

Practical Immunology
Lab No 2

Basic Serologic Laboratory Techniques



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Basic Serologic Laboratory Techniques

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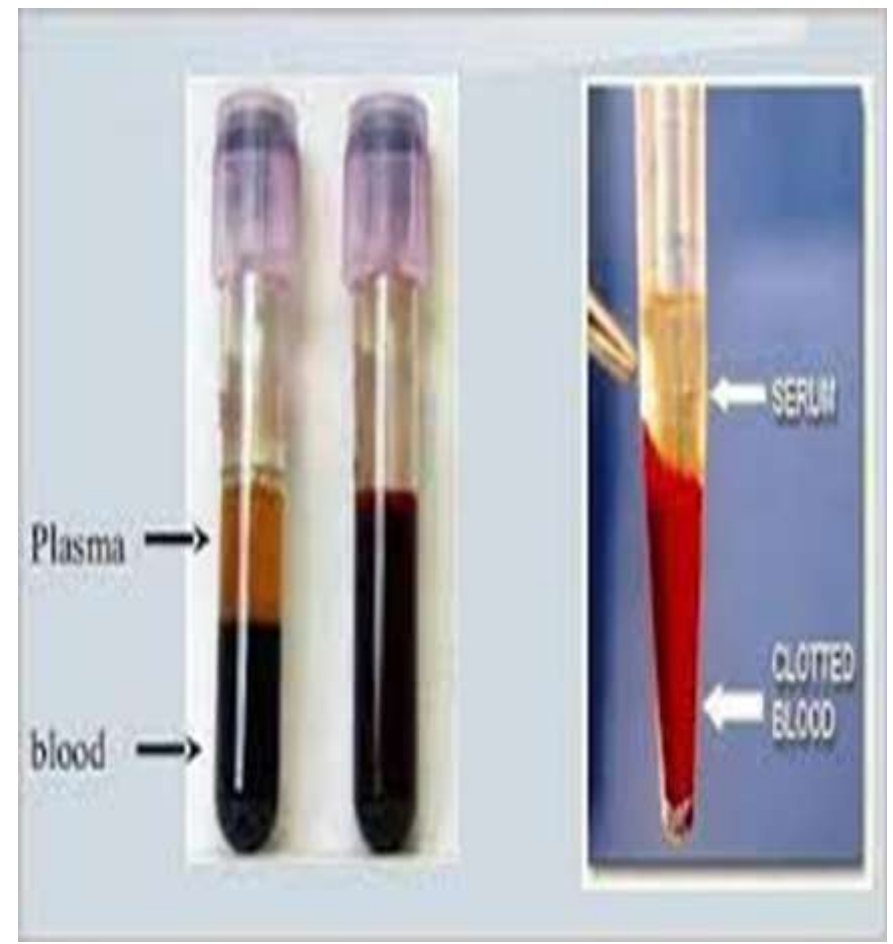
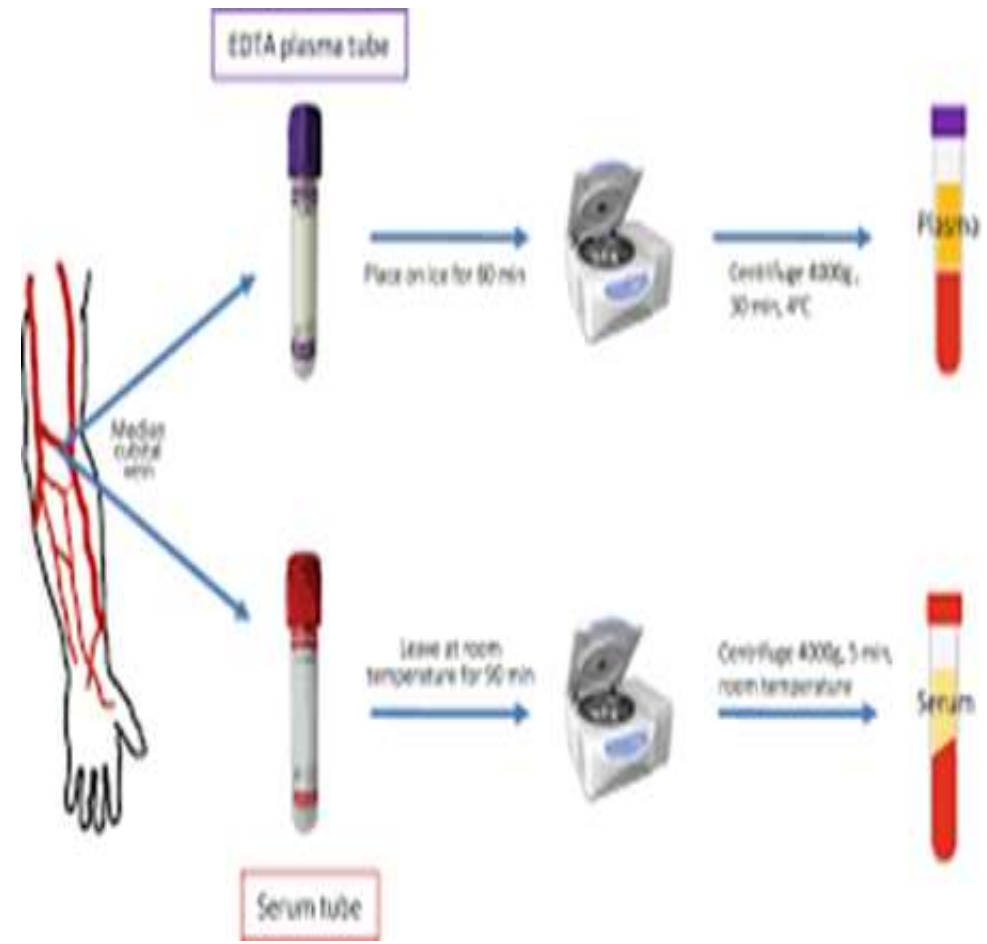
Procedures Manual

