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Visual scoring of milk mixed with blood

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Sorting of normal and abnormal milk at time of milking is done visually for conventional milking systems, but more concrete standards are needed when milking is done in automatic milking systems (AMS). Several panel tests were carried out to find out how different consumer groups, milkers and advisors look at and respond to the visual appearance of milk mixed with blood, in order to set a limit for what they think is acceptable. It is concluded from the test panel results that milk samples with 0·4% or more of blood all will be scored as pink and samples with 0·1% blood (about 6 μM-haemoglobin or 100 mg/l) can be visually detected if they are compared with milk samples without blood. The consumer group scored fewer of the samples with 0·1% blood as normal than did the professional groups. The test panel scored 65% of the samples with 1% blood as normal when milk was presented in a black strip cup, which is the reference method when foremilking takes place in a conventional parlour. Only 2% of the milk samples with 2% blood (about 120 μM-haemoglobin or 2000 mg/l) were scored as normal in a black strip cup and should consequently be detected by conventional as well as automatic systems. One model of AMS was tested for its ability to detect and separate milk coloured by blood. The model separated milk with $\geqslant 6$ μM-haemoglobin. Milk mixed with blood injected into the milk stream for a short time at the beginning of milking was not separated. We lack data on how blood is naturally expelled into milk and in what amount. We propose that cow composite milk with >6 μM-haemoglobin should be separated because at this level milk will have a red tinge.

Keywords: Automatic milking systems, blood, haemoglobin, visual scoring, milk.

Precursors of milk have to be transported by the blood to the udder and then either be used directly or indirectly. Red blood cells do not enter the milk unless the udder is injured or a blood vessel is leaking, and as such the number of red blood cells in normal milk is very low. The occurrence of visible blood in milk is rare but unacceptable to most consumers and dairy factories. The general conditions for hygienic milk production in the EU are defined in Commission Directive 89/362/EEC (1989) and Chapter III-4 states that the milker must inspect the appearance of the milk before milking of the individual cow and withhold milk from delivery if abnormalities are present. This includes that milk visibly changed in colour must be withheld. A workshop was held at the Danish Institute of Agricultural Sciences in 2002 with the objective of defining normal and abnormal milk (Rasmussen, 2002) and inputting to the text of the coming EU Hygiene Directive. Participants in the workshop (scientists, legislators, veterinarians and people from the milking machine and dairy factory industry) obtained consensus in that milk changed in colour because of the content of red blood cells should be regarded as abnormal (Rasmussen, 2004). The proposal for the coming Hygiene Directive part B, *Hygiene during milking, collection and transport* (Official Journal of the EU 2004/C 48 E/23), states that milk with physicochemical abnormalities should not be used for human consumption. This leaves a need for an exact definition of an acceptable level since a total avoidance of red blood cells in milk is probably not achievable. In order not to have double standards, such a definition has to apply to conventional as well as automatic milking.

Large amounts of blood in milk may originate from internal or external wounds in teats or udder. Blood from internal wounds can be detected during forestripping and external teat injuries may be seen at the same time. Forestripping is normally done in a black strip cup in order to detect clinical mastitis appearing as white or yellowish clots but such a background may not be the best for detection of milk coloured by blood. Additionally, conventional milkers can check the milk during milking through transparent parts of the milking equipment. For automatic milking systems (AMS), the 'normality' of the milk has to be measured directly or indirectly during the

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milking process. Conductivity is widely used for automatic detection of mastitic quarters since blood components pass through leaky tight junctions in the inflamed part of the udder and the ion concentration in milk increases (Bruckmaier et al. 2004). A 10% increase in conductivity has been used to initiate antibiotic treatment of induced mastitis (Milner et al. 1997) and an increase of 15% of one quarter compared with the lowest quarter has been used to indicate clinical mastitis (Maatje et al. 1992). Assuming an average electrical conductivity of 5·5 mS/cm in milk from healthy quarters (Hamann & Zecconi, 1998) and a conductivity in blood of 7–9 mS/cm, then a mixture of a large amount of blood in the milk is required to obtain a 15% increase in milk conductivity. Consequently, more direct measurements must be used.

