



# Instruction Manual

REF 5001

April 19<sup>th</sup> 2006

## INTENDED USE

**Human IgA is used for the quantitative determination of human immunoglobulin A in human serum for the diagnosis of immunoglobulin A deficiency.**

# Human IgA

- 96 determinations -

IVD

*In-vitro diagnostic device*



Enzyme immunoassay for the determination of immunoglobulin A in human serum

Immunoglobulin A (IgA) is the second most common immunoglobulin in human serum (after IgG) and is the predominant immunoglobulin found in mucosal secretions. It is synthesized with a mean rate of 60 mg/Kg and day by plasma cells. The half-life of IgA in serum is 4.5 to 5.9 days. Structurally, IgA has 2 different forms. Serum IgA is mainly a monomer with molecular weight of 160000. Secretory IgA is a dimer connected by a J chain and containing a secretory component, a glycoprotein with a molecular weight of 7000. This characteristic makes this unique immunoglobulin resistant to the proteolytic enzymes found in many human secretions.

IgA class antibodies can neutralize viruses, bind toxins, agglutinate bacteria, prevent bacteria from binding to mucosal epithelial cells, and bind to various food antigens. In aggregated form IgA activates complement on the alternative way.

IgA does not pass the placental barrier and is missing in fetal blood. Children reach normal adult IgA serum concentration with an age of about 12. Adult reference value for serum IgA ranges from 0.7 to 5 g/l.

Immunoglobulin A deficiency is a primary immunodeficiency disease and is the most common of the primary antibody deficiencies. Total IgA deficiency is defined as undetectable serum IgA at a value of 0.05 g/l in humans. Partial IgA deficiency refers to detectable but decreased IgA levels, more than 2 standard deviations below normal age-adjusted means.

Daele J, Zicot AF: Humoral immunodeficiency in recurrent upper respiratory tract infections. Some basic, clinical and therapeutic features. Acta Otorhinolaryngol Belg 2000; 54(3): 373-90

REF

Catalogue number

LOT

Batch code



Consult accompanying documents



Manufactured by



Temperature limitation



Use by



Consult operating instruction



Biological risk

## PRINCIPLE OF THE TEST

Human IgA is an enzyme immunoassay based on isotype-specific high-affine polyclonal antibodies raised in sheep. The assay is used for the quantitative determination of immunoglobulin A in human serum.

Human immunoglobulin A from standards, control and diluted patient samples react with anti-human IgA antibodies immobilized on the solid phase of microtiter plates. Following an incubation period of 30 min at room temperature (RT), unbound components are removed by a wash step.

The bound human IgA react specifically with polyclonal anti-human IgA antibodies conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at RT. Excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the antibody concentrations of the standards (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve.



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## PATIENT SAMPLES

### Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic or contaminated samples should not be run.

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

### Sample preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

**Note:** *Serum samples have to be diluted 1 + 5.000 (v/v) prior to the assay in two dilution steps*

e.g. 10 µl serum + 0,5 ml sample diluent (C) (1+50) and  
10 µl (1+50) + 1.0 ml sample diluent (C) (1+5.000)

## TEST COMPONENTS FOR 96 DETERMINATIONS

<b>A</b>	<b>Microtiter plate</b> , 12 breakable strips per 8 wells coated with polyclonal antibodies (sheep) to human IGA	1 vacuum sealed with desiccant
<b>Ag</b> <b>96</b>		
<b>B</b>	<b>Concentrated wash buffer</b> sufficient for 2000 ml solution	20 ml concentrate capped white
<b>BUF</b> <b>WASH</b>	<b>100 x</b>	
<b>C</b>	<b>Diluent</b>	2 x 100 ml ready for use capped black
<b>DIL</b>		
<b>D</b>	<b>Conjugate</b> containing anti-human-IgA (sheep) coupled with HRP	15 ml ready for use capped red
<b>CONJ</b>		
<b>E</b>	<b>Substrate</b> 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxid	15 ml ready for use capped blue
<b>SOLN</b> <b>TMB</b>		
<b>F</b>	<b>Stop solution</b> 0.25 sulfuric acid	15 ml ready for use capped yellow
<b>H2SO4</b>	<b>0.25 M</b>	
<b>1 - 5</b>	<b>Calibrators</b> (diluted serum) conc.: see leaflet enclosed	1 ml each ready for use capped white
<b>CAL</b>		
<b>P</b>	<b>Positive control</b> (diluted serum) conc.: see leaflet enclosed	1 ml ready for use capped green
<b>CONTROL</b>		

