

Virus Isolation and Identification

DIANE S. LELAND and MORRIS L. V. FRENCH

Cell Cultures: Monolayer cultures of primary, diploid, and continuous cell lines are the hosts of choice for virus isolation. Quality cell cultures are available commercially and are conveniently maintained in the laboratory. After proper decontamination and purification, each clinical sample is inoculated into several types of cell cultures; the preferred lines vary from virus to virus.

Alternative Cell Culture Techniques: New cell culture techniques have been developed to enhance virus isolation. Shell vials containing a monolayer of cells growing on a cover slip provide a virus isolation system that can be placed in the centrifuge and spun to enhance virus infection. Following incubation, the infected cover slip is withdrawn and stained to confirm virus identification. Shell vials are helpful in cytomegalovirus isolation. Herpes simplex isolation and identification systems are commercially available. A culture of herpes-susceptible cells and an immunostaining system are included. Inclusion of microspheres in cell culture has been shown to provide increased surface area for attachment of cells. Increased cell numbers provide for increased virus production. Several viruses can be isolated in suspensions of human lymphocytes rather than in traditional monolayer cultures.

Virus Quantitation: Viruses are quantitated by titration and plaquing methods. Formulas by Reed-Muench (1938) and Karber (1931) are used to determine the end point from titration data.

Virus Detection and Identification: Both immunologic and nonimmunologic methods are used in identification of isolated viruses and for detection of viral antigen within cell cultures or within clinical materials.

Nonimmunologic methods include histologic staining, electron microscopy, hemadsorption (HAD), hemagglutination (HA), challenge interference (CI), and DNA probes. HAD, HA, and CI are used primarily to detect non-cytopathogenic effect-producing viruses within cell cultures. DNA probes provide the most specific viral identification and will probably replace many traditional techniques.

Immunologic methods include immunofluorescence (IF), immunoperoxidase (IP), enzyme-linked immunosorbent assay (ELISA), neutralization (NEUT), hemagglutination inhibition (HAI), passive agglutination (PA), radioimmunoassay, and immune adherence hemagglutination. IF, IP, ELISA, and PA provide rapid and accurate virus identification, whereas NEUT and HAI, although accurate, are more costly and time-consuming.

Virus Isolation and Identification

In the past 5 to 10 years, progress in clinical diagnostic virology has been remarkable. Traditional techniques for virus isolation have been refined, and new alternative methods have been developed. All virus isolation and identification services have been simplified and enhanced by the commercial availability of quality cell cultures and antisera. Techniques previously reserved for research facilities are now performed on a routine basis in clinical virus-isolation facilities. New diagnostic tests for direct detection and identification of viral antigens in clinical samples are now available. These methods provide rapid, accurate, and cost-effective identification of many common viral pathogens, as well as confirmation of infections due to several viruses such as rotavirus and hepatitis, which, owing to their lack of proliferation in standard cell lines, have previously eluded the clinical virologist.

In this chapter, standard viral diagnostic methods are described. Guidelines are included for virus isolation as well as for direct methods in which virus isolation is not required. Traditional methods and contemporary technology are described; techniques useful in routine viral identification are emphasized, and those methods which are best reserved for full-service virology laboratories are outlined.

Virus Isolation

Cell Cultures

Because viruses lack ribosomes and systems for synthesis of energy and proteins, they are not capable of reproducing on artificial media. Viruses are obligate intracellular parasites and can replicate only within living cells. Several living cell systems have been used for viral proliferation. These include cell cultures, embryonated eggs, and animals. No single system will support the growth of all viruses; hence, the laboratory is faced with the problem of selecting culture environments suitable for isolation of the largest number of viral agents.

Cell cultures are the host system most frequently used for virus cultivation because of their broad spectrum of susceptibility and the relative ease with which cell cultures are maintained in the laboratory. Cell cultures are prepared with suspensions of cells which, if necessary, have been dissociated from the parent tissue by action of proteolytic enzymes and chelating agents. Dissociated cells, when placed in a culture vessel, adhere to the surface of the vessel and replicate to form a layer of cells (monolayer); adherence to the surface is an integral part of survival and

subsequent cell proliferation. This is the mode of culture common to most normal cells other than hemopoietic cells (Freshney, 1983). Other types of cells such as mature hemopoietic cells, transformed cell lines, or cells from malignancies may replicate in suspension. These cells can survive and proliferate without attachment (Freshney, 1983). Both monolayer and suspended cell cultures are useful in the isolation of viruses in the laboratory.

Monolayer cultures are observed microscopically without removing the monolayer from the wall of the vessel and without staining. Many types of virus-induced changes, collectively referred to as the cytopathogenic effect (CPE) of a virus and including any alteration in cellular characteristics that is virus induced, are visible when unstained cell monolayers are examined with the 10× objective of a standard light microscope. Other manifestations of CPE, such as inclusion bodies or chromosomal alterations, can be evaluated only in stained preparations. Suspended cell preparations are more difficult to evaluate microscopically, and the presence of a viral infection must be confirmed by alternative techniques.

Three types of monolayer cell cultures—primary, diploid, and established or continuous—are used in virus isolation. Primary cells are the first generation of cells that grow from the tissue of origin. Primary cell preparations contain predominantly epithelial cells, and the cells have normal diploid chromosomes of the same number as the parent tissue. Primary cell preparations are susceptible to a variety of human viral pathogens. One problem encountered occasionally with primary cultures is that of indigenous viral pathogens that may have infected the animal host prior to cell culture preparation. These indigenous viruses may produce CPE during cell culturing or may remain largely undetectable while reducing the susceptibility of the cultured cells to viral infection. Primary cell types include primary rhesus monkey kidney (pRMK) and primary human embryonic kidney (pHEK).

Diploid cell lines, as their name suggests, are those which continuously maintain their diploid chromosome number throughout serial passages; these cells usually die out after the 50th passage. Some well-known diploid cell strains are human lung fibroblasts (MRC-5 or WI-38) and human foreskin (MRHF).

Continuous (heteroploid, established) cell lines demonstrate heteroploid or aneuploid chromosome numbers during repeated subculturing. Continuous cell lines originate from malignancies; they replicate vigorously and are usually not difficult to culture, being capable of indefinite passaging. Continuous cell lines include human cervical carcinoma (HeLa) and human laryngeal carcinoma (HEp-2).