Ouweltjes & Hogeveen (2001) used a colour sensor based on reflection of the light generated by a light emitting diode (LED) to measure the colour of the milk. Three different wavelengths were used to measure reflections in the red, green, and blue area and they were all set at a value of 100 for normal milk. Milk samples with 0·0025% blood showed a slight decrease in colour values but appeared visually normal. Milk samples with 0·05% blood were denoted slightly pink and had a 21% reduction in the blue colour. A sample with 1% blood was visually pink and had clearly reduced colour values in all three wavelengths. Espada & Vijverberg (2002) used the same colour sensor to detect clinical mastitis, colostrum and blood in milk. Milk from cows with clinical mastitis showed colour patterns during milking that were different from those in normal milk, and colour measurements, especially within cow comparisons, seem valuable. The system was able to identify cows producing colostrum, their milk being more yellow. One cow had blood in the milk owing to a teat injury and it was mentioned that colour values were out of the normal range but specific values or graphs were not given for that cow. About 1% of the measurements done by Espada & Vijverberg (2002) were below the value 86 in the blue range corresponding to the colour reading of a milk sample with 0·03% blood interpolated from the data of Ouweltjes & Hogeveen (2001).

The red colour of blood and haemoglobin is due to the haem part containing an iron atom (Swenson, 1977). Four haem molecules unite with globin to form haemoglobin. Haemoglobin constitutes about 95% of the solids of erythrocytes (Swenson, 1977) and consequently, measurement of haemoglobin or counting of red blood cells will be highly correlated with the colour of blood. Cattle blood contains $(6–8) \times 10^6$ red blood cells per µl and bovine milk with $(2–4) \times 10^6$ red blood cells per ml has a visible red tinge and milk with 10×10^6 red blood cells per ml was clearly red (Whyte et al. 2004). Measurement of haemoglobin content or number of red blood cells gives more precise information about colourization than just a percentage. Whyte et al. (2004) developed an in-line sensor based on optical measurements of the colour of milk to detect the presence of blood. All six samples with $>10 \times 10^6$ red blood cells per ml were identified (sensitivity 100%) and out of 475 samples with a lower count, 473 were correctly identified as negative (specificity 99·6%). The 10×10^6 red blood cells per ml correspond to about 0·15% blood in the milk.

It is possible to give an estimate of the amount of blood mixed into the milk by measuring the colour of the milk, and the technical capabilities seem to exceed that of the human eye. To have the same standards for conventional and automatic milking and set a threshold for automatic diversion of abnormal milk, there is a need for an exact definition of what kind of milk is acceptable and of what is not.

The objectives were to (a) find out how different consumer groups, milkers, advisors, and people from the industry look at and respond to the visual appearance of milk mixed with blood; (b) find a limit for what these groups think is acceptable dependent on visual background and possibilities for within cow comparisons; and (c) test one AMS for its ability to discard milk mixed with blood.