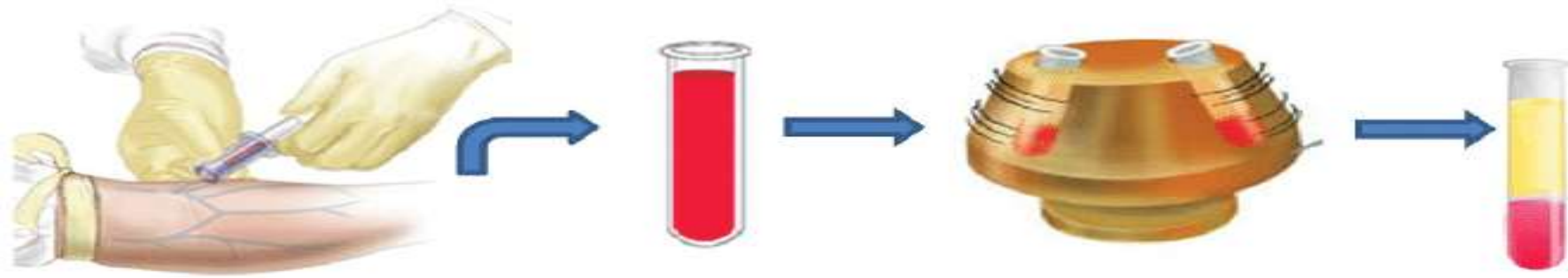
The procedures manual must be a complete document of current techniques and available at all times in the immediate bench area of laboratory personnel. Procedures used in immunology apply many techniques common to other scientific disciplines, such as chemistry. In the field of immunology, different serologic techniques are used to detect the interaction of antigens with antibodies.

Blood Specimen Preparation

After blood has been obtained from a patient, in a plain tube, without anticoagulant, it should be allowed to clot and the serum should be removed for testing. Clotting and clot retraction should take place at room temperature or in the refrigerator, depending on the protocol for the specific procedure. Complete clot retraction normally takes about 1 hour. After clot retraction, the clot should be loosened from the sides of the test tube with a stick and centrifuged for 10 minutes at a moderate speed.

- After centrifugation, serum can be transferred to a labeled tube with a Pasteur pipette and rubber bulb. If the serum is contaminated with erythrocytes, it should be recentrifuged. The serum-containing tube should be sealed.
- Excessive heat and bacterial contamination are avoided. Heat coagulates the proteins and bacterial growth alters protein molecules. If the test cannot be performed immediately, the serum should be refrigerated. In most cases, if the testing cannot be done within 72 hours, a serum specimen must be frozen at -20°C .
- For some testing, the serum complement must first be inactivated. If the protein complement is not inactivated, it will promote lysis of the red blood cells and other types of cells and can produce invalid results.

