



Technews

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RAPID MICRO-BIOLOGICAL TESTS

This bulletin includes technical information, latest development on products, systems, techniques etc. reported in journals, companies' leaflets and books and based on studies and experience. The technical information in different issues is on different areas of plant operation. It is hoped that the information contained herein, if employed in the dairy plant, will help in making it's operations more efficient.

Your contributions and suggestions will make the bulletin more useful and are welcomed.

*The theme of information in this issue is **Rapid Micro-biological Tests**. It may be noted that the information given here is by no means complete.*

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1. INTRODUCTION

Risk Management is an absolute necessity to survive and compete in the modern world of rapid, dynamic change. With the world-wide awareness of safe food for consumer, the Codex Alimentarius Commission has recommended employing the Hazard Analysis Critical Control Point (HACCP) System in food processing. Indeed, it has become essential to implement HACCP System in dairy processing (see Technews issues 11, 12, 17 and 20).

Preventive and timely corrective actions are essential to ensure product safety and also to facilitate cost control by minimizing downgraded product, rework and/or scrapped product and minimizing down-time and cleaning costs on process equipment.

Speed is now the vital factor for checking acceptable microbial levels, monitoring hygiene and releasing finished products from storage. Traditional microbiological tests, however, take 2 to 5 days. Rapid test methods have, therefore, become very important to be employed in dairy plants.

Introduction of rapid micro-biological test methods in dairy plants in India is very recent. This issue provides some useful information on instruments available for rapid microbiological tests of milk/milk products and of equipment surfaces for their hygienic condition.

2. TECHNOLOGIES

Rapid microbiological methods can be grouped in four broad categories:

1. modified and automated traditional methods;
2. direct cell counting methods;
3. methods based on the measurement of metabolic activity; and
4. methods detecting bio-chemical components of micro-organisms.

Modified and Automated Traditional Methods: These methods offer simplified, more user friendly, convenience devices or automatic systems for simpler preparation, plating procedures and counting. However, there is no benefit in the time taken to obtain the results.

Direct Cell Counting Methods: These methods include the Direct Epifluorescent Filter Technique (DEFT) or automatic versions using the same principle, and flow cytometry.

They offer the potential of very rapid analysis and give results in 20-30 minutes. They however require complex apparatus, skilled operator and substantial sample preparation. DEFT was designed for the analysis of raw milk and it has been successfully automated and the technique is limited to other homogenous filterable non-heat treated materials. These methods also have the potential to detect and enumerate the

specific groups of micro-organisms although few are in routine use.

Methods based on the Measurement of Metabolic Activity: This can be subdivided into two groups.

- (1) simple methods or
- (2) sophisticated methods

The simple methods are based on the old detection principles and tests take 2-8 hours. Although simple and cheap, they lack sensitivity and reliability. The sophisticated methods include impedance, micro-calorimetry, radiometry and biosensors which rely on the ability of actively metabolizing micro-organisms to produce detectable signal such as electrical conductance, heat, radio-labelled carbon dioxide or pressure changes. Impedance has found several industrial applications and gives results in 8 to 18 hours. Biosensors hold the promise of small portable device but have not been developed with sufficient sensitivity and reliability.

Methods Detecting Biochemical Components of Micro-organisms: These methods include ATP (adenosine triphosphate) bioluminescence and several other technologies such as ELISA (Enzyme Linked Immunosorbant Assays) and LAL (Limulus Amoebocyte Lysate) tests detecting specific organisms in a matter of hours. The LAL test detects endotoxin of gram negative bacteria and ELISA detects pathogenic bacteria *Salmonella*. They reduce overall detection time by 2-3 days by speeding up the final

detection and identification steps. The most recent advances have produced simple, user friendly, single shot dipstick formats for added components and no capital investment.

In recent times, ATP bioluminescence technique has gained wide popularity and acceptance. All living cells contain ATP and ATP bioluminescence test detects ATP using firefly enzyme luciferase. The firefly test is based on the measurement of light generated by luciferin/luciferase reaction in the presence of ATP to quantify the biological residues present in the test sample. It is very rapid and sensitive and has an ability to detect both micro-organisms and food residues, giving results in minutes. A wide range of applications include portable hygiene devices to monitor the efficacy and performance of cleaning procedures, instruments to test raw milk and automated instruments to verify product quality after processing.

The most widely accepted and used rapid methods in microbiology abroad are automated traditional methods, impedance, ATP bioluminescence and ELISA.

