
Archives of Razi Institute Journal

Volume 80, NO. 3, 2025

Journal homepage: https://archrazi.areeo.ac.ir

Original Article

Authentication of RBK (Razi Bovine Kidney) Cell Line Derived from Primary Kidney Cell

Masoumeh Maghami[1], Mohsen Lotfi[2*], Fattah Sotoodehnejadnematalahi[1], Ashraf Mohammadi[3]

1. *Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.*
2. *Department of Quality Control, Razi Vaccine and Serum Research Institute, Agricultural Research, Education, and Extension Organization (AREEO), Karaj, Iran.*
3. *Department of Human Viral Vaccines, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.*

Article Info:

Received: 19 October 2024
Revised: 16 November 2025
Accepted:19 November 2024

Keywords:

Authentication, Bovine, Cell Line, Identity, PCR, PCR-RFLP, Real Time PCR, Primary Cell, RBK.

ABSTRACT

RBK cell line, derived from primary bovine kidney cell, was established and introduced by Razi Vaccine and Serum Research Institute. RBK cell line has been transformed into continuous cell line through serial passaging of primary cell. Based on experiments conducted, the RBK cell line demonstrates high sensitivity to viruses. As cell line identification is crucial to confirm the validity of experimental results, and can ensure their credibility, accurate authentication helps prevent unreliable findings. Misidentification of the cell line or its contamination can lead to unreliable results as well as waste of resources. Forecasting the origin of a cell line, as it directly contributes to its value; the more detailed the origin, the more valuable the cell line is considered. Contamination is a serious concern in cell culture because it can significantly affect the physiology and viability of cell lines. Therefore, based on standard guidelines, it is necessary to implement various assays to ensure contaminant-free cultures and confirm the identity of cell lines. For accurate identification, verification and authentication of a cell line, various methods must be applied simultaneously. In this study, purification and identification of RBK cell line were investigated using molecular tests such as PCR, PCR-RFLP and real time PCR based on mitochondrial DNA. The present study provides the authentication of RBK cell line and demonstrates that the RBK is free of adventitious agents, including Bovine Leukemia Virus (BLV) and Bovine Viral Diarrhea (BVDV). Considering the RBK cell line's capability for unlimited proliferation, stability and sensitivity to viruses, the present study introduces this cell line as a reliable tool for viral research, which can contribute to numerous important scientific advances.

Corresponding Author:

m.lotfi@rvsri.ac.ir

https://orcid.org/0000-0001-7438-9192
https://orcid.org/0000-0001-9902-7999
https://orcid.org/0000-0002-4287-9792
https://orcid.org/0000-0001-5744-767X

How to cite this article: **Maghami M, Lotfi M, Sotoodehnejadnematalahi F, Mohammadi A. Authentication of RBK (Razi Bovine Kidney) Cell Line Derived from Primary Kidney Cell. *Archives of Razi Institute*. 2025;80(3):859-868.** DOI: 10.32592/ARI.2025.80.3.859

1. Introduction

One of the most important scientific discoveries of the past century is Hela cell line, introduced by Dr. George Otto Gey (1951), which has been extensively used in various scientific research fields. Since then, cell lines have gained prominence and have been widely employed in numerous areas of research, diagnostics, and scientific studies (1). Today, they play a key role in biological experiments, especially those involving the diagnosis of infectious or viral diseases through in vitro systems.

The validity and characterization of a cell line, whether derived from a primary cell or cell line, is necessary. One of the most important elements for determining cell identity is isoenzyme analysis and molecular methods, which are used to study and determine the intra-species origin of the cells dedicated to research and scientific work. These are methods authorized by the World Health Organization and form the basis of WHO guidelines for identifying different cells and preventing cross-contamination. Modern clinical virology emphasizes fast and accurate virusidentification of to control infection or treat diseases. Therefore, the quality and standardization of cell substrate is extremely important and affects the success of experiments; therefore, standardizing the cell lines to produce and conduct virology experiments can lay the ground for developing modern cell culture. As during a research precision in results and proper operation depend on cell lines in vitro, identification of cell lines and detection of cell contamination are crucial for scientific research and replicability of results. Generally, choosing the appropriate cell line for research is essential to obtain reliable and relevant results (2).

Previous studies have indicated that RBK cell line has a high proliferation capacity and high stability as an infinite cell..Furthermore, our findings revealed that the average doubling time of the RBK cell line was approximately 21 hours (3). $\mu=0.6931/TD = 0.33$

The RBK cell line exhibit high sensitivity to virus, and results demonstrated the RBK can serve as a proper cell line to identify such viruses. Notably, RBK cell growth rate is much higher than Vero or MRC-5 cell lines (3-6).

Our results suggested that the RBK cell line is a valuable tool in virology research, serving as a reliable model for diagnostic tests and experimental investigations. At this current study, in order to authenticate the RBK cell line, cell identity and purity were confirmed using different methods: DNA fingerprinting, PCR-RFLP and real time PCR (Figures 1_4).

