New insights in mechanism of action of the California Mastitis Test

J. Hamann, D. Kleinschmidt, F. Reinecke, R. Redetzky

Institute for Food Quality and Safety, University of Veterinary Medicine, Foundation, Hanover, Germany

Session 24; Abstract-No: 0953; jha911@hamann-kronhagen.de

Introduction

The California-Mastitis-Test (CMT) is a simple, fast, cheap and accurate cow side tool using an indirect method to determine the somatic cell count of milk from individual udder quarters. The test includes the addition of an anionic surfactant to milk and the subsequent alteration of the viscosity of the originated mixture according to the content of cells. Until now, the correct reaction mechanism is not yet resolved definitively.

This study details the influence of different cell types (polymorphonuclear leucocytes (PMN); lymphocytes (LYM)), the vitality of PMN and the integrity of the DNA on the reaction.

Material and methods

To determine the influence of cell types, LYM and PMN were isolated out of blood samples of six cows. These cells were diluted with PBS buffer to get a dilution series in five steps between 10,000 and 3,000,000 cells/ml. CMT and Fossomatic® were used for the cytological analysis.

In 191 quarter milk samples, the vitality of PMN was measured by means of flow cytometry (FACS): SYTO® 13 dyes vital as well as dead eukaryotic cells via link to the DNA, so that they have different deflexion patterns.

CMT and Fossomatic® were used as well for the cytological analysis of the samples.

The third trial consist of the determination of the somatic cell count in 20 quarter milk samples (CMT, Fossomatic®) the subsequent ultrasonic treatment (two times ten seconds), again CMT and Fossomatic®, a second ultrasonic treatment (two times, ten seconds) and a final analysis with CMT and Fossomatic®.

Results

PMN and LYM showed identical results in the CMT reaction. Neither the cell types as such nor the dilution series caused different CMT results (table 1).

The comparison of vital and necrotic PMN did not result in a significant difference between the CMT results.

The CMT scores after first ultrasonic treatment were significant higher than the results of the original samples.

The CMT results after the second application of ultrasonography were differing significantly from those after first treatment but not from the original ones (figures 1 and 2).

Tab. 1: Means CMT results: comparison between LYM and PMN at different dilution steps

Dilution levels (cells/ml)	CMT-results (n = 6/level)		Signific.
	LYM ± dev.	PMN ± dev.	LYM versus PMN
0 = 10 000	**0,33** ± 0,41	**0,42** ± 0,49	ns
0,5 = 20 000	**0,67** ± 0,26	**0,50** ± 0,45	ns
1 = 60 000	**1,91** ± 3,99	**1,67** ± 2,00	ns
2 = 180 000	**3,17** ± 1,33	**4,17** ± 0,41	ns
3 = 400 000	**4,33** ± 0,52	**4,33** ± 0,52	ns
4 = 960 000	**4,33** ± 0,52	**5,00** ± 0,00	ns
5 = 3 000 000	**5,00** ± 0,00	**5,00** ± 0,00	ns

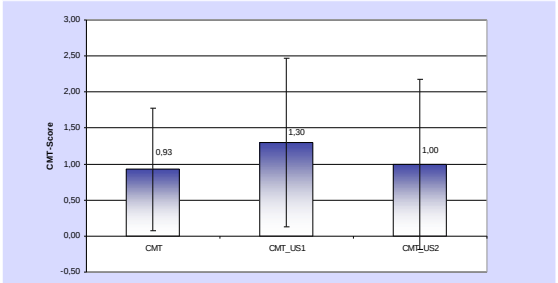

Fig. 1: Means of CMT reullts: CMT, CMT_US1 and CMT_US2 (n = 20)

Cutline Fig. 1, 2:
CMT = CMT-Score of the original samples;
CMT_US1 = CMT-Score of samples after 1st ultrasonic treatment;
CMT_US2 = CMT-Score of samples after 2nd ultrasonic treatment
LOG_{SCC} = Somatic cell count LOG 10;
LOG_{SCC}_US2 = Logarithmus basis 10 of SCC_US2

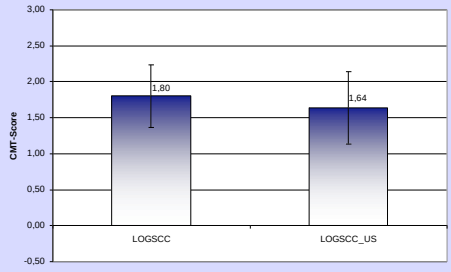

Fig. 2: Means of log SCC (LOG_{SCC} and LOG_{SCC}_US2) (n = 20)

Conclusion

Although the somatic cell count as such is determining the CMT result, the vitality of the cells is without influence. Therefore the CMT is suitable as a rapid test for different stages of mastitis. Furthermore, there were no cell type-based alterations of CMT-results: The comparison between the CMT results of 6 cell amount dilution levels of lymphocytes and PMN showed no significant difference.

As the CMT scores after the first ultrasonic treatment were significant higher than the results of the original samples, there is the assumption that the treatment destroyed the milk cell membrane with the consequence that the CMT test fluid was able to link easier to the DNA than compared to the original samples.

According to the results after the second ultrasonic treatment one can say that this second application leaded not only to the destruction of the cell membrane but also of the DNA of the cells as such, so that the intensity of the CMT reaction was reduced.