# Hepatitis B Antigen Virus Test

# Clinical Trial

Management of the study: R&D Department
Quality control department

Place of the study: Beijing China

**Abstract**

- **Background**
  Hepatitis B virus is a partially double-stranded circular DNA virus and is a member of the Hepadnaviridae family. The virus consists of a core capsid which contains viral DNA and this is surrounded by an envelope containing surface antigen (HBsAg). Both whole, intact virions and incomplete virus particles, consisting entirely of HBsAg, are produced during replication of HBV. The HBsAg particles vary greatly in morphology and are found in high concentrations in early acute infection and continue to be produced in chronic disease.
  Rapid diagnostic tests have been developed for the detection of hepatitis B surface antigen (HBsAg). They represent a promising alternative to enzyme immunoassays and a powerful tool for large-scale screening and diagnosis of HBV infection, especially in regions without easy access to serological and molecular testing.
- **Method**
  A multi-center prospective study was conducted to evaluate the diagnostic sensitivity and specificity of HBsAg Rapid Test manufactured by Atlas Link (Beijing) Technology Co., Ltd in serum or plasma specimens from the patients clinically diagnosed as HBV infected.
- **Result**
  The comparison study result confirmed high diagnostic sensitivity and diagnostic specificity of HBsAg Test.
- **Conclusion**
  The HBsAg test kit is a simple, rapid, easy-to-run and highly sensitive assay that can be used in both high- and low-risk populations for the diagnosis of HBsAg carriage. It appears to be a promising new tool for large-scale screening and diagnosis of HBV infection.

**Clinical Study**

Hepatitis B Surface Antigen (HBsAg) Test clinical trials were conducted to determine the rate of diagnostic sensitivity and specificity across seroconversion panels and HBsAg positive probands including known HBV serotypes as defined in the CTS for in vitro diagnostic medical devices in conformance to Decisions 2009/108/EC.

**1. Clinical Trial Location:**

**Study site for Diagnostic Sensitivity:**

| Evaluation Site | Probands |
|---|---|
| PLA 302 Hospital | 30 Commercial HBsAg seroconversion Panels( BBI and ZMC) |
| Sichuan Center for Disease Control and Prevention | 275 HBsAg positive serum or plasma samples including 79 specimens known HBV Serotypes. |
| Key Laboratory of Molecular Biology on Infectious Diseases, | 279 HBV positive serum or plasma samples including 82 specimens known HBV serotypes. |

| | |
|---|---|
| Chongqing University of Medical Sciences | |

**Study site for Diagnostic Specificity:**

| Evaluation Site | Probands |
|---|---|
| PLA 302 Hospital | 200 hospitalized patients |
| PLA 302 Hospital | 200 pregnant women |
| Key Laboratory of Molecular Biology on Infectious Diseases, Chongqing University of Medical Sciences | 151 specimens from patients of potential interference diseases |
| Sichuan Center for Disease Control and Prevention | 90 specimens from patients of potential interference diseases |
| Key Laboratory of Molecular Biology on Infectious Diseases, Chongqing University of Medical Sciences | 593 specimens from blood donors |
| Blood Center of Beijing Red Cross Society | 523 specimens from blood donors |

**2. Diagnostic Sensitivity Study**

**Summary**

A multi-center prospective study was conducted to evaluate the diagnostic sensitivity of HBsAg Rapid Test manufactured by Atlas Link (Beijing) Technology Co., Ltd in serum or plasma specimens from the patients clinically diagnosed as HBV infected. Total 554 positive serum samples including all the 4 HBV serotypes adw, ayw, ayr and adr,(Among the 554 there were 275 from SCCDC and 279 from Chongqing Medical University) were selected to evaluate the diagnostic sensitivity of HBsAg Rapid Test and compare the results with CE marked Beijing Wantai HBsAg EIA. From study results of SCCDC (Table 3), 275 of 275 positive samples were tested positive by both Atlas Link HBs Ag Rapid Test and Wantai HBsAg EIA. The diagnostic sensitivity of Atlas Link HBs Ag Rapid Test was 100% (275/275). From study results of Chongqing Medical University (Table 4), 278 of 279 HBsAg positive sera were tested positive by both Atlas Link HBsAg Rapid Test and Wantai HBsAg EIA, the sample showed false negative at Atlas Link product also gave false negative at Wantai HBsAg EIA product. The sensitivity of HBsAg Rapid Test was 99.64% (278/279). All the samples with known serotype were tested as positive by both assays.

