Effectiveness of Automatic Cleaning of Udder and Teats and Effects of Hygiene Management

Protocol for Evaluation of Teat Cleaning Systems

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Information

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Protocol for Evaluation of Teat Cleaning Systems

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Abstract

The cleaning of udder and teats before milking contributes to the image of hygienic food production. Teat and udder surfaces belong to the main sources of bacteria in raw milk. Therefore appropriate cleaning procedures are necessary to reduce the microbial contamination of raw milk. As part of workpackage 6 within the European project "Implications of the Introduction of Automatic Milking on Dairy Farms" a protocol was developed to evaluate mechanised teat cleaning devices that are in operation in automatic milking systems.

The evaluation of teat cleaning procedures will be based on a combination of four methods: visual inspection, sediment test on teat swabs and determination of total bacterial counts (TBC) and ATP in teat swabs. The results of visual inspection are largely dependent on the evaluating person and also vary within testing person when the same material is evaluated repeatedly. Nevertheless, visual inspection and sediment tests are suitable to determine teat cleanliness if cleanliness is defined as the absence of visible contamination with dirt and/or manure. For cleaned teats identical scoring by the two methods was determined in 95.8 % (n=96). The total impression of udder cleanliness seemed to be mainly influenced by the status of the udder basis.

By comparison of TBC and ATP in teat swabs taken before and after teat cleaning a differentiation of cleaning efficiency of manual teat cleaning methods was possible. By wet cleaning with subsequent drying a better teat cleaning effect was achieved: the reduction of TBC in Log10 units was 1.50 (wet cleaning with subsequent drying of the udder) versus 1.11 (dry cleaning), the reduction of ATP in Log10 RLU was 1.26 versus 0.56. The efficiency of manual cleaning methods will serve as a reference for the following evaluation of mechanised cleaning devices applied in practice during the second part of the study.

Another approach for the evaluation of teat cleaning efficiency was based on artificial contamination of teats with cobalt and subsequent determination of its carry over into milk, but could not be validated yet. The loss of cobalt after application on teats was high. Although carry over from teats and from teat cleaning devices into milk was found, no difference between cleaning and no cleaning of teats could be determined when the method was applied on an automatic milking system. Additional experiments are necessary to validate the cobalt method.

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1 Introduction

Automatic milking (AM) systems have been introduced on dairy farms in increasing numbers in several European countries. An important problem during practical use of AM systems that is still not completely solved is how to maintain the high level of milk quality that has been achieved in conventional dairy production in recent years. High quality of milk is a prerequisite for production of high quality products.

An increase of bacterial counts in bulk tank milk after introduction of AM systems has been reported from several countries (van der Vorst et al., 2002; Everitt et al., 2002; Billon and Tournaire, 2002). As udder and teat surfaces belong to the major sources of bacterial contamination of bulk tank milk, workpackage 6 within the European project "Implications on the Introduction of Automatic Milking on Dairy Farms" focuses on the evaluation of mechanised teat cleaning procedures in AM systems.

Teat cleaning is necessary to prevent milk from contamination with manure, dirt and included bacteria. The restriction of the initial contamination of raw milk is especially important with automatic milking because long milking times without cleaning and sometimes storage of uncooled milk in parts of the milking system may provide good conditions for bacterial growth. Furthermore, psychrotrophic bacteria originating from teat contamination can also multiply during refrigerated storage of milk.

Several studies have already been performed to investigate the effect of teat cleaning on milk quality in conventional milking systems. Different methods to assess the cleanliness of teats before milking and to determine the cleaning efficiency of various cleaning methods have been applied. In a first step of workpackage 6 a reliable method for assessment of mechanised cleaning devices had to be developed. The method will be used to evaluate the cleaning devices of different AM systems on dairy farms in practice during a follow up study. That study will be completed in the summer of 2003.

2 Literature review

2.1 Teat cleaning - Aspects of milk quality

According to Jørgensen (1990) the three main purposes of teat and udder cleaning are:

1. to add to the image of hygiene methods in food production
2. to remove all visible soil from teats and the base of the udder
3. to reduce contamination of milk

Although prevention of contamination of milk is essential with regard to milk quality the first two aspects should not be forgotten, because the conditions of food production become more important for consumers´ estimation of food quality today.

After introduction of automatic milking systems an increase of total bacterial counts in bulk tank milk was observed on farms in several European countries (van der Vorst et al., 2002, Everitt et al., 2002, Billon and Tournaire, 2002). Billon and Tournaire (2002) found an increase in the number of butyric acid bacteria spores in milk by about 30 % after introduction of AM. Factors that may have contributed to high bacterial counts in bulk tank milk are the level of teat contamination on dairy farms and insufficient performance of mechanised teat cleaning methods. Teat and udder surfaces together with mastitis pathogens and surfaces of milking and storage equipment are the main sources for bacterial counts in bulk tank milk exceeding 10 000 cfu/ml (Sumner, 1996).

Teat contamination mainly consists of bedding and faecal material. Bacterial counts in used bedding material can reach 10^7-10^9 cfu/g (Hogan et al., 1989). Although high carry over rates of bedding into milk would be necessary to enhance the bacterial count in bulk tank milk significantly, individual cows with heavily soiled teats can contribute milk with more than 10^4 cfu/ml (Cousins, 1972). It also has to be taken into account that gram-negative rods and aerobic spore-formers may be psychrotrophic, and therefore being able to multiply during refrigerated storage of milk. Faecal material contains high total bacterial counts and high numbers of coliform bacteria (10^4-10^6/g), and may also harbour bacteria which are potentially pathogenic for humans e.g. verotoxigenic *Escherichia coli*, *Campylobacter spp*., *Mycobacterium paratuberculosis*, and should therefore be prevented from entering the bulk tank.

In addition, the teat surface is the main source of anaerobic sporeformers (Jørgensen, 1990). Teat skin contamination with *Clostridium* (*Cl.*) *tyrobutyricum* spores is usually higher during the housing and beginning grazing period compared to the later grazing period (Bramley and McKinnon, 1981). Spores from low quality grass silage contaminate the alimentary duct and enter the milk via faecal material if udder preparation is not effective (De Vries and Stadhouders, 1977). The clostridia content in milk is mainly influenced by spore content in faeces and method of udder preparation (Bertilsson et al., 1996).

The most contaminated part of the teat and the part which comes into contact with the milk during milking is the apex (Jørgensen, 1990). Slaghuis (1996) concluded that by udder preparation contamination of milk can be reduced by a maximum of 90%, dependent on the initial level of contamination and the way of udder preparation. With high contamination levels this tenfold reduction might not be reached. Higher bacterial counts in milk were reported when teats were not dried after wet teat preparation (McKinnon et al.,1983; McKinnon et al., 1985; Galton et al., 1986; Nakano et al., 1995). Wet teat cleaning followed by drying prevents milk from contamination with bacteria such as *Cl. tyrobutyricum*.

Wet cleaning without drying has also negative effects on udder health. This explains udder preparation effects on the somatic cell count of bulk tank milk. Randy et al. (1990) did a survey of milking management practices on New York and Vermont DHI herds with low somatic cell counts. One result was that an essential factor in long term production of high quality milk is milking of dry clean udders. Therefore in general dry teat cleaning and for dirty cows wet teat cleaning followed by drying is advised to farmers (NMC, 1999).

2.2 Teat cleaning - Regulatory aspects

In the European Community regulatory aspects regarding milk production and marketing are laid down in the Milk Hygiene Directive 92/46/EEC. It is stated that "Milking must be carried out hygienically and under the conditions established by Directive 89/362/EEC" (Annex A, Chapter III, A, No. 1). Directive 89/362/EEC on general conditions of hygiene in milk production holdings gives concrete requirements on performance of the milking procedure. Regarding teat cleaning it is said: "Before the milking of a cow is started the teats, udder and if necessary adjacent parts of the groin, thigh and abdomen of the cow must be clean" (Chapter III, No. 3). These directives are the basis for national legislation on milk hygiene applicable for all milking systems.

Even in the current proposal for the recast of the European Food Hygiene Legislation going along with a simplification, still concrete requirements on hygiene on milk production holdings are involved: "Milking must be carried out hygienically, ensuring in particular that before milking is started, the teats, udder and if necessary adjacent parts are clean" (Doc 2000/C365E/03). In the documents no clear definition of "cleanliness" is given, presuming that a common view on cleanliness exists.

In some countries additional requirements for farms with automatic milking systems have been set. An international group consisting of members of the European Cooperation Group on Automatic Milking produced a "Proposal for a GMP code for milking with Automatic Milking Systems" (Jepsen et al., 2001). Here the requirements are more specified: "There should be a cleaning system to remove all visible contamination of the teats and the adjacent area of the udder" (Chapter 1.4). This demand implies that "cleanliness" means the absence of visible contamination.

In some other countries the special requirements for farmers working with AM systems refer also to the management that should aim for keeping cows clean (Everitt, 2000; BMVEL, 2001)

2.3 Teat cleaning - Methods applied in conventional milking systems

2.3.1 Manual methods

Through the years several manual teat cleaning methods have been compared in numerous experiments. Two big differences can be distinguished: dry and wet cleaning. The different methods of dry cleaning are 1) with paper for single or multiple use and 2) wipes/towels made of diverse materials, also used for one or multiple cows. The different methods of wet cleaning are 1) with water without drying 2) with water and drying and 3) with a disinfectant (liquid or gel) followed by drying. In order to judge the effect of the different teat cleaning methods on milk quality total bacterial count, preliminary incubation count, coliform count or butyric acid bacteria spores are used as standards. To prevent mastitis dry cleaning is advised, but as mentioned above wet cleaning followed by drying is favourable to prevent milk from contamination with bacteria like *Cl. tyrobutyricum*.

