Anti-HAV Elisa

For qualitative in-vitro detection of antibodies to hepatitis A virus (Anti-HAV) in human serum or plasma

OKDA00059

FOR RESEARCH USE ONLY

Aviva Systems Biology, 5754 Pacific Center Blvd., Suite 201, San Diego, CA 92121, Tel: 1-858-552-6979

1. INTENDED USE

Anti-HAV Elisa is an enzyme immunoassay for qualitative detection of antibody to hepatitis A virus (Anti-HAV) in human serum or plasma (heparin, EDTA or citrate).

2. SUMMARY AND TEST EXPLANATION

The hepatitis A virus (HAV) is a single-stranded RNA-containing virus without an envelope and with a diameter of 27 nm that belongs to the family of Picornaviridae [*1]. Hepatitis A - the most common form of acute viral hepatitis - is an infection of fecal-oral transmission produced in humans after an average incubation period of 28 days (range, 15-50 days). The illness caused by HAV infection typically has an abrupt onset of symptoms that can include fever, malaise, anorexia, nausea, abdominal discomfort, dark urine, and jaundice [*2].

Total anti-HAV and especially IgM anti-HAV is positive at the onset of a hepatitis A infection. After natural infection, anti-HAV-IgG antibodies can usually be detected for a lifetime providing protection against the disease [*3-4]. The detection of anti-HAV is indicative of current immunity and helps in deciding whether active immunization should be supplied by vaccination or immunoglobulins should be administered for post-exposure prophylaxis in at-risk situations [*5-6].

Anti-HAV Elisa is a fast test for the qualitative detection of antibodies to Hepatitis A virus in serum or plasma (heparin, citrate or EDTA) specimens. This is an enzyme linked immunosorbent assay (ELISA) which utilizes HAV Ag on microtiter wells and human Peroxidase-conjugated Anti-HAV in a competition principle to detect Anti-HAV levels in serum or plasma.

Specimens with absorbance values greater than the Cutoff Value are considered **NONREACTIVE** for Anti-HAV

Specimens with absorbance values lower or equal than the Cutoff Value are considered **REACTIVE** for Anti-HAV.

The test has to be repeated in duplicate for specimens with absorbance value within the retest range (Cutoff Value ± 10 %) and interpreted as above.

If the absorbance of any of the specimens retested in duplicate is still within the retest range, it is suggested to test follow-up samples of the patient.

3. TEST DESCRIPTION

Anti-HAV Elisa is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immunosorbent assay) based on a competitive principle. The solid phase of the microtiter plate is made of polystyrene wells coated with HAV Ag and the liquid phase of human Peroxidase conjugated Anti-HAV.

When a serum or plasma specimen containing Anti-HAV is added to the HAV Ag-coated wells together with the human Peroxidase conjugated Anti-HAV and incubated, a competition will take place for the binding to the HAV Ag on the wells. (HAV Ag)-(Anti-HAV • Peroxidase) complex and/or (HAV Ag)-(Anti-HAV) complex will form on the wells. After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. Due to the competitive principle a color develops inversely proportional to the amount of Anti-HAV bound

to HAV Ag deriving from the specimen. The Peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450 nm with a selected reference wavelength within 620 to 690 nm[*7] .

A Specimen containing Anti-HAV:

1. Plate well (HAV Ag) + specimen (Anti-HAV) + Anti-HAV·Peroxidase
   → Plate-HAV Ag-Anti-HAV complex and/or Plate-HAV Ag-Anti-HAV·Peroxidase complex
2. Washing to remove unbound material
3. Add TMB substrate solution → blue color to light pale blue color/even colorless
4. Add 2N sulfuric acid to stop the color development → Read OD at 450nm with a selected reference wavelength within 620 to 690nm[*7]

B Specimen without Anti-HAV:

1. Plate well (HAV Ag) + specimen (without Anti-HAV) + Anti-HAV·Peroxidase
   → Plate-HAV Ag-Anti-HAV·Peroxidase complex
2. Washing to remove unbound material
3. Add TMB substrate solution → colorless to blue color
4. Add 2N sulfuric acid to stop the color development, read OD at 450nm with a selected reference wavelength within 620 to 690nm[*7]

4. DESCRIPTION OF MATERIALS PROVIDED

- **Storage Conditions:** Item 1 - 6 on the following reagent table should be refrigerated at +2 to 8+ °C and the others stored at room temperature (+20 to +30 °C).