### Materials required

- variable micropipettes
- multi-channel pipette or multi-pipette
- trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- distilled or de-ionized water
- glassware
- Eppendorf reaction tubes (2 ml)
- incubator (37 °C)
- microplate reader with wavelength for 450nm and 620 nm or 690 nm

### Size and storage

Human IgA has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the Human IgA assay have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

### Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 100 times (1+99) with de-ionized or distilled water. For example, dilute 2 ml of the concentrate with 198 ml of distilled water.

The diluted solution prepared is stable at 2 - 8 °C up to 30 days.

Make sure the soak time of the wash buffer in the wells is 10 to 15 seconds per wash cycle and that the remaining fluid is completely drained off in every wash cycle.

Avoid exposure of the TMB substrate solution to light!

## ASSAY PROCEDURE

- Dilute samples with sample diluent (C) 1 + 5.000 (v/v), e.g. 10 µl serum + 0.5 ml sample diluent (C) (1+50) and 10 µl (1+50) + 1.0 ml sample diluent (C) (1+5.000)
- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently, avoid foam.
2. Dispense  
100 µl calibrators 1 - 5  
100 µl positive control P  
100 µl diluted patient samples  
into the respective wells.
3. Seal plate, incubate **30 min** at room temperature (18-25°C).
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Seal plate, incubate **30 min** at room temperature (18-25°C).
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 min** at room temperature (18-25°C) protected from light.
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

## DATA PROCESSING

**We recommend log / lin processing for best results.**

The standard curve is established by plotting the mean OD-values of the standards 1 - 5 on the ordinate, y-axis, (lin scale) versus their respective human IgA concentrations on the abscissa, x-axis, (log scale).

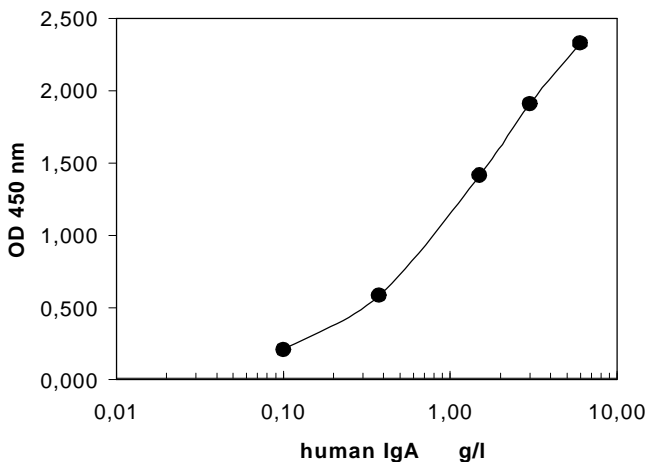
Using the recommended serum dilution of 1 + 5.000 (v/v) human IgA concentrations of the unknown samples are directly read off in g/l against the respective OD values from the standard curve. The concentrations of the standards are stated with regard to the recommended sample dilution. By use of other sample dilutions their IgA concentrations have to be calculated considering the dilution factor.

Human IgA may be used also with Computer Assisted Analysis using software able to plot log/lin curves with four-parameter fit.

### Example of Typical Assay Results

Well	OD (a)	OD (b)	OD (mean)	IgA g/l
Standard 1	0.196	0.220	0.208	<b>0.100</b>
Standard 2	0.575	0.591	0.583	<b>0.375</b>
Standard 3	1.408	1.424	1.415	<b>1.500</b>
Standard 4	1.893	1.911	1.910	<b>3.000</b>
Standard 5	2.251	2.408	2.330	<b>6.000</b>
Positive control	0.858	0.899	0.878	<b>0.650</b>

### TYPICAL STANDARD CURVE



### Test validity

The test run is valid if:

- the mean OD of the standard 1 is  $\leq 0.50$
- the mean OD of the standard 5 is  $\geq 1.20$

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

### Limitations of Method

As the absorbance values could vary from test to test, the standard curve has to be included in every test run.

