# Hepatitis C Virus Test

# (Colloidal Gold)

## Diagnostic Sensitivity and Specificity Study Report

**Final report date**: **2016-06-20**

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# Study Director Signature and Verification Dates

**This study meets with the technical requirements of the protocol. The study also meets with technical specification for the test.**

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**Position:** **Head of R & D Department**

**Verification Dates:2016-06-20**

# Study Summary

The purpose of this study was to obtain accurate information about the diagnostic sensitivity and specificity of the Hepatitis C Virus Test (Colloidal Gold), and we have evaluated the clinical effects of the Hepatitis C Virus Test (Colloidal Gold). The clinical evaluation was carried out for the clinical performance of Hepatitis C Virus Test (Colloidal Gold). Total 2138 samples were tested, of which the diagnostic sensitivity is 99.76%; diagnostic specificity is 99.59%; total compliance is 99.63%.

## 1. Purpose

To validate the diagnostic sensitivity and specificity of Hepatitis C Virus Test (Colloidal Gold).

## 2. Reference and Compliance

FDA guidance for In vitro diagnostic medical device

SFDA guidance

The present study conformed to all applicable laws and regulations.

## 3. Reagent and Materials

- Hepatitis C Virus Test (Colloidal Gold), Lot No.: HCV-1.
- CE Marked Ortho HCV 3.0 EIATest, LOT: 20160502
- Patient samples collected by

| Evaluation Site | Negative sample | Positive sample |
|---|---|---|
| PLA 302 Hospital | 200 hospitalized patients | 10 Commercial HCV seroconversion Panels( BBI and ZMC) |
| | 200 pregnant women | |
| Key Laboratory of Molecular Biology on Infectious Diseases, Chongqing University of Medical Sciences | 146 specimens from patients of potential interference diseases | 20 public HCV seroconversion Panels |
| Sichuan Center for Disease Control and Prevention | 90 specimens from patients of potential interference diseases | 267 HCV positive serum or plasma samples including 94 specimens known HCV genotype |
| Key Laboratory of Molecular Biology on Infectious Diseases, Chongqing University of Medical Sciences | 472 specimens from blood donors | 144 HCV positive serum or plasma samples including 81 specimens known HCV genotype |
| Blood Center of Beijing Red Cross Society | 619 specimens from blood donors | N/A |

## 4. Study Design:

### 4.1 Making evaluation for patient samples.

- Subject was a patient admitted to the hospital due to a respiratory virus infection;
- Total about 1727 negative samples and 411 positive samples were collected;

### 4.2 Examiner and clinical laboratories

- PLA 302 Hospital
- Key Laboratory of Molecular Biology on Infectious Diseases, Chongqing University of Medical Sciences
- Sichuan Center for Disease Control and Prevention

### 4.3 Sample requirement:

All the samples were confirmed.

Samples were to be randomly chosen and double-black labeled.

### 4.4 Test conduction:

- All tests were performed by the clinical technicians in each clinical laboratory according to the

manufacturer's instructions using the confirmed samples.

- Visual interpretations of the results of Hepatitis C Virus Test were made independently by the clinical technician.
- The testing center was responsible to summarize the result and send to Atlas Link

## 5. Evaluation Criteria

Positive(+):Both C and T lines appear regardless of color intensity.
Negative(-) :Only C line appears.
Undeterminable (±): C line appears; T line is so weak that it cannot be determined as positive.
Invalid result(/):Neither C line nor T line appears.

## 6. Results

Total 30 seroconversion panels including 20 from public source and 10 from commercial source were used to determine the relative diagnostic sensitivity of Hepatitis C Virus Test. The relative diagnostic sensitivity was determined by comparing the earliest detection date of serum conversion of the patient to HCV seropositive with the HCV serological status of each series bleed with that of CE-market HCV product or with the data provided by BBI or ZMC. For the 20 seroconversion panels from public source, Hepatitis C Virus Test showed earlier detection of seroconversion on panel HCV-989 than CE approved Ortho HCV 3.0, the value was -1. Later detection of seroconversion on panel HCV-041 than Orhto 's HCV products, the values was -1. Compared the relative diagnostic sensitivity of Hepatitis C Virus Test with that of Ortho HCV 3.0 EIA on HCV seroconversion panels, The total value was 0(Table 2). For the 10 commercial seroconversion panels, Hepatitis C Virus Test showed earlier detection of seroconversion on panel HCV6228 than Abbot's products, the value was -1. Earlier detection of seroconversion on panel PHV913 and HCV6224 than Orhto 's HCV products, the values were -2 and -2 respectively; later detection of seroconversion on panel PHV919 , the value was +1, the total value was -3(Table 3).