Total 554 positive samples were tested 553 positive by HBsAg Test and CE marked Wantai HBsAg EIA; the one (No.216 from ChongYi) tested negative by both HBs Ag Rapid Test and Wantai HBsAg EIA, was further confirmed positive by PCR. The serotype of the false negative sample was unknown. The diagnostic sensitivity of Atlas Link HBsAg Rapid Test was 99.82% (553/554) and

could identify the 4 HBV serotypes.

**Study Method and Results**

3 studies were carried out at different sites in China.

Total 30 commercial seroconversion panels including 20 from ZMC (ZeptoMetrix Corporation, ZMC, US) and 10 from BBI (SeraCare, US) were used to determine the relative diagnostic sensitivity of Atlas Link HBsAg Rapid Test. The relative diagnostic sensitivity was determined by comparing the earliest detection date of serum conversion of the patient to HBsAg seropositive with the HBsAg serological status of each series bleed with the data of CE-market HBs Ag products-Abbott HBsAg provided by ZMC or BBI. Atlas Link HBsAg Rapid Test showed earlier detection of seroconversion on panel 6275 and 11001 than Abbot's products, the value were -1, and -4 respectively; later detection of seroconversion on panel 11000, 11028 and PHM929, all the values were +1; the total value was -2. Overall, Atlas Link HBsAg Rapid Test demonstrated an ability to detect earlier in the conversion of a patient from seronegative to seropositive in comparison with Abbot HBsAg EIA products. (Table below)

Table Summary of Seroconversion Panels Detection at Atlas Link HBsAg Rapid Test

| **Panel Code** | **Period of Detection from the First Bleed Day** | | **Atlas Link results value compared with Abbot** |
|---|---|---|---|
| | **Abbot HBsAg EIA** | **Atlas Link HBsAg Rapid Test** | |
| 11064 | 68 | 68 | 0 |
| 6271 | 7 | 7 | 0 |
| 6273 | 25 | 25 | 0 |
| 6274 | 0 | 0 | 0 |
| 6275 | 22 | 9 | -1 |
| 6276 | 27 | 27 | 0 |
| 6280 | 13 | 13 | 0 |
| 6286 | 32 | 32 | 0 |
| 6288 | 29 | 29 | 0 |
| 6291 | 27 | 27 | 0 |
| 6293 | 17 | 17 | 0 |
| 11000 | 14 | 19 | +1 |
| 11001 | 44 | 0 | -4 |
| 11002 | 7 | 7 | 0 |
| 11003 | 142 | 142 | 0 |
| 11004 | 46 | 46 | 0 |
| 11016 | 27 | 27 | 0 |

| | | | |
|---|---|---|---|
| 11028 | 22 | 27 | +1 |
| 11058 | 38 | 38 | 0 |
| 11059 | 27 | 27 | 0 |
| PHM906 | 137 | 137 | 0 |
| PHM912 | 42 | 42 | 0 |
| PHM918 | NA | NA | 0 |
| PHM924 | 29 | 29 | 0 |
| PHM926 | 20 | 20 | 0 |
| PHM929 | 18 | 21 | +1 |
| PHM934 | 7 | 7 | 0 |
| PHM936 | 5 | 5 | 0 |
| PHM937 | 9 | 9 | 0 |
| PHM938 | 0 | 0 | 0 |

