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A comprehensive review on cell culture principles and its applications on laboratory diagnosis

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Abstract
Cell culture entails intricate steps for removing cells from their natural environments (*in vivo*) and then allowing them to develop in a controlled, artificial environment (*in vitro*). In order to isolate dangerous viruses, it is necessary to have access to authorised cell cultures. Cells from certain tissues or organs are cultivated as short-term or established cell lines, which are frequently used for research and diagnosis. By using virus isolation in cell culture as the "gold standard" for virus discovery, cell culture provides the necessary environment for the detection and identification of a variety of diseases. The opinions of scientists on the current application of cell culture technologies in the diagnosis of diseases are compiled in this review. Recent technical advancements, from the creation of monoclonal antibodies to molecular methods, have made it possible to detect the presence of viral infection. They serve as a starting point for developing quick testing for recently discovered diseases. The discovery of previously unknown viruses still depends on a mix of virus isolation in cell culture and molecular techniques. As a result, cell culture should be viewed as a crucial step in determining the probable infectious viral pathogen.

Keywords: Pathogen discovery, recombinant protein, transgenic cell line, viral isolation

Introduction
Early in the 20th century, cell culture technique was created as a way to examine animal cell behaviour *in vitro* [1]. When Roux, an embryologist, kept a chicken embryo alive in warm saline for several days, he developeds tissue culture principle, which is the basis for cell culture [2]. As a result, cell culture has been described as the removal of animal cells and their subsequent proliferation and cultivation *in vitro* in an artificial environment that is suitable for its growth [3]. Typically, this begins with primary culture that aims to produce a monolayer of cells in a culture flask with the necessary nutrients and growth factors added. Once confluence is reached, the cells are passaged or subcultured from the primary to secondary and then to tertiary levels until a continuous cell line is produced [4]. Virus isolation in cell culture is labour intensive and consumes lot of time [5, 6]. In tissues culture, many clinically significant viruses are still either difficult to grow or do not grow at all, while others may demand for a complex culture system that is either unsuitable for use in diagnostic laboratories or not available at all. This could lessen tissue culture's influence on clinical diagnosis, making it less desirable for diagnosing infections [5, 7]. Tissue culture, on the other hand, has been viewed as largely unbiased by some scientists, with the virus's capacity to grow on the chosen cell lines serving as its sole real limitation [8, 9]. Yet, Vero E6 cells were thought to be the most tolerant of all cell lines since they offered a flexible media for the recovery of new infections and the use of electron microscopy (EM) to identify and classify unknown agents [10, 11].
Cell culture observation by EM can offer early cues on the aetiologic agent and subsequently direct laboratory and epidemiologic research. Knowing the aetiologic agent can help public health officials implement a timely reaction and prevent or limit the further spread of the causative agent, which is why it is clinically significant, especially during illness outbreaks [12, 13]. Hence, it is claimed that the employment of traditional methods of viral isolation in tissue culture and inspection under EM is essential for the discovery of previously undetected viruses. Contrary to what was previously believed, if an infectious viral agent is detected, cell culture is a crucial procedure that can be carried out in hospital diagnostics and microbiology laboratories. In multiple investigations, this method was utilised to identify the Ebola virus in a suspected yellow fever patient and vice versa [14-17].
Deep sequencing technologies and recent advancements in metagenomics have made it possible to analyse the genome of microorganisms without isolating the virus via cell culture. This is accomplished through high-throughput sequencing employing randomly amplified

