

FIRST RESULTS OF THE VALIDATION OF THE COPAN MILK TEST FOR THE DETECTION OF RESIDUES OF ANTIBIOTICS AND SULPHONAMIDES IN MILK



INTRODUCTION

The CMT-Copan Milk Test (Copan Italia S.p.a., Italy) is a microbiological broad spectrum inhibitor test, using *Bacillus stearothermophilus* var. *calidolactis* as test organism and bromocresol purple as pH-indicator. The CMT, existing as single test (individual vials) and in a microplate format, can be used for screening milk and milk products on the presence of residues of antimicrobials. This poster shows some first results of the validation study of the CMT-Copan Milk Test in microplate format (CMT MICROPLATE), performed according to Commission Decision 2002/657/EC.

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CMT-MICROPLATE TEST PRINCIPLE

The CMT- Microplate is the microplate version of the Copan Milk Test. The microplates are filled with an agar medium. The agar is pre-seeded with spores of *Bacillus stearothermophilus* var. *calidolactis* and incorporates a fermentable sugar (glucose) and a pH indicator (bromocresol purple). The test is ready to use with no need to activate the product by adding a nutrient tablet.

After pipetting 100 µl of milk onto the surface of the agar, the microplate is incubated during 3 hours at 64°C in a waterbath. The milk diffuses throughout the agar medium. If there are no antimicrobial substances in the milk sample or if the concentration is below the limits of detection the *Bacillus* spores germinate, grow and metabolise the sugar. The acid produced changes the colour of the indicator in the medium to a yellow colour. Alternatively, if antimicrobial residues are present in the milk sample, then germination and growth of the *Bacillus* spores is inhibited, no acid is produced and the colour of the pH indicator remains purple.

REFLECTOMETRIC READING

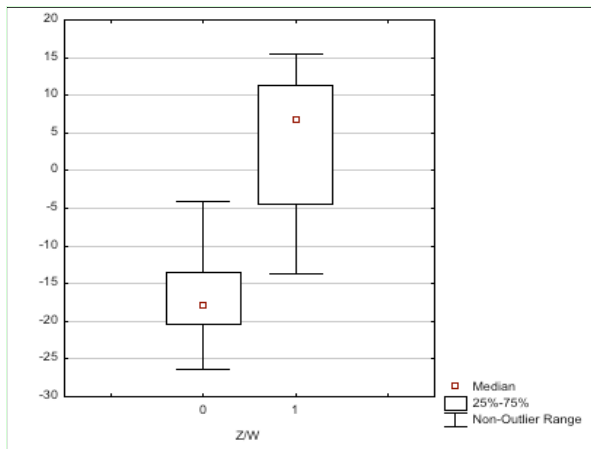


Figure 1. Determination of outliers: comparison of the visual interpretation of the colour of the agar medium in the cups of the microplate (0 = negative; 1 = positive) and a reflectometric reading using a flatbed scanner Delvoscanner (results expressed in z-values). Results for 2969 blank and doped milk samples.

For the cut off value ($z = -4.05$), all negative milk samples were also giving a negative result with the scanner. 11.11% of the milk samples were classified as negative with the scanner while interpreted as positive by visual reading.

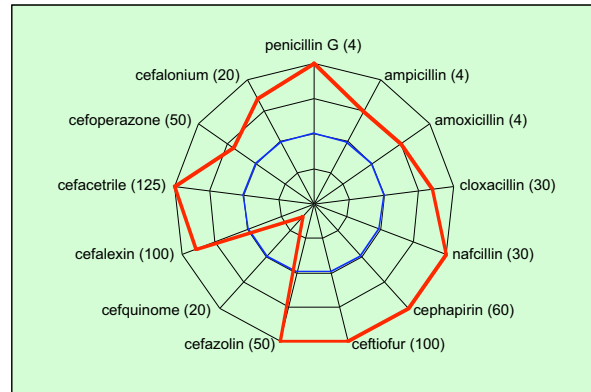
For a z-value = -9 the percentage of wrongly classified samples drops to 6.46%.

Making a same study for the Delvotest MCS (DSM-Food Specialties b.v., The Netherlands) a less good agreement was obtained due to the difficult visual interpretation of the in between colours.

CONCLUSION

All tested β -lactam compounds (except for cefquinome) and some sulpha drugs were detected at a level below their respective MRL. However, the compounds of the other groups were not detected at MRL. Using the test in monitoring programmes, no false positive or false negative results were noticed for the CMT. Also a reflectometric colour reading of the microplates was tested. In comparison with the Delvotest MCS the colours are more pronounced (less in between colours) what makes the visual interpretation of the test result easier.

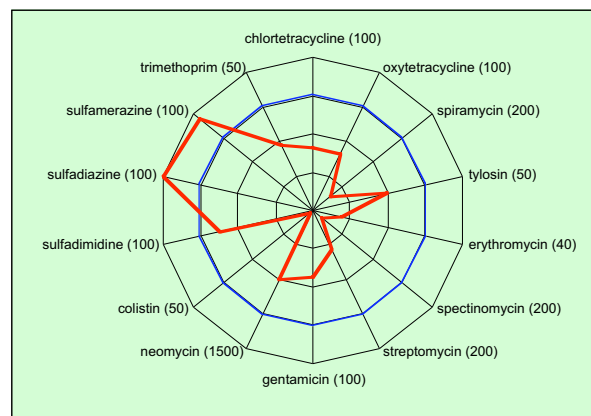
TEST SENSITIVITY FOR β -LACTAM COMPOUNDS



Inner circle = 2 MRL, circle 2 = MRL
circle 3 = 0.5 MRL, outer circle = 0.25 MRL

Figure 2. Test sensitivity of the CMT-Microplate for β -lactam compounds (visual reading). MRL (Maximum Residue Limit in milk ($\mu\text{g/kg}$), Council Regulation (EEC) No 2377/90) is mentioned in between brackets after the name of each substance.

TEST SENSITIVITY FOR NON β -LACTAM COMPOUNDS



Inner circle = 10 MRL, circle 2 = 2 MRL
circle 3 = MRL, outer circle = 0.5 MRL

Figure 3. Test sensitivity of the CMT-Microplate for non β -lactam compounds (visual reading). MRL (Maximum Residue Limit in milk ($\mu\text{g/kg}$), Council Regulation (EEC) No 2377/90) is mentioned in between brackets after the name of each substance.

REFERENCES

Anonymous, 1990. Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. Official Journal of the European Communities N° L224, p. 1-8 (and amendments).