



HBsAg Elisa

KAPG4SGE3

LOT : 090515/1



HBsAg Elisa

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For in-vitro qualitative detection of hepatitis B surface antigen (HBsAg) in human serum or plasma.

KAPG4SGE3

IN VITRO DIAGNOSTIC USE

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1) INTENDED USE

HBsAg Elisa is an enzyme immunoassay diagnostic kit for in-vitro qualitative detection of hepatitis B surface antigen (HBsAg) in human serum or plasma (heparin, citrate or EDTA).

2) SUMMARY AND TEST EXPLANATION

The hepatitis B surface antigen (HBsAg) is the first marker that appears in the blood following infection with hepatitis B virus (HBV) some days or weeks before clinical symptoms manifest. It is a lipoprotein polypeptide which constitutes the external envelope of the HB virus. The detection of HBsAg in human serum or plasma indicates an ongoing HBV infection, either acute or chronic. Testing of additional HBV markers is needed to define the specific disease state. HBsAg assays are used not only to diagnose HBV infections but also to monitor the course of the disease and the efficacy of antiviral therapy.

HBsAg Elisa is a fast test for the qualitative detection of the presence of HBsAg in serum or plasma (heparin, citrate or EDTA) specimen. The test utilizes monoclonal and polyclonal (anti-guinea pig) antibodies to selectively detect elevated levels of HBsAg in serum or plasma.

Specimens which are non-reactive by **HBsAg Elisa** are considered negative for HBsAg. Specimens with positive reaction should be retested in duplicate.

In case of a reactive repeat reaction, the specimen should be confirmed for HBsAg reactivity with validated confirmatory reagents .

Only confirmed positive specimens are considered to contain HBsAg.

3) TEST DESCRIPTION

HBsAg Elisa is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immuno-sorbent assay) based on the "sandwich principle".

The solid phase of the microtiter plate is made of polystyrene wells coated with mouse monoclonal antibodies specific for HBsAg; whereas guinea pig polyclonal antibody purified by affinity chromatography is used to prepare the anti-HBs•peroxidase (horseradish) conjugate in the liquid-phase.

When a serum or plasma specimen containing HBsAg is added to the anti-HBs antibody-coated wells together with the peroxidase conjugated anti-HBs antibody and incubated, an antibody-HBsAg-antibody-peroxidase 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. A color develops in proportion to the amount of HBsAg bound to Anti-HBs. The peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 620 to 690nm^{*1}.

The test principle is shown also in the following figure.

A Specimen containing HBsAg:


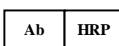


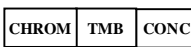
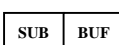
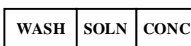
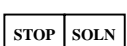
1. Plate well (Anti-HBs) + specimen (HBsAg)+ Anti-HBs•peroxidase → Anti-HBs•HBsAg•(Anti-HBs•peroxidase) sandwich complex
2. Sandwich complex + TMB substrate solution → Light blue to blue color
3. Add sulfuric acid to stop the color development → Read OD at 450nm (reference wavelength 620-690 nm^{*8})

B Specimen without HBsAg:

1. Plate well (Anti-HBs) + specimen (no HBsAg) + Anti-HBs•peroxidase → Anti-HBs (on the well)
2. Anti-HBs (on the well) + TMB substrate solution → Colorless to light blue color
3. Add sulfuric acid to stop the color development → Read OD at 450nm (reference wavelength 620-690 nm^{*8})

4) DESCRIPTION OF MATERIALS PROVIDED

- Item 1 - 6 in the following reagent table should be refrigerated at 2-8°C . Washing Solution D (20X) and stop solution can be stored at 2-30°C.

ITE MS	Components	Description	Qt. per 96 tests
(1)	Anti-HBs Plate 	Microtiter plate coated with mouse monoclonal anti-HBs.	1 plate
(2)	Anti-HBs Peroxidase Solution 	Polyclonal Anti-HBs HRPO conjugate, diluted in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal. Dye: phenol red.	1 bottle, 7 ml
(3)	HBsAg Positive Control 	Inactivated human serum positive for HBsAg but non-reactive for anti-HCV and anti-HIV1/2, diluted in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1 bottle, 1.1 ml
(4)	HB Negative Control 	Serum non-reactive for HBV markers, anti-HCV and anti-HIV1/2, diluted in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1 bottle, 1.6 ml
(5)	Chromogenic TMB concentrate 	0.6 mg/ml of 3,3',5,5'-tetramethylbenzidine (TMB) in 40% methanol with DMSO.	1 bottle, 10 ml
(6)	Substrate buffer 	Citrate Acid Buffer containing 0.03% H ₂ O ₂ .	1 bottle, 10 ml
(7)	Conc. Washing Solution (20X) 	Concentrated Phosphate buffer with Tween-20	1 bottle 52 ml
(8)	Stop Solution 	2N sulfuric acid	1 bottle 12 ml

- OTHER REQUIRED MATERIALS, BUT NOT PROVIDED

ITEMS	Components
(1)	50µl, 100µl micropipettes and tips are needed
(2)	Water bath or incubator with temperature control at +37°C.
(3)	Plate washing equipment.
(4)	ELISA Microwell Reader: Dual wavelength 450nm with 620-690nm as reference wavelength, bandwidth 10nm ^{*1} .
(5)	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 condition and Stability of the kit and components

