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マイコプラズマ否定試験に用いるマイコプラズマ参照品に関する研究（第２報†）第十七改正日本薬局方収載 NBRC 由来マイコプラズマの核酸増幅法（NAT）による検出感度に関する共同比較研究

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Studies on Reference Strains for Mycoplasma Testing (Part II) Collaborative Study on Detection Sensitivities of NAT-based Mycoplasma Testing with NBRC Mycoplasma Reference Strains in the Japanese Pharmacopoeia 17th Edition

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Summary

Mollicutes bacteria are silent contaminants of cell cultures, and mycoplasma testing should be routinely performed for the assessment of master/ working cell banks and cell cultures during the manufacture of biotechnological/ biological products. For application of the nucleic acid amplification test (NAT) as an alternative to other methods, the Japanese Pharmacopoeia 17th Edition (JP17) General Information requires suitable validation with mycoplasma reference strains to meet a limit of detection (LOD) value. The LOD value should not exceed certain limits: 10 CFU (Colony Forming Unit) /mL corresponding to the Culture Method and 100 CFU/mL corresponding to the Indicator Cell Culture Method. For validation of the LOD, JP17 General Information requires the use of mycoplasma reference strains prepared from a panel of 6 mycoplasma species and *Acholeplasma laidlawii*. As mycoplasma reference strains, 6 NBRC strains, as well as ATCC strains, are listed in JP17 General Information, based on Pharmaceutical and Medical Device Regulatory Science, 45 (5), 442-451, 2014. However, that work showed limited data for the LOD values of the NBRC strains. In order to verify the appropriateness of the NBRC mycoplasma reference strains, we conducted repeated tests with 4 NAT-based detection kits: MycoTOOL PCR, MycoTOOL Real-Time PCR, Myco Finder and MycoSEQ. In addition to mycoplasma reference strains listed in JP17 General Information, *Mycoplasma arginini* NBRC 111899, which was confirmed to be usable as a reference strain in the part I report, was also examined. We spiked prepared mycoplasma reference strains at 100, 10, and 1 CFU/mL into CHO DG44 cells as models of contaminated cell lines. The test results showed LOD values corresponding to less than 10 CFU/mL for all of the prepared mycoplasma reference strains. These results confirmed that all of the NBRC mycoplasma reference strains in JP17, as well as *M. arginini* NBRC 111899, can be used as positive controls for validation of NAT-based mycoplasma testing.

Key words

Mycoplasma, Mycoplasma testing, Mycoplasma reference strain, Nucleic acid amplification test, NAT, PCR, Validation

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