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The influence of technical factors on differential cell count in milk

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Differential cell count of milk is a traditional parameter for the evaluation of udder health. The literature shows great variation in differential cell counts of the milk of healthy mammary glands: macrophages range from 0% to 80%, lymphocytes from 1·5% to 79·5%, polymorphonuclear neutrophils from 3% to 95%, and epithelial cells from 1% to 19%. We conducted three studies to seek explanations for such variation. In the first, we evaluated the impact of polyethylene and glass sampling bottles. The aim of the second study was to compare the results of differential cell counts performed by three different technicians. The third study evaluated two methods of smear preparation. When polyethylene plastic bottles were used, the macrophage population was minimized but lymphocytes remained unaffected. This was shown by an exemplary flow cytometric analysis using four monoclonal antibodies against three lymphocyte surface structures. There were significant differences in the differential cell counts of 40 smears made by three technicians despite identical operating procedures. For the sediment smear, milk was centrifuged once and the sediment spread by eye on a glass slide. For the ''coffee grinder'' smear method, the sample was subjected to four centrifugations and then placed on a cover glass in order to spread the sediment using centrifugal force. The coffee grinder procedure led to a reduction of lymphocytes and an enrichment of polymorphonuclear neutrophils without affecting the macrophage population. Both methods made it possible to distinguish different udder health classes. It can be concluded that differential cell counts are a useful tool for comparing and monitoring udder health only if: samples are taken in a glass bottle; smears are prepared with the identical technique; and the differential cell counts are performed by a single person.

Keywords: Differential cell count, bovine, milk, technical factors.

Mastitis diagnosis worldwide is based on the cyto-bacteriological examination of foremilk samples. Owing to the relatively large variability of cell counts and microbiological diagnosis, this procedure is of only limited reliability, particularly if it is based on only the analysis of a single milk sample. Differential cell count provides a distinct improvement in the reliability of mastitis diagnosis by the detection of inflammation-related changes in the milk cell populations. It has been reported that, in contrast to somatic cell count (SCC) and bacteriology, cytology can differentiate nonmastitic, early inflammatory and late inflammatory animals (Rivas et al. 2001). A flow cytometric analysis revealed a selective recruitment of T cell subsets, depending on the mastitis pathogen (Soltys & Quinn, 1999). Aditionally, Leitner et al. (2000) and Pilai et al. (2001) stated the importance of studying the particular leucocyte population pattern for each mastitis pathogen. Apart from the pathogen, the well-documented influence of lactation stage and number should also be kept in mind (Blackburn, 1966, 1967; Dohoo et al. 1981; Burvenich et al. 1995; Morgante et al. 1996; Labohm et al. 1998; Dosogne et al. 2003). However, the use of differential cell count as a criterion for mastitis diagnosis requires that the method be submitted to extensive evaluation. A review of the literature shows that the variation in the results with microscopy and flow cytometry as well as the differences between the two should not be underestimated: macrophages (MAC) range from 0% to 80%, lymphocytes (LYM) from 1·5% to 79·5%, polymorphonuclear neutrophils (PMN) from 3% to 95%, and epithelial cells from 1% to 19% (Table 1).

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Table 1. A survey of values taken from the literature for differential cell count in milk from healthy udders

	Cell type†				
Publication	% MAC	% LYM	% PMN	% EPI	Method
Paape et al. 1979	0 cow 3 heifer	5 cow 22 heifer	95 cow 75 heifer		Microscopy
Lee et al. 1980	80	16	3	1	
Paape et al. 1981	60	28	10	2	
Kurzhals et al. 1985	63	1·5	34	2·4	
Miller et al. 1991	30	24	26	19	
Vangroenweghe et al. 2001	35	7	58		
Wever & Emanuelson 1989	48	15	37		Flow Cytometry
Östensson 1993	74	14	12		
Leitner et al. 2000	13		29	45	
Dosogne et al. 2003	10	60	30		

†MAC, macrophages; LYM, lymphocytes; PMN, polymorphonuclear neutrophils; EPI, epithelial cells

Table 2. Grouping of cows and quarters according to somatic cell count (SCC) in study 3

Cows	SCC × 10^{-3}/ml milk	All quarters <100	At least one quarter 100–400	At least one quarter >400
	Group	A	B	C
	n	13	9	17

Quarters	SCC × 10^{-3}/ml milk						
	Group	A	B1	B2	C1	C2	C3
	n	45	21	12	15	20	33

In this context, the aim of this paper is to analyse the variation of differential milk cell count resulting from three sources: the material of the sampling bottle (study 1); the subjective interpretation of the technicians performing the cell differentiation (study 2); and the number of centrifugations used for smear preparation (study 3).

