# Cell Culture, Technology: Enhancing the Culture of Diagnosing Human Diseases

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## Abstract

The complicated processes of removing cells from them native environment (in vivo) and growing them in an artificially controlled environment (in vitro) are all part of cell culture. Because the availability of appropriate cell cultures is a prerequisite for the isolation of harmful viruses, cells from particular tissues or organs are cultivated as short-term or established cell lines that are frequently utilized for research and diagnostics, particularly in the context of viral infection. The necessary environment is provided by cell culture for the detection and identification of many human diseases. The & quot;gold standard & quot; for virus discovery is virus isolation in the cell culture. Researchers opinions about the current application of cell culture technologies in the diagnosis.

**Keywords**: Pathogen discovery, Recombinant protein, Transgenic cell line, Viral isolation

# Cell Culture, Technology: Enhancing the Culture of Diagnosing Human Diseases

The early 20th century saw the development of the cell culture technique, which was used to examine animal cell behavior in vitro [1]. When embryologist Roux maintained chicken embryos in warm saline for several days, he developed the tissue culture concept, which laid the foundation for cell culture [2]. Thus, the term "cell culture" refers to the process of isolating animal cells and growing them in vitro in a controlled setting that is conducive to their growth [3, 4]. In order to achieve confluence, or the creation of a cell monolayer in a culture flask provided with the necessary nutrients and growth factors, this normally starts with a primary culture. After confluence is reached, the cells are either passage through or subculture from the the necessary development factors and nutrition. The cells are passaged or subculture from the primary to secondary and subsequence to tertiary until confluence is reached, until a continuous cell line is created. It takes time and a lot of labor to isolate a virus in a cell culture [5, 6]. Many clinically significant viruses are still either difficult to grow in tissues culture or do not grow at all, while others may require a sophisticated culture technique that is either unavailable or not suited for use in diagnostic laboratories. These could lessen the usefulness of tissue culture in clinical diagnosis, which would make it less appealing for the diagnosis of human illnesses [5,7]. However, other scientists discovered that tissue culture is a reasonably objective method whose sole drawback is the virus's capacity to proliferate on the chosen cell lines [8,9]. The ability to analyze a microorganism's genome without isolating the virus through cell culture has been made possible by recent developments in metagenomics and deep sequencing techniques. In order to finally identify the detected agent, this is accomplished using high-throughput sequencing employing a random amplified DNA product and comparing the sequences with a large bank of sequences. Because random primers can precisely amplify the template for sequencing without requiring prior knowledge about the suspected agent, this is made achievable [10–11]. In the field of pathogen detection, this approach is progressing quickly. It has been used indefinitely to find viruses, including the Schmallenberg, Bas-Congo, and Livia viruses [12, 13]. It is crucial to determine the infection's causal agent when dealing with critically ill individuals or outbreaks of infectious diseases. In order to identify the causal agent and recognize developing diseases, this study aims to describe some of the events in which viruses are isolated using molecular, serological, and electron microscope (EM) techniques, among other laboratory diagnosis assays.

## Methodology:

Utilizing the University Putra Malaysia online subscribed databases, searches for peer-reviewed journal articles in the field of health sciences and medicine were carried out utilizing search engines including Google Scholar, SCOPUS, and Medline. Except in cases where an older publication might be taken into consideration, all searches were restricted to those published between 2000 and 2015. Duplicates were eliminated, and all publications were in English. Articles published up until May 31, 2015, were the last ones that were searched. 2473 publications from the online database search were found; these were filtered based on the relevancy of the title and abstract, excluding conference abstracts, remarks, and brief messages, leaving 260 for full text review studies

## Cell Culture and Electron Microscopy in Diagnosis:

When identifying the cause of an uncommon clinical presentation, electron microscopy (EM) and cell culture separation play key roles. In a patient with a history of tick bites, the Bunya virus was isolated, according to one study [15]. Leukocytes from the patient who was initially suspected of having Ehrlichia pneumonia were inoculated into the DH82 cell line, which is a canine monocyte cell line, and the results showed some cytological alterations [Table/Fig-1]. The predicted bacterium was not found when the cells were processed for EM inspection; instead, a Bunya virus was detected. Bunya virus particles are detected as a bud in vesicles and extracellular areas of infected cells [Table/Fig-2]. With a little projection on the surface of the viral particles, the spherical virus envelope and the virus has granular core.

