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THE DIAGNOSIS OF BOVINE MASTITIS: A CRITICAL EVALUATION OF A POLYVALENT RADIAL IMMUNODIFFUSION TEST AND OTHER METHODS

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ABSTRACT

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A critically comparative investigation was performed on 228 quarter samples from 26 Friesland-type dairy cows respectively to evaluate the diagnostic value of conventional direct and indirect cytological methods and a single radial immunodiffusion test based on a polyvalent antiserum.

Data obtained suggest considerable diagnostic inaccuracies for all the methods tested. The lack of an adequate "mastitis standard" prevented a precise determination of the magnitude of the diagnostic inaccuracies.

INTRODUCTION

Various procedures for the diagnosis of mastitis by examination of the mammary secretion have been described, the most recent being a single radial immunodiffusion method based on a polyvalent antiserum (Morris & Hobbs, 1971). As their polyvalent mastitis test (PMT) has not yet been subjected to comprehensive evaluation, a critical comparison between it and other well known methods was undertaken to obtain data regarding:

1. Coefficient of correlation
2. Within-sample variation, i.e. repeatability
3. Influence of overnight sample storage
4. Influence of daily fluctuations
5. Coefficient of variation, i.e. accuracy and sensitivity
6. Correlation with clinical symptomatology
7. Correlation with conclusions reached after combination of various routine mastitis diagnostic methods
8. Influence of stage of lactation
9. Correlation with desoxyribonucleic acid (DNA) content of milk
10. Correlation with various somatic cells in milk

MATERIALS AND METHODS

1. *Experimental animals*

The 26 Friesland-type cows used in the investigation differed in age, number of lactations, daily milk yield and stage of lactation. Seven cows were used twice during this investigation. Some of the animals had a history of clinical or subclinical mastitis as established by detailed monthly examinations of their udders over a period of 3 years before the investigation began. All cows were free from tuberculosis and brucellosis, clinically healthy in other respects and in good condition. They were kept under identical conditions of feeding and management.

2. *Design of the experiments*

Three series of trials were carried out over a period of 8 months as follows:

SERIES I: Six cows in early to middle lactation sampled on 3 consecutive days (Day 1, 2 and 3). Samples obtained on Day 1 were divided into four aliquots (A, B, C and D).

SERIES II: Six cows in early to middle lactation and six cows in late lactation, sampled once.

SERIES III: Fifteen dry cows, sampled once.

3. *Sampling*

Before samples were taken the condition of the udders was recorded. After the usual premilking disinfection the udders were thoroughly dried with disposable paper towels and the teats, especially the tips, were vigorously swabbed with a pledget of cotton wool moistened with 70% alcohol. After discarding the first three jets of milk, approximately 150 to 200 ml of foremilk were aseptically milked from each quarter of lactating cows into sterile McCartney bottles. From the dry cows about 20 ml of secretion were collected after discarding the first jet.

4. *Laboratory examinations of milk*

A total of 228 samples was subjected to the following laboratory examinations immediately after sampling:

a. *The California Mastitis Test* (CMT) was performed according to standard methods described by the American Public Health Association (Anon., 1967), using a commercial test reagent(1).

b. *The Direct Microscopic Count* (DMC) was established from whole milk smears processed by the standard methods of the American Public Health Association (Anon., 1967), using a calibrated platinum wire loop. The smears were stained by the Broadhurst-Paley method (Schalm, 1962). All clearly recognizable somatic cells were counted in 20 microscopic fields selected vertically and horizontally at random across the smear; 40% of the fields were in the central area of the smear and 60% in the marginal area. The general cytological and bacteriological appearance of the microscopic picture was noted.

c. The direct microscopic cytological examination of *sediment smears* was carried out on smears prepared according to the method of Lerche (1966) and stained with Giemsa. Twenty microscopic fields, randomly selected vertically and horizontally across the smear, were evaluated, taking into account all clearly recognizable somatic cells and the general cytological and bacteriological appearance of the microscopic field. First a *Sediment Smear Estimate* (SSE) was established (Obiger, 1962). The results obtained were recorded by

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symbols equivalent to the number of cells per microscopic field as summarised in Table 1.