Table 2 Summary of Seroconversion Panels ( Public Source) Detection at Hepatitis C Virus Test

| Panel Code | Period of Detection from the First Bleed Day | | |
|---|---|---|---|
| | Ortho HCV 2.0/3.0 | Hepatitis C Virus Test | Hepatitis C Virus Test results value compared with Ortho 3.0 |
| HCV-973 | 10 | 10 | 0 |
| HCV-976 | 10 | 10 | 0 |
| HCV-982 | 41 | 41 | 0 |
| HCV-983 | 20 | 20 | 0 |
| HCV-986 | 41 | 41 | 0 |
| HCV-989 | 6 | 3 | -1 |
| HCV-991 | 4 | 4 | 0 |
| HCV-011 | 27 | 27 | 0 |
| HCV-012 | 24 | 24 | 0 |
| HCV-032 | 14 | 14 | 0 |
| HCV-033 | 16 | 16 | 0 |
| HCV-041 | 19 | 39 | +1 |

| | | | |
|---|---|---|---|
| HCV-042 | 27 | 27 | 0 |
| HCV-043 | 29 | 29 | 0 |
| HCV-072 | 11 | 11 | 0 |
| HCV-074 | 14 | 14 | 0 |
| HCV-081 | 15 | 15 | 0 |
| HCV-102 | 4 | 4 | 0 |
| HCV-103 | 9 | 9 | 0 |
| HCV-111 | 7 | 7 | 0 |

Table 3 Summary of Seroconversion Panels ( Commercial Source) Detection at Hepatitis C Virus Test

| Panel Code | Period of Detection from the First Bleed Day | | | |
|---|---|---|---|---|
| | Abbot HCV 2.0/4.0 EIA | Ortho HCV 2.0/3.0 | Hepatitis C Virus Test | Hepatitis C Virus Test results value compared with Abbot /Orhto |
| PHV911 | 13 | 13 | 13 | 0/0 |
| PHV913 | 7 | Not detected | 7 | 0/-2 |
| PHV919 | 32 | 28 | 32 | 0/+1 |
| HCV6213 | 43 | 43 | 43 | 0/0 |
| HCV6215 | 20 | 20 | 20 | 0/0 |
| HCV6224 | 23 | Not detected | 23 | 0/-2 |
| HCV6226 | 39 | 39 | 9 | 0/0 |
| HCV6227 | 74 | 74 | 74 | 0/0 |
| HCV6228 | 36 | 31 | 31 | -1/0 |
| HCV6241 | 61 | 61 | 61 | 0/0 |

Total 411 positive serum samples including 6 genotypes (Among the 411 specimens, there were 267 from SCCDC and 144 from Chongqing Medical University) were selected to evaluate the diagnostic sensitivity of Hepatitis C Virus Test and compare the results with CE marked Ortho HCV 3.0 EIA. From study results of SCCDC (Table 4), 267 positive samples were tested 267 positive by both Hepatitis C Virus Test and Ortho HCV 3.0. The diagnostic sensitivity of Hepatitis C Virus Test was 100% (267/267). From study results of Chongqing Medical University (Table 5), 143 of 144 HCV positive sera were tested positive by Hepatitis C Virus Test, the negative one tested by Hepatitis C Virus Test showed positive by Ortho HCV EIA. The sensitivity of Hepatitis C Virus Test was 99.31% (143/144). All the samples with known genotype 1-6 were tested as positive by both assays. Total 411 positive samples were tested 410 positive, 1 tested negative by Hepatitis C Virus Test which showed inconsistent results with CE marked Ortho HCV 3.0 EIA and was further confirmed positive by PCR. The genotype of the false negative sample was unknown. The diagnostic sensitivity of Hepatitis C Virus Test was 99.76% (410/411) and could identify HCV genotype 1-6.

