Instructions for Use

IgA Saliva ELISA

Enzyme immunoassay for the in-vitro determination of human IgA
IgA in saliva.

REF DM59171

Σ 96

2-8°C

For research use only.
Not for use in diagnostic procedures.

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

1. 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.

2. 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 determined. 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.

3. REAGENT, MATERIAL AND INSTRUMENTATION
3.1. Reagent and material supplied in the kit
1. IgA Standards 5x (1 vial = 1 mL)
   STD0
   STD1
   STD2
   STD3
   STD4
2. IgA Conc. Assay Buffer (5x) (1 bottle) 40 ml
   Hepes buffer 25 mM pH 7.4; BSA 0.5 gr/L
3. Conjugate (1 vial) 0.7 mL
   Conjugated anti-IgA-HRP
4. Coated Microplate (1 microplate breakable)
   Anti-IgA adsorbed on microplate
5. TMB-substrate (1 bottle) 12 mL
   H₂O₂,TMB 0.25gr/L (avoid any skin contact)
6. Stop solution (1 bottle) 12 mL
   Sulphuric acid 0.15 mol/L (avoid any skin contact)
7. Conc. Wash Solution (50x) (1 bottle) 20 ml
   NaCl 9 g/L, Tween 1 g/L, proclin-300 0.1 mL/L

3.2. Reagents necessary not supplied
Distilled water.

3.3. Auxiliary materials and instrumentation
Automatic dispenser.
Microplates reader

Note
Store all reagents between 2–8°C 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.
4. PRECAUTION
- The reagents contain Proclin 300R 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 500 µg/mL.

5. PROCEDURE

5.1. Preparation of the Standard (S₀, S₁, S₂, S₃, S₄)
The standard has approximately the following concentration: 0; 6.9; 62; 132, 400 ng/mL. Once open, the standards are stable six months at 2–8°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:

<table>
<thead>
<tr>
<th>µg/ml</th>
<th>S₀</th>
<th>S₁</th>
<th>S₂</th>
<th>S₃</th>
<th>S₄</th>
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<tr>
<td></td>
<td>0</td>
<td>6.9</td>
<td>62</td>
<td>132</td>
<td>400</td>
</tr>
</tbody>
</table>

5.2. Preparation of IgA Assay Buffer
Dilute contents of IgA Assay Buffer Conc. with 160 ml with distilled or deionized water in a suitable storage container. Store at room temperature 20–27°C for up to 60 days.

5.3. Preparation of Conjugate
Prepare immediately before use. Add 50 ml stock solution (reagent 3) to 1.0 mL of Diluted IgA Assay Buffer (reagent 2). 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.

5.4. 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 20–27°C for up to 60 days.

5.5. Preparation of the Sample
For sample collection is advised to use a centrifuge glass tube and a plastic straw. Let the saliva flow down through 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. Prepare the A solution by dilution of sample supernatant liquid 1:20 with diluted Assay Buffer (50 µL at 1 mL); after that mix gently the A solution by leaving it for at least 5 minutes on a rotating shaker and dilute this 1:50 with diluted Assay Buffer (20 µL at 1 mL). Finally dilution obtained 1:1000. Mix gently by leaving it for at least 5 minutes on a rotating shaker.

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

5.6. 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.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Standard</th>
<th>Sample</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>25 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard S₀–S₄</td>
<td>25 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diluted conjugate</td>
<td>100 µL</td>
<td>100 µL</td>
<td></td>
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</table>

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

<table>
<thead>
<tr>
<th>Substrate</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubate at room temperature 22–28°C for 15 minutes in the dark</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop solution</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

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

6. 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.

7. LIMITATION OF PROCEDURE
7.1. Assay Performance
Sample(s), which are contaminated microbically, 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.

7.2. 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.

8. RESULTS
8.1. Mean Absorbance
Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample.

8.2. Standard Curve – Automatic method
To use the method: 4 parameter logistic, sigmoid logistic or smoothed cubic spline like calculation algorithm.

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).

9. REFERENCE VALUE
Based on the literature data and on the results obtained with the h-IgA IBL kit, an highly summarized range of normality is:
40-170 µg/mL

10. PERFORMANCE AND CHARACTERISTICS
10.1. 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 %  |

10.2. 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.

10.3. Correlation with RIA
The IBL h-IgA ELISA was compared to another commercially available h-IgA assay. 22 samples were analysed according in both test systems. The linear regression curve was calculated:
y = 1.5865x – 7.614
r= 0.9478 (r² = 0.8984)

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

11. WASTE MANAGEMENT
Reagents must be disposed off in accordance with local regulations.

BIBLIOGRAPHY


Ed 01/08
Symbols / Symbole / Symbôles / Símbolos / Σύμβολα

<table>
<thead>
<tr>
<th>REF</th>
<th>Cat.-No.:</th>
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<th>N.-Cat.:</th>
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<td>Chargen-Bez.:</td>
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<td>Lot-No.:</td>
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<td>Usado por:</td>
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<td>No. de Determ.:</td>
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<td>Concentré</td>
<td>Concentrar</td>
<td>Concentrado</td>
<td>Συμπύκνωμα</td>
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<td></td>
<td>Lyophilized</td>
<td>Lyophilisat</td>
<td>Lyophilisé</td>
<td>Liofilizado</td>
<td>Liofilizzato</td>
<td>Λυοφιλιασμένο</td>
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<tr>
<td></td>
<td>Keep away from heat or direct sun light.</td>
<td>Vor Hitze und direkter Sonneneinstrahlung schützen.</td>
<td>Garder à l’abri de la chaleur et de toute exposition lumineuse.</td>
<td>Manténgase alejado del calor o la luz solar directa.</td>
<td>Non esporre ai raggi solari.</td>
<td>Φυλάσσεται μακριά από θέρμανση και άμεση επαφή με το φως του ηλίου.</td>
</tr>
<tr>
<td></td>
<td>Store at:</td>
<td>Lagern bei:</td>
<td>Stocker à:</td>
<td>Almacene a:</td>
<td>Armazenar a:</td>
<td>Conservare a:</td>
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<td></td>
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<td>Caution!</td>
<td>Vorsicht!</td>
<td>Attention!</td>
<td>¡Precaución!</td>
<td>Cuidado!</td>
<td>Attenzione!</td>
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Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλεύετε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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<td>WEB: <a href="http://www.IBL-Transatlantic.com">http://www.IBL-Transatlantic.com</a></td>
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**LIABILITY:** Complaints will only be accepted in written and if all details of the test performance and results are included (complaint form available from IBL or supplier). Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

Symbols Version 3.5 / 2008-10-01