DNA product and comparison of sequences with huge banks of sequences that are readily available for the purpose of identifying the detected agent. Because random primers can precisely amplify the template for sequencing without knowing beforehand what the suspected agent is, this is conceivable [18-20]. In terms of pathogen discovery, this technique is quickly advancing. It has always been used to find viruses such the Lioviu virus [21] Schmallenberg virus [22] and Bas –Congo virus [23]. Identification of the infection's causal agent is crucial in cases of critically ill individuals or infectious disease outbreaks. As a result, this study aims to describe some of the instances in which viruses are isolated for determining the causal agent and recognising developing diseases by further laboratory diagnosis assays such Electron Microscope (EM), serological, and molecular techniques. Inoculating clinical samples from a patient onto the culture cells allows for biological amplification of the virus to the point where it can be detected or viewed under EM and further confirmed by other techniques like serology, immunohistochemistry, as well as fluorescence antibody assays and molecular methods, further characterising the species and strain of the virus [24-26]. Consequently, the "gold standard" for diagnosing viral infections in clinical virology has been a culture-based approach for viral isolation, which has been useful for the laboratory for decades [27]. However, due to the development of quick and precise molecular approaches, the use and relative importance of virus culture have been on the decline [28-30]. In light of this, the purpose of this review is to critically summarise the opinions of researchers on the application of cell culture technology to the diagnosis of human diseases.

Applications of cell culture

A) Cell culture and electron microscopy in diagnosis

Using cell culture separation and electron microscopy (EM), it is possible to identify the cause of a unique clinical presentation. In one study, a patient with a history of tick bites was found to have the Bunya virus [31]. Ehrlichia spS was initially suspected, thus leukocytes from the suspected patient were implanted into DH82 cell (canine monocytes cell line), and it demonstrated some cytological alterations. The expected bacteria not found after the cells were prepared for electron microscopy (EM) analysis; instead, a Bunya virus was found. The Bunya virus can be discovered as a bud in vesicles and extracellular spaces in infected cells. The virus core is granular, and the virus's envelope is spherical with considerable protrusion on its surface.

B) Cell Culture and RT– PCR

Real-time reverse transcription polymerase chain reaction (qRT-PCR) and cell culture have both been widely employed in clinical settings to detect influenza viruses [32, 33]. Although time- and labor-intensive, it required highly skilled workers, specialised lab setups, and other requirements that made it unsuitable for use in primary healthcare settings or in low-income nations. Cell culture is still crucial for identifying the infection that caused an outbreak, though. Cell culture and RTPCR were used to confirm the currently listed H7 N9 influenza cases [34].

C) Cell Culture – Metabolomics

Cell culture metabolomics can be utilised to find the metabolic pathways that produce a pathological condition's biomarkers as well as the biomarkers themselves. By finding new cancer biomarkers, metabolites have a significant impact on cancer diagnosis, recurrences, and prognosis. The creation of prognostic models that will aid in the early detection of cancer is made possible by the detection of a modest alteration in metabolism in cellular process products. The propensity of human cancer cells to release volatile organic molecules was investigated in several research [35, 36], a few of which were capable of identifying the release of acetaldehyde from the lung cancer cell lines CALU-1 and SK-MES [37, 38].

D) Rapid Detection Cell Culture

Due to the development of commercially available, grown cell lines for the quick identification of a range of viruses, such as R-Mix (Diagnostic Hybrid, Inc.), a combination of monolayers of cells chosen for their capacity to isolate several viruses that cause respiratory tract infection. R-Mix contains lung tissue (MV1LU) and A549 cells in fresh, immediately usable form, frozen cell suspension that can be aliquoted by the laboratory, or frozen monolayers in shell vials that are immediately usable. Since no technical knowledge is needed, R mixed has thus far been found to give a quick and time-sensitive method of identifying viruses that frequently cause respiratory infections [39]. Table 1depict cytopathogenic effect in standard cell cultures of human viral pathogens common in the United States [40].

E) Transgenic Cell Lines and Viral Detection

Stable genetic materials are incorporated into cells using transgenic technology in cell culture such that when a certain virus enters the cell, it triggers the creation of virus-specific enzymes that are readily quantifiable [41, 42]. The genetic materials, known as "virus inducible reporter gene segment," can have bacterial, viral, or cellular origins [43, 44]. Transgenic cells can only be helpful in diagnostic laboratories if they have the desired promoter, which is quiet in infected cells but significantly upregulated by viral trans-activator protein in a manner that is specific, but not allowing heterologous viral transactivation protein to stimulate the promoter. For a transgenic system to function, the virus that is to be identified must be able to cling to the cell wall and prime its replication cycle without reaching the ending point, but sufficient to activate the gene through the promoter. This makes it possible to employ genetically modified cell lines to promote virus development, making it easier to identify virus-infected cells and creating a detection method that is highly accurate, sensitive, and user-friendly [45, 46]. Using HeLa cells that are susceptible to transformation, this approach was successfully used to identify the polio virus [47]. Nevertheless, monoclonal antibody staining is required for its identification, and it takes 16 to 24 hours after inoculation to identify it [48, 49]. In contrast, a quicker transgenic system that can quickly and readily identify HSV within 24 hours was created in a way that it does not require costly monoclonal antibodies or specialised medical knowledge. UL39-derived HSV promoter, which codes for big ribonucleotide reeducates subunit, is used in this process [50, 51].

