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Study of association between the electrical conductivity of milk and subclinical mastitis in dairy cows

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List of abbreviations

AUC – Area Under Curve

CM – Clinical Mastitis

IMI – Intra-mammary Infection

OR – Odd Ratio

ROC – Receiver Operating Characteristic

SC – Somatic Cell

SCC – Somatic Cell Count

SCM – Subclinical Mastitis

SE – Standard Error

Introduction

The single biggest problem facing dairy farmers, it has been reported that the prevalence of mastitis can be as high as 78.54% in dairy cows (Sharma, 2011). The average farmer faces substantial economic losses as a result of mastitis; a recent study suggests that annually losses of 1.5-3 billion USD can be expected as a result of infections in the US (F.J. Ferrero, 2013).

Mastitis is defined as an inflammation of the mammary gland, predominantly due to the effects of infection by bacterial pathogens (Merck , 2016, p. 1358). The most common mastitis pathogens include Staphylococci, Streptococci and E.coli and can be classified as either contagious or environmental pathogens (Sharma, 2011)

Contaminated milk has been long been associated with the zoonotic spread of many diseases including Tuberculosis, Leptospirosis and Brucellosis etc (Sharma, 2011). Therefore, if milk is found to be abnormal it must be discarded and prevented from entering the human food chain; EU Milk Hygiene Directive (EC/92/46) regulates this.

In the past almost every farmer kept a small number of dairy cows to supply their own family and neighbours with milk and cheese. Visual inspection during manual milking was the only way for mastitis infections to be identified and treated. Nowadays farming has changed dramatically, farms are fewer but bigger and more industrial, and milking is carried out in large parlours or by automatic robots.

In Figure 1 below it is possible to observe some data collected by the Agriculture and Horticulture Development Board (AHDB), the data shows an overall trend in the UK that the number of dairy farms is decreasing whilst the average herd size is increasing. Statistics published in April 2017 suggest a 1.5% decrease in the number of UK dairy farms from the previous year whilst the average herd size is increasing, currently 143.4 cows per farm (Agriculture and Horticulture Directives Board - Dairy, 2017)

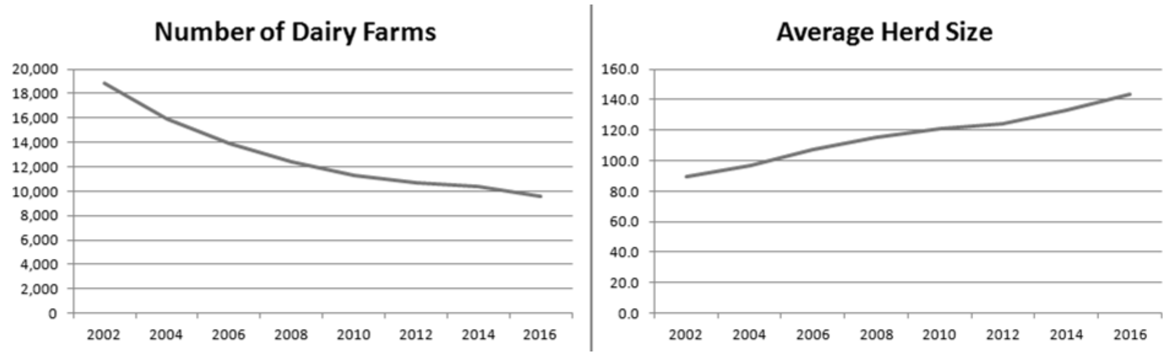

Figure 1 - Graphs indicating number of UK dairy farms and the average herd size (Agriculture and Horticulture Directives Board - Dairy, 2017)

Cows are no longer seen as individuals on a family farm, but rather as a number in a large milk producing facility. As a result there is great pressure placed on cows to produce high yields of milk. It has been suggested that even subclinical mastitis infections can see a reduction in yield by up to 20% (Reddy, 2014). The importance for farmers to detect changes in milk early cannot be underestimated. Early detection is vital to enable the timely and efficient treatment of the infected animal, prevent contamination and spread to the rest of the herd as well as eliminating any unfit milk from the food chain.

Since the mid-1980s methods to automatically detect mastitis with sensors has been under development, with the widespread introduction of automatic milking systems during the 1990s the demand for such sensors grew. In a recent report it was estimated that in the Netherlands approximately 10% of farms use automatic milking systems (Hogeveen, 2010), the use of such automatic milking systems allows for more efficient use of the parlour and reduces labour costs allowing for cheaper production costs in the long term.

Previous methods used to identify mastitis include:

- **Visual inspection of the foremilk** – manual drawing or 'stripping out' of the first milk from each quarter and observing for clots or flakes within the milk
- **California Mastitis Test (CMT)** – drawing milk from each quarter onto a specially designed paddle, adding CMT reagent and mixing, a result may be seen after 15-20 seconds, results are based on the quantity of gel precipitation in the paddle (negative = no precipitation, positive = precipitation or gel formation)

- **Somatic Cell Counters** – milk is placed into a specially designed machine which will give a result indicating the number of Somatic Cells/ml, the machine may take up to a minute to produce a result
- **Monthly SCC tests** are common place on many farms, where an external company enters the farm and takes milk samples from each cow. These samples are processed in a lab and the farmer receives a report regarding the SCC for each cow and his overall herd.

The majority of these methods are time consuming (SC counters and monthly testing) require a high degree of manual involvement (CMT) and some methods such as visual inspection is not accurate enough to detect SCM. An ideal method for mastitis detection would involve a test that is:

- Quick - carried out in just a few seconds
- Automatic - carried out by the milking machine
- 'In-line' – carried out within the milking machine
- Instant alert system highlighting the need for treatment
- Automatic milk diversion, taking contaminated milk away from the bulk tank
- Recorded automatically on computer

In recent years electrical conductivity has been the focus of many research studies, proving that there is correlation between the levels of conductance and the degree of mastitis infection. The theory behind EC involves increased numbers of sodium ions being able to pass through the tight junctions of the udder when the udder is inflamed during a mastitis infection. (M.Janzekovic, 2009). Sensors are used to detect the electrical conductance of the milk; mastitis milk containing higher levels of sodium ions gives a higher conductance.

The aim of this study is to identity if an association exists between the electrical conductivity of milk and the presence of subclinical mastitis.

Literature review

Electrical Conductance is the ability of a solution to conduct an electrical current between two electrodes; the unit of measurement is mS (milisiemensov) the SI unit of conductance. (M.Janzekovic, 2009)

The normal Electrical Conductance of milk is between 4.0 and 6.0mS/cm (F.J. Ferrero, 2013), in cows affected by mastitis due to the change in ion composition of the milk the EC increases. The same paper stated that when the EC was between 6.5-13.0mS/cm at 18·C the presence of mastitis was indicated.

