Original Article

Characterization of Razi Bovine Kidney (RBK) Cell Line as a Sensitive Cell to Bovine Herpesvirus-1 (BoHV-1)

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ABSTRACT

Viruses are obligate parasites that completely rely on host cells for survival and replication. Razi Bovine Kidney (RBK) cell line was introduced and developed by the Razi Vaccine and Serum Research Institute. It has been successfully established as a continuous cell line over successive passages. As demonstrated in this experimental study, the RBK cells have shown suitable sensitivity to certain viruses, including Bovine Herpesvirus-1 **(**BoHV-1) virus. In the present research, the RBK cell line characteristics were analyzed using molecular and karyotype methods and growth specifications. Cloning of the RBK cell line was performed using the limited dilution method, and each cell clone was quantitatively and qualitatively characterized. Four cell clones were compared based on their sensitivity to the BoHV-1 virus. Then, the RBK-D5 clone was selected as the most appropriate cell line for further studies. The RBK-D5 was subjected to tests for identity, chromosomal analysis, and doubling time. In the end, the origin of this cell line was confirmed by the PCR method. It was observed that the cell line exhibited karyotype diversity due to aneuploidy, which can be responsible for the procreation of chromosomal instability. This diversity represents chromosomal changes in the continuous cell line that carries the characteristic of an immortalized cell line. The RBK-D5 was found to be more sensitive to the BOHV-1 virus. Surprisingly, its titer was evaluated at $10^{8.5}$ CCID50/ml. The obtained results suggested that the RBK cell line is suitable for the BoHV-1 virus and can be useful for virus detection, propagation, and quality control or viral titration.

Keywords: BoHV-1 virus, Karyotyping, RBK cell line, RBK-D5

1. Introduction

The Razi Bovine Kidney (RBK) cell line is a Continuous cell line derived from the primary kidney cell of a two-day-old male Holstein calf. It has been successfully established through successive passages based on standard protocols and appears more epithelial-like with clear cytoplasm arranged in a single layer. This cell line has unlimited proliferation potential.

Chromosomal abnormalities or aneuploidy are associated with the immortality of the cell lines permitting them to divide continually (1). In this study, cytogenetic tests were conducted to determine chromosomal aberrations and aneuploidy in the RBK cell line.

A suitable cell line is essential for viral diagnosis and research, as viruses are extreme obligate intracellular parasites, they are strictly dependent on Sensitivity of cell cultures to certain viruses for isolation and proliferation of viruses that is one of the success factors in viral laboratories. In addition, disadvantages of primary cells, such as cost, time-consuming procedures, and primary/secondary contamination of cells with different types of cells or latent viruses, have led to using continuous cell lines to solve these problems (21, 15, 14). Nowadays, cell lines play an important role in the studies of virology, developmental biology, genetics, immunology, physiology, toxicology, and pharmacology (4).

Infectious Bovine Rhinotracheitis (IBR), caused by the Bovine Herpesvirus-1 (BoHV-1) virus, is a disease of domestic and wild cattle that can cause a persistent infection in the host and create reactivity periods, leading to immune suppression and severe health complications. This virus contains double-stranded DNA and hijacks endocytosis pathways to deliver its genetic material to the subcellular localization to replicate inside the living cells (19, 7).

Cytopathic effects (CPE) and changes in cell morphology occur in cells due to viral infection. The appearance of CPE is related to the type of virus. The cells may appear round, granule-like, and separate from each other, or multinucleated giant cells (24, 15).

Cell cultures decrease the use of experimental animals and provide a desirable environment for detection and identification. Virus isolation in cell cultures has long served as the "gold standard" for virus detection and can be useful for veterinary virology in particular and virus-host relationships in general (15). Currently, a variety of cell lines derived from different organs are available to identify viruses. In addition, the ability to successfully propagate viruses into standard cell lines is important when a sufficient virus titer is required for susceptibility testing or strain typing, which is needed for epidemiologic studies and vaccine strain selection (15, 18).

According to a previous study, the RBK cell line has shown significant sensitivity to BOHV-1 virus proliferation compared to other cell lines, such as CEF, BT, MDBK, and Vero cell lines. (23).

In this study, the RBK cell line characterization was evaluated based on the World Health Organization (WHO), TRS No 987. In addition, our results shown that the RBK as sensitive cell line to the BoHV-1 virus is a suitable cell line for viral diagnosis and research.

2. Materials and Methods

2.1. Cell Culture

The RBK cell line and local isolated BOHV-1 virus were prepared from the Biobank Department at the Razi Vaccine and Serum Research Institute, Karaj, Iran. Briefly, RBK cell was grown in accordance with the standard instructions in 25 cm^2 flask containing culture medium, that Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (Fetal Bovine Serum) serum, 100 IU/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B. Cells were incubated at 37°C in a 5% CO_2 incubator (9). The BOHV-1 virus was inoculated into the RBK cell line, then the harvest was aliquoted and stored at -70°C. Eventually, one of them was used each time.

2.2. Determining Optimal Multiplicity of Infection for Razi Bovine Kidney Cells

Different dilutions of virus were inoculated into cell cultures (within range of 0.005 to 0.1). For this purpose, RBK cells were cultured in five 25cm^2 flasks. Moreover, one flask was considered as a control. Flasks were infected with virus at different Multiplicity of infections (MOI of 0.005, 0.01, 0.02, 0.05, and 0.1), and then the flasks were incubated at 37°C Next, CPE degree on cells was estimated. In addition, the virus titer was compared in various situations, then appropriate MOI selected. Subsequently, the BOHV-1 virus was propagated on RBK cells based on obtained MOI. Finally, all of the results of titration was evaluated by the Kerber method and was stored at -70°C.

2.3. Razi Bovine Kidney Cell Cloning by Limiting Dilution Method

The 96-well micro plate was used to cloning the cell. First, 10 ml of cell suspension was prepared, containing 300 cells with 20% FBS and 100 µl/ml of antibiotics. Then, 100 µl cell suspension was seeded into each of the wells of micro plate, and after that, the micro plate incubated at 37°C in a 5% CO_2 incubator. The micro plate was carefully checked every day for the formation of cell clones originating from a single cell**,** as well as, the cell and proliferation quality of each clone. Afterward, they were compared with each other and the best cell clones were selected according to growth rate, proliferation, and morphological characteristics. Finally, the selected clones were transferred to a 24-well micro plate that was prepared with DMEM and 10% calf serum. Following three days, they were transferred to 25 cm^2 flasks, when they obtained proper growth and proliferation. Furthermore, the cell clones were individually propagated. Eventually, they were stored in a nitrogen tank for further procedures.

