



Definition of normal and abnormal milk at time of milking

*Consequences of definitions of acceptable milk quality
for the practical use of automatic milking systems*

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Information

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Definition of normal and abnormal milk at time of milking

*Consequences of definitions of acceptable milk quality
for the practical use of automatic milking systems*

Morten Dam Rasmussen

*Department of Animal Health and Welfare,
Danish Institute of Agricultural Sciences*

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Correspondence:

Morten Dam Rasmussen
Research Centre Foulum,
DK-8830 Tjele
Denmark

E-mail: MortenD.Rasmussen@agrsci.dk

Abstract

The general conditions of hygiene in milk production in the EU are defined by the Commission Directive 89/362/EEC (1989) but not all elements apply to automatic milking. The following text is proposed to be included in the coming EU Hygiene Directive:

Milking must be carried out hygienically ensuring in particular: - that milk from an animal is checked for abnormalities by the milker or a method achieving similar results and that only normal milk is used for human consumption and that abnormal, contaminated, and undesirable milk is excluded.

This text is based on the following definitions of normal, abnormal, contaminated, and undesirable milk:

- *Normal milk*: Milk suitable for human consumption.
- *Abnormal milk*: Milk which differs from normal milk in respect of colour or homogeneity.
- *Contaminated milk*: Milk which, prior to the milking of the animal, is known to be unfit for human consumption following treatment of the animal with antibiotics or other veterinary products which have a requirement that the milk must be withheld from sale for such use.
- *Undesirable milk*: Milk which, prior to the milking of the animal, is known to be unsuitable for human consumption, e.g. colostrum, high somatic cell count.

The definition of abnormal milk caused by clinical mastitis is proposed to be based on the homogeneity of the milk and not on the colour since the colour of the milk changes with breed, stage of lactation, feed stuff etc. The reference method is suggested as filtration of the milk through a filter with a pore size of 0.1 mm and milk where clots are clearly visible in such a filter is then defined as being abnormal. Incidences of watery and yellowish milk may or may not be detected by this method. Requirements of sensitivity and specificity have not been determined yet but should be equal for all milking systems. It is recommended that the specificity should be >99% to be well accepted by farmers. A sensitivity of 80% may apply. The frequency of visible blood in the milk is rare but regarded as abnormal. Secretion from the udder in the first 3 days after calving is mainly colostrum, which is not regarded as “normal” milk. A high cell count is a clear indicator of inflammation in the udder but cannot be required to be measured at every milking for determination of abnormal milk.

A workshop was held on 27th November 2002 on definition of normal and abnormal milk at time of milking. The main purpose of the workshop was to present background material for a definition, discuss the intention and consequences of the definition, and finally outline agreements and disagreements. There was consensus at the workshop that:

- There should be no double standards.
- If clots appear, the milk is abnormal.
- Milk that has changed in colour because of the level of red blood cells present is regarded as abnormal milk.
- The cell count should not be included in the definition of abnormal milk.

The workshop was not convinced about the reference method for detecting abnormal milk and had question marks on the reference for blood in milk. The colostrum period was stated as at least 72 hours with no further requirements. Sensitivities and specificities of reference methods were not established.

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1. Introduction

The general conditions of hygiene in the milk production in the EU are defined by the Commission Directive 89/362/EEC (1989) and Chapter III-4 reads:

Before the milking of the individual cow the milker must inspect the appearance of the milk. If any physical abnormality is detected, milk from the cow must be withheld from delivery.

Fulfilment of this directive is presently a problem with automatic milking systems because normally a human is not present and visual inspection of foremilk is not performed. Technical solutions may replace visual inspection for detection of abnormal milk either before or during milking, and subsequent separation. However, unequivocal and generally accepted definitions of normal and abnormal milk are not available. In order for AMS companies to develop sensors to detect abnormal milk, a precise definition of abnormal or unacceptable milk is needed. The definition has to apply not only to automatic milking but to conventional milking as well. A workshop was held at the Danish Institute of Agricultural Sciences on 27th November 2002 in order to give input to the coming EU-hygiene directive concerning this matter (Rasmussen, 2002a). Participants from outside of the EU were invited to this workshop to make the definition applicable worldwide and give a broad input to the present paper. This deliverable consists of the conclusions from the workshop, spin off of the discussion at the workshop, some of the background material, and the consequences of the proposed definition.

1. Changes in milk during intramammary infection

Healthy quarters are regarded as those, which are free from infection by pathogen. Those, which are infected by pathogens, but show no visible signs, are regarded as subclinically infected. Where the milk, or the cow, show signs of abnormality, the quarter is regarded as clinically diseased.

There is a continuous and overlapping area between these three stages mainly depending on the number and kind of markers used. Mammary inflammation affects the integrity of the gland structure, damaging the secretory epithelia and the blood-milk barrier. This damage is causing a decrease in the synthesis of milk-specific constituents while blood-borne substances and leakage products from udder tissue cells are released into the milk. For example, increased permeability of blood capillaries results in an increased concentration of serum albumin.

Many milk components are influenced by mastitis. Major components such as fat, protein, and lactose are reduced and the concentrations of protein fractions, caseins and whey proteins are altered. Furthermore, the concentrations of minerals and trace elements, enzymes and vitamins are changed. An overview of these changes is given in Table 1. A detailed description of the changes in milk composition is given by Kitchen (Kitchen, 1981) and the reader is referred to this review for details. With all these changes occurring in milk during inflammation of the udder, a definition based on one single factor is unlikely to be sufficiently comprehensive. Currently laboratory methods used for diagnosis of mastitis are primarily based on SCC and the differentiation of bacterial pathogens in milk samples. However, no distinct threshold can be set so far for any of the milk components that will clearly distinguish between healthy and diseased quarters.

Table 1. Effect of mastitis on the composition of milk (Korhonen and Kaartinen, 1995).

