**INTENDED USE**

WanTai Hepatitis E Virus ELISA is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgG-class antibodies against human hepatitis E virus (HEV) in human serum or plasma.

**SUMMARY**

Hepatitis E virus (HEV) is a non-enveloped, single-stranded RNA virus identified in 1990. Infection with HEV induces acute or sub-clinical liver diseases similar to hepatitis A. HEV infections, endemic and frequent in developing countries, are seen in also in developed countries in persons of all age groups at risk of consumption of contaminated food or water. The overall case-fatality is 0.5~3%, and much higher (15~25%) among pregnant women. A hypothesis that HEV is zoonosis was presented in 1999. Then a HEV and later an HEV variant were identified and sequenced separately in 1997 and 2001. Since then, HEV infection includes anti-HEV, viremia and feces excretion of HEV was seen in a wide variety of animals, i.e. sows, rodents, wild monkeys, deer, cows, goats, dogs and chickens in both developing and developed countries. A direct testimony was reported that the consumption of uncooked deer infected with hepatitis E virus led to acute hepatitis E in humans. And HEV genome sequences can be detected in pork livers available in the supermarkets in Japan.

**PRINCIPLE OF THE TEST**

WanTai Hepatitis E Virus ELISA kits employ polyclonal antibody to hepatitis E virus antigen to recognize the IgG class antibodies in human serum or plasma. The HEV ELISA Kit uses a two-step incubation procedure. The coated antigen-antibody complexes are formed and detected by HRP-conjugated antibodies. The test involves a Blocking, Wash, Anti-IgG, HRP, Chromogen, Color Stop sequence in this order. The color developed is directly proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells samples negative for anti-HEV IgG remain colorless.

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Pre-washed polystyrene microwell strips.
- Washing solution.
- Plate reader.
- Microplate reader.
- Reference wavelength at 650 nm.
- Wash buffer.
- Stop solution.
- Sodium hypochlorite solution.
- Distilled/deionized water.
- Microscope.
- Pipettes.
- Pipette tips.
- Laboratory gloves.
- Safety glasses.
- Specimen diluent.
- Preservative.
- Storage tank.
- Test plate.
- Centrifuge.

**SPECIMEN COLLECTION, TRANSPORTING AND STORAGE**

***Wells are washed to remove unbound serum proteins and then, rabbit anti-human IgG antibodies attached to the solid phase carrier to provide the antigen-antibody interactions (HRP)-conjugated to horseradish peroxidase (HRP-Conjugate) are added. During the secondary incubation step, these interacting antibodies and peroxidase enzyme conjugate complex are washed off the solid phase and the unbound HRP-Conjugate is then removed by washing. Chromogen solutions containing Tetramethylbenzidine (TMB) and urea solutions are then added to the wells and color develops.

**TO BE USED EXCLUSIVELY FROM QUALIFIED PROFESSIONAL**

The WanTai Hepatitis E Virus ELISA are time and temperature sensitive. To avoid incorrect result, strictly follow the test procedures and do not modify them.

1. Do not exchange reagents from different lot or use reagents from other commercially available kits.
2. The concentrations of standards and quality control samples should be within the valid range.
3. Never use the reagents that are beyond their expiration date.
4. Do not use the lot in which any one of the negative control A values does not meet the criteria.
5. Do not use the lot in which any one of the positive control A values does not meet the criteria.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before they are wasted in an appropriate way.

**PRECAUTIONS AND SAFETY**

- Avoid assay steps long time interruptions. Assure same working conditions for all wells.
- Calibrate the pipette frequently to assure the accuracy of sample/reagents dispensing. Use different disposable pipette tips for each specimen/reagent in order to avoid cross-contaminations.
- Avoid pipeting in the interior of the wells. Fingers or scratches may interfere with the reading. When reading the results, assure that the plate bottom is dry and there are no air bubbles inside the wells.
- Never mix the microplate with any other microplate or experimental performance.
- Should be free from contamination with microorganism or chemicals.

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**PROCEDURE**

1. Reagents preparation: Make sure the reagents are within the validity indicated on the kit box and of the same lot.
2. Add 50 μl of Specimen Diluent into each well of Specimen Diluent into each well except for the Blank.
3. Add 50 μl of Chromogen B solutions into each well including the Blank well.
4. Stop solution into each well and only clean vessels to dilute the buffer. All other reagents are taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the specimen should be removed by centrifugation at 3000 RPM (round per minute for 30 minutes) at room temperature.
5. Incubating: Do not eat and drink during incubating. Place the plate in 37°C for 30-60 seconds. After the washing cycle, turn the plate onto blotting paper or clean towel, and tap it to remove any remainders.
6. Stop solution into each well and only clean vessels to dilute the buffer.
7. Wash with distilled/deionized water. For enclosing the strips not in use protect the reagents from contamination with microorganism or chemicals.

