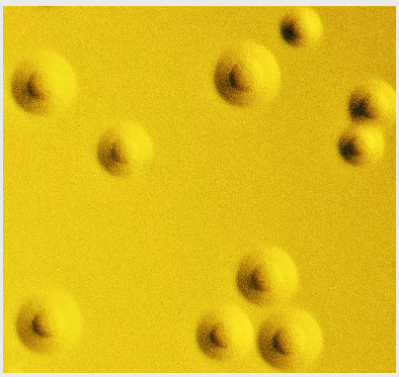
mycosafe®

Mycosafe® Viable Mycoplasma Culture Reference Standards

Validation Standards & Controls
for NAT- & Culture-based Mycoplasma
Detection Methods according to
EP 2.6.7., USP <63> & JP G3
for Application in Pharma QC, Cell & Gene
Therapies, and Regenerative Medicine

17 Pharmacopoeia Mycoplasma Reference Strains



Titer Formats
1000-100-10 CFU
per 100 µL

4 Product-Relevant Non-Pharmacopoeia Mycoplasma Reference Strains

User Guide

Low-GC/CFU
Live Mid-log Phase Cell Preparations of
Certified Mycoplasma Reference Strains

October 2021

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Cover Images
10 single mid-log phase mycoplasma cells corresponding to 10 genome copies (GC) and 10 colony forming units (CFU), as confirmed for low-GC/CFU Mycosafe® Mycoplasma Culture Reference Standard preparations by a GC/CFU ratio of 1.
Mycosafe® Viable Mycoplasma Culture Reference Standards are live mycoplasma cell preparations with a high ratio of viable/total mid-log phase mycoplasma cells after freezing/thawing that are suitable for a wide range of mycoplasma testing applications, including mycoplasma testing by both direct Real-Time PCR analysis and by media enrichment/Real-Time PCR hybrid assays, as well as by the compendial Culture and Indicator Cell Culture Methods.

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Validation Standards & Controls for NAT- & Culture-based Mycoplasma Detection Methods according to EP 2.6.7., USP <63> & JP G3

User Guide
Low-GC/CFU Live Mid-log Phase Cell Preparations of Certified Mycoplasma Reference Strains
Titer Formats 1000 - 100 - 10 CFU per 100 µL

October 2021

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1. Mycosafe® Viable Mycoplasma Culture Reference Standard Products

This User Guide refers to the products of low-GC/CFU live mycoplasma cell preparations listed below. These are flash-frozen suspensions of culture-grown mid-log phase cells of all pharmacopoeia and important product-relevant non-pharmacopoeia mycoplasma reference strains and are provided as Mycosafe® Viable Single-Strain Reference Standards and convenient Mycosafe® Viable Multi-Strain Reference Standard Validation & Control Sets in low- and high-titer product formats. They are available in product units of 250 µL in cryovials. All Mycosafe® Viable Mycoplasma Culture Reference Standard Products are traceable to authentic reference cultures of the National Collection of Type Cultures (NCTC), the American Type Culture Collection (ATCC), the European Directorate for the Quality of Medicines & HealthCare (EDQM), and the PHE Mycoplasma Reference Laboratory with Mycoplasma Experience Ltd as supply source, and are at low passage level that is ≤ 15 passages from the reference culture to comply with EP 2.6.7. (1), USP <63> (2) and JP G3 (3).

1.1 Mycosafe® Viable Single-Strain Culture Reference Standards

Mycosafe® Viable Single-Strain Culture Reference Standards
Product Formats

Product Format	1000	100	10
Calibrated Total Cell Titer	1000 CFU/100 µL	100 CFU/100 µL	10 CFU/100 µL
Product Unit	250 µL	250 µL	250 µL
Mid-log Phase Mycoplasma Cells	●	●	●
Specified Low GC/CFU Ratio	●	●	●

Product/Order Numbers

Mycoplasma Reference Strain	Product/Order No.
Pharmacopoeia Type Strains (T)	
Acholeplasma laidlawii **PG8^T** NCTC 10116 ATCC 23206	Al-T-1000
	Al-T-100
	Al-T-10
Mycoplasma arginini **G230^T** NCTC 10129 ATCC 23838	Ma-T-1000
	Ma-T-100
	Ma-T-10
Mycoplasma fermentans **PG18^T** NCTC 10117 ATCC 19989	Mf-T-1000
	Mf-T-100
	Mf-T-10
Mycoplasma gallisepticum **PG31^T** NCTC 10115 ATCC 19610	Mg-T-1000
	Mg-T-100
	Mg-T-10
Mycoplasma hyorhinis **BTS7^T** NCTC 10130 ATCC 17981	Mh-T-1000
	Mh-T-100
	Mh-T-10
Mycoplasma orale **CH19299^T** NCTC 10112 ATCC 23714	Mo-T-1000
	Mo-T-100
	Mo-T-10
Mycoplasma pneumoniae **FH^T** NCTC 10119 ATCC 15531	Mp-T-1000
	Mp-T-100
	Mp-T-10
Mycoplasma salivarium **PG20^T** NCTC 10113 ATCC 23064	Msa-T-1000
	Msa-T-100
	Msa-T-10
Mycoplasma synoviae **WVU 1853^T** NCTC 10124 ATCC 25204	Ms-T-1000
	Ms-T-100
	Ms-T-10
Spiroplasma citri **R8-A2^T** NCTC 10164 ATCC 27556	Sc-T-1000
	Sc-T-100
	Sc-T-10