Component	Normal content	Direction and degree of change *)
Major component		
Fat %	4.3	-
Protein %	3.3	-
Lactose %	4.8	-
Fat components		
Free fatty acids (mEqv/l)	0.7	++
Fatty acid composition mg/g fat		
C 4 - C 12	126.4	+
C 16 - C 18	708.4	-
Protein fractions (mg/ml)		
Total casein	27.9	--
Total whey protein	8.5	+++
Caseins (mg/ml)		
α_{S1} -casein	13.3	---
β -casein	10.6	---
κ -casein	1.6	++ ?
Whey proteins (mg/ml)		
β -lactoglobulin	3.3	---
α -lactalbumin	1.2	---
Immunoglobulins	0.6	+++
Proteose-peptones	0.5	++
Serum albumin	0.3	+++
Lactoferrin	0.1	+++
Minerals and trace elements (μg/ml)		
Sodium	470	++
Chloride	1030	+++
Potassium	1500	-
Calcium	1210	---
Magnesium	120	---
Phosphorus	950	---
Iron	0.53	+
Copper	0.12	+
Zinc	3.60	-
Enzymes		
Catalase (μ mol O ₂ /min/ml)	0.08	++++
Lactate dehydrogenase (mU/ml)	300-500	+++
Alkaline phosphatase (U/ml)	191	+++
Acid phosphatase (μ mol/min/ml)	0.06	++
Carboxylesterase (μ g/ml)	0.004	++++
Arylesterase (μ g/ml)	0.03	+++
β -glucuronidase (μ g/ml)	0.01	+++
Lactoperoxidase (μ g/ml)	0.02	+
Lipase (μ g/ml)	1.5	++
Lysozyme (μ g/ml)	0.0004	++++
Xanthine oxidase (μ g/ml)	12	+/-
Plasmin (U/ml)	73.5	+++
Ornithine decarboxylase (U/ml)	984	--
N-acetyl- β -D-glucosaminidase (NAGase %)	7.3	+++
Vitamins (μg/ml)		
Vitamin A	0.37	+/-
β -carotene	0.21	+/-
B ₁ (thiamin)	0.42	-
B ₂ (riboflavin)	1.72	--
Vitamin C	18	---

*) Explanation

+=	10x increase	--	10 % decrease
++=	11-100x increase	---	11-25 % decrease
+++ =	101-1000x increase	----	26-75 % decrease
++++ =	>1000x increase	-----	> 75 % decrease

Somatic cell count (SCC) has long been used as the main indicator of inflammation and as the key parameter in monitoring udder health in mastitis control programs (Dohoo and Meek, 1982; Schukken and Kremer, 1996; Erskine, 2001). During inflammation SCC is increased as a host immune response (diapedesis of leucocytes) to neutralize the pathogens causing the inflammation (Harmon, 1994). The rapid increase in SCC following inflammation makes SCC a valuable indicator of intramammary infection along with a number of enzymes and milk constituents (Kitchen, 1981; Hamann and Kromker, 1997).

The golden standard criterion most often used to determine recovery from an intramammary infection is milk samples that are free of bacteria. However, bacteriological methods are not completely reliable because bacteria cannot always be isolated, especially not from just a single sampling.

Indirect mastitis tests may provide information about the inflammatory status of a quarter and aid correct determinations of diseased or recovered quarters, which is important for several reasons. The initial increase in indicators of mastitis following an infection is valuable for the detection of new infections, as a management tool, and as an indicator of considering antibiotic treatment. Secondly, farmers have to decide upon discard of the milk unsuitable for human consumption, which is normally based on the clinical appearance of the foremilk. Thirdly, a high cow SCC may affect bulk milk SCC and violate the accepted threshold. Fourthly, the knowledge on mammary inflammatory status of a cow may be included in culling decisions. Farmers normally consider a quarter or cow cured from mastitis if the cow shows no clinical symptoms and the milk appears normal. At present no automated systems are available to accurately predict when intramammary infection has developed or been cured nor to differentiate between subclinical and clinical mastitis.

The structural developments in dairy farming have led to increased farm size. Farm staff therefore has to identify production diseases and other problems on a considerably larger number of animals than before and consequently much less time is available for the individual animal. Due to this situation, and automatic milking systems, there is a need for developing new automated technology to help monitor changes in udder health status. From the vast amount of literature available, it is clear that a number of potential indicators of udder health status are available and new indicators will probably be found. However, much development (sensors, biology, biometry, technology) is still needed in creating cost effective methods and systems to automatically determine udder health status and to discriminate between normal and abnormal milk. Very sophisticated methods are probably needed if very high sensitivity and specificity is a demand. However, the golden standard has to be clearly defined and unequivocal in order to test and calculate sensitivities and specificities for both conventional and automatic milking systems.

2. Responses to visual appearance of milk

Very few scientists have dealt with an exact definition of the visual appearance of milk in relation to the hygienic quality. This chapter is an attempt to objectively define how different groups of evaluators assess normal and abnormal milk. Many papers deal with treatment of clinical mastitis, prevalence and incidence rates, and effect on milk yield and milk quality. However, the actual appearance of the clinical mastitis is seldom presented. The same appears to be the case for blood in the milk where no clear threshold is available either. A high content of colostrum in the delivered milk may cause problems for the dairy factories because the IgG-content makes the milk coagulate during pasteurisation. Colostrum is normally withheld from delivery and fed to the calves during the first 3 or 4 days after calving. Still, there is no clear threshold defining when milk is acceptable after calving, although several countries impose definite time spans. Several panel tests were carried out to find out how different consumer groups, farmers, and advisors look at and respond to the visual appearance of milk in order to set a limit for what they think is acceptable.

2.1. Visual scoring of clinical mastitis

A test panel of 15 persons comprising 5 milk quality inspectors, 5 milkers, and 5 consumers scored the visual appearance of normal milk and of milk from cows with clinical mastitis and high SCC. The panel scored a total of 120 dishes with milk 4 at a time (simulating scoring of milk from 4 quarters). The milk samples were milked out and presented to the panel for scoring within 15 min. The scoring was not performed immediately at foremilk as usual, which may have caused the milk to curdle. The samples were scored as being normal, watery, containing clots, blood, or colostrum. The test panel did not agree on the scoring and only 10% of the samples had exactly the same score by everybody. Milk quality inspectors agreed the most and consumers the least. Consumers are only accustomed to looking at milk taken directly from the fridge and as an example a high fat percentage made them score normal milk as colostrum (Figure 1). There was no conclusive score of the milk samples but the scoring could be compared with the SCC and a colour scanning. Milk samples that most of the test panel scored as having clots had high SCCs. However, 25% of the samples scored as normal milk had $SCC > 10^6/ml$ and some were even above $10^7/ml$. The main conclusion is that it is not possible to differentiate between high and low SCC milk samples just by looking at the visual appearance.

