



# **Effectiveness of automatic cleaning of udder and teats and effects of hygiene management**

*Report on effectiveness of cleaning procedures applied  
in different automatic milking systems*

**January 2004**

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# **Effectiveness of automatic cleaning of udder and teats and effects of hygiene management**

*Report on effectiveness of cleaning procedures applied  
in different automatic milking systems*

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## Abstract

Teat cleaning is necessary to prevent milk from contamination with manure, dirt and included bacteria. Clean udder and teats before milking is also a requirement according to Council Directive 89/362/EEC. A lot of research has already been done to investigate the effect of teat cleaning on milk quality. In automatic milking (AM) systems the mechanisation of teat cleaning has become part of the procedure. Still missing is the online control of teat cleaning efficiency. The assessment of the teat cleaning efficiency of different types of cleaning devices in AM systems was the subject of this research.

The methods for evaluating teat cleaning efficiency had to be developed for this purpose. Part of these methods was presented in deliverable D13, another part is presented in this deliverable.

Two different approaches were chosen for evaluation of teat cleaning devices:

In the first approach teats were artificially contaminated with a tracer substance and carry over of the tracer into milk was determined. The protocol consisted of applying a mixture of poppy seed and manure (20% w/w) on teats of ten cows per farm. Five cows were milked after teat cleaning and five cows were milked without a previous teat cleaning. Milk per cow was filtered through a cotton filter. The filters were dried and the poppy seed was counted.

Two farms per brand of AM system were selected by the producers/suppliers. Six brands including 12 farms and two farms with conventional milking systems were tested.

Results showed significant effects of teat cleaning versus no teat cleaning and between different brands. From three brands teat cleaning was effective (more than 85% reduction in poppy seed in milk versus no cleaning) and from three other brands teat cleaning was less effective (50-70% reduction in poppy seed in milk). Conventional teat cleaning gave a reduction of 99%.

Teat cleaning in practice might be less effective, because the waiting time after application was less than one hour and the adhesion of poppy seed is probably less strong than of dried manure and/or bacterial spores.

In the second approach a combination of visual evaluation of teats before and after teat cleaning, sediment tests after teat cleaning and the determination of total bacterial counts and ATP in teat swabs taken before and after cleaning was applied. 6 brands with 3 farms per brand were tested. On every farm 50 cows were sampled on two teats each.

Results showed large variation within and between individual farms. An analysis of variance showed that significant differences existed between teat cleaning efficiency of 4 brands versus 2 other brands. The ranking of brands regarding teat cleaning efficiency was similar independent of the method applied and also consistent with the results from part 1 of this study. Only for one brand deviating results were found by the combination of 4 methods. This was probably due to the fact, that no samples could be taken from teats directly after teat cleaning. The cleaning effect determined in this case was at least in part due to teat washing during milking.

The comparison of teat cleaning devices with brushes with those working with cups showed different effectiveness dependent on teat contamination level. Brushes seemed to be less effective with low level contamination, leading to a higher bacterial load on teat surfaces when teat contamination was low. With high contamination levels, brushes were more effective than cups in removing bacteria and organic material.

An analysis of variance was carried out to find out which of the factors brand, farm, position of quarter and initial contamination of teats was most important for teat cleaning efficiency. Although significant differences between brands were found, the most important factors were the individual farm and the initial contamination before teat cleaning. These findings underline the importance of management to ensure an effective teat cleaning. In the last deliverable of this work package different management factors will be evaluated regarding their influence on teat cleanliness.

From some observations during this study it can be concluded that good maintenance of the cleaning device is important for an effective teat cleaning procedure.

Based on the findings from our study a pragmatic approach for evaluation of teat cleaning efficiency on farms is suggested. The procedure is based on ATP measurements with HyLite<sup>®</sup> 2 in teat swabs suspended in 8.0 ml sodium-peptone solution. 50 cows per farm should be sampled after teat cleaning at two teats each. Not more than 25 % of teats should exceed a proposed value of 3.2 Log<sub>10</sub> RLU after cleaning. This approach can also be applied on farms with conventional milking systems.

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## 1 Introduction

High quality of milk is important for the dairy industry. Achieving this high quality level of milk starts at the dairy farm. The quality of feed, hygiene of housing as well as hygiene before, during and after milking can influence this (Slaghuis, 1996). The milking process is one of the most important activities in dairy farming with regard to milk quality.

Udder and teat surfaces are an important source for contamination of milk. Therefore teat cleaning is necessary to prevent milk from contamination with manure, dirt and included bacteria. The cleaning of udder and teats before milking is also a requirement according to Council Directive 89/362/EEC.

A lot of research has already been done to investigate the effect of teat cleaning on milk quality as well as on the positive stimulating effect of teat cleaning on cows' milk let down (Jørgensen, 1990, Kingwill, 1980 and see D13).

The methods of teat cleaning are several. In conventional milking systems they can be distinguished in manual and mechanised methods, but mechanised methods are of low importance. In automatic milking systems the mechanisation of teat cleaning has become a fact. Still missing is the online control of teat cleaning efficiency. Usually only a standard protocol for teat cleaning is applied on all cows of a herd independent of teat contamination. In all systems it is possible to modify cleaning intensity on herd level and in some systems also facilities are available to introduce more intensive cleaning for certain cows.

The following procedures are applied for teat cleaning by the different automatic milking (AM) manufacturers (table 1).

**Table 1: Teat cleaning procedures in AM systems**

<b>Company (Country): Milking Robot</b>	<b>Teat cleaning system</b>
DeLaval (SE): Voluntary Milking System (VMS)	Separate cleaning device: application of warm water, vacuum and air-pressure, foremilk simultaneously with teat cleaning, drying of teats with warm air, cleaning water separated together with foremilk
Insentec (NL): Galaxy	Separate cleaning device: cleaning with warm water, foremilk after drying of teats with warm air, cleaning water and foremilk separated
Lely Industries (NL): Astronaut	Cleaning by wet rotating brushes
Fullwood (UK): Merlin	Cleaning by wet rotating brushes
Prolion Sales (NL): AMS Freedom, AMS Liberty; Manus (NL): Solos, Miros; Gascoigne Melotte (NL): Zenith	Cleaning within teat cups used for milking: water inlet at the head of the liner, application of high pulsation rate, cleaning water separated together with foremilk
Westfalia Landtechnik GmbH (DE): Leonardo	Cleaning in a preparation box by wet rotating brush, removing residual water (drying) from brush by high speed rotation, subsequently brushing with dried brush

Some investigations have been performed on the teat cleaning efficiency of individual AM systems. Schuiling (1992) used lithium mixed into sterilised manure as tracer for artificial contamination of teats. According to the amount of indicator recovered in the collected milk, 69% of the manure was removed comparing udder treatment with no udder treatment by using half brushes per cow.

Melin et al. (2002) based their investigation on artificial contamination with spores and carry over of spores into milk. They found that the teat cleaning procedure of the VMS of DeLaval was superior to manual cleaning (reduction of spore counts in milk of 98.0 versus 66.5 %).

TenHag and Leslie (2002) used a swab method with a simplified determination of bacterial counts based on a score count system according to the number of colonies per swab. In a preliminary investigation to examine automatic teat cleaning in comparison to manual cleaning no significant difference was found between the two methods of teat preparation.

However, no approach has been made so far to determine the teat cleaning efficiency of different systems with the same method.

## 1.1 Aim of this study

The aim of this study was the evaluation of teat cleaning systems used in present-day automatic milking systems of different manufacturers. A common approach on all AM systems was made in order to determine teat cleaning efficiency of the individual systems.

Before testing the effectiveness of the udder preparation of automatic milking systems, a procedure for testing had to be developed. Part of the procedure was presented in Deliverable D13.

A two step approach was chosen. In the first part of the investigation the teat cleaning efficiency was evaluated by using a standardized artificial contamination of teats with a tracer substance and measuring the carry over of the tracer into milk. This part is directly related to milk quality.

By using the tracer cobalt an attempt was made to develop a protocol for evaluation of teat cleaning systems. Cobalt as tracer did not succeed in a good protocol (D13), so poppy seed was introduced as tracer. By replacing cobalt by poppy seed a protocol was developed for testing the effectiveness of udder cleaning by different automatic milking systems.

In the second part the current systems were investigated by a combination of methods without any special preparation of cows. This approach consisted of four different methods: visual inspection, sediment tests on teats, measuring total bacterial count (TBC) and adenosine tri-phosphate (ATP) in teat swabs.

The results of the evaluation of teat cleaning systems are presented in this deliverable. Differences between individual systems might be used by the manufacturers to improve their systems if necessary.

During farm visits for the second part of the investigation a questionnaire and the checklist was used in order to find reasons for differences in teat cleanliness between individual farms. The evaluation of management factors will give an indication which factors are most important to keep teats clean. The results of that part of the study will be presented in deliverable D15.

## 2 Materials and Methods

### 2.1 Investigation based on artificial contamination of teats

The investigation based on artificial contamination of teats was performed by the Research Institute for Animal Husbandry, Lelystad, NL.

#### 2.1.1 Preliminary experiments with poppy seed

For this study poppy seed was used as a tracer because the results of a previous study using cobalt as tracer were not satisfying (D13). Poppy seed is easy to mix with manure, attaches on teats, stays attached and is easy to analyze (by counting). Mixing and attaching qualities were tested in laboratory circumstances with different percentages of manure/poppy seed mixture on artificial teats.

The use of poppy seed as a tracer was tested on an experimental farm. Per cow a mixture of 0.5 g poppy seed and 4.5 g manure (10% w/w) was put on the teats. Ten cows were used. These cows had to wait for at least half an hour before they were milked, without teat cleaning. Pre squirts, milk and unit flush water were collected separately and filtered through a cotton filter, held between two metal riddles. In former times these filters were used for filtering milk after milking with a bucket machine or by hand.

After leaving the robot the teats of the cows were cleaned with paper cloth (Kleencare®). The poppy seed on this paper cloth and on the cotton filter was counted. The cotton filter was put in a tray and photographed (before and after drying) and dried two days in a forced air oven at 70°C.

In the second experiment on an experimental farm ten cows were fixed at the fence in the waiting room near the AM system in order to put on poppy seed/manure mixture on teats. In the first experiment loss of poppy seed/manure could not be excluded because a number of cows started to run in the stable when they were picked up for milking. The mixture was now made of 2 g poppy seed and 8 g manure (20% w/w) in order to improve the chance that poppy seed gets into the milk. The teats were photographed after putting on the mixture. Cows were milked after a waiting period of half an hour. Brush water, pre squirts and milk were filtered. Milk was filtered twice; the first time with cotton filter between metal riddles and the second time only with metal riddles.

The cotton filters were dried in the same way as in the first experiment. Unit flush water was not filtered this time and the cow was released right after milking without cleaning the teats with paper cloth. The number of poppy seed on the cotton filters was counted.

#### 2.1.2 Set-up of experiments

The experiment was done at two farms of each AM brand (six brands were included: DeLaval, Fullwood, Insentec, Lely Industries, Prolion (now: Gascoigne Melotte) and Westfalia Landtechnik GmbH and at two farms with a conventional milking parlor. Farms per brand were selected by the producers/suppliers of the AM systems. Brands were coded with numbers.

Per farm one AM box was used for the experiment. Some farms had more boxes.

At each farm ten cows, with an in between milking time of at least 6 hours, were at random selected. Five cows were treated with a poppy seed/manure mixture and were milked with teat cleaning. Another five cows were treated with poppy seed/manure and milked without teat cleaning.

#### 2.1.3 Testing effectiveness of cleaning procedures

##### 2.1.3.1 Preparation and application of manure/poppy seed mixture

Two gram (20% w/w) poppy seed was mixed in a Petri dish with 8 gram sterilized manure (30 min., 121 °C, 1 atm.) just before the mixture was put on the teats of a cow. One Petri dish was used per cow. This mixing was done with a long brush. Application on the teats was performed with the same long brush.

The ten Petri dishes (one per cow) per day were weighed before and after application of the mixture for calculation of the amount of poppy seed and manure being put on the teats.

### 2.1.3.2 Testing effectiveness of cleaning procedures

The mixture of poppy seed/manure was applied on the teats of ten cows; one Petri dish per cow. As much as possible was applied, avoiding accidental dripping off of mixture. About 5-6 grams per cow were added in this way. From the cows also teat length and thickness were estimated. After application, the cows had to wait for at least half an hour before testing.

The first five cows were treated. After the waiting time the teats of these five cows were cleaned, by the AM teat cleaning system or manually in conventional milking systems, before they were milked. Depending on the brand the teat cleaning was done by brushes, a cleaning and pre milking teat cup or in the milking teat cups. Manual cleaning was performed with a humid washable towel on one farm and with a dry paper towel on the other farm. Teats of cows were cleaned in about 10 seconds per cow. Milk (and in some cases also pre squirts of milk) was separated and filtered two times, the first time with cotton filter (Kroon melkfilters and Hygia Supra II, 22 cm, NL) between metal riddles and the second time only with metal riddles. The poppy seed on the metal riddles was counted immediately after filtering. Each milking was followed by a unit flush, to clean the AM system and to prevent carry over between cows, before the next cow was milked.

