Laboratory Procedure Manual

Analyte: Hepatitis D Antibody

Matrix: Serum

Method: ETI-AB-DELTAK-2
DiaSorin (REF p2808)

Method No.:

First Published: February 24, 2011
Revised: N/A

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Important Information for Users

The National Center for HIV/AIDS, Hepatitis, STD and TB Prevention (NCHHSTP) periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

February 24, 2011

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
HEPBD_F	LBDHD	Hepatitis D (anti-HDV)

February 24, 2011

ETI-AB-DELTAK-2 (p2808)

For Research Use Only

ENZYME IMMONOASSAY FOR QUALITATIVE DETERMINATION OF TOTAL ANTIBODIES TO HEPATITIS DELTA ANTIGEN (anti-HD) IN SERUM OR PLASMA SAMPLES

1. INTRODUCTION

   The Delta antigen/antibody system (HDAg/Anti-HD) is related to HBV infection but immunologically distinct from its known reactivities; it is the expressionof the Delta virus (HDV.Hepatitis D Virus), couse of severe liver disease in HBsAg carriers.

   HDV is a 35-37nm particle containing loe molecular weight RNA and HDAg, with an outer coat of HBsAG obtained from HBV. HDV is a defective virus and its replication requires helper functions provided by HBV.

   HDAg has been detected in liver and in serum and induces a specific antibody response (anti-HD antibodies) both IgG and IgM class.

2. PRINCIPLE OF THE ASSAY

   The method for qualitative anti-HD determination is s simultaneous competitive assay.

   Anti- HD present in the sample and labelled anti-HD antibodies complete for a fixed quantity of HDAg bound to the solid phase. The quantity of enzyme tracer bound to the solid phase and consequently the enzyme activity are inversely propotional to the anti-HD concentration present in samples or controls.

   Enzyme activity is measured by adding a colourless chromogen/substrate3 solution. The enzyme action on chromogen/substrate produces a colour which is measured with a photometer.

3. REAGENTS AND ACCESSORIES

3.1 Reagents provided in the kit

Coated strips	12-8 well strips
Enzyme tracer	1 vial. 0.5 ml
Negative Control	1 vial. 2ml
Positive Control	1 vial. 2ml
Tracer diluent	1 vial. 17.5 ml
Wash Buffer	1 vial. 40 ml
Chromogen	1 vial. 9 ml
Substrate	1 vial. 9ml
Blocking reagent	1 vial . 30Ml
Number of tests	96

STORAGE:- Upon receipt, store all reagents at 2-8'C, away from intense light. Do not freeze. Once opened the reagents of this kit are stable for eight weeks when properly stored, unless otherwise

stated. The kit is stable for one week when used throughout the day for eight hours at room temperature and stored overnight at 2-8'C.
Reagents should not be used past the expiry date. The expiry date of the kit is reported on the external label. The expiry date of each component is reported on the respective vial label.

Specific reagents (see 4.a) from different batches must not be mixed. Common reagents (see. 4b) are interchahgeable between batches.

3.2 Materials provided with the kit

--- 2 precut cardboard sealers suitable for 1 to 12 strips
---2 cardboard sealers suitable for 12 strips (one plate)
--- pouch sealer.

3.3 Equipment and materials required but not supplied

--Vertical reading photometer with the following instrument specifications wavelength: dual wavelength,450nm and 620-630nm bandwidth: < 10nm. Absorbance range: 0 absorbance units to > 2.5 absorbance units repeatability: better than or equal to 0.005 absorbance units, or1 % whichever is greater. Drift less than 0.005 absorbance units per hour.
-- Thermostatically-controlled humid chamber with the following specifications: tempreture:37'C+/-1'C
-- Manual or automatic equipment for rinsing wells with the following instrument specifications. Volume dispensed: 300-370ul number of wash cycles: 5 . soak time: 30 seconds. Aspirate the last aliquot or dispensed liquid: yes.
-- Micropipettes with disposable tips (50, 100ul) (50ul: trueness +/-3%, precision 2%
100ul : trueness +/- 2%, precision 1%)
--Glassware
-- Distilled water.

4. COMPOSITION AND PREPARATION OF REAGENTS

All serum and plasma units to produce the components provided in this kit have been tested for the presence of HBsAg, and anti-HCV and anti-HIV ½ and found to be non reactive, except for the material positive for anti-HD, which is reactive for HBsAg and anti-HCV. The presence of HBsAg and HCV in the final reagents is however excluded. Because HBsAg is removed by purification and HCV-RNA is found negative. However, as no test method can offer absolute assurance the pathogens are absent; all specimens of human origin should be considered potentially infectious and handled with care.

a) SPECIFIC KIT REAGENTS

4.1 Coated strips

Wells are coated with biotinylated anti-HD IgG (human) and recombinant HDAg. Ready to use, the strips should be kept at 2-8'C

Bring the coated strips to room temperature before opening the pouch to avoid development of condensed water in the wells. Place unused strips in the pouch, securely reseal and store at 2-8'C.

