

1. Validations and Field Trials

In-vitro bacteria and micro-bacteria biocidal tests have been carried out by independent, accredited laboratories, in accordance with the international AFNOR (Association Française de Normalisation) Standards.

To determine the effectiveness of pre-washing of teats, a field study was undertaken on ten cows during a four month period. Five of the group had selected quarters dipped daily before milking, using a solution strength of 1400ppm (treated group). The remaining five had their teats washed only with water (water only group). Swabs were taken from teat skin 10-15 minutes after washing or dripping.

A period of 20 days was allowed to elapse before new collections were made, using identical procedures, over a representative sample of the ten cows (control). Isolated from the quarters were the following: *Corynebacterium sp*; *Staphylococcus sp*; *Streptococcus sp*; *Bacillus sp*; *Micrococcus sp*; *Nocardia sp*; *Pasturella sp*; *E coli*; *Proteus vulgaris*. Comparing the results from the study showed a dramatic decrease in the number of quarters colonised by bacteria after washing with AgriseptMC®

Number of colonies	Percentage of Quarters		
	Treated group (13 teats; 18 sample periods)	Water only group (11 teats, 18 sample period)	Control (19 teats, 4 sample periods)
Too numerous to count	10%	30%	61%
≥100	7%	37%	32%
<100	33%	21%	7%
Nil	50%	12%	0%

With the AgriseptMC® treated group there is a considerably higher percentage of negative and low colony count swabs (and with no isolation of enterobacteria), than with other groups.

Many field studies using Sodium Troclosene as a post-milk teat dip have been undertaken and all confirm their effectiveness in reducing populations of mastitis-causing bacteria on teat skin. Evaluations were undertaken against *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli* and *Pseudomonas aeruginosa*. Results showed reductions in bacteria counts from 81.3% to 99.99% from undipped control groups. For example, healthy lactating cows were selected out of a herd of 16 cows at random. Suspensions of *Strep. agalactiae* and *Staph. aureus* were prepared and the four teats of the cow then dipped into this mixed pathogen suspension and allowed to air-dry. After 45 minutes the teats were dipping using a sodium troclosene solution 3000ppm. Control teats were not dipped. Swabs were taken 90 minutes after dipping in the pathogen suspension. The pathogens recovered were compared to the control group. There was an 81.3% reduction for *Strep agalactiae* and 95.2% reduction for *Staph aureus*.

In a further study, tablets containing NaDCC, which released 2800ppm available chlorine, were tested against new *Staphylococcus aureus* and *Streptococcus agalactiae* IMI using an experimental challenge model in a 5-9 week farm trial. During the afternoon milking, (Monday to Friday), all teats were immersed in a challenge suspension of *Staph. aureus* and

Strep. agalactiae immediately after the milking machines were removed. Two selected teats were then dipped into the NaDCC solutions, with the remaining two teats used as controls. When compared to the controls, the products reduced the number of new Staph. aureus infections by 69.0 -73.6% and new Strep. agalactiae infections by 63.5-65.1%. This study also evaluated the skin condition of the teats, and no adverse effects were observed.

These studies and other published data on the effectiveness of sodium troclosene clearly demonstrate AgriseptMC®'s effectiveness against a wide range of micro-organisms and particularly where there is organic contamination, which will be a feature of farming applications.