Jørgensen (1990) described and evaluated three methods of teat/udder cleaning and analysed various disinfectants. The method of washing in tepid water from a hose should be combined with careful drying of the teats in order to minimize the bacterial contamination of the milk (Cousins, 1972; Galton et al., 1986; McKinnon et al.,1983; McKinnon et al., 1985; according to Jørgensen, 1990). Dry-wipe cleaning with single service towels shows less effect on reduction of milk contamination with bacteria than other methods (Hansen, 1973; Galton et al., 1986; Horvath and Møller Madsen, 1984 according to Jørgensen, 1990). Premilking dip in a disinfectant followed by drying with paper towels was found more effective than no cleaning but involves a risk of residues in the milk (Galton et al., 1986 according to Jørgensen, 1990). Different detergents/disinfectants have been tested on cleaning effects and reduction of bacterial contamination (Adkinson et al., 1991; Ingawa et al., 1992). If disinfectants are used in udder preparation, thorough wiping is necessary to prevent contamination of milk with residues (Galton, 1984).

Wiping with single service towels soaked in a tepid solution of disinfectant prevents carry over of pathogens via towels from cow to cow and the method minimises the milker´s risk of carrying pathogens on the hands compared to using dry paper towels. Wiping with a multiple service towel soaked in a disinfectant and wrung before use is cheap, because the towel is used in a number of cows. However, it involves the risk of carrying pathogens from cow to cow as the disinfectant loses effect due to inactivation by organic matter. Wiping with cotton towels applied on single service basis but used again at the next milking after washing in a mechanical washer involves a very limited risk of carrying patho-

gens from cow to cow. The towel material makes it possible to clean the teats well and it ensures good milk let down. The towel should be wrung so well that the teats are not left wet.
De Vries and Stadhouders (1977) proved that effective udder preparation also decreases the contamination of milk with butyric acid bacteria spores substantially. Cleaning the udder with water, by hand shower or bucket, and drying with a clean towel showed better results than cleaning with a dry paper or moistened cotton towel.
Jørgensen (1990) pointed out that the success of teat/udder cleaning does not depend only on what method is chosen but to a very high degree on how carefully the method is carried out.

2.3.2 Mechanised methods

In order to reduce labour for milkers several approaches for mechanisation of teat cleaning and preparation have been made. Kingwill (1980) mentioned the use of sprays in collecting yards in warm climates. The udders of a large batch of cows were washed at the same time. They dry in the sun before milking. Kingwill (1980) also mentioned systems of automatic udder spraying immediately prior to the entry to the milking parlour. Correct coverage of the udder seems to be a problem as well as the effectiveness of the method.
Bodini (1989) cleaned individual teats with rotating brushes and Mottram (1997) used a device with rubber fingers. Taverna et al. (2001) described the effect of the use of Lava-teat® system, a handheld machine for washing and drying of teats, on bacterial counts compared to two different premilking udder preparation methods. These treatments were 1) teat washing with water hose, no drying and 2) teat washing with water hose, drying with paper towel. The Lava-teat® system had a similar effect on standard plate counts as treatment two but was superior to both manual cleaning methods in reduction of butyric spore counts.

2.4 Teat cleaning - Methods applied in automatic milking systems

In AM systems different procedures are applied for cleaning of udder and teats:

Table 1 Teat cleaning procedures in AM systems

Company (Country): Milking Robot	Teat cleaning system
DeLaval (SE): Voluntary Milking System (VMS)	Separate cleaning device: application of warm water, vacuum and air-pressure, foremilking 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, foremilking after drying of teats with warm air, cleaning water and foremilk separated
Lely Industries (NL): Astronaut; 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

In most systems, only the teat surfaces are cleaned whereas the udder and adjacent parts of the body are not implied. At present, none of the systems is able to check the degree of teat contamination before or after cleaning. The cleaning intensity is set by cleaning time or number of cleaning cycles. An adjustment of cleaning intensity to contamination level in the form of a feedback control requires additional sensor systems and may lead to extended contact times with dirty cows disturbing the milking routine (Mottram, 1997). All systems use wet teat cleaning methods with only three of them providing techniques for drying teats afterwards.

2.5 Methods for the evaluation of teat cleanliness

Assessing the cleanliness of teats has been done by several methods based on different approaches.

2.5.1 Visual evaluation

In conventional parlours the herdsman inspects the teats visually and tactile before milking and checks milk filters after milking. By several investigators visual inspection is applied to determine cow cleanliness including udders and teats (Mottram, 1997; Snell et al., 2000; Ten Hag and Leslie, 2002)

2.5.2 Sediment tests

Sediment testing for milk is a standard method used to assess cleanliness of milk production since the sixties of the last century. The main cause of sediment in milk is improper cleaning of teats and udders. Sediment tests can be performed on all levels of milk production from single cows milk to processed milk. According to Roman (1971) the glass bottle was first accepted with reservation by those selling milk since it made every consumer a potential milk inspector.
The tests are usually applied on bulk tank milk. A defined volume of milk is filtered through a special filter disc and the appearance and quantity of sediment is assessed in comparison to filter discs prepared with certain amounts of reference material (Wright, 1978).

Today the conditions of milk production have improved significantly. In addition the proportion of sediment in milk decreases with increased milk yield. The sediment test on bulk tank milk as a very rough estimate is nowadays routinely performed in selected countries only. No differences in sediment in bulk tank milk were found between AM farms and conventional farms in the Netherlands (Van der Vorst, 2001).

McKinnon et al. (1983) used sediment tests on cow composite milk to compare different teat preparation methods and different housing conditions. 500 ml of milk per cow were taken from the recorder jar because milk from inline milk samplers was insufficient for determination of sediment. Considerable variation of sediment amounts was determined under the same housing conditions and teat washing treatments. Drying of teats after washing had no additional effect on sediment amounts in milk compared to washing without drying. A relationship between results of sediment tests and bacterial counts was only found for samples from uncleaned teats. According to Galton et al. (1984) the combination of wet cleaning and physical manipulation of teats is most effective for removal of sediment.
The amount of sediment is also dependent on the milking technique. If during milking no teat washing occurs, the transfer of sediment into milk is restricted.

Phillips et al. (1981) correlated the amount of sediment on milk filters to the cleanliness of cows and the efficiency of teat preparation methods. Although the milk was filtered still unacceptable amounts of very fine sediment in bulk milk were detected with poor environmental conditions ("mud hole conditions") indicating that by the milk filter only large particles are withheld from milk.

2.5.3 Bacteria on teat surfaces

The effects of different cleaning procedures on the bacterial population on teat surfaces have been investigated by different approaches. The determination of bacteria in bulk tank milk is useful to locate possible causes if problems with milk quality exist (Reinemann et al., 1997). Coliform counts between 100 and 1000 cfu/ml are an indication that teat preparation is insufficient, but higher coliform counts probably originate from milk contact surfaces.

McKinnon et al. (1983) determined bacterial counts in cow composite milk samples to compare teat preparation methods. Steam sterilised milking clusters were used to prevent contamination of milk from milking equipment. Milk from heavily soiled, uncleaned udders contained high total bacterial counts with more than 10 000 cfu/ml, but not more than 20 cfu/ml of coliforms. For this kind of experiments the udder health status of cows has to be determined in advance and cows with intramam-

mary infections - shedding high numbers of mastitis pathogens in milk – have to be excluded from the experiments (McKinnon et al., 1983, Galton et al., 1984).
Rasmussen et al. (1991) determined the anaerobic spore count in milk to compare manual teat cleaning methods. Difficulties with this method can be based on variable contamination levels of teats with anaerobes dependent on feeding and housing conditions on farms.
Significantly lower bacterial counts during pasture can make it impossible to distinguish between teat preparation methods based on bacterial counts in milk (McKinnon et al., 1990). According to Hansen (1973) teats from well-bedded cows are less contaminated than teats from cows kept on minimal bedding when the same cleaning method is applied.

McLarty et al. (1981) showed that the direct detection of bacteria on teat surfaces is dependent on the method applied. Higher numbers were identified by teat rinsing instead of using teat swabs. The flora on teat surfaces consisted mainly of micrococci and aerobic sporeformers, other species represented only 10-12 % of total bacterial load. According to Jørgensen (1990) the relation of staphylococcci: streptococci:gram-negative bacteria on teat skin is 100:10:1, but the composition of the microbial population is also related to the flora in bedding material and to total counts on teat skin (Rendos et al., 1975).
McLarty et al. (1981) were seldom capable of detection of coliforms in teat swabs or teat rinses in numbers exceeding 100 cfu/ml. These results also comply with the findings of McKinnon et al. (1983) with low coliform counts in cow composite milk. Thermoduric bacteria in teat rinses were used by McKinnon and Pettipher (1984) to detemerine the origin of heat resistant bacteria.
The use of teat swabs is the most common method to determine the bacterial load on teat surfaces, because this is a quick method (Mottram, 1997).
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.

Leslie et al. (2002) suggested cowside bioluminescence tests for an objective measurement of teat cleanliness. However, a poor correlation between ATP values and TPC was obtained when the method was applied to swabs from milking equipment or to milk from transport tankers (Nieuwenhof, 1996) or to bulk tank milk (Samkutty, 2001).

2.5.4 Use of tracer substances

Artificial contamination of teats with tracer substances is used to determine carry over of contaminating material into milk. If the amount of material applied on teats is known, a better calculation on carry over rates can be made.
Schuiling (1992) applied lithium as indicator mixed into sterilised manure to the teats before cleaning and measured lithium in milk of cows. A rotating brush cleaned udders and 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.