Mycoplasma Reference Strain	Product/Order No.
Pharmacopoeia Reference Field Strains (RFS)	
Acholeplasma laidlawii **RFS** EDQM Y0000693	Al-RFS-1000
	Al-RFS-100
	Al-RFS-10
Mycoplasma fermentans **RFS** EDQM Y0000692	Mf-RFS-1000
	Mf-RFS-100
	Mf-RFS-10
Mycoplasma hyorhinis **RFS** EDQM Y0000690	Mh-RFS-1000
	Mh-RFS-100
	Mh-RFS-10
Mycoplasma orale **RFS** EDQM Y0000691	Mo-RFS-1000
	Mo-RFS-100
	Mo-RFS-10
Mycoplasma synoviae **RFS** EDQM Y0000689	Ms-RFS-1000
	Ms-RFS-100
	Ms-RFS-10
Mycoplasma pneumoniae **RFS 5167** Mycoplasma Experience	Mp-RFS-1000
	Mp-RFS-100
	Mp-RFS-10
Pharmacopoeia 'Non-Cultivable' Cultivar α Reference Strain (Alpha)	
Mycoplasma hyorhinis **DBS 1050** ATCC 29052	Mh-Alpha-1000
	Mh-Alpha-100
	Mh-Alpha-10

Mycoplasma Reference Strain	Product/Order No.
Product-Relevant Non-Pharmacopoeia Type Strains (T)	
Mycoplasma arthritidis **PG6^T** NCTC 10162 ATCC 19611	Mart-T-1000
	Mart-T-100
	Mart-T-10
Mycoplasma bovis **PG45^T** NCTC 10131 ATCC 25523	Mb-T-1000
	Mb-T-100
	Mb-T-10
Mycoplasma hominis **PG21^T** NCTC 10111 ATCC 23114	Mho-T-1000
	Mho-T-100
	Mho-T-10
Ureaplasma urealyticum **T960^T** NCTC 10177 ATCC 27618	Uu-T-1000
	Uu-T-100
	Uu-T-10

1.2 Mycosafe® Viable Multi-Strain Culture Reference Standard Validation & Control Sets

Categories of Mycosafe® Multi-Strain Reference Standard Validation & Control Sets	
EPUSP10	**All-in-One Validation Set according to EP 2.6.7. and USP <63>,** consisting of all 9 EP/USP mycoplasma type strains, including the type strains of *M. gallisepticum*, *M. synoviae* and *S. citri,* plus the 'non-cultivable' *M. hyorhinis* cultivar α reference strain
EPUSP7	**Complete Basic Validation Set according to EP 2.6.7. and USP <63>,** consisting of all 6 non-avian and non-plant EP/USP mycoplasma type strains, plus the 'non-cultivable' *M. hyorhinis* cultivar α reference strain
JP10	**All-in-One Validation Set according to JP G3**., consisting of all 9 JP mycoplasma type strains including the type strains of *M. synoviae* and *S. citri,* plus the 'non-cultivable' *M. hyorhinis* cultivar α reference strain
JP7	**Complete Basic Validation Set according to JP G3**, consisting of all 7 non-avian and non-plant JP mycoplasma type strains
EPUSPJP5	**Small Basic Validation Set according to EP 2.6.7., USP <63> and JP G3.,** consisting of the 5 EP/USP/JP mycoplasma type strains of *A. laidlawii, M. arginini, M. fermentans*, *M. hyorhinis* and *M. pneumoniae*
EPUSPJP3	**Control Set for Routine Mycoplasma Testing according to EP 2.6.7., USP <63> and JP G3,** consisting of the 3 EP/USP/JP mycoplasma type strains of *A. laidlawii, M. orale* and *M. pneumoniae*
EP-EDQM5	**Complete Validation Set** of **all 5 EP EDQM mycoplasma reference field strains according to EP 2.6.7.**
EPUSP-RFS5	**Basic Validation Set of 5 mycoplasma reference field strains according to EP 2.6.7. and USP <63>**, including the 4 non-avian EP EDQM reference field strains and the *M. pneumoniae* reference field strain 5167 originating from the PHE Mycoplasma Reference Laboratory
ATMP8	**Complete Basic Validation Set for Blood- and Placenta Cell-derived Advanced Therapy Medicinal Products (ATPMs),** consisting of (i) the 6 EP/USP/JP mycoplasma type strains of *A. laidlawii, M. arginini, M. fermentans, M. orale, M. pneumoniae* and *M. salivarium*, and (ii) the 2 product-relevant non-pharmacopoeia mycoplasma type strains of *M. hominis* and *U. urealyticum*
ATMP9	**Extended Validation Set for Blood- and Placenta Cell-derived ATMPs,** consisting of the same mycoplasma reference strain combination as comprising the ATMP Complete Basic Set, plus the non-pharmacopoeia type strain of *M. bovis* that is required if bovine serum is used during the manufacturing process

Mycosafe® Viable Multi-Strain Culture Reference Standard Validation & Control Sets
Product Formats

Product Format	1000	100	10
Calibrated Total Cell Titer	1000 CFU/100 µL	100 CFU/100 µL	10 CFU/100 µL
Product Unit	250 µL	250 µL	250 µL
Mid-log Phase Mycoplasma Cells	●	●	●
Specified Low GC/CFU Ratio	●	●	●