Materials and Methods

Animals

Ten high-yielding mid-lactation cows in their first to fifth lactations were selected using data from the Dairy Performance Test for two studies: the sampling container material (study 1); and the subjectivity of technicians conducting the laboratory analysis (study 2). Of the 40 sampled quarters, 23 had SCC of <100 000 cells/ml with a geometrical mean (Xg) SCC of 23 500 cells/ml. The Xg SCC of the remaining 17 quarter milk samples was 927 000 cells/ml, the Xg SCC of all sampled quarters was 93 000 cells/ml. Thus, these samples covered a wide range of SCC.

The impact of the preparation technique (study 3) was conducted on 39 high-yielding primi- and multiparous cows in midlactation, sampled following cytobacteriological screening on quarter level, according to DVG (German Veterinary Society, DVG 1994) standards. These data were grouped according to the udder health status of the cows as indicated by quarter milk samples (Table 2). Udder health was defined as proposed by the German Veterinary Society (DVG 1994) using a threshold of 100 000 cells/ml.

Whenever it was not possible to count 100 cells for the cell differentiation, the whole data set of those samples was excluded from further analysis. The milk samples in group A were free of mastitis pathogens throughout the cytobacteriological screening and on the sampling day itself as proven by the cytobacteriological analysis described below. Therefore group A could be used as physiological reference.

Milk sampling

Sampling was conducted at the quarter level according to NMC standards (National Mastitis Council, 1999) during morning milking. A sample (10 ml) of milk was taken in sterile glass tubes for cytobacteriologic analysis and 500 ml was handmilked into a glass bottle. For studies 1 and 2, the 500-ml milk sample was gently mixed directly after milking and transferred in equal parts of 250 ml to

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Table 3. Comparison of the differential cell count of milk samples taken in plastic and glass bottles

		Technician 1		Technician 2		Technician 3	
Cell type†	Material	plastic (p)	glass (g)	plastic (p)	glass (g)	plastic (p)	glass (g)
PMN	X ± SD	61·37 ± 24·87	58·53 ± 24·70	67·30 ± 18·43	63·56 ± 21·29	67·10 ± 19·20	65·49 ± 20·51
	p–g (X ± SD)	3·17 ± 10·78		5·33 ± 12·55*		1·58 ± 9·20	
LYM	X ± sd	13·47 ± 13·32	12·00 ± 11·29	14·83 ± 10·99	14·44 ± 13·59	5·10 ± 4·42	5·67 ± 4·99
	p–g (X ± SD)	1·39 ± 6·41		0·75 ± 7·31		−0·55 ± 6·94	
MAC	X ± sd	23·11 ± 16·42	28·10 ± 16·42	16·63 ± 11·52	21·23 ± 11·70	27·49 ± 17·39	28·38 ± 17·77
	p–g (X ± SD)	−5·21 ± 8·96*		−4·08 ± 12·89*		−0·88 ± 15·03	
n		38	39	40	39	39	39

† MAC, macrophages; LYM, lymphocytes; PMN, polymorphonuclear neutrophils
* $P < 0{\cdot}05$

two different bottles, one of plastic (polyethylene) and one of glass.

Cytobacteriology

SCC was determined with a Fossomatic 360 (Foss Electric, Hillerød, Denmark) in accordance with IDF standards (International Dairy Federation, 1984); the microbiological status was evaluated in accordance with NMC standards (National Mastitis Council, 1999).

