Detection of specific antibodies

IMMUNE RESPONSE

Immune response to an infection agent can be **innate** (nonadaptive) or **adaptive** (acquired) and could be schematically demonstrated as in the figure 1 (ref. 1).

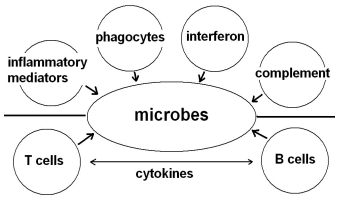


Fig.1. The components of the innate immunity characterised by physiological barriers and fast responses (top) and the adaptive immune system consists of cells displaying recognition molecules and has the capacity for long-term memory (ref.1).

ANTIGEN & ANTIBODIES

Antigens (Ag) are to the body foreign substances (e.g. structural parts of microbes) that creates an antibody production (fig. 2).
Antibodies (Ab) - immunoglobulins (Ig) are produced by plasma cells to which B cells are precursors. They make up about 20% of plasma proteins (ref.1).

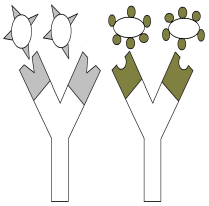


Fig.2. After processed by an antigen presenting cell Ag activate B and T cells through receptors for that specific Ag on their surface (displayed Ig molecules in the case of B cells) (ref.1). Activated T cell stimulate clonal selection of plasma cells which produce and secrete specific Ab. There are a few Ig classes (IgG, IgA, IgE, IgM, IgD) from which IgM is produced in early stage of an infection. Production of Ab is significantly higher if the host encounters the same Ag a second time because of memory cells.

BASIC SEROLOGICAL METHODS

Serologic reactions can be used to identify **antigens** or **antibodies** in patient sera, if either of these reagents is known. The detection of antigens is a direct method, while the detection of antibodies is indirect. Quantification (titration) is keeping one the reagent constant and diluting the other. The methods and application for antigen detection was described in a previous course (*Direct detection of infectious agent*). Many of serological methods and are known but only the basic ones are described here.

Agglutination reaction: Specific antigens and antibodies tend to adhere in groups (coagulate) when mixed. This could be visualized by binding either of the components onto erythrocytes (hemagluttination) or latex particles (latexagglutination) (fig.3). Sensitivity of the method is not high but its simple and low-cost.

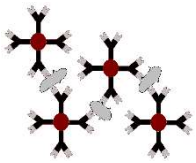


Fig.3. A schematic diagram of latex agglutination: Ag (grey particles) is mixed with labeled specific antibodies (black with grey hypervariable region) from patient sera to create complex. The aggregation crates large, visible clumps, leaving the supernatant clear. In agglutination both IgM and IgG react in the same way and could therefore not be distinguished.

Complement fixation reaction: The proteins of the complement system binds to Ag-Ab complexes. If specific Ab are present in the serum of the patient they will bind to corresponding Ag and consume the complement. Added RBCs sensitised by anti-erythrocytic Ab will therefore not undergo hemolysis as complement no longer is avaliable.
ELISA (*Enzyme-linked immunosorbent assay*): A specific Ab is attached to the bottom of a container, and binds specific antigens that may be present in the serum of a patient. Nonbinding Ag is washed away. The bound Ag allows a Ab labeled with enzymatic activity resulting in color change to bind to the Ab-Ag complex. (fig. 4). The method has high sensitivity and specificity. It could also be used oppositely by attaching Ag to the bottom. Then particular Ig classes could be differentiated. The method is technically and financially more demanding.

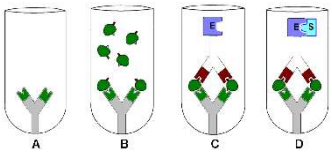


Fig.4. The principle of the ELISA method. The secondary Ab could by labeled by an enzyme creating a chromophore and detected spectrophotometrically or by an fluorochrome and detected by immunofluorescent microscopy

Western blott (Immunoblott) (fig.5): Ag (proteins) are electrophoretically separated in a gel (A) and blotted (transferred) onto a membrane (B). If the patient's sera contain specific primary Ab (C1) to the Ag using secondary (C2) enzyme labeled Ab (C3) and substrate (C4) they are detected as bands with particular size (D).

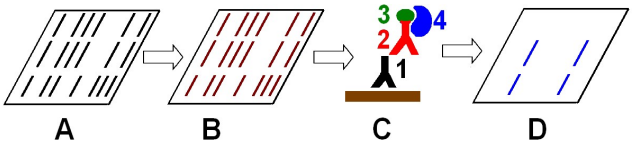


Fig.5. Schematical diagram of Western blott analysis

Inhibition reaction: The principle is blocking of biological properties of the Ag (hemolysis, hemaglutination, cytopatic effect) by Ab in patient sera (e.g. virus neutralization test).

SEROLOGICAL METHODS & APPLICATION

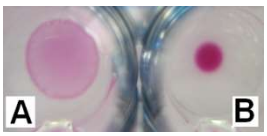


Fig.6. Agglutination assay for detection of specific Ab to Treponema pallidum. Gelatine particles are senzitized with the purified agent. The sensitized particles agglutinate if specific Ab are present in the patient's sera (right). Negative control (B).

