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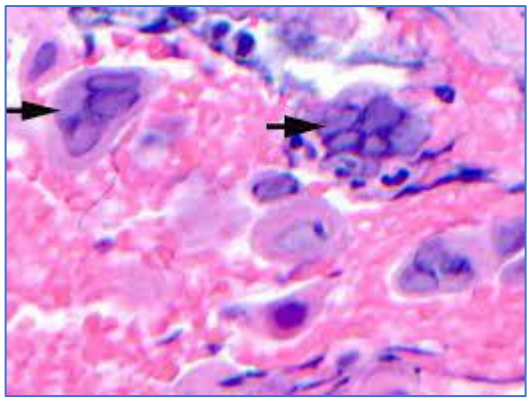
Rapid Diagnostic Methods

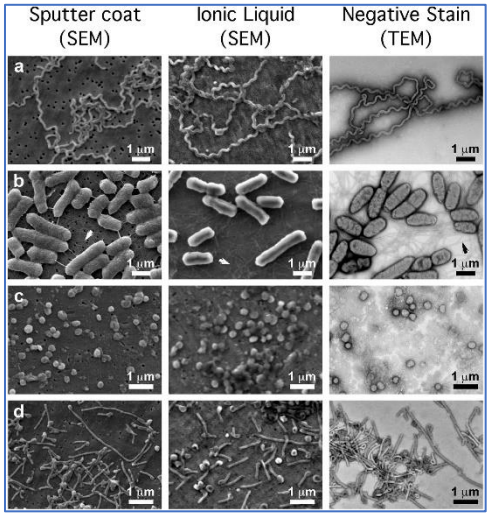
The rapid viral diagnosis is a "directed approach" that requires prior consideration of the virus suspected. Viral isolation, in contrast, is an "open-ended approach" that may yield interesting, unanticipated results.

1-Viral Cytopathology



The analysis of viral cytopathology is the oldest form of rapid diagnosis. An example of this form of testing is the Tzank preparation used to diagnose herpes virus infections. The technique is performed by scraping the base of a skin vesicle and transferring the scraping to a microscope slide. The slide is allowed to air dry and then stained with Giemsa or Wright stain. Slides are viewed under a standard microscope. The finding of multinucleated giant cells is diagnostic of a herpesvirus infection.

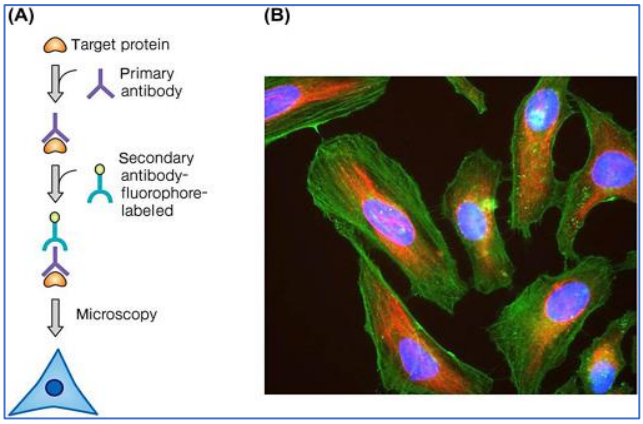
2- Electron microscopy (EM)



It has been used for many years for the rapid detection of viruses in clinical specimens. This technique relies on the identification of viruses by their characteristic morphology. One limitation of EM is that virus must be present in sufficient quantity (approximately 105–106 particles/mL) in order to be detected. The most potent usefulness lies in detecting viruses in fecal contents; EM is not used widely for routine diagnosis because it is expensive, cumbersome, and insensitive. Newer rapid tests are available for most viruses that previously were diagnosed by EM.

3- Immunofluorescence (IF)

It has been used for rapid diagnosis of respiratory tract infections, and vesicular exanthems and examination of tissues. The method is rapid, precise, and sensitive when careful attention is paid to be the technique of obtaining and processing the specimens, using appropriate controls, and having a well-trained laboratory staff to interpret the results. Clinical specimens are applied to a slide, dried, fixed, and stained. A fluorescence microscope is used to read the slides for either fluorescing organisms or infected cells. Staining may be direct, using a specific antimicrobial antibody with attached fluorescence dye, or indirect, using an unlabeled specific antimicrobial antibody followed by fluorescein-labeled antibody directed against the initial antibody.

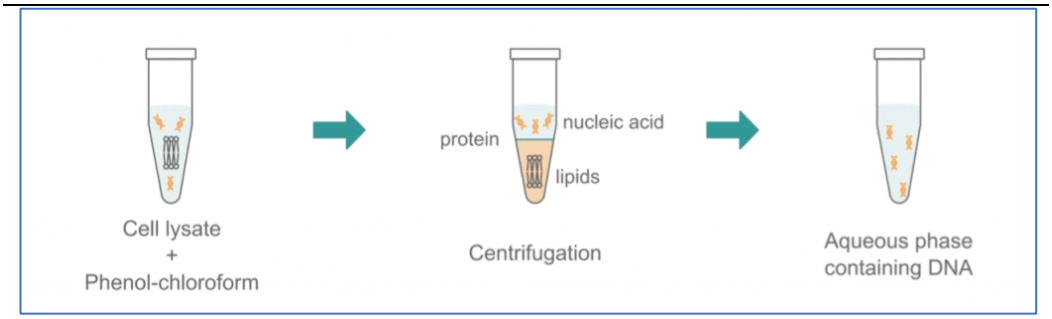


DNA EXTRACTION METHOD

1- Guanidinium Thiocyanate Phenol-Chloroform Extraction

After the cellular structure of the nucleic acid has been disrupted, DNase and RNase is used. The desired nucleic acids can then be separated from the cell debris.

