

Wantai Hepatitis E Virus Diagnostics

WANTAI HEV-Ag ELISA ^{Plus}

Diagnostic Kit for Hepatitis E Virus Antigen (ELISA)



WE-7596



V. 2016-01 [Eng.]



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Read the package insert carefully and completely before performing the assay. Follow the instructions and do not modify them. Only by strict adherence to these instructions, the erroneous results can be avoided and the optimal performance of WANTAI HEV-Ag ELISA ^{Plus} achieved.

INTENDED USE

WANTAI HEV-Ag ELISA ^{Plus} is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of hepatitis E virus antigen in human serum or plasma specimens. It is intended for use in clinical laboratories for diagnosis and management of patients related to infection with hepatitis E virus.

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 frequently epidemic in developing countries, is seen also in developed countries in a sporadic form with or without a history of traveling to endemic area. The overall case-fatality is 0.5–3%, and much higher (15–25%) among pregnant women. A hypothesis that HEV infection is a zoonosis was presented in 1995. Then a swine HEV and later an avian HEV were identified and sequenced separately in 1997 and 2001. Since then, HEV infection include anti-HEV, viremia and feces excretion of HEV was seen in a wide variety of animals, i.e., swine, rodents, wild monkeys, deer, cow, goats, dogs and chicken in both the developing and developed countries. A direct testimony was reported that the consumption of uncooked deer meat infected with HEV led to acute hepatitis E in human. And HEV genome sequences can be detected in pork livers available in the supermarkets in Japan.

PRINCIPLE OF THE TEST

This kit is a two-step incubation, solid-phase antibody "sandwich" ELISA assay in which polystyrene microwell strips are pre-coated with anti-HEV antibodies directed against the viral antigen. Patient's serum, or plasma specimen is added into the microwells. In case of presence of HEV Ag in the specimen, the pre-coated antibodies will bind to the viral antigen and during the first incubation step, the specific immunocomplex formed is captured on the solid phase. After the incubation, second anti-HEV antibody conjugated to the enzyme Horseradish Peroxidase (HRP) is added into the wells then the plate is incubated for second time. During the second incubation, this HRP-conjugated antibody will bind to the anti-HEV-HEV Ag complexes immobilized onto the wells during the first incubation step. The unbound HRP conjugate is removed during washing and Chromogen solutions containing Tetramethyl benzidine (TMB) and urea peroxide are then added into the wells. In presence of the antibody-antigen-antibody (HRP) "sandwich" immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP-conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antigen captured in the wells, and to its amount in the specimen respectively. Wells containing specimens negative for HEV Ag remain colorless.

IVD In Vitro Diagnostic Use Only

This kit contains reagents sufficient for testing of maximum of 91 specimens in a test run.

UUI PLATE

Code 5 (1x12wells)
8x12/12x8-well per plate

CONTROL -

Code 8 (1x1ml per vial)
preserv.0.1% ProClin™ 300

CONTROL +

Code 7 (1x1ml per vial)
preserv.0.1% ProClin™ 300

HRP CON

Code 6 (1x12ml per vial)
preserv.0.1% ProClin™ 300

DIL SPE

Code 9 (1x5ml per vial)
preserv.0.1% ProClin™ 300

WASH BUF 20X

Code 1 (1x50ml per bottle)
DILUTE BEFORE USE!
detergent Tween-20

CHROMI SOL A

Code 2 (1x6ml per vial)

CHROMI SOL B

Code 3 (1x6ml per vial)

STOP SOL

Code 4 (1x6ml per vial)

STOP SOLUTION:

Colorless liquid in a white vial with yellow screw cap.
Diluted sulfuric acid solution (0.5M H₂SO₄).
Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.

- PLASTIC SEALABLE BAG: For enclosing the strips not in use 1 unit
- PACKAGE INSERT 1 copy
- CARDBOARD PLATE COVER 2 sheets

To cover the plates during incubation and prevent evaporation or contamination of the wells.

MATERIALS REQUIRED BUT NOT PROVIDED

Freshly distilled or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing system and/or pipette, disposable pipette tips, absorbent tissue or clean towel, dry incubator or water bath, 37±1°C, plate reader, single wavelength 450nm or dual wavelength 450/600–650nm, microwell aspiration/wash system.