Magnusson et al. (2002) used high numbers of spores of *Cl. tyrobutyricum* in manure water slurry for artificial contamination of teats and determination of spores in milk to evaluate the efficiency of manual cleaning methods. The advantage of these spores is that no aerobic growth occurs and the spores are not killed by cleaning agents. The spores are non-pathogenic for humans and animals. Applying high numbers overcomes the problem with variable contamination under natural conditions. A similar method was applied by Melin et al. (2002) to compare automatic and manual cleaning. In that study the cleaning of the milking robot was found more effective in removing spores than manual cleaning (98.0 versus 66.5 %)

2.5.5 Automated methods

Mottram (1993) interpreted photographs of teats to determine the percentage area covered with contaminants. Shadows from other teats, however, could be interpreted as dirt and gave inconsistent results. Bull et al. (1995) used a gated monochromator to analyse the levels of light reflected from teats at different wavelengths. Although it is possible to distinguish white teats from black teats or teats coated with manure or soil, this method does not give a reliable index for discriminating between black teats and those covered with soil contaminants.

Mottram and Persaud (2000) proved that conducting polymer odour sensors could discriminate between different contaminants on a teat within a teat cup in a laboratory situation. For practical use, however, the sampling device needs to be re-engineered to control incoming airflow and reckon with the variability of ambient humidity.

3 Material and Methods

3.1 Visual inspection of udder and teats

Visual inspection is a method that can easily be performed under all circumstances without interfering with the process of teat preparation. However, the evaluation of teat contamination is subjective and the value of the method may be restricted due to dependence of the results on the evaluating person.
To evaluate the repeatability of visual inspection and to perform comparisons between different observers a set of 70 photographs of udders was used. Photos of uncleaned and cleaned udders with different levels of contamination were taken with a digital camera (Nikon Coolpix 990).
A scoring system with 4 points was used for judgement:
1 = completely clean
2 = slightly splashed
3 = partially contaminated
4 = heavily soiled

A presentation of photos by Microsoft® PowerPoint® 2000 allowed 20 seconds for judgement of each photo. The following areas per photo were defined for separate scoring:

1 total impression of udder and teats
2-5 teats enumerated in succession from left to right in order to facilitate assessment for unexperienced testing persons
6 udder basis

13 testing persons - 4 milkers and 9 others with different experience regarding husbandry of dairy cattle - evaluated the photos individually from a PC screen. A set of 6 photographs was used to inform testing persons on different degrees of cleanliness (Figure 1).

Figure 1 Photographs for instruction of testing persons on degrees of teat/udder contamination

In order to determine repeatability within testing person 5 of 65 photos were used repeatedly without knowledge of the testing person. In addition one testing person evaluated the photos 3 times in intervals of 1 and 3 weeks.

The results were stored in a data base using Microsoft® Access 2000 and statistical evaluations were made by SAS® System, release 8.01 (Proc Means, Proc Univariate).
As for teat cleanliness respectively degree of teat contamination no reference value is available the mode as the score with the highest agreement within the test panel was used as the reference. Modes were calculated for each area of each photo. If two different scores occurred with the same frequency, according to the settings of SAS® System the lower score was defined as the mode.

3.2 Sediment tests on teats

Sediment tests are usually applied on bulk tank milk to evaluate the hygiene of milk production. The test on bulk milk only gives a rough indication on teat preparation. With automatic sampling devices only a small volume of milk is sampled per milking which is usually not sufficient for sediment testing. The proportion of sediment in milk decreases with increasing milk yield.
Therefore a different approach was chosen. For a more direct measurement of teat cleanliness sediment was determined in swabs taken from single teats´ surfaces.
All experiments were performed on German Holstein cows on the experimental farm Schaedtbek of the Federal Dairy Research Centre in Kiel, Germany. Cows were tied up during the winter period with straw bedding. Samples were taken before milking during regular milking times in a tandem (2 x 3)-milking parlour. Cows were randomly assigned to sampling. The two teats of a cow facing the investigator were sampled separately as follows:
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 and placed on a laboratory shaker for 30 minutes with 100 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 in correspondence to the scoring system for visual evaluation of udder and teats (see 2.1). For each scoring level a set of 3 filters representing a certain range of teat contamination was used as a reference for visual comparisons.

Figure 2 Reference for visual evaluation of results from sediment tests

To determine the correlation of sediment tests with visual inspection, swabs were taken from uncleaned teats (n=104) as well as from manually cleaned teats (n=96). Manual cleaning was performed after premilking by use of dry paper towels. Visual scoring was performed in the milking parlour directly before the swab was taken. During one milking time teats were only subjected to one sediment test, either before or after cleaning.

3.3 Bacterial counts in teat swabs

3.3.1 Determination of bacterial counts by standard procedures

An additional approach on determination of teat cleanliness was based on determination of bacterial counts on teats. As teat preparation leads to milk let down in some cows and milk from cows with clinical or subclinical mastitis can contain high numbers of bacteria, bacterial counts in swabs from the teat apex may be influenced significantly by the udder health status of the cows. Based on preliminary experiments comparing bacterial counts in swabs taken from teat sides and from the teat apex it was decided to swab only the teat sides for further investigations.
The experiments were performed during routine milking times as described in 2.2. Two teats per cow, one front and one hind teat of the same side were sampled.
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 and was cooled until laboratory investigation. Before start of analysis the test tube with the swab was vigorously shaken on a vortexer for 10 seconds and the swab was removed.

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
- Coliforms - IDF standard 73A:1985
- Thermoduric bacteria - heating of 3.5 ml of swab solution for 30 min at 62.8 °C, placing immediately on ice, then determination of bacterial counts according to IDF standard 100B:1991 (Frank et al., 1985)
- Staphylococci - plating of 2 x 0.1 ml per dilution on Mannitol-NaCl-Agar (Merck, 64293 Darmstadt, DE) containing sodium-azide 0.05 g/l; incubation of plates for 24 h at 37 °C
  The selectivity of Mannitol-NaCl-Agar is based on high NaCl-content. Growth of other salt-tolerant bacteria than staphylococci is not excluded, but no identification of colonies was made.

Results are presented in cfu (colony forming units)/ml swab solution.

The determination of TBC started with the first decimal dilution. For staphylococci, coliforms and thermoduric bacteria the undiluted solution was implied. This procedure results in detection limits of 100 cfu/ml for TBC and staphylococci and of 10 cfu/ml for coliform and thermoduric in swab solution. Results of TBC below the detection limit were set to the detection limit (100 cfu/ml) for calculation of differences between TBC before and after cleaning. This can lead to an underestimation of cleaning efficiency of the respective method.
As manual reference the following cleaning methods for the teats were used:

- dry cleaning with paper towels
- wet cleaning by application of warm water with a water hose followed by thorough drying with paper towels

The intensity of the cleaning procedure was as routinely adjusted to the contamination status of the cow. Sampling of teats after cleaning was performed on 10 days for dry cleaning (134 samples) and on 7 days for wet cleaning (88 samples). For comparisons of bacterial counts before versus after cleaning sampling was performed on 4 (dry cleaning, 39 samples) respectively 6 (wet cleaning, 50 samples) days.
The determination of cleaning efficiency was based on the difference between Log10 TBC before versus Log10 TBC after cleaning expressed as reduction of bacterial counts in Log10 units.
Statistical evaluations were made by SAS® System, release 8.01 (Proc Univariate, Proc TTest).

3.3.2 *Determination of bacterial counts by ATP measurement*

In addition to the determination of bacterial counts with standard procedures it was tested if ATP (Adenosin-tri-phosphate)-measurements could be used to replace the time-consuming determination of TBC in teat swabs. 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® 2 (Merck, 64293 Darmstadt, DE) was applied to the swab solution of a total number of 216 samples of uncleaned and cleaned teats (see 3.3.1) before determination of bacterial counts started.

3.4 Use of a marker substance

For this study manure was mixed with cobalt solution. Cobalt was used as a tracer, because in a recent study (Slaghuis et al., 2002) this method was developed for recovery and analysis in milk. Under normal circumstances cobalt levels usually are very low in milk (about 1-10 µg/kg). Therefore, cobalt in elevated levels is suitable to act as tracer and can be analysed in milk after application. The experiments were performed by the Research Institute for Animal Husbandry, Lelystad, The Netherlands.
The research for this method development was split up in three experiments.

1. Defining the loss of manure/cobalt mixture
2. Defining the carry-over of cobalt
3. Determining the effect of teat cleaning

Before coming to a protocol, the loss of manure/cobalt during waiting time between application of the mixture and milking, the carry-over of cobalt from cow to cow by means of the milking equipment or from cow into milk was determined. The effect of teat cleaning was determined by applying the cleaning to one group of cows and no teat cleaning to another group of cows.

3.4.1 *Preparation of manure/cobalt mixture*

On one farm manure was collected from at least ten cows. Cobalt solution was added (100 g/kg) to obtain a manure/cobalt mixture with a concentration of 5 g/kg. Amounts of about 300 g of manure/cobalt mixture were sterilised (30 min., 121 °C, 1 atm.) in glass bottles. After sterilisation, the mixture was put in smaller plastic bottles with screw cap and brush (100 ml, CFS Products). The brushes were used to put the mixture on the teats of the cows. Before and after application of the mixture on the teats, the bottles were weighed to determine the amount being applied to the teats.

3.4.2 *Defining the loss of manure/cobalt mixture*

Fifteen cows were divided at random into three groups of five cows. Each group was prescribed a waiting time between application and milking. Waiting time was the experimental treatment. The experiment was carried out during one day. The chosen three waiting times were: 15 minutes, 6 and 12 hours. The teats of these cows were treated as described in 3.4.1.
After the prescribed waiting time the teats were cleaned with a moistened paper cloth (Calgonit Soft). The cloths of five cows were put in a jar and filled with 1000 ml tap-water, giving a pooled sample of 4 teats x 5 cows. After this the cows were milked in a 10-stand open milking parlour. Of each cow milk was proportionally sampled to make a pooled sample per waiting time (5 cows per group). During the next milking, the milk of the 15 cows was sampled in the same way as an extra control on carry-over to this next milking.
The measured amount of cobalt in the pooled samples was compared with the amount of cobalt that was applied on teats. The difference was the loss of cobalt during waiting time, loss of cobalt to the milking equipment and the not removable rest on the teats.