2. Materials and Methods

2.1. DNA Fingerprinting

Determination and choice of cells: For the present study, six cells were chosen with the following specifications: The cell line from bovines' kidney (RBK) , the Madin-Darby bovine kidney (MDBK), the primary cells from lambs' kidney (LK),the cell line from pigs' kidney (IBRS-2), the diploid cell from human embryo (MRC-5), the cell line from African green monkeys' kidney (Vero), and the primary cells from goats' lymphocytes (GL) (Figure 2). Of these, five are monolayer that can grow and attach to the surface of flask; the cells of goats lymphocytes are cultured in suspension. The selected cells were subjected to PCR analysis on extracted DNA. The PCR mix for each sample contained:

2.1.1. PCR

The selected cells were subjected to perform PCR on extracted DNA. 0.5µl of the PCR Master Mix, which contains dNTPs, PCR buffer, Mgcl2, and Taq DNA polymerase, 0.5µl of the F primer, and 0.5µl of the R primer (according to Table 1, species-specific primers used for amplification of a part of cytochrome c oxidase subunit I (COI) gene), 5µl of DNA, and for each, and up to 25ml for each micro tube, and deionized water added to every sample of 12.5µl. The heat cycle to amplify COI gene in the thermal cycler machine is as follows: The initial denaturation step performed at 95°C for 5 min, and then 30 to 35 cycles, including three stages of denaturing, annealing and extension was done in 1 min, then again denaturation at 94°C for 1 min, the stage of annealing at 53°C for 50 sec, and the stage of extension at 72°C were all performed. The final stage, the extension step was performed for 10 min at 72°C. At the end, the PCR products were then stored at 4°C and subjected to electrophoresis for analysis

2.2. PCR-RFLP

2.2.1. Amplifying part of the cytochrome b (cyt-b) gene

To assess cross-contamination and generate cell line fingerprints through PCR-RFLP, part of the cyt-b gene was amplified using the reverse and forward primers designed by Parson, which target a specific segment of DNA (358bp). PCR was performed on DNA extracted from the RBK and MDBK cell lines.

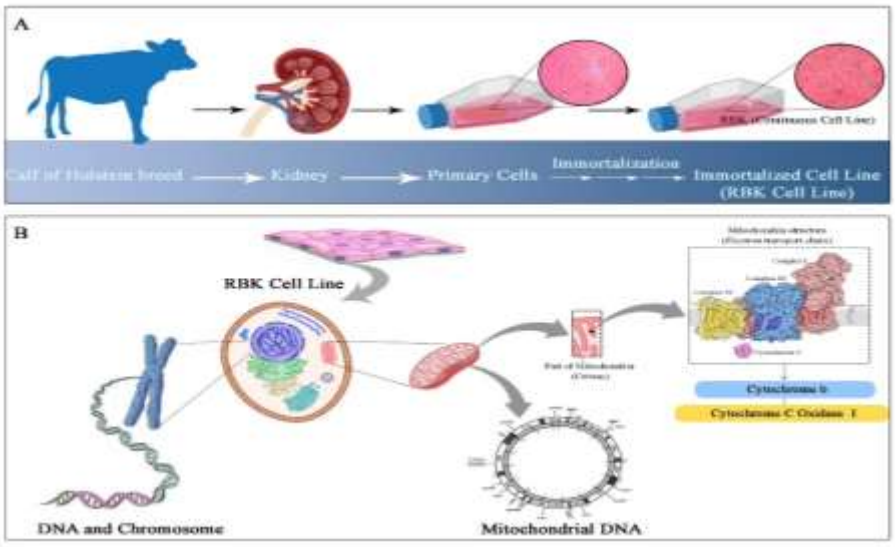


Figure 1. RBK cell line. **A)** Primary cells obtained from kidney of two-day-old male calf of Holstein breed, serial passaging cause chromosomal variation in cell, so primary cell became immortalized cell line. **B)** Schematic of RBK cell line, mitochondria, mitochondrial DNA (mtDNA), mitochondrial cytochrome b (cyt-b) and cytochrome c oxidase subunit I (COI). We evaluated the RBK cell line from different aspects by different assays (chromosomes of RBK cell line, and its cyt-b and COI).

