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FACULTY OF MEDICINE & ALLIED SCIENCE
MEDICAL TECHNOLOGY PROGRAM

Laboratory Procedure Handout

IMMUNOGLOBULIN A

"IgA"

Immunodiffusion "single diffusion" precipitation immunoassay

INTRODUCTION

IgA is present in **two forms**: one in the **serum** and the other in **various body secretions.**

1. Serum IgA, represents about **15%** to **20%** of the total serum immunoglobulins and is present on **normal serum** at a **concentration** of **approximately 200 mg/dl**.

A. Structure

B.

(a) In humans, over **80%** of **serum IgA** exists in a **monomeric form** with an **S value** of 7S.
(b) The **rest** exists as **dimers**, **trimers**, or **tetramers**. In these **polymeric forms** of **IgA**, the **monomeric units** are **linked** by **disulfide bonds and J chains**.

C. Biological and chemical properties

(a) IgA has a **half-life** of approximately **6 days**.
(b) IgA does not bind complement via the classical pathway but may do so via the altrnative pathway.
(c) IgA can be **inactivated by** an **IgA protease produced by** gonococci, meningococci, pneumococci, and Haemophilus influenzae

2. Secretory IgA (sIgA) is the predominant immunoglobulin in various secretions (saliva; tears; colostrum, bronchial, genitourinary, and intestinal secretions).

A. Structure

(1) The sIgA molecule consists of two monomeric units plus J chain and secretory component. It has an S value of 11 S.

Secretory component (secretory piece) is a polypeptide chain with a
(a) molecular weight of about 70 kDa.
(b) It is joined to sIgA by disulfide bonds.
(c) It serves as a receptor for IgA on the surface of epithelial cells lining exocrine glands and is important in the secretion of sIgA.

(2) The dominant subclass of sIgA is **sIgA2**. The sIgA2 molecule is unique for its absence of H-L bonds; this subclass instead has L-L bonds.

A. Biological and chemical properties

(1) Secretory component is synthesized by exocrine epithelial cells and enables dimeric IgA to pass through the mucosal tissues into the secretions.

a) The epithelial cells bear an IgA-specific receptor.
b) After binding IgA, the receptor-IgA complexis internalized by endocytosis, transported across the cell cytoplasm, and extruded into the external secretions.
c) As the complex is extruded, proteolytic cleavage of the receptor leaves a fragment, the secretory component, attached by a dislufide obnd to the IgA dimer.
d) Secretory component appears to protect IgA from mammalian proteases.

(1) Secretory IgA functions in several ways

a) It protects mucosal surfaces by reacting with adhesion molecules on the suface of potential pathogens and interfering with their adherence and colonization.
b) It may also opsonize foreign particles, as polymorphonuclear neutrophils (PMNs) have Fc α R in their membranes.

Diagnostic Significance

IgA is elevated in the serum in the case of chronic infectious diseases (e.g., chronic hepatitis). Cirrhoses of liver are associated with a marked increase in IgA which correlates with the degree of severity of the toxic inflammatory liver damage. In the case of alcoholic cirrhosis the IgA rises to about fourfold the normal mean value. In IgA-myeloma the IgA is greatly increased, while the remaining immunoglobulins are reduced, thus giving rise to the symptoms of an antibody deficiency. The various forms of the antibody deficiency syndrome are associated with low concentrations of one or more immunoglobulin classes. In the case of autoimmune diseases (e.g., lupus erythematosus, progressive rheumatoid arthritis) IgA is increased in the serum.

PRINCIPLE

Single (one component is **fixed**) radial immunodiffusion is a precipitation reaction, where the antigen is soluble (**in this case the antigen is the immunoglobulin** with the **α chain "IgA"**). **The antigen-antibody interaction take places** in a **semisolid medium** (e.g., **agarose-gel layer**), "**bands" of precipitation will form**.

REAGENTS

Petri dish contains agarose-gel layer, contains **monospecific antiserum** to **human IgA (α chain)**. These **antisera** is **obtained** by the **immunization** of **rabbits**, **sheep**, **horses, pigs** or **goats**. The **preservatives** used in the agar is approximately 1g/l **sodium azide**, and **sodium p-ethyl-mercury-mercapto-benzene-sulfonate** (max. 0.1g/l) The used peti dish is supplied by the **NOR-Partigen company**. The kit is supplied with a **control** and **standards** to **be run along with the specimens** . The **assay range**: **0.365-5.52 g/l (IFCC) or 0.42-6.34 g/l (Behring)**.

Stability & Storage

The NOR Partigen used up to the date given on the label when stored in the original unopened pack at +2 to + 8 C. It is imperative to protect t he plate from freezing (e.g., in the vicinity of the deep-freeze compartment of a refrigerator). Once opened, a plate should be used within a maximum of 4 weeks.

