Applicant: CHIMERTECH PRIVATE LIMITED Total no. of sheets:11

Title: A DEVICE FOR EARLY DETECTION OF SUBCLINICAL MASTITIS Sheet no.:01

IN THE MILK OF DAIRY ANIMAL

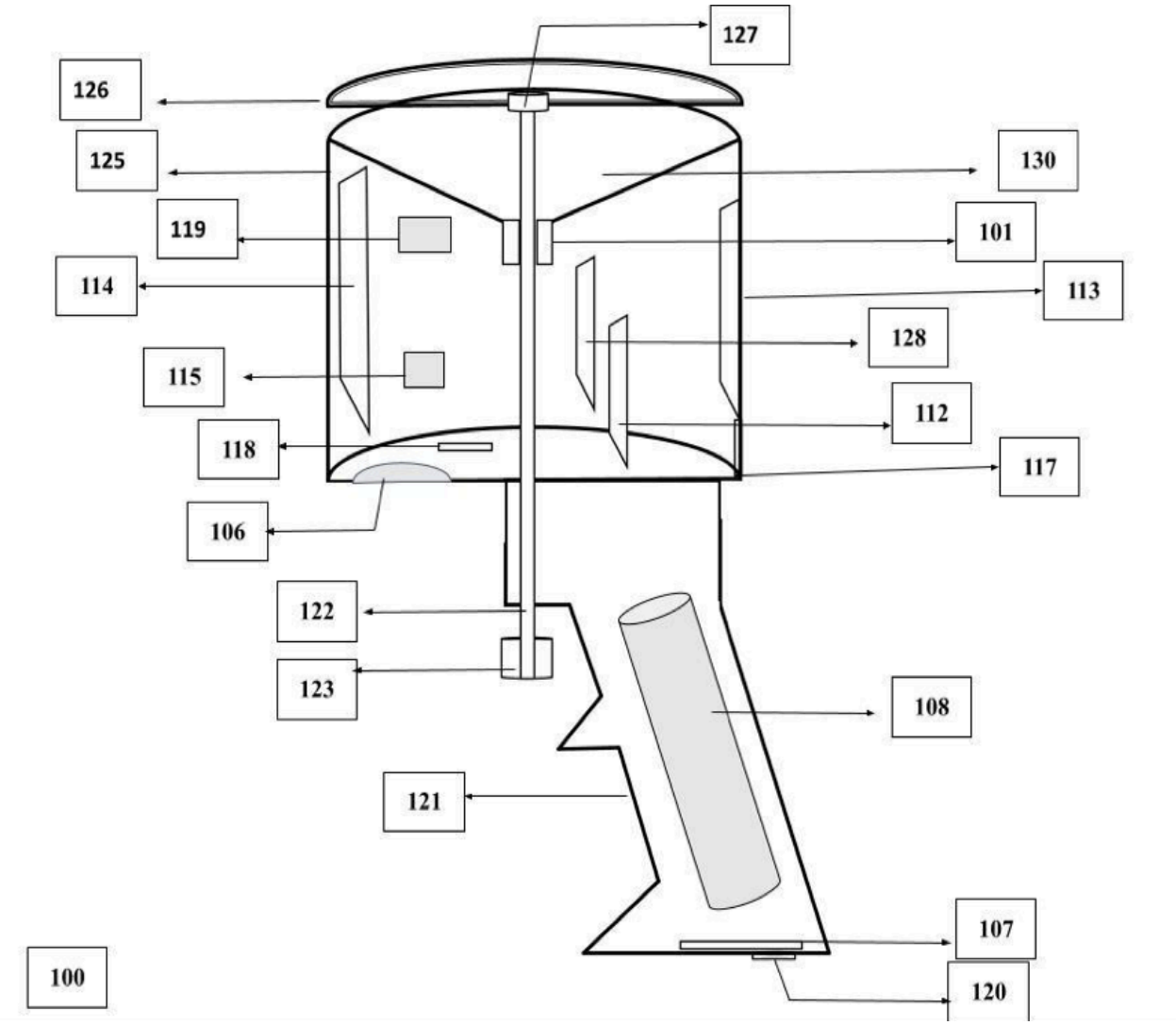


Figure 1

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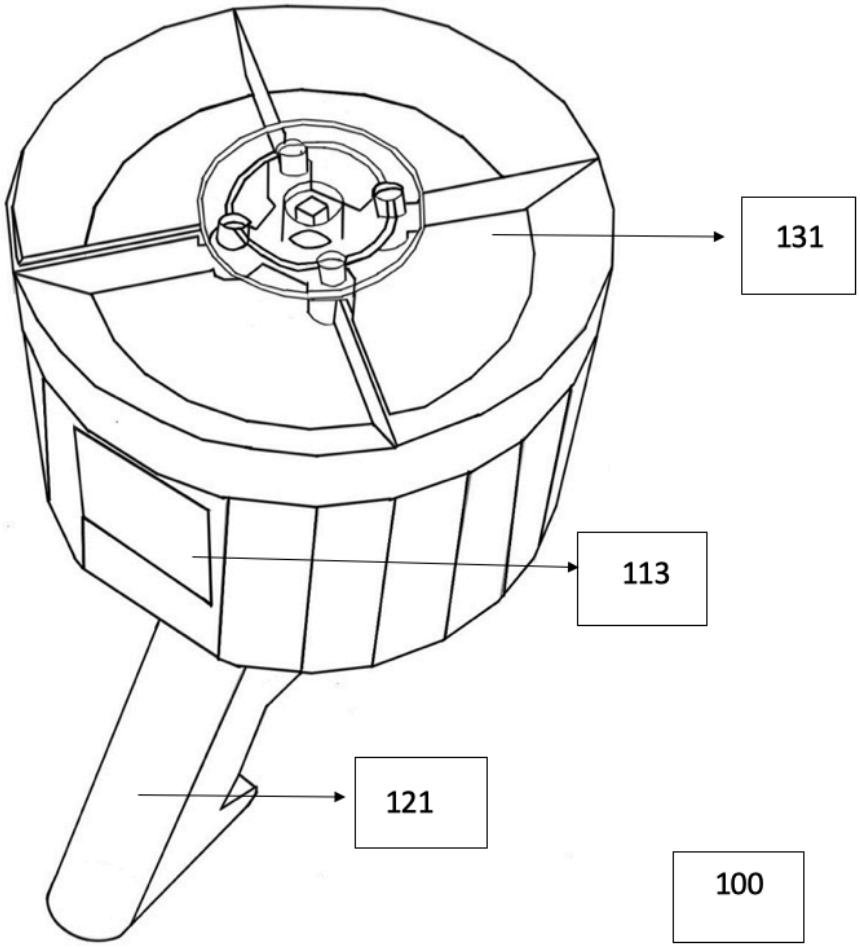


Figure 2

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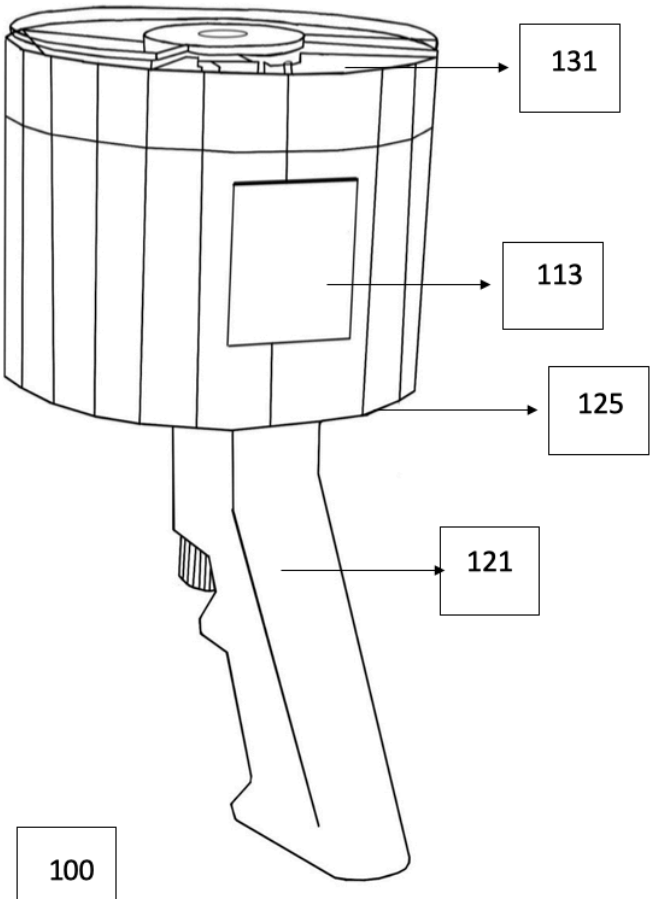