3. RAPID TESTS FOR MILK & MILK PRODUCTS

Most of the instruments available for rapid microbiological tests of milk and milk products use either direct cell counting methods (DEFT, flow

cytometry) or methods detecting biochemical components of microbes (ATP, ELISA). Instruments are available for measuring total bacterial counts (TBCs) and/or specific microbe count. Information is provided here only on instruments which measure TBC.

BACTOSCAN: Bactoscan FC, from FOSS, is a direct cell counting instrument based on flow cytometry. This is a very stable optical system which gives an improved signal/noise ratio increasing the sensitivity. The instrument uses a laser source with a life time in excess of 10,000 hrs. The instrument is used for milk. It takes a 4.5 ml milk sample, which passes through a filter to be thoroughly mixed and homogenized. The incubation reagent is added and the mix incubated at 42°C for 8 minutes. During this time, the solution passes several times through fine mesh filters to ensure disintegration of somatic cells, protein and fat globules and thorough staining of bacteria with the fluorescent stain (Ethidium bromide). The mixture then passes through a flow cell where a laser beam excites a single light pulse per organism, is converted into electronic pulse and counted. The result is expressed as individual bacterial cells per ml.

The instrument is available in three sizes, with throughputs of 50, 100 and 150 samples per hour.

Another version is Bactoscan 8000, which has lower throughput capacity than Bactoscan FC. This direct cell

counting instrument uses the fluoro-optics (Rotating Wheel) principle. It is used to measure total viable counts (TVCs) of bacteria in milk.

CELSIS-LUMAC: The Lumac's Raw Milk Microbial Kit with Biocounter M1800 or M2500 is based on the ATP technique. When raw milk arrives at the dairy, a sample can be tested in around 5 minutes. The system measures the bacterial count in terms of Relative Light Units (RLU), and then calibrated in terms of Colony Forming Units (CFU) per ml.

For testing the sterility of the UHT milk, Lumac's instrument Dairy Product Sterility Kit with Autobiocounter M 4000 is also based on ATP bioluminescence technique. The sample needs to be incubated for a period of only 48 hours. Then the results are obtained from the instrument within minutes. The instrument measures the bacterial count as RLU and is calibrated to display results as pass or fail.

Another instrument based on ATP Bioluminescence technique from Celsis.Lumac is the Pasteurized Milk Screen Kit with Biocounter M 1800, M 2800 or Autobiocounter M 4000 (Fig-1). After a 24 hr pre-incubation period, a pasteurized milk sample is tested in the instrument in 5 minutes.

Lumac also offers a rapid testing kit for Salmonella, Path-Stik One Step Salmonella Test.

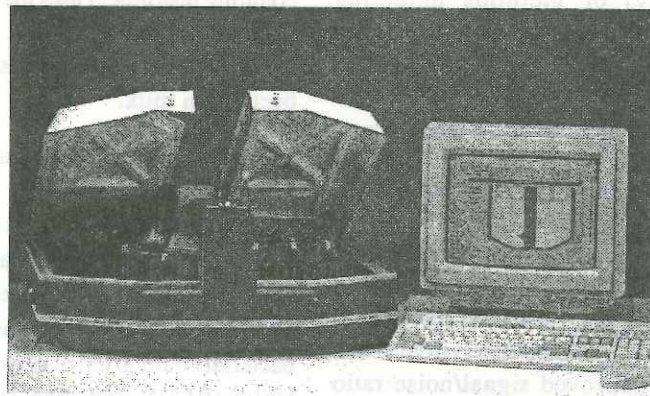


Figure 1: The Autobiocounter M 4000

CHEM FLOW: The **Chemflow Autosystem 3** of Paris-based company Chemunex is also a direct cell counting instrument based on flow cytometry. The instrument has high sensitivity, and throughput of 48 samples per hour. Even without pre-enrichment, it can detect levels of 100 organisms per ml in less than 30 minutes. Coliform organisms can be detected within 10 hrs and yeasts in less than 24 hrs.

In the instrument, the labelled cells emit a fluorescent signal when illuminated by the laser beam so each cell is counted directly by sensitive optical detectors.

Chemflow 3 has been used for microbial tests for milk, sterility tests for UHT milk and other tests.

BIOCOM: Another direct cell counting instrument available is **Asterias 100** from French Company Biocom. Asteria is a filter cytometry-based technique but uses an advanced system. Sample preparation is automatic. The

instrument analyses samples of milk for total bacterial counts (TBCs) and specific cell numbers. Asterias have the capacity to carry out 400 analysis per hour.

