Cell Culture, Cytopathic Effect and Immunofluorescence Diagnosis of Viral Infection

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Abstract

Viruses are obligate intracellular parasites that require living cells in order to replicate. Cell culture for propagation and identification of viruses is an important component of the clinical virology laboratory. In general, diagnostic tests can be grouped into three categories: direct detection, virus isolation and serology. Direct examination methods can usually give a result either within the same or the next day. Immunofluorescence is widely used for the rapid diagnosis of virus infections by detection of virus antigen in clinical specimens and detection of virus-specific antibodies.

Cell culture is the complex process by which cells are grown under controlled conditions and support the widest range of viruses. Identification of virus is usually done by cytopathic effect and haemadsorption changes. Cytopathic effect induces cellular changes that are noticed as the monolayer cells deteriorate as a result of the viral infection. These changes include swelling or shrinkage of cells, the formation of multinucleated giant cells and the production of inclusions in the nucleus or cytoplasm of the infected cell.

There are three types of cell cultures including primary cells, semi-continuous cells and continuous cells. Primary cultures are derived directly from excised, normal animal tissue and cultures either as an explant culture or following dissociation into a single cell suspension by enzyme digestion. Continuous cultures are comprised of a single cell type that can be serially propagated in culture either for a limited number of cell divisions or otherwise indefinitely.

Keywords: Cell culture; Virus; Cytophatic effect; Immunoflueresence; ELISA

List of Abbreviations: CFT: Complement fixation tests; CMV: Cytomegalovirus; CPE: Cytopathic effect; ELISA: Enzyme linked immunosorbent assay; HAI: Haemagglutination inhibition tests; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HIV: Human immune virus; HTLV: Human T lymphocyte virus; IF: Immunofluorescence; PCR: Polymerase chain reaction; RIA: Radioimmunoassay; RNA: Ribonucleic acid; RSV: Respiratory synctia virus; VZV: Varicella zoster virus

Introduction

Cell culture for propagation and identification of viruses is an important component of the clinical virology laboratory. Since viruses are obligate intracellular parasites, they require living cells to replicate. In general, diagnostic tests can be grouped into three categories: direct detection, indirect examination (virus isolation), and serology. In direct examination, the clinical specimen is examined directly for the presence of virus particles, virus antigen or viral nucleic acids. In indirect examination, the specimen will be inoculated into cell culture, eggs or animals in an attempt to grow the virus: this is called virus isolation. Serology actually constitutes by far the bulk of the work of any virology laboratory. A serological diagnosis can be made by the detection of rising titers of antibody between acute and convalescent stages of infection, or the detection of Immunoglobulin M (IgM). The majority of common viral infections can be diagnosed by serological tests [1]. This paper reviewed the evolution of cell culture methods and demonstrates why cell culture is a preferred method for identification of viruses.

Direct Examination of Specimen

1. Electron microscopy for detection of morphology
2. Light microscopy histological appearance - e.g. inclusion bodies

3. Antigen detection immunofluorescence, ELISA, etc.
4. Molecular techniques for the direct detection of viral genomes

Indirect Examination

1. Cell Culture - CPE, haemadsorption, confirmation by neutralization, immunofluorescence, etc.
2. Eggs pocks on CAM - haemagglutination, inclusion bodies

Serology

Detection of rising titers of antibody between acute and convalescent stages of infection, or the detection of IgM in primary infection can be done by the following methods.

1. Complement fixation tests (CFT)
2. Enzyme linked immunosorbent assay (ELISA)
3. Haemagglutination inhibition tests (HAI)
4. Immunofluorescence techniques (IF)
5. Recombinant immunoblot assay (RIA)

Viral Diagnosis Methods

Direct examination methods are often also called rapid diagnostic methods because they can usually give a result either within the same or the next day. This is extremely useful in cases when the clinical management of the patient depends greatly on the rapid availability of laboratory results e.g. diagnosis of RSV infection in neonates, or severe CMV infections in immunocompromised patients. However, it is important to realize that not all direct examination methods are rapid, and conversely, virus isolation and serological methods may sometimes give a rapid result. With the advent of effective antiviral chemotherapy, rapid diagnostic methods are expected to play an increasingly important role in the diagnosis of viral infections [1,2].

