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PAPER

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Development and test of a portable device to monitor the health status of Sarda breed sheep by the measurement of the milk electrical conductivity

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ABSTRACT

The electrical conductivity (EC) of milk is a parameter which is often used for identifying sub-clinical mastitis in dairy animals. It is widely used for cattle, and is measured either by means of probes integrated into the milking machine or by means of portable devices. However this is not the case for small ruminants, where the available devices are few. The aim of this study is to deepen the knowledge of about the relationship between EC and certain constituents of Sarda sheep milk, and thus to develop a portable device specifically designed for on-site measurement of conductivity and to estimate the somatic cell count (SCC) of Sarda sheep milk. Therefore, the device allows a rapid test for checking the acceptability of milk to monitor the effects of udder infection. The receiver operating characteristic (ROC) method was used to evaluate how efficacious EC was in discriminating between animals with a somatic cell level higher or lower of a threshold value previously defined. The cut-off values, sensitivity, specificity and the area under the ROC curve for EC were, respectively, 4.835 mS/cm, 73.08, 75.46 and 0.804, using a threshold of 700 000 cells/ml. Our results gave a positive evaluation of the portable device that we had designed for estimating the SCC in sheep milk. Only 8.8% of the samples were incorrectly identified as negative. A portable device for EC measurement is a useful tool for monitoring the somatic cell level individually, and allows early and efficacious action to contrast new intramammary infections.

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Electrical conductivity; milk; ROC curve; sheep; somatic cell count

Introduction

The somatic cell count (SCC) is widely used for determining subclinical mastitis and evaluate udder health in dairy cattle (Dürr et al. 2008). Moreover, SCC is a useful predictor of intramammary infection in dairy ewes (Gonzalo et al. 2002) and it could be used as an estimator in dairy goats (Bergonier et al. 2003). A high SCC is also linked to a deterioration of milk quality and frequently to a loss in milk production (Gonzalo et al. 1994, 2002; Ying et al. 2002, 2004; Nudda et al. 2003; Leitner et al. 2004a, 2004b; Dürr et al. 2008; Hagnestam-Nielsen et al. 2009; Hand et al. 2012). EC is generally used to detect health status, studies in dairy goats showed that daily measurements of EC may represent a useful method to detect intra-mammary infections (Díaz et al. 2012; Zaninelli et al. 2014). The electrical conductivity (EC) is one of the indirect systems for determining the quantity of somatic cells in milk (Peris et al. 1991; Barth et al. 2008; Tangorra et al. 2010; Romero et al. 2012a). This parameter is widely used for cattle, where the probes for measuring it are often integrated into the milking machine and the EC is continually monitored during milking (Maatje et al. 1992; Zecconi et al. 2004; Norberg 2005). There are also various portable devices for cattle that by measure of the EC of the milk provide an indication of the SCC (Ferrero et al. 2002). In small ruminants the SCC is usually measured in the bulk tank milk, by laboratory analysis, which commonly uses Fossomatic SCC method. At present there is a portable device for SCCs in ovine milk (DeLaval cell counter, DCC). Swift intervention in sub-clinical and early clinical mastitis before clinical signs appear, and early treatment, has obvious benefits in terms of the yield and quality of the milk and the health of the animals (Milner et al. 1997).

The aim of this study was first to study the relationships between the EC and the SCC and between the EC and various constituents of the Sarda sheep milk. Thus, the second part of the study was to design and create a portable device for measuring conductivity specific for Sarda sheep milk. This would be able to monitor the SC level by the EC of the milk of each individual sheep. The receiver operating characteristic (ROC) method was used to identify the EC threshold value that would yield the optimal mix of false positive and false negatives and to evaluate the diagnostic

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effectiveness of discriminating potentially infected udders in Sarda dairy sheep.

Materials and methods

Ewe milk characteristics

In the first phase of the study information was obtained on the composition of the milk from Sarda breed sheep. This was before the design and creation of the prototype. A total of 540 samples of half udder milk of 300 ewes, randomly selected, were collected before milking and after discarding the first streams of milk (in sterile containers) for analysis from 12 different flocks in the north of Sardinia from February to June 2013. The samples were used to determine the composition of the milk from each individual animal during morning milking; unfortunately, some samples were lost or damaged during sampling procedure. The EC (LF 92, WTW GmbH, Weilheim, Germany), freezing point, chlorides, pH, fat, lactose, protein, (Milkoscan FT 6000, Foss Electric, Hillerød, Denmark), SCC (Fossomatic 5000, Foss Electric, Hillerød, Denmark) of the milk were analysed at the ARA certified laboratory (Associazione Regionale Allevatori) in Oristano (Sardinia, Italy).

