

## СОБЛЮДЕНИЕ ЭТИЧЕСКИХ СТАНДАРТОВ

Работа была выполнена без использования животных или участия людей в качестве объектов исследования.

## КОНФЛИКТ ИНТЕРЕСОВ

Авторы заявляют об отсутствии конфликта интересов.

## СПИСОК ЛИТЕРАТУРЫ

- Ёлшин Н.Д., Петров А.В. 2017. Изучение возможности использования метода qPCR для контроля отсутствия микоплазменной контаминации в клеточных культурах. БИОпрепараты. Профилактика, диагностика, лечение. Т. 63. С. 173. (Yolshin N.D., Petrov A.V. 2017. Possibilities of qPCR control of mycoplasma contamination of cell cultures. BIOPreparations. V. 63. P. 173.)
- Adeniji J.A., Faleye T.O.C., Adewumi O.M., Olayinka O.A., Donbraye E., Oluremi B., George U.E., Arowolo O.A., Omoruyi E.C., Ifeorah M.I., Akande A. 2018. Draft genome sequence of *Mycoplasma arginini* strain NGR\_2017. Genome Announcements. V. 6. P. 26. <https://doi.org/10.1128/genomeA.00577-18>
- Bruchmüller I., Pirkl E., Herrmann R., Stoermer M., Eichler H., Klüter H., Bugert P. 2006. Introduction of a validation concept for a PCR-based Mycoplasma detection assay. Cytotherapy. V. 8. P. 62.
- Dumke R., Schurwanz N., Lenz M., Schuppler M., Lück C., Jacobs E. 2007. Sensitive detection of *Mycoplasma pneumoniae* in human respiratory tract samples by optimized real-time PCR approach. J. Clin. Microbiology. V.45. P. 2726.
- Ishikawa Y., Takaharu K., Morita H., Saida K., Oka S., Masuo Y. 2006. Rapid detection of *Mycoplasma* contamination in cell cultures using SYBR Greenbased real-time polymerase chain reaction. In Vitro Cell Dev. Biol. Animal. V. 42. P. 63.
- Jurstrand M., Jensen J.S., Fredlund H., Falk L., Mölling P. 2005. Detection of *Mycoplasma genitalium* in urogenital specimens by real-time PCR and conventional PCR assay. J. Med. Microbiology. V. 54. P. 23.
- Kong F., Gordon S., Gilbert G.L. 2000. Rapid-cycle PCR for detection and typing of *Mycoplasma pneumoniae* in clinical specimens. J. Clin. Microbiology. V. 38. P. 4256.
- Kong F., James G., Gordon S., Zelynski A., Gilbert G.L. 2001. Species-specific PCR for identification of common con-
- taminant Mollicutes in cell culture. Appl. Environ. Microbiology. V. 67. P. 3195.
- Langdon S.P. 2004. Cell culture contamination. In: Cancer cell culture. Humana Press. P. 309.
- Lazarev V.N., Levitskii S.A., Basovskii Y.I., Chukin M.M., Akopian T.A., Vereshchagin V.V., Kostrjukova E.S., Kovaleva G.Y., Kazanov M.D., Malko D.B., Vitreschak A.G., Sernova N.V., Gelfand M.S., Demina I.A., Serebryakova M.V. et al. 2011. Complete genome and proteome of *Acholeplasma laidlawii*. J. Bacteriology. V. 193. P. 4943.
- Morozova A.V., Borchsenius S.N., Vishnyakov I.E., Malinin A.Y. 2017. Testing the purity of cell cultures using clinical diagnostic PCR kits. Cell Tiss. Biol. V. 11. P. 250.
- Ossewaarde J.M., de Vries A., Bestebroer T., Angulo A.F. 1996. Application of a *Mycoplasma* group-specific PCR for monitoring decontamination of *Mycoplasma* infected *Chlamydia* sp. strains. Appl. Environ. Microbiology. V. 62. P. 328.
- Perepelitschouk M., David S.W., Bhattacharya B., Volokhov D.V., Chizhikov V. 2011. Detection of mycoplasma contamination in cell substrates using reverse transcription-PCR assays. J. Appl. Microbiol. V. 110. P. 54.
- Störmer M., Vollmer T., Henrich B., Kleesiek K., Dreier J. 2009. Broad-range real-time PCR assay for the rapid identification of cell-line contaminants and clinically important mollicute species. Int. J. Med. Microbiology. V. 299. P. 291.
- Sykes P.J., Neoh S.H., Brisco M.J., Hughes E., Condon J., Morley A.A. 1992. Quantitation of targets for PCR by use of limiting dilution. Biotechniques. V. 13. P. 444.
- van Kuppeveld F.J., Johansson K.E., Galama J.M., Kissing J., Bölske G., van der Logt J.T., Melchers W.J. 1994. Detection of mycoplasma contamination in cell cultures by a *Mycoplasma* group-specific PCR. Appl. Environ. Microbiology. V. 60. P. 149.
- van Kuppeveld F.J., van der Logt J.T., Angulo A.F. Van Zoest M.J., Quint W.G., Nieters H.G., Galama J.M., Melchers W.J. 1992. Genus-species-specific identification of mycoplasmas by 16S rRNA amplification. Appl. Environ. Microbiology. V. 58. P. 2606.
- Volokhov D.V., Graham L.J., Brorson K.A., Chizhikov V.E. 2011. mycoplasma testing of cell substrates and biologics: review of alternative non-microbiological techniques. Mol. Cell Probes. V. 25. P. 69.

## DETECTION OF MYCOPLASMAS IN EUKARYOTIC CELL LINES BY REAL-TIME PCR USING DIFFERENT METHODS OF CONCENTRATION OF THE SAMPLE

**A. A. Chaplenko<sup>a,\*</sup>, O. V. Merkulova<sup>a</sup>, I. S. Semyonova<sup>a</sup>, A. R. Sayfutdinova<sup>a</sup>,  
E. V. Melnikova<sup>a</sup>, and V. A. Merkulov<sup>a</sup>**

<sup>a</sup>FSBI "Scientific Centre for Expert Evaluation of Medicinal Products" of the Ministry of Health of the Russian Federation,  
Moscow, 127051 Russia

\*e-mail: a.a.chaplenko@yandex.ru

Detection of mycoplasma contamination in eukaryotic cell lines is an important step in the quality control of these lines. Eukaryotic cell lines are used in manufacturing of biotechnological drugs and biomedical cell products. The methods of detection that proposed in the State Pharmacopoeia are microbiological (cultural) and cytochemical

(indicator cell culture) methods. These methods take several days to complete, they are difficult to standardize, and their result can be subjectively interpreted by the operator. Molecular methods for the detection of mycoplasmas can be used as an alternative. The most widely used molecular method is PCR; it is much more express, but less sensitive. Using pre-concentration samples can solve this problem. In this study, we compared the effectiveness of different cell line concentration methods, assessed the impact of sample composition on the parameters of the concentration process, and also validated the combined detection technique using two kinds of mycoplasmas (*M. arginini* and *A. laidlawii*) as examples.

**Keywords:** mycoplasma, PCR, biomedical cell products, cell lines contamination