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**QUALITY CONTROL AND CALCULATION OF THE RESULTS**

Each microplate should be considered separately when calculating and interpreting the results of the assay. Regardless of the number of plates concurrently processed. The results are calculated by relating each specimen to the Cut-off value and use the Cut-off value of the control (C) of the plate. If the Cut-off result is based on single filter plate reader, the results should be calculated by subtracting the Blank well A value from the print report values of the specimen in order to calculate the Cut-off value. If the Cut-off result is based on dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and controls.

**Calculation of the Cut-off value (C) = Nc × 0.18 (Nc = the mean absorbance value for three negative controls).**

**In Vitro Diagnostic Use Only**

**PRODUCT INFORMATION**

**Conflict of Interest**

We hereby declare that we have no conflict of interest in publishing this product.

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**WanTai Hepatitis E Virus ELISA**

**Diagnostic Kit for IgG Antibodies to Hepatitis E Virus**

**CODE | VOLUME | UNIT | STORAGE AND STABILITY**

- **CHROM | SOL | A**
- **CHROM | SOL | B**
- **CHROM | SOL | C**
- **CHROM | SOL | D**
- **CHROM | SOL | E**
- **CHROM | SOL | F**
- **CHROM | SOL | G**
- **CHROM | SOL | H**
- **CHROM | SOL | I**
- **CHROM | SOL | J**

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**INSTRUCTIONS FOR WASHING**

1. A good washing procedure is essential in order to obtain correct and precise analytical data.
2. It is therefore, recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 300-well washing cycle, turn the plate onto blotting paper or clean towel, and tap it to remove any remainders.

**REAGENTS AND CONTROL**

**POSITIVE CONTROL:**

Red-colored liquid in a white vial with red screw cap.

**NEGATIVE CONTROL:**

Blue-colored liquid filled in a vial with green screw cap.

**WASH SOLUTION:**

Blue-colored liquid in a white vial with dark blue screw cap.

**STOP SOLUTION:**

Colorless liquid in a white vial with grey cap.

**CHROMOGEN B SOLUTION A:**

Colorless liquid filled in a white vial with green screw cap.

**CHROMOGEN B SOLUTION B:**

Colorless liquid filled in a white vial with brown screw cap.

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**Contradiction of Instability**

Values of the Positive or Negative control should be calculated again using the remaining two values. If more than one Negative control A values do not meet the criteria, the lot is not validated.

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**Note:** Use different disposal pipette tips for each specimen/reagent in order to avoid cross-contaminations.

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**Calculation of the Cut-off value (C) = Nc × 0.18 (Nc = the mean absorbance value for three negative controls).**

**In Case of Using a Dual Filter Plate Reader,** the results should be calculated by subtracting the Blank well A value from the print report values of the specimen in order to calculate the Cut-off value.
INTERPRETATIONS OF THE RESULTS

Negative Results (A / C.O. ˂1). Samples giving A value less than the Cut-off value are negative for this assay, which indicates that no IgG to HEV have been detected with WANTAI HEV-IgG ELISA kit, therefore there are no pathological indications for infection to HEV.

Positive Results (A / C.O. ˃1). Samples giving A value which is equal to, or greater than the Cut-off value are considered initially reactive, which indicates that IgG to HEV have probably been detected using the WANTAI ELISA kit. Reretesting in duplicates of any initially reactive sample is recommended. Reretest reactive samples could be considered positive for IgG to HEV and therefore the patient is probably infected with HEV.

Limitations

- If, after reretesting of the initially reactive samples, both wells are negative results (A/C.O. ˂1), these samples should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, non-repeatable false positive results may occur due to several reasons, mostly of which are related but not limited to inadequate washing step. For more information regarding WANTAI ELISA Troubleshooting, please refer to WANTAI’s “ELISAs and Troubleshooting Guide”.

- If after reretesting, one or both wells are positive results, the final result from this ELISA test should be recorded as reactive result. Reretests, non-reactive specimens could be considered positive for IgG antibodies to HEV.

- After reretesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.