After milking the first group, poppy seed/manure mixture was put on the teats of the second group of five cows. These cows also had to wait for at least half an hour before they were milked without a previous teat cleaning. Depending on the technical possibilities of the AM brand pre milking was not applied (brands Delaval and Insentec), applied fully (brands Lely, Fullwood and Westfalia) or partially (brand Prolion: one second without water). Milk was also separated and filtered two times.

Information about cow number, in between milking time, milking time, kg milk, time of applying poppy seed/manure mixture, teat length, teat thickness was recorded.

All cotton filters were put in a tray and dried two days in a forced air oven at 70 °C.

## 2.1.4 Analysis

### 2.1.4.1 Poppy seed analysis

Poppy seed on the dried cotton filters was counted. For comparison of cleanliness all milk filters per farm were photographed.

### 2.1.4.2 Statistical analysis

The levels of number of poppy seed in milk were analysed for the fixed effects of brand and teat cleaning efficiency using a mixed logistic model. In the model the relationship between the probability  $p$  ( $0 < p < 1$ ) of observing the poppy seed and the explanatory variables were described using the logit-link function. For these data the logit function was:

$$\text{Logit}(p_{ijk}) = \ln(p_{ijk} / (1 - p_{ijk})) = c + \text{farm}_i + \text{brand}_j + \text{teat cleaning effect}_k + \text{brand}_j * \text{teat cleaning effect}_k$$

Where  $c$  represents the constant term and is the mean for the combination with all factors at the lowest level:  $\text{farm}_i$  = random effect of farm  $i$ , considered to be normally distributed with mean 0 and variance equal to  $\sigma^2_{\text{farm}}$ ,  $\text{brand}_j$  = fixed effect of brand  $j$  and  $\text{teat cleaning effect}_k$  = fixed effect of teat cleaning effect  $k$ . The model assumed that the variance of the observed counts  $Y$  can be adequately described by  $\text{variance}(Y | p) = np(1-p)$ . Estimates of model parameters and components of variance were obtained using the Generalized Linear Mixed Model (GLMM) Genstat procedure of S. Welham (2002). Fixed effects were assessed using chi-squares for the Wald statistics.

## 2.2 Investigation based on combination of methods

The evaluation of teat cleaning systems by a combination of methods was performed by the Federal Dairy Research Institute, Kiel, DE.

### 2.2.1 *Set up of experiments*

The experiments for determination of teat cleanliness under practical conditions were performed with a combination of methods that was described in detail in D13. In the following only a short description is given.

The method was applied on 3 farms per AM manufacturer. The following brands were included: DeLaval, Fullwood, Insentec, Lely Industries, Prolion/Gascoigne Melotte, Westfalia Landtechnik GmbH. Addresses of farms were supplied by the manufacturers. All farms were located in Germany, except the farms working with an AM system of Insentec. Two of those farms were also used for the evaluation based on artificial teat contamination in part 1. The brands were coded according to part 1 of this study.

On each farm 50 cows were sampled. If fewer cows were available as many cows as possible were sampled during a period of approximately 8 hours. The cows were sampled in the milking robot. From each cow the sampling was performed on two teats, one front and one hind teat of the side facing the investigator. The teats were sampled before and after the cleaning procedure without interrupting the actions of the robot. Only for DeLaval the robot actions were started manually.

For Prolion/Gascoigne Melotte it was not possible to define correctly the end of the teat cleaning procedure and the beginning of milking per teat because both is performed within the same cup. In addition, a detachment of the milking cluster would have been necessary to take samples after teat cleaning. Therefore the cows were sampled before manual attachment of the cluster and after the end of the milking procedure.

All scorings of teat cleanliness and sediment tests as well as the teat swabbing were performed by the same person.

### 2.2.2 *Visual inspection*

Teats were visually inspected before and after teat cleaning. A scoring system with 4 points was used for judgement of teat cleanliness:

- 1 = completely clean
- 2 = slightly splashed
- 3 = partially contaminated
- 4 = heavily soiled

### 2.2.3 *Sediment test*

Half a gauze swab (10x10cm, gauze type 17, 12-ply, cotton; Lohmann & Rauscher Int., 56579 Rengsdorf, DE) was moistened in tap water and used for a thorough cleaning of a single teat. The gauze swab was placed in a disposable cup with screw cap (100 ml, 76x60 cm; Sarstedt, 51588 Nümbrecht, DE) filled with 70 ml tap water. Samples were refrigerated for 24 to 48 h before further handling.

Cups were placed on a laboratory shaker for 30 minutes with 120 rpm. The swab was rinsed with 250 ml of tap water and the entire fluid was filtrated at room temperature by using a sediment tester (Schmutzprober Sedilab, Funke-Gerber, 12105 Berlin, DE) and respective filter discs of the same manufacturer with a diameter of 28 mm. 500 ml of tap water were used for rinsing of the apparatus.

Filters were air-dried and visually evaluated using a score system of 4 scores (scores 1, 2, 3, 4). For each scoring level a set of 3 filters representing a certain range of teat contamination was used as a reference for visual comparisons.

#### 2.2.4 *Total bacterial counts in teat swabs*

Plastic screw capped test tubes with sterile cotton wool swabs (Heiland, 22006 Hamburg, DE) were prepared with 8.0 ml sterile solution of 0.85 % NaCl, 0.1 % peptone one day before sampling and kept refrigerated until use.

3 strokes (basis to apex) from each of two opposite sides of a teat were taken. The swab was rotated during sampling. For comparisons before versus after cleaning the front and hind surface of the same teat before cleaning and the inner and outer surface after cleaning were sampled. The swab was replaced into the test tube. The tube was cooled until ATP measurement. The test tube with the swab was vigorously shaken on a vortexer for 10 seconds and the swab was removed. About 1.5 ml of the solution was used for ATP measurements immediately on the farm. The remaining solution was cooled for max 24 h until further analysis.

The determination of bacterial counts in the remaining swab solution was according to the following standard procedures:

- Total bacterial count (TBC) - IDF Standard 100B:1991

Results were  $\text{Log}_{10}$  transformed for further analysis and are presented in  $\text{Log}_{10}$  cfu/ml swab solution.

#### 2.2.5 *ATP in teat swabs*

Measurements of ATP were performed on the same swab solution that was used for determination of TBC. The measurement was carried out within a time period of 4 hours after sampling. The test principle is based on an enzymatic reaction resulting in the emission of light (bioluminescence) dependent on the amount of ATP in the test sample. Test results are given in Relative Light Units (RLU). The test system HyLite<sup>®</sup> 2 (Merck, 64293 Darmstadt, DE) for determination of ATP in fluids was used. ATP was measured before determination of bacterial counts started.

Results were  $\text{Log}_{10}$  transformed for further analysis and are presented in  $\text{Log}_{10}$  RLU.

#### 2.2.6 *Statistical evaluation*

Statistical evaluations were made with SAS<sup>®</sup> System, release 8.01.

Teat cleaning efficiency was expressed as the difference between contamination before cleaning minus contamination after cleaning based on visual evaluation (Scores 1 to 4) respectively TBC ( $\text{Log}_{10}$  cfu/ml) or ATP ( $\text{Log}_{10}$  RLU) measurements. A normal distribution of contamination before cleaning was assumed.

For comparisons of cleaning efficiency of brushes versus cups a two sample t-test was performed for different levels of contamination before cleaning.

Data on distribution of results from TBC determination and ATP-measurement are presented as box-plots. Two different styles of box plots were used with the following interpretation:

**Table 2: Interpretation of box plots**

Feature of Box-and-Whisker Plot	Group summary statistics	
	Figures 11 to 22	Figures 24 to 30
Symbols (Squares)	-	Single observations above upper fence
Endpoint of upper whisker	Maximum observation	Maximum observation below upper fence
Upper edge of box	Third quartile (75th percentile)	Third quartile (75th percentile)
Line inside box	Median (50th percentile)	Median (50th percentile)
Symbol (cross)	Mean (arithmetic)	Mean (arithmetic)
Lower edge of box	First quartile (25th percentile)	First quartile (25th percentile)
Endpoint of lower whisker	Minimum observation	Minimum observation above upper fence
Symbols (Squares)	-	Single observations below lower fence

IQR = interquartile range=25th percentile to 75th percentile;  
Upper fence = 1.5 IQR above 75th percentile, Lower fence = 1.5 IQR below 25th percentile (not shown)

### 2.2.6.1 Analysis of variance

In order to determine which factors have a systematic influence on teat cleaning efficiency, an analysis of variance was carried out. For this purpose the General Linear Model (GLM) procedure of the statistic package SAS, release 8.01, was used.

The teat cleaning efficiency as difference of TBC before cleaning ( $\text{Log}_{10}$  cfu/ml) minus TBC after cleaning ( $\text{Log}_{10}$  cfu/ml) resp. ATP before cleaning ( $\text{Log}_{10}$  RLU) minus ATP after cleaning ( $\text{Log}_{10}$  RLU) was used as dependent variable ( $y$ ). The following influence factors were included into the analysis: brand (1, 2, 3, 4, 5, 6), farm per brand (A to R), quarter (front or hind). The initial contamination as TBC before cleaning ( $\text{Log}_{10}$  cfu/ml) resp. ATP before cleaning ( $\text{Log}_{10}$  RLU) was used as covariate.

The linear model had the following form:

$$Y_{ijkl} = \mu + br_i + fm_{ij} + qu_k + b(X_{ijkl}) + e_{ijkl}$$

With:

$$Y_{ijkl} = \text{dependent variable - teat cleaning efficiency}$$

(for TBC:  $\text{Log}_{10}$  cfu/ml before cleaning minus  $\text{Log}_{10}$  cfu/ml after cleaning  
for ATP:  $\text{Log}_{10}$  RLU before cleaning minus  $\text{Log}_{10}$  RLU after cleaning)

$$\mu = \text{overall mean}$$

$$br_i = \text{effect of the } i^{\text{th}} \text{ brand}$$

$$fm_{ij} = \text{effect of the } j^{\text{th}} \text{ farm of the } i^{\text{th}} \text{ brand}$$

$$qu_k = \text{effect of the } k^{\text{th}} \text{ quarter}$$

$$b = \text{slope for TBC (} \text{Log}_{10} \text{ cfu/ml) resp. ATP (} \text{Log}_{10} \text{ RLU) before cleaning}$$

$$e_{ijkl} = \text{random residual error}$$

Teat cleaning intensity was not included as a factor because no or low variation was seen between the three farms per brand.

### 3 Results and Discussion

#### 3.1 Investigation based on artificial contamination of teats

##### 3.1.1 Preliminary experiments with poppy seed

Poppy seed and manure mixed together and put on artificial teats showed no losses of falling off after drying of the mixture.

Experiences with the thickness of the mixture resulted in a need for a rather high moisture manure because otherwise the needed amount of dry poppy seed (10%) resulted in a thick mixture, being not easy to apply on teats.

Half a gram of poppy seed was counted six times and resulted in a mean count of 1203 with a standard deviation of 19,6 in 0,501 g of seed. For calculations a count of 1200 per half a gram was used.

Putting 5% poppy seed/manure mixture on 10 artificial teats and washing with water resulted after filtration in a mean recovery of 86% with a standard deviation of 6%.

Results of the first study on an experimental farm (figure 1) showed differences between cows, but in all cases countable amounts of poppy seed in milk were found. Here 10 % w/w of poppy seed was mixed with manure. A mean amount of mixture of 3.4 gram per cow was applied. After application the Petri dishes were weighed again and the number of remaining poppy seed was counted. This resulted in mean count of 400 seed. Calculating this amount on weight (1200 seed per 0.5 gram) resulted in a mean count of 393 seed. Mixing seemed to be accurate.

A mean count of 801 seed was applied on the teats. Mean waiting time was 73 min and the mean in between milking time was 9.5 hours.

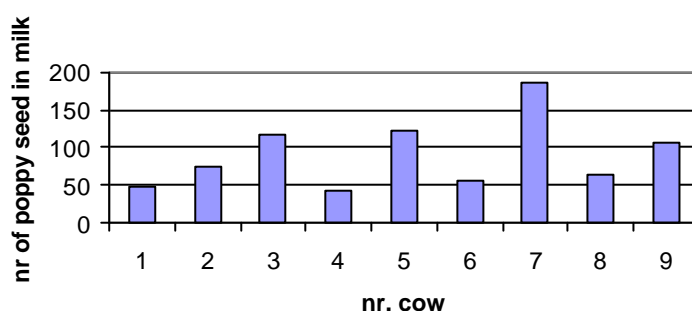


Figure 1: Results of poppy seed in milk from 9 cows on one AM-system without udder cleaning

The mean number of poppy seed in milk for these 9 cows was 91 with a standard deviation of 46.

Also the recovery of the poppy seed was calculated. The mean total recovery was 24% of the amount of seed put on the teats with a standard deviation of 9.1%. From this 24%, 13% was found in the milk, 6% was found on the teats after milking, 3% was found in the pre squirts and 1% in the unit flush water. Cows were not fixed during the waiting time of at least half an hour, so probably some losses of mixture off the teats and part of the variability might be due to this free movement of animals in the barn. During the rest of the experiments cows were fixed at the fence.

