Challenge

Correct detection and measurement of *Mycoplasma bovis* in infected animals.

Solution

Combing UV/Vis spectrophotometric and qualitative real-time PCR analysis, it is possible to correlate the results of both techniques to support the correct detection and measurement of harmful pathogens.

Detection and Measurement of *Mycoplasma bovis*: Correlation of Results using ScanDrop² with CHIPCUVETTE and qTOWER³

Introduction

Mycoplasma and especially *Mycoplasma bovis* is a harmful pathogen for cows and calves, which can lead to inflammation and mastitis. Mastitis in turn may result in the reduction of milk production, an important economic factor for farmers in addition to their concern for the health and welfare of their livestock. In veterinary diagnostics it is important to, first, isolate the harmful bacteria from the milk and, second, to detect it correctly. Due to *Mycoplasma bovis*' absent cell wall and therefore its natural resistance against antibiotics, therapy is not possible. In essence, spreading of the disease may be stopped only by segregating the infected cows.

As Mycoplasma has no cell wall, a special bacteria kit is recommended for the extraction of bacterial nucleic acids from milk. The innuPREP Bacteria DNA Kit by Innuscreen GmbH allows for the isolation of the nucleic acids, offering high purity of the DNA and RNA.

Prior to using qualitative real-time PCR to confirm the nature of the extracted nucleic acids, it is helpful to check the quality of the samples. This can be done with a relatively simple, fast and inexpensive method using a UV/Vis spectrophotometer. The ScanDrop² from Analytik Jena has been especially designed for these applications, as it gives users the possibility to measure samples spanning a wide range of concentrations.

The CHIPCUVETTE is also particularly well suited for this application, as it allows for the simultaneous measurement of two path lengths for one sample, 0.1 mm and 1 mm. The software can then choose which path length measurement should be used for evaluation, based on a defined set of criteria. Shortly summarized, for samples with concentrations resulting in absorbance values of < 2.0, the 1 mm path length measurement is used. For samples with concentrations resulting in absorbance values >2.0, the 0.1 mm path length measurement is used. This eliminates the need of sample dilution and saves valuable time in the lab.

For this particular example, samples with high concentrations of extracted bacterial DNA may be expected from infected animals. Furthermore, the information concerning the concentration of the target DNA provided by the SanDrop^2 can help to eliminate false negative results of the qualitative real-time PCR analysis.

Materials and Methods

The isolation of bacterial DNA from milk was realized with the innuPREP Bacteria DNA Kit.
The extracted nucleic acids samples were then measured with the ScanDrop^2 using the CHIPCUVETTE adapter before the qualitative real-time PCR was started. The detection of the mycoplasma was carried out with the Venor GeM qOne Step Kit from Minerva Biolabs® in combination with Analytik Jena's qTOWER^3.

Samples and Reagents

- innuPREP Bacteria DNA Kit (IST Innuscreen GmbH)
- Venor GeM qOne Step (Minerva Biolabs®)

Instrumentation

The UV/Vis-spectrophotometric measurements were made with ScanDrop^2 using the FlashSoftPro^2 software, and the CHIPCUVETTE Adapter from Analytik Jena. The qualitative real-time PCR measurements were carried out with the Analytik Jena qTOWER^3 using the qPCR soft 3.4 software.

Table 1: qPCR Protocol

Step	Cycle	Profile	Temperature	Holding time	Ramp rates
1	1	Initial denaturation	95 °C	3 min	max
2	45	Denaturation	95 °C	30 sec	max
		Annealing	55 °C	30 sec	max
		Elongation*	60 °C	45 sec	max

* Data acquisition: Color Module 1 (470 – 520 nm) and 3 (353 – 580) with Gain 5

Results and Discussion

The results of the photometric measurements performed with the ScanDrop² in combination with the CHIPCUVETTE adapter are presented below in Table 2. These devices are particularly well suited for this application, as they allow for measuring samples spanning a wide range of concentrations.

The results show that some samples contain concentrations of extracted DNA which are below the detection limits (Sample 16, Table 2), which for ScanDrop² in combination with the CHIPCUVETTE adapter is 7.5 ng/µL for a 1 mm path length. It must be noted, that the low concentration can also be due to low DNA purity of the samples.

For samples with DNA concentrations higher than 1000 ng/µL (Sample 6, Table 2), corresponding to an absorption higher than 2.0, the software automatically uses the results for 0.1 mm path length, instead of 1 mm, thus sample dilution is not required.