There are marked differences among dairy ruminants with respect to SCC in milk (Souza et al. 2012). The average of SCC threshold values for discriminating between healthy and infected halves differ among species and breeds (Pengov 2001; Berthelot et al. 2006; Lafi 2006; Ruegg 2011). Therefore, a threshold of 700 000 cells/ml was set for this study, based on our experience and knowledge in Sarda breed.

The results of the analysis allowed us to identify the EC cut-off value, based on whether the number of cells was greater (or equal too) or less than 700 000 cells/ml.

Prototype design

In this stage a portable prototype was developed to measure the EC of milk. The instrument was designed by arriving at a compromise between the differing demands of functionality, precision, speed in taking the samples and cost. Before construction the instrument was modelled in 3D using SketchUp software (version 14.0.4900, 2014; Figure 1).

The block diagram of the device is shown in Figure 2. The EC probe (k 1.0, Atlas Scientific, New York, NY) was connected to the EC integrated circuit (v 3.0, Atlas Scientific, New York, NY) by a BNC connector. The k 1.0 probe can measure EC in a range from 1.3 mS/cm to 40 mS/cm with a precision of ±5

Figure 1. The final prototype of the portable device for measuring conductivity in Sarda sheep milk.

μS/cm. The micro-controller used was an ATmega32U4 (Arduino Pro Micro – 5V/16MHz), characterised by: low power consumption, a high performance 8 bit CMOS and low cost. The conductivity readings were compensated for temperature using the DS18B20 (Dallas Semiconductor, Dallas, TX) digital sensor. This can measure temperatures in a range from −55 °C to 125 °C with a precision of ±0.5 °C with a resolution of between 9 and 12 bits, which corresponds to a temperature resolution of 0.5 °C, 0.25 °C, 0.125 °C or 0.0625 °C, respectively. The temperature of the milk is a critical variable when measuring EC, as an increase in temperature results in greater ionic movement and thus influences the measurement of the EC. The following relation was used to compensate the milk temperature:

$$\sigma_{25} = \frac{\sigma_T}{1 + \alpha \cdot (T - 25)}$$

Where σ_{25} is the EC of milk at 25 °C; σ_T is the EC of the milk at sample temperature; α is the temperature

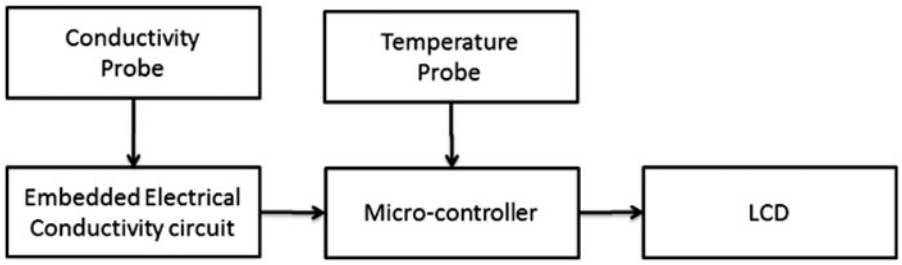

Figure 2. Block diagram of the device for estimating the SCC in sheep milk.