Product/Order Numbers

Mycoplasma Reference Standard Strain Set Category	Product/Order No.	Mycoplasma Reference Standard Strain Set Category	Product/Order No.	Mycoplasma Reference Standard Strain Set Category	Product/Order No.
EPUSP10	EPUSP10-1000	**EPUSPJP5**	EPUSPJP5-1000	**EP-EDQM5**	EP-EDQM5-1000
	EPUSP10-100		EPUSPJP5-100		EP-EDQM5-100
	EPUSP10-10		EPUSPJP5-10		EP-EDQM5-10
EPUSP7	EPUSP7-1000	**EPUSPJP3**	EPUSPJP3-1000	**EPUSP-RFS5**	EPUSP-RFS5-1000
	EPUSP7-100		EPUSPJP3-100		EPUSP-RFS5-100
	EPUSP7-10		EPUSPJP3-10		EP-USP-RFS5-10
JP10	JP10-1000			**ATMP8**	ATMP8-1000
	JP10-100				ATMP8-100
	JP10-10				ATMP8-10
JP7	JP7-1000			**ATMP9**	ATMP9-1000
	JP7-100				ATMP9-100
	JP7-10				ATMP9-10

2. Scope of Use

Mycosafe® Viable Mycoplasma Culture Reference Standards are flash-frozen low- and high-titer live mycoplasma cell preparations with low GC/CFU ratio

(i) for use in quality control of biopharmaceuticals and cell & gene therapy products as **Validation Standards** and as **External or Positive Controls** in NAT- and culture-based mycoplasma testing applications,
(ii) for use in growth promotion testing of culture media batches used in mycoplasma testing, and
(iii) for laboratory *in vitro* research purposes.

Mycosafe® Viable Mycoplasma Culture Reference Standards are not for any human or animal therapeutic or diagnostic use.

3. Intended Use

Mycosafe® Viable Mycoplasma Culture Reference Standards are flash-frozen low-GC/CFU live mycoplasma cell preparations at low and high titer that have been originally developed for use as control QC reference material for appropriate validation and routine application of rapid Real-Time PCR-based mycoplasma testing methods, as well as for the traditional culture-based methods, to ensure their regulatory compliance.

Mycosafe® Viable Mycoplasma Reference Standards are therefore particularly intended for use as reference material and controls in in-process and release mycoplasma testing by **NAT- and Culture-based mycoplasma detection methods** according to EP 2.6.7. (1), USP <63> (2) and JP G3 (3), including both direct Real-Time PCR analysis and media enrichment/Real-Time PCR hybrid assays according to the forthcoming revised Mycoplasma Testing Guidelines of EP 2.6.7. (4), as well as for the compendial Culture and Indicator Cell Culture Methods. They are also suitable for use in growth promotion testing of mycoplasma culture media batches used in mycoplasma testing and in other quantitative applications such as disinfectant challenge testing.

For the generic and product-specific validation of NAT-based mycoplasma detection methods, such as the currently commercially available Real-Time PCR mycoplasma detection systems, Mycosafe® Viable Mycoplasma Reference Standards are ideally suited for use as **Validation Standards**, as spiked test samples may simulate a 'real' mycoplasma contamination caused by viable mycoplasma cells**.** Likewise, they are ideally suited as **External Controls** during routine application of validated Real-Time PCR mycoplasma test methods.

Mycosafe® Viable Mycoplasma Reference Standards are also suitable for use as **Validation Standards** and **Positive Controls** in the culture-based mycoplasma testing methods, such as the Culture Method and Indicator Cell Culture Method according to EP 2.6.7., USP <63> and JP G3, and in the Real-Time PCR Hybrid Method that involves prior to Real-Time PCR analysis a short enrichment step in a suitable liquid medium, such as the Mycosafe® ABO™ Universal Mycoplasma Rapid Enrichment Medium or the Mycosafe® UREA™ Ureaplasma Rapid Enrichment Medium.

4. Composition & Specifications

Mycosafe® Viable Mycoplasma Reference Standards are comprised of cultured mid-log phase cells of certified mycoplasma reference strains suspended at low or high titer in FRIIS, FREY, Mycosafe® ABO™ or Mycosafe® UREA™ medium.

Mycosafe® Viable Mycoplasma Culture Reference Standard Products are supplied flash-frozen in product units of 250 µL contained in 1.5- or 2-mL plastic cryovials.

The traceability of each Mycosafe® Viable Mycoplasma Culture Reference Standard Product to an authentic reference culture of a specified mycoplasma reference strain from the NCTC, the ATCC, the EDQM or from the

PHE Mycoplasma Reference Laboratory with Mycoplasma Experience Ltd as supply source, the mycoplasma species identity, as well as the low passage level from the reference culture, the calibrated CFU titer of the total mycoplasma cells, the low GC/CFU ratio and the initial high post-freezing viability rate are stated and specified in a corresponding **lot-specific Certificate of Analysis (CoA)**.

5. Materials for Optional Use but not Provided

Diluent

Particularly for the high-titer product format (1000 CFU/100 µL), a diluent might be required to obtain the desired concentration of total mycoplasma cells that is suitable for the intended use.

For the preparation of diluted viable mycoplasma cell suspensions, the same mycoplasma culture medium that was originally used as growth medium (FRIIS, FREY, Mycosafe® ABO™ or Mycosafe® UREA™) is - if available - the optimal choice as diluent, or - if not available -, an equivalent alternative mycoplasma culture medium.