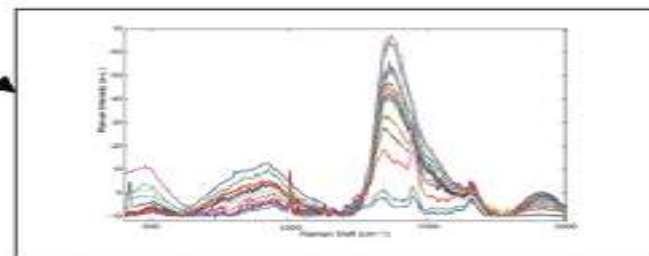




AgNPs



Serum



Types of Specimens Tested

- Most immunology tests are done on serum, although body fluids may also be tested. **Lipemia, hemolysis**, or any **bacterial contamination** can make the specimen unacceptable. **Icteric** or **turbid** serum may yield valid results for some tests but may interfere with others.
- Other specimens include **urine** for pregnancy tests and tests for urinary tract infection.
- Any specimen must be collected into a suitable container to prevent in vitro changes that could affect the assay results. Proper handling and storage of the specimen until testing are essential. Immunologic assays are also done on **cerebrospinal fluid (CSF)**, other **body fluids**, and **swabs of various body exudates and discharges**.

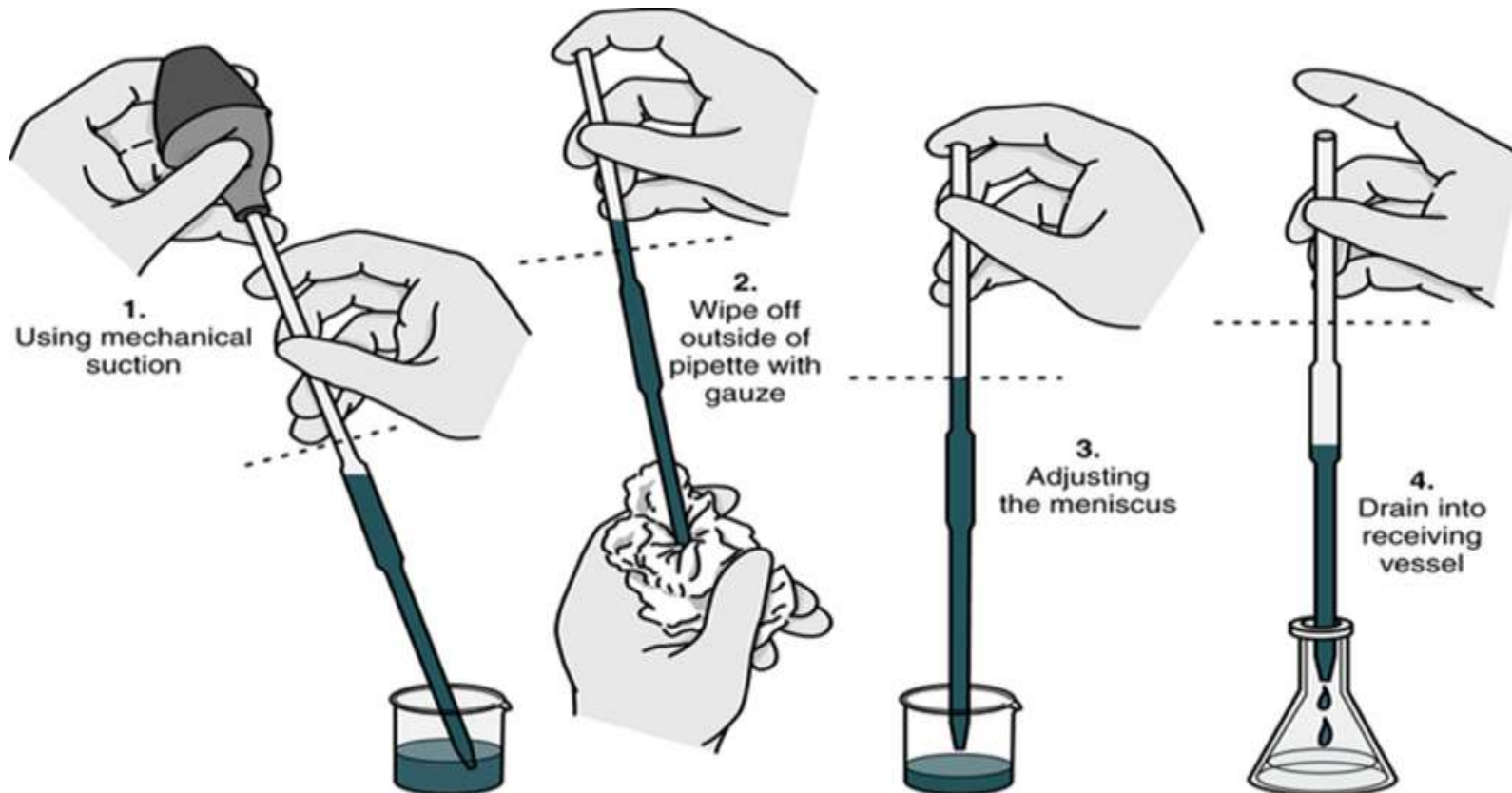
Inactivation of Complement

- Some procedures require the use of inactivated serum. Inactivation is the process that destroys complement activity. Complement may also cause lysis of the indicator cells in hemagglutination assays.
- Complement in body fluids can be inactivated by heating to 56° C for 30 minutes. When more than 4 hours has elapsed since inactivation, a specimen can be reactivated by heating it to 56° C for 10 minutes.
- **Pipettes**
- Pipettes are used in the immunology-serology laboratory for the quantitative transfer of reagents and the preparations of serial dilutions of specimens such as serum. Although semi automated micropipettes have replaced traditional glass pipettes in the laboratory, traditional methods may still be needed at times.

Pipetting Techniques

Manual Pipettes

- With practice, it is important to develop a good technique for handling pipettes . The same general steps apply to pipetting with all manual pipettes , with few exceptions.



Pipetting technique. (From Turgeon ML: Linné & Ringsrud's clinical laboratory science: the basics and routine techniques, ed 6, St Louis, 2012, Mosby.)

Automatic Pipettes