Phenol alone is a flammable, corrosive and toxic carbolic acid. But a mixture of phenol, chloroform and a small amount of isoamyl can be used to extract DNA. When phenol and chloroform are added to the sample, an emulsion forms containing a layer of DNA at the top, as a result of its hydrophilic nature. The DNA can then be collected and precipitated by centrifugation. The resulting DNA pellet can then be dissolved with sterile water.



A diagram showing phenol-chloroform extraction steps: adding the phenol-chloroform mixture to the cell lysate, centrifugation, followed by washing with water to obtain the isolated DNA.

The guanidinium thiocyanate-phenol-chloroform technique can then be used to extract RNA in a single step. RNA is separated from DNA after extraction using an acidic solution consisting of guanidinium thiocyanate, sodium acetate, phenol and chloroform.

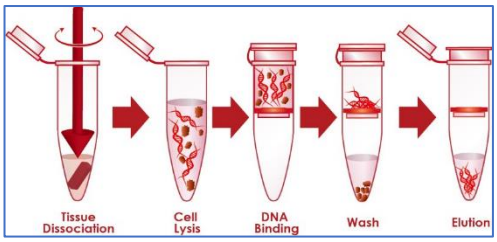
2- Cesium Chloride / Ethidium Bromide Gradient Centrifugation

Cesium Chloride / Ethidium Bromide gradient centrifugation has been used in research labs since 1950. The method exploits the differing densities between the caesium ions and water, along with the intercalation of ethidium bromide to interfere with DNA replication, transcription, repair and recombination.

This gradient centrifugation is a complicated, expensive and time-consuming method compared to other isolation protocols. It requires a large amount of sample and so is not suitable for all types of sequencing. Also, ethidium bromide is harmful. Therefore, this method is not used in the clinical lab due to its limitations.

3- Solid-Phase Extraction

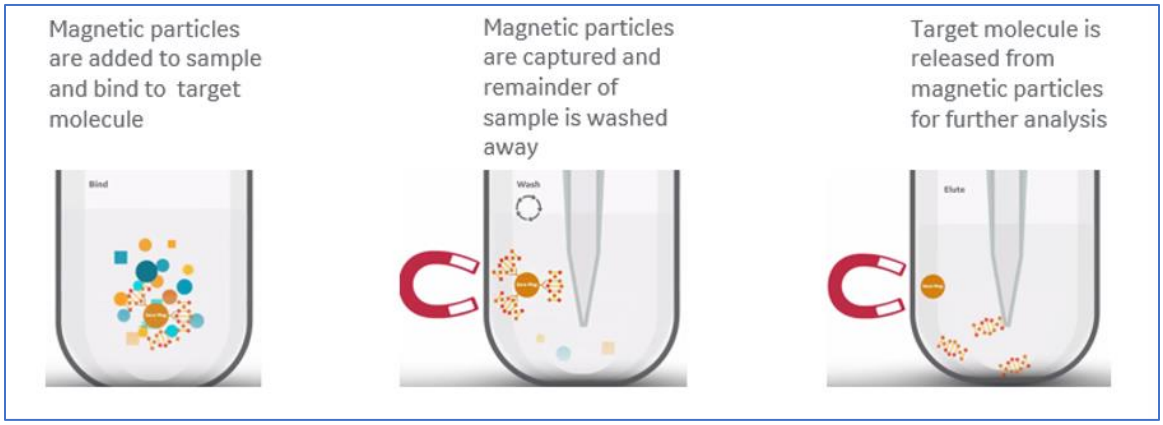
Solid-phase nucleic purification can be found in most of the commercial extraction kits available on the market today. It is normally performed using a spin column that is operated under centrifugal force, allowing the DNA to be



purified rapidly and efficiently. The column must first be conditioned for sample absorption, which can be done using a buffer at a certain pH. After the cells have been disrupted, the desired nucleic acids absorb to the column because of the pH of the binding solution. Contaminants are then removed by washing with a competitive agent, and water is introduced to release the desired nucleic acids from the column.

4- Magnetic bead-based purification

Magnetic separation is now deemed a simple and efficient method used in the purification of nucleic acids. It is a modification of solid-phase extraction. The beads have a negative surface charge and selectively bind to proteins, such as DNA. The binding process may sometimes be assisted by a magnet being applied to the side of the tube as this aggregates the particles near the wall. The remainder of the sample, consisting of cellular debris and unwanted material, can then be poured away. The nucleic acids are removed from the magnetic particles with a buffer and any remaining contaminants are washed away.



A diagram showing the magnetic bead-based purification protocol. Image credit

This method certainly has advantages – it doesn't need repeated centrifugation, vacuum filtration or column separation, making it time and cost effective.