TABLE 1 Estimation of Cell content in milk sediment smears (SSE) (according to Obiger, 1962)

Number of cells per microscopic field	Symbol	Cell content
0 to 10	0	Normal
11 to 20	+	Slightly increased
More than 20; slight floccule formation	++	Distinctly increased
Uncountable; cell conglomeration, extensive floccule formation	+++	Markedly increased

Subsequently the *Sedimented Smear Count* (SSC) was obtained using the method described by Dedié & Kielwein (1961). A differential count of the various types of cells present in the sediment smear was made.

d. *The Cream Smear Estimate* (CSE) was established according to the method of Meara (1951). The cream smears were prepared from the gravity cream layer of samples stored overnight at 4°C. The smears were prepared in the same way as DMC whole milk smears but Newman's method was used for fixation, defatting and staining (Charlett, 1954). The number of all clearly recognizable somatic cells was estimated in 20 microscopic fields randomly selected vertically and horizontally across the smear. Results of the CSE were recorded by symbols equivalent to the number of cells per microscopic field as summarised in Table 1.

e. *The Whiteside Mastitis Test* (WMT) was done 1 to 3 hours after sampling according to the standard recommendations of the American Public Health Association (Anon., 1967).

f. *The Electronic Cell Count* (ECC) was performed with a Model B Coulter Counter equipped with a 70μ aperture tube (Tolle, Zeidler & Heeschen, 1966). The milk was processed for counting with a Counter Dual Diluter and commercially available fat solvent(2).

g. *Desoxyribonucleic acid* (DNA - μg/ml of milk) was determined according to Dische (1955).

h. *The Polyvalent Mastitis Test* (PMT) was performed as described by Morris & Hobbs (1971). After inoculation the PMT plates were left for 24 hours at room temperature, then the diameter of the largest precipitin ring was measured by graduated callipers before and after staining with amido black. Comparison of the PMT with other methods was effected by correlating the size of the largest ring with data obtained from other tests. The frequent presence of additional precipitin rings was recorded. The antiserum-agarose slides or plates required for the investigation were produced by Dr N. M. Morris(3) and Mr M. H. Viljoen(4).

5. *Laboratory examination of colostrum.*

All samples were processed and examined immediately after collection. To determine the effect of colostrum on the PMT, the Series III samples were only subjected to the following tests:

a. Cultural and microscopic bacteriology
b. The SSE
c. The PMT

6. Data were processed for computerization and preliminary statistical analysis after consultation with Drs C. A. E. G. De Ville De Goyet(5) and I. F. P. Willers(5). The final statistical analysis was performed by Dr J. P. Jooste(6).

7. The cytological diagnoses of mastitis were confirmed by carrying out the following tests on the samples:

a. Catalase flotation test (Willits & Babel, 1965)
b. Cultural bacteriology (Giesecke, Nel & Van den Heever, 1968)
c. Electrometric pH measurement
d. Chloride determination (Hawk, 1965)
e. Calcium and potassium determinations by atomic-absorbtion photometry with a Techtron Model AA-4.

The data obtained from these tests were not included in the comparative evaluations of the indirect cytological methods.

8. The abbreviations used for the various tests are summarised as follows:

PMT - Polyvalent Mastitis Test
CMT - California Mastitis Test
DMC - Direct Microscopic Count
SSE - Sediment Smear Estimate
SSC - Sediment Smear Count
CSE - Cream Smear Estimate
WMT - Whiteside Mastitis Test
ECC - Electronic Cell Count
DNA - Desoxyribonucleic acid determination

RESULTS AND DISCUSSION

1. *The coefficient of correlation*

By subjecting aliquots A and B of the 24 Day 1, Series I samples to the PMT, ECC and DMC the coefficients of correlation for these tests shown in Tables 2 and 3 were obtained. These results are significant at all levels analysed.