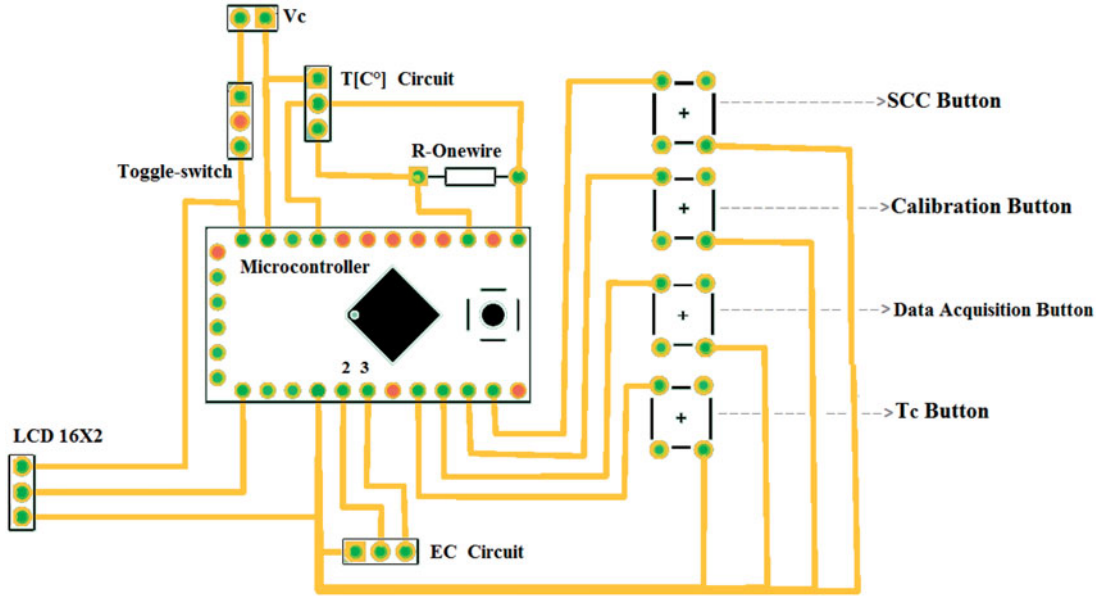

Figure 3. The printed circuit board layout.

coefficient and is near to 0.989%/°C, that expressed the rate of EC changes with temperature; *T* is the sample temperature (Ferrero et al. 2014).

Erroneous temperature compensations can result in errors in EC measurements and invalidate the results for the predictive diagnosis of mastitis (Romero et al. 2012b). The three-point calibration of the instrument was carried out following the instructions provided by Atlas Scientific, using two standard buffers (standardised against NIST-certified references) of 10 500 µS and 40 000 µS. The accuracy of the calibration of the instrument was tested by comparing the results with those of a commercial EC-measuring device (WTW LF 92, WTW GmbH, Weilheim, Germany). The results were more than satisfactory ($R^2 = 0.987$).

Figure 3 shows the electronic circuit of the instrument. The Rx (receiving) and Tx (transmitting) channels of the EC integrated circuit are connected, respectively, to pins two and three of the micro-controller. The LCD 16X2 (Sparkfun Electronics, Boulder, CO) screen includes an integrated micro-circuit based on PIC 16F88, which allows a serial connection to be made with the micro-controller. The resistance (indicated by R_Onewire) of 4.7 kΩ between the positive pin and signal pin ensures that the DS18B20 temperature probe functions correctly. There are four switches which are normally open (NO): the 'Tc' switch for compensating for milk temperature, the 'Data Acquisition' switch for reading the EC, the 'Calibration' switch for calibrating the device and the 'SCC' switch for estimating SCC. The instrument includes a container with a clearly visible stud, which has to be filled with 50 ml of milk so that a correct EC reading is obtained.

The firmware loaded in the micro-controller is written in C/C++, the size of the binary file of the programme is 14.034 kbytes. The device is powered by a 9V battery.

Evaluation of the prototype

In the third phase the prototype was evaluated in the field. A total of 68 half udder milk samples taken at two farms between May and June 2014 were analysed. The prototype was used to measure the EC of the samples directly in the field, and further measurements were then taken in the laboratory, the EC being

measured with the LF 92 and the SCC with the Fossomatic 5000.

Statistical analysis

Statistical analyses were carried out using RStudio (version: 0.98.50), and in particular the ROCR (Sing et al. 2005) and pROC libraries (Robin et al. 2011). The values of the different traits measured in the milk (SCC, chlorides, freezing point, fat, EC, lactose, pH and protein) are presented as arithmetic mean values and standard deviation. In addition, the Spearman rank correlations between the parameters were calculated.

A non-parametric approach was used to fit the ROC curve to the continuously distributed EC. ROC analysis was used to determine the optimal EC cut-off point for distinguishing between positive (milk with SC $\geq$700 000 cells/ml) and negative (milk with SC $<$700 000 cells/ml) results. The point on the ROC curve closest to the top left-hand corner was used as the cut-off value, since this represents the closest point at the curve indicating the 100% of sensitivity and 100% of specificity (gold standard; Dastjerdi et al. 2013). The sensitivity/specificity pair nearest to the top left-hand corner gives the most accurate threshold values (Sasse 2002). The area under the ROC curve (AUC), which can be used to measure the accuracy of the test, was also calculated. A value of 1.0 for the AUC indicates that there is a cut-off point for the variable at which there is perfect discrimination between cases and non-cases. A value of 0.5 would be obtained, if discrimination at every cut-off point occurred purely by chance. For an imperfect, but better than casual discriminator, the AUC would be in the range 0.5–1.0.