Kit/components	Storage temp.	State	Stability
HBsAg Elisa KIT	+2 to +8°C	Original	15 months
		Once open	1 month
HBsAg Positive Control	+2 to +8°C	Original	15 months
		Once open	1 month
HB Negative Control	+2 to +8°C	Original	15 months
		Once open	1 month
Anti-HBs Plate	+2 to +8°C	Original	24 months
		Once open	1 month
Anti-HBs· HRPO Conjugate Solution	+2 to +8°C	Original	15 months
		Once open	1 month
Concentrated Washing Solution (20X)	Room temp.	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	18 months
		Once open	1 month
Substrate buffer	+2 to +8°C	Original	18 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 professional use only.
- 5.1.2) This reagent kit is for *in vitro* diagnosis only.
- 5.1.3) Bring all kit reagents and samples to room temperature (+20 to +30°C) and mix carefully before use.
- 5.1.4) Do not use reagent beyond its expiration date.
- 5.1.5) Do not interchange reagents between different lots.
- 5.1.6) Do not put pipette in mouth.
- 5.1.7) Do not smoke or eat in areas where specimens or reagents are handled.
- 5.1.8) All kit components and specimens should be regarded as potential health hazards. It should be used and discarded according to your laboratory's safety procedures. Such safety procedures probably include the wearing of protective gloves and avoiding the use of aerosols.
- 5.1.9) Potential infectious specimens and non-acid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with your practice for potential bio-hazard control.
- 5.1.10) Prior to disposing used specimens and kit reagents as general waste; it should be treated in accordance with the local practice of potential bio-hazardous waste or treated as follows:
Both liquid and solid waste should be autoclaved at +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.
- 5.1.11) Stop Solution is an irritant to skin, eyes, respiratory tract and mucous membranes. Avoid contact of the stop solution with skin and mucous membranes. In case of contact, flush immediately with abundant amounts of water. In case of inhalation, find fresh air and seek medical attention in case of pain.
- 5.1.12) Chromogenic TMB concentrate contains 40% methanol which is toxic: danger of serious irreversible effects through inhalation, in contact with skin and if swallowed. Chromogenic TMB concentrate contains dimethyl sulfoxide, an irritant to skin and mucous membranes.
- 5.1.13) Although all human sourced material are tested non reactive for Anti-HCV and Anti-HIV, and inactivated at +56°C for one hour, the reagent shall be handled as potential infectious material.*2

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 specimens can be used with this test kit. Whole blood specimen 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 avoid 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 homogeneously 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 sera and microbial-contaminated specimens should not be used.

5.3) Storage conditions and Stability of Reagents

- 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 one month after opening the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and taped tightly.
- 5.3.3) Return reagents to +2 to +8°C immediately after use.
- 5.3.4) Washing Solution (20X) Concentrate can be stored at room temperature to avoid crystallization, because the kits are stored and shipped at +2 to +8°C. If crystals have been precipitated before use, warm up the solution at 37°C in a water bath until the crystals are disappeared.

5.4) Plate Washing Procedure

- 5.4.1) Preparation of washing solution:
Dilute Washing Solution (20X) Concentrate with distilled or de-ionized water to obtain a 1:20 dilution. Do not use tap water.
- 5.4.2) Plate washing:
(a) For plate washer with overflow aspirating function: 6 cycles with at least 0.5 ml washing buffer per well per cycle.
or
(b) For plate washer without overflow aspirating function: 8 cycles with at least 0.35 ml washing buffer per well per cycle.
- 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

- 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) Reserve one well for blank. Add 50 µl of each control or specimen to appropriate wells of the microtiter plate (3 Negative Controls and 2 Positive Controls).
NOTE:
 - a. Use a clean pipette tip for each sampling to avoid cross-contamination.
 - b. Each plate needs respective negative controls, positive controls and blank well.
 - c. Do not use any cut-off value established for other plates of HBsAg Elisa.
- 5.5.3) Add 50 µl of Anti-HBs Peroxidase Solution to each well except the blank.
NOTE: Do not touch the wall of the plate wells to prevent contamination.
- 5.5.4) Gently tap the plate.
- 5.5.5) Remove the protective backing from the Adhesive Slip and press it onto the reaction plate, so that it is tightly sealed.
- 5.5.6) Incubate the reaction plate at +37±1°C in a water bath or incubator for **80 minutes**.
- 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) Select one of the following two methods for color development:

- A. Mix equal volumes of **Chromogenic TMB concentrate and Substrate Buffer** in a clean container immediately prior to use. Add **100 ml** of the mixture solution to each well including the blank well.
- B. Add **50ml of Chromogenic TMB concentrate** first, and then add **50 ml of Substrate buffer** into each well including the blank. Mix well carefully.

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 mix. The mixture should be kept away from intense light.

5.5.9) Cover the plate with a 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 30 minutes with a precision spectrophotometer at 450/620-690nm (450nm reading wavelength with 620 – 690 nm reference wavelength)*².

Use the blank well to blank spectrophotometer.

NOTE: The color of the blank should be colorless to light yellow; otherwise, the test results are invalid.

Substrate blank : absorbance value must be less than 0.100.

5.6) Calculations of Results

5.6.1) Calculation of the NC (Mean Absorbance of Negative Control).

Example: Sample No.	Absorbance
1	0.022
2	0.025
3	0.023

$$NC = (0.022 + 0.025 + 0.023) / 3 = 0.023$$

NCx should be \leq 0.1, otherwise, the test is invalid.

5.6.2) Calculation of the PC (Mean Absorbance of Positive Control)

Example: Sample No.	Absorbance
1	1.432
2	1.508

$$PC = (1.432 + 1.508) / 2 = 1.470$$

PC should be \geq 0.6, otherwise, the test is invalid.

5.6.3) Calculation of the P - N Value

$$P - N = PC - NC$$

Example: NC = 0.024

PC = 1.470

$$P - N = 1.470 - 0.024 = 1.446$$

P - N Value must be \geq 0.5, otherwise, the test is invalid.

5.6.4) Calculation of the Cutoff Value

$$\text{Cutoff Value} = NC + 0.025$$

Example:

$$\text{Cutoff Value} = 0.023 + 0.025 = 0.048$$

5.7) Validity of Test Runs

5.7.1) NC should be \leq 0.1; otherwise, the test is invalid.

5.7.2) PC should be \geq 0.6, otherwise, the test is invalid.

5.7.3) P - N Value must be \geq 0.5, otherwise, the test is invalid.

NOTE: Negative Control: absorbance value must be less than or equal to 0.100 after subtracting the blank.

5.8) Interpretation of Results

5.8.1) Specimens with absorbance values **LESS** than the **Cutoff Value** are **NON-REACTIVE** and are considered **NEGATIVE for HBsAg**.

