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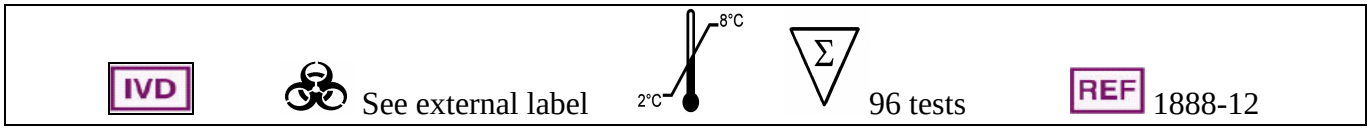


AccuDiag™ HEV-IgG ELISA

Hepatitis E Virus

Cat # 1888-12

Test	HEV-IgG ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Indirect ELISA: Antigen coated Plate
Detection Range	Qualitative Positive; Negative control & Cut off
Sample	10ul Serum
Specificity	85.4%
Sensitivity	93.8%
Total Time	~ 75 min
Shelf Life	12 -18 Months from the manufacturing date

***** *Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

INTENDED USE

The purpose of the HEV IgG ELISA Test is for use as an aid in supplementary diagnosis up to acute hepatitis E infection, and in research studies regarding its recurring infection rate in patients. The HEV IgG ELISA test is an enzyme-linked immunosorbent assay for the qualitative identification of IgG-class antibodies to hepatitis E virus in human serum/plasma.

SUMMARY

Hepatitis E is a recurring infection in developing countries. In less frequent, isolated cases, it has also been reported in developed countries. Identified in 1990, Hepatitis E (HEV) is a non-enveloped, single-stranded RNA virus where infection can lead to acute (or subclinical) liver disease, like that associated to hepatitis A infection. Fatality rates are generally low - averaging 0.5-3 percent for most patients, while pregnant women hold elevated averages around 15-25 percent. In 1995 a theory was introduced that HEV was transmitted to humans from animals (zoonosis). Then, in 1997 and 2001, respectively, HEV was identified as transmitted from swine and from birds. After that time, HEV infection transmission has been associated with several animals, for example, wild monkeys, deer, cow, goats, rodents, dogs, and chickens. Found in both developing and developed countries, these HEV infections (including anti-HEV) consisted of viremia and feces excretion of HEV-affected animals, such as those listed above. A direct link to acute hepatitis E in humans from eating uncooked deer meat, was also reported. Even in supermarkets in Japan, HEV genome sequences have been identified in pork livers. Serology has been enhanced in relation to the discovery of conformational epitopes in HEV. Diagnosis, epidemiology, zoonosis-related studies, and development of a vaccine have all been strengthened by the occurrence of long-lasting and protective HEV antibodies.

PRINCIPLE OF THE ASSAY

The principle of the HEV IgG ELISA is a solid phase, indirect ELISA system for identification of IgG-class antibodies to HEV (anti-HEV). This is accomplished in a two-step incubation formula. Recombinant, highly immunoreactive antigens related to the structural regions of HEV (ORF-2) are pre-coated on the polystyrene microwell strips. Anti-HEV specific antibodies (if present) will be bound to the solid phase pre-coated HEV antigens during the first incubation stage. Next, it is important to wash the wells so that any unbound serum proteins can be removed. Added after this are rabbit anti-human IgG antibodies (anti-IgG) conjugated to horseradish peroxidase (HRP-Conjugate). These HRP-conjugated antibodies will be bound to any antigen-antibody (IgG) complexes previously formed. This occurs during the second incubation phase. Also at this time, the unbound HRP-conjugate is removed by washing. Added to the wells are the chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide. This is done in the presence of the antigen-antibody-anti-IgG(HRP) immunocomplex. A blue-colored product appears when the colorless chromogens are hydrolyzed by the bound HRP conjugate. After stopping the reaction with sulfuric acid, the blue color turns yellow. The color intensity can be gauged proportionally to the amount of antibody captured in the wells, and to the sample, respectively. Colorless wells appear when samples are negative for HEV-IgG.