Milk samples were colour scanned. The R^2 -value of $\log SCC$ regressed on the outcome of the colour scanner was 0.26 but 0.65 for the visual mean score of each sample regressed on the colour. There seems to be a possibility of using colour scanning as aid in the differentiation between normal and abnormal appearance of the milk as confirmed by Ouweltjes & Hogeveen (2001) and Espada & Vijverberg (2002). However, if high SCC is included in the definition of abnormal milk at time of milking, this property has to be measured more directly.

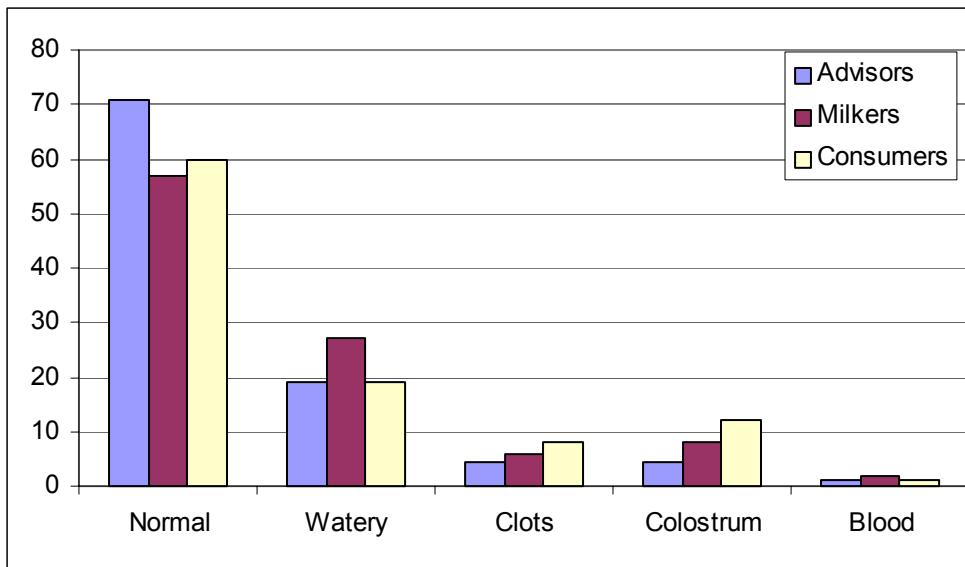


Figure 1. Percentages of samples scored visually as normal, watery, containing clots or blood of dishes with normal milk and milk from quarters with clinical mastitis. Milk quality advisors, milkers (operators), and consumers formed the test panel.

2.2. Visual scoring of milk mixed with blood

The test panel scored 120 dishes, 4 at a time with different percentages of blood mixed homogeneously with milk containing either 0.5 or 3.5% fat. Milk samples were scored as being normal, slightly pink, or pink. The test panel was able to detect milk samples with 0.1% blood. The consumer group did best and scored 37% of the samples with 0.1% blood as normal versus 60-70% by the other two groups (Figure 2).

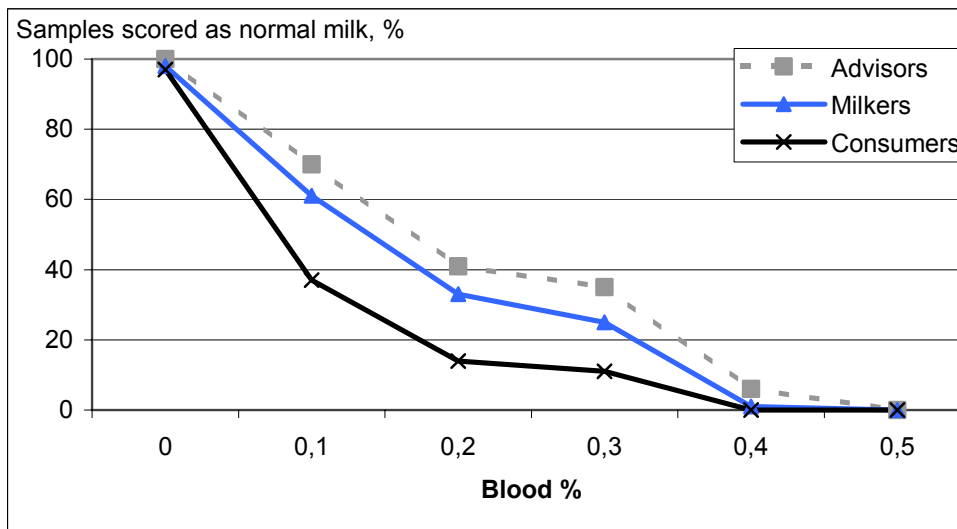


Figure 2. Visual scoring of dishes with normal milk and milk containing blood. Milk quality advisors, milkers (operators), and consumers formed the test panel.

The consumers typically scored samples with 0.1% blood as normal if all 4 samples had the same percentage of blood or some were higher. This was seen for higher percentages as well. All members of the panel scored milk with 0.5% blood as being pink. Out of the samples with

0.1% blood, the panel scored 67% of the samples with 0.5% fat as being normal versus 45% of the samples with the 3.5% fat in the milk.

Foremilk normally has a low fat percentage, which makes it more difficult to point out milk samples with a low content of blood. It is concluded from the test panel results that milk samples with 0.4% or more blood all will be scored as pink and samples with 0.1% blood can be visually detected if they are compared with milk samples without blood.

To explore the lower limit, an additional test was carried out with scoring of pictures of milk containing blood. Containers with 0.05% blood were not scored to be different from containers without blood. If we accept 0.1% blood in the milk as the upper limit at quarter level and that this will happen in a maximum of 3 out of 300 lactation days, then we will always have <1 ppm blood in the bulk tank. Discarding of milk with >0.1% blood will ensure that bulk milk will be visually free of blood. This statement concerns the red blood cells only because all precursors of the milk come directly or indirectly from the blood. Colour scanning was excellent in detecting low amounts of blood in the milk. The R^2 -value of blood percentage regressed on the outcome of the colour scanner was 0.998 and the $SD=0.009\%$, which makes it possible to detect 0.02% blood in the milk and then of course discard milk with more than 0.1% blood. However, the Red Blood Cell content of blood can vary considerably. A concentration of 0.1% raw bovine blood in milk may contain 5-10 m RBC/mL (Baines and Mein, pers. com.). Therefore, a standard specification based on a fixed percentage is flawed and should include Red Blood Cell count. As an indicator of the level for the standard, milk containing 2-4 m RBC/mL has a visible reddish tinge. Milk with 10 m RBC/mL is clearly red. Moreover, blood occurring in milk is not mixed homogeneously and colouring of the milk will vary throughout the milking.