## Cell Culture and RT– PCR:

For the identification of influenza viruses, real-time reverse transcription polymerase chain reaction (qtr.-PCR) and cell culture have been widely employed in clinical settings [16, 17]. However, it is labor-intensive, time-consuming, and requires highly skilled workers with specialized laboratory equipment and conditions, which makes it unsuitable for primary healthcare settings and low-income nations. Cell culture is still crucial for identifying the infection's causal agent in an outbreak, though. Cell culture and RTPCR were used to confirm the H7 N9 influenza cases that are now being reported [18].

## Cell Culture:

metabolomics Metabolomics in cell cultures is a useful tool for determining both the metabolic pathways that lead to the production of disease-related biomarkers and the biomarkers themselves. Through the discovery of new cancer biomarkers, metabolites are crucial in the diagnosis, recurrence, and prognosis of cancer. The construction of predictive models that will aid in the early detection of cancer is possible due to the detection of a modest alteration in metabolism in the products of cellular processes.

## Transgenic Cell Lines and Viral Detection:

In cell culture, transgenic technology entails introducing stable genetic elements into the cell so that, upon infection, the virus induces the creation of readily detectable enzymes specific to the virus [19, 20]. But monoclonal antibody staining allows for its detection, and it can be found 16–24 hours after inoculation [21, 22]. On the other hand, a quicker transgenic system that can quickly identify HSV within 24 hours was created without the need for pricey monoclonal antibodies or medical training. The big rib nucleotide re-educates sub unit is coded for by the HSV promoter derived from UL39 [23, 24].

## Expression of Recombinant Protein for Detection of Influenza Virus Antibody:

Recombinant protein technology has proven helpful for serological surveys of infections and is crucial in satisfying the need for quick, simple, and accurate tests in diagnostic laboratories [25]. It is possible to generate recombinant protein and utilize it to identify influenza virus antibodies.

SDS-PAGE was used to analyze the expressed protein's expression [Table/Fig-4]. Western blotting was then used to confirm the expression of the predicted 13 KDa protein, which was detected by polyclonal anti-NS antibody [26]. This demonstrated that Elisa, which offers a significant advantage over other methods for detecting particular antibodies, could be utilized

to identify specific antibodies against influenza viruses utilizing the antigen. This demonstrated that Elisa could be used to identify certain antibodies against influenza viruses based on the antigen.

## Issues arising from this review:

uniformity in contrast to molecular techniques, the outcomes of cell culture might vary significantly based on the methods used for collection, transportation, and handling of [Table/Fig-3]. [Table/Fig. 4]:

the sample to preserve healthy inoculated cells and virus vitality [28]. Scholars engaged in debates on the value of cell culture in medical labs. While some people thought that there would be circumstances where using tissue culture in a diagnostic virology laboratory would be appropriate, others felt that this may not always be the case at the point of care, which would alter the role that cell culture plays in diagnostics [29, 30]. When necessary for a specific reason, viral isolation can be carried out by national laboratories with the necessary knowledge and cell culture systems, as well as by certain local regions [31].

## Time Consuming:

In this day and age, when quick and precise clinical diagnosis is essential for prompt and efficient action, cell culture is quickly losing ground and importance in the diagnosis of human diseases. However, molecular methods offer a reliable and ageless means of diagnosis. As a result, molecular techniques are quickly replacing the conventional cell culture-based, early, and accurate diagnostic techniques, which have a substantial impact on patient care by limiting the extent of diseases through prompt treatment and, consequently, lowering the need for unnecessary hospital stays, the use of antibiotics, and the costs associated with them [32].

## Conclusion:

To sum up, cell culture is a vital technique in contemporary medicine with countless uses in the identification of human infections. The only restriction on cell culture techniques is the virus's capacity to proliferate in a certain cell line. But with the development of transgenic cell culture technologies, this has been resolved. Thus, we advise: Cell culture should be utilized to track the sensitivity and specificity of quick testing based on yearly antigen assays, and the findings should be reported to the clinicians; Additionally, cell culture should be promoted for both positive results during low prevalence and negative fast test results received from patient features infection during high prevalence or outbreak: For the diagnosis of an unidentified virus, cell culture can also be utilized in conjunction with immunohistochemistry, histopathology, and PCR serological testing. Additionally, they help create a quick test for recently identified pathogens.



