Anti-HAV ELISA

For the quantitative determination of Anti-HAV in serum and plasma.

For Research use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 22-HAVHU-E01
Size: 96 Wells
Version: Version 4 - ALPCO 2.0

1. Intended Use

The anti-HAV ELISA is an enzyme immunoassay that detects antibodies against Hepatitis A virus in human serum. For research use only. Not for use in diagnostic procedures.

2. Principle of the Test

The Anti-HAV ELISA is a pseudo-competitive enzyme immunoassay. Serum or plasma samples are added to the wells of a microtiter plate, which has been previously coated with inactivated HAV antigen, and incubated for 2 hours at 37°C. Anti-HAV antibodies bind to the antigen. The conjugate (peroxidase labeled anti-HAV) is added and incubated again for 1 h at 37°C. Free binding sites of the antigen are bound with conjugate. Excess conjugate is washed off the plate and the substrate is added and incubated for 30 min at room temperature. The bound conjugate changes the color of the subtrate to blue. The reaction is terminated by adding the stop solution. The color turns yellow. The absorbance of the colored reaction product is measured on a microtiter plate reader. The absorbance is reciprocal to the anti-HAV titer. For **quantitative determination** use the included serum standard for preparation of a titration curve.

3. Warnings and Precautions

This kit is for research use only, not for internal applications in humans or animals. This product must be used as described in the enclosed package insert provided with the kit. Use the valid version of the package insert provided with the kit. Be sure that the everything has been understood. ALPCO is not liable or responsible for any damage or loss, caused by non-compliance with the product instructions. A Material Safety Data Sheet is available on request.

• Do not use obviously damaged, microbially contaminated, or spilled material.

• Caution: This kit contains material of human and/or animal origin. Therefore, all components and samples should be treated as potentially infectious.

• Appropriate precautions and good laboratory practices must be used in the storage, handling,
and disposal of the kit reagents. The disposal of the kit components must be made according
to the local regulations.

Human Serum

The following components contain human serum: **PK, NK, KK, STD 1-3**

The microplate is coated with inactivated antigen, which was negative in the rest infectivity test. Source human serum for the control sera provided in this kit was tested and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV). No known methods can offer total security of the absence of infectious agents; therefore, all components and samples should be treated as potentially infectious.

Reagents KK, VP, WP

Contain as preservative a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (< 0.015%)

H317	May cause an allergic skin reaction.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P302+P352	IF ON SKIN: Wash with plenty of soap and water
P501	Dispose of contents/ container in accordance with local/ regional/ national/international regulations.

Substrate (S)

The TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbenzidine (< 0.05%)

H315	Causes skin irritation.

H319 Causes serious eye irritation.
H335 May cause respiratory irritation.
P261 Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Stop Solution (SL)

The Stop solution contains 0.2 M Sulfuric Acid (H_2SO_4)

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301+P330+P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P305+P351+P338 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P310 IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor.

3.1 General First Aid Procedures

• Skin contact: Wash affected area rinse immediately with plenty of water at least 15 minutes.
Remove contaminated clothes and shoes.
• Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. To ensure effective rinsing, hold the eyelids open.
• Ingestion: After swallowing the product, if the affected person is conscious, rinse out the mouth with plety of water. Seek medical advice immediately.

4. Samples

4.1 Sample Type

Serum and Plasma
Serum and Heparin/EDTA-Plasma yield comparable values.

4.2 Sample Collection

Use standard venipuncture for the blood sampling. Hemolyzed samples are to be avoided.

4.3 Required Sample Volume: 25 µL

4.4 Sample Stability

In firmly closable sample vials
• Storage at 4°C max. 3 days
• Freeze-thaw cycles max. 3

Freezing and thawing of samples should be minimized. 3 Freeze-thaw cycles showed no effect on samples.