3.4.3 *Defining the carry-over of cobalt*

In this experiment the carry over of cobalt from cow to cow during milking was defined. Bulk tank milk samples were taken before and after the experiment. Of five cows the teats were treated as given in 3.4.1. An automatic milking system milked the cows following premilking and teat cleaning by the system. The milk was separated. Milking was followed by a unit flush. After the unit flush an untreated cow was milked and the milk proportionally sampled (2 or 3 ml/kg milk). From five untreated cows, each following a treated cow, milk was pooled to one sample.

3.4.4 The effect of teat cleaning

To determine how many cows for how many days should be sampled, three groups of 10 cows were used. During the first of in total four experimental days, the cows were chosen at random. During the next three consecutive days these cows were also used.
The effect of teat cleaning was determined by splitting each group of 10 cows into two subgroups of 5 cows: one with and one without teat cleaning (with 1 sec fore-milking).
The day before the experiment the bulk milk tank was emptied. On each day the treatment (5 cows with teat cleaning and 5 cows without teat cleaning within one group) was the reverse to the day before; 5 cows with teat cleaning on day 1 had no teat cleaning on day 2.
Bulk milk samples were taken before and after milking to define the carry over of cobalt: the milk of the treated cows was excluded from the tank.
The teats of the cows were treated according to 3.4.1. After a waiting time of at least 6 hours an automatic milking system milked the cows of a subgroup consecutively. Dependent on the treatment cleaning water and foremilk of a cow was collected and saved. From the five samples of a subgroup one pooled sample was made, resulting in 6 samples.
Milking a treated cow was followed by a unit flush and the milking of an untreated cow. Every experimental day resulted in one sample from manure/cobalt mixture, 6 pooled milk samples, 3 cleaning water (including foremilk) pooled samples (3 groups with teat cleaning) and 2 bulk milk samples (before and after the experiment).

3.4.5 Analysis

All samples were frozen until transport to NIZO Food Research for analysis.

Chemical analysis

Samples of manure/cobalt, cloths in water, milk and cleaning water were dried in a quartz crucible and programmed ashed until maximal 500°C. In case of not fully ashing, the residue was smoked with HNO_3 and ashed again. The ash was diluted in nitric acid, the solution was filtrated and cobalt was measured with Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) at 230 and 786 nm wavelengths. Cobalt content was calculated by external calibration with standard solutions. Samples with higher signals than the highest standard were diluted and measured again.
All types of samples (milk, manure, cleaning water and ‘cloth water’) were used for standard addition for control on matrix effects. If these effects were present, all samples of the same type were quantified by standard addition.
The cobalt contents at two wavelengths should not differ more than 5%. During measuring after ten samples, a standard was measured for control on drift. The detection limit for this method was 1 microgram/kg for all samples. The precision was at contents of 1-10 microgram/g 10% relative and at contents >10 microgram/kg 5% relative.

Statistical analysis

Analysis of variance was carried out on the results of the experiments.

4 Results and Discussion

4.1 Visual inspection of udder and teats

13 testing persons evaluated 70 photos of udders representing different contamination levels. For one unexperienced person the time for each photo had to be increased from 20 to 30 seconds. Two persons mentioned that in practice udders with much higher contamination levels than presented in the show can be observed. Both gave this as a reason for seldomly using score 4 during the evaluation, which means that they did not score according to the standards presented in this study. One testing person seemed to score the photos not according to his own perception but in a way he thought he was expected to score.
Figure 3 summarises the scoring results of visual evaluation of 70 photos by the 13 testing persons.

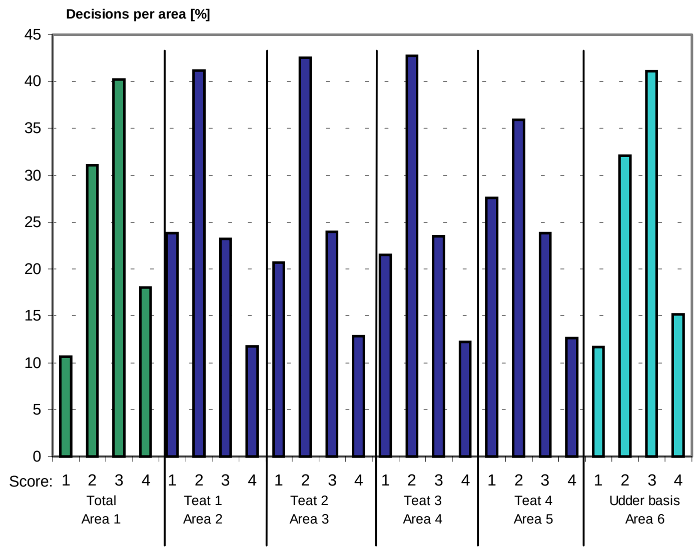

Figure 3 Visual evaluation of cleanliness – distribution of scores per area (70 photos with 6 areas each, results of 13 testing persons)

Whereas for the 4 teats a similar distribution of scoring results occurs with score 2 as the most frequently used one, for areas 1 and 6 (total impression and udder basis) score 3 was most often applied by all testing persons.
Large differences occurred between the scores of single testing persons. For all areas the mode was calculated as the reference value. As the score value with the highest agreement in the group of testing persons the mode is probably closest to the real degree of contamination.

In order to find out which area(s) mainly influence the total impression of the udder (area 1) the difference between mode of total impression and the other areas was calculated for each photo. The results are summarized in table 2.

Table 2 Equivalence of mode for total impression with modes of other areas within the same photo, results of 13 testing persons

Difference to mode of total impression	Area: Teat 1 n	Teat 1 %	Teat 2 n	Teat 2 %	Teat 3 n	Teat 3 %	Teat 4 n	Teat 4 %	Udder basis n	Udder basis %
-2	0	-	1	1.4	0	-	0	-	0	-
-1	3	4.3	4	5.7	4	5.7	5	7.1	4	5.7
0	**33**	**47.1**	**30**	**42.9**	**36**	**51.4**	**32**	**45.7**	**55**	**78.6**
1	28	40.0	34	48.5	23	32.9	25	35.7	11	15.7
2	6	8.6	2	2.9	6	8.6	8	11.4	0	-
3	0	-	0	-	1	1.4	0	-	0	-

With almost 80 % the mode values for udder basis (area 6) show the highest degree of equivalence with the modes for total impression. Only deviations of plus/minus 1 score point occur between the two areas, whereas between total impression and teats deviations upto 3 score points are observed. These results indicate that the status of cleanliness of the udder basis is very important for the overall impression and evaluation of udder cleanliness.

To test the variability of decisions between different testing persons for each person the number of decisions deviating from the mode was calculated (Figure 4).

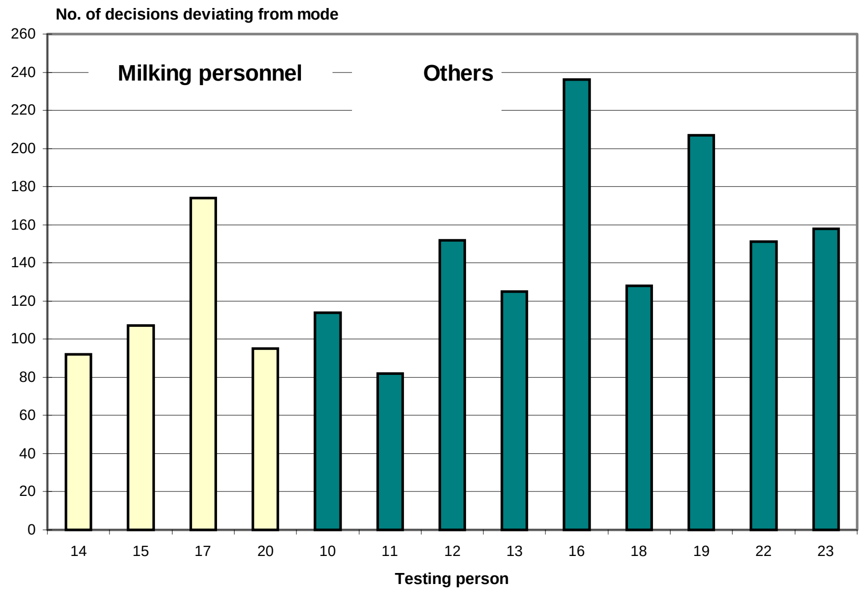

Figure 4 Number of decisions deviating from mode based on decisions of 13 testing persons; No. of decisions per testing person: 420

Figure 4 makes the differences between the testing persons obvious. Testing person 16 deviated from the mode in more than 50 % of decision whereas testing person 11 agreed with the mode in more than 80 % of decisions. If visual inspection shall be applied as a method for assessment of teat cleaning procedures a high consensus of evaluating persons is a prerequisite.

For further analysis the 3 persons with the highest number of decisions deviating from the mode were excluded and the mode for all areas was recalculated. The number of deviations from the mode for the remaining 10 testing persons after exclusion of testing persons 16, 17 and 19 is shown in figure 5.

