009 *(110)* | Bivariate | China | Retrospective cohort | 20,000 | PCR (unknown manufacturer) | ELISA (Unknown manufacturer) | No | 80 | Unknown | Unknown | N/A |
| Zheng H, 2010 *(111)* | Bivariate | China | Case-control | 6 log10 | Real-Time Fluorescence quantitative PCR (Kehua Bio-engineering) | ELISA (Kehua HBeAg test) | No | 478 | Mean (SD): 27 (6.2) | 0/478 (0.0%) | N/A |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Zhu B, 2013 *(112)* | Bivariate | China | Retrospective cohort | 5 log10 | PCR COBAS Taqman (Roche) | CLIA (Elecsys Roche) | Yes | 53 | Mean (SD): 26 (4.1) | 0/53 (0.0%) | 53 |

### 4.2.1. Summary of included studies

There were 82 original studies, including 71 allowing estimation of both sensitivity and specificity, using bivariate analysis, and 11 that can be only used for the univariate analysis to estimate sensitivity only (n=10) or specificity only (n=1). Except for one randomized controlled trial, all were observational studies: 28 cross-sectional, 50 cohorts (31 prospective and 19 retrospective) and 3 case–control studies (Table 2).

Table 3 summarizes the characteristics of studies included in the PICO2A. Across all 82 studies, recruitment took place as early as 1989 and up until 2018. Most studies took place in the WHO Western Pacific Region (n=54); including China (n=47), Cambodia (n=1), Democratic Republic of Korea (n=1), Japan (n=1), Lao People's Democratic Republic (n=1), Singapore (n=1) and Australia (n=2). Fifteen studies took place in the WHO European Region: the United Kingdom (n=6), Sweden (n=2), France (n=1), Germany (n=1), Ireland (n=1), the Netherlands (n=1), Greece (n=1), Spain (n=1) and Turkey (n=1). Six studies took place in the WHO African Region: Ivory Coast (n=1), Uganda and Rwanda (n=1), Ethiopia (n=1), Malawi (n=1), South Africa (n=1), and Cameroon (n=1). Five studies took place in WHO region of the Americas: United States of America (n=4) and Canada (n=1). One study took place in the WHO South-East Asia Region in Indonesia, and one study took place in the WHO Eastern Mediterranean Region in Egypt (Table 2).

Of 82 studies, 52 reported maternal age; mean/median maternal age was higher in EUR/AMR regions (7/10 studies reporting a mean/median maternal age ≥28 years in EUR and 4/4 in AMR) and lower in the AFR Region (only n=1/4 reporting mean/median maternal age ≥28 years). EIA was most frequently used to assess pregnant women's HBeAg status (n=35/72, 48.6%) followed by CLIA (n=29/72, 40.3%) for all WHO regions except EUR where CLIA was more frequently used than EIA (8 using CLIA vs 4 using EIA). FIA (time-resolved fluoroimmunoassay) was used only in Chinese studies (n=3/72, 4.2%). Only 1 study (1.4%) used a rapid diagnostic test (*Ségéral O et al., 2018*). Most studies exclusively included women without coinfection: n=40/60 (66.7%)

for HIV, n=41/50 (82.0%) for HCV and n=26/30 (86.7%) for HDV). Only a few studies reported HBV genotypes (n=24/82) and, of this, the majority had mixed genotypes (n=18/24, 75.0%). Only two studies reported the frequency of indeterminate HBeAg results: *Kubo A et al., 2014* (n=4/835 (0.5%)) and *Tang A et al., 2018* (n=3/200 (1.5%)).

Table 3. Characteristics of studies included in PICO2A

| Subgroup variables | Subgroup category | Studies (N) | % |
|---|---|---|---|
| **Overall** | | 82 | 100.0 |
| **WHO region (n=82)** | **WPR** | 54 | 65.9 |
| | **SEAR** | 1 | 1.2 |
| | **EUR** | 15 | 18.3 |
| | **EMR** | 1 | 1.2 |
| | **AFR** | 6 | 7.3 |
| | **AMR** | 5 | 6.1 |
| **HBeAg assay type (n=72)** | **EIA** | 35 | 48.6 |
| | **FIA** | 3 | 4.2 |
| | **CLIA** | 29 | 40.3 |
| | **RDT** | 1 | 1.4 |
| | **Multiple** | 3 | 4.2 |
| | **CIA/RIA** | 1 | 1.4 |
| **Mean / median maternal age (n=52)** | **<28 years** | 24 | 46.2 |
| | **≥28 years** | 28 | 53.8 |
| **HIV status (n=60)** | **All uninfected** | 40 | 66.7 |
| | **All infected** | 11 | 18.3 |
| | **Mixed population** | 9 | 15.0 |
| **HCV status (n=50)** | **All uninfected** | 41 | 82.0 |
| | **All infected** | 0 | 0.0 |
| | **Mixed population** | 9 | 18.0 |
| **HDV status (n=30)** | **All uninfected** | 26 | 86.7 |
| | **All infected** | 0 | 0.0 |

| | | | |
|---|---|---|---|
| | **Mixed population** | 4 | 13.3 |
| **Genotype (n=24)** | **Genotype A** | 2 | 8.3 |
| | **Genotype B** | 0 | 0.0 |
| | **Genotype C** | 1 | 4.2 |
| | **Genotype D** | 3 | 12.5 |
| | **Genotype E** | 0 | 0.0 |
| | **Mixed population** | 18 | 75.0 |

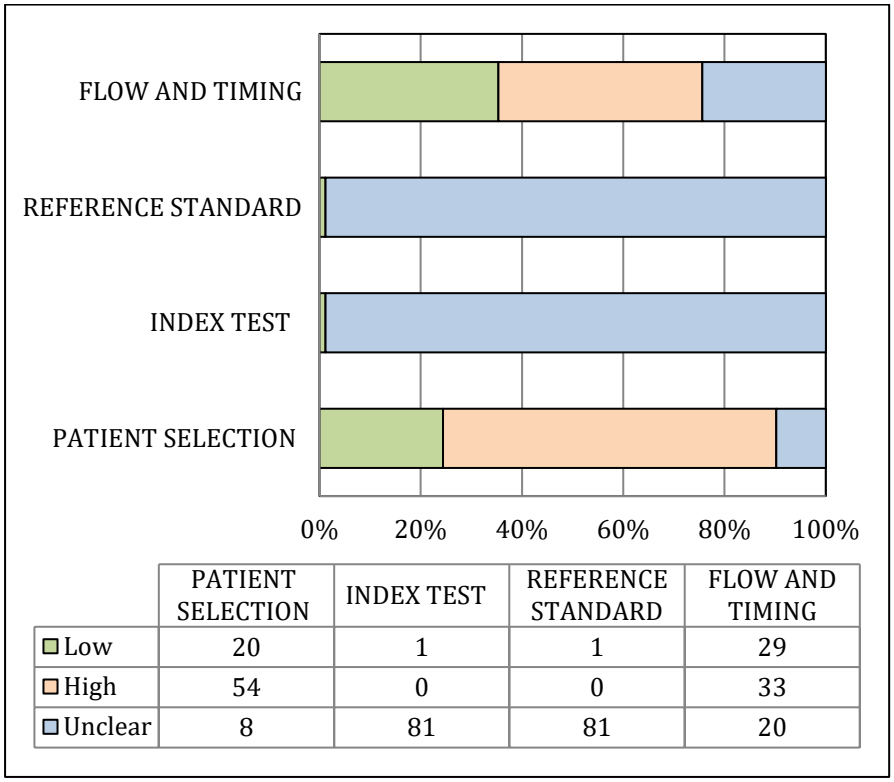
### 4.2.2. Risk of bias assessment

The risk of bias assessment is presented in Fig. 2 and 3 (full details are provided in **Appendix D**).

Only one study was designed to evaluate the diagnostic performance of an HBeAg test in pregnant women, and had the same objective as that of the PICO2A *(85)*. Therefore, for the other diagnostic studies included, essential information was often missing. Except for the one study that shared the same objectives as PICO2A *(85)*, all were rated as having an unclear risk of bias for both the reference standard and the index tests due to the fact that they did not state whether or not blinding was performed (Fig. 2). The risk of selection bias was high in most studies (n=54/82, 65.9%), mainly due to restrictive exclusion criteria (e.g. exclusion of HIV-, HCV-, or HDV-coinfected pregnant women).

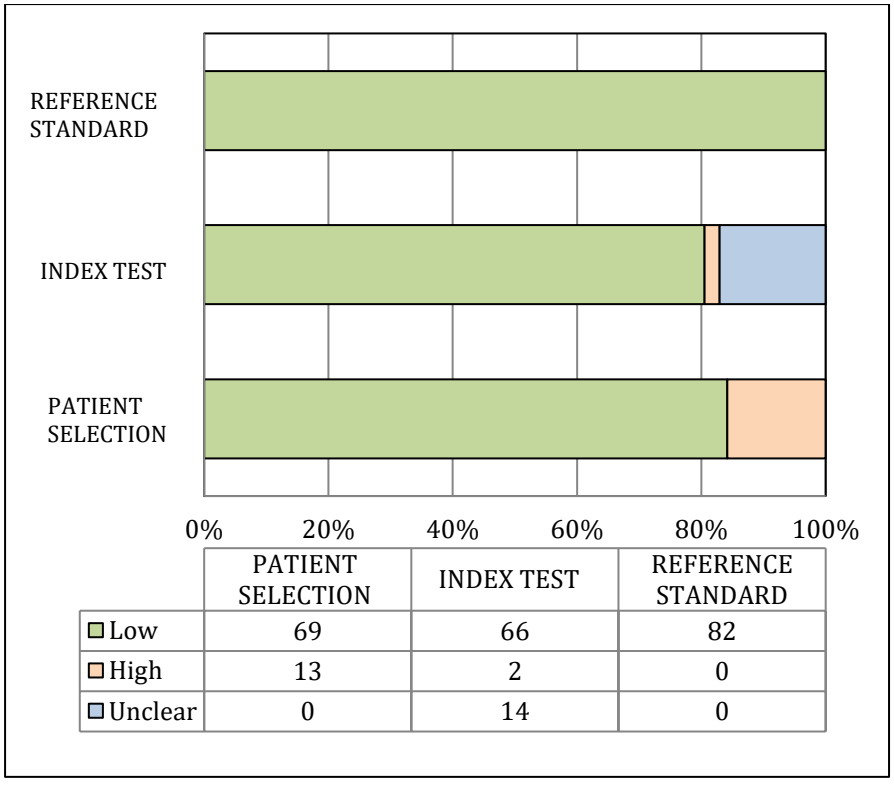
Concerns about applicability were low for most studies (Fig. 3). There was high concern for patient selection applicability in 13 studies (15.9%), due to the enrolment of pregnant women based on their HBV viral load. For two studies there was concern regarding the applicability of the index test, because HBeAg status was not systematically ascertained during the pregnancy. This was unclear for a further 14 studies (17.1%) that did explicitly report the timing of HBeAg testing or type of HBeAg assay test used.

Fig. 2. Risk of bias results for PICO2A




| | PATIENT SELECTION | INDEX TEST | REFERENCE STANDARD | FLOW AND TIMING |
|---|---|---|---|---|
| Low | 20 | 1 | 1 | 29 |
| High | 54 | 0 | 0 | 33 |
| Unclear | 8 | 81 | 81 | 20 |

Fig. 3. Concerns about applicability results for PICO2A




| | PATIENT SELECTION | INDEX TEST | REFERENCE STANDARD |
|---|---|---|---|
| Low | 69 | 66 | 82 |
| High | 13 | 2 | 0 |
| Unclear | 0 | 14 | 0 |

### 4.2.3. Primary analysis, narrative descriptions and forest plots

#### 4.2.3.1. Overall performance of HBeAg to identify pregnant women with high HBV DNA levels

Tables 4 and 5 present detailed performance results for PICO2A. Detailed results of the performance of HBeAg to identify pregnant women with high viral load ≥5, 6 and 7 log IU/mL are presented in the following sections.

Table 4. Performance of HBeAg to identify pregnant women with high viral load, stratified by HBV DNA threshold

| HBV DNA threshold (IU/mL) | Meta-analysis | Studies (N) | Subjects (N) | TP (N) | FN (N) | TN (N) | FP (N) | Se (95% CI) or Range Se | Sp (95% CI) or Range Sp |
|---|---|---|---|---|---|---|---|---|---|
| **20,000** | **Bivariate** | 19 | 4506 | 1121 | 224 | 2598 | 563 | 91.0 (79.2-96.4) | 91.8 (83.6-96.1) |
| | **Univariate** | - | - | - | - | - | - | - | - |
| **5 log10** | **Bivariate** | 38 | 13734 | 3486 | 669 | 8684 | 895 | 84.2 (80.2-87.4) | 92.3 (89.5-94.5) |
| | **Univariate** | 42 | 14231 | 3719 | 695 | 8917 | 900 | 82.5 (79.0-85.5) | 91.4 (88.9-93.5) |
| **6 log10** | **Bivariate** | 27 | 9651 | 2109 | 160 | 6691 | 691 | 92.0 (88.2-94.6) | 92.7 (90.3-94.5) |
| | **Univariate** | 34 | 10196 | 2514 | 300 | 6691 | 691 | 88.9 (84.0-92.4) | 91.7 (89.5-93.5) |
| **7 log10** | **Bivariate** | 24 | 8825 | 1416 | 62 | 5972 | 1375 | 98.0 (93.3-99.4) | 88.5 (80.7-93.4) |
| | **Univariate** | - | - | - | - | - | - | - | - |
| **8 log10** | **Bivariate** | 9 | 3786 | 575 | 7 | 2578 | 626 | 98.5 (88.1-99.8) | 83.9 (77.0-89.1) |
| | **Univariate** | - | - | - | - | - | - | - | - |

Table 5. Post-test probabilities of having high viral load depending on HBeAg test results in pregnant women

| HBV DNA threshold (IU/mL) | WHO region | Studies, N | Pre-test probability (%) | PLR (95% CI) | Post-test probability of having high viral load after positive HBeAg result (%) | NLR (95% CI) | Post-test probability of having high viral load after negative HBeAg result (%) |
|---|---|---|---|---|---|---|---|
| **20,000** | **Overall** | 19 | 34 | 11.1 (5.7-21.7) | 79.1 | 0.10 (0.04-0.23) | 3.2 |
| | **WPR** | 14 | 36 | 8.9 (4.3-18.6) | 76.2 | 0.08 (0.03-0.24) | 3.0 |
| | **AFR** | 3 | 21 | 31.4 (18.3-53.9) | 86.8 | 0.15 (0.10-0.22) | 3.1 |
| **5 log10** | **Overall** | 38 | 31 | 11.0 (7.9-15.3) | 77.3 | 0.17 (0.14-0.22) | 5.0 |
| | **WPR** | 26 | 37 | 7.9 (5.7-10.8) | 74.4 | 0.18 (0.14-0.24) | 6.3 |
| | **AFR** | 4 | 19 | 26.0 (14.5-46.8) | 83.2 | 0.12 (0.05-0.33) | 2.3 |
| **6 log10** | **Overall** | 27 | 22 | 12.6 (9.5-16.7) | 73.5 | 0.09 (0.06-0.13) | 1.9 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **WPR** | 19 | 24 | 10.4 (8.0-13.6) | 71.4 | 0.08 (0.05-0.13) | 1.9 |
| | **AFR** | 4 | 16 | 24.90 (16.3-38.0) | 79.9 | 0.07 (0.03-0.13) | 1.0 |
| **7 log10** | **Overall** | 24 | 14 | 8.5 (5.0-14.6) | 54.4 | 0.02 (0.01-0.08) | 0.3 |
| | **WPR** | 12 | 14 | 4.6 (2.6-8.3) | 39.3 | 0.03 (0.01-0.10) | 0.4 |
| | **AFR** | 2 | 18 | 5.7-19.3 | 50.5-77.7 | 0.00-0.05 | 0.0-21.6 |
| **8 log10** | **Overall** | 9 | 11 | 6.1 (4.2-9.0) | 40.3 | 0.02 (0.00-0.16) | 0.2 |
| | **WPR** | 7 | 11 | 5.7 (3.7-8.6) | 38.4 | 0.01 (0.00-0.49) | 0.1 |
| | **AFR** | 2 | 13 | 4.5-11.1 | 36.9-59.0 | 0.00-0.03 | 0.0-10.5 |

#### 4.2.3.2. Performance of HBeAg to identify pregnant women with HBV DNA ≥5 log10 IU/mL

- Overall pooled sensitivity = 84.2% (95% CI: 80.2–87.4)
- Overall pooled specificity = 92.3% (95% CI: 89.5–94.5)

There was no evidence of publication bias (*P*=0.48) (Appendix G.1). However, the funnel plots for the assessment of publication bias were thought to be not very informative because only one study had the same objective as that of the PICO2A *(85)*. We identified one outlier study (*Lin J, 2002*) through visual assessment of the overall coupled forest plot (Fig. 4) and the summary scatter plot (Fig. 5). This retrospective cohort study from China reported low sensitivity (40.5%) and high specificity (95.6%) of HBeAg to identify women with HBV viral load ≥5 log10 IU/mL. Recruitment was from 1999 to 2000. HBV DNA was measured using a commercial PCR (real-time fluorescence quantitative PCR [Biotromics technologies]). HBeAg was determined by tne ELISA test (WB-2496 Wantai). Maternal age and HIV status were not reported.

Fig. 4. Coupled forest plot of the performance of HBeAg to identify pregnant women with HBV viral load ≥5 log10 IU/mL

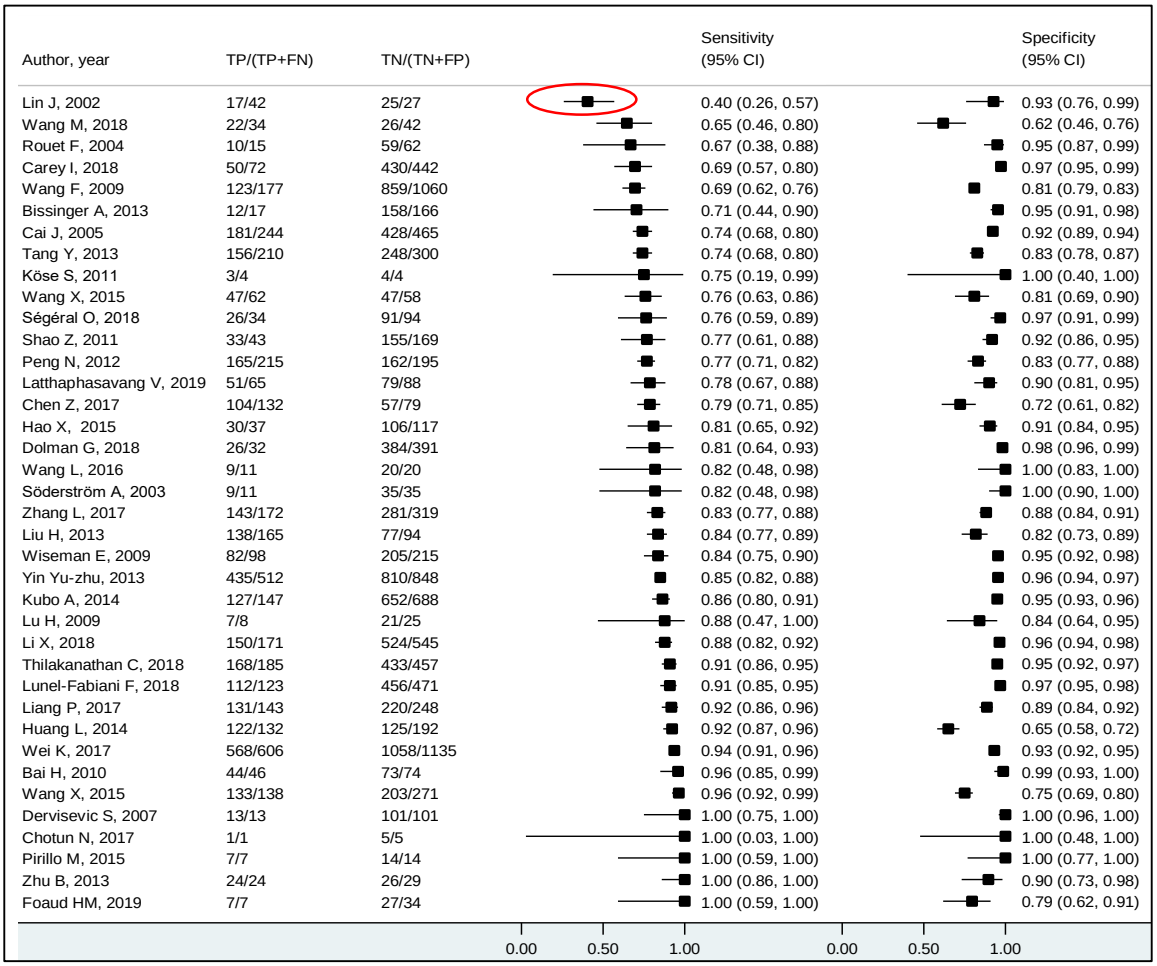
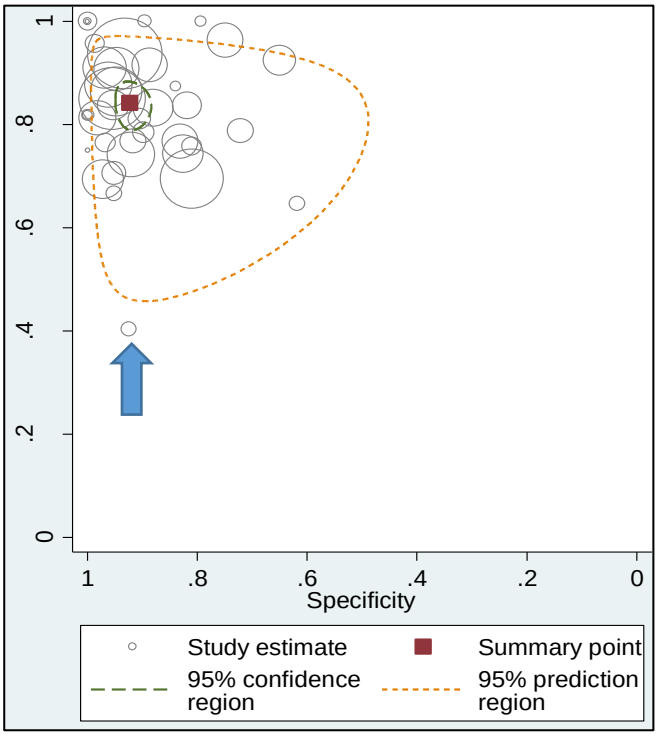


Fig. 5. Summary scatter plot of the performance of HBeAg to identify maternal HBV DNA ≥5 log10 IU/mL



**Subgroup analysis**

Of the variables predefined as potential sources of heterogeneity, it was not possible to do a subgroup analysis by HCV- or HDV-coinfection status, nor by maternal HBV genotype as too few studies reported information on these factors.

Table 6 and Fig. 6 to 11 present detailed results of the performance of HBeAg to identify pregnant women with HBV DNA ≥5 log10 IU/mL, stratified by the potential sources of heterogeneity.

There was some evidence of interaction by maternal age (*P*=0.02), with a higher sensitivity and lower specificity in studies of young mean/median maternal age (<28 years) (Fig. 10). There was strong evidence that the performance of HBeAg to identify pregnant women with HBV DNA ≥5 log IU/mL varied according to the WHO region (*P*=0.001), some evidence for HBeAg assay type (*P*=0.02), weak evidence for maternal HIV status (*P*=0.09) and no evidence for HBV DNA type of assay.

Table 6. Performance of HBeAg to identify pregnant women with HBV viral load ≥5 log10 IU/mL, stratified by potential sources of heterogeneity

| **Subgroup variables** | **Subgroup category** | **Studies (N)** | **Se (95% CI) or Range Se** | **Sp (95% CI) or Range Sp** | ***P*-value** |
|---|---|---|---|---|---|
| **Overall** | | 38 | 84.2 (80.2-87.4) | 92.3 (89.5-94.5) | N/A |
| **WHO region** | **WPR** | 26 | 83.9 (79.2-87.7) | 89.3 (85.6-92.1) | 0.001* |
| | **SEAR** | 0 | N/A | N/A | |
| | **EUR** | 6 | 79.9 (65.4-89.3) | 98.2 (95.7-99.3) | |
| | **EMR** | 1 | 100.0 | 79.4 | |
| | **AFR** | 4 | 88.0 (70.9-95.7) | 96.6 (94.3-98.0) | |
| | **AMR** | 1 | 86.4 | 94.8 | |
| **HBeAg assay type** | **EIA** | 19 | 79.4 (74.0-84.0) | 90.4 (86.3-93.3) | 0.02** |
| | **FIA** | 3 | 91.4 (78.5-96.9) | 96.6 (81.2-99.5) | |
| | **CLIA** | 12 | 89.1 (83.2-93.0) | 92.8 (88.1-95.8) | |
| | **RDT** | 1 | 76.5 | 96.8 | |
| | **Multiple** | 1 | 83.7 | 95.3 | |
| | **Commercial PCR** | 34 | 83.7 (79.5-87.1) | 91.9 (89.0-94.0) | 0.58 |

| | | | | | |
|---|---|---|---|---|---|
| **HBV DNA assay type** | **In-house or unknown test** | 4 | 87.8 (76.4-94.2) | 95.0 (80.4-98.9) | |
| **Mean / median maternal age** | **<28 years** | 12 | 89.7 (83.6-93.7) | 91.8 (86.1-95.3) | 0.02 |
| | **≥28 years** | 10 | 82.3 (77.6-86.2) | 95.3 (92.8-97.0) | |
| **HIV status** | **All uninfected** | 12 | 89.4 (82.7-93.7) | 89.0 (81.7-93.6) | 0.09*** |
| | **All infected** | 3 | 82.6 (55.8-94.7) | 98.2 (74.7-99.9) | |
| | **Mixed population** | 3 | 83.6 (71.8-91.0) | 94.5 (89.9-97.1) | |

*comparison of WPR/EUR/AFR

**comparison CLIA/EIA/FIA

***comparison all HIV uninfected vs all HIV infected

## Fig. 6. Coupled forest plot of the performance of HBeAg to identify maternal HBV DNA ≥5 log10 IU/mL by WHO region

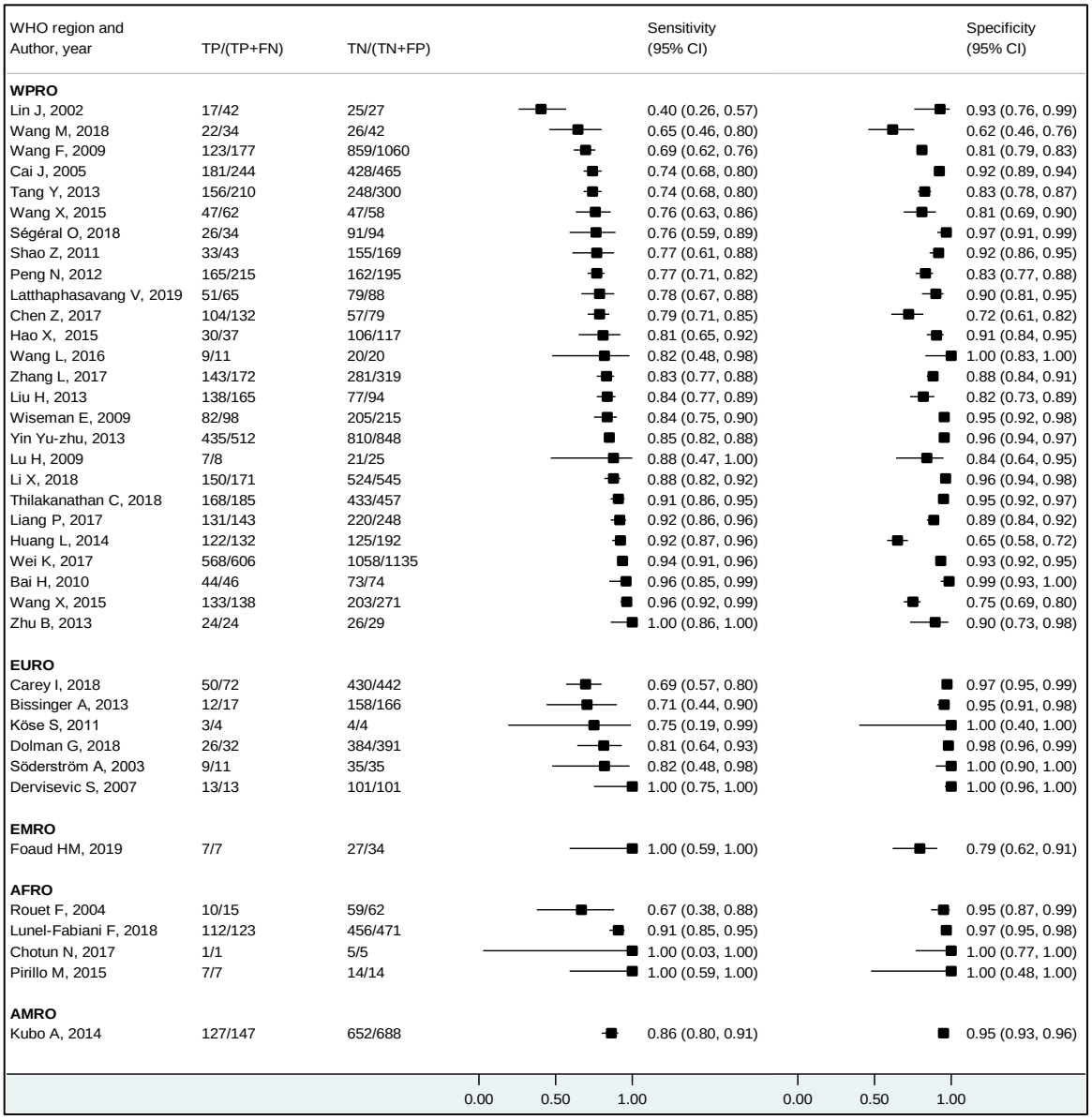


Fig. 7. Coupled forest plot of the performance of HBeAg to identify maternal HBV DNA ≥5 log10 IU/mL by type of HBeAg assay