4.5 Sample Dilution

Qualitative Test: **1:10** with **Dilution Buffer VP**
Quantitative Test: **1:10** with **Dilution Buffer VP, > 1:10 with VPN**

Use a 10% solution of **anti-HAV antibody negative serum** in **Dilution Buffer to make VPN** for sample dilutions >1:10

Example: **Dilution 1:10**
25 µl Sample is added to **225 µL** Dilution Buffer **VP**

Example: **Dilution 1:50**
1 mL anti-HAV antibody negative serum is mixed with 9 mL Dilution Buffer **VP to make VPN**

10 µl Sample is added to **490 µL** of the VPN, including negative serum

5. Materials
5.1 Materials Provided

MTP	**Microtiter plate,** ready for use, coated with inactivated HAV-antigen. Well strips are separable.	**8 x 12 wells**
KK	**Conjugate Concentrate KK, 100-fold concentrated** (peroxidase labeled Anti-HAV IgG)	**1 x 100 µL**
PK	**Positive Control, PK,** anti-HAV positive control serum >500 mIU/ml, ready for use.	**1 x 1 mL**
NK	**Positive Control, PK,** anti-HAV positive control serum >500 mIU/ml, ready for use.	**1 x 1 mL**
VP	**Dilution Buffer,** ready for use. Please shake before use!	**1 x 120 mL**
WP	**Wash Buffer (WP)** 20-fold concentrated solution.	**1 x 50 mL**
S	**Substrate (S),** ready for use, horseradish-peroxidase-(HRP)-substrate	**1 x 12 mL**
SL	**Stop Solution (SL),** ready for use, 0.2 M sulfuric acid	**1 x 12 mL**
-	Sealing tape	**2**

Additionally for the Semi-Quantitative Test

STD1	**Serum Standard 1**(anti-HAV titer 50 mIU/mL) (STD1) ready for use	**1 x 1 mL**
STD2	**Serum Standard 2**(anti-HAV titer 30 mIU/mL) (STD2) ready for use	**1 x 1 mL**
STD3	**Serum Standard 3**(anti-HAV titer 10 mIU/mL) (STD3) ready for use	**1 x 1 mL**

MATERIALS REQUIRED BUT NOT PROVIDED
Distilled or deionized water for dilution of the Wash Buffer (WP)
Precision pipettes, micropipettes and multichannel pipettes with disposable plastic tips
Polyethylene PE/Polypropylene PP tubes for dilution of samples
Anti-HAV antibody negative serum (for dilutions >1:10 to the semi-quantitative measurement)
Incubator or water bath with adaptor for microtiter plates
Vortex-mixer
Microtiter plate washer (recommended)
Microplate reader ("ELISA-Reader") with filter for 450 and ≥590 nm

6. Technical Notes
Storage Conditions
Store the kit at 2-8°C until its expiration date. Avoid thawing and freezing.

Sorage Life
The shelf life of the components after initial opening is 4 weeks. Store the unused strips and microtiter wells airtight together with the desiccant at 2-8°C in the clip-lock bag. Use in the frame provided. The 1:20 diluted Wash Buffer WP is stable for up to 4 weeks at 2-8°C.
Preparation of Reagents
Bring all reagents to room temperature (20°C -25°C) before use. Possible precipitates in the buffers must be dissolved before usage by mixing and/or warming. Reagents with different lot numbers cannot be mixed.

Dilution

Use the Dilution Buffer **VP** for dilution of the Conjugate concentrate **KK.** Prepare only the amount required. The 1X working conjugate can be stored for up to one week at **4 °C**.

The required volume of Wash Buffer **WP** is prepared by 1:20 dilution of the provided 20-fold concentrate with distilled or deionized water. Please dilute only according to daily requirements.

Incubation
Incubation at room temperature means incubation at 20 – 25 °C. The Substrate Solution S is photosensitive, store and incubate in the dark.