F) Expression of recombinant protein for detection of influenza virus antibody

Recombinant protein technology is crucial for satisfying the requirement for simple, quick, and accurate tests in diagnostic laboratories and has proved helpful for serological survey of

infection [52]. In order to find influenza virus antibodies, recombinant protein can be expressed. For instance, the NSI gene was effectively isolated and cloned into a vector [pCR2.1 TOPO TA cloning (3.9 kb)], and competent cells (TOPOIO F' E. coli strain) were distributed on LB agar and cultured at 37°C overnight. PCR was used to screen the positive colonies that contained the NSI gene. The agarose gel test findings revealed the expected 690 bp band [53]. After sequencing, it was determined that it was in the right orientation and in frame with the N-terminal. The host cell strain B12 (DE3) pLysS was then used to convert the recombinant plasmids into for expression. Using the thermal shock approach, transformation was accomplished. SDS-PAGE was used to analyse the expressed protein, and western blotting was then used to confirm it. The polyclonal anti-NS antibody was used to create the predicted 13 KDa protein [54]. Elisa, which has a significant advantage over other techniques for detecting particular antibodies, was able to be utilised to detect specific antibodies against influenza viruses utilising the antigen, as was confirmed.

G) Biopesticides

Due of growing concerns about agrochemicals and their residues in the environment and food, biopesticides have become more significant in recent years. Insect and plant disease can be controlled effectively and environmentally safely with the use of biopesticides. The most commonly used cell lines in biopesticide production are the Sf21 and Sf9 cell lines, which are derived from ovarian tissues of the fallarmy worm (*Spodoptera frugiperda*). Sf9 cells show a faster growth rate and higher cell density than Sf21 cells and are preferred. High Five cell lines (designated BTI-Tn-5BI-4) established from Trichoplusiani embryonic tissue are also being used.

H) Gene therapy

Gene therapy clinical trials and studies have already received approval and are being carried out all around the world. Over 500 clinical studies have been reported from 1989 to the present, with 70% of these research focusing on the treatment of cancer. Gendicine, a drug made in China by Shenzhen Sibiono Genetech, was the first item intended for gene therapy. Gendicine is used to treat head and neck cancer. Recombinant adenoviruses that express the tumour 4 suppressing gene p53 produce the protein p53, which helps to regulate and eradicate tumours. Gendicine has been produced using the SBN-cel cell line, which was subcloned from the human embryonic kidney (HEK) cell line 293.

Constraints of cell culture

A) Standardization

Contrary to molecular methods, cell culture outcomes might vary significantly depending on specimen collection, transport, and treatment to maintain viral viability and healthy inoculated cells [55]. Both proponents and opponents of cell culture in clinical laboratories have been discussed by researchers. While some people anticipated that there would be instances where using tissue culture in a diagnostic virology lab would be appropriate, others believe that this may be true to some extent but not at the point of care, changing the significance of cell culture in diagnostics [56, 57]. However, molecular quantitative assay is still highly variable as such required standardization [58-59]. Certain local areas and national laboratories that have the necessary knowledge and maintain cell culture systems could do viral isolation when necessary for a specific reason [60].

B) Time Consuming

In this era of seeking a prompt and accurate clinical diagnosis required for early and successful intervention, cell culture is gradually losing its position and relative value in the diagnosis of human diseases. Nevertheless, molecular approaches offer a reliable and ageless diagnostic approach. Therefore, molecular techniques are quickly replacing traditional cell culture-based, early and accurate diagnostic methods that have a significant impact on patient care by limiting the extent of diseases through timely treatment, thereby reducing unnecessary hospitalisation, antimicrobial use and their associated cost.

