

On-site Quick Measurement of Calcium Ion in Blood of Dairy Cow with Flat Ion Sensor

Rika UTSUMI

Hypocalcemia due to decreases of calcium ions (iCa) in blood of dairy cows is a common disease that affecting many perinatal dairy cows. The iCa measurement and quick treatment are strongly required in order to keep dairy cow healthy and maintain milk production. However, the iCa concentration cannot be measured quickly on site because accurate measurement of iCa requires strict anaerobic environment and temperature control. In this paper, we introduce that the iCa concentration in blood can be measured quickly on site by the use of the LAQUAtwin calcium ion meter.

Introduction

A calcium ion (iCa) concentration in blood of perinatal dairy cows tends to decrease physiologically.^[1] There is a possibility that a significant drop of iCa concentration causes symptoms such as decreased consciousness and postparturient paraplegia,^[1] and it also is the cause of other perinatal diseases.^[2, 3] These series of symptoms called hypocalcemia are one of the major diseases of dairy cows.^[1] The subclinical hypocalcemia in these symptoms that does not show various symptoms is a common disease affecting about 50% of perinatal dairy cows, and one of the problems in the health management of dairy cows in order to maintain milk production.^[4]

It is necessary to analyze the iCa concentration that has the physiological activity in the total calcium (tCa) contained in blood in order to understand the health condition of dairy cows.^[4, 5]

For analysis of the tCa concentration, some reagents are added to a sample and reacts with calcium of various forms, and the measurement method with the detector of the optical system is used. This optical method in a laboratory can provide the stable, easy, and inexpensive measurement. However, the optical analyzer for the tCa concentration is large and cannot be easily used on site, and further, it does not offer quick results because of the necessity of the pretreatment for sample. In addition, the prediction of the variation of the iCa concentration with the tCa concentration has a difficulty, and the tCa is also influenced by the variation of albumin concentration due to hydration condition and physiological change of dairy cows.^[4] Therefore, the tCa concentration cannot be easily measured on site, and the iCa concentration cannot be

estimated from the tCa concentration to appropriately understand the health condition of the dairy cow.

On the other hand, the measurement of the iCa concentration doesn't require pretreatment of the sample, and whole blood can be measured as it is. However, the iCa concentration easily varies by the variation of the pH value in the sample. In order to determine the iCa concentration accurately without the influence by the pH value variation, strict anaerobic conditions and temperature control are required. In order to realize these conditions, a flow-through cell is generally utilized in the measurement system of the iCa concentration, but the device becomes complicated and large, and the measurement cost also becomes expensive.^[7] Therefore, it is difficult to measure the iCa concentration quickly on site and perform appropriate treatment to dairy cows.

The LAQUAtwin series are a compact water quality meter based on an ion selective electrode. Figure 1 shows a

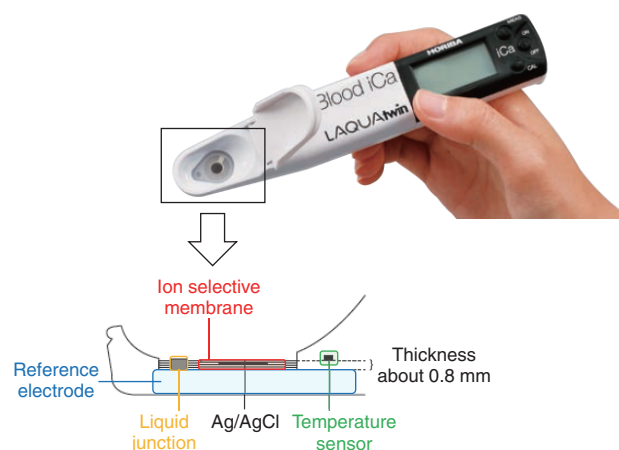


Figure 1 Schematic diagram of the ion flat sensor of LAQUAtwin

schematic diagram of the ion sensor used for LAQUAtwin series. A flat sheet of about 0.8 mm in thickness is utilized in the measurement part. A liquid junction of the reference electrode and an ion selective membrane are located on the same sheet. This flat sensor enables us to measure as little as 0.3 mL of sample volume required for measurement. In addition, since the unit where a measurement part and display are integrated has a size that can be carried by one hand, the ion concentration can be easily measured on site.