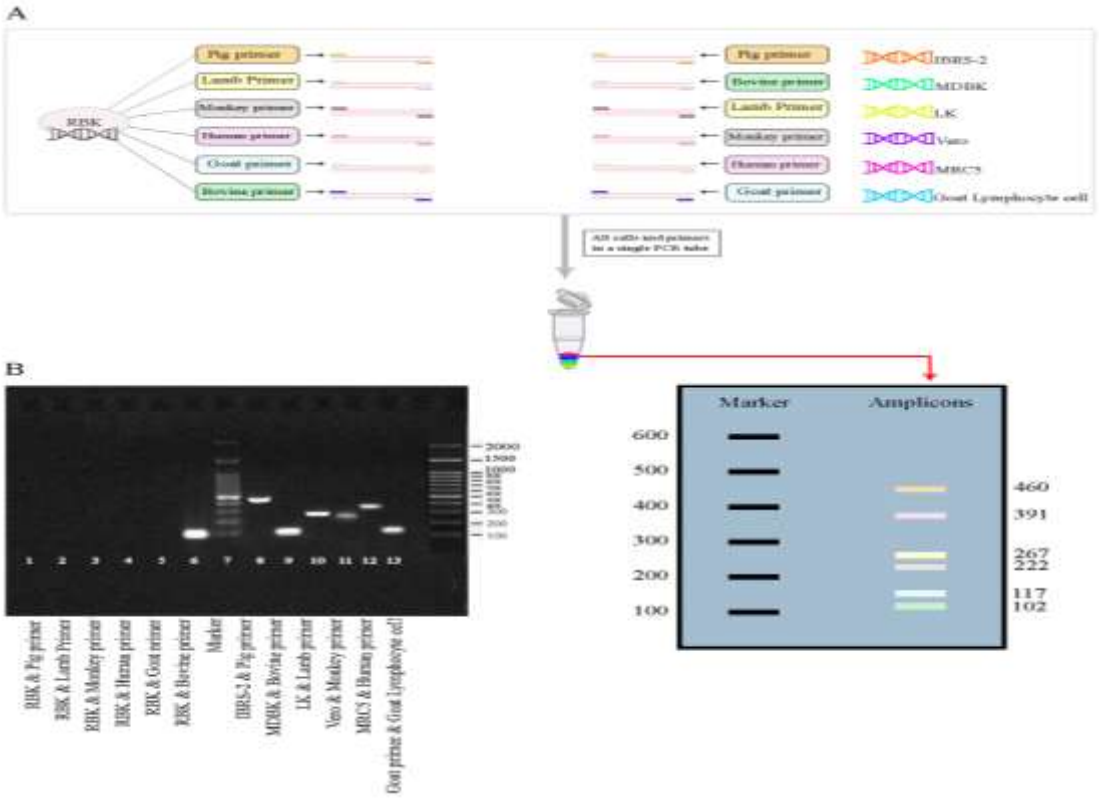


Figure 2. Electrophoresis of part of mitochondrial COI gene of the RBK. **A)** Cells and primers that used in this study. **B)** The figure above proves free-contamination with the cells of other species. Line 1 to 6 are related to the extracted DNA from RBK with the primers of 5 other species, where no band was observed. In lines 8 to 13, the extracted DNA from all cells with their own dedicated primers, where a sizeable band was observed, such as the following: Line 1: RBK cell line with pig primer of 460bp. Line 2: RBK cell line with lamb primer. Line 3: RBK cell line with monkey primer. Line 4: RBK cell line with human primer. Line 5: RBK cell line with goat primer. Line 6: RBK cell line with bovine primer. Line 7: marker. Line 8: IBRS-II cell with pig primer 460bp. Line 9: MDBK cell with bovine primer 102bp. Line 10: LK cell with lamb primer 267bp. Line 11: Vero cell with monkey primer 222bp. Line 12: MRC5 cell with human primer 391bp. Line 13: Goat Lymphocyte cell with Goat primer 117bp.

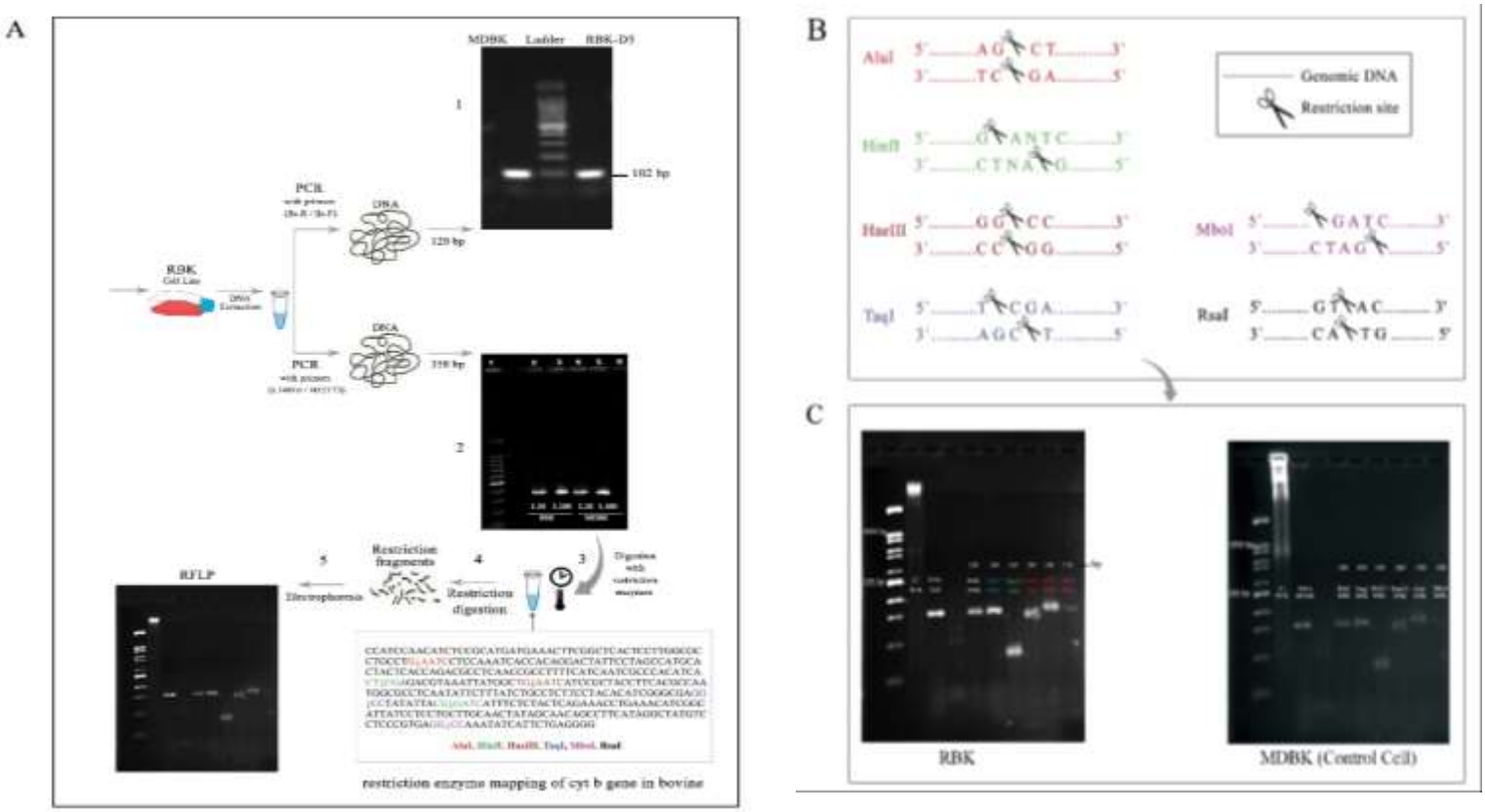
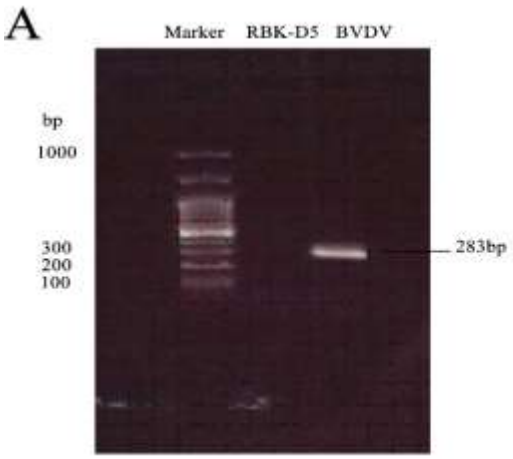
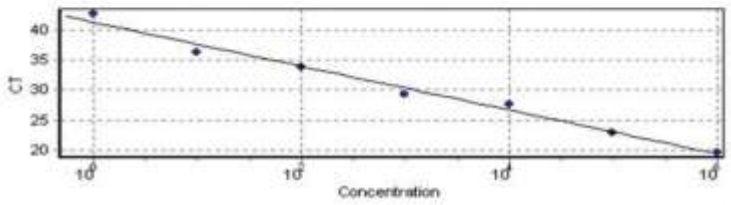