Table 1. Milk composition of Sarda breed sheep examined in the present study (n = 540).

Item	Mean and standard deviation	Minimum	Maximum
SCC (10^3 cells/ml)	1.144 ± 3.675	26	26 317
Chlorides(mg/L)	143.9 ± 66.26	50.6	693.6
Freezing point (°C)	575.9 ± 13.37	479.0	606.0
Fat (%)	6.45 ± 1.25	2.84	12.52
EC (mS/cm)	4.73 ± 0.54	3.40	7.60
Lactose (%)	4.75 ± 0.50	0.94	5.49
pH	6.59 ± 0.14	5.42	6.91
Protein (%)	5.48 ± 0.67	3.68	11.00

Results and discussion

The average and the standard deviations of the milk parameters under investigation are shown in Table 1. The results for fat, protein, lactose, freezing point and the pH content, all fall within the range reported for Sarda sheep (Nudda et al. 2002). The arithmetic mean of the SCC agrees with the national figures (Rosati et al. 2005), while the mean value of EC was higher than results found by Serra et al. (1997), which ranged from 4.21 to 4.51 mS/cm.

Table 2 shows the values of the correlation coefficients for the variables under examination. The number of somatic cells of the entire sample had a significant positive correlation with the chlorides, fats, proteins and the EC, while there was a significant negative correlation with the freezing point and the lactose. Over all possible combinations of parameters, the highest coefficients were found for EC with chlorides (0.893), lactose with chlorides (−0.843), and lactose with EC (−0.592).

The correlation between SCC and EC (r = 0.306) was lower than that found for cattle (r = 0.399; Kasikci et al. 2012), goats (r = 0.380; Díaz et al. 2011) and sheep (r = 0.455–0.471; Serra et al. 1997). The freezing point and the EC (r = −0.143) had lower values and opposite signs when compared with the results in studies on cattle (0.228; Kasikci et al. 2012). However no significant difference was found in the correlation between SCC and freezing point in cows (Kasikci et al. 2012), while in our study the opposite was true.

The data on EC were elaborated taking into consideration the number of somatic cells, or rather adopting 700 000 cells/ml as the threshold value for discriminating animals with suspected sub-clinical mastitis (Table 3). There were far fewer samples (n = 104) with cell values of $\geq$700 000 cells/ml than samples with values $<$700 000 cells/ml (n = 436), with the medium values of conductivity of 5.20 and 4.63 mS/cm, respectively. The minimum and maximum levels for both

Table 2. Spearman correlation coefficients among milk variables in Sarda breed sheep milk (n = 540).

Item	SCC	Chlorides	Freezing point	Fat	EC	Lactose	pH	Protein
SCC	1.000							
Chlorides	0.407*	1.000						
Freezing point	−0.171*	−0.153*	1.000					
Fat	0.131*	0.077	0.112*	1.000				
EC	0.306*	0.893*	−0.143*	−0.216*	1.000			
Lactose	−0.384*	−0.843*	0.232*	−0.430*	−0.592*	1.000		
pH	0.015	−0.186*	0.318*	−0.209*	−0.050	0.435*	1.000	
Protein	0.249*	0.066	0.161*	0.451*	−0.167*	−0.343*	−0.132*	1.000

*$p < 0.01$

groups showed that conductivity varied greatly. This can be explained by the fact that other factors other than mastitis are related to conductibility (individual variation of EC, farm, parity and stage of lactation; Baumgartner et al. 1992; Nudda et al. 2002; Díaz et al. 2011).

The ROC is one sensitive and specific tool for evaluating the adequacy of a diagnostic test. This allows to identify the best cut-off, or, in other words, the test value which minimises the number of false positives and negatives (Figure 4). The ROC curve was elaborated from the conductivity data, which corresponded to the value of the cells, divided up as in Table 3. The cut-off value (closest top-left index) was 4.835 mS/cm, which corresponds to a sensitivity of 73.08% and a specificity of 75.46% (Table 4).

The AUC, which measured the diagnostic accuracy of the test, was 0.804 ($p < 0.0001$). This indicated that the test was moderately accurate (Swets 1988; Greiner et al. 2000; Table 5), or rather indicated that the EC levels were different in the two groups, and thus discriminated sufficiently well between them. In practice, a diagnostic test with an AUC of ≥80% is considered adequate (D'Arrigo et al. 2011).

