

# Principle and Practice of Milk Checker



Tokyo, Japan

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## Principle and Practice of Milk Checker

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## *Introduction*

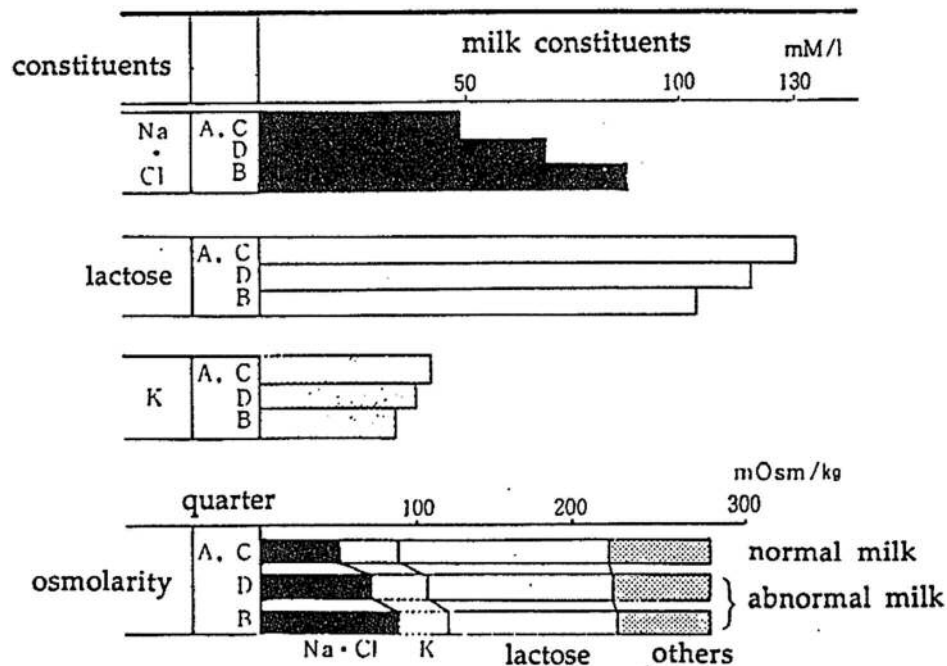
Bovine mastitis causes a big loss of milk in the dairy industry. Our Milk Checker is a handy device which measures the electrical conductivity of milk from cow's quarter and helps diagnose mammary disorders of an early stage.

Such disorders are accompanied by changes in concentration of electrolytes contained in the milk, such as  $\text{Na}^+$  and  $\text{Cl}^-$ , due to the exudation of plasma constituents. A rise in sodium and chloride content results in increased electrical conductivity in the milk.

The Milk Checker has been designed based on the principle. It enables the rapid and simple detection mastitis.

## Principle and Practice of Milk Checker

### 1. Changes of milk constituents infected by mastitis.



A, C : normal milk / B, D : abnormal milk

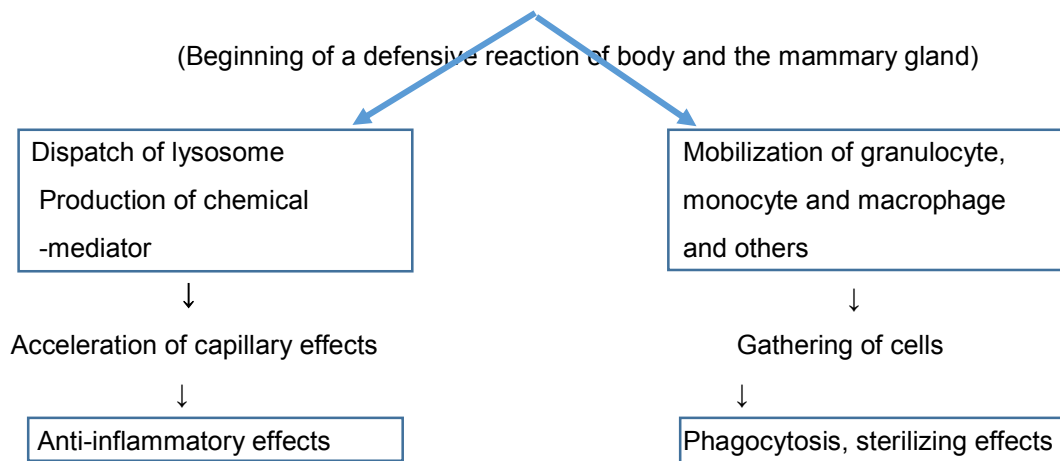
Contribution of milk constituents to osmolality of the normal and abnormal quarter milk.

It appeared that the rise in osmolality in the abnormal milk due to the increase of  $\text{Na}^+$  and  $\text{Cl}^-$  was counterbalanced mainly by the decrease of lactose and K (potassium).