Somatic Cell Count (SCC) is the term which describes the levels of somatic cells found per millilitre of milk. As an indicator of mammary gland inflammation, the levels of somatic cells can be used to identify cows which may be suffering from clinical or subclinical mastitis (Sharma, 2011). Inflammation of the udder causes an increase in the capillary permeability; this allows the passage of somatic cells (leukocytes and erythrocytes) into the milk (Reddy, 2014).

Milk from cows affected by mastitis leaves it unsuitable for human consumption (Sharma, 2011). With regards to human consumption of milk, there have been several directives laid out by governments detailing the maximum levels of somatic cells at which the milk is still acceptable.

According to EU directive 92/46CEE a SCC of 400,000 cells/ml is acceptable for products made with raw milk (Council Directive, 1992), this limit is also applied in several other countries including Norway, Switzerland, Australia and New Zealand (More, 2009). Within the USA the upper acceptable SCC limit is set at 750,000 cells/ml (G. van Schaik, 2002) while in Canada the limit is 500,000 cells/ml (Norman, 2000).

There is much debate within the scientific community as to the levels of somatic cells which indicate subclinical mastitis. In order to identify the level at which a cow is said to be affected by SCM it must be known the SCC of a healthy cow. Several authors have estimated that uninfected dairy cows can have a SCC of between 22,000-68,000 cells/ml (Djabri B, 2002) (Madouasse A. H., 2010).

It is thought that if a cow has a SCC <100,000 cells/ml she can be considered free from SCM. Whilst in current literature it is generally accepted that <200,000 cells/ml indicates a healthy udder (Madouasse A. , 2011), whilst above this level SCM is present.

Somatic Cell Count (cells/ml)	Comment
<100,000	Uninfected cow, no significant production losses
>200,000	Cow is affected by SCM
>300,000	Significant pathogen infection, cow affected by CM
>400,000	Milk unfit for human consumption (EU regulations) Norway, Switzerland, Australia and New Zealand also
500,000[1]	Upper limit in Canada, above this limit the milk is deemed unfit for human consumption
750,000	Upper limit in USA, above this limit the milk is deemed unfit for human consumption

Figure 2 - Summery of the various SCC limits across different countries

Whilst SCC is a good indicator of mastitis infection, the count can also be influenced by several other factors including:

- **Age of the cow** – SCC increases with increasing age (Beckley, 1966)
- **Stage of lactation** – in freshly calved cows the SCC can exceed 1,000,000 cells/ml but within 2 weeks this decreases to 100,000 cells/ml (Jensen, 1981)
- **Season** – Winter generally has the lowest SCC, whilst it peeks in the warm summer months (Khate, 2010) due to increased bacterial growth with the warmer humid weather
- **Diurnal variance** – studies have found daily fluctuations in SCC of up to 40% within the same cow (Sharma, 2011)

Being able to quickly and accurately detect cows that are affected by CM or SCM is of utmost importance to the modern dairy farmer. The use of SCC is a useful tool for this, but cannot be used alone. With other factors such as those listed above influencing the levels of somatic cells in the milk, human examination of the animal along with other diagnostic tools must still be employed.

[1] Since 2012 Canada has been implementing a change in the SCC limit with the introduction of the Canadian Quality Milk Program, reducing the SCC from 500,000 to 400,000 cells/ml (Poirier, 2011)

Materials and Methods

Data

The data was gathered from a large scale dairy farm. The milking data (milk yield, conductivity, flow) were available for each cow for each milking time. In a different database the monthly reported individual somatic cell count data was available for the sampling day. We used the milking data if the certain cow had three milkings on the day.

We have analysed the association of classes based on the somatic cell count and the three independent variables. These variables were the daily milk yield, the daily average conductivity and flow rate. In the case of all independent variables we calculated a reference value for each cow for different time periods. The periods were three, five and ten days length. The period data were involved just for the preceding days of the cell count data dates.

<u>Statistical analysis</u>

The association was analyzed by logistic regression (Venables & Ripley, 2002; Gelman & Hill, 2006). Beside the association analysis we were interested wheter any of the variables could be used as a classifier for cell count classes.

The classification performance was analyzed by receiver operating characteristic (ROC) curve (Fawcett, 2006), and it was expressed by the area under the curve (AUC).

A Receiver Operating Characteristic Curve plots the sensitivity, true positive result, against the specificity, false positive result. Each point on the curve represents a sensitivity/specificity pair equivalent to a certain decision threshold.

If we take a value which we have decided is significant based on its statistical association, determined by the Beta value, standard error (SE) and p.values, we can produce a curve which details the predictive value of this threshold value. The predictive value is given by the area under the curve (AUC).

For example, an AUC value of 0.5 indicates there is 50% chance of obtaining the desired result, a true positive result, at a given threshold; therefore there is also a 50% chance of not obtaining the desired result. In an ideal situation we would aim to achieve an AUC of a much higher value, perhaps 0.95, indicating there is a 95% chance of obtaining a true positive result for this given example.

Based on our work the AUC would allow us to determine the predictive value of a threshold somatic cell count. This would facilitate us to recognize a cow or milk sample suffering from subclinical mastitis.

All data processing and statistical analyses were performed by using the R environment (Dinya & Solymosi, 2016; R Core Team, 2017).

Method

The milk yield was calculated by:

Milk Yield(kg) = $Yield_{IMd} - Yield_{3d}$

- $Yield_{IMd}$ - In Milking Day, day on which an external milk recording company takes milk samples from all cows, the SCCs recorded on this day are used as the reference values
- $Yield_{3d}$ -The average milk yield for the previous 3 days milkings prior to the In Milking Day

Results

Depicted below are the results obtained from our statistical analysis, the results have been listed in a set of 9 tables, each table lists the SCC threshold together with the associated Beta value, SE, p.value and AUC. It is possible to observe from these tables whether the results can classified as significant based on the p.value, results <0.05 are significant results. The AUC is also available which indicates the predictive value of each SCC threshold.

The results have been recorded over three different time scales, the results in tables 1-3 have been collected over a 3 day time period. The comparison is made between 'In-Milking day' values and the median result of the 3 day period.

A 5 day time period is shown in tables 4-6, with the comparison being made between 'In-Milking day' values and the median result of the 5 day period.

Tables7-9 use data collected over a 10 day period, the comparison is made between 'In-Milking day' values and the median result of the 10 day period.