Assay principle scheme: Indirect ELISA

Ag(p)+Ab(s)→[Ag(p)–Ab(s)+ENZ]→[Ag(p)–Ab(s)–ENZ]→blue→yellow （+）

Ag(p)+ → [Ag(p) +ENZ]→[Ag(p)]→no color （-）

Incubation1	Incubation 2	Immobilized Complex	Coloring	Results
30min.	30min.		15min	

Ag(p)–pre-coated HEV antigens(ORF-2);

Ab(s)–HEV antibodies in sample(IgG);

ENZ–HRP conjugated rabbit anti-human IgG;

COMPONENTS

96 Tests

- **MICROWELL PLATE** 1plate
  Blank microwell strips fixed on white strip holder.
  The plate is sealed in aluminium pouch with desiccant.
  8×12/12×8-well strips wells per plate.
  Each well contains recombinant HEV antigens.
  The microwell strips can be broken to be used separately.
  Place unused wells or strips in the plastic sealable storage bag together with desiccant and return to 2~8 ℃
- **NEGATIVE CONTROL** 1vial
  Blue-colored liquid filled in a vial with green screw cap.
  0.5ml per vial.
  Protein-stabilized buffer tested non-reactive for anti-HEV.
  Preservatives: 0.1% ProClin 300.
  Ready to use as supplied.
  Once open, stable for one month at 2-8 ℃
- **POSITIVE CONTROL** 1vial
  Red-colored liquid filled in a vial with red screw cap.
  0.5ml per vial.
  anti-HEV IgG antibodies diluted in protein-stabilized buffer
  Preservatives: 0.1% ProClin 300.
  Ready to use as supplied.
  Once open, stable for one month at 2-8 ℃
- **SPECIMEN DILUENT** 1vial
  Green-colored liquid filled in a white vial with blue screw cap.
  12ml per vial.
  Protein-stabilized buffer, casein, and sucrose solution.
  Ready to use as supplied.
  Once open, stable for one month at 2-8°C
- **HRP-CONJUGATE REAGENT** 1vial
  Red-colored liquid filled in a white vial with red/orange screw cap.
  12ml per vial.
  Horseradish peroxidase-conjugated rabbit anti-human IgG antibodies.
  Ready to use as supplied.
  Once open, stable for one month at 2-8°C
- **STOCK WASH BUFFER** 1bottle
  Colorless liquid filled in a clear bottle with white screw cap.
  50ml per bottle.
  PH 7.4 20 × PBS. (Contains Tween-20 as a detergent)
  DILUTE BEFORE USE -The concentration must be diluted 1 to 20 with distilled/deionized water before use.
  Once diluted, stable for one weeks at room temperature or for two weeks at 2-8^{o}C.
- **CHROMOGEN SOLUTION A** 1vial
  Colorless liquid filled in a white vial with green screw cap.
  7ml per b vial.
  Urea peroxide solution.
  Ready to use as supplied.

Once open, stable for one month at 2-8℃

- **CHROMOGEN SOLUTION B** 1vial
  Colorless liquid filled in a brown vial with brown/black screw cap.
  TMB solution (Tetramethyl benzidine dissolved in citric acid).
  7ml per vial.
  Ready to use as supplied.
  Once open, stable for one month at 2-8℃
- **STOP SOLUTION** 1vial
  Colorless liquid filled in a white vial with white/yellow screw cap.
  7ml per vial.
  Diluted sulfuric acid solution (2.0M H_2SO_4).
  Ready to use as supplied.
- **PLASTIC SEALABLE BAG** 1unit
  For enclosing the strips not in use.
- **CARDBOARD PLATE COVER** 2sheets
  To cover the plates during incubation and prevent evaporation or contamination of the wells.
- **PACKAGE INSERTS** 1copy

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED BUT NOT PROVIDED

- Freshly distilled or deionized water.
- Disposable gloves and timer.
- Appropriate waste containers for potentially contaminated materials.
- Disposable V-shaped troughs.
- Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
- Absorbent tissue or clean towel.
- Dry incubator or water bath, 37±0.5 ℃
- Microshaker for dissolving and mixing conjugate with samples.
- Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
- Microwell aspiration/wash system.

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

1. **Sample Collection:** Either fresh serum or plasma samples can be used for 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 hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.
2. **Transportation and Storage:** Store samples at 2-8 ℃. Samples not required for assaying within 3 days should be stored frozen (-20 ℃ or lower).Mult iple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

SPECIAL INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential 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 sample or HRP-conjugate, after incubation do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. 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 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 liquids are wasted in an appropriate way.
7. The concentrated Washing solution should be diluted **1 to 20** before use. For one plate, mix 50ml of the concentrate with 950ml of water for a final volume of 1000ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

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 ℃, **do not freeze**. To assure maximum performance of this HEV-IgG ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

This kit is intended **FOR IN VITRO USE ONLY** IVD

FOR PROFESSIONAL USE ONLY

The ELISA assay is a time and temperature sensitive method. 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 as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature (18-30℃) before use.
4. Shake reagent gently before, and return to 2-8℃ immediately after use.
5. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
6. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
7. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
8. 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.
9. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
10. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37℃ inside the incubator.