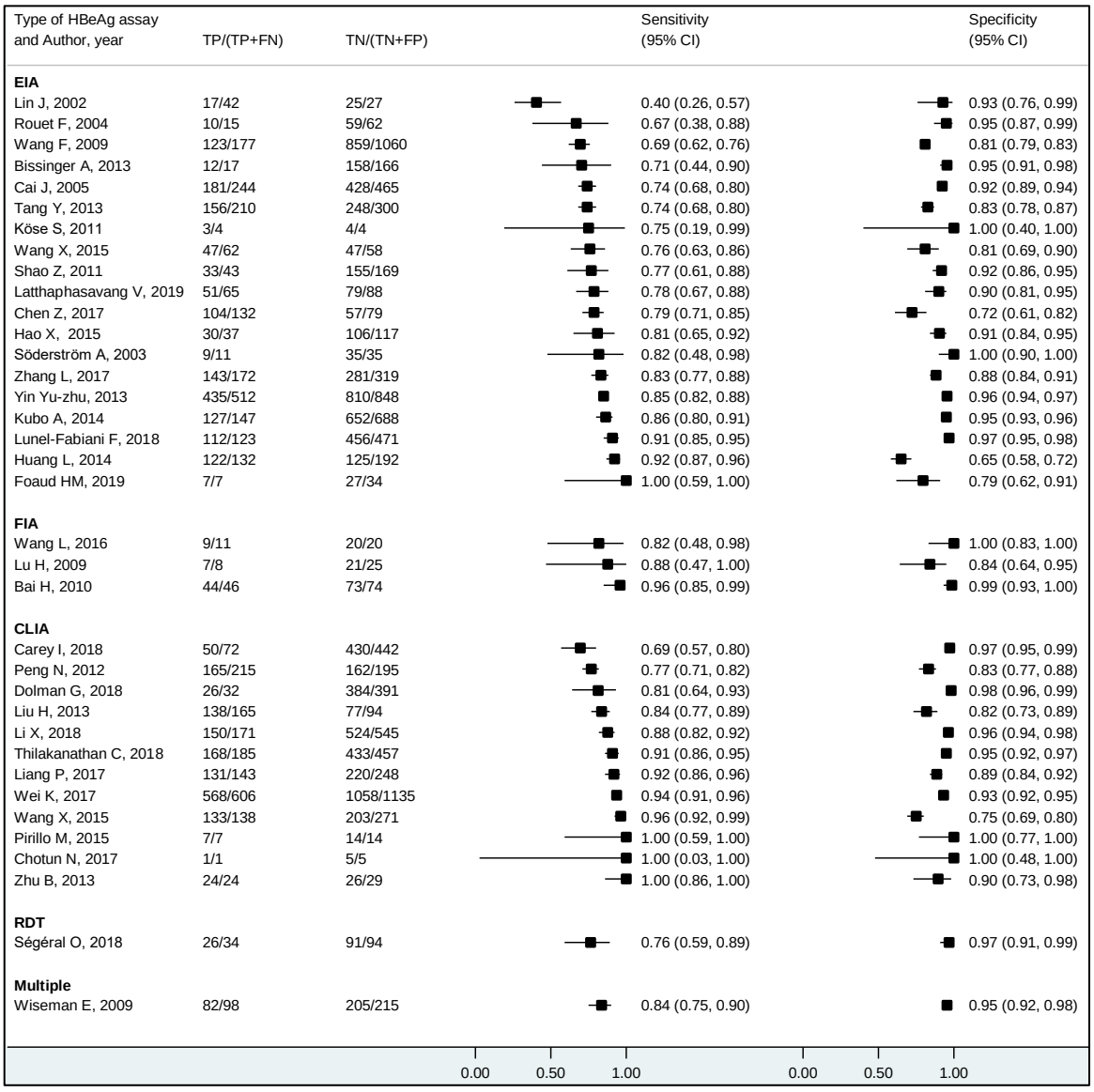


Fig. 8. Coupled forest plot of the performance of HBeAg to identify maternal HBV DNA ≥5 log10 IU/mL by HBeAg assay commercial name

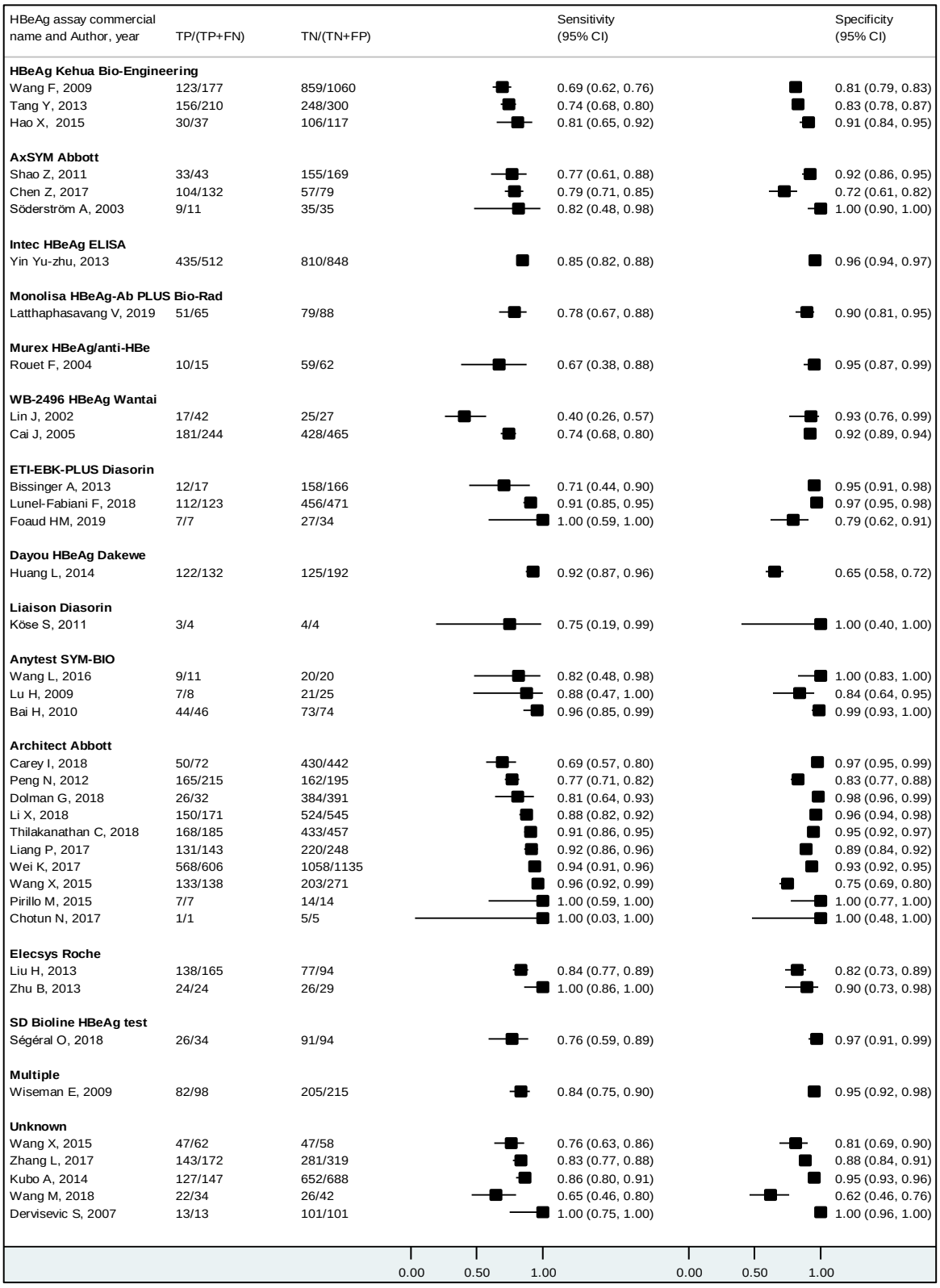


Fig. 9. Coupled forest plot of the performance of HBeAg to identify maternal HBV DNA ≥5 log10 IU/mL by type of HBV DNA assay

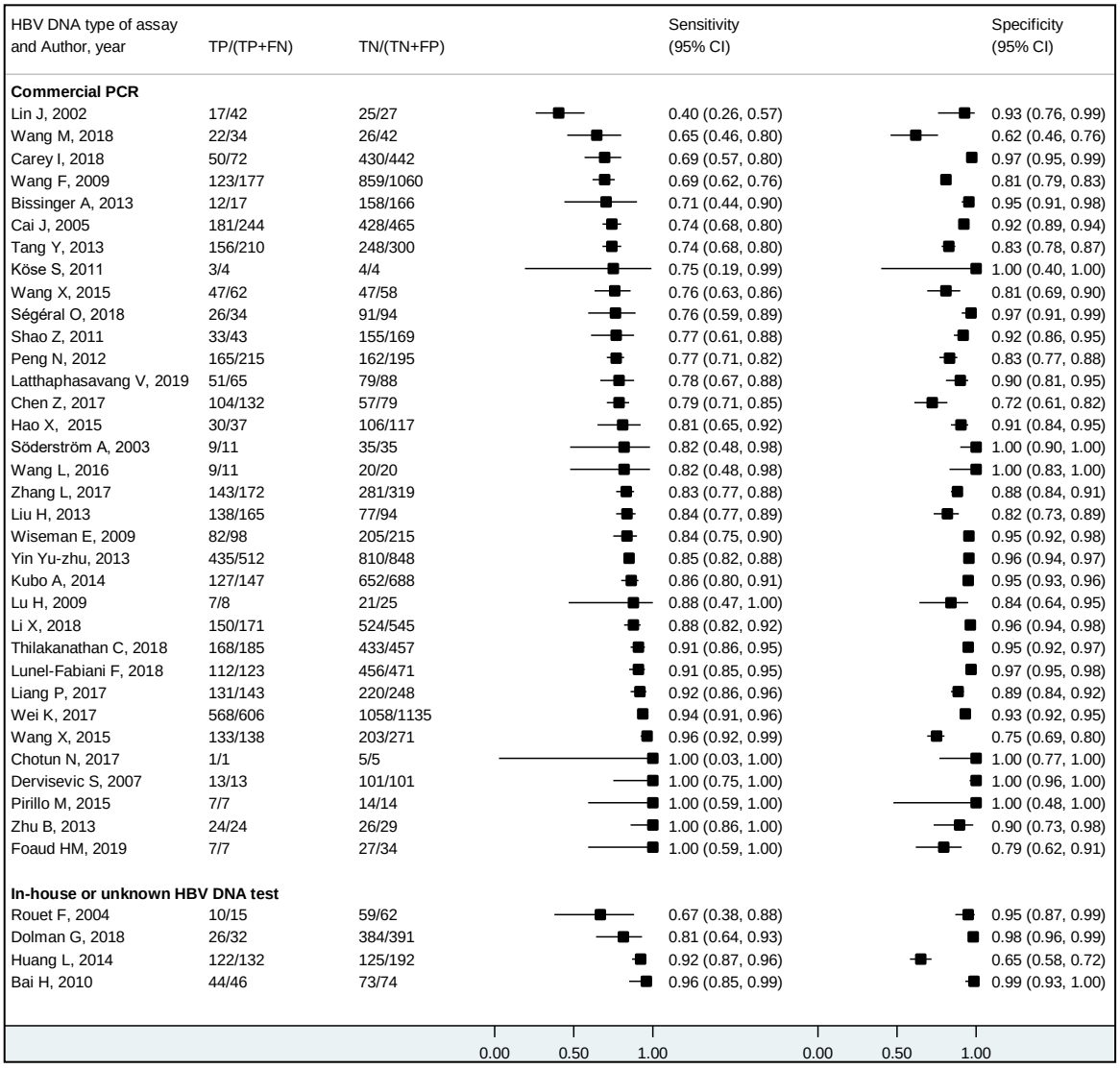
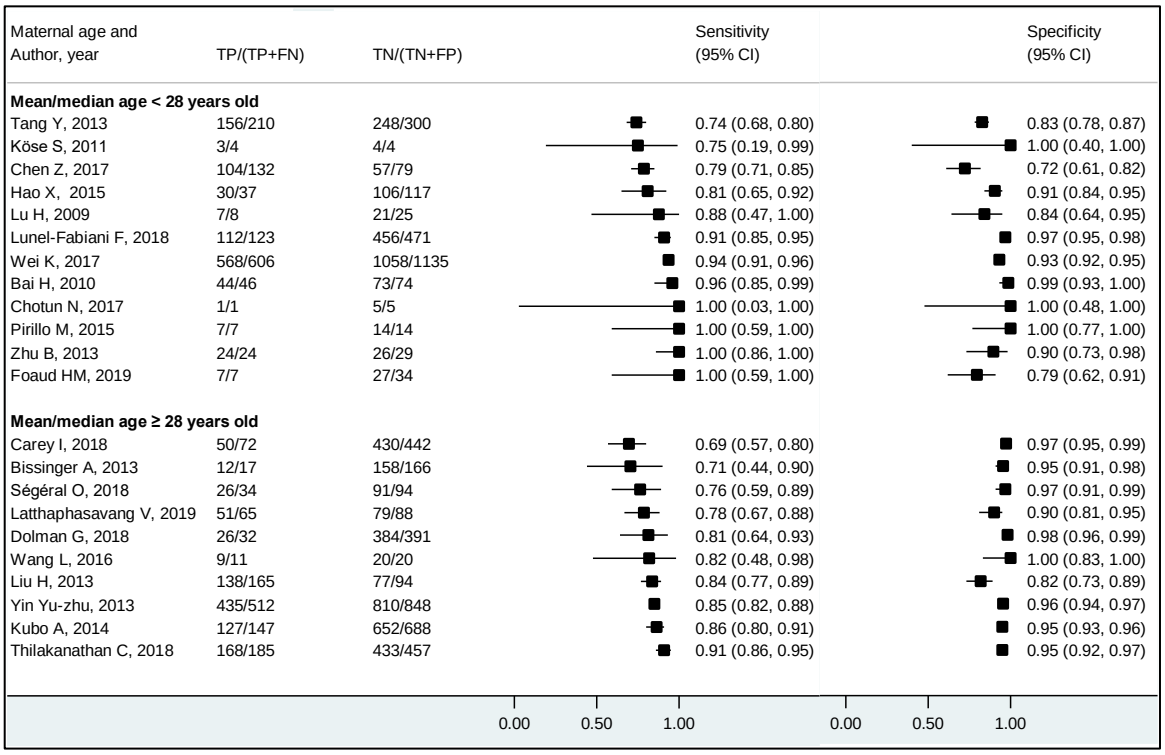
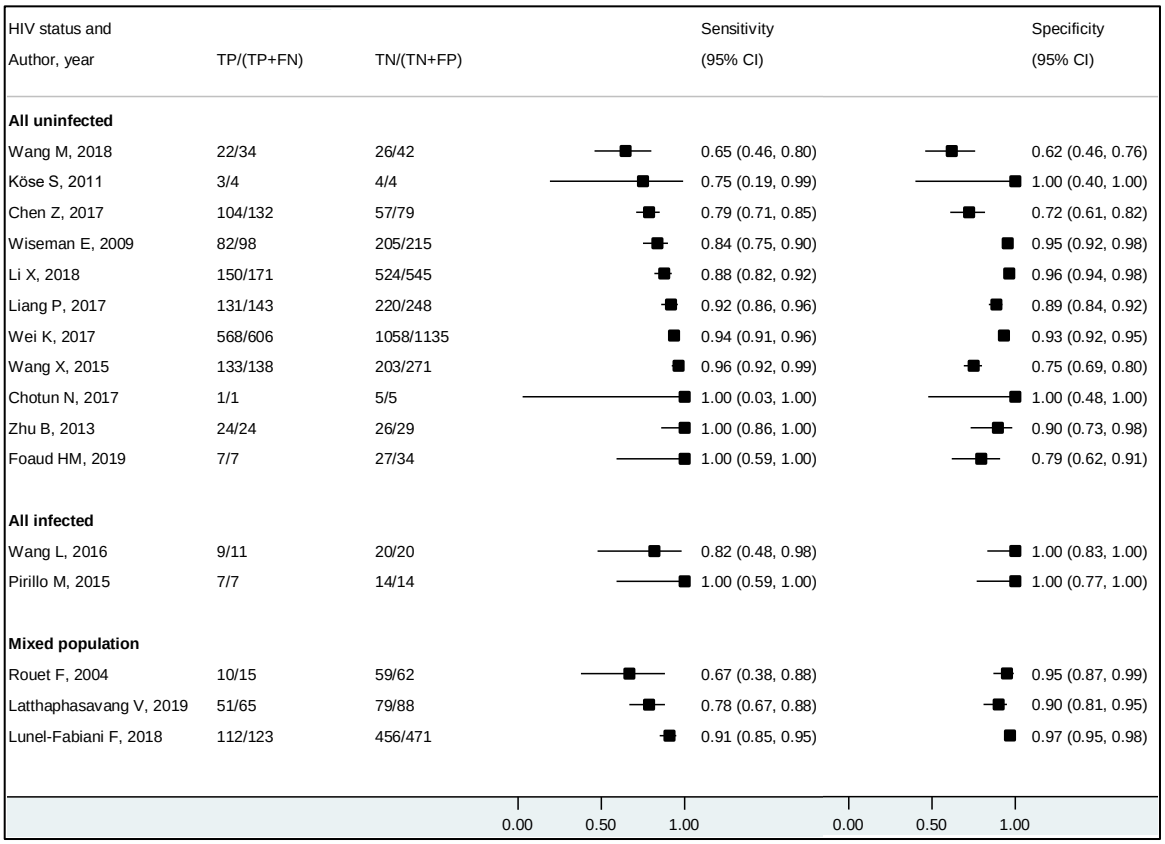


Fig. 10. Coupled forest plot of the performance of HBeAg to identify maternal HBV DNA ≥5 log10 IU/mL by mean or median maternal age



## Fig. 11. Coupled forestplot of the performance of HBeAg to identify maternal HBV DNA ≥5 log10 IU/mL by maternal HIV status



**Sensitivity analysis for the performance of HBeAg to identify pregnant women with HBV DNA ≥5 log10 IU/mL (Table 7)**

Sensitivity and specificity were slightly lower in univariate analysis compared to the bivariate analysis. However, there was no evidence of a statistically significant difference between the bivariate and univariate analysis estimates as 95% CI overlapped. Results were unchanged when the outlier study (*Lin J, 2002*) was excluded from the bivariate analysis or when studies with an overall "high" risk of bias as per QUADAS-2 assessment were excluded.

Table 7. Results of the sensitivity analysis for the performance of HBeAg to identify pregnant women with HBV DNA ≥5 log10 IU/mL

| **Sensitivity analysis** | **Studies, N** | **Se (95% CI) or Range Se** | **Sp (95% CI) or Range Sp** |
|---|---|---|---|
| Overall bivariate | 38 | 84.2 (80.2-87.4) | 92.3 (89.5-94.5) |
| Overall univariate | 42 | 82.5 (79.0-85.5) | 91.4 (88.9-93.5) |
| Bivariate without outlier study | 37 | 84.8 (81.4-87.7) | 92.4 (89.4-94.5) |
| Bivariate without studies with an overall "high" risk of bias | 28 | 85.0 (79.9-89.0) | 93.0 (89.5-95.4) |

#### 4.2.3.3. Performance of HBeAg to identify pregnant women with HBV DNA ≥6 log10 IU/mL

- Overall pooled sensitivity = 92.0% (95% CI: 88.2-94.6)
- Overall pooled specificity = 92.7% (95% CI: 90.3-94.5)

There was no evidence of publication bias (p=0.15) (Appendix G.2). We did not identify any outlier study through visual assessment of the overall coupled forest plot (Fig. 12) and the summary scatter plot (Fig. 13).

Fig. 12. Coupled forestplot for all studies included in the meta-analysis of the performance of HBeAg to identify maternal HBV DNA ≥6 log10 IU/mL

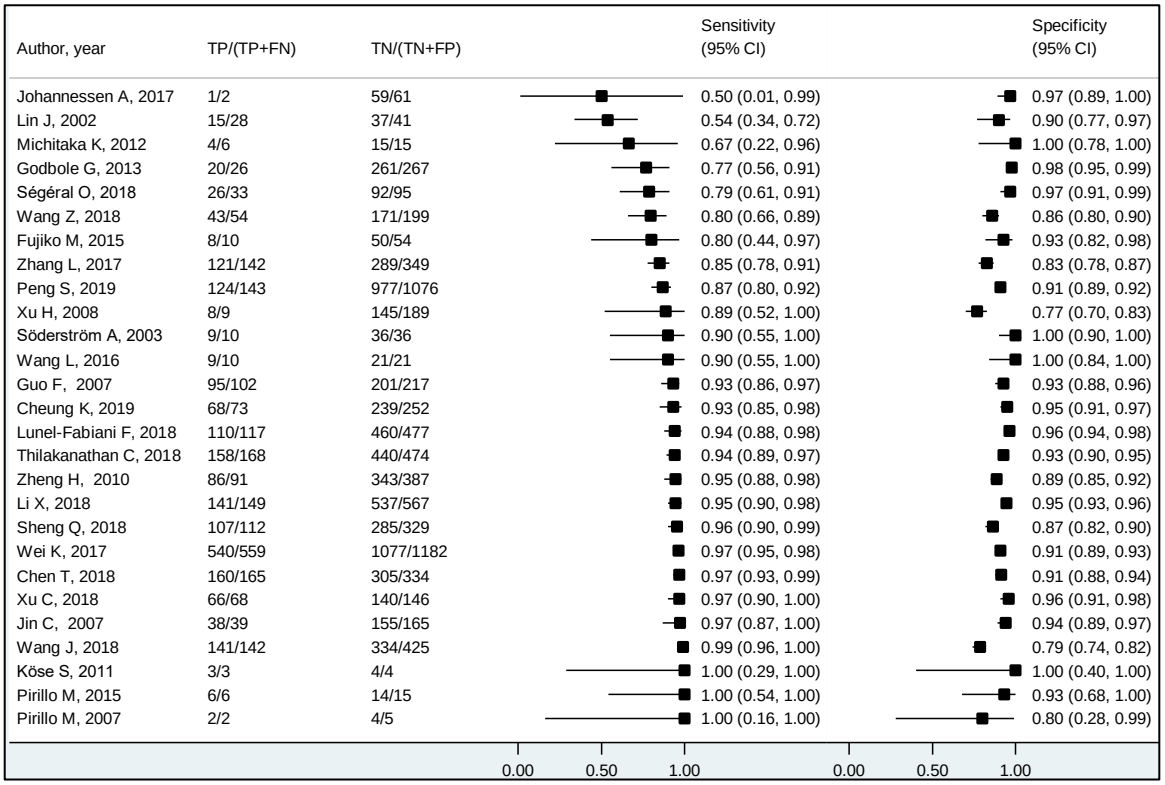
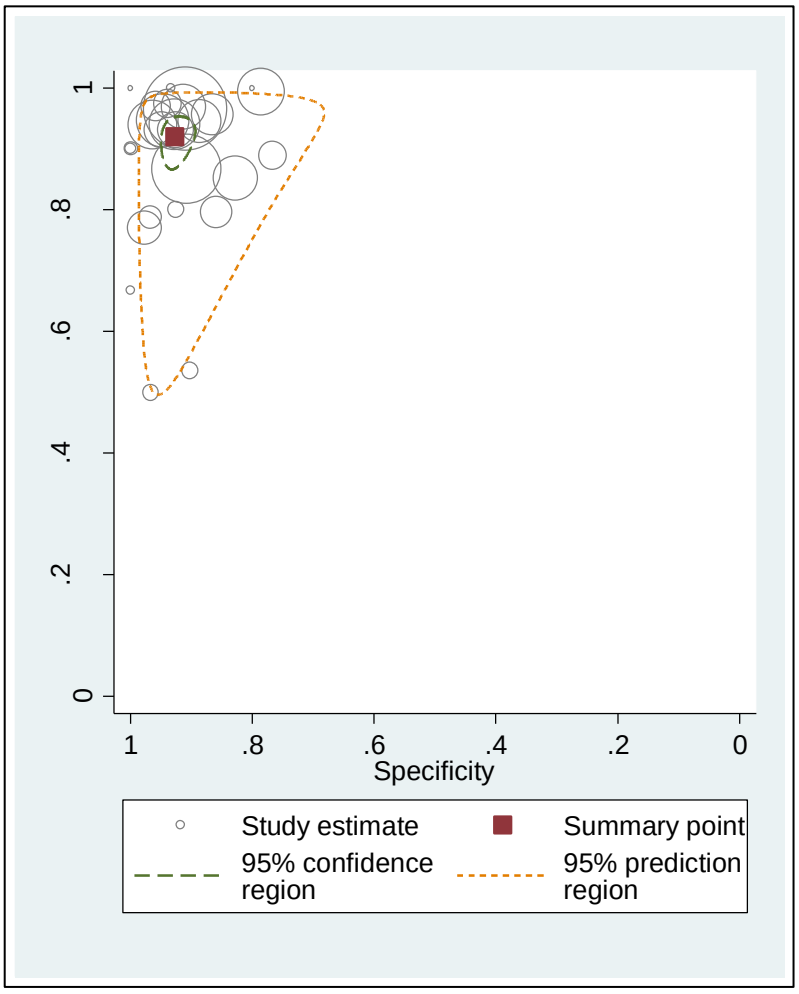


Fig. 13. Summary scatter plot of the performance of HBeAg to identify maternal HBV DNA ≥6 log10 IU/mL



**Subgroup analysis**

Table 8 and Fig. 14 to 19 present detailed results of the performance of HBeAg to identify pregnant women with HBV DNA ≥6 log10 IU/mL stratified by the potential sources of heterogeneity.

There was strong evidence that the performance differed according to mean/median maternal age; higher sensitivity and lower specificity were observed in younger age group (*P*=0.001) (Fig. 18). There was some evidence that performance of HBeAg varied according to the WHO region (*P*=0.04), weak evidence for HBeAg assay type (*P*=0.08), and no evidence for HIV status.

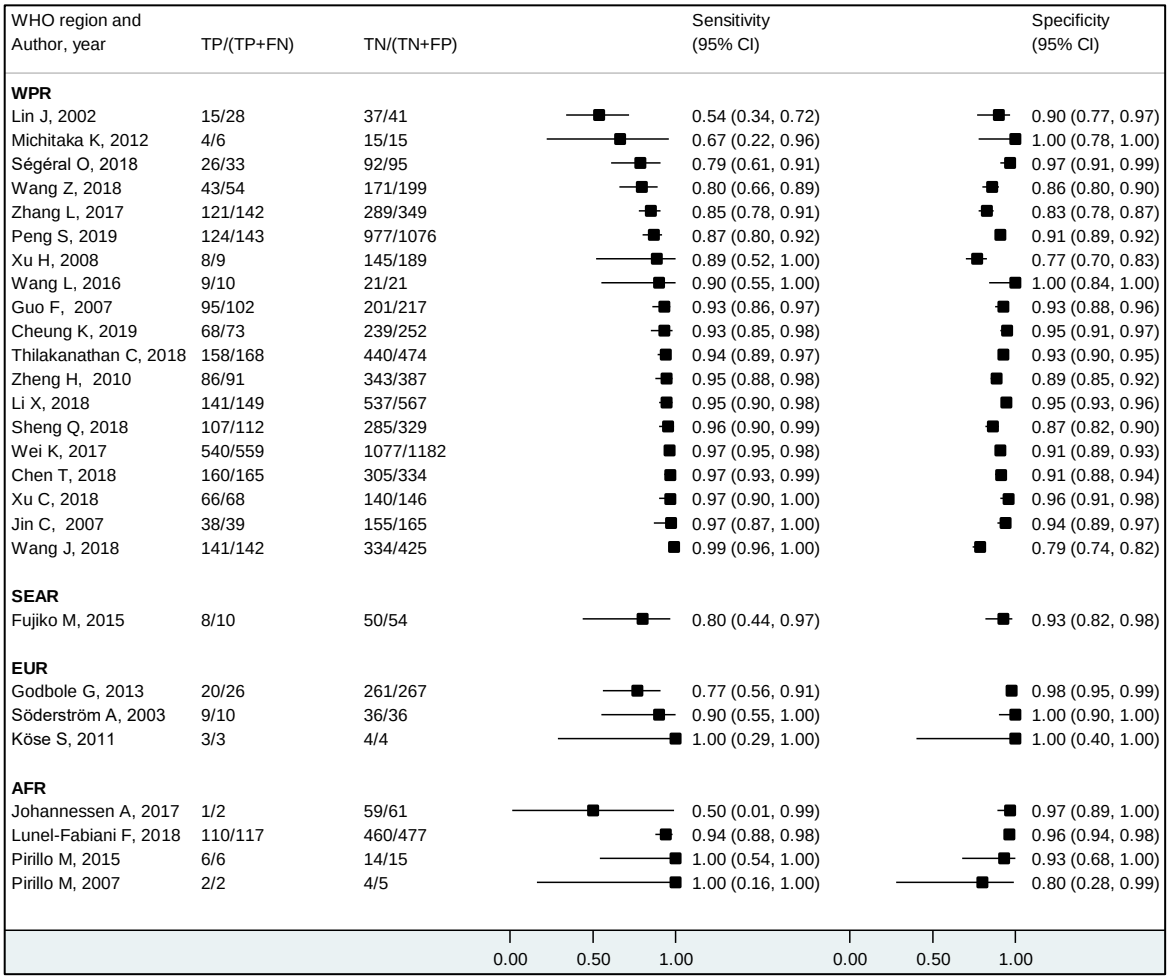
Table 8. Performance of HBeAg to identify pregnant women with HBV viral load ≥6 log10 IU/mL, stratified by potential sources of heterogeneity

| **Subgroup variables** | **Subgroup category** | **Studies (N)** | **Se (95% CI) or Range Se** | **Sp (95% CI) or Range Sp** | ***P*-value** |
|---|---|---|---|---|---|
| **Overall** | | 27 | 92.0 (88.2-94.6) | 92.7 (90.3-94.5) | N/A |
| **WHO region** | **WPR** | 19 | 92.6 (88.4-95.3) | 91.1 (88.4-93.2) | 0.04* |
| | **SEAR** | 1 | 80.0 | 92.6 | |
| | **EUR** | 3 | 82.1 (66.9-91.2) | 98.0 (95.7-99.1) | |
| | **EMR** | 0 | N/A | N/A | |
| | **AFR** | 4 | 93.7 (87.9-96.8) | 96.2 (94.3-97.5) | |
| | **AMR** | 0 | N/A | N/A | |
| **HBeAg assay type** | **EIA** | 12 | 88.2 (80.9-93.0) | 91.5 (87.0-94.6) | 0.08** |
| | **FIA** | 1 | 90.0 | 100.0 | |
| | **CLIA** | 9 | 94.0 (89.9-96.5) | 93.9 (90.9-95.9) | |
| | **RDT** | 1 | 78.8 | 96.8 | |
| | **Multiple** | 1 | 97.1 | 95.9 | |
| | **RIA/CIA** | 0 | N/A | N/A | |
| **Type of HBV DNA assay** | **Commercial PCR** | 25 | 92.3 (88.4-94.9) | 93.1 (90.7-94.9) | N/A |
| | **In-house or unknown test** | 2 | 79.6-100.0 | 80.0-85.9 | |
| **Mean / median** | **<28 years** | 8 | 96.6 (94.8-97.8) | 91.7 (87.0-94.8) | 0.001 |
| | **≥28 years** | 11 | 86.9 (80.2-91.6) | 94.1 (90.9-96.3) | |

| | | | | | |
|---|---|---|---|---|---|
| **maternal age** | | | | | |
| **HIV status** | **All uninfected** | 7 | 94.2 (90.3-96.6) | 92.2 (89.9-94.0) | 0.77*** |
| | **All infected** | 3 | 95.2 (56.3-99.7) | 95.2 (80.1-99.0) | |
| | **Mixed population** | 1 | 94.0 | 96.4 | |