4.2 Enzyme Tracer (conjugate)
The vial contains 0.5 ml human anti-HD Fab fragments conjugated to horseradish peroxidase (HRP), phosphate buffer, BSA stabilizers and preservatives. The solution should be diluted 1:50 with tracer diluents (4.5) (e.g 100ul tracer + 4.9 ml diluents). Prepare only amount of working enzyme tracer needed for the run and keep the concentrated enzyme tracer at 2-8'C
The working enzyme tracer can be stored for one week at 2-8"c after preparation.

4.3 Negative Control
The vial contains 2ml human serum/plasma non-reactive for anti-HD and preservatives. Ready to use, the reagent should be stored at 2-8'C up to expiry date.

4.4 Positive Control
The vial contains 2ml human anti-HD antibodies, PBS buffer and preservatives. Ready to use, the reagents should be stored at 2-8'C.

4.5 Tracer diluents
The vial contains 17.15ml human serum/plasma. PBS buffer, newborn calf serum, and preservatives. Ready to use, the reagent should be stored at 2-8'C up to the expiry date. The solution is used to dilute the enzyme tracer (4.2)

b) REAGENTS COMMON TO OTHER KITS

4.6 Wash buffer (25x)
The vial contains 40ml PBS buffer, Tween 20 and preservatives. Dilute the vial contents to one litre with distilled water and store fot one week at 2-8'C. The reagents is used to rinse wells.
If crystallization occurs at 2-8'C, warm the wash buffer to 37'C and mix well before diluting.

4.7 Chromogen
The vial contains 9ml tetramethylbenzidine derivative in buffer solution. Mix the solution 1: 1 with substrate (4.8) (e.g 1 ml chromogen + 1 ml substrate). After dilution chromogen/ substrate can be stored for 8hours at room temperature, away from the light.

4.8 Substrate
The vial contains 9ml buffer solution containing H2O2. The solution should be mixed 1:1 with chromogen (4.7)

4.9 Blocking reagent.
The vial contains 30ml IN sulfuric acid (R 36/38, S 26). Ready to use, the reagent should be stored at 2-8'C up to expiry date.

5 SPECIMEN COLLECTION AND PREPARATION

Either human serum or plasma may be used. The anticoagulants citrate. EDTA and heparin have been tested may be used with the assay. Borderline or low-positive results obtained from EDTA-plasma specimens should be evaluated with care. Blood should be collected aseptically by veinpuncture, allowef to clot. And the serum separated from the clot as soon as possible. Samples having particulate matter, turbidity, lipaemia, or erythrocyte debris may require clarification by filtration or centrifugation before testing. Grossly haemolyzed or lipaemic samples as wellas samples containing particulate matter or exhibiting obvious microbial contamination should bnot be tested. If the assay is performed within 48 hrs of sample collection, the samples should be kept at 2-8'C; otherwise they should be aliquoted and stored deep-frozen (-@0'C or below), If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freeze-thaw cycles.

Samples containing sodium azide should not be assayed.

6. ASSAY PROCEDURE

Bring reagents to room temperature (20-25'C) before assaying. Perform all assay steps without stepping. Number sufficient strips to run 3 negative controls, 2 positive controls and unknowns in singlicate. Samples and controls should be subjected to the same process and incubation time. Prepare one blank well containing chromogen/ substrate only. A disposable tip should be used for dispensing each sample and control. Dispense the samples and controls os illustrated in the scheme below.

Adjust the thermostatically-controlled humid chamber to 37'+/- 1'C/

1. Dispense 50ul negative control and positive control I into their respective wells.
2. Dispense 100ul diluted enzyme tracer (4.2) into all wells except for the blank well
3. Apply a cardboard sealer in order to prevent evaporation.
4. Incubate for three hours +/_ 15min at 37'C)+/1'C.
5. Prepare chromogen/substrate just before the end of incubation.
6. When incubation has been completed, discard the cardboard sealer and rinse the strips. Use a suitable automatic or semiautomatic washer for wahing strips. Aspirate the liquid and rinse each well five times with a volume of wash buffer ranfing from 0.30 to 0.37 ml. Avoid overflow from the reaction wells. When either an automatic or semiautomatic washer is used, the soak time between each rinse should be 30 seconds.
7. Dispense 100ul chromogen/substrate solution into wells.
8. Incubate for 30+/- 2 min at room temperature, away from intense light.
9. Dispense 100ul blocking reagent into all wells in the same order and at the same rate as for chromogen/substrate.
10. Measure the absorbance of specimens with a photometer at 450/630nm within one hour of adding the block reagent. When dedicated equipment is used, absorbance values are provided automatically after selecting the suitable protocol. When another vertical reading

photometer is used, blank the instrument with the blank, record the absorbance at 450/630nm for each specimen and substrate the 630nm absorbance value from 450nm absorbance value.