Figure 3

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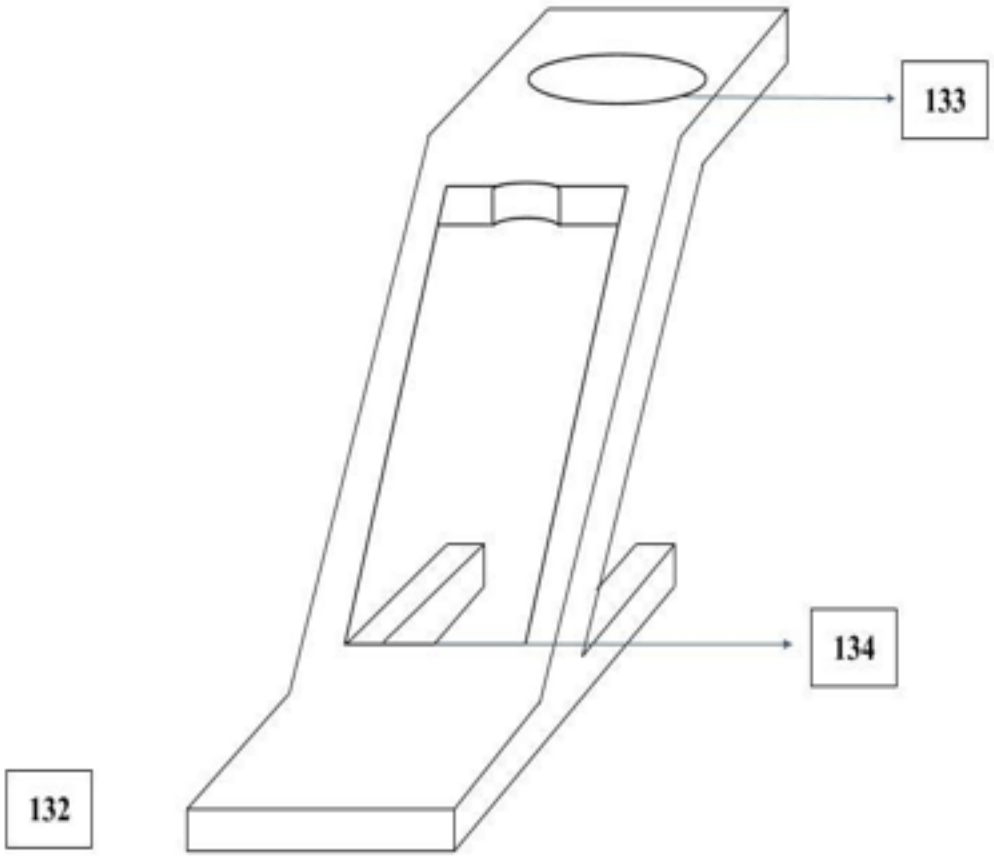


Figure 4

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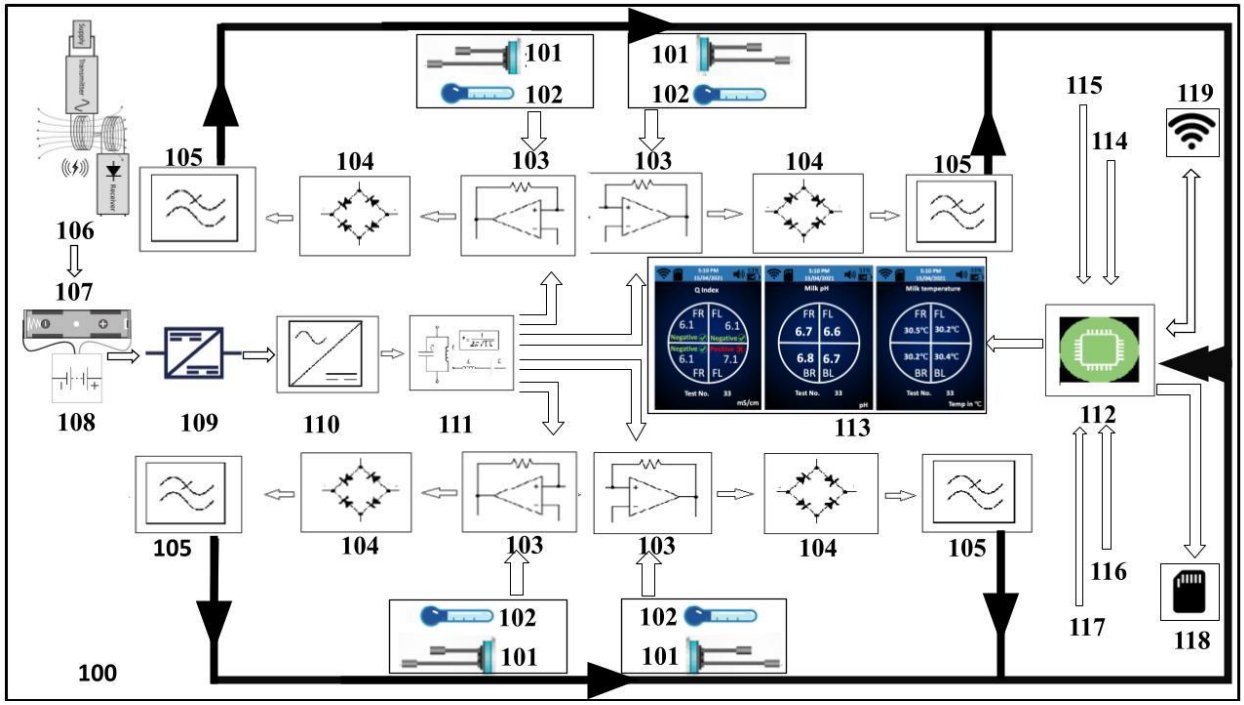


Figure 5

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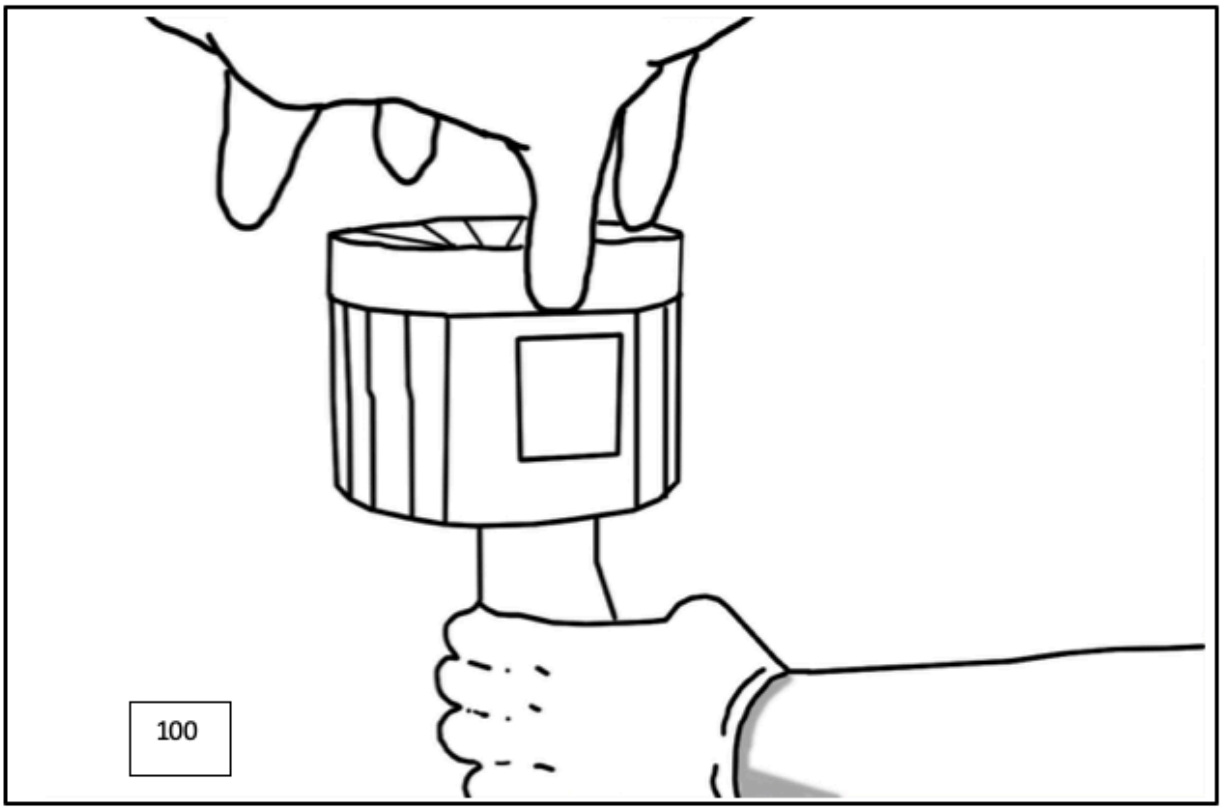


Figure 6

AROKYA NITHYA. D
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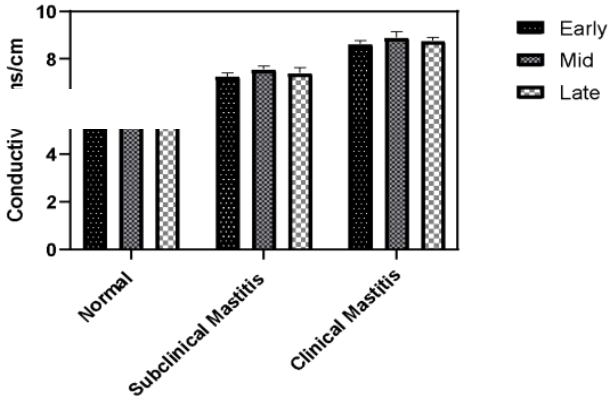