Preparation techniques

Milk (200 ml) was diluted with 200 ml phosphate-buffered saline (PBS). The cream layer and supernatant were discarded after centrifuging at 1000 ***g*** for 15 min. The cells were then washed three times in PBS and the cell suspension (CS) was adjusted to a concentration of 4×10^6 cells/ml. To prepare the 'coffee grinder' (CG) smear, 10 µl of blood plasma was spread on a cover glass and 40 µl of CS was added. The cover glass was centrifuged at approx. 200 ***g*** for 5 s and then glued onto a microscope slide with the cell side turned upwards. After air drying, the smear was stained with eosin-giemsa (Hemacolor, Merck Eurolab GmbH, 64293 Darmstadt, Germany). The CG technique was used in all three studies.

In study 3, an additional 10 ml of milk from the hand-milked samples was centrifuged at 1400 ***g*** for 10 min, and the cream layer and supernatant were discarded. One drop of saline was added and the sediment was spread over an area of 2 cm^2 on a microscope slide. After air drying, the sediment smear (SS) was stained with toluidine blue (2 mg/l). This technique was used in study 3 only. A total of 146 smears were prepared using both the SS and the CG to evaluate the impact of the preparation technique.

Microscopic differential cell count

Oil immersion was used to count 100 cells, which were then differentiated into PMN, LYM and MAC. In studies 1 and 3, the cells were counted by only one person, whereas in study 2 all slides were counted three times by three different technicians.

Monoclonal antibodies (study 1 only)

Additionally, in order to corroborate the findings for LYM subpopulations in study 1, flow cytometric analysis was also performed using four monoclonal antibodies against three different lymphocyte surface structures. The four antibodies used were: CC8 (Serotec GmbH, 40210 Düsseldorf, Germany) and CACT83A (Immunology Unit, School of Veterinary Medicine Hannover, Germany) against CD4, CC101 (Serotec GmbH) against WC1, and GB21A (vmrd, Inc., Pullman, WA 99163, USA) against TcR1-N24.

The secondary antibody was a fluorescein isothiocyanate (FITC)-conjugated F(ab')2 rabbit anti-mouse IgG, crossreacting with IgM (Serotec GmbH).

Statistical analysis

Statistical tests were made with the SAS 8 software (SAS, 1999). Data were tested on a normal distribution and a Greenhouse-Geisser two-factor analysis of variance was conducted on matching pairs followed by a verification of the normal distribution of the residues. Multiple comparisons were done with the Ryan-Einot-Gabriel-Welsh-Test with $\alpha = 0{\cdot}0125$.

Results

Effect of the material of the sampling bottle

Table 3 shows the differential cells counts of the three technicians and the difference between the results with the plastic (p) and glass (g) bottles (p−g). The analysis of variance revealed a general influence of the plastic material, which led to a reduction of MAC. Microscopic examination revealed no effects on the LYM due to the material of the sampling bottle. There were no significant differences

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Table 4. Comparison of the percentage of positive cells (%) and mean fluorescence intensity (Xm) of the lymphocyte antibodies tested in plastic and glass bottles

	Plastic				Glass			
MAB†	CC8	CACT 83A	CC101	GB21A	CC8	CACT 83A	CC101	GB21A
%	31·8±9·9	4·5±3·3	4·6±3·0	12·8±6·5	31·1±10·7	4·9±3·0	5·0±2·7	13·7±6·9
Xm	128·0±18·9	287·3±205·0	305·3±276·8	337·7±73·2	127·0±17·3	346·6±309·3	236·0±151·4	343·4±153·2
n	40	40	39	40	40	40	33	39

† MAB, monoclonal antibody

Table 5. Comparison of the differential cell count performed by three different technicians (T1, T2, T3)

		Plastic			Glass		
Cell type†	Material	T1–T2	T1–T3	T2–T3	T1–T2	T1–T3	T2–T3
PMN	X±SD	−7·08±13·41**	−5·32±9·97**	1·88±11·62	−5·09±8·58***	−7·33±8·58***	−1·92±9·98
LYM	X±SD	−2·03±12·07	8·03±11·92***	9·85±11·54***	−1·29±7·03	6·66±10·11***	8·77±11·51***
MAC	X±SD	5·33±12·34**	−4·97±8·96**	−10·18±13·76***	5·76±9·97	−0·66±10·34**	−7·15±11·43***
n		38	39	40	39	39	39

† MAC, macrophages; LYM, lymphocytes; PMN, polymorphonuclear neutrophils
**$P<0·01$; ***$P<0·001$

Table 6. Total and differential cell count obtained by sediment smear (SS) and coffee grinder (CG) smear