## 2. Inflammation process and clinical changes on mastitis

### Progress of Inflammation

Adhesion of bacteria to epithelial cell (then fixture) growth of bacteria (then production of toxin or enzyme) damages to epithelial cells mammary (alveoli, duct, interstitium)



### Changes for inflammation

<u>Changes</u>	<u>Circulatory lesion</u> .....	Hyperemia
	<u>Exudation</u> .....	<ul style="list-style-type: none"><li>Only plasma</li><li>Mixed a plenty of blood cells (neutrophil, lymphocyte, monocyte, erythrocyte)</li></ul>
	<u>Proliferation of tissue</u> ....	<ul style="list-style-type: none"><li>Infiltration and proliferation of wandering cells (monophagocyte and lymphocyte system)</li><li>Proliferation of fixed cells (fibroblast, endothelium)</li></ul>

## Progress

Acute.....

Degeneration of tissue

Circulatory lesions

Exudation.....Exudation inflammation



Serious inflammation, Fibrinous-exudative inflammation, Hemorrhagic inflammation, Necrotic inflammation, Gangrenous inflammation

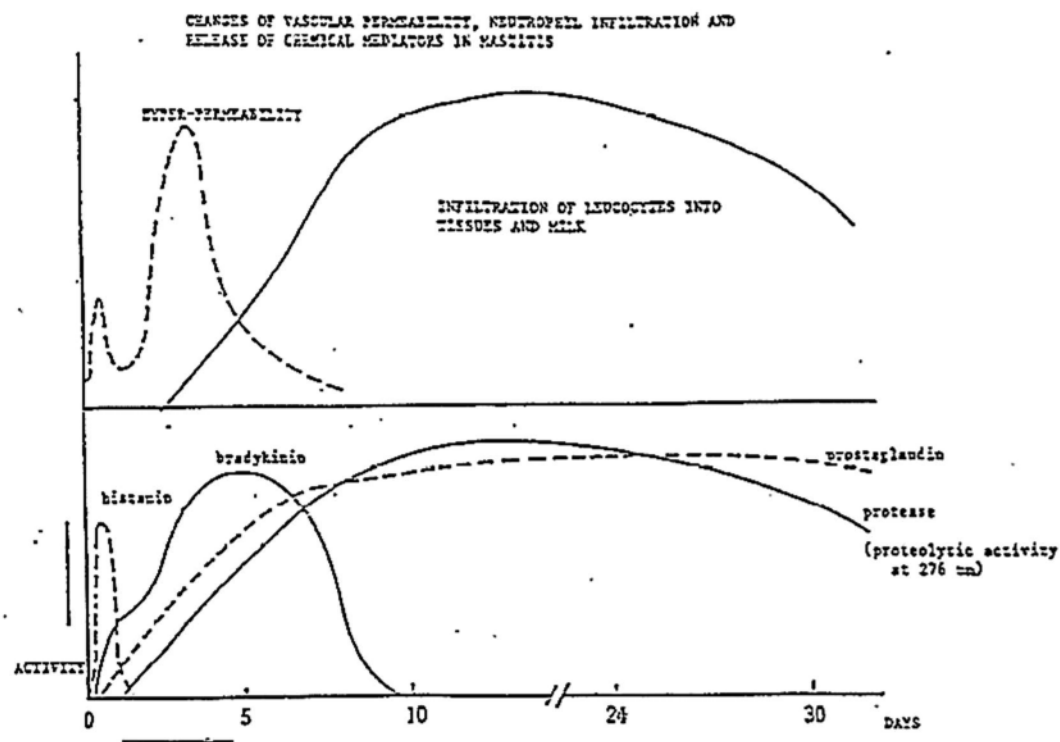
Chronic.....

Proliferation of tissue

→ Proliferative inflammation

→ Granulom formation

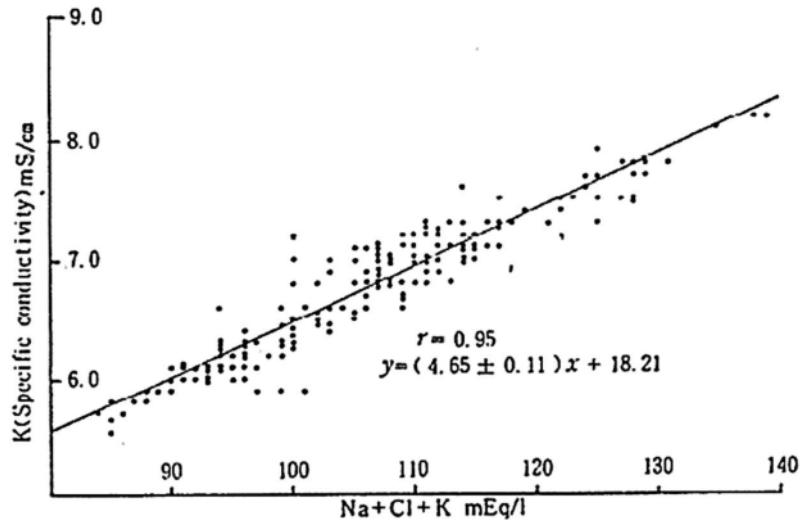
Bovine udder was caused various stimulation from the circumstances, subclinical mastitis progressed on the increase of permeability and / or that of somatic cell count.



