

Testing for Mycoplasma species using Polymerase Chain Reaction Techniques

All regulatory guidelines specify that products manufactured using cells must be tested for the presence of Mycoplasma species. This includes all biologics and some vaccines. The testing is prescribed for master and working cell banks, virus seed lot and bulk harvests. Traditionally the tests for Mycoplasma have included cell culture or growth on media (broth and agar) however nucleic acid amplification techniques (NAT) may be used as an alternative to one or both of the other methods after suitable validation by certain guidelines. The reason for the acceptance of the NAT techniques by the European Pharmacopoeia (EP) is that they have recognised that some samples are difficult to test for Mycoplasma either due to cytotoxicity or due to the rapid turnaround of samples required for particular products.

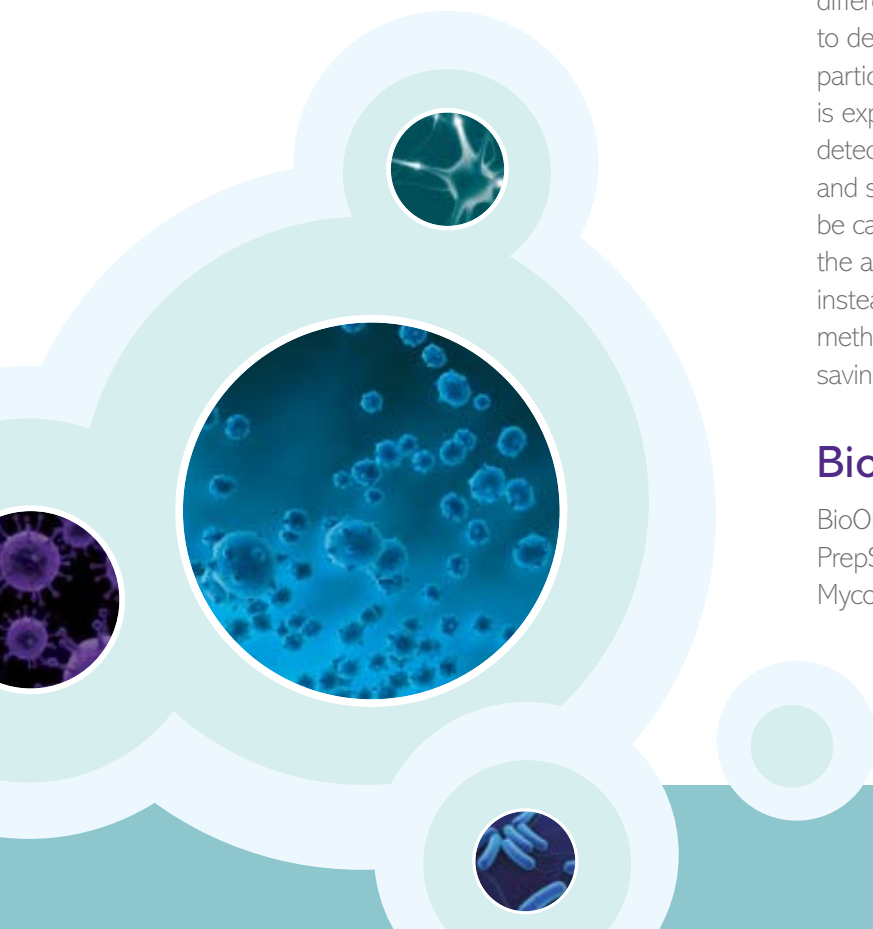
Polymerase Chain Reaction

SECTION 2.6.21 of the European Pharmacopoeia states that Nucleic acid amplification, such as PCR, techniques may be used for detection of mycoplasmas. NAT indicate the presence of a particular nucleic acid sequence and not necessarily the presence of viable mycoplasma. This presents some advantages and disadvantages; the

principal advantage is the sensitivity and specificity of these assays. The main disadvantage is the detection of non viable Mycoplasma sequences and these sequences may be present in many of the reagents and culture material used in the production process. This is because, whilst the manufacturing of the reagents will have eliminated the viable organisms, some DNA will remain due to it being very difficult to remove. A number of different techniques are available which can be applied to detect this presence and the EP does not specify a particular method but does outline the validation which is expected. The EP also indicates that commercial PCR detection kits will be suitable for use for release assays and specifies that certain elements of the validation may be carried out by the manufacturer to facilitate use of the assay commercially. The PCR technique may be used instead of the culture method and the indicator cell culture method after suitable validation as a release test, thus saving time and cost.