Table 4 Summary of HCV positive specimens' detection from SCCDC at Hepatitis C Virus Test

| Genotype | | Results of Hepatitis C Virus Test | | Results of CE Marked Test | | Subtotal |
|---|---|---|---|---|---|---|
| | | Positive | Negative | Positive | Negative | |
| 1 | 1a | 2 | 0 | 2 | 0 | 23 |
| | 1b | 21 | 0 | 21 | 0 | |
| 2 | 2a | 10 | 0 | 10 | 0 | 18 |
| | 2b | 5 | 0 | 5 | 0 | |
| | 2a/2b | 3 | 0 | 3 | 0 | |
| 3 | 3a | 8 | 0 | 8 | 0 | 15 |
| | 3b | 7 | 0 | 7 | 0 | |
| 4 | 4 | 5 | 0 | 5 | 0 | 16 |
| | 4a | 5 | 0 | 5 | 0 | |
| | 4c/4d | 6 | 0 | 6 | 0 | |
| 5a | | 4 | 0 | 4 | 0 | 4 |
| 6 | 6a | 12 | 0 | 12 | 0 | 16 |
| | 6e | 4 | 0 | 4 | 0 | |
| 1b/2a | | 2 | 0 | 2 | 0 | 2 |
| Unknown genotype | | 173 | 0 | 173 | 0 | 173 |
| Subtotal | | 267 | 0 | 267 | 0 | 267 |

Table 5 Summary of HCV Positive Specimens' Detection from Chongqing Medical University at Hepatitis C Virus Test

| Genotype | | Results of Hepatitis C Virus Test | | Results of Ortho HCV EIA | | Subtotal |
|---|---|---|---|---|---|---|
| | | Positive | Negative | Positive | Negative | |
| 1 | 1a | 2 | 0 | 2 | 0 | 28 |
| | 1b | 26 | 0 | 26 | 0 | |
| 2 | 2a | 15 | 0 | 15 | 0 | 19 |
| | 2b | 4 | 0 | 4 | 0 | |
| 3 | 3a | 9 | 0 | 9 | 0 | 13 |
| | 3b | 4 | 0 | 4 | 0 | |
| 4 | 4 | 2 | 0 | 2 | 0 | 4 |
| | 4a | 2 | 0 | 2 | 0 | |
| 5a | | 2 | 0 | 2 | 0 | 2 |
| 6 | 6a | 10 | 0 | 10 | 0 | 14 |
| | 6e | 4 | 0 | 4 | 0 | |
| 1b/2a | | 1 | 0 | 1 | 0 | 1 |
| Unknown genotype | | 62 | 1 | 63 | 0 | 63 |
| Subtotal | | 143 | 1 | 144 | 0 | 144 |

Table 6 Summary of HCV Positive Specimens' Detection at Hepatitis C Virus Test

| Genotype | | Results of Hepatitis C Virus Test | | Results of CE Marked Test | | Subtotal |
|---|---|---|---|---|---|---|
| | | Positive | Negative | Positive | Negative | |
| 1 | 1a | 4 | 0 | 4 | 0 | 51 |
| | 1b | 47 | 0 | 47 | 0 | |
| 2 | 2a | 25 | 0 | 25 | 0 | 37 |
| | 2b | 9 | 0 | 9 | 0 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 2a/2b | 3 | 0 | 3 | 0 | |
| 3 | 3a | 17 | 0 | 17 | 0 | 28 |
| | 3b | 11 | 0 | 11 | 0 | |
| 4 | 4 | 7 | 0 | 7 | 0 | 20 |
| | 4a | 7 | 0 | 7 | 0 | |
| | 4c/4d | 6 | 0 | 6 | 0 | |
| 5a | | 7 | 0 | 7 | 0 | 7 |
| 6 | 6a | 22 | 0 | 22 | 0 | 30 |
| | 6e | 8 | 0 | 8 | 0 | |
| 1b/2a | | 3 | 0 | 3 | 0 | 3 |
| Unknown genotype | | 234 | 1 | 235 | 0 | 235 |
| Subtotal | | 410 | 1 | 411 | 0 | 411 |

Total 200 specimens with known un-HCV positive from hospitalized patients in PLA 302 Hospital, PRC, were used to evaluate the diagnostic specificity among inpatients. 200 of 200 specimens were tested anti-HCV negative by both Hepatitis C Virus Test and Ortho HCV 3.0 EIA. The diagnostic specificity of Hepatitis C Virus Test in inpatients was 100% (200/200). (Table 7)

Table 7 Summary of Diagnostic Specificity of Hepatitis C Virus Test among Inpatients

| 200 inpatients | Results of Hepatitis C Virus Test | | Results of Ortho HCV 3.0 EIA | | Subtotal |
|---|---|---|---|---|---|
| | Negative | Positive | Negative | Positive | |
| True Negative | 200 | 0 | 200 | 0 | 200 |
| True Positive | 0 | 0 | 0 | 0 | 0 |
| Subtotal | 200 | 0 | 200 | 0 | 200 |