5.8.2) Specimens with absorbance value **GREATER** than or **EQUAL** to the **Cutoff Value** are considered **INITIALLY REACTIVE**. The original specimens must be retested in duplicate.

5.8.3) If both absorbance values in the retest are **less** than the cutoff value, the specimens are considered **NEGATIVE for HBsAg**.

If in the retest at least one of the two absorbance values is **GREATER** than or **EQUAL** to the **Cutoff Value** then the specimens are considered as **repeated HBsAg positive**. The repeated positive specimen shall be confirmed with validated confirmatory reagents.

5.9) Troubleshooting

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

- 5.9.1) Improper washing procedure.
- 5.9.2) Contamination with positive specimen.
- 5.9.3) Wrong volume of sample, conjugate or substrates.
- 5.9.4) Contamination of the well rim with conjugate.
- 5.9.5) Improper specimen, such as hemolyzed serum or plasma, specimen containing sediments and specimen not thoroughly mixed 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 only.
- 5.10.2) The reagent kit has not been validated for use with cadaveric samples.
- 5.10.3) A negative HBsAg result without other evidence should not be used to exclude an HBV infection.
- 5.10.4) Interfering Substances:
The following results were obtained in respective experiments:
 1. No interferences with different anticoagulants such as lithium heparin, EDTA, citrate have been observed.
 2. Heat-treated specimens (+60°C, 10 hours) exhibited diminished HBsAg titer.
 3. No cross reactivity was detected using specimens deriving from patients with a) other infections by HAV, EBV, CMV, HSV, VZV, Lyme Borreliosis, HCV, HIV, b) other disease states such as chronic renal failure, hemodialysis, autoimmune hepatitis, liver cirrhosis, and c) presence of certain antibodies like HAMA, GAD, IA2, APS).
 4. Samples containing potential interfering substances [e.g. triglycerides (lipemia), hemoglobin (hemolysis), bilirubin (icterus), monoclonal immunoglobulin components, elevated levels of autoimmune antibodies (rheumatoid factor-RF, antinuclear antibodies-ANA, or antimitochondrial antibodies-AMA)] and samples from pregnant women did not interfere with the HBsAg Elisa assay.

5.11) Performance Characteristics

5.11.1) Diagnostic Specificity

Results from the European Performance Evaluation for DIAsource HBsAg Elisa - Reactivity of HBV Negative "Donor" and "Clinical" Specimens.

Total No. of Specimens	DIAsource HBsAg Elisa					
	N	Neg	*IR	**RR	Confirmed	False Positive
HBV negative (clinical specimen)	213	211	2	2	0	2
HBV negative (donor specimen)	5501	5479	22	22	0	22
Total	5714	5690	24	24	0	24

*IR: initial reactive **RR: repeat reactive

Diagnostic specificity = $5690/5714 = 99.58\%$

5.11.2) Diagnostic Sensitivity

1. The diagnostic sensitivity determined in the European performance evaluations yielded the following results:

Sample	No. of sample	Reactive	Sensitivity
HBsAg positive sera	400	400	100%

Diagnostic sensitivity = $400/400 = 100\%$

2. The evaluation of the HBsAg Sensitivity Panel PHA807 (BBI, USA) yielded the following results:

Panel	No. of panel members	Results
BBI HBsAg Sensitivity Panel PHA807	PHA807-01 ~ 21	Equivalent to or better than the competitors.

3. The evaluation of the HBsAg Mixed Titer Performance Panel PHA205 (BBI, USA) yielded the following results:

Panel	No. of panel members	Results
BBI HBsAg Mixed Titer Performance Panel PHA205	PHA205-01 ~ 25	Equivalent to or better than the competitors.

4. The HBsAg Mixed Titer Performance Panel VHA601 (BBI, USA) evaluation yielded the following results:

Panel	No. of panel members	Results
BBI HBsAg Mixed Titer Performance Panel VHA601	VHA601-01 ~ 06	Met BBI's expected results.

5. The evaluation of the HBsAg Mixed Titer Performance Panel QHA711 (BBI, USA) yielded the following results:

Panel	No. of panel members	Results
BBI HBsAg Mixed Titer Performance Panel QHA711	QHA711-01 ~ 06	Met BBI's expected results.

6. INTS HBsAg Subtype Panel Test Result:

The results of the evaluation demonstrate that all known HBsAg serotypes (HBsAg subtypes) available in the INTS HBsAg subtype panel can be detected by both HBsAg Elisa and the reference assay up to 10^4 x ~ 10^6 x dilutions.

7. Summary table of the evaluation of all tested seroconversion panels:

PPanel ID	HBsAg Positive Result From Date of First Blood Collection			
	Reference HBsAg Assay (A in days)	HBsAg Elisa (B in days)	Difference in Bleed Days (A-B)	Difference in Bleed #s (panel member No.)
PHM907(ay)	50	50	0	0
PHM916(ay)	62	65	-3	-1
PHM920(ad)	26	26	0	0
PHM921(ad)	0	0	0	0
PHM930(ad)	3	3	0	0
PHM933(ad)	7	9	-2	-1
PHM934(ad)	0	0	0	0
PHM935A (ad)	9	21	-12	-3
PHM935B (ad)	231	217	14	-1
NabiSB0411	6	6	0	0
PHM903(ad)	6	10	-4	-1
PHM904(ad)	7	18	-11	-1
PHM906(ad)	137	0	137	1
PHM909(ad)	9	9	0	0
PHM910(ad)	35	18	17	1
PHM912(ad)	42	0	42	7
PHM914(ad)	0	146	-146	-1
PHM915(ind)	12	28	-16	-5
PHM917(ind)	36	>43	->7	->2
PHM918 (ad)	7	7	0	0
PHM923 (ay)	15	21	-6	-1
PHM924 (ad)	29	35	6	1
PHM925(ind)	8	14	-6	-1
PHM926(ind)	13	15	-2	-1
PHM927(ind)	4	7	-3	-1
PHM928 (ad)	9	9	0	0
PHM929 (ad)	14	18	-2	-1
PHM931(ind)	19	21	-2	-1