2.4. Selection of the Most Sensitive Cell Clone than Bovine Herpesvirus-1

The selected clones were cultured in 25 cm^2 flasks at the same time with equal numbers per unit volume, and after 48 h, they were inoculated by BOHV-1 virus with the same MOI.

In this study, the amount of cell destruction was monitored, and the required time to destroy 100% of cells was evaluated. The flasks were frozen and thawed after complete cell destruction, and the supernatant was used in virus titration, after centrifuging at 3000 rpm for 10 min.

The above process was repeated three times, and the results of the experiments were statistically analyzed. According to the obtained data, the most sensitive cell clone was selected and used to determine the characteristics.

2.5. Polymerase Chain Reaction Test for Species Identification of Razi Bovine Kidney Cell Line

Investigation of cell identity was performed based on the amplification amplification of a 102 bp fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI) (Table 1).

Polymerase Chain Reaction (PCR) method was performed using extracted DNA of selected RBK cell clone. Moreover, the DNA of the MDBK cell line (ATCC; CCL-22) was used as control.

A 12.5 µl of PCR master mix consisted of Taq DNA polymerase, dNTPs, MgCl2, buffer, F primer (0.5 µl), R primer (0.5 µl), extracted DNA (5 µl), and non-ionized distilled water (25 µl) for each microtube. The PCR cycling conditions were included: initial denaturation for 5 min at 95°C, then 30-35 cycles, including three stages of denaturation, binding of primers, and extension. Respectively, denaturation at 94°C for 1 min, primers connection step at 53°C for 50 s, and extension at 72°C for 10 min to complete the polymerization process. Next, PCR products were placed at 4°C. Ultimately, the PCR products were electrophoresed.

2.6. Razi Bovine Kidney Growth Studies

In order to measure the cell doubling time of the selected clone, a standard protocol was applied and the experiment was repeated three times (9). The cells were grown in culture flasks and incubated for 10 days. Then, flasks of cells were harvested and counted daily until day 10 (respectively, 24, 48, 72, 96, 120, 144, 168, 192,

216, and 240 h). Then, cell doubling times for RBK-D5 were calculated at different passages. Furthermore, after 24 h, the cells were checked microscopically for shape, adhesion to flask surface, growth, size, arrangement, and uniform distribution. Then, cell counting was done by hemocytometer to determine an accurate number of viable cells in flask. Checking cell growth in the flasks was followed until the number of cells per flask remained constant for three consecutive days. Finally, growth curve and doubling time were evaluated for RBK-D5 cell line.

2.7. Cytogenetic

Cytogenetic analysis is an essential part of characterizing and identifying cell lines, that is the study of chromosomes during metaphase of the cell cycle. In this study the metaphase chromosomes were analysed to determine chromosome abnormalities of RBK-D5 cell line. Firstly, cells were arrested in metaphase by colchicine, then the cells were harvested with trypsin and treated with KCL. Later, the cells were fixed by methanol/acetic acid. After preparing the slides, they were stained by Giemsa. Lastly, the metaphase cells were photographed and analyzed by microscope.

To investigate chromosomal diversity, cell stability, and numbers of chromosomes, karyological study was conducted by standard and banding methods at 27^{th}, 30^{th}, 36^{th}, 42^{th}, 46^{th}, 54^{th}, and 60^{th} passages (Table 2). In the direction of analyzing the chromosomes, around 120 cells were counted at each passage.

Table 1. Primer sequences from Cooper 2007

Size(bp)	Sequence	primers	Species
102	GCTATTCC AAC CGG GGT AAA AGT C GAAAAT AAA GCC TAG GGC TCA C	Bt-F Bt-R	Bos taurus (cow)

Table 2. Data were obtained from chromosomal changes of RBK-D5 cell line, which collected at different passages via karyotype analysis

the number of cells counted at each passage	chromosomes Number 58-60	48	46	44	42	40	38≤	Cell passage number
120	4	5	6	49	3	5	16	P27
120	2	4	28	57	23	2	4	P30
120	3	6	24	65	17	3	2	P36
120	1	3	19	58	29	6	4	P42
120	3	4	11	47	30	8	17	P48
120	-	-	2	35	54	10	19	P54
120	-	-	-	18	56	18	28	P60
840	13	22	90	329	244	52	90	In total

2.8. Sterility Tests

Sterility testing of RBK-D5 cell lines was conducted to ensure the absence of microbial, mycoplasma, fungal, and yeast contamination in cells. This test was performed by a direct method under aseptic conditions. In this research, the following media were used to culture thioglycolate medium for cultivating aerobic, microaerophilic, and anaerobic bacteria. Tryptic Soy Broth (TSB) medium supports the growth of various microorganisms, especially common aerobic, facultative anaerobic bacteria, and fungi. PPLO Broth and PPLO Agar were used to detect mycoplasma contamination. Additionally, the cell line was tested for the presence of mycobacterium species; thus, the cell line was inoculated on Lowenstein's medium containing glycerin in parallel with the Culture medium containing pyruvate to detect any contamination of human and animal tuberculin. Then,

they were investigated and evaluated for any evidence of growth.

2.9. Detection of Adventitious Agents

Virology testing assures of adventitious free agents. According to WHO guidelines (TRS 987), investigating adventitious viruses contamination in RBK-D5 extract should be carried out by direct inoculation method on three cell lines (such as Vero, IBRSII, and MDBK), hem adsorption test, injection of extracted cells into embryonated SPF-eggs and egg allantoic fluid, as well as injection of extracted cells into adult and infant mice.

3. Results

3.1. Cell Culture

In the present study, morphological observations of the RBK cells were made using an inverted microscope (Figure 1). The RBK cells appeared epithelial-like in a suitable culture, with clear cytoplasm, and grew attached to a substrate in the flask. The cell passages were performed at a 1:3 ratio every 48 h.