Assay Procedure
When performing the assay, reagents and the samples should be pipetted as quickly as possible (e.g., 15 minutes). To avoid distortions due to differences in incubation times, the diluted Conjugate Concentrate **KK**, as well as the Substrate **S** should be added to the plate in the same order and in the same time interval as the samples. Stop Solution **SL** should be added to the plate in the same order as the Substrate **S**. All determinations should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

Washing
Proper washing is of basic importance for a secure, reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate washing problems. All washing must be performed with the provided wash buffer **WP** diluted to 1X working concentration. Washing volume per washing cycle and well must be at least 300 µl. The danger of handling potentially infectious material must be taken into account. When using an automatic microtiter plate washer, the respective instructions for use must be carefully followed. Device adjustments, e.g. for plate geometry and the provided washing parameters, must be performed. Dispensing and aspirating manifold must not scratch the inside well surface. Provisions must be made that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, this could be controlled, and possible remaining fluid could then be removed, by inverting the plate and repeatedly tapping it dry on lint-free absorbent tissue.

Manual washing is an adequate alternative option. Wash Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamically swinging the microtiter plate over a basin. If aspirating devices are used, care must be taken that the inside well surface is not scratched. After every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on lint-free absorbent tissue.

7. Procedure

Reagent Preparation			
Before use bring all reagents to room temperature: 20°C -25°C			
KK	Conjugate Concentrate	in Dilution Buffer **VP** (e.g. 10 µL KK + 990 µL **VP**)	1:100
WB	Wash Buffer	in deionized/distilled water (DI water) (e.g. 50 mL WP + 950 mL DI water)	1:20
-	Samples	In Dilution Buffer **VP** (1 mL negative serum + 9 mL **VP**)	-
VPN	VP + 10% negative Serum*	In Dilution Buffer **VP** + Serum **VPN** (e.g. 10 µL Sample + 490 µL **VPN**)	>1:10

Assay Procedure in Double Determination			
Pipette	**Reagents**		**Position**
100 µL	-	-	A1/A2
100 µL	Positive Control	PK	B1/B2
100 µL	Negative Control = Standard 4, 0 mIU/mL	NK	C1/C2
100 µL	Standard 1, 50 mIU/mL (only in semi-quantitative measurement)	STD1	D1/D2
100 µL	Standard 2, 30 mIU/mL (only in semi-quantitative measurement)	STD2	E1/E2
100 µL	Standard 3, 10 mIU/mL (only in semi-quantitative measurement)	STD3	F1/F2
100 µL	Samples diluted 1:10 into appropriate wells	According to demand	
Cover the wells with the sealing tape			
Incubation: 2 h at 37°C			
50 µL	1:100 diluted Conjugate Concentrate	From B1 on in each well	
Cover the wells with the sealing tape			
Incubation: 1 h at 37°C			
3 x 300 µL	Aspirate the contents of the wells and wash 3x with 300 µL each 1X working Wash Buffer /well	in each well	
100 µL	Substrate Solution S	in each well	
Substrate S Incubation: 30 minutes in the dark at 20 – 25 °C			
100 µL	Stop Solution **SL**	in each well	
Measure the absorbance within **30 min** at **450 nm** with **≥ 590 nm** as reference wavelength.			

***not included in the test**

Instead of standards contained in the test, an internal standard can be used. This may be the dilution of a reference preparation or prepared by titration of serum against a reference preparation.

For more information on reference standards for this assay contact ALPCO Technical Support at techsupport@alpco.com

8. Quality Control

GLP requires that controls be run with each calibration curve. A statistically significant number of controls should be frequently assayed to establish mean values and acceptable ranges to assure proper performance.

8.1 Quality Criteria

In order to evaluate the results it should be ensured that the absorbance of the blank (A1/A2) **0.1** do not exceed the positive control **PK 0.15** OD_{450} units. The signal of the Negative Control **NK** must be greater than 1.0 OD_{450} units. The difference between the absorbance of the Negative Control **NK** and Positive Control **PK** must be at least 0.4 OD_{450} units.