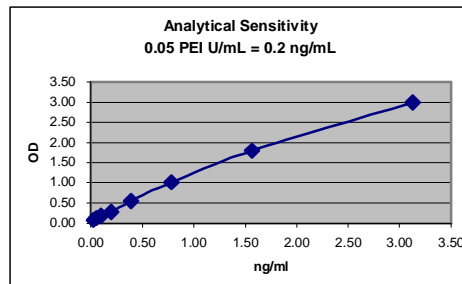
PHM932 (ad)	61	61	0	0
PHM935A (ad)	21	28	-7	-2
Over all	-----	-----	Sum = -13	-----
		Average	-13/30 = 0.4 days	-----

Summary of the evaluation of all tested seroconversion panels:

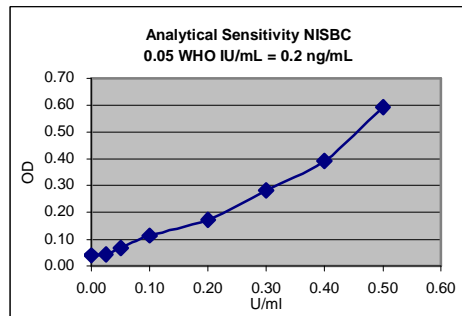
The results have shown that the HBsAg Elisa is nearly equivalent to the reference assay. On average the HBsAg Elisa test is only 0.4 days later in the 30 tested seroconversion panels in comparison to the reference assay.

5.11.3) Analytical Sensitivity and Linearity on Dilution

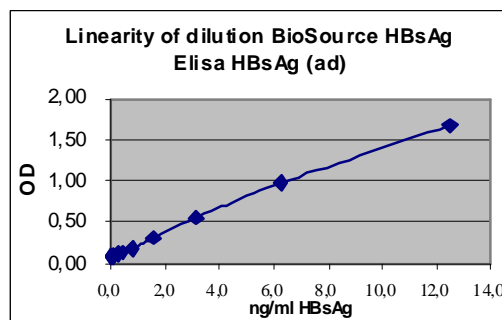
1. Analytical sensitivity determined using the PEI HBsAg standard (subtype ad)



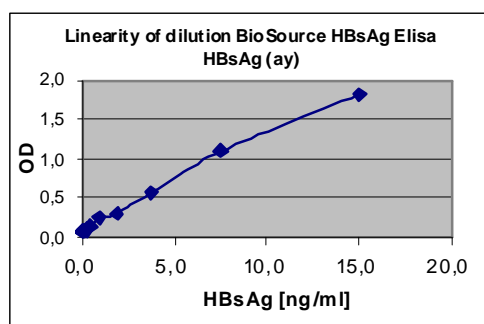
2. Analytical sensitivity determined using the 1st WHO International HBsAg Standard (NIBSC)



3. Analytical sensitivity determined using a patient sample with higher HBsAg (subtype ad) concentration



4. Analytical sensitivity determined using a patient sample with higher HBsAg level



The minimal detectable dose (analytical sensitivity) of HBsAg Elisa was determined as 0.2 ng/ml for all tested standards and patient samples.

5.11.4) Precision

1. Intra-assay reproducibility

Intra-assay precision was determined using one positive control sample and two patient serum samples of different HBsAg concentration (slightly above the cutoff level and at medium level) which were analyzed in replicates of 20 in a single run over 3 days.

Run	Sample ID	Mean	Standard Deviation	Coefficient of Variation
1	PC 1 :32	0.7541	0.0857	11.36
1	PS 1 :32	2.2766	0.1571	6.90
1	PS 1 :64	1.3159	0.1168	8.88
2	PC 1 :32	0.9671	0.0358	3.70
2	PS 1 :32	2.7325	0.1025	3.75
2	PS 1 :64	1.5822	0.0888	5.61
3	PC 1 :32	0.9669	0.0909	9.40
3	PS 1 :32	2.6088	0.2367	9.07
3	PS 1 :64	1.6502	0.1499	9.08

The calculated CV's ranged between 3.7 % and 11.36 %. (= acceptable value for an immunoassay in microtiter plate format).

2. Inter-assay reproducibility

The precision evaluation experiments were performed over 10 operating days is using five serum samples (with borderline positive and clearly above cutoff value HBsAg levels).

Sample ID	N	Mean	Standard Deviation	Coefficient of Variation
F1	10	0.0609	0.0185	30.41
F2	10	0.0770	0.0189	24.56
F3	10	0.1000	0.0245	24.51
F4	10	0.1786	0.0462	25.87
F5	10	0.6107	0.1412	23.12

The calculated CV's ranges between 30.4 for an HBsAg negative sample and 23.1 % for an HBsAg low positive sample (= acceptable values for the inter-assay imprecision of an immunoassay in microtiter plate format).

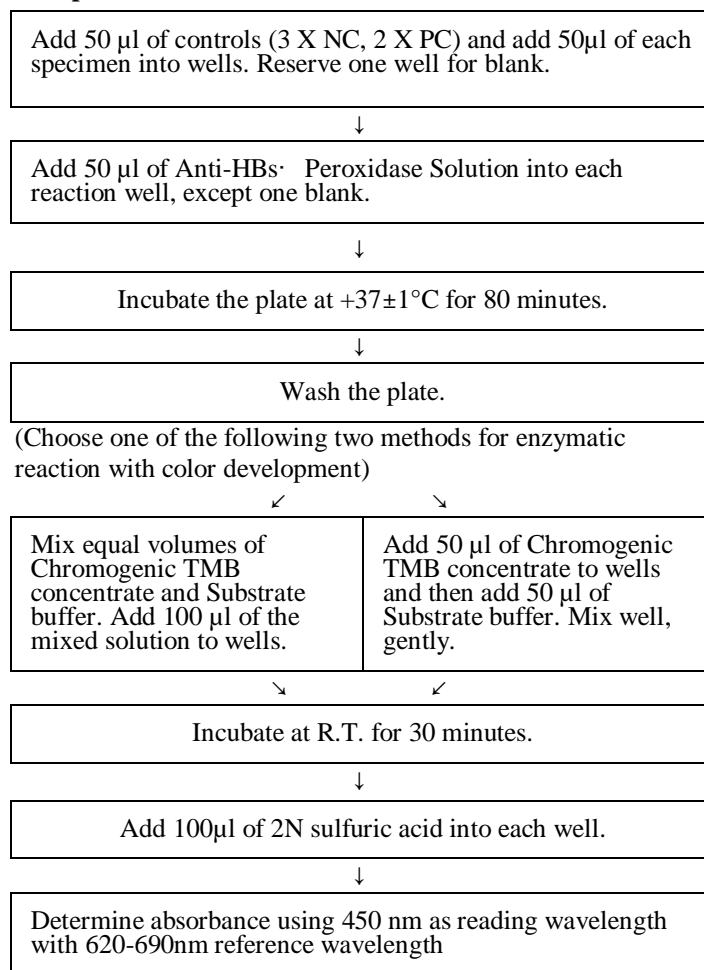
5.11.5) Antigen Excess/High-dose hook effect