*comparison of WPR/EUR/AFR
**comparison CLIA/EIA
***comparison all uninfected vs all infected

Fig. 14. Coupled forest plot of the performance of HBeAg to identify maternal HBV DNA ≥6 log10 IU/mL by WHO region



## Fig. 15. Coupled forest plot of the performance of HBeAg to identify maternal HBV DNA ≥6 log10 IU/mL by type of HBeAg assay

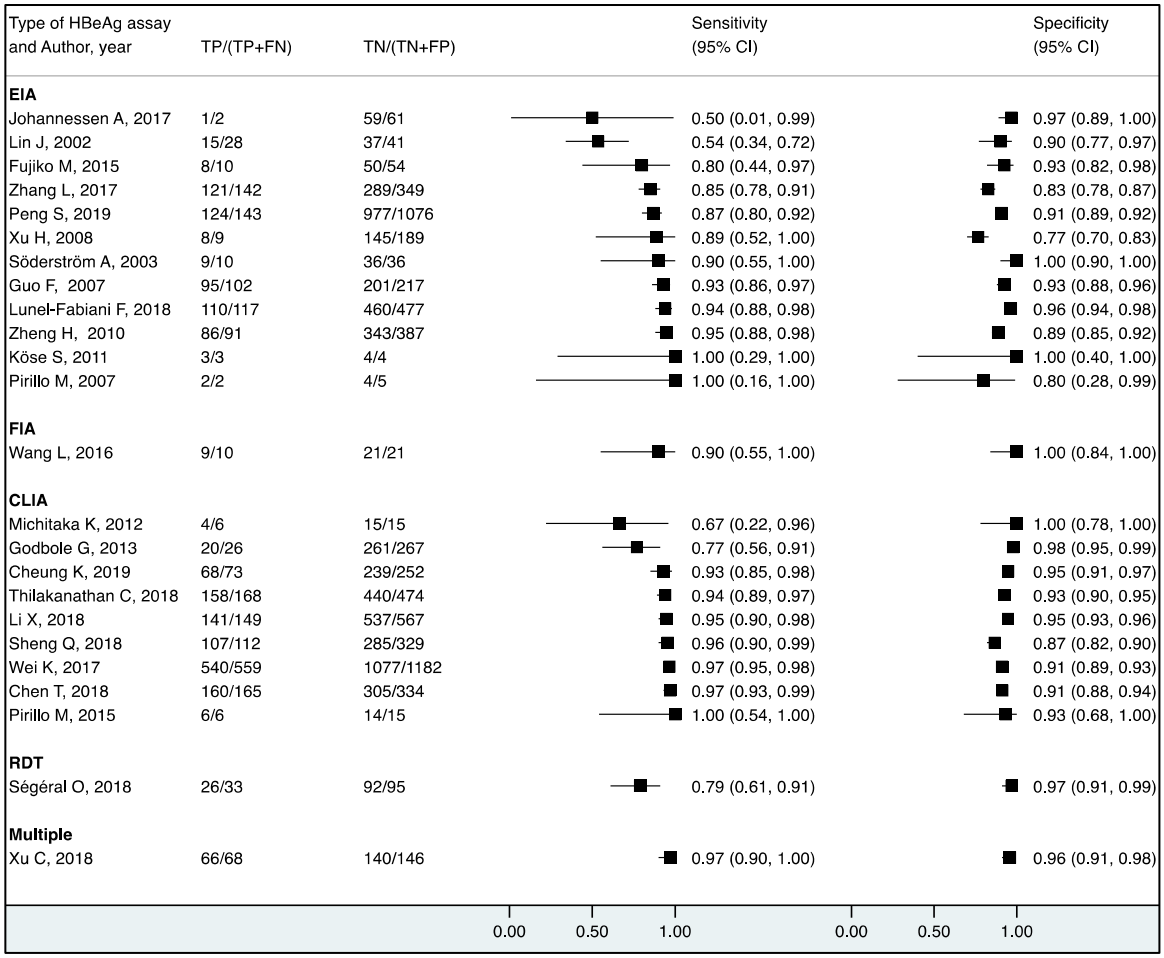
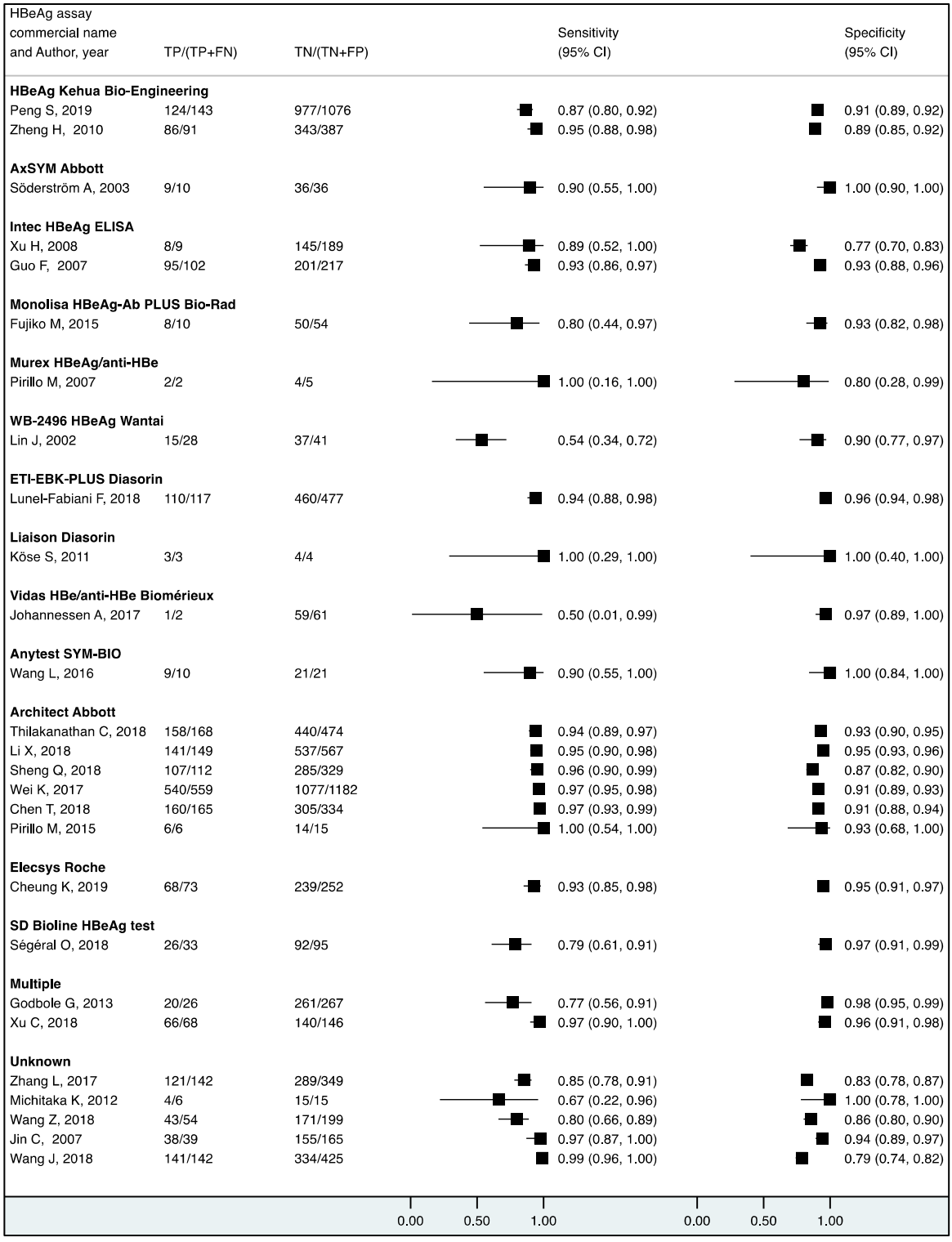
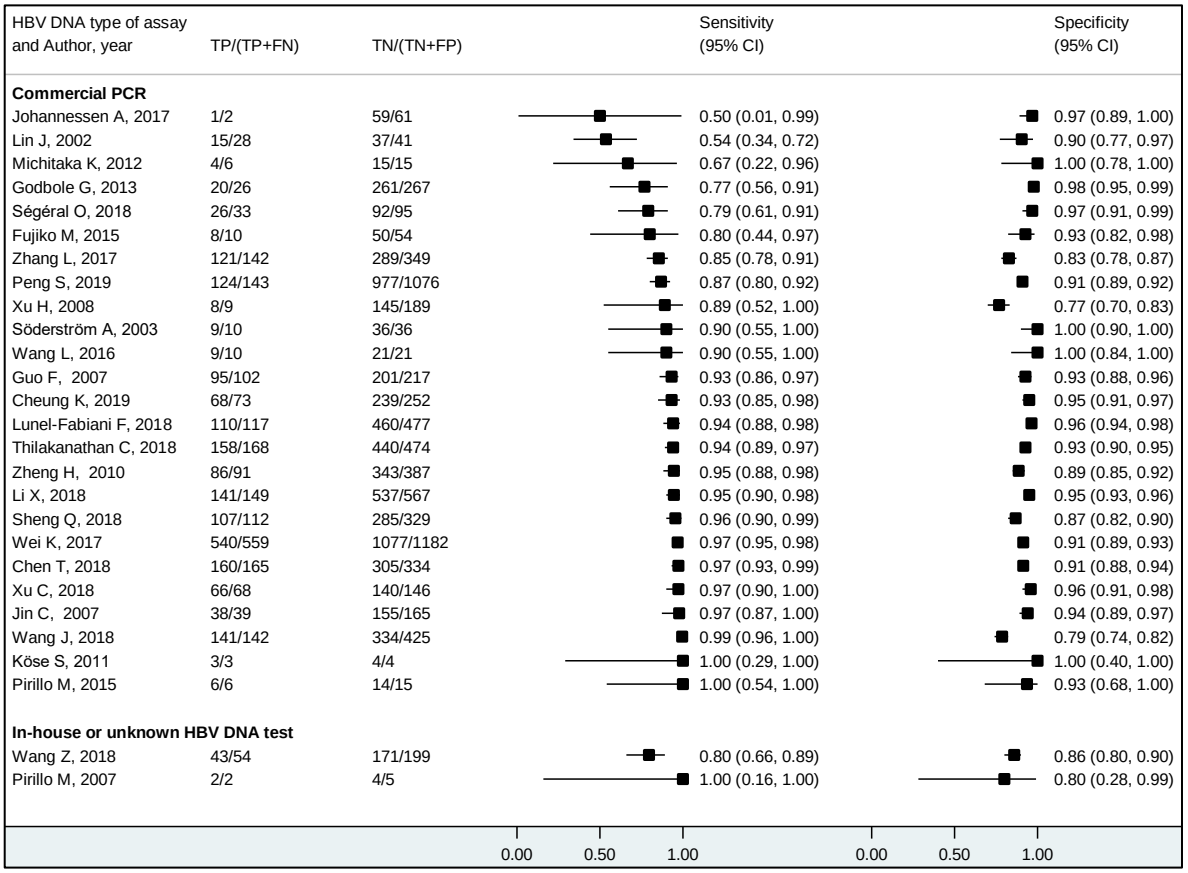


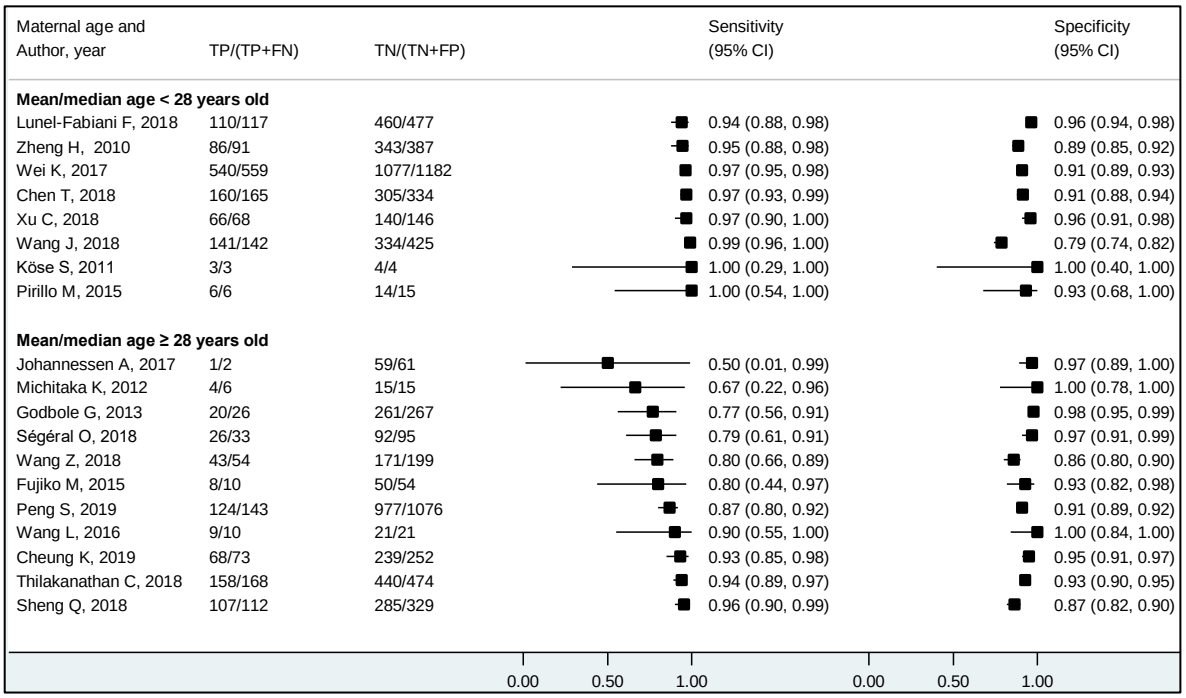
Fig. 16. Coupled forestplot of the performance of HBeAg to identify maternal HBV DNA ≥6 log10 IU/mL by HBeAg assay commercial name



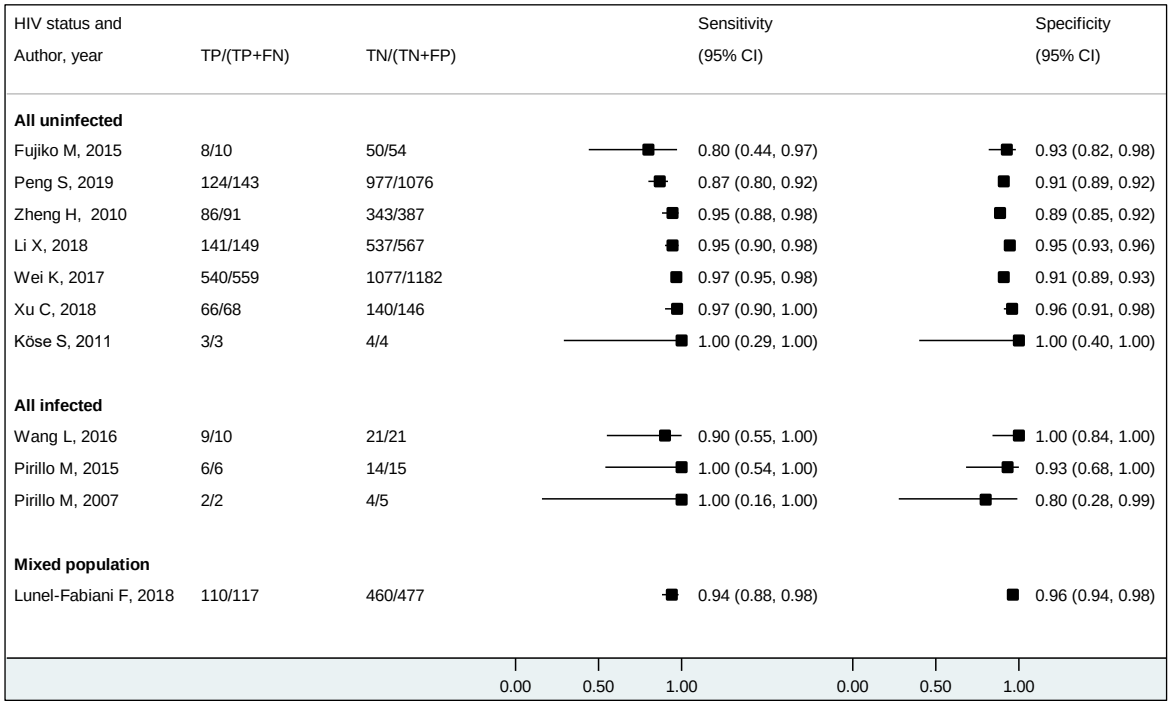
## Fig. 17. Coupled forestplot of the performance of HBeAg to identify maternal HBV DNA ≥6 log10 IU/mL by type of HBV DNA assay



## Fig. 18. Coupled forestplot of the performance of HBeAg to identify maternal HBV DNA ≥6 log10 IU/mL by maternal age




## Fig. 19. Coupled forestplot of the performance of HBeAg to identify maternal HBV DNA ≥6 log10 IU/mL by maternal HIV status



**Sensitivity analysis for the performance of HBeAg to identify pregnant women with HBV DNA ≥6 log10 IU/mL (Table 9)**

As there was no outlier study identified for this HBV DNA threshold, we did not perform sensitivity analysis on this factor.

When excluding from the analysis studies with an overall "high" risk of bias as per QUADAS-2 assessment or performing univariate analysis, there was no evidence of a difference in pooled sensitivity and specificity compared with those of the main bivariate analysis (95% CI overlapped).

Table 9. Results of the sensitivity analysis for the performance of HBeAg to identify pregnant women with HBV DNA ≥6 log10 IU/mL

| **Sensitivity analysis** | **Studies, N** | **Se (95% CI) or Range Se** | **Sp (95% CI) or Range Sp** |
|---|---|---|---|
| **Overall bivariate** | 27 | 92.0 (88.2-94.6) | 92.7 (90.3-94.5) |
| **Overall univariate** | 34 | 89.2 (83.9-92.8) | 91.7 (89.5-93.5) |
| **Bivariate without studies with an overall "high" risk of bias** | 21 | 90.5 (85.8-93.7) | 93.6 (91.0-95.5) |

#### 4.2.3.4. Performance of HBeAg to identify pregnant women with HBV DNA ≥7 log10 IU/mL

- Overall pooled sensitivity = 98.0% (95% CI: 93.3-99.4)
- Overall pooled specificity = 88.5% (95% CI: 80.7-93.4)

There was no evidence of publication bias (*P*=0.25) (Appendix G.3). We identified three outliers (*Elefsiniotis I, 2007*; *Ding Y, 2013* and *Li Y, 2013*) through visual assessment of the overall coupled forest plot (Fig. 20) and the summary scatter plot (Fig. 21).

The first outlier (*Elefsiniotis I, 2007*) was a cross-sectional study in Greece that reported a very low sensitivity (12.5%) and high specificity (100.0%). Recruitment was from 2003 to 2005. HBV DNA was measured using a commercial PCR (COBAS Amplicor HBV Monitor test (Roche Diagnostics)). HBeAg was tested using an ELISA (AxSYM Abbott). Maternal age and HIV status were unknown, and all women were HCV negative.

The second outlier (*Ding Y, 2013*) was a prospective cohort in China that reported a low specificity (38.7%) and high sensitivity (100.0%). Recruitment was from 2010 to 2011. HBV DNA was measured using a commercial PCR (COBAS Taqman (Roche Diagnostics). HBeAg was tested using a CLIA (Architect Abbott). Maternal age was unknown, but 2/249 women were younger than 20 years and 247/249 women were older than 20 years. Maternal HIV status was unknown.

The third outlier (*Li Y, 2013*) was a prospective cohort in China that reported a low specificity (25.4%) and high sensitivity (100.0%). Recruitment was in 2006. HBV DNA was measured using a commercial PCR (Real-Time Fluorescence quantitative PCR (Toyobo)). HBeAg was tested using an ELISA (manufacturer not stated). Maternal age was unknown, and all recruited women were HIV negative.

Fig. 20. Coupled forestplot for all studies included in the meta-analysis of the performance of HBeAg to identify maternal HBV DNA ≥7 log10 IU/mL

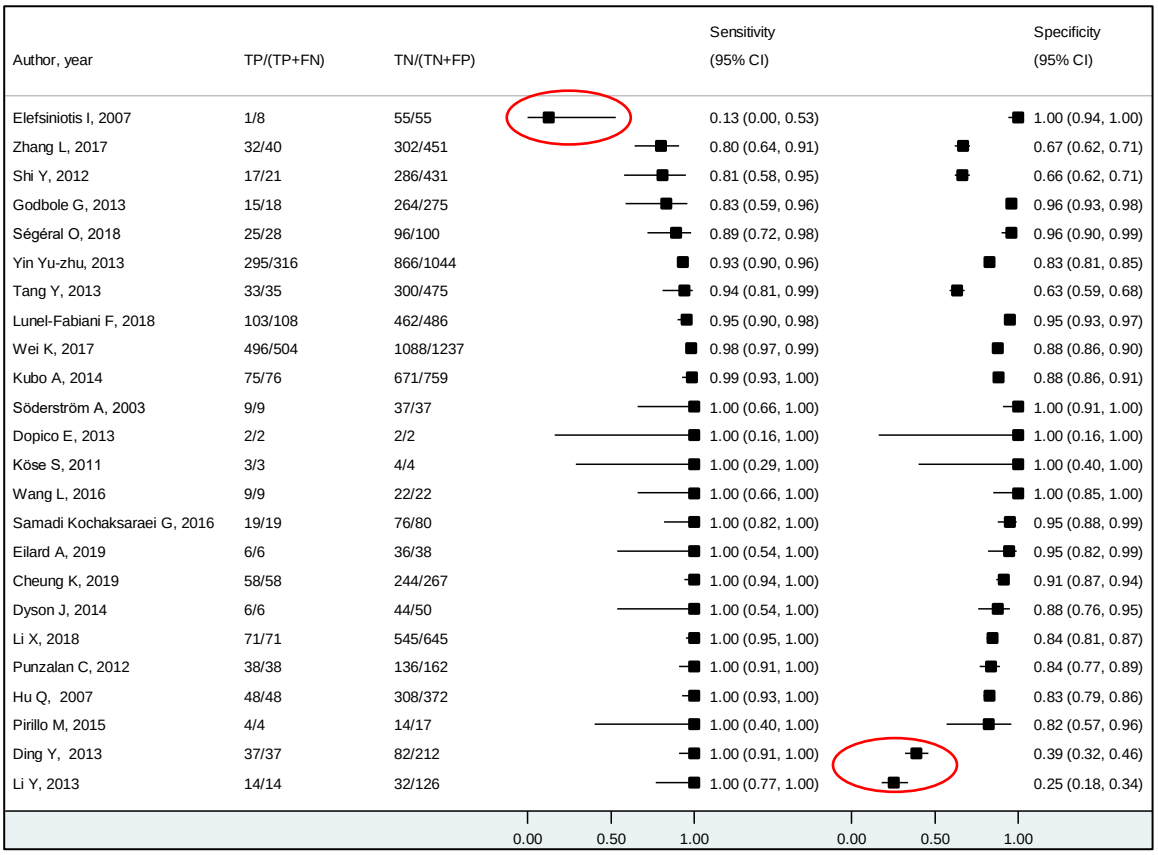
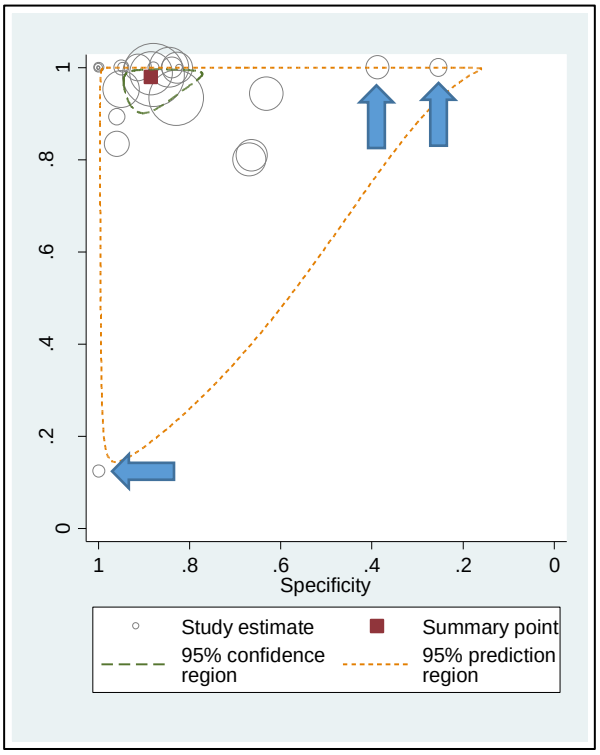


Fig. 21. Summary scatter plot of the performance of HBeAg to identify maternal HBV DNA ≥7 log10 IU/mL



**Subgroup analysis**

Of the variables predefined as potential sources of heterogeneity, it was not possible to do a subgroup analysis by HCV- or HDV-coinfection status, nor by maternal HBV genotype as too few studies reported information on these factors. Additionally, as all studies had an important risk of bias, we did not perform a subgroup analysis on risk of bias.

Table 10 and Fig. 22 to 27 present detailed results of the performance of HBeAg to identify pregnant women with HBV DNA ≥7 log10 IU/mL stratified by the potential sources of heterogeneity identified.

There was some evidence that performance of HBeAg to identify pregnant women with HBV DNA ≥7 log IU/mL varied according to the WHO region (*P*=0.01), and weak evidence that it varied with HBeAg assay type (*P*=0.07). There was no evidence that its performance differed according to maternal age.

Table 10. Performance of HBeAg to identify pregnant women with HBV viral load ≥7 log10 IU/mL, stratified by potential sources of heterogeneity

| Subgroup variables | Subgroup category | Studies (N) | Se (95% CI) or Range Se | Sp (95% CI) or Range Sp | *P* value |
|---|---|---|---|---|---|
| **Overall** | | 24 | 98.0 (93.3-99.4) | 88.5 (80.7-93.4) | N/A |
| **WHO region** | **WPR** | 12 | 97.6 (92.2-99.3) | 78.9 (64.2-88.6) | 0.01* |
| | **SEAR** | 0 | - | - | |
| | **EUR** | 7 | 96.7 (36.7-99.9) | 95.6 (91.2-97.8) | |
| | **EMR** | 0 | - | - | |
| | **AFR** | 2 | 95.0-100.0 | 82.0-95.0 | |
| | **AMR** | 3 | 99.2 (94.9-99.9) | 88.2 (86.1-90.1) | |
| **HBeAg assay type** | **EIA** | 11 | 94.4 (81.3-98.5) | 86.9 (67.9-95.4) | 0.07** |
| | **FIA** | 1 | 100.0 | 100.0 | |
| | **CLIA** | 9 | 98.8 (94.8-99.7) | 87.9 (77.2-94.0) | |
| | **RDT** | 1 | 89.3 | 96.0 | |
| | **Multiple** | 0 | - | - | |
| **Type of HBV DNA assay** | **Commercial PCR** | 22 | 97.5 (92.2-99.2) | 89.0 (80.3-94.1) | N/A |
| | **In-house or unknown test** | 2 | 100.0 | 83.4-88.0 | |

| | | | | | |
|---|---|---|---|---|---|
| **Mean / median maternal age** | **<28 years** | 6 | 98.0 (96.3-98.9) | 85.9 (74.7-92.7) | 0.16 |
| | **≥28 years** | 9 | 96.8 (89.5-99.1) | 92.5 (88.5-95.2) | |
| **HIV status** | **All uninfected** | 7 | 98.9 (97.0-99.6) | 85.3 (66.4-94.4) | N/A |
| | **All infected** | 2 | 100.0 | 82.4-100.0 | |
| | **Mixed population** | 2 | 95.4-100.0 | 88.0-95.1 | |