Table 3. Descriptive statistics of electrical conductivity (mS/cm) calculated for somatic cell counts of less than 700 000 and more than or equal to 700 000 cells/ml.

	$EC_{SCC < 700 \times 10^3}$	$EC_{SCC \geq 700 \times 10^3}$
n	436	104
Mean	4.63	5.20
Median	4.60	5.10
Standard deviation	0.40	0.69
Minimum	3.40	4.00
Maximum	6.10	7.60

Table 4. Sensitivity, specificity and confidence interval (CI) for coordinates of the ROC curve.

Cut-off (mS/cm)	Sensitivity, %	95% CI[a]	Specificity, %	95% CI[a]
4.835	73.08	63.5–81.3	75.46	71.1–79.4

The EC was found to be well able to estimate the number of somatic cells, as can be seen by the fact that the confidence interval (CI) of the ROC curve (CI at 95%: 0.768–0.837), not included 0.5 (the threshold for diagnostic lack of difference). According to these results, the cut-off value obtained was used in the prototype.

The flow diagram in Figure 5 shows how the device works. Once the device is switched on, the micro-controller displays a welcoming message on the LCD and then awaits instructions. Once the container integrated in the device is filled with 50 ml of milk, one must press the 'T_c' button. This takes the temperature of the milk, which is shown on the LCD screen and is sent to the automatic temperature compensation circuit. The 'Data Acquisition' button allows one to measure the EC value of the milk, and this is displayed on the LCD screen and memorised by the device. At this point the 'SCC' button, allows the micro-controller to elaborate the EC values, comparing them with the pre-set

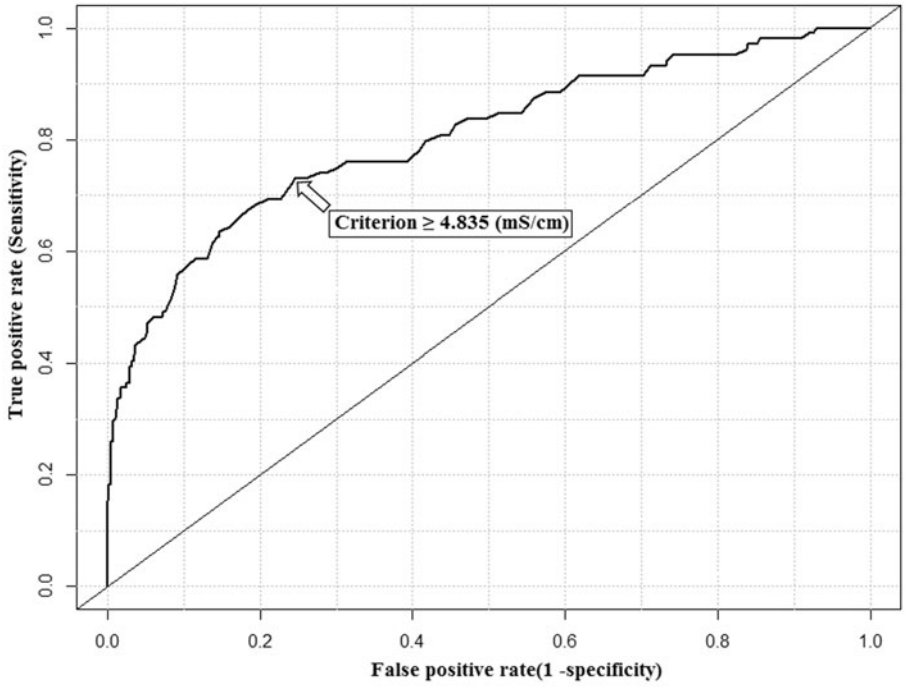

Figure 4. Receiver operating characteristic (ROC) curve between the true positive rate and the false positive rate. The optimal threshold, selected using the closest top-left method, is indicated by the arrow.

Table 5. Significance level, standard error (SE) and confidence interval (CI) for area under the curve (AUC).

			Asymptotic 95% CI	
AUC	SE	*p*	Lower bound	Upper bound
0.804	0.0265	<0.0001	0.768	0.837

Table 6. 2 × 2 Contingency table relating probability of disease status by electrical conductivity (cut-off at EC = 4.835 mS/cm) and disease status predicted from gold standard ().

