CELL & GENE THERAPY INSIGHTS

CELL THERAPY BIOPROCESSING & ANALYTICS: TODAY'S KEY TOOLS & INNOVATION REQUIREMENTS TO MEET FUTURE DEMAND

SPOTLIGHT

WEBINARS ON DEMAND

EXPERT COMMENTARY

Celyad Oncology's case study: validation approach for accelerated mycoplasma testing

Sarah Snykers

Underneath the development and launch of cell therapy products lies a highly complex supply chain, and clinical manufacturers of cell therapies must ensure quality control from the starting material through to product delivery to the patient. Before a product can make it to the clinic, it must meet predefined criteria that confirm safety, quality and efficacy. Quality management and release criteria, including microbiological safety, form a crucial part of this process. Within this, mycoplasma testing can pose a challenge, as the classical analytical methods documented within the US and European guidances involve challenging manual techniques with significant turnaround times. Alternative testing approaches are allowed, but they must be validated and studied in comparison to established mycoplasma testing methods. In this case study, the benefits and performance of the Roche MycoTOOL qPCR test are discussed.

Cell & Gene Therapy Insights 2021; 6(10), 1755–1764

DOI: 10.18609/cgti.2020.193

ACCELERATED MYCOPLASMA TESTING: THE NEED

As a company, Celyad Oncology is working to discover and develop innovative allogeneic and autologous CAR T products to fight cancer, with a number of pioneering CAR T approaches under development. Of course, before a cell therapy can be brought into the clinic it must meet predefined acceptance criteria that confirm safety, quality, and efficacy. Microbiological testing, and more specifically mycoplasma testing, is a crucial part of the characterization and release process.

Both the European Pharmacopeia 2.6.7 and the US Pharmacopeia 63 recommend two different methods; the culture method, and the indicator cell culture method.

The culture method is based on an extension in broth culture, and a detection in agar. While the limit of detection (LOD) is sensitive (0.1 CFU/mL), the turnaround time for this test is about 28 days. Only culturable mycoplasmas can be detected, and often a very large volume is required. The test is manual and not automated.

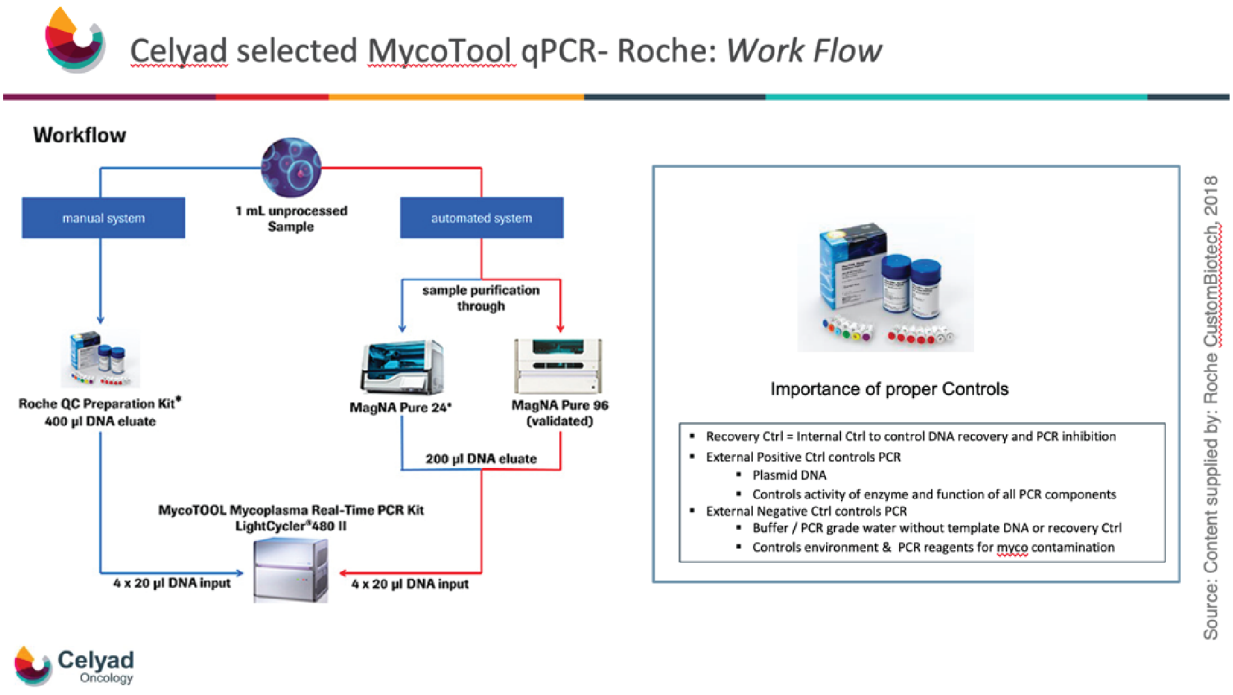
The other option is the indicator cell culture method, which involves an extension in cell culture, and afterwards characteristic fluorescent staining of the DNA. Using this method mycoplasma can be detected by its characteristic pattern, and non-cultivable mycoplasma can also be detected. The limitation is again a long turnaround time, in this case of about 10 days. The LOD is only 100 CFU/mL, and this approach poses a challenging manual method.