This was performed testing a serum sample with a very high HBsAg concentration of 125 mg/L in serial dilution with the DIAsource HBsAg Elisa assay. No high-dose hook effect was observed.

5.11.6) Traceability

The DIAsource HBsAg Master Calibrator has been calibrated against the British Working Standard for HBsAg (NIBSC-Code: 01/476-006) using the **HBsAg Elisa** assay. The relative potency (ratio) of the British Working Standard for HBsAg versus the DIAsource HBsAg Master Calibrator is 4.081 (3.853-4.325 95% CI). The concentration of the Positive Control of **HBsAg Elisa** assay has been determined against the DIAsource HBsAg Master Calibrator and was established with 42 IU/ml \pm 20%.

5.12) Flow chart of the test procedure



6) NOTES

- *1 The reference wavelength of the photometer to be used can be 620 nm to 690 nm. However, the user should validate the photometer in combination with **HBsAg Elisa** before use.
- *2 Incomplete inactivation of hepatitis B virus after heat treatment at +60°C for 10 hours, J. Infect. Dis. 138:242-244.

7) BIBLIOGRAPHY

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	<u>Used symbols</u>	<u>Symboles utilisés</u>
	Consult instructions for use	Consulter les instructions d'utilisation
	Storage temperature	Température de conservation
	Use by	Utiliser jusque
	Batch code	Numéro de lot
	Catalogue number	Référence de catalogue
	Control	Contrôle
	In vitro diagnostic medical device	Dispositif médical de diagnostic in vitro
	Manufacturer	Fabricant
	Contains sufficient for <n> tests	Contenu suffisant pour <n> tests
	Wash solution concentrated	Solution de lavage concentrée
	Zero calibrator	Calibreur zéro
	Calibrator #	Calibreur #
	Control #	Contrôle #
	Tracer	Traceur
	Tracer	Traceur
	Tracer concentrated	Traceur concentré
	Tracer concentrated	Traceur concentré
	Tubes	Tubes
	Incubation buffer	Tampon d'incubation
	Acetonitrile	Acétonitrile
	Serum	Sérum
	Specimen diluent	Diluant du spécimen
	Dilution buffer	Tampon de dilution
	Antiserum	Antisérum
	Immunoabsorbent	Immunoabsorbant
	Calibrator diluent	Diluant de calibrateur
	Reconstitution solution	Solution de reconstitution
	Polyethylene glycol	Glycol Polyéthylène
	Extraction solution	Solution d'extraction
	Elution solution	Solution d'élution
	Bond Elut Silica cartridges	Cartouches Bond Elut Silica
	Pre-treatment solution	Solution de pré-traitement
	Neutralization solution	Solution de neutralisation
	Tracer buffer	Tampon traceur
	Microtiterplate	Microplaque de titration
	HRP Conjugate	HRP Conjugué
	HRP Conjugate	HRP Conjugué
	HRP Conjugate concentrate	HRP Conjugué concentré
	HRP Conjugate concentrate	HRP Conjugué concentré
	Conjugate buffer	Tampon conjugué
	Chromogenic TMB concentrate	Chromogène TMB concentré
	Chromogenic TMB solution	Solution chromogène TMB
	Substrate buffer	Tampon substrat
	Stop solution	Solution d'arrêt
	Incubation serum	Sérum d'incubation
	Buffer	Tampon
	AP Conjugate	AP Conjugué
	Substrate PNPP	Tampon PNPP
	Biotin conjugate concentrate	Biotine conjugué concentré
	Avidine HRP concentrate	Avidine HRP concentré
	Assay buffer	Tampon de test
	Biotin conjugate	Biotine conjugué
	Specific Antibody	Anticorps spécifique
	Streptavidin HRP concentrate	Concentré streptavidine HRP
	Non-specific binding	Liant non spécifique
	2nd Antibody	Second anticorps
	Acidification Buffer	Tampon d'acidification