3.2. Determining Optimal Multiplicity of Infection for Razi Bovine Kidney Cells

This test was repeated three times. Five cell flasks were prepared under the same conditions and infected with the virus at different MOI ratios. The flasks were compared with each other to determine the optimal MOI. The highest titer was obtained at MOI = 0.005, and the highest cytopathic effect was observed at this MOI.

In this study, a titer of 10^7 CCID50/ml was observed after BOHV-1 replication in infected RBK cells.

3.3. Razi Bovine Kidney Cell Cloning by Limiting Dilution Method

Cell cloning was performed for the RBK cell line (Figure 2). After cloning was completed successfully, the selection and isolation of suitable cell clones was an important step. Thus, between 12 **cell clones** (RBK-C9, RBK-C12, RBK-F7, RBK-F3, RBK-D1, RBK-D5, RBK-D7, RBK-E8, RBK-E9, RBK-E11, and RBK-G8), four elite clones were selected based on their growth traits and morphological appearance (RBK-C9, RBK-D1, RBK-D5, and RBK-E11).

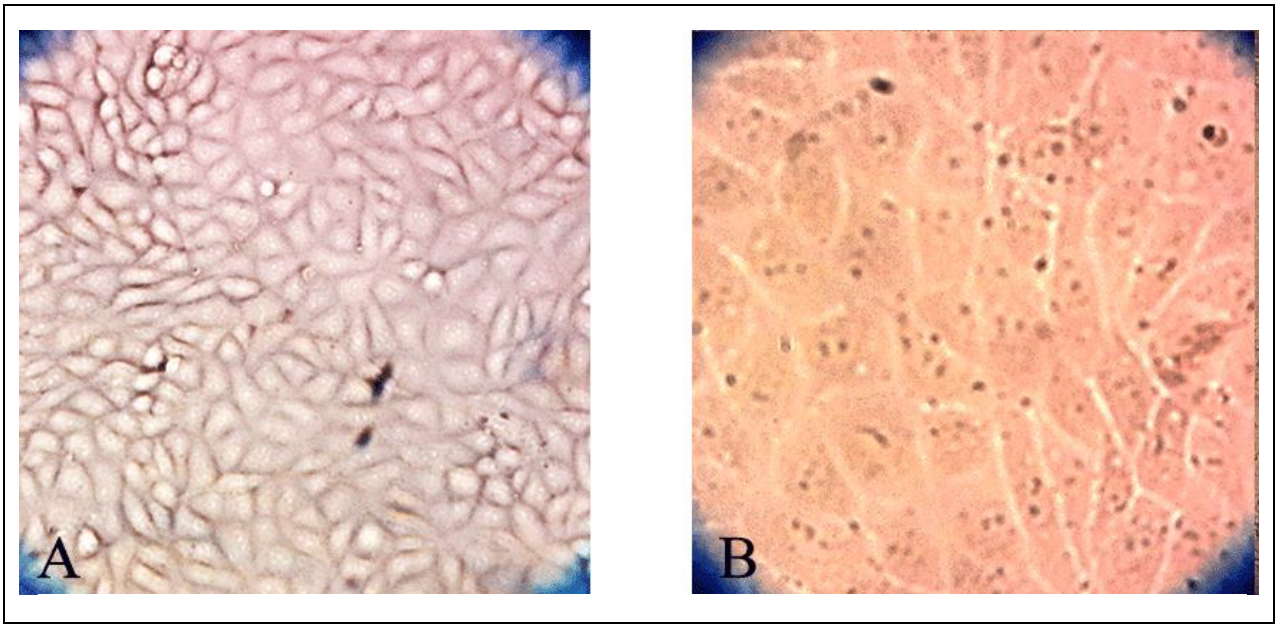

Figure 1. Microscopic images: A) The RBK cell line appears epithelial-like with clear cytoplasm arranged in a single layer, at 20x magnification. B) At 40x magnification

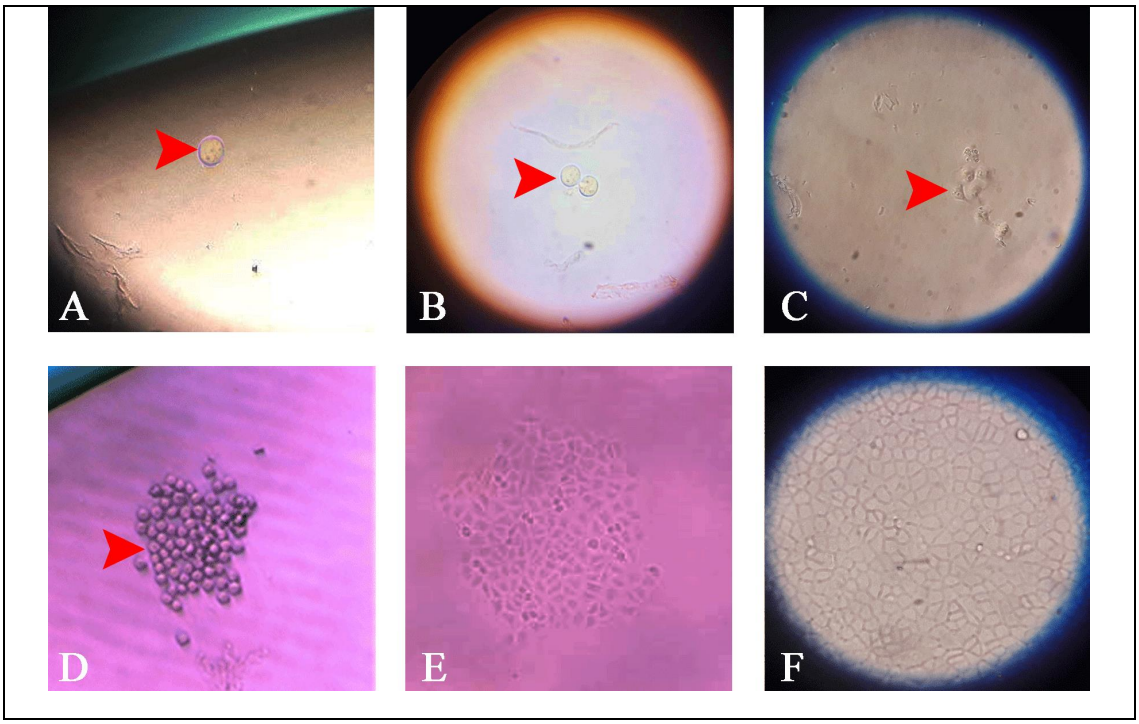

Figure 2. Microscopic images: A to F) appearance of the first cells obtained from RBK cloning in 96 well micro plate; 10X magnification. A) Single cell after cloning. B) After 24h. C) After 48 h. D) After 72 h. E) After 96 h. F) After 120 h