12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from animal origin should be considered as potentially infectious.
15. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121℃ or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (2M H_2SO_4) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.
20. Materials Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system, 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.

ASSAY PROCEDURE

Step1 **Reagents preparation:** Allow the reagents and samples to reach room temperature **(18-30°C)** for at least 15-30minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37℃ until crystals dissolve. Dilute the stock Wash Buffer **1 to 20** with distilled or deionized water. Use only clean vessels to dilute the Wash Buffer.

Step2 **Numbering Wells:** Set the strips needed in strip-holder and number sufficient number of wells including three Negative control **(e.g. B1, C1, D1)**, two Positive control **(e.g. E1, F1)** and one Blank (**e.g. A1**, neither samples 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.

Step3 **Adding Diluent:** Add **100µl** Specimen Diluent into each well.

Step4 **Adding Sample:** Add **10µl** of Positive control, Negative control, and Specimen into their respective wells. **Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination**. Mix by tapping the plate gently.

Step5 **Incubating(1):** Cover the plate with the plate cover and incubate for **30minutes at 37°C**. It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

Step6 **Washing (1):** At the end of the incubation, remove and discard the plate cover. Wash each well **5times** with diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the plate onto blotting paper or clean towel, and tap it to remove any remainders.

Step7 **Adding HRP-Conjugate:** Add **100µl** HRP-Conjugate to each well except the Blank.

Step8 **Incubating(2):** Cover the plate with the plate cover and incubate the plate for **30minutes at 37°C**

Step9 **Washing(2):** After the end of the incubation, remove and discard the plate cover. Wash each well 5times with diluted Wash buffer as in **Step6**.

Step10 Coloring: Dispense **50µl** of Chromogen A and **50µl** Chromogen B solution into each well

including the **Blank** and mix by tapping the plate gently. Incubate the plate at **37℃ for 10minutes avoiding light**. The enzymatic reaction between the Chromogen A/B solutions produces blue color in Positive control and anti-HEV/IgG positive sample wells.

Step11 Stopping Reaction: Using a multichannel pipette or manually, add **50µl** Stop Solution into each well and mix gently by tapping the plate. Intensive yellow color develops in Positive control and anti-HEV/IgG positive sample wells.

Step12 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 **630nm**. Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within 5minutes after stopping the reaction)

INTERPRETATION OF RESULTS AND QUALITY CONTROL

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample's optical density (OD) 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 OD value from the print report values of samples and controls. In case the reading is based on Dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

1. **Calculation of <u>Cut-off value (C.O.) = *Nc + 0.16</u>**

***Nc** = the mean absorbance value for three negative controls.

Important: If the mean OD value of the negative control is lower than 0.03, take it as 0.03. If higher than 0.03 see the Quality Control Range.

Example:
1. Calculation of Nc:

Well No	B1	C1	D1
Negative controls OD value	0.02	0.012	0.016

Nc=0.016 (Nc is lower than 0.03 so take it as 0.03)
2. Calculation of Cut-off (C.O.) = 0.03 + 0.16 = 0.190

If one of the Negative control values does not meet the Quality control range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

2. Quality control range:

The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
2. The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.
3. The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.