	<u>Gebruikte symbolen</u>	<u>Gebrauchte Symbolen</u>
	Raadpleeg de gebruiksaanwijzing	Gebrauchsanweisung beachten
	Bewaartemperatuur	Lagern bei
	Houdbaar tot	Verwendbar bis
	Lotnummer	Chargenbezeichnung
	Catalogusnummer	Bestellnummer
	Controle	Kontrolle
	Medisch hulpmiddel voor in-vitro diagnostiek	In Vitro Diagnostikum
	Fabrikant	Hersteller
	Inhoud voldoende voor <n> testen	Ausreichend für <n> Ansätze
	Wasoplossing, geconcentreerd	Waschlösung-Konzentrat
	Nulkalibrator	Null kalibrator
	Kalibrator #	Kalibrator #
	Controle #	Kontrolle #
	Tracer	Tracer
	Tracer	Tracer
	Tracer geconcentreerd	Tracer Konzentrat
	Tracer geconcentreerd	Tracer Konzentrat
	Buisjes	Röhrchen
	Incubatiebuffer	Inkubationspuffer
	Acetonitrile	Azetonitril
	Serum	Humanserum
	Specimen diluent	Probenverdünner
	Verdunningsbuffer	Verdünnungspuffer
	Antiserum	Antiserum
	Immunoabsorbent	Immunoabsorbens
	Kalibratorverdunner	Kalibratorverdünnung
	Reconstitutieplossing	Rekonstitutionslösung
	Polyethyleen glycol	Polyethylenglykol
	Extractieplossing	Extraktionslösung
	Elutieoplossing	Eluierungslösung
	Bond Elut Silica kolom	Bond Elut Silikakartuschen
	Pre-behandelingsoplossing	Vorbehandlungslösung
	Neutralisatieoplossing	Neutralisierungslösung
	Tracerbuffer	Tracer-Puffer
	Microtiterplaat	Mikrotiterplatte
	HRP Conjuaat	HRP Konjugat
	HRP Conjuaat	HRP Konjugat
	HRP Conjuaat geconcentreerd	HRP Konjugat Konzentrat
	HRP Conjuaat geconcentreerd	HRP Konjugat Konzentrat
	Conjuaat buffer	Konjugatpuffer
	Chromogene TMB geconcentreerd	Chromogenes TMB Konzentrat
	Chromogene Oplossing TMB	Farblösung TMB
	Substraatbuffer	Substratpuffer
	Stopoplossing	Stopplösung
	Incubatieserum	Inkubationsserum
	Buffer	Puffer
	AP Conjuaat	AP Konjugat
	Substraat PNPP	Substrat PNPP
	Geconcentreerd Biotine conjuaat	Biotin-Konjugat-Konzentrat
	Geconcentreerd Avidine-HRP conjuaat	Avidin-HRP-Konzentrat
	Assay buffer	Assaypuffer
	Biotine conjuaat	Biotin-Konjugat
	Specifiek antilichaam	Spezifischer Antikörper
	Streptavidine-HRP concentraat	HRP Streptavidinkonzentrat
	Aspecifieke binding	Unspezifische Bindung
	2de antilichaam	Sekundärer Antikörper
	Verzuringbuffer	Ansäuerungspuffer

	<u>Simboli utilizzati</u>	<u>Símbolos utilizados</u>
	Consultare le istruzioni per l'uso	Consultar las instrucciones de uso
	Limitazioni di temperatura	Limitación de temperatura
	Utilizzare entro	Fecha de caducidad
	Numero di lotto	Código de lote
	Numero di catalogo	Número de catálogo
	Controllo	Control
	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico in vitro
	Fabbricante	Fabricante
	Contenuto sufficiente per <n> saggi	Contenido suficiente para <n> ensayos
	Tampone di lavaggio concentrato	Solución de lavado concentrada
	Calibratore zero	Calibrador cero
	Standard #	Calibrador #
	Controllo #	Control #
	Marcato	Trazador
	Marcato	Trazador
	Marcato concentrato	Trazador concentrada
	Marcato concentrato	Trazador concentrada
	Provette	Tubos
	Tampone incubazione	Tampón de incubación
	Acetonitrile	Acetonitrilo
	Siero	Suero
	Diluyente campione	Diluyente de Muestra
	Tampone diluizione	Tampón de dilución
	Antisiero	Antisuero
	Immunoassorbente	Immunoabsorbente
	Diluyente calibratore	Diluyente de calibrador
	Soluzione di ricostituzione	Solución de Reconstitución
	Polietilenglicole	Glicol Polietileno
	Soluzione di estrazione	Solución de extracción
	Soluzione di eluizione	Solución de elución
	Cartucce di silice bond elut	Cartuchos Bond Elut Silica
	Soluzione di pretrattamento	Solución de Pre-tratamiento
	Soluzione di neutralizzazione	Solución de Neutralización
	Tracer Buffer	Tampón de trazador
	Piastra di microtitolazione	Placa de microvaloración
	HRP Coniugato	HRP Conjugado
	HRP Coniugato	HRP Conjugado
	HRP Coniugato concentrato	HRP Conjugado concentrada
	HRP Coniugato concentrato	HRP Conjugado concentrada
	Buffer coniugato	Tampón de Conjugado
	Cromogena TMB concentrato	Cromógena TMB concentrada
	Soluzione cromogena TMB	Solución Cromógena TMB
	Tampone substrato	Tampón de sustrato
	Soluzione di arresto	Solución de Parada
	Incubazione con siero	Suero de Incubación
	Buffer	Tampón
	AP Coniugato	AP Conjugado
	Substrato PNPP	Sustrato PNPP
	Concentrato coniugato con biotina	Concentrado de conjugado de biotina
	Concentrato avidina HRP	Concentrado avidina-HRP
	Soluzione tampone per test	Tampón de ensayo
	Coniugato con biotina	Conjugado de biotina
	Anticorpo Specifico	Anticuerpo específico
	Streptavidina-HRP concentrata	Estreptavidina-HRP Concentrado
	Legame non-specifico	Unión no específica
	2° Anticorpo	Segundo anticuerpo
	Tampone Acidificante	Tampón de Acidificación