Changes of vascular permeability, neutrophil and release of chemical medications in mastitis

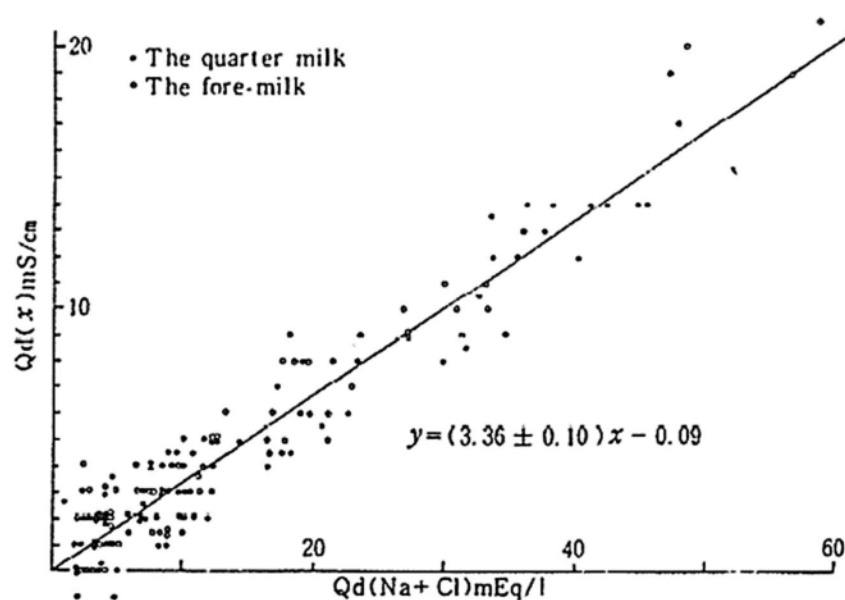
### 3. Inflammation process and clinical changes on mastitis

#### 1) Relationship between Electrolytes and Electrical Conductivity (EC)



Detecting abnormal quarter milk by the EC measurement was set up a criterion the basis of the concentration of Na, K, and Cl.

#### 2) Relationship between the quarter difference of Na + Cl and that of EC of the quarter milk



The regression equation of them were

For quarter milk:  $y = (3.34 \pm 0.10)x - 0.09$   $r = 0.96$

For fore milk:  $y = (3.30 \pm 0.10)x - 0.28$   $r = 0.95$



#### 4. Inflammation process and clinical changes on mastitis

##### 1) Absolute Electrical Conductivity (EC)

Frequency distribution on absolute EC in normal 4077 quarters.

class	< 4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4
frequency	58	47	89	151	240	244	264	306	242	249	320
cumulative	58	105	194	345	585	829	1,093	1,399	1,641	1,890	2,210

class	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4 <
frequency	367	398	326	256	224	151	71	42	18	14
cumulative	2,577	2,975	3,301	3,557	3,781	3,932	4,003	4,045	4,063	4,077

Criteria by the absolute EC is resulted in 6.2mS/cm which includes more than 99% of normal quarters.

##### 2) Difference Electrical Conductivity (EC)

Frequency distribution on absolute EC in normal 2415 quarters.

	mS/cm									
class	0	.1	.2	.3	.4	.5	.6	.7	.8	.9
class range	0 ~	.1 ~	.2 ~	.3 ~	.4 ~	.5 ~	.6 ~	.7 ~	.8 ~	.9 ~
(mS/cm)	<.1	<.2	<.3	<.4	<.5	<.6	<.7	<.8	<.9	<1.0
frequency	1,313	707	247	81	35	11	7	8	3	1
cumulative	1,313	2,020	2,269	2,350	2,385	2,396	2,403	2,411	2,414	2,415

The amount of permeated fluid could be estimated in the milk by the determination on Na + CL:

quarter difference	amount of extracellular fluid mixed in milk(%)	judgement
< .3	< 5	-, ±
.5	10	+
1.0	20	++

## 5. Relationship between Electrical Conductivity (EC) and Somatic cell count

### 1) Correlation between the quarter difference of EC and CMT score (1)

diff.conduct 10 <sup>-4</sup> mho	C M T				
	-	±	+	++	+++
0	327	67	20	1	1
1	163	40	21		2
2	115	41	44	6	1
3	51	29	19	5	2
4	5	14	10	9	4
5	3	11	6	5	4
6	4	2	3	2	2
7	3	1	2	2	3
8	2	4	3	1	2
9	2	4	3	1	4
10		1	2	1	4
11	1		1		1
12				1	4
13					5
14					3
15					2
15<					11
計	676	214	134	34	55
conformability	89%	31%	37%	79%	93%

In quarter difference of electrical conductivity (Qd) method, the quarter milk with conductivity higher by more than 0.3 mS than the lowest quarter milk within an udder at a milking is graded abnormal. Distribution of Qd values in each CMT class has a wide range. In the quarter milk with CMT score of (-), 89% of their Qd (EC) value was smaller than 3mS. In the milk with score of (++) or (+++), 79 and 93% of Qd (EC) value were larger than 3 mS or (+), respectively.