3. Interpretations of the results:

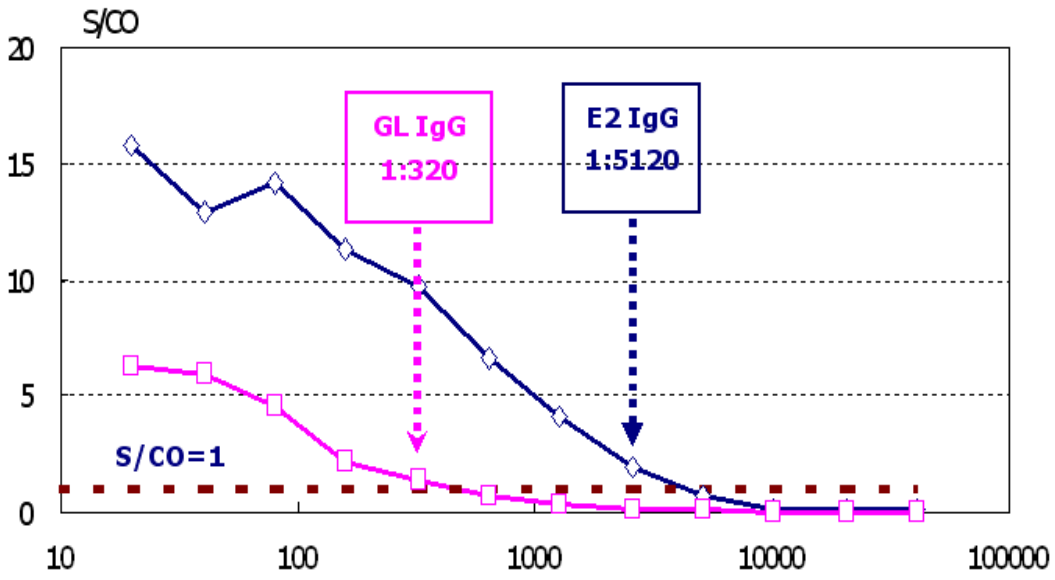
(S = the individual absorbance (OD) of each specimen)

Negative Results (S/C.O. < 1) : samples giving absorbance less than Cut-off value are negative for this assay, which indicates that no IgG-class antibodies to hepatitis E virus have been detected with this anti-HEV/IgG ELISA kit, therefore there are no serological indications of current or past infection with hepatitis E virus.
Positive Results (S/C.O. ≥1): samples giving an absorbance greater than or equal to the Cut-off value are considered initially reactive, which indicates that IgG-class antibodies to hepatitis E virus have probably been detected using this ELISA kit. Retesting in duplicates of any reactive sample is recommended. Repeatedly reactive samples could be considered positive for IgG-class antibodies to HEV and therefore there are serological indications of current or past infection with hepatitis E virus.
Borderline (S/C.O. =0.9-1.1): Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these samples in duplicates is recommended to confirm the results. Repeatedly positive samples could be considered positive for HEV.

TEST PERFORMANCE ANC EXPECTED RESULTS

Experiments data from laboratory testing: C.O.=0.19. The Absorbance (OD) Values are given below:

Negative sample			Positive sample		
1	2	3	Control	Weak	Strong
0.030	0.008	0.042	1.934	2.410	2.562



Analytical sensitivity on WHO HEV antibody standard: HEV IgG ELISA (E2 IgG – lowest detection limit 1:5120) and a reference HEV IgG ELISA (Gl IgG – lowest detection limit 1:320)

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

Reagents	Samples	Pos. rate%	Cut-off	Positive samples OD			Avr. pos S/CO
				lowest	avr.	highest	
HEV IgG	50	86	0.148	0.532	1.368	2.327	9.24
EIA 1	50	36	0.512	0.514	1.018	2.415	1.98
EIA 2	50	30	0.228	0.229	0.457	1.094	2.08

Reproducibility		Within Run		Between Run	
Sample	No	MeanS/CO	CV%	Mean S/CO	CV%
Weak positive	10	4.69	9.1%	4.58	9.5%
Moderate positive	10	11.21	7.0%	10.49	7.5%
Strong positive 1	10	16.42	4.2%	16.07	4.4%
Strong positive 2	10	13.31	3.8%	13.12	4.0%

LIMITATIONS

1. Non-repeatable positive result may occur due to the general biological characteristics of ELISA assays. The assay is designed to achieve very high performance characteristics of sensitivity and specificity and the “indirect model” minimizes the unspecific reactions, which may occur due to interference between unknown meters in sample and the rabbit anti-human IgG used as a conjugate. Antibodies may be undetectable during the early stages of the disease and in some immunosuppresed individuals.
2. If, after retesting 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, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
3. Positive results must be confirmed with another available method. Any positive result must be interpreted together with the patient clinical information and other laboratory results.
4. Common sources for mistakes: Kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure 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.
6. 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.
7. This is a qualitative assay and the results can not be used to measure antibodies concentrations.

INDICATIONS OF INSTABILITY OR DETERIORATION OF THE REAGENTS

1. Values of the Positive or Negative controls ,which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones.
2. If after mixing of the Chromogen A and B solutions into the wells, the, the color of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

VALIDITY

Please do not use this kit beyond the expiry date indicated on the kit box and reagent labels.

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