Virus Isolation

Cell culture is an important component of the clinical virology laboratory to isolate virus. Several laboratories have been successful in the primary culture of adult rat hepatocytes. Cultured cells, eggs and laboratory animals may be used for virus isolation. Although embroyonated eggs and laboratory animals are very useful for the isolation of certain viruses, cell cultures are the sole system for virus isolation in most laboratories. The development of cultivating animal cells has been essential for progress to animal virology [1,3].

Isolation of viruses in cell culture has been the standard procedure in diagnostic laboratory for a long time. Viruses that can be commonly isolated in standard cell cultures include: adenovirus; cytomegalovirus; many of the enteroviruses (coxsackie B virus, echovirus, poliovirus types 1, 2 and 3); herpes simplex virus types 1 and 2; influenza virus A and B; measles virus, mumps virus; parainfluenza virus types 1,2,3 and 4; respiratory syncytial virus; varicella virus. To prepare cell cultures, tissue fragments are first dissociated, usually with the aid of trypsin or collagenase. The cell suspension is then placed in a flat-bottomed glass or plastic container together with a suitable liquid medium. After a variable lag, the cells will attach and spread on the bottom of the container and then start dividing, giving rise to a primary culture. Attachment to a solid support is essential for the growth of normal cells [2,3].

Cell Cultures

In the 1900s, embryonated eggs and laboratory animals were used for isolation of viruses. Typically, cell cultures are developed from tissue samples and then disaggregated by mechanical, chemical, and enzymatic methods to extract cells suitable for isolation of viruses. With the utilization of cell culture technique, use of laboratory animals in experiments has decreased significantly [3]. Cell culture is the complex process by which cells are grown under controlled conditions, generally outside of their natural environment. In practice, the term "cell culture" now refers to the culturing of cells derived from multi-cellular eukaryotes, especially animal cells. The historical development and methods of cell culture are closely interrelated to those of tissue culture and organ culture [4,5].

Cells can be isolated from tissues for *ex vivo* culture in several ways. Cells can be easily purified from blood; however, only the white cells are capable of growth in culture. Mononuclear cells can be released from soft tissues by enzymatic digestion with enzymes such as collagenase, trypsin or pronase, which break down the extracellular matrix. Alternatively, pieces of tissue can be placed in growth media, and the cells that grow out are available for culture. Cells are grown and maintained at an appropriate temperature and gas mixture (typically, 37 degrees Celsius, 5% CO_2 for mammalian cells) in a cell incubator. Culture conditions vary widely for each cell type and variation of conditions for a particular cell type can result in different phenotypes [3,4]. There are three types of cell cultures:

Primary Cells: These are essentially normal cells obtained from freshly killed adult animals. These cells can only be passaged (sub-cultured) once or twice e.g., primary Monkey Kidney cells (MK), primary rabbit kidney cells (RK) and mice fibroblasts.

Semi-Continuous Cells: Semi-continuous cells have diploid number of chromosomes and can be passaged for several times e.g. Human embryonic kidney (HEK) and skin fibroblast cells (MRC-5 and WI-38). These are cells taken from embryonic tissue, and may be passaged up to 50 times.

Continuous Cells: Continuous or transformed cell lines are immortalized cells that can be "passaged" without limit e.g., HeLa (Henrietta Lacks), Vero, Hep-2, LLC-MK2 and BGM. These are immortalized cells i.e., tumor cell lines and may be passaged indefinitely.