2.3. Visual scoring of slides with abnormal milk

It was difficult to keep milk in a homogeneous state in the small dishes and have many people score the milk samples one at a time. Consequently, a new series of pictures was taken of normal (visually normal and low SCC), watery (appeared thin and shiny), blood, and clotty milk (quarters with clinical mastitis). Pictures were taken during simulation of pre-milking into a black strip cup using a laboratory syringe. Sixty slides of 4 pictures each were shown. The 4 pictures illustrated pre-milking going from a full strip cup to an almost empty one. Twelve slides were repeated. The first 40 slides did not contain blood and the test panel was told that. The last 20 pictures included 8 pictures with a blood content of 0.03 to 1.0%. Out of the 60 slides, 26 were from quarters with the foremilk appearing normal, 8 were watery, 18 were from quarters with clinical mastitis where clots appeared in the foremilk, and 8 were intentionally mixed with blood. These scorings are regarded as the true status. The slides were scored by 20 milk quality inspectors, 24 farmers, 25 veterinarians from the Danish Food and Veterinary Administration, and by a consumer group representing 13 students, 6 technicians, and 15 housewives with no direct relation to dairy farming.

Only about 50% of the normal samples were scored as normal milk (Figure 3), but about 35% were scored as being watery. The change from having much milk on the strip cup to being almost empty could be interpreted as if the milk was watery when the layer of milk became thin (see Figure 3). Droplets of milk on the plate of the strip cup may have been interpreted as clots since 12% of the normal samples were scored as having clots. Close to 80% of the watery samples were scored correctly as watery and this high score was probably due to the fact that these samples were very thin and watery. Milk from cows with clinical mastitis

changed from having few flakes to being abnormal in both homogeneity and colour. The panel scored 63% of the abnormal samples correctly and about 20% were scored as being normal. It turned out to be very difficult to point out samples with blood where less than 10% were placed correctly. Blood does not show well on a black plate in comparison with scoring of a container with a small percentage of blood being next to a container with white milk.

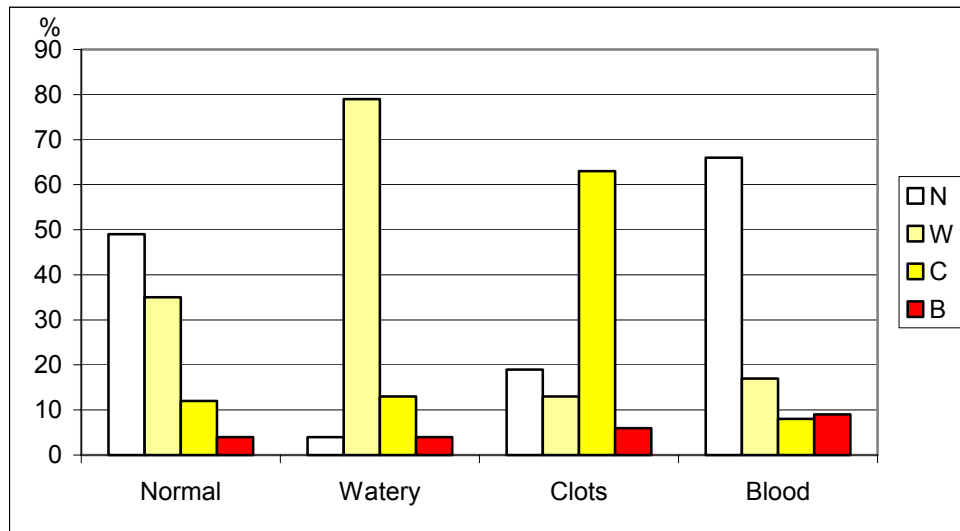


Figure 3. Visual scoring of slides with normal (N), Watery (W), Clots (C), or Blood (B) milk as percentage of the true status.

The different groups scored slides differently in relation to the true status of the milk sample (Figure 4). Milk quality inspectors had the highest percentage of normal milk samples scored correctly and farmers the least ($P < 0.001$), which was mainly due to the fact that a high percentage was scored as watery. Milk quality inspectors and veterinarians scored well on the true watery samples. Veterinarians had the highest percentage of samples with visually abnormal milk scored correctly closely followed by the farmers. Although not impressively, veterinarians and farmers were better at detecting samples with blood than the other two groups. However, these slides were difficult to score correctly even knowing the true result, and the technical condition for the presentation surely influenced the results. It was estimated that the technical condition played a minor role in pointing out quarters with normal, watery, and abnormal milk. Milk quality inspectors and veterinarians were the most consistent in repeating their score of the 12 repeated slides and they also had the highest percentage of correct answers for these slides.

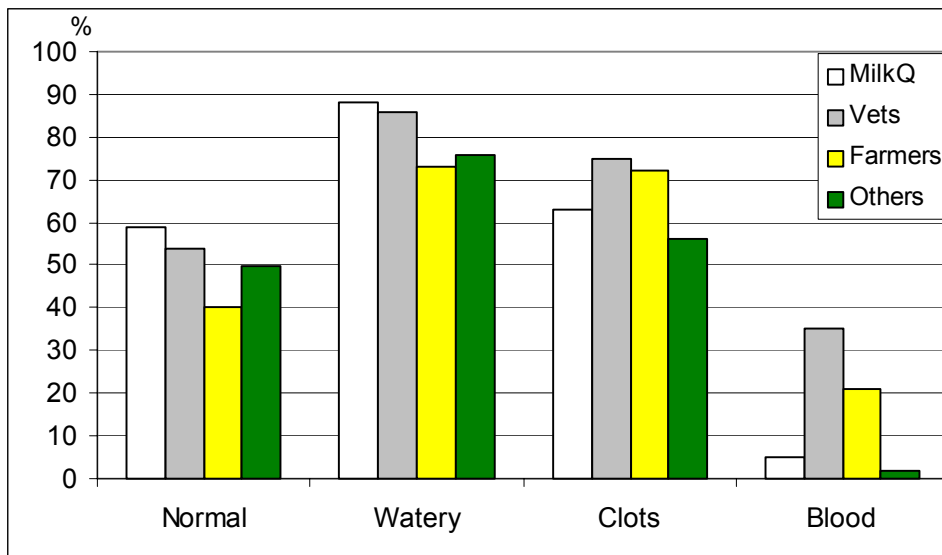


Figure 4. The percentage of samples (slides) with normal, watery, clots, or blood in the milk scored correctly by groups of milk quality inspectors, veterinarians, farmers, and other consumer groups.