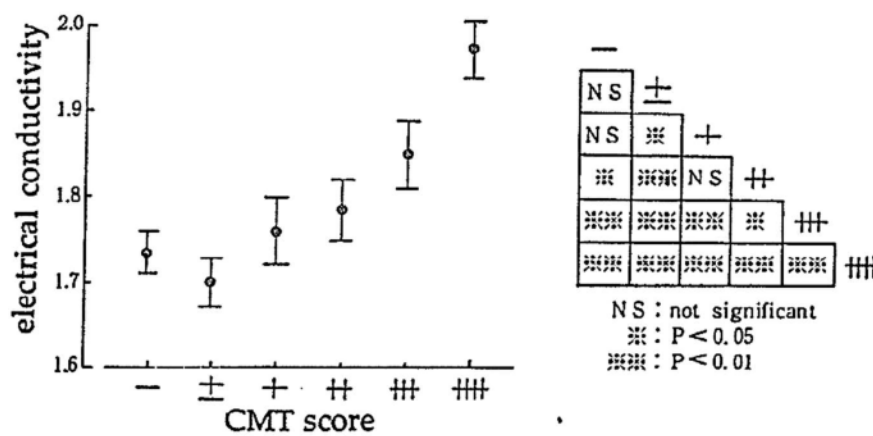
Agreements were fairly good in these classes. On the other hand, in the classes with CMT scores of (±) or (+), the agreements was low; 31 and 37%, respectively.

CMT examines the number of somatic cells in milk, but the EC has no direct relation with the number of the cells. In an infected quarter, EC measures the extent if the damage caused by the bacteria and their toxins to secretory epithelia and duct while SCC measures something entirely different – the combating while blood cell response of the cow to the infection.

Appearance of both SCC and EC in milk should not be agreed on the stage concurrently. Oshima concluded that combination of CMT with EC have been practiced for more accurate detection of abnormal quarters and for the presume of extent lesion.

## 2) Correlation between the quarter difference of EC and CMT score (2)

Log: 10-4s / cm



The absolute EC in this evaluation is agreement with CMT score more than (+++).

## 3) Appearance of EC and CMT score in milk

category	quarter no.	percentage	
		each (%)	total (%)
a	325	52.3	72.2
b	120	19.4	
c	133	21.6	21.1
d	6	1.0	6.2
e	32	5.2	
total	616	100.0	100.0

Categorized manner

category	description
a	EC(Qd)positive but CMT negative *
b	EC(Qd)positive & later CMT positive
c	EC(Qd)&CMT positive at a same time
d	CMT positive & later EC(Qd)positive
e	CMT positive but EC(Qd) negative *

\*During experiment period

The study was conducted measuring EC and CMT scores on foremilk samples of 162 cows at 2weeks interval for months. The result shows that EC could be

detected quarter disorders caused by subclinical mastitis at earlier stage than CMT scores.

#### 4) Relationship between EC and SCC

A high accuracy was obtained in normal quarters and severity of infection, however moderate infection was not proportional.

Overall changes in the composition in the abnormal quarter milk could be understood, if one postulates that a body fluid, presumably an exudate, inflows and mixes with milk in the quarter. On the bases of the common properties of exudate, it is inferable that the degree of changes in the composition of abnormal milk will depend on the amount of inflow such exudate into milk.

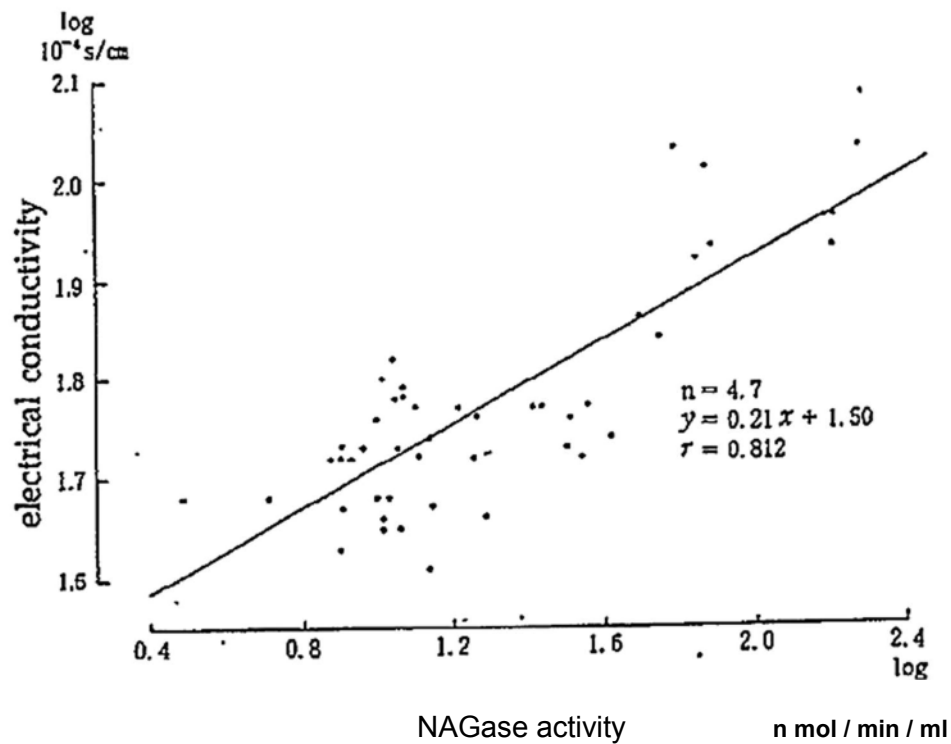
On the other hand, SCC measures the disease combating white blood cell response of the cow to the infection.

