

平成 18 年度「日本薬局方の試験法に関する研究」研究報告*4
—医薬品製造用細胞基材に対するマイコプラズマ否定試験法の問題点—

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**Some Problems in Compendial Requirements for the Detection of
Mycoplasmas from Cell Cultures**

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Summary

Mycoplasmas are common contaminants of cell cultures, and may affect the normal functions of cells in many ways. It is therefore essential to use mycoplasma-free cell lines for the production of biological products. Referring to four compendial requirements, VICH, WHO, JP and EP, for the detection of mycoplasma contamination from cell cultures, we point out some problems in these requirements. In these four compendia, common detection methods are a culture method and an indicator cell-culture method using Vero cells. JP and EP allow PCR (NAT), but PCR primers are not defined in the EP. In the case of the indicator cell-culture method, *M. hyorhinitis* and *M. orale* are specified as positive control organisms. However, *M. hyorhinitis* DBS 1050 can not grow on agar media, so that it is impossible to count an inoculum size of not more than 100 cfu. *M. orale* does not show a positive reaction even if the inoculum size is 1,000 cfu. Therefore, these mycoplasma strains are not suitable as positive control organisms. Since the PCR amplifies DNA from both viable and nonviable mycoplasmas, PCR is not used as a first-line detection method, like the culture method or indicator cell-culture method, in JP. If a positive result is obtained only by the indicator cell-culture method, PCR can be used to determine whether mycoplasma is actually present in a tested sample. However, it is possible to evaluate mycoplasma contamination by a reliable PCR method. We demonstrated the reliability of PCR as a sterility test for mycoplasmas from cell cultures and would like to propose PCR as a first-choice testing method for mycoplasma contamination in cell cultures. We also showed that some Vero cells used in Japan are contaminated with unculturable *M. orale* (registered as AB353273).

Key words

Mycoplasma contamination, cell culture, sterility test, PCR, VICH, JP, EP