In this paper, we introduce that the problem of measuring the iCa concentration quickly on site which the conventional measurement method could not solve by can be solved by the use of the LAQUAtwin calcium ion meter.

Forms of Calcium in Blood

The tCa exists in blood mainly in three forms (Figure 2). 50% of tCa is bound to a protein such as albumin and 5% is bound to an organic acid such as citric acid or phosphoric acid, and the remaining 45% is present in an ionized state (iCa).^[5] The iCa contributes to neuromuscular excitation, blood coagulation, enzyme reaction, hormone and neurotransmitter release reaction and so on. Only iCa has physiological activity.^[5, 6, 8] This is a reason why analysis of the iCa concentration is needed to understand the health condition of dairy cows.^[4] The iCa concentration in blood of dairy cows is considered to be normal between 1.05 and 1.30 mmol dm⁻³, although there are some differences between study examples, and hypocalcemia is diagnosed at 0.9 mmol dm⁻³ or less.^[5]

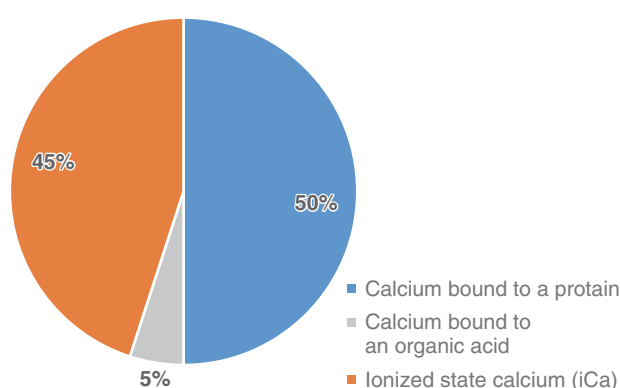
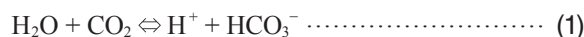


Figure 2 Ratio of forms of calcium in blood

Change of the iCa Concentration with pH Change

The pH value in blood of a living body is strictly maintained between 7.3 and 7.4.^[9] The pH value in blood fluctuates according to the equilibrium state of bicarbonate

ion (Equation 1), and this bicarbonate ion buffer system is the most important biological system in the buffer systems that maintain the homeostasis of the living body.^[10]



However, the pH value in the sample after blood collection easily changes due to the influence of various factors such as the method of sample pretreatment, an ambient temperature and time under aerobic conditions and so on.^[7] Although 50% of tCa is bound to proteins such as an albumin, this binding state varies with the pH value in blood. A schematic diagram of the equilibrium state of iCa, pH and albumin in blood is shown in Figure 3. For example, when a sample temperature rises, the solubility of carbon dioxide decreases, then the reaction of Equation 1 moves to the left, and the pH value increases because H⁺ concentration decreases. The reduced H⁺ is maintained in equilibrium by dissociation of H⁺ bound to albumin, but albumin that supplied H⁺ binds to iCa and shifts to a stable state. Therefore, the pH value increases, consequently, the iCa concentration decreases. As described above, since the pH value in the sample changes due to various factors, the pH value change, that is, the iCa concentration change easily occurs.