However, both the Pharmacopeia and USP leave the door open for alternative testing. The European Pharmacopeia is very precise – a validated nucleic acid amplification technique (NAT) test is recommended. It must be validated, and a comparability study against the classical methods must be done.

The benefit of a NAT over classical approaches is that it is significantly faster, no cultivation is needed, and the LOD of the technique is at least 10 CFU/mL. There are currently several commercially available alternative approaches for mycoplasma testing available.

FIGURE 1

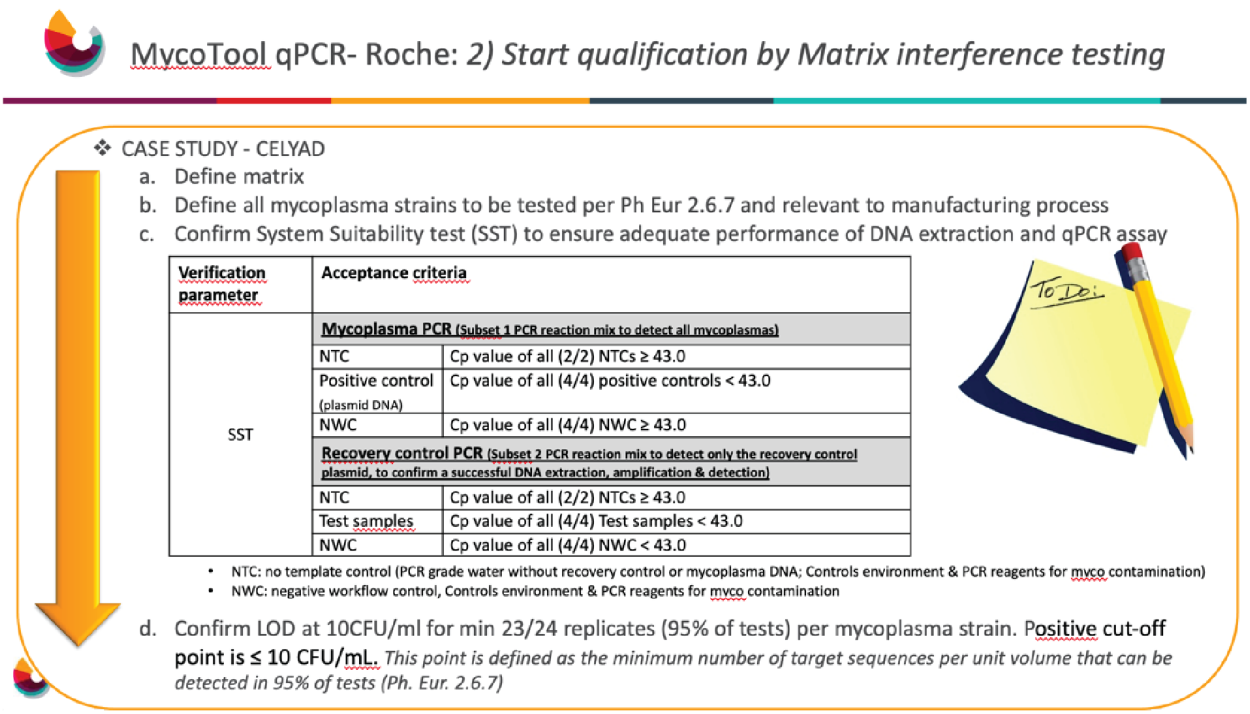
MycoTOOL qPCR workflow.