*comparison of WPR/EUR/AMR

**comparison of CLIA/EIA

## Fig. 22. Coupled forest plot of the performance of HBeAg to identify maternal HBV DNA ≥7 log10 IU/mL by WHO region

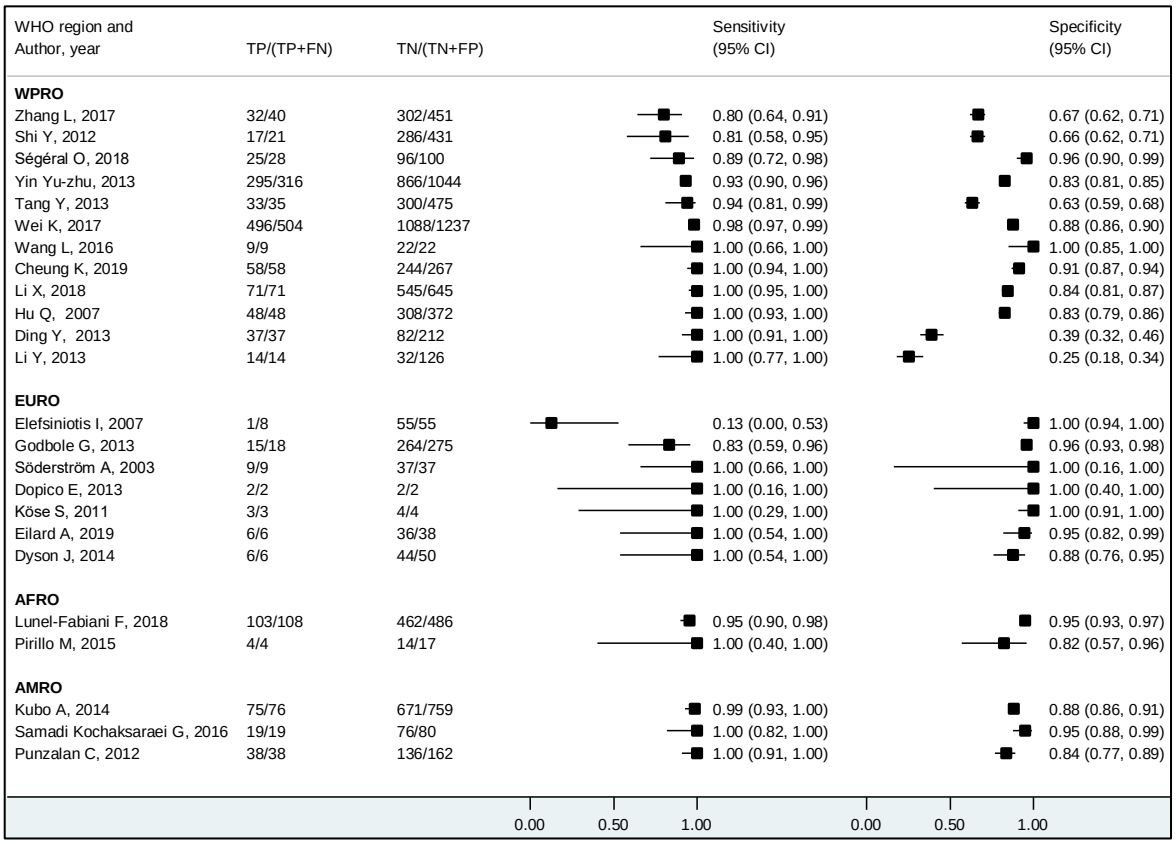
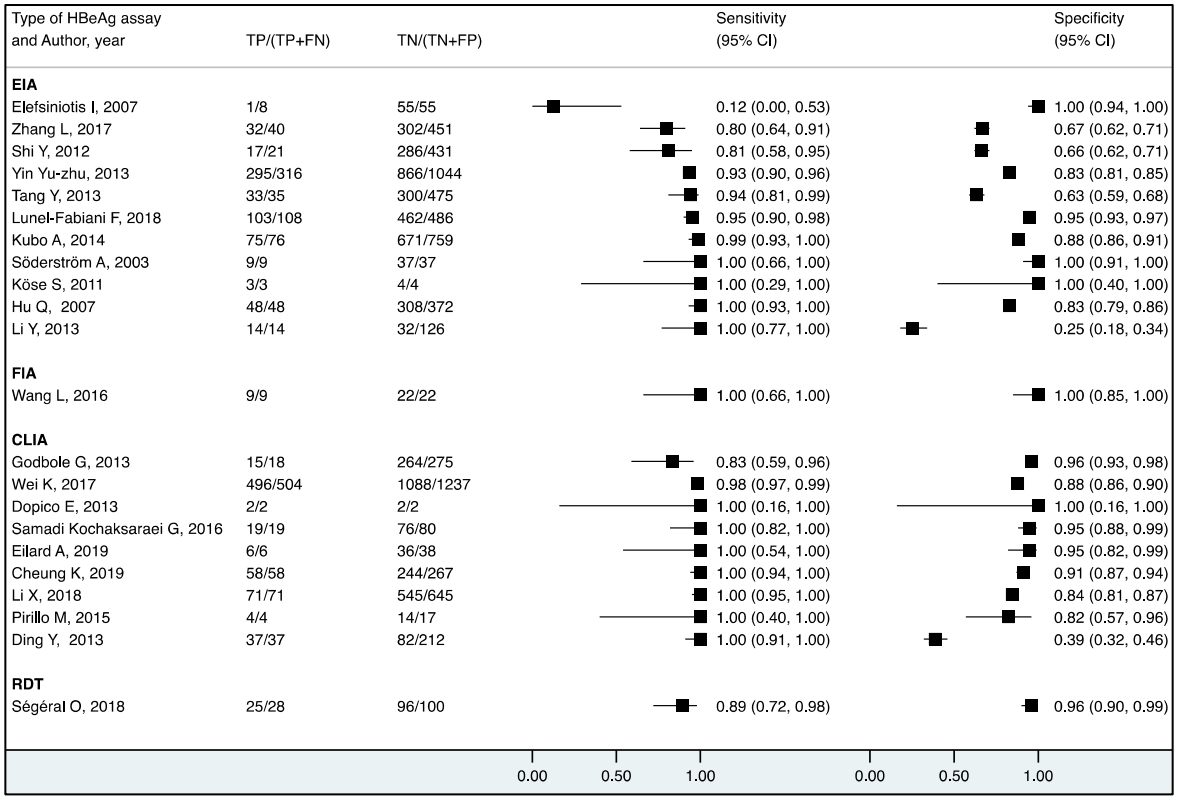
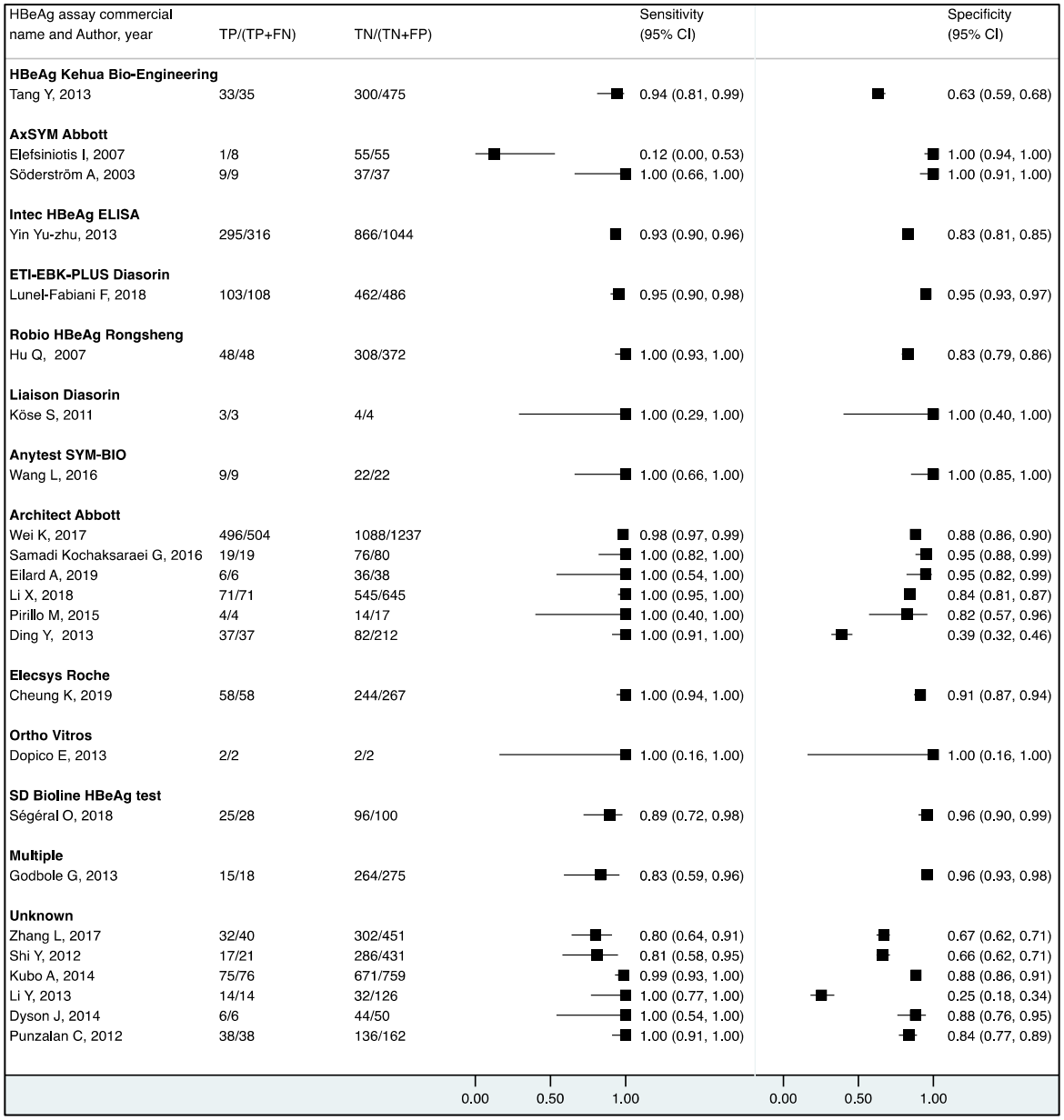


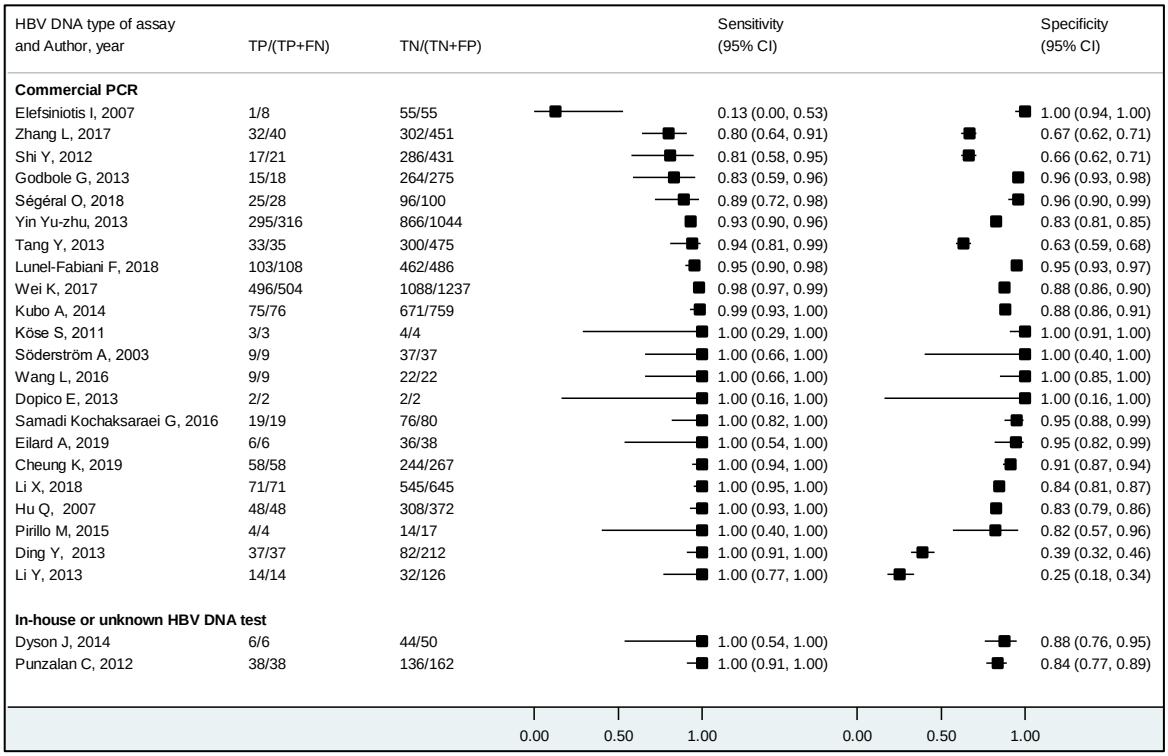
Fig. 23. Coupled forest plot of the performance of HBeAg to identify maternal HBV DNA ≥7 log10 IU/mL by HBeAg assay type



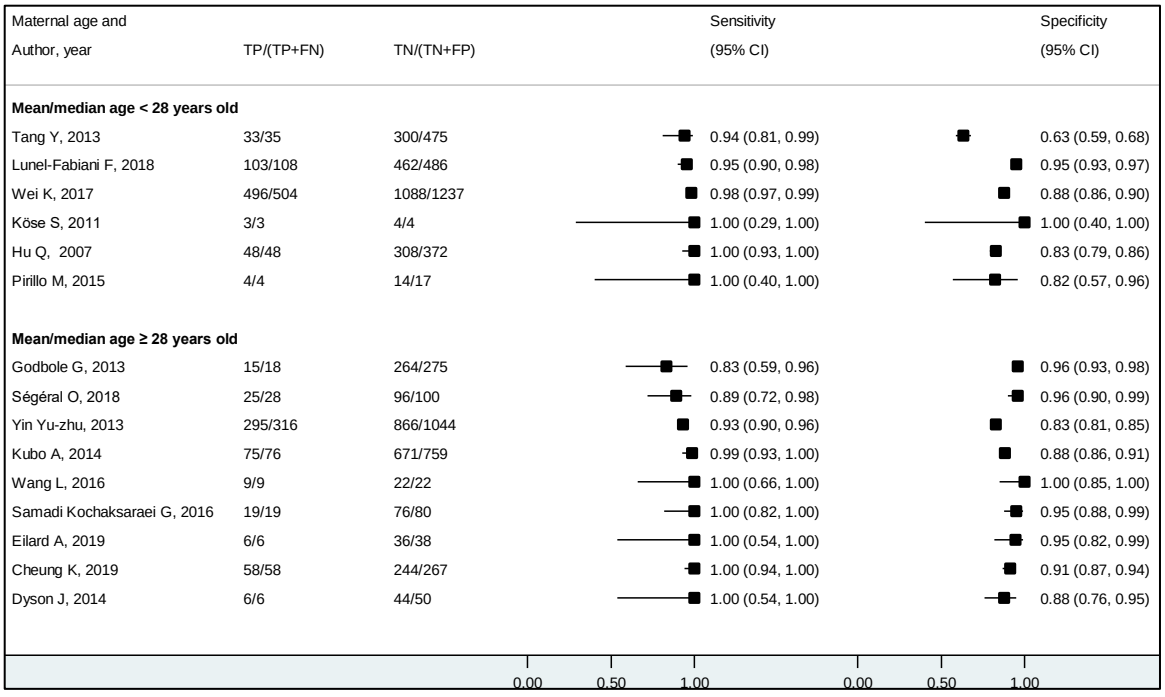
## Fig. 24. Coupled forest plot of the performance of HBeAg to identify maternal HBV DNA ≥7 log10 IU/mL by HBeAg assay commercial name



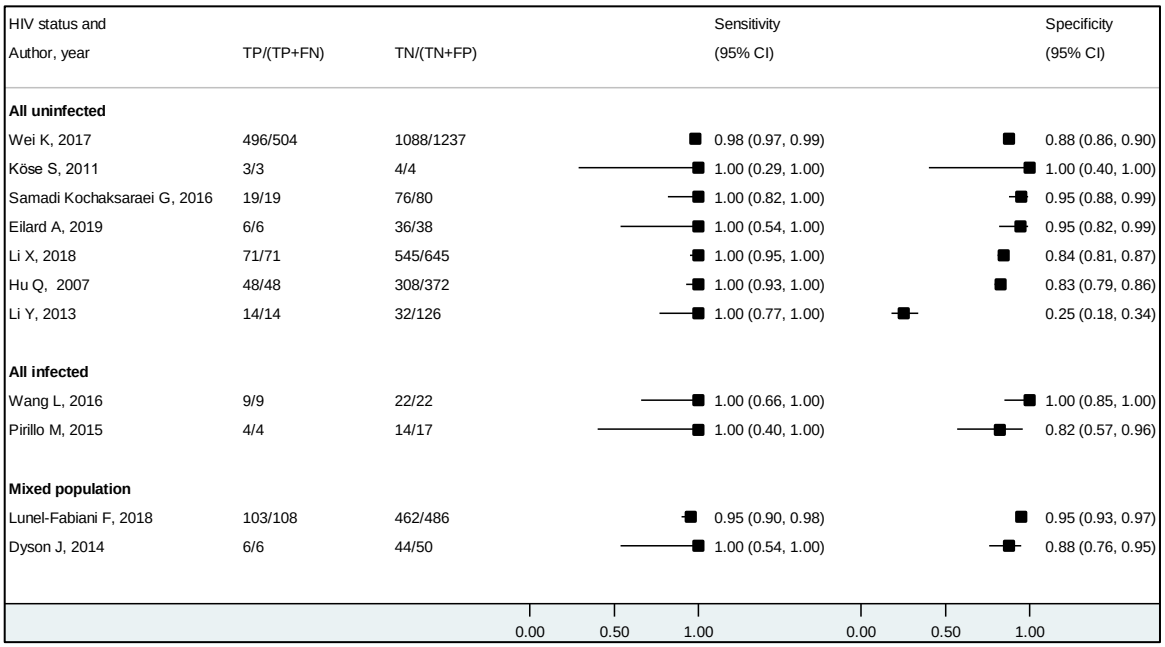
## Fig. 25. Coupled forest plot of the performance of HBeAg to identify maternal HBV DNA ≥7 log10 IU/mL by HBV DNA assay type




## Fig. 26. Coupled forest plot of the performance of HBeAg to identify maternal HBV DNA ≥7 log10 IU/mL by mean or median maternal age



## Fig. 27. Coupled forest plot of the performance of HBeAg to identify maternal HBV DNA ≥7 log10 IU/mL by maternal HIV status



**Sensitivity analysis for the performance of HBeAg to identify pregnant women with HBV DNA ≥7 log10 IU/mL (Table 11)**

As all the included studies provided sufficient data to conduct bivariate analysis, univariate analysis was not conducted.

When excluding the three outlier studies from the analysis or those with an overall "high" risk of bias as per QUADAS-2 assessment, there was no evidence of a difference in pooled sensitivity and specificity compared with those of the main bivariate analysis (95% CI overlapped).

Table 11. Results of sensitivity analysis for the performance of HBeAg to identify pregnant women with HBV DNA ≥7 log10 IU/mL

| Sensitivity analysis | Studies, N | Se (95% CI) or Range Se | Sp (95% CI) or Range Sp |
|---|---|---|---|
| **Overall bivariate** | 24 | 98.0 (93.3-99.4) | 88.5 (80.7-93.4) |
| **Bivariate without outlier studies** | 21 | 97.6 (94.1-99.0) | 89.3 (84.3-92.9) |
| **Bivariate without studies with an overall "high" risk of bias** | 20 | 98.6 (92.3-99.8) | 90.2 (81.3-95.1) |

### 4.2.4. GRADE evidence profile for PICO2A

Table 12. GRADE evidence profile table of the performance of HBeAg to identify pregnant women with an HBV viral load ≥5 log IU/mL, and ≥6 log IU/mL, ≥7 log IU/mL

| Test performance | Reference standard | Studies, n | Risk of bias | Inconsistency | Indirectness | Imprecision | Patients, n | Strength of evidence[a] |
|---|---|---|---|---|---|---|---|---|
| **HBeAg** | **HBV DNA 5 log10 IU/mL** | Sens: 41<br>Spec: 39 | No serious | No serious | No serious | No serious | Sens: 4414<br>Spec: 9817 | Sens: High<br>Spec: High |
| **HBeAg** | **HBV DNA 6 log10 IU/mL** | Sens: 34<br>Spec: 27 | No serious | No serious | No serious | No serious | Sens: 2814<br>Spec: 7382 | Sens: High<br>Spec: High |
| **HBeAg** | **HBV DNA 7 log10 IU/mL** | Sens: 24<br>Spec: 24 | No serious | No serious | No serious | No serious | Sens: 1478<br>Spec: 7347 | Sens: High<br>Spec: High |

[a] Although there was no evidence of publication bias, we did not take this factor into account to grade the strength of evidence because most studies were not designed to assess the diagnostic accuracy and there are no reliable methods to assess publication bias in such a context.

## 4.3. PICO2B: Performance of HBeAg to predict MTCT

Detailed characteristics of each included study are presented in Table 13 below.

Table 13. Characteristics of the 15 studies included for the PICO2B

| Author, year | PICO2B | Country | Original study design | HBeAg assay | Maternal age (years) | Maternal HIV, n (%) | Measure of prevention for MTCT | Children tested, n | Children age at testing (months) | Marker tested in children |
|---|---|---|---|---|---|---|---|---|---|---|
| **Chen Z, 2017** (40) | Bivariate | China | Prospective cohort | ELISA (AxSYM Abbott) | Mean: 26.96 | 0/211 (0.0%) | TBD + HBIg | 168 | 7 | HBsAg & HBV DNA |
| **Chotun N, 2017** (43) | Univariate (specificity) | South Africa | Prospective cohort | CLIA (Architect Abbott) | Mean (Range): 27 (21-34) | 0/6 (0.0%) | TBD only | 4 | 7 | HBsAg |
| **Ding Y, 2013** (45) | Bivariate | China | Prospective cohort | CLIA (Architect Abbott) | Unknown (Range: 16-45) (n=4536) | Unknown | TBD + HBIg | 249 | 7 | HBsAg & HBV DNA |
| **Foaud HM, 2019** (51) | Univariate (Specificity) | Egypt | Prospective cohort | ELISA (ETI-EBK PLUS Diasorin) | Mean: 27.3 | 0/41 (0.0%) | TBD + HBIg | 39 | 12 | HBV DNA |
| **Latthaphasavang V, 2019** (65) | Bivariate | Lao PDR | Prospective cohort | ELISA (Monolisa HBeAg-Ab PLUS Bio-Rad) | Median (IQR): 28 (24-30) | 1/153 (0.7%) | TBD only | 120 | 6 | HBsAg |
| **Lee L, 2015** (66) | Bivariate | Singapore | Prospective cohort | CLIA (Architect Abbott) | Mean (SD): 32.4 (4.7) | Unknown | TBD + HBIg | 154 | 9 | HBsAg & HBV DNA |
| **Liu CP, 2015** (71) | Univariate (Sensitivity) | China | Retrospective cohort | ELISA (HBeAg Santa-Cruz) | Mean: 25.7 | 0/256 (0.0%) | TBD + HBIg | 256 | 6 | HBsAg |
| **Liu Y, 2016** (73) | Bivariate | China | Prospective cohort | CLIA (Architect Abbott) | Mean 27.7 | 0/110 (0.0%) | TBD + HBIg | 78 | 12 | HBsAg & HBV DNA |
| **Lunel-Fabiani F, 2018** (75) | Bivariate | Cameroon | Prospective cohort | Unknown | Median (IQR): 24 (20-30) | 1/592 tested (0.2%) | TBD only | 133 | 24 | HBsAg |
| **Peng S, 2019** (79) | Bivariate | China | Prospective cohort | ELISA (Kehua HBeAg) | Mean: 29.17 | 0/1219 (0.0%) | TBD + HBIg | 93 | 7 | HBsAg |
| **Shao Z, 2011** (88) | Univariate (Sensitivity) | China | Prospective cohort | ELISA (AxSYM Abbott) | Unknown | Unknown | TBD + HBIg | 158 | 7 | HBsAg |
| **Thilakanathan C, 2018** (94) | Univariate (Sensitivity) | Australia | Retrospective cohort | CLIA (Architect Abbott) | Median (Range): 30 (15-44) | Unknown | TBD + HBIg | 469 | 9 | HBsAg |
| **Wiseman, E, 2009** (105) | Bivariate | Australia | Prospective cohort | CLIA or ELISA (AxSYM Abbott or Architect Abbott) | Unknown | 0/313 (0.0%) | TBD + HBIg | 138 | 9 | HBsAg |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Yin Y, 2013** (108) | Bivariate | China | Retrospective cohort | ELISA (Intec HBeAg) | Mean (SD): 28.83 (3.92) | Unknown | TBD + HBIg | 1360 | 12 | HBsAg & HBV DNA |
| **Zhu B, 2013** (112) | Univariate (Specificity) | China | Retrospective cohort | CLIA (Elecsys Roche) | Mean (SD): 26 (4.1) | 0/53 (0.0%) | TBD + HBIg | 53 | 12 | HBsAg |

### 4.3.1. Summary of included studies

There were 15 original studies, including 9 that allowed for the estimation of both sensitivity and specificity using bivariate analysis, and for the remaining 6, only univariate analysis was possible to estimate sensitivity (n=3) or specificity (n=3). All were observational cohort studies (11 prospective and 4 retrospective) (Table 13).

Recruitment took place from 2002 (*Shao Z, 2011 and Wiseman E, 2009*) to 2017 (*Latthaphasavang V, 2019*). Most studies took place in the Western Pacific Region (n=12); including China (n=8), Lao People's Democratic Republic (n=1), Singapore (n=1) and Australia (n=2). Two studies took place in WHO African Region: Cameroon (n=1) and South Africa (n=1); and one study in the WHO Eastern Mediterranean Region in Egypt (Table 13). No study took place in WHO South-East Asian, European or American regions.

Table 14 describes the characteristics of studies included in PICO2B. Of 15 studies, 12 reported maternal age: 7 (58.3%) with a mean/median maternal age below 28 years; and 5 (41.7%) with ≥28 years. EIA and CLIA were most frequently used to assess HBeAg (8/15=53.3% and 6/15=40.0, respectively). No study reported the use of a rapid diagnostic test. Most studies included women not infected with HIV, HCV or HDV (n=7/9 (77.8%), n=5/6 (83.3%) and n=2/2 (100.0%), respectively). Four studies reported HBV genotypes: one with genotype D only and 3 with mixed genotypes. Among studies included in PICO2B. All studies reported giving timely birth dose of HBV vaccine and 12 studies (80.0%) reported giving HBIG to infants. Lastly, all 15 studies reported that infants had received at least 3 doses of HBV vaccine.

Table 14. Characteristics of studies included in the PICO2B

| PICO2B | | Overall (n=15) | | TBD + HBIG (n=12) | | TBD only (n=3) | |
|---|---|---|---|---|---|---|---|
| **Subgroup variables** | **Subgroup category** | **Studies (N)** | **%** | **Studies (N)** | **%** | **Studies (N)** | **%** |
| **WHO region (n=15)** | **WPRO** | 12 | 80.0 | 11 | 91.7 | 1 | 33.3 |
| | **SEARO** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **EURO** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **EMRO** | 1 | 6.7 | 0 | 0.0 | 0 | 0.0 |
| | **AFRO** | 2 | 13.3 | 1 | 8.3 | 2 | 66.7 |
| | **AMRO** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **HBeAg assay type (n=15)** | **EIA** | 8 | 53.3 | 6 | 50.0 | 2 | 66.7 |
| | **FIA** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **CLIA** | 6 | 40.0 | 5 | 41.7 | 1 | 33.3 |
| | **RDT** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Multiple** | 1 | 6.7 | 1 | 8.3 | 0 | 0.0 |
| **Maternal age (n=12)** | **<28 years** | 7 | 58.3 | 5 | 55.6 | 2 | 66.7 |
| | **≥28 years** | 5 | 41.7 | 4 | 44.4 | 1 | 33.3 |
| **HBIg at birth (n=15)** | **No** | 3 | 20.0 | 0 | 0.0 | 3 | 100.0 |
| | **Yes** | 12 | 80.0 | 12 | 100.0 | 0 | 0.0 |
| **First dose of HBV vaccine (n=15)** | **Timely birth dose** | 15 | 100.0 | 12 | 100.0 | 3 | 100.0 |
| | **Delayed** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **Three doses of HBV vaccine (n=15)** | **No** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Yes** | 15 | 100.0 | 12 | 100.0 | 3 | 100.0 |
| **HIV status (n=9)** | **All uninfected** | 7 | 77.8 | 6 | 100.0 | 1 | 33.3 |
| | **All infected** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Mixed population** | 2 | 22.2 | 0 | 0.0 | 2 | 66.7 |
| **HCV status (n=6)** | **All uninfected** | 5 | 83.3 | 5 | 83.3 | 0 | 0.0 |
| | **All infected** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Mixed population** | 1 | 16.7 | 1 | 16.7 | 0 | 0.0 |
| **HDV status (n=2)** | **All uninfected** | 2 | 100.0 | 2 | 100.0 | 0 | 0.0 |
| | **All infected** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Mixed population** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **Genotype (n=4)** | **Genotype A** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Genotype B** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Genotype C** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Genotype D** | 1 | 0.0 | 0 | 0.0 | 1 | 50.0 |
| | **Genotype E** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Genotype F** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Genotype I** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Mixed population** | 3 | 100.0 | 2 | 100.0 | 1 | 50.0 |

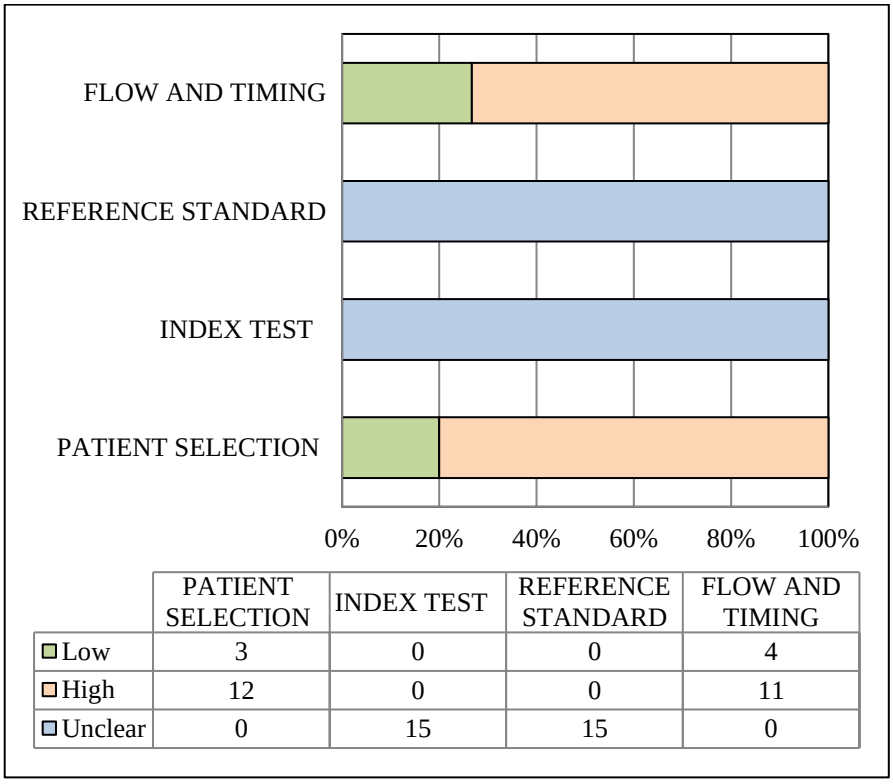
### 4.3.2. Risk of bias assessment

The risk of bias assessment is presented in Fig. 28 & 29 (and details in **Appendix E**).

None of the studies had the same objective as that of the PICO2B. Therefore, the essential information for the diagnostic studies were often missing. All studies were rated as unclear risk of bias for the reference standard and for the index tests because whether blinding was performed was not mentioned (Fig. 28). Risk of selection bias was high in most studies (n=12/15, 80.0%), mainly due to restrictive exclusion criteria (e.g. exclusion of HIV-, HCV-, or HDV-coinfected pregnant women).

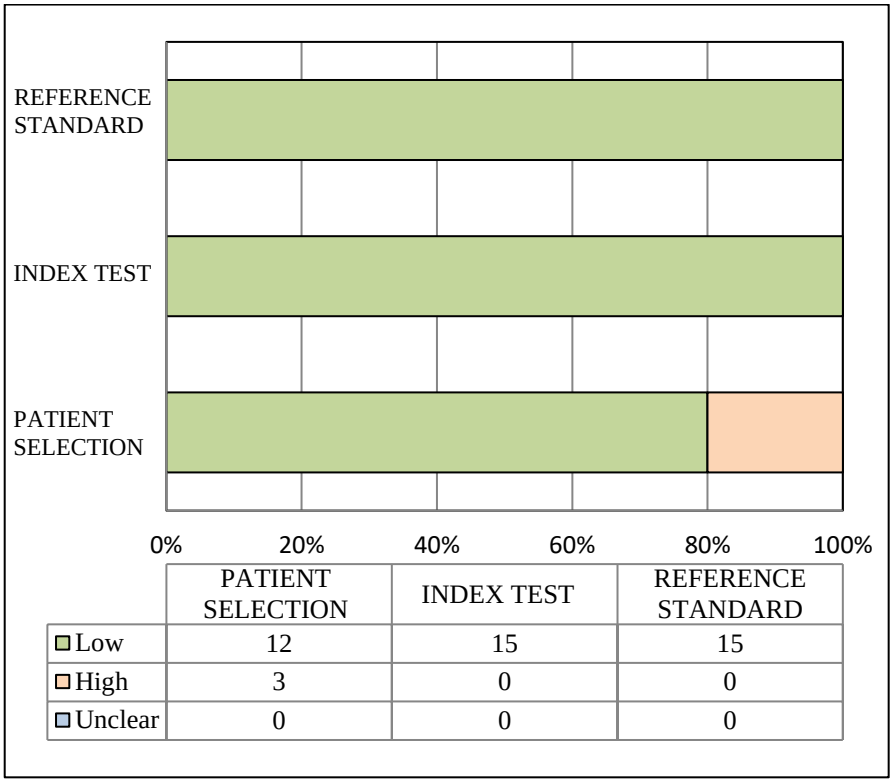
Concerns about applicability were low for most studies (Fig. 29). There was high concern for patient selection applicability in 3 studies (20.0%), due to the enrolment of pregnant women based on their HBV viral load. There was no concern about the applicability of the index test or about the reference standard.