	<u>Símbolos utilizados</u>	<u>Använda symboler</u>
	Consulte instruções de utilização	Läs instruktionerna före användning
	Temperatura de conservação	Förvaringstemperatur
	Utilizar antes de	Används av
	Código de lote	Lotnummer
	Número de catálogo	Katalognummer
	Controlo	Kontroll
	Dispositivo médico de diagnóstico in vitro	In vitro diagnostiskt kit
	Fabricante	Tillverkare
	Conteúdo suficiente para <n> testes	Innehållet räcker till <n> prover
	Solução de lavagem concentrada	Tvättlösning, koncentrerad
	Calibrador zero	Nollkalibrerare
	Calibrador #	Kalibrator #
	Controlo #	Kontroll #
	Marcador	Radioisotop, antigen
	Marcador	Radioisotop, antikropp
	Marcador concentrada	Radioisotop, antigen koncentrerad
	Marcador concentrada	Radioisotop, antikropp koncentrerad
	Tubos	Rör
	Tampão de incubação	Inkuberingsbuffert
	Acetonitrilo	Acetonitril
	Soro	Serum
	Diluidor de espécimes	Spädningsbuffert för prover
	Tampão de diluição	Spädningsbuffert
	Anti-soro	Antiserum
	Imunoadsorvente	Immunoadsorberare
	Diluyente do calibrador	Kalibratordiluent
	Solução de Reconstituição	Rekonstitutionslösning
	Poliétileno-glicol	Polyetylen glykol
	Solução de Extração	Extraktionslösning
	Solução de Eluição	Elueringslösning
	Cartuchos de sílica Bond Elut	Silikonpatroner för elueringsbindning
	Solução de pré-tratamento	Förbehandlingslösning
	Solução de neutralização	Neutraliseringslösning
	Tampão Marcador	Tracerbuffert
	Placa de micro titulação	Microtiterplatta
	HRP Conjugação	HRP-konjugat
	HRP Conjugação	HRP-konjugat
	HRP Conjugação concentrada	HRP-konjugat-koncentrat
	HRP Conjugação concentrada	HRP-konjugat-koncentrat
	Conjuge o tampão	Konjugatbuffert
	Cromogénica TMB concentrada	Kromogeniskt TMB-koncentrat
	Solução Cromogénica TMB	Kromogenisk TMB-lösning
	Tampão de substrato	Substratbuffert
	Solução de Paragem	Stopplösning
	Soro de incubação	Inkubationsserum
	Tampão	Buffert
	AP Conjugação	AP-konjugat
	Substrato PNPP	Substrat-PNPP
	Concentrado conjugado de biotina	Biotinkonjugat koncentrat
	Concentrado HRP de avidina	Avidin HRP-koncentrat
	Tampão de ensaio	Provbuffert
	Conjugado de biotina	Biotinkonjugat
	Anticorpo específico	-
	Estreptavidina HRP concentrado	-
	Ligações não específicas	-
	Anticorpo secundário	-
	Tampão de acidificação	-

	<u>Επεξήγηση συμβόλων</u>	<u>Anvendte symboler</u>
	Συμβουλευτείτε τις οδηγίες χρήσης	Læs brugsvejledningen
	Θερμοκρασία αποθήκευσης	Opbevaringstemperatur
	Ημερομηνία λήξης	Anvend inden
	Αριθμός παρτίδας	Batchkode
	Αριθμός καταλόγου	Katalognummer
	Πρότυπο ελέγχου	Kontrol
	In Vitro Διαγνωστικό Ιατροτεχνολογικό προϊόν	Medicinsk udstyr til in vitro-diagnosticering
	Κατασκευαστής	Fabrikant
	Περιεχόμενο επαρκές για «n» εξετάσεις	Indeholder nok til <n> test
	Συμπυκνωμένο διάλυμα έκπλυσης	Koncentreret vaskeopløsning
	Μηδενικός βαθμονομητής	Nul-kalibrator
	Βαθμονομητής #	Kalibrator nr.
	Ορός ελέγχου #	Kontrol nr.
	Ιζηθέτης	Markør
	Ιζηθέτης	Markør
	Χρωμογόνος Ιζηθέτης	Koncentreret markør
	Χρωμογόνος Ιζηθέτης	Koncentreret markør
	Σωληνάριο	Tuber
	Ρυθμιστικό διάλυμα επώασης	Inkubationsbuffer
	Ακετονιτρίλιο	Acetonitril
	Ορός	Serum
	Διάλυμα αραιώσης δειγμάτων	Prøvediluent
	Ρυθμιστικό διάλυμα αραιώσης	Fortyndingsbuffer
	Αντιορός	Antiserum
	Ανοσοπροσροφητικό	Immonoadsorbent
	Αραιωτικό βαθμονομητών	Kalibratordiluent
	Διάλυμα ανασύστασης	Rekonstitueringsopløsning
	Πολυαιθυλενογλυκόλη	Polyetylenglykol
	Διάλυμα εκχύλισης	Ekstraktionsopløsning
	Διάλυμα έκλυσης	Elueringsopløsning
	Φύσιγγες πυριτίου Bond Elut	Patroner med bindingselueringssilica
	Διάλυμα προεπεξεργασίας	Forbehandlingsopløsning
	Διάλυμα εξουδετέρωσης	Neutraliseringsopløsning
	Ρυθμιστικό διάλυμα	Markørbuffer
	Πλάκα μικροτιτλοδότησης	Mikrotiterplade
	HRP Σύζευγμα	HRP-konjugat
	HRP Σύζευγμα	HRP-konjugat
	Χρωμογόνος HRP Σύζευγμα	HRP-konjugat-koncentreret
	Χρωμογόνος HRP Σύζευγμα	HRP-konjugat-koncentreret
	Ρυθμιστικό διάλυμα συζεύγματος	Konjugatbuffer
	Χρωμογόνος TMB	Kromogen TMB-koncentreret
	Διάλυμα χρωμογόνου TMB	Kromogen TMB-opløsning
	Ρυθμιστικό διάλυμα υποστρώματος	Substratbuffer
	Ανασχετικό αντιδραστήριο	Stopopløsning
	Ορός επώασης	Inkubationsserum
	Ρυθμιστικό διάλυμα	Buffer
	AP Σύζευγμα	AP-konjugat
	PNPP υποστρώματος	Substrat PNPP
	Συμπυκνωμένο αντιδραστήριο συζευγμένο με βιοτίνη	Biotin konjugat koncentrat
	Συμπυκνωμένο διάλυμα αβιδίνης-HRP	Avidin HRP koncentrat
	Ρυθμιστικό διάλυμα προσδιορισμού	Prøvebuffer
	αντιδραστήριο συζευγμένο με βιοτίνη	Biotin konjugat
	Ειδικό Αντίσωμα	-
	Συμπυκνωμένη στρεπταβιδίνη συνεξευγμένη με HRP	-
	μη-ειδική δέσμευση	-
	2ο Αντίσωμα	-
	Ρυθμιστικό Διάλυμα όξινο	-