Preparation of Cell Culture

1. Take a piece of tissue/organ
2. Subject to the action of certain proteolytic enzymes plus mechanical shock
3. Isolate, wash and count cells
4. Suspend in growth media which contains buffered isotonic solution, amino acid, fatty acids, carbohydrates, vitamins, precursor of nucleic acids, penicillin/streptomycin and keep pH between 7.1-7.4
5. Place in tube/bottle or petri dish
6. Multiply a single monolayer of cells, then it is ready to be inoculated with virus of interest to cultivate

Primary Cultures

Primary cultures are derived directly from excised, normal animal tissue/ healthy tissue and cultures either as an explant culture or following dissociation into a single cell suspension by enzyme digestion. Such cultures are initially heterogeneous but later become dominated by fibroblasts. Primary cell cultures are widely acknowledged as the best cell culture systems available since they support the widest range of viruses. Primary cells are cells straight from the tissue with no passages. Primary cultures by definition have not been passaged; as soon as they are passaged they become a cell line and are no longer primary. Cell lines have at least one passage [1,6].

Steps to prepare a primary cell culture

• Mince organ and treat with a protease to separate cells.
• Wash, count and dilute cells in growth medium, let settle on flat surface.
• Incubate under 5% CO_2 and about 37 °C.

Cell cultures vary greatly in their susceptibility to different viruses. It is of utmost importance that the most sensitive cell cultures are used for a particular suspected virus. Specimens for cell culture should be transported to the laboratory as soon as possible upon being taken. Swabs should be put in a vial containing virus transport medium. Bodily fluids and tissues should be placed in a sterile container [7] (Figure 1).
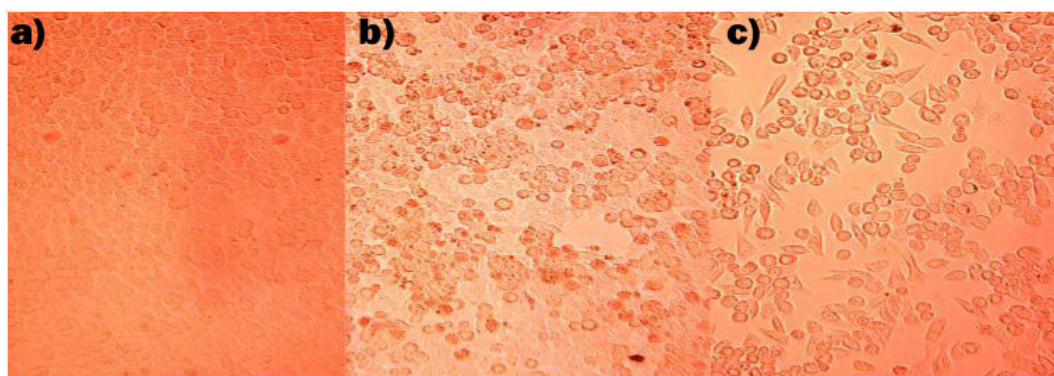
Adenovirus (Source: CDC)
Figure 1: a) Uninfected epithelial cell **b)** Early infected epithelial cell **c)** Late infected epithelial cell

Continuous Cultures

Continuous cultures are comprised of a single cell type that can be serially propagated in culture either for a limited number of cell divisions (approximately thirty) or otherwise indefinitely. Cell lines of a finite life are usually diploid and maintain some degree of differentiation. The fact that such cell lines senesce after approximately thirty cycles of division means it is essential to establish a system of master and working banks in order to maintain such lines for long periods. Continuous cell lines can be propagated indefinitely because they have been transformed into tumor cells [3]. Tumor cell lines are often derived from actual clinical tumors, but transformation may also be induced using viral oncogenes or by chemical treatments. Transformed cell lines present the advantage of almost limitless availability, but the disadvantage of having retained very little of the original *in vivo* characteristics. It is good to grow fastidious types of viruses [8].