The absorbances of the samples should be within the standard curve **(NK-STD3)** in the semiquantitative method. If the absorbances are outside this range, for reliable determinations, they should be determined again at higher dilutions in a second test.

9. Evaluation of Results

First, the absorbance determined with the chosen reference wavelength (>590 nm) is subtracted from each absorbance value at 450 nm, regardless of the chosen evaluation method. Following this, the absorbance of the blanks (A1/A2) is also subtracted from all values. The measured values thus obtained are the basis for all further analysis.

9.1 Qualitative Test Analysis

For the qualitative determination of anti-HAV antibodies in human serum samples, a method was created in the context of product development which enables the distinction of positive and negative samples with a sensitivity of 98.3 % (n=801). For this purpose, a cut-off value is calculated from the signal of the positive control and negative control according to the following formula:

$$\text{Cut-off} = \frac{(\text{mean absorbance positive control} + \text{mean absorbance of negative controls})}{2}$$

Samples with mean absorbance values higher than the cut-off value are *negative*. Samples with absorbance values less than the cut-off are *positive*. Samples around ±10 % of the cut-off absorbance should be determined again.

An exemplary test result is shown in Table 1. The absorbance difference between negative and positive control is greater than 0.4 (1.390 to 0.24). The calculation of the cut-off value gives the following result:

$$\text{Cut-off} = \frac{(NK + PK)}{2} = \frac{1.390 + 0.024}{2} = 0.707$$

The cut off value is therefore 0.707. Thus, all samples whose signal is higher than this are classified as negative (containing no anti-HAV antibody) and all samples in which the signal is lower than 0.707 are assessed as positive (containing anti-HAV antibody).

The absorbance of serum sample 1 is greater than the cut-off value, thus, it is negative. The absorbance of serum sample 2 is less than the cut-off value, thus, it is positive. In serum sample 3, the absorbance is in the range of 0.778 – 0.636 (±10 % of 0.707), the determination must be repeated in the above example.

Table 1: Results of a qualitative anti-HAV determination as an example.

Sample:	Absorbance	Mean
Negative Control	1.372	1.390
Negative Control	1.408	
Positive Control	0.024	
Serum Sample 1	1.461	1.387
Serum Sample 1	1.312	
Serum Sample 2	0.025	0.023
Serum Sample 2	0.021	
Serum Sample 3	0.735	0.739
Serum Sample 3	0.743	

The test is valid because the following condition is complied with:
The Extinctions difference between Negative Control (**NK**) and Positive Control (**PK**) > 0.4

9.2 Semi-Quantitative Test Analysis

Antibody titer calculation

In addition to the calculation of the antibody content in mIU/mL by using a defined standard, a semi-quantitative analysis can be carried out by the determination of the antibody titer. For this the unknown sample is used in various dilutions in this ELISA. Depending on the expected level of antibodies, the sample is diluted. For these dilutions, the dilution buffer, including 10% negative serum (VPN) is used. To determine the titer of the (cut-off) value the following formula is used:

$$\text{Cut-off} = \frac{(\text{mean absorbance positive control} + \text{mean absorbance of negative controls})}{2}$$

The serum dilution with an absorbance directly below the cut-off value represents the antibody titer.

Analysis with Standards

A more accurate determination of the serum titer can be carried out with a semi-quantitative test method. For this purpose, the test kit includes 3 standards, which contain a defined amount of anti-HAV antibodies. These are ready-to-use and can be tested directly in the assay.

These samples are used to estimate the anti-HAV titer based on a calibration curve. The absorbance values of each serum standard (STD1-STD3) and the negative control (NK) as a standard with the concentration of 0 mIU/mL are given on the y-axis against the concentration (mIU/mL) of the anti-HAV antibody on the x-axis and the regression line is placed through the points.