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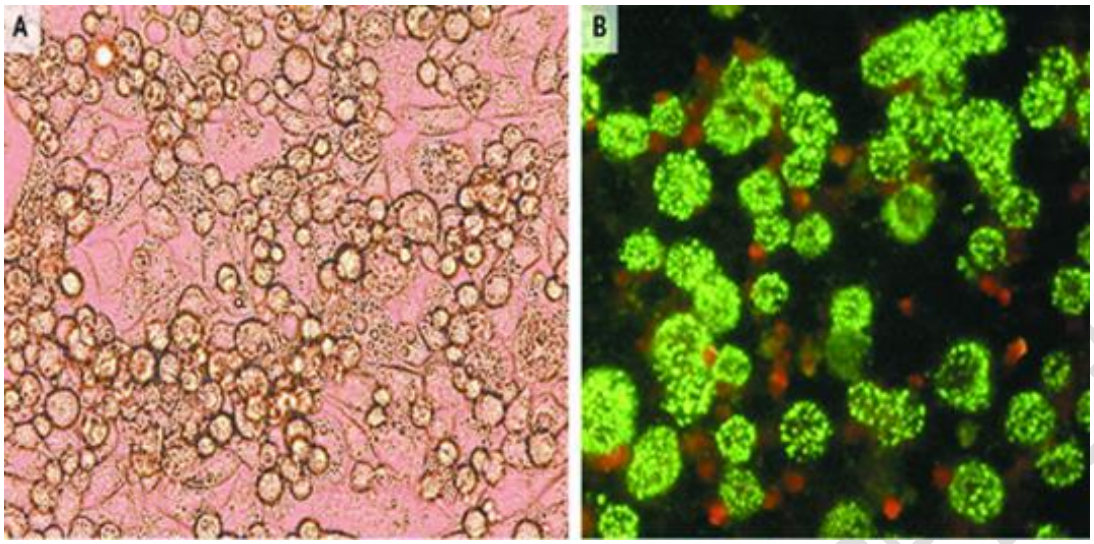
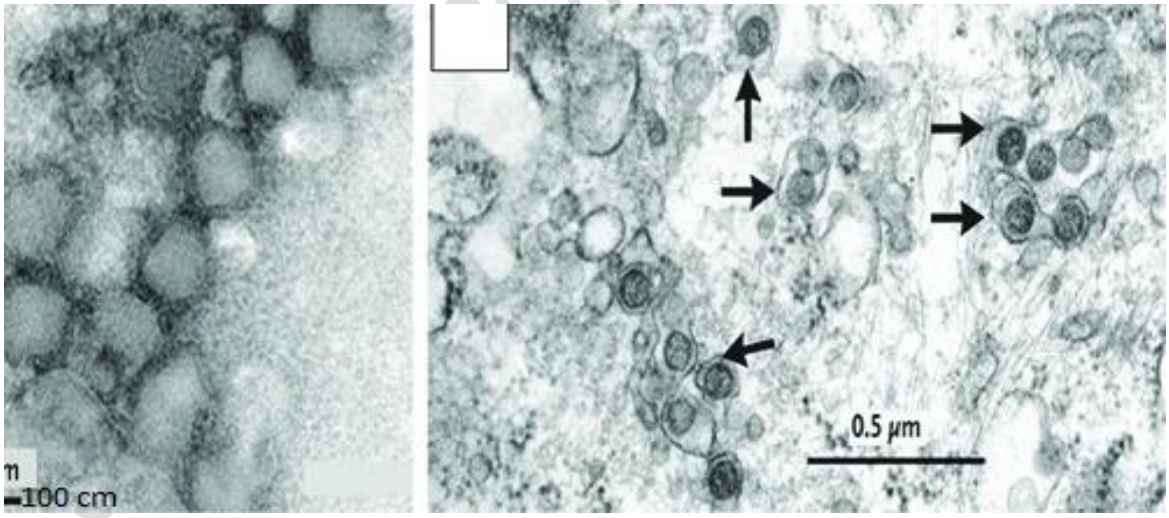


