# Challenge

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

# Solution

Combing photometric 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 have no cell well, a special bacteria kit is recommended for the extraction of bacterial nucleic acids from milk. The innuPREP Bacteria DNA Kit from Analytik Jena allows for the isolation of the NA, offering high purity of the DNA and RNA.

Prior to using qualitative real-time PCR to confirm the nature of the extracted NA, it is helpful to check the quality of the samples. This can be done with a relatively simple, fast and inexpensive method using a 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.

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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 for diluting the sample and saves valuable time in the lab.

For this particular example, samples with high concentrations of extracted bacterial NA may be expected from infected animals. Furthermore, the information concerning the concentration of the target DNA provided by the SanDrop² can help to eliminate false negative results of the qualitative real-time PCR analysis.

## Materials and Methods
The isolation of bacterial NA from milk was realized with the Analytik Jena innuPREP Bacteria DNA Kit.
The extracted NA samples were then measured with the ScanDrop² 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 qTOWER³.

### Samples and Reagents
- innuPREP Bacteria DNA Kit (Analytik Jena)
- Venor GeM qOne Step (Minerva Biolabs®)

### Instrumentation
The photometric measurements were made with ScanDrop² using the FlashSoftPro² software, and the CHIPCUVETTE® Adapter from Analytik Jena. The qualitative real-time PCR measurements were carried out with the Analytik Jena qTOWER³ using the qPCR soft 3.4 software.

Table 1: qPCR Protocol

| Step | Cycle | Profile | Temperature | Holding time | Ramp rates |
|------|-------|---------|-------------|--------------|------------|
| 1 | 1 | Initial denaturation | 95 ℃ | 3 min | max |
| 2 | 45 | Denaturation | 95 ℃ | 30 sec | max |
| | | Annealing | 55 ℃ | 30 sec | max |
| | | Elongation* | 60 ℃ | 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 NA 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, eliminating the need for diluting the samples.

Table 2: Results of the photometric 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 photometric and PCR measurement, it can be seen that the results from the two techniques are correlated. For example, for Sample 16, with a very low dsDNA concentration, no Ct-value is expected, which is the case. 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 |
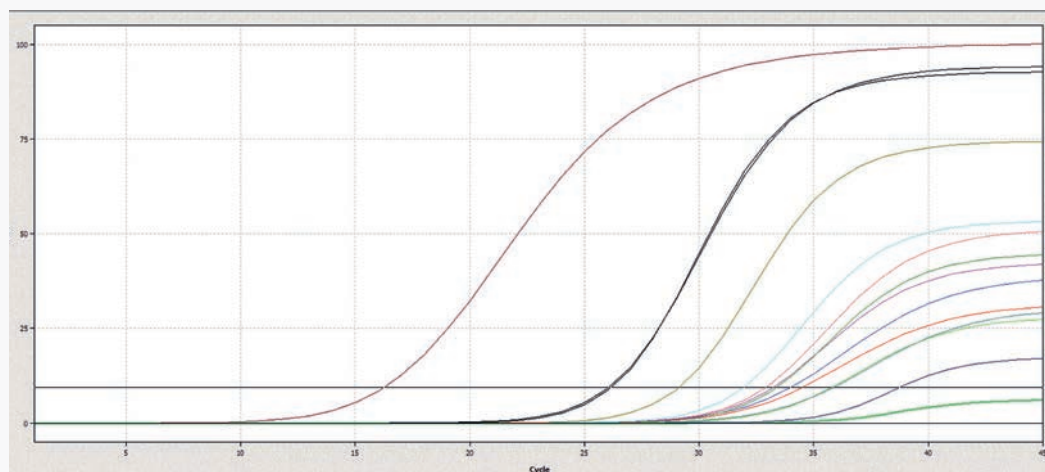


Fig. 1: Display of the qPCR curves

## Conclusion

The results as presented above show that the results of the photometric and qualitative real-time PCR analysis are correlated: samples with low DNA concentrations show the absence of a Ct-value and samples with high DNA concentration show 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.