C) Labour Intensive

Cell culture needs specialised equipment, experienced technologists, and experts. So, it is crucial to utilise the existing technology based on a certain circumstance in order to produce more beneficial results. Pathogen identification has been accomplished with the use of transgenic technology, but it takes a lot of work and requires knowledge. The requirement for intensive labour was reduced with the invention of quick cell culture technology that uses fluorescence staining, in which colour change is utilised to identify infections because the technician does not need to be proficient in maintaining CPE in cells [62]. As a result, laboratories should assess the necessary equipment, facilities, degree of training, and competence.

D) Sensitivity

This review makes it obvious that some scientists believe that cell culture is less sensitive than molecular methods like PCR and has a much smaller viral spectrum. Cell culture would then be less useful for viruses that are "non culturable," reducing its sensitivity for use in diagnostics (63-65). However, there are several issues with molecular techniques like PCR, including the potential for false negative results because of PCR inhibitors and the genetic variety of the viruses, as well as false positive results because of contamination, latent infection, and viral co-infection. Determining the sensitivity and specificity of molecular approaches requires cell culture, which is why doing so is crucial.

Table 1: Cytopathogenic effect in standard cell cultures of human viral pathogens common in the United States

Virus	Cytopathogenic effect in				Final identification of isolates
	Fibroblasts	A549 cells	RhMK cells	Other	
Adenovirus	Some produce clusters	Grape-like clusters or "lacy" pattern; 5–8 days	Some produce clusters	HNK: grape-like clusters; 5–7 days	IF for group, neutralization for type
CMV	Foci of contiguous rounded cells; 10–30 days	None	None	Use shell vials for rapid detection	CPE alone
Enteroviruses	Some produce CPE, same as in RhMK cells; 2–5 days	Infrequent, degenerative	Small, round cells with cytoplasmic tails; 2–5 days	-----	IF for groups, neutralization for type
HSV	Rounded large cells; 2–6 days	Rounded large cells; 1–4 day	Some produce CPE, same as in A549 cells, 4–8 days	RK or HNK: rounded large cells; 1–4 day	IF
Influenza virus	None	None	Undifferentiated CPE, cellular granulation; 4–8 days	HAD-positive with GP HAD-positive with GP	IF
Parainfluenza virus	None	None	Rounded cells, some syncytia; 4–8 days	HAD-positive with GP HAD-positive with GP	IF
Rhinovirus	Degeneration, rounding; 7–10 days	None	None	Incubate fibroblasts at 33°C	CPE only f (difficult to differentiate from enteroviruses)
RSV	Infrequent, granular degeneration	Infrequent	Syncytia; 4–10 days	HEp-2d: syncytia; 4–10 days	IF
VZV	Some CPE; small, round cells; 6–8 days	Small, round cells; 6–8 day	None	HNK: small, round cells; 6–8 days	IF

*Leland and Ginocchio, 2007 [5]

Conclusion

Present study inferred that cell culture is a vital tool in contemporary medicine with countless uses for diagnosing infections. Cell culture techniques are somewhat unbiased and are only constrained by the virus's capacity to proliferate in a certain cell line. With the development of transgenic cell culture techniques, this was however overcome. Thus, we advise every year, quick tests based on antigen assays should be monitored for specificity and sensitivity using cell culture and the results should be communicated to the practitioners. Cell culture should be encouraged both for positive results during low prevalence and for both positive and negative quick test results obtained from patient features infection during high prevalence or outbreak. PCR serological testing, histopathology, and immunological histochemistry can all be used in conjunction with cell culture to diagnose undetermined viruses. In developing a quick test for recently discovered infections, they are also utilised.

