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Unit-II

Serodiagnostics

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Unit II:

Serodiagnostics- Define Acute & Convalescent sera, collection of serum specimen, storage, preparation of dilutions. Serodiagnosis of various infectious diseases- Detection of antibodies to microbial antigen- Syphilis, typhoid, streptococci infections, HIV, Hepatitis B and C- Comments on respective clinically specific antigens, Clinical significance of autoantibodies in the diagnosis of autoimmune diseases.

PRESENTATION: 1

Serodiagnostics

- Serodiagnostics, or serodiagnosis, refers to a diagnostic method that relies on the analysis of a patient's blood serum to identify the presence of specific antibodies or antigens related to various diseases.

Key Features:

1. **Antibody Detection:** Serodiagnostics primarily focuses on identifying antibodies produced by the immune system in response to pathogens (such as bacteria, viruses, or parasites). The presence of specific antibodies can indicate current or past infections.
2. **Antigen Detection:** In some cases, serodiagnosis can also involve the detection of antigens (substances that induce an immune response) in the serum, which can help identify active infections.
3. **Types of Tests:** Common serological tests include enzyme-linked immunosorbent assays (ELISA), Western blotting, and immunofluorescence assays. These tests are designed to measure the concentration of antibodies or antigens in the serum.

ACUTE AND CONVALESCENT SERA

1. **Acute Sera:** Acute sera refer to blood serum samples collected during the early phase of an infection, typically within the first week after the onset of symptoms. This serum contains antibodies that the immune system produces in response to the infection. The level of these antibodies is often low at this stage, as the body is still in the process of mounting an immune response.

2. **Convalescent Sera:** Convalescent sera are blood serum samples collected after the acute phase, usually 7 to 14 days later, when the patient has started to recover from the infection. At this stage, the antibody levels are expected to be significantly higher, reflecting the immune system's response to the pathogen. The increase in antibody concentration can help confirm a diagnosis of the infection.

COLLECTION OF SERUM SPECIMEN

Collecting serum specimens is a critical procedure in laboratory diagnostics, enabling the analysis of various biomarkers for disease diagnosis, monitoring, and research.

Materials Needed

1. Serum Separator Tube: Options include: Red-top tubes (no anticoagulant)
2. Gold-top tubes (serum separator)
3. Grey/red-top SST (serum separator tube)
4. Transfer Pipette: For transferring serum to storage vials.
5. Cryovials: Recommended sizes are 1.0 mL or 1.8 mL for aliquoting serum.
6. Disposable Gloves: For safety and to prevent contamination.
7. Centrifuge: A standard centrifuge for separating serum from blood cells.

Blood collection tubes

Tube	Colour	Name	Anticoagulant	Test
	Light Blue	Sodium Citrate	3.8% or 3.2%Sodium Citrate	Blood Clotting Factor PT/INR (Plasma)
	Red	Red or Plain	No Additive	Immunology, Drug, serology
	Gold	SST (Serum Separating Tube)	Clot Activator with gel	All Biochemistry test (LFT,KFT, TFT.....)
	Green	Heparin Tube	Sodium or Lithium Heparin	Special test, Trace elements
	Lavender	EDTA	EDTA	CBC'ESR, Blood Bank (Whole Blood)
	Gray	Sodium Fluoride	Sodium Fluoride or Potassium Oxalate	Blood Sugar (Plasma)

Collection Procedure

1. **Blood Collection:** Collect blood from the patient using a sterile technique. Venipuncture is the most common method, where a needle is inserted into a vein, typically in the arm.
2. **Inversion and Clotting:** After collecting blood into the serum separator tube, gently invert the tube 8-10 times to mix the blood with any clotting factors.
3. Allow the tube to sit upright at room temperature for 15-30 minutes to enable the blood to clot.
4. **Centrifugation:** Within 2 hours of collection, centrifuge the tube at 1000 G for 10-15 minutes. This process separates the serum from the clot and blood cells.

5. Serum Recovery: Using a transfer pipette, carefully recover the serum without disturbing the clot.

6. Aliquoting: Place approximately 0.5 mL of serum into each cryovial.

7. Labeling: Clearly label each vial with the appropriate study code, date of collection, and the amount of serum. Importantly, do not include any identifying patient information on the label.