- Automatic pipettes allow fast, repetitive measurement and delivery of solutions of equal volumes. The sampling type measures the substance in question. The sampling-diluting type measures the substance and then adds the desired diluent. The sampling type of automatic pipette is mechanically operated and uses a piston-operated plunger. These are adjustable so that varying amounts of reagent or sample can be delivered with the same device. Disposable and exchangeable tips are available for these pipettes. Automatic pipettes and micropipettors must be calibrated before use.

Micropipettes

- Automatic micropipetting devices allow rapid repetitive measurements and delivery of predetermined volumes of reagents or specimens. The most common type of micropipette used in many laboratories is one that is automatic or semiautomatic, called a micropipettor.
- Many micropipettors are continuously adjustable so that variable volumes of liquids can be dispensed with the same device. . Different types or models are available, which allow volume delivery ranging, for example, from 0.5 to 5000 μL .
- The piston, usually in the form of a thumb plunger, is depressed to a stop position on the pipetting device. The tip is placed in the liquid to be measured, and then the plunger is slowly allowed to rise back to the original position

Micropipettes



Antibody Testing, Antibody titer

- Antibody serology tests check for the presence or level of specific antibodies in the blood. Antibodies are proteins that your immune system makes to fight foreign substances. These substances are often pathogens, such as viruses and bacteria. When you have an infection, your body makes antibodies that are targeted to those pathogens. These antibodies may protect you from getting another infection or from getting severe symptoms. A vaccine can also provide protection by triggering your immune system to make antibodies to pathogens.
- Antibody serology tests are used to look for antibodies to specific diseases. These include:
 - COVID-19
 - Measles and mumps
 - Varicella zoster virus, which includes chickenpox
 - Hepatitis
 - An autoimmune disease.
- **An antibody titer:** the highest dilution of serum that gives a positive reaction with the antigen

Why do I need an antibody test?

- To find out if you've had a recent or past infection.
- To check your vaccination status.
- To find out if a vaccine is effective.
- As a requirement for your school or job.
- To find out if you have an autoimmune disease, such as lupus.