DOI: 10.18609/cgti.2020.193

FIGURE 2

Matrix interference testing approach.



CHOOSING AN ALTERNATIVE MYCOPLASMA TEST: THE MYCOTOOL KIT

Celyad Oncology made the decision to move forward with the MycoTOOL qPCR test from Roche, due to several advantages it offered. The method is fully validated by Roche, and validation reports can be accessed under an NDA. The set-up and validation is performed on a LightCycler® system that is already in use at Celyad Oncology. The method is fit for automation from the DNA extraction to the PCR steps, making it sustainable in the long term. Finally, it is applicable for both cellular and non-cellular matrices, so can also be used for raw material release.

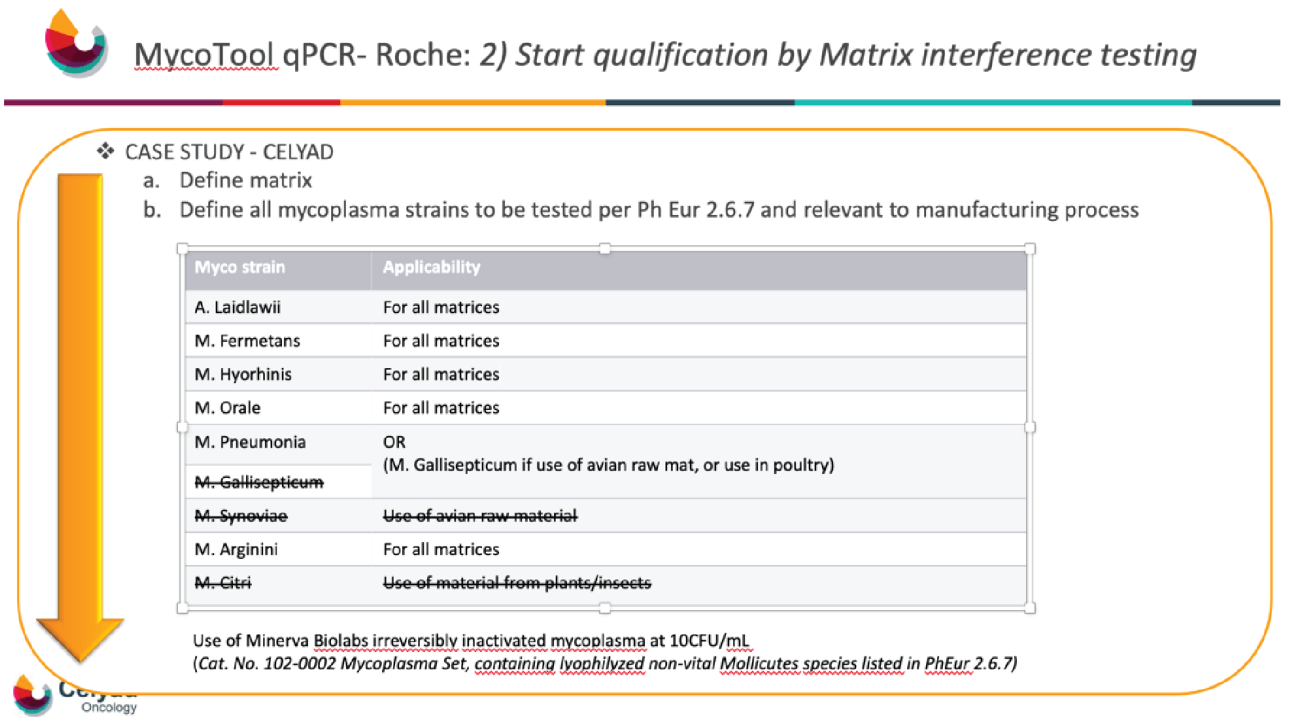
However, these advantages are contrasted against some limitations. Currently the test is only validated for up to 5 million cells per mL, which means an alternative sampling strategy may be required depending on product volume and concentration. The automated extraction method is validated on the Roche MagNA Pure 96 system. Both the MagNA Pure 24 system and the QC Preparation Kit are functionally tested, but not validated. If another system is used, it would first need to be validated.

The workflow of the MycoTOOL is shown in Figure 1. Considering the specific needs of Celyad Oncology, currently the manual system is used. The workflow is straightforward, and the automated system can then be used at times when a large number of samples need to be tested.