While some correlation between SCC and EC is expected, the agreement would not always expected to very close.

Relationship between EC and SCC

EC(Qd)	< 500	501 ~ 1500	1500 ~ 3000	3001 ~ 6000	6000 <	total	accuracy (%)
0 ~ 0.2	43	11	6	2	1	63	68.3
0.3 ~ 0.4	5	4	4	3	0	16	31.3
0.5 ~ 0.8	2	3	4	6	2	17	13.3
0.9 ~ 1.4	0	2	5	8	6	21	100
1.5 <	0	1	9	8	43	61	100
total	50	21	28	27	52	178	
accuracy	96.0	28.6	64.3	81.5	98.0		

## 6. Relationship between EC and NAGase



NAGase is an enzyme which is found in high levels in bovine mammary gland secretory and epithelial cells. Milk secreted from healthy udders has very low level of NAGase present, but damage to the tissue cells caused by toxins and metabolites of pathogenic bacteria results in marked increases in the level of the enzyme in milk.

## 7. Relationship between EC and milk constituents

### 1) Measurement of EC in fore – and quarter milk (normal milk)

	Morning milk	Evening milk	Mix mor.&even.milk
n	207	216	423
EC(A)quarter milk	5.08 ± 0.41	5.04± 0.40	5.06±0.40
EC(B)foremilk	5.19±0.41	5.06±0.40	5.12±0.41
A-B(C)	-0.11	-0.02	-0.06
[C/A] × 100%	2.2	0.4	1.2
coefficientcy( $\gamma$ )	0.906	0.941	0.919

EC value in foremilk was little higher than quarter milk, however the difference was very small, both measures were close relation. The results suggested that measuring EC in foremilk could be presumed that in quarter milk.

### 2) Measurement of EC and milk constitution in quarter milk, foremilk and individual milk

Coefficient correlation in quarter milk

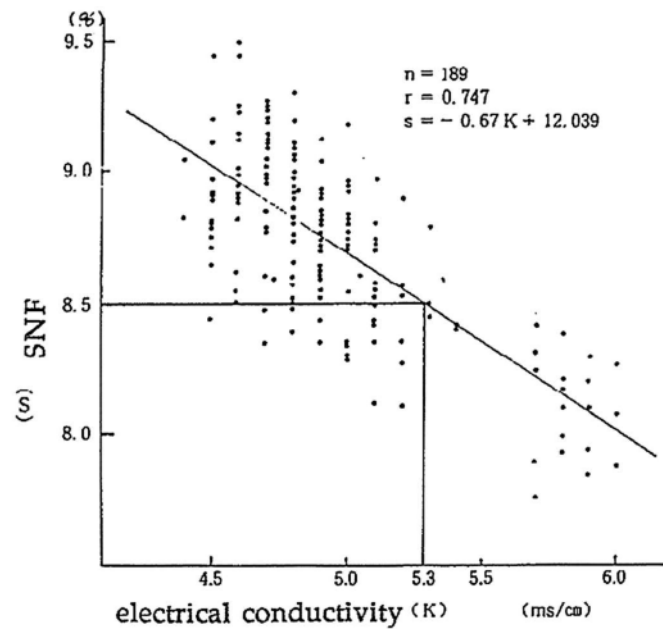
Milk constituents	Morning milk	Evening milk	Mor.+Even milk
n	103	102	205
SNF(S)	- .779	- .868	- .820
TMS	- .687	- .773	- .719
Fat	- .322	- .507	- .402
Protein(P)	- .439	- .622	- .530
S-P	- .758	- .811	- .759

