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Detection of contaminants in cell cultures, sera and trypsin

Tatiana Flávia Pinheiro de Oliveira [a], Antônio Augusto Fonseca Jr. [a], Marcelo Fernandes Camargos [a], Anapolino Macedo de Oliveira [a], Ana Cláudia Pinto Cottorello [a], Antonizete dos Reis Souza [a], Iassudara Garcia de Almeida [a], Marcos Bryan Heinemann [b, *]

[a] Laboratório de Biologia Molecular/Laboratório de Diagnóstico de Doenças Virais, Laboratório Nacional Agropecuário de Minas Gerais, Pedro Leopoldo, Minas Gerais, Brazil
[b] Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

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ABSTRACT

The aim of this study was standardization and application of polymerase chain reaction (PCR) for the detection of contaminants in cell cultures, sera and trypsin. Five PCR protocols were standardized to assess the presence of genetic material from mycoplasma, porcine circovirus 1 (PCV1), bovine leukemia virus (BLV) or bovine viral diarrhea virus (BVDV) in cell culture samples. PCR reactions for the genes GAPDH and beta-actin were used to evaluate the efficiency of nucleic acid extraction. The PCR protocols were applied to 88 cell culture samples from eight laboratories. The tests were also used to assess potential contamination in 10 trypsin samples and 13 fetal calf serum samples from different lots from five of the laboratories. The results showed the occurrence of the following as DNA cell culture contaminants: 34.1% for mycoplasma, 35.2% for PCV1, 23.9% for BVDV RNA and 2.3% for BLV. In fetal calf sera and trypsin samples BVDV RNA and PCV1 DNA was detected. The results demonstrated that cell culture, sera and trypsin used by different laboratories show a high rate of contaminants. The results highlight the need for monitoring cell cultures and controlling for biological contaminants in laboratories and cell banks working with these materials.

1. Introduction

Contamination of cell cultures is not easily eliminated and causes delays, financial loss and requires effort to detect and eliminate such contamination [1]. The discovery of contaminants can place past or current results in question and cause cell cultures to be lost [2]. Scientific credibility may become threatened by reports of contamination, which is capable of invalidating results and devaluing products and medicines [3]. Moreover, vaccines produced in infected cell cultures can lead to seroconversion or disease in vaccinated humans and animals [4,5].

Vaccines against classical swine fever virus, infectious bovine rhinotracheitis, bovine respiratory syncytial virus infected with the bovine viral diarrhea virus (BVDV) have been responsible for infection in pigs vaccinated or by the origin of disease outbreaks in cattle [6]. There are reports of vaccines against Aujeszky's disease virus produced in cells from sheep infected with Border disease virus, causing infection and consequently producing antibodies that are cross-reactive for Classical Swine Fever Virus (CSFV), generating false-positive results in serological diagnosis of classical swine fever [7].

Classical Swine Fever (CSF) in Brazil presents controlled without vaccination. According to the World Organization for Animal Health (OIE), the final report of this disease was in 2009. The BVDV is one of differential virus in the diagnosis of CSF, which assigns high importance to it. In this sense, it is of great importance to use of cell cultures and fetal bovine serum free of contaminants, mainly for BVDV, thus avoiding false-positive results in the diagnosis of diseases of major impact as the CSF.

Recently, the porcine circovirus type 1 (PCV1) was detected in a commercial vaccine against human rotavirus and it was confirmed that it was present in the cell stock used for vaccine production [8]. The case of vaccine contamination with PCV1 reinforces the need for continued efforts to reduce the likelihood of introducing viruses

* Corresponding author. Escola de Veterinária, Universidade Federal de Minas Gerais, UFMG. Av. Antônio Carlos 6627 Caixa Postal 567, Campus da UFMG CEP 30123-970, Belo Horizonte – Minas Gerais, Brazil. Tel.: +55 31 3409 2077; fax: +55 31 3409 2080/2077.
E-mail addresses: oliveiratfp@yahoo.com.br (T.F. Pinheiro de Oliveira), mabryan@ufmg.br, mabryan@vet.ufmg.br (M.B. Heinemann).

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of animal origin in materials used in the manufacture of biological products [9].

Cell culture contamination is caused by biological and chemical agents. The biological contaminants most commonly found in cells are mycoplasma, viruses, bacteria and yeasts. In most cases, contamination can occur without the knowledge of the analyst [2,10]. Contamination with mycoplasma and viruses may go unnoticed, causing changes in infected cells, such as a reduction in growth rate, as well as alterations in cell morphology, chromosomes and metabolism of amino acids and nucleic acids [11].

Mycoplasma and viruses can originate from contaminated cultures or supplies, such as serum and trypsin that are commonly used in the maintenance of cell cultures [12] or from inadequate handling procedures [13].

Methodologies used to monitor cell culture contamination include the PCR, virus culture and measuring the reactivity of antibodies against specific agents [5,10].

Because many infectious agents are not easily cultivable [5] PCR has been shown to be an efficient methodology for the detection of biological contaminants in cell culture and its supplies. This technique has attracted much attention in the detection of cell culture contaminants because it is fast, robust, highly sensitive and specific compared to traditional techniques [14–16].

The importance of working with the detection of contaminants has been demonstrated by studies in Brazil [17–21], however, the laboratorial practice of cell contaminants is not carried out regularly.