ITEMS	Components	Description	Qt. per 96 tests
(1)	HAV Ag Plate	Microtiter Plate Coated with HAV Antigen.	1 plate
(2)	Anti-HAV·Peroxidase Solution Ab | HRP	Anti-HAV (mouse monoclonal) ·Peroxidase (horseradish) conjugate dissolved in buffer with protein stabilizers. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.	1bottle, 12 ml
(3)	Anti-HAV Positive Control CONTROL | H	Human plasma positive for antibody to HAV in buffer with protein stabilizers. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.	1 bottle, 1 ml
(4)	HA Negative Control CONTROL | L	Human plasma non-reactive for antibody to HAV with protein stabilizers. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.	1 bottle, 1 ml
(5)	Chromogenic TMB concentrate CHROM | TMB | CONC	0.6 mg/ml of 3,3',5,5'-tetramethylbenzidine (TMB) in an organic base.	1 bottle, 12 ml
(6)	Substrate buffer SUB | BUF	Citric Acid Buffer containing 0.03% H_2O_2.	1 bottle, 12 ml
(7)	Conc. Washing Solution D (20X) WASH | SOLN | CONC	Concentrated phosphate buffer with Tween-20.	1 bottle 58 ml
(8)	Stop Solution STOP | SOLN	2N H_2SO_4 (Sulfuric Acid)	1 bottle 12 ml

- OTHER MATERIALS AND DEVICES REQUIRED, BUT NOT PROVIDED

ITEMS	Components
(1)	10µl, 100µl and 1.0 ml micropipettes and tips are needed
(2)	Incubator or waterbath with temperature control at +37 °C.
(3)	Plate washing equipment.
(4)	ELISA microwell reader: Dual wavelength 450nm with 620-690nm as reference wavelength[*7], bandwidth 10nm
(5)	Purified water: distilled or deionized water.
(6)	Fully automatic EIA micro-plate analyzer is optional. User should validate the automatic EIA micro-plate analyzer in combination with the kit.

4.1. Storage Conditions and Stability of Kit and Components *

Kit/Components	**Storage condition**	**State**	**Stability**
Anti-HAV Elisa KIT	+2 to 8 °C	Original	15 months
		Once open	1 month
Anti-HAV Positive Control	+2 to 8 °C	Original	15 months
		Once open	1 month
HA Negative Control	+2 to 8 °C	Original	15 months
		Once open	1 month
HAV Ag Plate	+2 to 8 °C	Original	15 months
		Once open	2 months
Anti-HAV·Peroxidase Conjugate Solution	+2 to 8 °C	Original	15 months
		Once open	1 month
Concentrated Washing Solution D (20x)	+2 to 8 °C	Original	24 months
		Once open	1 month
20x Diluted Washing Solution	Room temp.	Diluted	2 days
	+2 to 8 °C	Diluted	1 week
Chromogenic TMB concentrate	+2 to 8 °C	Original	24 months
		Once open	1 month
Substrate buffer	+2 to 8 °C	Original	24 months
		Once open	1 month
2N Sulfuric Acid	Room temp.	Original	24 months
		Once open	1 month

5. INSTRUCTIONS FOR USE

5.1. Warnings

5.1.1. This reagent kit is for research use only.

5.1.2. Bring all kit reagents and samples to room temperature (+20 to +30°C) and mix gently before use.

5.1.3. Do not use reagent beyond its expiration date.

5.1.4. Do not interchange reagents between different lots.

5.1.5. Do not pipette in the mouth.

5.1.6. Do not smoke or eat in areas where specimens or reagents are handled.

5.1.7. The positive control, negative control, conjugate solution and specimens should be regarded as potential hazards to health. They shall be used and discarded according to the user's laboratory safety procedures. Such safety procedures probably shall include wearing protective gloves and avoiding aerosols generation.

5.1.8. Potential infectious specimens and nonacid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with the laboratory's practice for potential bio-hazard control.