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

1. **Serum / Plasma Specimen:** No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be 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 minutes) for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, but **highly lipaemic, icteric, or hemolytic specimens should not be used** as they can give false results in the assay. **Do not heat inactivate specimens.** This can cause deterioration of the target analyte. Specimens with visible microbial contamination should never be used.
3. This kit is intended ONLY for testing of individual serum or plasma specimens. Do not use the assay for testing of cadaver specimens, saliva, urine or other body fluids, or pooled (mixed) blood.
4. **Transportation and Storage:** Store specimens at 2-8°C. Specimens not required for assaying within 1 week should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, specimens should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical specimens and ethological agents.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, do not freeze. To assure maximum performance of this kit, during storage, protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

TO BE USED ONLY BY QUALIFIED PROFESSIONALS

The ELISA assays are time and temperature sensitive. To avoid incorrect result, **strictly follow the test procedure steps and do not modify them.**

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.
4. Use only sufficient volume of specimen as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.
5. Do not touch the exterior bottom of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
7. Avoid long time interruptions of assay steps. Assure same working conditions for all wells.
8. Calibrate the pipette frequently to assure the accuracy of specimens/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.
9. Assure that the incubation temperature is 37°C inside the incubator.
10. When adding specimens, do not touch the well's bottom with the pipette tip.
11. When measuring with a plate reader, determine the absorbance at 450nm or at 450/600–650nm.
12. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.
13. If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.
14. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
15. **WARNING:** Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for HBsAg and antibodies to HIV 1/2, HCV, TP. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
16. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
17. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.
19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the

20. Chromogens, and the Wash buffer.
The Stop solution 0.5M H₂SO₄ is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.
21. ProClin™ 300 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

INDICATIONS OF INSTABILITY DETERIORATION OF THE REAGENT: Values of the Positive or Negative controls, which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the specimens must be retested. In case of constant erroneous results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one contact Wantai technical support for further assistance.



Warning:
H317, P280, P333+P313, P363
ProClin™ 300



Danger:
H360D, P201, P280, P308+P313
N,N- dimethylformamide

PROCEDURE

Reagents preparation: Allow the reagents to reach room temperature (18-30°C). Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and only clean vessels to dilute the buffer. All other reagents are **READY TO USE AS SUPPLIED.**

- Step 1 Preparation:** Mark three wells as Negative control (e.g. B1, C1, D1), two wells as Positive control (e.g. E1, F1) and one Blank (e.g. A1, neither specimens nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.
- Step 2 Adding Diluent:** Add 20µl of Specimen Diluent into each well except the Blank.
- Step 3 Adding Specimen:** Add 50µl of Positive control, Negative control, and Specimen into their respective wells except the Blank. **Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination.** Mix by tapping the plate gently.
- Step 4 Incubating:** Cover the plate with the plate cover and incubate at 37°C for 60 minutes.
- Step 5 Adding HRP-Conjugate:** At the end of the incubation, remove and discard the plate cover. Add 100µl of HRP-Conjugate into each well except the Blank.
- Step 6 Incubating:** Cover the plate with the plate cover and incubate at 37°C for 30 minutes.
- Step 7 Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
- Step 8 Coloring:** Add 50µl of Chromogen Solution A and then 50µl of Chromogen Solution B into each well including the Blank, mix gently. Incubate the plate at 37°C for 15 minutes avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and HEV antigen positive specimen wells.
- Step 9 Stopping Reaction:** Using a multichannel pipette or manually, add 50µl of Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and HEV antigen positive specimen wells.
- Step 10 Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 600–650nm. Calculate the Cut-off value and evaluate the results. **(NOTE: read the absorbance within 10 minutes after stopping the reaction).**

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 5 automatic washing cycles of 350-400µl/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with specimen or HRP-conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to carry out 5 washing cycles, dispensing 350-400µl/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
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.
7. The concentrated Wash buffer should be diluted 1 to 20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

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 absorbance (A) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading 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 specimens and controls. In case the reading 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.O.) = Nc + 0.16
(Nc = the mean absorbance value for three negative controls).

Quality control (assay validation): The test results are valid if the Quality Control criteria are fulfilled. It is

recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient specimen being analyzed.