Fig. 28. Risk of bias results for PICO2B




| | PATIENT SELECTION | INDEX TEST | REFERENCE STANDARD | FLOW AND TIMING |
|---|---|---|---|---|
| Low | 3 | 0 | 0 | 4 |
| High | 12 | 0 | 0 | 11 |
| Unclear | 0 | 15 | 15 | 0 |

Fig. 29. Concerns about applicability results for PICO2B




| | PATIENT SELECTION | INDEX TEST | REFERENCE STANDARD |
|---|---|---|---|
| Low | 12 | 15 | 15 |
| High | 3 | 0 | 0 |
| Unclear | 0 | 0 | 0 |

### 4.3.3. Primary analysis, narrative descriptions and forest plots

Overall performance of HBeAg to identify pregnant women at high risk of mother-to-child transmission of HBV, stratified by prevention of MTCT strategy, was as below.

- Overall pooled sensitivity = 99.1 (95% CI: 61.8-100.0)
- Overall pooled specificity = 55.7 (95% CI: 34.0-75.5)

Tables 15 and 16 present detailed performance results for PICO2B. There was no evidence of publication bias (*P*=0.97) (Appendix G.4). We identified one outlier study (*Liu Y, 2016*) through visual assessment of the coupled forest plots (Fig. 30 and 31) and the summary scatter plot with SROC curve (Fig. 32).

The outlier study was a prospective cohort in China that reported high sensitivity (100.0%) and very low specificity (3.0%) that recruited high viral load (HBV DNA ≥6 log IU/mL) pregnancy women from 2010 to 2013. HBeAg status in pregnant women was determined using CLIA (Architect Abbott). Mean maternal age was 27.7 years and all enrolled women were HIV negative. In this study, infants received a timely birth dose vaccine, HBIg at birth and 2 subsequent doses of infant vaccine at 1 month and 6 months of age.

Table 15. Performance of HBeAg during pregnancy to predict MTCT of HBV, stratified by HBV MTCT prevention strategy

| Prevention of MTCT strategy | Meta-analysis | Studies (N) | Subjects (N) | TP (N) | FN (N) | TN (N) | FP (N) | Se (95% CI) or Range Se | Sp (95% CI) or Range Sp |
|---|---|---|---|---|---|---|---|---|---|
| **Overall** | **Bivariate** | 9 | 3619 | 92 | 9 | 2401 | 1117 | 99.1 (61.8-100.0) | 55.7 (34.0-75.5) |
| | **Univariate** | 15 | 3720 | 106 | 9 | 2454 | 1151 | 84.8 (74.8-91.3) | 59.9 (48.0-70.7) |
| **TBD + HBIg** | **Bivariate** | 7 | 3366 | 84 | 9 | 2220 | 1053 | 98.8 (52.0-100.0) | 49.2 (25.1-73.7) |
| | **Univariate** | 12 | 3465 | 98 | 9 | 2271 | 1087 | 86.5 (74.3-93.4) | 55.2 (41.4-68.2) |
| **TBD only** | **Bivariate** | 2 | 253 | 8 | 0 | 181 | 64 | 100.0 | 60.0-86.2 |
| | **Univariate** | 3 | 255 | 8 | 0 | 183 | 64 | 100.0 | 76.1 (47.8-91.7) |

Table 16. Post-test probabilities of having MTCT of HBV after positive or negative maternal HBeAg result

| HBeAg to identify MTCT | Studies, N | Pretest probability (%) | PLR (95% CI) or Range PLR | Posttest probability of having MTCT of HBV after positive HBeAg result (%) | NLR (95% CI) or Range NLR | Post-test probability of having MTCT of HBV after positive HBeAg result (%) |
|---|---|---|---|---|---|---|
| **Overall** | 9 | 4 | 2.2 (1.4-3.7) | 8.2 | 0.02 (0.00-1.0) | 0.07 |
| **TBD + HBIG** | 2 | 3 | 2.5-7.2 | 7.0-17.8 | 0.00 | 0.00 |
| **TBD only** | 9 | 4 | 2.2 (1.4-3.7) | 8.2 | 0.02 (0.00-1.0) | 0.07 |

Fig. 30. Coupled forest plot of the performance of HBeAg during pregnancy to predict MTCT of HBV by prevention strategy for MTCT

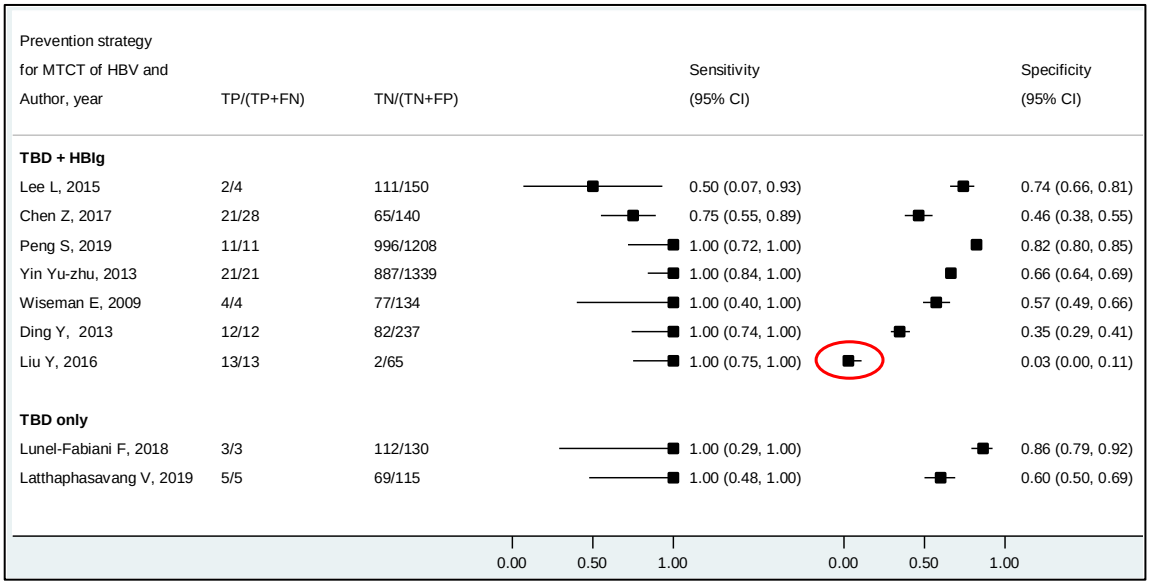


Fig. 31. Coupled forest plot of the performance of HBeAg during pregnancy to predict MTCT of HBV by HBeAg assay

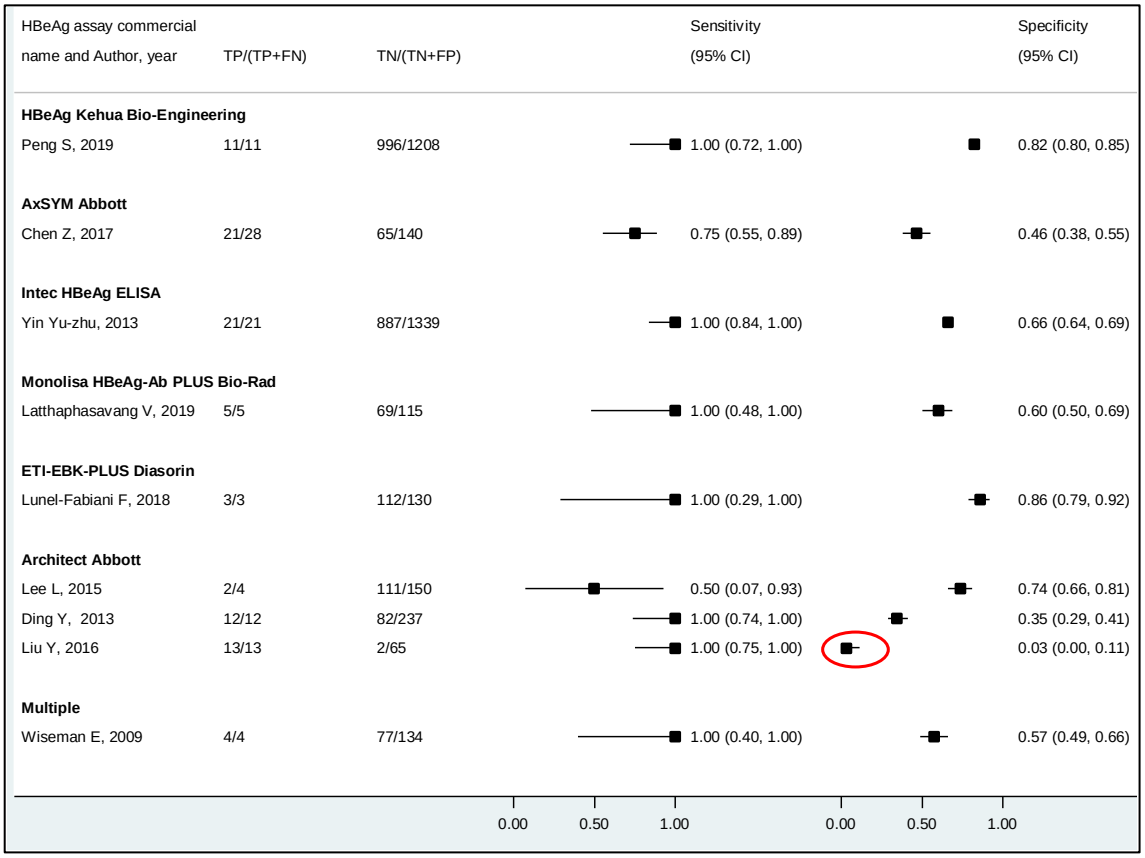
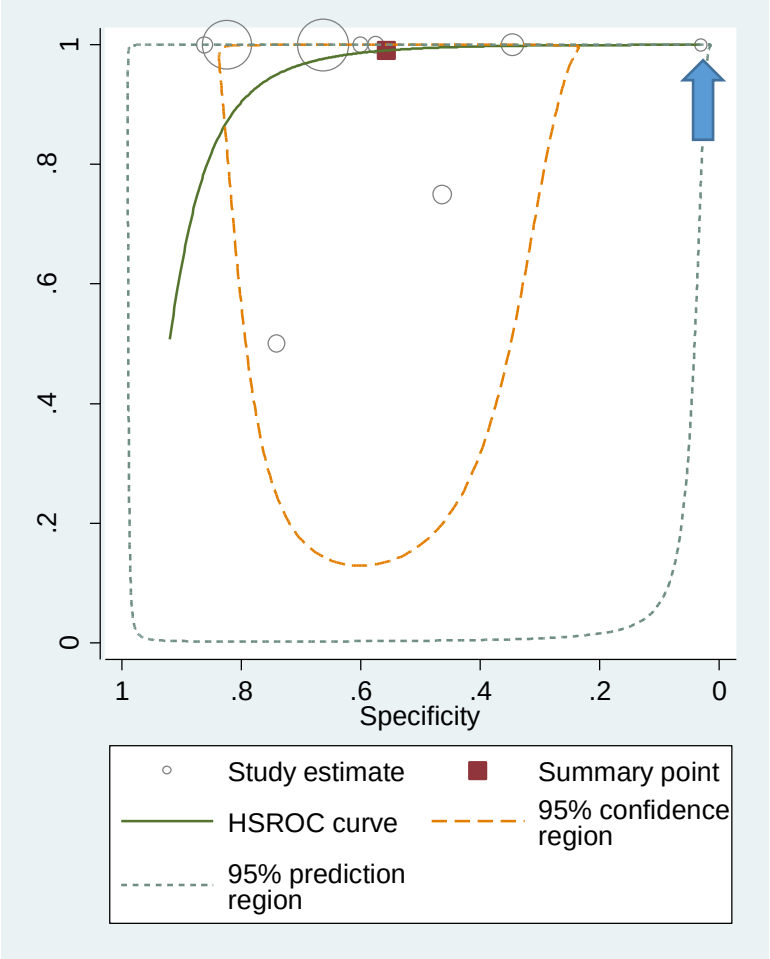


Fig. 32. Summary scatter plot with SROC curve of the performance of HBeAg during pregnancy to predict MTCT



**Subgroup analysis**

As the number of studies was low, we were not able to perform subgroup analysis of the performance of HBeAg during pregnancy to predict MTCT.

**Sensitivity analysis for the performance of HBeAg to predict MTCT (Table 17)**

When performing univariate analysis and excluding the outlier study from the analysis or those with an overall "high" risk of bias, results remained unchanged compared with that of the main bivariate analysis (95% CI overlapped).

Table 17. Results of sensitivity analysis for the performance of HBeAg during pregnancy to predict MTCT

| | **Studies, N** | **Se (95% CI) or Range Se** | **Sp (95% CI) or Range Sp** |
|---|---|---|---|
| **Overall bivariate** | 9 | 99.1 (61.8-100.0) | 55.7 (34.0-75.5) |
| **Overall univariate** | 15 | 84.8 (74.8-91.3) | 59.9 (48.0-70.7) |
| **Bivariate without outlier studies** | 8 | 98.6 (59.0-100.0) | 65.1 (52.2-76.2) |
| **Bivariate without studies with an overall "high" risk of bias** | 6 | 100.0 | 34.6-86.2 |

### 4.3.4. GRADE evidence profile for PICO2B

Table 18. GRADE evidence profile table of the performance of HBeAg during pregnancy to predict MTCT, stratified by prevention strategy for MTCT

| Test performance | Reference standard | Prevention strategy for MTCT | Studies, n | Risk of bias | Inconsistency | Indirectness | Imprecision | Patients, n | Strength of evidence[a] |
|---|---|---|---|---|---|---|---|---|---|
| **HBeAg** | **MTCT** | **Overall** | Sens: 12<br>Spec: 12 | Serious | No serious | No serious | Serious | Sens: 115<br>Spec: 3605 | Sens: Low[b]<br>Spec: Low[b] |
| **HBeAg** | **MTCT** | **TBD + HBIg** | Sens: 10<br>Spec: 9 | Serious | No serious | No serious | Serious | Sens: 107<br>Spec: 3358 | Sens: Low[c]<br>Spec: Low[c] |
| **HBeAg** | **MTCT** | **TBD only** | Sens: 2<br>Spec: 3 | Serious | Serious | No serious | Serious | Sens: 8<br>Spec: 247 | Sens: Insufficient data[d]<br>Spec: Very low[d] |

[a] Although there was no evidence of publication bias, we did not take this factor into account to grade the strength of evidence because none of the included studies was designed to assess the diagnostic accuracy and there are no reliable methods to assess publication bias in such a context.

[b] Downgraded from high to low strength of evidence because 7/12 studies that evaluated sensitivity and 8/12 studies that allowed estimation of specificity had an overall high risk of bias, and there was serious imprecision because 95% confidence range for pooled specificity was 22.7%.
[c] Downgraded from high to low strength of evidence because 6/10 studies that allowed estimation of sensitivity and 6/9 studies that evaluated specificity had an overall high risk of bias, and there was serious imprecision in pooled specificity estimate (95% CI range was 26.8%);
[d] We did not rate evidence for sensitivity because there were only two studies available and results could not be pooled. Concerning specificity, we downgraded from high to very low the strength of evidence because 2/3 studies that allowed estimation of specificity had an overall high risk of bias, there was serious inconsistency as 1/3 study was not within 20% of the pooled estimate, and there was serious imprecision in pooled specificity estimate (95% CI range was 40.0%);

## 4.4. PICO2C: Performance of HBV DNA to predict MTCT

Detailed characteristics of each included study are presented in Table 19 below.

Table 19. Characteristics of 13 studies included in the PICO2C

| Author, year | PICO2C | Country | Original study design | HBV DNA threshold(s) (IU/mL) | HBV DNA assay | Maternal age (years) | Maternal HIV, n (%) | Measure of prevention for MTCT | Children tested, n | Children age at testing (months) | Marker tested in children |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Chen Z, 2017** (40) | Bivariate | China | Prospective cohort | 5 log10 | Unknown | Mean: 26.96 | 0/211 (0.0%) | TBD + HBIg | 168 | 7 | HBsAg & HBV DNA |
| **Chotun N, 2017** (43) | Univariate (Specificity) | South Africa | Prospective cohort | 20,000 & 5 log10 | PCR Amplicor HBV Monitor test (Roche) | Mean (Range): 27 (21-34) | 0/6 (0.0%) | TBD only | 4 | 7 | HBsAg |
| **Ding Y, 2013** (45) | Bivariate | China | Prospective cohort | 7 log10 | Real-Time Fluorescence quantitative PCR (Kehua Bio-engineering) | Unknown (Range: 16-45) (n=4536) | Unknown | TBD + HBIg | 249 | 7 | HBsAg & HBV DNA |
| **Latthaphasavang V, 2019** (65) | Bivariate | Lao PDR | Prospective cohort | 5 log10 | PCR Amplicor HBV Monitor test (Roche) | Median (IQR): 28 (24-30) | 1/153 (0.7%) | TBD only | 120 | 6 | HBsAg |
| **Lee L, 2015** (66) | Bivariate | Singapore | Prospective cohort | 20,000 | PCR COBAS Taqman (Roche) | Mean (SD): 32.4 (4.7) | Unknown | TBD + HBIg | 154 | 9 | HBsAg & HBV DNA |
| **Liu CP, 2015** (71) | Univariate (Sensitivity) | China | Retrospective cohort | 6, 7 & 8 log10 | PCR COBAS Taqman (Roche) | Mean: 25.7 | 0/256 (0.0%) | TBD + HBIg | 256 | 6 | HBsAg |
| **Lunel-Fabiani F, 2018** (75) | Bivariate | Cameroun | Prospective cohort | 8 log10 | PCR Da'an Gene | Median (IQR): 24 (20-30) | 1/592 tested (0.2%) | TBD only | 133 | 24 | HBsAg |
| **Peng S, 2019** (79) | Bivariate | China | Prospective cohort | 6 log10 | Real-Time Fluorescence quantitative PCR (Kehua Bio-engineering) | Mean: 29.17 | 0/1219 (0.0%) | TBD + HBIg | 93 | 7 | HBsAg |
| **Shao Z, 2011** (88) | Univariate (Sensitivity) | China | Prospective cohort | 5 & 8 log10 | Real-time PCR (Fast-track Diagnostics) | Unknown | Unknown | TBD + HBIg | 158 | 7 | HBsAg |
| **Thilakanathan C, 2018** (94) | Bivariate | Australia | Retrospective cohort | 5 & 6 log10 | PCR COBAS Taqman (Roche) | Median (Range): 30 (15-44) | Unknown | TBD + HBIg | 469 | 9 | HBsAg |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Wiseman, E, 2009** (105) | Bivariate | Australia | Prospective cohort | 8 log10 | PCR artus HBV PCR Kits, QIAGEN | Unknown | 0/313 (0.0%) | TBD + HBIg | 138 | 9 | HBsAg |
| **Yin Yu-zhu, 2013** (108) | Bivariate | China | Retrospective cohort | 5 &7 log10 | PCR Da'an Gene | Mean (SD): 28.83 (3.92) | Unknown | TBD + HBIg | 1360 | 12 | HBsAg & HBV DNA |
| **Zhu B, 2013** (112) | Univariate (Specificity) | China | Retrospective cohort | 5 log10 | PCR COBAS Taqman (Roche) | Mean (SD): 26 (4.1) | 0/53 (0.0%) | TBD + HBIg | 53 | 12 | HBsAg |

### 4.4.1. Summary of included studies

There were 13 original studies, including 9 for which bivariate analysis was performed, and 4 for which only univariate analysis to estimate sensitivity (n=2) or specificity (n=2) was possible. All were observational cohort studies (9 prospective and 4 retrospective) (Table 19).

Recruitment took place from 2002 (*Shao Z, 2011* and *Wiseman E, 2009*) to 2017 (*Latthaphasavang V, 2019*). Most studies took place in the Western Pacific Region (n=11); including China (n=7), Lao People's Democratic Republic (n=1), Singapore (n=1) and Australia (n=2). Two studies took place in the African Region: Cameroon (n=1) and South Africa (n=1). None of the studies took place in AMR, EUR, EMR or SEAR (Table 20).

Among studies included in PICO2C, 10/13 studies (76.9%) reported giving HBIg at birth, and all gave timely birth dose of HBV vaccine to infants. Table 20 describes the characteristics of studies included in the PICO2C.

Table 20. Characteristics of studies included in the PICO2C

| | | Overall (n=13) | | TBD + HBIG (n=10) | | TBD only (n=3) | |
|---|---|---|---|---|---|---|---|
| **Subgroup variables** | **Subgroup category** | **Studies (N)** | **%** | **Studies (N)** | **%** | **Studies (N)** | **%** |
| **WHO region (n=13)** | **WPR** | 11 | 84.6 | 10 | 100.0 | 1 | 33.3 |
| | **SEAR** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **EUR** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **EMR** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **AFR** | 2 | 15.4 | 0 | 0.0 | 2 | 66.7 |
| | **AMR** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **HBeAg assay type (n=13)** | **EIA** | 7 | 53.8 | 5 | 50.0 | 2 | 66.7 |
| | **FIA** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **CLIA** | 5 | 38.5 | 4 | 40.0 | 1 | 33.3 |
| | **RDT** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Multiple** | 1 | 7.7 | 1 | 10.0 | 0 | 0.0 |
| **Maternal age (n=10)** | **<28 years** | 5 | 50.0 | 3 | 42.9 | 2 | 66.7 |
| | **≥28 years** | 5 | 50.0 | 4 | 57.1 | 1 | 33.3 |
| **HBIg at birth (n=13)** | **No** | 3 | 23.1 | 0 | 0.0 | 3 | 100.0 |
| | **Yes** | 10 | 76.9 | 10 | 100.0 | 0 | 0.0 |
| **First dose of HBV vaccine (n=15)** | **Timely birth dose** | 13 | 100.0 | 10 | 100.0 | 3 | 100.0 |
| | **Delayed** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **Three doses of HBV vaccine (n=15)** | **No** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Yes** | 13 | 100.0 | 10 | 100.0 | 3 | 100.0 |
| **HIV status (n=11)** | **All uninfected** | 9 | 81.8 | 7 | 100.0 | 2 | 50.0 |
| | **All infected** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Mixed population** | 2 | 18.2 | 0 | 0.0 | 2 | 50.0 |
| **HCV status (n=7)** | **All uninfected** | 6 | 85.7 | 6 | 85.7 | 0 | 0.0 |
| | **All infected** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Mixed population** | 1 | 14.3 | 1 | 14.3 | 0 | 0.0 |
| **HDV status (n=3)** | **All uninfected** | 3 | 100.0 | 3 | 42.9 | 0 | 0.0 |
| | **All infected** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Mixed population** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **Genotype (n=3)** | **Genotype A** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Genotype B** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Genotype C** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Genotype D** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Genotype E** | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| | **Mixed population** | 3 | 100.0 | 1 | 100.0 | 2 | 100.0 |

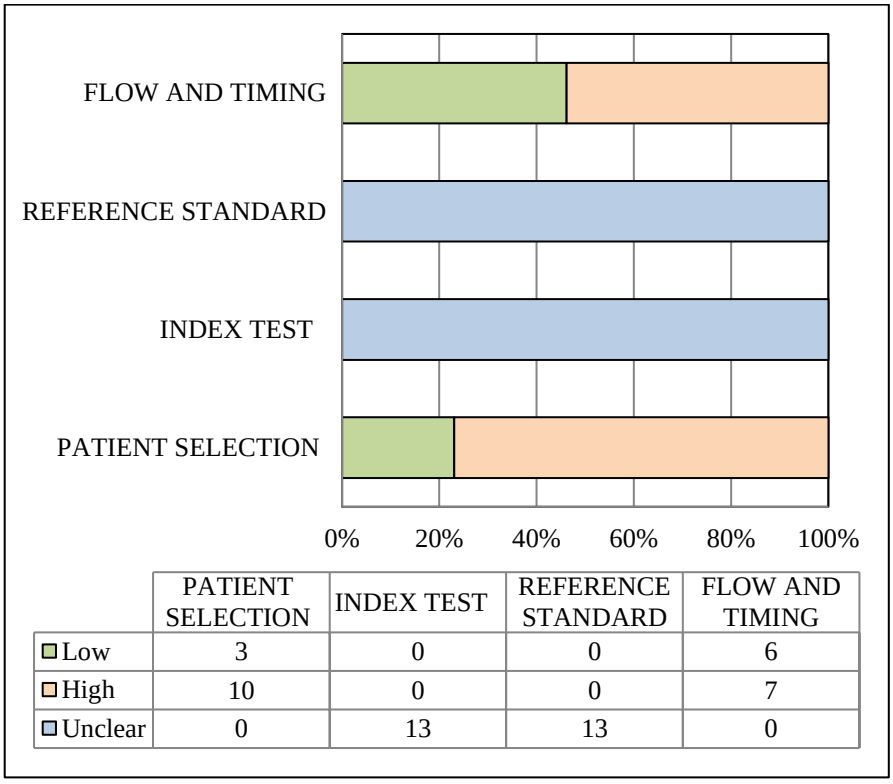
### 4.4.2. Risk of bias assessment

The risk of bias assessment is presented in Fig. 33 & 34 (and details in **Appendix F**).

Overall, none of the studies included had the same objective as that of the PICO2C, and therefore, essential information was often missing. All studies were rated as having an unclear risk of bias for the reference standard and for the index tests because they did not mention whether or not blinding was performed (Fig. 33). Risk of selection bias was high in most studies (n=10/13, 76.9%), mainly due to restrictive exclusion criteria (e.g. exclusion of HIV-, HCV-, or HDV-coinfected pregnant women).

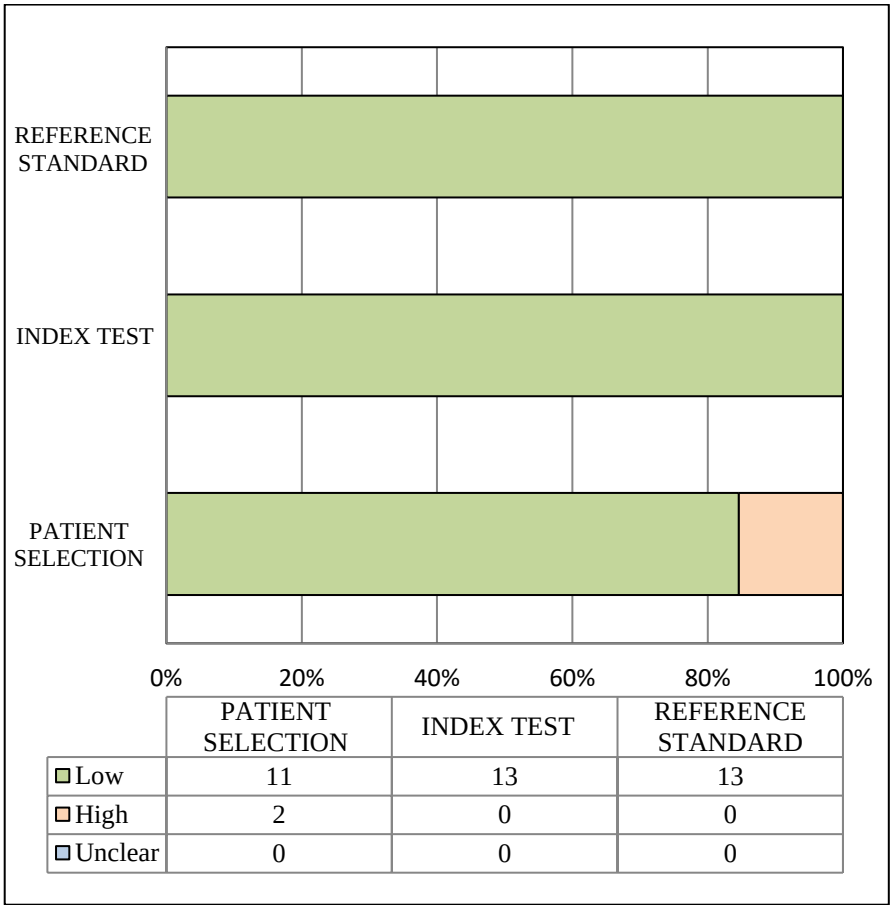
Concerns about applicability were low for most studies (Fig. 34). There was high concern for patient selection applicability for two studies (15.4%), due to the enrolment of pregnant women based on their HBV viral load. There was no concern about applicability of index test or HBV DNA assay test used.

Fig. 33. Risk of bias results for PICO2C




| | PATIENT SELECTION | INDEX TEST | REFERENCE STANDARD | FLOW AND TIMING |
|---|---|---|---|---|
| Low | 3 | 0 | 0 | 6 |
| High | 10 | 0 | 0 | 7 |
| Unclear | 0 | 13 | 13 | 0 |

Fig. 34. Concerns about applicability results for PICO2C




| | PATIENT SELECTION | INDEX TEST | REFERENCE STANDARD |
|---|---|---|---|
| Low | 11 | 13 | 13 |
| High | 2 | 0 | 0 |
| Unclear | 0 | 0 | 0 |

### 4.4.3. Primary analysis, narrative descriptions and forest plots

#### 4.4.3.1. Overall performance of HBV DNA during pregnancy to predict MTCT

Tables 21 and 22 present detailed performance results for the PICO2C. In the following sections, we present detailed results of the performance of HBV DNA ≥5 and 7 log IU/mL to predict MTCT.