Total 554 positive serum samples including all the 4 HBV serotypes adw, ayw, ayr and adr,(Among the 554 there were 275 from SCCDC and 279 from Chongqing Medical University) were selected to evaluate the diagnostic sensitivity of Atlas Link HBsAg Rapid Test and compare the results with CE marked Beijing Wantai HBsAg EIA. From study results of SCCDC (Table 1), 275 of 275 positive samples were tested positive by both Atlas Link HBs Ag Rapid Test and Wantai HBsAg EIA. The diagnostic sensitivity of Atlas Link HBs Ag Rapid Test was 100% (275/275). From study results of Chongqing Medical University (Table 2), 278 of 279 HBsAg positive sera were tested positive by both Atlas Link HBsAg Rapid Test and Wantai HBsAg EIA, the sample showed false negative at Atlas Link product also gave false negative at Wantai HBsAg EIA product. The sensitivity of HBsAg Rapid Test was 99.64% (278/279). All the samples with known serotype were tested as positive by both assays. Total 554 positive samples were tested 553 positive by Atlas Link HBsAg Test and CE marked Wantai HBsAg EIA; the one (No.216 from ChongYi)tested negative by both Atlas Link HBsAg Rapid Test and Wantai HBsAg EIA, was further confirmed positive by PCR. The serotype of the false negative sample was unknown. The diagnostic sensitivity of Atlas Link HBsAg Rapid Test was 99.82% (553/554) and could identify the 4 HBV serotypes.

Table 1 Summary of HBsAg positive specimens' detection from SCCDC at Atlas Link HBsAg Rapid Test

| Serotype | Results of Atlas Link HBs Antigen Rapid Test | | Results of CE Marked Wantai HBsAg EIA | | Subtotal |
|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | |
| adw2 | 51 | 0 | 51 | 0 | 51 |
| ayw1 | 18 | 0 | 18 | 0 | 18 |
| adr | 5 | 0 | 5 | 0 | 5 |
| ayw2 | 3 | 0 | 3 | 0 | 3 |
| ayr | 1 | 0 | 1 | 0 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| ayw3 | 1 | 0 | 1 | 0 | 1 |
| Unknown serotype | 196 | 0 | 196 | 0 | 196 |
| Subtotal | 275 | 0 | 275 | 0 | 275 |

Table 2 Summary of HBsAg Positive Specimens' Detection from Chongqing Medical University at Atlas Link HBsAg Rapid Test

| Serosubtype | Results of Atlas Link HBs Antigen Rapid Test | | Results of Wantai HBsAg EIA | | Subtotal |
|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | |
| adw2 | 67 | 0 | 67 | 0 | 67 |
| ayw1 | 15 | 0 | 15 | 0 | 15 |
| Unknown serosubtype | 196 | 1 | 196 | 1 | 197 |
| Subtotal | 278 | 1 | 278 | 1 | 279 |

Table 3 Summary of HBsAg Positive Specimens' Detection at Atlas Link HBsAg Rapid Test

| Serotype | Results of Atlas Link HBs Antigen Rapid Test | | Results of CE Marked Wantai HBsAg EIA | | Subtotal |
|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | |
| adw2 | 118 | 0 | 118 | 0 | 118 |
| ayw1 | 33 | 0 | 33 | 0 | 33 |
| adr | 5 | 0 | 5 | 0 | 5 |
| ayw2 | 3 | 0 | 3 | 0 | 3 |
| ayr | 1 | 0 | 1 | 0 | 1 |
| ayw3 | 1 | 0 | 1 | 0 | 1 |
| Unknown serotype | 392 | 1 | 392 | 1 | 393 |
| Subtotal | 553 | 1 | 553 | 1 | 554 |

Comparison of the sensitivity of Atlas Link HBsAg Rapid Test with that of Wantai HBsAg EIA

| | | Atlas Link HBsAg Rapid Test | | subtotal |
|---|---|---|---|---|
| | | Positive | Negative | |
| Results of CE Marked Wantai HBsAg EIA | Positive | 553 | 0 | 553 |
| | Negative | 0 | 1 | 1 |
| Subtotal | | 553 | 1 | 554 |

Sensitivity of Atlas Link HBsAg Test: 99.82% (553/554).
Sensitivity of CE Marked HBsAg Test: 99.82% (553/554).