For computational evaluation various regression mehods are useful. The method with the best curve fit should be selected. Ideally, the calculation of the antibody content of the sampleis performed by means of an evaluation program. An exemplary standard curve determined by 4-parameter logistics is shown in Figure 1.

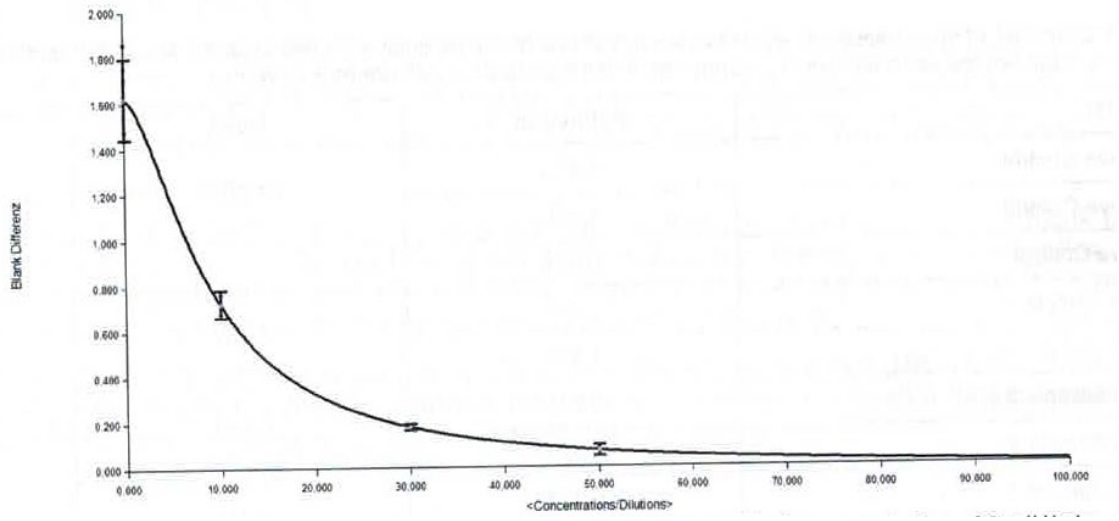

Figure 1. Exemplary standard curve with negative control as standard with the concentration of 0 mIU/mL.

In Table 2, exemplary results of anti-HAV ELISA are shown.

Table 2: An example of absorbance readings with the semi-quantitative method using Standards including negative control as standard with 0 mIU/mL. The sample dilution >1:10 was carried out in dilution buffer with 10% negative serum (VPN). The calculation of the sample was performed using an evaluation program.

		Blank Corrected $OD_{450-620}$	**Anti-HAV Titer in the Diluted Probe (mIU/mL)**	**Anti-HAV Titer in the undiluted sample (mIU/mL)**
Positive Control		0.01	-	-
Negative Control Standard 4		1.631	0	-
Standard 1		0.083	50	
Standard 2		0.190	30	-
Standard 3		0.735	15	-
Sample	Dilution			
Serum 1	1:10	0.089	48.1	481
Serum 2	1:10	0.294	22.2	222
Serum 3	1:10	1.264	4.2	42
Serum 4	1:10	0.598	12.4	618
Serum 5	1:10	0.121	40.0	1998

Analysis with Reference Preparation
In addition, it is also possible to prepare standards in the desired concentrations from reference material to quantify the anti-HAV antibody.

The reference material should be diluted with the Dilution Bufer with 10% anti-HAV antibody negative serum (VPN). An exemplary result is shown in Table 3. The cut-off value for the evaluation of the results shown here is 0.724 OD_{450} units ((0.02 + 1.427)/2). The dilution of the sample, with an absorbance directly below the cut-off value, is chosen for the calculation of the antibody content.