References

1. Thorpe TA. History of plant tissue culture. Molecular Biotechnology. 2007;37(2):169-180.
2. Fung S, Wong F, Hussain M, Lok A. Sustained response after a 2-year course of lamivudine treatment of hepatitis B e antigen-negative chronic hepatitis B. Journal of Viral Hepatitis. 2004;11(5):432-438.
3. Ganem D, Schneider RJ. Hepadnaviridae: the viruses and their replication. Fields Virology. 2001;2:2923-2969.
4. Willmer EN. Cells and tissues in culture: methods, Biology and Physiology: Elsevier; c2013.
5. Leland DS, Ginocchio CC. Role of cell culture for virus detection in the age of technology. Clinical Microbiology Reviews. 2007;20(1):49-78.
6. Oberste MS, Nix WA, Maher K, Pallansch MA. Improved molecular identification of enteroviruses by RT-PCR and amplicon sequencing. Journal of Clinical Virology. 2003;26(3):375-77.
7. Griffith LG, Naughton G. Tissue engineering--current challenges and expanding opportunities. Science. 2002;295(5557):1009-1014.
8. Khademhosseini A, Vacanti JP, Langer R. Progress in tissue engineering. Scientific American. 2009;300(5):64-71.
9. Ransohoff RM, Perry VH. Microglial physiology: unique stimuli, specialized responses. Annual Review of Immunology. 2009;27:119-145.
10. Abiko C, Mizuta K, Itagaki T, Katsushima N, Ito S, Matsuzaki Y, *et al*. Outbreak of human metapneumovirus detected by use of the Vero E6 cell line in isolates collected in Yamagata, Japan, in 2004 and 2005. Journal of Clinical Microbiology. 2007;45(6):1912-1919.
11. Weidmann M, Sanchez-Seco MP, Sall AA, Ly PO, Thiongane Y, Lô MM, *et al*. Rapid detection of important human pathogenic Phleboviruses. Journal of Clinical Virology. 2008;41(2):138-142.
12. Goldsmith CS, Ksiazek TG, Rollin PE, Comer JA, Nicholson WL, Peret TC, *et al*. Cell culture and electron microscopy for identifying viruses in diseases of unknown cause. Emerging Infectious Diseases. 2013;19(6):864.
13. Alberts B, Bray D, Hopkin K, Johnson A, Lewis J, Raff M, *et al*. Essential cell biology: Garland Science; c2013.
14. Kann M, Bischof A, Gerlich WH. *In vitro* model for the nuclear transport of the hepadnavirus genome. Journal of Virology. 1997;71(2):1310-1316.
15. McMullan LK, Frace M, Sammons SA, Shoemaker T, Balinandi S, Wamala JF, *et al*. Using next generation sequencing to identify yellow fever virus in Uganda. Virology. 2012;422(1):1-5.
16. Muyembe-Tamfum J-J, Mulangu S, Masumu J, Kayembe J, Kemp A, Paweska JT. Ebola virus outbreaks in Africa: past and present. Onderstepoort Journal of Veterinary Research. 2012;79(2):06-13.
17. Kuno G. 23 Yellow Fever Virus. Manual of Security Sensitive Microbes and Toxins; c2014. p. 265.
18. Mokili JL, Rohwer F, Dutilh BE. Metagenomics and