Within each time period we examined three different criteria, tables 1, 4 and 7 relate to milk yield, it is well understood that a cow suffering from mastitis will produce milk of lesser quality and quantity (F.J. Ferrero, 2013). Therefore milk yield can be considered a significant indicator in relation to udder health.

Tables 2, 5 and 8 are concerned with the EC, the normal EC of milk is between 4.0 and 6.0mS/cm (F.J. Ferrero, 2013), EC increases in cows affected by mastitis due to the change in ion composition within the milk.

The third criteria is shown in tables 3, 6 and 9 which shows the results in relation to the milk flow model, with increasing levels of somatic cells in the milk, there is a noted alteration in the consistency or density of the milk, thus denser milk flows more slowly giving a lower flow rate (Sharma, 2011).

Table 1: Results of daily milk yield model. The reference is the median of the preceding three days.

	SCC	Beta	SE	p.value	AUC
1	100	0.033	0.010	0.00166019	0.523
2	150	0.025	0.010	0.01216186	0.518
3	200	0.031	0.010	0.00277491	0.524
4	250	0.029	0.010	0.00581258	0.522
5	300	0.030	0.011	0.00607166	0.522
6	350	0.035	0.011	0.00173171	0.528
7	400	0.040	0.011	0.00043336	0.532
8	450	0.051	0.012	0.00001752	0.541
9	500	0.060	0.012	0.00000070	0.548
10	550	0.063	0.012	0.00000032	0.550
11	600	0.073	0.013	0.00000001	0.557
12	650	0.078	0.013	0.00000000	0.561
13	700	0.080	0.013	0.00000000	0.561
14	750	0.089	0.014	0.00000000	0.570
15	800	0.092	0.014	0.00000000	0.571
16	850	0.096	0.014	0.00000000	0.576
17	900	0.101	0.014	0.00000000	0.579
18	950	0.109	0.015	0.00000000	0.586
19	1000	0.119	0.015	0.00000000	0.594

Table 2: Results of conductivity model. The reference is the median of the preceding three days.

	SCC	Beta	SE	p.value	AUC
1	100	-0.024	0.087	0.78250533	0.504
2	150	0.006	0.086	0.94628625	0.502
3	200	0.090	0.087	0.29984792	0.513
4	250	0.060	0.090	0.50355336	0.510
5	300	0.122	0.093	0.19207449	0.514
6	350	0.205	0.097	0.03537434	0.521
7	400	0.156	0.100	0.11991207	0.517
8	450	0.156	0.104	0.13305125	0.516
9	500	0.228	0.107	0.03313663	0.524
10	550	0.222	0.110	0.04345732	0.523
11	600	0.180	0.113	0.11068327	0.518
12	650	0.186	0.116	0.11090233	0.519
13	700	0.184	0.119	0.12197273	0.519
14	750	0.152	0.121	0.21052582	0.515
15	800	0.150	0.125	0.22968562	0.514
16	850	0.142	0.128	0.26498586	0.514
17	900	0.208	0.131	0.11193014	0.518
18	950	0.248	0.134	0.06548272	0.520
19	1000	0.222	0.136	0.10378400	0.517

Table 3: Results of flow model. The reference is the median of the preceding three days.

	SCC	Beta	SE	p.value	AUC
1	100	0.215	0.127	0.09182316	0.513
2	150	0.160	0.125	0.19986985	0.510
3	200	0.230	0.128	0.07255269	0.515
4	250	0.183	0.132	0.16437467	0.512
5	300	0.117	0.136	0.39145072	0.510
6	350	0.047	0.141	0.74077979	0.506
7	400	0.151	0.146	0.30329546	0.516
8	450	0.210	0.152	0.16620648	0.518
9	500	0.174	0.156	0.26470973	0.517
10	550	0.173	0.161	0.28091724	0.518
11	600	0.212	0.166	0.20048172	0.519
12	650	0.343	0.172	0.04677842	0.527
13	700	0.364	0.176	0.03881955	0.528
14	750	0.461	0.181	0.01097068	0.533
15	800	0.439	0.186	0.01840745	0.533
16	850	0.535	0.191	0.00515733	0.537
17	900	0.584	0.195	0.00278646	0.540
18	950	0.653	0.201	0.00118961	0.545
19	1000	0.783	0.205	0.00013631	0.551

Table 4: Results of daily milk yield model. The reference is the median of the preceding five days.

	SCC	Beta	SE	p.value	AUC
1	100	0.041	0.012	0.00055281	0.538
2	150	0.036	0.011	0.00183758	0.535
3	200	0.037	0.012	0.00151378	0.537
4	250	0.033	0.012	0.00475801	0.533
5	300	0.035	0.012	0.00403490	0.534
6	350	0.040	0.013	0.00147655	0.538
7	400	0.041	0.013	0.00161439	0.538
8	450	0.049	0.013	0.00021538	0.546
9	500	0.056	0.014	0.00003409	0.551
10	550	0.058	0.014	0.00002942	0.552
11	600	0.067	0.014	0.00000287	0.557
12	650	0.073	0.015	0.00000051	0.565
13	700	0.075	0.015	0.00000045	0.567
14	750	0.083	0.015	0.00000004	0.574
15	800	0.088	0.016	0.00000001	0.578
16	850	0.090	0.016	0.00000001	0.579
17	900	0.094	0.016	0.00000000	0.583
18	950	0.101	0.016	0.00000000	0.590
19	1000	0.109	0.017	0.00000000	0.599

Table 5: Results of conductivity model. The reference is the median of the preceding five days.

	SCC	Beta	SE	p.value	AUC
1	100	-0.080	0.107	0.45799659	0.506
2	150	-0.055	0.105	0.59964908	0.500
3	200	0.054	0.107	0.61252735	0.513
4	250	0.039	0.110	0.72060756	0.511
5	300	0.140	0.115	0.22309673	0.518
6	350	0.267	0.120	0.02619270	0.528
7	400	0.208	0.123	0.09248369	0.523
8	450	0.247	0.128	0.05338569	0.527
9	500	0.318	0.132	0.01611560	0.533
10	550	0.331	0.136	0.01503604	0.533
11	600	0.260	0.140	0.06302400	0.527
12	650	0.211	0.144	0.14246805	0.523
13	700	0.223	0.146	0.12871897	0.525
14	750	0.186	0.150	0.21466828	0.521
15	800	0.205	0.155	0.18640211	0.522
16	850	0.199	0.159	0.20940258	0.521
17	900	0.287	0.163	0.07689387	0.528
18	950	0.335	0.167	0.04572880	0.529
19	1000	0.296	0.171	0.08250203	0.526

Table 6: Results of flow model. The reference is the median of the preceding five days.