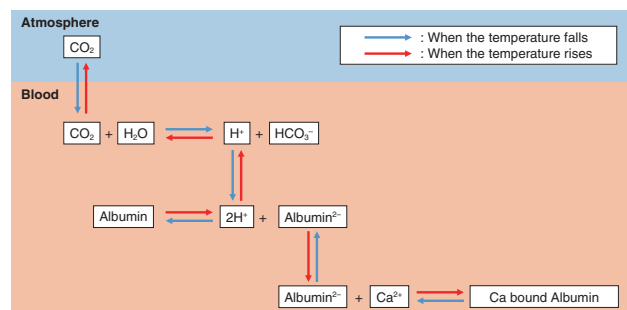


Figure 3 Schematic diagram of the equilibrium state of iCa, pH and albumin in blood with temperature change

Problem of iCa Measurements by the Use of Flat Sensor

An instrument with a flow-through cell is generally used to suppress the variation of the pH value in samples in order to measure the iCa concentration. These instruments enable calibration and measurement under an anaerobic environment and at a constant temperature, thus can minimize degradation of the sample being measured. Therefore, these instruments enable the high accurate measurement of the iCa concentration, and are widely used in a clinical site not only for dairy cows but also for humans. This instrument is not suitable for on-site use to measure the iCa concentration and determine the

treatment for each dairy cows because the instrument for laboratory use is complicated and difficult to handle in order to realize high accurate measurement.

On the other hand, the instrument equipped with a flat sensor in open system can be miniaturized because the amount of samples required are small, and the measurement can be conducted anywhere. The instrument based on the flat sensor in open system is suitable for measuring the iCa concentration in blood of dairy cows simply on site. However, an equilibrium reaction of carbon dioxide between an air and a sample and a temperature change due to an influence of the outside air temperature always occur in flat sensor system because the sample cannot be sealed and an amount of the sample is small. As a result, the pH value in the sample where such open system is very variable. The instrument with an open sensor cannot realize the high accurate iCa measurement, therefore it cannot be utilized for the diagnosis of hypocalcemia.

The iCa concentration influence a health of dairy cows and ultimately affects milk production. Accordingly, the judgment of the health condition of dairy cows and quick treatment is strongly required at dairy farm. Although the conventional analysis instrument enables the high accurate iCa measurement, the iCa concentration cannot be measured on site quickly. If the screening of dairy cows for the detection of hypocalcemia with iCa concentration can be easily done on site, a dairy cow that needs a detailed examination can be detected even if it is a flat sensor that cannot measure the iCa concentration with high accuracy. In addition, the time for the treatment and the expense of examination and treatment can be reduced. In this paper, the feasibility of the screening of dairy cows for the detection of hypocalcemia is demonstrated with a flat sensor and contents of evaluation are introduced below.

Customize to Make a Device Dedicated to iCa Measurement

In the ion measurement by the use of an ion selective electrode, an activity of ions is obtained by the measured cell voltage between ion selective membrane and reference electrode in a sample. Since the flat sensor which has been used in the conventional LAQUAtwin series was intended for measurements in various aqueous solutions, the iCa concentration in an internal solution of the sensor has been adjusted to 50 mmol dm⁻³. In this paper, the iCa concentration in the internal solution of the sensor was adjusted to 1 mmol dm⁻³ in order to reduce a temperature effect of the sample during measurement. The 1 mmol dm⁻³ concentration was determined from an average value of the iCa concentration in blood of a dairy cow.

The calibration of a sensor with standard solutions is necessary before every measurement. A solution containing

target ions to measure is commonly used for calibration of an ion sensor. However, sodium ions, potassium ions and other ions contained in blood influence the iCa concentration. In order to reduce this influence the calibration was performed by the use of solutions with composition similar to an electrolyte composition of blood.

Furthermore, there is a concern that a surface of a flat sensor may be contaminated due to adhesion of solid components in blood when measuring blood. The flat sensor has a larger area that contacts the sample than the flow-through cell. Consequently, dirt easily adheres to the surface, which causes a decrease in sensor sensitivity. This was prevented by washing the surface with a dedicated cleaning solution which a proteolytic enzyme was added.