Among the 200 pregnant women, there were 10 multiparas. 199 of 200 specimens were tested anti-HCV negative by Hepatitis C Virus Test and Ortho HCV 3.0 EIA. The reactive samples were tested repeatedly by these two Tests. 2 specimens showed disagreement results between Hepatitis C Virus Test and Ortho. Result from Hepatitis C Virus Test was negative while that from Ortho was positive at sample No. 22; Result from Nova was positive while that from Ortho was negative at sample No. 181. Further Real-time PCR (COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, Roche Molecular Systems, Inc ) was carried out at these two samples. PCR showed negative results with both samples. The diagnostic specificity of Hepatitis C Virus Test in pregnant women was 99.5% (199/200). (Table 8)

Table 8 Summary of Diagnostic Specificity of Hepatitis C Virus Test among Pregnant Women

| 200 Pregnant Women | Results of Hepatitis C Virus Test | | Results of Ortho HCV 3.0 EIA | | Subtotal |
|---|---|---|---|---|---|
| | Negative | Positive | Negative | Positive | |
| True Negative | 199 | 1 | 199 | 1 | 200 |
| True Positive | 0 | 0 | 0 | 0 | 0 |
| Subtotal | 199 | 1 | 199 | 1 | 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 Hepatitis C Virus Test. The potential interference diseases status were HAV, HBV, HIV, Dengue, EBV, CMV, HTLV, Rubella and E.coli infections, CRF, ASOT, RF positive. There were at least 15 specimens for each interference status. 146 specimens from Chongqing Medical University included the interference status of HAV, HBV, HIV, EBV, RF, CMV, ASOT, CRF, Dengue, Rubella and HTLV positive. These 146 specimens had no interference with Hepatitis C Virus Test (Table 9). 90 specimens from SCCDC included the interference status of HAV, HIV Dengue, HTLV and E.coli positive. 89 of the 90 specimens showed negative results by Hepatitis C Virus Test. The one tested as false positive by Nova is Anti-E.coli positive serum No. 89(Table 10). Among the 236 interference specimens, 235 showed no interference with Hepatitis C Virus Test. The diagnostic specificity among potential interference disease status was 99.58%(235/236)(Table 11)

Table 9 Summary of Diagnostic Specificity of Hepatitis C Virus Test among Interference Disease Status(Chongqing Medical University)

| Interference Diseases | Results of Hepatitis C Virus Test | | Results of CE Marked Ortho HCV 3.0 Test | | Subtotal |
|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | |
| HAV | 0 | 12 | 0 | 12 | 12 |
| HBV | 0 | 20 | 0 | 20 | 20 |
| HIV | 0 | 15 | 0 | 15 | 15 |
| Dengue | 0 | 5 | 1 | 4 | 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 | 1 | 146 | 146 |

Table 10 Summary of Diagnostic Specificity of Hepatitis C Virus Test among interference Disease Status(SCCDC)

| Interference Diseases | Results of Hepatitis C Virus Test | | Results of CE Marked Ortho HCV 3.0 Test | | Subtotal |
|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | |
| HAV | 0 | 20 | 0 | 20 | 20 |
| HIV | 0 | 20 | 0 | 20 | 20 |
| Dengue | 0 | 16 | 1 | 15 | 16 |
| HTLV | 0 | 13 | 0 | 13 | 13 |
| E.coli | 1 | 20 | 0 | 21 | 21 |
| Subtotal | 1 | 89 | 1 | 89 | 90 |

Table 11 Summary of Diagnostic Specificity of Hepatitis C Virus Test among Interference Disease Status

| Interference Diseases | Results of Hepatitis C Virus Test | | Results of CE Marked Ortho HCV 3.0 Test | | Subtotal |
|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | |
| HAV | 0 | 32 | 0 | 32 | 32 |

| | | | | | |
|---|---|---|---|---|---|
| HBV | 0 | 20 | 0 | 20 | 20 |
| HIV | 0 | 35 | 0 | 35 | 35 |
| Dengue | 0 | 21 | 1 | 20 | 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 |
| E.Coli | 1 | 20 | 0 | 21 | 21 |
| Subtotal | 1 | 235 | 1 | 235 | 236 |