- The A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450nm.
- The A values of the Positive control must be ≥ 0.800 at 450/600-650nm or at 450nm after blanking.
- The A values of the Negative control must be ≤ 0.100 at 450/600-650nm or at 450nm after blanking.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded, and the mean value should be calculated by using the remaining two values. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Example:

1. Quality Control

Blank well A value: A1= 0.025 at 450nm (Note: blanking is required only when reading with single filter at 450nm)

Well No.: B1 C1 D1
 Negative control A values after blanking: 0.012 0.010 0.011

Well No.: E1 F1
 Positive control A values after blanking: 2.363 2.436

All control values are within the stated quality control range

2. Calculation of Nc: = $(0.012+0.010+0.011) = 0.011$

3. Calculation of the Cut-off: (C.O.) = $0.011 + 0.16 = 0.171$

INTERPRETATIONS OF THE RESULTS

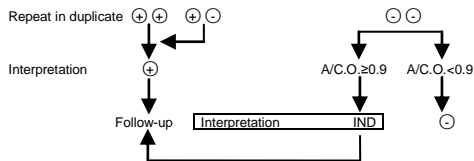
Negative Results (A / C.O. < 1): Specimens giving A value less than the Cut-off value are negative for this assay, which indicates that no HEV antigen have been detected with this kit, therefore there are no serological indications for infection with HEV.

Positive Results (A / C.O. ≥ 1): Specimens giving A value equal to or greater than the Cut-off value are considered initially reactive, which indicates that HEV antigen have probably been detected with this kit. Retesting in duplicate of any initially reactive specimen is recommended. Repeatedly reactive specimens could be considered positive for HEV antigen and therefore there are serological indications of current infection with HEV.

Borderline (A / C.O. = 0.9-1.1): Specimens with A value to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicate is required to confirm the initial results.

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

INITIAL RESULTS INTERPRETATION AND FOLLOW-UP ALL INITIALLY REACTIVE OR BORDERLINE SPECIMENS



IND = non interpretable

- If, after retesting of the initially reactive specimens, both wells are negative results (A/C.O.<0.9), these specimens should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, 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 retesting in duplicate, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for HEV antigen and therefore the patient is probably infected with HEV.
- After retesting in duplicate, specimens with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone specimen, or uninterpretable for the time of testing.

PERFORMANCE CHARACTERISTICS

Specificity: The specificity of this kit was determined by testing of 1494 clinical specimens including specimens from patients infected with hepatitis A, hepatitis B, hepatitis C and Syphilis. Specimens from chronic hepatitis patients with unknown nature of infection (AH) were also tested. The specificity was over 99.93%.

Testing Centers	Specimens	Positive(%)	Negative(%)	Total
Center#1	HAV	0(0)	36(100)	36
	HBV	0(0)	40(100)	40
	HCV	0(0)	40(100)	40
	AH	0(0)	99(100)	99
	Total	0	215	215
Center#2	HBV	0(0)	250(100)	250
	AH	1(0.2)	400(99.8)	401
Center#3	Total	1	650	651
	ALT (elevated)	0(0)	224(100)	224
	TP	0(0)	79(100)	79
	HBV/HCV	0(0)	185(100)	185
	Total	0	488	488
	HAV	0(0)	3(100)	3

Center#4	HBV	0(0)	87(100)	87
	unknown hepatitis	0(0)	50(100)	50
	Total	0	140	140
	Total	1(0.07)	1493(99.93)	1494

No interference was observed from rheumatoid factors up to 2000U/ml.

The assay performance characteristics are unaffected from elevated concentrations of bilirubin (up to 1.71mmol/L), hemoglobin (up to 400mg/L), and triglyceride (up to 170mmol/L).

Frozen positive/negative specimens have been tested to check for interferences due to collection and storage. The performance characteristics of this kit were not affected.

Panels of specimens with elevated levels of anti-E.coli antibodies, specimens from pregnant women and individuals with auto-immune diseases were tested. The performance characteristics of this kit were not affected.

Sensitivity: 251 specimens collected from three testing centers were tested with this kit and the results confirmed by HEV PCR. The positive agreement between this kit and PCR was 66.7% (30/45) and the negative agreement was 95.1% (196/206).