Examples could easily have been chosen of normal and abnormal milk that would have a high probability of being scored correctly. However, many of the samples with abnormal milk were selected from quarters where foremilk was truly abnormal and ejected milk instantly appeared normal until milk washed off the strip cup and left clots behind. The focus of the panel test was abnormal milk and this may have moved the panel to score more samples as being abnormal than normal in order to be sure to find all abnormal milk samples. The percentage of abnormal milk samples (57%) was much higher than during normal milking, which gives a better evaluation but probably overestimates the percentage of correct answers for abnormal milk. The large differences between percentages of correctly scored samples within groups of observers highlight the need for a more objective method of categorising foremilk.

2.4. Colostrum

Colostrum from the first milking after calving is normally a creamy, thick fluid with a yellow to brown colour. The milk composition and properties of this fluid change radically during the first days after calving. Immunoglobulins are the major constituents of colostrum and the content drops about 10-fold during the first four milkings but has not even reached the minimum one week after calving. The content may change due to differences in milking frequency, which may be higher or lower than two daily milkings. It is a normal procedure to deliver the milk for human consumption about 4-5 days after calving whenever the colour seems “normal”.

An experiment was carried out to study the physiological, physical and chemical changes in milk during early lactation, and how these changes were affected by leaving out one quarter in either first or second milking in order to study local and systemic regulating factors. Milk samples were collected from each quarter of 17 cows during the first five days after calving and on the two following Thursday evenings. The samples were analysed for SCC, fat, protein, casein, lactose, IgG₁, IgA, α -Lactalbumin, β -Lactoglobulin, bovine serum albumin plasmin, plasminogen, colour, pH, and coagulation properties.

The results indicated that maturation of the secretory cells, including closure of the tight junctions, was nearly complete at parturition. Colostrogenesis, active transport of Igs, appeared to end five days after calving, while lactogenesis, synthesis of α -La and lactose, seemed to start before calving, causing an overlap between the two physiological phases. From the effects of leaving out one quarter in one milking, it seemed that local regulation of the synthesis of most of the components was important but seemed to be of little importance to the physical properties. Vast variations occurred in both chemical and physical properties throughout the studied period (Table 2). Within six milkings, the concentration of casein decreased by 60%, IgG₁ by 94%, and lactose increased by 34%. At milking six, rennet coagulating time was lowest and curd firmness highest. The pH increased from 6.4 to 6.7 over the following period and fell to within the range of normal milk after five milkings. All samples could stand pasteurisation after the 3rd milking. The colour was measured in three dimensions: lightness (L) appeared normal after the 6th milking; redness (a) appeared normal at the 2nd milking and; yellowness (b) appeared normal after milking 10. SCC is naturally high at calving and SCCs of uninfected quarters decrease during the first days after calving and reach <400,000 cells/ml after about 3 days of milking. Measurement of the colour of the milk was able to explain 85% of the variation in immunoglobulin content.

Table 2. Changes in milk composition (SCC geometric means x 1000) and colour (lightness; L, redness; a, and yellowness; b), density, pH, pasteurisation (% of coagulated samples) and rennet coagulation time (Log₁₀R (min)) during the first milkings after calving. The milk was considered “normal” when no further change occurred.

Milking no	IgG, %	Protein, %	SCC	L	a	b	Density	pH	Past	R
1	6,22	13.90	854	34.1	-1.97	12.2	1,048	6.37	80	1.23
2	4,13	10.89	947	35.3	-2.45	10.2	1,042	6.42	43	1.30
3	1,56	6.89	571	36.3	-2.75	6.9	1,038	6.42	5	1.15
4	0,77	5.56	570	37.1	-2.65	7.1	1,034	6.45	0	1.12
5	0,45	5.06	449	37.5	-2.62	6.1	1,033	6.49	0	1.09
6	0,29	4.78	364	38.0	-2.48	6.3	1,032	6.50	0	1.05
7	0,25	4.65	254	38.1	-2.50	5.0	1,033	6.54	0	1.08
8	0,18	4.47	235	38.2	-2.44	4.9	1,031	6.56	0	1.09
9	0,15	4.40	198	38.3	-2.36	4.2	1,032	6.59	0	1.13
10	0,13	4.32	164	38.3	-2.44	3.6	1,032	6.63	0	1.14
11†	0,11	4.07	132	38.1	-2.45	2.8	1,032	6.68	0	1.15
12†	0,09	3.55	85	38.0	-2.34	2.4	1,030	6.73	0	1.19
Normal at milking	12	12	?	6	2	11	6	5	4	3

†Note that milking numbers 11 and 12 are the evening milkings on the two Thursdays following milking 10.

3. Consequences of definitions

3.1. The relation between visual appearance and cell count

Classification of the inflammatory status of a quarter was earlier based on a threshold of 500,000 cells/ml and bacteriological investigation, but lately Hillerton (1999) has suggested that the threshold of 200,000 cells/ml could be used as a simple and cheap tool to discriminate between infected and uninfected quarters. Based on the likelihood of infection and altered manufacturing properties, Smith et al. (2001) conclude that milk from quarters with SCC >200,000 cells/ml, with or without clinical signs, is abnormal milk. SCC at cow level may not always be the best determinant of the mastitis status of a quarter or cow. SCC is currently the only parameter that can be interpreted from quarter to bulk tank milk and it is widely used for this purpose and as an indicator of the milk quality. As a result, it is often used as the main indicator of subclinical mastitis and as a determinant of the need to dump milk.

Regulations on bulk milk SCC are not a matter of human safety but of suitability. The European Union enforces a maximum bulk milk SCC of 400,000 cells/ml. This level is meant as an indirect control of the number of infected cows delivering milk for consumption. Eberhart et al. (1982) estimated that 13% of the cows were infected at this bulk milk SCC level. Rasmussen (2002b) found that the bulk milk SCCs were likely to be <400,000 cells/ml if less than 5% of the cows had visually abnormal foremilk.

Composite milk samples were collected during automatic milking and the appearance of foremilk from 2835 cow milkings scored (Rasmussen, 2002a). Cows, where all quarters had normal milk, had low cell counts in the composite milk and only 3.6% of the samples had >10⁶ cells/ml (Table 3). Cows that had at least one quarter with clots in the foremilk had a geometric mean SCC of about 10⁶ cells/ml and 53.7% had >10⁶ cells/ml. Even when all quarters had visually normal foremilk, 11.8% of the composite milk samples exceeded 400,000 cells/ml but not all samples (73%) were above this threshold if one or more quarters had clots in the foremilk. Cows with blood or small flakes in the foremilk had intermediate cell counts in composite milk.

