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# Information on the new EP 2.6.7 (12.2) and updated Minerva Biolabs' product portfolio


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A revised version of the European Pharmacopoeia (EP) chapter 2.6.7 has been published in Issue 12.2 in October 2025 and will enter into force on April 01, 2026. This revision establishes, for the first time, a clear regulatory framework for NAT-based mycoplasma detection methods (e.g. PCR, qPCR, dPCR) as stand-alone assays.

Recognising the specific challenges of ATMPs – such as cell-containing matrices, complex manufacturing processes, limited sample volumes and high matrix variability – the revised chapter explicitly allows NAT methods as equivalent alternatives to culture and indicator cell methods, provided appropriate validation and product-specific suitability testing are performed.
The chapter also further specifies requirements for mycoplasma reference standards and now explicitly includes genome copy (GC)-based standards alongside traditional CFU-based standards. A key addition is the "test for inhibitory substances", ensuring that the product matrix does not interfere with the NAT assay and that the required sensitivity is achieved under real conditions.

To address these requirements, Minerva Biolabs developed a coordinated system comprising the **Venor® Mycoplasma PCR** assays, the **Venor® Mycoplasma Extraction** Kit, and the **100GC®**, **10CFU®** and **100CFU® Mycoplasma Standards**.

This document summarizes the key regulatory updates, outlines Minerva Biolabs' adapted product portfolio, and provides guidance on implementing and validating NAT-based mycoplasma testing in accordance with EP 2.6.7.

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## SECTION 1: REGULATORY FRAMEWORK – EP 2.6.7 (EDITION 12.2)

### 1.1 What has changed with the EP 2.6.7 (12.2)?

- The requirements for mycoplasma material used for validation studies has been specified, in particular:
- Colony forming units (CFU) must be determined at the time of harvest, without prior freezing of the culture.
- The ratio of genome copies to colony forming units (GC/CFU) must not exceed 10.
- Genomic copies are mentioned for the first time. A LOD of < 100 GC/ml has been added as acceptable sensitivity alongside the LOD of ≤10 CFU/ml. Therefore, reference standards may consist of native mycoplasmas (CFU) or nucleic acids (GC).
- Product- or matrix-specific suitability testing is clearly defined (referred to in the EP as the "test for inhibitory substances")
- Testing of both cells and supernatants is recommended whenever possible
- Clearly defined controls are required, including an external positive control

### 1.2 Can PCR replace the culture method?

Yes. NAT-based methods (e.g. PCR, qPCR, dPCR) can replace the culture method and/or the indicator cell culture method, provided they are validated in accordance with EP 2.6.7.

During the manufacturers validation of our mycoplasma PCR kits, viable mycoplasma spike material was used. The comparison with viable material has therefore already been established. The user only needs to perform a product-specific validation (see section 4 for further details).

### 1.3 What are the new LOD requirements?

The limit of detection (LOD) is clearly defined as ≤10 CFU/ml or <100 GC/ml.

### 1.4 What is the "test for inhibitory substances"?

The term "test for inhibitory substances" means the same as „matrix-specific validation", „product-specific validation" or „method suitability test".

All of these terms describe the same regulatory concept: demonstrating that the product matrix does not inhibit the NAT method and that the required sensitivity is achieved in the presence of the product.

### 1.5 What is the "external positive control "?

The external positive control (EPC) is an additional positive control used independently of the detection kit. According to EP 2.6.7 (12.2), it must contain a defined number of target-sequence copies or CFUs and is set close to the cut-off, to demonstrate that the validated sensitivity is achieved.

Minerva Biolabs' mycoplasma detection kits only contain an internal control and an internal positive control. The EPC is not included in our mycoplasma PCR detection kits. For EPC we recommend to use 100GC® or 100CFU® Mycoplasma Standards.