Figure 7a

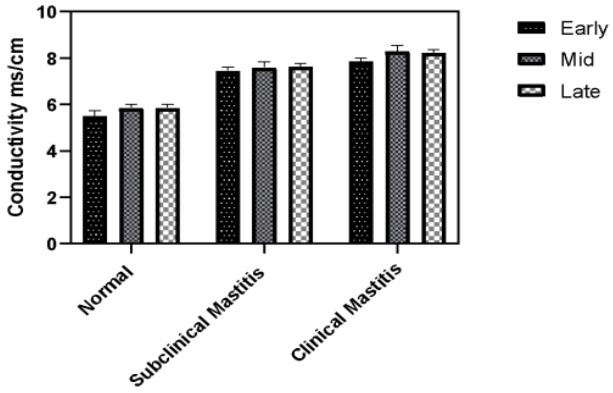


Figure 7b

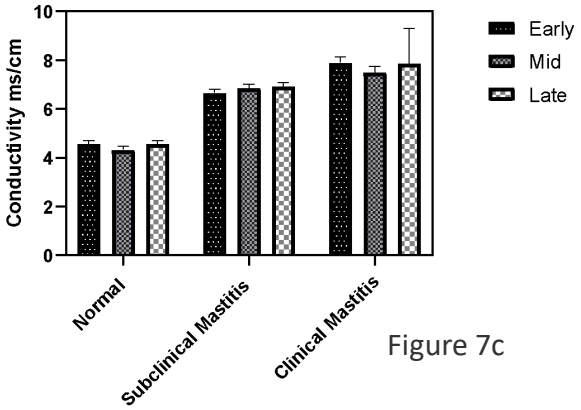


Figure 7c

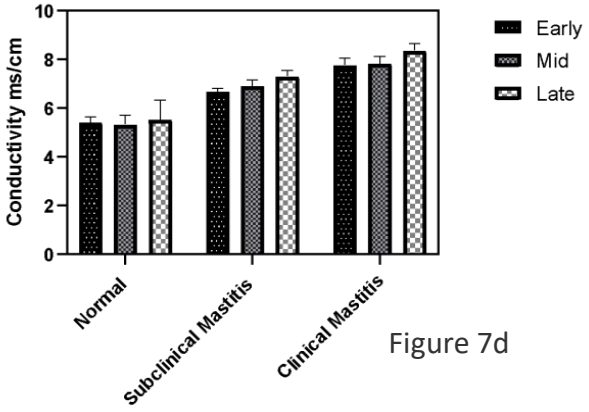


Figure 7d

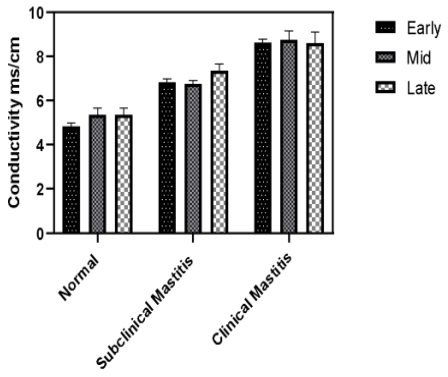


Figure 7e

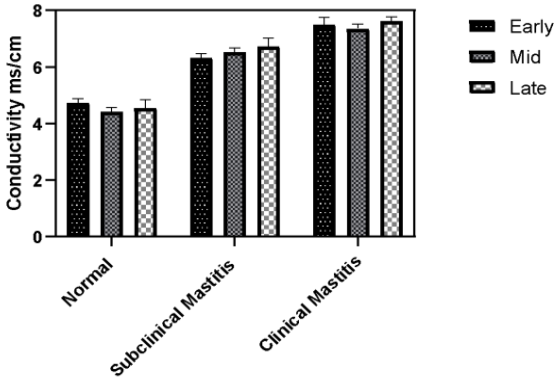


Figure 7f

AROKYA NITHYA. D
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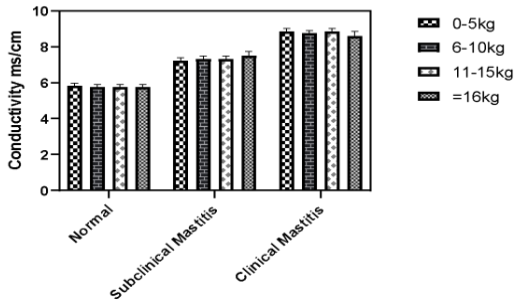


Figure 8a

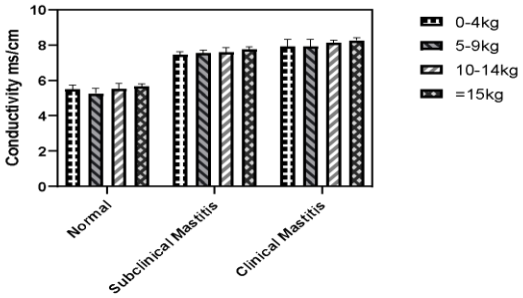


Figure 8b

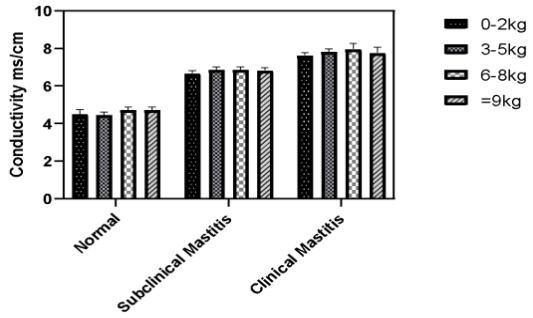


Figure 8c

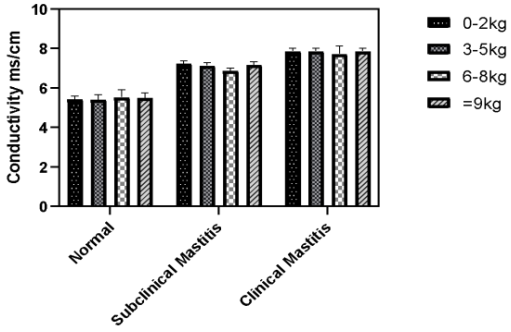


Figure 8d

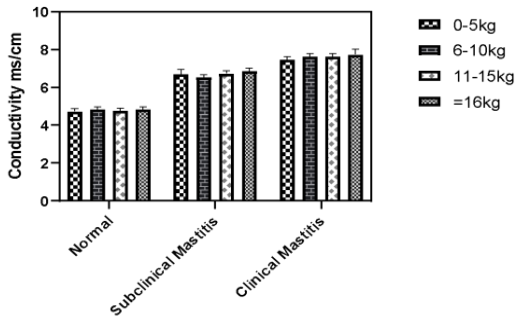


Figure 8e

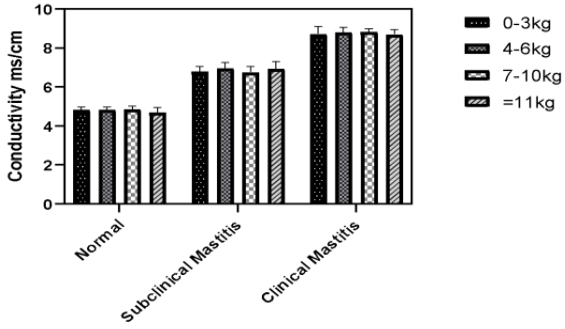


Figure 8f

AROKYA NITHYA. D
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On behalf of the applicant