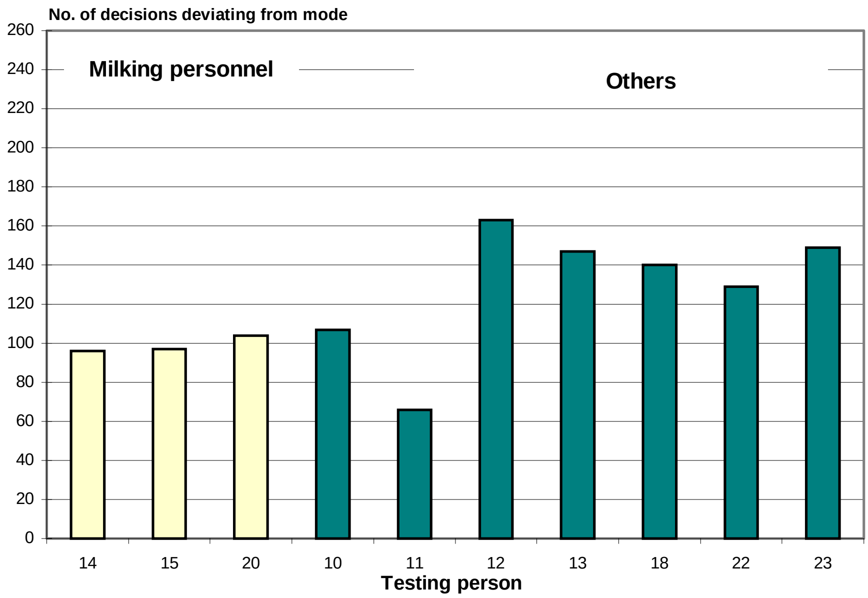

Figure 5 Number of decisions deviating from mode based on decisions of 10 testing persons; No. of decisions per testing person: 420

The exclusion of the three testing persons led to more homogeneity within the test panel. Especially the testing persons belonging to the milking personnel scored close to the mode although their evaluation contributed only 30 % to the mode. A selection of the testing persons can reduce variability of decisions. Nevertheless, it has to be taken into account, that some testing persons show a higher proportion of deviations from the newly calculated mode than before.

The mode values for different areas are based on decisions of a variable number of testing persons. If the mode is 1 or 4 it is on average supported by a higher number of testing persons compared to score 2 or 3 (Table 3). These data could be interpreted in a way suggesting that a more common view on complete cleanliness or heavy contamination exists. But it also has to be regarded that for score 1 and 4 only deviations in one direction are possible whereas for score 2 and 3 deviations in both directions can be chosen.

Table 3 Identical decisions of testing persons with mode in dependence of mode level, results of 10 testing persons

Mode [Score]	No. of areas	Total no. of decisions in agreement with mode [%]
1	81	77.2
2	170	72.5
3	131	65.0
4	38	77.1

To check for repeatability of decisions within testing person 5 of 65 different photos had to be evaluated two times (Figure 6).

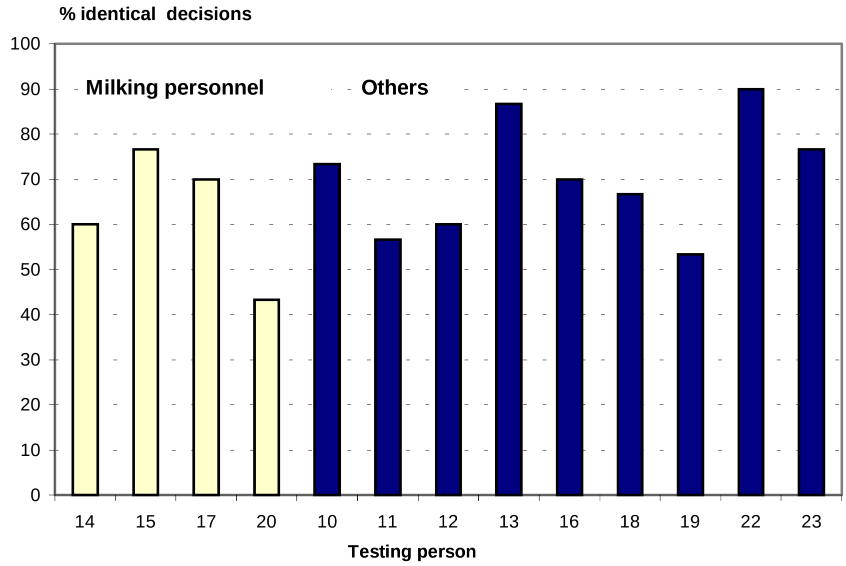

Figure 6 Percent of identical decisions per testing person
Double evaluation of 5 photos à 6 areas = 30 decisions

The results from figure 5 with the 3 testing persons belonging to the milking personnel judging very close to the mode could be interpreted in a way that their decisions were based on a more precise idea of cleanliness and are made less casually than by other testing persons. Therefore a high repeatability within person was to be expected for the milking personnel. However, this assumption is not supported by the results presented in figure 6. The repeatability in testing persons 14 and 15 is not significantly higher than in other testing persons and testing person 20 has the lowest repeatability observed in the test panel with less than 50 % identical decisions.

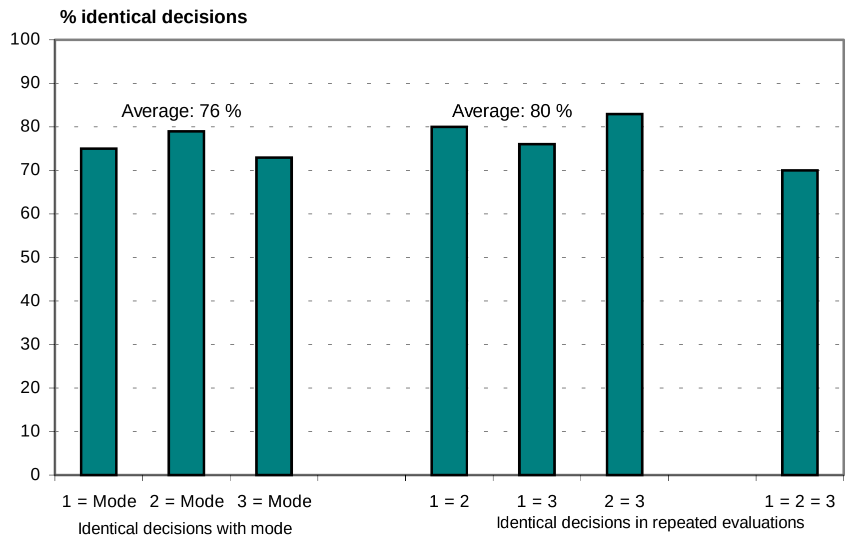

Figure 7 Repeated evaluation of cleanliness by one testing person – 3 repetitions à 420 decisions

Figure 7 shows that even in the same testing person the percentage of identical decisions between two evaluations is usually not more than 80 %. If more than two evaluations are compared the number of identical decisions decreases even more.
The results on visual evaluation of udder and teat cleanliness show clearly that the method is very subjective and that repeatability between testing persons and even within testing persons is highly variable. It has to be regarded that the testing persons in this study had no special training for visual

evaluation of teat cleanliness. If the method shall be applied for routine evaluation in practice efforts have to be made to enhance the reliability and to exclude as much variation as possible between as well as within testing person in order to make results of different evaluations comparable. Preferably the evaluation should be performed by the same person or by a limited number of persons to reduce variation between persons. By special training it should be possible to enhance the repeatability within person.

The results also underline the influence of the status of the udder basis on the total impression of cleanliness and this should be taken into account with regard to consumers´ acceptance.

4.2 Sediment tests on teats

In a next step comparisons between visual inspection of teats and sediment tests on teat swabs were made. The visual evaluation of teats and the swabbing for the sediment test was performed by the same person in all cases. The results are summarised in table 4.

Table 4 Comparison of visual inspection and sediment test for evaluation of teat cleanliness

Visual score minus sediment score	Cleaned teats		Uncleaned teats	
	n	%	n	%
1	0	-	22	21.2
0	92	95.8	66	63.5
-1	4	4.2	15	14.4
-2	0	-	1	1.0
∑	96	100.0	104	100.0

If applied on cleaned teats a high consensus of results from the two methods is observed. The negative results of visual score minus sediment score can be explained by overlooking of residual contamination on the back of the teats or on pigmented teats during visual inspection but subsequent detection by the sediment test. For uncleaned teats the results of the two methods show more differences with also higher visual scores than sediment scores for 22 teats, indicating that the system used for the scoring of filter discs from sediment tests evaluates not in the same range as the visual evaluation. Nevertheless the sediment test combined with visual inspection is very useful to determine a standard for a clean teat.

As the necessary equipment is transportable the sediment test can easily be performed on the farm as a cowside test. The test has a demonstration effect for the farmer because results are available immediately at the time of evaluation. The filters can be subjected to revaluation under standardised conditions and are stored for documentation of results.

4.3 Bacterial counts in teat swabs

4.3.1 Comparison of cleaning methods

To determine teat cleanliness based on bacterial counts in teat swabs different bacterial groups were determined after performance of two standard manual cleaning procedures. Table 5 summarises the results for TBC and staphylococci.

Table 5 Determination of differential bacterial counts in teat swabs after manual cleaning of teats [cfu/ml swab solution]

Percentiles for bacterial counts in teat swab solution	Dry cleaning with paper towel		Wet cleaning with water hose and drying with paper towel	
	TBC (n=134)	Staphylococci (n=133)	TBC (n=87)	Staphylococci (n=88)
Min	$\leq 10^2$	$\leq 10^2$	$\leq 10^2$	$\leq 10^2$
25 %	5.0×10^2	1.2×10^2	$\leq 10^2$	$\leq 10^2$
50 %	1.3×10^3	3.8×10^2	2.1×10^2	$\leq 10^2$
75 %	3.2×10^3	8.8×10^2	8.4×10^2	2.6×10^2
Max	8.0×10^4	1.2×10^4	1.4×10^4	1.0×10^4

After wet cleaning of teats with subsequent drying, in general, lower numbers of TBC and staphylococci were detected on the teat skin than after dry cleaning.
With both teat cleaning methods only a small percentage of samples taken after cleaning contained thermoduric and coliform bacteria in numbers exceeding the detection limit of 10 cfu/ml. Samples from dry cleaned teats contained thermoduric bacteria in 35.0 % (max 2.6 x 10^2/ml) and coliforms in 11.2 % (max. 1.4 x 10^3/ml) whereas samples from wet cleaned teats were positive for thermoduric bacteria in 21.6 % (max. 7.7 x 10^2/ml). Only in one sample coliforms were detected after wet cleaning (5.6 x 10^2/ml).