## SECTION 2: NEW PRODUCT PORTFOLIO

### 2.1 Overview of new EP-compliant portfolio

#### 2.1.1 Which components are required for EP 2.6.7 (12.2) compliant NAT testing?

For EP 2.6.7 compliant use of a NAT-based mycoplasma test, the following components are typically required:

1. A validated mycoplasma PCR assay, including an internal control and a positive control, e.g. Venor® Mycoplasma qPCR or Venor® Mycoplasma dPCR.
2. A mycoplasma extraction kit to isolate DNA and RNA for sample purification and reduction of potential PCR inhibitors, e.g. Venor® Mycoplasma Extraction kit
3. Mycoplasma standards for sensitivity determination and as external positive control, e.g. 100GC® Mycoplasma Standard, 10CFU® Mycoplasma Standard or 100CFU® Mycoplasma Standards

#### 2.1.2 Can the new Venor® Mycoplasma PCR assays be used for new validations according to EP 2.6.7 (12.2)?

Yes. Venor® Mycoplasma PCR assays can be used for new validations as well as re-validations according to EP 2.6.7.


### 2.2 Why were new mycoplasma standards and PCR kits introduced?

#### 2.2.1 The critical role of mycoplasma reference material

The performance evaluation of a NAT-based Mycoplasma assay according to EP 2.6.7 requires the use of well-characterized mycoplasma suspensions of defined concentration. Validation is performed by spiking the relevant sample matrices with known quantities of mycoplasma organisms.

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Consequently, the validity and interpretability of the entire assay validation are directly dependent on the quality and correct characterization of the spike material used. Any inaccuracy in the preparation or quantification of this material would directly impact the assessment of assay performance.

This is why the preparation and characterization of compliant mycoplasma material represents the most critical and technically demanding part of the validation processes.

### 2.2.2 Production and characterization of former 10CFU®/100CFU® Sensitivity Standards

Minerva Biolabs' 10CFU®/100CFU® Sensitivity Standards were developed in accordance with the best scientific knowledge available at the time, outlined in EP 2.6.7 (01/2008). These standards are fully compliant with the EP 2.6.7 (Editions 6.1-12.1).

Genome copy number (GC) is determined using a quantitative PCR (qPCR) assay validated against the culture method. The target Ct value used for quality control is derived from the average value observed during kit validation with viable mycoplasmas at a concentration of 10 CFU/ml. Only the Ct value is used as release criteria. The standards are manufactured and tested to achieve Ct values comparable to those obtained during initial validation with viable mycoplasmas.

At customer's requests, a GC/CFU ratio was determined using the state-of-the-art methodology available at the time, applying linear regression following photometric quantification of the calibrator in qPCR, although no regulatory requirement was in place.

The native material used to produce the former 10CFU®/100CFU® Sensitivity Standards was harvested in the exponential growth phase and subsequently diluted down to a target concentration of 10 CFU/ml. Although dilutions were performed assuming theoretical linearity, this linearity could never be verified in practice. Dilution errors – whether systematic or random – cannot be identified retrospectively based on plating results. In addition, mycoplasma species may exhibit aggregation or clustering behavior, further complicating homogeneous dilution.

### 2.2.3 Regulatory considerations

With the most recent revision of the EP 2.6.7 (12.2), the requirements for mycoplasma material used in validation studies have been specified, in particular:

- CFU must be determined at the time of harvest, without prior freezing of the culture.
- The ratio of GC/CFU must not exceed 10.
- LOD of <100 GC/ml has been added as acceptable sensitivity alongside the LOD of ≤10 CFU/ml.

These requirements are intended to ensure that the material used for validation appropriately reflects viable organisms and does not contain disproportionately high amounts of amplifiable nucleic acid that could potentially lead to an overestimation of assay sensitivity.

### 2.2.4 Use of emerging technologies

To establish compliance with the GC/CFU requirement, genome copy numbers must be determined. The EP does not prescribe a specific methodology for genome copy quantification. Various approaches are scientifically justifiable. For the validation of our kits, digital PCR (dPCR) was selected as the quantification method of choice. This decision reflects our interpretation of the regulatory intention to avoid the use of material containing excessive amounts of amplifiable nucleic acid relative to viable organisms.

Digital PCR, enabling absolute quantification, may yield GC/CFU ratios that differ from those obtained using traditional methods. These deviations are typically attributable to mycoplasma aggregates, which may not be thoroughly dispersed by sample processing techniques such as sonication or vortexing. In the culture method, a single agglomerate forms only one colony, but it contains an unpredictable number of mycoplasma cells, which break up during sample preparation in NAT assays, each generating one GC (Ackermann et al., 2019, Journal of Microbiological Methods, 165).

Absolute quantification by dPCR demonstrated that the assigned values of the former 10CFU®/100CFU® Sensitivity Standards were overestimated.