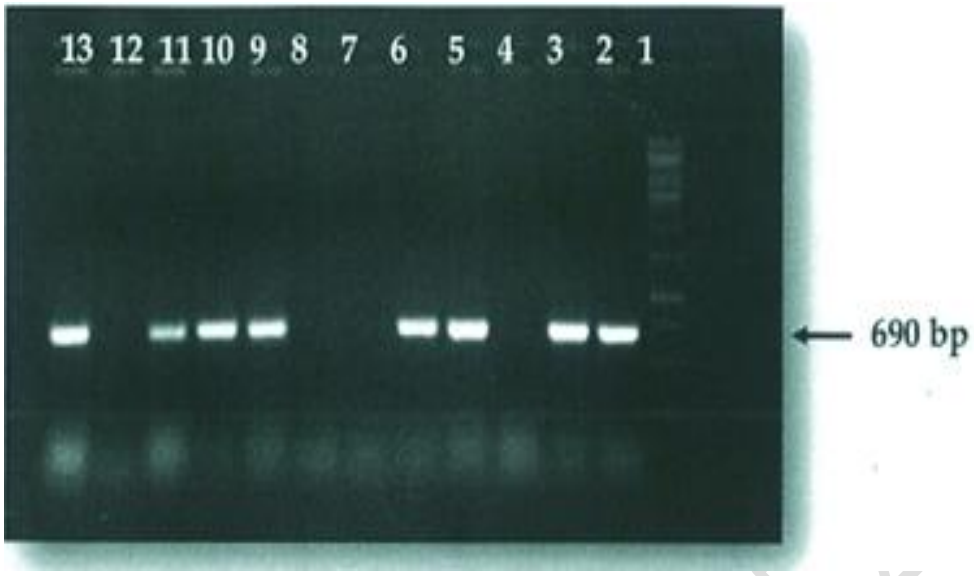

[Table/Fig-1]:

**a)** Visible virus induced cytopathic effect in DH82 cells 8 days' post infection with Bunya virus. The infected cells show visible granular particles and differentiated into macrophages with elongated pseudopodia. **b)** Bunya virus grown in Vero cells, detected on immunofluorescence assay [15].



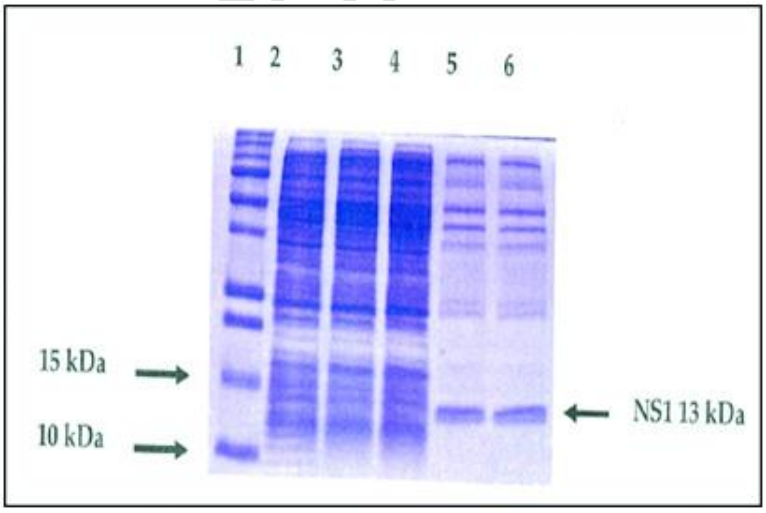

[Table/Fig-2]:

**a)** Negative stain Bunya virus purified from infected Vero cells. **b)** Transmission electron microscopy of virus infected cells (DH82) shown by black arrows [15].




[Table/Fig-3]

Result of successful positive recombinant colonies ligation. Lane 1: DNA ladder 1kb; Lane 2-13: positive colonies with NS1; Lane 4, 7, 8 and 12: negative colonies with no NS1 inserted [27]




[Table/Fig-4]:

SDS-PAGE profile of un-purified expressed NS1 (13KDa) protein and un-induced plasmid. Lane 1: protein ladder; Lane 2 - 4: un-induced recombinant plasmid at zero hour; Lane 5 and 6: un-purified recombinant NS1 (13KDa) protein [26].