SNF (S) : Solid Non Fat

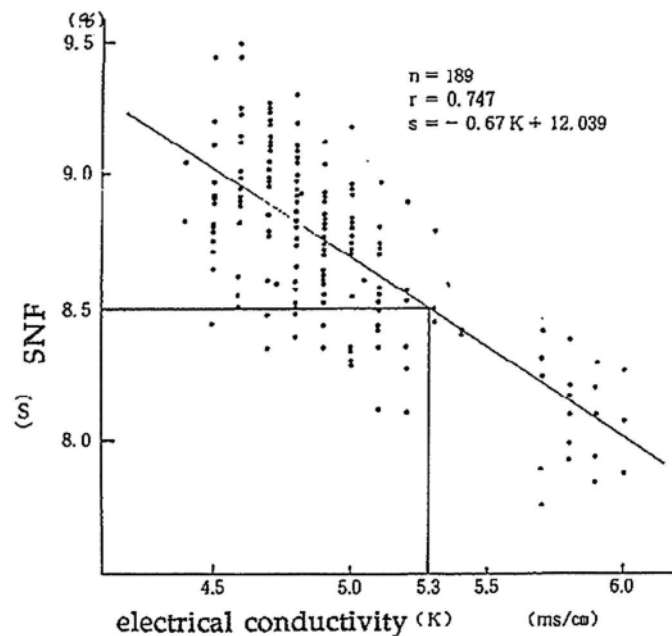
TMS : Total Milk Solid

S-P : Lactose + Ash

Inverse relationship between EC and SNF in normal individual milk



Inverse relationship between EC and SNF in normal individual milk



The method for prediction of milk solids content in normal individual milk by the EC can be fully utilized for the evaluation on milk quality of quarter, fore-milk and / or individual milk solid content in normal individual milk is feasible by measuring EC of the fore-milk.

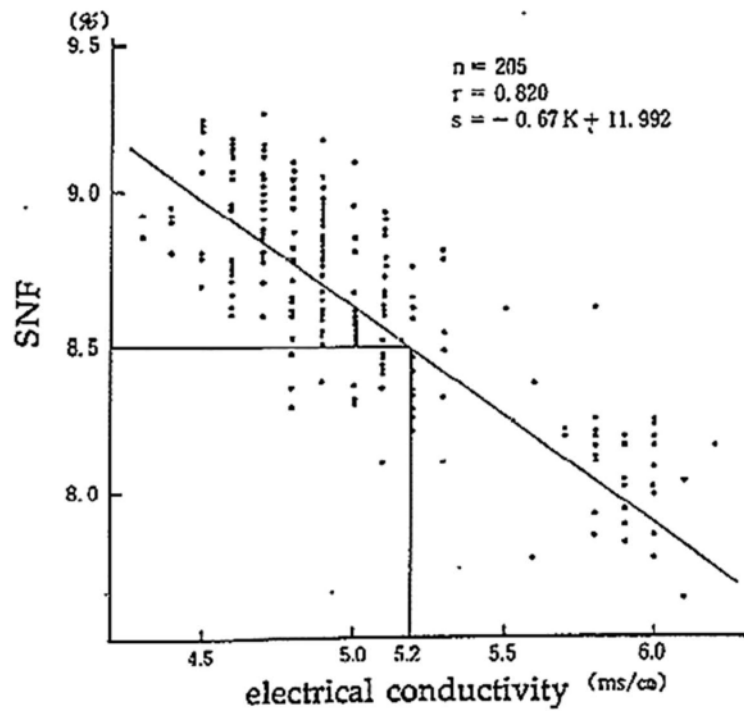
Coefficient correlation in individual milk

Milk constituents	Morning milk	Evening Milk	Mor. + Even. milk
N	113	113	226
SNF	-.772	-.767	-.759
TMS	-.702	-.592	-.667
Fat	-.497	-.227	-.407

Coefficient correlation in fore milk

Milk constituents	Morning milk	Evening Milk	Mor. + Even. milk
N	90	99	189
SNF	-.756	-.752	-.747
TMS	-.572	-.382	-.468
Fat	-.306	-.012	-.140

Inverse relationship between EC and SNF in normal quarter milk





## 8. Relationship between EC and milk constituents

Fernando et al : J. Dairy Sci., 68,449, 1983

### 1) Means of several indirect measures of subclinical mastitis in foremilk, stripping and bucket milk in presence or absence of infection

Variable	Uninfected		Infected			
			Secondary pathogen		Primary pathogen	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
<b>Foremilk<sup>1</sup></b>						
Conductivity, (μmho/cm)	5217	382	5339	664	5624	493
Cl, mg %	103	19	105	29	122	21
Na, mg %	42	12	51	19	55	15
K, mg %	148	15	150	17	147	15
Lactose, %	5.1	.38	5.1	.38	4.8	.57
Blood serum albumin, mg %	.21	.10	.25	.10	.3	.3
Log somatic cell counts	5.52	.97	5.73	.93	5.92	1.13
<b>Strippings<sup>1</sup></b>						
Conductivity, (μmho/cm)	5078	607	5710	910	6411	825
Cl, mg %	112	25	130	33	160	32
Na, mg %	53	19	76	34	94	33
K, mg %	131	24	126	23	121	22
Lactose, %	4.7	.51	4.5	.57	4.1	.61
Blood serum albumin, mg %	.19	.13	.26	.38	.38	.26
Log somatic cell counts	6.15	1.17	6.29	1.06	6.48	1.36
<b>Bucket milk<sup>2</sup></b>						
Conductivity, (μmho/cm)	5061	367	5070	261	5443	403
Cl, mg %	102	20	100	14	118	17
Na, mg %	42	11	48	12	54	12
K, mg %	144	15	147	13	148	15
Lactose, %	5.1	.26	5.0	.42	4.8	.32
Blood serum albumin, mg %	.19	.08	.22	.08	.3	.47
Log somatic cell counts	6.04	1.01	5.50	.77	6.19	.95

