Advanced version of the Diralec system: a technology for the analysis of the quality of milk

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ABSTRACT

The National Center of Scientific Researches (CNIC) and the National Center of Agricultural Health (CENSA) have being working jointly for more than a decade on the development of the Diralec® system to evaluate the bacteriological quality of milk. Diralec-02 is the most recent version (in developing phase) of the family of Diramic® equipments; it is an autonomous system and it will guarantee the communication with a computer through a USB port. It will allow having a database that includes all the elements for the statistical analysis of the behavior of the bacteriological quality of milk. The new equipment will have as a main element a microcontroller that will control all its operations and will allow determining: the level of contamination by *Staphylococcus sp.*, by coliform bacteria, the presence of bacterial growth inhibitors, pH and to diagnose the subclinical mastitis in milk, fitted to the requirements of the international regulation. Also, this system will have to classify the raw milk in "excellent", "good", "fair" and "bad", and the pasteurized milk, "complying the standard" and "Not complying with the standard". This classification will be done as from the total count of cells present in the sample.

Keywords: quality, diagnosis, Diralec-02, milk, mastitis

RESUMEN

Versión avanzada del sistema Diralec: una tecnología para el análisis de la calidad de la leche. El Centro Nacional de Investigaciones Científicas (CNIC) y el Centro Nacional de Sanidad Agropecuaria (CENSA) han venido trabajando conjuntamente por más de una década en el desarrollo del sistema Diralec® para evaluar la calidad bacteriológica de la leche. Diralec-02 es la versión (en fase de desarrollo) más reciente de la familia de equipos Diramic®; es un sistema autónomo y asegurará la comunicación con una computadora a través de un puerto USB. Ello permitirá contar con una base de datos que incluya todos los elementos para el análisis estadístico del comportamiento de la calidad bacteriológica de la leche. El nuevo equipo tendrá como elemento principal un microcontrolador que gobernará todas sus operaciones y permitirá determinar: el nivel de contaminación por *Staphylococcus sp.*, por bacterias coliformes, la presencia de inhibidores del crecimiento bacteriano, el pH y diagnosticar la mastitis subclínica en la leche, ajustado a las exigencias de la normativa internacional. Además, este sistema deberá clasificar la leche cruda en "excelente", "buena", "regular" y "mala", y la leche pasteurizada, "en norma" y "fuera de norma". Esta clasificación se hará a partir del conteo total de células en la muestra.

Palabras clave: calidad, diagnóstico, Diralec-02, leche, mastitis

Introduction

Milk constitutes one of the fundamental foods for man, and its demand increases in the course of time up to the present days. The increase of the indicators of its quality is fundamental, due to the importance of giving a more nourishing product to the consumer and with fewer risks for his health. The safety of the quality of the milk begins in the dairy farm, thanks to the requirements with the quality of milk products. In addition to the nourishing requirements, the hygiene parameters, the presence of residues of medicines and the affectation by mastitis, they are decisive in the determination of the quality of milk as raw material [1].

In Cuba there are technological limitations and obsolescence in the laboratory equipments dedicated to the study of the quality of milk, which restricts the scope and the analytical possibilities, and does not allow the establishment of all the Good Practices principles [2]. More than 150 of these laboratories do not have analytical technologies that allow determining the basic indicators of quality of milk, as the detection of the subclinical mastitis, the residues of antibiotics and the presence of pathogenic microorganisms [3], and so there is not a real evaluation of the situation of these indicators in the country. A similar problem appears in countries from the Third World, which have the need to improve their competitive capacity on the market of the milk sector, both in the national scope and in the international market [4].

The Diralec® system, developed by the National Center of Scientific Researches of Cuba (CNIC), is based on the detection of the bacterial growth, which happens in a culture medium inoculated with the sample that we want to study. The detection of the

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bacterial growth is carried out from the measurement of the changes of turbidity that take place in a culture medium inoculated with the sample under analysis in a period of 8 hours. The magnitude of the growth is measured in cfu/mL, by means of the application of photometric methods, developed specifically for this purpose [5].