	Expected positive	Expected negative
Positive screening	11 (17)	12
Negative screening	6	39 (51)

threshold values (4.835 mS/cm), and showing on the LCD screen whether they are greater than or inferior to 700 000 cells/ml.

In the last step the prototype was tested in the field, and 68 milk samples were analysed (Table 6). The device found 11 of the 17 samples, which had SCC greater than 700 000 cells/ml (64.7% success rate). When it identified negative samples, its results were confirmed in the laboratory in 39 cases out of 51, with a success rate of 76.5%. Obviously it is most important to reduce the number of false negatives by as much as possible, as these do not recognise animals which have SCC values greater than the threshold value, and thus the animal itself could suffer because a deeper analysis was not carried out. With our device the number of false negatives was 6 out of 68 samples (8.8%).

Conclusions

The study showed that measuring the EC is a useful way for identifying sheep with levels of SSC greater than 700 000 (cells/ml) and potentially with not healthy glands, and thus reducing the costs of cyto-bacteriological analyses of the individual milk samples. The portable device described here, specifically designed for sheep milk, gave a good accuracy (73.5%), expressed as number of correct assessments/number of all assessments. It allows an initial screening of the SCC to be carried out, based on the threshold value of the EC. Increasing the amount of data available for each animal provides useful information to monitor health status of their udders, and is also helpful when making decisions on the management of the whole flock.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

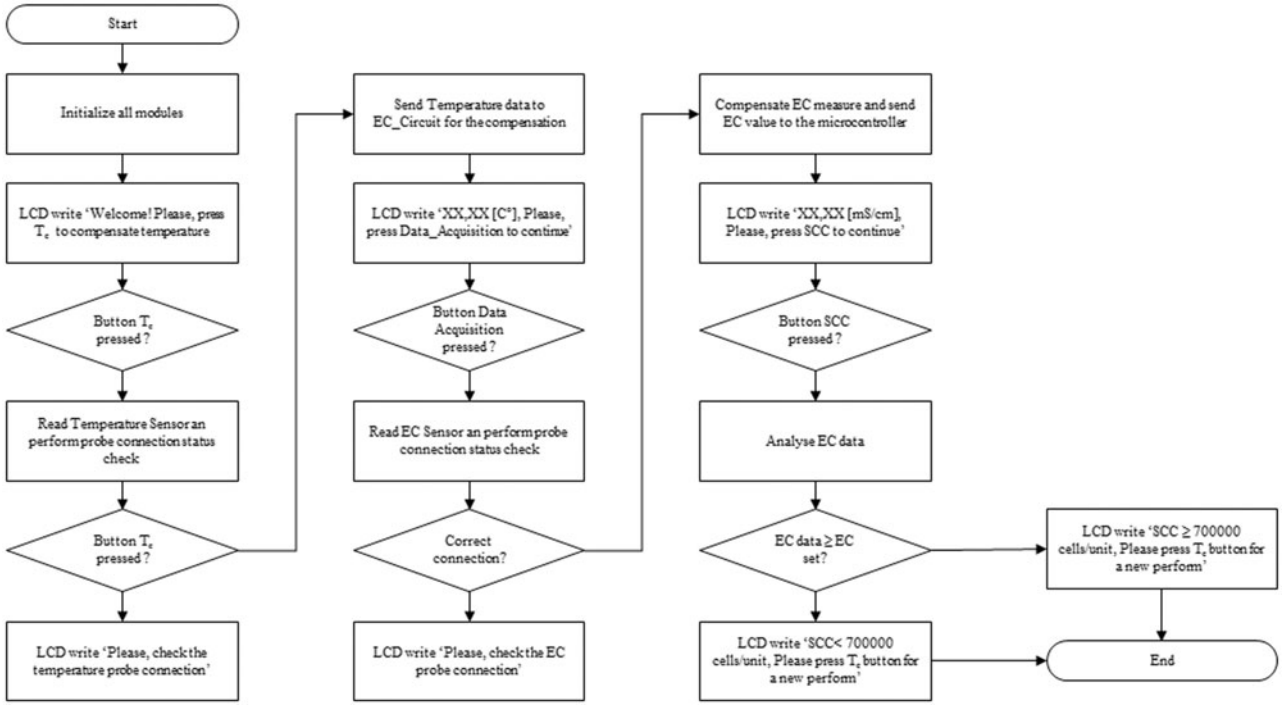

Figure 5. Flowchart of the device.

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