3.4. Selection of the Most Sensitive Razi Bovine Kidney Cell Clone to Bovine Herpesvirus-1

The RBK Cell clones were infected with BOHV-1 virus at an MOI of 0.005; after inoculating the mentioned virus, the first CPEs were observed in 24 h (Figure 3). The CPEs were checked microscopically every day for seven days (Figure 4). The results showed that in three repetitions of the experiment, the measured virus titer in the RBK-D5 clone was significantly higher than in other clones (Figure 5). Consequently, we selected RBK-D5 as the most sensitive clone. Moreover, further investigations were carried out on RBK-D5 cell clones (Figures 3 and 4).

The present research revealed that the highest titers were related to RBK-D5 and RBK-C9 cell clones (Figure 5). Furthermore, there was a significant difference between the two cells.

In this research, studies have been performed based on statistical analysis and cell characteristics analysis (including morphology, growth rate, and sensitivity of RBK-D5 to BOHV-1 virus); in the following, the results demonstrated that RBK-D5 was more sensitive to BOHV-1 virus. Surprisingly, its titer was evaluated at $10^{8.5}$ CCID50/ml. Moreover, the proliferation of this infectious virus was estimated at the highest rate (Figure 5).

3.5. Polymerase Chain Reaction Test for Species Identification of Razi Bovine Kidney Cell Line

According to the observation of a single band at 102 bp for COI gene, it approved that the RBK-D5 cell line originated from bovine species (Figure 6).

3.6. Growth Studies

The doubling time experiment was repeated three times for RBK-D5 cells under the same condition, and the average doubling time was 21 h. The cell growth curve for RBK-D5 plotted based on results of cell counting over time (Figure 7) and their calculation. A growth pattern was observed during the incubation for

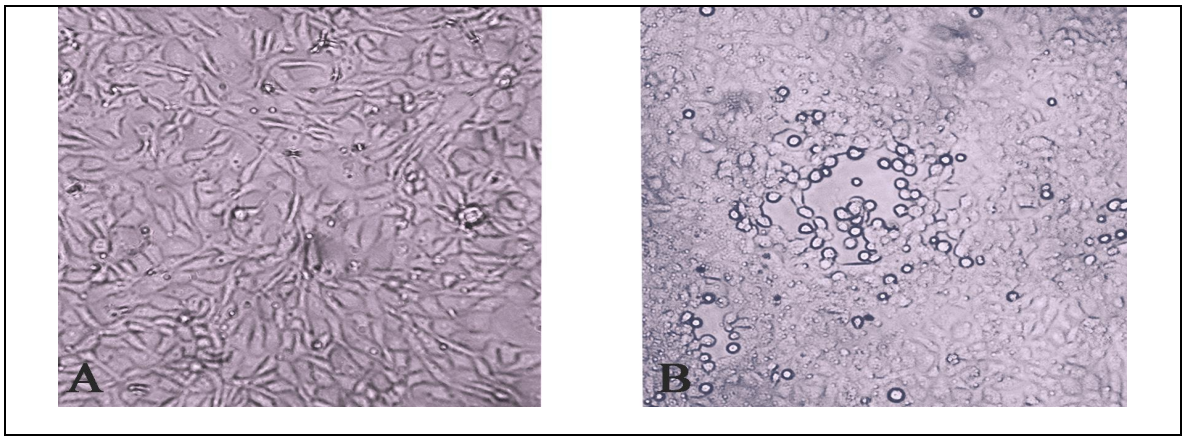

Figure 3. Microscopic images: A) RBK-D5 cell line as negative control, with 10X magnification. B) RBK-D5 cell line after inoculation of BOHV-1 virus (cytopathic effects were caused by virus replication in the cell line after two days)