Follow-up confirmation and supplementary testing of any positive specimen with other analytical system is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

PERFORMANCE CHARACTERISTICS

1. Detection of HEV antibodies in samples from patients with 10 years of HEV post-infection history:

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Samples</th>
<th>Pos</th>
<th>Cut-off</th>
<th>Positive samples OD</th>
<th>Ave. pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT IgG*</td>
<td>50</td>
<td>68</td>
<td>0.148</td>
<td>0.532</td>
<td>1.368</td>
</tr>
<tr>
<td>EIA 1**</td>
<td>50</td>
<td>38</td>
<td>0.152</td>
<td>0.514</td>
<td>1.018</td>
</tr>
<tr>
<td>EIA 2**</td>
<td>50</td>
<td>30</td>
<td>0.228</td>
<td>0.229</td>
<td>0.457</td>
</tr>
</tbody>
</table>

*Beijing Wantai BP HEV IgG ELISA (WT IgG)

**Commercially available HEV IgG ELISA tests.

2. Detection of serial serum samples from acute HEV phase

Since no golden standard for hepatitis E is available, the reference HEV IgG assay served as control reagent. Parallel comparison testing was performed with acute hepatitis E samples (Testing Center 1). If the true positive status was defined as positive for any of the two tests used in this study, the sensitivity of WANTAI HEV-IgG and the reference HEV IgG was 97.96% and 91.36% respectively.

In the evaluation of 120 serial sera samples obtained from 30 hepatitis E patients (Testing Center 2), the sensitivity of WANTAI HEV-IgG were 100%, while the sensitivity of the reference HEV IgG were 93.33%. The sensitivity of WANTAI HEV IgG was 99.06% in the parallel testing of a total of 218 serum samples of acute hepatitis E, which was significantly higher than the reference HEV IgG tests (92.6%).

3. Testing 10587 blood samples from blood donors, the results showed in the Fig.1 from the frequency distribution map, it existed two peaks of WT-IgG, the first peak was higher, of which the center was near the OD value 0.0126, representing the people who did not infect HEV. The OD logarithm of the first peak was similar to the log-normal distribution. Considering the first peak as the center, calculate the standard deviation of the data on the left. The corresponding OD values in accordance with 99.5% and 99.9% of the right respectively were 0.089 and 0.120 and the probability value at the cutoff value 0.185 was 99.99%. The second peak concentrated near the OD value 0.32, representing the HEV infection population, and its logarithm behaved as negative skewed distribution. As a result, the specificity of WT-IgG was much higher, with false positive rate at 0.01%.

LIMITATIONS

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of ELISA methods. The test is designed to achieve very high performance characteristics of high sensitivity and specificity.

2. Any positive results must be interpreted in conjunction with patient clinical information and other laboratory testing results.

3. If, after reretesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, non-repeatable false positive results may occur due to the several reasons, mostly of which are related but not limited to inadequate washing step. For more information regarding WANTAI ELISA Troubleshooting, please refer to WANTAI’s “ELISAs and Troubleshooting Guide” or contact Beijing Wantai technical support for further assistance.

4. Common sources for mistakes: klo beyond the equity date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, false to add samples or reagents, equipment, timing, volumes, sample nature and quality.

5. The prevalence of the marker will affect the assay’s predictive values.

This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

This is a qualitative assay and the results cannot be use to measure antibodies concentrations.

REFERENCES


SUMMARY OF THE MAJOR COMPONENTS OF THE KIT:

Use this summary only as a reference and always follow the comprehensive method sheet when performing the assay. Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

SUMMARY OF THE ASSAY PROCEDURE:

Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

SUMMARY OF THE DETERMINATION OF THE KIT:

Add Sample Dilution 1.0 ml Positive Control Code 3, and Incubate 15min

Add Substrate B Code 9, and Incubate 15min

Stop the reaction 50 μl stop solution

Read the absorbance

Log2 IgG+3

Reproducibility

Within Run

Between Run

Sample | No | Mean SD/ CO | CV% | Mean SD/ CO | CV% |

Weak positive | 10 | 4.69 | 9.1% | 4.58 | 9.5% |

Moderate positive | 10 | 11.2 | 7.3% | 10.49 | 7.5% |

Strong positive | 10 | 16.42 | 4.2% | 16.07 | 4.4% |

Strong positive 2 | 10 | 13.31 | 3.9% | 13.12 | 4.0% |

CE MARKING SYMBOLS:

In Vivo diagnostic medical device

+2°C +8°C Storage conditions

Batch

EU Authorized Representative

Content sufficient for non-tax tests

Catalog Number

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Fr e quen  c y

0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40

0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

0.00 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00