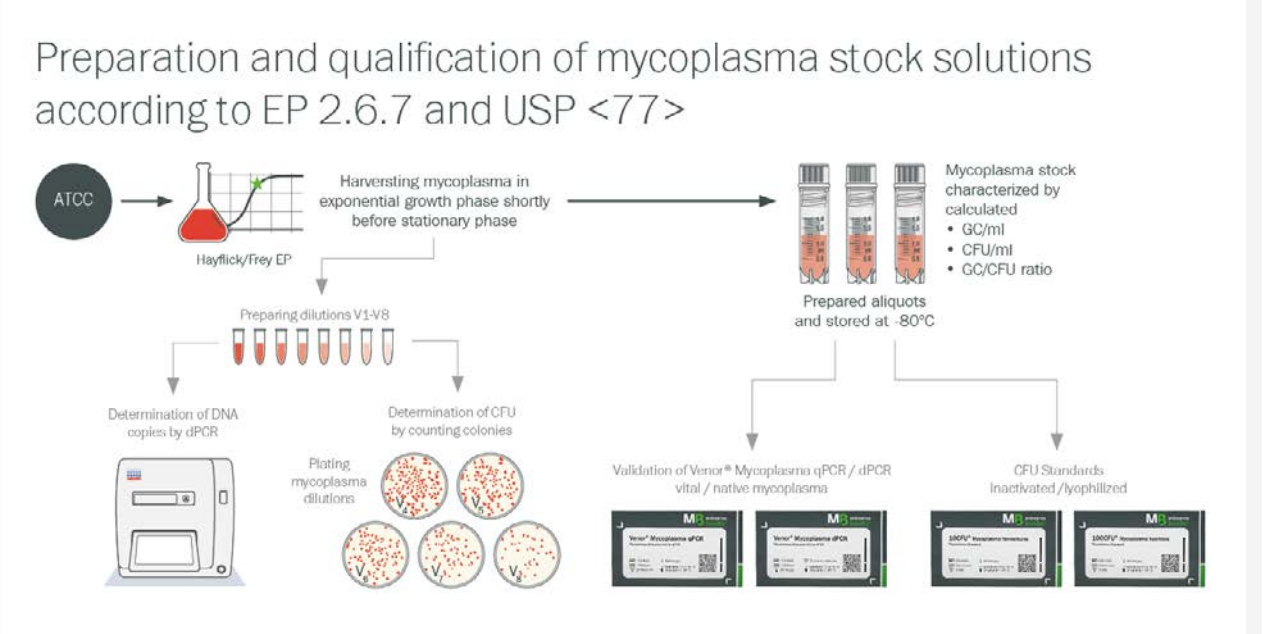
### 2.2.5 Updated mycoplasma standard portfolio

In response to the revised EP 2.6.7 and to maintain methodological state-of-the-art alignment, Minerva Biolabs developed new mycoplasma standards covering all relevant species in accordance with the updated requirements.

- 100GC® Mycoplasma Standard
- 10CFU® Mycoplasma Standard
- 100CFU® Mycoplasma Standard

For the production of the new standards, enhanced control measures were implemented. Amongst others, the mycoplasma material was absolutely quantified using dPCR. In addition, extracts of the dilutions used for CFU enumeration were analysed by qPCR. This analysis was not used for absolute quantification but served to evaluate dilution linearity and to detect potential systematic or random dilution errors.

With the implementation of dPCR technology, the new standards could be calibrated with significantly greater precision, resulting in improved alignment between culture-based and NAT-based results.




These standards are characterized as follows:

- Manufactured from native mycoplasma material
- Absolute quantification by validated dPCR (QC release criterion)
- Defined GC/CFU ratio < 10 in accordance with EP 2.6.7 (12.2), documented in the CoA
- Optimized cultivation and improved dispersion (reduced cluster formation)
- Characterization in both CFU and GC in line with the requirements of the revised EP
- Gentle, proprietary inactivation process (replacing former heat inactivation)
- Preservation of stable DNA/RNA-containing inactivated mycoplasma standards to support NAT and RT-NAT methods
- Manufacturing process based on precisely defined master batches in accordance with new regulatory expectations

The new standards are suitable for method validation, re-validation and future regulatory applications.