8. Freezing: Freeze the serum at -80°C until ready for analysis or shipping.

Shipping Procedure

1. **Preparation for Shipment:** Contact the designated laboratory or facility to ensure proper receipt of samples.
2. Ship samples on dry ice using overnight shipping, preferably from Monday to Thursday, to avoid delays.
3. **Documentation:** Include a shipping log with details such as study code, date collected, and aliquot amounts. Email this log to the receiving laboratory for verification.
4. **Avoid Thawing:** Ensure that the serum does not thaw during transport, as this can compromise the integrity of the samples.

STORAGE AND PREPARATION OF DILUTIONS

Preparation of Dilutions

1.Use high-quality chemicals to avoid contamination when preparing dilution buffers.

2.Sterilize the dilution buffer if it will be used for microbiological experiments to eliminate microbial contamination.

3.Label the buffer with the name, concentration, and date of preparation.

4.Prepare dilutions by weight or volume. Avoid using glass volumetric flasks to eliminate contamination issues.

5.Add acid and most of the water first before adding individual element concentrates when preparing mixtures to avoid precipitates.

6.Allow diluted solutions to cool to room temperature and adjust to the final volume before use.

Storage of Dilutions

1.Know the chemical stability of the standard and perform stability studies to avoid issues. Strictly adhere to preparation methodology.

2.Maintain storage temperature around 20°C. Some standards require refrigeration or freezing.

3.Use the container material selected for the stability study. Avoid using volumetric flasks due to expense, contamination and transpiration issues.

4.Protect photosensitive standards from light. Photosensitivity increases with higher energy light and trace organics.

5.Use LDPE (Low Density Polyethylene) containers that will not contribute to contamination of inorganic standards.

6.Weigh the standard solution before and after storage to check for transpiration.

Following proper protocols for preparation, labeling, and storage is essential to maintain the stability and accuracy of diluted solutions for various applications in chemistry, biology and medicine.

PREPARATION OF DILUTIONS

General Steps:

1.Materials Needed:

1. Serum sample or antigen/antibody solution
2. Dilution buffer (e.g., PBS or saline)
3. Micropipettes and pipette tips
4. Microtiter plates (e.g., 96-well plates)
5. Sterile test tubes or microcentrifuge tubes
6. Marker and lab notebook for recording dilutions

2. Determine the Dilution Factor:

- A **dilution factor** indicates how much of a sample you dilute into a diluent. Common dilutions in serodiagnostics include 1:2, 1:10, 1:100, and 1:1000.

3. Prepare Serial Dilutions:

Serial dilutions are often performed to get different concentrations of a serum sample or antigen/antibody solution. The basic idea is to progressively dilute the sample in a stepwise manner.

- For example, to perform serial 1:2 dilutions:
- Label a series of tubes (e.g., Tube 1, Tube 2, Tube 3).
- Add a fixed volume of diluent (e.g., 500 µL) to each tube.
- To Tube 1, add a specific volume of the sample (e.g., 500 µL).
- Mix thoroughly, and then transfer 500 µL from Tube 1 into Tube 2.
- Continue transferring and mixing through the series of tubes until the desired dilution range is achieved.

4. Record Dilutions:

Keep a detailed record of the exact dilutions you perform and the corresponding locations (wells or tubes).

5. Applying the Dilution to Serodiagnostic Tests:

•In tests like ELISA, the diluted serum or antigen is applied to wells coated with antigen/antibody. After incubation, you can detect the binding of antigen-antibody complexes using enzyme-linked secondary antibodies.

•For agglutination assays, the diluted sera may be mixed with antigen-coated particles, and visible agglutination is noted at specific dilutions.

•In complement fixation tests, serum dilutions are incubated with antigen and complement to assess whether complement fixation occurs, indicating the presence of antibodies.

6. Analyzing Results:

The endpoint of serodiagnostic assays often corresponds to the highest dilution at which a visible or measurable reaction occurs. This is often reported as the **titer** of the sample.

Example of 1:10 Serial Dilution:

- Step 1: Add 9 mL of diluent to a test tube.
- Step 2: Add 1 mL of the serum sample to the test tube. This creates a 1:10 dilution.
- Step 3: To create a 1:100 dilution, take 1 mL of the 1:10 dilution and add it to 9 mL of diluent.
- Continue this process if further dilutions are needed.

Considerations:

- Use sterile techniques to avoid contamination.
- Ensure consistent mixing of samples to maintain accurate dilutions.
- Depending on the assay, the appropriate buffer, temperature, and incubation time will vary.

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