From the results of the second experiment on an experimental farm (figure 2) an effect of teat cleaning on carry over of poppy seed into milk can be concluded. The mean count in milk from cows without teat cleaning was 342 (standard deviation 82) and in milk from cows with teat cleaning was 41 (standard deviation 44). So a reduction of poppy seed in milk of 88% could be calculated. Also the recovery of poppy seed in milk was 13% for cows without teat cleaning, which is comparable to the results from the first experiment (figure 1) Recovery of poppy seed in milk was 2% for cows with teat cleaning. From two cows the number of poppy seed in pre squirts was counted and resulted in a mean recovery of 11%.

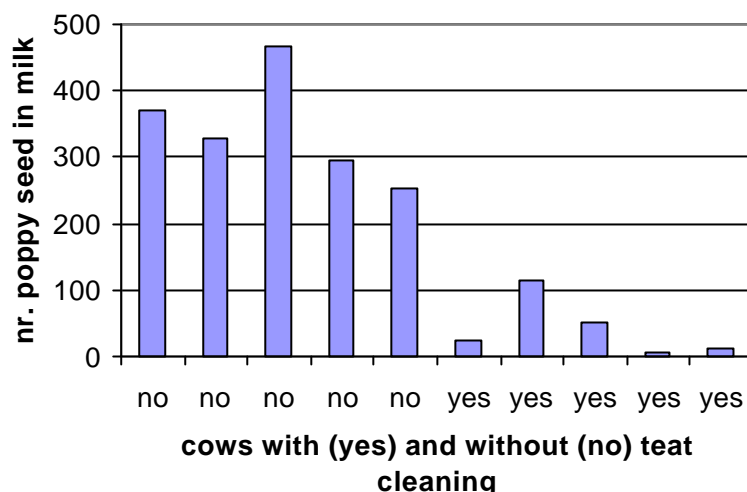


Figure 2: Results of poppy seed in milk from 5 cows with teat cleaning and 5 other cows without teat cleaning on the same AM-farm.

These results indicated that the method of applying poppy seed/manure mixtures on the teats was suitable as a method to determine the efficiency of teat cleaning of different AM-systems.

### 3.1.2 Effectiveness of cleaning procedures

#### 3.1.2.1 Results per brand

The most important results of the statistical analysis are given in table 3. Because a significant interaction of effect of teat cleaning and brand was found, the results per brand, with and without teat cleaning are presented. In all cases an effect of teat cleaning was found, but differences between brands could be shown. Teat cleaning of brand 4 was comparable with conventional teat cleaning. Brand 2, 3 and 6 seemed to be less effective in teat cleaning (50-70% reduction). In figure 3 the effect is visualised.

Differences between brands may be due to the different methods of teat cleaning, but brands with less effective teat cleaning did not all belong either to AM with cup or with brush cleaning devices. A better cleaning by either system could not be detected.

The level of reduction of the manual teat cleaning was comparable with the results from Magnusson et al., (2002) regarding removal of bacterial spores where a maximal reduction of 96% (moist washable towel + dry paper (10 + 10s)) compared to no cleaning was found. Melin et al. (2002) found better removal of spores with a VMS™ teat cleaning procedure (98% reduction) than with conventional manual cleaning (66.5% reduction). In that study the manual cleaning was performed according to normal preparation procedure in the milking parlour. In our study the manual teat cleaning was also a normal preparation procedure (about 10 sec per cow with a moist washable towel on one farm and on the other farm with dry paper during about 10 sec per cow). Our reduction of 99% was better than for spores, probably because of better removal properties of poppy seed or less adhesion of poppy seed to the teats. Also less poppy seed than spores could be applied to the teats because of different diameters of the seed and spores. Vries and Stadhouders (1977) found a maximal reduction of butyric acid spores of 90% with intensive udder preparation and not too high levels of spores.

Obvious is also the difference between brands in level of poppy seed in milk from cows without teat cleaning. These differences may be partly caused by some practical problems occurring during the experiments.

In some cases the pre squirting could not be excluded, because the AM-system could not apply teat cups to the teats without pre squirting (see 2.1.3.2). Sometimes the pre squirts came into the milk or were added to the milk, sometimes these were separated. But in all cases the pre squirts coming into the milk or not have been treated equal per brand for both teat cleaning and no teat cleaning.

In some cases applying the teat cups to the teats was not done automatically, because when the teat cleaning was switched off, the AM system could only work by applying teat cups by hand. Also when teat cups were applied automatically and the location of the teats was difficult to find, teat cups were applied manually. So applying teat cups to the teats was in general comparable for all farms. One can imagine that during attachment of teat cups at the teats and not finding the teat immediately, contact between teat cup and teat with mixture of manure/poppy seed might result in loss of poppy seed.

**Table 3: Back-transformed mean percentages of probability of poppy seed in milk from cows with and without teat cleaning. Reduction of effect of teat cleaning is calculated from these probabilities**

Teat cleaning/ brand	No (%)	Yes (%)	Reduction (%)
Conventional	8.3	0.1	99
1	10.5	1.3	88
2	15.0	6.6	56
3	23.2	8.4	65
4	18.1	0.2	99
5	24.6	2.5	90
6	11.0	5.3	51

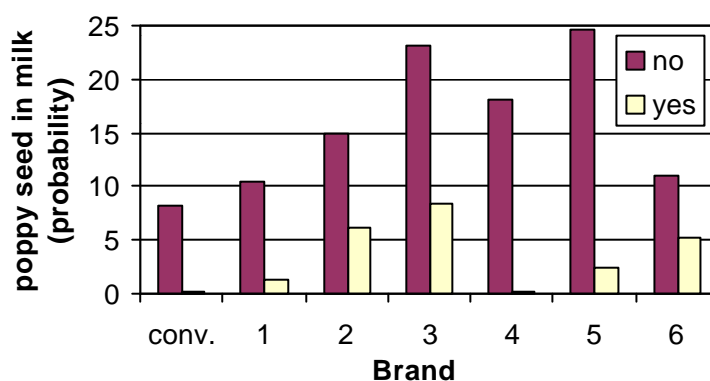


Figure 3 Percentage of mean probability of poppy seed in milk from cows with (yes) and without (no) teat cleaning per brand.

The mean reduction of poppy seed by teat cleaning over all brands was 78%. Schuiling (1992) found in a comparable experiment a mean reduction with lithium as tracer on one AM preliminary system of 69%.

### 3.1.2.2 Results per farm

Results per farm are presented in figure 4 and in table 4. Farms and brands are mixed in these results. The recovery of poppy seed in milk showed differences between farms. Possible explanations have been discussed (3.1.2.1.) These results were not based on the statistical analysis; during statistical analysis differences between farms in the amount of mixture of manure/poppy seed put on the teats have been corrected. A positive relation between size of teat surface and amount of mixture applied to the teats has been found.

However, the reduction after teat cleaning was in the same order for the two farms per brand, especially for the higher reduction brands (>90% reduction: table 3). The reduction showed more variation between farms when this reduction was lower than 90%.

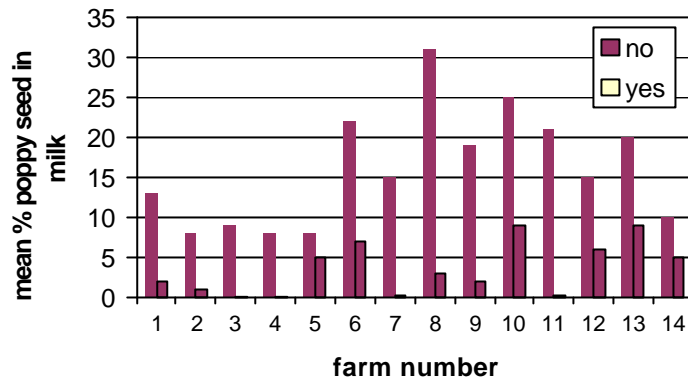


Figure 4: Mean percentage of poppy seed in milk of five cows per farm for teat cleaning (yes) and five cows per farm for no teat cleaning (no).

**Table 4: Results per farm of recovery of poppy seed in percentage in milk**

Far m	Re- duc- tion (%)	No teat cleaning (% re- covery)	Teat cleaning (% re- covery)	Mean recov- ery (%) in milk	Mean milk pro- duction (kg)	Applied on teats per cow (mea- n g mix- ture)	Mean wait- ing time (min)	In be- tween milking time (h)	Recovery in other samples
1	86	13	2	7	12.8	5.17	72	10:51	24% total
2	90	8	1	4	12.3	5.01	49	9:30	11% in premilking
3	99	9	0,1	4	14.5	3.68	64	10	
4	100	8	0,1	5	10.9	3.27	121	10	
5	29	8	5	6	13.9	4.68	73	10:04	10% in pre squirts
6	66	22	7	15	15.6	5.12	75	11:54	
7	99	15	0,2	8	12.5	3.86	101	11:22	
8	91	31	3	17	10.9	5.32	93	11:20	
9	89	19	2	11	12.0	5.70	72	10:28	
10	62	25	9	17	10.0	5.22	78	9:08	
11	99	21	0,3	11	14.7	4.95	82	10:43	
12	61	15	6	10	14.5	5.48	96	10:18	
13	55	21	9	16	9.3	4.38	121	10:28	70% total
14	56	10	5	7	11.4	4.76	55	10:34	44% total
Mean	90	16	4	10	12.5	4.46	82	10:29	

The results of farm number 5 showed less reduction through teat cleaning. This was due to the maintenance of the teat cleaning system, which was not in good order. Normally brushes have to be replaced yearly, but because of high costs this is sometimes delayed.

On other farms with less reduction not due to the maintenance of the system, this resulted from the teat cleaning method itself. Maybe the water was not flowing fast enough for a good removal of mixture.

Translation of these results to practice may indicate that teat cleaning in practice might be less effective, although the waiting time after application was in this study less than normal waiting times and the adhesion of poppy seed is probably less strong than dried manure and/or bacterial spores.

When teats are soiled with butyric acid bacteria spores with better adhesion to the teats and with very high counts of these spores, a better reduction after teat cleaning may result in better milk quality with lower spore counts (De Vries and Stadhouders, 1977).

Therefore maintenance of teat cleaning devices of AM systems is very important for effective cleaning of teats.

## 3.2 Investigation based on combination of methods

In the second part of the investigation a combination of 4 methods was applied for evaluation of teat cleaning efficiency. The results are presented for the individual methods as well as for combination of methods.

### 3.2.1 *Visual inspection of teats and sediment tests*

The results of visual inspection before and after cleaning as well as the results of sediment tests after teat cleaning are summarized per farm in figures 5, 6 and 7.

A large variation in the distribution of results from visual inspection was found between individual farms. Before teat cleaning on most farms between 25 and 50 % of teats were visually clean (Score 1). After cleaning more variation was found between the individual farms by visual inspection and sediment tests on teats.

The differences between brands (figures 8, 9, 10) are at least partially due to the very different results of individual farms. Cows on farm B had extremely dirty teats before cleaning and also after cleaning more than 40 % of teats were scored 3 or 4.

The results of sediment scores were similar to those of visual inspection after teat cleaning (Figure 6 and 7, table 6).