5.1.9. **Prior to dispose the waste of used specimens and kit reagents as general waste, it should be treated in accordance with the local procedures for potential bio-hazardous waste or treated as follows:**

Both liquid and solid waste should be autoclaved maintaining +121 °C for at least 30 minutes.

Solid waste can also be incinerated.

Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%.

Acidic liquid wastes must be neutralized before treatment with sodium hypochlorite as mentioned above and should stand for 30 minutes to obtain effective disinfection.

5.1.10. 2N sulfuric acid is an irritant to skin, eyes, respiratory tract and mucous membranes. Avoid contact of the 2N sulfuric acid with skin and mucous membranes. In case of contact, clean with large lots of water immediately. In case of inhalation, supply fresh air and seek medical advice in case of complaints.

5.1.11. Chromogenic TMB concentrate contains methanol, which is flammable (and toxic, depends on the concentration).Chromogenic TMB concentrate contains dimethyl sulfoxide, an irritant to skin and mucous membranes.

5.2. Specimen Collection and Preparation for Analysis

5.2.1. No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques.

5.2.2. Either serum or plasma can be used with this kit. Whole blood specimens should be separated as soon as possible in order to avoid hemolysis. Any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen should be removed prior to use.

5.2.3. Specimens must be stored at +2 to +8 °C and avoided heat-inactivation to minimize deterioration. For long-term storage, they should be frozen below -20 °C. Storage in self-defrosting freezer is not recommended.

5.2.4. Frozen specimens must be thoroughly thawed and mixed homogenously before test.

5.2.5. Avoid multiple freeze-thaw procedures

WARNING
1. The specimen must not contain any compounds of AZIDE, which inhibits the peroxidase activity.
2. Incompletely coagulated serum samples and microbial-contaminated specimens should not be used.

5.3. Reagents storage

5.3.1. The kit must be stored at +2 to +8°C. Do not freeze.

5.3.2. Strips of the plate should be used within 1 month after open the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and taped the opening tightly.

5.3.3. Return the reagents to +2 to +8 °C immediately after use.

5.3.4 Washing Solution D (20x) Concentrate should be stored at room temperature to avoid crystallization. If the crystal has been precipitated before use, warm up the solution in a +37 °C water bath till the crystal is dissolved.

5.4. Plate washing procedure

5.4.1. Preparation of washing solution:

Dilute Washing Solution D (20x) Concentrate with distilled or de-ionized water to 1:20 dilution. Do not use tap water.

5.4.2. Plate washing:

Any commercial automatic micro-plate washer or other liquid aspirating/ dispensing devices can be used for washing purpose. The user should test the devices to determine the proper volume of water and wash cycles to insure proper washing.

It is suggested to wash 6 cycles with at least 350µl washing buffer per well per wash and soaking at least for 10 seconds.

5.4.3. Blot dry by inverting the plate and tapping firmly onto absorbent paper. Too much residual wash buffer will cause false results.

WARNING Improper washing will cause false results.

5.5. Test procedure

Assay process can be performed by an automatic EIA micro-plate immunoanalyzer,. Please set up the program according to the following test procedure.

5.5.1. Bring all reagents and specimens to room temperature (+20 to +30°C) before assay. Adjust water bath or incubator to +37±1°C.

5.5.2. Prepare the needed number of wells, including 2 wells for blanks, 3 wells for Negative Control, 2 wells for Positive Control, and one well for each specimen.

Reserve 2 wells for blanks (**Do not add any specimen or conjugate)**.

Add 10µl of each control or specimen to the appropriate wells of HAV Ag coated plate, except the 2 blanks.

NOTE:

a. Use a new pipette tip for each sampling to avoid cross-contamination
b. Each plate needs its own negative controls, positive controls and blank wells.
c. Do not use cut-off values established for other plates of Anti-HAV Elisa.

5.5.3. Add 100µl of Anti-HAV·Peroxidase solution to each of the above wells except the 2 blanks.

Note: Do not touch the cuvette wall for preventing contamination.

5.5.4. Gently tap the plate.

5.5.5. Seal the plate with an adhesive slip.

5.5.6. Incubate the reaction plate in +**37±1 °C** water bath or incubator for **one hour**.

5.5.7. At the end of the incubation period, remove and discard the adhesive slip and wash the plate in accordance with **5.4) Plate washing procedure.**

5.5.8. Choose one of the following two methods for color development:

NOTE: Chromogenic TMB concentrate should be colorless to light blue, otherwise, it should be discarded. The mixture of Chromogenic TMB concentrate and Substrate buffer should be used within 30 minutes after mixing. The mixture should be avoided from intense light.