		A†	B1†	C1†	B2†	C2†	C3†
log SCC/ml		4·35	4·45	4·54	5·36	5·36	5·89
	SS	19·54±17·31	28·90±16·67	47·67±20·16	57·00±12·99	63·35±23·48	62·00±22·70
% PMN‡	CG	33·03±20·17	43·15±21·84	66·42±17·35	68·18±14·50	67·00±26·20	79·91±16·07
	SS–CG	−13·50±17·04***	−14·25±15·34***	−11·07±13·33**	−18·75±19·63**	−3·65±15·96	−17·91±16·22***
	SS	45·56±22·10	30·14±17·60	26·75±10·38	17·73±8·22	19·75±15·07	16·94±14·39
% LYM‡	CG	24·82±15·35	19·20±9·75	11·23±10·99	7·20±3·00	11·05±10·13	6·33±9·42
	SS–CG	20·74±17·96***	10·94±13·67**	10·53±8·43***	15·52±12·68****	8·70±9·94***	10·61±7·91***
	SS	34·53±14·89	36·71±13·46	25·50±14·29	24·40±8·22	16·85±10·31	20·73±10·83
% MAC‡	CG	39·05±17·60	34·95±18·54	19·84±8·45	24·53±14·55	21·75±16·65	12·94±8·36
	SS–CG	−4·52±17·83	1·76±17·07	−0·13±12·56	5·66±14·40	−4·90±13·19	7·79±10·52***
	n	45	21	15	12	20	33

† for definitions see Table 2
‡ MAC, macrophages; LYM, lymphocytes; PMN, polymorphonuclear neutrophils
**$P<0·01$; ***$P<0·001$

between the samples in the percentage of positive cells or in the intensity of fluorescence (Table 4).

Effect of the technician performing the cell differentiation

The analysis of variance of the differential cell count performed by three different persons revealed a general influence of this factor, and showed that the individual differences between the persons applied to all cell types involved (Table 5).

Effect of the method of smear preparation

To include udder health status in the analysis, the data were divided into six udder health classes (see Table 2). Mean values of the SS were subtracted from those of the CG to confirm which cell types were increased or reduced by a particular method (see Table 6). Application of the SS for differential cell counting revealed the presence of significantly more LYM and fewer PMN in all udder health groups except one. Significant differences in MAC due to the two methods were detected only in the case of severe mastitis (group C3).

Discussion

At present the broad variation in published results on differential cell count in milk (see Table 1) makes it impossible to establish this technique as a tool for monitoring

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udder health. To evaluate physiological and pathological changes in the differential cell count, it is important to analyse the effect of laboratory methods on cell differentiation.

As shown in study 1, this impact began in the milking parlour with the sampling bottle. The plastic material of bottles significantly influenced the percentage of phagocytes in milk by reducing the number of MAC. This result can be explained considering that the primary function of MAC is adherence. Phagocytes, especially macrophages, adhere even to smooth surfaces like glass (Desiderio & Campbell, 1980; Lee et al. 1980; Mielke, 1980; Garrouste et al. 1982; Ellis et al. 1988). Ours is the first study focused on the actual significance of this MAC property for the differential cell count in milk.

LYM were not affected by the bottle material probably because the main function of these cells in the mammary gland is immunomodulation (Paape et al. 2000; Riollet et al. 2000).

A factor well known to all laboratory personnel is the subjective component of the technician at the microscope (Heeschen, 1975; Kitchen, 1981) and that effect was evident in the present study. The present results of this paper demonstrate that it is quite difficult for technicians to distinguish between MAC and EPI, and between MAC and LYM, and in some cases also between PMN and LYM.

Another important point was the centrifugation of milk samples. Literature reports that MAC can be found in the cream layer and in the supernatant of centrifuged milk (Lee et al. 1980; Dulin et al. 1982). Whereas today's standard method (cytospin) uses a single step, as does the SS, the preparation of milk samples for flow cytometry in combination with monoclonal antibodies requires four centrifugation steps. On the other hand, the methods of Hageltorn & Saad (1986), Miller et al. (1993), Pilai et al. (2000) and Dosogne et al. (2003) do not include any centrifugation of the milk at all. But our study showed that the number of centrifugations significantly influenced the differential cell count. Interestingly, the population of MAC was least affected by this factor, which, according to the literature mentioned above, was unexpected. Further studies are necessary to investigate this finding further.

