PCR-based Mycoplasma detection in biopharmaceutical production:

Development of an integrated sample preparation and detection work-flow

Matthias Hornschuh[1], Dirk Vollenbroich[1], Jens Beator[2], Birgit Höding[2], Hans Joos[2]

[1]Minerva Biolabs GmbH, Berlin, Germany; [2]STRATEC Molecular GmbH, Berlin, Germany

ABSTRACT

The presence of Mycoplasma in biopharmaceuticals or cell therapeutics presents a serious problem for patient's health. Therefore, product quality assessment is highly regulated, e.g. according to European Pharmacopoeia (EP) 2.6.7, which is a time consuming process based on cultivation methods requiring up to 28 days. The introduction of rapid test methods like PCR-based detection offers a significant improvement to the biopharmaceutical industry by saving time and, thus, money. When using high performance molecular diagnostic tools providing ultra-sensitive results, the quality and consistency of sample preparation is of major concern in means of robustness, DNA concentration and overall suitability of the extracted sample with the requirements of the subsequent PCR.

Here we present an integrated workflow using the InviGenius® for fully automated sample extraction with total in-process control. The instrument incorporates a unique magnetic separation tool combined with reliable pipetting technology without system liquid. An integrated heat incubator enables on-board lysis of samples and the easy-to-use software reduces human error to a minimum. Up to 4 ml of sample volumes can be processed using standard plastic consumables.

The extracted DNA is subsequently used for Mycoplasma detection using the Venor®*GeM* Prime PCR-based detection kit which enables simultaneous detection of more than 79 Mycoplasma species including all species listed in the EP 2.6.7. Utilizing the innovative qPCR probe technology, this kit is characterized by a high specificity to avoid false-positive results and works, in combination with the InviGenius®, with any relevant matrix from research and biopharmaceutical industry. Validation data on sensitivity of detection and inter/intra assay variability for these different sample materials for all EP-listed mycoplasma species are presented.

The workflow presented here provides several benefits to industrial customers in pharmaceutical and biotechnical manufacturing: large sample volume and high total DNA yield for highest sensitivity of detection; fast, reliable and robust for various sample matrices.

METHODS

1) Samples

Samples were EDQM Reference Standards spiked in TE80 media. Serial dilutions were prepared; 1:10 in TE80 Media.

2) Extraction Kits

InviMag® Universal Kit/ IG (STRATEC Molecular GmbH, Berlin, Germany)
Sample volume: 200 µl; elution Volume: 200 µl
All extraction procedures were performed using standard scripts on the InviGenius® instrument.

3) Real-time PCR

- Template amount 12.5 µl, VenorPrime Mycoplasma Detection Kit for qPCR (Minerva Biolabs, Berlin, Germany)
- Instrument: Mx3005p (Agilent Technologies)

RESULTS

Dilution series and reproducibility

18 samples from *Mycoplasma fermentans*, 10 samples from *Mycoplasma* synoviae, 18 samples from *Mycoplasma hyorhinis*, 16 samples from *Mycoplasma orale* were extracted on the InviGenius®. All samples were analyzed in repeated extractions using dilution series.

- *Mycoplasma fermentans:* 3 extractions in parallel, 6 dilutions
- *Mycoplasma synoviae:* 2 extractions in parallel, 5 dilutions
- *Mycoplasma hyorhinis*: 3 extractions in parallel, 6 dilutions
- *Mycoplasma orale*: 4 extractions in parallel, 4 dilutions

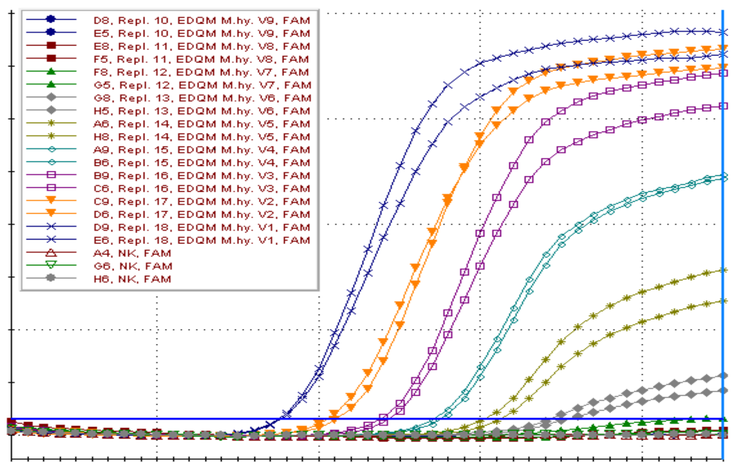

Fig. 1: Real-time PCR results for *Mycoplasma hyorhinis*

All sample types delivered highly reproducible results. A representative real-time PCR result of *Mycoplasma hyorhinis* is shown in Fig. 1. The CT results showed a low standard deviation. However the lowest dilution step of two samples resulted in a standard deviation of >5 % due to statistical effects when only low numbers of PCR templates are present in the reaction mix. The sample volume of 200 µl is sufficient for detection of *Mycoplasma synoviae* down to 20 cfu/ ml (see table 1, right). All dilution series showed strict linearity (see Fig 2a-c, right). In the case of *Mycoplasma fermentans* and *Mycoplasma hyorhinis* the linearity is shown for 6 log steps, showing excellent sensitivity and a wide concentration range for the quantification of Mycoplasma.