Confirming and decision of the following three points is necessary in order to measure the iCa concentration in blood of a dairy cow with LAQUAtwin with customized flat sensor, standard solutions and cleaning solutions and prove that the screening by using of LAQUAtwin is feasible.

1. Accuracy must be within an acceptable degree that does not cause misdiagnosis when screening the iCa concentration
2. The iCa concentration of a dairy cow suffering from hypocalcemia is lower than that of a normal dairy cow
3. The determination of the optimum temperature condition considering the changes in the iCa concentration due to differences in sample temperature.

Confirmation of Accuracy by Measurement of Standard Substance of NIST

Standard substances of NIST (STANDARD REFERENCE MATERIAL 956 d Electrolytes in Frozen Human Serum) was measured in order to confirm whether the accuracy was within an acceptable degree that would not cause misdiagnosis. The measured value by LAQUAtwin was compared with the predetermined concentration of standard substance. Concentrations of standard substances are three types of 1.17 mmol dm⁻³, 1.47 mmol dm⁻³, and 1.77 mmol dm⁻³. Measurement results are shown in Figure 4. ○ in the figure indicates the measured value by LAQUAtwin, and error range from the concentration of standard substances are indicated by a dotted line. Measured values by LAQUAtwin were within the range of ±20% of concentrations of all three standard substances. Concentrations of standard substances is predetermined by an instrument with a flow-through cell. The reason of the difference between measured values by LAQUAtwin and concentrations of standard substances was changes of the pH value that occurred during the measurement due to the difference of measurement

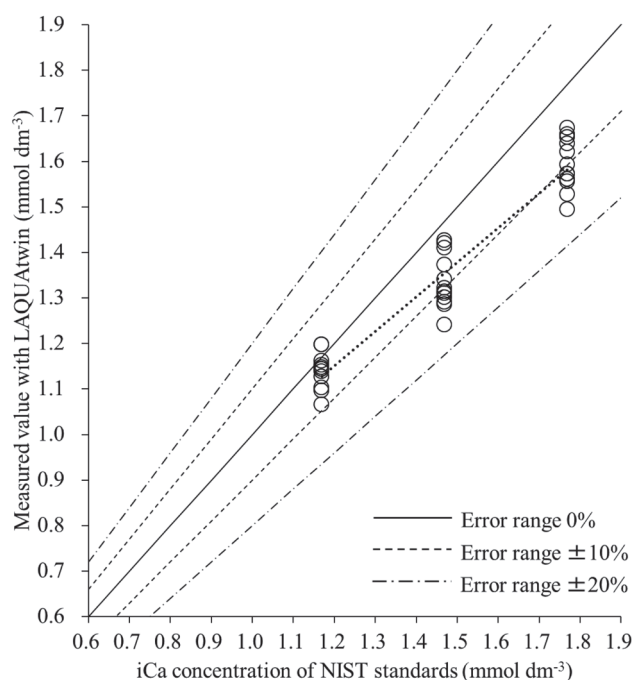


Figure 4 Measurement results by LAQUAtwin of standard substances of NIST

system. A normal value of the iCa concentration in blood of a dairy cow is about 1.2 mmol dm^{-3} , and hypocalcemia is suspected when the iCa concentration is 0.9 mmol dm^{-3} or less. Even if LAQUAtwin has an error of $\pm 20\%$, a risk of misdiagnosis is low, and LAQUAtwin has a satisfactory accuracy as a checker for the screening.

