Microbial Testing: Five Myths Vs. Reality

American Pharmaceutical Review™

MYTH:

Regulatory agencies require a 28-day test for lot release

REALITY:

- In 2007, the European Pharmacopoeia accepted nucleic acid tests, such as real-time PCR, as an alternative method for traditional Mycoplasma detection after validation
- FDA states PCR-based assays may be used to detect mycoplasma, provided that such an assay can be shown to be comparable to the agar and broth media procedure and the indicator cell culture procedure

MYTH:

For validation, it is adequate to use a mycoplasma stock with a GC:CFU Ratio of more than 50

REALITY:

- The GC-to-CFU ratio depends on the growth phase.
- Only during the log phase of the growth curve are GC and CFU roughly the same (GC/CFU ~ 1)
- If measurement is taken in the log phase, 1GC=1CFU
- Regulators expect you to use stocks with a GC-to-CFU ratio close to one, which means this is dependent of the stage of growth phase[1]

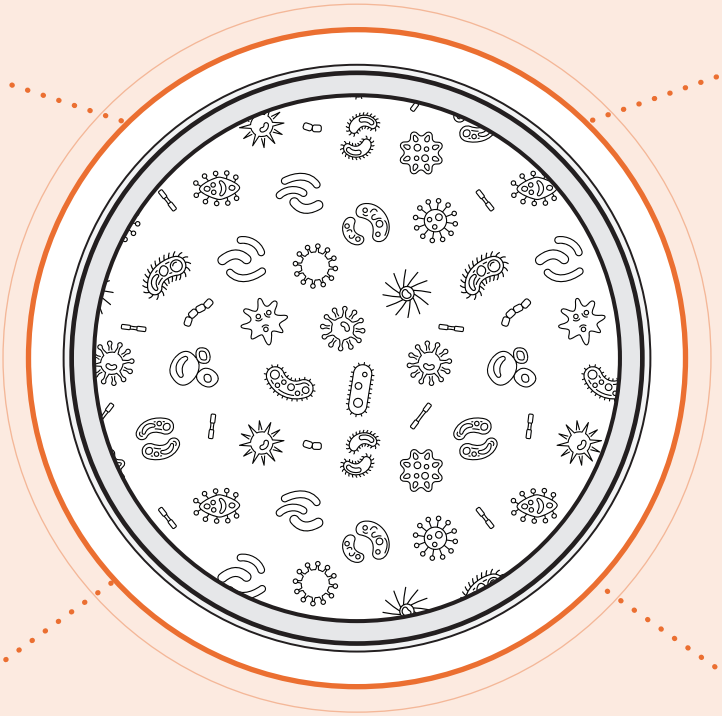
[1] *Journal of Applied Microbiology 111, 904–914.*

MYTH:

Starting input volume of 1 ml can be used to demonstrate a LOD of 10 CFU/ml

REALITY:

- Assay sensitivity is a function of the sample input volume
- To achieve a LOD of 10 CFU/ml, the input sample volume needs to be 10ml
- A starting volume of 1ml does not translate to a final LOD of 10 CFU/ml - it translates to 0.1 CFU/ul



MYTH:

There is no need for genomic equivalent measurement if you can demonstrate a level of detection (LOD) in CFU/ml

REALITY:

- The EP 2.6.7 states that LOD in genomic equivalent should be demonstrated
- During validation it is necessary to demonstrate equivalent sensitivity in CFU/ml and GC/ml

MYTH:

A Rapid Mycoplasma Assay used in early clinical phase (Phase 1/2) is considered a fully validated method

REALITY:

- For IND (Investigation New Drug) Submission, test method does not require extensive validation
- For Biological License Application (BLA)/New Drug Application (NDA) submission the test method is reviewed at highest level of detail/scrutiny
- Full validation design must meet regulatory expectations that were not required in early phase trials