With the use of the Diralec® system, the quality of milk is estimated from:

a) The level of contamination of the raw milk at 8 hours from the turbidity changes.

b) According to the sensitivity that is being searched for, the pasteurized milk is preincubated for 6 or 15 hours and the samples are classified into two categories, "Complying with standard" or "Not complying with standard" [6]. In none of two cases, the readings are destructive and it allows continuing sampling for a better interpretation.

Antecedents and the current state of the topic

The CNIC has developed the Diralec® system to evaluate the bacteriological quality of milk from samples of raw or pasteurized milk. The first version of this system was set up in the Diramic 03c equipment at the beginning of the 90s (Figure 1). The Diramic 03c is based on the Z80 microprocessor. At the end of this decade arose a new version of the Diralec® system, and a personal computer was used as support.

The Diralec® system, installed in the Diramic 03c equipment, had pronounced limitations for the processing and storage of the data, characteristic of the microprocessor technology, so as soon as the initial production was exhausted, this model was not produced any more.

The computerized Diralec® system uses a data input card as communication interphase with a personal computer (Figure 2). This ISA input card is placed in a bus, present in the ancient computers. Due to the rapid technological development, this type of bus has been eliminated from the current computers. Also, the constructive characteristics of the new models have changed notably, so it's more difficult the assembling and the manipulation of the system inside the computer.

Figure 1. Diralec® System connected to the Diramic 03c equipment.

Figure 2. Computerized Diralec® System.

In Cuba there is a network of equipments, integrated by ten milk complexes, which use the Diralec® system installed in the Diramic 03c equipment. Also, the Ministry of Higher Education (MES) has a network of the computerized Diralec® system, integrated by eight universities.

Systems to determine the quality of milk

MK 2.5 Fat meter

The MK 2.5 fat meter allows determining rapidly and with precision the percentage of fat in milk and in its derivatives.

MK 540 Electronic digital Cryoscope and MC 5400 electronic cryoscope

The MK 540 and MC 5400 electronic cryoscopes are used for the determination of the freezing point of the milk. The normal freezing point of the milk is constant, although are differences depending on the habitat of the cow, of the race and of its feeding, among other reasons. When determining this parameter, it might be necessary, even quantitatively, if the milk was adulterated by the addition of water, since if it is so, the freezing point is close to 0 °C [7].

Bax System

Bax System analyzes the presence of pathogenic bacteria in the food, by means of the detection of the DNA [8].

Bentley 150 Combi, Bentley 2300 Combi, Bentley 2500

Bentley 150 Combi, Bentley 2300 Combi and Bentley 2500 is a family of infrared precision analyzers to determine fat, protein, lactose, count of somatic cells and total solids in milk samples and other milk products [9].

The family of equipments LactoScope Filter-Model C3, LactoScope Filter-Model C4+ and LactoScope FTIR Auto 400, based on the technology of infrared absorption, allows determining the content of fat, protein and total fatty solids and non fatty in samples of fluid milk, ice creams, concentrated milk, evaporated milk and concentrated serum [10].

SomaScope-Model MKII Auto 200 and SomaScope-Model MKII Auto 400 are counters of somatic cells

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8. http://www.foodqualitynews.com/ news/ng.asp?id=13242-bax-system- adopted. Consultado 29 de marzo del 2007.

9. http://www.bentleyinstruments.com/ combi.html. Consultado 29 de marzo del 2007.

10. http://www.deltainstruments.com/. Consultado 29 de marzo del 2007.

are, and they base on the technology of flow cytometry of fluorescence. The flow cytometry is an analytical technique recognized by the International Federation of milk products (IDF). The precision of this system allows determining between 10^5 and 10^6 cells/mL in samples of raw milk. CombiScope-Model FTIR 400 is an integrated system of analysis of raw milk, which consists of a spectrometer LactoScope FTIR and a flow cytometer of SomaScope, and it allows determining the composition of milk, carrying out the somatic cells count and determining its freezing point [10].