The choice of the medium is particularly important if the current concentration of viable mycoplasma cells after thawing, or the number of viable cells contained in the spike inoculum, is determined by plating and colony counting (post-freezing titer), since depending on the mycoplasma culture medium used, the values obtained may vary significantly. Particularly if the spike inoculation is like for the Real-Time PCR Hybrid Method based on the viable mycoplasma cells, it is recommended to first determine the mycoplasma growth efficiency (recovery rate) in the culture media used for enrichment and viable counting.

For application of the diluted viable reference standard preparations as Validation Standards or External Controls in direct Real-Time PCR testing, Phosphate-Buffered Saline (molecular biology grade PBS) can alternatively be used as diluent.

6. Instructions for Use

6.1 Preparation for Use

Rapid Thawing

(i) For thawing, the flash-frozen viable mycoplasma reference standard material is rapidly warmed at 36 ± 1°C (< 1 minute in a water bath or heating block) until there is just a small bit of ice left in the cryovial, and then kept on ice prior to use for direct spike inoculation without further dilution, or for preparation of an appropriate working dilution.

Optional Preparation of Working Dilutions

(ii) After rapid thawing of the flash-frozen viable mycoplasma reference standard material at 36 ± 1°C it may be - if required or desired - diluted with the corresponding or an alternative appropriate mycoplasma growth medium as diluent, or alternatively - if the intended use is for direct Real-Time PCR testing -, with molecular biology grade PBS, to obtain the desired number of total mycoplasma cells in an appropriate spike inoculation volume that is suitable for the intended use.

(iii) The final dilution and the required dilution steps must be based on the titer of the total mycoplasma cell number, which is specified in the corresponding CoA in CFU.

(iv) The dilution steps with the culture medium or PBS as diluent must be carried out on ice immediately after thawing of the flash-frozen viable mycoplasma reference standard material.

(v) The diluted viable mycoplasma reference standard material must be kept on ice if not immediately used.

Spike Inoculation

(vi) The product sample material to be spiked must be ready for spike inoculation as soon as the viable mycoplasma reference standard material is ready to be used in the desired total cell concentration – either undiluted or in the desired working dilution.

(vii) The ready-to-use undiluted or diluted viable mycoplasma reference standard material must be mixed prior to use by vortexing the vial for 10 seconds at full speed to obtain an optimal homogeneous suspension of the viable mycoplasma cells.

(viii) The spike inoculation with the ready-to-use undiluted or properly diluted suspension of mycoplasma cells should be completed **within 1-2 hours following the thawing step**.

6.2 Spike Inoculation Doses

Mycosafe® Viable Mycoplasma Culture Reference Standard Products are designed for single, few, several or multiple spike inoculations at the same time, depending on the titer format, the target sample spike level and the applied spiking procedure (direct spiking of test sample replicates, or spiking of a larger test sample volume prior to aliquoting into identical sample replicates), including the volume of the product sample material and the number of sample replicates to be spiked. Examples for different Spike Inoculation Dose size ranges per mL test sample replicate are given below.

Product Format		1000	100	10
Spike Inoculation Dose 100 CFU Total Cells per mL Test Sample				
Spike Inoculation Volume Size Range per Dose Undiluted Ready-to-Use*		10 µL	100 µL	
Number Doses per Product Unit* **	**Single (1-5)**	●	●	
	Few (6-25)	●		
	Several (26-100)			
	Multiple (> 100)			
Spike Inoculation Dose 10 CFU Total Cells per mL Test Sample				
Spike Inoculation Volume Size Range per Dose Undiluted Ready-to-Use*		1 µL	10 µL	100 µL
Number Doses per Product Unit* **	**Single (1-5)**	●	●	●
	Few (6-25)	●	●	
	Several (26-100)	●		
	Multiple (> 100)	●		
Spike Inoculation Dose 1 CFU Total Cells per mL Test Sample				
Spike Inoculation Volume Size Range per Dose Undiluted Ready-to-Use*			1 µL	10 µL
Appropriate Dilution Required for Spike Inoculation**		●		
Number Doses per Product Unit* **	**Single (1-5)**	●	●	●
	Few (6-25)	●	●	●
	Several (26-100)	●	●	
	Multiple (> 100)	●	●	

*The exact volume of spike inoculum needs to be individually calculated for each test or test series
**To be determined
***Undiluted or diluted

6.3 Repeated Use after Storage & Disposal

(i) In mycoplasma testing applications by **direct Real-Time PCR analysis**, the unused undiluted or diluted viable mycoplasma reference standard material may be **refrozen and stored at ≤ - 60°C for further single use** (see under Section 7 below), or disposed by autoclaving (see under Section 8 below). For long-term storage and multiple use in direct Real-Time PCR applications it is recommended to divide the undiluted or diluted live mycoplasma cell preparations into several aliquots, each for separate use as required.

(ii) Typically, the low-titer product formats (100 and 10) are used only once in direct Real-Time PCR testing. The remaining unused undiluted or diluted mycoplasma reference standard material is therefore usually disposed immediately.

(iii) For application in **Real-Time PCR hybrid assays** involving a short enrichment step and in **mycoplasma testing by the pure culture-based methods**, as well as in **growth promotion tests** of mycoplasma culture media batches, the undiluted or diluted viable mycoplasma reference standard preparation should be **used only once**, as repeated freezing and thawing will deplete the viability of the mycoplasma cells and must therefore strictly be avoided. The remaining unused mycoplasma reference standard material must be disposed by autoclaving.