Table 21. Performance of HBV DNA during pregnancy to predict MTCT, stratified by HBV DNA threshold and prevention of MTCT strategy

| **HBV DNA threshold (IU/mL)** | **Prevention of MTCT strategy** | **Meta-analysis** | Studies, N | Subjects (N) | TP (N) | FN (N) | TN (N) | FP (N) | Se (95% CI) or Range Se | Sp(95% CI) or Range Sp |
|---|---|---|---|---|---|---|---|---|---|---|
| **≥5 log** | **Overall** | **Bivariate** | 4 | 1999 | 46 | 10 | 1298 | 645 | 97.7 (42.9-100.0) | 68.4 (48.6-83.2) |
| | | **Univariate** | 7 | 2056 | 48 | 10 | 1329 | 669 | 81.5 (62.4-92.1) | 66.6 (51.3-79.0) |
| | **TBD + HBIg** | **Bivariate** | 3 | 1879 | 41 | 10 | 1231 | 597 | 97.9 (30.3-100.0) | 71.1 (46.0-87.7) |
| | | **Univariate** | 5 | 1934 | 43 | 10 | 1260 | 621 | 81.1 (57.8-93.1) | 67.5 (47.8-82.6) |
| | **TBD only** | **Bivariate** | 1 | 120 | 5 | 0 | 67 | 48 | 100.0 | 58.3 |
| | | **Univariate** | 2 | 122 | 5 | 0 | 69 | 48 | 100.0 | 58.3-100.0 |
| **≥6 log** | **Overall (All TBD + HBIG)** | **Bivariate** | 2 | 1570 | 13 | 0 | 1404 | 153 | 100.0 | 89.1-94.0 |
| | | **Univariate** | 3 | 1580 | 23 | 0 | 1404 | 153 | 93.3 (72.5-98.7) | 89.1-94.0 |
| **≥7 log** | **Overall (All TBD + HBIG)** | **Bivariate** | 2 | 1609 | 31 | 2 | 1254 | 322 | 90.5 -100.0 | 77.8-89.5 |
| | | **Univariate** | 3 | 1619 | 37 | 6 | 1254 | 322 | 84.4 (52.0-96.4) | 77.8-89.5 |
| **≥8 log** | **Overall** | **Bivariate** | 2 | 271 | 7 | 0 | 210 | 54 | 100.0 | 67.9-91.5 |
| | | **Univariate** | 4 | 283 | 9 | 10 | 210 | 54 | 57.8 (13.3-92.4) | 82.1 (49.0-95.6) |
| | **TBD + HBIg** | **Bivariate** | 1 | 138 | 4 | 0 | 91 | 43 | 100.0 | 67.9 |
| | | **Univariate** | 3 | 150 | 6 | 10 | 91 | 43 | 45.9 (6.1-91.7) | 67.9 |
| | **TBD only** | **Bivariate** | 1 | 133 | 3 | 0 | 119 | 11 | 100.0 | 91.5 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Univariate | - | - | - | - | - | - | - | - |

Table 22. Post-test probabilities of MTCT if pregnant women have high or low HBV viral load, according to various HBV DNA thresholds and prevention of MTCT strategies

| **HBV DNA threshold (IU/mL)** | **HBV DNA to diagnose MTCT** | **Studies, N** | **Pre-test probability (%)** | **LR+ (95% CI) or Range LR+** | **Post-test probability of having MTCT after high viral load result for HBV DNA test (%)** | **LR- (95% CI) or Range LR-** | **Post-test probability of MTCT after low viral load result for HBV DNA test (%)** |
|---|---|---|---|---|---|---|---|
| **5 log10** | **Overall** | 4 | 4 | 3.1 (1.6-5.8) | 11.0 | 0.03 (0.00-2.1) | 0.13 |
| | **TBD + HBIg** | 3 | 4 | 3.4 (1.5-7.8) | 11.9 | 0.03 (0.00-3.6) | 0.12 |
| | **TBD only** | 1 | 4 | 2.4 | 8.7 | 0.00 | 0.00 |
| **6 log10** | **Overall** | 2 | 1 | 9.2-16.6 | 9.2-16.6 | 0.00 | 0.00 |
| **7 log10** | **Overall (All TBD + HBIg)** | 2 | 2 | 4.1-9.5 | 4.1-9.5 | 0.00-0.12 | 0.00-0.24 |
| **8 log10** | **Overall** | 2 | 3 | 3.1-11.8 | 3.1-11.8 | 0.00 | 0.00 |
| | **TBD + HBIg** | 1 | 3 | 3.1 | 3.1 | 0.00 | 0.00 |
| | **TBD only** | 1 | 2 | 11.8 | 11.8 | 0.00 | 0.00 |

#### 4.4.3.2. Performance of HBV DNA ≥5 log10 IU/mL during pregnancy to predict MTCT

- Overall pooled sensitivity = 97.7 (95% CI: 42.9-100.0)
- Overall pooled specificity = 68.4 (95% CI: 48.6-83.2)

There was no evidence of publication bias (p=0.63) (Appendix G.5). We did not identify any outlier through visual assessment (Fig. 35 and 36) and summary scatter plot with the SROC curve (Fig. 37).

Fig. 35. Coupled forest plot of the performance of HBV DNA ≥5 log10 IU/mL during pregnancy to predict MTCT of HBV by type of prevention of MTCT strategy

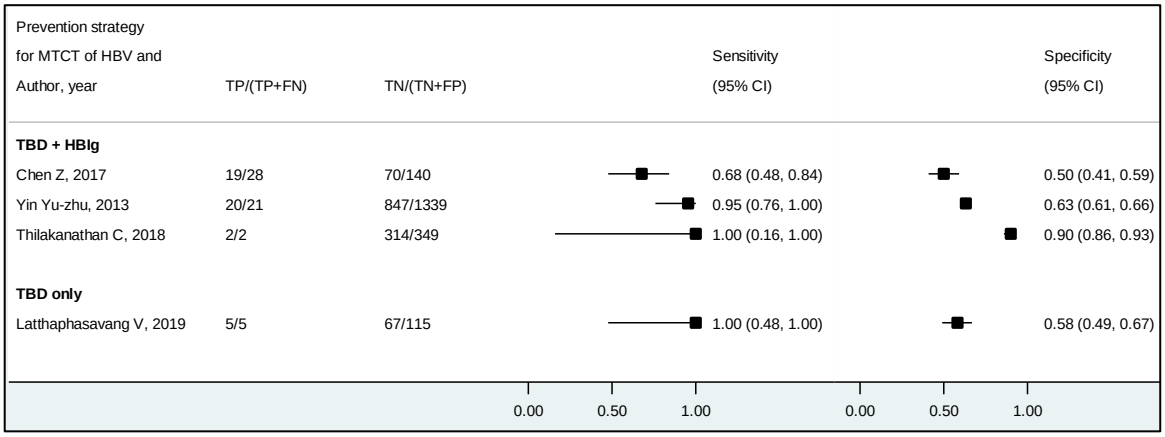



Fig. 36. Coupled forest plot of the performance of HBV DNA ≥5 log10 IU/mL during pregnancy to predict MTCT of HBV by type of HBV DNA assay

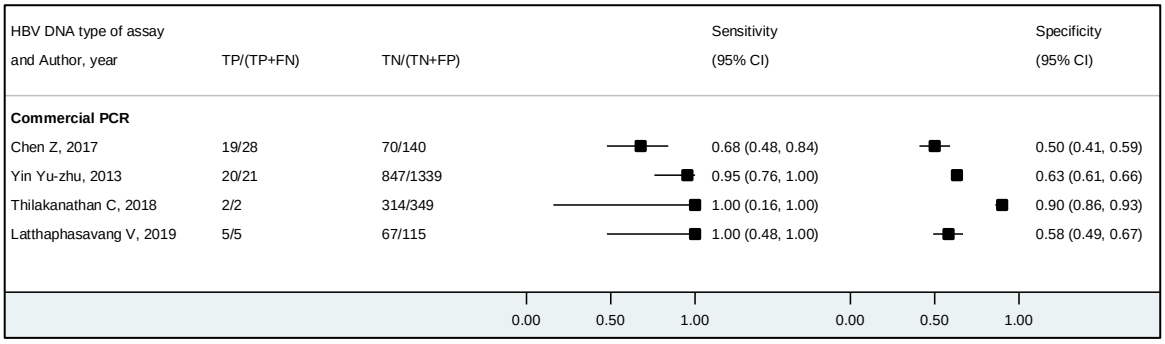
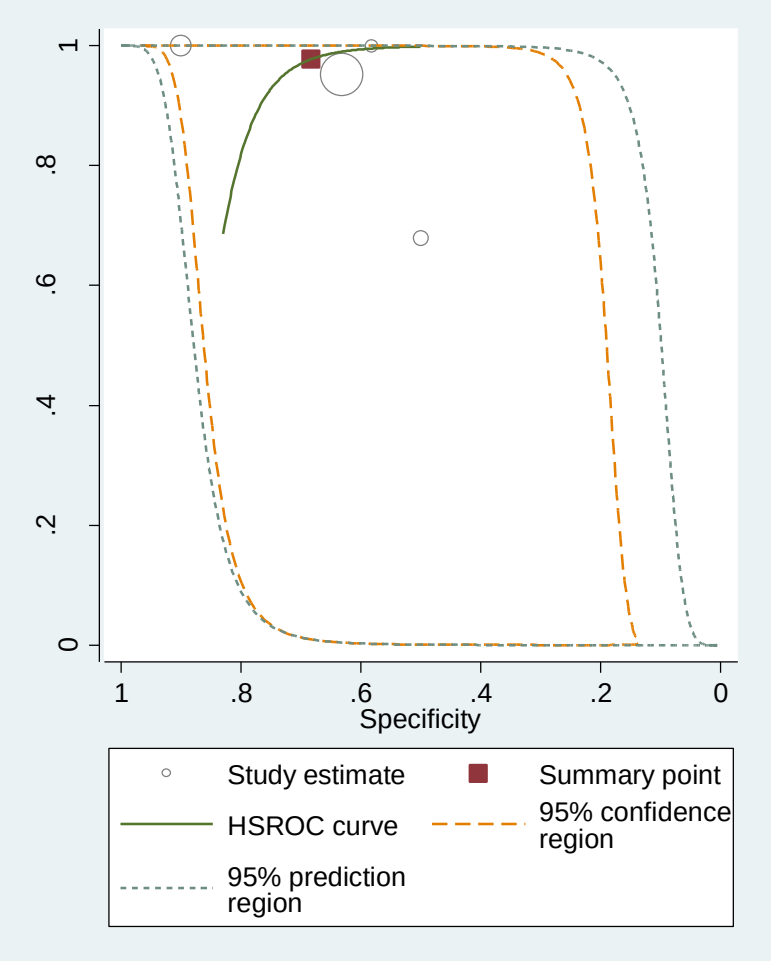


Fig. 37. Summary scatter plot and SROC curve of the performance of HBV DNA ≥5 log10 IU/mL during pregnancy to predict MTCT



**Subgroup analysis**

The number of included studies was very low. Therefore, we were not able to perform subgroup analysis of the performance of HBV DNA ≥5 log10 IU/mL to predict MTCT of HBV.

**Sensitivity analysis for the performance of HBV DNA ≥5 log10 IU/mL to predict MTCT**

As no outlier study was identified, we did not conduct sensitivity analysis on this factor
Results of sensitivity analysis are presented in Table 23. Sensitivity was lower in univariate analysis compared with bivariate analysis and the specificity remained unchanged. However, there was no evidence of a statistical difference between the bivariate and univariate analysis because the 95% CIs overlapped. Moreover, results remained unchanged when excluding studies with a high risk of bias.

Table 23. Results of sensitivity analysis for the performance of HBV DNA ≥5 log10 IU/mL during pregnancy to predict MTCT of HBV

| | **Studies, N** | **Se (95% CI) or Range Se** | **Sp (95% CI) or Range Sp** |
|---|---|---|---|
| **Overall bivariate** | 4 | 97.7 (42.9-100.0) | 68.4 (48.6-83.2) |
| **Overall univariate** | 7 | 81.5 (62.4-92.1) | 66.6 (51.3-79.0) |
| **Bivariate without studies with overall "high" risk of bias** | 3 | 97.3 (34.7-100.0) | 73.6 (52.2-87.7) |

#### 4.4.3.3. Performance of HBV DNA ≥7 log10 IU/mL during pregnancy to predict MTCT

- Range sensitivity = 90.5–100.0
- Range specificity = 77.8–89.5

As the number of studies was too low (n=2), we could not assess publication bias or present a summary scatter plot with SROC curve. The coupled forest plots (Fig. 38 and 39) are presented below. Both included studies reported giving timely birth dose of HBV vaccine and HBIG at birth to infants.

Fig. 38. Coupled forest plot of the performance of HBV DNA ≥7 log10 IU/mL during pregnancy to predict MTCT of HBV by type of prevention of MTCT strategy

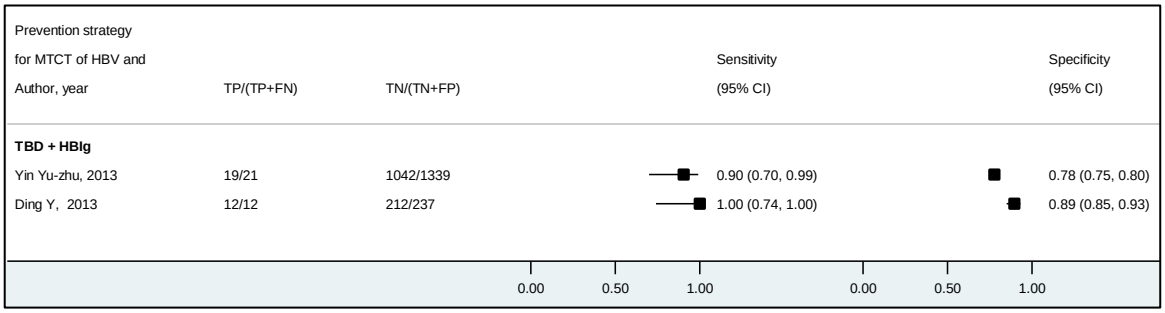
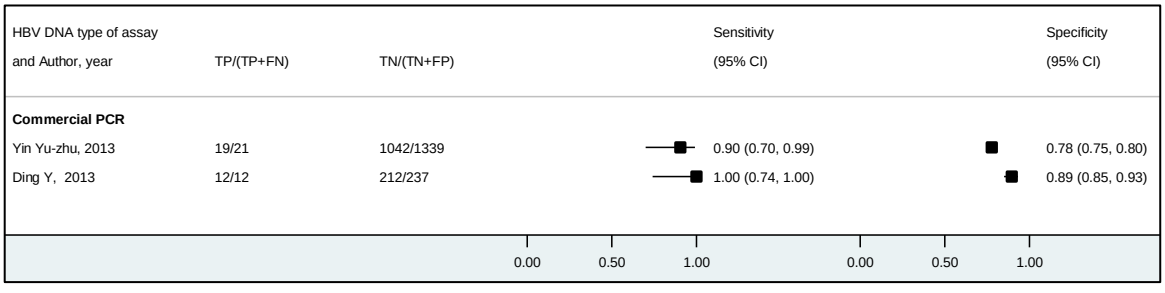



Fig. 39. Coupled forest plot of the performance of HBV DNA ≥7 log10 IU/mL during pregnancy to predict MTCT of HBV by type of HBV DNA assay



**Subgroup analysis**

As per the HBV DNA threshold ≥5 log10 IU/mL, we were not able to perform subgroup analysis of the performance of HBV DNA ≥7 log10 IU/mL during pregnancy to predict MTCT, given the small number of included studies.

**Sensitivity analysis for the performance of HBV DNA ≥7 log10 IU/mL during pregnancy to predict MTCT** (Table 24)

As no study had an overall "high" risk of bias, we did not perform sensitivity analysis on this factor. When performing univariate analysis, the results remained unchanged.

Table 24. Results of sensitivity analysis for the performance of HBV DNA ≥7 log10 IU/mL during pregnancy to predict MTCT

| | **Studies, N** | **Se (95% CI) or Range Se** | **Sp (95% CI) or Range Sp** |
|---|---|---|---|
| **Overall bivariate** | 2 | 90.5 –100.0 | 77.8–89.5 |
| **Overall univariate** | 3 | 84.4 (52.0–96.4) | 77.8–89.5 |

### 4.4.4. GRADE evidence profile for PICO2C

Table 25. GRADE evidence profile table of the performance of HBV DNA ≥5 log10 IU/mL during pregnancy to predict MTCT, stratified by prevention strategy for MTCT

| Test performance | Reference standard | Prevention strategy for MTCT | Studies, n | Risk of bias | Inconsistency | Indirectness | Imprecision | Patients, n | Strength of evidence[a] |
|---|---|---|---|---|---|---|---|---|---|
| **HBV DNA 5 log 10 IU/mL** | **MTCT** | **Overall** | Sens: 5<br>Spec: 6 | No serious | Serious | No serious | Serious | Sens: 58<br>Spec: 1998 | Sens: Low[b]<br>Spec: Low[b] |
| **HBV DNA 5 log 10 IU/mL** | **MTCT** | **TBD + HBIg** | Sens: 4<br>Spec: 4 | No serious | Serious | No serious | Serious | Sens: 53<br>Spec: 1881 | Sens: Low[c]<br>Spec: Low[c] |
| **HBV DNA 5 log 10 IU/mL** | **MTCT** | **TBD only** | Sens: 1<br>Spec: 2 | Insuff. data | Insuff. data | Insuff. data | Insuff. data | Sens: 5<br>Spec: 117 | Sens: Insufficient data[d]<br>Spec: Insufficient data[d] |

[a] Publication bias was not considered because none of the included studies was designed to evaluate diagnostic accuracy, and the assessment of publication bias in such a context may not be reliable.
[b] Downgraded from high to low strength of evidence because of serious inconsistency (8/11 studies were within 20% of the pooled estimate) and serious imprecision (the range in 95% CI was 29.7% for sensitivity and 27.7% for specificity)
[c]Downgraded from high to low strength of evidence because of serious inconsistency (3/4 studies were within 20% of the pooled estimate) and serious imprecision (the range in 95% CI was 35.3% for sensitivity and 34.8% for specificity)
[d]There was not enough data to rate the evidence for the timely birth dose only group because the number of studies was too low (n=2)

Table 26. GRADE evidence profile table of the performance of HBV DNA ≥7 log10 IU/mL during pregnancy to predict MTCT, stratified by prevention strategy for MTCT

| **Test performance** | **Reference standard** | **Prevention strategy for MTCT of HBV** | **Studies, n** | **Risk of bias** | **Inconsistency** | **Indirectness** | **Imprecision** | **Patients, n** | **Strength of evidence[a]** |
|---|---|---|---|---|---|---|---|---|---|
| **HBV DNA 7 log 10 IU/mL** | **MTCT** | **Overall (All TBD + HBIg)** | Sens: 3<br>Spec: 2 | No serious | No serious | No serious | Very serious | Sens: 43<br>Spec: 1576 | Sens: Low[b]<br>Spec: Insufficient data[a] |

[a] We did not rate publication bias because there were too few studies and its assessment may not be reliable

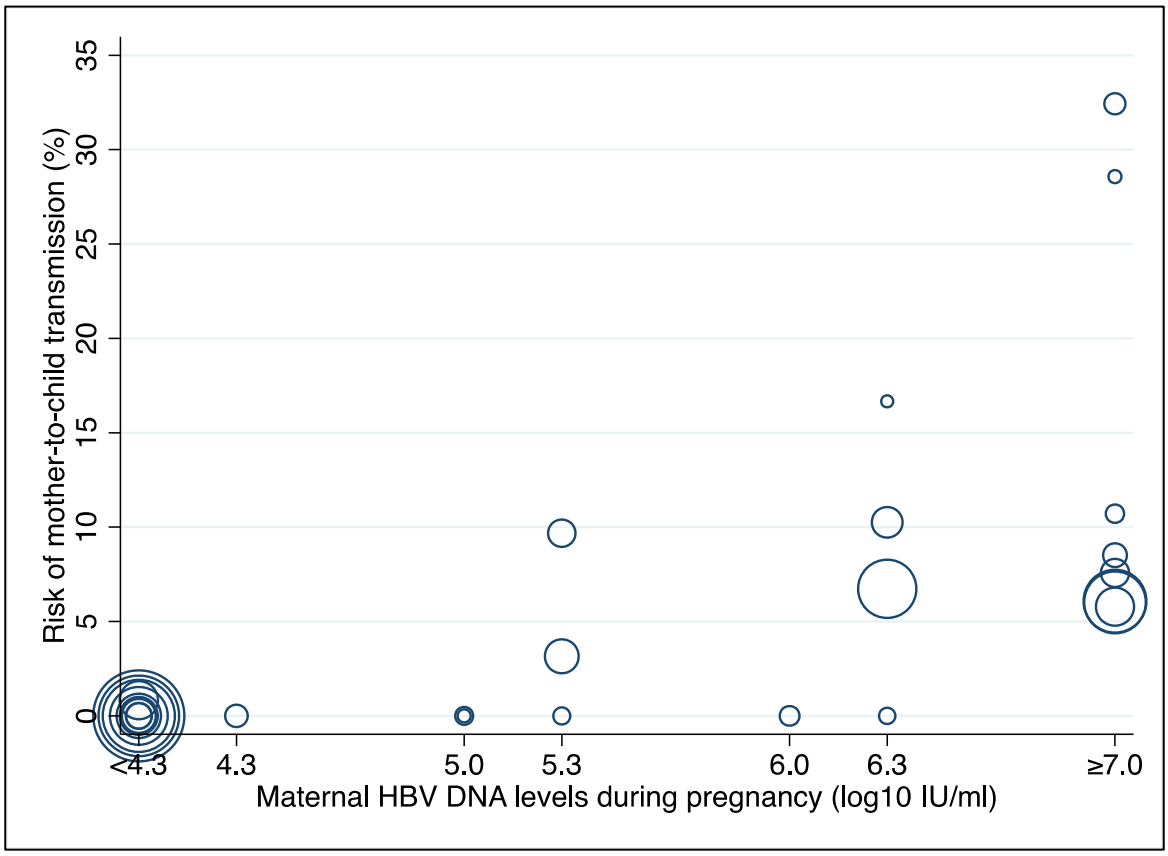
[b] Downgraded from high to low strength of evidence because of very serious imprecision in pooled sensitivity (95% CI range was 44.4%)

## 4.5. Post-hoc analysis

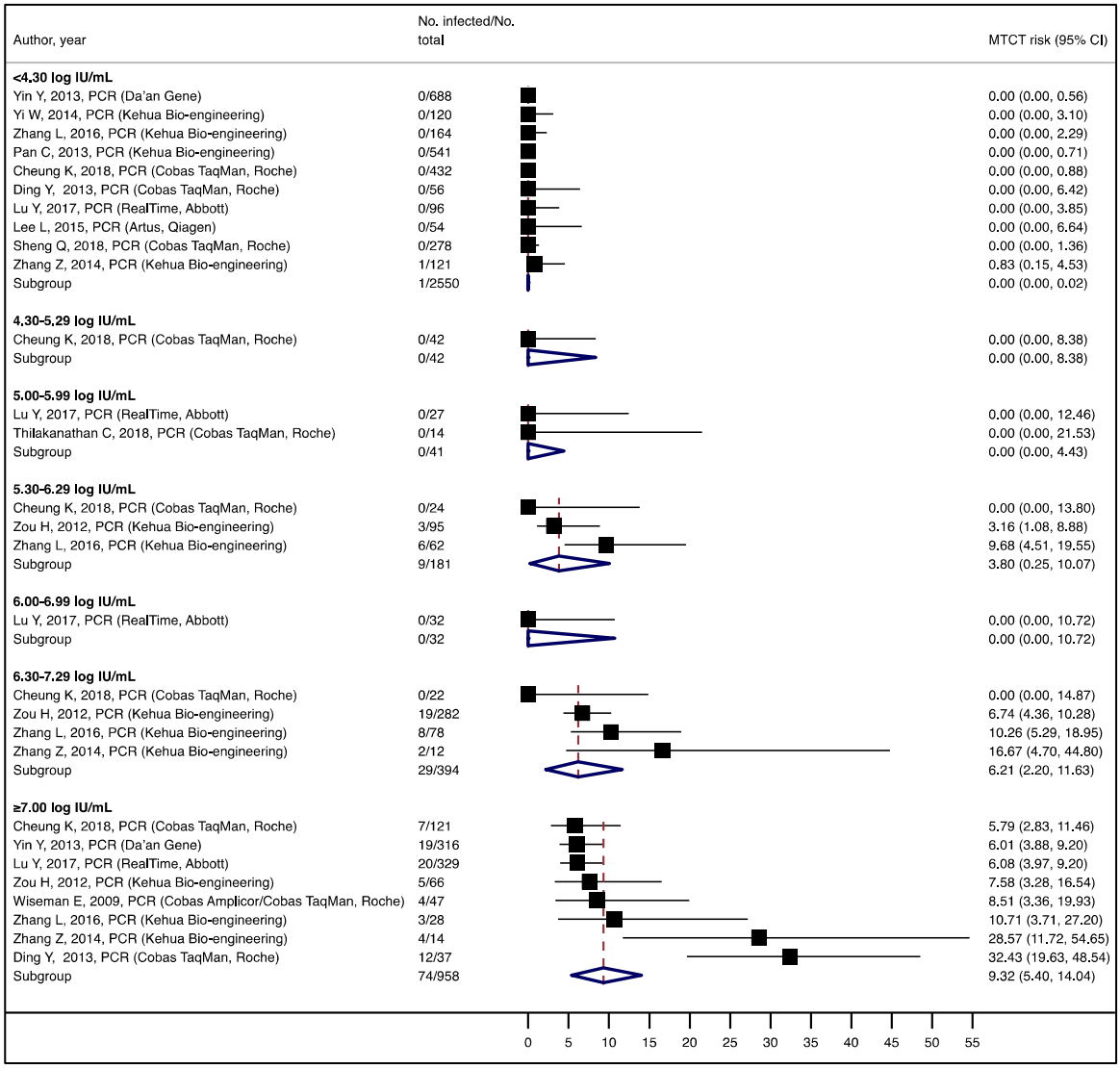
### 4.5.1. HBV DNA level during pregnancy from which the MTCT risk increases despite infant's immunoprophylaxis

Thirteen studies were included in this analysis *(45,66,89,94,105,108,113–119)*. All these studies provided timely birth dose and HBIG at birth. Fig. 40 and 41 show that the risk of HBV MTCT starts to increase from a maternal HBV DNA level during pregnancy of 5.3 log IU/mL (200 000 IU/mL), despite passive–active immunoprophylaxis. There were three studies that examined the risk of MTCT from pregnant women with a viral load of 5.3–6.2 log IU/mL; the risk of passive–active immunoprophylaxis failure was 0% (0/24) in the study of Cheung K et al., 3.2% (3/95) in Zou H et al., and 9.7% (6/62) in Zhang L et al. The pooled risk was 3.8% (95%CI: 0.3–10.1, Fig. 41). The exact viral loads (log IU/mL) of pregnant women who transmitted the virus to their infants despite passive–active immunoprophylaxis in this viral load range (5.3–6.2 log IU/mL) were: 5.7, 6.1, and 6.2 in the study by Zou H et al.; and 5.8, 5.8, 5.9, 5.9, 6.1, and 6.2 in the study by Zhang L et al. Both studies used PCR assay of Kehua Bio-engineering to quantify HBV DNA levels *(115,116)*.

Fig. 40. Risk of HBV MTCT according to maternal HBV DNA level during pregnancy



## Fig. 41. Forest plot of the risk of HBV MTCT according to maternal HBV DNA level during pregnancy

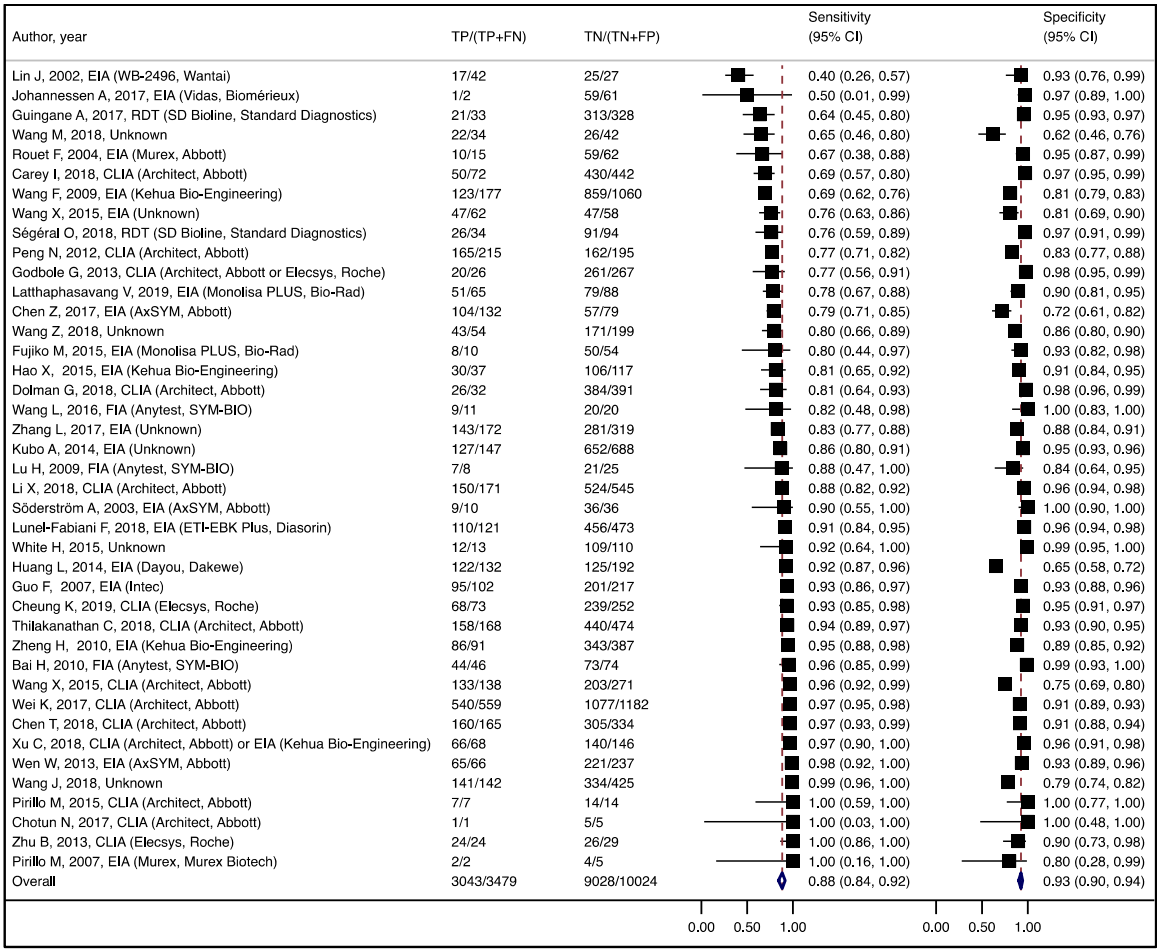


### 4.5.2. Performance of HBeAg to identify pregnant women with HBV DNA ≥5.3 log10 IU/mL

A total of 41 studies were included for this meta-analysis (Fig. 42).

- Overall pooled sensitivity = 88.2% (95% CI: 83.9-91.5)
- Overall pooled specificity = 92.6% (95% CI: 90.0-94.5)

Fig. 42. Coupled forest plot for all studies included in the meta-analysis of the performance of HBeAg to identify maternal HBV DNA ≥5.3 log10 IU/mL



**Subgroup analysis**

Of the variables predefined as potential sources of heterogeneity, it was not possible to do a subgroup analysis by HCV- or HDV-coinfection status, or by maternal HBV genotype as too few studies reported information on these factors.

