Validation of the MycoTOOL Mycoplasma Real-Time PCR Kit
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Abstract
Mycoplasma contamination of biopharmaceuticals – as a consequence of cell culture contamination in the manufacturing process – poses a potential health risk to patients. To minimize this risk, regulatory agencies require manufacturers of biopharmaceuticals to ensure the absence of mycoplasmas in the manufacturing process and end product. To test for mycoplasmas, key regulatory guidelines such as EP 6.7, USP<803>, and JP<1> have in the past recommended protocols that relied exclusively on the culture method or indicator cell culture method. These protocols suffer from several limitations, such as lengthy overall testing time (28 days) and difficult-to-cultivate or non-cultivable mycoplasma species. Hence, many regulatory agencies now additionally accept rapid nucleic acid amplification techniques (NAT) such as real-time quantitative polymerase chain reaction (qPCR) for mycoplasma testing. The MycoTOOL Mycoplasma Real-Time PCR Kit (MycoTOOL RT) is a commercially available qPCR assay developed in-house by Roche Pharma Biotech that has now been validated according to the EP 6.7 NAT validation guidelines. In the following, we present the MycoTOOL RT work flow and validation results. The full validation report is available upon request and under confidential disclosure agreement.

1 – MycoTOOL RT Kit Design

Work Flow

Control Concept
MycoTOOL RT uses the following fluorescent dyes to detect mycoplasma DNA and a recovery control plasmid, respectively, fluorescein amidite (FAM), and Yellow 565. All controls are included in the MycoTOOL RT Kit.
Each biological sample to be tested for mycoplasma contamination requires the following separate qPCR reactions on a 96-well PCR plate:
1. Cell sample spiked with recovery control plasmid prior to DNA preparation (4 technical replicates [no] per fluorescent dye)
2. Plasmin containing mycoplasma DNA (positive PCR control, 4 no)
3. Water (negative PCR control 2 no per fluorescent dye)
4. Mycoplasma-free cell sample spiked with recovery control plasmid prior to DNA preparation (negative process control, 4 no per fluorescent dye).

Validation Design
The validation of the MycoTOOL RT followed the criteria as mentioned in the EP 6.7 and ICH Q2(R1) guidelines:
1. Limit of Detection (LOD)
We spiked CHO cell culture samples with each mycoplasma reference strain shown in Table 2 in a dilution series ranging from 10 – 0.1 CFU/mL. The MycoTOOL RT assay was run in eightfold determination and repeated on three different days, yielding 24 results per dilution. The LOD was defined as the lowest CFU of that could be detected in 23 out of 24 samples. Acceptance criteria by EP 6.7 were met if:
   • the LOD was ≤ 0.1 CFU/mL.
2. Specificity
We spiked CHO cell culture samples with three gram-positive bacterial species (Streptococcus bovis, Lactobacillus acidophilus, Clostridium spongiosum) in a tenfold dilution series ranging from 10^6 – 10^0 CFU/mL. The MycoTOOL RT assay was run in eightfold determination and repeated on three different days, yielding 24 results per dilution. Acceptance criteria by EP 6.7 were met if:
   • the spiked samples showed a qPCR quantification cycle (Cq) value at or above the respective calculated LOD of Table 1.

2 – Materials and Methods

Materials – Mammalian and Mycoplasma Cells
For each sample, we used 5 x 10^6 cells/mL of a standard CHO cell culture that had been confirmed to be mycoplasma-free in a fourfold determination. We obtained stocks of each different mycoplasma reference strains from three different sources and determined their generic copy per colony forming units (CFU) ratio using an in-house method (Table 1). The mycoplasma strains chosen represent all strains required by the EP 6.7 and additionally include M. orale, M. salivarium and M. hominis.

3 – Results

Determination of GC/CFU Ratio
To ensure that the viability of the mycoplasma reference strains is sufficiently high, a GC/CFU ratio of ≤ 100 is recommended. All tested mycoplasma reference strains showed GC/CFU ratios ≤ 100 (Table 1).

4 – Summary and Discussion
This validation study demonstrates the performance of MycoTOOL RT with the EP 6.7 NAT validation guideline. The results demonstrate that MycoTOOL RT is sensitive, specific, robust, precise, and comparable to the conventional mycoplasma methods. Thus, it fulfills all requirements as given by EP 6.7 to detect mycoplasma contamination during CHO manufacturing process of biopharmaceuticals. Like Roche Pharma Biotech Penzberg in Germany many manufactures of biopharmaceuticals are moving rapid NAT methods and we believe that this trend will continue in the future.

5 – References