	SCC	Beta	SE	p.value	AUC
1	100	0.292	0.153	0.05533521	0.521
2	150	0.299	0.151	0.04708896	0.522
3	200	0.343	0.154	0.02559571	0.526
4	250	0.312	0.158	0.04846481	0.524
5	300	0.317	0.164	0.05362772	0.526
6	350	0.233	0.170	0.16981412	0.522
7	400	0.298	0.176	0.09022628	0.529
8	450	0.410	0.182	0.02454712	0.533
9	500	0.363	0.187	0.05282117	0.531
10	550	0.339	0.192	0.07802228	0.529
11	600	0.393	0.199	0.04810080	0.531
12	650	0.604	0.206	0.00344581	0.544
13	700	0.643	0.211	0.00228292	0.547
14	750	0.730	0.216	0.00074268	0.551
15	800	0.764	0.224	0.00064411	0.555
16	850	0.774	0.229	0.00072859	0.555
17	900	0.815	0.233	0.00048075	0.557
18	950	0.871	0.240	0.00028633	0.563
19	1000	1.000	0.245	0.00004590	0.570

Table 7: Results of daily milk yield model. The reference is the median of the preceding ten days

	SCC	Beta	SE	p.value	AUC
1	100	0.025	0.018	0.15955089	0.536
2	150	0.022	0.017	0.19444028	0.532
3	200	0.022	0.017	0.19940549	0.525
4	250	0.022	0.018	0.20682134	0.524
5	300	0.020	0.018	0.27645204	0.522
6	350	0.035	0.019	0.06334007	0.536
7	400	0.027	0.019	0.16000466	0.533
8	450	0.039	0.020	0.04918979	0.548
9	500	0.038	0.020	0.06050644	0.545
10	550	0.051	0.021	0.01364876	0.557
11	600	0.064	0.021	0.00285055	0.570
12	650	0.070	0.022	0.00140608	0.579
13	700	0.068	0.022	0.00222297	0.582
14	750	0.077	0.023	0.00064801	0.593
15	800	0.076	0.023	0.00102591	0.590
16	850	0.080	0.024	0.00085600	0.590
17	900	0.075	0.024	0.00176671	0.585
18	950	0.090	0.025	0.00025347	0.601
19	1000	0.099	0.025	0.00007973	0.613

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Table 8: Results of conductivity model. The reference is the median of the preceding ten days.

	SCC	Beta	SE	p.value	AUC
1	100	-0.005	0.173	0.97798450	0.509
2	150	0.068	0.169	0.68713652	0.516
3	200	0.134	0.172	0.43702890	0.523
4	250	0.076	0.178	0.66911827	0.517
5	300	0.162	0.185	0.37916861	0.526
6	350	0.195	0.191	0.30901888	0.529
7	400	0.220	0.198	0.26662841	0.532
8	450	0.256	0.206	0.21276776	0.532
9	500	0.232	0.210	0.26948165	0.531
10	550	0.179	0.216	0.40821091	0.525
11	600	0.028	0.222	0.89793086	0.509
12	650	0.038	0.227	0.86612533	0.511
13	700	0.084	0.232	0.71756319	0.514
14	750	-0.005	0.237	0.98233856	0.508
15	800	0.072	0.247	0.77194348	0.515
16	850	0.057	0.255	0.82291798	0.514
17	900	0.053	0.259	0.83826655	0.514
18	950	0.114	0.269	0.67253388	0.513
19	1000	0.056	0.275	0.83881919	0.509

Table 9: Results of flow model. The reference is the median of the preceding ten days.

	SCC	Beta	SE	p.value	AUC
1	100	0.192	0.241	0.42494571	0.532
2	150	0.232	0.236	0.32746608	0.532
3	200	0.291	0.241	0.22700623	0.540
4	250	0.358	0.250	0.15202883	0.539
5	300	0.384	0.258	0.13687827	0.543
6	350	0.367	0.267	0.16887102	0.545
7	400	0.320	0.274	0.24392563	0.546
8	450	0.641	0.288	0.02638761	0.560
9	500	0.576	0.294	0.04999968	0.558
10	550	0.567	0.303	0.06153358	0.562
11	600	0.758	0.317	0.01680979	0.572
12	650	0.769	0.324	0.01764215	0.574
13	700	0.721	0.329	0.02852778	0.572
14	750	0.838	0.340	0.01365616	0.578
15	800	0.938	0.352	0.00773135	0.590
16	850	0.887	0.363	0.01450844	0.585
17	900	0.853	0.367	0.02007725	0.584
18	950	0.871	0.379	0.02143195	0.588
19	1000	1.279	0.391	0.00107741	0.610

The table below, Table 10, is a summary illustrating the SCC levels at which the p-values become significant, these figures have been taken from the results shown in tables 1-9.

Table 10 – Comparison of significant results

	SCC ($x10^3$cells/ml) level at which results became significant ($p<0.05$)		
	3 day	5 day	10 day
Milk Yield	All	All	450
Conductance	350, 500, 550	350, 500, 550, 950	None
Milk flow	650	450	450

Based on the results obtained and the values which are considered important in literature I chose to examine the ROC curves of certain SCC thresholds.

When choosing which time period to examine in more detail I based my decision on the figures combined in Table 10. When making this decision I observed the fact that none of the results in the 10 day period for conductivity were significant based on p.values.

The 3 day time period had a fewer number of significant results compared to the 5 day time period, therefore I chose to produce ROC curves based on the 5 day time period results.

If we consider the threshold value of $100x10^3$cells/ml, a value that is generally considered a healthy udder we can examine the milk yield, conductivity and flow model and find the predictive value of this threshold based on the AUC values.

The results of the $100x10^3$cells/ml threshold during the 5 day reference range for the three given criteria (yield, EC and Flow) as depicted graphically below in the ROC curves shown in figures 3, 4 and 5.