### 2.2.6 Updated mycoplasma PCR assay portfolio

Due to the updated requirements on the preparation of the new 10CFU®, 100CFU® und 100GC® Mycoplasma Standards, the sensitivity of DNA-based PCR assays (Venor®GeM Classic and Venor®GeM qEP) was no longer adequate to reliably detect 10 CFU/ml or 100 GC/ml. Consequently, new PCR kits featuring combined DNA and RNA detection through reverse transcriptase (RT) technology were developed:

- Venor® Mycoplasma qPCR
- Venor® Mycoplasma dPCR
- Venor® Mycoplasma gelPCR

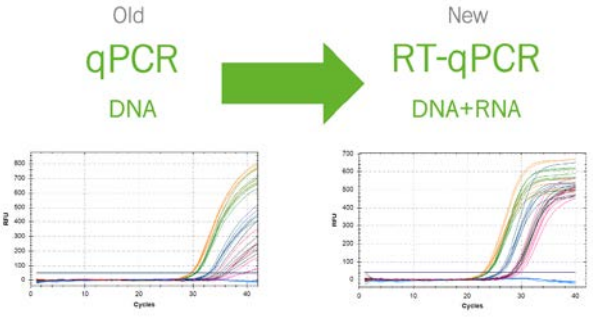
The new PCR kits were manufactured in accordance with EP 2.6.7 (12.2) and are aligned with RT-PCR technology, providing an improved sensitivity and robustness in the range of 10 CFU/ml and 100 GC/ml.

## SECTION 3: COMPARISON OLD VS. NEW PRODUCTS AND TRANSITION STRATEGY

### 3.1 PCR detection kits

**3.1.1 Why are the new Venor® Mycoplasma PCR kits reverse transcriptase assays (detection of RNA)? Are there any regulatory requirements?**

No, detection of RNA and DNA (reverse transcriptase PCR; RT-PCR) is not a regulatory requirement.
With the new Mycoplasma Standards, developed in accordance with the updated requirements for reference material preparation, the required sensitivity and robustness can only be achieved by detecting both DNA and RNA using RT-PCR.



### 3.1.2 Can existing authority-approved systems (Venor®GeM qEP / Venor®GeM Classic) continue to be used?

Venor®GeM qEP or Venor®GeM Classic remain available until April 2029 and may continue to be used for existing, authority-approved applications.

We currently have no generally applicable, officially binding statement that stipulates that existing validations must be revalidated after EP 2.6.7 (12.2) comes into force.

Our interpretation is as follows: Validations carried out under EP 2.6.7 (01/2008) remain valid within the scope of the regulatory requirements applicable at that time. Existing validations are not automatically invalidated retroactively. Whether revalidation or verification is required is a case-specific issue and depends on the authority, country, product, and risk assessment.
We recommend discussing this with your local authority and planning to switch to the new Venor® Mycoplasma PCR product line by the end of April 2029.

### 3.1.3 How long will the older kits Venor®GeM qEP / Venor®GeM Classic remain available?

Until end of April 2029.

### 3.1.4 Why is it recommended to switch to the new Venor® Mycoplasma PCR assays?

A transition to the new Venor® Mycoplasma PCR assays is particularly recommended when:

- New validations or re-validations according to EP 2.6.7 (12.2) are performed
- Significant changes to the method, product matrix or reference standards are planned
- Unstable or borderline results are observed with older PCR assays
- New EP-compliant Mycoplasma Standards are to be implemented


### 3.1.5 Do the new Venor® Mycoplasma PCR assays come with a manufacturers validation?

Yes. Venor® Mycoplasma PCR assay and Venor® Mycoplasma Extraction kit has extensively been validated together. Sensitivity, specificity and robustness are covered in the validation report, that is available on request. During the manufacturers validation, viable mycoplasma spike material was used. The comparison with viable material has therefore already been established. The user only needs to perform a product-specific validation (see section 4 for further details).

### 3.1.6 Can the older kits Venor®GeM qEP / Venor®GeM Classic be used for new validations in accordance with EP 2.6.7 (12.2)?

For new method validations or re-validations according to EP 2.6.7 (12.2), we recommend using the new Venor® Mycoplasma PCR assays. Venor®GeM qEP and Venor®GeM Classic are not intended for this purpose.