A. Mix equal volumes of Chromogenic TMB concentrate and Substrate buffer in a clean container immediately prior to use. Add 100 µl of the mixture solution to each well including the 2 blank wells.

B. Add 50µl of Chromogenic TMB concentrate first, then add 50µl of Substrate buffer into each well including the 2 blanks. Mix well gently.

5.5.9. Cover the plate with black cover and incubate at room temperature for 30 minutes.

5.5.10. Stop the reaction by adding 100 µl of stop solution to each well including the blank.

5.5.11. Determine the absorbance of controls and test specimens within 15 minutes with a photometer at 450nm with a selected reference wavelength within 620 to 690nm[*7].

Use the blank well to blank the photomer.

NOTE: The color of the blank should be colorless to light yellowish; otherwise, the test result is invalid. In this case the test must be repeated.

Substrate blank : absorbance value must be less than 0.100.

5.6.	**Calculation of Test Results**
5.6.1.	Calculation of the NCx (Mean Absorbance of Negative Control). Example: Sample No. Absorbance 1 1.263 2 1.305 3 1.290 NCx = (1.263 + 1.305 + 1.290)/3=1.286 **NCx must be ≥ 0.4, otherwise, the test is invalid.**
5.6.2.	Calculation of PCx (Mean Absorbance of Positive Control) Example: Sample No. Absorbance 1 0.054 2 0.060 PCx = (0.054 + 0.060) /2 = 0.057 **PCx must be ≤0.1, otherwise, the test is invalid.**
5.6.3.	Calculation of the **N-P Value** **N-P = NCx – PCx** Example: N - P = 1.286 - 0.057 = 1.229 **N-P Value must be ≥ 0.3, otherwise, the test is invalid.**
5.6.4.	Calculation of the **Cutoff Value** **Cutoff Value = (NC**x **+ PC**x**)/2** Example: Cutoff Value =(1.286 + 0.057)/2 = 0.672
5.6.5.	Calculation of the **Retest Range** **Retest Range = Cutoff Value ±10%** Example: Cutoff Value = 0.672 Retest Range = (0.672 - 0.067) to (0.672 + 0.067) = 0.605 to 0.739
5.7.	**Validity of Test Runs**
5.7.1.	**NCx must be ≥0.4, otherwise, the test is invalid.**
5.7.2.	**PCx must be ≤0.1, otherwise, the test is invalid.**
5.7.3.	**N-P Value must be ≥0.3, otherwise, the test is invalid.**

5.8. Interpretation of Results

5.8.1. Specimens with O.D. values **GREATER** than the **Cutoff Value** are considered **non-reactive** for Anti-HAV.

5.8.2. Specimens with O.D. values **LOWER** than or **EQUAL** to the **Cutoff Value** are considered **reactive** for Anti-HAV.

5.8.3. If the data is within the **Retest Range**, the test must be repeated in duplicate and interpreted as above. If the retested absorbance still within the retest range, it is suggested to test follow-up-samples.

5.9. Troubleshooting

If the result cannot be reproduced, a preliminary troubleshooting should be performed by checking the possibilities listed below:

5.9.1. Improper washing procedure.

5.9.2. Contamination with positive specimens.

5.9.3. Wrong volume of sample, conjugate or substrates.

5.9.4. Contamination of well rim with conjugate.

5.9.5. Improper specimen such as hemolyzed serum or plasma, specimen containing precipitate and specimen not being mixed well before use.

5.9.6. Wrong incubation time or temperature.

5.9.7. Obstructed or partial obstructed washer aspirate/dispense head and needles.

5.9.8. Insufficient aspiration.

5.10. Limitations and Interferences

5.10.1. **This reagent kit is to be used for un-pooled human serum or plasma samples only.**

5.10.2. **Non-repeatable reactive results may be obtained with any** enzyme immunoassay **kit,** largely due to technical error e**i**ther on the part of the operator or malfunction of apparatus used.