7. CALCULATION OF RESULTS

7.1 Calculation of cut-off-value

The cut-off value is determined by adding the mean absorbance for negative control values (NCx) multiplied by 0.5 to the mean absorbance for the positive controls values (PCx)multiplied by0.5

Cut-off value = 0.5 NCx + 0.5 PCx

7.2 Run validation criteria

Always validate quality control with the following steps when evaluating results.

The mean negative control absorbance value must be greater than or equal to

0.600 NCx > 0.600

The mean positive control absorbance value must be less than or equal to

0.080 PCx < 0.080

The difference between the mean negative control absorbance value and the mean positive control absorbance value (N – P) must be greater than or equal to 0.500

NCx – PCx > 0.500

If not, the run is invalid and must be repeated.

7.3 Interpretion of results

The presence or absence of anti HD is determined by comparing the absorbance of the unknown samples to that of the cut-off value

The unknown samples with the absorbance values less than or equal to the cut-off value should be considered reactive for anti-HD. The unknown samples with absorbance values greater than the cut-off value should be considered non-reactive.

Samples with absorbance values within +/- 10% of cut-off value must be retested in order to confirm the initial result. Samples which are repeatedly reactive should be considered positive. Samples which are non-reactive at the second test should be considered negative.

7.4 Calculation example

The following data must only be considered an example and should not be employed instead of the data obtained by the user

Absorbance for the negative control

Negative control	Net absorbance at 450/630nm
1	1.273
2	1.253

3	1.289

Mean absorbance, NCx = 1.272

Absorbance for positive control

Positive control	Net absorbance at 450/630nm
1	0.016
2	0.018

Mean Absorbance, PCx =0.017
Cut-off value (0.5 NCx = 0.5 PCx) = 0.5 x 1.272 = 0.5x 0.017 = 0.645
Negative - Positive control difference (N- P) = 1.272-0.017 =1.255
The N – P difference is greater than 0.500; thus the technique is acceptable and data should be considered valid.

Screening of unknown samples
Sample 1 = absorbance 0.182
Sample 2 = absorbance 1.083
Sample no.! should be considered reactive for anti-HD and sample no.2 non-reactive as cut-off value is 0.645

8. LIMITATIONS OF THE PROCEDURE
Bacterial contamination, repeated freezes-thaw cycles or heat inactivation of the specimens mat affect the absorbance values of the samples mwith consequence alteration of IgM anti-HD levels

9. WARNING AND PRECAUTIONS
A skillful technique and strict adherence to the instructions are necessary to obtain reliable results
Non-repeatedly reactive samples might derive from various methodological factors, such as
--cross- exchange of vial caps
-- use of the same tip when withdrawing from different vials or dispensing different samples
--exposure of reagents or samples to intense heat or heavy sources of bacterial contamination
--inadequate rinsing of wells
--contamination of well rims by tracer or samples
--use of reagents from different master lots
In addition the following precautions are required:
--To avoid contamination, use a clean dedicated dispenser for the enzyme tracer solution
-- wash buffer should be stored in clean containers to prevent contamination with enzyme – inactivating substances
--it is essential that clean labware be used for chromogen/substrate preparation.

10 .SAFGETY PRECAUTIONS

--Handle with care chromogen, substrate and blocking reagent. Avoid chromogen, substrate and blocking reagent coming into contact with oxidizing agents of metallic surface

--- Do not eat, drink smoke or apply cosmetics in the assay laboratory

---Do not pipette solutions by mouth.

---Avoid direct contact with all potentially infectious materials by using articles such as lab coats , protective glasses and disposable gloves . Wash hands thoroughly at the end of assay.

---Avoid splashing or forming an aerosol. Ant reagent spills should be washed with a 5% sodium hypochloride solution and disposed of as though potentially infectious.

---All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each Country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Liquid waste containing acid must be neutralized before treatment. Any materials to be reused must be autoclaved using an overkill approach (USP 24,200,p. 2143). A minium of one hour at 121'C is usually considered adequate though the usere must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.

--- Blocking reagent (Council Directive 99/45/EC):

R 36/38 ---- Irritating to eyes and skin

S 26 ---- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

SCHEME OF THE ASSAY

ADJUST THE THERMOSTATICALLY- CONTROLLED HUMID CHAMBER TO 37'C

1. DISPENSE REAGENTS INTO THE STRIP WELLS ACCORDING TO THE FOLLOWINF SCHEME. LEAVING AN EMPTY WELL FOR THE BLANK
2. –INCUBATE FOR 3 HOURS +/- 15 MIN AT 37'C
3. – ASPIRATE THE LIQUID REPEATEDLY WASH WITH WASH BUFFER
4. – DISPENSE 100UL CHROMOGEN/SUBSTRATE INTO ALL WELLS
5. – INCUBATE FOR 30+/- MIN AT ROOM TEMPERATURE IN THE DARK
6. – DISPENSE 100UL BLOCKING REAGENT INTO ALL WELLS
7. –READ ABSORBANCE WITH A PHOTOMETER AR 450/630NM