<sup>1</sup> Quarter samples.

<sup>2</sup> Weigh jar sample; infection status is on a cow basis.

## 2) Comparison of accuracy diagnosing mastitis

Variable	Uninf	Inf	Threshold <sup>1</sup>	False +	False -
	(no.)				(%)
Foremilk					
Conductivity, mmho/cm	400	96	5.50	16.5	47.7
Chloride, mg %	373	89	113	26.8	34.8
Sodium, mg %	290	74	52	19.6	41.9
Potassium, mg %	297	74	154	44.1	54.0
Lactose, %	279	79	4.8	16.5	59.4
Blood serum albumin, mg %	354	82	20	35.0	51.2
Log somatic cell counts	382	81	6.05	25.0	59.7
Strippings					
Conductivity, mmho/cm	390	90	5.80	11.2	15.5
Chloride, mg %	368	84	145	15.5	26.2
Sodium, mg %	287	71	77	10.4	35.2
Potassium, mg %	293	71	126	38.6	43.6
Lactose, %	258	76	4.4	17.4	35.5
Blood serum albumin, mg %	351	80	30	7.4	56.2
Log somatic cell counts	322	77	6.85	27.0	59.7
Bucket milk					
Conductivity, mmho/cm	73	37	5.26	24.6	40.5
Chloride, mg %	72	33	110	20.8	42.4
Sodium, mg %	55	28	47	23.6	42.9
Potassium, mg %	55	28	147	45.5	42.8
Lactose, %	43	32	4.7	20.9	46.8
Blood serum albumin, mg %	68	31	17.5	29.4	70.2
Log somatic cell counts	66	33	6.26	29.0	51.5

<sup>1</sup> Infections (inf) are by primary pathogens.

<sup>2</sup> Value where the probability is equal for a quarter being classified as infected or uninfected by discriminant analysis.

## 9. Threshold and Practices

### 1) Threshold

The threshold values for evaluating infected milk by means of the MILK CHECKER. Differential conductivity is more important to detect mastitis.

Electrical Conductivity	Infected
Absolute Conductivity	>6.2 mS / cm
Differential Conductivity	>0.5 mS / cm

#### **Remarks:**

As cow milk conductivity varies with breeds, lactation stage or individual cow's pathological conditions, it is recommended to interpret the differences in absolute conductivities, based on the lowest value of same cow. It will help to detect mastitis early and properly. MILK CHECKER is a screening device for the detection of mastitis and proper treatment should be discussed with veterinarian or specialists.

### 2) Evaluation of abnormal milk by both absolute and differential EC

Absolute EC (mS / cm)	Differential EC (mS / cm)	Evaluation
6.2>	0.5>	Normal
6.2>	0.5<	Mastitis
6.2<	0.5>	Low component or physiologically abnormal
6.2<	0.5<	Low component or physiologically abnormal and mastitis

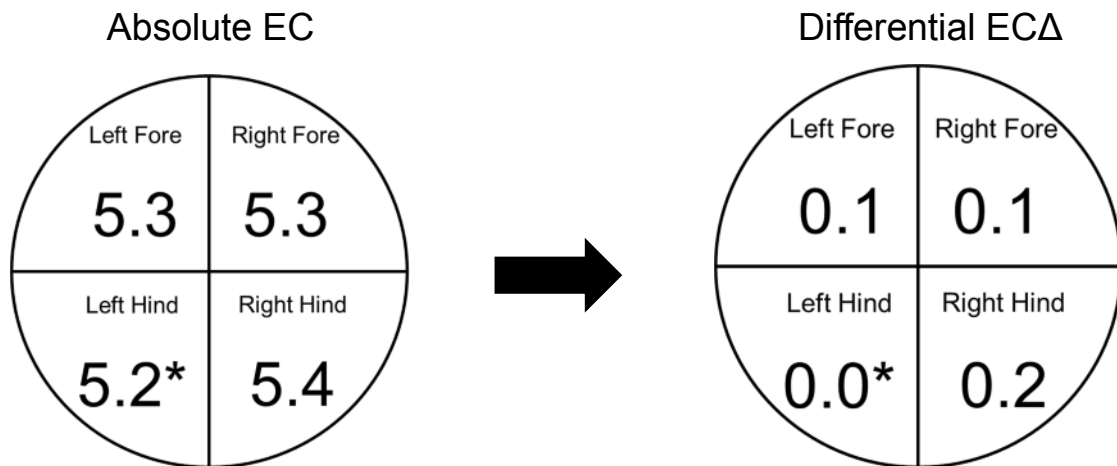
Careful observation of the quarter is necessary when the differential EC value exceeds 0.4, as it is an indication of mastitis.