TABLE 2 Coefficients of correlation for Aliquot A (Day 1, Series I)

	PMT	ECC	DMC
PMT	1,00	0,74	0,76
ECC	0,74	1,00	0,82
DMC	0,76	0,82	1,00

TABLE 3 Coefficients of correlation for Aliquot B (Day 1, Series I)

	PMT	ECC	DMC
PMT	1,00	0,73	0,64
ECC	0,73	1,00	0,79
DMC	0,64	0,79	1,00

2. *The within-sample variation*

The within-sample variation was established from data obtained from aliquots A and B and is expressed in terms of the coefficients of correlation in Table 4. It was lowest for the PMT, i.e. the repeatability of the PMT was superior to that of the ECC and DMC when taking the largest PMT precipitin rings into account.

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TABLE 4 The within-sample variation of data obtained from Aliquots A and B (Day 1, Series I)

Variables	Coefficient of correlation
PMT_A: PMT_B	0,90
ECC_A: ECC_B	0,84
DMC_A: DMC_B	0,71

3. *The influence of overnight storage*

Aliquot C of the Day 1, Series I samples was stored at 4°C for 24 hours, thoroughly shaken and then subjected to the tests already applied to aliquots A and B. Comparison of the mean values of data obtained from the fresh aliquots (A and B) and from the refrigerated aliquot (C) gave the coefficients of correlation shown in Table 5.

TABLE 5 Coefficients of correlation for data obtained from Series I Aliquots examined before (A/B) and after (C) Cold Storage

Variables	Coefficient of correlation
$PMT_{A/B}$: PMT_C	0,88
$ECC_{A/B}$: ECC_C	0,85
$DMC_{A/B}$: DMC_C	0,68

Table 5 indicates that the PMT displays the highest coefficient of correlation while the DMC shows the lowest. The coefficient of correlation between the PMT on the samples before cold storage (Table 4) and the ECC and DMC on the sample after storage (Table 5) decreased appreciably. These findings suggest that the repeatability of the PMT is less influenced by cold storage than is that of the ECC and DMC.

4. *The influence of daily fluctuations*

Aliquot D of the 24 Day 1, Series I samples and the samples collected on Days 2 and 3 were subjected to the PMT, ECC, DMC, CMT and WMT immediately after sampling. The correlations of these samples collected on 3 successive days are detailed in Tables 6, 7 and 8.

TABLE 6 Coefficients of correlation for Aliquot D of Day 1, Series I samples

	PMT	ECC	DMC	CMT	WMT
PMT	1,00	0,82	0,78	0,86	0,90
ECC	0,82	1,00	0,86	0,78	0,81
DMC	0,78	0,86	1,00	0,74	0,77
CMT	0,86	0,78	0,74	1,00	0,85
WMT	0,90	0,81	0,77	0,85	1,00

TABLE 7 Coefficients of correlation for Day 2, Series I samples

	PMT	ECC	DMC	CMT	WMT
PMT	1,00	0,85	0,82	0,84	0,91
ECC	0,85	1,00	0,81	0,76	0,86
DMC	0,82	0,81	1,00	0,79	0,83
CMT	0,84	0,76	0,79	1,00	0,92
WMT	0,91	0,86	0,83	0,92	1,00

TABLE 8 Coefficients of correlation for Day 3, Series II samples

	PMT	ECC	DMC	CMT	WMT
PMT	1,00	0,78	0,74	0,90	0,89
ECC	0,78	1,00	0,95	0,83	0,76
DMC	0,74	0,95	1,00	0,79	0,70
CMT	0,90	0,83	0,79	1,00	0,83
WMT	0,89	0,76	0,70	0,83	1,00

The information given in Tables 6 to 8 supports the data already presented in Tables 2 and 3 concerning the coefficients of correlation between the PMT, ECC and DMC. Furthermore, coefficients of correlation between the CMT and WMT and the other methods tested are shown to be highly significant at all levels analyzed.

It is therefore concluded that daily fluctuations in the samples affected all the data obtained. These fluctuations were, however, not great enough to alter the significance of the coefficients of correlation. This becomes particularly obvious when the results from Series I samples collected on Day 1 are compared with the mean values obtained for samples collected on Days 2 and 3 (Table 9).