The FOSS Company provides a versatile scale of solutions, from the laboratory line up to solutions in production line to analyze raw milk and finished milk products. The solutions include analysis of the composition, inventory of somatic cells and hygienic quality of the raw milk, as well as composition and microbiological analysis of finished products like cheese and yogurt. Some of the systems produced by FOSS are: BactoScan ™ FC, Fossomatic ™ 5000, MicroFoss ™, MilkoScan Minor, MilkoScan ™ FT2 and CombiFoss ™ 5000 [11].

Due to the technology that they use, the processing speed and the high volumes of samples that these systems allow to analyze, they are very expensive; their price ranges between 50 000 and 150 000 dollars. Generally there are solutions directed to the production line of big plants or milk complexes, as well as for concentrating laboratories of big quantities of samples, which does not correspond with the cattle infrastructure of Third World countries or of limited development in the dairy sector.

Diralec-02 System: new applications

Diralec-02 is an autonomous equipment and it will assure a communication through a USB port with a personal computer, which will allow having an extensive database that includes all the elements for the statistical analysis of the behavior of the bacteriological quality of the milk. Among its new applications, this system allows determining the presence of bacterial growth inhibitors, as well as the pH of the sample and the diagnosis of the subclinical mastitis, fitted to the requirements of the international regulation: variables of big importance to evaluate the quality of milk. Also, as the previous versions, it will allow determining the level of contamination by coliform bacteria and the total count of bacteria in the milk.

The electrical conductivity and the mastitis

The bovine mastitis is the most common disease of the milch cow. Its damage implies a huge economic expense, not only due to the losses that it causes to the primary manufacturers, but also to the dairy industry; among other reasons, because of the decrease in the yield and the quality of milk, associated with the increase of the number of somatic cells [12].

The milk has conductive properties due to its components, especially the mineral salts. The electrical conductivity owes to the total ionic content of the milk, mainly of chlorine, sodium and potassium, and in smaller extent, of calcium, magnesium and phosphorus. It is determined, mainly, by the ions of sodium and chloride [13-15].

The increase of the cellular permeability associated with the bacterial infections and with other physiological or traumatic changes are the cause of a decrease of the lactose and of the increase of sodium and chlorides [16].

The use of the electrical conductivity as indicator of the health state of the udder has been perfected in the last 30 years, and from the 90's of the XXth century, new criteria have been established [17]. The design of computerized systems of measurement coupled to the milking equipments allow obtaining the information from the electrical conductivity, the temperature and the volume of milk of every fourth, and to detect possible changes, before the clinical symptoms of the mastitis appear. The rapidness and possibility of integration of several indicators make this method have a practical value superior to other well-known procedures of diagnosis [18].

Bacterial growth inhibitors in milk

Due to its wide use in the veterinary medicine, the antibiotics are the main residues in milk. They are used in the cattle as therapeutic and prophylactic medicines. Together with the detergents and disinfectants, the antibiotics are included in the group of substances that inhibit the bacterial growth, known commonly as "inhibitors". In this context, they have a fundamental role. The biggest source of contamination of the milk is the intramammary treatments [19].

The presence of antimicrobial agents in the milk creates numerous disadvantages to the milk industry, since the majority of them exercise their action against the lactic bacteria. The inhibitors then prevent or slow down the development of these bacteria, or modify the relation between the microorganisms present in the ferment, with the respective adverse technological consequences.

It induces products with serious defects, which should be discarded, and needs major costs of manufacture, expenses of raw material and alteration of the production program, which implies a loss with non-profitability for the company [20].