Material and Methods

Visual scoring of dishes containing milk mixed with blood

A test panel of 15 persons comprising 5 milk quality advisors, 5 milkers, and 5 consumers not dealing professionally with raw milk scored the visual appearance of normal milk and milk mixed with blood. The test panel scored a total of 120 dishes with milk four at a time to simulate scoring of milk from the four quarters. Blood from one cow was used and five different percentages of blood were mixed homogeneously with milk containing either 0·5 or 3·5% fat. The milk was pasteurized and homogenized to avoid agglutination of fat globules. Each series of four dishes was mixed differently using either four samples of the same blood percentage or a mixture of different samples. The same protocol was used for the two fat percentages. Out of 60 such dishes containing milk mixed with blood 11, 11, 15, 12, 8, and 3 dishes contained 0, 0·1, 0·2, 0·3, 0·4, and 0·5% blood, respectively. Each dish was filled with a 10-ml mixture. The dishes were scored within 15 min of preparation. The panel had 30 s to score each series, write their scoring on the sheet provided, along with their initials, drop the sheet in a closed box, and move to the next booth. Milk samples were scored as normal, slightly pink, or pink. Colour was measured using a Chroma meter (CR-300, Minolta Co., Osaka 541-8556, Japan) where 2 ml of a mixture was transferred to a black capsule and colour was measured immediately. Colour was expressed on three scales within the visible spectrum using the CIE (Commision Internationale de L'Enclairage) Lab scale (D65): 'L', a light/dark scale that runs from 0 (black) to 100 (white), 'a', a red/green scale (– is green, and + is red), and 'b', a blue/yellow scale (– is blue, and + is yellow). The haemoglobin content of the blood samples was not measured in the first three series of experiments.

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Scoring of multiple samples

To explore the lower limit and overcome the logistic problems of having many people scoring the same samples, an additional test was carried out with scoring of pictures of milk containing blood. A slideshow with 20 slides each showing 4 vials was scored by 16 persons comprising 6 mastitis researchers, 4 scientists not dealing with milk, and 5 from the milking machine industry. Out of the 80 vials with milk mixed with blood from a cow, 12, 10, 14, 17, 14, 9, 3, and 1 contained 0, 0·05, 0·1, 0·2, 0·3, 0·4, 0·5 and 5% blood, respectively. The different percentages of blood were mixed homogeneously with milk containing 1·5% fat. The panel had 14 s to evaluate each slide and write their scoring on the sheet of paper provided. Vials were scored as normal, slightly pink, or pink.

Scoring of single samples on a neutral background and on a black surface

A slideshow with 40 slides showing one dish with milk each on a light grey background was scored by 13 persons comprising 6 milking machine researchers and 7 from the milking machine industry. Out of the 40 dishes containing milk mixed with blood from a cow, 6, 6, 6, 6, 3, 3, 3, 3, 2, and 2 contained 0, 0·06, 0·12, 0·25, 0·5, 1, 2, 3, 4, and 5% blood, respectively. The different percentages of blood were mixed homogeneously with milk containing 1·5% fat. Additionally, the test panel scored 40 slides showing milk in a strip cup with a black surface. The same percentages of blood were used and the test panel scored four slides of each percentage in a random order. The panel had 5 s to evaluate each slide and write their scoring on the sheet provided. Samples were scored as normal, slightly pink, or pink.

Test of automatic detection systems for coloured milk

A Lely Astronaut model MQC (Lely Industries NV, Maasland, The Netherlands) was used in one Danish herd to carry out this test. Fresh blood was drawn from one cow of the herd into a bottle containing heparin to avoid clotting. The haemoglobin (measured as Fe-atoms) content of the blood was 8·0 mM (Hb 201+, HemoCue AB, Ängelholm, Sweden). Since this meter measures each haem group, the molecular weight of 16 700 kDa was used to calculate the haemoglobin content of the samples. Composite milk from one cow was used to mix 4 × 1 l of milk homogeneously with 0, 3, 6, and 120 µmol (about 0, 50, 100, and 2000 mg) haemoglobin, respectively. These concentrations corresponded to 0, 0·05, 0·1 and 2% blood in the milk using blood with 6 mM-haemoglobin (average content of cow blood (Kaneko et al. 1997)). This milk was sucked into the AMS through an artificial udder. The flow was adjusted to about 0·5 l/min per quarter. This test method was set up in order to work with known concentrations of blood in the milk. However, the AMS had difficulties with the attachment owing to the 'shape' of the artificial udder and manual operation was not possible. Consequently, the test protocol was modified so that blood was injected into an additional 1-m looped hose of one of the 'short' milk hoses before milk entered the sensor. A milk separator was inserted immediately after the sensor to be able to measure the milk yield and avoid the added blood mixing with milk for delivery. Blood was injected at a flow of about 0·5, 1·0 and 10 ml/min in order to reach the three planned concentrations of haemoglobin in the milk. This was done on 11 cows. Additionally, 1, 2, and 20 ml of blood were injected 15–30 s after milking of the three cows had started, again to reach the planned levels of haemoglobin for the quarter milk but over a short time period to simulate milking of an injured teat.