TABLE 9 Coefficients of correlation between results obtained from Aliquot D (D) of samples collected on Day 1 and the mean values obtained from samples on Days 2 and 3, (M), (Series I)

Variables	Coefficient of correlation
PMT_D: PMT_M	0,94
ECC_D: ECC_M	0,93
DMC_D: DMC_M	0,79
CMT_D: CMT_M	0,84
WMT_D: WMT_M	0,90

The data given in Tables 4 and 9 indicate the superior repeatability of the PMT and the ECC compared with the DMC. It can also be seen from Table 9 that daily fluctuations influenced the daily repeatability of the CMT and WMT less than that of the DMC. From the information in Tables 6 to 8 and particularly Table 9 it is obvious that a significant correlation exists between the methods tested.

5. *The coefficient of variation*

Good repeatability of results may be due to either high or low extremes of diagnostic accuracy and sensitivity of the method concerned. As there is no absolute standard against which to evaluate a cytological method used for diagnosing mastitis reliance must be placed on the coefficient of variation to indicate its accuracy and sensitivity.

From data obtained from all samples collected in Series I and II the coefficients of variation were established for the PMT, ECC and DMC and are summarized in Table 10.

TABLE 10 The coefficients of variation for PMT, ECC and DMC as established from all samples collected in Series I and Series II

Method	Coefficient of variation
PMT	50,10%
ECC	173,56%
DMC	179,60%

These results show that the coefficient of variation which is lowest for the PMT and highest for the DMC, is extremely high for all these methods. Against an absolute standard any diagnostic method should have a coefficient of variation of 15% or less. The data obtained therefore suggest that none of the evaluated methods are truly adequate for the diagnosis of mastitis. In the absence of an adequate standard, and considering repeatability, accuracy and sensitivity, it seems that the PMT is superior to the ECC and the DMC in that order.

6. *The relationship between clinical changes in udder tissues, various cytological methods and the PMT*

During the examination of Series I and II samples, it was established that both clinically normal and asymmetric udders with palpable chronic parenchymal or cisternal induration may produce milk of either normal or pathological cellular content. Similarly, milk from patently normal and chronically indurated quarters produced both small and large precipitin rings in the PMT. No significant correlation could be established between the clinical state of a chronically mastitic udder or quarter and the cell content of the milk or the size of the PMT ring.

7. *The relationship between the PMT and mastitis-negative or positive quarter samples*

Using standards laid down by the International Dairy Federation (Kästli, 1967), Series I and II individual samples were classified as being derived from mastitis-negative or positive quarters on the basis of the clinical, cytological and cultural findings. The PMT reactions on corresponding samples were grouped according to the size of the outer rings. These results are presented in Table 11.

Application of the chi-square test (χ^2) disclosed a highly significant difference ($\chi^2 = 59{,}53$) between the sizes of the PMT precipitin rings obtained on testing mastitis-negative and positive milk. However, a breakdown of the data resulting from the examination of milk obtained from cows in various stages of lactation and in the dry period (see Tables 12, 14 and 16) shows that the PMT, in its present form, is influenced by the stage of lactation and does not differentiate between aseptic and septic mastitis.

TABLE 11 Chi-square test on PMT results of all samples collected during Series I and II

		Size of PMT ring (mm)		
		6	6-10	10
Mastitis Negative	Observed value f	42	14	5
	Expected value f	22,75	17,58	20,34
Mastitis Positive	Observed value f	2	20	35
	Expected value f	21,25	16,42	19,32

Final result: $\chi^2 = 59{,}53$

8. *The influence of the stage of lactation on the PMT*

Data on the health of the udder and on the diameter of the PMT ring obtained from samples from cows in early to mid-lactation and in late lactation, plus analyses of these data by the chi-square test, are presented in Tables 12 to 15. Data established from samples from dry cows appear in Table 16; these were not analysed by the chi-square test.

From Tables 13 and 15 it is concluded that the application of the chi-square test to data from cows in early to mid-lactation indicates that $\chi^2 = 55{,}55$, while for cows in later stages of lactation, $\chi^2 = 9{,}68$. To be statistically significant the χ^2-value for a 3×2 table must be greater than or equal to 5,99; results are highly significant where $\chi^2 = 9{,}21$. Thus it may be concluded that the results obtained by application of the PMT to cows in various stages of lactation are highly significant.