Figure 3. A-1) PCR for RBK and MDBK cells, Marker 100bp. a single band in 120bp, that confirmed the origin of RBK cell is bovine (Part of COI gene was amplified by Bt-R and Bt-F primers). **A-2)** The extracted DNAs from both RBK and MDBK were prepared in two dilutions of 1.10 and 1.100. Using L14816 and H15173 primers, cyt-b gene of RBK and MDBK was amplified (358bp) by PCR. **A-3)** RFLP process: Digestion of PCR products by restriction enzymes after 16 hours. **A-4)** restriction enzymes (Taq I, HinfI, HaeIII, AluI, Rsa and MboI) cut DNA. **A-5)** Electrophoresis separates the restriction fragments in agarose gel. **B)** Restriction enzymes and restriction sites. **C)** PCR-RFLP for RBK and MDBK, Electrophoresis separates the restriction fragments in Agarose gel, Marker 1000bp.



B

	Name	Type	Ct	Given Conc (Copies)	Calc Conc (Copies)	% Var
	P	Standard	19.58	1.000000000E+06	8.845024379E+05	11.5%
	-1	Standard	22.93	1.000000000E+05	1.062899403E+05	6.3%
	-2	Standard	27.61	1.000000000E+04	5.479232401E+03	45.2%
	-3	Standard	29.34	1.000000000E+03	1.833520042E+03	83.4%
	-4	Standard	33.81	1.000000000E+02	1.075632560E+02	7.6%
	-5	Standard	36.19	1.000000000E+01	2.394866056E+01	139.5%
	-6	Standard	42.60	1.000000000E+00	4.110155483E-01	58.9%
	e-	Negative Control				



