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Laboratory Procedure Handout
IMMUNOGLOBULIN G

"IgG"

Immunodiffusion "single diffusion" precipitation immunoassay

INTRODUCTION

IgG is the **major immunoglobulin in human serum**, accounting for approximately **75%** of the total normal immunoglobulin pool at a **concentration** of approximately **1200 mg/dl**. .

A. Structure

- **a)** **IgG** is a **monomer** consisting of **identical pairs of H and L chains linked** by **disulfide bridges**.
- **b)** **Four subclasses** of IgG have been identified, **based on H chain differences**: subclasses **IgG1, IgG2, IgG3**, and **IgG4** correspond to **H chains** γ_1, γ_2, γ_3 , and γ_4.

B. Biological and chemical properties

(a) Most IgG subclasses have a molecular weight of 150 kDa and an S value of 7S; IgG3 is slightly larger, at 170 kDa.
(b) Most serum IgG is IgG1

- **c)** IgG is the only immunoglobulin that can cross the placenta in humans; therefore, maternal IgG provides most of the protection of the newborn during the first months of life [secretory IgA (sIgA) in colostrum protects the infant's gastrointestinal tract].
- **d)** IgG molecules are capable of binding complement by the classical pathway (except for IgG4, which activates by the alternative pathway). The binding site for complement component C1q is the C_H2 domain.
- **e)** IgG is the major antibody produced in the secondary immune response

(1) IgG has a half-life of approximately 21 days (IgG3 has a half-life of only 7 days).
(2) Effective antitoxic immunity is exclusively IgG.

(f) IgG is the major opsonizing immunoglobulin in phagocytosis; neutrophil have receptors for the Fc fragments of IgG1 and IgG3.

Diagnostic Significance

Elevated serum IgG concentrations are to be found especially in the chronic stage of infectious diseases. In active alcoholic cirrhosis the IgG attains values twice as high as the normal mean value. Patients with IgG-myeloma have greatly elevated IgG values, while the remaining immunoglobulins are reduced, thereby giving rise to the symptoms of an antibody deficiency. The various forms of the antibody deficiency syndrome are associated with low concentration of one or more immunoglobulin classes. In the case of autoimmune diseases (e.g., lupus erythematosus, progressive rheumatoid arthritis) IgG is increased in serum. In the nephritic syndrome a severe depletion of serum IgG is detectable in consequence of the loss of protein through the kidneys.

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 **γ chain "IgG"**). **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 IgG (γ 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**: **2,3-34,7 g/l (IFCC) or 2,50-37,7 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 IgG-paraproteinaemia, the sample should be examined in a dilution from about 1 + 2 to 1 + 10), control , and the standards.**

A. Procedure A. (Table of calibration values / 1 control serum).

For checking the accuracy of NOR-Partigen IgG, introduce neither control serum for NOR Partigen into well 1. Wells 2 to 12 are intended for the specimens to be examined.

B. Procedure B. (Reference curve / 3 standard solutions).

As an alternative to the routine determination in accordance with procedure A the IgG determination may also be effected by plotting a reference curve. Behring supplies three prediluted standard solutions for this purpose. Introduce neither 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 neither 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 Partingen; 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 IgG concentrations corresponding to the precipitate diameters of the patient sera are ascertained from this reference curve.

Evaluation after 18 hours diffusion

Procedure A

At IgG concentration up to about 16.0 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 IgG-paraproteinaemia (monoclonal IgG) the IgG determined can differ from the results with other methods because of a possible difference form the physicochemical and immunochemical properties of the polyclonal IgG

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
IgG content in the serum of healthy central European men (15 to 64 years old)	11.5	12.50	100	7.36-16.56	8.00-18.00	64-144
Children (11 to 15 years old)	11.02	11.98	96	± 3.31	± 3.60	± 29
Neonates (2 to 8 days old)	10.71	11.64	93	± 2.77	± 3.01	± 24
Infants 5-6 months old	3.19	3.47	28	± 1.26	1.37	±11

References

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