Table 3. Log. SCC of composite milk and percentage of samples > 200,000, 400,000, and 10⁶ cells/ml by visual appearance of foremilk of the worst quarter.

Worst quarter	No.	Log SCC	SCC > 200, %	SCC > 400, %	SCC > 1,000, %
Normal	2450	4.99	23.8	11.8	3.6
Blood	25	5.41	54.2	29.2	8.3
Watery and flakes	126	5.36	52.5	30.0	11.7
Clots	220	6.01	82.3	73.0	53.7

Hillerton (1999) suggested that bulk milk quality is acceptable if <10% of the cows have SCC between 200-400,000 cells/ml and none above this level. This is not achievable by discarding milk from cows with visually abnormal foremilk even if consecutive milkings are taken into account. It is necessary to perform tests on foremilk more directly associated with SCC on composite milk if milk from cows with SCC > 400,000 cells/ml is considered undesirable.

SCC was measured on 1424 milk samples from 287 quarters. Four percent of the quarters with foremilk appearing normal had a cell count above 1 million per ml, which was mainly from newly calved cows. Out of the quarters with clots in the foremilk, 88% had a SCC >10⁶

cells per ml in the milk produced specifically from that quarter (Table 4). According to Smith et al. (2001), discarding the milk from quarters with SCC >200,000 cells/ml would dump the milk of 204 quarters (15% of 1362 (Table 4)) with visually normal foremilk and this number should be compared with 40 being visually abnormal. Such a low SCC threshold on the quarter basis will ensure that most visually abnormal milk is withheld from delivery but also cause large amounts of milk with an otherwise normal appearance to be dumped. Consequently, SCC should not be included in the definition of abnormal milk.

Table 4. Log. SCC of quarter milk and percentage of quarters > 200,000, 400,000, and 10⁶ cells/ml by visual appearance of foremilk.

Appearance of the foremilk	No.	Log. SCC	SCC/ml	SCC>200, %	SCC>400, %	SCC>1 mill., %
Normal	1362	4.8	295,000	15	9	4
Watery and flakes	22	6.1	3,573,000	86	77	59
Clots in the milk	40	6.8	12,269,000	93	93	88

3.2. Sorting of milk based on a filter method

Experiments were set up to find an objective method to score the appearance of milk (Rasmussen, 2002a). Filters of size 0.05, 0.07, 0.1, 0.2, 0.5, 1.0, and 2.0 mm were used to filter milk from cows with visually abnormal foremilk. The idea was that clots on the filter could then be scored instead of the visual appearance in a strip cup. A strip cup was formed out of black plastic that could drain through the filter and into a container. In this manner, filters could be changed with the container and the sampled milk analysed. About 10 ml of milk from each quarter was foremilked into the modified strip cup. Foam turned out to disturb the scoring but pouring about 10 ml of water through the filter solved this problem. Milk of different breeds and fat percentages was filtered. Milk did not pass the two smaller size filters as willingly as the larger ones. The larger filter sizes were more difficult to read than the smaller ones and also difficult to mount in the modified strip cup. The 0.1 mm filter was the most convenient filter to work with and additionally, this pore size is about the particle size that is visible to the human eye.

Milk from quarters with clinical mastitis was filtered through the largest down to the smallest filter and clots were visible on all filters. The filters were easily blocked by milk from clinical mastitis. Milk from a few cases of clinical mastitis was filtered from the smallest to the largest size filter and again clots were visible on all filters. Obviously, there were factors in the milk that made it clot again when some clots were removed, and the pore size of the filter seems to be of less importance.

A herd with 3 automatic milking units and about 130 cows was foremilked once weekly during 11 weeks and the milk scored for visual appearance, CMT-score, and appearance of the filter. Totally, 5167 foremilk samples were scored. Foremilk was scored as normal, watery, clots, yellowish, or containing blood. CMT was scored on a scale of 1-5 having <150,000 cells/ml for score 1 to an expected >3 mill. cells/ml for score 5. The filter was scored as normal, very few (<4) and small flakes (< 2 mm), or clots. Visually abnormal milk was found in 2.3% of the samples, CMT-score 5 in 0.9%, and clots on the filter in 2.1% of the samples. About 1% of the samples appearing visually normal showed as clots on the filter (Table 5) and additionally 2% were found with small flakes. Watery milk was not detected on the filter in 74% of the cases. All except two of the samples scored visually as clots did not

show as clots on the filter, one was scored normal and one as having few small flakes. The one sample being normal on the filter was marked as being yellow in visual appearance and the other sample contained blood at the next scoring. Consequently, we conclude that all milk samples with clots will show on the filter as well. About half of the samples being yellowish could be detected on the filter, but only 2 out of 9 samples with blood in the milk, and the filter method is not useful to detect these abnormalities. CMT-score 5 was given to 24% of the samples appearing visually abnormal versus 29% of the samples showing clots on the filter. Out of the group with CMT-score 5, 62% of the samples were visually abnormal but 69% showed as clots on the filter and 6% as small flakes. Visual appearance of the milk from an infected quarter will depend on the physiological status of the cow and may or may not show as distinct clots. Out of 89 quarters with visually abnormal milk, 30% kept this status throughout the 11 weeks. Out of 83 quarters with clots on the filter, 41% kept their status. The immediate conclusion from this is that the filter method is at least as stable (if not better) to categorise the visual appearance of the foremilk as the visual method. However, if few and small flakes are included in the scoring of the filter, then 76% changed status, which is a poorer result than the normal method.

In conclusion, the filter method is not reliable to point out quarters with watery, yellowish, or bloody milk whereas the method seems consistent and better than visual appearance in finding clots in the milk. Clots should show clearly on the filter to be counted as abnormal milk. Further documentation of the filter method will be provided in the near future.

Table 5. The visual appearance of foremilk in a strip cup and on a filter of size 0.1 mm, no. (% of the row).