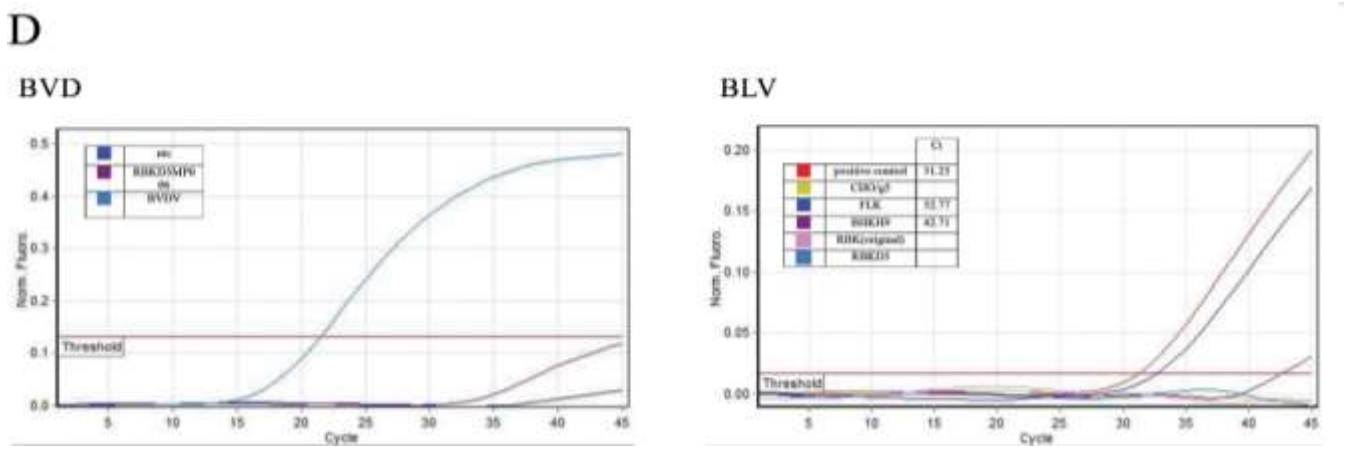


Figure 4. RT-PCR. **A)** No bands in agarose gel electrophoresis for RBK and the presence of band for BVDV. **B)** The number of copies of the virus have been determined in 6 serial dilutions of the BVDV with the primary titer of 10^6PFU/ml. **C)** curve obtained by quantitative analysis based on 6 serial dilutions of the BVDV with the primary titer of 10^6PFU/ml (6 virus diluters (from 1 to 10^6) copies in each ml is shown on the horizontal axis, and Ct on the vertical axis, here R2 values was obtained to be 0.95592. Therefore, the experiment can identify and report as low as 100 copies of the virus). **D- BVD)** RBK determined contamination-free with BVD virus. **D- BLV)** Due to absence of Ct in the RBK, this cell is BLV contamination-free.

Table 1. Primers and probes used in PCR reactions.

Species	Forward Primer	Sequence	Reverse Primer	Sequence	Siz (bp)	Referance
Sus scrofa(Pig)	Ss-F	CT ACT ATC CCT GCC AGT T	Ss-R	GAA TAG GAA GAT GAA GCC	460	
Capra hircus(goat)	Ch-F	ATA TCA ATC GGG TTT CTA GGA TTT ATT	Ch-r	AGT TGG GAT AGC GAT AAT TAT GGT AGT	117	
Bos taurus (cow)	Bt-F	GCTATTCC AAC CGG GGT AAA AGT C	Bt-R	GAAAAT AAA GCC TAG GGC TCA C	102	
Ovis aris (sheep)	Oa-F	CGA TAC ACG GGC TTA CTT CAC G	Oa-R	AAA TAC AGC TCC TAT TGA TAA T	267	**Cooper 2007**
Homo sapiens (human)	Hs-F	TAG ACA TCG TAC TAC ACG ACA CG	Hs-R	TCC AGG TTT ATG GAG GGT TC	391	
Cercopithecus Aethiops (Gr. Monkey)	Ca-F	CTTCTTTCCTGCTGCTAATG	Ca-R	TTTGATACTGGGATATGGCG	222	
Cyt b gene	L14816	CCATCCAACATCTCCGCATGATGAAA	H15173	CCCCTCAGAATGATATTTGGCCTCA	358	**Bravia 2004**
BVDV	BVDco-F	CAT GCC CAT AGT AGG AC	BVDco-R	CCA TGT GCC ATG TAC AG	283	**Ridpath 1998**
BVDV	BVD190-F	GRA GTC GTC ART GGT TCG AC	V326-R	TCA ACT CCA TGT GCC ATG TAC	208	**OIE 2018**
	Probe: TQ-pesti	FAM-TGC YAY GTG GAC GAG GGC ATG C-TAMRA				
BLV	MRBLVL	CCT CAA TTC CCT TTA AAC TA	MRBLVR	GTA CCG GGA AGA CTG GAT TA	120	**OIE 2018**
	MRBLV probe	FAM GAA CGC CTC CAG GCC CTT CA BHQ1				

0.5µl of the PCR Master Mix, which contains dNTPs, PCR buffer, Mgcl2, and Taq DNA polymerase, 0.5µl of the F primer, and 0.5µl of the R primer, 5µl of DNA, and for each, and up to 25ml for each micro tope, de-ionized distilled water were added to every sample of 12.5µl. To develop cyt-b gene, the heat cycle in the thermal cycler machine was set as follows: First, DNA was denatured for 5 minutes at 95°C, and then 30 to 35 cycles, including three stages of denaturation, annealing and extension was done in 1 minute, again denaturation at 94°C for 1 minute, then annealing stage at 53°C for 50 seconds, and the stage of extension at 72°C for 10 min were all performed to complete polymerization.

2.2.2. Restriction enzyme digestion of PCR products

Briefly, The 358 bp product of cyt-b gene of RBK cell line was purified and digested with six restriction enzymes (Tables 2 and 3). Similarly, the *MDBK* PCR products served as control and were digested with six restriction enzymes (Figure 3).

In this experiment, first 10µl of the PCR product is poured into a 0.5ml micro tube. DNA density must be between 0.1 to 0.5 µg. Then 3µl of the buffer was added. Later, 16µl of distilled water was added to microtubes except HinfI microtube (16.75µl of distilled water was added to HinfI micro tube). Water was heated to 37°C, and kept at 37°C incubator. Then 1µl of MboI, Rsa, HaeIII and AluI enzymes was added to each of microtube from 1 to 4, and then 0.25 of HinfI enzyme was added to tube 5.

All microtubes were placed at 37°C incubator for 16 hours. 1µl of TaqI was poured into microtube 6, and kept at 65°C incubator for 16 hours. The volume of the reaction solution was 30µl. Digestion results were visualized using the electrophoresis gel.

2.3. Adventitious VirusesAassay

Viral nucleic acid extracted from RBK cell was analyzed to detect the adventitious viruses in cell line (especially BVDV and BLV), to assess possible contamination.