The more steps of centrifugation (i.e., the CG method) there were, the fewer LYM were lost, as these are the cells with the lowest density. PMN were enriched, although quite a number of them have phagocytosed milk fat and are thus less dense, especially in nonmastitic milk (Paape et al. 1979; Lee et al. 1980).

To permit the best possible comparison of published results of microscopic evaluations, the material of the sample bottles should be considered as well as the preparation technique used for microscopic or flow cytometric cell differentiation. Furthermore, microscopic evaluation should be performed by only one person. But, as proven by haematology, the subjective influence of the technician does not avert the use of differential cell count in routine diagnosis.

In conclusion, it is difficult to compare scientifically the differential cell counts of milk obtained by different methods and/or persons, because the results are influenced by the material of which the sample bottle is made; the technician at the microscope; and the method of preparation. When publishing results, one should indicate not only the method of smear preparation but also the material of the sampling bottle. Provided that a common definition of a healthy mammary gland can be found, e.g., a threshold of 100 000 cells/ml as first proposed by Tolle (1970) and confirmed by Hamann (2002), attention can be turned to the role of the number of lactations and days in milk as well as the mastitis-causing pathogen in order to establish differential cell count as a tool for monitoring udder health. The differential cell count then could give the option not only to support bacteriology in identifying the pathogen but also to define the stage of infection. The latter is of help in deciding between therapy and culling.

References

Blackburn PS 1966 The variation in the cell count of cows' milk throughout the lactation and from one lactation to the next. *Journal of Dairy Research* **33** 193–198

Blackburn PS 1967 The cell count of cows' milk and the micro-organisms cultured from milk. *Journal of Dairy Research* **35** 59–65

Burvenich C, Guidry AJ & Paape MJ 1995 Natural defence mechanisms of the lactating and dry mammary gland. *Proceedings of the 3rd International Mastitis Seminar, Tel Aviv, Israel* pp. 3–13

Desidero JV & Campbell SG 1980 Bovine mammary gland macrophage: isolation, morphologic features, and cytophilic immunogloulins. *American Journal of Veterinary Research* **41** 1595–1599

Deutsche Veterinärmedizinische Gesellschaft e.V. (DVG) Giessen 1994 [Guidelines for the control of bovine mastitis as a herd problem.] 3. Aufl., Deutsche Veterinärmedizinische Gesellschaft e.V., Frankfurter Strasse 89, 3539. Giessen

Dohoo IR, Meek AH, Martin SW & Barnum DA 1981 Use of total and differential somatic cell counts from composite milk samples to detect mastitis in individual cows. *Canadian Journal of Comparative Medicine* **45** 8–14

Dosogne H, Vangroenweghe F, Mehrzad J, Massart-Leen AM & Burvenich C 2003 Differential leucocyte count method for bovine low somatic cell count milk. *Journal of Dairy Science* **86** 828–834

Dulin AM, Paape MJ & Weinland BT 1982 Cytospin centrifuge in differential counts of milk somatic cells. *Journal of Dairy Science* **65** 1247–1251

Ellis JA, Davis WC, MacHugh ND, Emery DL, Kaushal A & Morrison WI 1988 Differentiation antigens on bovine mononuclear phagocytes identified by monoclonal antibodies. *Veterinary Immunology and Immunopathology* **19** 325–340

Garrouste F, Capo C, Benoliel A-M, Bongrand P & Depieds R 1982 Nonspecific binding by macrophages: different modulation of adhesive properties of rat peritoneal cells after plating on a glass or plastic surface. *Journal of the Reticuloendothelial Society* **31** 415–422

Hageltorn M & Saad MA 1986 Flow cytofluorometric characterization of bovine blood and milk leucocytes. *American Journal of Veterinary Research* **47** 2012–2016

Hamann J 2002 Relationships between somatic cell count and milk composition. *Bulletin of the IDF* **37** 256–59

Heeschen W 1975 Determination of somatic cells in milk (technical aspects of counting). *Bulletin of the IDF* **85** 79–92