future perspectives in virus discovery. Current opinion in virology. 2012;2(1):63-77.
19. Willner D, Hugenholtz P. From deep sequencing to viral tagging: recent advances in viral metagenomics. Bioessays. 2013;35(5):436-442.
20. Lagier J-C, Edouard S, Pagnier I, Mediannikov O, Drancourt M, Raoult D. Current and past strategies for bacterial culture in clinical microbiology. Clinical microbiology reviews. 2015;28(1):208-236.
21. Mehedi M. Ebola virus RNA editing: Characterization of the mechanism and gene products; c2011.
22. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. Nature Reviews Immunology. 2010;10(2):103-110.
23. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. Journal of Interferon & Cytokine Research. 2009;29(6):313-326.
24. Gardner PS, McQuillin J. Rapid virus diagnosis: Application of immunofluorescence: Butterworth-Heinemann; c2014.
25. Straus D. Rapid and sensitive detection of cells and viruses. Google Patents; c2011.
26. Kango N. Textbook of Microbiology: IK International Pvt Ltd; c2010.
27. De Serres G, Skowronski D, Wu X, Ambrose C. The test-negative design: validity, accuracy and precision of vaccine efficacy estimates compared to the gold standard of randomised placebo-controlled clinical trials. Euro Surveill. 2013;18(37).
28. Emerson SU, Purcell RH. Hepatitis E virus. Reviews in Medical Virology. 2003;13(3):145-154.
29. Yang S, Rothman RE. PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. The Lancet infectious diseases. 2004;4(6):337-348.
30. Niesters HG. Clinical virology in real time. Journal of Clinical Virology. 2002;25:3-12.
31. Yu X-J, Liang M-F, Zhang S-Y, Liu Y, Li J-D, Sun Y-L, *et al*. Fever with thrombocytopenia associated with a novel bunyavirus in China. New England Journal of Medicine. 2011;364(16):1523-1532.
32. Templeton KE, Scheltinga SA, Beersma MF, Kroes AC, Claas EC. Rapid and sensitive method using multiplex real-time PCR for diagnosis of infections by influenza A and influenza B viruses, respiratory syncytial virus, and parainfluenza viruses 1, 2, 3, and 4. Journal of Clinical Microbiology. 2004;42(4):1564-1569.
33. Rahman M, Vandermause MF, Kieke BA, Belongia EA. Performance of Binax NOW Flu A and B and direct fluorescent assay in comparison with a composite of viral culture or reverse transcription polymerase chain reaction for detection of influenza infection during the 2006 to 2007 season. Diagnostic Microbiology and Infectious Disease. 2008;62(2):162-166.
34. Xia J, Liu L, Wang L, Zhang Y, Zeng H, Liu P, *et al*. Experimental infection of pregnant rabbits with hepatitis E virus demonstrating high mortality and vertical transmission. Journal of Viral Hepatitis; c2015.
35. Zhao W-D, Chen J, LIU F-G, Wang M, LI J-M. Poster Abstracts–Liver. Chinese Journal of Digestive Diseases. 2005;6:A31-A51.
36. Beebe K, editor Employing metabolomics in cell culture and bioprocessing to gain greater predictability, control and quality. Annual Meeting and Exhibition. 2014 (July 20-24, 2014); 2014: Simb.
37. Smith D, Wang T, Sulé-Suso J, Španel P, Haj AE. Quantification of acetaldehyde released by lung cancer cells *in vitro* using selected ion flow tube mass spectrometry. Rapid communications in mass spectrometry. 2003;17(8):845-850.
38. Kalluri U, Naiker M, Myers M. Cell culture metabolomics in the diagnosis of lung cancer—the influence of cell culture conditions. Journal of breath research. 2014;8(2):027109.
39. Tatematsu K, Tanaka Y, Kurbanov F, Sugauchi F, Mano S, Maeshiro T, *et al*. A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype. Journal of Virology. 2009;83(20):10538-10547.
40. Diane S. Leland and Christine C. Ginocchio. Role of Cell Culture for Virus Detection in the Age of Technology. Clinical microbiology reviews. 2007;20(1):49-78.
41. Deisseroth K, Airan RD. Cell line, system and method for optical control of secondary messengers. Google Patents; c2014.
42. Dimitrov DS. Virus entry: molecular mechanisms and biomedical applications. Nature Reviews Microbiology. 2004;2(2):109-122.
43. Lutz A, Dyall J, Olivo PD, Pekosz A. Virus-inducible reporter genes as a tool for detecting and quantifying influenza A virus replication. Journal of Virological Methods. 2005;126(1):13-20.
44. Li Y, Larrimer A, Curtiss T, Kim J, Jones A, Baird-Tomlinson H, *et al*. Influenza virus assays based on virus-inducible reporter cell lines. Influenza and Other Respiratory Viruses. 2009;3(5):241-251.
45. Palmer AE, Jin C, Reed JC, Tsien RY. Bcl-2-mediated alterations in endoplasmic reticulum Ca2+ analysed with an improved genetically encoded fluorescent sensor. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(50):17404-17409.
46. Kang S, Ren D, Xiao G, Daris K, Buck L, Enyenihi AA, *et al*. Cell line profiling to improve monoclonal antibody production. Biotechnology and Bioengineering. 2014;111(4):748-760.
47. Mowafi F. Chemokines and chemokine receptors during viral infections in man: Institutionen för mikrobiologi, tumör-och cellbiologi/Department of Microbiology, Tumor and Cell Biology; c2007.
48. Anderson NW, Buchan BW, Ledeboer NA. Light microscopy, culture, molecular, and serologic methods for detection of herpes simplex virus. Journal of Clinical Microbiology. 2014;52(1):2-8.
49. Atmar RL. Immunological Detection and Characterization. Viral Infections of Humans: Springer; c2014. p. 47-62.
50. Ma JZ, Russell TA, Spelman T, Carbone FR, Tscharke DC. Lytic gene expression is frequent in HSV-1 latent infection and correlates with the engagement of a cellintrinsic transcriptional response. PLoS pathogens. 2014;10(7):e1004237.
51. Fan F, Day S, Lu X, Tang Y-W. Labouratory diagnosis of HSV and varicella zoster virus infections. Future Virology. 2014;9(8):721-731.