**3. Diagnostic specificity Study**

**Summary**

A multi-center prospective study was conducted to evaluate the diagnostic specificity of the HBsAg Rapid Test manufactured by Atlas Link (Beijing) Technology Co., Ltd in serum or plasma specimens from different populations including blood donors, inpatients, pregnant women and other potentially interfering diseases. From the 6 diagnostic specificity studies among different populations, total 1752 negative samples including 1116 from blood donors, 200 from inpatients, 200 from pregnant women, 236 from potentially interfering diseases were selected to evaluate the specificity of HBsAg Rapid Test and Compare the results with Wantai HBsAg EIA. Of the 1752 samples, 1750 were tested negative, 2 tested positive (both from blood donors) by HBsAg Rapid Test. All the results which showed inconsistent with CE marked Wantai HBsAg EIA were further confirmed negative by PCR. The diagnostic sensitivity of HBsAg Rapid Test was 99.89% (1750/1752), false positive rate is 0.11% .

**Study Method and Results**

6 studies were carried out at different sites in China.

Total 200 specimens with known non-HBs Antigen positive from hospitalized patients in PLA 302 Hospital, PRC, were used to evaluate the diagnostic specificity among inpatients. 200 of 200 specimens were tested HBsAg negative by both HBs Antigen Test and Wantai HBsAg EIA. The diagnostic specificity of HBsAg Rapid Test in inpatients was 100% (200/200).

Table Summary of Diagnostic Specificity of HBsAg Rapid Test among Inpatients

| 200 inpatients | Results of HBsAg Rapid Test | | Results of Wantai HBsAg EIA | | Subtotal |
|---|---|---|---|---|---|
| | Negative | Positive | Negative | Positive | |
| True Negative | 200 | 0 | 200 | 0 | 200 |

Among the 200 pregnant women, there were 17 multiparas. 200 of 200 specimens were tested HBs Ag negative by both HBsAg Test and Wantai HBsAg EIA. The diagnostic specificity of HBsAg Rapid Test in pregnant women was 100% (200/200). (Table below)

Table Summary of Diagnostic Specificity of HBsAg Rapid Test among Pregnant Women

| 200 Pregnant Women | Results of HBsAg Rapid Test | | Results of Wantai HBsAg EIA | | Subtotal |
|---|---|---|---|---|---|
| | Negative | Positive | Negative | Positive | |
| True Negative | 200 | 0 | 200 | 0 | 200 |

Total 236 specimens (146 from Chongqing Medical University and 90 from SCCDC) from patients with potential interference diseases were tested to evaluate the diagnostic specificity of HBsAg

Rapid Test. The potential interference diseases status were HAV, HIV, HCV, Dengue, EBV, CMV, HTLV, Rubella and E.coli infections, CRF, ASOT, RF positive. There were at least 15 specimens for each interference status. 151 specimens from Chongqing Medical University included the interference status of HAV, HIV, HCV, EBV, RF, CMV, ASOT, CRF, Dengue, Rubella and HTLV positive. These 146 specimens had no interference with HBsAg Rapid Test (Table 4). 90 specimens from SCCDC included the interference status of HAV, HCV Dengue, HTLV and E.coli positive. 90 of the 90 specimens showed negative results by HBsAg Rapid Test (Table 5). Among the 236 interference specimens, 236 showed no interference with HBsAg Rapid Test. The diagnostic specificity among potential interference disease status was 100% (236/236)(Table 6)

Table 4 Summary of Diagnostic Specificity of HBsAg Rapid Test among Interference Disease Status (Chongqing Medical University)

| Interference Diseases | Results of HBs Ag Rapid Test | | Results of CE Marked Wantai HBsAg EIA | | Subtotal |
|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | |
| HAV | 0 | 12 | 0 | 12 | 12 |
| HIV | 0 | 15 | 0 | 15 | 15 |
| HCV | 0 | 20 | 0 | 20 | 20 |
| Dengue | 0 | 5 | 0 | 5 | 5 |
| CMV | 0 | 15 | 0 | 15 | 15 |
| EBV | 0 | 15 | 0 | 15 | 15 |
| HTLV | 0 | 4 | 0 | 4 | 4 |
| Rubella | 0 | 15 | 0 | 15 | 15 |
| RF | 0 | 15 | 0 | 15 | 15 |
| CRF | 0 | 15 | 0 | 15 | 15 |
| ASOT | 0 | 15 | 0 | 15 | 15 |
| Subtotal | 0 | 146 | 0 | 146 | 146 |