TABLE 12 Cows in early to mid-lactation: diameter of outer PMT ring (O.R.) in relation to mastitis-negative and positive samples and the frequency of additional precipitin rings (A.R.) (Series I & II)

Diagnosis	Diameter (mm) of outer precipitin ring												
	2	3	4	5	6	7	8	9	10	11	12	13	14
Mastitis negative:													
Number of samples with O.R. —diameter of:		5	17	13	3	1	1						
Number of additional rings (A.R.) for corresponding O.R. *AR* 1		5	17	13	3	1	1						
Mastitis positive:													
Septic mastitis:													
Number of samples with O.R. —diameter of:				1	3	2	2	3	3	5	3	2	10
Number of A.R. for corresponding O.R. *AR* 1				1	3	2	2	3	2	5	3	2	9
2									1				1
Aseptic mastitis:													
Number of samples with O.R. —diameter of:								2					
Number of A.R. for corresponding O.R. *AR* 1								2					
Mastitis suspected:													
Number of samples with O.R. —diameter of:	1	2	4	5									
Number of A.R. for corresponding O.R. *AR* 1	1	2	4	5									

TABLE 14 Cows in late lactation: diameter of outer PMT ring (O.R.) in relation to mastitis-negative and positive samples and the frequency of additional precipitin-rings (A.R.) (Series II)

Diagnosis		Diameter (mm) of outer precipitin ring																								
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Mastitis negative:																										
Number of samples with O.R.																										
—diameter of:			1	3	3	1	3	2	1	2	3	1		1												
Number of additional rings (A.R.) For corresponding O.R.	*AR*																									
	1		1	3	3	1	3	1	1		2															
	2							1			1			1												
	3									2		1														
Mastitis positive:																										
Septic mastitis:																										
Number of samples with O.R.																										
—diameter of:					1	3		1	2	1	2		3	2	2		2		1	1		1				1
Number of A.R. for corresponding O.R.	*AR*																									
	1				1																					
	2					3		1	2				2		1											
	3									1	2		1							1						
	4													1												
	5													1	1		2		1			1				
	6																									1
Aseptic mastitis:																										
Number of samples with O.R.																										
—diameter of:							1							1		1										
Number of A.R. for corresponding O.R.	*AR*																									
	1																									
	2						1																			
	3													1												
	4															1										
Mastitis suspected:																										
Number of samples with O.R.																										
—diameter of:												1														
Number of A.R. for corresponding O.R.	*AR*																									
	6											1														

TABLE 13 Chi-square test on results of PMT on Series I & II samples collected from cows in early to mid-lactation

		Size of PMT ring (mm)		
		6	6–10	10
Mastitis Negative	Observed value f	35	5	0
	Expected value f	19,4680	0,7290	10,8100
Mastitis Positive	Observed value f	1	13	20
	Expected value f	16,5420	8,2710	9,1900

Final result: $\chi^2 = 55{,}55$

TABLE 15 Chi-square test on results of PMT on Series II samples collected from cows in late lactation

		Size of PMT ring (mm)		
		6	6–10	10
Mastitis Negative	Observed value f	7	9	5
	Expected value f	3,8184	7,6363	9,5460
Mastitis Positive	Observed value f	1	7	15
	Expected value f	4,1816	8,3632	10,4540

Final Result: $\chi^2 = 9{,}68$

TABLE 16 Dry cows: diameter of outer PMT ring (O.R.) in relation to mastitis negative and positive samples and the frequency of additional precipitin-rings (A.R.) (Series III)

Diagnosis	Diameter (mm) of outer precipitin ring																	
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Mastitis negative																			
Number of samples with the O.R. —diameter of:											2		3	2	12	10	5	5	
Number of additional rings (A.R.) for the corresponding O.R. *AR* 1														1		2	1	1	
2											1		2	1	9	6	2	3	
3													1		3	2	1	1	
4																	1		
Mastitis positive:																			
Septic mastitis:																			
Number of samples with O.R. —diameter of:											1		2	5	3	2	1		1
Number of A.R. for the corresponding O.R. *AR* 1														1	1	1			1
2											1		1	3	2	1	1		
3													1	1					
4																			
Mastitis suspected:																			
Number of samples with O.R. —diameter of:																1		1	2
Number of A.R. for the corresponding O.R. *AR* 1																		1	
2																1			2

When evaluated against quarters of known mastitic status, the accuracy of the PMT obviously decreases as lactation proceeds to the extent that results obtained at the colostral stage are unreliable (Table 16).