Table 27 present detailed results of the performance of HBeAg to identify pregnant women with HBV DNA ≥5.3 log10 IU/mL stratified by the potential sources of heterogeneity.

Table 27. Performance of HBeAg to identify pregnant women with HBV viral load ≥5.3 log10 IU/mL, stratified by potential sources of heterogeneity

| Subgroup variables | Subgroup category | Studies (N) | Se (95% CI) or Range Se | Sp (95% CI) or Range Sp | *P* value |
|---|---|---|---|---|---|
| **Overall** | | 41 | 88.2 (83.9-91.5) | 92.6 (90.0-94.5) | N/A |
| **WHO region (N=41)** | **WPR** | 27 | 90.0 (85.0-93.5) | 89.1 (85.6-91.8) | <0.0001* |
| | **AFR** | 7 | 82.0 (65.1-91.7) | 96.0 (94.4-97.1) | |
| | **EUR** | 5 | 78.9 (67.3-87.1) | 98.0 (96.8-98.8) | |
| | **AMR** | 1 | 86.4 (79.8-91.5) | 94.8 (92.8-96.3) | |
| | **SEAR** | 1 | 80.0 (44.4-97.5) | 92.6 (82.1-97.9) | |
| | **EMR** | 0 | - | - | |
| **HBeAg assay type (N=36)** | **EIA** | 18 | 84.6 (77.4-89.8) | 90.5 (86.4-93.4) | 0.11** |
| | **CLIA** | 13 | 91.4 (85.2-95.1) | 93.9 (90.2-96.3) | |
| | **FIA** | 3 | 91.4 (78.5-96.9) | 96.6 (81.2-99.5) | |
| | **RDT** | 2 | 70.1 (58.2-79.9) | 95.7 (93.3-97.3) | |
| **Mean / median maternal age (N=28)** | **<28 years** | 14 | 94.2 (88.8-97.1) | 91.9 (87.8-94.8) | 0.02 |
| | **≥28 years** | 14 | 85.3 (79.0-90.0) | 95.6 (93.6-97.0) | |
| **HBV DNA assay (N=41)** | **Commercial PCR** | 35 | 88.1 (83.3-91.8) | 92.5 (90.0-94.4) | 0.98 |
| | **In-house or unknown test** | 6 | 88.8 (78.6-94.5) | 93.5 (79.8-98.1) | |
| **HIV status (N=22)** | **All uninfected** | 11 | 93.5 (87.2-96.9) | 89.2 (82.4-93.6) | 0.30*** |
| | **All infected** | 4 | 95.1 (50.7-99.7) | 96.6 (82.8-99.4) | |
| | **Mixed population** | 7 | 82.7 (70.5-90.6) | 95.6 (94.3-96.6) | |

*comparison of WPR/EUR/AMR

**comparison of CLIA/EIA

***comparison of uninfected/infected

# 5. CONCLUSION

To our knowledge, this systematic review is the first to examine the diagnostic accuracy of the HBeAg test to diagnose high HBV DNA levels in pregnant women. Our results suggest that the risk of HBV MTCT, despite passive–active immunoprophylaxis, starts to increase at a maternal viral load of 5.3 log IU/mL (i.e. 200 000 IU/mL). The pooled sensitivity and specificity of HBeAg, obtained by the bivariate analyses, were: 84.2% (95% CI: 80.2–87.4%) and 92.3% (89.5–94.5%) to diagnose viral load of ≥5 log10 IU/mL; 92.0% (88.2–94.6%) and 92.7% (90.3–94.5%) for ≥6 log10 IU/mL; and 98.0% (93.3–99.4%) and 88.5% (80.7–93.4%) for ≥7 log10 IU/mL, respectively. We evaluated the performance of HBeAg for three other different HBV DNA thresholds (≥20 000, ≥200 000 and ≥8 log10 IU/mL). Irrespective of these different HBV DNA cut-off levels, pooled sensitivity and specificity were constantly higher than 80%, with the lower boundary of the 95% CI exceeding 75%. The univariate analyses provided similar results, supporting the robustness of these estimates. As expected, the sensitivity improved with increasing HBV DNA threshold whereas the specificity decreased.

We found evidence that the performance of HBeAg during pregnancy to identify pregnant women with a high viral load differed according to the WHO region, type of HBeAg and maternal age. However, there was no evidence that its performance varied according to HBV DNA NAT or maternal HIV status.

The performance of HBeAg differed significantly between lower (<28 years) and higher (≥28 years) mean/median maternal age reported in each study; younger maternal age was associated with higher sensitivity and lower specificity to diagnose HBV DNA levels of ≥5 and ≥6 log10 IU/mL, compared to higher maternal age (≥28 years). Although the difference was not significant, a similar tendency was observed for the cut-off of ≥7 log10 IU/mL. Since spontaneous loss of HBeAg occurs over time, the prevalence of HBeAg is higher in younger HBsAg-positive women than in older HBsAg-positive women *(120)*, and this may explain the difference in performance of HBeAg according to maternal age.

The natural history of CHB varies according to geographical area, particularly between Asia and sub-Saharan Africa, which both carry a high HBV-related disease burden. Historically in both areas, the majority of CHB occurs during childhood either perinatally from infectious mothers or horizontally from household members. In Asia, a substantial proportion (about 40%) of children who are chronically infected with HBV continue to carry HBeAg and high viral load beyond their adolescence *(121)* while in Africa, spontaneous HBeAg loss often occurs at younger age and only 10–20% of HBV-infected women of childbearing age carry HBeAg *(122).* This difference might be due to varying frequency of emerging basal core promoter (BCP) or precore (PC) variants, which abolish or reduce HBeAg production without affecting the capacity of HBV to replicate *(123)*. We found a similar high sensitivity of HBeAg to diagnose high viraemia in both regions: 83.9% (79.2–87.7%) in Asia and 88.0% (70.9–95.7%) in Africa for HBV DNA levels ≥5 log10 IU/mL, respectively; 92.6% (88.4–95.3%) and 93.7% (87.9–96.8%) for HBV DNA levels ≥6 log10 IU/mL, respectively. However, the specificity of HBeAg to diagnose high maternal viral loads tended to be lower in Asia (Western Pacific) compared with Africa: 89.3% (85.6–92.1%) and 96.6% (94.3–98.0%) for HBV DNA levels ≥5 log10 IU/mL; 91.1% (88.4–93.2%) and 96.2% (94.3–97.5%) for HBV DNA levels ≥6 log10 IU/mL, respectively.

More recent types of HBeAg assays such as CLIA tended to have a higher sensitivity for diagnosing viraemia than EIA: 89.1% (95% CI: 83.2–93.0%) and 79.4% (74.0–84.0%) to diagnose viral load ≥5 log10 IU/mL; and 94.4% (81.3–98.5%) and 98.8% (93.7–99.8%) to diagnose viraemia ≥7 log10 IU/ml, respectively. This might be related to improved analytical sensitivity of CLIA, in comparison with EIA, to detect HBeAg. Although there was only one study that evaluated RDT, this had lower clinical sensitivity compared to the laboratory-based immunoassays (76.5% and 89.3% to diagnose high HBV DNA levels of ≥5 log10 and ≥7 log10 IU/mL, respectively). Low clinical sensitivity of commercially available HBeAg RDT was also found to be related to its low analytical sensitivity compared to CLIA *(17).*

A few outlying studies were identified: one study for ≥5 log10 IU/mL and three studies for ≥7 log 10 IU/mL. Compared to other studies, these outliers tended to have either "lower sensitivity and higher specificity" *(50,70)*, or "lower specificity with higher sensitivity" *(45,68)*. Although we did not perform further assessment, this might be related to multiple factors such as low HBeAg prevalence (1.6%) in HBsAg-positive pregnant women in a Greek study *(50)* or the difference in analytical sensitivity of the HBeAg tests used in these outlier studies *(68,70)*. The sensitivity analyses excluding these outliers did not alter the interpretation of these results.

The systematic review addressed two additional questions: the performance of HBeAg detection and HBV DNA quantification during pregnancy to predict an MTCT event, defined as HBsAg positivity in infants aged 6–12 months. These analyses were stratified by the type of preventive measures provided to mother–child pairs. We found that the pooled sensitivity and specificity of maternal HBeAg during pregnancy to predict MTCT despite infant immunoprophylaxis were 99.1% (95% CI: 61.8–100%) and 55.7% (34.0–75.5%). The pooled sensitivity and specificity of maternal HBV DNA levels ≥5 log IU/mL was 97.7% (95% CI: 42.9–100.0%) and 68.4% (95% CI: 48.6–83.2%); and the sensitivity and specificity range of maternal HBV DNA levels ≥7 log IU/mL were 90.5% to 100.0% and 77.8% to 89.5%, respectively. Although no formal assessment was performed, these results might indicate that: (i) both HBeAg and high HBV DNA levels, measured during pregnancy, have high sensitivity to predict the risk of immunoprophylaxis failure; and (ii) the specificity is low for HBeAg and moderate for high HBV DNA levels.

The strength of the evidence for the performance of HBeAg to identify pregnant women with viral loads ≥5, ≥6, and ≥7 log 10 IU/mL were high. Although most studies had excluded women coinfected with either HIV, HCV or HDV, this did not constitute a serious risk of bias. There was no evidence of publication bias; however, only one study was designed to evaluate diagnostic accuracy, which might have made the conventional assessment of publication bias, through assessing small sample effects, less meaningful. Moreover, since essential information for diagnostic studies (e.g. blinding) were missing

in the majority of studies, the assessment of the quality of studies was difficult for the index test and reference standard sections of the QUADAS2.

For the performance of HBeAg to predict MTCT, the strength of evidence was low due to high risk of bias in most studies and serious imprecision of the pooled estimates, probably due to the small number of included studies. When stratified by prevention of MTCT regimen, evidence was low for the timely birth dose plus HBIG regimen and could not be graded for timely birth dose only regimen (without HBIG) because there was not enough data available.

The strength of evidence for the overall performance of maternal HBV DNA levels ≥5 log 10 IU/mL was low due to inconsistency in the estimates and serious imprecision in the pooled estimates. When stratifying by prevention of MTCT strategy, evidence was low for timely birth dose plus HBIG for the same reasons as stated above, and there was not enough data to grade the evidence for the timely birth dose only strategy. For maternal HBV DNA levels ≥7 log 10 IU/mL, all included studies reported using the timely birth dose plus HBIG strategy and there was not enough data to grade evidence for specificity. Concerning sensitivity, evidence was low because of very serious imprecision in the pooled estimate, which may be due to the low number of studies included to answer this objective.

As a strength of this study, the literature was systematically searched through both English- and Chinese-language databases, and independently reviewed by two investigators for each language (a total of four reviewers). Duplicate publications were carefully checked and excluded from the analysis to avoid biased estimates.

As a limitation, this systematic review was primarily designed to accomplish the primary objective (PICO2A); we thus included only studies that measured both HBeAg and HBV DNA during pregnancy to answer the secondary objectives. Of the studies eligible for the PICO2A, we further selected those that followed infants to ascertain MTCT end-points to answer two additional questions (PICO2B and PICO2C). Consequently, we might have missed a few of the eligible studies for PICO2B (e.g. a study evaluating maternal HBeAg

during pregnancy and infant HBsAg at 6 months without doing maternal HBV DNA) or PICO2C (e.g. a study evaluating maternal HBV DNA and infant MTCT end-point, without assessing HBeAg during pregnancy). Because of these limitations, care must be taken when interpreting PICO2B and PICO2C.

## 5.1. Implications for practice

This study suggests that risk of HBV MTCT, despite passive–active immunoprophylaxis, starts to increase at a maternal viral load of 5.3 log IU/mL. This threshold should be theoretically lower when the PMTCT strategy does not include HBIG (e.g. timely birth dose alone).

With the high strength of the evidence observed, this study suggests that HBeAg might be a good alternative marker to HBV DNA NAT to diagnose high HBV viral load during pregnancy. Moreover, although the strength of the evidence was low, the systematic review found high sensitivity (99.1% (95% CI: 61.8–100%)) of HBeAg during pregnancy to predict immunoprophylaxis failure in infants (MTCT despite administration of birth dose vaccine and HBIG), with a poor specificity of around 55%.

The findings are particularly relevant in countries where there is limited access to HBV DNA NAT. Even though the HBeAg test may perform less well than HBV DNA NAT to identify pregnant women with an elevated risk of MTCT, other parameters (lower costs, improved access to testng and uptake, better linkage to care, and greater feasibility) may favour its use in certain contexts.

## 5.2. Implications for research

The vast majority of the included studies were from the Western Pacific Region (WPR: 65.9%), followed by the European Region (EUR: 18.3%), African Region (AFR: 7.3%), the Americas (AMR: 6.1%), and only one study each from South-East Asia (SEAR: 1.2%) and Eastern Mediterranean Region (EMR: 1.2%). We need additional research, particularly

outside East Asia. There was no study that included only HCV- or HDV-coinfected women. Only a few studies provided the estimates for HIV-coinfected mothers; we did not find any difference in performance of HBeAg to diagnose high viraemia according to HIV status. As there were only a few studies that assessed viral genotype, we could not investigate the performance of HBeAg during pregnancy in different HBV genotypes.

The use of RDT is more attractive than laboratory-based immunoassays, because the former may be less expensive, faster, easier to perform, and thus more feasible than the latter in a peripheral laboratory in resource-limited contexts. We identified only one study evaluating the performance of RDT during pregnancy, and its sensitivity tended to be lower than that of EIA or CLIA. We need additional studies to evaluate the performance of RDT in pregnant women; but we may also need improvement of analytical sensitivity of RDT to detect HBeAg. The development and evaluation of other low-cost molecular assays or serological markers (e.g. hepatitis B core-related antigen (HBcrAg)) is also highly warranted *(124)*.

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# 8. APPENDICES

## 8.1. Appendix A: Search strategies

Database: PubMed
Date searched: From 1 January 2000 to 3 April 2019
Search strategy:

| Item | Search words | # Records |
|---|---|---|
| 1 | "hepatitis b"[MeSH] OR "hepatitis b virus"[MeSH] | 36043 |
| 2 | hepatitis b[Text] OR type b hepatitis[Text] OR hepatitis type b[Text] OR hbv[Text] OR vhb[Text] OR hep b[Text] OR hbsag[Text] OR hbs ag[Text] OR hbs antigen*[Text] | 58981 |
| 3 | 1 OR 2 | 58981 |
| 4 | "viral load"[MeSH] OR "viremia"[MeSH] OR "DNA, viral"[MeSH] OR "nucleic acid amplification techniques"[MeSH] | 404088 |
| 5 | viral load*[Text] OR viremi*[Text] OR viraemi*[Text] OR DNA[Text] OR nucleic acid test*[Text] OR nucleic acid amplification*[Text] OR NAT[Text] OR polymerase chain reaction*[Text] OR PCR[Text] | 1469530 |
| 6 | 4 OR 5 | 1470454 |
| 7 | "pregnancy"[MeSH] OR "pregnant women"[MeSH] OR "maternal-fetal relations"[MeSH] OR "infectious disease transmission, vertical"[MeSH] OR "pregnancy complications, infectious"[MeSH] OR "prenatal diagnosis"[MeSH] | 374590 |
| 8 | pregnan*[Text] OR trimest*[Text] OR gestation*[Text] OR antepartum[Text] OR ante-partum[Text] OR prepartum[Text] OR pre-partum[Text] OR intrapartum[Text] OR intra-partum[Text] OR peripartum[Text] OR peri-partum[Text] OR antenatal*[Text] OR ante-natal*[Text] OR prenatal*[Text] OR pre-natal*[Text] OR perinatal*[Text] OR peri-natal*[Text] OR intrauterine[Text] OR intra-uterine[Text] OR inutero[Text] OR in utero[Text] OR transplacental*[Text] OR placenta*[Text] OR vertical*[Text] OR congenital*[Text] OR mother*[Text] OR matern*[Text] OR fetomaternal*[Text] OR foetomaternal*[Text] OR fetal*[Text] OR foetal*[Text] OR fetus[Text] OR foetus[Text] OR offspring[Text] OR MTCT[Text] OR TME[Text] | 920702 |
| 9 | 7 OR 8 | 924605 |
| 10 | 3 AND 6 AND 9 | 1131 |

Database: Embase via Ovid
Date searched: from 1 January t 2000 to 3 April 2019
Search Strategy:

| Item | Search words | # Records |
|---|---|---|
| 1 | exp hepatitis B/ OR exp Hepatitis B virus/ | 88165 |
| 2 | (hepatitis b OR type b hepatitis OR hepatitis type b OR hbv OR vhb OR hep b OR hbsag OR hbs ag OR hbs antigen*).mp. | 114021 |
| 3 | 1 OR 2 | 114021 |
| 4 | exp virus load/ OR exp viremia/ OR exp virus DNA/ OR exp nucleic acid amplification/ | 124590 |
| 5 | (viral load* OR viremi* OR viraemi* OR DNA OR nucleic acid test* OR nucleic acid amplification* OR NAT OR polymerase chain reaction* OR PCR).mp. | 2014356 |
| 6 | 4 OR 5 | 2031867 |
| 7 | exp pregnancy/ OR exp pregnant women/ OR exp mother fetus relationship/ OR exp vertical transmission/ OR exp pregnancy complication/ OR exp prenatal diagnosis/ | 462746 |
| 8 | (pregnan* OR trimest* OR gestation* OR antepartum OR ante-partum OR prepartum OR pre-partum OR intrapartum OR intra-partum OR peripartum OR peri-partum OR antenatal* OR ante-natal* OR prenatal* OR pre-natal* OR perinatal* OR peri-natal* OR intrauterine OR intra-uterine OR inutero OR in utero OR transplacental* OR placenta* OR vertical* OR congenital* OR mother* OR matern* OR fetomaternal* OR foetomaternal* OR fetal* OR foetal* OR fetus OR foetus OR offspring OR MTCT OR TME).mp. | 1348015 |
| 9 | 7 OR 8 | 1351584 |
| 10 | 3 AND 6 AND 9 | 2696 |

Database: Scopus
Date searched: From 1 January 2000 to 3 April 2019
Search Strategy:

| Item | Search words | # Records |
|---|---|---|
| 1 | TITLE-ABS-KEY (“hepatitis b” OR “type b hepatitis” OR “hepatitis type b” OR “hbv” OR “vhb” OR “hep b” OR “hbsag” OR “hbs ag” OR “hbs antigen*”) | **88699** |
| 2 | TITLE-ABS-KEY (“viral load*” OR “viremi*” OR “viraemi*” OR “DNA” OR “nucleic acid test*” OR “nucleic acid amplification*” OR “NAT” OR “polymerase chain reaction*” OR “PCR”) | **2077123** |
| 3 | TITLE-ABS-KEY (“pregnan*” OR “trimest*” OR “gestation*” OR “antepartum” OR “ante-partum” OR “prepartum” OR “pre-partum” OR “intrapartum” OR “intra-partum” OR “peripartum” OR “peri-partum” OR “antenatal*” OR “ante-natal*” OR “prenatal*” OR “pre-natal*” OR “perinatal*” OR “peri-natal*” OR “intrauterine” OR “intra-uterine” OR “inutero” OR “in utero” OR “transplacental*” OR “placenta*” OR “vertical*” OR “congenital*” OR “mother*” OR “matern*” OR “fetomaternal*” OR “foetomaternal*” OR “fetal*” OR “foetal*” OR “fetus” OR “foetus” OR “offspring” OR “MTCT” OR “TME”) | **1632184** |
| 4 | #1 AND #2 AND #3 | **1851** |

Database: CENTRAL Database (The Cochrane Library)
Date searched: From 1 January 2000 to 3 April 2019
Search strategy:

| Item | Search words | # Trials |
|---|---|---|
| 1 | hepatitis b [MeSH, exp] OR hepatitis b virus [MeSH, exp] | 1744 |
| 2 | "hepatitis b" OR "type b hepatitis" OR "hepatitis type b" OR hbv OR vhb OR "hep b" OR hbsag OR “hbs ag” OR “hbs antigen” OR “hbs antigens” | 6913 |
| 3 | 1 OR 2 | 6913 |
| 4 | “viral load”[MeSH, exp] OR “viremia”[MeSH, exp] OR “DNA, viral”[MeSH, exp] OR “nucleic acid amplification techniques”[MeSH, exp] | 4212 |
| 5 | “viral load*” OR viremi* OR viraemi* OR DNA OR “nucleic acid test*” OR “nucleic acid amplification*” OR NAT OR “polymerase chain reaction*” OR PCR | 29632 |
| 6 | 4 OR 5 | 29637 |
| 7 | pregnancy [MeSH, exp] OR pregnant women [MeSH, exp] OR maternal-fetal relations [MeSH, exp] OR infectious disease transmission, vertical [MeSH, exp] OR pregnancy complications, infectious [MeSH, exp] OR prenatal diagnosis [MeSH, exp] | 5294 |
| 8 | pregnan* OR trimest* OR gestation* OR antepartum OR ante-partum OR prepartum OR pre-partum OR intrapartum OR intra-partum OR peripartum OR peri-partum OR antenatal* OR ante-natal* OR prenatal* OR pre-natal* OR perinatal* OR peri-natal* OR intrauterine OR intra-uterine OR inutero OR “in utero” OR transplacental* OR placenta* OR vertical* OR congenital* OR mother* OR matern* OR fetomaternal* OR foetomaternal* OR fetal* OR foetal* OR fetus OR foetus OR offspring* OR MTCT OR TME | 76201 |
| 9 | 7 OR 8 | 76271 |
| 10 | 3 AND 6 AND 9 | 218 |

Database: Wanfang
主题: ("乙型肝炎"+"乙肝"+"乙型病毒性肝炎"+"乙型肝炎病毒"+"乙肝病毒"+"HBV"+"乙型肝炎表面抗原"+"乙肝表面抗原"+"乙型肝炎病毒表面抗原"+"乙肝病毒表面抗原"+"HBsAg") and 主题: ("病毒载量"+"病毒血症"+"病毒 DNA"+"DNA"+"核酸扩增技术"+"核酸检测"+"核酸扩增"+"NAT"+"多聚酶链反应"+"多聚酶链式反应"+"聚合酶链反应"+"PCR") and 主题: ("妊娠"+"孕妇"+"孕期"+"母胎"+"母亲"+"胎儿"+"新生儿"+"婴儿"+"子代"+"子女"+"母婴传播"+"垂直传播"+"妊娠并发症"+"产前筛查"+"产前诊断"+"出生前诊断"+"先天"+"产前"+"产时"+"围产"+"出生前"+"围生"+"宫内"+"跨胎盘"+"胎盘")

Database: CNKI

SU='乙型肝炎'+'乙肝'+'乙型病毒性肝炎'+'乙型肝炎病毒'+'乙肝病毒'+'HBV'+'乙型肝炎表面抗原'+'乙肝表面抗原'+'乙型肝炎病毒表面抗原'+'乙肝病毒表面抗原'+'HBsAg' AND SU='病毒载量'+'病毒血症'+'病毒 DNA'+'DNA'+'核酸扩增技术'+'核酸检测'+'核酸扩增'+'NAT'+'多聚酶链反应'+'多聚酶链式反应'+'聚合酶链反应'+'PCR' AND SU='妊娠'+'孕妇'+'孕期'+'母胎'+'母亲'+'胎儿'+'新生儿'+'婴儿'+'子代'+'子女'+'母婴传播'+'垂直传播'+'妊娠并发症'+'产前筛查'+'产前诊断'+'出生前诊断'+'先天'+'产前'+'产时'+'围产'+'出生前'+'围生'+'宫内'+'跨胎盘'+'胎盘'

## 8.2. Appendix B: QUADAS2 protocol

**Domain 1: Patient Selection (PICO2A, 2B and 2C)**

- *Risk of Bias: Could the selection of patients have introduced bias?*

• Signaling question 1: Was a consecutive or random sample of patients or specimens enrolled?

Answer "yes" if the study enrolled a consecutive or random sample of eligible patients or specimens; "no" if the study selected patients or specimens by convenience; and "unclear" if the study did not report the sample selection methods.

• Signaling question 2: Was a case-control design avoided?

Answer "no" for a case-control study (i.e., study in which the chance of being included differs between cases (with high viral load for PICO2A and with MTCT for PICO2B & 2C) and controls (with low viral load for PICO2B and with no MTCT for PICO2B & 2C)); and "yes" for a retrospective/prospective cohort or a cross-sectional study.

• Signaling question 3: Did the study avoid inappropriate exclusions?

Answer "no" if the study excluded HBsAg-positive pregnant women with a criterion other than concomitant anti-HBV therapy (e.g., HCV/HDV co-infection, sever liver disease, having HBsAg-positive spouse, etc…); and "unclear" if unable to tell.

Risk of bias is rated as "low" if selection was done in a random or consecutive manner and the study was not a case-control design and did not exclude HBsAg-positive pregnant women with a criterion other than concomitant anti-HBV therapy (i.e., all answers to signaling questions rated "yes"); "high" if any signaling question was answered "no"; and '"unclear" if insufficient data were reported to permit a judgment (i.e., at least one signaling question rated "unclear" and no question rated "no").

- *Applicability: Are there concerns that the included patients and setting do not match the review question?*

Our primary objective is to assess the performance of HBeAg as an alternative to HBV DNA to assess eligibility for antiviral therapy initiation in pregnant women who are identified to carry HBsAg. Therefore, if a study included only selected pregnant women on

the basis of HBV DNA levels or HBeAg sero-status, it may not be relevant to the study question. We will rate concerns about applicability as "low" if patients were selected irrespective of HBV DNA levels, "high" if study participants were only those with high (or low) HBV DNA levels, and "unclear" if the eligibility for inclusion in the study was not well described. Of note, if a study was designed to only include HBeAg-positive women or HBeAg-negative women, the study should be excluded from our systematic review.

**Domain 2: Index Test**

**PICO2A and PICO2B:**

- *Risk of Bias: Could the conduct or interpretation of the index test have introduced bias?*

• Signaling question 1: Were the index test results interpreted without knowledge of the results of the reference standard?

Consider "yes" if the examiner of the index text was blinded to the results of the reference standard, "no" if reference standard results were not blinded, and "unclear" if no mention of blinding, or if it is not known when the test was done.

• Signaling question 2: If a threshold was used, was it pre-specified?

Consider "yes" unless the study explicitly mentioned the use of a specific HBeAg threshold (PEIU/mL or cut-off index (COI)) which differs from its limit of detection.

Risk of bias is rated as "low" if both signaling questions were answered "yes"; "high" if at least one signaling question was answered "no"; and "unclear" if at least one question was rated as "unclear" and no question rated "no".

- *Applicability: Are there concerns that the index test, its conduct, or its interpretation differ from the review question?*

Rate concerns about applicability as "high" if the test was done outside pregnancy or if the type of assay used was not one of these: lateral flow immunochromatographic rapid diagnostic test (RDT), Enzyme immunoassay (EIA, ELISA), chemiluminescence immunoassay (CLIA), Radioimmunoassay (RIA), Counting immunoassay (CIA),

Fluoroimmunoassay (FIA); "unclear" if some information was missing on the test (not know what type of assay was used or when it was done); "low" otherwise.

**PICO2C:**

- *Risk of Bias: Could the conduct or interpretation of the index test have introduced bias?*

• Signaling question 1: Were the index test results interpreted without knowledge of the results of the reference standard?

Consider "yes" if the examiner of the index text was blinded to the results of the reference standard, "no" if reference standard results were not blinded, and "unclear" if no mention of blinding, or if it is not known when the test was done.

• Signaling question 2: If a threshold was used, was it pre-specified?

Consider "yes" as mentioning HBV DNA threshold is part of inclusion criteria for this review.

- *Applicability: Are there concerns that the index test, its conduct, or its interpretation differ from the review question?*

Rate concerns about applicability as "low", or "unclear" if the type of test used is not reported.

**Domain 3: Reference Standard**

**PICO2A:**

- *Risk of Bias: Could the reference standard, its conduct, or its interpretation have introduced bias?*

• Signaling question 1: Is the reference standard likely to correctly classify the target condition?

Answer "no" if the reference test is a non-validated "in-house" method (without citing a previous study validating the method). Answer "unclear" if the reference test used is not described. For the other studies, answer "yes" because although there are several different methods of NAT, the test results are reliable particularly for the high HBV DNA thresholds that the current review targets.

• Signaling question 2: Were the reference standard results interpreted without knowledge of the results of the index test?

Answer “yes” if the examiner of the reference standard was blinded to the results of the index test; “no” if index test results were not blinded. Answer “unclear” if it is not known when the test was done or no notion of blinding was reported.

Risk of bias is rated as “low” if both signaling questions were answered “yes”; “high” if at least one signaling question was answered “no”; and “unclear” if at least one question was rated as “unclear” and no question rated “no”.

- *Applicability: Are there concerns that the target condition as defined by the reference standard does not match the question?*

Since our target condition is high HBV DNA levels defined by the NAT, all studies will be rated as “low concern”.

**PICO2B and PICO2C:**

- *Risk of Bias: Could the reference standard, its conduct, or its interpretation have introduced bias?*

• Signaling question 1: Is the reference standard likely to correctly classify the target condition?

Answer “yes” if the reference test is performed in infants aged 6-12 months, extended to 24 months in infants who received three doses of HBV vaccine. Answer “unclear” if no mention of timing of testing.

• Signaling question 2: Were the reference standard results interpreted without knowledge of the results of the index test?

Answer “yes” if the examiner of the reference standard was blinded to the results of the index test; “no” if index test results were not blinded. Answer “unclear” if no notion of blinding was reported.

Risk of bias is rated as “low” if both signaling questions were answered “yes”; “high” if at least one signaling question was answered “no”; and “unclear” if at least one question was rated as “unclear” and no question rated “no”.

- *Applicability: Are there concerns that the target condition as defined by the reference standard does not match the question?*

Since our target condition is clinical mother-to-child transmission of HBV defined by the HBsAg or HBV DNA positivity in infants, all studies will be rated as “low concern”.

**Domain 4: Flow and Timing**

**PICO2A:**

- *Risk of Bias: Could the patient flow have introduced bias?*

• Signaling question 1: Was there an appropriate interval between the index test and reference standard?

Answer “yes” if both HBeAg and HBV DNA tests were done using the same sample. Answer “no” if the sample used for HBeAg test and the sample used for HBV DNA test were not collected on the same day. Answer “unclear” if information on when HBeAg or HBV DNA testing was performed is missing.

• Signaling question 2: Did all patients in the study receive the same reference standard?

Answer “no” if different NAT methods were used for different patients; and “unclear” if NAT method was not defined.

• Signaling question 3: Were all patients included in the analysis?

Answer “yes” if the number of patients enrolled was the same as the number of individual patient data obtained; “no” if the number of individual patient data obtained was smaller than the number of patients enrolled; and “unclear” if the number of patients enrolled was not presented in the original study.