Table 5 Summary of Diagnostic Specificity of HBsAg Rapid Test among interference Disease Status (SCCDC)

| Interference Diseases | Results of HBsAg Rapid Test | | Results of CE Marked Wantai HBsAg EIA | | Subtotal |
|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | |
| HAV | 0 | 20 | 0 | 20 | 20 |
| HIV | 0 | 20 | 0 | 20 | 20 |
| Dengue | 0 | 16 | 0 | 16 | 16 |
| HTLV | 0 | 13 | 0 | 13 | 13 |

| | | | | | |
|---|---|---|---|---|---|
| Anti-E.coli | 0 | 21 | 0 | 21 | 21 |
| Subtotal | 0 | 90 | 0 | 90 | 90 |

Table 6 Summary of Diagnostic Specificity of HBsAg Rapid Test among Interference Disease Status

| Interference Diseases | Results of HBsAg Rapid Test | | Results of CE Marked Wantai HBsAg EIA | | Subtotal |
|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | |
| HAV | 0 | 32 | 0 | 32 | 32 |
| HIV | 0 | 35 | 0 | 35 | 35 |
| HCV | 0 | 20 | 0 | 20 | 20 |
| Dengue | 0 | 21 | 0 | 21 | 21 |
| CMV | 0 | 15 | 0 | 15 | 15 |
| EBV | 0 | 15 | 0 | 15 | 15 |
| HTLV | 0 | 17 | 0 | 17 | 17 |
| Rubella | 0 | 15 | 0 | 15 | 15 |
| RF | 0 | 15 | 0 | 15 | 15 |
| CRF | 0 | 15 | 0 | 15 | 15 |
| ASOT | 0 | 15 | 0 | 15 | 15 |
| Anti-E.Coli | 0 | 21 | 0 | 21 | 21 |
| Subtotal | 0 | 236 | 0 | 236 | 236 |

Total 1116 specimens from blood donors (523 from Blood Center of Beijing Red Cross Society and 593 from Blood Transfusion Department, the Secondary Affiliated Hospital of Chongqing Medical University) were used to evaluate the diagnostic specificity of HBsAg Rapid Test. The 1116 specimens consisted of 81 specimens from consecutive blood donations. Among the 523 specimens from Blood Center of Beijing Red Cross Society, 522 showed negative results by HBsAg Rapid Test, the diagnostic specificity was 99.81% (Table 7). Among the 593 specimens from Chongqing Medical University, 592 showed negative results by HBsAg Rapid Test, the diagnostic specificity was 99.83%(Table 8). Combined these two results together, total 1116 specimens from blood donors, 1114 showed negative results by HBsAg Rapid Test, 2 gave false positive; the diagnostic specificity among blood donors was 99.82%(Table 9)

Table 7 Summary of Diagnostic Specificity of HBsAg Rapid Test among Blood Donors (Blood Center of Beijing Red Cross Society)

| Blood donors | Results of HBsAg Rapid Test | | Results of Wantai HBsAg EIA | | Subtotal |
|---|---|---|---|---|---|
| | Negative | Positive | Negative | Positive | |
| True Negative | 522 | 1 | 521 | 2 | 523 |

Table 8 Summary of Diagnostic Specificity of HBsAg Rapid Test among Blood Donors (Chongqing Medical University)

| Blood donors | Results of HBsAg Rapid Test | | Results of Wantai HBsAg EIA | | Subtotal |
|---|---|---|---|---|---|
| | Negative | Positive | Negative | Positive | |
| True Negative | 592 | 1 | 592 | 1 | 593 |