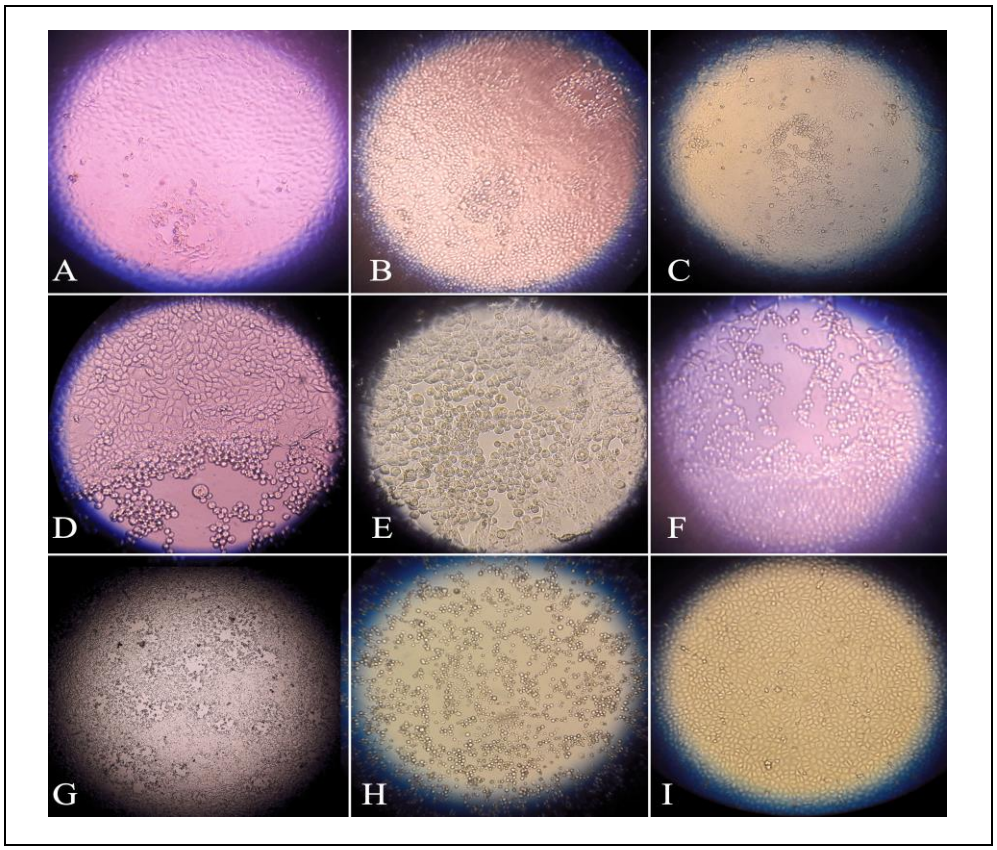

Figure 4. Microscopic images: A to H) show cytopathic effects (CPE) of BOHV-1 virus that caused morphological changes in RBK-D5 cell line, and cells became smaller and rounded. I) the control cell (without infection)

10 days. We also found that cell density was hit its maximum after seven days, where cells entered their

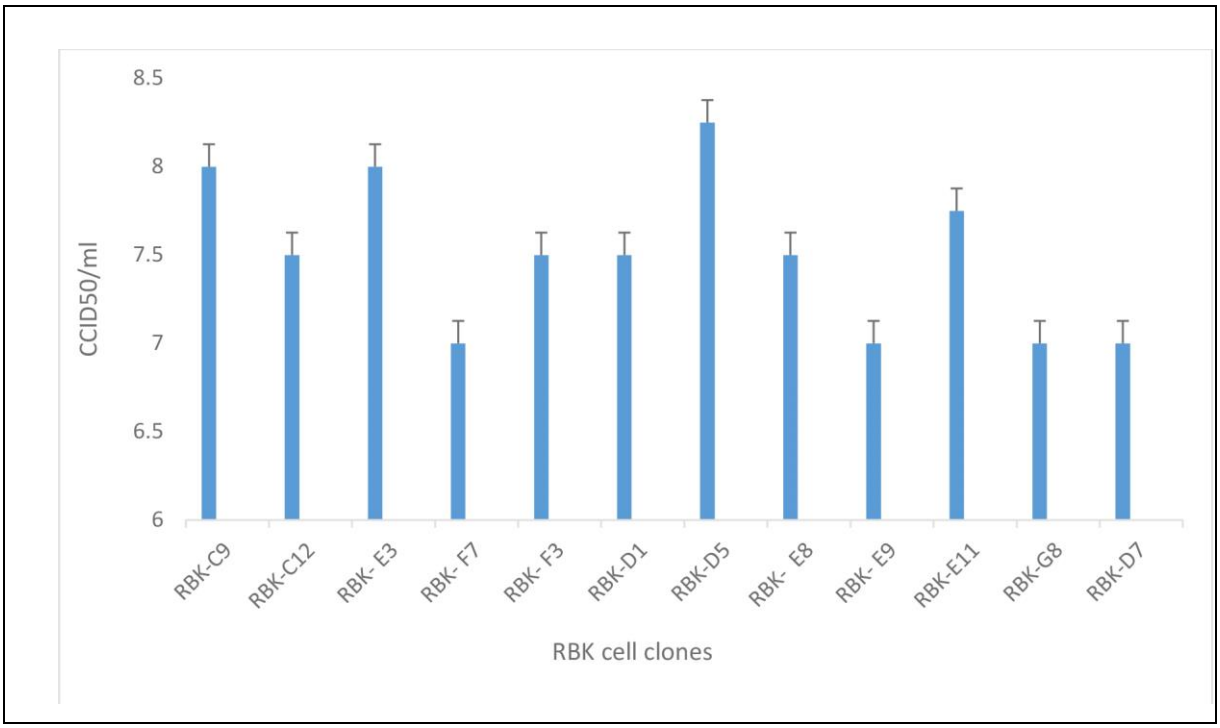

Figure 5. Comparison of BOHV-1 virus titer in each RBK cell clones, that shows more sensitivity of RBK-D5 clone than other clones

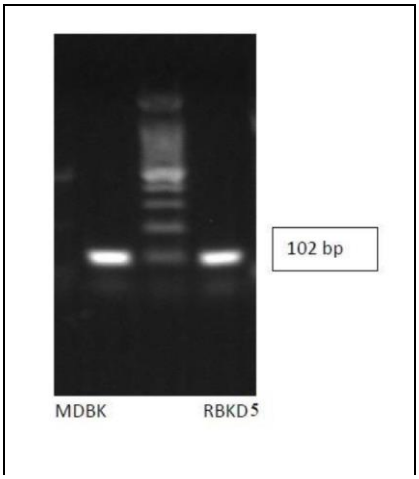

Figure 6. PCR test to confirm the origin of RBK-D5. MDBK cell line as a positive control. 100 bp DNA ladder

stationary phase (Figure 8).

In this research, after 72 h of incubation, a logarithm growth was observed in parallel with the calculation of cell doubling time (Figure 7). Finally, the cells entered the linear growth phase after 144 h (Figure 8).

3.7. Cytogenetic

Aneuploidy was observed during karyotyping (Figures 9 and 10 and Table 2). At the 27th passage, 42% of RBK-D5 cells had 44 chromosomes, which consisted of acrocentric, metacentric, sub-telocentric, and telocentric chromosomes (Figures 9 and 10).