SPECIMINS

Plasma or **serum** sample which are **as fresh as possible or** have been **stored deep-frozen**.

METHOD

1. Remove the plastic container, and allow the opened plate to stand for about 5 minutes at room temp for evaporation of any condensed water which may have penetrated into the wells.

2. Dispense exactly 5 µl = 0.005ml (volume required per well) [use Hamilton Microtiter syringe, Eppendorf Micropipette, Behring dispenser, or Partigen dispenser] undiluted patient's serum (except if a suspected IgA-paraproteinaemia, the sample should be examined in a dilution from about 1 + 5 to 1 + 20), control , and the standards.

1st. Procedure A. (Table of calibration values / 1 control serum).
For checking the accuracy of NOR-Partigen IgA, introduce control serum for NOR Partigen into well 1. Wells 2 to 12 are intended for the specimens to be examined.

2nd. Procedure B. (Reference curve / 3 standard solutions).
As an alternative to the routine determination in accordance with procedure A the IgA determination may also be effected by plotting a reference curve. Behring supplies three prediluted standard solutions for this purpose. Introduce Ig/C3c standard serum (human) for NOR Partigen (solutions 1, 2, 3) into wells 1 to 3. Wells 4 to 12 are intended for the specimens to be examined. The accuracy of the method may be checked by introducing the control serum for NOR Partigen into one well. After introduction of the specimens allow the plate to stand tightly closed at room temperature.

EVALUATION

Measurement of the diameters

After expiration of a diffusion period of **2 days measure** the **diameters D** of the **precipitates** to **an accuracy of 0.1 mm using a suitable device** such as the measurement template for NOR Partigen, **a scaled magnifying glass against a black background with lateral illumination**, or the Behringwerke Measuring Viewer for immunoanalysis. **In the case of precipitate ring diameters D > 8.0 mm, the result should be rechecked later, to enable a correction to be made where there has been further diffusion**. **In the case of deviations in the precipitate ring diameter of ± 0.4 mm or more, the error for the result is of a magnitude greater than ± 15 %. The absolute error** is **greater** in the **lower assay range**, **precipitin ring diameters D < 5.5 mm**, **than in the upper range**.

Evaluation after attainment of the diffusion end-point

Procedure A

The corresponding assay results may be ascertained by reading the values from the appended Table of Calibration Values for the precipitate ring diameters measured. The accuracy of these results is checked by means of Control serum for NOR Partigen; in this connection the batch-dependent precipitate ring diameter given in the Table of Assigned Value must be confirmed within the confidence range (D = ± 0.3 mm). Confirmation of the assigned values for the control serum for NOR Partigen also guarantees the accuracy of the assay results for the specimens examined.

Procedure B

The squares of the diameters of the precipitates from the standard solutions (wells 1 to 3) are plotted on linear millimeter graph paper as a function of the standard concentrations;

Abscissa: antigen concentration in g/l (Behring) or g/l (IFCC)
Ordinate: squares of the ring diameters in mm

The result is a straight line whose intersection with the ordinate should lie between 8.5 and 13.5 mm^2. The IgA concentrations corresponding to the precipitate diameters of the patient sera are ascertained from this reference curve.

Evaluation after 18 hours diffusion

Procedure A

At IgA concentration up to about 2.60 g/l (D = 6.5 mm) the diffusion end-point is already attained after 18 hours. With diameters D> 6.5 mm an enlargement of the precipitate is to be expected at a later reading. The readings obtained after 18 hours have the following inferential value for an early diagnosis:

---- concentrations 20 to 125 % of the normal = hypoproteinaemia or normal finding
---- concentration > 125 % of the normal = normal finding or hyperproteinaemia

The exact results in the hyperproteinemic range can be ascertained after the diffusion end-point has been reached.

Procedure B

The diameters of the 3 standard solutions are plotted on semilogarithmic millimeter graph paper as a function of the standard concentration:

Abscissa: log antigen concentration in g/l (Behring) or g/l (IFCC)
Ordinate: ring diameter in mm

The result is a straight line which permits a relatively exact early evaluation. In the case of an IgA-paraproteinaemia (monoclonal IgA) the IgA determined can differ from the results with other methods because of a possible difference form the physicochemical and immunochemical properties of the polyclonal IgA.

Reference values

	Mean value			Range of variation		
	g / l (IFCC)	g / l (Behring)	% of the normal	g / l (IFCC)	g / l (Behring)	% of the normal
IgA content in the serum of healthy central European men (15 to 64 years old)	1.83	2.10	100	0.783-3.92	0.90-4.50	43-214
Children (8 to 10 years old)	1.31	1.51	72	± 0.497	± 0.55	± 28
Neonates (2 to 8 days old)	0.0026	0.003	<0.2	± 0.0087	± 0.01	

References

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