Visual appearance	Appearance on the filter		
	Normal	Few, small flakes	Clots
Normal	4894 (97)	110 (2)	43 (1)
Watery	34 (74)	4 (9)	8 (17)
Clots	1 (2)	1 (2)	50 (96)
Yellowish	6 (46)	2 (15)	5 (39)
Blood	7 (78)	0 (0)	2 (22)

4. Economic considerations

The data presented in chapter 4.1 were used to evaluate the economic consequences of sorting milk based on a number of different decision rules. In each case the milk price was calculated according to the current Danish scheme where a bonus is paid for keeping the bulk milk SCC < 300,000 cells/ml and deductions are made for SCC > 400,000 cells/ml. The 5 herds tested had bulk milk SCC of 250-420,000 cells/ml.

Sorting of milk at the quarter level obtained the highest milk price if milk from all quarters was ranked after cells/ml and milk removed until the expected bulk tank SCC was within the Danish bonus quality limit of 300,000 cells/ml (different levels will apply in other countries but calculations be similar). The total milk price was 0.9% higher than when sorting at composite milk level and 2.5% higher than when including all milk. If milk from quarters where the foremilk appeared visually abnormal was excluded, the total amount of milk was reduced with 3.2%. If the milk was excluded at composite milk level, the reduction in volume was 10.7% and the reduction in total milk price 8.3% compared to the price of all the milk. Sorting of milk at composite level resulted in the highest milk price if milk with cell counts > $5 \cdot 10^6$ cells/ml was discarded.

The sorting and removal of milk at quarter level result in a higher total milk price than sorting of composite milk. Sorting of milk according to visual appearance of foremilk is not economically profitable in the current study when milk is removed at composite milk level because the total milk price was reduced using these sorting criteria. However, the economic calculations do not include risks of spreading mastitis pathogens among cows by not removing milk but are concentrated solely on milk produced on the days of measurements. This study shows that the Danish payment scheme will *not* be an incentive for farmers to discard visually abnormal milk. Higher deductions for high bulk milk SCC or higher bonuses for lower bulk milk SCC will improve the economics for sorting based on cow SCC and reduce the probability of including abnormal milk but will still not ensure that all abnormal milk is dumped.

There is not much research carried out on the basic economics (costs versus benefits) of milk quality regulation. When governments make regulations or dairy factories impose certain requirements, it is important to know what the consequences are in terms of costs versus the benefits, and in terms of reduced human health risk. The benefits of a good milk quality for the dairy processors can be increased product yield, extended shelf life and a lower risk of calamities. The effects of better milk quality in terms of image improvement and increased consumption of milk and milk products are not known. Although many farmers are proud to deliver first class milk, financial incentives are important to direct the behaviour of the farmers. This will become more important when the margins on milk production become lower because of decreasing milk prices. Finally, when using sensors to detect abnormal milk and separate automatically based upon the outcome of the sensors, one should be aware that a very high specificity is needed in order to reduce the losses of incorrectly discarded milk.

5. Requirements of sensitivity and specificity

Normally only cows without clots or blood in the foremilk deliver milk for consumption. For conventional milking, detection of clots in the foremilk depends on the skills of the milker and on the practical conditions under which the foremilk is performed. Hillerton (2000) states that the sensitivity is 80% for detecting cows with clinical mastitis during foremilk but the specificity is 100%. If clots can be found in the milk at any time during milking, the quarter suffers from clinical mastitis. Analysis of data from chapter 4.1 revealed that the sensitivity of detecting a cow with abnormal foremilk by CMT based on the highest score depended on the time interval between milkings. The sensitivity dropped from 71 to 43% going from an interval at least 3 hours shorter than the average to an interval at least 3 hours longer. Parallel to this decrease, specificity of CMT for detection of visually abnormal milk increased from 78 to 93%. However, the visual appearance was independent of time interval between milkings and since CMT-score was not, the decrease in sensitivity is due to a decrease in number of correct positives and the increase in specificity is due to an decrease in number of false positives. Multiple scorings of visual appearance will help in classifying a cow as having normal or abnormal foremilk. The sensitivity of using CMT-score with multiple milkings as a tool to detect cows with visually abnormal foremilk was considerably higher than for one milking only (Table 6). So, if cows truly have clinical mastitis, CMT-scores will increase and predict better. The sensitivity seemed to decrease with increasing time interval between milkings reflecting a dilution of the cells in the foremilk with the longer interval and higher milk yield.

Table 6. Sensitivity and specificity at cow level. True state: No abnormal visual appearance, versus More than 50% abnormal visual appearance on worst quarter. Test state: All quarters CMT<4, versus CMT>3 of worst quarter.

Deviation from average milking time, hours	-3 or less	-3 to -1	-1 to +1	+1 to +3	+3 or more	All
Sensitivity %	80.5	93.4	83.7	81.7	75.6	84.2
Specificity %	78.0	85.2	90.0	89.4	92.7	88.0

Sensitivity and specificity using CMT-score to detect quarters with abnormal or normal appearance of the foremilk was higher than for the cow level (Table 7) but still seem to be dependent on time interval between milkings. The confidence intervals for specificity's are reasonably small reflecting a much higher proportion of quarters being normal than abnormal.

Table 7. Sensitivity and specificity on quarter level. True state: Free: No abnormal visual appearance, Infected: More than 50% abnormal visual appearance. Test state: Free: CMT<4, Infected: CMT>3.

Deviation from average milking time, hours	-3 or less	-3 to -1	-1 to +1	+1 to +3	+3 or more	All
Sensitivity %	95	88	86	88	*94	88
(95% conf. int.)	(88;100)	(80;95)	(79;93)	(79;98)	(82;100)	(83.8;91.8)
Specificity %	90	94	96	96	97	95
(95% conf. int.)	(88;92)	(93;95)	(96;97)	(95;97)	(96;99)	(94.5;95.5)

* Only 1 observation

Correct sorting of milk does not only make heavier demands on the detection system, but requires that the true status of normal or abnormal is precise. A sensitivity of 90% means that out of 10 cows with abnormal milk, 9 are detected. A combination with a specificity of 99% means that 1 out of 90 normal cows is detected as false positive and in total, 10 out of the 100 cows will be pointed out (Table 8). In a herd having one cow with abnormal milk out of 100 cows, a sensitivity of 90% and a specificity of 99% will point out 2 cows. Any reduction in the specificity will have a major effect on number of false positives which becomes a factor 20 to the one having truly abnormal milk. Consequently, the sensitivity is the most important factor in a herd with a high prevalence of clinical mastitis, and specificity is the most important factor in herds with low prevalence. Although related to the visual appearance of foremilk, SCC and CMT score are not detecting abnormal foremilk with a high enough precision and it seems that the detection system should be more closely related to the property of clots in the milk. Sensitivities and specificities using the filter method have not yet been calculated and need to be established in order to test different milking systems.

