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Instructions for use

IgA Saliva ELISA

REF **SA E-6800**

96

+8 +2 °C

IVD

CE

IgA Saliva ELISA

INTENDED USE

Immunoenzymatic colorimetric method for quantitative determination of h-IgA in saliva.

CLINICAL SIGNIFICANCE

IgA represents about 15% to 20% of immunoglobulins in the blood, although it is primarily secreted across the mucosal tract into the stomach and intestines. This prevents microbes from binding to epithelial cells in the digestive and respiratory tracts. This immunoglobulin helps to fight against pathogens that contact the body surface, are ingested, or are inhaled. It exists in two forms, IgA1 (90%) and IgA2 (10%) that differ in the structure. IgA1 is found in serum and made by bone marrow B cells, however IgA2 is made by B cells located in the mucosae and has been found to secrete into, colostrum, maternal milk, tears and saliva. The IgA found in secretions have a special form. They are dimeric molecules, linked by two additional chains. One of these is the J chain (from join), which is a polypeptide of molecular mass 1.5 kD, rich with cysteine and structurally completely different from other immunoglobulin chains. The dimeric form of IgA in the outer secretions also has a polypeptide of the same molecular mass (1.5 kD) called the secretory chain and is produced by epithelial cells. Decreased or absent IgA, termed selective IgA deficiency, can be a clinically significant immunodeficiency.

PRINCIPLE

The h-IgA saliva ELISA test is based on simultaneous binding of human IgA to two antibodies, one monoclonal immobilized on microwell plates, the other, polyclonal conjugates with horseradish peroxidase (HPR). After incubation the bound/free separation is performed by a simple solid-phase washing, then the substrate solution (TMB) is added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbance is determinated.
The h-IgA concentration in the sample is calculated based on a series of standard. The color intensity is proportional to the h-IgA concentration in the sample.

REAGENT, MATERIAL AND INSTRUMENTATION

Reagent and material supplied in the kit

Standards

	Cat. no.	Standard	Concentration	Volume/Vial
STANDARD A	**SA E-6801**	Standard 0	0 µg/ml	1 ml
STANDARD B	**SA E-6802**	Standard 1	6.9 µg/ml	1 ml
STANDARD C	**SA E-6803**	Standard 2	62 µg/ml	1 ml
STANDARD D	**SA E-6804**	Standard 3	132 µg/ml	1 ml
STANDARD E	**SA E-6805**	Standard 4	400 µg/ml	1 ml

Conversion: pg/mL x 3.5 = pmol/L

ASSAY-BUFF 5x **SA E-6813 Assay Buffer concentrate**

1 vial, 40 ml, Hepes buffer 25 mM pH 7.4; BSA 0.5 g/L, see „Preparation of Reagents".

CONJUGATE-CONC **SA E-6840 Enzyme Conjugate concentrate**

1 vial, 0.7 mL, Conjugated anti-IgA, see „Preparation of Reagents".

96 **SA E-6831 Coated Microplate**

Anti-IgA adsorbed on microplate

WASH-CONC 50x **SA E-0030 Conc. Wash Solution 50X**

1 vial, 20 mL (50X concentrated); contains Phosphate buffer 50 mM pH 7.4; Tween20 1gr/l, see „Preparation of Reagents".

SUBSTRATE **MS E-0055 TMB-substrate**

(1 bottle) 12 mL; H_2O_2-TMB 0.26 g/L (avoid any skin contact)

STOP-SOLN **MS E-0080 Stop solution**

(1 bottle) 12 mL; Sulphuric acid 0.15 mol/L (avoid any skin contact)

Reagents necessary not supplied

Distilled water.

Auxiliary materials and instrumentation

Automatic dispenser. Microplates reader

Note

Store all reagents between 2°C - 8C° in the dark.
Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close immediately after use. Do not remove the adhesive sheets on the strips unutilized.

PRECAUTION

- The reagents contain Proclin 300 as preservative.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Use only reagents supplied in the kit.
- This method allows the determination of IgA from 0.5 µg/mL to 400 µg/mL.

PROCEDURE

Preparation of the Standard (S0, S1, S2, S3, S4)

The standards have approximately the following concentration: 0; 6.9; 62; 132, 400 **ng/mL**.
Once open, the standards are stable six months at 2°C - 8°C.
The standard concentration are 1000 times lower than the values reported in the reference range because in this method the samples are diluted 1/ 1000 while the standards are not diluted.
The concentrations to be entered in the instruments for calculations are:

	S0	S1	S2	S3	S4
µg/mL	0	6.9	62	132	400

Preparation of IgA Assay Buffer

Dilute contents of IgA Assay Buffer Conc. with 160 mL of distilled or deionized water in a suitable storage container.
Store at 2°C - 8°C until expiration date printed on label.