Statistical methods

The statistical procedure PROC GENMOD (SAS Institute, 1999) was used to test the frequency of samples scored as normal milk. Model 1 was used for Experiment 1:

$$[1]\quad Y_{ijkm} = \mu + Blood_i + Fat_j + Person(Group)_k + Group_l + Blood * Group_{il} + \varepsilon_{ijkm}$$

where
Y_{ijkm} = Blood score,
μ = overall mean,
$Blood_i$ = the effect of blood percentage (i = 0, 0·1, 0·2, 0·3, 0·4, 0·5),
Fat_j = the effect of fat percentage (j = 0·5, 3·5),
$Person(Group)_k$ = the effect of person within group (k = 1, …, 15),
$Group_l$ = the effect of Group (l = consumer, milk quality inspector, milker),
$Blood * Group_{il}$ = the interaction between blood percentage and group,
ε_{ijkm} = residual error.
Model 2 used for Experiment 2 was as model 1 except that the effect of Fat was not included. The model used for Experiment 3 was:

$$[3]\quad Y_{ijkm} = \mu + Blood_i + Background_j + Person(Group)_k + Group_l + Blood * Background_{ij} + \varepsilon_{ijkm}$$

where explanations are as for model 1 and
$Background_j$ = the effect of background (j = light grey, black),
$Blood * Background_{ij}$ = the interaction between blood percentage and background.

Results

Visual scoring of dishes containing milk mixed with blood

All samples without blood were scored as normal milk by the test panel. All members of the panel scored milk with 0·5% blood as pink (Fig. 1 and Table 1). Samples with

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Table 1. Percentage of samples scored as normal milk depending on the lowest percentage of blood in the reference samples within a series

	Lowest reference				
Blood, %	0	0·1	0·2	0·3	0·4
0	98	99	100	100	100
0·1	37	58	68	100	100
0·2	8	12	46	39	100
0·3	1	0	18	35	52
0·4	0	0	0	7	3
0·5	0	0	0	0	0

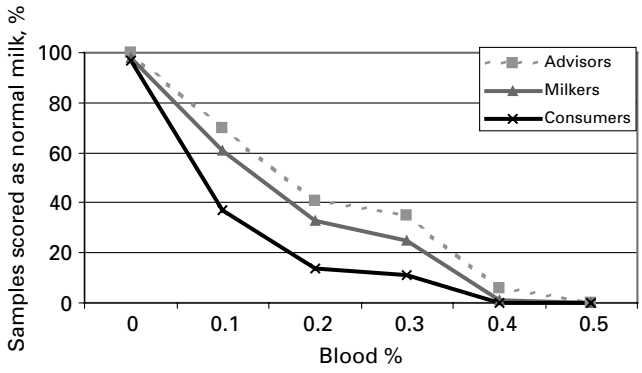

Fig. 1. Visual scoring of dishes with normal milk and milk containing blood. Milk quality advisors, milkers (operators), and consumers comprised the test panel.

0·4% blood were scored as pink except for one of the milk quality inspectors, who scored the series with four samples containing 0·4% blood as normal. However, it turned out that he was red-green colour blind. He only differed from his group in this series and otherwise used the lightness of the sample to distinguish between percentages of blood. The consumer group scored fewer of the samples with 0·1–0·3% blood as normal than the other groups ($P<0·01$). The consumers scored 37% of the samples with 0·1% blood as normal as against 60–70% by the other two groups (Fig. 1).