As the bacterial counts on teats after cleaning are influenced by the contamination level before cleaning (McKinnon et al., 1983), the results of the two cleaning methods are not fully comparable. In the following bacterial counts in teat swabs taken from the same teat before cleaning and after cleaning were compared.

The results for TBC are shown in figure 8. The comparison between dry cleaning with a paper towel and wet cleaning with warm water followed by drying with paper towels shows significant differences ($p<0.05$) between the two cleaning methods regarding reduction of TBC in Log units.
Significant differences between the two methods were also obtained for reduction of staphylococci on teat surfaces ($p<0.05$, data not shown).
Coliform and thermoduric bacteria were only present on teat surfaces in low numbers before cleaning. In 71 of 100 samples the coliform count was at or below the detection limit of 10 cfu/ml. Bacterial counts exceeding 10^3 cfu/ml were only detected in 1 of 100 samples for coliforms and 3 of 93 samples for thermoduric bacteria. The low bacterial counts of thermoduric and especially of coliform bacteria on teats limits the value of these bacterial groups for determination of cleaning efficiency. The reduction of coliforms and thermoduric bacteria (in Log10 units) was not significantly different between the two cleaning methods.
However, coliforms on teat skin are an indicator for faecal contamination and higher thermoduric bacterial counts, e.g. resulting from bedding material, may be observed under different housing conditions. The determination of the two bacterial groups in teat swabs may give additional information on the hygiene status of farms in practice.

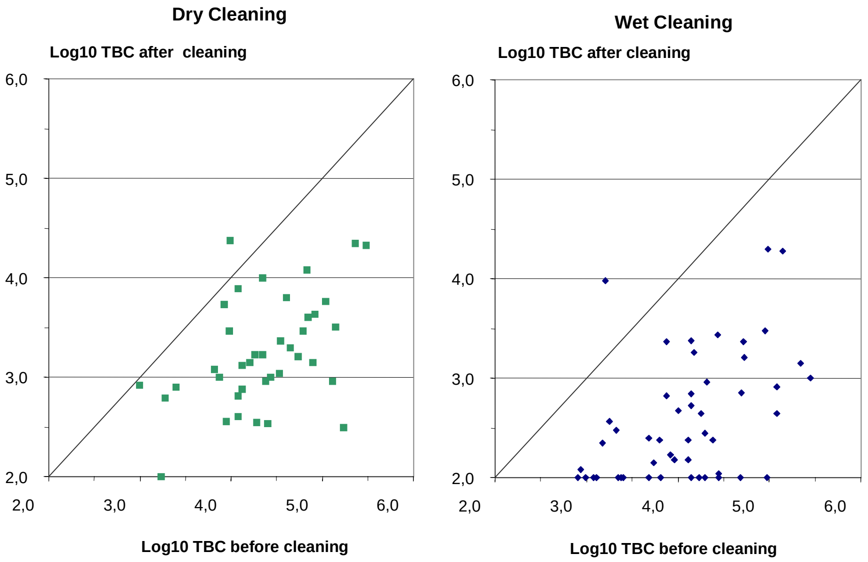

Figure 8 Efficiency of manual teat cleaning methods – TBC in teat swabs before and after teat cleaning; Comparison of single teat results

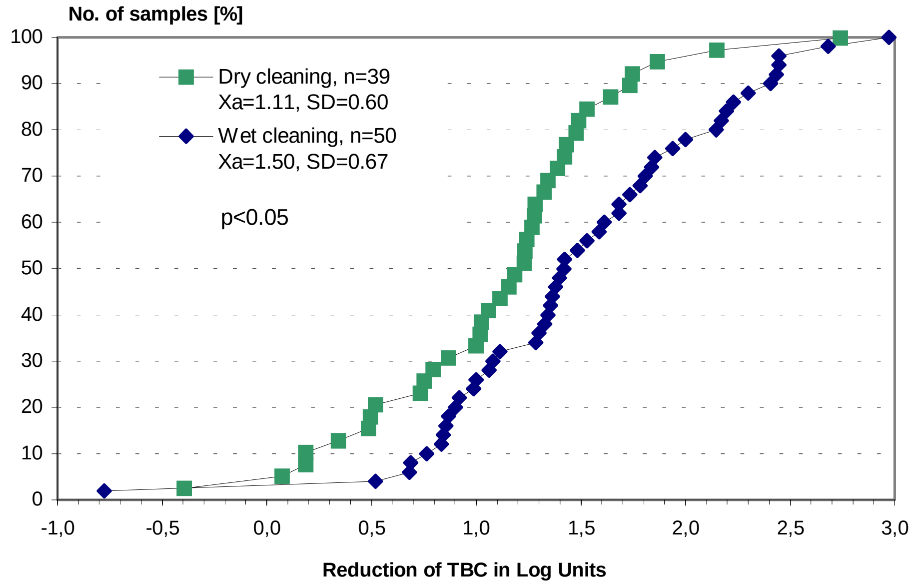

Figure 9 Efficiency of manual teat cleaning methods – Reduction of TBC on teat surfaces; Comparison of cumulative distributions of results

The findings in figures 8 and 9 together with the results in table 5 indicate that wet cleaning followed by drying with a paper towel leads to a better reduction of bacterial contamination of teats than the use of dry paper towels only. These findings are in accordance with results from earlier investigations (McKinnon et al., 1983; Galton et al., 1984; Galton et al., 1986). The determination of cleaning efficiency can be based on reduction of TBC on teat skin as was demonstrated on the two manual cleaning methods. A clear differentiation of cleaning efficiency was possible, although only a limited number of samples was investigated. In addition the two manual cleaning methods, performed as recommended by mastitis control schemes, can serve as references for comparisons with automated teat cleaning methods.

4.3.2 Determination of cleanliness based on ATP measurement

To facilitate the determination of cleanliness the method of ATP measurement was examined as a possible means to replace the determination of TBC. The correlation between TBC and ATP determined from the same teat swab solution is shown in figure 10.

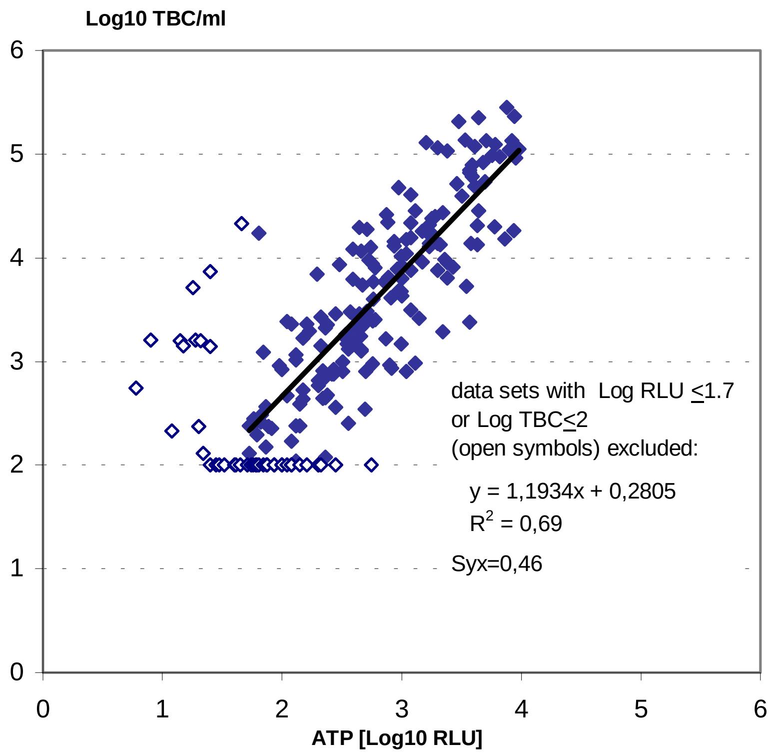

Figure 10 Correlation between ATP and TBC in teat swab solution from cleaned and uncleaned teats (n=216)

Figure 10 shows a high correlation between ATP (in Log RLU) and TBC (in Log TBC) in teat swabs. Data sets with RLU<50 (Log RLU < 1.7) or TBC ≤100 (Log TBC≤ 2) were excluded. Results with RLU<50 are probably due to failures in measurement and may occur if the test stick is not completely covered with liquid. In recent experiments the measurement was repeated when the result was below 50 RLU and in most cases the result of the second measurement was higher. For routine use values below 50 RLU should be repeated. Values with TBC ≤ 100 cfu/ml and high ATP values may occur when other sources of ATP like somatic cells or other organic material are present. In addition, bacteria which are not detected with the cultural method applied may have been present in the samples.
By Samkutty et al. (2001) a lower correlation with R^2 =0.58 was found between Log10 values of ATP and TBC in raw milk but this may be due to the different composition of microbial flora.

The determination of ATP in teat swabs before and after cleaning with regard to the two manual cleaning methods applied is demonstrated in figures 11 and 12.