Several different types of cell cultures are inoculated with each viral specimen and are cultivated in tubes or Roux flasks made of a special glass or plastics mostly in stationary way at +37 degrees Celsius. Cultivation media contain proteins, vitamins, minerals and amino-acids and can be enriched with fetal calf serum. Bacterial contamination is suppressed with combination of antibiotics added to the medium pH is set up with solution of NaHCO3. Cell passaging or splitting is a technique that enables an individual to keep cells alive and growing under cultured conditions for extended periods of time. Cells should be passed when they are 90%-100% confluent [7,9].

Commonly used monkey kidney cells are used for recovery of myxoviruses, paramyxoviruses and many enteroviruses. Human fetal diploid cells are fibroblastic cells that support the growth of herpesviruses, cytomegalovirus, varicella-zoster virus, adenoviruses and picornaviruses. Hep2 cell derived from a human cancer are used for isolation of respiratory syncytial virus and adenoviruses. Some other viruses, as HIV, coxsackie A, togaviruses require special procedures-either co-cultivation with mononuclear blood cells or inoculating to a suckling mouse or special tissue cultures (9).

Specimen Processing and Inoculation

Performing a viral culture requires the following steps: processing of the specimen and inoculation onto the cell culture, maintenance of the inoculated cell culture, and detection of viral growth. Normally sterile body fluids, such as CSF, may be inoculated directly onto cell cultures. Urine specimens should have the pH adjusted toward neutrality before inoculation. Specimens from body sites typically contaminated with bacteria, such as respiratory or genital specimens, are treated with antibiotics before inoculation to prevent bacterial or fungal overgrowth. After inoculation, cultures are incubated at 35 to 37 °C and inspected periodically, for example, once daily or every other day. Respiratory cultures directed at detection of influenza or rhinoviruses may be incubated at 33 °C [10].

Storage Temperature

Proper storage of specimens is important as it can take several days before some specimens are finally inoculated onto isolation systems. Most viruses can be stored at +4 to +8 °C for several days before there is an excessive loss of infectivity. Freezing specimens to -70 °C and bellow will preserve residual viral infectivity of most specimens, although a significant loss of infectivity may occur with repeating freeze-thaw cycle. Many viruses lose their infectivity rapidly when stored at -15 to -20 °C. For a long time storing of viral strains the liquid oxygen system (-196 °C) is used [9].

The inoculated tubes should be read at least every other day for the presence of cytopathic effect. Certain specimens, such as urine and feces, may be toxic to cell cultures that may produce a CPE-like effect. If toxic effects are extensive, it may be necessary to passage the inoculated cells. Cell cultures that are contaminated by bacteria should either be put up again or passed through a bacterial filter. Cell cultures should be kept for at least one to two weeks (longer in the case of CMV). Cell cultures should be reefed with fresh maintenance medium at regular intervals or if required should the culture medium become too acidic or alkaline. When CPE is seen, it may be advisable to passage infected culture fluid into a fresh culture of the same cell type. For cell-associated viruses such as CMV and VZV, it is necessary to trypsinize and passage intact infected cells. Other viruses such as adenovirus can be sub-cultured after freezing and thawing infected cells [3,11,12].

Identification of Growing Virus

Viral growth in cell cultures is most often detected based on the development of microscopically visible cytopathic effect (CPE) and haemadsorption changes. Some viruses, however, can grow to high titer without producing visible CPE and must be detected by other means [10].

Cytopathic Effect (CPE): The term "cytopathic effect" is frequently applied to virus-induced cellular changes that are visible by light microscopy. Cytopathic effect is noticed as the monolayer cells deteriorate as a result of the viral infection. These changes include swelling or shrinkage of cells, the formation of multinucleated giant cells (syncytia), and the production of "inclusions" (made visible by staining) in the nucleus or cytoplasm of the infected cell. It may be specific or non-specific e.g. HSV and CMV produces a specific CPE, whereas enteroviruses do not [13] (Figure 2). Cytopathic effects include:

- Rounding
- Detachment (plaques)
- Clumping
- Ballooning (Giant cell)
- Fusion (syncytium formation)
- Inclusion body formation

Haemadsorption: Cells acquire the ability to stick to mammalian red blood cells. Haemadsorption is mainly used for the detection of influenza and parainfluenza viruses. Confirmation of the identity of the virus may be carried out using neutralization, haemadsorption- inhibition, immunofluorescence, or molecular tests [13].
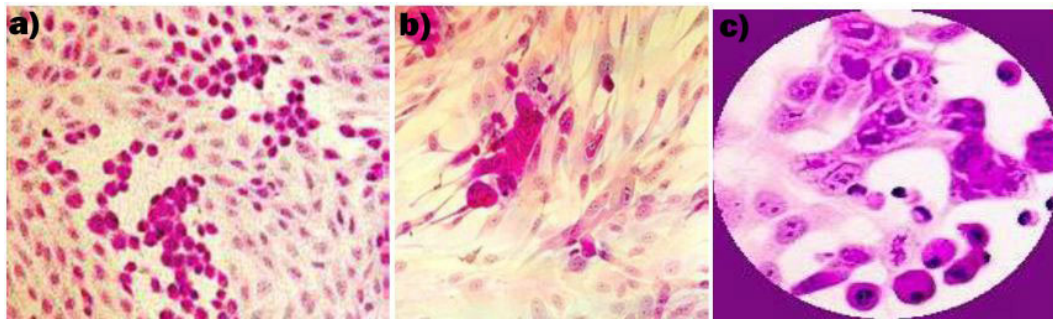
(Source: Courtesy of Linda Stannard, University of Cape Town, S.A.)
Figure 2: a) Cytopathic effect of HSV on cell culture **b)** Cytopathic effect of CMV **c)** Adenoviral inclusion bodies

Shell Vial Culture

Shell vial culture refers to a modified cell culture in which the specimen is centrifuged onto the cell monolayer and viral growth is detected by antigen detection procedures, whether or not CPE is visible. The method was developed originally for CMV but has subsequently been applied to many other viruses, including herpes simplex virus (HSV), VZV, respiratory viruses, and enteroviruses. The main advantage of the shell vial culture method is that it can decrease the time required to detect the presence of the virus being sought. For a slow-growing virus such as CMV, this difference can be dramatic. For some viruses, either the centrifugation step or the antigen detection component of the full shell vial procedure can be omitted, although for CMV, inclusion of both components is required to maximize the sensitivity of the method [10].

In a common application of the shell vial method, human fibroblast cells are grown on a cover slip that is placed at the base of a 1-dram vial (these thin-walled vials are also known as shell vials). After inoculation of the specimen, the vial is centrifuged at low speed (i.e., 700 × g for 45 minutes) and then incubated for at least 16 hours. After the incubation period, the cover slip is removed and subjected to FA staining using a monoclonal antibody specific for an immediate early antigen of CMV. The presence of one or more fluorescent nuclei is evidence of the presence of CMV in the specimen [10] (Table 1 and 2).

Cell Lines for Growth of Selected Viruses

	Type of virus	Cell lines
1	Herpes Simplex	Vero, Hep-2, human diploid (HEK-293 and HELA), human amnion
2	VSV	human diploid (HELA, HEK)
3	CMV	human diploid fibroblasts (HEF)
4	Adenovirus	Hep-2, HEK,
5	Poliovirus	MK, BGM, LLC-MK2, Vero, Hep-2, Rhadomyosarcoma, HELA, HEK
6	Coxsackie B	MK (Monkey Kidney cell), BGM, LLC-MK2, Vero, hep-2
7	Echo	BGM (Buffalo green monkey), MK, LLC-MK2, human diploid, Rd
8	Influenza A	MK, LLC-MK2, MDCK(Madin-Darby canine kidney cells)
9	Influenza B	MK, LLC-MK2 (Rhesus Monkey Kidney Epithelial Cells), MDCK
10	Parainfluenza	MK, LLC-MK2
11	Mumps	MK, LLC-MK2, HEK, Vero
12	RSV	Hep2 (Human epidermoid cancer cells), Vero
13	Rhinovirus	human diploid (HEK, HEL (Henrietta Lacks))
14	Measles	MK, HEK(Human embryo kidney), Vero, B95a
15	Rubella	Vero, RK13 (Rabbit kidney epithelial cells)
16	Rabies	WI-38 (Human lung fibroblast cell line), human diploid cells
17	HCV	(Huh-7) human hepatocellular carcinoma cell line