Scoring of samples depended on references within a series (Table 1). Consumers scored 10% and the other groups 50% of the samples with 0·1% blood as normal when they had the 0% blood as reference ($P<0·01$). When all four samples had 0·1% blood or some were higher, the consumers scored 48% of the samples as normal compared with 61% of the other groups ($P<0·01$). Consumers scored 21% and the other groups 53% of the samples with 0·2% blood as normal when they had milk with the same or a higher blood concentration for comparison ($P<0·001$).

Samples with blood were detected better when there was more fat in the milk sample ($P<0·001$). Out of the samples containing 0·1% blood, the panel scored 66% of the samples with 0·5% fat as being normal *v.* 46% of the samples with 3·5% fat in the milk. Foremilk normally has

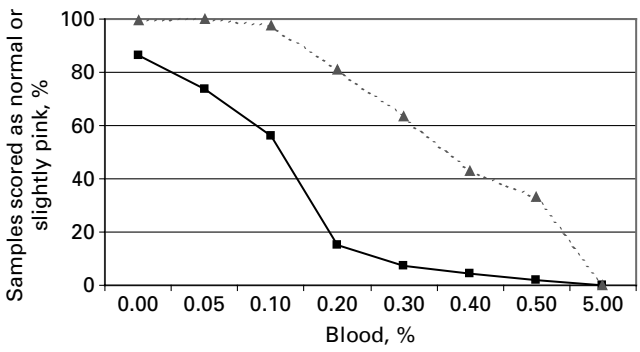

Fig. 2. Visual scoring of milk samples containing different amounts of blood. Samples were scored as normal (■), slightly pink or pink (▲). Mastitis researchers, other scientists, and people from the milking machine industry constituted the test panel.

a low fat percentage, which then makes it more difficult to identify milk samples with a low content of blood. Colour-scanning far exceeded the ability of the panel to detect blood in milk, explaining 99·8% of the variation in blood percentages (SD = 0·009%). The red/green scale of course provided the best differentiation between percentages of blood and the blue/yellow scale provided the best differentiation between fat percentages.

Scoring of multiple samples

The test panel scored some of the vials containing 0 and 0·05% blood as slightly pink (Fig. 2), which could have been due to difficulties in distinguishing between almost white (milk is not purely white) and slightly coloured. The panel scored 56% of the samples with 0·1% blood as normal but only about 15% of the samples with 0·2% blood were scored as normal. However, when a comparison was made with vials containing no blood, only 5% of the samples were scored as normal ($P<0·001$). There was no significant difference between groups but individuals scored differently ($P<0·001$). This panel seemed to give the same conclusion as in the first experiment, where milk containing 0·2% or more of blood is clearly identified as abnormal.

Scoring of single samples on a neutral background and on a black surface

All samples containing 0 or 0·06% blood were scored as normal milk with the neutral background (Fig. 3). A few of the samples containing 0·12% blood were scored as slightly pink and none of the samples with 1% or more blood were scored as normal. However, the test panel scored 65% of the samples with 1% blood as normal when the milk was in a black strip cup. The panel scored 2, 50, and 48% of the samples containing 2% blood in the black strip cup as normal, slightly pink or pink, respectively, but all samples with 2% blood on a neutral background were

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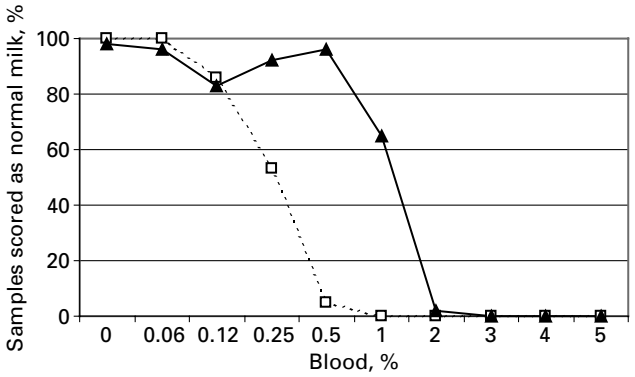

Fig. 3. Visual scoring of milk samples containing different amounts of blood. Samples were scored as normal, slightly pink, or pink on a light grey (□) or black (▲) background. Milking machine researchers and people from the milking machine industry constituted the test panel.

scored as pink. The scorings of the panel of the samples with 0·25–2% blood were significantly different depending on the background ($P<0·001$). There was no significant difference between groups but individuals scored differently ($P<0·001$).