The use of proper controls are critical to the workflow, and all required controls are provided within the kit. The recovery control is used to control DNA extraction, recovery, and PCR formation. The external positive control again controls PCR, allowing control of the activity of the enzyme and also the function of all the PCR components. The external negative control is a buffer or PCR grade water without any templates, to control for the environment and equipment, and also for PCR reagents, to ensure that all components are mycoplasma free.

FIGURE 3

Relevant mycoplasma strains to be tested as per Ph Eur 2.6.7.



MYCOTOOL VALIDATION

When choosing an alternative method for mycoplasma testing, the first step is validation. The MycoTOOL from Roche, as discussed above, is fully validated according to the requirements of pharmacopoeia 2.6.7. and also ICH Q2 R1, in terms of robustness, precision, specificity, sensitivity, cross-contamination, and comparability versus classical methods.

Additionally, parts of the validation from the manufacturer can replace the validation by the user, as per Ph Eur 2.6.7:

"When the analytical procedure is fully or partly implemented using commercial kits, aspects of validation already supported by the manufacturer, supporting documentation being available, can be omitted by the user. Nevertheless, this one must demonstrate the performances of the kit related to the intended use."

From the guidance, it is clear that the user must demonstrate the performance of the kit related to the intended use. This involves assessing the matrix inhibitory or interference properties at the length of detection, i.e., 10 CFU/mL.

Figure 2 lists a test flow approach developed by Celyad Oncology in accordance with the recommendations provided by Roche and the Pharmacopeia 2.6.7. Firstly, the user needs to define the matrix. Once this is done, mycoplasma strains to be tested must be defined as per Pharmacopeia 2.6.7, with the caveat that only those that are relevant to the manufacturing process being used need to be selected.

A system suitability test must be performed to ensure adequate performance of the DNA extraction and qPCR asset. With MycoTOOL there are two different subsets – one focuses on mycoplasma, and the other focuses on recovery control. The recovery control is added to all samples except for the non-template control and positive control. This provides assurance of a successful DNA extraction and mycoplasma detection, so that in case of a negative result the user can be confident this is not a false negative.

The positive control, a plasmid DNA, is to ensure any mycoplasma present is being

DOI: 10.18609/cgti.2020.193

detected. There is also the non-template control, which as discussed above, is needed to ensure that the environment, equipment and PCR reagents are indeed free from mycoplasma.

Once testing is completed, the next step is to confirm a LOD of 10 CFU/mL for a minimum of 23 out of 24 replicates, i.e., a detection capability of 95% per mycoplasma strain. In Celyad Oncology's case, a target of 24 out of 24 was chosen in order to achieve 100% detection capability.

Celyad Oncology Oncology produces some cryopreserved products at concentrations higher than 5 million cells per ml. In order to increase sensitivity, a fresh sample was used that consisted of cells and cell culture supernatants at harvest. Cells were included in the testing to increase sensitivity, as mycoplasma may also be present within them.

Next, the mycoplasma strains that are recommended per the pharmacopoeia, but were also relevant to the manufacturing process, had to be defined. As Celyad Oncology does not use avian raw material or material from a plant or insect source, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and *Mycoplasma citri* were excluded, and the other six strains listed in the Ph Eur 2.6.7 were tested (Figure 3).

In order to be able to do the whole qualification at Celyad Oncology in the GMP QC laboratory of Celyad Oncology, and not contaminate the laboratory with mycoplasma, inactivated mycoplasma reconstituted at 10 CFU/mL was chosen.

With the mycoplasma and matrix selected and the system suitability test completed, the next significant task was the LOD. The 6 spiked mycoplasmas were detected at 10CFU/mL, and detection was achieved for 4 out of 4 replicates, resulting in a pass (Figure 4). Next, verification was performed on two selected mycoplasmas with a higher Cp value (so most difficult to detect), and again, for all the replicates detection was seen.

FIGURE 4

Results of qualification by Matrix interference testing.