6.4 Summary of Instructions for Use

(i) If necessary, determination of the working dilution required to obtain the desired spike concentration for the product sample material to be spiked, particularly when using the higher-titer product format 1000.
(ii) Removal of the cryovial containing the undiluted flash-frozen mycoplasma reference standard material from the ≤ -60°C freezer.
(iii) Rapid thawing of the flash-frozen material by warming at 36 ± 1°C (< 1 min) and keeping it on ice after thawing.
(iv) Conduction of the required dilution steps - if required - on ice, using the corresponding or an alternative appropriate mycoplasma growth medium as diluent, or alternatively - if the intended use is for direct Real-Time PCR testing -, molecular biology grade PBS.
(v) Keeping the ready-to-use undiluted or diluted viable mycoplasma reference standard material on ice if not immediately used.
(vi) Mixing the ready-to-use undiluted or diluted viable mycoplasma reference standard material prior to use by vortexing the vial for 10 seconds at full speed.
(vii) Keeping the product sample material to be spiked ready for spike inoculation with the ready-to-use undiluted or diluted viable mycoplasma reference standard material.
(viii) Termination of the spike inoculation with the undiluted or properly diluted suspension of viable mycoplasma cells within 1-2 hours following the thawing step.
(ix) Storage of the unused undiluted or diluted viable reference standard material at ≤ - 60°C for further single use in direct Real-Time PCR testing or disposing by autoclaving.

6.5 Additional Notes

(i) For successful use of Mycosafe® Viable Mycoplasma Culture Reference Standards, they should not be used beyond their shelf life indicated in the corresponding CoA.
(ii) Deviations from the described Instructions for Use may affect the results.

See also under Section 9 below.

7. Storage & Stability

The flash-frozen Mycosafe® Viable Mycoplasma Reference Standard Products have a high stability during long-term storage at ≤ -60°C.

Once received, they must be stored properly at ≤ - 60°C prior to thawing for use up to the expiration date specified in the corresponding lot-specific CoA. Long-term exposure to higher temperatures than ≤ - 60°C can adversely affect the stability of the live mycoplasma cell preparations.

After use for spike inoculation, the rest of the undiluted or diluted viable mycoplasma reference standard material may be stored at ≤ - 60°C for further single use in direct Real-Time PCR mycoplasma testing. For application in mycoplasma testing by Real-Time PCR hybrid assays involving a short enrichment step and by the pure culture-based methods, for instance in side-by-side comparability studies, the undiluted or diluted viable mycoplasma reference standard preparations are for immediate single use only and should therefore not be refrozen at ≤ - 60°C for further use, as this will ultimately lead to a reduction of the concentration of viable mycoplasma cells (see also above under Section 6.3).

If the viable mycoplasma reference standard material, particularly the higher-titer product format 1000, is diluted and not immediately used after dilution in direct Real-Time PCR testing, users should determine the shelf life of the diluted preparations according to their own assessment based on experience, method of preparation, storage conditions and use. When stored at ≤ - 60°C, a shelf life of 1 year for single use from the date of

preparation, i.e., the date of dilution, is recommended to be issued for the freshly prepared diluted viable mycoplasma reference standard material.

8. Precautions

Mycosafe® Viable Mycoplasma Culture Reference Standards consist of flash-frozen mid-log-phase mycoplasma cells of which a portion is viable after thawing. As this viable portion of mycoplasmas contained in these reference standard products that are derived from authentic reference cultures of all pharmacopoeia mycoplasma reference strains and from the most important product-relevant non-pharmacopoeia mycoplasma reference strains may cause human or animal disease, most of these reference standard preparations are considered as biohazard material with the Biosafety Level 2 (see below under 8.1). The frozen mycoplasma cell suspensions must therefore be processed in a laboratory environment which, as defined by national regulations or guidelines, is suitable for the handling of bacteria classified as group 2 biological agents, as appropriate.

Each laboratory that orders Mycosafe® Viable Mycoplasma Culture Reference Standards must therefore have the facilities and be equipped to receive, store, process and dispose bacteria classified as group 2 biological agents, and must be aware of and comply with the proper disposal of biohazard material according to the national and local regulations. Only trained laboratory personnel that has experience in the handling of infectious biological material should use these live mycoplasma cell preparations, and proper techniques in accordance with Biosafety Level 2 practices as described in corresponding guidelines must be employed.

There is no or only a minimal risk of infection if the required laboratory precautions are employed and Biosafety Level 2 practices are observed in the handling of these live mycoplasma cell preparations by (i) wearing proper personal protective equipment, such as an appropriate laboratory coat, protective eye wear and disposable gloves; (ii) using a microbiology safety cabinet conforming to EN12469:2000 Biotechnology - Performance Criteria for Microbiological Safety Cabinets; (iii) avoiding hand-to-mouth contact while working with this mycoplasma reference standard material; and (iv) applying normal handwashing and disinfection procedures relating to the handling of infectious biological material.

Users of Mycosafe® Viable Mycoplasma Reference Standards assume all risk and responsibility in connection with the receipt, handling, storage, disposal, transfer and use of Mycosafe® Viable Mycoplasma Reference Standards. Refer to the corresponding **Material Safety Data Sheet (MSDS)** for more detailed information.