9. *The relationship between the PMT, direct and indirect cytological methods used for the diagnosis of mastitis and the DNA - content in milk*

It was found that samples from *cows in early to mid-lactation* showed a more defined, relatively narrow range in the size of the outer PMT ring (Table 12), as well as differences in the results of the various cytological tests employed, if compared with samples obtained from *cows in late lactation* (Table 14) or *dry cows* (Table 16). Such differences are possibly responsible for an important inherent error in methods which are presently widely employed in the diagnosis of mastitis. To obtain more conclusive information, the DNA-content of Series I and II milk samples, established concurrently with all the other data (Table 17), was processed statistically (Tables 18 and 19).

The DNA-content of milk samples which had been classified on the basis of the combined results of cytological, bacteriological, chemical and clinical examinations is indicated in Table 17.

TABLE 17 DNA content of mastitis-negative and -positive milk samples from cows in various stages of lactation ($\pm$ = plus or minus range μg DNA/ml of milk)

Stage of lactation	Mastitis Negative	Mastitis Positive
Early to mid-lactation	892 $\pm$ 316	2803 $\pm$ 1390
Late-lactation	1725 $\pm$ 1340	3804 $\pm$ 2248

The coefficients of correlation between the DNA content of milk and other direct or indirect cytological mastitis diagnostic methods are provided in Tables 18 and 19.

It was concluded earlier that the diagnostic value of the PMT varies according to the stage of lactation. The data given in Tables 18 and 19 indicate that all the direct and indirect cytological diagnostic methods tested are even less reliable, and thus of very doubtful value diagnostically, when applied to milk samples derived from cows in late lactation. This is particularly clear when the DNA content of milk is expressed in terms of cellular-DNA-equivalence on the basis that one diploid bovine cell contains $6{,}5 \times 10^{-6}$ μg DNA (Boivin, Vendrely & Vendrely, 1948). This calculation

TABLE 18 Coefficient of correlation between DNA in milk and other diagnostic means for cytological examination of Series I & II milk samples derived from cows in early to mid-lactation

	PMT	ECC	DMC	CSE	SSC	SSE	CMT	WMT	DNA
PMT	1,00	0,78	0,72	0,48	0,78	0,52	0,90	0,83	0,87
ECC	0,78	1,00	0,80	0,27	0,62	0,37	0,83	0,77	0,75
DMC	0,72	0,80	1,00	0,43	0,70	0,50	0 86	0,73	0,53
CSE	0,48	0,27	0,43	1,00	0,55	0,41	0,44	0,61	0,41
SSC	0,78	0,62	0,70	0,55	1,00	0,78	0,83	0,81	0,59
SSE	0,52	0,37	0,50	0,41	0,78	1,00	0,53	0,77	0,32
CMT	0,90	0,83	0,86	0,44	0,83	0,53	1,00	0,77	0,78
WMT	0,83	0,77	0,73	0,61	0,81	0,77	0,77	1,00	0,72
DNA	0,87	0,75	0,53	0,41	0,59	0,32	0,78	0,72	1,00

TABLE 19 Coefficient of correlation between DNA in milk and other diagnostic means for cytological examination of Series II milk samples derived from cows in late lactation