2.3.1. Assay for BVDV

2.3.1.1. RNA Extraction

According to kit instructions.

2.3.2.2. Two-step RT-PCR

After extraction of the RNA, the two-step RT-PCR method was used to amplify 5′UTR gene, i.e. part of the BVD. Primers used are introduced in Table 1.

cDNA Synthesis*:* In this stage, to provide cDNA from the extracted RNA for amplification of 5′UTR gene, i.e. part of the BVD for a volume of 10µl (2.25% distilled water, 2µl M-MuLV buffer (5x), 2µl dNTPs (10mmol), 10.5µl reverse, 10.5µl forward of the primers (10pmol), 10.25µl of RNase Inhibitor, 10.5µl of Reversed Replication Enzyme M-MuLV, and 3µl of RNA) were poured into a tube, and then the microtube was placed inside a thermocycler. Thermal cycle for amplification of 5′UTR gene of cDNA is as follows: First, at 42°C for 60 min., then 94°C for 4 min.

Table 2. Expected length of the pieces cut by restriction enzymes.

Species	AluI	HinfI	HaeIII	TaqI	RsaI	MboI
Bovine (Cow)	358	198 160	231 106 21	358	358	358

Table 3. Each restriction endonuclease targets different nucleotide sequences in DNA strand, then cuts at different sites.

Restriction Enzyme	Degree of activity	Buffer	Restriction Site
AluI	37℃	Tango	**5′…A G↓C T…3′** **3′…T C↑G A…5′**
HinfI	37℃	R	**5′…G↓A N T C…3′** **3′…C T N A↑G…5′**
HaeIII	37℃	R	**5′…G G↓C C…3′** **3′…C C↑G G…5′**
TaqI	65℃	TaqI	**5′…T↓C G A…3′** **3′…A G C↑T…5′**
RsaI	37℃	Tango	**5'… G T↓A C… 3'** **3′... C A↑T G … 5'**
MboI	37℃	R	**5′…↓G A T C…3′** **3′…C T A G↑…5′**

2.3.2.3. PCR

To amplify cDNA obtained we need PCR reaction for 20µl of every sample: 8.4µl of the PCR master mix, 6.0µl of forward primer, 6.0µl of reverse primer, 20µl of cDNA, and 8.4µl of unionized distilled water were added to each microtube (Enzyme used for amplification in this stage was Taq DNA Polymerase). The Thermocycler heat cycle for the second stage of the Two-Step RT-PCR reaction for amplification of 5′UTR gene, i.e. part of the BVD is as follows: 30 cycles including 3 stages of denaturation, annealing, and extension over 55 sec. Denaturation at 94°C for 10 sec., annealing at 50°C for 10sec., and finally extension for 30 sec. at 72°C. Ultimately, another cycle of 72°C for 10 min was performed to complete polymerization. Then the PCR products were electrophoresed.

2.3.2.4. Real Time PCR for *BVDV*

To do this, master mix (RealQ Plus 2X Master Mix for Probe, High ROX Amplicon, Denmark) was used. 9 various dilutions of cDNA obtained from RNA (10^6 copies in each ml) were prepared. The amplification reaction at 20ml volume was done using Corbet 6000 Real Time PCR at the following thermal pattern, including: 10µl of master mix, 5µl of cDNA, obtained from serial dilutions, 1µl of initiator, 6µl of probe, and 2.4µl of water. Here is the thermal cycle: 95°C for 15 min., and then 45 cycles including: 95°C for 15 sec., and 60°C for 60 seconds. The sequence of the primers and probe as well as the procedure were done in accordance with OIE 2018 presented in Table 1.

2.3.2. Assay for BLV

Real Time PCR method on the nucleic acid obtained was performed. The sequence of the primers and probe as well as the procedure were done in accordance with OIE 2018 (Table 1). Here is the thermal cycle: 94°C for 60 seconds., then 35 cycles including: 94°C for 60 seconds., 60°C for 60 seconds and 72°C for 60 seconds. Ultimately, 1 cycle of 72°C for 5 minutes was performed.

3. Results

3.1. DNA fingerprinting

In order to determine and select the right cells, COI, species-specific gene was amplified to analyze cell identity and confirm the absence of contamination with other cells (7). By using specific primers designed for bovine species, a 102bp fragment of COI gene was amplified (Figure 2).

3.2. PCR-RFLP

In this study, cell line identification and confirmation of the RBK Cell Species were done using the PCR and PCR-RFLP methods based on mitochondrial cyt-b gene. The PCR-RFLP has identified the animal species origin with 100% accuracy. After a segment of DNA wascut into pieces with restriction enzymes, the fragments were compared to verify species origin and to assess the absence of contamination by other species (Figure 3).

Using the PCR-RFLP technique, through analysis of restriction sites (through enzymes mentioned earlier) and Mitochondrial DNA (mtDNA) length, we managed to determine cell line contamination or, otherwise, with undesirable cell species in addition to identification of the origin of the cell species. This study confirms the origin of RBK cell line (bovine species), revealing that it is contamination- free. By relying on the test under investigation, it can be concluded that cell line identity is confirmed (8, 9).

