Challenge

Detection of four pathogens of mastitis diseases.

Solution

Using qTOWER³ in combination with extraction and detection systems for DNA diagnostics.

Detection of Mastitis Pathogens in Raw Milk of Symptomatic Cows

Introduction

Mycoplasma and especially *Mycoplasma bovis* as well *Staphylococcus aureus* and *Streptococcus agalastiae* are harmful pathogens for cows and calves, which can cause inflammation and the Mastitis disease. Mastitis leads to a reduction in the quantity of milk, which, in addition to animal health and welfare, is also an important economic factor for farmers. In veterinary diagnostics it is important to isolate the harmful bacteria from the milk and to detect them precisely. Since *Mycoplasma bovis* has no cell wall and is therefore naturally resistant to antibiotic therapy, therapy is particularly difficult. Only isolation of the symptomatic cows can prevent it from spreading.

The extraction of bacterial DNA from milk is a challenge due to the diversity of ingredients, such as fat, sugar and proteins. That's why a special extraction kit or procedure is recommended, for instance the Mastit 4C Kit by DNA Diagnostic which is perfectly optimized for the extraction of bacterial DNA from milk.

The extracted nucleic acid can then be detected using the qTOWER³ for quantitative real-time PCR and the Mastit 4C Kit from DNA Diagnostic. The detection kit Mastit 4C Kit detects up to four targets in parallel like *Staphylococcus aureus*, *Streptococcus agalastiae* and *Mycoplasma bovis* and *Mycoplasma species* and provides an amplification control in addition.

This application note describes a method for the detection of different pathogens with the Mastit 4C Kit by DNA Diagnostic on the qTOWER³ and proves its specificity.

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Materials and Methods

The Mastit 4C Kit from DNA Diagnostic was used to isolate bacterial DNA from milk. The number of samples of the protocol was adapted. All steps were performed in a 1.5 ml reaction tube. After the transfer of the Pre-lysis buffer from the 96 Deep Well Plate to the 1.5 ml reaction tube, 500 µl of raw milk were added. One sample was contaminated with different pathogens to test the performance of the extraction and detection.
To detect Mycoplasma, Staphylococcus and Streptococcus in the samples, the detection part of the Mastit 4C kit of DNA Diagnostic in combination with the qTOWER³ by Analytik Jena were used. The qPCR protocol was adapted to the qTOWER³.

Samples and Reagents

- Mastit 4C Kit (DNA Diagnostic)
- Raw Milk (Dairy farm)

Instrumentation

- qTOWER³ (Analytik Jena)
- qTOWER³ Software: qPCR soft 3.4

Table 1: qPCR Protocol

Step	Cycle	Profile	Temperature	Holding time	Ramp rates
1	1	Initial denaturation	95 °C	1 min	2.5 °C/sec
2	40	Denaturation	95 °C	10 sec	2.5 °C/sec
		Annealing/ Elongation*	60 °C	25 sec	2.5 °C/sec

* Data aquisition: Color Module 1 (470 – 520 nm) with Gain3, 3 (553 – 580) with Gain 2, 4 (565 – 605) with Gain 5, 5 (630 – 670) with Gain 3 and Color Module 6 (660 – 705) with Gain 5

Results and Discussion

Before the values were checked for the different pathogens, the internal amplification control was applied to verify the results. The criteria for valid results is an Internal Amplification Control (IAC) lower than a Ct-value of 32. All samples show Ct-values between 23 and 31 so the result of the other targets are acceptable.

Samples number 2 and 3 show positive IAC, but are negative for all the other channels, resulting in a negative diagnosis. Samples number 1 and 4 show a positive signal for the ROX channel, resulting in contamination with *Streptococcus agalactiae*, whereas the the signal for sample number 4 is very low.

Sample 5 shows Ct-values for HEX (*Staphylococcus aureus*), ROX (*Streptococcus agalactiae*) as well for Cy5 (*Mycoplasma species*).

Table 2: Results of the qPCR detection based on the Mastit 4C Assay (DNA Diagnostic) and qTOWER³ (Analytik Jena)

Sample	Target				
	IAC Quasar 705	*Mycoplasma bovis* FAM	*Staphylococcus aureus* HEX	*Streptococcus agalactiae* ROX	*Mycoplasma species* Cy5
Sample 1	29.29	no Ct	no Ct	21.13	no Ct
Sample 2	30.18	no Ct	no Ct	no Ct	no Ct
Sample 3	31.59	no Ct	no Ct	no Ct	no Ct
Sample 4	29.59	no Ct	no Ct	35.7	no Ct
Sample 5	24.87	no Ct	14.79	10.65	18.33
Positiv control	23.47	19.15	19.67	14.14	18.19
NTC	28.44	no Ct	no Ct	no Ct	no Ct

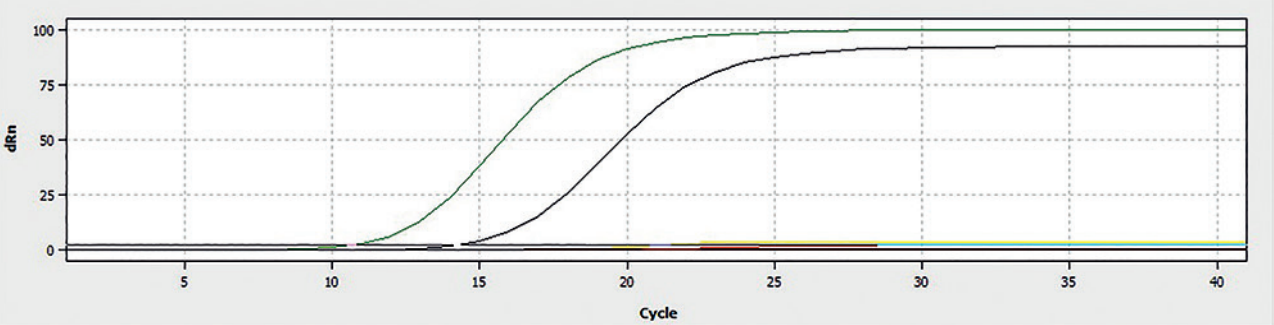

Fig. 1: Display of the qPCR curves for ROX (green – Sample 5, black – Postiv control)

Conclusion

The results of the IAC show, that the amplification of the Mastit 4C Kit in Analytik Jena's qTOWER³ is working perfectly together. First of all we resume, that the whole experiment is valid due to the results of the NTC (No template control) and Positive control. Furthermore the Internal Amplification Control is present in all samples so that results of the different raw milk samples from cows with symptoms of Mastitis are positive or negative for the several pathogens. To summarize this experiment the combination of qTOWER³ (Analytik Jena) and Mastit 4C kit (DNA Diagnostic) could be a solution for customers, which want to detect pathogens in milk.

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