	Stosowane symbole	Használt szimbólumok
	Przed zastosowaniem zapoznać się z instrukcją	Olvassa el a használati útmutatót
	Temperatura przechowywania	Tárolási hőmérséklet
	Zużyć przed	Lejáratí idő
	Kod serii	Gyártási kód
	Numer katalogowy	Katalógus szám
	Kontrola	Kontrol
	Urządzenie medyczne do diagnostyki in vitro	In vitro diagnosztikai eszköz
	Producent	Gyártó
	Zawartość wystarczająca do <n> testów	Tartalma <n> teszt elvégzésére elegendő
	Roztwór płuczący stężony	Mosó folyadék koncentrátum
	Kalibrator zerowy	Zero kalibrátor
	Kalibrator nr	Kalibrátor #
	Kontrola nr	Kontrol #
	Znacznik izotopowy	Nyomjelző izotóp
	Znacznik izotopowy	Nyomjelző izotóp
	Znacznik izotopowy stężony	Nyomjelző izotóp koncentrátum
	Znacznik izotopowy stężony	Nyomjelző izotóp koncentrátum
	Probówki	Csővek
	Wymagana inkubacja buforu	Inkubáló puffer
	Acetonitryl	Acetonitril
	Surowica	Szérum
	Rozcieńczalnik próbki	Mintahígító
	Bufor do rozcieńczania	Hígító puffer
	Antysurowica	Antiszérum
	Immunoabsorbent	Immunadsorbens
	Rozcieńczalnik kalibratora	Kalibrátor hígító
	Roztwór do rozcieńczania	Mintaelőkészítő oldat
	Glikol poli(oksy)etylenowy	Polietylén glikol
	Roztwór ekstrakcyjny	Extraktív oldat
	Roztwór elucyjny	Eluáló oldat
	Kolumny krzemionkowe Bond Elut	Bond Elut Silica szilikagél patronok
	Roztwór do przygotowania wstępnego	Előkezelő oldat
	Roztwór neutralizujący	Semlegesítő oldat
	Bufor znacznika	Nyomjelző izotóp hígító puffer
	mikroplytka	Mikrotiter lemez
	Koniugat peroksydazy chrzanowej	HRP konjugátum
	Koniugat peroksydazy chrzanowej	HRP konjugátum
	Koncentrat koniugatu peroksydazy chrzanowej	HRP konjugátum koncentrátum
	Koncentrat koniugatu peroksydazy chrzanowej	HRP konjugátum koncentrátum
	Bufor do koniugacji	Konjugátum puffer
	Koncentrat chromogenu TMB (czterometrylobenzodyny)	Kromogén TMB koncentrátum
	Roztwór chromogenu TMB (czterometrylobenzodyny)	Kromogén TMB oldat
	Bufor substratu	Szubsztrát puffer
	Roztwór zatrzymujący reakcję	Stop oldat
	Wymagana inkubacja surowicy	Inkubációs szérum
	Bufor	Puffer
	Koniugat AP (fosfatazy alkalicznej)	AP konjugátum
	p-nitrofenylofosforan substratowy	Szubsztrát PNPP
	Koncentrat koniugatu biotyny	Biotin konjugátum koncentrátum
	Koncentrat peroksydazy chrzanowej z awidyną	Avidin HRP koncentrátum
	Bufor do oznaczania	Vizsgáló puffer
	Koniugatu biotyny	Biotin konjugátum
	Przeciwciało swoiste	Specifikus ellenanyag
	Koncentrat streptawidyny HRP	Sztreptawidin HRP koncentrátum
	Wiązanie nieswoiste	Nem-specifikus kötődés
	Drugie przeciwciało	Másodlagos ellenanyag
	Bufor zakwaszający	Savas puffer

	<u>Използвани символи</u>
	Вижте инструкцията за работа
	Температура на съхранение
	Използвайте с
LOT	Партиден код
REF	Каталожен номер
CONTROL	Контрол
IVD	Ин витро диагностично медицинско изделие
	Производител
	Съдържание достатъчно за <n> теста
WASH SOLN CONC	Концентриран измиващ разтвор
CAL 0	Нулев калибратор
CAL N	Калибратор #
CONTROL N	Контрол #
Ag 125I	Трейсър
Ab 125I	Трейсър
Ag 125I CONC	Концентриран маркер
Ab 125I CONC	Концентриран маркер
	Епруветки
INC BUF	Инкубационен буфер
ACETONITRILE	Ацетонитрил
SERUM	Серум
DIL SPE	Разредител за пробите
DIL BUF	Буфер за разреждане
ANTISERUM	Антисерум
IMMUNOABSORBENT	Имуноабсорбент
DIL CAL	Разредител за калибратора
REC SOLN	Пресъздаващ разтвор
PEG	Полиетилен гликол
EXTR SOLN	Екстрактов разтвор
ELU SOLN	Разтвор за елюиране
GEL	Силикагелни пълнители
PRE SOLN	Пред-лечебен разтвор
NEUTR SOLN	Неутрализиращ разтвор
TRACEUR BUF	Маркерен буфер
ULI	Микротигърна пластина
Ab HRP	HRP конюгат / Конюгат на хрянова пероксидаза
Ag HRP	HRP конюгат / Конюгат на хрянова пероксидаза
Ab HRP CONC	HRP конюгиран концентрат
Ag HRP CONC	HRP конюгиран концентрат
CONJ BUF	Буфер за конюгата
CHROM TMB CONC	Хромогенен ТМВ концентрат
CHROM TMB	Хромогенен ТМВ разтвор
SUB BUF	Субстратен буфер
STOP SOLN	Стоп разтвор
INC SER	Инкубационен серум
BUF	Буфер
Ab AP	AP конюгат / конюгат на алкална фосфатаза
SUB PNPP	Субстрат PNPP / пара нитрофенил фосфат
BIOT CONJ CONC	Биотин конюгиран концентрат
AVID HRP CONC	Авидин HRP концентрат
ASS BUF	Буфер за пробите
Ab BIOT	Биотин конюгат
Ab	специфично антитяло
SAV HRP CONC	стрептавидин HRP концентрат
NSB	не специфично свързване
2nd Ab	второ антитяло
ACID BUF	киселинизиращ буфер