Table 9 Summary of Diagnostic Specificity of HBsAg Rapid Test among Blood Donors

| Blood donors | Results of HBsAg Rapid Test | | Results of Wantai HBsAg EIA | | Subtotal |
|---|---|---|---|---|---|
| | Negative | Positive | Negative | Positive | |
| True Negative | 1114 | 2 | 1113 | 3 | 1116 |

From the 6 diagnostic specificity studies among different populations, total 1752 negative samples including 1116 from blood donors, 200 from inpatients, 200 from pregnant women, 236 from potentially interfering diseases were selected to evaluate the specificity of HBsAg Rapid Test and Compare the results with Wantai HBsAg EIA. Of the 1752 samples, 1750 were tested negative, 2 tested positive (both from blood donors) by HBsAg Rapid Test. All the results which showed inconsistent with CE marked Wantai HBsAg EIA were further confirmed negative by PCR. The diagnostic sensitivity of HBsAg Rapid Test was 99.89% (1750/1752), false positive rate is 0.11% (Table 10).

Table 10 Summary of Diagnostic Specificity of HBsAg Rapid Test

| | Results of HBsAg Rapid Test | | Results of CE Marked Wantai HBsAg EIA | | Subtotal |
|---|---|---|---|---|---|
| | Negative | Positive | Negative | Positive | |
| Blood Donors | 1114 | 2 | 1113 | 3 | 1116 |
| 200 clinic al specimens | 200 | 0 | 200 | 0 | 200 |
| 200 Pregnant Women | 200 | 0 | 200 | 0 | 200 |
| 236 potentially interfering samples | 236 | 0 | 236 | 0 | 236 |
| Subtotal | 1750 | 2 | 1749 | 3 | 1752 |

Table 11 Comparison of the specificity of HBsAg Test with that of Wantai HBsAg EIA

| | | Altas Link HBsAg Test | | subtotal |
|---|---|---|---|---|
| | | Positive | Negative | |
| Results of CE Marked | Positive | 0 | 3 | 3 |
| | Negative | 2 | 1747 | 1749 |

| | | | |
|---|---|---|---|
| Wantai HBsAg EIA Subtotal | 2 | 1750 | 1752 |

Specificity of HBsAg Test: 99.89% (1750/1752)
Specificity of CE Marked HBsAg Test: 99.83% (1749/1752)
When evaluated the diagnostic sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and 95% Confidence limits (CL) of the calculated sensitivity and specificity of HBsAg Test with total 554 true positive and 1752 true negative HBsAg serum samples, the sensitivity was 99.82% (553/554), the specificity was 99.89% (1750/1752), PPV was 99.64%(553/555), NPV was 99.94%(1750/1751), 95% CL of sensitivity was 99.65%-99.99%; 95% CL of specificity was 99.84%-100%.

| | Altas Link HBsAg Rapid Test | | subtotal |
|---|---|---|---|
| | Positive | Negative | |
| True Positive | 553 | 1 | 554 |
| True Negative | 2 | 1750 | 1752 |
| Subtotal | 555 | 1751 | 2306 |

Comparison of the sensitivity and specificity of HBsAg Test with those of CE Marked HBsAg EIA , the agreement of the two HBsAg test was 99.78%((553+1748)/2306), Statistical significance of sensitivity and specificity between HBsAg Test and CE Marked HBsAg EIA was determined by $x^2$ test, and no significant difference between these two assays. ($P > 0.01$)

| | | Altas Link HBsAg Rapid Test | | subtotal |
|---|---|---|---|---|
| | | Positive | Negative | |
| Results of CE Marked Wantai HBsAg EIA | Positive | 553 | 3 | 556 |
| | Negative | 2 | 1748 | 1750 |
| Subtotal | | 555 | 1751 | 2306 |

**4. Conclusion**

By analysis the data collected in these clinical investigations, it is concluded that Hepatitis B Surface Antigen Test (Serum/Plasma) manufactured by Atlas Link (Beijing) Technology Co., Ltd has relatively high sensitivity, specificity and accuracy for a lateral flow One Step test. This test is convenient, fast and simple to operate. The test has been determined to be safe and effective. No special instrument is needed to read the test result. The background is clear and interpretation of the test result is very easy.