5.10.3. The reagent kit has not been validated for use with cadaveric samples.

5.10.4. Potential interfering substances: By addition tests the following results were obtained:

1. The anticoagulants heparin, citrate and EDTA had no effect on the test result.
2. Hemoglobin up to 8.0 g/l had no effect on the test result.
3. Bilirubin up to 0.3 g/l: had no effect on the test result.
4. Triglyceride up to 5.0 g/l had no effect on the test result.
5. A rheumatoid factor high positive specimen exhibited a false positive result.

Pregnancy did not effect the test result.

Performance Characteristics

5.11.1. **Specificity**

1. Specimens from hospitalized patients:

		Aviva Anti-HAV Elisa		
		Negative	Positive	Total
Comparison assay	Negative	984	5	989
	Positive	1	551	552
	total	985	556	1541

sensitivity = 100% x 551/552 = 99.8%

specificity = 100% x 984/989 = 99.5%

2. Samples with acute hepatitis A:

		Aviva Anti-HAV Elisa		
		Negative	Positive	Total
Comparison assay	Negative	42	0	42
	Positive	0	9	9
	total	42	9	51

Conformity = 100%

3. Hepatitis A samples in convalescent period:

		Aviva Anti-HAV Elisa		
		Negative	Positive	Total
Comparison assay	Negative	10	0	10
	Positive	0	18	18
	total	10	18	28

Conformity = 100%

4. Hepatitis B carriers:

		Aviva Anti-HAV Elisa		
		Negative	Positive	Total
Comparison assay	Negative	85	0	85
	Positive	0	22	22
	total	85	22	107

Conformity = 100%

5. Auto-immune samples:

		Aviva Anti-HAV Elisa		
		Negative	Positive	Total
Comparison assay	Negative	4	0	4
	Positive	0	16	16
	total	4	16	20

Conformity = 100%

6. Samples with HAV infection:

		Aviva Anti-HAV Elisa		
		Negative	Positive	Total
Comparison assay	Negative	0	0	0
	Positive	0	19	19
	total	0	19	19

specificity = 100%

sensitivity = 100%

7. Patients with other viral infections:

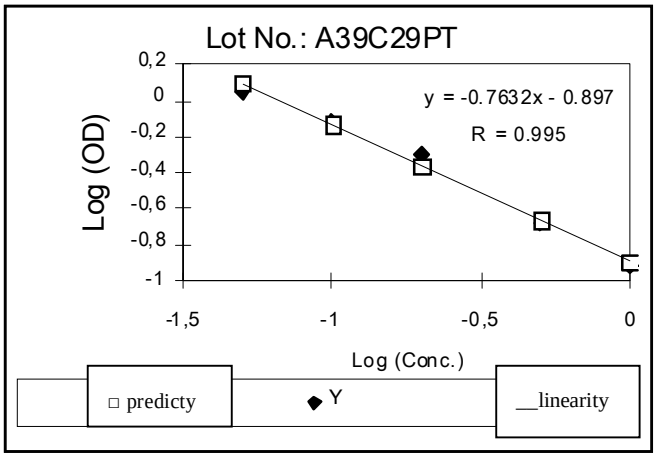
		Aviva Anti-HAV Elisa		
		Negative	Positive	Total
Comparison assay	Negative	15	0	15
	Positive	0	20	20
	total	15	20	35

Conformity = 100%

5.11.2. Analytical Sensitivity

Analytical sensitivity = 0.121 PEI U/ml ≒ 0.157 IU/ml.

Conc. (E/ml)	OD	Log (Conc.)	Log (OD)
1	0.121	0	-0.9172146
0.5	0.207	-0.30103	-0.6840297
0.2	0.499	-0.69897	-0.3018995
0.1	0.77	-1	-0.1135093
0.05	1.125	-1.30103	0.0511525
Cutoff	0.636	-0.91776471	-0.1965429
Sensitivity		0.121	PEI U/ml