ROC Curves

Figure 3 - Results of daily milk yield model when SCC equals 100 x10^3cells/ml with the 5 day reference range

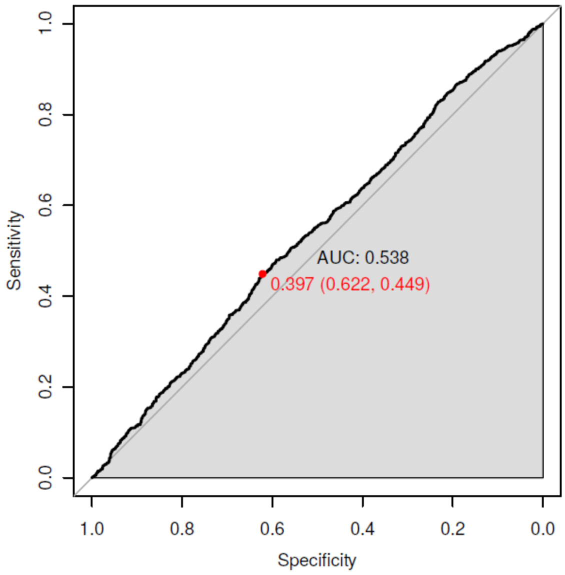


Figure 4 - Results of conductivity model when SCC equals 100 x10^3cells/ml with the 5 day reference

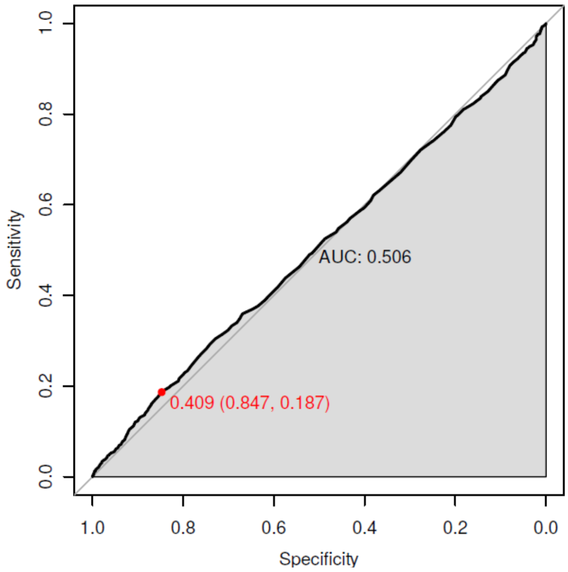


Figure 5 – Results of milk flow model when SCC equals 100 x10^{3}cells/ml with the 5 day reference range

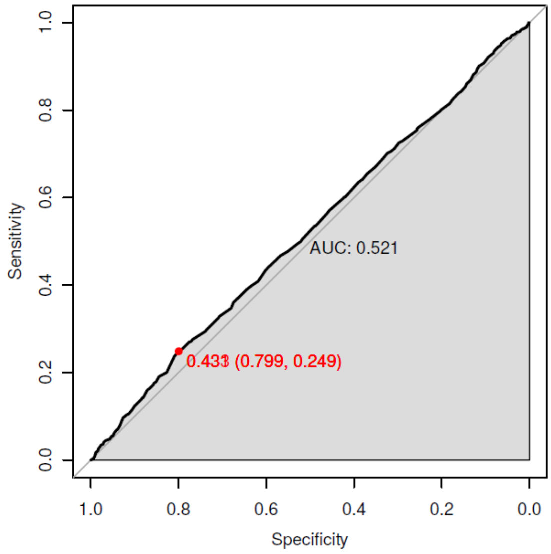


Figure 6 - Results of daily milk yield model when SCC equals 400x10^{3}cells/ml with the 5 day reference

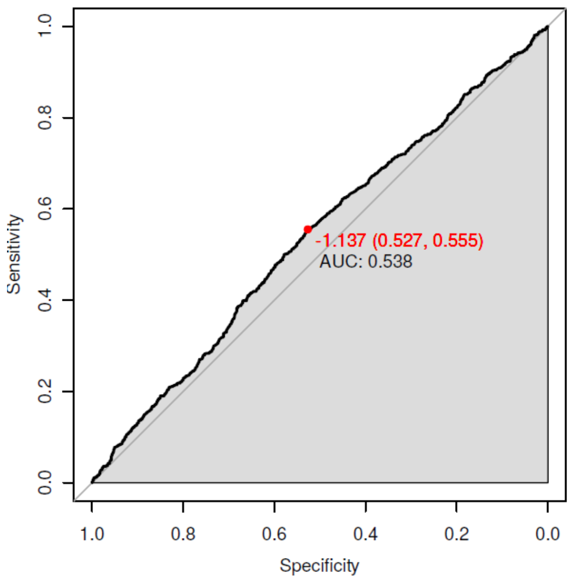


Figure 7 - Results of conductivity model when SCC equals 400 x10^3cells/ml with the 5 day reference

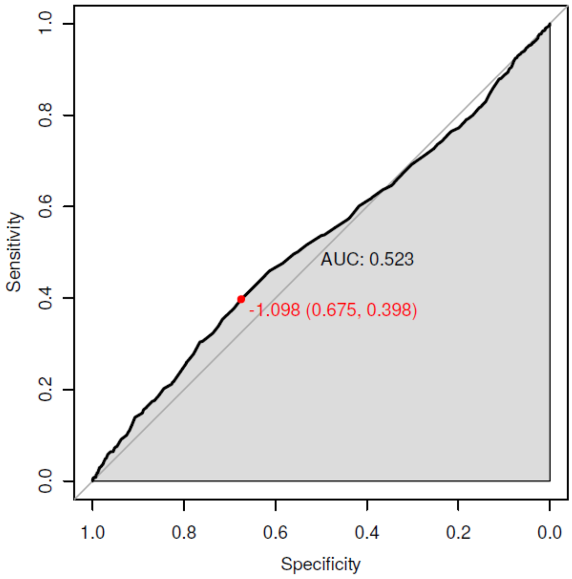


Figure 8 - Results of milk flow model when SCC equals 400x10^3cells/ml with the 5 day reference

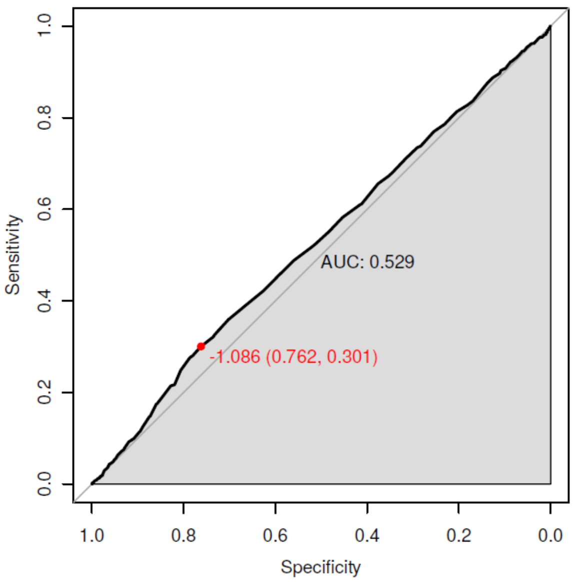


Figure 9 - Results of daily milk yield model when SCC equals 750x10^{3}cells/ml with the 5 day reference range.