In serum 1 the absorbance of the 1:400 diluted sample is directly below the cut-off. The anti-HAV titer in the diluted sample is 8.8 mIU/mL. The anti-HAV titer of the serum is therefore 8.8 x 400 = 3,520 mIU/mL.
In serum 2, the absorbance of 1:1600 diluted sample is directly below the cut-off. The anti-HAV titer in the diluted sample is 8.5 mIU/mL. The anti-HAV titer in the undiluted serum is therefore 8.5 x 1600 = 13,600 mIU/mL.

Table 3. Exemplary absorbance measurements by the semi-quantitative method using the reference preparation (NIBSC 97/646): The reference preparation and the unknown samples were diluted in Dilution Buffer with 10% negative serum (VPN) to the concentration as given in the table and used in the assay. The calculation of the antibody content of the antibody content of the sample was performed using an evaluation program.

	Mean Absorbance	**anti-HAV titer in the diluted sample (mIU/ml)**
Positive Control:	0.020	-
Negative Control:	1.427	-
Standard 1*	0.068	50
Standard 2*	0.157	30
Standard 3*	0.334	15
Standard 4*	0.542	10
Standard 5*	0.838	5
Standard 6*	0.953	3
Standard 7*	1.245	1
Serum 1:		
1: 100 diluted	0.110	
1: 200 diluted	0.262	
1: 400 diluted	0.571	8.8 x 400= 3520
1: 800 diluted	0.761	
1: 1600 diluted	1.048	
Serum 2:		
1: 100 diluted	0.013	
1: 200 diluted	0.036	
1: 400 diluted	0.088	
1: 800 diluted	0.228	
1: 1600 diluted	0.532	8.5 x 1600 = 13600

*not included in the test kit

10. Limitation of the Procedure
The influence of the heterophilic antibodies, rheumatoid factors, and anti-species antibodies is reduced by the assay design but can not be completely excluded.

11. Performance Characteristics
11.1 Assay Calibration
The assay is calibrated to the International Standard WHO 97/646 material. The traceability of the measured results is shown in Figure 2. Exemplary results for the International Standard NIBSC 97/646 are shown for 30 and 10 mIU/mL for 1997 to 2014.

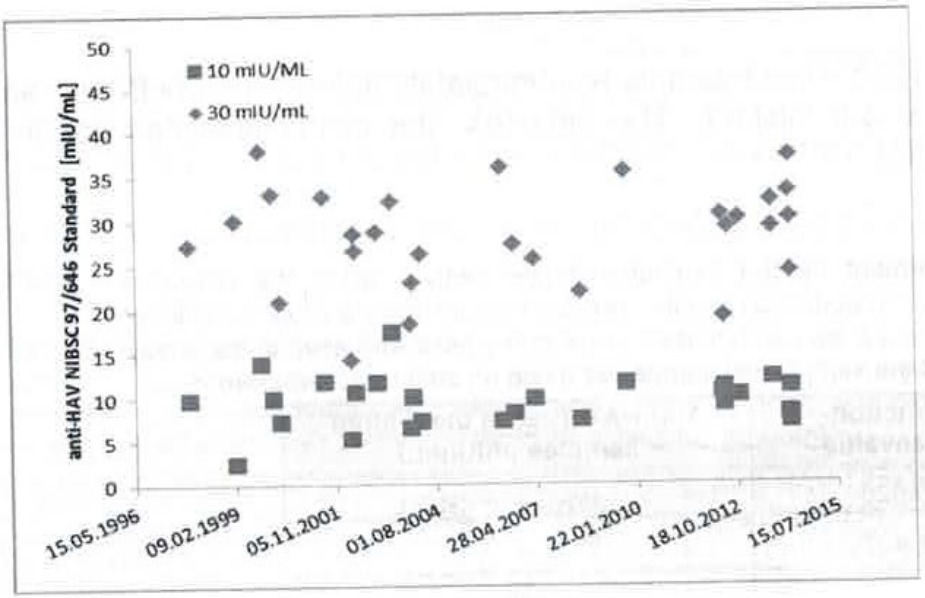

Figure 2 Tracebility of Mediagnost anti HAV Antibody ELISA,

11.2 Sensitivity

The sensitivity of the test system was evaluated by the International Standard NIBSC 97/646. The standard material was diluted and treated as a sample in the kit. The measured signals (OD_{450}) are shown in Table 4.