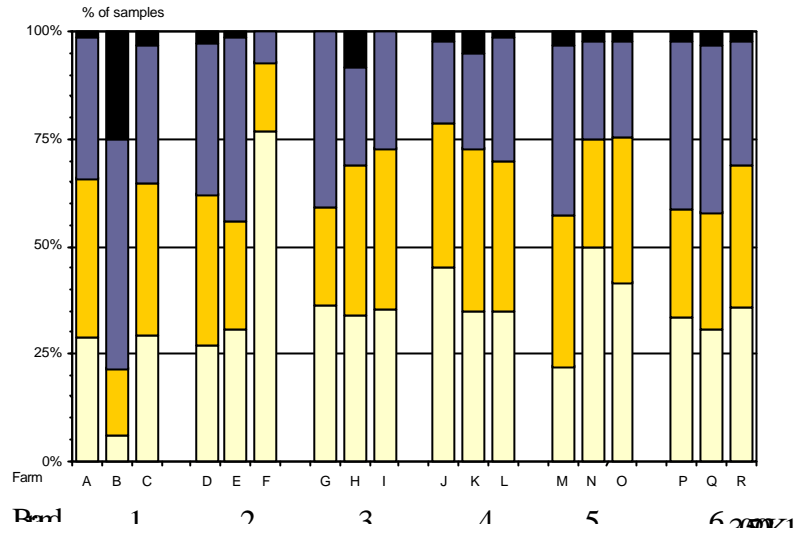


Figure 5: Results of visual evaluation of teats before cleaning - summarized per farm

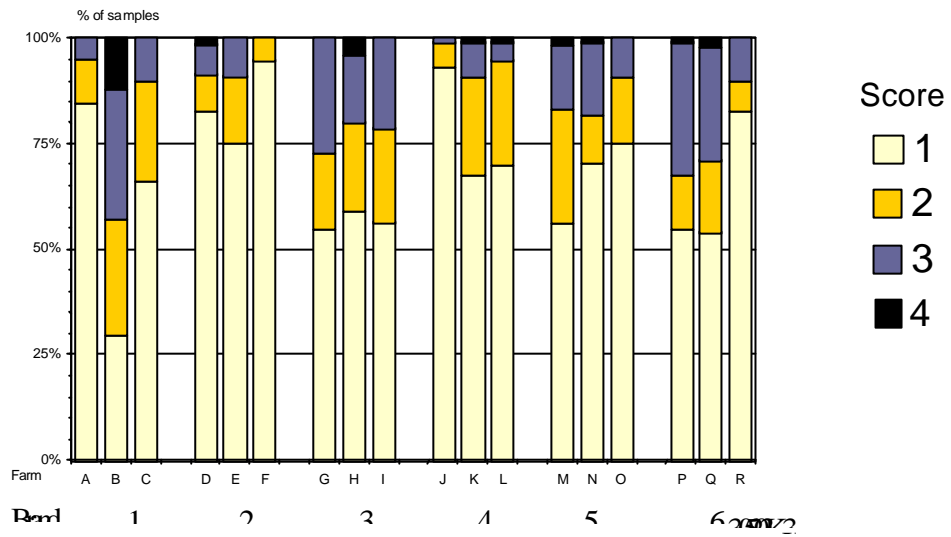


Figure 6: Results of visual evaluation of teats after cleaning - summarized per farm

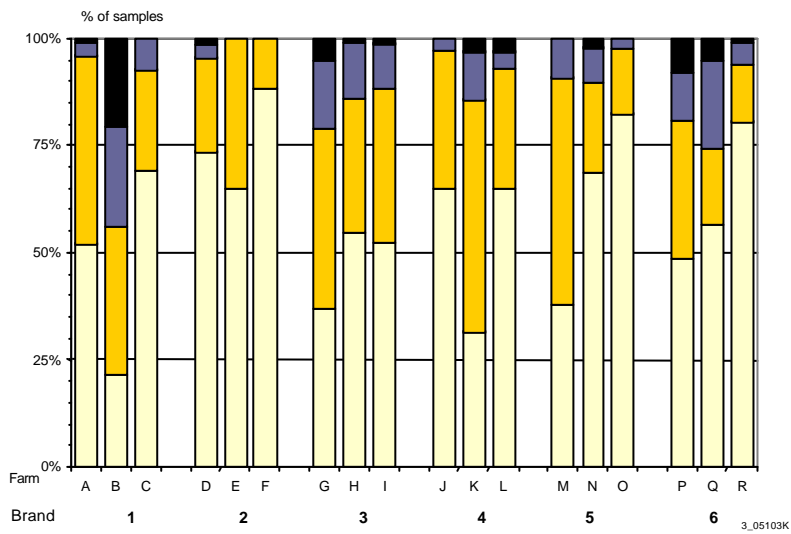


Figure 7: Results of sediment test of teats after cleaning - summarized per farm

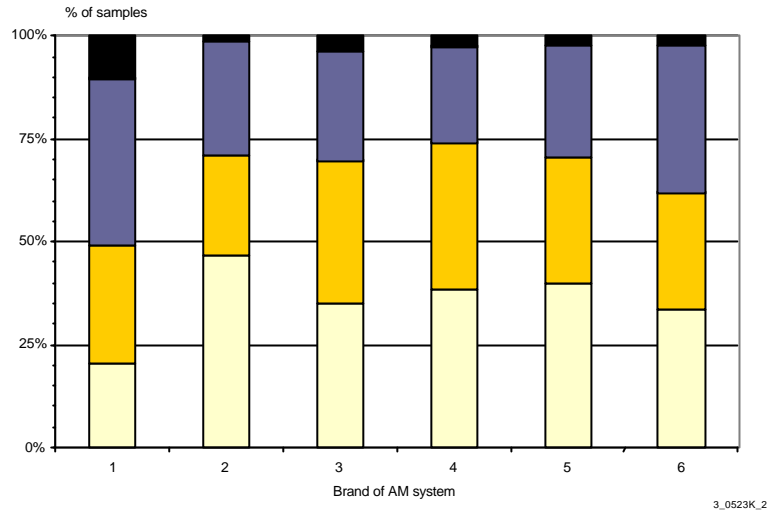


Figure 8: Results of visual evaluation of teats before cleaning - summarized per brand

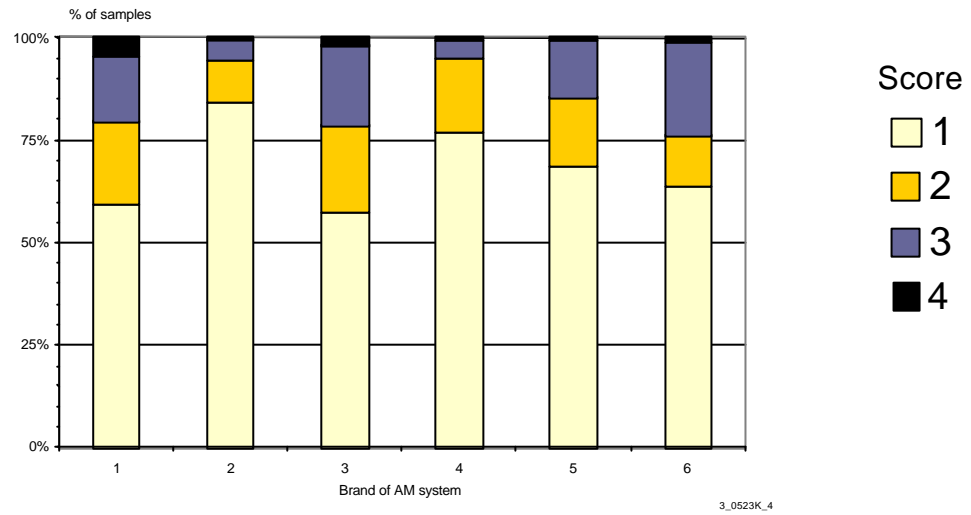


Figure 9: Results of visual evaluation of teats after cleaning - summarized per brand

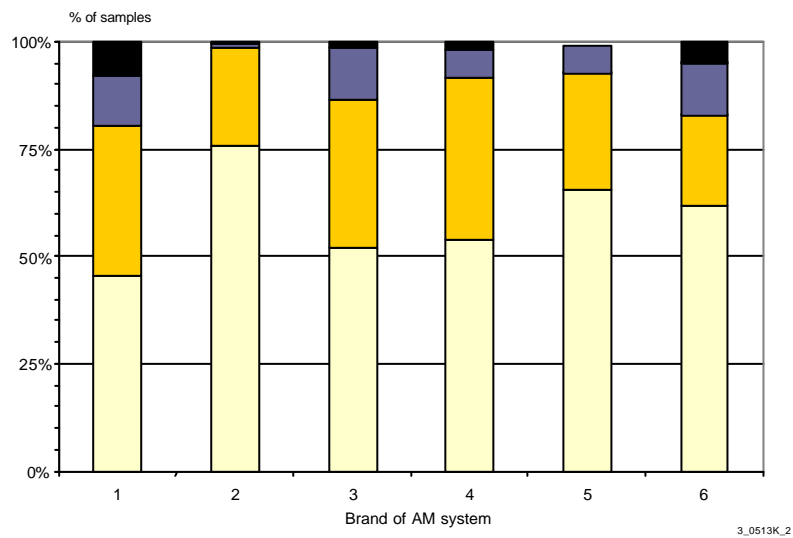


Figure 10: Results of sediment test of teats after cleaning - summarized per brand

In table 5 teat cleaning efficiency is shown for the different brands based on visual inspection of teats before and after cleaning. Teats which were visually clean before cleaning were excluded from the analysis.

**Table 5: Teat cleaning efficiency as reduction of visual contamination**

Brand	No. of teats with score 2, 3 or 4 before cleaning	Difference in scores before versus after cleaning (in % of teats scored >1 before cleaning)	
		0*	≥1*
1	207	26.6	73.4
2	141	14.9	85.1
3	140	53.6	46.6
4	182	18.7	81.3
5	155	34.2	65.8
6	198	41.9	58.1

0 = no reduction of visual contamination, ≥1 = reduction of visual contamination by at least one score point

Brand 2 and 4 showed the best cleaning results regarding reduction of visual contamination. A reduction was observed in more than 80 % of teats. For brands 3 and a removal of dirt was observed in less than 60 % of teats. In interpreting these results it has to be taken into account, that the level of contamination before cleaning was not considered in these calculations. Therefore the results give only a tendency.

In table 6 test results of visual inspection after teat cleaning are compared with the results of sediment tests over all teats sampled after cleaning.

**Table 6: Comparison of results of visual inspection and sediment test on teats after cleaning, data of all farms**

Frequency Percent	Visual Score				S	
	1	2	3	4		
Sediment test	1	824 53.4	79 5.1	10 0.7	1 0.1	914 59.2
	2	216 14.0	148 9.6	93 6.0	1 0.1	458 29.7
	3	18 1.2	24 1.6	80 5.2	4 0.3	126 8.2
	4	3 0.2	4 0.3	23 1.5	16 1.0	46 3.0
S	1061 68.7	255 16.5	206 13.3	22 1.4	1544 100.0	

69.2 % of all teats were scored identical by visual inspection and sediment test (table 6, grey fields). The sediment test scored higher in 18.7 %, visual inspection scored higher in 12.2 %. Deviation of more than one score point occurred in only 2.4 % of samples. The scales of visual inspection and sediment test do not necessarily correspond, because they were not harmonized.

Summarizing the results of all farms 31 % of all teats did not appear visually clean after the teat cleaning procedure. With the sediment test in about 40 % of teats residual dirt was detected after teat cleaning.

### 3.2.2 Total bacterial counts resp. ATP in teat swabs

The distribution of TBC ( $\text{Log}_{10}$  cfu/ml) in teat swabs before and after cleaning is shown per farm in figure 11 and 12. The cleaning effect is expressed as the difference of TBC before cleaning ( $\text{Log}_{10}$  cfu/ml) minus TBC after cleaning ( $\text{Log}_{10}$  cfu/ml) (Figure 13). Values below zero indicate that after cleaning a higher contamination was found than before cleaning.

It is obvious that the initial bacterial load of teats varies largely within and between the individual farms as does the cleaning efficiency.

When teat cleaning efficiency is calculated as the difference between contamination before and after cleaning farms B, G, H, I, K, L, N and Q have a high percentage of negative results indicating higher contamination after cleaning. Most of these are farms had a relatively low initial contamination level.

The results are summarized per brand of AM in figures 14 to 16. The distribution is more equal per brand. Brand 3 and 4 have high percentages of negative results with higher contamination levels after cleaning than before (figure 16). For these two brands the respective means of cleaning efficiency are close to zero.

The corresponding results for ATP measurements are shown in figures 17 to 22. Here also a large variation is seen between the individual farms. For teat cleaning efficiency calculated from ATP measurements (ATP before cleaning ( $\text{Log}_{10}$  RLU) minus ATP after cleaning ( $\text{Log}_{10}$  RLU)) slightly different effects compared to teat cleaning efficiency based on TBC are observed per farm. Not more than 25 % of increased teat contamination after teat cleaning (negative values for teat cleaning efficiency) occurred.

It seems that with low bacterial contamination before cleaning the bacterial load is increased by cleaning. This could be due to the wet cleaning procedure by which bacteria are suspended and more easily detectable after cleaning. Another explanation is that by insufficient disinfection of the teat cleaning device bacteria might be added to the teat surface during the cleaning procedure. Considering only TBC with low level contamination no cleaning would probably be preferable.

If calculation of teat cleaning efficiency per brands is based on ATP measurements a better teat cleaning efficiency is calculated than compared to evaluation based on TBC (figures 16 and 22). ATP measures contamination by organic material also apart from bacteria. For example skin cells could be a source for ATP. Probably also with low level contamination some organic material is removed which explains the difference between TBC- and ATP-based evaluation.

The calculation of teat cleaning efficiency based only on the difference between before and after cleaning does not take into account the different contamination levels on the farms. That is why the comparability between manufacturers is limited. For these purposes an analysis of variance was carried out (see chapter 3.2.4).