52. Marks LV. The Lock and Key of Medicine: Monoclonal Antibodies and the Transformation of Healthcare: Yale University Press; c2015.
53. Portela A, Melero JA, Martínez C, Domingo E, Ortín J. A primer vector system that allows temperature dependent gene amplification and expression in mammalian cells: regulation of the influenza virus NSI gene expression. Nucleic acids research. 1985;13(22):7959-7977.
54. Schägger H. Tricine–SDS-PAGE. Nature Protocols. 2006;1:16-22.
55. Kao J-H, Chen P-J, Lai M-Y, Chen D-S. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology. 2000;118(3):554-559.
56. Sacks G. Reproductive immunology: the relevance of labouratory research to clinical practice (and vice versa). Human Reproduction. 2014:deu325.
57. Viswanathan S, Keating A, Deans R, Hematti P, Prockop D, Stroncek DF, *et al*. Soliciting strategies for developing cell-based reference materials to advance mesenchymal stromal cell research and clinical translation. Stem cells and development. 2014;23(11):1157-1167.
58. Nagy A, Jirinec T, Cerníková L, Jirincová H, Havlícková M. Large-Scale Nucleotide Sequence Alignment and Sequence Variability Assessment to Identify the Evolutionarily Highly Conserved Regions for Universal Screening PCR Assay Design: An Example of Influenza A Virus. PCR Primer Design; c2015. p. 57-72.
59. Barnett D, Louzao R, Gambell P, De J, Oldaker T, Hanson CA. Validation of cell-based fluorescence assays: Practice guidelines from the ICSH and ICCS– part IV–postanalytic considerations. Cytometry Part B: Clinical Cytometry. 2013;84(5):309-314.
60. Stacey GN. The Challenge of Standardization in Stem Cell Research and Development. Stem Cell Banking: Springer; c2014. p. 11-18.
61. Franco E, Bagnato B, Marino MG, Meleleo C, Serino L, Zaratti L. Hepatitis B: Epidemiology and prevention in developing countries. World Journal of Hepatology. 2012;4(3):74.
62. Yu M-W, Yeh S-H, Chen P-J, Liaw Y-F, Lin C-L, Liu C-J, *et al*. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. Journal of the National Cancer Institute. 2005;97(4):265-272.
63. Kao J-H, Wu N-H, Chen P-J, Lai M-Y, Chen D-S. Hepatitis B genotypes and the response to interferon therapy. Journal of Hepatology. 2000;33(6):998-1002.
64. Koo ES, Yoo C-H, Na Y, Park SY, Lyoo HR, Jeong YS. Reliability of nonculturable virus monitoring by PCR-based detection methods in environmental waters containing various concentrations of target RNA. Journal of Microbiology. 2012;50(5):726-734.
65. Doerr H. Replacement of biologic by molecular techniques in diagnostic virology: Thirty years after the advent of PCR technology—do we still need conventional methods? Med Microbiol Immunol. 2013;202(6):391-392.