Table 4. Analytical Sensitivity. Shown are the measured signals (OD_{450})

NIBSC [mIU/mL]	0	2	5	10	20
Replicates	1.577	1.327	1.136	0.925	0.564
	1.495	1.337	1.159	0.83	0.475
	1.587	1.297	1.108	0.898	0.557
	1.329	1.373	1.142	0.79	0.51
	1.562	1.071	1.111	0.819	0.505
	1.278	1.219	1.083	0.854	0.398
		1.225	1.093	0.812	0.464
		1.331	1.199	0.865	0.45
Mean	1.47	1.27	1.13	0.85	0.49
SD	0.13	0.10	0.04	0.05	0.06
CV%	9	8	3	5	11

The recalculated analytical sensitivity resulting from the 2-fold standard deviation of the negative control (n=6) is 1.10 mIU/mL. In an additional experiment the theoretically calculated analytical sensitivity was proven by assessing 1 mIU/mL diluted NIBSC 97/646. Here the negative control showed a signal of 2.5 (SD 0.18) and the diluted international standard had a signal of 2.12 (SD 0.2).

11.3 Specificity

Assessment of the specificity of this ELISA kit was done with 25 samples negative for anti-HAV IgG antibody, but positive for IgG antibodies against different viruses.

Sample Number	Tested Positive For	Antibody	Result with 22-HAVHU-E01
1	Anti-HBc, anti-HBs	IgG	Negative
2	Anti-HBs, anti-HCV	IgG	Negative
3	Anti-HCV	IgG	Negative
4	Anti-HEV	IgG	Negative
5	Anti-HCV, anti-HEV	IgG	Negative
6	Anti-HCV	IgG	Negative
7	Anti-HCV	IgG	Negative
8	Anti-HBe, anti-HBc	IgG, IgG & IgM	Negative
9	Anti-HBs	IgG	Negative
10	Anti-HBs	IgG	Negative
11	Anti-HBs	IgG	Negative
12	Anti-HBs, anti-HBc	IgG, IgG & IgM	Negative
13	Anti-HCV	IgG	Negative
14	Anti-HEV	IgG	Negative
15	Anti-HBs	IgG	Negative
16	Anti-HBs	IgG	Negative
17	Anti-HBs	IgG	Negative
18	Anti-HBs	IgG	Negative
19	Anti-HBe,anti-HBc	IgG, IgG	Negative
20	HBsAG,anti-HBs	IgG, IgG & IgM	Negative
21	Anti-HEV	IgG	Negative
22	Anti-HBs	IgG	Negative
23	Anti-HBs,anti-HBe,anti-HEV	IgG, IgG,IgG	Negative
24	HBsAG,Anti-HBe,anti-HBc	IgG,IgG,IgG & IgM	Negative
25	Anti-HBc, anti-HBs	IgG, IgG	Negative

11.4 Precision Data

Serum samples were measured in at least 13 independent assays and the variation of the measurement result was calculated (Table 6).

Table 6. Inter-assay variation. SD = Standard deviation, CV% = Coefficient of Variation in %. Number = Number of independent determinations.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
Mean	19303	3077	297	378	46	1210	45	27	9	5
SD	2557	378	36	54	13	156	5	3	2	1
CV%	13	12	12	14	29	13	11	13	17	19
Number	20	21	16	19	19	18	30	21	20	13

11.5 Linearity

The linearity of the assay was evaluated by the dilution of two human sera samples with high anti-HAV antibody titer. The dilution of the samples was carried out until 1:10 in Dilution Buffer (VP) and for all dilutions >1:10 in Dilution Buffer + 10% anti-HAV antibody negative serum (VPN). In figure 3 the dilutions of 1:2.5 to 1:80 from serum 1 and 1:5 to 1:320 for serum 2 are shown. Linearity regression of the data demonstrated high linearity.