Risk of bias is rated as “low” all signaling questions are answered “yes”; “high” if at least one question is answered “no”; and “unclear” if at least one question was answered “unclear” and no question rated “no”.

**PICO2B:**

- *Risk of Bias: Could the patient flow have introduced bias?*

• Signaling question 1: Was there an appropriate interval between the index test and reference standard?

Answer "yes" if maternal HBeAg was measured during pregnancy and MCTC was measured between 6 and 12 months. Answer "no" if different from this. Answer "unclear" if timing of HBeAg testing in mothers or MTCT timing of testing is unknown.

• Signaling question 2: Did all patients in the study receive the same reference standard?

Answer "yes" if the markers used to assess MTCT and timing of its assessment were the same for all infants.

• Signaling question 3: Were all patients included in the analysis?

Answer "yes" if the number of patients enrolled was the same as the number of individual patient data obtained; "no" if the number of individual patient data obtained was smaller than the number of patients enrolled; and "unclear" if the number of patients enrolled was not presented in the original study.

Risk of bias is rated as "low" all signaling questions are answered "yes"; "high" if at least one question is answered "no"; and "unclear" if at least one question was answered "unclear" and no question rated "no".

**PICO2C:**

- *Risk of Bias: Could the patient flow have introduced bias?*

• Signaling question 1: Was there an appropriate interval between the index test and reference standard?

Answer "yes" if maternal HBV DNA was measured during pregnancy and MCTC was measured between 6 and 12 months. Answer "no" if different from this. Answer "unclear" if timing of HBV DNA testing in mothers or MTCT timing of testing is unknown.

• Signaling question 2: Did all patients in the study receive the same reference standard?

Answer "yes" if the markers used to assess MTCT and timing of its assessment were the same for all infants.

• Signaling question 3: Were all patients included in the analysis?

Answer “yes” if the number of patients enrolled was the same as the number of individual patient data obtained; “no” if the number of individual patient data obtained was smaller than the number of patients enrolled; and “unclear” if the number of patients enrolled was not presented in the original study.

Risk of bias is rated as “low” all signaling questions are answered “yes”; “high” if at least one question is answered “no”; and “unclear” if at least one question was answered “unclear” and no question rated “no”.

## 8.3. Appendix C: Data extraction sheet protocol

- **Publication details**
  - ID screening
  - First author
  - Year
  - Journal
  - Language

- **Methods**
  - Country
  - Study main objective
  - Study design
  - Recruitment period
  - Recruitment setting (center or regional details, number of study sites)
  - Inclusion criteria
  - Exclusion criteria

  - Maternal HBV DNA: Type of sample
  - Maternal HBV DNA: When sample was taken
  - Maternal HBV DNA: Type of assay, commercial name
  - Maternal HBV DNA: Qualitative or quantitative

  - Maternal HBeAg: Type of sample
  - Maternal HBeAg: When sample was taken
  - Maternal HBeAg: Type of assay, commercial name
  - Maternal HBeAg: Qualitative or quantitative
  - Maternal HBeAg : Limit of detection of the assay used

  - Clinical endpoint for MTCT is reported (Yes/No)

- Infant HBsAg: Type of sample
- Infant HBsAg: When sample was taken
- Infant HBsAg: Type of assay, commercial name
- Infant HBV DNA: Type of sample
- Infant HBV DNA: When sample was taken
- Infant HBV DNA: Type of assay, commercial name
- Infant HBV DNA: Qualitative or quantitative
- Infant HBV DNA: Limit of detection of the assay used

- **Preventive measures**
  - Maternal antiviral therapy during pregnancy (type of drug, duration of treatment)
  - First dose of HBV vaccine: timely (≤24h) or delayed (>24h)
  - HBIG at birth
  - 3 doses of hepatitis B vaccine completed
  - Other

- **No. of participants at enrolment**
  - No. of women eligible for HBsAg screening
  - No. of women screened for HBsAg
  - No. of women tested positive for HBsAg
  - No. of women who had both HBV DNA & HBeAg tested
  - No. of women tested for HBV DNA
  - Maternal HBV DNA threshold used
  - No. of women with high viral load
  - No. of women with low viral load
  - No. of women screened for HBeAg
  - No. of women tested positive for HBeAg
  - No. of women tested negative for HBeAg

- **Women's characteristics**

- Define the population for the following characteristics (ideally this should be women who had both HBV DNA & HBeAg tested, but this may be often those who tested positive for HBsAg, irrespective of subsequent HBeAg/HBV DNA tests)
- Mean (SD) or median (IQR) maternal age
- Numerator/denominator by HBV viral genotypes
- Numerator/denominator by HIV status
- Numerator/denominator by HCV status
- Numerator/denominator by HDV status

- Outcomes 1: sensitivity and specificity of HBeAg to diagnose high viremia
- Maternal HBV DNA: i) For quantitative test: HBV DNA threshold to dichotomize into high or low viral load; ii) For qualitative test: Limit of detection
- No. with TP1 (high VL & HBeAg-pos)
- No. with FN1 (high VL & HBeAg-neg)
- No. with TN1 (low VL & HBeAg-neg)
- No. with FP1 (low VL & HBeAg-pos)
- Sensitivity of HBeAg to identify mothers with a high HBV viral load (TP / (TP+FN))
- No. of women with indeterminate result for HBeAg (if any)

- **Infants' characteristics**
  - No. of infants tested for HBsAg at the age of 6-12 months
  - No. of infants tested for HBV DNA at the age of 6-12 months

  - Outcomes 2-1: sensitivity and specificity of maternal HBeAg during pregnancy to predict MTCT (only those reporting MTCT endpoint)
  - Definition of MTCT: infant HBsAg or HBV DNA, & in which period for the following data
  - No. with TP2-1 (MTCT & maternal HBeAg-pos)

- No. with FN2-1 (MTCT & maternal HBeAg-neg)
- No. with TN2-1 (No MTCT & maternal HBeAg-neg)
- No. with FP2-1 (No MTCT & maternal HBeAg-pos)
- Sensitivity of maternal HBeAg to predict MTCT (TP/ (TP+FN))
- Specificity of maternal HBeAg to predict MTCT (TN / (TN+FP))

- Outcomes 2-2: sensitivity and specificity of maternal high HBV DNA levels during pregnancy to predict MTCT (only those reporting MTCT endpoint)
- Definition of MTCT: infant HBsAg or HBV DNA, & in which period for the following data
- Maternal HBV DNA: i) For quantitative test: HBV DNA threshold to dichotomize into high or low viral load; ii) For qualitative test: Limit of detection
- No. with TP2-2 (MTCT & maternal high VL)
- No. with FN2-2 (MTCT & maternal low VL)
- No. with TN2-2 (No MTCT & maternal low VL)
- No. with FP2-2 (No MTCT & maternal high VL)
- Sensitivity of maternal high VL to predict MTCT (TP / (TP+FN))
- Specificity of maternal high VL to predict MTCT (TN / (TN+FP))

- **Other**
  - Funding by industry
  - Comments
  - Need to contact an author for individual data
  - Need to contact an author for aggregated data

  - Only when there is a need to contact the author
  - Name of the author contacted
  - Title of the author contacted (e.g. PhD)
  - Affiliation of the author contacted
  - Email address of the author contacted

- Question to ask to the author contacted
- Was the author contacted?
- Reply from the author contacted

- **Decision**
  - Eligibility for the review
  - Reason for non-eligibility

## 8.4. Appendix D: Detailed QUADAS2 results for studies included in PICO2A

☺ Low Risk ☹ High Risk ? Unclear Risk

| Study | RISK OF BIAS | | | | APPLICABILITY CONCERNS | | |
|---|---|---|---|---|---|---|---|
| | PATIENT SELECTION | INDEX TEST | REFERENCE STANDARD | FLOW AND TIMING | PATIENT SELECTION | INDEX TEST | REFERENCE STANDARD |
| Bai H, 2010 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Bissinger, A , 2013 | ? | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Cai J, 2005 | ☺ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Cai S, 2009 | ☺ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Carey I, 2017 | ☹ | ? | ? | ☺ | ☹ | ☺ | ☺ |
| Carey I, 2018 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Chang C , 2017 | ☹ | ? | ? | ? | ☹ | ? | ☺ |
| Chen T, 2018 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Chen T, 2018 | ☹ | ? | ? | ? | ☹ | ☺ | ☺ |
| Chen ZX, 2017 | ☹ | ? | ? | ☹ | ☹ | ☺ | ☺ |
| Cheng S, 2006 | ☹ | ? | ? | ? | ☺ | ☺ | ☺ |
| Cheung K, 2019 | ☺ | ? | ? | ☹ | ☺ | ? | ☺ |
| Chotun N, 2017 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Dervisevic S, 2007 | ☺ | ? | ? | ☺ | ☺ | ? | ☺ |
| Ding Y, 2013 | ☺ | ? | ? | ? | ☺ | ☺ | ☺ |
| Dolman G, 2018 | ☹ | ? | ? | ? | ☺ | ? | ☺ |
| Dopico E, 2013 | ☹ | ? | ? | ? | ☺ | ☺ | ☺ |
| Dyson J, 2014 | ☺ | ? | ? | ☹ | ☺ | ? | ☺ |
| Eilard A, 2019 | ☹ | ? | ? | ? | ☺ | ☺ | ☺ |
| Elefsiniotis I, 2007 | ☹ | ? | ? | ☹ | ☺ | ? | ☺ |
| Foaud H, 2019 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Fujiko M, 2015 | ☹ | ? | ? | ? | ☺ | ☺ | ☺ |
| Godbole G, 2013 | ☺ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Guo F, 2007 | ☺ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Han Y, 2007 | ? | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Hao X, 2015 | ? | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Hu Q, 2007 | ☺ | ? | ? | ? | ☺ | ☺ | ☺ |
| Huang L, 2014 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Jackson V, 2015 | ☹ | ? | ? | ☹ | ☹ | ? | ☺ |
| Jin C, 2007 | ? | ? | ? | ☺ | ☺ | ? | ☺ |
| Johannessen A, 2017 | ? | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Köse, S, 2011 | ☹ | ? | ? | ? | ☹ | ? | ☺ |
| Kubo, A, 2014 | ☹ | ? | ? | ☹ | ☺ | ☹ | ☺ |
| Lao, T , 2012 | ☹ | ? | ? | ? | ☺ | ? | ☺ |
| Latthaphasavang, V, 2019 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Lee, L, 2015 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Li Xue-Li, 2018 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Li Yan, 2013 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Liang Pei-Song, 2017 | ☺ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Lin Jian, 2002 | ? | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Liu CP, 2015 | ☹ | ? | ? | ? | ☺ | ☺ | ☺ |
| Liu Hui-Yuan, 2013 | ☹ | ? | ? | ? | ☺ | ☺ | ☺ |
| Liu, Yingxia, 2016 | ☹ | ? | ? | ☹ | ☹ | ☺ | ☺ |
| Lu Hui-Fen, 2009 | ☺ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Lunel-Fabiani, F, 2018 | ☺ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Ma Yan-Hua, 2014 | ☹ | ? | ? | ? | ☺ | ☺ | ☺ |
| Michitaka, Kojiro, 2012 | ☺ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Peng Ning, 2012 | ? | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Peng, Songxu, 2019 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Pirillo, M, 2007 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Pirillo, MF, 2015 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Punzalan, C M, 2012 | ☹ | ? | ? | ? | ☺ | ? | ☺ |
| Rouet, F, 2004 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Samadi Kochaksaraei, G, 2016 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Ségéral, O, 2018 | ? | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Sellier, PO, 2017 | ☹ | ? | ? | ☹ | ☹ | ☺ | ☺ |
| Seo, Kwang Il, 2018 | ☹ | ? | ? | ☹ | ☹ | ☺ | ☺ |
| Shao, Z, 2011 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Sheng, Q, 2018 | ☺ | ? | ? | ? | ☺ | ☺ | ☺ |
| Shi Yan-Yan, 2012 | ☺ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Söderström, A, 2003 | ☹ | ? | ? | ? | ☺ | ☺ | ☺ |
| Tang Yong-Chun, 2013 | ☹ | ? | ? | ☹ | ☹ | ☹ | ☺ |
| Tang, A, 2018 | ☺ | ? | ? | ? | ☺ | ☺ | ☺ |
| Thilakanathan, C, 2018 | ☺ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| van Zonneveld, M , 2003 | ☹ | ? | ? | ☹ | ☹ | ? | ☺ |
| Wang Feng, 2009 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Wang Jing, 2018 | ☹ | ? | ? | ☹ | ☹ | ☺ | ☺ |
| Wang Miao, 2018 | ☺ | ? | ? | ? | ☺ | ? | ☺ |
| Wang Xing-Xing, 2015 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Wang Xue-Ling, 2015 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Wang Zhi-Qiang, 2018 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Wang, Jing, 2015 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Wang, L, 2016 | ☺ | ? | ? | ☹ | ☺ | ? | ☺ |
| Wei Kai-Ping, 2017 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Wiseman, E, 2009 | ☹ | ? | ? | ☹ | ☹ | ☺ | ☺ |
| Xu Hui-Li, 2008 | ☹ | ☺ | ☺ | ☺ | ☺ | ☺ | ☺ |
| Xu, Chenyu, 2018 | ☺ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Yin, Yu-zhu, 2013 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Zhang Li, 2017 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Zhang Qing, 2009 | ☹ | ? | ? | ? | ☺ | ☺ | ☺ |
| Zheng Hong, 2010 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Zhu Bao-Shen, 2013 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |

## 8.5. Appendix E: Detailed QUADAS2 results for studies included in PICO2B

☺Low Risk ☹High Risk ? Unclear Risk

| Study | RISK OF BIAS | | | | APPLICABILITY CONCERNS | | |
|---|---|---|---|---|---|---|---|
| | PATIENT SELECTION | INDEX TEST | REFERENCE STANDARD | FLOW AND TIMING | PATIENT SELECTION | INDEX TEST | REFERENCE STANDARD |
| Chen Z, 2017 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Chotun N, 2017 | ☹ | ? | ? | ☹ | ☺ | ? | ☺ |
| Ding Y, 2013 | ☺ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Foaud H, 2019 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Latthaphasavang, V, 2019 | ☹ | ? | ? | ☹ | ☹ | ☺ | ☺ |
| Lee, L, 2015 | ☹ | ? | ? | ☹ | ☺ | ☹ | ☺ |
| Liu CP, 2015 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Liu, Yingxia, 2016 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Lunel-Fabiani, F, 2018 | ☺ | ? | ? | ☹ | ☹ | ☺ | ☺ |
| Peng, Songxu, 2019 | ☹ | ? | ? | ☹ | ☹ | ? | ☺ |
| Shao, Z, 2011 | ☹ | ? | ? | ☹ | ☹ | ☺ | ☺ |
| Thilakanathan, C, 2018 | ☺ | ? | ? | ☹ | ☹ | ☺ | ☺ |
| Wang, Jing, 2015 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Wiseman, E, 2009 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Yin, Yu-zhu, 2013 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Zhu Bao-Shen, 2013 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |

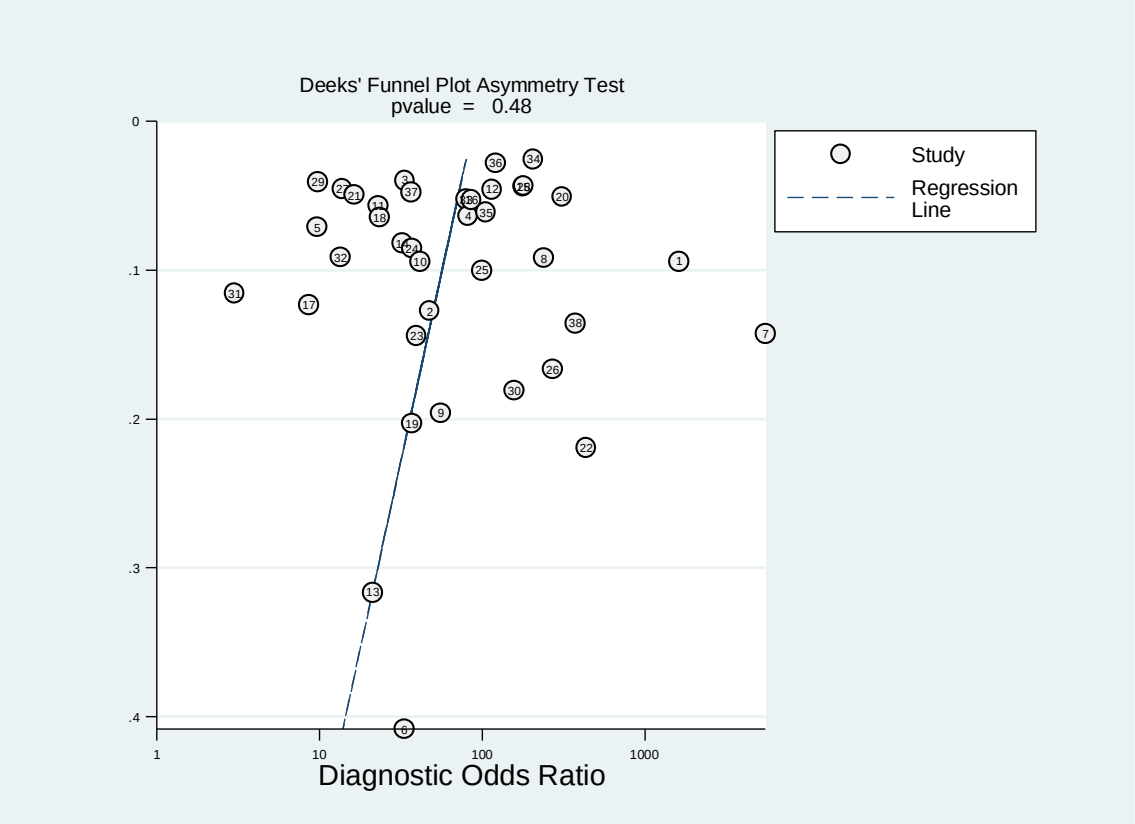
## 8.6. Appendix F: Detailed QUADAS2 results for studies included in PICO2C

☺Low Risk ☹High Risk ? Unclear Risk

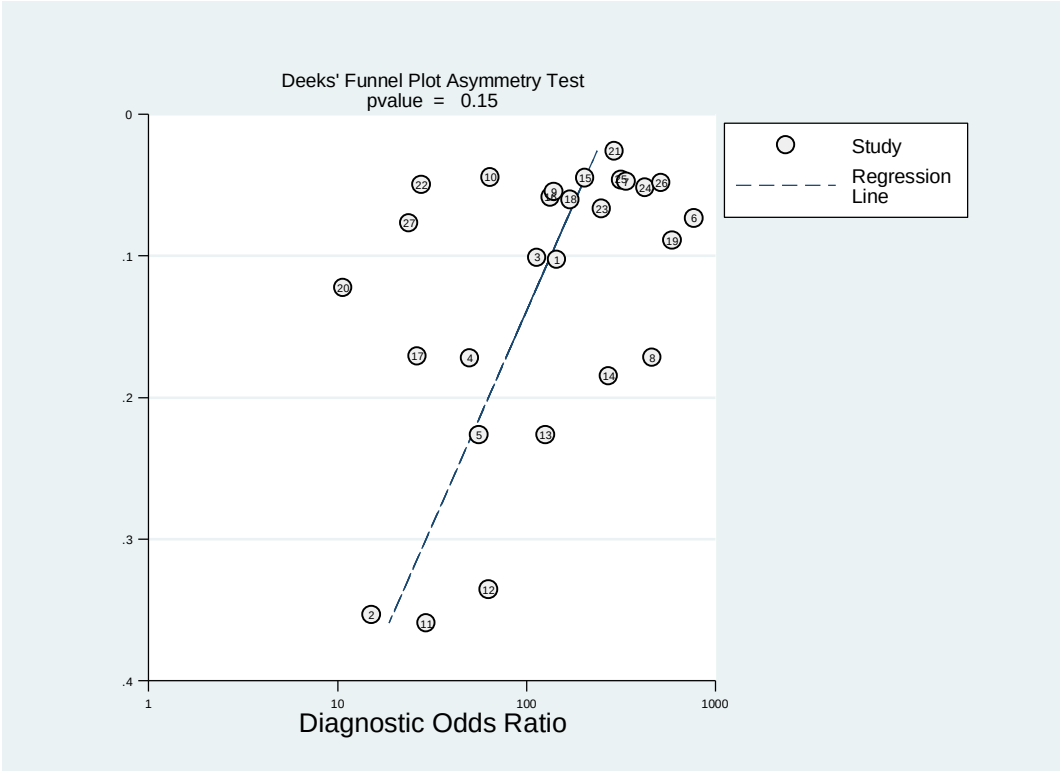
| Study | RISK OF BIAS | | | | APPLICABILITY CONCERNS | | |
|---|---|---|---|---|---|---|---|
| | PATIENT SELECTION | INDEX TEST | REFERENCE STANDARD | FLOW AND TIMING | PATIENT SELECTION | INDEX TEST | REFERENCE STANDARD |
| Chen Z, 2017 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Chotun N, 2017 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Ding Y, 2013 | ☺ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Latthaphasavang, V, 2019 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Lee, L, 2015 | ☹ | ? | ? | ☹ | ☹ | ☺ | ☺ |
| Lui CP, 2015 | ☹ | ? | ? | ☺ | ☺ | ? | ☺ |
| Lunel-Fabiani, F, 2018 | ☺ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Peng, Songxu, 2019 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Shao, Z, 2011 | ☹ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Thilakanathan, C, 2018 | ☺ | ? | ? | ☹ | ☺ | ☺ | ☺ |
| Wiseman, E, 2009 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Yin, Yu-zhu, 2013 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |
| Zhu Bao-Shen, 2013 | ☹ | ? | ? | ☺ | ☺ | ☺ | ☺ |

## 8.7. Appendix G: Funnel plots for publication bias

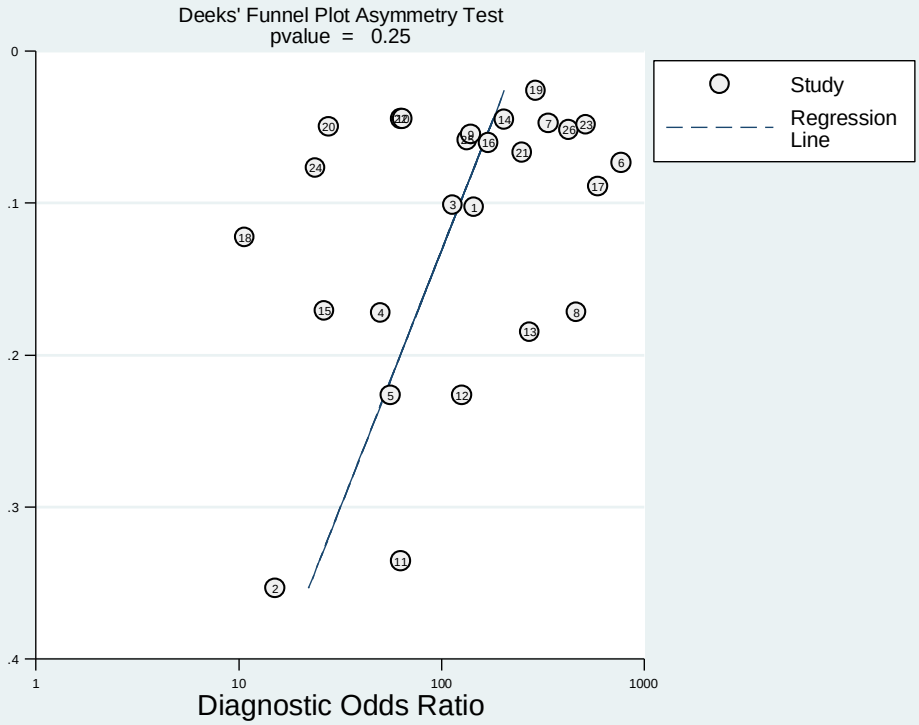
*Appendix G.1. Funnel plot for all studies included in the bivariate meta-analysis of the performance of HBeAg to identify maternal HBV DNA ≥5 log10 IU/mL*



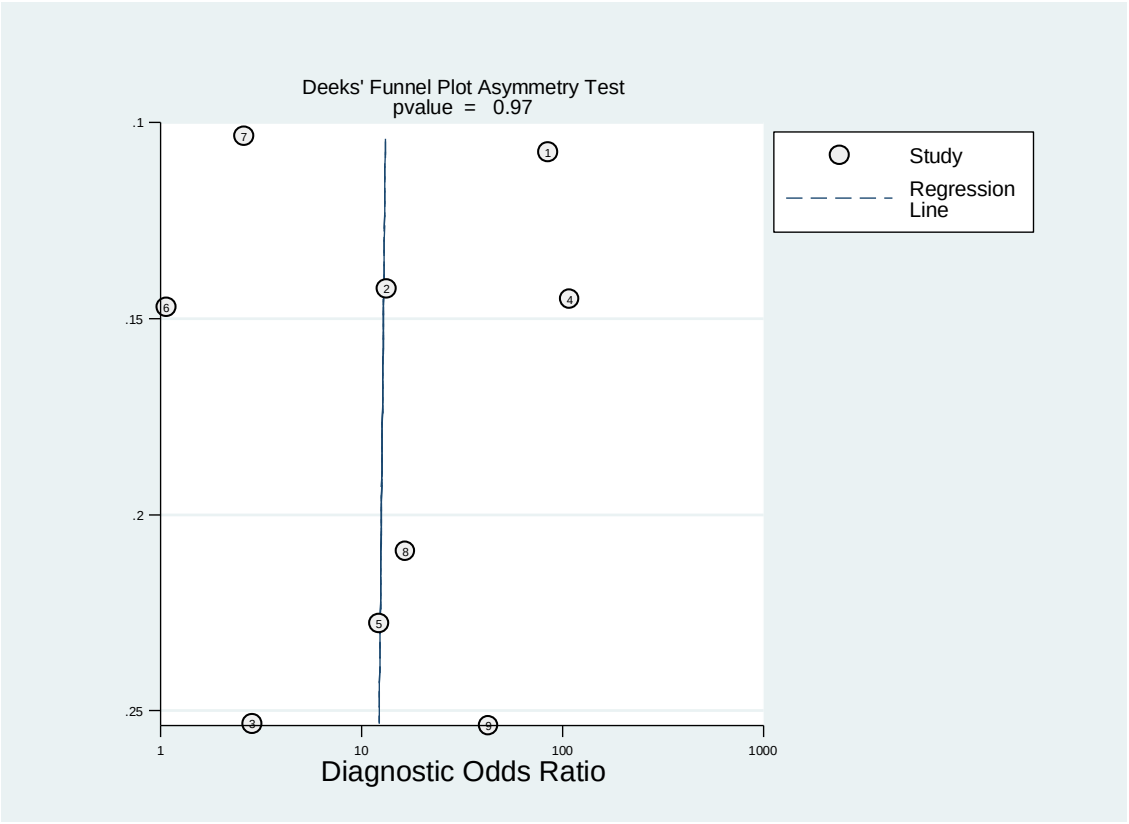
*Appendix G.2. Funnel plot for all studies included in the bivariate meta-analysis of the performance of HBeAg to identify maternal HBV DNA ≥6 log10 IU/mL*



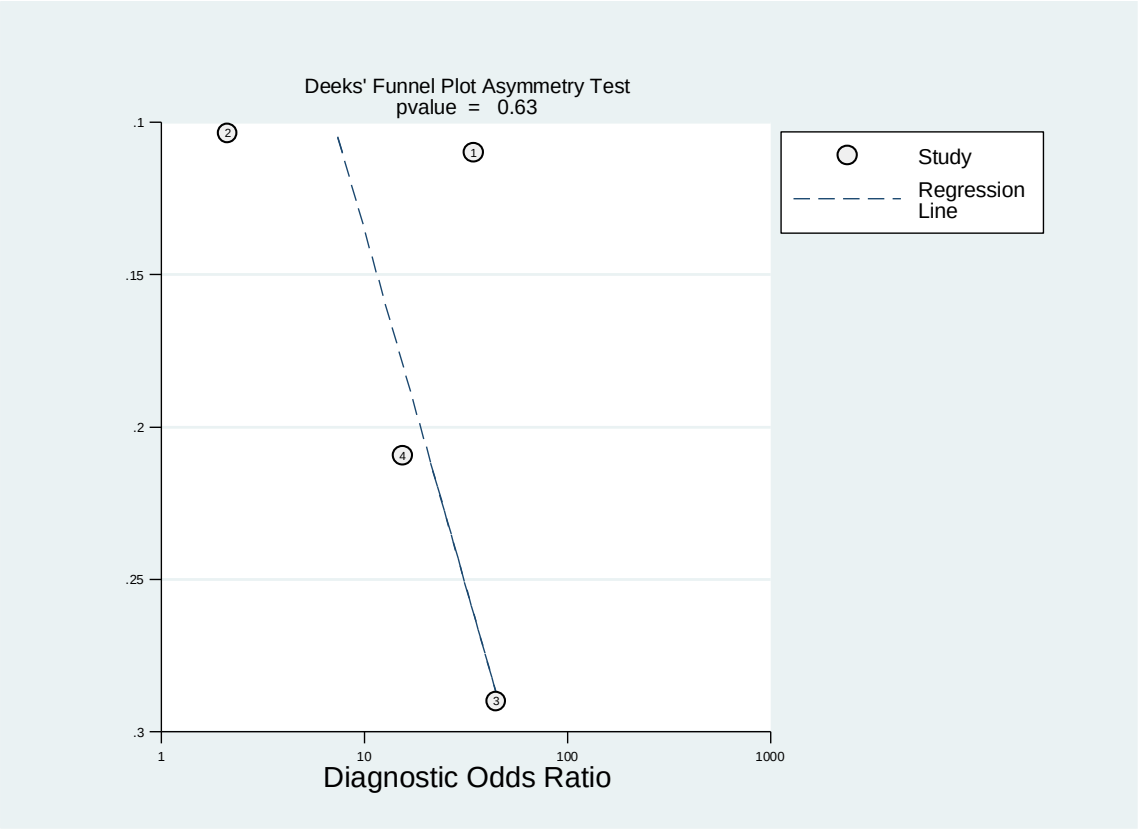
*Appendix G.3. Funnel plot for all studies included in the bivariate meta-analysis of the performance of HBeAg to identify maternal HBV DNA ≥7 log10 IU/mL*

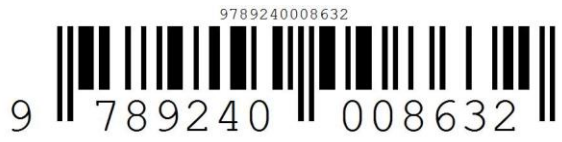


*Appendix G.4. Funnel plot for all studies included in the bivariate meta-analysis of the performance of HBeAg during pregnancy to predict MTCT of HBV*



*Appendix G.5. Funnel plot for all studies included in the bivariate meta-analysis of the performance of maternal HBV DNA ≥5 log10 IU/mL during pregnancy to predict MTCT of HBV*




9789240008632
9 789240 008632