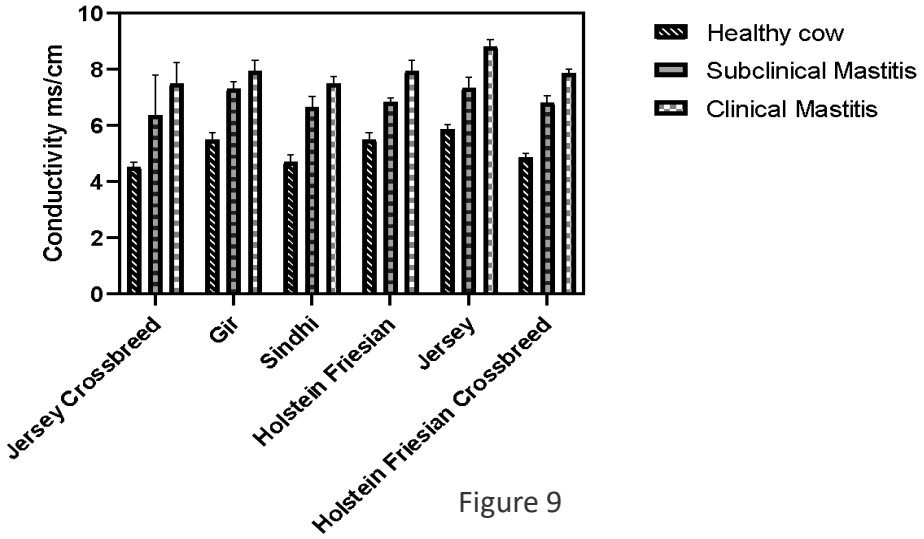


Figure 9

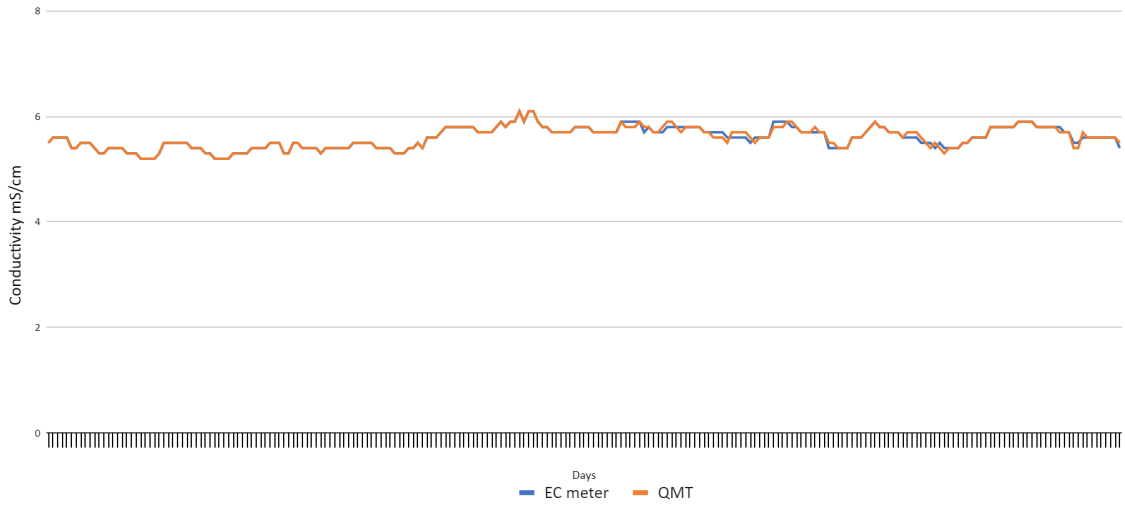


Figure 10

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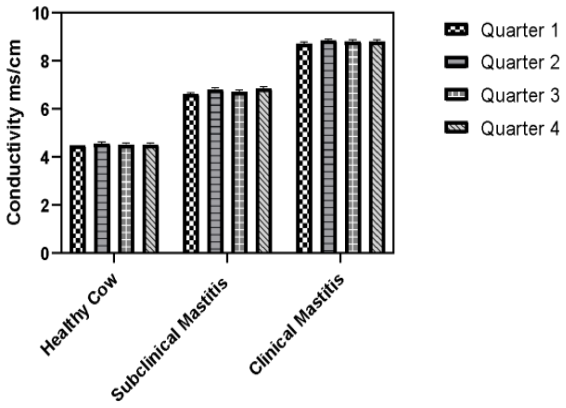


Figure 11a

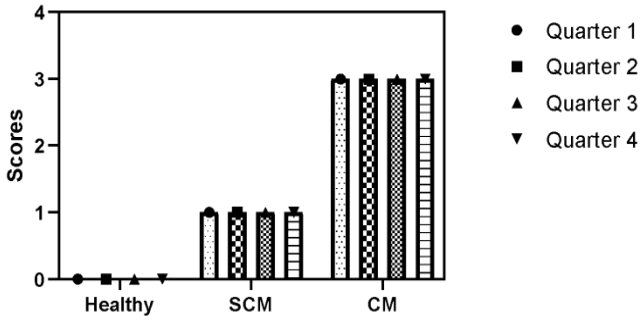


Figure 11b

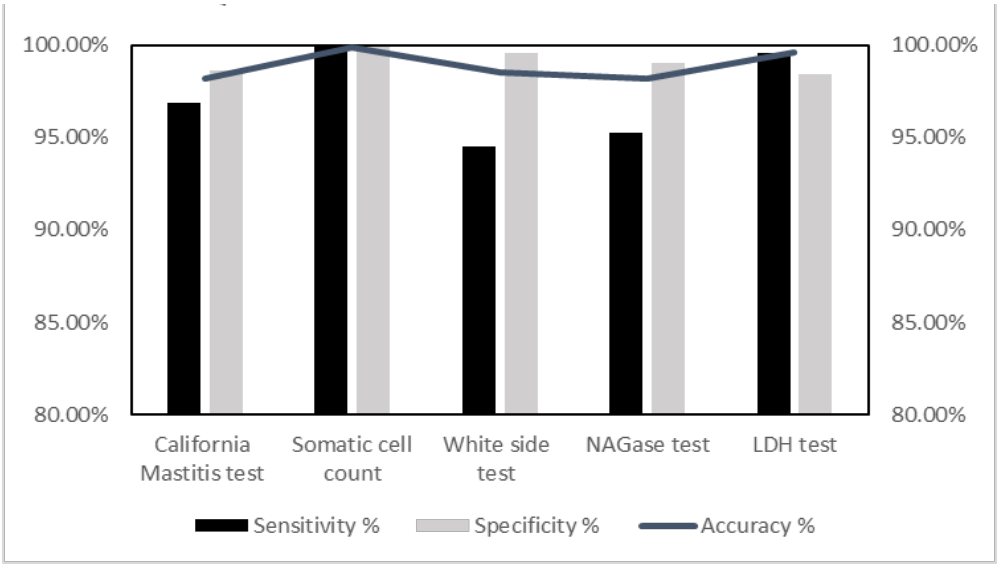


Figure 12

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On behalf of the applicant

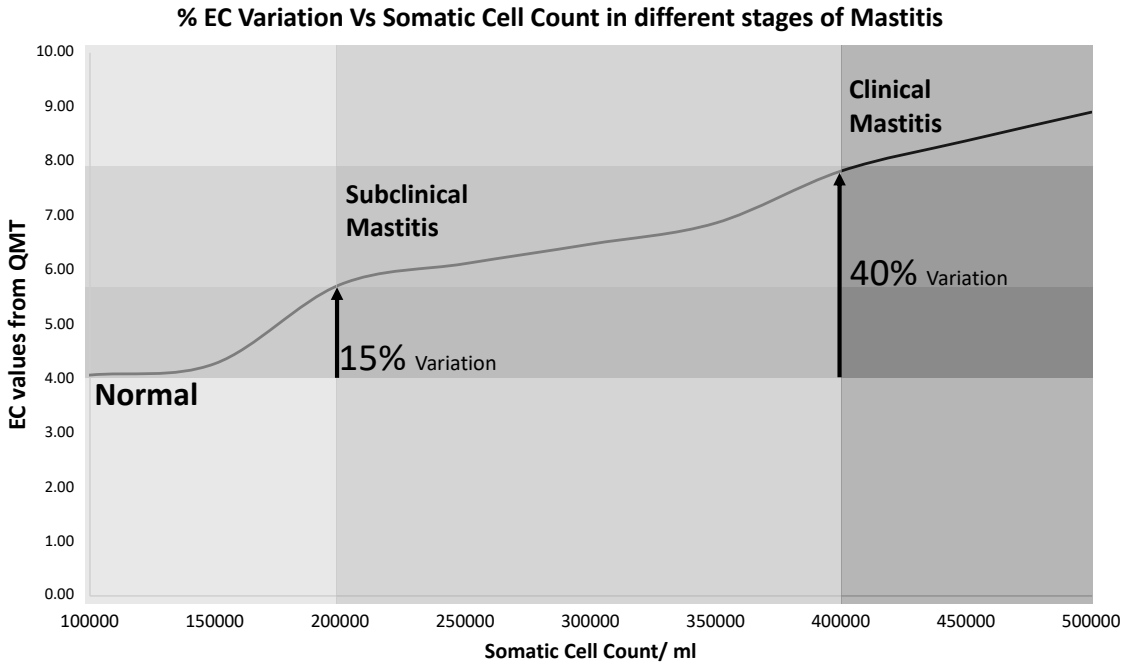


Figure 13

AROKYA NITHYA. D
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On behalf of the applicant

FORM 2

THE PATENTS ACT 1970

(39 OF 1970)

&

THE PATENTS RULES, 2003

COMPLETE SPECIFICATION

(See Section 10 and Rule 31)

1.TITLE OF THE INVENTION

A DEVICE FOR EARLY DETECTION OF SUBCLINICAL MASTITIS IN THE MILK OF DAIRY ANIMAL

2.APPLICANTS

a. Name: CHIMERTECH PRIVATE LIMITED

b. Nationality: Indian

c. Address: 16, Sindu Garden, Gopalapuram, Kazinjur, Vellore TN 632006 IN.

3.PREAMBLE TO THE DESCRIPTION

The following specification particularly describes the invention and the manner in which it is to be performed

FIELD OF INVENTION

The present invention relates to device for early subclinical mastitis detection useful in Animal husbandry and Dairy technology, particularly relate to a device for mastitis detection from the milk sample of cattle. More particularly the present invention further detects subclinical mastitis along with pH, the temperature of milk.

BACKGROUND OF THE INVENTION

Mastitis is an infectious disease condition that causes inflammation in the mammary gland and udder tissue of the cow. It is the most prevalent disease in cows, where it has different degrees as mild, moderate and severe. It is considered to be one of the upscaled diseases which affects the profitability of rearing animals. Mastitis results in reduction of milk yield and milk compositions. The current market-based technologies for detecting infection are unable to identify mastitis effectively at the pre-clinical stage. Electronic instruments for detecting somatic cell count (SCC) can be used to confirm mastitis early on, but for milk producers, this is not possible.

2807/DEL/2014 disclosed immunoassay system for the detection of subclinical mastitis in cows and buffaloes using the polyclonal antibodies against a mammary gland specific glycoprotein. The mammary gland proteins (MGP) were isolated from the milk of buffaloes by a series of chromatographic procedures and were identified by mass spectroscopy. The purified MGP were used for the production of polyclonal antibodies in Rabbit. Polyclonal antibody against the protein was purified by rabbit antisera by protein an affinity column chromatography. To detect MGP in milk, the milk was diluted 10-fold and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out. This was transblotted in polyvinylidene fluoride (PVDF) membrane. Further, it was detected by colour development using HRP conjugated secondary antibody. In normal cases, it could not develop colour. This invention claims that MGP were identified and MGP polyclonal antibody was used as a biomarker for the detection

of clinical and subclinical mastitis in lactating animals. Highly sensitive ELISA is developed to detect subclinical mastitis.

201641024968 discloses the biomarker-based detection and also provides a kit to monitor subclinical mastitis in bovine. This invention shows there are about 5 biomarkers in mastitis milk. Using computational biology, major epitopes for protease peptone, complement component 3, L-selectin, IL-8, pentraxin coding sequences were custom synthesized, the biomarker protein termed as a polyvalent immunogenic bovine biomarker (PIBB) was then expressed, affinity purified and used for antiserum production. Liquid phase blocking (LPB) ELISA using PIBB antigen could be a diagnostic test for the diagnosis of mastitis in cows and the cytokines used in LPB ELISA was highly specific.