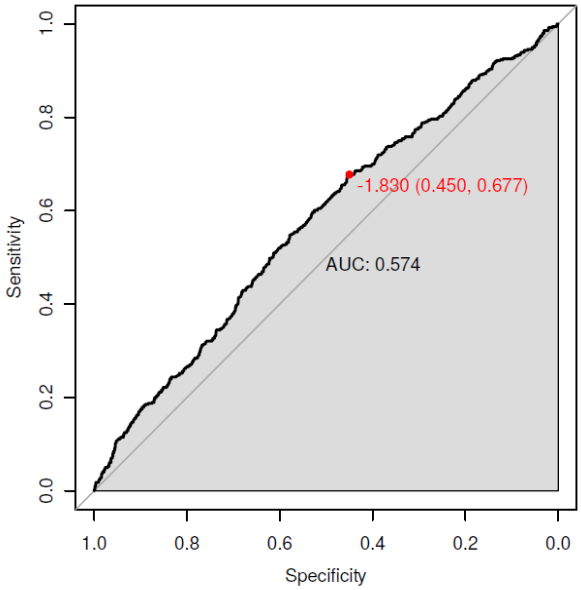


Figure 10 - Results of conductivity model when SCC equals 750 x10^{3}cells/ml with the 5 day reference range.

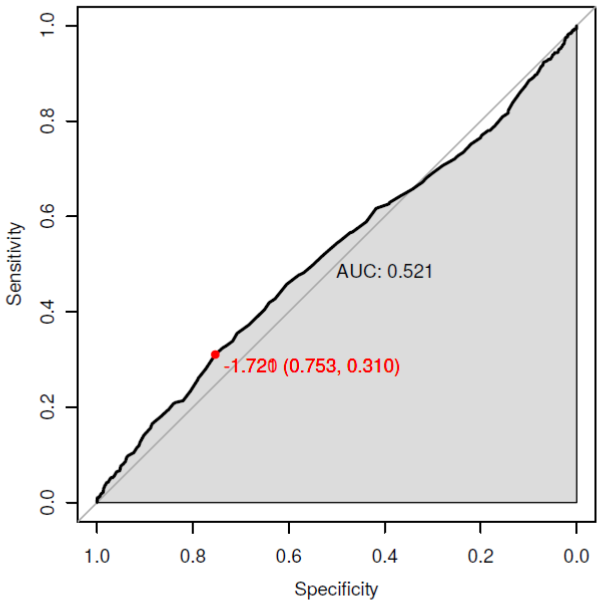
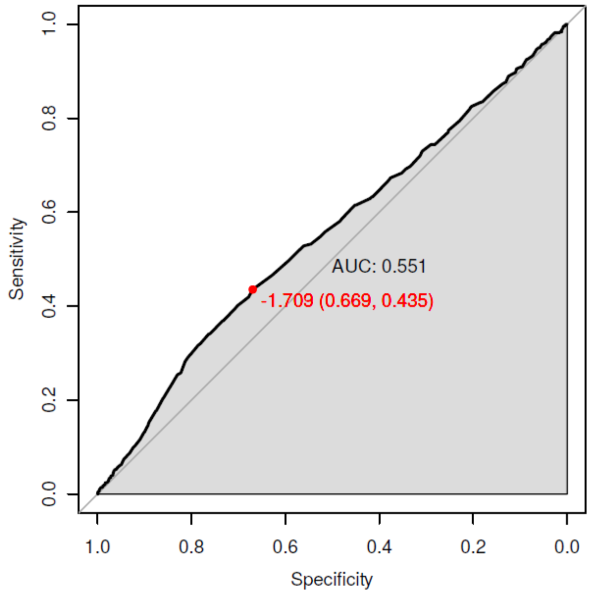


Figure 11 – Results of milk flow model when SCC equals 750x10^{3}cells/ml with the 5 day reference range



The table below summarizes the AUC values and the significant results for the selected threshold values for the 5 day time period for the 3 monitored criteria.

Table 11 – 5 day time period summary

SCC threshold value	Milk Yield		EC		Milk Flow	
	AUC	Significant	AUC	Significant	AUC	Significant
100x10^{3}cells/ml	0.538	Yes	0.506	No	0.521	No
400x10^{3}cells/ml	0.538	Yes	0.523	No	0.529	No
750x10^{3}cells/ml	0.574	Yes	0.521	No	0.551	Yes

Discussion

A cow suffering from mastitis, an IMI, inflammation of the udder; will produce milk of lesser quality and quantity (F.J. Ferrero, 2013). This is a well understood concept which has been noted by numerous researchers. This theory is confirmed by the results in Table 1 which gives a Beta value; the Beta value indicates the OR of the cow meeting the SCC threshold.

Therefore as the milk yield decreases, the Beta value increases and as a result the OR increases indicating the cow is more likely to reach the SCC threshold. Since the p-values for all the results on Table 1 are <0.05 this indicates that these results are significant.

The meaning of these results is further established when considering a paper produced by Sharma et al. 2011. It was noted that reductions in milk yield were first observed when the SCC exceeded 100,000 cells/ml, as the SCC increased further the milk yield losses also increased considerably.

When considering the changing electrical conductivity, cows with mastitis are proven to have higher levels of electrical conductance; the inflammation causes destruction of the tight junctions within the udder, leading to the movement of Na^+ and Cl^- ions into the milk (M.Janzekovic, 2009). As the conductance value increases the OR also increases, indicating the cow has higher odds of reaching the SCC threshold. The results on Table 2 indicate several significant results (p.value <0.05) for SCCs of 350, 500 and 550 ($x10^3$cells/ml)

It is interesting to note for these values the Beta value is also higher compared to the neighbouring results. This is a positive indicator that EC and SCC do share a relationship; despite this the AUC for these results is still quite low, 0.521, 0.524 and 0.523 respectively.

Table 3 shows results for the flow rate of milk, in our results no significant change is observed until the SCC reaches $650x10^3$cells/ml, from this point onwards all p-values are below 0.05.

The flow rate of milk has been studied by many other researchers, including an article which was published in the Journal of Dairy Science in 2007. The results concluded that cows with SCC >500,000 cells/ml had a lower peak flow rate compared to cows who had a SCC <200,000 cells/ml (Tančin, 2007).