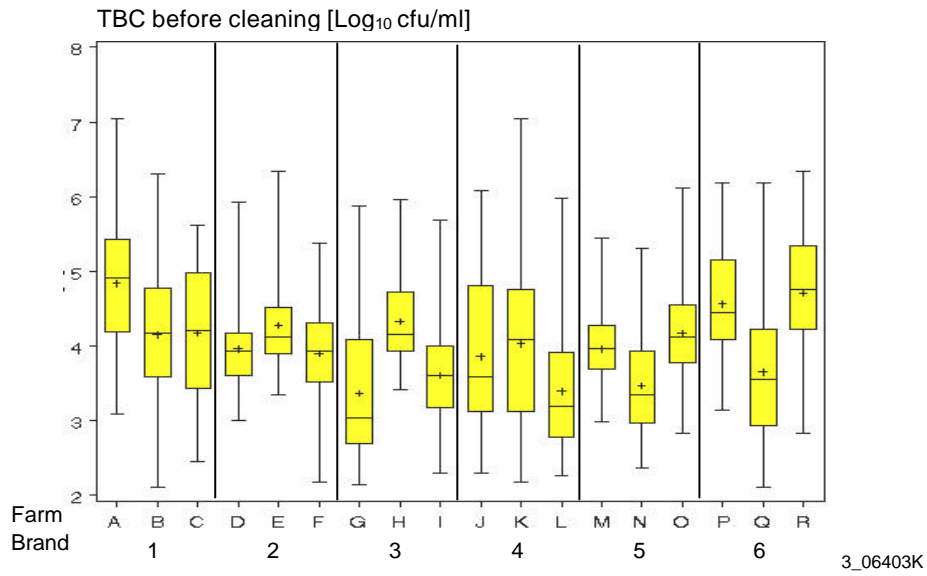


Figure 11: Results of TBC in teat swabs before cleaning - summarized per farm

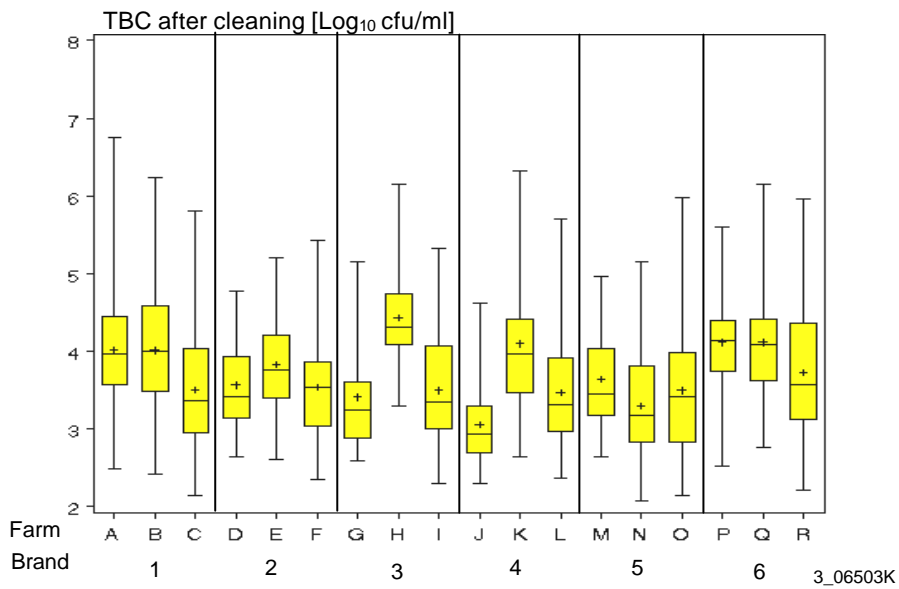


Figure 12: Results of TBC in teat swabs after cleaning - summarized per farm

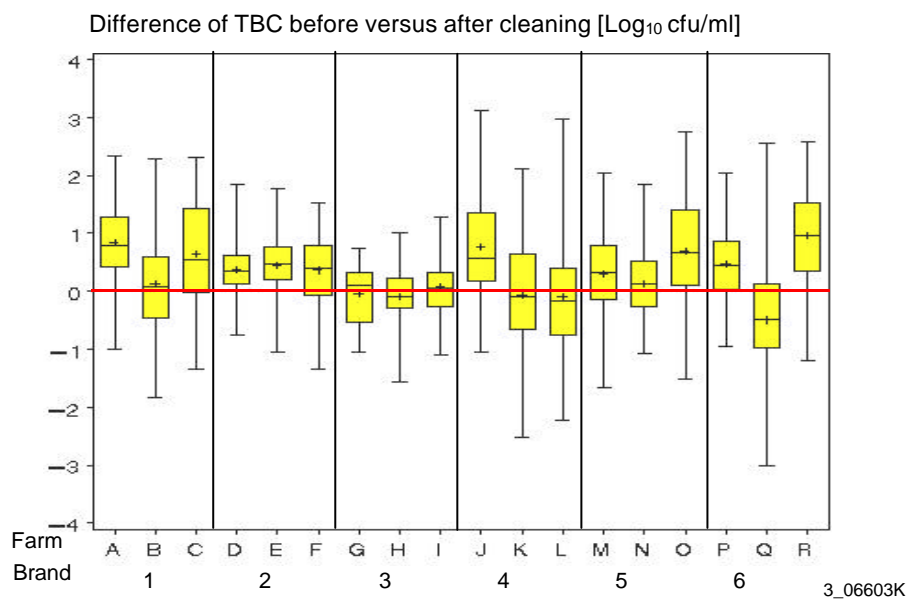


Figure 13: Teat cleaning efficiency based on TBC - summarized per farm

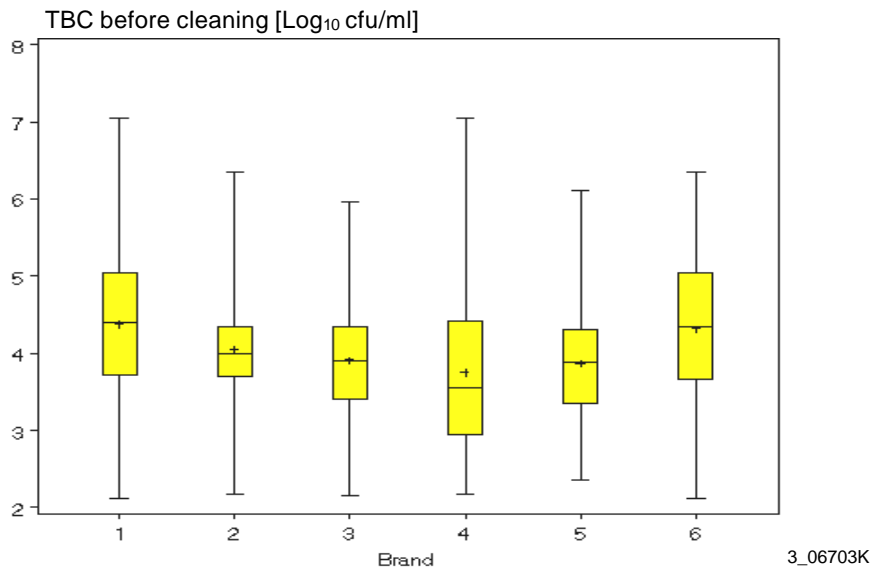


Figure 14: Results of TBC in teat swabs before cleaning - summarized per brand

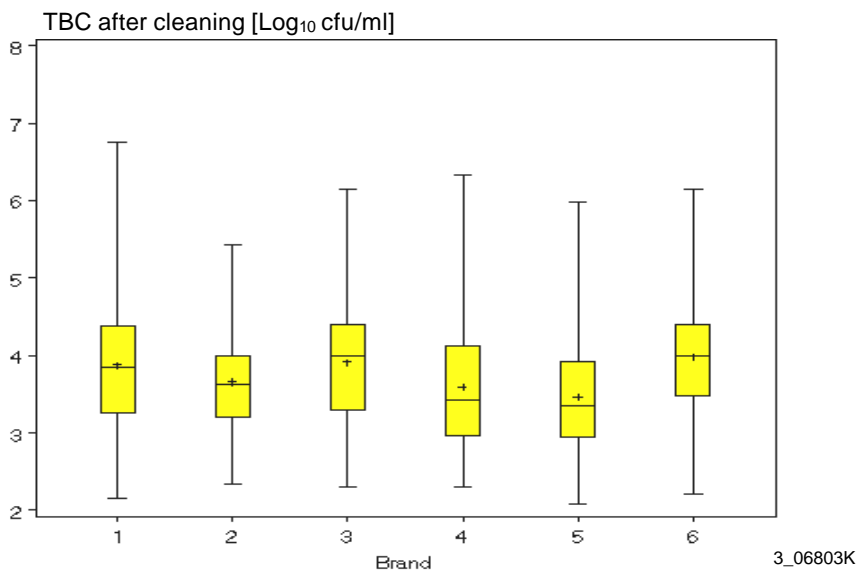


Figure 15: Results of TBC in teat swabs after cleaning - summarized per brand

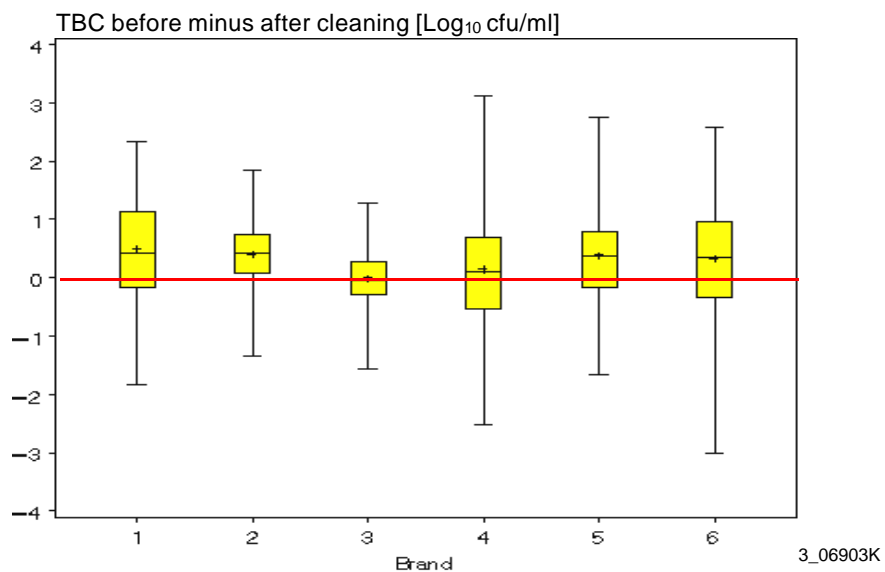


Figure 16: Teat cleaning efficiency based on TBC - summarized per brand

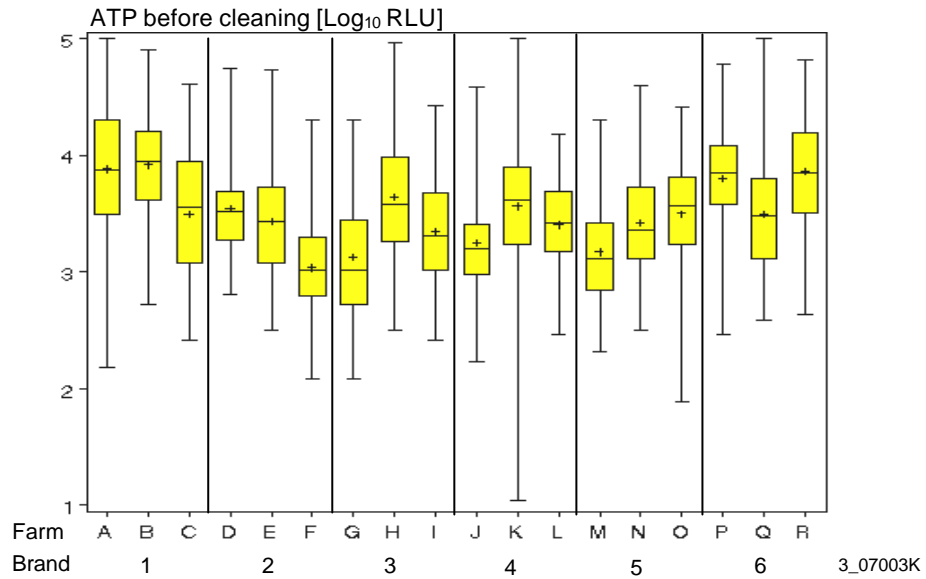


Figure 17: Results of ATP in teat swabs before cleaning - summarized per farm

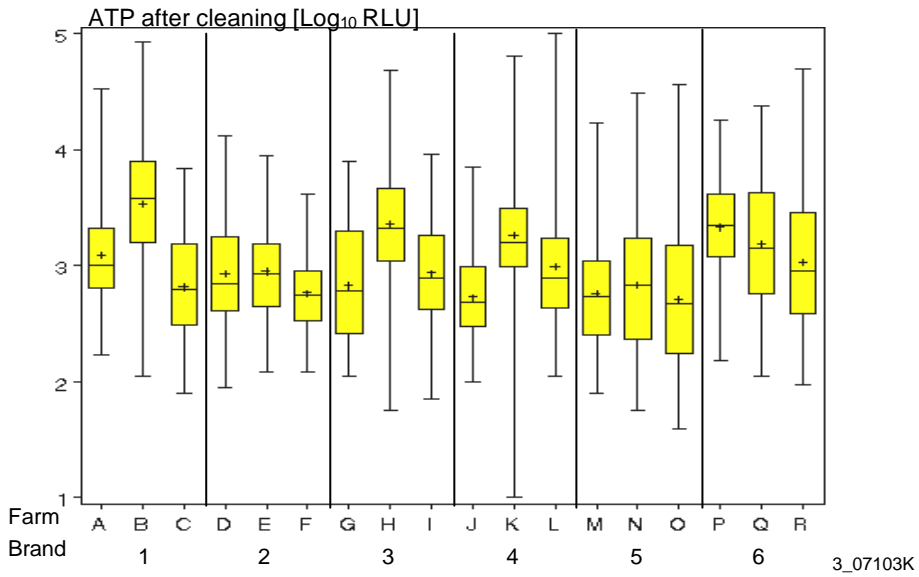


Figure 18: Results of ATP in teat swabs after cleaning - summarized per farm

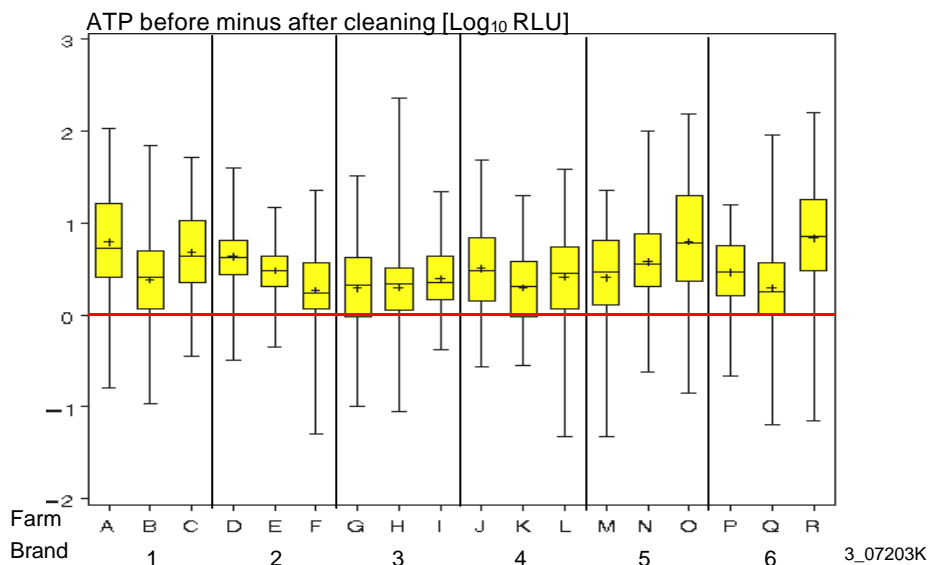


Figure 19: Teat cleaning efficiency based on ATP - summarized per farm

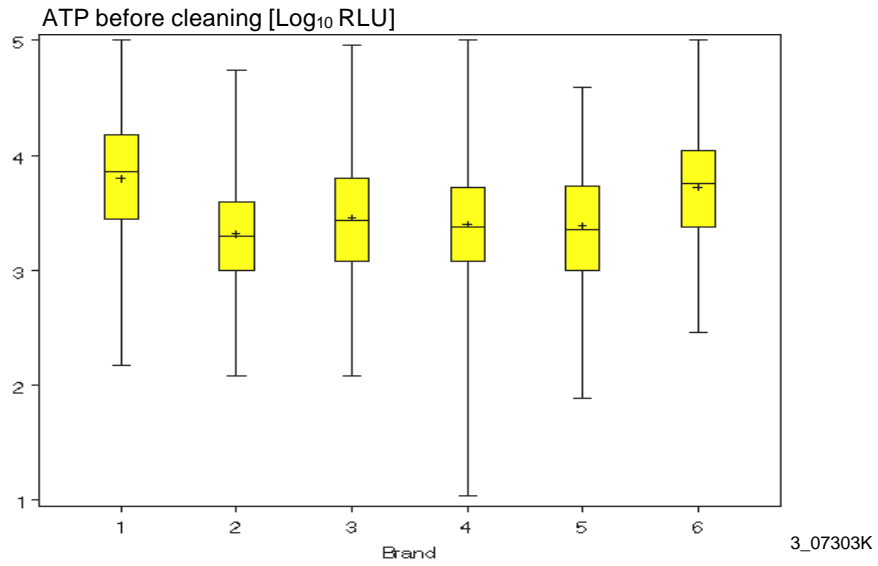


Figure 20: Results of ATP in teat swabs before cleaning - summarized per farm

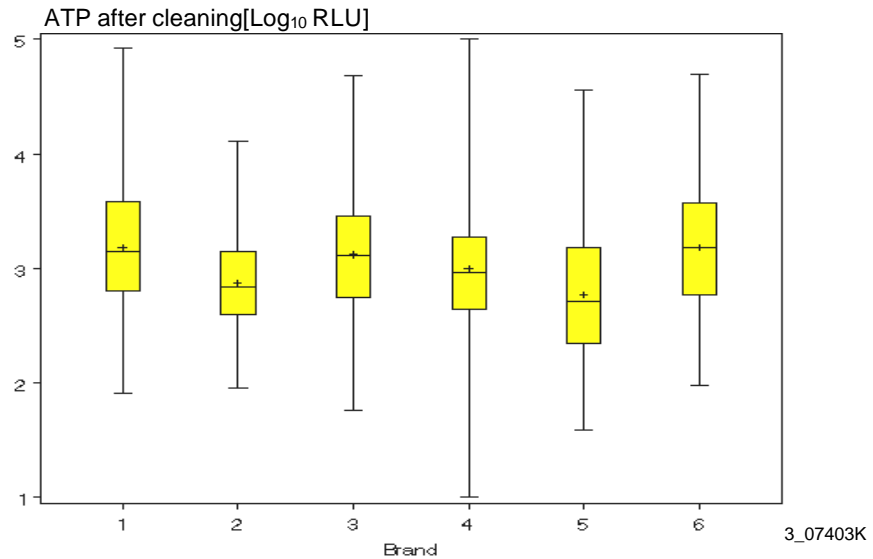


Figure 21: Results of ATP in teat swabs after cleaning - summarized per brand

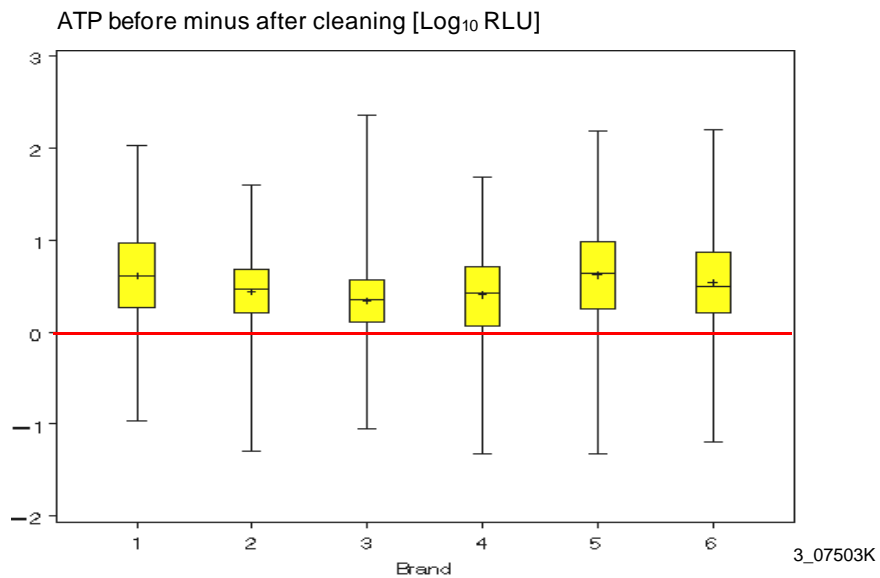


Figure 22: Teat cleaning efficiency based on ATP - summarized per brand

### 3.2.3 Correlation between different parameters for determination of teat cleanliness

In figure 23 the correlation between ATP and TBC in teat swabs is shown for samples taken after cleaning.