	PMT	ECC	DMC	CSE	SSC	SSE	CMT	WMT	DNA
PMT	1,00	0,77	0,75	—0,11	0,62	0,69	0,76	0,70	0,12
ECC	0,77	1,00	0,82	—0,38	0,57	0,47	0,88	0,74	0,30
DMC	0,75	0,82	1,00	—0,05	0,60	0,52	0,84	0,67	0,30
CSE	—0,11	—0,38	—0,05	1,00	—0,02	0,18	—0,31	—0,31	0,00
SSC	0,62	0,57	0,60	—0,02	1,00	0,83	0,48	0,54	0,30
SSE	0,69	0,47	0,52	0,18	0,83	1,00	0,43	0,44	0,34
CMT	0,76	0,88	0,84	—0,31	0,48	0,43	1,00	0,56	0,10
WMT	0,70	0,74	0,67	—0,31	0,54	0,44	0,56	1,00	0,27
DNA	0,12	0,30	0,30	0,00	0,30	0,34	0,10	0,27	1,00

TABLE 20 Coefficient of correlation between cellular-DNA-equivalence and diagnostic methods (Series I & II) at various stages of lactation

Cellular-DNA-Equivalence of milk samples derived from	Diagnostic methods used for the diagnosis of mastitis								
	PMT	ECC	DMC	CSC	SSE	SSC	CMT	WMT	Catalase test
Cows in early to mid-lactation	0,87	0,75	0,53	0,41	0,59	0,32	0,78	0,72	—0,62
Cows in late-lactation	0,12	0,30	0,30	0,00	0,30	0,34	0,10	0,27	—0,27

may be affected by the presence of bacterial DNA in milk from infected udders but this influence is assumed to be negligible (Hauke & Lüttigh, 1966). The coefficients of correlation between cellular-DNA-equivalence and other diagnostic methods are summarized in Table 20.

The information given in this table confirms that the diagnostic value of all the methods tested is greatly influenced by the stage of lactation. The ECC appears to be slightly more reliable than the WMT and PMT. All the other methods tested are less reliable than the ECC, WMT or PMT.

10. *The relationship between the PMT and the cellular constituents of milk*

The basis for the comparison of the various methods discussed above, with the possible exception of the PMT, is the presence of somatic cells in milk. The mean values of differential counts of the cells present showed that the coefficients of variation for *epithelial cells, lymphocytes* and *polymorphonuclear neutrophilic leucocytes* during the entire period of lactation were 134,90%, 427,88% and 312,07% respectively. Comparison of similar data obtained in respect of cows in early to mid-lactation and cows in late-lactation indicates that the coefficients of variation for clearly recognizable *epithelial cells* were 117,34% and 150,78% respectively: for *lymphocytes* the figures were 149,80% and 567,59%, while for *polymorphonuclear neutrophilic leucocytes* the figures were 164,54% and 587,58%. Such changes in the total numbers of cells/ml of milk, and changes in the relative proportions of its various cellular constituents, will undoubtedly influence the efficacy of the diagnostic methods tested considerably, particularly when there is a significant correlation between an indirect cytological test and a certain cell type.

The coefficients of correlation between the PMT and the number of cells of various types were established for samples derived from cows in early to mid-lactation and from cows in late-lactation. The results are summarized in Table 21.

TABLE 21 Coefficients of correlation between PMT and the number of individual cell types in milk from cows in various stages of lactation (Series I & II)

Diagnostic method	Cell types in milk during					
	Early to mid-lactation			Late-lactation		
	Epithelials	PMN*	Lymphocytes	Epithelials	PMN*	Lymphocytes
PMT	0,01	0,68	0,58	0,20	0,21	0,21

*PMN=polymorphonuclear neutrophilic leucocytes

From these data it appears that the good results obtained with the PMT in early to mid-lactation are closely related to the presence of *lymphocytes* and *polymorphonuclear neutrophilic leucocytes,* which vary only slightly in numbers in milk from normal udders at this period of lactation. The decrease of reliability observed on examining milk samples from cows in late-lactation may result to some extent from the wide variation in the number of *lymphocytes and polymorphonuclear neutrophilic leucocytes* but is probably mainly attributable to the increase in barely recognizable cellular constituents and cellular detritus. It is presumed that other direct or indirect cytological tests are similarly influenced. Thus one could conclude that the examination of milk samples by means of the PMT, ECC, CMT and WMT is a relatively reliable way of diagnosing mastitis during early to mid-lactation whereas in late-lactation these tests become completely unreliable. The data summarised in Table 21 also suggest a low coefficient of correlation between PMT and the cell content of milk although earlier (see Tables 2, 3, 4, 18, 19 and 20) a high coefficient of correlation was repeatedly confirmed.