Tables 4-6 consider the same 3 independent variables, yield, conductance and flow rate, this time the reference value is the median of the previous 5 days. Milk yield again remains a significant indicator, the Beta values show an increasing OR, for that reason a cow with decreased yield will be more probable to reach the SCC level, all results are significant due to the p-values being <0.05.

The values recorded in the case of Table 5 when considering EC over the previous 5 days show a range of results, several results are indicated as significant, 350, 500, 550 and 950x10^3cells/ml again highlighting that there is a relationship between EC and SCC, unfortunately the AUC for these values are still not ideal.

The SCC level of 450 x10^3cells/ml on Table 6 is the level at which the results become significant, beyond this point, with the exception of 550 x10^3cells/ml, all the p-values are <0.05. These values indicate that cows with reduced milk flow have a higher OR, which in turn means they are more likey to reach the SCC thresholds.

Table 7 indicates significant results above the SCC of 450x10^3cells/ml, p-values <0.05. When observing Table 8 it is found that none of the obtained p-values are significant. Several negative Beta values signify that the OR is decreasing, indicating that there is less probability that the cows will reach the SCC threshold. The AUC values on Table 8 gradually increase from SCC 100 (0.509), peaking at SCC 400-450 (0.532) before decreasing again.

Finally Table 9 in relation to the flow rate shows results becoming significant from 450 x10^3cells/ml onwards, p-values <0.05. The increasing AUC is also increasing as the SCC increases.

When taking into consideration the significant results shown in table 10 together with the information gathered during my literature review I have decided to focus on the ROC curves for the following somatic cell counts:

- 100 x10^3cells/ml – considered a healthy udder
- 400 x10^3cells/ml – EU upper limit for consumable milk
- 750 x10^3cells/ml – US acceptable upper limit for consumable milk

When considering previous studies relating to mastitis detection an article published in the International Journal of Veterinary Science (Reddy, 2014) discussed the use of various diagnostic tests and their accuracy. The study compared the CMT, EC and SCC whilst using bacterial culture as a reference.

The California Mastitis Test can be used as a simple cow side test for the detection of mastitis. The test is able to indicate based on the degree of colour change samples which have higher levels of somatic cells.

The test reagent interferes with the cell membrane of the SCs releasing the DNA, the DNA is then able to react with the test reagent producing a colour and consistency change. In case of a high SCC the colour turns dark purple whilst the consistency forms an almost solid gel.

Despite the test being slow to perform requiring a test to be carried out manually for each cow the results produced tend to have a high degree of sensitivity. The test is undertaken by using a specially designed paddle containing four individual wells. A milk sample from each udder quarter is placed in the corresponding well and an equal amount of test reagent is added to the milk sample and mixed.

The results are based on a visual interpretation of the colour and consistency changes and generally scored on a zero to three point scale. Zero indicating little or no change while three points indicates the formation of a semi-solid gel of dark purple colour. The results are qualitative; you are able to report yes or no as to whether the cow has mastitis rather than quantatitivly being able to report a SCC value.

In general the SCC must be greater than $300x10^3$cells/ml in order to give a reliable result using the CMT. The test is favoured due to its ease of use and real-time results whilst also being able to determine which quarter is affected is also a benefit.

The results determined by the aforementioned study performed by Reddy and published in the International Journal of Veterinary Science indicates that the CMT had the highest degree of sensitivity at 71.01%, whilst the EC test was the least sensitive at 56.52% compared to the bacterial culture which was considered 100% sensitive.

Despite this, due to the high specificity, 84.84%, the study concluded that EC had the best Predictive Value of obtaining a positive test. The final conclusion stated that EC could be used as a decision criterion on farms when choosing whether to cull or treat cows with SCM. (Reddy, 2014)

When examining the values in Figure 3, based on the 5 day median reference range, the AUC has been given as 0.538 meaning there is a 53.8% predictive value based at this threshold. When considered together with the Beta value, SE and p.value (<0.05) this result is found to be a significant. Despite this being a significant result the predictive value is still relatively low, so the practical value of this threshold is uncertain.

A similar result is observed in Figure 4, this illustrates the electrical conductivity of the milk with a SCC threshold of $100x10^3$cells/ml with a 5 day reference range. For the conductivity at this low SCC level, the AUC is 0.506 (50.6%); the statistical association looking at the p.value for this threshold indicates that this result is not significant. Overall the predictive value is too low to be considered as a useful practical tool in this case.

Figure 5 depicts the milk flow model again when the SCC is 100 $x10^3$cells/ml compared the 5 day reference range, the AUC here is slightly improved, 0.521 (52.1%) meaning the predictive value here may be more useful. When taking into consideration the statistical associations of this threshold the p.value of 0.09 indicates that this result is still not significant.

Within the EU directive 92/46CEE states that a SCC of 400 $x10^3$cells/ml is acceptable for products made with raw milk (Council Directive, 1992), when considering this threshold in relation to our results the following ROC curves can be produced.

When inspecting the daily milk yield for the SCC threshold value of $400x10^3$cells/ml the AUC is found to be 0.538 giving a predictive value of 53.8%. When the statistical association of this value is taken into consideration, the p.value (0.0016) indicates that this result is significant. Whilst this result is significant, the practical value of this result is showing promising signs, but is currently of little use.

Figure 7 gives the results from the EC at the threshold SCC level of $400x10^3$cells/ml when compared to the 5 day reference range. The AUC value of 0.523 indicates the predictive value of 52.3%. The overall value of this result is low. When taking into consideration the p.value (0.092) this value is again not significant.

Results from the milk flow model at the SCC threshold value of 400 $x10^3$cells/ml produces an AUC of 0.529 with a predictive value of 52.9%. This means there is a 52.9% chance that using this threshold will enable us to correctly identify a cow with SCM. In contrast there is still a 47.1% that this threshold could fail to identify such a cow. The predictive value together with the p.value (0.09) indicates that this result is not significant.

The United States has a much higher acceptable upper limit for SCC when examining milk. In America milk is considered suitable for human consumption up to a level of $750x10^3$cells/ml. Again I have examined all three of the criteria, milk yield, conductance and flow model and produced the corresponding ROC curves for the $750x10^3$cells/ml threshold.

The milk yield model for the $750x10^3$cells/ml threshold is depicted as Figure 9. Here the AUC is found to be 0.574 giving a predictive value of 57.4% the highest predictive value observed thus far.

This indicates that for higher SCC thresholds milk yield may have a higher practical value when compared to lower thresholds.

The p.value also indicates this result is significant. Despite these promising characteristics the predictive value of 57.4% is much lower than we would like

Within Figure 10 the AUC is 0.521, this provides the predictive value of 52.1%. The p.value given for this threshold, 0.214, indicates that this result does not have a statistical significance.