201841010518 disclosed a device to detect mastitis in mammals. This device comprises an imaging unit, a controller to adopt the process and a milk sample holder. In this embodiment, both the camera and the illumination sources are assembled at the sample holder to inspect the milk sample where the image captured calculates the number of somatic cells present in the milk.

916/DEL/2012 involves the estimation of alpha 1 acid glycoprotein (AAG) in healthy and subclinical mastitis milk and also provides a diagnostic kit and biomarker for the detection of subclinical mastitis. This is a simple, highly sensitive and cost-effective process.

3012/KOLNP/2010 disclosed the milk analysis for detecting mastitis in milk by isolating somatic cells in the milk by using centrifugal sedimentation which forms pellets. This invention further relates to the milk analysis apparatus for cytometer incorporation. In addition to the detection of mastitis in the milk sample, it also determines the quantity of cream or fat within the milk sample and the determination of protein and enzymatic analysis of the milk sample.

565/CHE/2007 discloses the prognostic kits for testing infection in milk-secreting glands where the base plate comprises a layer of film coated with protein analogues of leucocyte, a testing reagent that has an anionic electrolyte based confirmatory reagent. This reacts with the milk to form gel-like white strands in the medium. This also provides an udder care kit which consists

of a sanitizer powder (source of sulphonyl derivatives) used for external spray that arrests the infections at a pre-clinical state. Moreover, it is rapid, easy to carry and competitively priced.

917/DEL/2012 presents testing the quality of milk by analysing and estimating the concentration of calcium. This invention detects mastitis by identifying the significantly decreased concentration of calcium in milk.

919/DEL/2012 analyses and estimates the concentration of magnesium in the milk of dairy animals like cows and buffaloes by colorimetric method. This invention extends to detecting mastitis in dairy animals by colorimetric based method and provides a biochemical alteration in milk caused by mastitis.

920/DEL/2012 presents the process of testing the quality of milk by analysing the concentration of urea. It also provides a kit to detect mastitis in dairy animals in which the urea reacts with diacdyl in hot solution at nearly 100◦C released by diacetyl monoxime by oxidatible condensation reaction. The color intensity shows the concentration of urea present in the milk

These prior art shows different methods of detection of subclinical mastitis but none shows the teat-to-teat variation in somatic cell count with variation in Electrical Conductivity for the presence of infection irrespective of breed, temperature, lactation, pregnancy, feed and water. Also, none of the above prior art searches included a rapid, point of care, robust, easy to handle, eco-friendly, highly sensitive and cost-effective device for subclinical mastitis detection in the cattle. Hence, there is a need for the device to be designed effectively to detect mastitis in cattle.

The principal objective of this invention is eliminating the prior art problem and giving an alternative approach.

Another objective of this invention is to develop a 4-quadrant prototype that determining Electrical conductivity variations, pH and temperature between teats.

Yet another object of this invention is to compare with market available technologies and gold standards for subclinical mastitis detection.

Still another object of this invention is to provide a device that improves the accuracy of detection of mastitis.

A further objective of this invention is to validate the test and test performance at different variable conditions like parity, lactation period, milk yield, disease progression conditions on different types of breeds.

SUMMARY OF THE INVENTION

Accordingly, present invention provides a hand held device for early subclinical mastitis detection in the teat to teat milk of dairy animal comprising; a main circular body (125) configured to attach with handle (121) at the bottom end of the said circular body adapted to hold the device; the top end of the circular body configured to be closed with a rotatable lid (126); plurality of sample holders (131) disposed in the said circular body (125) closed at the bottom and opened at the top configured to retain the milk sample within a defined area of the udder of dairy animal for testing sub-clinical mastitis; rotatable lid (126) attached to magnet configured to collect the milk from each teat avoid mixing and enable easy washing; top end of the shaft (122) provided with magnet connected to the rotatable lid (126) and the bottom end is attached to the knob(123) adapted to rotate the lid(126); displaying means (113) configured to display the pH, temperature and Q index results fixed on the front side wall of the main circular body (125) and a navigation button (117) provided thereby adapted to navigate between the display; reading means (114) configured to read the coded tag provided on the cattle being milked to identify the animal and store the information for future reference; sensing means configured to detect sample and connected with a amplifying mean and controlling means; a Real-Time Clock (RTC) (115) module coupled to the microcontroller to provide a time-stamp; the controlling means configured to communicate the detected value for each sample holder to the said displaying means for visualization (119), storing means for data storage (118), and IoT Connectivity for data transmission (119).

Another embodiment of the invention to provide a method for detecting mastitis using the device of the present invention comprising;

a) collecting milk sample from four teat of the cattle into four sample holder each teat milk sample separately;
b) transmitting the sensing signal and other signals from sensing means to amplifying means to amplify the signal before communicating to controlling means;
c) receiving the amplified signal and convert the signals into values and send to displaying means, storing means and IoT;
d) sensing means the temperature and pH
e) displaying means converting the values into different colours corresponding to higher and lower value of the cell count for respective teat, temperature and pH;
f) determining the somatic cell count with electrical conductivity for each teat with respect to the standard value to confirm the presence and absence of subclinical mastitis.

BRIEF DESCRIPTION OF DRAWINGS

Further objects and features of the present invention will be understood from the following detailed description of preferred, but non-exclusive, embodiments of the hand-held device for detecting subclinical in the teat-to-teat milk of dairy animal according to the invention, when taken in conjunction with the accompanying drawings in which like reference numerals designate like parts and wherein:

FIGURE 1: Illustrates a sectional view of the device

FIGURE 2: Illustrates top view of the device

FIGURE 3: Illustrates an isometric view of the device

FIGURE 4: Illustrates wireless charging stand for device

FIGURE 5: Illustrates a block diagram of the device and its components

FIGURE 6: Illustrates the user operating view of device for milking from teat

FIGURE 7a – 7f: Illustrates the graphs represent the EC value of Jersey, Gir, Sindhi, Holstein Friesian, Jersey crossbreed, Holstein Friesian crossbreed cows' milk respectively at different stages of lactation.

FIGURE 8a – 8f : Illustrates the graphs represent the EC value of Jersey, Gir, Sindhi, Holstein Friesian, Jersey crossbreed, Holstein Friesian crossbreed cows' milk yield respectively.

FIGURE 9 : The graphs represent a comparative analysis of that EC value of 6 different breeds such as Jersey (14 cows), Gir (31 cows), Sindhi(23 cows), Holstein Friesian(32 cows), Jersey (102 cows) and Holstein Friesian crossbreed (89 cows) that accounts for a total of 291 cows' health condition as the sensed EC values are higher for Jersey followed by Gir, Holstein Friesian, Holstein Friesian crossbreed, Sindhi and Jersey crossbreed

FIGURE 10: The graphs represent the comparison of the device with the EC meter for a period of 231 days where the data were more accurate in every aspect.

FIGURE 11a: The graphs represent the difference in 4 quarters of the device with different health conditions such as clinical, subclinical mastitis and normal cows. The result are found to be similar and accurate.

FIGURE 11b: The graphs represent the difference in quarters with respect to the CMT in cows with different health conditions such as clinical, subclinical mastitis and normal cows. The results are found to be similar and accurate.

FIGURE 12: The graphs represent the comparison of devices with other different tests for mastitis detection such as CMT, SCC, WST, NAGase test and LDH test, in which device is 100% sensitive with 99.9% specificity and accuracy.

FIGURE 13: Illustrates the Percentage (%) variation in Electrical conductivity and its coordination with somatic cell count in different stages of mastitis.

DETAILED DESCRIPTION OF THE INVENTION

The following descriptions are exemplary embodiments only, and are not intended to limit the scope, applicability or configuration of the invention in any way. Rather, the following description provides a convenient illustration for implementing exemplary embodiments of the invention. Various changes to the described embodiments may be made in the function and arrangement of the elements described without departing from the scope of the invention as set forth in the appended claims.

According to the main embodiment of the invention, it provides technology for detecting subclinical mastitis earlier in milk which is the 2nd largest cause of death in the dairy industry. Subclinical mastitis is 30-40% more prevalent when compared to clinical mastitis. However, subclinical mastitis can only be detected by diagnostics like California mastitis test (CMT), Whiteside test (WST) and SCC count. Mastitis is an inflammation of the mammary gland caused by bacterial infection - *Staphylococcus* (*S. aureus and coagulase-negative Staphylococci* (CNS)) and *Streptococci* usually *Streptococcus* (*S. uberis, S. dysgalactiae* and *S. agalactiae*). Mastitis is of extreme zoonotic importance since milk is unsafe for human consumption. Subclinical mastitis infections don't cause any visible changes in milk or udder appearance, making it difficult to detect. Physical, chemical, bacteriological changes in the milk and pathological changes in glandular tissue of the udder which reduces yield, quality of milk (~ 10%) and milk components - protein, lactose, fat leading to economic loss and often culling. The pH of mastitis milk was significantly higher and has an elevated level of SCC (score 3-5). The SCC provides a good quantitative estimate of the degree of inflammation. Subclinical form of mastitis was found to be more common in India (varying from 10-50% in cows and 5-20% in buffaloes) when compared to clinical mastitis (1-10%). In India over the past 4 decades, it was observed that 25% of cows and 15% of buffaloes are affected by a subclinical form of mastitis. With respect to mastitis prevalence, West Bengal and Tamil Nadu shows a very high prevalence. Every 3 out of 4 cows is infected in West Bengal and every 1 out of 2 cows is infected in Tamil Nadu with subclinical mastitis. Bovine mastitis is associated with a daily loss that varied from 1.0 to 2.5 kg of milk during the first two weeks and a total loss of 110 to 552 kg during the entire lactation

period. The total cost of mastitis treatment contributes towards the major proportion of the economic loss.