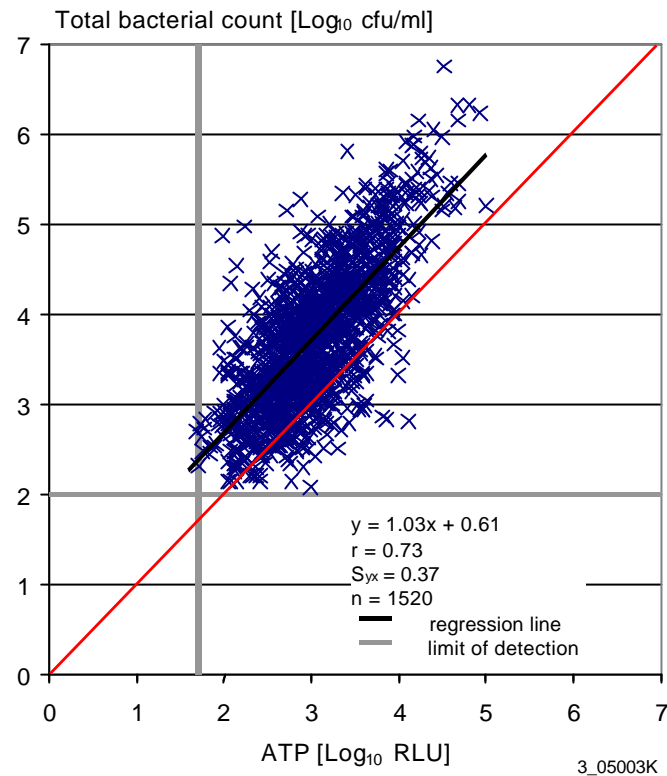


Figure 23: Correlation between ATP measurement and TBC in teat swabs after cleaning

The correlation of  $r^2 = 0.53$  between ATP [Log<sub>10</sub> RLU] and TBC [Log<sub>10</sub> cfu/ml] is lower than that determined by Finger and Sicho (2001) with  $r^2 = 0.64$ . This is probably due to the fact that in their investigation ATP and TBC were both measured per cm<sup>2</sup> of teat surface. They stated that ATP can be used to show average changes in teat cleanliness due to pre-milking preparation. Other investigators could not find a close relationship (Johnson et al., 2003) which might be due to a different solution used to moisten the swab. In our investigation peptone water was used in which no major changes of bacterial counts occur if the solution is cooled appropriately.

The relationship between visual evaluation resp. sediment tests and TBC resp ATP in samples taken after cleaning is shown in figures 24 - 27.

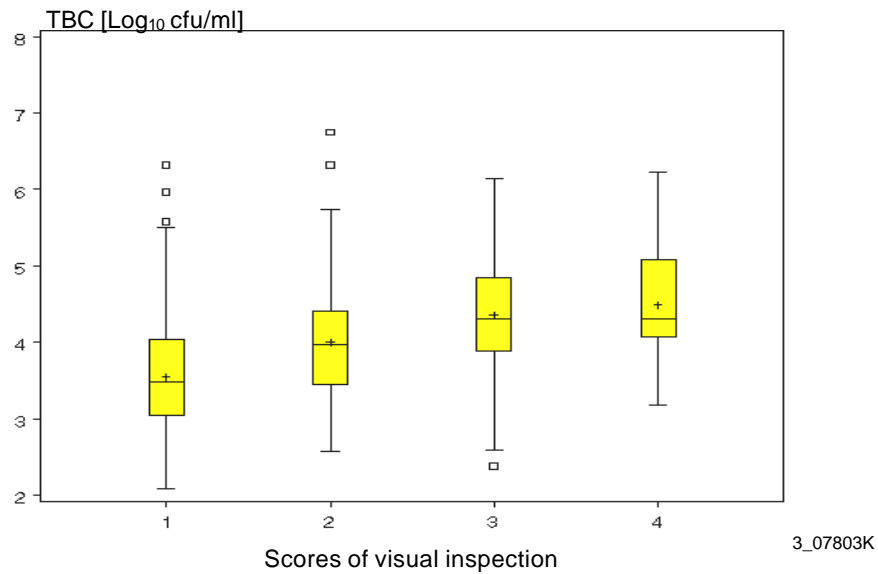


Figure 24: Results of TBC in teat swabs with regard to visual inspection after cleaning (Score 1: n=1019, Score 2: n=255, Score 3: n=207, Score 4: n=23)

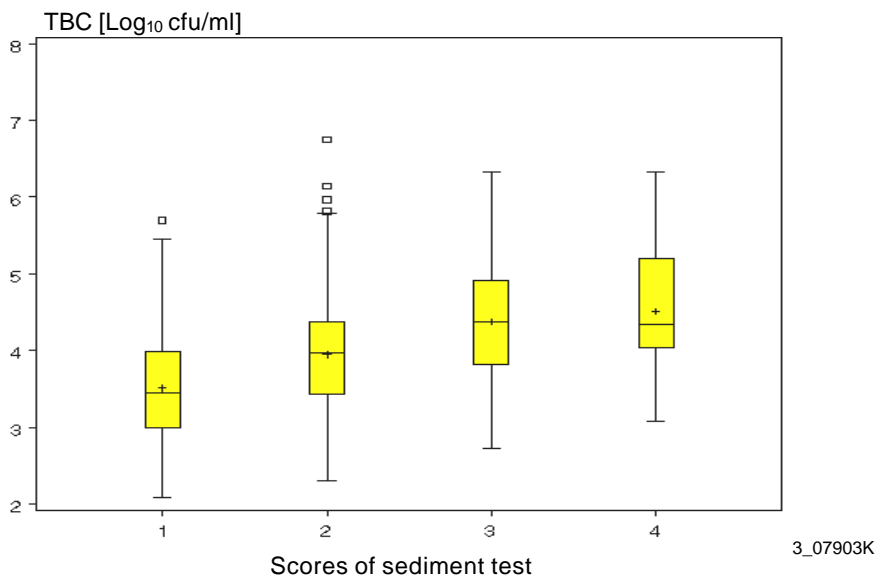


Figure 25: Results of TBC in teat swabs with regard to sediment test after cleaning (Score 1: n=883, Score 2: n=435, Score 3: n=125, Score 4: n=46)

When TBC [Log<sub>10</sub> cfu/ml] is compared to visual evaluation or sediment tests a large variation and overlapping of the TBC values corresponding to visual scores is observed. The means of TBC within score 3 and 4 are almost the same.