3.8. Sterility Test

Sterility testing is a process that ensures the cells are free from viable microorganisms. In this research, sterility tests on the RBK-D5 cell clone demonstrated that viable contaminating microorganisms such as

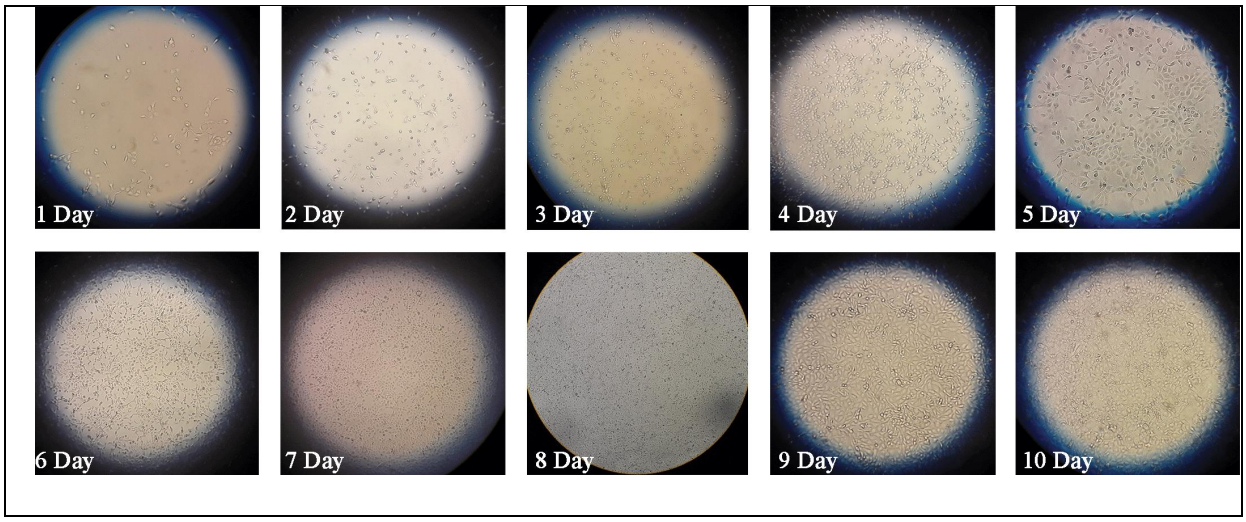

Figure 7. Microscopic images: After 24 hours of incubation, Cell growth was checked by microscope for ten days (magnification 4X)

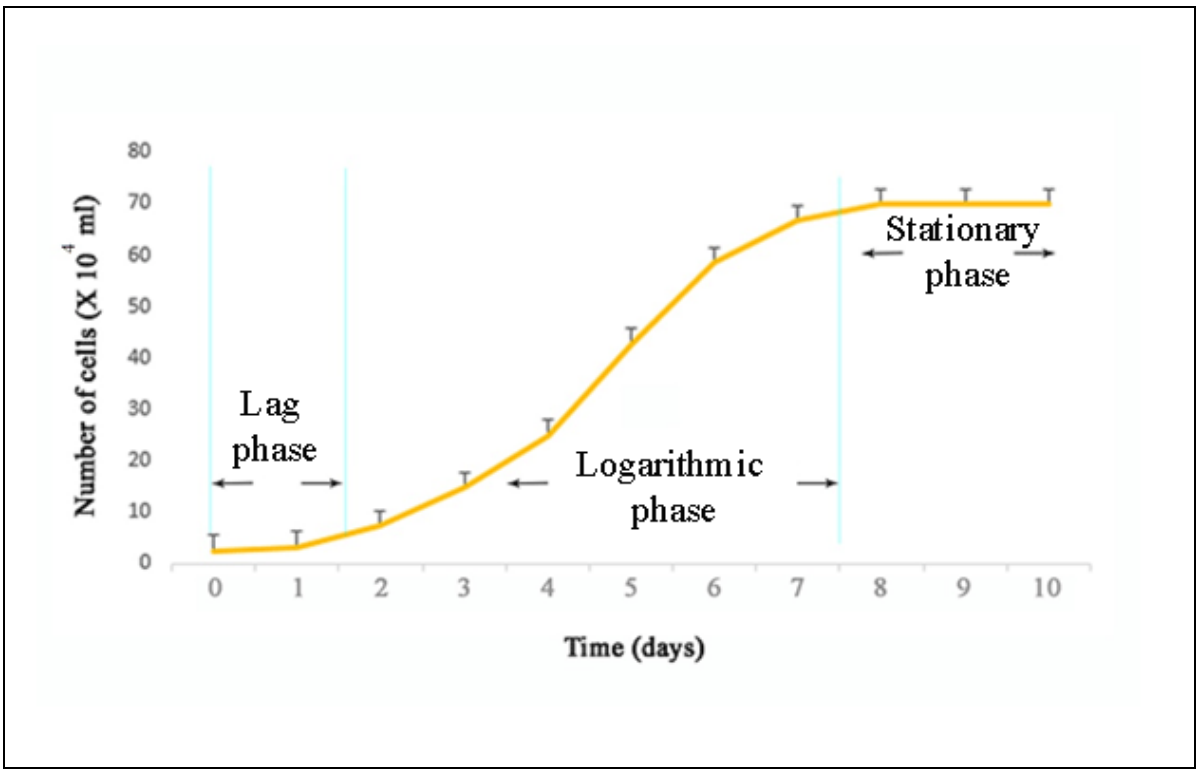

Figure 8. Cell growth curve of RBK-D5 cell line in terms of cell doubling time that showed the suitable growth for RBK-D5. The horizontal axis is related to consecutive times of cell counting in the cell culture and the Y-axis is related to number of cells on different days

bacteria, fungi, mycoplasma, and mycobacterium were not evident in this cell. After inoculating the cell suspension into thioglycolate and TSB culture media, the results demonstrated the absence of turbidity on the 14th day of incubation. The results showed that RBK-D5 cell was free from bacterial and fungal contamination.

Mycoplasma contamination was evaluated by culture method as the gold standard; first, RBK-D5 cell suspension culture in PPLO agar plates, which