3.3. Adventitious viruses assay

RT-PCR result shows no contamination of RBK cell with BVD or BLV viruses. Representative fluorescence acquisition graph showednegative control, BVD ,and RBK (Based on OIE, positive sample must be Ct value< 40). In this assay, BVD virus was used as the positive control , with Ct= 21.45. Negative control samples must have no Ct value. Based on the results of the experiment, RBK determined contamination-free with BVD virus. Real time-PCR was used to analyze contamination RBK cell line with BLV (Number of copies is according to RT-PCR for BLV contamination). Based on the OIE, cell infected with BLV or positive control must be $Ct \leq 40.95$. In this experiment, BLV virus sample and FLK cell (infected with virus) were used as positive control. Moreover, negative control must be $Ct > 40.95$. Due to absence of Ct in the RBK, this cell is BLV contamination-free (Figure 4) (10).

4. Discussion

In order to authenticate the cell line, confirmation of its origin and free- contamination are important and crucial to forecast the value of a cell line. Therefore, based on standard protocols, it is necessary to establish a cell line origin and its purification than cross-contamination and interspecific contamination (2, 3, 11-14).

There are numerous reports on using isoenzymes, which analyze cell isoenzymes as well as assurance of their cleanness from impurities at different stages.

Isoenzymes analysis not only is useful in identification and contamination detection of the cell lines, it also constitutes part of the tests for acceptance of new cell line. However, it would be better if isoenzymes analysis were associated with karyotyping or molecular techniques (15, 16).

mtDNA is a powerful tool for tracing generations through mothers and differentiating species due to its genetic variability; making it a robust tool for species identification, understanding population structures, and detecting contamination among closely related species (17).

As cell lines are valuable models and essential resources for research, the more information in terms of the origin of a cell line is important. Moreover, DNA barcoding is one of the best methods in identifying cells, species, contaminations, and their identification of conserved genes, setting the code for mitochondrial proteins (13, 14, 18, 19).

Therefore, in this project, we investigated a multifaceted molecular approach, focusing on mitochondrial genomes perspectives. In the present study, Initially, a 102 bp fragment of COI gene, containing conserved sequences, was used to identify the species (Figure 3 A-1).

DNA fingerprinting is a reliable tool for rapid identification of cell cultures and tracing cross-contaminations among different species. Comparing their DNA profiles of cells can serve as a useful tool for researchers to identify the origin of a cell (17, 18).

In this research, a part of COI gene of RBK cell line was experimented and analyzed using species-specific primers for 6 cells ,including, GL, Vero, MRC5, IBRS-2, LK, and RBK (Table 1) simultaneously, and the identity of the RBK cell line and its absence of contamination with other species were confirmed (Figure 2).

PCR-RFLP utilized mitochondrial DNA markers, serving as a rapid and reliable markers that can be a powerful and valuable tool for genotyping and genetic fingerprinting to guarantee the precision and reliability of the cell line used in research. Additionally, PCR-RFLP is a different isoenzyme analysis system for confirmation of species origin and lack of interspecies contamination (20, 21). Among intergenic regions of mDNA, genes of COI, COII and cyt-b are more often investigated. Among mitochondrial genes, cyt-b gene has multiple advantages in identification and discovering cross- contamination of cell lines (8, 22).

In the current study, cyt-b was used to assess the PCR-RFLP identification power of the bovine species and its sensitivity to identifying interspecies and cross-contamination. Based on the results obtained, the cell samples have no cross-contamination with other species (Figure 3).

Real-time PCR is one of the techniques that used target amplification widely and was developed to identify cell lines among species such as: mammalian, human, drosophila, pig and chicken cell lines (23-26).

Real-time PCR method is also useful in assessing cell lines with adventitious agents such as viruses. This method utilizes special primers and probes, is able to use its special features to target the intended section reliably, and serves as a sensitive test for identification of intended items in diluted solutions (23). Overall, Real-time PCR is a reliable and economical approach that can validate all research and diagnostic activities (27).

In this research, RBK cell line was studied for contamination with BLV and BVDV using the Real-time PCR method.

Our results indicated absence of contamination with the above-mentioned viruses (Figure 4). Considering that one of the key factors in isolating viruses is multiplication of viruses in cell cultures; selecting the proper cell line with high sensitivity to the target virus is crucial extremely important to obtain the most reliable results. As such, in this project, based on the research conducted and the results obtained, RBK cell line is confirmed to be contamination-free by adventitious agents. Overall, our investigations confirm high capability of RBK to support replication and stability. Therefore, RBK cell line can be used widely used in research, diagnosis, virus proliferation and quantitative control of the virus (Titration).

Acknowledgment

We hereby thank the staff at the Quality Control Department of Razi Vaccine and Serum Research Institute. Special thanks also go Morteza Kamalzdeh for his valuable assistance. Thanks are also due to the staff at the Human Virus Vaccines Department, as well as all the others who assisted us in the course of this research.

Authors' Contribution

Study concept and design: M. L.
Acquisition of data: M. M, M. L, A. M, F.S.
Analysis and interpretation of data: M. M, M. Li.
Drafting of the manuscript: M. M, M. Li.
Critical reversion of the manuscript for important intellectual content: M. L.
Statistical analysis: M. M.

Ethics

All experimental protocols and animals were approved by the Biomedical Research Ethics Committee and evaluated by Research Ethics Committees of Islamic Azad University-Science and Research Branch (Approval ID: IR.IAU.SRB.REC.1402.235 , Approval date: 2023-09-04), and all experiments were carried out in accordance with relevant guidelines and regulations.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

Not Applicable.