## 3.2 Mycoplasma standards (100GC®, 10CFU®, 100CFU®)

### 3.2.1 Are previous 10CFU®/100CFU® Sensitivity Standards still EP-compliant with Edition 12.2?

No. The previous 10CFU®/100CFU® Sensitivity Standards will no longer meet the revised regulatory requirements. The new EP (12.2) provides direct guidelines for the production of reference materials, forming the basis for the development of the new 10CFU®/100CFU® Mycoplasma standards. For details, please refer to point 2.2.
10CFU®/100CFU® Sensitivity Standards are only compliant with the EP 2.6.7 (Editions 6.1-12.1).

### 3.2.2 How long will the older 10CFU®/100CFU® Sensitivity Standards remain available?

The 10CFU®/100CFU® Sensitivity Standards remain available until the end of April 2029.

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### 3.2.3 We are concerned if RNA may be degraded under 2–8 °C. What is the shelf life of the new standards?

Shelf lives were assigned based on experience and risk assessment: 18 months for the 100GC® Mycoplasma Standards and 12 months for the 10CFU® and 100CFU® Mycoplasma Standards, when stored in lyophilized form at 2–8 °C. After rehydration, the standards must be used immediately. Stability studies are ongoing. Please subscribe to our Notification of Change to stay informed about any updates to the shelf life.

### 3.2.4 Can both 10CFU® Mycoplasma Standards and 100GC® Mycoplasma Standards be used for EP-compliant validation?

Yes. EP 2.6.7 (12.2) explicitly allows ≤10 CFU/ml or <100 GC/ml. Both units are regulatorily acceptable.

### 3.2.5 Why are 100GC® Mycoplasma Standards recommended for NAT-based methods?

10CFU® Standards are regulatorily acceptable under EP 2.6.7. However, 100GC® Standards are the more appropriate choice for NAT-based validation for the following reasons:

- NAT assays measure nucleic acids regardless of whether mycoplasmas are viable, making GC the more appropriate unit for molecular assays.
- CFU originates from culture-based methods and reflects colony-forming capability, whereas GC ensures clearer interpretation and comparability of PCR results.
- In inactivated materials, colony formation is no longer possible, so CFU values can only be assigned indirectly, while GC remains analytically valid and independent of culturability.

Furthermore, a level of 100 GC corresponds to the highest possible titer (assuming a maximum GC/CFU ratio of 10) and thus represents the most favorable scenario for validation. CFU-based materials, however, pose a greater challenge.

### 3.2.6 Can the previous 10CFU®/100CFU® Sensitivity Standards be used with the new Venor® Mycoplasma PCR assays?

In principle, yes. However, this is not advisable in light of the revised EP 2.6.7 (12.2).
The previous 10CFU®/100CFU® Sensitivity Standards were not manufactured in accordance with the revised EP. They are therefore not recommended for new method validations or re-validations under the updated guideline. They may still be used for existing applications, transition phases, or comparative studies.

For EP-compliant new validations, the use of 100GC®, 10CFU® or 100CFU® Mycoplasma Standards is mandatory, as these have been characterized in accordance with EP 2.6.7 (12.2) and are aligned with the new Venor® Mycoplasma PCR assays.


### 3.2.7 Can the new 10CFU®, 100CFU® or 100GC® Mycoplasma Standards be used with the older Venor®GeM qEP & Venor®GeM Classic?

In principle, yes. However, this is not advisable. The new 100GC® and 10CFU® Mycoplasma Standards were not validated together with Venor®GeM qEP and Venor®GeM Classic and cannot be robustly detected.

## 3.3 Nucleic acid extraction

### 3.3.1 What is the difference between the Venor® Mycoplasma Extraction kit and Venor®GeM Sample Preparation Kit?

Compared to the Venor®GeM Sample Preparation Kit (spin column based), the Venor® Mycoplasma Extraction Kit (magnetic bead based) offers the following advantages:

- Validated in combination with the new Venor® Mycoplasma PCR assays
- Extraction of both DNA and RNA
- Higher extraction efficiency
- Higher method robustness and reproducibility
- Improved compatibility with complex sample matrices
- Proteinase K included in the kit
- Manual or automated processing (e.g. using extraction instruments) possible

### 3.3.2 Must the Venor® Mycoplasma Extraction kit be used, or is Venor®GeM Sample Preparation Kit still permitted?

EP 2.6.7 does not require a specific extraction kit. For the use of Venor® Mycoplasma PCR assays, it is essential that DNA and RNA are reliably extracted, relevant inhibition is avoided, and the required sensitivity is achieved. The Venor® Mycoplasma Extraction kit is strongly recommended, as it was validated together with the Venor® Mycoplasma PCR assays and provides robust and reproducible performance. The Venor®GeM Sample Preparation Kit may in principle be used, but was not validated together with the Venor® Mycoplasma PCR assays and therefore requires full user-side verification.