Test of automatic detection systems for coloured milk

Milk yields of the sampled quarters were 1·0–5·8 l and concentrations of blood were 0–180 μmol haemoglobin per l milk. Nine samples with <6 μmol haemoglobin per l (<100 mg/l) milk were not separated. Out of the eight samples containing ⩾6·0 μmol haemoglobin per l milk, six were separated and two were not. These samples contained 7·6 and 39·0 μmol haemoglobin per l milk, respectively, but were the two samples that had blood injected for a short time just after attachment of the milking machine.

Discussion

Visual detection of blood in milk

Consumers expect the colour of the milk to be as when they take it out of the fridge. Our consumer group of the test panel reacted to lower levels of blood in milk than did milk quality inspectors and milkers used to evaluate raw milk. With 0% blood in the milk as reference, only 10% of the samples containing 0·1% blood were scored as normal by the consumer group compared with 50% by the other groups. Our test panels did not clearly describe samples with 0·05% blood as slightly pink, which was the level at which Ouweltjes & Hogeveen (2001) and Whyte et al. (2004) found a red tinge. Differences in haemoglobin content may explain this. The test panel scored some of the vials containing 0 and 0·05% blood as slightly pink, which could be due to difficulties in distinguishing between almost white (milk is not purely white) and slightly coloured or that colours may appear different in a picture than in real life. Moreover, the panel knew that they should look for milk samples coloured by blood and maybe wanted to take pride in detecting all the positive samples. In most cases, our test panels scored milk samples containing 0·2% blood as being coloured no matter whether they had a reference without blood or not. We can then expect that milk coloured by 0·1% or more of blood may be detected at the quarter level if the milk can be clearly observed during milking and the colour compared through transparent hoses or other visible parts. Composite milk in a recorder jar will probably be scored as pink or slightly pink if it holds 0·2% or more of blood. However, even 1% of blood does not show clearly in a black strip cup, which is the reference method when foremilking takes place in a conventional parlour. The entire test panel mainly identified milk containing 2% blood as coloured when evaluated in the black strip cup, but such a high percentage of blood would be unacceptable when visible in other parts of the milking equipment.

We tested only a limited number of basic milk samples and used homogenized milk for some of our tests. Milk differs in the basic colour depending on stage of lactation, breed and feeding regime. Milk that has a high content of beta-carotene will be more yellowish and it will be more difficult for the human eye to detect small amounts of blood in such a single sample. The possibility of detecting the blood improves if adjacent samples do not contain blood but originate from the same cow. A high beta-carotene content or colostrum from Jersey cows (Madsen et al. 2004) seem not to disturb the detection of milk coloured by blood when measured with a colour scanner. The colour of homogenized milk differs slightly from that of raw milk but changes in fat content cause bigger differences. We used homogenized milk in our panel tests in order to avoid fat segregation, which does not take long when milk is stored at room temperature. Although not covering all basic colours of milk, we consider our tests adequate as a basis for general conclusions on the detection of milk coloured by blood.

Better comparisons between our own experiments and those in the literature could have been made if all had measured the amount of haemoglobin in the blood used to mix with milk. However, there seems to be consistency in the overall conclusions from the different experiments and we can assume that the haemoglobin content of the blood was neither extremely high nor low.