Disposal Measures
Mycosafe® Viable Mycoplasma Culture Reference Standard Products are considered as biohazard material. The remaining unused Mycosafe® Viable Mycoplasma Culture Reference Standards Product material or the remaining unused material derived therefrom by dilution must be - unless not stored at ≤ -60°C for further single use in direct Real-Time PCR testing (see under Section 7 above) - disposed by using an autoclave
(i) in accordance with the implemented laboratory protocol for disposal of biohazard materials, and
(ii) in accordance with all national and local regulations.

8.1 Regulatory Information Concerning the Biosafety Level of Mycoplasma Reference Strains

Mycosafe® Viable Mycoplasma Culture Reference Standard Products are derived from 21 pharmacopoeia and product-relevant non-pharmacopoeia mycoplasma reference strains of the genus *Acholeplasma* (1 species, 2 reference strains), *Mycoplasma* (11 species, 17 reference strains), *Spiroplasma* (1 species, 1 reference strain) and *Ureaplasma* (1 species, 1 reference strain) as source organisms, of which all except two species are classified according to Annex III of Directive 2000/54/EC - amendment of 31.10.2019 (5) - and/or according to TRBA 466 'Classification of Prokaryotes (Bacteria and Archae) into Risk Groups' - amendment of 14.05.2020 (6) - as group 2 biological agents/risk group 2 bacteria. *Mycoplasma orale* (2 reference strains) and *Spiroplasma citri* (1

reference strain), which are not listed in Annex III of Directive 2000/54/EC, are defined according to TRBA 466 (6) as risk group 1 bacteria.

Group 2 Biological Agents/ Risk Group 2 Bacteria (5,6)	**18 Certified Reference Strains of the Genus *Acholeplasma* (*A.*), *Mycoplasma* (*M.*) and *Ureaplasma* (*U.*) as Source Organisms of Mycosafe® Viable Mycoplasma Culture Reference Standard Products**	
	***A. laidlawii* PG8^T** NCTC 10116 ATCC 23206	***M. salivarium* PG20^T** NCTC 10113 ATCC 23064
	***A. laidlawii* RFS** EDQM Y0000693	***M. pneumoniae* FH^T** NCTC 10119 ATCC 15531
	***M. arginini* G230^T** NCTC 10129 ATCC 23838	***M. pneumoniae* RFS** 5167 Mycoplasma Experience
	***M. fermentans* PG18^T** NCTC 10117 ATCC 19989	***M. synoviae* WVU 1853^T** NCTC 10124 ATCC 25204
	***M. fermentans* RFS** EDQM Y0000692	***M. synoviae* RFS** EDQM Y0000689
	***M. gallisepticum* PG31^T** NCTC 10115 ATCC 19610	***M. arthritidis* PG6^T** NCTC 10162 ATCC 19611
	***M. hyorhinis* BTS7^T** NCTC 10130 ATCC 17981	***M. bovis* PG45^T** NCTC 10131 ATCC 25523
	***M. hyorhinis* RFS** EDQM Y0000690	***M. hominis* PG21^T** NCTC 10111 ATCC 23114
	***M. hyorhinis* DBS 1050** ATCC 25902	***U. urealyticum* T960^T** NCTC 10177 ATCC 27618
Group 1 Biological Agents/ Risk Group 1 Bacteria (6)	**3 Certified Reference Strains of the Genus *Mycoplasma* (*M.*) and *Spiroplasma* (*S.*) as Source Organisms of Mycosafe® Viable Mycoplasma Culture Reference Standard Products**	
	***M. orale* CH19299^T** NCTC 10112 ATCC 23714	***S. citri* R8-A2^T** NCTC 10164 ATCC 27556
	***M. orale* RFS** EDQM Y0000691	

9. Warranty & Limitations

Mycosafe® Viable Mycoplasma Reference Standards are warranted to meet the specifications given in the CoAs and related Mycosafe® Product Information Material. The warranty, expressed or implied, is limited and the stated characteristics and specifications in the corresponding CoAs cannot be guaranteed when

(i) Mycosafe® Viable Mycoplasma Reference Standards are employed for applications other than the intended use described in this User Guide and cited in the Mycosafe® Mycoplasma Reference Standards Product Flyer,
(ii) the procedures employed are different to the directions and instructions given in this User Guide,
(iii) Mycosafe® Viable Mycoplasma Reference Standards are repeatedly thawed and refrozen,
(iv) Mycosafe® Viable Mycoplasma Reference Standards are stored improperly at higher temperatures than ≤ - 60°C, and
(v) Mycosafe® Viable Mycoplasma Reference Standards are used after the expiration date has passed.

Mycosafe® Viable Mycoplasma Reference Standard Products are supplied with the condition that users are responsible for their safe storage, handling, and use. Prof Rosengarten and Mycosafe® Consulting assume no liability for any direct, indirect, consequential, or incidental damages resulting from the use, the results of use, or the inability to use Mycosafe® Viable Mycoplasma Reference Standard Products.

10. Restrictions

Mycosafe® Viable Mycoplasma Culture Reference Standards contain trade secrets and intellectual property of Prof Renate Rosengarten and may not be modified, copied or replicated by users, or not be transferred by users to any third party in whole or in part.

The rights granted to the user by purchasing Mycosafe® Viable Mycoplasma Culture Reference Standard Products are personal to the user. The user may not resell or distribute Mycosafe® Viable Mycoplasma Culture Reference Standard Products or any part thereof in any way.