PEI = Paul Ehnlich Institute

5.11.3. Precision

Intra-assay reproducibility: Intra-assay CV% < 20

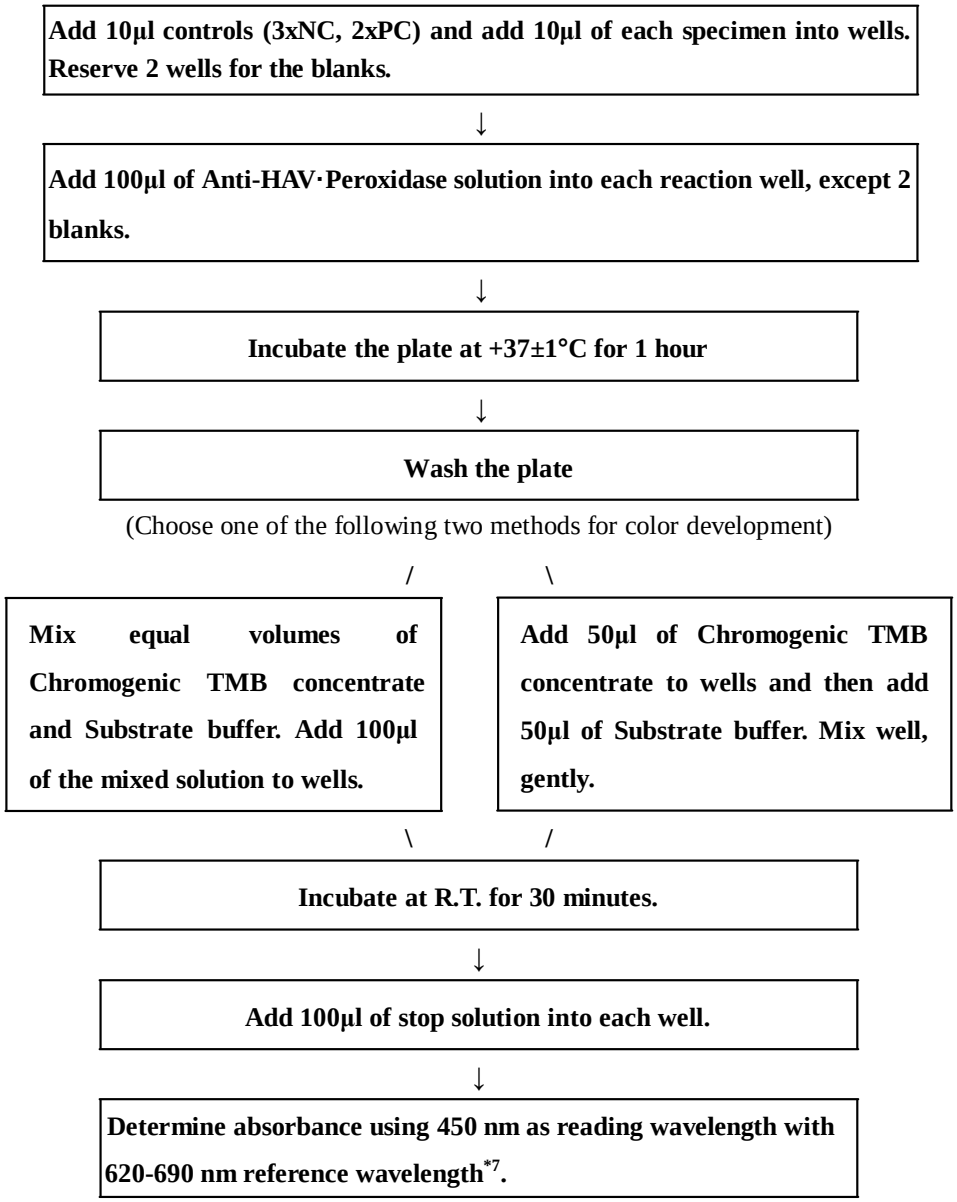
Inter-assay reproducibility: Inter-assay CV% <25

5.11.4. Traceability:

Concentration of Anti-HAV Positive Control = 7 ±4 PEI U/ml = 9.1 ±5.2 IU/ml

5.12. Flow chart of the test procedure

The simplified procedure should be used only by experienced users. New users are advised to read and follow the detailed test procedure carefully.