Frequency of appearance of blood in milk

Data on the frequency and amount of visible blood in milk are not available in the literature. The frequency of blood appearing in milk is very low but may occur owing to teat injuries and internal leakage in the udder during the colostrum period. Occasionally, whole bulk tanks of milk are dumped owing to colouring with blood and most dairy factories will not accept such milk. Withholding of milk

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from quarters with more than 2% blood in the milk (conventional detection) will on average keep the bulk milk below 0·05% for a herd with one quarter containing blood in the milk per 10 cows. If we accept 0·1% blood in the milk as the upper limit at the quarter level and this happens on a maximum of 3 out of 300 lactation days, then we will have <1 ppm blood in the bulk tank. Consequently, discarding of milk with >0·1% blood will ensure that bulk milk will be visually free of blood.

Automatic diversion of coloured milk

Milk may change in colour for many different reasons but only colouring by blood will be discussed since the participants of the workshop at the Danish Institute of Agricultural Sciences were in favour of not considering the colour caused by mastitis (Rasmussen, 2004). Colostrum differs from normal milk in colour as well but participants at the workshop defined the first 3 d after calving as the colostrum period and colour changes from that time onward are minor (Madsen et al. 2004). 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 was 0·009%, which makes it possible to detect 0·02% blood in the milk and then of course discard milk containing more than 0·1% blood.

The automatic colour sensor of the AMS tested here worked precisely when measuring a continuous flow of milk mixed with blood. Quarter milk with up to 5·5 μmol haemoglobin per l milk was not separated and milk mixed with ⩾6 μM-haemoglobin (⩾100 mg/l) had a visible red tinge and was separated. However, two exceptions occurred when the amount of blood was injected at the beginning of the milking for a short time. These samples contained 7·6 and 39·0 μmol haemoglobin per l milk when averaged for the full milking and would have been separated if the blood had been injected continuously over the entire milking of these quarters. These findings raise the question whether coloured milk should be separated based on single high concentrations of blood or on the endpoint result after mixing with milk from the rest of the quarter and with milk from the other quarters. We think that composite milk with >6 μM-haemoglobin (100 mg/l or about 0·1% blood) should be separated because at this level, milk will have a red tinge, which could be noted by an average producer or consumer. When milking in a conventional system with dark hoses and no recorder jar, such redness would not be noted.

Blood occurring in milk is probably not mixed homogeneously and colouring of the milk will vary throughout the milking. The test protocol was set up to test quarters with a continuous flow of coloured milk but does not simulate the situation where blood is coming in as squirts over short time periods. Presently, we do not have the data to show how blood is mixed into milk whenever the teat has an external wound, internal wound, or a punctured blood vessel. Foremilking in conventional systems will be able to detect external wounds of the teat and note if the first squirts contain >120 μmol haemoglobin per l milk (>2000 mg/l or about 2% blood in the milk). A small, leaking blood vessel will not be detected in a conventional system but will probably be discovered by an AMS such as the one tested here. The occurrence of visible blood in milk is rare and we believe that systems capable of detecting blood in milk according to the proposed protocol will also be able to detect most of the naturally occurring cases of blood in milk. A better use of the software for detection of coloured milk could probably account for different natural ways of mixing blood into milk and calculate an average concentration. It is important for milk quality as well as animal welfare to identify cows with blood in their milk.

It is concluded from the test panel results that all milk samples containing 0·4% or more of blood will be scored as pink and samples with 0·1% blood (about 6 μM-haemoglobin or 100 mg/l) can be visually detected if they are compared with milk samples free of blood. Milk samples with <6 μM-haemoglobin are scored as visually white with a sensitivity of >80% by a representative group of people when compared with milk containing no haemoglobin. One percent of blood does not show clearly in a black strip cup, which is the normal reference method when foremilking takes place in a conventional parlour. About 2% of blood (about 120 μM-haemoglobin or 2000 mg/l) in milk is scored as visually red with a sensitivity of >80% by a representative group of people when scoring the coloured milk on a black surface. Consequently, this amount of blood in milk should be detected by conventional as well as AMS. When mixed with milk from the other quarters, the composite milk should not show redness.

We propose that cow composite milk with >6 μM-haemoglobin (>100 mg/l) should be separated because at this level milk will have a red tinge.

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