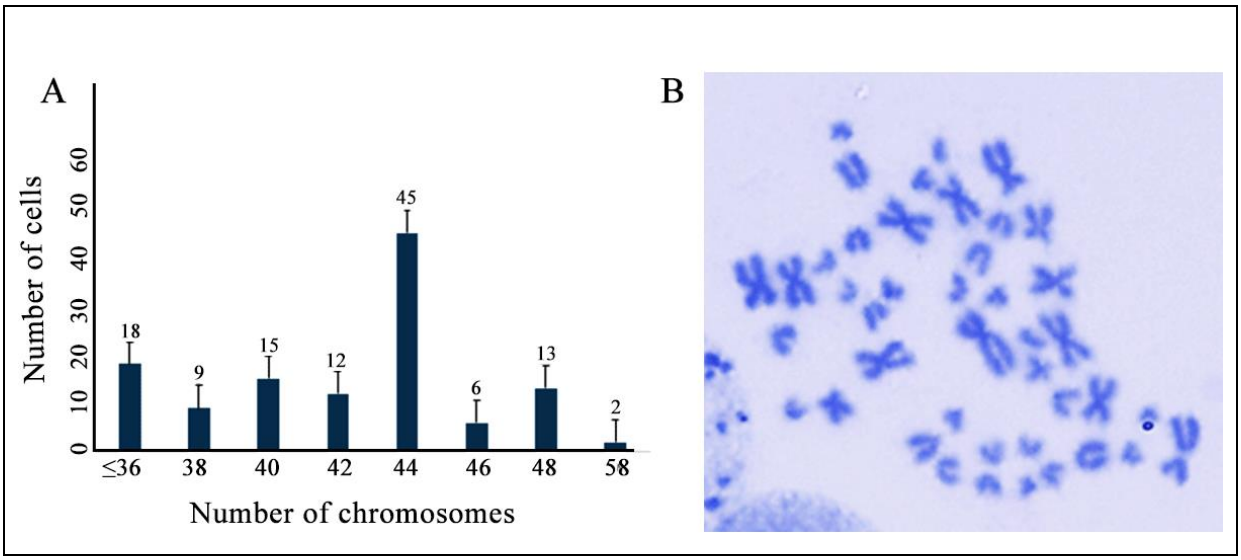

Figure 9. Chromosome analysis of RBK-D5 cell at passage 27. (A) Chromosome number distribution in metaphase cells (B) Cell chromosomes at passage 27

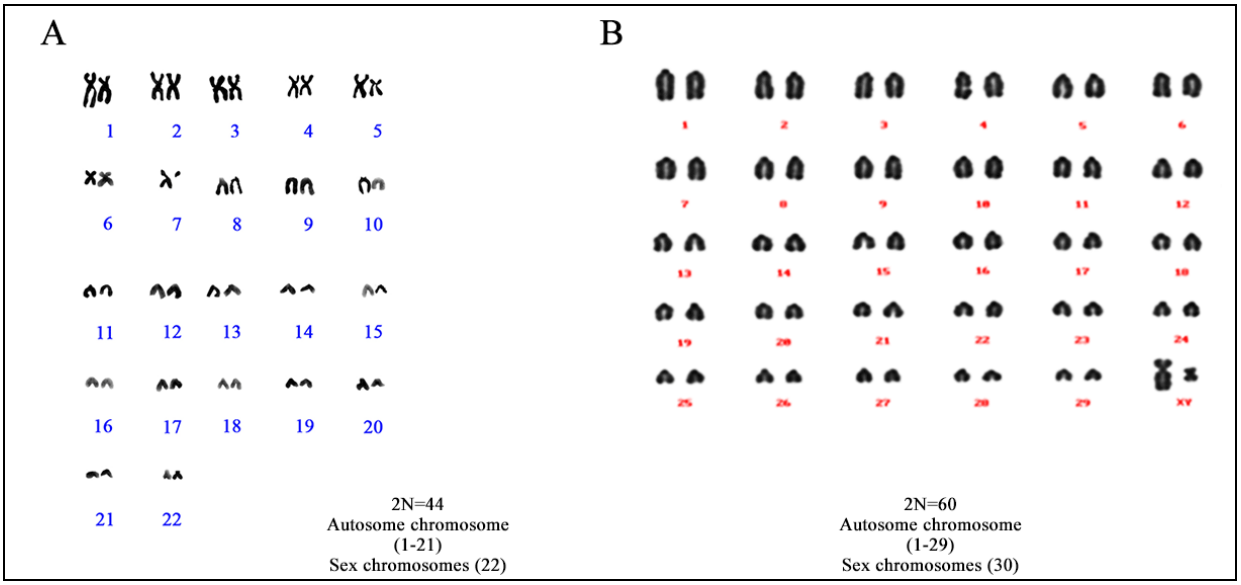

Figure 10. (A) RBK cell line with 44 chromosomes at passage 27, which consisted of a pair of XY chromosomes (mediocentrics); that also have 21 pairs of autosomal chromosomes, including types of chromosomes: acrocentric, submetacentric, metacentric and telecentric. This population showed favourable diversity in terms of chromosomal characteristics. (B) A sample of bovine cell with 60 chromosomes as a normal karyotype that is composed of 58 acrocentric autosomes and two subtelometric sexchromosomes (8)

were incubated in both aerobic and anaerobic conditions. Then, an indication of the growth of Mycoplasma was determined by a color change in PPLO broth. After three days, further confirmation was performed by subculturing from broth to PPLO agar, as well as after 14 days. Finally, checked to observe typical fried egg colonies under a light microscope after 21 days, and the absence of colony growth on PPLO agar plates indicated non-contamination.

Based on the TRS987 investigation of cell contamination for Mycobacterium tuberculosis, no colonies were present in the Lowenstein medium during the incubation, indicating that the cell was free from animal and human mycobacteria contamination.

3.9. Detection of Adventitious Agents

During inoculation of RBK-D5 into sensitive cell lines (Vero, IBRS II, and MDBK) and performing hemoadsorption, no CPE was observed.

Moreover, in this study, extracted cell inoculation was performed in 10 embryonated eggs, intraperitoneal injection in 10 adult mice and 10 infant mice. It was confirmed that the cell clone was free from adventitious viruses.

4. Discussion

Due to the disadvantages of primary cells and their limited lifespan, continuous cell lines have been developed in the field of biological experiments (21, 15, 14). A cell line is developed by continuous passaging of primary cells to obtain a cell line, and the cell line might have mutations or genetic alterations during sub-culturing. In general, the cell lines in culture are prone to genetic instability. In this study, the RBK cell line was derived from primary bovine kidney cells.

As shown in Figure 10, chromosomal abnormalities in numerical aberrations were found in the RBK-D5 cell line. We identified a variable number of chromosomes due to the presence of aneuploidy in cells. Aneuploidy is associated with a reduced number of chromosomes leading to the development of chromosomal abnormalities (22). In addition, analysis of cytogenetic further clarified changes caused by aneuploidy in RBKD5 cell line, which improves their proliferative capacity. The results revealed that aneuploidy has a causative role in creating the continuous RBK cell line. As a matter of fact, cell line lacks normal biological growth regulation and can divide continuously. Subsequently, immortalized cell line grown for prolonged periods *in vitro*.