Table 1: Some selected viruses and their cell cultures

Name of Virus	Cell Culture	Origin of cell	Specimen type	Incubation period (CPE)	Storage temperature
CMV	HEL	Human embryonic lung fibroblasts	CSF/Blood	5 - 14 days	-70 °C to -196 °C
Poliovirus(1,2,3)	Rhadomyosarcoma (Rd)	Human cancer of connective tissue	Stool	1 - 7days	-86 °C to -196 °C

Name of Virus	Cell Culture	Origin of cell	Specimen type	Incubation period (CPE)	Storage temperature
VZV	HeLa	Human fibroblasts	Vesicle fluid and scrapings	5 - 14 days	-70 °C to -196 °C,
RSV	HeP-2	Human cervical cancer cells	Nasopharyngeal aspirates, nasal washes	4 - 5 days	-70 °C to -196 °C
Influenza A	MDCK	Madin-Darby canine kidney cells	Throat swab	2 - 5 days	-70 °C to -196 °C
HSV	HEK-293	Human diploid cell	Genital and tissue lesions	1 - 4 days	-70 °C to -196 °C
Adenovirus	HEK	Human embryo kidney	Stool/eye swab	2 - 7 days	-80 °C to -196 °C
Mumps	MK	Monkey kidney cell	pharyngeal exudates, saliva	3 - 10 days	-20 °C to -70 °C
Rubella virus	RK-13	Rabbit kidney cell	Dermal lesion	3 -10 days	-70 °C to -196 °C
Measles	MK	Monkey kidney cells	Blood, throat swabs	3 - 10 days	-70 °C to -196 °C
Rabies	WI-38	Human lung fibroblast	Neck skin biopsy	5-14 days	-20 °C to -80 °C
Rhinovirus	HeLa	Human fibroblast	Nasal swab	4-5 days	-20 °C to -196 °C

(Source: http://www.mayomedicallaboratories.com/.html)
Table 2: Different viruses with their culture, specimen type incubation period and storage temperature

Rapid Culture Techniques

Rapid culture techniques are available whereby viral antigens are detected 2 to 4 days after inoculation. Examples of rapid culture techniques include shell vial cultures and the CMV DEAFF test. In the CMV DEAFF test, the cell sheet is grown on individual cover slips in a plastic bottle. After inoculation, the bottle then is spun at a low speed for one hour (to speed up the adsorption of the virus) and then incubated for 2 to 4 days. The cover slip is then taken out and examined for the presence of CMV early antigens by immunofluorescence [1].

Limitations of Cell Culture

The main problem with cell culture is the long period (up to 4 weeks) required for a result to be available. Also, the sensitivity is often poor and depends on many factors, such as the condition of the specimen, and the condition of the cell sheet. Cell cultures are also very susceptible to bacterial contamination and toxic substances in the specimen. Lastly, many viruses will not grow in cell culture at all *e.g.*, Hepatitis B and C, diarrheal viruses, Epstein-Barr virus, JC virus, parvovirus etc.