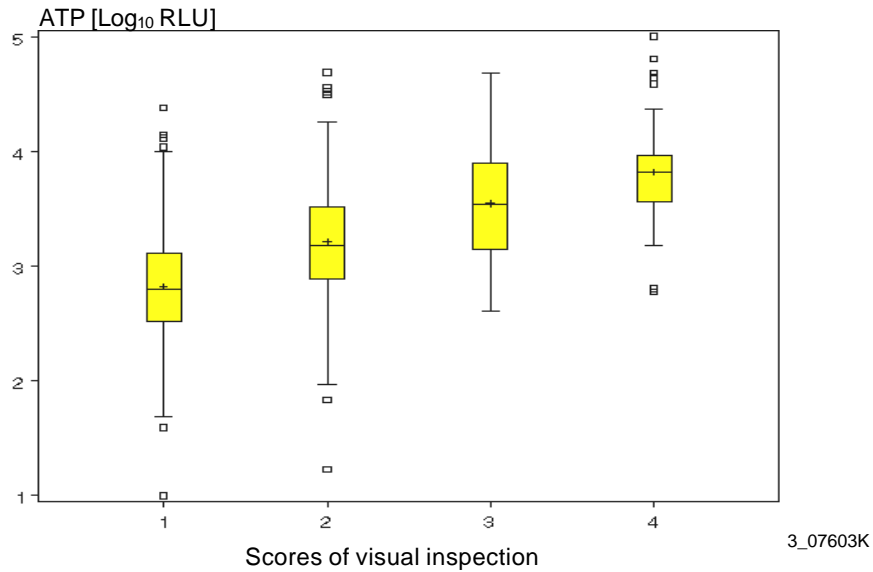


Figure 26: Results of ATP in teat swabs with regard to visual inspection after cleaning (Score 1: n=1080, Score 2: n=257, Score 3: n=211, Score 4: n=23)

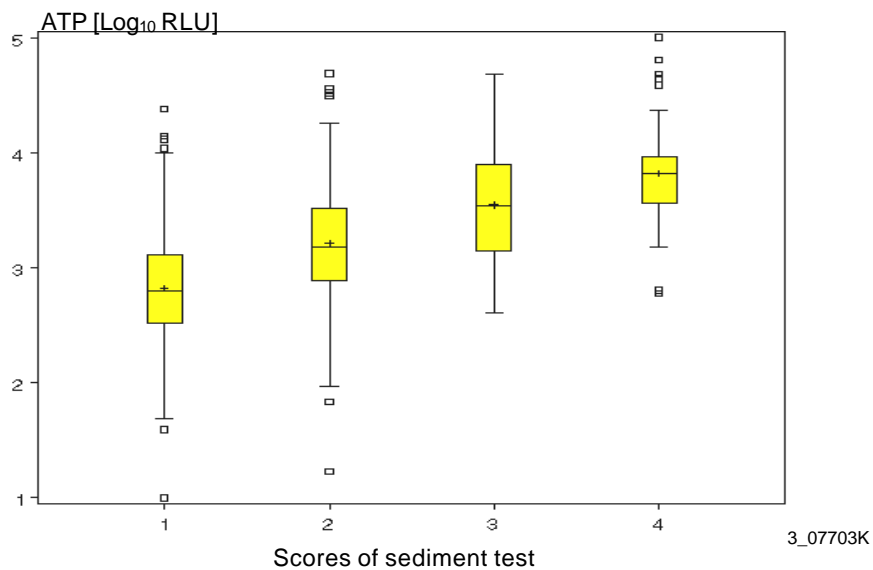


Figure 27: Results of ATP in teat swabs with regard to sediment test after cleaning (Score 1: n=924, Score 2: n=458, Score 3: n=126, Score 4: n=46)

With ATP measurements a slightly stronger differentiation is possible between the 4 scores of visual evaluation or sediment test, especially between scores 3 and 4. This could mean that ATP is more suitable for differentiation of teat cleanliness than TBC. In addition, with ATP also other sources of organic contamination than bacteria e.g. cells from the teat surface or from milk are detected.

### 3.2.4 Analysis of variance

The analysis of variance based on determination of TBC ( $\text{Log}_{10}$  cfu/ml before cleaning minus  $\text{Log}_{10}$  cfu/ml after cleaning) explained 50 % of variance. Factors with significant influence on teat cleaning efficiency were brand of robot, farm and initial contamination. Between front and hind quarters no significant difference was detected. The main factors of influence were the initial contamination of teats and the individual farm. When the factor brand was excluded these two factors together still accounted for 50 % of variance.

The analysis of variance based on determination of ATP explained only 32 % of variance. In addition to brand of robot, farm and initial contamination (as ATP) also the quarter had a significant influence on teat cleaning efficiency. Again the main influence factors were the initial contamination of teats and the individual farm, together accounting for 32 % of variance after excluding brand as a factor. The cleaning efficiency on the front quarters was significantly higher ( $\text{LSQ}_M=0.54$ ) than on the hind quarters ( $\text{LSQ}_M=0.46$ ) with  $p<0.05$ .

Part of the variance that could not be explained by the model is probably due to the fact that no standardized area of the teat surface was sampled. If values were corrected e.g., per  $\text{cm}^2$  probably a better comparison could be made. However, a standardized sampling procedure would only be applicable if the robot actions could be started manually. Otherwise time for sampling without interfering with the robot actions is too short.

It has also to be taken into account, that teat contamination is not equally distributed over the whole surface of the teat. Probably also the cleaning effect is not the same at all sides of the teats, for example brushes only move in a certain direction and cups have certain locations for water inlet. This could also explain a number of negative results when teat cleaning efficiency is calculated as the difference before versus after cleaning.

The Least Square Means ( $\text{LSQ}_M$ ) for the different brands derived from the analysis of variance are summarized in table 7.

**Table 7: Cleaning efficiency per brand based on TBC resp. ATP measurements in teat swabs**

Brand	Cleaning efficiency - Least Square Means ( $\text{LSQ}_M$ ) $\pm$ standard error	
	TBC*	ATP*
1	0.36 <sup>a</sup> $\pm$ 0.04	0.51 <sup>bc</sup> $\pm$ 0.03
2	0.41 <sup>a</sup> $\pm$ 0.04	0.55 <sup>b</sup> $\pm$ 0.03
3	0.15 <sup>b</sup> $\pm$ 0.05	0.40 <sup>d</sup> $\pm$ 0.04
4	0.37 <sup>a</sup> $\pm$ 0.04	0.46 <sup>cd</sup> $\pm$ 0.03
5	0.48 <sup>a</sup> $\pm$ 0.04	0.67 <sup>a</sup> $\pm$ 0.03
6	0.17 <sup>b</sup> $\pm$ 0.04	0.44 <sup>cd</sup> $\pm$ 0.03

\* Different superscripts within the same column indicate significant differences ( $p<0.05$ )

TBC in  $\text{Log}_{10}$  cfu/ml before cleaning minus  $\text{Log}_{10}$  cfu/ml after cleaning

ATP in  $\text{Log}_{10}$  RLU before cleaning minus  $\text{Log}_{10}$  RLU after cleaning

The ranking of brands according to teat cleaning efficiency was the same for evaluation based on ATP and TBC with the exception that Brand 1 and 4 changed places with ATP.

The total cleaning efficiency was much lower compared to manual cleaning methods as determined under experimental conditions with the reference methods. Cleaning with a dry paper towel reduced TBC on average by 1.1 Log-units, wet cleaning with subsequent drying led to a reduction by 1.5 Log-units (D13).

Only a few ATP measurements were available for the reference method, but still the average cleaning efficiency was a reduction of 0.56 resp. 1.26 Log RLU which means that the cleaning efficiency of some brands was similar to or even better than manual dry cleaning, but not comparable to wet manual cleaning.

The Least Square Means (LSQ<sub>M</sub>) for the different farms within brand are summarized in table 8:

**Table 8: Cleaning efficiency per farm based on TBC resp. ATP measurements in teat s wabs**

Brand	Farm	Cleaning efficiency - Least Square Means (LSQ <sub>M</sub> ) ± standard error	
		TBC [Log <sub>10</sub> cfu/ml before cleaning minus Log <sub>10</sub> cfu/ml after cleaning]	ATP [Log <sub>10</sub> RLU before cleaning minus Log <sub>10</sub> RLU after cleaning]
1	A	0.41 <sup>a</sup> ± 0.07	0.62 <sup>a</sup> ± 0.04
	B	0.08 <sup>b</sup> ± 0.06	0.20 <sup>b</sup> ± 0.04
	C	0.58 <sup>a</sup> ± 0.08	0.69 <sup>a</sup> ± 0.05
2	D	0.44 <sup>a</sup> ± 0.07	0.62 <sup>a</sup> ± 0.05
	E	0.33 <sup>a</sup> ± 0.06	0.52 <sup>a</sup> ± 0.04
	F	0.45 <sup>a</sup> ± 0.06	0.50 <sup>a</sup> ± 0.04
3	G	0.35 <sup>a</sup> ± 0.13	0.48 <sup>a</sup> ± 0.09
	H	-0.24 <sup>b</sup> ± 0.06	0.23 <sup>b</sup> ± 0.04
	I	0.34 <sup>a</sup> ± 0.06	0.48 <sup>a</sup> ± 0.05
4	J	0.88 <sup>a</sup> ± 0.07	0.64 <sup>a</sup> ± 0.04
	K	-0.06 <sup>b</sup> ± 0.06	0.28 <sup>b</sup> ± 0.04
	L	0.27 <sup>c</sup> ± 0.06	0.46 <sup>c</sup> ± 0.04
5	M	0.36 <sup>a</sup> ± 0.07	0.57 <sup>a</sup> ± 0.05
	N	0.48 <sup>ab</sup> ± 0.06	0.63 <sup>a</sup> ± 0.04
	O	0.61 <sup>b</sup> ± 0.06	0.81 <sup>b</sup> ± 0.04
6	P	0.18 <sup>a</sup> ± 0.06	0.33 <sup>a</sup> ± 0.04
	Q	-0.25 <sup>b</sup> ± 0.06	0.31 <sup>a</sup> ± 0.04
	R	0.60 <sup>c</sup> ± 0.06	0.67 <sup>b</sup> ± 0.04

Different superscripts of farms per brand within the same column indicate significant differences (p<0.05)

For almost all brands significant differences were found between teat cleaning efficiencies on individual farms as measured by TBC or ATP. For almost all farms the derived ranking for the three farms per brand was the same for the evaluation based on ATP or TBC. Main differences between the two evaluation methods were only observed for farms H and Q which may be explained by certain management aspects:

As the only one of the three farms of brand 3 farm H used cold water for teat cleaning which may explain the low teat cleaning efficiency regarding TBC.

In contrast to farms P and R farm Q did not use any disinfectant for the teat cleaning device which explains the negative values for teat cleaning efficiency based on TBC. For ATP the teat cleaning efficiency is comparable to farm P. Obviously bacteria from the brush are smeared over the teat surface during the process intended for cleaning.

Some differences between farms of one brand may be explained by the following observations:

Farm B had extremely dirty cows. The teat cleaning device had been in use for about two years without changes.

Farm O was only working for 2 months with the AM system: one explanation for the better results compared to farms M and N might be that the teat cleaning devices were older on the other farms.

The age of the teat cleaning device seems to play a role for the efficiency of teat cleaning. Unfortunately not on each farm information was available on the age of the devices.

As the initial contamination is one of the major influence factors the teat cleaning efficiency of the 3 brands working with brushes was compared to the 3 brands working with cups for teat cleaning for different teat contamination levels (table 9).