Sample Measurement of Dairy Cow Suffering from Hypocalcemia

Blood samples of a dairy cow suffering from hypocalcemia which is the actual measurement target were measured in order to confirm to show the change in the iCa concentration. A measured value by an ion electrode used

for a blood gas analyzer ABL 800 FLEX (manufactured by Radiometer Medical ApS, hereinafter ABL) was used as a standard of the iCa concentration. This ion electrode of ABL uses a flow-through type cell, and performs calibration and measurement at 37°C . Blood samples were collected from a tail of the dairy cow and lithium heparin was added as an anticoagulant. Results of measurement of blood sample by LAQUAtwin and ABL were shown in Figure 5. In this figure, an average value measured three times by two sensors of LAQUAtwin was indicated by \circ , and maximum and minimum values were indicated by error bars. Measured values of ABL was indicated by \triangle . In this dairy cow, the iCa concentration was $1.25 \text{ mmol dm}^{-3}$ before parturition, then the iCa concentration decreased to $0.94 \text{ mmol dm}^{-3}$ immediately after parturition. As a result, this dairy cow presented with symptoms of hypocalcemia and suffering from diseases caused by hypocalcemia. After that, surgery and treatment were performed, and the iCa concentration recovered to $1.18 \text{ mmol dm}^{-3}$. The measured value of LAQUAtwin was $0.10 \text{ mmol dm}^{-3}$ lower on average than the measured value of ABL. As described above, the measurement system and the measurement temperature are different between the flat sensor of LAQUAtwin and ABL. When the measurement is performed with a flat sensor, the carbon dioxide concentration in the sample decreases during measurement and the pH value increases, and as a result, the iCa concentration decreases. This is a cause of the difference between measured values by LAQUAtwin and ABL. On the other hand, measured values by LAQUAtwin was able to show changes of the iCa concentration depends on the health condition of this dairy cow. In the conventional analysis of the iCa concentration, it is necessary to return to a laboratory where the device is installed after collecting blood samples, and perform measurement after pre-treatment of blood samples. The iCa concentration by

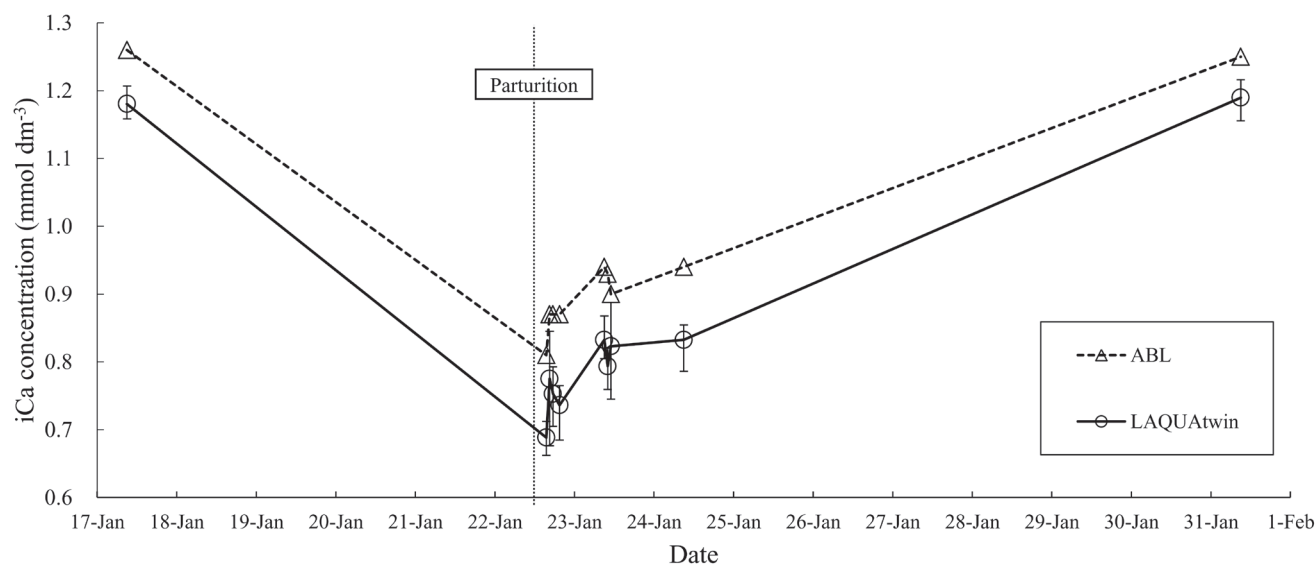


Figure 5 Measurement results by LAQUAtwin and ABL of blood samples of dairy cow suffering from hypocalcemia

using LAQUAtwin can be easily checked on site without such a pretreatment and sample transportation. LAQUAtwin cannot be used for diagnosis of hypocalcaemia but it can find a dairy cow with possibility of hypocalcaemia. LAQUAtwin was sufficiently useful as a screening checker in order to lead to a close examination.