These variable results inevitably point to possible deficiencies of the PMT antiserum. Such results may be basically influenced by

a. changes in the cell content and in the cell-bound proteins of milk;

b. changes in the cell-free proteins of milk; and

c. the presence of several precipitin rings resulting from various antigen-antibody reactions which follow the use of a polyvalent antiserum.

Tables 12, 14 and 16 record the multiplicity of precipitin rings encountered during application of the PMT. The large outer rings probably result from either cell-bound or cell-free proteins in the test milk, and as these two types of outer rings are visually indistinguishable, the results may be confusing. This does not, however, mean that the correlations which have been established are necessarily invalid. Cell-bound and cell-free antigens are apparently closely related during the first half of lactation but this relationship is lost later in lactation. From this it follows that the good correlation coefficients which were shown to exist between the results of the PMT and cytological examinations during the first half of the lactation period probably concern both the cell-bound and cell-free fractions in the test milk. On the other hand, the poor correlation coefficients which were obtained with milk of mid- to late lactation and with dry udder secretions apply only to cells and cell-bound antigenic fractions. It is, however, abundantly clear that the cell content of milk in late-lactation and of dry udder secretion is of no significance as an indicator of mastitis and consequently, purely cytological methods for diagnosing mastitis in late lactation are unreliable.

When the DNA content of milk and the DNA content per individual diploid bovine cell is considered it is obvious that the international standard and results obtained by various direct or indirect cytological tests do not refer to the true number of cells present in milk but instead only to a small fraction of the entire cell population.

From the variability of comparative data obtained by means of the PMT and cytological methods it also is apparent that the antiserum used for the polyvalent mastitis test has to be made monovalent and specific.

Conclusions

The following conclusions can be drawn from the results obtained in this investigation:

1. There is a high degree of correlation between the PMT, ECC and DMC, and also between the PMT, SSC, SSE, CMT and WMT.
2. The within-sample variation was smaller for the PMT than for the ECC and DMC, i.e. its repeatability was significantly better.
3. The overnight cold storage of milk affected the MT and ECC less than the DMC.
4. Although daily fluctuations occurring in the composition of the milk seem to have little effect on the PMT, ECC and WMT, they have a noticeable effect on the CMT and DMC.
5. The coefficients of variation established for the PMT, ECC and DMC suggest that none of the methods is truly adequate for the diagnosis of mastitis.
6. No significant correlation could be established between chronic clinical changes in the udder and the results obtained with the various diagnostic methods investigated.
7. A highly significant difference was observed in the size of the outer PMT precipitin ring when testing mastitis-negative and mastitis-positive milks.
8. The differences in the size of the outer PMT rings were most obvious in milk obtained from cows in early to mid-lactation and were still significant in mastitis-negative and positive samples in late-lactation. However, there were no significant differences present when applied to colostrum.
9. Evaluation of the reliability of the PMT and other direct or indirect cytological methods in terms of the DNA content of milk showed that during early to mid-lactation the PMT was superior to other methods such as the ECC, CMT, WMT and catalase test. The DMC, CSE, SSC and SSE were much less reliable. None of these methods is adequate for testing milk from cows in the later stages of lactation.
10. Quantitative and qualitative changes in the cellular constituents of milk, including the increase in cellular detritus, must have an appreciable effect on the diagnostic reliability of all methods in the later stages of lactation. Thus it is apparent that throughout the entire period of lactation the WMT, PMT and ECC are relatively more reliable than the other methods tested.

From the data presented it may be concluded finally that both the conventional and the new polyvalent immunodiffusion test recommended for the diagnosis of mastitis in dairy cows leave much to be desired as regards their diagnostic repeatability and accuracy. Due to the lack of adequate standards the magnitude of diagnostic inaccuracies concerned cannot be determined precisely.

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