MycoTool qPCR- Roche: *3) Results of qualification by Matrix interference testing*

a. System Suitability Test: PASS
b. LOD: PASS

Verification parameter	Sample description	Acceptance criteria	Results	Acceptance
LOD	All 6 spiked mycoplasmas should be detected at 10 CFU/mL (= LOD)	Target LOD: ≤ 10 CFU/mL: Cp value of minimum 4/4 replicates < 43.0	Cp value of all mycoplasma replicates (4/4) < 43.0 for all 6 mycoplasma strains	PASS
	Confirm LOD at 10CFU/ml for min 23/24 replicates (95% of tests) per mycoplasma strain. Done for 2 selected mycoplasma strains with highest Cp	Target LOD: ≤ 10 CFU/mL Cp value of minimum 23/24 replicates < 43.0	Cp value of all mycoplasma replicates < 43.0 for all selected mycoplasma strains	PASS

Celyad Oncology

CONCLUSION

Fast-track mycoplasma tests offers a range of advantages and can be useful for both autologous and allogeneic cell therapies. Their rapid outcomes allow for both quick in-process control and product release, and they can assist manufacturers in overcoming technical limitations. They are also suitable for in-house and automated testing.

When considering whether to adopt an alternative mycoplasma testing approach, a risk-based strategy is the best option. It is also important to fully understand what regulatory bodies expect – for example, the guidance discussed above lists a number of mycoplasma strains, but it is only necessary to test those which are relevant to your manufacturing process. It is crucial to work in partnership with regulatory authorities, in order to ensure a reliable and sensitive alternative method which will enable the development of high-quality products.

Sarah Snykers
Cell Therapy Manufacturing
Unit Director, Celyad Oncology

Q & A

Q What are the chief considerations for Celyad Oncology's autologous and allogeneic cell therapy product candidates in terms of biological testing?

SS: In my personal opinion, it is critical to do a risk assessment for ICHQ9.

It is true that for all microbiologic testing, the general rule remains that you have to follow the compendial monographs. But for cell therapy products, this is not always feasible. The good news is that the regulatory landscape is moving forward, and it is going in the right direction and opening new doors for us around rapid alternative testing. This is especially the case for short lived products and also for cell therapy products that cannot be assessed by compendial methods due to technical limitations such as volume, size, or concentration.

It is important that these fast alternative methods are fully validated, that proper controls are used, and that the methods and validation are fit for purpose – and you also have to look into the matrix interference properties. We fully validate in terms of method sensitivity, specificity, and also reproducibility.

Q Can you go deeper into the specific drivers behind Celyad Oncology's search for an alternative mycoplasma testing solution?

DOI: 10.18609/cgti.2020.193

SS: There are three main drivers. Firstly, having a very sensitive test that can be easily validated and also easily transferred. Secondly, having a test that allows rapid in-process detection, but also rapid release of our autologous products. Finally, having also a method that is sustainable in the long term, that can be internalized, and is also fit for automation.

Q Which sample types are most suitable for the test?

SS: This is a very individual answer, because you have to know your product. For instance, if you have a cell therapeutic product, personally I would recommend both cell culture supernatants and also the cells themselves, because the mycoplasma can actually also be present within the cells.

On the other hand, if you have a cryopreserved method you want to have a detection that is as sensitive as possible, so here we recommend to do the testing on a fresh sample.

Q How long can your sample for testing be stored?

SS: This is again very individual, and you have to test that for yourself. I would highly recommend when you do this kind of validation that you also include stability shelf life testing.

Q Having now successfully qualified an alternative mycoplasma testing approach, what advice you would have for other companies that are interested in implementing a rapid microbial testing approach for their therapy?

SS: You have to follow a risk-based approach, I cannot emphasize that enough. Ensure that your method as well as your validation is fit-for-purpose. You have to use the proper controls – don't just read the guidelines; ensure they are relevant for your manufacturing process. Don't be afraid to start this kind of validation, but use it wisely.

BIOGRAPHY

Sarah Snykers

Sarah is Director of the Cell Therapy Manufacturing Unit at Celyad, a clinical-stage biotechnology company focused on the discovery and development of chimeric antigen receptor T cell (CAR T) therapies for cancer. She has over 10 years of experience in cell therapy, biomedical research, development and quality control. She is a pharmacist from education and has a PhD in pharmaceutical and biomedical sciences, specialised in stem cell biology. In 2010, she joined Promethera BioSciences, a biopharmaceutical company dedicated to the development of cell therapy products based on allogeneic adult stem cell technology to treat liver diseases. She headed the R&D, QC and Preclinical Departments. In 2015, she joined Celyad. Sarah initially led the immune-oncology program at R&D level, focused on technology transfer, product characterization and optimization. In 2016, she became Head of the QC Department, covering release of clinical products and raw material, analytical method development and translational research activities. In 2020, all operational activities including Production, QC, Validation and Tech transfer of processes, methods, equipment, raw material and viral vector became under her responsibility.