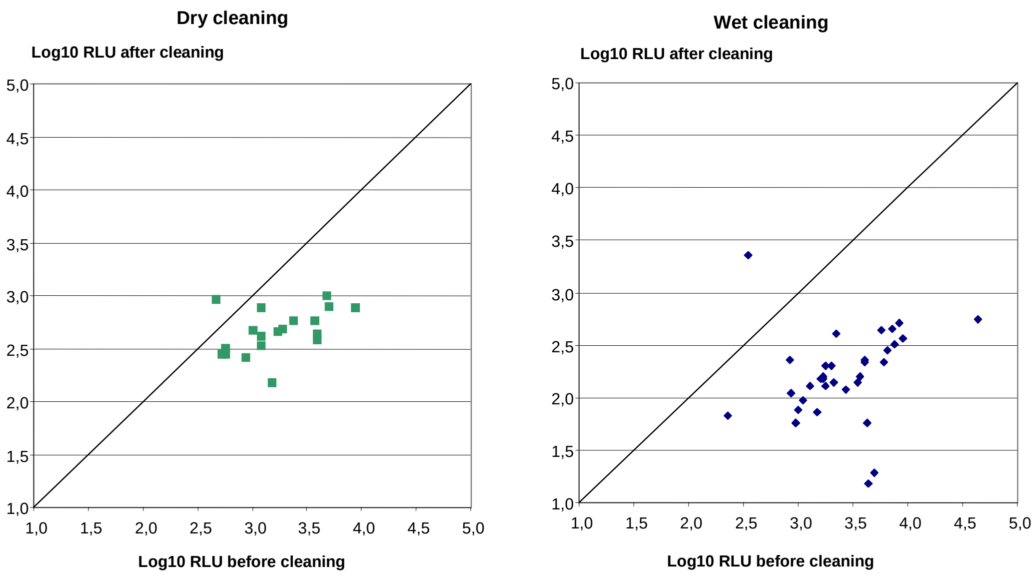

Figure 11 Efficiency of manual teat cleaning methods – ATP measurement [Relative light units = RLU] in teat swabs; Comparison of single teat results

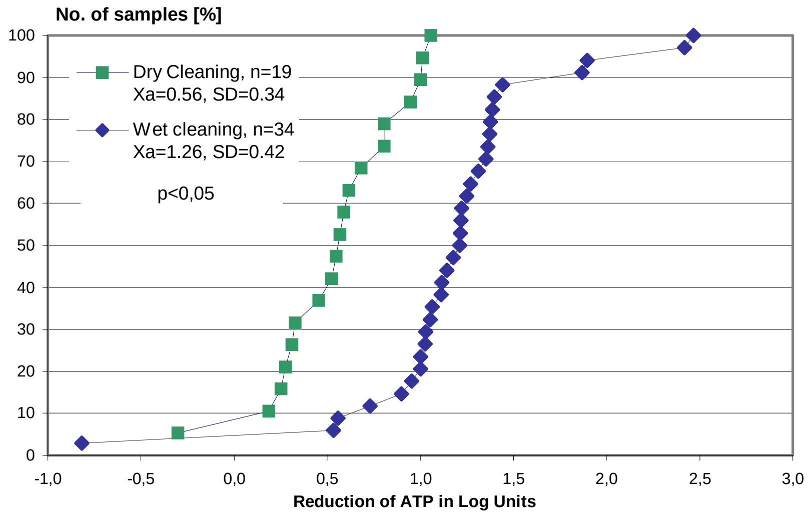

Figure 12 Efficiency of manual cleaning methods - Reduction of ATP on teat surfaces; Comparison of cumulative distributions of results

Based on ATP reduction in Log10 units an even better differentiation between the two cleaning methods seems to be possible than by use of TBC. However, it has to be taken into account that very limited data were available for this kind of comparison.
The method of ATP measurement is preferable to determination of TBC because it is very fast, easy to perform and can be used as a cowside test. As with sediment tests the immediate availabilty of results can be used for demonstrations to the farmer. Documentation of results is possible.

In general all experiments have been performed on a single farm (experimental farm, Schaedtbek), where a comparable bacterial flora over time can be expected. It has to be taken into account that on other farms different results may be obtained due to varying housing conditions and differing bacterial flora composition.

4.4 Use of a marker substance

4.4.1 Defining the loss of manure/cobalt mixture

The results of the first experiment are tabulated in table 6. Comparing the calculated cobalt in the manure-solution with the measured cobalt in the removed manure-solution showed losses of cobalt into the environment of the cow, even after only 15 minutes waiting time. Possibly not all of the cobalt could be removed, as can be seen by the residues of cobalt found in the first milking after the removal of cobalt/manure mixture. Nevertheless, most of the cobalt could not be recovered, even after 15 minutes.
In addition to the samples of milk and cleaning water, samples of the tap water, cloths and gloves used during the experiment were analysed on cobalt too. Also bedding material was sampled at random. No high levels of cobalt were measured in these samples.
The carry-over from teats into the milk has been calculated and the highest percentage was found with the longest waiting time between application of cobalt and the next milking.
The next milking (morning milking) showed no residues of cobalt anymore.

Table 6 Influence of waiting time on cobalt in pooled removed manure/cobalt solution and in pooled milk from 5 cows per waiting time

Waiting time	Cobalt put on 5x4 teats	Calculated cobalt in manure-solution	Measured cobalt in removed manure-solution		Cobalt in milk after removal of manure/cobalt		Cobalt in milk of the next milking
(hour)	(µg)	(µg/kg)	(µg/kg)	Recovery (%)	(µg/kg)	Carry-over (%)	(µg/kg)
0.25	67 000	67 000	7 800	12	42	2	1
6	48 800	48 800	900	2	11	1	<1
12	41 500	41 500	1 200	3	80	10	<1

From the results of this experiment it can be concluded that cobalt/ manure put on teats was recovered with a maximum of only 14 % in removed manure and in milk. Removal after 6 and 12 hours waiting time was less effective than after 15 minutes of waiting time. Carry-over in milk was highest after 12 hours of waiting time. This may suggest the sticking of cobalt to the teats once the manure is dried up and release during milking. Compared to Schuiling (1992) the levels found were similar. However, Schuiling (1992) put more manure on the teats (about 2 gram per teat versus about 1 gram per teat in this study) and added higher amounts of lithium compared to cobalt into the manure (2.7% against 0.5% in this study).

4.4.2 Defining the carryover of cobalt

Carry-over from treated cows to untreated cows (Table 7) showed differences between treated cows and not treated cows. The teats of treated cows were contaminated with manure/cobalt mixtures and the untreated cows were milked directly after the treated cows were milked. A unit flush was performed after milking the treated cows. Still some carry-over into the milk of the next milking was found. Possibly the teat cleaning system was contaminated.

Table 7 Cobalt in bulk tank milk before and after the experiment and in pooled milk from cows treated with manure/cobalt mixture and cows not treated with manure/cobalt

	Cobalt (µg/kg)		Cobalt (µg/kg)
Bulk tank milk before experiment	4	Pooled milk sample of 5 treated cows	800
Bulk tank milk after experiment	1	Pooled milk sample of 5 not treated cows milked after the treated cows	13

4.4.3 The effect of teat cleaning

The effect of the teat treatment was determined by comparison of milk from cows with and without teat cleaning (Table 8 and 9). Differences between teat cleaning and no teat cleaning were small and sometimes conflicting with the expected results. Group 3 for example showed lower average levels for the uncleaned cows than for the cows with cleaned teats. No teat cleaning was supposed to give more cobalt in the milk than when teats were cleaned. As a result, analysis of variance with day and group as block structure showed no significant differences between the two teat cleaning treatments. One of the reasons for these contradictory results may be that the average waiting times between application of the manure/cobalt mixture and milking varied (Table 10). Especially group 3 had longer waiting times, due to practical performance of the experiment. Analysis of the effect of waiting time before sampling and milking also showed no influence on the results: no differences between teat cleaning and no teat cleaning were seen. Some similarity between waiting time and milk production can be seen (Table 10 and 11)

Compared to the treated cows in table 7 (800 µg/kg) the levels of cobalt in the milk in table 8 are rather low (mean <80 µg/kg).

Some remarks have to be made in accordance to the practice of udder and teat cleaning of the automatic milking system. Although to part of the cows no teat cleaning was applied, still the first squirts of milk were separated, because the system used could not exclude this from its software. So only one-second squirts of milk were removed. On the first day of our experiment, these squirts were sampled. The amount of cobalt found in these samples were from the same magnitude as in the cleaning solution of the samples from the teat treated cows. So probably the difference between the treated and untreated (read uncleaned) cows were not distinguishing enough. Perhaps with other automatic milking systems the difference will be more pronounced. During the other experimental days these first squirts of milk were mixed with the milk from these cows of which the teats were not cleaned.

Comparing the levels with the study of Schuiling (1992) with lithium as indicator, gives similar levels. However, more manure was put on the teats and higher concentrations of lithium were used, so perhaps increasing the level of cobalt in manure and putting more manure on the teats might improve this method.