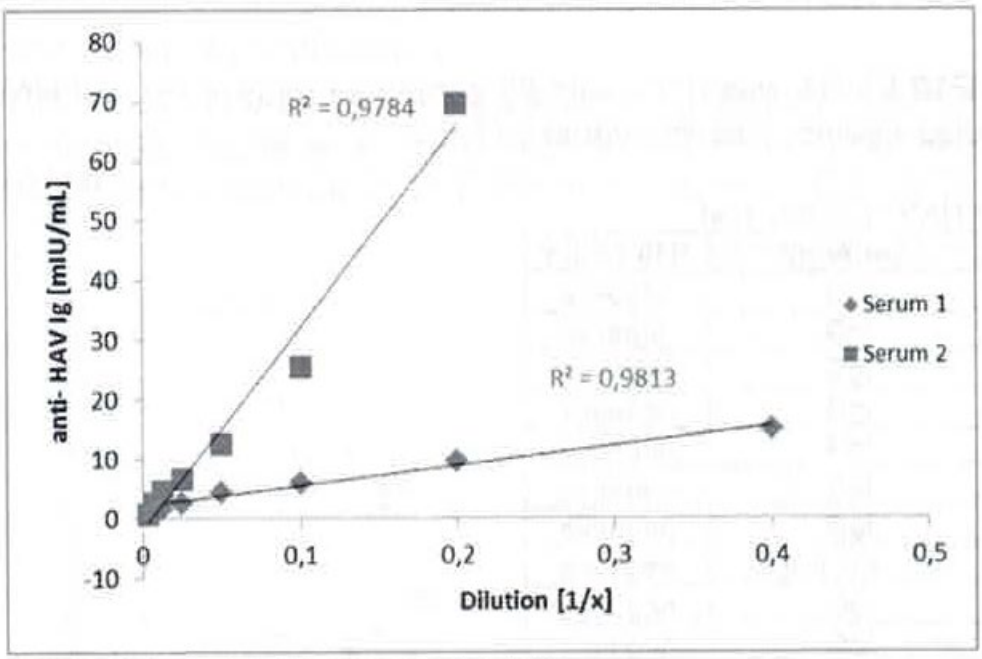

Figure 3 Linearity of the sample dilution.

11.6 Interference

Anticoagulants like EDTA and heparin in the usual concentrations do not influence the test. Bilirubin concentrations do not interfere up to 200 µg/mL and triglyceride concentrations up to 5 mg/mL. Strongly hemolyzed samples (hemoglobin > 10 mg/mL) can result in false negative or false low values. Strongly hemolyzed samples should not be used.

12. LITERATURE

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13. SUMMARY - anti-HAV ELISA

KK	**Conj**	**1:100 in Dilution Buffer VP**
WP	**Washbuf 20X**	**1:20 in distilled water**
Sample		**1:10 in Dilution Buffer VP**
20 - 25 °C		

-	-	A1/A2
100 µl	Positive Control	B1/B2
100 µl	Negative Control	C1/C2
100 µl	Sample Dilution	in the rest of the wells according to requirements
Cover the wells with the sealing tape		
Incubate: 2 h at 37°C		
50 µl	**Conj KK**	Each well except A1/A2
Cover the wells with the sealing tape		
Incubation: 1 h at 37°C		
3 x 300 µl	Aspirate the contents of the wells and wash 3x with **300 µl** 1X working Wash Buffer **WP**.	Each well
100 µl	Substrate Solution **S**	Each well
Incubation: 30 min in the dark at RT		
100 µl	Stop Solution **SL**	Each well
Measure the absorbance within **30 min** at (450/≥590 nm)		