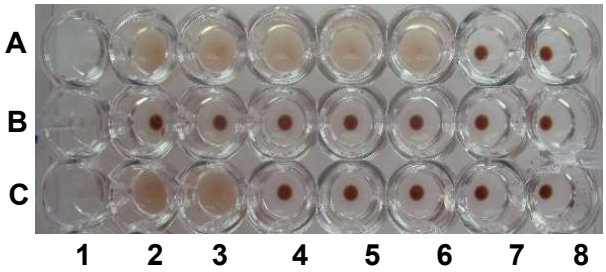


Fig.7. Hemagglutination assay for detection of specific somatic antigens of Yersinia enterocolitica (serotype 03, enteric pathogen). Erythrocytes are senzitized with purified Ag of Y. enterocolitica. The sensitized erythrocytes agglutinate if specific Ab are presented in patient sera. Patients´ sera (A1, B1,C1) are diluted geometrically from dilution 1:10. Patient A has titre of specific Ab 1:160, patient B negative, patient C has titre 1:20.

Fig.8. Reagin (RPR) nontreponemal test for detection of Treponema pallidum infection. Reagin is a cardiolipin (diphosphatydylglycerol from an animal heart). Positive patient sera react with the reagin containing charcoal producing visible agglutination.

Fig.9. Western blott analysis of patient sera containing specific Ab against Borrelia burgdorferi Ag. Positive control (above, start – blue line) with bands of Ab specific for particular Ag. Bands specific for Ag p17 and p19 (left).

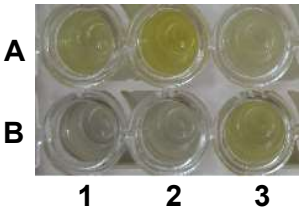


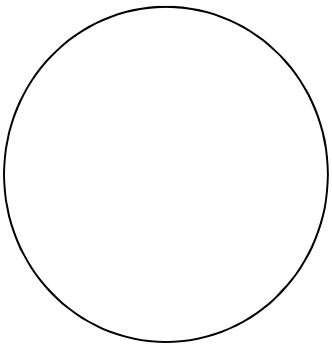
Fig.10. ELISA for detection of specific IgG againt Mycoplasma pneumoniae in patient sera. Positive (A1), negative (B1) controls and cut off value (B2) should be included also in the test. Positive (A2, B3) and negative patient samples.

reference:

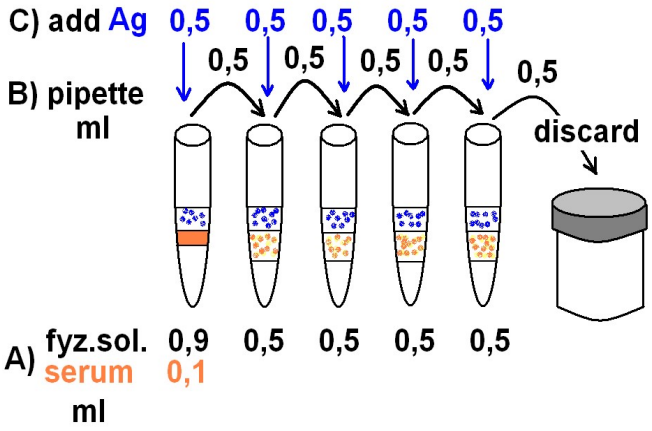
1. Brooks G F et al. Jawetz, Melnick & Adelbergs medical Microbiology, 24th edition, 2007
2. Žampach P. Interpretation of serological analysis, Klin mikrob inf lék, 2007, 13:5-8

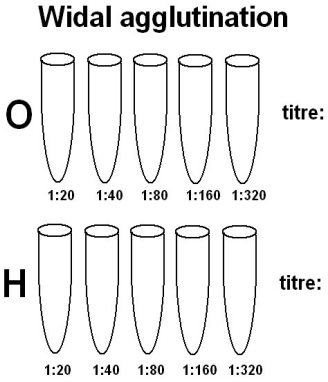
PRACTICAL PART – DETECTION OF SPECIFIC ANTIBODIES

1. VLDR is nontreponemal test for detection of specific Ab against *Treponema pallidum* with reagin (the principle is analogous to RPR test, more information described above). Labeled reagin react with the specific Ab in patient serum to develop agluttination. Mix well the labeled Ag with positive patient sera on slide and observe agglutination. Write notes below and/or draw a figure.



2. Widal agglutination reaction was introduced as a serologic technique to aid in diagnosis of typhoid fever. The test is based on demonstrating the presence of agglutinin (antibody) in the serum of an infected patient, against the H (flagellar) and O (somatic) antigens of *Salmonella typhi* but also *Salmonella paratyphi* and other Salmonella species (and salmonelosis). Dilute patient sera geometrically, add the H and O Ag (following steps A, B and C indicated in the schema below), incubate overnight at 37˚C and 45˚C, respectively. Observe positive reaction and determine specific antibodies titre.





LAB QUIZ

1. Are specific antibodies part of innate or acquired immune response?

1. Describe the principle of agglutination method.

3. Describe the principle of ELISA method.

4. Describe the principle of complement fixation reaction.

4. Describe the principle of Western blott (Immunoblot) analysis.