Characteristics of the Diralec-02 system

Electrical design

In the electrical design of the Diralec-02 system there is used the MSP430FG438 microcontroller from the Texas Instruments; one of the most recent microcontrollers of the MSP430 family. Together with related software, this microcontroller guarantees the control of the system modules: pH, electrical conductivity, temperature and optical, thermal printer, graphic screen, as well as the attention to the keyboard, the USB communication and the storage of the data. The electronic card is made from the superficial assembling technology, which guarantees a much more compact and trustworthy design, which includes the shielding and guard techniques, needed to achieve an accuracy of ± 0.01 units of pH and electrical conductivity.

Inoculum gage

Using an inoculum gage based on the optical principle of measurement, it is possible to determine the level

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of contamination by *Staphylococcus sp.*, by coliform bacteria, the presence of bacterial growth inhibitors, and can be carried out the total count of cells present in the sample.

Measurement of pH

The users of the system will have the possibility of carrying out measurements of pH to samples of milk and milk derivatives. The pH electrode includes a sensor of the temperature of the milk sample and that in turn allows compensating its measurement automatically. It guarantees that the values have an accuracy of ± 0.01 units of pH.

Measurement of electrical conductivity

The users will be able to carry out measurements of electrical conductivity to milk samples and milk derivatives for which it will bear in mind the automatic compensation of the temperature and will be an indicator of the contamination by subclinic mastitis. According to reports, 70% of the milch cows in our country endures this disease, hence the importance of its diagnosis. The mastitis constitutes the disease of major prevalence in the international scope [21]. The measurements of electrical conductivity will have an accuracy of ± 0.01 units.

Membrane keyboard and graphic screen

The user will be able to interact with the equipment through a membrane keyboard with tactile effect that will help you gain access to the different services. On a graphic screen (10 cm x 8 cm) of liquid crystal (LCD) there will be shown different routines, as well as the result of the measurements.

Capacity of communication with a computer

The communication with a computer is established by means of a USB 2.0 port, there is executed a Windows application that will make possible to unload the complete table of the state of the samples from the equipment. This application in Windows will allow locating the original data of the samples: their origin, dairy, route, if it is milk from a platform or from a tank, the type of milk (raw, pasteurized, etc.), the specialist who carried out the analysis, the date and the time in which it was carried out.

Capacity of thermal printing

The system has a printing module coupled to it, with a roll of paper of 2 inches wide incorporated. The manufacturer guarantees its good functioning up to 50 km from printed paper. In a laboratory, where the result of 200 daily samples as average is printed, the useful life of the printer would surpass 8 years.

Calendar

The calendar will allow showing the date and the hour in which the measurements, indispensable requirement are carried out in the fulfillment of the good laboratory practices.

Total capacity of samples

The equipment will have a capacity of storage sufficient to safe the result of the analysis of more than 10 000 samples.

Advantages and disadvantages of Diralec-02 compared to homologous equipments in the market

Advantages

1. It is a cheaper equipment: at least five times less than the least costly homologous equipment.

2. It has good level of integration: it allows carrying out several analyses.

3. It reduces the consumption of culture media between 85 and 90 %.

4. It is made in Cuba.

5. It is easy to handle.

6. It fits to the current situation of the dairy. A very modern and sensitive equipment is usually a problem.

Disadvantages

1. Diralec-02 is a less sensitive equipment, that is to say, it does not distinguish levels below 100 000 cfu/mL.

2. Time of detection between 2 and 8 hours.

3. Less automated. It needs to carry out two readings to give the result.

Conclusions

The new possibilities of analysis incorporated into the Diralec-02 system will allow the Cuban Milk Industry to equip its laboratories, complexes and dairies with a modern system, according to the requirements and needs of the national industry, as well as to increase the range of the techniques of analysis and to improve its good practice principles.

The determination of basic indicators of quality of the milk, as well as the subclinical detection of the mastitis, the bacterial growth inhibitors and presence of pathogenic microorganisms, will allow having a real evaluation of its situation in Cuba, in addition to the obtaining of a raw material of major quality.

The Diralec-02 system also will represent for our company the possibility of being inserted ion the market of the systems dedicated to determining the quality of milk, mainly in Latin America.

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