\*Absolute electrical conductivity value: Figure display on the Milk Checker

\*\*Differential electrical conductivity value: Take the absolute EC value as the base and subtract it from the other figures.

## A. Normal Milk

The absolute electrical conductivity of bovine Milk usually ranges from 4.5 – 5.5 mS/cm.



Δ: To calculate diff. EC

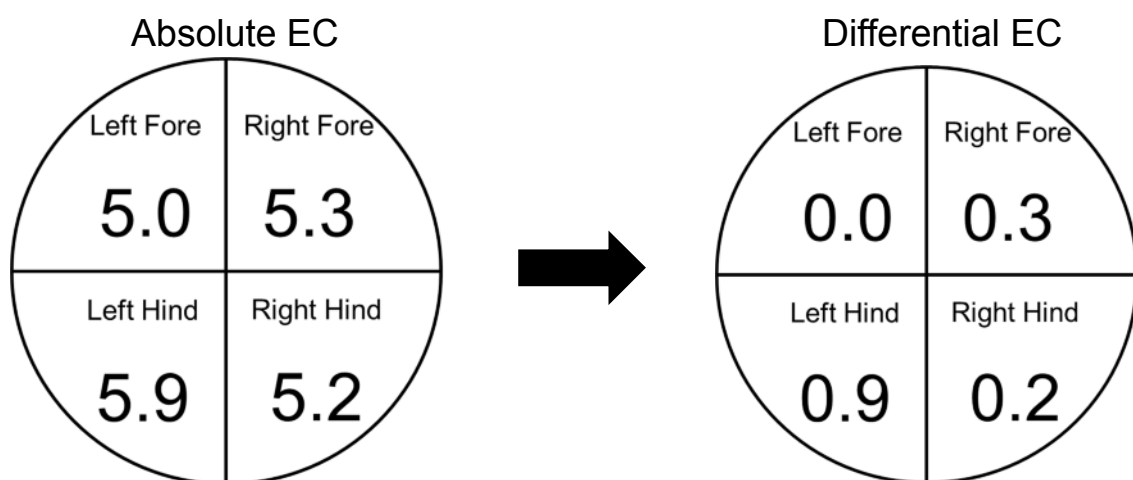
Left fore:  $5.3 - 5.2 = 0.1$

Right fore:  $5.3 - 5.2 = 0.1$

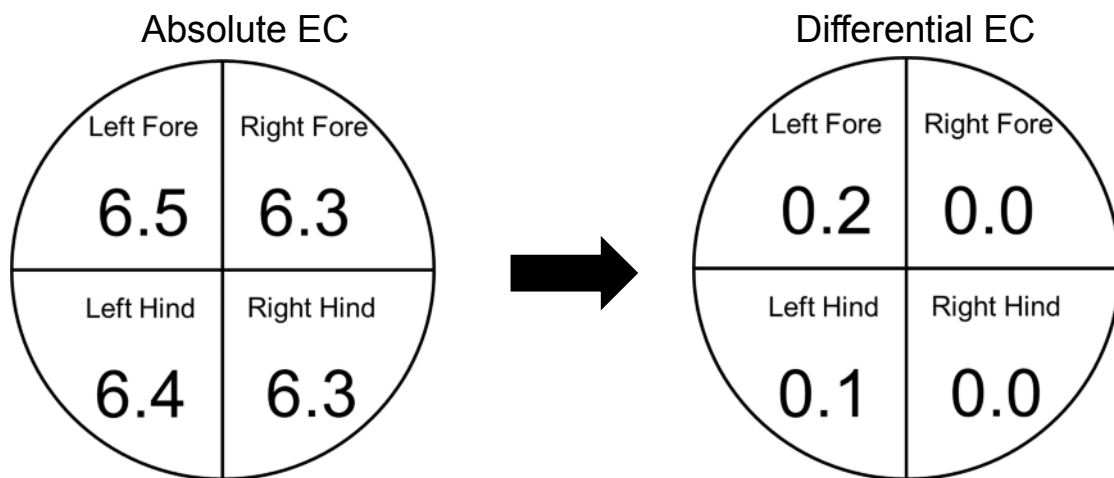
Right hind:  $5.4 - 5.2 = 0.2$

\*Left hind:  $5.2 - 5.2 = 0.0$  / Min absolute value

## B. Mastitis Milk



C. Low component or physiologically abnormal milk



D. Mastitis Milk (combined with low components or physiological abnormality)