Tab. 1: Real-time PCR statistics of the whole study

Dilution series from	cfu/ml	mean CT	standard deviation %
Mycoplasma fermentans ***n = 18***	9.55E+06	17.12	1.0
	9.55E+05	20.38	2.1
	9.55E+04	24.20	4.0
	9.55E+03	27.45	1.6
	9.55E+02	30.41	1.3
	9.55E+01	35.80	10.8
Mycoplasma synoviae ***n = 10***	1.86E+05	17.28	1.6
	1.86E+04	20.20	0.2
	1.86E+03	22.99	0.1
	1.86E+02	26.65	0.5
	1.80E+01	30.10	0.5
Mycoplasma hyorhinis ***n = 18***	1.17E+07	17.80	1.4
	1.17E+06	21.12	2.0
	1.17E+05	24.09	0.9
	1.17E+04	27.24	1.0
	1.17E+03	30.35	2.4
	1.17E+02	33.51	5.8
Mycoplasma orale ***n = 16***	4.90E+04	24.38	1.2
	4.90E+03	27.39	0.5
	4.90E+02	30.36	0.6
	4.90E+01	34.10	2.6

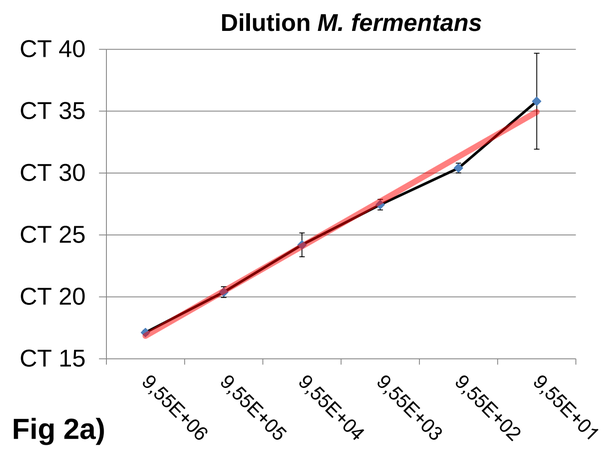

Fig 2a)

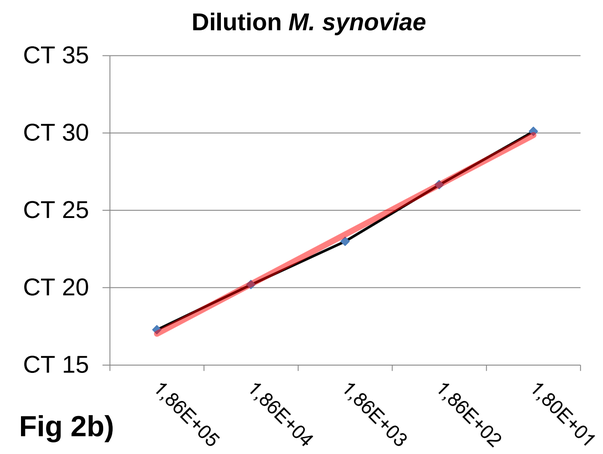

Fig 2b)

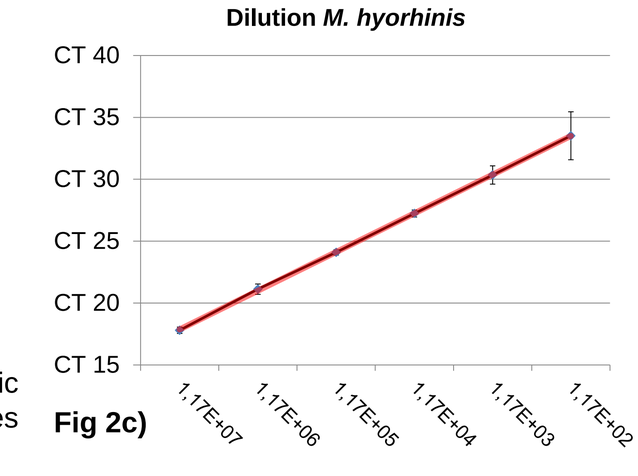

Fig 2c)

Fig. 2: Linearity plots of characteristic dilution series

SUMMARY

Repeated dilution series of Mycoplasma were purified using the InviGenius® magnetic particle processor. A standard sample volume of 200 µl was used for all extractions. The PCR detection was done using the VenorPrime Mycoplasma Detection Kit. In this study of 62 extractions no isolation or detection failures were observed. All repeats showed low standard deviations and all dilutions showed strict linearity. A sensitivity of down to 20 cfu per ml was obtained. The use of the InviGenius® with the InviMag® Universal Kit/ IG in combination with the VenorPrime Mycoplasma Detection Kit demonstrates a high sensitivity in detection of Mycoplasma. Therefore this extraction and detection method leads to improved process safety for biopharmaceutical production and quality control.