Immunofluorescence Virus Detection

Detection of viral antigens by immunofluorescence methods directly on clinical samples was used as early as the 1970s. IF is widely used for the rapid diagnosis of virus infections by detection of virus antigen in clinical specimens, as well as the detection of virus-specific IgG or IgA or IgM antibody. The technique makes use of a fluorescein-labeled antibody to stain specimens containing specific virus antigens, so that the stained cells fluoresce under UV illumination. In the case of direct IF, the specimen is probed directly with a specific labeled antibody against a particular virus antigen. In the case of indirect IF, the specimen is first probed with a non-labeled specific antibody, followed by a labeled antibody against the first antibody. Direct or indirect IF can be used for the detection of virus antigen, whereas indirect IF is virtually always used for the detection of antibody. Indirect IF possess the advantage of an extra amplification step for the signal, however, it requires an extra step in comparison to direct IF [13,14].

Viral respiratory tract infections are the most common contagious diseases responsible for considerable morbidity and mortality in humans. Nasopharyngeal aspirates are the best specimens to use and usually collected from babies less than 12 months old. A number of respiratory viruses can be detected by direct or indirect IF, including RSV, influenza A and B, adenoviruses and parainfluenza viruses. The method is most useful in the case of RSV where antiviral treatment is available for severely ill babies. IF is also widely used for the detection of HSV infections, from vesicle lesions and brain lesions, for VZV and CMV infections [14].

A typical indirect IF procedure for the detection of viral antigens is as follows; -cells from the clinical specimen are immobilized onto individual wells on a slide. Specific polyclonal or monoclonal sera are then added to each well and the slide is incubated at 37 degrees Celsius for 30 to 60 minutes. The slide is then washed 3 times for 5 minutes each with PBS and fluorescein labeled antibody against the first antibody is added. The slide is further incubated at 37 degrees Celsius for 30 to 60 minutes and washed again. The slide is then prepared for microscopy. Specific monoclonal or polyclonal sera raised against the viral antigen can be used. Monoclonal sera offer the advantage of increased sensitivity and specificity. However, one must be certain that it can detect all the different strains of the virus [14,15].

Detection of viral antibodies: It simply requires virally infected cells that express viral antigens and a fluorescein-labeled antiserum against human immunoglobulin. IF can be used to detect IgG, IgM and IgA. Being very easy to set up, it is often the first and only serological assay available for newly discovered viruses, in particular arboviruses. IF is extensively used for the diagnosis of EBV infections and is also routinely used for other viruses such as VZV [16].

Detection of viral antigens: Antigen detections include immunofluorescence testing of nasopharyngeal aspirates for respiratory viruses e.g. RSV, flu A, flu B, and adenoviruses, detection of rotavirus antigen in feces, the pp65 CMV antigenaemia test, the detection of HSV and VZV in skin scrapings and the detection of HBsAg in serum. The main advantage of these assays is that they are rapid to perform with the result being available within a few hours. The quality of the specimen obtained is of utmost importance in order for the test to work properly [16,17] (Figure 3).
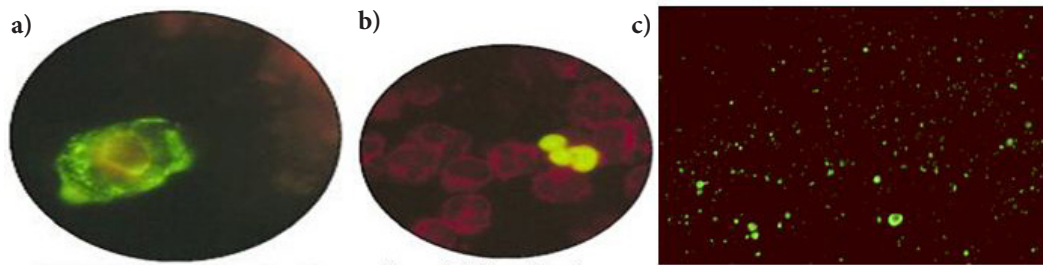

Figure 3: Positive immunofluorescence tests for **a)** HSV antigen from epithelial cell **b)** CMV pp65 antigen from peripheral blood neutrophils and **c)** Test for rabies virus antigen (Source: CDC)

Conclusion

Diagnosis and identification of viruses is an important component of the clinical virology laboratory. In general, diagnostic tests can be grouped into three categories: direct detection, indirect examination (virus isolation), and serology. Many different types of serological tests are available to diagnose different viral diseases. IF is widely used for the rapid diagnosis of virus infections by detection of virus antigen in clinical specimens and detection of virus-specific antibodies.