Table 2: Results of the UV/Vis spectrophotometric measurement using ScanDrop²

Name	DNA purity (A_{260}/A_{280})	dsDNA concentration [ng/µL]	Path length
Reference	-		
Sample 1	1.43	3.15	1.0 mm
Sample 2	1.62	3.48	1.0 mm
Sample 3	1.81	11.02	1.0 mm
Sample 4	1.78	6.54	1.0 mm
Sample 5	2.02	4.37	1.0 mm
Sample 6	1.89	1318.22	0.1 mm
Sample 7	2.67	5.47	1.0 mm
Sample 8	2	12.42	1.0 mm
Sample 9	2.07	2.21	1.0 mm
Sample 10	2.04	10.78	1.0 mm
Sample 11	1.87	12.86	1.0 mm
Sample 12	1.92	13.33	1.0 mm
Sample 13	2.29	7.44	1.0 mm
Sample 14	2.19	16.72	1.0 mm
Sample 15	2.7	2.81	1.0 mm
Sample 16	-	-	-
Sample 17	2.47	4.16	1.0 mm

The results of the qualitative real-time PCR measurements are shown below in Table 3 (see also Table 1, for the qPCR Protocol). Comparing the UV/Vis spectrophotometric and PCR measurement, it can be seen that the results from the two techniques nicely correlate. For example, for Sample 16, the dsDNA concentration is negligible whereas no Ct-value is observed. The absence of a Ct-Value for Sample 15 also correlates to the low dsDNA concentration of this sample. Sample 6, with a very high dsDNA concentration, shows, as expected, a low Ct-value. The same is true for Sample 8, a high dsDNA concentration correlates with a low(er) Ct-value.
In general, the photometric measurements can be used to give an indication whether a qualitative real-time PCR measurement is necessary for a specific sample. They can also help to eliminate a false negative result of the qualitative real-time PCR analysis.

Table 3: Results of the qualitative real-time PCR measurements made with the qTOWER³

Name	DNA purity (A_{260}/A_{280})	dsDNA concentration [ng/µL]
Sample 1	Unknown	No Ct
Sample 2	Unknown	33.2
Sample 3	Unknown	35.78
Sample 4	Unknown	No Ct
Sample 5	Unknown	32.88
Sample 6	Unknown	16.24
Sample 7	Unknown	31.93
Sample 8	Unknown	29.11
Sample 9	Unknown	34.01
Sample 10	Unknown	33.34
Sample 11	Unknown	34.48
Sample 12	Unknown	No Ct
Sample 13	Unknown	38.74
Sample 14	Unknown	35.84
Sample 15	Unknown	No Ct
Sample 16	Unknown	No Ct
Sample 17	Unknown	No Ct
	Positive control	26.06
	Positive control	26.15
	NTC	No Ct
	Negative control	No Ct

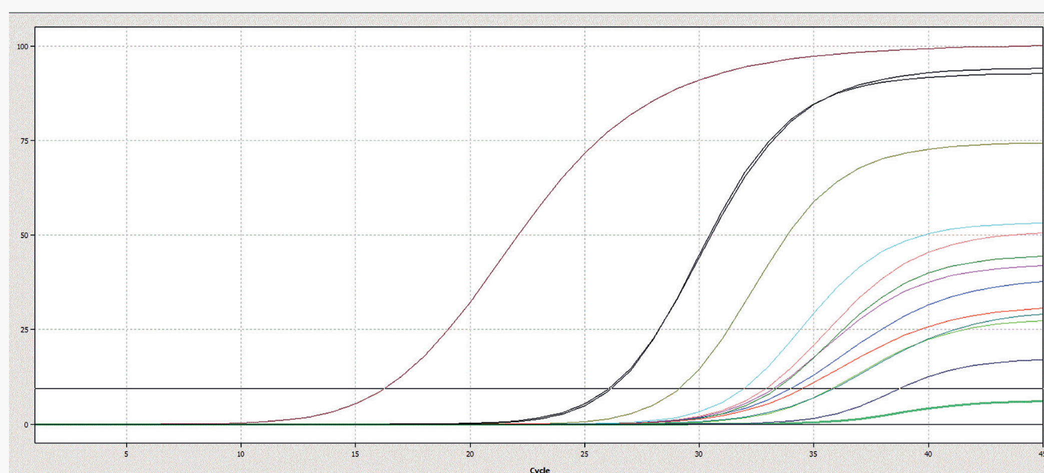

Fig. 1: Display of the qPCR curves

Conclusion

The results as presented above show that UV/Vis spectrophotometric and qualitative real-time PCR analysis correlate samples with low DNA concentrations that show the absence of a Ct-value and samples with high DNA concentrations showing low Ct-values. This correlation can be used to give an indication whether a qualitative real-time PCR measurement is necessary for a specific sample, which can save time and money. Additionally, the correlation can be used to help eliminate a false negative result of the qualitative real-time PCR analysis.

The CHIPCUVETTE offers the possibility to measure a wide range of unknown concentrations, as it is possible to measure both the 0.1 mm and 1 mm path length concentrations simultaneously. The software chooses which path length measurement should be used for evaluation, based on a defined set of criteria. This unique feature eliminates the need for dilution, resulting in further potential time and cost savings.

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Headquarters
Analytik Jena GmbH
Konrad-Zuse-Strasse 1
07745 Jena · Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279

info@analytik-jena.com
www.analytik-jena.com

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