Contaminated test reagents as well as contaminated samples can cause false results. Also cross contaminations of the kit reagents and samples can cause false results.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

## REFERENCE VALUES

Human IgA	g/l
Normal adult serum	<b>0.7 – 5.0</b>
total IgA deficiency (adult)	<b>&lt; 0.05</b>

Normal IgA concentrations in children are lower:

$< 6$ months	0.05 - 0.35 g/l
7 - 24 months	0.1 - 1.5
3 - 12 years	0.3 - 3.0
13 - 16 years	0.5 - 3.5

## PERFORMANCE CHARACTERISTICS

### Linearity

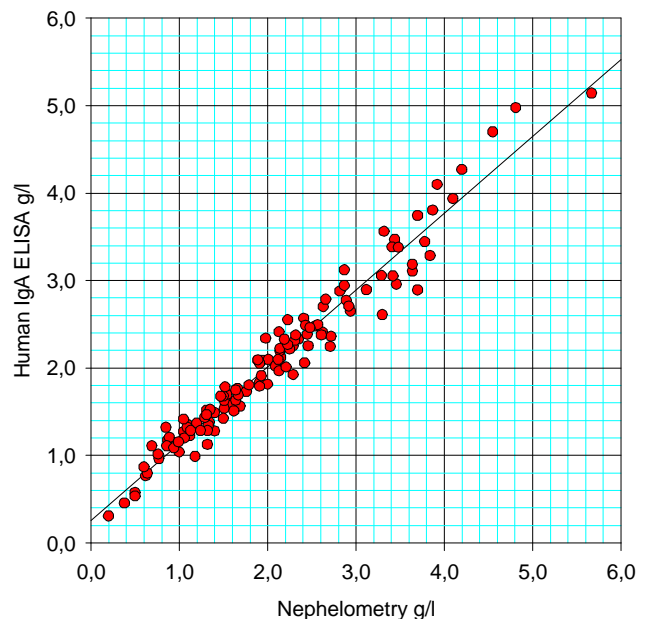
Positive serum samples have been tested by this assay and found to dilute linearly. However, due to the heterogeneous nature of human sera there might be samples that do not follow this rule.

### Precision

Intraassay (n = 8)		Interassay (n = 4 x 8)	
mean (g/l)	CV %	mean (g/l)	CV %
1.702	4.55	1.924	6.96
1.679	2.89	1.794	4.46
1.588	2.69	1.659	4.50
1.323	2.14	1.258	4.86

### Method correlation

Comparison of IgA determination in 123 human serum samples using the Human IgA ELISA and Nephelometry:



Coefficient of correlation:  $r = 0.9785$

## INCUBATION SCHEME

# Human IgA (5001)

**Dilute patients sample 1 + 5.000  
in 2 steps**

**10 µl sample + 0.5 ml sample diluent (C) (1+50)  
10 µl (1+50) + 1.0 ml sample diluent (C) (1+5.000)**

1	Bring all ready for use reagents to room temperature (18-25°C) before use.				
2	Pipette	Calibrators (1 - 5) Positive Control (P) prediluted patient sera	100 µl	100 µl	100 µl
3	Incubate 30 minutes at room temperature (18-25°C)				
4	Wash Decant, Dispense 3 x 300 µl (made of B)				
5	Pipette conjugate (D)		100 µl	100 µl	100 µl
6	Incubate 30 minutes at room temperature (18-25°C)				
7	Wash Decant, Dispense 3 x 300 µl (made of B)				
8	Pipette substrate (E)		100 µl	100 µl	100 µl
9	Incubate protected from light 15 minutes at room temperature (18-25°C)				
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm				

## SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.