### 3.3.3 Is an extraction step mandatory under EP 2.6.7?

EP 2.6.7 describes extraction as part of the analytical procedure, particularly for NAT-based methods.
In practice, when using NAT based detection methods, DNA and RNA extraction is strongly recommended and often necessary, because:

- Both cells and supernatants should be tested whenever possible
- RNases and DNases may be present in the product matrix
- PCR inhibition occurs more frequently without extraction
- The required sensitivity is often not reliably achieved without extraction.

### 3.3.4 Can the Venor®GeM Sample Preparation Kit in principle extract RNA?

In principle, Venor®GeM Sample Preparation Kit is also able to extract RNA. But there are no internally validated data confirming that the Venor®GeM Sample Preparation Kit provides RNA extraction of sufficient quality and quantity for use with the Venor® Mycoplasma PCR assays. For this reason, its use is not recommended.

Due to the advantages of the Venor® Mycoplasma Extraction kit (refer to point 3.3.1) and for a robust, validated workflow in regulated environments, we recommend using the Venor® Mycoplasma Extraction kit.

## SECTION 4: VALIDATION AND METHOD SUITABILITY

### 4.1 Which test do I need to perform as a user before I can proceed with routine testing?

You only have to perform product-specific suitability test (referred to in the EP as the “test for inhibitory substances”). For this purpose, we recommend our new mycoplasma standards.

### 4.2 What should the experimental setup look like for the test of inhibitory substances (product-specific validation)?

EP 2.6.7 describes, among others, the following acceptable approach:

- Spiking selected mycoplasma species at a defined level (e.g. <100 GC/ml or ≤10 CFU/ml)
- 5 replicates per batch
- 3 different batches
- All replicates must be positive

In the presence of the product, the cut-off must not exceed the required sensitivity. Alternative approaches are acceptable, provided the rationale and acceptance criteria are documented.

### 4.3 Which mycoplasma species should be selected for EP-2.6.7-compliant validation?

EP 2.6.7 (12.2) defines suitable mycoplasma species based on contamination frequency and phylogenetic relevance. Listed species include:

- *Acholeplasma laidlawii*
- *Mycoplasma fermentans*
- *Mycoplasma hyorhinis*
- *Mycoplasma orale*
- *Mycoplasma pneumoniae*
- *Mycoplasma arginini*
- *Mycoplasma salivarium*
- *Mycoplasma gallisepticum* (relevant for avian materials)
- *Mycoplasma synoviae* (relevant for avian materials)
- *Spiroplasma citri* or another Spiroplasma species (relevant for insect or plant materials)

Not all species must be tested. The chapter requires a risk-based selection, considering the manufacturing process (e.g. mammalian, avian or insect materials), the product category (e.g. biopharmaceuticals, ATMPs, vaccines) and known contamination risks (literature or process history).

In practice, 3–5 relevant species are typically selected to support EP-compliant validation or product-specific suitability testing.

## Support

For further information, technical assistance or to request product samples, please contact:
support@minerva-biolabs.com

## Related Products

| Product | Content | Cat.No. |
|---|---|---|
| **Venor® Mycoplasma Extraction** | 10 extractions<br>100 extractions | 56-3010<br>56-3100 |
| **Venor® Mycoplasma qPCR** | 25 reactions<br>100 reactions | 111-9025<br>111-9100 |
| **Venor® Mycoplasma dPCR** | 35 µl primer/probe mix<br>4 x 35 µl primer/probe mix | 111-0001<br>111-0002 |
| **100GC® Mycoplasma Standard** | 3 vials with 100 GC/vial | 104-XX01 |
| **10CFU® Mycoplasma Standard** | 3 vials with 10 CFU/vial | 105-XX01 |
| **100CFU® Mycoplasma Standard** | 3 vials with 100 CFU/vial | 106-XX01 |

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