International Dairy Federation 1984 Recommended methods for somatic cell counting in milk. *Bulletin of the IDF* **168**

Kitchen BJ 1981 Review of the progress of dairy science: bovine mastitis: milk compositional changes and related diagnostic tests. *Journal of Dairy Research* **48** 167–188

Kurzhals P, Klima H & Manz D 1985 [The relationship between cell count, differential cell count and bacteriological findings in subclinical bovine mastitis.] *Milchwissenschaft* **40** 6–9

Labohm R, Götz E, Luhofer G, Hess RG & Bostedt H 1998 Factors influencing the somatic milk-cell-count in dairy cows. 1. Influence of bacteriological findings, stage and number of lactation. *Milchwissenschaft* **53** 63–66

Lee C-S, Wooding FBP & Kemp P 1980 Identification, properties, and differential count of cell populations using electron microscopy of dry cow secretions, colostrum and milk from normal cows. *Journal of Dairy Research* **47** 39–50

Leitner G, Shoshani E, Krifucks O, Chaffer M & Saran A 2000 Milk leucocyte population patterns in bovine udder infection of different aetiology. *Journal of Veterinary Medicine Series B* **47** 581–589

Mielke H 1980 [The mononuclear phagocytosis system and macrophages in bovine milk.] *Monatsheft für Veterinärmedizin* **35** 370–376

Miller RH, Paape MJ, Filep R & Link S 1993 Flow cytometric analysis of neutrophils in cows' milk. *American Journal of Veterinary Research* **54** 2975–1979

Miller RH, Paape MJ & Fulton LA 1991 Variation in milk somatic cells of heifers at first calving. *Journal of Dairy Science* **74** 3782–3790

Morgante M, Ranucci S, Pauselli M, Casoli C & Duranti E 1996 Total and differential cell count in milk of primiparous Comisana ewes without clinical signs of mastitis. *Small Ruminant Research* **21** 265–271

National Mastitis Council 1999 *Laboratory Handbook on Bovine Mastitis*. Madison, WI, USA: National Mastitis Council

Östensson K 1993 *Trafficking of leucocytes and immunoglobulin isotypes in the bovine udder*. Dissertation, Swedish University of Agricultural Sciences, Uppsala

Paape MJ, Shafer-Weaver K, Capuco AV, van Oostveldt K & C Burvenich 2000 Immune surveillance of mammary tissue by phagocytic cells. *Advances in Experimental Medicine and Biology* **480** 259–277

Paape MJ, Wergin WP, Guidry AJ & Pearson RE 1979 Leucocytes – Second line of defense against invading mastitis pathogens. *Journal of Dairy Science* **62** 135–153

Paape MJ, Wergin WP, Guidry AJ & Schultze WD 1981 Phagocytic defense of the ruminant mammary gland. *Advances in Experimental Medicine and Biology* **137** 555–578

Pillai SR, Kunze E, Sordillo LM & Jayarao BM 2000 Application of differential inflammatory count as a tool to monitor udder health. *Journal of Dairy Science* **84** 1413–1420

Riollet C, Rainard P & Poutrel B 2000 Cells and cytokines in inflammatory secretions of bovine mammary gland. *Advances in Experimental Medicine and Biology* **480** 247–258

Rivas AL, Quimby FW, Blue J & Coksaygan O 2001 Longitudinal evaluation of bovine mammary gland health status by somatic cell counting, flow cytometry and cytology. *Journal of Veterinary Diagnostic Investigation* **13** 399–407

SAS Institute Inc 1999 SAS OnlineDoc® Version 8. Cary, NC: SAS Institute Inc. 1999

Soltys J & Quinn MT 1999 Selective recruitment of T-cell subsets to the udder during staphylococcal and streptococcal mastitis: analysis of lymphocyte subsets and adhesion molecule expression. *Infection and Immunity* **67** 6293–6302

Vangroenweghe F, Dosogne H, Mehrzad J & Burvenich C 2001 Effect on milk sampling techniques on milk composition, bacterial contamination, viability and functions of resident cells in milk. *Veterinary Research* **32** 565–579

Wever P & Emanuelson U 1989 Effects of systematic and intramammary infection on differential and total systematic cell counts in quarter milk samples from dairy cows. *Acta Veterinaria Scandinavica* **30** 465–474