6. Bibliography

1. Melnick JL. History and epidemiology of hepatitis A virus. J Infect Dis 1995;171(Suppl 1):2-8.
2. Koff RS. Hepatitis A. Lancet 1998;341:1643–49.
3. Lemon SM, Binn LN. Serum neutralizing antibody response to hepatitis A virus. J Infect Dis. 1983;148**:** 1033-1039.
4. Duermeyer W., Van der Veen J., Koster B. "ELISA in Hepatitis A". Lancet. 1978; 1(8068).:823-824.
5. Lemon SM. Inactivated hepatitis A virus vaccines. Hepatology.1992;15:1194-1197.
6. Craig AS, Schaffner W. Prevention of hepatitis A with the hepatitis A vaccine N Engl J Med 2004; 350:476-481
7. The reference wavelength of spectrometer can be 620nm to 690nm. However, user should validate the photometer in combination with this kit before use.

Revision date : 2011-06-28

	Usedsymbols	**Symbolesutilisés**
	Consult instructions for use	Consulter les instructions d'utilisation
	Storage temperature	Température de conservation
	Use by	Utiliser jusque Batch
LOT	code	Numéro de lot
REF	Catalogue number	Référence de catalogue
CONTROL	Control	Contrôle
IVD	In vitro diagnostic medical device	Dispositif médical de diagnostic in vitro
	Manufacturer	Fabricant
	Contains sufficient for <n> tests	Contenu suffisant pour <n> tests
WASH SOLN CONC	Wash solution concentrated	Solution de lavage concentrée
CAL 0	Zero calibrator	Calibrateur zéro
CAL N	Calibrator #	Calibrateur #
CONTROL N	Control #	Contrôle #
Ag 125I	Tracer	Traceur
Ab 125I	Tracer	Traceur
Ag 125I CONC	Tracer concentrated	Traceur concentré
Ab 125I CONC	Tracer concentrated	Traceur concentré
	Tubes	Tubes
INC BUF	Incubation buffer	Tampon d'incubation
ACETONITRILE	Acetonitrile	Acétonitrile
SERUM	Serum	Sérum
DIL SPE	Specimen diluent	Diluant du spécimen
DIL BUF	Dilution buffer	Tampon de dilution
ANTISERUM	Antiserum	Antisérum
IMMUNOADSORBENT	Immunoadsorbent	Immunoadsorbant
DIL CAL	Calibrator diluent	Diluant de calibrateur
REC SOLN	Reconstitution solution	Solution de reconstitution
PEG	Polyethylene glycol	Glycol Polyéthylène
EXTR SOLN	Extraction solution	Solution d'extraction
ELU SOLN	Elution solution	Solution d'elution
GEL	Bond Elut Silica cartridges	Cartouches Bond Elut Silica
PRE SOLN	Pre-treatment solution	Solution de pré-traitement
NEUTR SOLN	Neutralization solution	Solution de neutralisation
TRACEUR BUF	Tracer buffer	Tampon traceur
	Microtiterplate	Microplaque de titration
Ab HRP	HRP Conjugate	HRP Conjugué
Ag HRP	HRP Conjugate	HRP Conjugué
Ab HRP CONC	HRP Conjugate concentrate	HRP Conjugué concentré
Ag HRP CONC	HRP Conjugate concentrate	HRP Conjugué concentré
CONJ BUF	Conjugate buffer	Tampon conjugué
CHROM TMB CONC	Chromogenic TMB concentrate	Chromogène TMB concentré
CHROM TMB	Chromogenic TMB solution	Solution chromogène TMB
SUB BUF	Substrate buffer	Tampon substrat
STOP SOLN	Stop solution	Solution d'arrêt
INC SER	Incubation serum	Sérum d'incubation
BUF	Buffer	Tampon
Ab AP	AP Conjugate	AP Conjugué
SUB PNPP	Substrate PNPP	Tampon PNPP
BIOT CONJ CONC	Biotin conjugate concentrate	Biotine conjugué concentré
AVID HRP CONC	Avidine HRP concentrate	Avidine HRP concentré
ASS BUF	Assay buffer	Tampon de test
Ab BIOT	Biotin conjugate	Biotine conjugué Specific
Ab	Antibody	Anticorps spécifique
SAV HRP CONC	Streptavidin HRP concentrate	Concentré streptavidine HRP
NSB	Non-specific binding	Liant non spécifique
2nd Ab	2nd Antibody	Second anticorps
ACID BUF	Acidification Buffer	Tampon d'acidification