The developed device helps in early detection of subclinical mastitis in cattle, thereby reducing cattle death and increasing milk production. The hand-held device comprises of a four-quadrant sample collector with rotatable lid; a four-quadrant display connected to the quadrants, along with sensors, RFID reader, IoT Connectivity, battery, charging unit to process the sample, and a device holder positioned below the four-quadrant sample collector. The device of the present invention determine the quantity of the somatic cell count through electrical conductivity for each teat simultaneously and displays variation between teats regardless of breed, temperature, lactation, pregnancy, feed and water. The of the present invention detects subclinical mastitis at an early stage with 200,000 somatic cells/ml, which is equivalent to the gold standard SCC. The device also monitors the pH and temperature of milk from each teat in a steady state. The device displays results within 10 seconds and it doesn't require any expertise to interpret the results. The device includes an RFID reader for reading the RFID tag on the cow being milked and receiving the associated ID details. The device is reagent-free, thus there are no recurrent costs or consumables for the farmer. It is portable, easy to use, rechargeable, eco-friendly, and cost-effective for detecting subclinical mastitis in a variety of breeds, allowing for the procurement of high-quality milk. It minimizes milk loss by 50 to 80 %, as well as financial losses associated with milk production, and costs of keeping the herd in good condition, allowing for an earlier treatment and a shortened grace period. ·

A subclinical mastitis milk detection device and a method thereof of the present disclosure is described with reference to Figure 1 through Figure 12.

Figure 1, 2 and 3 illustrates a device for determining somatic cell count with electrical conductivity in the milk of dairy animal comprising; a main circular body (125) configured to attach with handle (121) at the bottom end of the said circular body adapted to hold the device; the top end of the circular body configured to be closed with a rotatable lid (126); plurality of sample holders (131) disposed in the said circular body (125) closed at the bottom and opened at the top configured to retain the milk sample within a defined area of the udder of dairy

animal for testing sub-clinical mastitis; rotatable lid (126) attached to magnet configured to collect the milk from each teat avoid mixing and enable easy washing; top end of the shaft (122) provided with magnet connected to the rotatable lid (126) and the bottom end is attached to the knob(123) adapted to rotate the lid(126); displaying means (113) configured to display the pH, temperature and Q index results fixed on the front side wall of the main circular body (125) and a navigation button (117) provided thereby adapted to navigate between the display; reading means (114) configured to read the coded tag provided on the cattle being milked to identify the animal and store the information for future reference; sensing means configured to detect sample and connected with a amplifying mean and controlling means; a Real-Time Clock (RTC) (115) module coupled to the microcontroller to provide a time-stamp; the controlling means configured to communicate the detected value for each sample holder to the said displaying means for visualization (119), storing means for data storage (118), and IoT Connectivity for data transmission (119). In an embodiment, before sensing the milk, the sensors are checked to be in a steady-state for providing better values of the milk.

The device (100) comprises a base (125) detachably attached lid (126) with magnetic attachment (127), a handle (121) with a rotating knob (123), and communicatively coupled to the IoT Connectivity (119). The displaying means, the LCD Display (113) is configured to indicate the following:

a) the disease condition, positive or negative with colors;
b) the status of the battery level to the user (116);
c) Time and date using RTC module (115);
d) Test number
e) Displays pH, temperature and Q Index; and
f) power on/off (120).

In an embodiment, the sensing means (130) senses the parameters such as real-time pH, temperature and Q Index of the milk sample from each teat and display in the displaying means.

In an embodiment, after power on, the subclinical mastitis milk device (100) will take 10 seconds to analyse the sample for mastitis disease and indicate the result for individual teat of the cow as following:

I. FL - Front left teat
II. FR - Front right teat
III. BL - Back left teat
IV. BR - Back right teat

The red light indicates the cattle affected by mastitis which is a positive case with a cross symbol and green light indicates the cattle without mastitis infection which is a negative case with a tick symbol

In an embodiment, the device (100) for the detecting of sub-clinical mastitis in an udder of a dairy animal by determining mastitis infected teat of the cattle. The device comprises four sample holders (131) for each individual teat. Each sample holder hold the milk directly collected from each teat of the udder of the dairy animal to be tested for sub-clinical mastitis. The device further comprises a sensing means (130) associated with a sample holder (131) to detect subclinical mastitis in the collected milk sample. An amplifying means (128) is coupled with a sample holder and sensing means for amplification, calibration and noise cancellation. Further, controlling means comprising a microcontroller (112) receives a value indicative of the collected milk samples from the amplifying means and determine subclinical mastitis in the udder of the dairy animal based on the value of the collected milk sample. The device sensing means also comprises a temperature sensor (102) coupled to the sample collector to measure the temperature of the collected milk sample and communicate a value indicative of the temperature of the collected milk sample to the microcontroller. The device also includes a Real-Time Clock (RTC) (115) module coupled to the microcontroller to provide a time-stamp, a power unit coupled to the sensing unit to provide current to the sensors, and a display unit coupled to the microcontroller to display the measured value and a diagnosis output of subclinical mastitis in the dairy animal. The device diagnosis output can be an indication of the

presence or absence of subclinical mastitis in the udder of the dairy animal obtained after the determination of the diagnosis by the microcontroller.

Accordingly, the sensing means (130) comprises a number of sensors selected, but not limited to, a temperature sensor (102) and probes (101). A 2mm probe (316l stainless steel make) is placed 1 cm apart with negative temperature co-efficient (NTC) in the middle for thermal compensation. For the calibration (104) and noise cancellation (105) of the detected values, the sensed values are transmitted through an operational amplifier (128) and processed by a microcontroller (112). An AC signal is passed to the probe placed in the milk sample. The output from the probe is amplified using the amplifier (TL074) which is converted to DC signal and passed onto the Arduino programming. Furthermore, the results of four teats are transmitted to the LCD for visualization (119), SD card for data storage (118), and IoT Connectivity for data transmission (119).

In an embodiment, the device (100) includes an RFID reader (114) for reading the RFID tag provided on the cattle being milked to receive the associated ID and to store the data of the ID associated with the corresponding cattle.

In an embodiment, the device (100) is powered by a rechargeable battery (3.7 V). On a completely charged battery, the device (100) can operate for 18 hours. After each test, the device (100) must be cleaned by removing the detachable lid (126) and flowing water through sample collectors (131). To avoid sensor damage, hot water with a temperature of more than 60 degrees Celsius must be avoided, and only RO water should be used.

In an embodiment Figure 4 illustrates a wireless charging stand comprising a transmitter (133) and a power cable (134). The device (100) is charged by a wireless charger (132) which comprises a transmitter (133) and receives Direct Current (DC) from a power source through a power cable (134). When a power receiver (106) in the device (100) is placed near the transmitter, it gets charged. The battery (108) inside the device can be charged using a wireless charger (106) by placing the device nearby to a wireless power transmitter (129). An input DC rail of 5 V powers the wireless charging transmitter, which is usually provided from a USB port

or an AC/DC power adapter. The transmitter is coupled to the wireless power receiver (106) in the device.

Figure 5 illustrates the block diagram of the controlling means comprising probes (101), temperature sensor (102), loop gain (103), low pass filter (105), operational amplifier (128), RTC module (115) and microcontroller (112). The controlling means (130) connected to the sample holders (131) configured to detect the transmitted value of the respective samples of the sample holder and communicate to the microcontroller through an amplifying means (128) adapted to amplify, calibrate and noise cancellation of the signal into a value indicative of the respective milk samples configured to determine subclinical mastitis in the udder of the dairy animal based on the value of the collected milk sample; a temperature sensor (102) configured to measure the temperature of the milk sample in the respective sample holder and communicate a value indicative of the temperature to the microcontroller; a power unit coupled to the sensing means to provide current comprising a wireless receiver (106) configured to receive power from the wireless transmitter (133) connected with a power source (134) and charge the battery (108) that provides power to the controlling means through DC booster and oscillator. The 2mm probe is placed 1 cm apart with negative temperature co-efficient in the middle for thermal compensation and the output from the probe is amplified using the amplifying means which is converted to DC signal and passed onto the Arduino module. Loop gain (103) adapted to couple with a sample collector and sensing unit for amplification of signal from the amplifying mean; precision rectifier (104) used for the calibration of the detected values; low pass filter (105) configured to cancel the noise of the detected values; oscillator (111) configured to produce a constant frequency of 9kHz to the device. Reading means (114) is an RFID reader for reading the RFID tag on the animal being milked and receiving the associated ID details. Displaying means (113) configured to display the pH, temperature and Q index results fixed on the front side wall of the main circular body (125) and a navigation button (117) provided thereby adapted to navigate between the display. Sensing means configured to detect sample and connected with an amplifying mean and controlling means; a Real-Time Clock (RTC) (115) module coupled to the microcontroller to provide a time-stamp; the controlling means configured to communicate the detected value for

each sample holder to the said displaying means for visualization (119), storing means for data storage (118), and IoT Connectivity for data transmission (119).

Figure 6 illustrates the user operating view of device for milking from teat. The device of the present invention configured to collect the milk sample directly from each teat of the udder of the cattle so as to detect the infection level of the teat to early identification of mastitis and also increase the milk production.