BioOutsource Service Offering

BioOutsource is pleased to offer the Applied Biosystems PrepSEQ™ *Mycoplasma* detection kit for use in detecting Mycoplasma to EP requirements. The PrepSEQ kit was



chosen as it has been shown to be compliant with the validation criteria as specified by the EP Guidelines. The technique starts with the extraction of DNA from the sample. This is a critical step and uses a lysis buffer as in standard DNA extraction techniques to release the DNA from the cells. This method can be applied to up to 10mL of sample, which is particularly useful for testing larger volume samples such as bulk harvests. Following release, the DNA is captured on magnetic beads which reduces the cross reactivity and inhibitors in the assay. The PCR technique is based on the TaqMan™ reaction conditions and also uses the SYBR Green which binds to double stranded DNA. The temperature annealing profile can be scrutinised should there be any positive reactions to verify if the reactive sample is truly a mycoplasma contamination.

In our hands this assay has been shown to be suitable to detect Mycoplasma species in cell free samples as well as cell harvest and cell bank preparation.

The speed at which the samples can be extracted and assayed is particularly useful. This can be accomplished within 48 hours of sample receipt. This allows BioOutsource to offer a rapid turnaround to result in a matter of days rather than weeks for the traditional assays.

Validation of ABI MicroSEQ Mycoplasma Detection Assay

The EP specifies a large number of Mycoplasma species which should have validation data acquired before use. The Presept kit has shown by the manufacturer to achieve a sensitivity (<10 CFU/mL) of the assay with *A. laidlawii*; *M. fermentans*; *M. hyorhinis*; *M. orale*; *M. pneumoniae*; *M. gallisepticum*; *M. synoviae*; *Mycoplasma arginini* and *Spiroplasma citri* to comply with the EP guidelines and in our hands the assay performs. The Mycoplasma species tested in the validation have been sourced from the EDQM where available. The assay has also been validated to demonstrate the specificity of the assay and has included include *Escherichia*, *Bacillus*, *Clostridium perfringens* and *sporogenes*, *Lactobacillus*, *Staphylococcus* and *Streptococcus*.

In our hands the assay has been qualified to achieve the same level of sensitivity as that specified by the

manufacturer and the specificity with contaminating DNA has also been established. Using numerous kits and samples, BioOutsource has also demonstrated the robustness of the assay.

Assay Controls Included in the Assay

The assay includes an inhibition control which verifies the absence of inhibition to the PCR reaction. This control is a DNA sequence which is unrelated to the Mycoplasma sequences detected and sample acceptance criteria specifies that this must be within an acceptable range for the sample result to be valid.

BioOutsource assays offer, to all of our clients, our specific Sample Extraction Control which is used to verify the extraction efficiency of every extraction. We would typically expect to achieve < 70% of DNA recovered from the extraction control.

External controls.

The positive control used in the assay is a specifically designed plasmid which is tested in reaction buffer and is diluted to contain a defined number of target-sequence copies of 10¹ to 10⁸. The negative control used is composed of buffer containing no Mycoplasma target sequences.

Replacing Existing Methods with PCR

To directly replace the traditional methods for Mycoplasma detection with PCR would require firstly a compatibility study and then a comparability study to be carried out. The EP suggests that this study should include mainly a comparison of the respective detection limits of the alternative method and traditional methods. The ABI Presept system has already been shown to be sensitive and specific to meet EP guidelines and therefore a relatively small well designed study would be required to satisfy the guidelines. This study will include the simultaneous testing of samples using both methods showing the detection limit of both methods using samples of calibrated strains (EDQM).