Preparation of Enzyme Conjugate

Prepare immediately before use.
Add 50 µl Enzyme Conjugate Conc. to 1.0 mL of diluted IgA Assay Buffer.
The quantity of diluted conjugate is proportional at the number of tests.
Mix gently for 5 minutes, with rotating mixer.
Stable for 3 hours at room temperature.

Preparation of Wash solution

Dilute contents of Wash Concentrate to 1000 ml with distilled or deionized water in a suitable storage container.
Store at room temperature 20°C - 27°C for up to 60 days.

Preparation of the Sample

For sample collection it is advised to use a centrifuge glass tube and a plastic straw. Let the saliva flow down trough the straw into the centrifuge glass tube; then centrifuge at 3000 rpm per 15 minutes.
Do not use plastic tube or commercially available devices for the saliva collection to avoid false results.

Dilution A (1:20):
50 µL liquid sample supernatant + 950 µL diluted Assay Buffer;
Mix gently by leaving it for at least 5 minutes on a rotating shaker

Dilution B (1:50):
20 µL Dilution A + 980 µL diluted Assay Buffer
Mix gently by leaving it for at least 5 minutes on a rotating shaker.

The finally dilution obtained is 1:1000.

If the assay is not carried out in the same day of collection store the saliva at -20°C.

PROCEDURE

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S0-S4), two for each sample, one for Blank.

Pipette:

	Standard	**Sample**	**Blank**
Diluted sample	---	25 µL	---
Standard S_0 –S_4	25 µL	---	---
Diluted Conjugate	100 µL	100 µL	---

Incubate at room temperature (22°C - 28°C) for *1 hour*.
Remove the contents from each well; wash the wells with 300 µL of diluted wash solution. Repeat for three times the washing procedure by draining the water completely.

Pipette:

	Standard	**Sample**	**Blank**
TMB-Substrate	100 µL	100 µL	100 µL

Incubate at room temperature (22°C - 28°C) for 15 minutes in the dark.

Pipette:

	Standard	**Sample**	**Blank**
Stop Solution	100 µL	100 µL	100 µL

Read the absorbance (E) at 450 nm against Blank.

QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of IgA for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

LIMITATION OF PROCEDURE

Assay Performance

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic or haemolysed specimen(s) should similarly not be used. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

Interpretation of results

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

RESULTS

Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample.

Standard Curve – Automatic method

To use the method: 4 parameter logistic, sigmoid logistic or smoothed cubic spline like calculation algorithm.

Standard Curve – Manual method

A dose response curve is used to ascertain the concentration of h-IgA in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader.
2. Plot the absorbance for each duplicate serum reference versus the corresponding h-IgA concentration in µg/ml on linear graph paper
3. Connect the point with a best-fit curve
4. To determine the concentration of h-IgA for unknown samples, locate the average absorbance of the duplicates for each unknown sample on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated).

REFERENCE VALUE

Based on the literature data and on the results obtained with the h-IgA ELISA, an highly summarized range of normality is: 40-170 µg/mL

PERFORMANCE AND CHARACTERISTICS

Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

h IgA	100.0 %
h IgA1	124.5 %
h IgA2	145.5 %
h IgG	<0.3 %
h IgM	<0.3 %

Sensitivity

The lowest detectable concentration of h-IgA that can be distinguished from the zero standard is 0.5 µg/mL at the 95 % confidence limit.

Correlation with RIA

The h-IgA ELISA was compared to another commercially available h-IgA assay.
22 Serum samples were analysed according in both test systems.
The linear regression curve was calculated $y = 1.5865x - 7.614$; $r = 0.9478$ ($r^2 = 0.8984$)

Hook Effect

The h-IgA ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to 600 µg/mL

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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TROUBLESHOOTING

POSSIBLE ERROR CAUSES / SUGGESTIONS

No colorimetric reaction
- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)
- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)
- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers
- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed)

too high within run (CV%)
- reagents and/or strips not pre-warmed to room temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run (CV%)
- incubation conditions not constant (time, temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

For actual literature, information about clinical significance or any other information please contact your local supplier.

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content		CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!