Table 8. Number of cows detected as having abnormal milk depending on sensitivity and specificity of the system.

Abnormal milk True status	Sensitivity / specificity			
	90/99	80/99	90/90	80/80
10 out of 100 cows	10 (9+1)	9 (8+1)	18 (9+9)	26 (8+18)
1 out of 100 cows	2 (1+1)	2 (1+1)	11 (1+10)	21 (1+20)

6. Definition of normal and abnormal milk at time of milking

A workshop was held on 27th November 2002 on this subject. The main purpose of the workshop was to present background material for a definition, discuss the intention and consequences of the definition, and finally outline agreements and disagreements. The parts of the definition where there was consensus at the workshop are clearly stated below. Based on the discussion at the workshop, the author recommends:

1. There should be no double standards. The requirements for milk quality produced under conventional and automatic milking conditions should be the same.
2. Milk at time of milking can be classified in four categories:
 - *Normal milk*: Milk suitable for human consumption.
 - *Abnormal milk*: Milk which differs from normal milk in respect of colour or homogeneity.
 - *Contaminated milk*: Milk which, prior to the milking of the animal, is known to be unfit for human consumption following treatment of the animal with antibiotics or other veterinary products which have a requirement that the milk must be withheld from sale for such use.
 - *Undesirable milk*: Milk which, prior to the milking of the animal, is known to be unsuitable for human consumption, e.g. colostrum, high somatic cell count.
3. If clots appear, the milk is abnormal. The reference method is proposed to be appearance of clearly visible clots on a filter with a pore size of 0.1 mm.
4. Milk that has changed in colour because of the level of red blood cells present is regarded as abnormal milk.
5. Milk from the first 72 hours after calving with at least two daily milkings (the colostrum period) is regarded as undesirable. Milk may be withheld for a longer period if it still appears discoloured.
6. The cell count of milk should not be included in the definition of abnormal milk at time of milking.

Re 1. There was consensus at the workshop that there should be no double standards. The requirements shall apply to all milking conditions and not be special for automatic milking. The author suggests that the following text for the coming EU Hygiene Directive may apply:

Milking must be carried out hygienically ensuring in particular: - that milk from an animal is checked for abnormalities by the milker or a method achieving similar results and that only normal milk is used for human consumption and that abnormal, contaminated, and undesirable milk is excluded.

This text implies that milk should be inspected and that abnormal, contaminated, and undesirable milk should not be delivered for human consumption. Contaminated and undesirable milk are conditions known prior to milking and may or may not be checked or monitored during milking. The sensors used for automatic milking systems to detect abnormal milk should be as good as the milker. The skills of the milker are not defined and the workshop proposed to use the level of an experienced milker to give the reference level for sensitivity and specificity. The author recommends that the specificity should be >99% to be well accepted by farmers. A sensitivity of about 80% has been reported for conventional milkers. It was noted that there are no educational requirements for becoming a milker, but this should not be used to alter the requirements of detection of abnormal milk. The dairy industry may have additional requirements of bulk milk.

Re 2. A classification and definition of milk was drafted at an ISO meeting for development of international standards for automatic milking (ISO/TC 23/WG 1 Automatic milking installations) in the week before the workshop. The text was further discussed and revised at the workshop. The benefit of the definition is to help to distinguish between conditions where milk is known to be unfit for human consumption either prior to the milking of the individual cow (undesirable and contaminated milk) or at the milking (abnormal milk). The definition of abnormal milk is not a food safety issue. It is the responsibility of the farmer that only normal milk is delivered but it can only be checked for the occurrence of abnormalities at time of milking. Consequently, reliable sorting mechanisms are needed for unattended milking methods. The workshop discussed rejection of abnormal milk at the quarter or cow level. The main opinion is that if milk from any quarter is abnormal, all milk from that cow should be considered abnormal. Discard of abnormal quarter milk only may be attractive but the thought of milk coming from cows carrying infection may harm the image of the milk.

Re 3. It is important that the reference method for classification of the milk is based on science, is applicable, repeatable, and objective. The workshop was in favour of defining abnormal milk caused by clinical mastitis on the homogeneity of the milk and not on the colour since the colour of the milk changes with breed, stage of lactation, feed stuff, etc. The author proposed a reference method to be based on filtration of the milk through a filter with a pore size of 0.1 mm. Milk where clots were clearly visible in such a filter is then defined as being abnormal. Incidences of watery and yellowish milk may or may not be detected by this method. The workshop questioned the reference method and additional information and consequences of using the filter as a reference method is needed and will be provided.

The current standard is to inspect the appearance of foremilk. Clinical mastitis develops due to invasion of pathogens through the teat canal. It may happen that clinical signs are not seen in the foremilk but will appear later on and the workshop proposed to include all milk from the quarter in the definition, i. e. if clots are detected at any stage of the milking the milk is abnormal. It is not practical to base the daily judgement on milk fractions other than the foremilk. However, the frequency of cows with no clots in the foremilk but clots appearing late into the milking is expected to be very low and is likely to show at foremilkings of coming milkings.

Re 4. There was consensus at the workshop that milk coloured by blood should be regarded as abnormal milk. The frequency of visible blood in the milk is rare. The reference level for detection has not been defined yet. Test panels of consumers and professionals can detect samples with about 0.1% of blood having a reference of white milk but even 1% of blood does not show clearly in a black strip cup being the conventional reference method at foremilkings. The percentage of haemoglobin, i.e. red cell count, in blood has to be taken into account when determining the reference.

Re 5. There is an overlap between the physiological phases of colostrum and milk production. Colostrum is a normal secretion of a postpartum udder in early lactation, but it cannot be regarded as “normal milk” in the context of the above definitions. At the fourth day after calving the secretion from most cows will appear as normal milk when milking twice daily. The workshop did not fully agree on the number of days to withhold colostrum milk but 3 full days is regarded as a minimum withholding period. Local or national regulations may require a longer withholding period.

Re 6. There was consensus at the workshop that the cell count of milk should not be included in the definition of abnormal milk at time of milking. A high cell count is a clear indicator of

inflammation in the udder but cannot be required to be measured at every milking for determination of abnormal milk. It is still recommended that cell count is part of the milk quality survey of bulk milk.

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