Referring to Figures 7a, 7b, 7c, 7d, 7e and 7f, the graphs are plotted with experimental results. Milk samples used for the testing from Jersey, Gir, Sindhi, Holstein Friesian, Jersey, and Holstein Friesian crossbreed. All the samples drawn from each teat of the sample is tested for somatic cell count in the milk. The graphs represent the differentiation of values in known milk samples of normal, sub-clinical and clinical mastitis stages as confirmed by SCC in 6 different breeds such as Jersey (14 cows), Gir (31 cows), Sindhi (23 cows), Holstein Friesian (32 cows), Jersey (102 cows), and Holstein Friesian crossbreed (89 cows) with a total of 291 cows at different lactation stages. The sensed values of sub-clinical and clinical mastitis were found to be higher than that of the EC values of normal cows.

Referring to Figures 8a, 8b, 8c, 8d, 8e and 8f, the graphs are plotted with experimental results. The graphs represent the differentiation of EC in known milk samples of normal, sub-clinical and clinical mastitis stages as confirmed by SCC in 6 different breeds such as Jersey (14 cows), Gir (31 cows), Sindhi (23 cows), Holstein Friesian (32 cows), Jersey (102 cows), and Holstein Friesian crossbreed (89 cows) with a total of 291 cows of different milk yield. The sensed EC values of sub-clinical and clinical mastitis was found to be higher than that of the EC values of normal cows.

Referring to Figure 9, the graphs are plotted with experimental results. The graphs represent the differentiation of EC in known milk samples of normal, sub-clinical and clinical mastitis stages as confirmed by SCC in 6 different breeds such as Jersey (14 cows), Gir (31 cows), Sindhi (23 cows), Holstein Friesian (32 cows), Jersey (102 cows), and Holstein Friesian crossbreed (89

cows) with a total of 291 cows. The sensed EC values are higher for Jersey followed by Gir, Holstein Friesian, Holstein Friesian Crossbreed, Sindhi and Jersey crossbreed.

Once the mastitis in milk is determined, the sample of the milk is sent to the lab for confirmation.

Referring to Figure 10, the graphs are plotted with experimental results. The graphs represent the comparison of the device (100) with EC meter for a period of 231 days where the data were more accurate in every aspect.

Referring to Figures 11a and 11b the graphs are plotted with experimental results. The graphs represent the comparison of 4 quarters in the device (100) with the CMT where it is similar and accurate.

Referring to Figure 12 the graphs are plotted with experimental results. The graphs represent the comparison of devices with other different tests for mastitis detection such as CMT, SCC test, WST, NAGase test and LDH test, in which device is 100% sensitivity, 99.9% specificity and accuracy in SCC.

Referring to Figure 13 illustrates the Percentage (%) variation in value and its coordination with somatic cell count in different stages of mastitis.

Table 1. Illustrates the result interpretation based on the Percentage (%) variation in Electrical conductivity and its coordination with somatic cell count and CMT

Sl. No	Cattle No	Quarters	Electrical Conductivity	Somatic Cell count	CMT Score	% EC Variation from least EC quarter	Interpretation
1	1	Quarter 1	4.9	100,423	N	0 %	Negative
2		Quarter 2	5.7	245,097	T	16.3 %	Sub Clinical Mastitis
3		Quarter 3	5.1	187,341	N	4.1 %	Negative
4		Quarter 4	6.1	376,213	T	24.5 %	Sub Clinical Mastitis
5	2	Quarter 1	5.6	176,983	N	9.8 %	Negative
6		Quarter 2	5.3	151,231	N	3.9 %	Negative
7		Quarter 3	5.1	119,092	N	0.0 %	Negative
8		Quarter 4	5.2	143,921	N	2.0 %	Negative
9	3	Quarter 1	5.4	172,012	N	12.5 %	Negative
10		Quarter 2	5.1	156,981	N	6.2 %	Negative
11		Quarter 3	4.8	94,234	N	0.0 %	Negative
12		Quarter 4	5.9	346,981	T	22.9 %	Sub Clinical Mastitis
13	4	Quarter 1	6.1	219,823	T	15.1 %	Sub Clinical Mastitis
14		Quarter 2	7.5	412,012	1	41.5 %	Clinical Mastitis
15		Quarter 3	5.9	185,760	N	11.3 %	Negative
16		Quarter 4	5.3	159,871	N	0.0 %	Negative
17	5	Quarter 1	6.3	159,023	N	3.2 %	Negative
18		Quarter 2	6.1	128,723	N	0	Negative
19		Quarter 3	7.9	325,009	T	29.5 %	Sub Clinical Mastitis
20		Quarter 4	7	187,072	N	14.7 %	Negative
21	6	Quarter 1	4.1	119,823	N	0	Negative
22		Quarter 2	6.3	459,871	1	53.6 %	Clinical Mastitis
23		Quarter 3	4.8	228,981	T	17%	Sub Clinical Mastitis
24		Quarter 4	5.1	372,345	T	24 %	Sub Clinical Mastitis

TECHNICAL ADVANCEMENTS

The present disclosure described hereinabove has several technical advantages including, but not limited to, the realization of, subclinical mastitis milk detecting device and a method thereof which:

i. Provides dairy cattle's mastitis detection by Q Index;
ii. Increases accuracy to predict and detect subclinical mastitis at early stage;
iii. A four-quadrant prototype detects conductivity variations, pH and temperature between teats;
iv. 100% sensitive and 99.9% Specific with respect to SCC at 200,000 – 8,000,000 somatic cells/ml stages;
v. 100% sensitive and 100% specific with respect to the CMT at all four CMT scores;
vi. Validated the test and test performance at different variable conditions like parity, lactation period, milk yield, disease progression conditions on different types of breeds;
vii. Performs easy monitoring of the dairy cattle health;
viii. Portable and waterproof for easy handling of the device;
ix. Rechargeable, eco-friendly and cost-effective; and
x. ZERO Milk wastage for testing of subclinical mastitis detection.

The device is beneficial to all three parties involved in the dairy industry: the farmer, the consumer, and the cattle.

To Dairy Farmers:

Farmers lose INR 7,824/cow in India, INR 14,530 to 35,023 /cow globally. Annual economic losses due to bovine mastitis are estimated to be Rs.7165.51 crores in India, out of which 57.93% (Rs.4151.16 crores losses) has been attributed to subclinical mastitis. Subclinical mastitis permanently damages the mammary gland, thereby causing a permanent loss in milk production which can be recovered if the animal is treated for mastitis. The occurrence of sub-clinical mastitis ranges up to 83% in cows. About 70-80% of economic loss in India has been

attributed due to subclinical mastitis. Thus, the development of the device will enable us to avoid a great economic loss to farmers as well as our country's GDP.

To Milk Consumers:

With the use of the device, subclinical mastitis is detected at a very early stage and thereby the need for usage of antibiotics for treatment of mastitis is largely reduced. The following ill effects of antibiotics can be avoided

Antibiotic resistance: The presence of a low level of antibiotic residues in milk and other dairy products causes microorganisms to be resistant to antibiotics. The resistant microbes may be transmitted among individuals via direct contact or indirectly by the exchange of resistant genes in the environment.

Allergic reactions: Residues of various antibiotics are associated with multiple types of allergic reactions, including serum sickness and anaphylaxis, especially in the case of penicillin.

Carcinogenicity: Residues of antibiotics possess potential carcinogenic impacts by interacting with cellular elements, such as DNA and RNA.

Mutagenicity: Mutagenic effect is another dangerous impact of ARs, which can cause mutation of DNA molecules or damage of chromosomes. The infertility of human beings may result from this mutation.

Teratogenicity: Various congenital anomalies may be seen in newborn children due to long term exposure to ARs during the gestation period.

Disturbances in the normal intestinal environment: Normal habitant of the intestine coexists with others and colonizes to prevent the pathogenic microbes from producing diseases. ARs in milk resulting from usage of broad-spectrum antibiotics may kill a wide range of microflora in the intestine including the non-pathogenic organisms, which can make the disease-causing microorganisms more prominent and disrupt the normal intestinal environment.

Effects on the dairy industry: The existence of ARs in milk, even in very low concentrations is of great concern in dairy industries. The residues of antibiotics can interfere with the fermentation process during the production of cheese and yoghurt by inhibiting the starter cultures.

To Cattle:

When a cow detects infection, it sends white blood cells into the udder tissue. The response by the cow's immune system may be seen in the milk as flakes or clots, or as watery milk. When the milk from cows with mastitis is tested, increases in SCCs may be seen. The large increase in SCC comes from the white blood cells flowing from blood vessels into milk. The clots in the milk are mainly formed of clumps of dead white blood cells. Blood flow to the udder increases to counteract the infection. This may result in heat, swelling, pain and redness. Mastitis also causes fever and tiredness. Mastitis adversely affects the reproductive performance of dairy cows and on average, it takes 40 days longer for the cows to get pregnant that have had a case of mastitis compared to herd mates that have not had a case of mastitis. Hence, with the developed device, early detection of subclinical mastitis is possible. This prevents the infection before it gets worse which leads to a better livestock health management.