11. References

(1) European Pharmacopoeia, 10th Edition, Chapter 2.6.7., Mycoplasmas, 2020.
(2) United States Pharmacopoeia, 43rd Edition, Chapter 63, Mycoplasma Tests, 2020.
(3) Japanese Pharmacopoeia, 18th Edition, Mycoplasma Testing for Cell Substrates used for the Production of Bio-technological/Biological Products, in: Chapter G3 Biotechnological/Biological Products, 2021.
(4) Karo J.-O. Mycoplasma Testing in the 21st Century. Trends, Revisions & Expectations. 2nd International Mycoplasma qPCR Testing User Day, PharmaLab 2019 Pre-Conference, November 11, 2019, Neuss-Düsseldorf, Germany.
(5) Annex III of Directive 2000/54/EC of the European Parliament and Council of 18.09.2000 on the Protection of Workers from Risks Related to Exposure to Biological Agents at Work. Official Journal of the European Communities No. L 262/21 of 17.10.2000, Amendment of 24.10.2019, published in OJ 279/54 of 31.10.2019.
(6) Technical Rules for Biological Agents (TRBA) 466, Classification of Prokaryotes (Bacteria and Archaea) into Risk Groups, Edition 2015, GMBl. No. 46-50 of 25.08.2015, 6th Amendment of 14.05.2020, GMBl. Nr. 18.

12. User Guide at a Glance

Mycosafe® Viable Single-Strain Culture Reference Standards	**21 Mycoplasma Reference Strains** • 10 pharmacopoeia type strains (EP 2.6.7., USP <63>, JP G3) • 6 pharmacopoeia reference field strains (EP 2.6.7., USP <63>) • 1 pharmacopoeia 'non-cultivable' cultivar α reference strain (EP 2.6.7., USP <63>, JP G3) • 4 product-relevant non-pharmacopoeia type strains • Traceability to authentic reference cultures of the NCTC, the ATCC, the EDQM, and the PHE Mycoplasma Reference Laboratory • Strain Passage Level: P2 to ≤ P15 • Confirmed low GC/CFU ratio: usually 1-5, can be slightly higher for some Mycosafe® Viable Culture Reference Standard lots of the pharmacopoeia type strains of *Mycoplasma fermentans, Mycoplasma pneumoniae*, *Mycoplasma salivarium, Mycoplasma synoviae* and *Spiroplasma citri,* and the reference field strain 5167 of *Mycoplasma pneumoniae* • For detailed information, see the Mycosafe® Reference Standards Product Flyer
Mycosafe® Viable Multi-Strain Culture Reference Standard Validation & Control Sets	**10 Strain Set Categories** • EPUSP10, EPUSP7, EP-EDQM5, EPUSP-RFS5 • JP10, JP7 • EPUSPJP5, EPUSPJP3 • ATMP8, ATMP9 For detailed information, see the Mycosafe® Reference Standards Product Flyer
Product/Titer Formats & Product Unit Size	**Product Formats** / **Calibrated Total Cells/100 µL** / **Product Unit Size** • 1000 / • 1000 / • 250 µL • 100 / • 100 • 10 / • 10 For detailed information, see the Mycosafe® Reference Standards Product Flyer
Scope of Use	• Validation Standards, and External/Positive Controls in NAT- and culture-based mycoplasma testing methods for quality control of biopharmaceuticals and cell & gene therapy products • Growth promotion testing of mycoplasma culture media batches used in mycoplasma testing • Laboratory *in vitro* research purposes
Intended Use	• Primarily intended for use as Validation Standards and External or Positive Controls for in-process and release mycoplasma testing by NAT- and culture-based mycoplasma detection methods according to EP 2.6.7., USP <63> and JP G3, including direct Real-Time PCR assays and media enrichment/Real-Time PCR hybrid assays according to the forthcoming revised Mycoplasma Testing Guidelines of EP 2.6.7.
Compositon & Specifications	• Cultured mid-log phase cells of certified mycoplasma reference strains suspended at low or high CFU titer in FRIIS, FREY, Mycosafe® ABO™ or Mycosafe® UREA™ medium • Supplied flash-frozen in product units of 250 µL contained in 1.5- or 2-mL plastic cryovials • Specifications stated in lot-specific Certificates of Analysis (CoAs): (i) the mycoplasma species identity; (ii) the traceability to an authentic reference culture of a certified mycoplasma reference strain from NCTC, ATCC, EDQM, or from Mycoplasma Experience Ltd; (iii) the passage level from the original reference culture; (iv) the calibrated CFU titer of the total mycoplasma cells; (v) the GC/CFU ratio; (vi) the initial post-freezing viability rate
Materials for Optional Use but not Provided	• A diluent particularly for the high-titer product format (1000) to obtain the desired concentration of mycoplasma cell suspension that is suitable for the intended use • Use of the corresponding growth medium (FRIIS, FREY, Mycosafe® ABO™, Mycosafe® UREA™) as diluent, or if not available, an equivalent alternative mycoplasma culture medium • For application in direct Real-Time PCR testing, Phosphate-Buffered Saline (molecular biology grade PBS) can alternatively be used as diluent