**Table 9: Comparison of cleaning effect: brushes versus cups, two sample t-test per class of contamination**

	Brushes		Cups	
	Mean	n	Mean	n
<b>Bacterial count - class</b>				
2	-0.82 <sup>a</sup>	110	-0.17 <sup>b</sup>	66
3	-0.06 <sup>a</sup>	219	0.13 <sup>b</sup>	320
4	0.54 <sup>a</sup>	265	0.45 <sup>a</sup>	274
5	1.20 <sup>a</sup>	172	0.92 <sup>b</sup>	50
<b>ATP - class</b>				
1	0.12 <sup>a</sup>	105	0.14 <sup>a</sup>	157
2	0.34 <sup>a</sup>	237	0.47 <sup>b</sup>	278
3	0.57 <sup>a</sup>	290	0.62 <sup>a</sup>	211
4	0.84 <sup>a</sup>	210	0.80 <sup>a</sup>	83

Classes of TBC (in Log<sub>10</sub> cfu/ml): class **2**:  $x < 3$ , class **3**:  $3 \leq x < 4$ , class **4**:  $4 \leq x < 5$ , class **5**:  $x \geq 5$ ,  
Classes of ATP (in Log<sub>10</sub> RLU): class **1**:  $x < 3$ , class **2**:  $3 \leq x < 3.5$ ; class **3**:  $3.5 < x < 4$ ; class **4**:  $x \geq 4$   
Different superscripts within the same row indicate significant differences ( $p < 0.05$ )

In general, the amount of contamination removed by teat cleaning effect was higher with high contamination levels. For ATP significant differences between the two cleaning method were only determined for ATP class 2, but more differences were observed for TBC. With low contamination levels the cleaning efficiency of cups was better, whereas with very high contamination level (bacterial class 5) the cleaning with brushes was more efficient.

### 3.3 Suggestion for the evaluation procedure of teat cleanliness in practice

The cleanliness of udder and teats before milking is a requirement according to Council Directive 89/362/EEC. Because individual ideas about what is clean may differ from person to person it would be preferable to have an objective method for determination of teat cleanliness.

Based on the findings of our studies a pragmatic approach is suggested to evaluate teat cleanliness in practice:

The evaluation should preferably be based on only one measurement after cleaning to reduce expenses of time and material. For this purpose ATP measurement is the method of choice. It is an objective and quick test that can be applied on the farm and the results can immediately be demonstrated to the farmer.

With the procedure used in this study an evaluation of teat cleanliness should be based on ATP measurement with a target value of 3.2. If this value is applied about 75 % of teats with visual score 1 (25 % false negatives), about 50 % of score 2 and not more than 25 % of score 3 or 4 are evaluated as clean (Figure 28). As the number of teats with scores 3 and 4 is limited, only a small number of visually dirty teats (score 3 and 4) would be evaluated as clean by ATP measurement. A similar result is found when ATP measurements are compared with the results of the sediment tests (Figure 29).

If the evaluation is based on sediment test and TBC a target value would result in 4.04 for TBC [Log<sub>10</sub> cfu/ml] (Figure 30). The target values of ATP [Log<sub>10</sub> RLU] and TBC [Log<sub>10</sub> cfu/ml] are closely related. A value of 3.2 for ATP corresponds to 3.94 for TBC according to the correlation equation in figure 23.

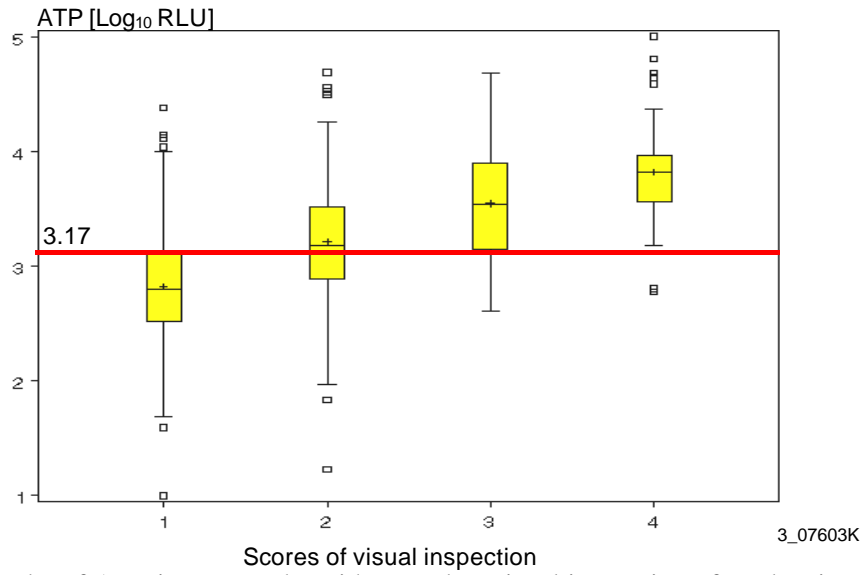


Figure 28: Results of ATP in teat swabs with regard to visual inspection after cleaning, derived target value for ATP: 3.17

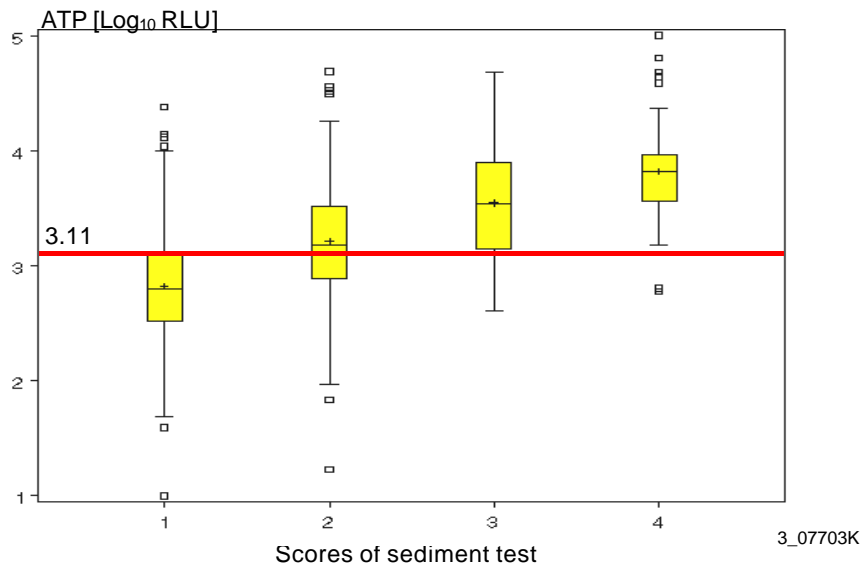


Figure 29: Results of ATP in teat swabs with regard to sediment tests after cleaning, derived target value for ATP: 3.11

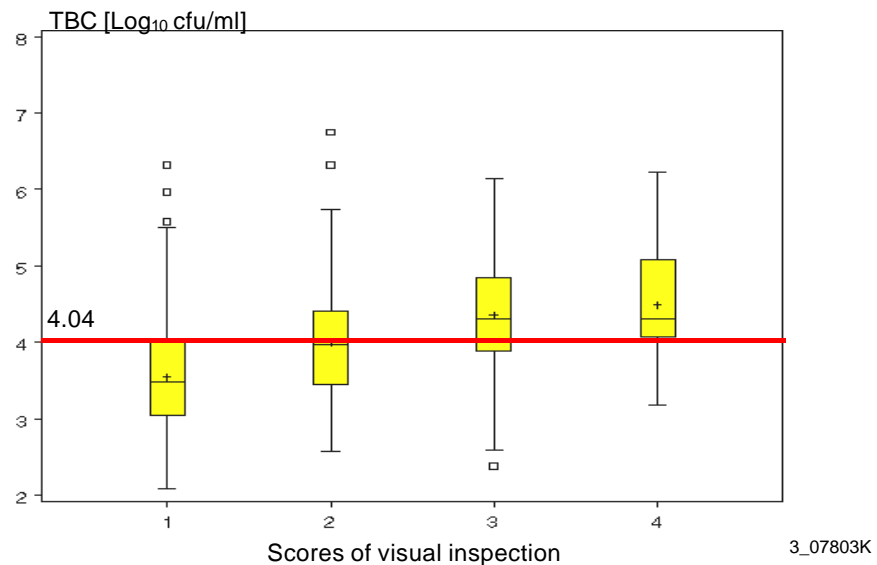


Figure 30: Results of TBC in teat swabs with regard to sediment tests after cleaning, derived target value for TBC: 4.04

Based on our results the following number of wrong judgements by ATP in percent of total number of evaluated teats is estimated:

- Sediment score 1 falsely determined as not sufficiently cleaned: 15 %
- Sediment score 2 falsely determined as sufficiently cleaned: 15 %
- Sediment score 3 falsely determined as sufficiently cleaned: 2 %
- Sediment score 4 falsely determined as sufficiently cleaned: < 0.5%

When a farm is evaluated, not more than 25 % of teats should have ATP values >3.2 after teat cleaning. This could serve as an orientation for individual farmers if the management is sufficient to ensure clean teats with the applied teat cleaning procedure. The evaluation can also be applied on farms with a conventional milking system.

However, it has to be taken into account, that the correlation between ATP and visual inspection resp. sediment test is dependent on performance of the method as described here. Any changes in the volume of swab solution or in the ATP test system applied would lead to changes in these relationships.

As an example the evaluation of the 18 farms based on the proposed procedure is shown in figure 31.

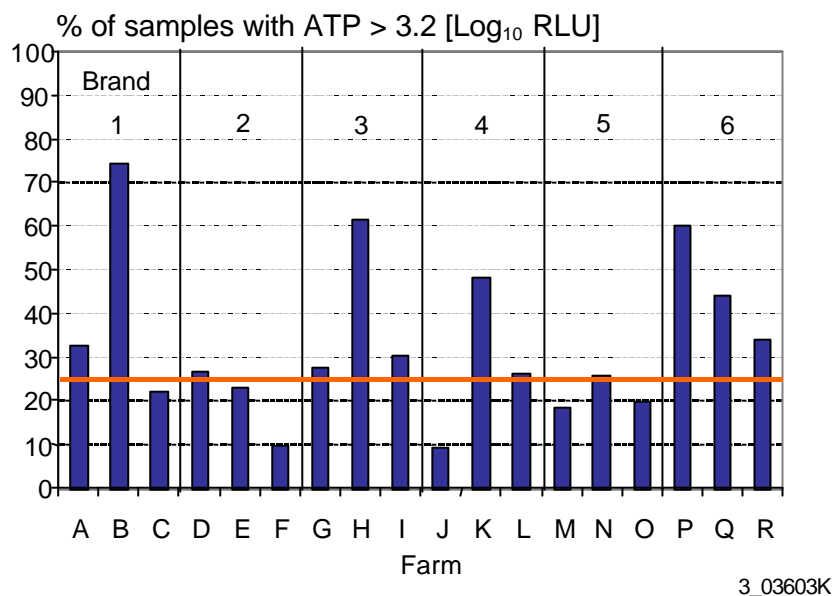


Figure 31: Evaluation of teat cleanliness based on ATP measurement in teat swabs after cleaning. Not more than 25 % of teats should have values > 3.2 Log<sub>10</sub> RLU.

For most brands the evaluation revealed at least one farm per brand that would pass the proposed test. The differences between farms working with the same brand of AM system indicates again that management is extremely important for teat cleaning efficiency. However, regarding brands 3 and 6 none of the farms would pass the test. This is an indication that the effectiveness of the teat cleaning system is not satisfying and that the test results are not only due to insufficient management.

## 4 Conclusions

Based on our investigation the following conclusions can be drawn:

- For all brands an effect of teat cleaning on the carry over of contaminating material into milk could be found based when the evaluation was based on artificial contamination of teats and comparison of cleaning with no cleaning of teats.
- Differences between teat cleaning efficiency of brands could be found. The ranking of brands was similar when different methods (visual evaluation, sediment tests, determination of TBC and ATP in teat swabs) were applied to evaluate teat cleaning efficiency.
- Only for one brand the ranking was different between an evaluation based on artificial contamination and an evaluation by a combination of methods. Because the combination was not applied after teat cleaning, but after milking it may be concluded that part of the teat cleaning effect determined in part 2 was due to cleaning of teats during milking (teat washing).
- Although significant differences between cleaning efficiency of brands were found, the most important influence factors on teat cleaning efficiency were initial contamination of teats before cleaning and the individual farms. These findings emphasize the importance of a good hygiene management to keep udders and teats clean.
- The position of the udder quarter had only a significant influence on teat cleaning efficiency when the determination was based on ATP measurement in teat swabs.
- When contamination level of teats was low teat cleaning led to an increase of bacterial counts on teat surfaces. In these cases no cleaning would be preferable. On the other hand also with low level contamination organic material such as skin debris is removed by teat cleaning which could be shown by ATP measurement.
- For the artificial contamination differences per farm and brand between levels of carry over of poppy seed into milk in cows milked without cleaning were partly due to practical problems like possibilities of excluding pre squirting or not.
- When cleaning methods with brushes and cups were compared, cups seemed to have a better cleaning effect when low level contamination was present whereas brushes were superior when high contamination levels were observed.
- Maintenance of the teat cleaning device of the AM system is important for an adequate function of this cleaning procedure.
- ATP measurement is a suitable method to evaluate teat cleaning efficiency on individual farms in practice. The test can be applied to detect management failures regarding teat cleanliness on individual farms.
- Significant differences between teat cleaning efficiency of individual brands of robots show that improvements are possible. The results of this study might be useful for the manufacturers to improve their systems.

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## Abbreviations

AM	Automatic Milking
ATP	Adenosin-tri-phosphate
cfu	Colony forming units
GLM	General Linear Model
GLMM	General Linear Mixed Model
h	hour(s)
LSQ <sub>M</sub>	Least Square Mean
min	minute
RLU	Relative Light Units
rpm	Rounds per minute
TBC	Total bacterial count