Noting the relevance of the effects caused by cell culture contaminants in virological diagnosis and vaccine production, and the fact that PCR may be a useful tool in the detection of these contaminants, the present study aimed to develop methods for routine use to detect mycoplasma, PCV1, BVDV and BLV and evaluate the occurrence of these agents in cell culture, trypsin and bovine serum samples of government laboratories and, research and teaching institutions.

2. Materials and methods

2.1. Cell cultures, sera and trypsin

We analyzed 88 cell culture samples from different species (cattle, pig, monkey, hamster, rat, mouse, rabbit, cat, sheep, canine, human, equine and insect) belonging to 32 cell cultures in suspension. The samples were obtained from eight laboratories, five of which were government laboratories (coded GL1 to GL5) where they were used in the diagnosis of viral diseases that affect animals and also for evaluation of vaccines and three of which were research and teaching institutions which were used in different experiments and also for diagnostic purposes (coded RTI1 to RTI3).

We analyzed samples from 10 different batches of trypsin, which came from six manufacturers and samples from 13 different batches of fetal calf serum, which came from 11 manufacturers. All of these samples were sent by the GL1, GL2, GL3, GL5 and RTI3 laboratories.

2.2. Extraction of genetic material

RNA extraction from different samples (cell cultures, trypsin and bovine serum) was performed using Trizol® (Life Technologies, Carlsbad, California, USA), according to the manufacturer's instructions for BVDV and beta-actin RNA detection. There was at least one negative control consisting of 375 μL of nuclease-free water to verify that the reagents are free from contaminating and to monitor and measures to prevent PCR cross-contamination. The quantities of sample subjected to extraction were as follows: 375 μL of cell suspension (concentration 10^6 cells/mL); 375 μL of fetal calf serum and 50 mg of trypsin diluted in 375 μL of nuclease-free water.

One ml of Trizol® was added to the samples and the mixtures were incubated at room temperature for 5 min. Then 200 μL of chloroform was added to each sample and the microtubes were shaken vigorously and centrifuged at 12,000 *g* for 15 min at 4 °C. The upper aqueous phase obtained was transferred to another Eppendorf tubes and the RNA was precipitated by the addition of 500 μL of isopropyl alcohol and centrifugation at 12,000 *g* for 10 min at 4 °C. The supernatant was then carefully discarded by inverting the tube and the precipitate was washed with 1 mL of ethanol 75%, homogenized and centrifuged at 7500 *g* for 5 min at 4 °C. The supernatant was again discarded and the microtubes were inverted on paper towels for a few minutes to complete drying of the ethanol. The precipitate was suspended in 20 μL of nuclease free water (DEPC water) and the product stored at −70 °C until use.

Extracted RNA was transcribed into complementary DNA (cDNA) for performing PCR using the Reverse Transcription System kit (Promega, Fitchburg, Massachusetts, USA) according to the manufacturer's protocol.

Two methods of DNA extraction were used, as follows: i) the phenol-chloroform method [22] for the extraction of DNA from samples of fetal calf serum and trypsin and ii) the protocol proposed in Fonseca Jr. [23] with modifications for the extraction of DNA from cell cultures. The quantities of sample subjected to DNA extraction were as follows: 200 μL of cell suspension (concentration 10^6 cells/mL); 500 μL of fetal calf serum and 50 mg of trypsin diluted in 500 μL of nuclease-free water.

For extracting DNA from the fetal calf serum and trypsin, 20 μL of Proteinase K (10 mg/mL) was added to the samples and the microtubes were incubated at 55 °C for 1 h. We used Eppendorf tubes containing 500 μL of nuclease-free water as a negative control for every extraction to verify that the reagents are free from contamination and to monitor cross-contamination during nucleic acid extraction. Two hundred microliters of saturated pH 8 phenol was added and the mixtures were homogenized by inverting the microtubes and immediately centrifuging at 434 *g* for 5 min at room temperature. The upper aqueous phase obtained was transferred to another microtube and 200 μL of chloroform/isoamyl alcohol at a ratio of 24:1 was added. The mixtures were homogenized and centrifuged again at 434 *g* for 5 min at room temperature and the upper aqueous phase was collected and transferred to a new microtube. It was added ethanol 95% in a volume twice that of the aqueous phase and ammonium acetate in a volume equal to that of the aqueous phase. The mixtures were homogenized and incubated at −80 °C for 1 h. The microtubes were then centrifuged at 18,327 *g* for 10 min at 4 °C. The supernatant was carefully discarded by inverting the microtubes and 500 μL of ethanol 70% was added to wash the DNA. The microtubes were again centrifuged at 18,327 *g* for 10 min at 4 °C. The supernatant was discarded and the microtubes were inverted on paper towels for complete drying of the ethanol. The precipitate was suspended in 50 μL of TE buffer (10 mM Tris HCl, 1 mM EDTA, pH8) and the product stored at - 20 °C until use.

For extracting DNA from the cell suspensions, cell DNA was extracted using a quantity of 20 μL of proteinase K (10 mg/ml) in 200 μL of cryopreserved cell suspension. We used Eppendorf tubes containing 200 μL of TE buffer (10 mM Tris HCl, 1 mM EDTA, pH8) as a negative control for every extraction to verify that the reagents are free from contamination and to monitor PCR cross-contamination. Samples were incubated at 55 °C for 1 h, then centrifuged at 16,000 *g* for 1 h at 4 °C and the supernatant discarded. The precipitate was resuspended in 50 μL of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) and incubated at 90 °C for 30 min. The product was stored at −20 °C until use.