References

1. Ambrose CT. The Tissue Culture Laboratory of Dr. George Otto Gey 60 yrs ago as recalled by a former student. In Vitro Cellular & Developmental Biology-Animal. 2017;53(5):467-73.
2. Chen X, Qian W, Song Z, Li Q-X, Guo S. Authentication, characterization and contamination detection of cell lines, xenografts and organoids by barcode deep NGS sequencing. NAR Genomics and Bioinformatics. 2020;2(3):lqaa060.
3. Maghami M, Lotfi M, Nematalahi FS, Mohammadi A. Characterization of Razi Bovine Kidney (RBK) Cell Line as a Sensitive Cell to Bovine Herpesvirus-1 (BoHV-1). Archives of Razi Institute. 2023;78(5):1528.
4. Masoudi S. Evaluating of Iran Razi Khedmati Bovine Kidney (IRKHBK) Cell Line for Isolation and Propagation of Viruses. Iranian Journal of Virology. 2014;8(4):20-4.
5. Muhsen M, Aoki H, Ikeda H, Fukusho A. Biological properties of bovine viral diarrhea virus quasispecies detected in the RK13 cell line. Archives of virology. 2013;158(4):753-63.
6. Hajia M, Mahmoodzadeh A, Morovati H. Use of Razi Bovine Kidney Cell Line for Proliferation of Pneumocystis Carinii. Iranian Journal of Pathology. 2008;3(4):197-202.
7. Cooper JK, Sykes G, King S, Cottrill K, Ivanova NV, Hanner R, et al. Species identification in cell culture: a two-pronged molecular approach. In Vitro Cellular & Developmental Biology-Animal. 2007;43(10):344-51.
8. Bravi CM, Lirón JP, Mirol PM, Ripoli MV, Peral-García P, Giovambattista G. A simple method for domestic animal identification in Argentina using PCR-RFLP analysis of cytochrome b gene. Legal Medicine. 2004;6(4):246-51.
9. Ridpath JF, Bolin SR. Differentiation of types 1a, 1b and 2 bovine viral diarrhoea virus (BVDV) by PCR. Molecular and cellular probes. 1998;12(2):101-6.
10. Oie A. Manual of diagnostic tests and vaccines for terrestrial animals. Office international des epizooties, Paris, France. 2008;2002:1092-106.
11. Eggleston H, Njoya K, Anderson CE, Holm I, Eiglmeier K, Liang J, et al. Molecular characterization and genetic authentication assay for Anopheles 'hemocyte-like'cell lines 4a-3A and 4a-3B. Parasites & Vectors. 2022;15(1):465.
12. Hay R. Cell line preservation and characterization. Animal cell culture: a practical approach. 1992:104-35.
13. Romano P, Manniello A, Aresu O, Armento M, Cesaro M, Parodi B. Cell Line Data Base: structure and recent improvements towards molecular authentication of human cell lines. Nucleic acids research. 2009;37(suppl_1):D925-D32.
14. Tong L, Corrigan A, Kumar NR, Hallbrook K, Orme J, Wang Y, et al. An automated cell line authentication method for AstraZeneca global cell bank using deep neural networks on brightfield images. Scientific reports. 2022;12(1):7894.
15. Halton DM, Peterson WD, Hukku B. Cell culture quality control by rapid isoenzymatic characterization. In vitro. 1983;19(1):16-24.
16. Steube KG, Grunicke D, Drexler HG. Isoenzyme analysis as a rapid method for the examination of the species identity of cell cultures. In Vitro Cellular & Developmental Biology-Animal. 1995;31(2):115-9.
17. Cabrera C, Cobo F, Nieto A, Cortés J, Montes R, Catalina P, et al. Identity tests: determination of cell line cross-contamination. Cytotechnology. 2006;51(2):45-50.
18. Rojas A. The imperative authentication of cell lines. Antimicrobial agents and chemotherapy. 2017;61(11):10.1128/aac. 01823-17.
19. PD H, editor Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. Proc Biol Sci; 2003.
20. Ferreira KM, e Silva OL, Arias MC, Del Lama MA. Cytochrome-b variation in Apis mellifera samples and

its association with COI–COII patterns. Genetica. 2009;135(2):149-55.
21. Rokas A, Williams BL, King N, Carroll SB. Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature. 2003;425(6960):798-804.
22. Marx V. Cell-line authentication demystified. Nature methods. 2014;11(5):483-8.
23. Das A, Xu L, Jia W. Development of conventional and real time PCR assays for rapid species authentication of mammalian cell lines commonly used in veterinary diagnostic laboratories. Research in Veterinary Science. 2019;126:170-7.
24. Corral-Vázquez C, Aguilar-Quesada R, Catalina P, Lucena-Aguilar G, Ligero G, Miranda B, et al. Cell lines authentication and mycoplasma detection as minimun quality control of cell lines in biobanking. Cell and tissue banking. 2017;18(2):271-80.
25. Mariyappa D, Rusch DB, Han S, Luhur A, Overton D, Miller DF, et al. A novel transposable element-based authentication protocol for Drosophila cell lines. G3. 2022;12(2):jkab403.
26. Pajurek M, Warenik-Bany M, Mikolajczyk S. Dioxin transfer simulation from feed to animal tissues and risk assessment. Chemosphere. 2023;313:137379.
27. Lung O, Candlish R, Nebroski M, Kruckiewicz P, Buchanan C, Moniwa M. High-throughput sequencing for species authentication and contamination detection of 63 cell lines. Scientific Reports. 2021;11(1):21657.