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Regulatory Disclaimer: *For use in quality control/manufacturing process only.*

LightCycler® 480 Instrument: For Life Science Research Use Only. Not for use in diagnostic procedures.

Trademarks: *MycoTOOL, MAGNA PURE, and LIGHTCYCLER are trademarks of Roche. All other product names and trademarks are the property of their respective owners.*

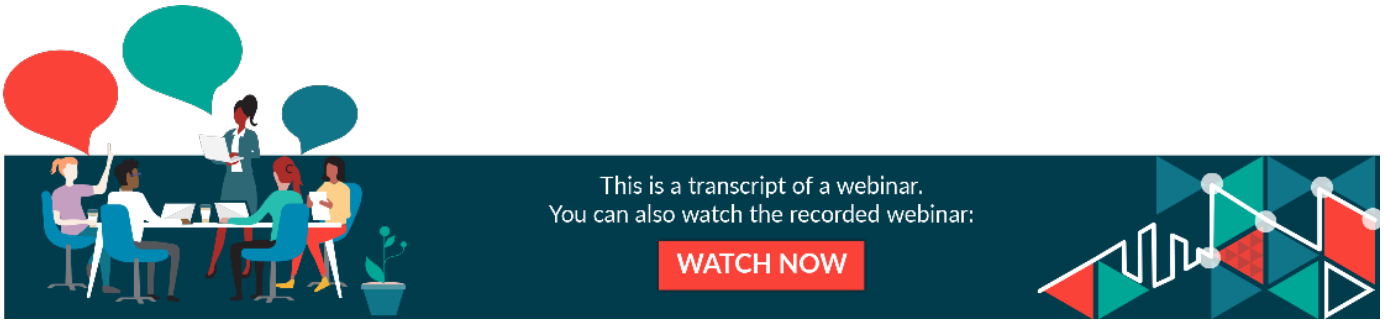
Disclosure and potential conflicts of interest*: The author declares that they have no conflicts of interest.*

Funding declaration: *Sarah Snykers has been paid a fee by Roche Diagnostics for her participation in this interview.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *This article is a transcript of a previously published webinar, which can be found here.*

Webinar published: *Jan 7 2021;* ***Publication date:*** *Feb 16 2021.*



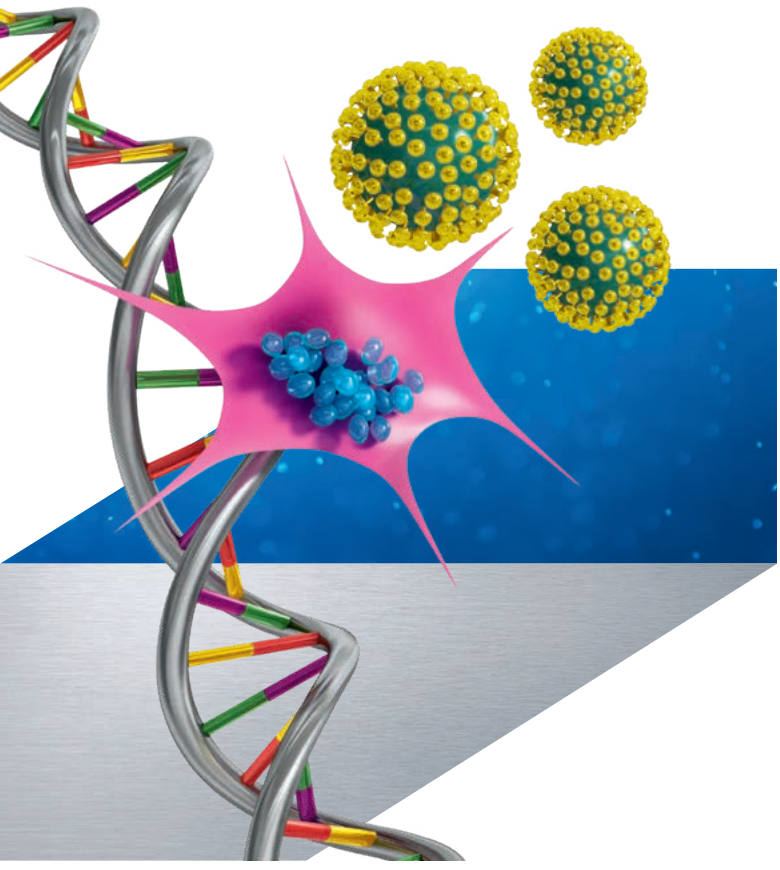
DOI: 10.18609/cgti.2020.193

MycoTOOL Mycoplasma Real-Time PCR Kit

Rapid and accurate testing

Mycoplasmas are a common source of contamination in bio-pharmaceutical production, cell therapy, and tissue engineering, with contamination rates reported between 15 and 35% according to recent industry data.*