The AUC for the 750 $x10^3$cells/ml threshold when considering the flow model shown in Figure 11 is 0.551. The predictive value of 55.1% shows a promising trend in that milk flow could be used as a SCM indicator. When taking into account the p.value of 0.00074, this also indicates that this result is statistically significant. Despite this the practical value of this result is less than ideal.

Overall the ROC curves provided some interesting results; milk yield appears to be the most consistent in terms of significant p.value results. Several of the threshold values for the given results in relation to the 5 day milk yield model have p.values <0.05 indicating they are statistically significant.

Unfortunately electrical conductivity provided results which failed to indicate any statistical significance. All predictive values were found to be >50%, despite this the practical value of these results is relatively low.

The results in relation to milk flow model only show significant results as the SCC threshold increases. The lower threshold SCC values of $100x10^3$cells/ml and $400x10^3$cells/ml are found not to be significant.

A positive trend can be observed in relation to the AUC for both milk yield and flow models, as the SCC threshold increases from $100x10^3$cells/ml to 750 $x10^3$cells/ml the AUC increases also. Overall as the SCC increases the predictive value of this criteria improves, 53.8% increases to 57.4%. As a result this means cows with higher SCC are more likely to be detected as having SCM when examining their milk yield.

It has been suggested that for results to be considered reliable there should be a sensitivity of 80% and a specificity of 99% (Hogeveen, 2010). Despite this promising trend the overall predictive value is still considered too low to have any beneficial practical value.

Conclusion

The biological changes associated with the changes in electrical conductivity when an udder is affected by mastitis have been well understood for many years. The changes to basement membranes and tight junction permeability due to inflammation have been widely reported, by researchers such as (M.Janzekovic, 2009) and (Kitchen, 1981).

Numerous studies have been able to prove an association between the electrical conductivity of milk and mastitis in cows while also achieving reliable sensitivity and specificity levels. Those such as (Claycomb, et al, 2009) as well as (De Mol & Woldt, 2001) were all able to achieve reliable results. It has been suggested that to be able to use a test to detect CM it should have a sensitivity of 80% and a specificity of 99% (Hogeveen, 2010).

The majority of studies have focused on detection of clinical mastitis. Since CM is often associated with higher levels of infection, thus resulting in more inflammation and therefore more changes in the EC consequently providing a more reliable set of results.

Our aim was to prove the association between the electrical conductivity of milk and subclinical mastitis in dairy cows. SCM often associated with lower levels of SCC, being able to detect SCM at an early stage is a key model that is currently being developed. The more discreet changes associated with SCM make providing a reliable detection method more challenging.

We were able to observe definite trends when taking into consideration the milk yield and flow models. A positive tendency can be seen; as the SCC increases the predictive value of the selected criteria (Milk yield or flow model) becomes more reliable.

The results achieved when observing the EC were not so dependable; the overall predictive values were low, not high enough to be considered reliable. We processed results based over 3 different time spans, 3 day, 5 day and 10 day reference ranges, during each time span we considered 19 possible SCC thresholds.

At the end of the data analysis for the EC we had 57 (3 time spans x19 SCC thresholds) individual sets of results, of the given p.values only 6 (10.5%) were found to be significant.

The low levels of significance combined with the low AUC values indicate the predictive value of the electrical conductivity detection method for SCM does not currently hold much practical use.

The scientific theory behind electrical conductivity supports EC as a valid method of detection for mastitis. EC is widely used by many large scale automatic milking systems, the Lely company who have customers in over 60 countries, produce several ranges of automatic milking machines that use EC as a mastitis detection (Lely, 2017). They spend much of their research and development time looking at ways to improve the mastitis detection.

In the future it is probable that all new milking machines will avail of some form of EC mastitis detection. Being able to detect mastitis from a subclinical stage is of utmost importance to making dairy farming a more profitable business, whilst also improving the overall welfare of the cows.

Our study failed to achieve any results which are of value to the farming industry, but we are confident that with further research and more clinical trials, EC will prove to be a major step forward in the detection of sub-clinical mastitis.

Summary

Mastitis has been a major problem facing the dairy industry from the very outset of farming, both in terms of an economic view point and the welfare of the cows. The stress that a mastitis infection can have on farmers cannot be neglected either.

Farmers, veterinarians and industry experts have been working tirelessly to make the detection of mastitis more efficient. Numerous methods of detection are available to the dairy industry, visual inspection of the milk together with cow side tests such as the California Mastitis Test and handheld SCC detectors whilst samples can be sent to labs for bacterial culture.

The aim of detecting mastitis at an early stage therefore enables the implementation of an early treatment program before any major economic impact can occur. In recent years the development of using electrical conductivity as a mastitis detection method has proved to be useful in terms of detecting clinical mastitis. With a higher degree of infection, the physical changes associated with CM are detected more reliably.

Our aim was to prove if EC could be used as a valid detection method for sub-clinical mastitis. We examined results taken from a large-scale dairy farm who has automatic detection and recording facilities within the milking parlour. We examined three variable factors for each sample the milk yield, EC and milk flow. We were able to examine these factors when observed at different SCC thresholds.

The data was processed using R environment software and results presented in a range of ROC curves (Figures 3-11), the AUC for each curve could then be analysed. The AUC indicated the predictive value of the test at the given variable and threshold.

When we examined the ROC curves produced we found the AUC of all curves to be low, all values were found to fall between 0.5 and 0.6, meaning there is only a 50-60% chance of the test returning a true positive result.

To make a final decision as to whether the results are reliable the p.value was also examined, when focusing on EC very few of the given results (10%) were found to be significant. The overall predictive value and therefore the practical value of the EC method in this case is of little use.

In conclusion the ability of this kind of detection and analysis has shown promising characteristics when examining the overall milk yield and flow models in relation to mastitis. A greater number of significant results were obtained, and the AUC values were marginally higher.

With most large-scale producers already employing the use of EC detection in the detection of CM, the sensitivity of these sensors must be advanced further in order to detect SCM with a high degree of accuracy. The use of EC as a sub clinical detection method requires more research and development, being able to detect the early sub-clinical changes in relation to an udder infection is a vital step forward in making dairy farms a more economically profitable business.

Our results were unfortunately of no benefit in this case, but with more advanced research it is only a matter of time before the technology is in place and better equipped to make early mastitis detection an everyday procedure.

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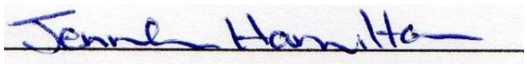

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