Total 1091 specimens from blood donors (619 from Blood Center of Beijing Red Cross Society and 472 from Blood Transfusion Department, the Secondary Affiliated Hospital of Chongqing Medical University) were used to evaluate the diagnostic specificity of Hepatitis C Virus Test. The 1091 specimens consisted of 83 specimens from consecutive blood donations. Among the 619 specimens from Blood Center of Beijing Red Cross Society, 616 showed negative results by Hepatitis C Virus Test, the diagnostic specificity was 99.52% (Table 12). Among the 472 specimens from Chongqing Medical University, 470 showed negative results by Hepatitis C Virus Test, the diagnostic specificity was 99.58%(Table 13). Combined these two results together, total 1091 specimens from blood donors, 1086 showed negative results by Hepatitis C Virus Test, 5 gave false positive; the diagnostic specificity among blood donors was 99.54%(Table 14)

Table 12 Summary of Diagnostic Specificity of Hepatitis C Virus Test among Blood Donors (Blood Center of Beijing Red Cross Society)

| Blood donors | Results of Hepatitis C Virus Test | | Results of Ortho HCV 3.0 EIA | | Subtotal |
|---|---|---|---|---|---|
| | Negative | Positive | Negative | Positive | |
| True Negative | 616 | 3 | 618 | 1 | 619 |
| True Positive | 0 | 0 | 0 | 0 | 0 |
| Subtotal | 616 | 3 | 618 | 1 | 619 |

Table 13 Summary of Diagnostic Specificity of Hepatitis C Virus Test among Blood Donors (Chongqing Medical University)

| Blood donors | Results of Hepatitis C Virus Test | | Results of Ortho HCV 3.0 EIA | | Subtotal |
|---|---|---|---|---|---|
| | Negative | Positive | Negative | Positive | |
| True Negative | 470 | 2 | 471 | 1 | 472 |
| True Positive | 0 | 0 | 0 | 0 | 0 |
| Subtotal | 470 | 2 | 471 | 1 | 472 |

Table 14 Summary of Diagnostic Specificity of Hepatitis C Virus Test among Blood Donors

| Blood donors | Results of Hepatitis C Virus Test | | Results of Ortho HCV 3.0 EIA | | Subtotal |
|---|---|---|---|---|---|
| | Negative | Positive | Negative | Positive | |
| True Negative | 1086 | 5 | 1089 | 2 | 1091 |
| True Positive | 0 | 0 | 0 | 0 | 0 |
| Subtotal | 1086 | 5 | 1089 | 2 | 1091 |

From the 6 diagnostic specificity studies among different populations, total 1727 negative samples including 1091 from blood donors, 200 from inpatients, 200 from pregnant women, 236 from potentially interfering diseases were selected to evaluate the specificity of Hepatitis C Virus Test and Compare the results with CE marked Ortho HCV 3.0. Of the 1727 samples, 1720 were tested negative, 7 tested positive( 5 from blood donors, 1 from potentially interfering diseases, 1 from pregnant women) by Hepatitis C Virus Test. All the results which showed inconsistent with CE marked Ortho HCV 3.0 were further confirmed negative by PCR. The diagnostic sensitivity of Hepatitis C Virus Test was 99.59% (1720/1727), false positive rate is 0.41% (Table 15).

Table 15 Summary of Diagnostic Specificity of Hepatitis C Virus Test

| | Results of Hepatitis C Virus Test | | Results of CE Marked Ortho HCV 3.0 EIATest | | Subtotal |
|---|---|---|---|---|---|
| | Negative | Positive | Negative | Positive | |
| Blood Donors | 1086 | 5 | 1089 | 2 | 1091 |
| 200 clinic al specimens | 200 | 0 | 200 | 0 | 200 |
| 200 Pregnant Women | 199 | 1 | 199 | 1 | 200 |
| 236 potentially interfering samples | 235 | 1 | 235 | 1 | 236 |
| Subtotal | 1720 | 7 | 1723 | 4 | 1727 |

Table 16 Summary of all data

| | | Hepatitis C Virus Test (Colloidal Gold) | | Total |
|---|---|---|---|---|
| | | POS | NEG | |
| HCV Samples | POS | 410 | 1 | 411 |
| | NEG | 7 | 1720 | 1727 |
| Total | | 417 | 1721 | 2138 |

Diagnostic Sensitivity:410/411×100%=99.76%

Diagnostic Specificity:1720/1727×100%=99.59%

Overall Agreement: (410+1720)/2138×100%=99.63%

## 7. Conclusion

The clinical evaluation was carried out for the clinical performance of Hepatitis C Virus Test (Colloidal Gold). Total 2138 samples were tested, of which the diagnostic sensitivity is 99.76%; diagnostic specificity is 99.59%; total compliance is 99.63%.

## 8. Report

8.1 Original raw data is archived at Quality Control Department

8.2 The original final report is archived in Quality Control Department.