According to the international standard, a suitable cell line should be confirmed in terms of some characteristics such as growth, survival, identity, stability, sterility, cytogenetics, microbial contamination, and other possible unwanted factors (25).

In current research, after culturing, cloning, and selection of an appropriate cell, our results revealed that all characteristics of the selected cell (RBK-D5) were acceptable (e.g., arrangement, size, and morphology). In the following, further investigations were carried out regarding the RBK-D5 cell.

Species identification of cell lines and detection of cross-contamination are crucial for scientific research accuracy and reproducibility. Therefore, researchers have to ensure the identity and the purity of their cell lines (13, 17, 6).

By standard, methods can determine evolutionary relationships between species. A DNA-based system of species identification using gene of *COI* was proposed by Hebert et al., who coined the term DNA barcoding. Barcode analyses can serve as a quick screening tool for those lineages with deep genetic divergence, aiding detection of overlooked species (11). DNA barcoding also enables taxonomic data to be gathered, analyzed, and synthesized into useful products in a timeframe that meets the challenge presented by the rate of biodiversity loss (23). In order to characterization and contamination detection of RBK-D5 cell line, we used PCR to amplify a 102 bp fragment from the COI gene. Implementation of this assay accurately determined the species of cell line and confirmed the origin of the cell line. As well as, reduced the problems of misidentification and cross-contamination that plague research efforts.

The population growth rate is an important characteristic of any cell culture (16). The most common measurement for cell culture growth rate is the so-called population doubling time, i.e., the time it takes for a population to double its size (1, 2).

Based on essential data, after the lag phase, the cells prepare to enter logarithmic phase, when cells reach their maximum density, the cells enter stationary phase; therefore, the cell growth curve attributes to specific characteristics of the cell. Typically, *in vitro* research is done at the exponential phase of cell cycle, that is also quite important (12).

In the current study, according to the growth characteristics, the RBK-D5 cell immediately shown a short "lag phase" after seeding (Figure 8); at this stage, cells do not divide and are adjusting to the culture condition and preparing for the cell division. The cell skeleton was reconstructed, and extracellular matrix was secreted facilitating communication between cells and their proliferation; and all these morphological features enabled the cell to enter a new cycle. Subsequently, the cells entered a logarithmic phase where the cell population doubled at a certain rate. Thus, on the third and fourth days of incubation, the doubling time of RBK-D5 cell was approximately equal to 21 h. Finally, when the cell population becomes very dense and the substrate is practically metabolized, the cells enter a stationary phase, where the growth rate is almost zero (Figure 8). As our results indicated, RBK-D5 divide rapidly with a generation time of 21 h and has a potential to subculture indefinitely. Moreover, the cell lines exhibit aneuploidy.

During culture, the karyotype of cells often changes. Consequently, many continuous cell lines are polyploid or heteroploid. The results of this study demonstrated that continuous cell lines sometimes show a certain number of chromosomes, or differences between the karyotypes of cell line at any passages. In general, the chromosome number is distributed over a wide range. This pattern of distribution may change at passages (Table 2).

Despite the rapid development of new molecular techniques, chromosome analysis remains a key method for chromosomal screening in cytogenetic field. Therefore, cytogenetic analysis plays an essential role in determining cell line characteristics. In addition, employment of cytogenetics can be a beneficial part of tests toward evaluation of chromosomal aberrations and structural changes on chromosomes (10).

Chromosomes in diploid cells are arranged in homologous pairs. The diploid chromosome number of a cell (2n) is the total number of chromosomes in a cell. In the case of cattle, 2n=60, meaning there are a total of 60 chromosomes in a diploid bovine cell. In addition, standard karyotype of cattle is composed of 58 acrocentric autosomes and two subtelomeric sex chromosomes (2n=60, XX or 2n=60, XY) (7). In the present study, we found chromosomal abnormalities and numerical aberrations at 27th passage of RBK-D5 cell line that described processes leading to aneuploidy.

Aneuploidy and chromosomal instability often co-exist. Aneuploidy is also recognized as a direct outcome of chromosomal instability. The relationship between aneuploidy and chromosomal instability can be envisioned as

a "vicious cycle," that lead to further karyotype diversity in cells. Additionally, according to research and studies, aneuploidy itself can be responsible for the procreation of chromosomal instability (20). In this research, a decreasing process was estimated at passage 27, where the majority of cells had 44 chromosomes (Figure 9 and Table 2); Based on this essential data, karyotype diversity represents chromosomal changes in the continuous cell line that carries the characteristic of immortalized cell line (Table 2). Contamination is a major concern in cell and tissue culture laboratories that have an impact on experiment conduction and causing false positive/negative errors. There are several ways to detect contamination, including PCR, enzymatic, indirect DNA DAPI staining and microbial culture methods (5). Based on standard procedures, lack of microbial contamination and other unwanted factors play essential role in terms of characteristics of the cell line. In this paper, the experiments confirmed that RBK-D5 cells are free of adventitious virus, bacterial, fungai, mycoplasma and mycobacterium contaminations. Overall, one of the key factors in virus isolation is multiplication speed in cell culture and high production efficiency. Therefore, it is absolutely necessary to select a suitable cell line with high sensitivity to a certain virus. In the present study, our results revealed that RBK-D5 cell line contains surprisingly high sensitivity to BOHV-1 virus at different passages, as well as our results confirmed the absence of contamination to other cells and unwanted factors. In total, the conducted investigations confirmed the capability of the studied cell. In conclusion, the RBK cell line can be widely used in research, detection, virus propagation, and virus quality control or titration.

Authors' Contribution

Study concept and design: M. lotfi Acquisition of data: M. Maghami & M. Lotfi & A. mohammadi & F. Sotoodehnejad nematalahi Analysis and interpretation of data: M. Maghami & M. Lotfi Drafting of the manuscript: M. Maghami & M. Lotfi Critical revision of the manuscript for important intellectual content: M. Lotfi Statistical analysis: M. Maghami

Ethics

This study was approved by the Ethics Committee of Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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