Cell culture is the complex process by which cells are grown under controlled conditions and support the widest range of viruses. There are various cell culture techniques used to isolate different virus types. The presence of growing (identification of) virus is usually detected by cytopathic effect and haemadsorption changes. Cytopathic effect induces cellular changes that are noticed as the monolayer cells deteriorate as a result of the viral infection. These changes include swelling or shrinkage of cells, the formation of multinucleated giant cells and the production of inclusions in the nucleus or cytoplasm of the infected cell.

Recommendations

From my article review on cell culture, cytopathic effect and immunofluorescence detection of virus, I thereby would like to recommend some points to laboratory professionals and other stakeholders: to apply cell culture techniques in diagnosis of viral diseases, demonstrate and practice these technologies in isolating virus, give attention for viral diagnosing methods by allocating adequate budget.

References

1. Overview of Diagnostic Methods for virus (2017).

2. Leland DS, Ginocchio CC (2007) Role of Cell Culture for Virus Detection in the Age of Technology. Clin Microbiol Rev 20: 49-78.

3. Freshney RI (2000) Culture of Animal Cells: A Manual of Basic technique. (4th edn). New York, 200-4.

4. Hematian A, Sadeghifard N, Mohebi R, Taherikalani M, Nasrolahi A, et al. (2016) Traditional and Modern Cell Culture in Virus Diagnosis. Osong Public Health Res Perspect 7: 77-82.

5. Klaunig JE, Goldblatt PJ, Hinton DE, Lipsky MM, Trump BF (1981) Mouse liver cell culture. II. Primary culture. In Vitro 17: 926-34.

6. Konduru K, Kaplan GG (2006) Stable growth of wild-type hepatitis a virus in cell culture. J Virol 80: 1352-60.

7. Olivo PD (1996) Transgenic cell lines for detection of animal viruses. Clin Microbiol Rev 9: 321-34.

8. Berson RE, Friederichs G (2008) A self-feeding roller bottle for continuous cell culture. Biotechnol Prog 24: 154-7.

9. Laboratory diagnosis of viral infections (2017).

10. Karupiah G (2002) Fields Virology (4th Ed.). Immunol Cell Biol 80: 314-5.

11. Liu L, Loutherback K, Liao D, Yeater D, Lambert G (2010) A microfluidic device for continuous cancer cell culture and passage with hydrodynamic forces. Lab Chip 10: 1807-13.

12. Yi M (2010) Hepatitis C virus: propagation, quantification, and storage. Curr Protoc Microbiol 15: D1.

13. Cytopathic effect (CPE) and haemadsorption of virus (2017).

14. Jaguś P, Chorostowska-Wynimko J, Radzikowska E, Gawryluk D (2011) The Effectiveness of Common Respiratory Viruses Detection by Indirect Immunofluorescence Test in Samples from Upper Respiratory Tract. Centr Eur J Immunol 36: 37-9.

15. CDC (2017) Guidelines for viral diagnosis and identification.

16. Sadeghi CD, Aebi C, Gorgievski-Hrisoho M, Mühlemann K, Barbani MT (2011) Twelve years' detection of respiratory viruses by immunofluorescence in hospitalised children: impact of the introduction of a new respiratory picornavirus assay. BMC Infect Dis 11: 41.

17. Mebatsion T, Cox JH, Frost JW (1992) Isolation and characterization of 115 street rabies virus isolates from Ethiopia by using monoclonal antibodies: identification of 2 isolates as Mokola and Lagos bat viruses. J Infect Dis 166: 972-7.