Table 8 Cobalt in milk determined in 3 groups during 4 days applying teat cleaning and no teat cleaning

Cobalt in pooled milk samples (µg/kg)	Group 1 (n=10)		Group 2 (n=10)		Group 3 (n=10)	
	Teat cleaning	No teat cleaning	Teat cleaning	No teat cleaning	Teat cleaning	No teat cleaning
Day 1	23	29	41	36	15	21
Day 2	79	89	35	99	53	39
Day 3	116	84	60	87	63	24
Day 4	50	104	44	46	98	25
Mean	67	78	45	76	57	27

Table 9 Carry-over (%) of cobalt from teats into milk determined in 3 groups during 4 days applying teat cleaning and no teat cleaning

Carry-over from teats in milk (%)	Group 1 (n=10)		Group 2 (n=10)		Group 3 (n=10)	
	Teat cleaning	No teat cleaning	Teat cleaning	No teat cleaning	Teat cleaning	No teat cleaning
Day 1	2.9	2.2	2.9	3.6	1.9	1.8
Day 2	4.9	7.6	2.9	6.5	2.7	4.2
Day 3	7.5	6.1	6.7	6.9	7.7	2.2
Day 4	5.2	3.9	3.5	3.9	6.7	2.5
Mean	5.1	4.9	4.0	5.2	4.7	2.7

Table 10 Waiting times after application of manure/cobalt mixtures on teats of cows until teat cleaning and milking

Waiting time (hours)	Group 1 (n=10)		Group 2 (n=10)		Group 3 (n=10)	
	Teat cleaning	No teat cleaning	Teat cleaning	No teat cleaning	Teat cleaning	No teat cleaning
Day 1	9.55	8.05	5.97	7.78	9.45	6.97
Day 2	5.90	8.55	9.47	7.55	9.62	12.93
Day 3	5.40	7.43	8.92	7.38	9.52	11.28
Day 4	8.27	4.98	7.27	6.68	8.12	10.45
Mean	7.28	7.25	7.91	7.35	9.18	10.41

Table 11 Total milk production per group after application of manure/cobalt mixtures on teats of cows, teat cleaning and milking

Milk production (kg)	Group 1 (n=10)		Group 2 (n=10)		Group 3 (n=10)	
	Teat cleaning	No teat cleaning	Teat cleaning	No teat cleaning	Teat cleaning	No teat cleaning
Day 1	66.9	47.5	48.5	42.9	71.3	48.1
Day 2	42.2	57.2	63.3	61.7	45.3	82.9
Day 3	47.8	43.7	65.3	52.4	73.8	67.4
Day 4	58.5	27.7	58.0	55.1	55.2	83.8
Mean	53.9	44.0	58.8	53.0	61.4	70.6

Results of the other samples than milk are shown in Table 12. The amount of cobalt in the manure was about what was added; during sterilisation the manure was concentrated so the added 5g/kg cobalt increased a little. The cobalt contents in bulk tank milk can be divided in two groups; the first and the last two days. Changing to another lot of silage may be a possible explanation for the increase of cobalt in milk. But despite changing to another feed lot, the last two days an increase of cobalt content of bulk tank milk can be concluded after the experiment compared to the first two days: This might indicate more carry-over of cobalt from cows taking part in the experiments into the bulk tank milk, but distinguishing this from the higher natural level is difficult.

Table 12 Cobalt content in different samples taken during experiment 3

Sample	Day 1	Day 2	Day 3	Day 4	Mean
Cobalt/manure (µg/kg)	5 400 000	5 700 000	5 500 000	5 200 000	5 500 000
Bulk tank milk before experiment (µg/kg)	2	1	10	8	5
Bulk tank milk after experiment (µg/kg)	3	1	21	17	11
Teat cleaning sample group 1 (µg/kg)	1700	2600	2550	2600	2350
Teat cleaning sample group 2 (µg/kg)	1900	1850	1450	850	1500
Teat cleaning sample group 3 (µg/kg)	500	1100	1600	1500	1200

Results on the residues in the solution which contained the removed manure/cobalt mixture from the teats can be evaluated from the teat cleaning samples. These samples consisted of the first squirts of milk and the teat cleaning residues separated after teat cleaning before milking. From the teats of group 1 more manure/cobalt was removed than from the other two groups. Considering the waiting times (Table 10) similar levels for group 1 and 2 would be expected. So the waiting time is not the most important effect on removal of manure/cobalt. Furthermore, considering the results in table 6, higher levels of cobalt in milk could be expected with longer waiting times. However, group 3 with longer waiting times until milking showed no significant higher levels in the milk and not in teat treatment samples (Table 12).
Although no differences between teat cleaning treatment could be found (Table 8), also no influence of day and group was concluded. Analysis was also performed on total amounts of cobalt in milk (data not presented), correcting for milk production: also no differences between teat cleaning treatments could be found.

4.5 Protocol for evaluation of teat cleaning systems

The evaluation of teat cleaning efficiency of cleaning devices used in AM systems will be based on a combination of methods:

Proposed protocol for evaluation of teat cleaning systems (1)

Before cleaning

1. Visual scoring of teat cleanliness
2. Teat swab for determination of ATP and TBC on teat surfaces

Cleaning of teats

After cleaning

3. Visual scoring of teat cleanliness
4. Teat swab for determination of ATP and TBC on teat surfaces
5. Teat swab for sediment test

Two teats per cow facing the investigator, one front and one hind teat will be sampled. In single box systems all cows that are milked within a time period of 6 hours will be sampled. In multibox systems an adequate proportion of cows will be included.

The cleaning efficiency of the mechanised cleaning devices will be evaluated based on reduction of contamination. The results will be compared to the present results of the two manual reference cleaning methods of dry cleaning with paper towels respectively wet cleaning with subsequent drying.
Three farms per manufacturer will be visited. If inconsistent results occur additional farms will be included.

An additional evaluation based on artificial contamination of teats with cobalt as tracer is dependent on the results of further experiments. Suggestions for a protocol are made, but could not be validated by the results of the respective experiments until now. Therefore the protocol should be tested on a farm with another brand of automatic milking system.

Proposed protocol for evaluation of teat cleaning systems (2)

1. Before and after the experiment a bulk tank samples should be taken to examine carryover effects and initial levels of cobalt.
2. For this protocol ten cows should be taken at random. On the teats of these ten cows a mixture of manure/cobalt with higher cobalt concentrations will be applied. Preferably about 2 gram per teat should be applied. Waiting time should be about 15 minutes.
3. Five cows will be milked without teat cleaning and five cows with teat cleaning. After milking of one ‘cobalt’ cow, the system should be cleaned by a unit flush or a similar cleaning. Before milking the next ‘cobalt’ cow, at least one normal cow must be milked to avoid carry-over effects. Milk samples should be taken from all ‘cobalt cows’ and pooled to one sample for the cows without teat cleaning and one for the cows with teat cleaning. The cobalt content of the manure/cobalt mixture is also controlled.
4. Careful cleaning of the teat cleaning system and the milking unit after milking treated cows is advised.

5 Conclusion

Different approaches for the evaluation of teat cleaning efficiency have been tested and a protocol consisting of a combination of methods has been developed which can be used on mechanised cleaning devices. A combination of at least two independent methods for evaluation of teat cleaning efficiency was already proposed by Mottram (1997). The combination of methods is expected to lead to a reliable evaluation taking into account different objectives like reduction of visible contamination as well as reduction in bacterial load of the teat skin.
The investigations on visual inspection of teat cleanliness by different testing persons showed the limits of this method due to dependence of results on evaluating person and restricted repeatability within person. Nevertheless, the visual inspection should be included in evaluation procedures because in a common sense cleanliness means the absence of visible contamination. In combination with sediment tests the results of visual inspection are useful at least to assess if a teat is free from visible dirt.
The determination of total bacterial counts in teat swabs before and after cleaning is suitable to define the cleaning efficiency of a teat cleaning method. The determination of staphylococci gives no additional information compared to TBC. Thermoduric and coliform bacteria are usually present on teat skin in low numbers and therefore their value for evaluation of cleaning efficiency is limited.
By ATP measurement in teat swabs before and after cleaning a comparable discrimination of cleaning methods can be obtained as with TBC. The possibility to determine ATP in samples as a cowside test enhances the usefulness of this method.
In table 13 a summary of the combination of test methods is provided.

Table 13 Characterisation of methods used in combination for evaluation of teat cleaning systems

	Visual inspection of teats	Sediment test on teat swabs	TBC in teat swabs	ATP in teat swabs
Objective measurement	-	-	+	+
Comparison before versus after cleaning	+	-	+	+
Documentation of status of teat cleanliness	-	+	+	+
Cowside test	+	+	-	+
Comparison with other studies	-	-	+	(+)[1]

[1] only limited data available

The two manual cleaning methods that were performed as recommended in mastitis control programmes will serve as reference methods for comparisons of mechanised teat cleaning methods. The cleaning efficiency of the reference methods is well characterised by the experiments on reduction of bacterial contamination and ATP. For the follow up experiments on farms in practice it has to be taken into account that the initial bacterial load as well as the flora composition may be significantly different from the situation on the experimental farm where the experiments were performed.
During farm visits housing and management conditions will be evaluated along a checklist and correlated to the contamination level of udders and teats in the herd in order to identify major factors influencing level and kind of teat contamination as well as teat cleaning efficiency. As mechanised cleaning of the udder basis is not desirable because of the possible increase of microbial contamination of raw milk – especially if wet cleaning without drying is applied – efforts have to be made to reduce contamination of udder and teats by other means.

A protocol based on artificial contamination of teats with cobalt could not yet be validated. The loss of cobalt after application on teats was rather high. Recovery with a maximum of 14 % was found. Carry-over from teats and from teat cleaning device into the milk was found.

The effect of teat cleaning on cobalt in the milk could not be determined in experiments where teats from five cows were cleaned and from five other cows were not cleaned after application of manure/cobalt mixture on the teats of the ten cows.
An additional experiment will be performed on carry over of cobalt into milk with a higher cobalt concentration in the manure mixture and a different automatic milking system. Aspects of toxicity shall be kept in mind.

As the evaluation of teat cleaning efficiency based on artificial contamination of teats is a completely different approach and in any case has to be performed separately the evaluation on farms in practice with methods as first described above is not affected by the delay through additional experiments on the tracer solution study. If the protocol based on cobalt measurement can be validated this method will be applied additionally to evaluate the teat cleaning efficiency in AM systems.

The presented protocol in chapter 4 is the result of the first sub-package of workpackage 6 of the EU project. Within the second sub-package the protocol will be applied on farms in practice to determine teat cleaning efficiency of automatic cleaning devices in AM systems. That report will be finished in the summer of 2003.

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Abbreviations

AM	Automatic Milking
ATP	Adenosin-tri-phosphate
cfu	Colony forming units
IDF	International Dairy Federation
NMC	National Mastitis Council
RLU	Relative Light Units
rpm	Rounds per minute
TBC	Total bacterial count