Instructions for Use	• Rapid thawing of the flash-frozen viable mycoplasma reference standard material at 36 ± 1°C (< 1 min) • If required, conduction of suitable dilution steps on ice using as diluent the corresponding growth medium, or an appropriate alternative mycoplasma culture medium, or alternatively - if applied in direct Real-Time PCR testing -, molecular biology grade PBS, to obtain the desired working dilution • Keeping the ready-to-use undiluted or diluted viable mycoplasma reference standard preparation on ice if not immediately used • Mixing the ready-to-use undiluted or diluted viable mycoplasma reference standard preparation prior to use by vortexing the vial for 10 seconds at full speed • Termination of the spike inoculation with the undiluted or properly diluted suspension of viable mycoplasma cells within 1-2 hours following the thawing step • If applied for direct Real-Time PCR testing, the unused undiluted or diluted viable mycoplasma reference standard material may be stored at ≤ - 60°C for further single use, or disposed by autoclaving • For application in mycoplasma testing by Real-Time PCR hybrid assays involving a short enrichment step, and by the pure culture-based methods, the unused undiluted or diluted viable mycoplasma reference standard preparation should be used only once, and the remaining material must be disposed by autoclaving
Storage & Stability	• High stability during long-term storage at ≤ -60°C • After application, the remaining undiluted or diluted viable mycoplasma reference standard material may be stored at ≤ - 60°C for further single use in direct Real-Time PCR mycoplasma testing • If the viable mycoplasma reference standard material is diluted and after dilution not immediately used in direct Real-Time PCR testing, users should determine the shelf life of the diluted preparation according to their own assessment based on experience, method of preparation, storage conditions and use. When stored at ≤ - 60°C, a shelf life of 1 year for single use from the date of preparation, i.e., the date of dilution, is recommended to be issued for the freshly prepared diluted viable mycoplasma reference standard material • For application in mycoplasma testing by Real-Time PCR hybrid assays involving a short enrichment step and by the pure culture-based methods, the undiluted or diluted live mycoplasma cell preparation is for immediate single use only
Precautions	• Mycosafe® Viable Mycoplasma Culture Reference Standards have the Biosafety Level 2 or 1 • Laboratory precautions and Biosafety Level 2 practices must therefore be employed, as described in corresponding guidelines: (i) Handling by trained laboratory personnel only, that has experience in the handling of infectious biological material and wears proper personal protective equipment; (ii) handling of all application procedures in a microbiology safety cabinet conforming to EN12469:2000 Biotechnology - Performance Criteria for Microbiological Safety Cabinets; (iii) avoiding hand-to-mouth contact while working with the mycoplasma reference standard material; (iv) normal handwashing and disinfection procedures relating to the handling of infectious biological material • The remaining unused viable mycoplasma reference standard material or the remaining used or unused material derived therefrom by dilution must be - unless not stored at ≤ -60°C for further single use in direct Real-Time PCR testing - disposed by using an autoclave in accordance with all national and local regulations • For detailed information, see the Mycosafe® Mycoplasma Reference Standards Material Safety Data Sheet (MSDS) for live mycoplasma cell preparations
Warranty & Limitations	• Product warranty is limited to the stated characteristics and specifications in the corresponding CoA • The stated characteristics and specifications cannot be guaranteed (i) for applications other than the intended use, (ii) when the procedures employed are different to the given directions and instructions, (iii) when repeated thawing/freezing cycles are applied, (iv) when higher temperatures than ≤ - 60°C are applied for long-term storage, (v) when the expiration date has passed
Restrictions	• Product changes, copies and replications as well as the transfer, resale and distribution in whole or in part by users to third parties are prohibited

Related Mycosafe® Products, Consultancy & Licensing Options

1. Alternative Mycosafe® Reference Standard Product Formats

Mycosafe® Inactivated Mycoplasma Culture Reference Standards
Total Cell Titer: 1000, 100, 10 CFU/100 µL
Product Unit: 250 µL

Mycosafe® Mycoplasma DNA Reference Standards
Titer: 100 GC/µL
Product Unit: 100 µL

For detailed information, refer to the Mycosafe® Reference Standards Product Flyer

2. Mycosafe® Mycoplasma Expert Consultancy

Scientific, Regulatory- & Business-Related Mycoplasma Expert Advisory Support Options
Risk Assessments & Mycoplasma Safety Concepts: Biopharmaceuticals · ATMPs · Raw Materials

For detailed information, please contact Prof Rosengarten at renate.rosengarten@mycosafe-consulting.com

3. Mycosafe® Licensing Options

Mycosafe® ABO™ Universal Mycoplasma Rapid Enrichment Medium
for Real-Time PCR Hybrid Assays according to the forthcoming revised Mycoplasma Testing Guidelines of EP 2.6.7.
Non-commercial product licensing options

Mycosafe® UREA™ Ureaplasma Rapid Enrichment Medium
for Real-Time PCR Hybrid Assays according to the forthcoming revised Mycoplasma Testing Guidelines of EP 2.6.7.
Commercial & non-commercial product licensing options

Mycosafe® ALPHA™ Selective Medium
for 'non-cultivable' cultivar α strains according to the forthcoming revised Mycoplasma Testing Guidelines of EP 2.6.7.
Commercial & non-commercial product licensing options

Mycosafe® SF™ Serum-Free Mycoplasma Culture Media
for preparation of mycoplasma antigens
Commercial & non-commercial product licensing options

Mycosafe® Mycoplasma Images
Selection of high-quality authentic mycoplasma images for any marketing project related to products for mycoplasma detection and culture- and NAT-based mycoplasma testing solutions
Exclusive, semi-exclusive & non-exclusive image licensing options

Mycosafe® Know-How
End-to-End Rapid Mycoplasma Testing Solutions
Commercial & non-commercial know-how licensing options

For detailed information, please contact Prof Rosengarten at renate.rosengarten@mycosafe-consulting.com

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