The Roche MycoTOOL Real-Time PCR Kit makes mycoplasma testing fast, easy and reliable. Avoid the 28-day culture test and obtain results in just a few hours.



Achieve high sensitivity and specificity through improved tests

- ≤10 CFU/ml, meeting regulatory requirements
- No cross reactivity with closely related bacterial species

Rely on an accurate, and robust test procedure

- Measures ~150 culturable and non-culturable mycoplasma species
- Optimized controls and validated reagents (mycoplasma free)

Save time with easy-to-use kit and proven real-time PCR technology

- Automated workflow using automated sample preparation and Real-Time PCR
- <5 hours, including sample preparation**

Characteristics

MycoTOOL Mycoplasma Real-Time PCR Kit	
Sample prep	1 ml cell culture from high throughput or manual sample preparations.
Control concept	Recovery of sample preparation is controlled using an internal control.
Enhanced specificity	~150 mycoplasma species can be detected. The probe format ensures that nonspecific amplicons are **not** detected**.
All reagents included	Supplied and ready-to-use reagents jump start your assay.
High cell densities	Unprocessed bulk of standard cell concentrations (*e.g.*, 5 x 10^6 CFU/ml) processed directly.
High quality	Roche is certified according to ISO 13485. Change notification available upon request.

*Armstrong SE, Mariano JA, Lundin DJ. The scope of mycoplasma contamination within the biopharmaceutical industry. Biologicals. 2010 Mar;38(2):211-3. https://www.ncbi.nlm.nih.gov/pubmed/20362237. Date accessed: Dec 11, 2020. **Data on file.

For use in quality control/manufacturing process only.

CUSTOMBIOTECH

The MycoTOOL Mycoplasma Real-Time PCR Kit is easily combined with the MagNA Pure 96 Automated Sample Preparation System and the LightCycler® 480 Real-Time PCR Instrument for high throughput automated workflows.

Application	Batch testing Flexible sample volume	Batch testing Flexible sample volume	Real Time PCR
Instrument	**MagNA Pure 96 Instrument** 	**MagNA Pure 24 Instrument** 	**LightCycler® 480 Instrument II (96 well version)** 
Catalog number	06 541 089 001	07 290 519 001	05 015 278 001 (96 well)
Kit	MagNA Pure 96 DNA and Viral NA Large Volume Kit	MagNA Pure 24 Total NA Isolation Kit	MycoTOOL Mycoplasma Real-Time PCR Kit (160 PCR reactions)
Catalog number	06 374 891 001	07 658 036 001	06 495 605 001

Ordering information

Product	Catalog number
MycoTOOL Mycoplasma Real-Time PCR Kit (160 PCR reactions)	06 495 605 001

Related products	Catalog number
QC Sample Preparation Kit	08 146 829 001
MycoTOOL Mycoplasma Detection Amplification Kit	05 184 240 001

Regulatory disclaimer
For use in quality control/manufacturing process only.
LightCycler® 480 Instrument: for life science research only. Not for use in diagnostic procedures.

custombiotech.roche.com

Please contact your local CustomBiotech representative

Europe, Middle East, Africa, Latin America
Phone +49 621 759 8580
Fax +49 621 759 6385
mannheim.custombiotech@roche.com

United States
Phone +1 800 428 5433 (toll-free)
Fax +1 317 521 4065
custombiotech.ussales@roche.com

Canada
Phone +1 450 686 7050
Fax +1 450 686 7012
custombiotech.can@roche.com

Japan
Phone +81 3 6634 1046
Fax +81 3 5479 0585
japan.custombiotech@roche.com

Asia Pacific
Phone +65 6371 6638
Fax +65 6371 6601
apac.custombiotech@roche.com

Published by
Roche Diagnostics GmbH
Sandhofer Straße 116
68305 Mannheim
Germany