Determine of the Optimum Sample Temperature Condition

A sample temperature was adjusted arbitrarily and measurement was performed in order to confirm changes in the iCa concentration due to changes of sample temperature and determine the optimum condition for measurement. Samples adjusted at any temperature using a water bath and were measured. Measurement results at each sample temperature were shown in Figure 6. The vertical axis in this figure shows the difference between measured values of the two devices obtained by subtracting measured values by ABL from measured values by LAQUAtwin. Error bars indicated maximum and minimum values of measured values by LAQUAtwin. When the sample temperature was 15°C or less, measured values by LAQUAtwin were about 0.3 to 0.4 mmol dm⁻³ higher than measured values by ABL. This fall in temperature caused the solubility of carbon dioxide to increase and the pH value decreased, as a result, the iCa concentration increased. On the other hand, when the sample temperature was 30°C or more, measured values by LAQUAtwin was about 0.1 mmol dm⁻³ lower than the measured value by ABL. Contrary to the previous temperature condition, the solubility of carbon dioxide decreased and the pH value increased as the temperature rose, and the iCa concentration became lower. When the sample temperature was 20 to 25°C, the difference between measured value by LAQUAtwin and ABL used as the reference of the iCa concentration was 0.1 mmol dm⁻³ or less. Therefore, the temperature change of samples causes the pH value

change, and consequently, the iCa concentration changes. In addition, in the measurement with LAQUAtwin, it was found that by adjusting the temperature of the sample to 20 to 25°C, a high correlation with ABL in which the measurement is performed with a flow-through type cell can be obtained.

Conclusions

The problem of quick measurement of the iCa concentration on site is solved by the use of a flat sensor. The method of measuring the iCa concentration in blood with a flat sensor that is an open system is a new idea that has never really been seen before. In addition, measurement of sodium ion or potassium ion in blood is also very important for health management of dairy cows, and there are many more needs besides iCa. We will keep to develop application that makes use of the advantages of LAQUAtwin that can be measured easily and quickly on site, and will contribute to diverse needs not only in the dairy industry but in all industries.

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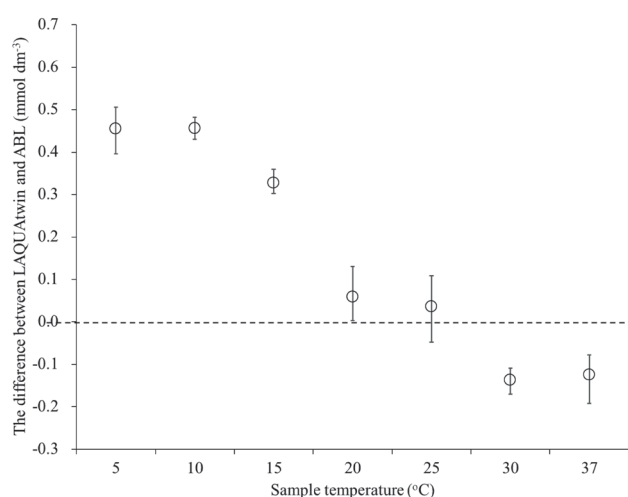


Figure 6 The difference between measured value by LAQUAtwin and ABL of blood samples with each temperature

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Rika UTSUMI

New Product Development Department 1
Research & Development Division
HORIBA Advanced Techno, Co., Ltd.