Summarizing the present invention, a device for determining subclinical mastitis in the milk of dairy animal comprising; a main circular body (125)configured to attach with handle (121) at the bottom end of the said circular body adapted to hold the device; the top end of the circular body configured to be closed with a rotatable lid (126);plurality of sample holders (131)disposed in the said circular body (125) closed at the bottom and opened at the top configured to retain the milk sample within a defined area of the udder of dairy animal for testing sub-clinical mastitis; rotatable lid (126) attached to magnet configured to collect the milk from each teat avoid mixing and enable easy washing; top end of the shaft (122) provided with magnet connected to the rotatable lid (126) and the bottom end is attached to the knob(123) adapted to rotate the lid(126); displaying means(113) configured to display the pH, temperature and Q index results fixed on the front side wall of the main circular body (125)and a navigation button (117) provided thereby adapted to navigate between the display; reading means (114) configured to read the coded tag provided on the cattle being milked to identify the animal and store the information for future reference; sensing means configured to detect sample and connected with a amplifying mean and controlling means; a Real-Time Clock (RTC) (115) module coupled to the microcontroller to provide a time-stamp; the controlling means

configured to communicate the detected value for each sample holder to the said displaying means for visualization (119), storing means for data storage (118), and IoT Connectivity for data transmission (119).

The controlling means comprising probes (101), temperature sensor (102), loop gain (103), low pass filter (105), operational amplifier (128), RTC module (115) and microcontroller (112).The four sample holders (131) adapted to hold milk sample from four udder of the animal separately incorporated in the four quadrants of the said circular body (125). The wall of the sample holder is transparent wall which is made up of glass, polymeric material, composite material and the like. The wall thickness of the sample holder is between 5mm and 45mm. The reading means (114) is an RFID reader for reading the RFID tag on the animal being milked and receiving the associated ID details.

The device (100) is detachably attached to a magnetic rotatable lid configured to close and remove the main base body of the said device (100). The controlling means (130) connected to the sample holders (131) configured to detect Q index of the respective samples of the sample holder and communicate to the microcontroller through an amplifying means (128) adapted to amplify, calibrate and noise cancellation of the signal into a value indicative of the respective milk samples configured to determine subclinical mastitis in the udder of the dairy animal based on the value of the collected milk sample; a temperature sensor (102) configured to measure the temperature of the milk sample in the respective sample holder and communicate a value indicative of the temperature to the microcontroller; a power unit coupled to the sensing unit to provide current to the sensor unit. The sample to be detected is milk of cattle/animal is cow, buffalo, camel, donkey, goat, horse, sheep, water buffalo and crossbreed thereof.

The 2mm probe is placed 1 cm apart with negative temperature co-efficient in the middle for thermal compensation and the output from the probe is amplified using the amplifying means which is converted to DC signal and passed onto the Arduino module. The handle (121) comprising power storing means (108) configured to store power; a wireless charger (106)

adapted to charge the said power storing means by placing the device near a wireless power transmitter (129) is coupled to the wireless power receiver (106) in the device where an input DC rail of 5V powers the wireless charging transmitter is adapted to provide from a USB port or an AC/DC power adapter; a charging point (107) provided at the bottom of the handle configured charge the power storing means (108); power control (120) configured to switch on/off the device.

The Loop gain (103) adapted to couple with a sample collector and sensing unit for amplification of signal from the amplifying mean; precision rectifier (104) used for the calibration of the detected values; low pass filter (105) configured to cancel the noise of the detected values; oscillator (111) configured to produce a constant frequency of 9kHz to the device. The charging unit (124) configured to charge the device using wireless charger with the wireless charging stand (132) comprising a transmitter adapted to transmit the power signals for charging the device.

The method for determining subclinical mastitis using the device comprising; collecting milk sample from four teat of the cattle into four sample holder each teat milk sample separately; transmitting the sensing signal and other signals from sensing means to amplifying means to amplify the signal before communicating to controlling means; receiving the amplified signal and convert the signals into values and send to displaying means, storing means and IoT; sensing means the temperature and pH; displaying means converting the values into different colours corresponding to higher and lower value of the cell count for respective teat, temperature and pH; determining subclinical mastitis for each teat with respect to the standard value to confirm the presence and absence of mastitis.

The foregoing disclosure has been described with reference to the accompanying embodiments which do not limit the scope and ambit of the disclosure. The description provided is purely by way of experiments and illustrations.

CLAIMS

We claim,

1. A device for determining subclinical mastitis in the milk of dairy animal comprising; a main circular body (125)configured to attach with handle (121) at the bottom end of the said circular body adapted to hold the device; the top end of the circular body configured to be closed with a rotatable lid (126);plurality of sample holders (131)disposed in the said circular body (125) closed at the bottom and opened at the top configured to retain the milk samplewithin a defined area of the udder of dairy animal for testing sub-clinical mastitis; rotatable lid (126) attached to magnet configured to collect the milk from each teat avoid mixing and enable easy washing; top end of the shaft (122) provided with magnet connected to the rotatable lid (126) and the bottom end is attached to the knob(123) adapted to rotate the lid(126); displaying means(113) configured to display the pH, temperature and Q index results fixed on the front side wall of the main circular body (125)and a navigation button (117) provided thereby adapted to navigate between the display; reading means (114) configured to read the coded tag provided on the cattle being milked to identify the animal and store the information for future reference; sensing means configured to detect sample and connected with a amplifying mean and controlling means; a Real-Time Clock (RTC) (115) module coupled to the microcontroller to provide a time-stamp; the controlling means configured to communicate the detected value for each sample holder to the said displaying means for visualization (119), storing means for data storage (118), and IoT Connectivity for data transmission (119).

2. The device as claimed in claim 1, wherein the controlling means comprising probes (101), temperature sensor (102), loop gain (103), low pass filter (105), operational amplifier (128), RTC module (115) and microcontroller (112).

3. The device as claimed in claim 1, wherein four sample holders (131) adapted to hold milk sample from four udder of the animal separately incorporated in the four quadrants of the said circular body (125).

4. The device as claimed in claim 1, wherein wall of the sample holder is transparent wall which is made up of glass, polymeric material, composite material and the like.

5. The device as claimed in claim 1, wherein the wall thickness of the sample holder is between 5mm and 45mm.

6. The device as claimed in claim 1, wherein reading means (114) is an RFID reader for reading the RFID tag on the animal being milked and receiving the associated ID details.

7. The device as claimed in claim 1, wherein the device (100) is detachably attached to a magnetic rotatable lid configured to close and remove the main base body of the said device (100).

8. The device as claimed in claim 1, whereincontrolling means (130) connected to the sample holders (131) configured to detect Q index of the respective samples of the sample holder and communicate to the microcontroller through anamplifying means (128) adapted to amplify, calibrate and noise cancellation ofthe signal into a value indicative of the respective milk samples configured to determine subclinical mastitis in the udder of the dairy animal based on the value of the collected milk sample; a temperature sensor (102) configured to measure the temperature of the milk sample in the respective sample holder and communicate a value indicative of the temperature to the microcontroller; a power unit coupled to the sensing unit to provide current to the sensor unit.

9. The device as claimed in claim 1, wherein the sample to be detected is milk of cattle/animal is cow, buffalo, camel, donkey, goat, horse, sheep, water buffalo and crossbreed thereof.

10. The device as claimed in claim 1, whereinthe 2mm probe is placed 1 cm apart with negative temperature co-efficient in the middle for thermal compensation and the output from the probe is amplified using the amplifying means which is converted to DC signal and passed onto the Arduino module.

11. The device as claimed in claim 1, wherein the handle (121) comprising power storing means (108) configured to store power; a wireless charger (106) adapted to charge the said power storing means by placing the device near a wireless power transmitter (129) is coupled to the wireless power receiver (106) in the device where an input DC rail of 5V powers the wireless charging transmitter is adapted to provide from a USB port or an AC/DC power adapter; a charging point (107) provided at the bottom of the handle configured charge the power storing means (108); power control (120) configured to switch on/off the device.

12. The device as claimed in claim 1, wherein Loop gain (103) adapted to couple with a sample collector and sensing unit for amplification of signal from the amplifying mean; precision rectifier (104) used for the calibration of the detected values; low pass filter (105) configured to cancel the noise of the detected values; oscillator (111) configured to produce a constant frequency of 9kHz to the device.

13. The device as claimed in claim 1, wherein the charging unit (124) configured to charge the device using wireless charger with the wireless charging stand (132) comprising a transmitter adapted to transmit the power signals for charging the device.

14. A method for determining subclinical mastitis using the device comprising;

a) collecting milk sample from four teat of the cattle into four sample holder each teat milk sample separately;

b) transmitting the sensing signal and other signals from sensing means to amplifying means to amplify the signal beforecommunicating to controlling means;

c) receiving the amplified signal and convert the signals into values and send to displaying means, storing means and IoT;

d) sensing means the temperature and pH

e) displaying means converting the values into different colours corresponding to higher and lower value of the cell count for respective teat, temperature and pH;

f) determining subclinical mastitis for each teat with respect to the standard value to confirm the presence and absence of mastitis.

Dated this the 06th day of June, 2022

Arokya Nithya. D (IN/PA-4159)

On behalf of the Applicant

ABSTRACT

A DEVICE FOR EARLY DETECTION OF SUBCLINICAL MASTITIS IN THE MILK OF DAIRY ANIMAL

A device for early detection of subclinical mastitis in the milk of dairy animal relates to a device for detecting early subclinical mastitis in milk of the cattle which is useful in Animal husbandry and Dairy technology, particularly relate to a device for determining somatic cells through electrical conductivity from the milk sample of cattle. More particularly the present invention further detects subclinical mastitis along with pH, the temperature of milk, point of care subclinical mastitis detection device for cattle thereof. The device (100) detects teat to teat milk variation for subclinical mastitis along with pH and temperature of the milk. The device helps in reducing milk loss and financial losses. It is simple to operate, portable, robust, reagent free, has no wastage of milk and works for all breeds of cattle.

Figure 1