Testing Centers	WANTAI	HEV RNA			
		+	-	Total	
Center#1		+	-	Total	
		+	19	4	23
		-	8	162	170
		Total	27	166	193
Center#2		+	-	Total	
		+	11	6	17
		-	7	19	26
		Total	18	25	43
Center#3		+	-	Total	
		+	0	0	0
		-	0	15	15
		Total	0	15	15
Total		+	-	Total	
		+	30	10	40
		-	15	196	211
	Total	45	206	251	

LIMITATIONS

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information. The assay is designed to achieve very high performance characteristics of sensitivity and specificity and the "sandwich model" minimizes the unspecific reactions due to interference with unknown matters in specimen (false positive results).
2. Antigen may be undetectable during the early stage of the disease. Therefore, negative results obtained with this kit are only indication that the specimen does not contain detectable level of HEV antigen and any negative result should not be considered as conclusive evidence that the individual is not infected with HEV.
3. If, after retesting of the initially reactive specimens, the assay results are negative, these specimens should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most 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 Wantai technical support for further assistance.
4. The most common assay mistakes are: using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
5. The prevalence of the marker will affect the assay's predictive values.
6. This kit is intended ONLY for testing of individual serum or plasma specimens. Do not use it for testing of cadaver specimens, saliva, urine or other body fluids, or pooled (mixed) blood.
7. This kit is a qualitative assay and the results cannot be used to measure antigen concentration.

REFERENCES

1. Reyes GR, Purdy MA, Kim JP, et al. Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis. Science 1990; 247: 1335-1339
2. Clayson E, Innis B, Myint K, et al. Detection of hepatitis E virus infections among domestic swine in the Kathmandu Valley of Nepal. Am J Trop Med Hyg, 1995;53:228-232
3. Meng XJ, Purcell RH, Halbur PG, et al. A novel virus in swine is closely related to the human hepatitis E virus. Proc Natl Acad Sci USA, 1997, 94: 9860-9865
4. Tei S, Kitajima N, Takahashi K, et al. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 2003; 362(9381):371
5. Zheng YJ, Zhang J, Xia NS. A debate about that hepatitis E is a zoonosis. Chinese J Zoonosis (in press)
6. Zhang J, et. Two Hepatitis E Virus Neutralization Sites analyzed by monoclonal antibodies raised against a recombinant peptide of virus capsid protein. J Virol 2003
7. Zhang J, et. Evaluation of antibody based and nucleic acid based assays for diagnosis of hepatitis E virus infection in a rhesus monkey model. J Med Virol 2003;71(4):518-26

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. Note: the components of individual kits are not lot- interchangeable.

1. Microwell plate	Code 5	one
2. Negative Control	Code 8	1x 1ml
3. Positive Control	Code 7	1x 1ml
4. HRP-Conjugate	Code 6	1x 12ml
5. Specimen Diluent	Code 9	1x 5ml
6. Wash Buffer	Code 1	1x 50ml
7. Chromogen Solution A	Code 2	1x 6ml
8. Chromogen Solution B	Code 3	1x 6ml
9. Stop Solution	Code 4	1x 6ml

SUMMARY OF THE ASSAY PROCEDURE:

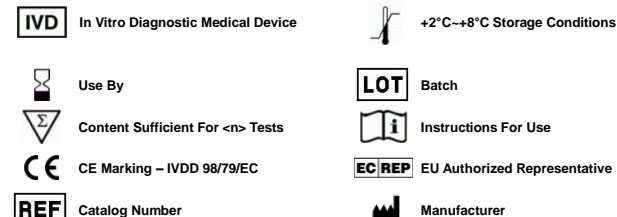
Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

Add Specimen Diluent	20µl
Add Specimen	50µl
Incubate	60 minutes
Add HPR-Conjugate	100µl
Incubate	30 minutes
Wash	5 times
Coloring	50µl A + 50µl B
Incubate	15 minutes
Stop the reaction	50µl stop solution
Read the absorbance	450nm or 450/600-650nm

EXAMPLE SCHEME OF CONTROLS / SPECIMENS DISPENSING:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	S3										
B	Neg.	...										
C	Neg.	...										
D	Neg.											
E	Pos.											
F	Pos.											
G	S1											
H	S2											

CE MARKING SYMBOLS:



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