Biocom's another similar, though less advanced, instrument for measuring total bacterial counts of milk is **Cobra** rapid microbiology system. This is the automated version of the DEFT technique.

BIOTRACE: The **Biotrace Dairy Kit and Microbial Luminescence System** is based on ATP bioluminescence technique to detect microbial contamination in UHT milk and related dairy products. The instrument has a highly sensitive state-of-the-art luminometer and results are recorded in the integral computer. UHT products under test are preincubated for 48-72 hrs at 28-30 Deg C. The instrument gives results within 20 minutes as simple YES or NO.

OTHER INSTRUMENTS: Some other instruments using ATP bioluminescence technique are from Quadra Chem Laboratories and Hughes Whitlock, both of U.K.

Quadra Chem has launched a new portable bioluminometer, **Charm LUM-T** (Fig-2), capable of a wide range of test for dairy products. Test kits are available for pasteurization of milk, somatic cells in milk, insecticides, aflatoxins, microbial quality and hygiene.



Figure 2: The Portable Bioluminometer

The LUM-T weighs only 0.9 kg, has an internal battery and a high intensity, simple to read, LCD display. It takes only 4 minutes to perform a 3-step ATP test. Results can be expressed as RLU, test specific units, plus pass/fail limits.

Bioprobe, with appropriate kits, from Hughes Whitlock, can measure bacterial counts (in terms of RLUs) in raw milk, ice cream and other dairy products.

4. RAPID HYGIENE MONITORING SYSTEMS

There are a number of rapid hygienic monitoring instruments available which are portable, simple and easy to use. Most of them are ATP bioluminescence based. Some of them are briefly mentioned here.

BIOTRACE: For assessing the cleanliness of equipment surfaces, the instruments of **Biotrace**, UK are the most popular ones. The instruments are based on ATP bioluminescence technique.

Its **Uni-Lite** (Fig-3) was among the first direct, portable hygiene swab test instruments to be introduced. It provides test results in a matter of seconds. Easy to use, robust and light weight, it delivers reliable results (pass/caution/fail) which can be displaced on its LCD (Liquid Crystal Display) screen or as hard copy on a microprinter. Powered by rechargeable built-in battery or mains/AC power, it is suitable for use in both production and remote areas.

Unit-Lite Xcel is an advanced portable rapid cleanliness testing instrument, see Fig-3.

The system has full PC compatibility and data logging capabilities. Rechargeable battery enables flexibility of operation. All tests are automatically recorded in seconds with the date, time, user and point of test. Results are shown as pass/



Fig. 3: Biotrace Uni-Lite and Uni-Lite XCEL

caution/fail with a built-in security that the user has to acknowledge a fail. Also it can perform trend analysis.

To make swabbing simple and rapid, Biotrace has introduced **Clean-Trace** (Fig-4), which is a self-contained single shot swab with all reagents inside it. The pre-moistened swab is removed from its holder, the test surface under test swabbed and the swab returned by depressing a clip on the Clean-Trace and pushing it firmly down. This activates the test for measurement. The Clean-Trace is inserted into the Uni-Lite/Uni-Lite Xcel sample chamber and result read. Test time is less than 30 seconds. Swab can be activated upto 4 hours after swabbing.

CELSIS.LUMAC: Celsis.Lumac's hand-held **Rinse Water Test Kit** can be used to check on the spot whether the CIP of processing equipment has been effective or not. It gives a result in minutes so that if there is a problem due to inadequate cleaning, corrective action can be taken immediately. A similar instrument is **Hygiene Monitoring Kit**. These kits are used in combination with



Figure 4: Biotrace Clean Trace

Lumac's **Biocounter M1500 or M2500 /M4000**. The results are shown in Relative Light Units (RLU) and calibrated in Pass/Fail. The instruments are ATP based.

Another hand-held hygiene monitoring instrument from Celsis is **SpotCheck**. This single shot system uses patented colour change technology to detect the presence of ATP. The output is a simple colour change that is visible to the naked eyes. SpotCheck employs a novel patented enzyme packaged into a self-contained, single shot disposable pen-shaped device. The device is easy to use. The results are interpreted as a simple colour change from transparent to dark green that occurs within 1 to 10 minutes,

depending on the level of contamination present.

System Sure is still another ATP based portable instrument. It weighs less than 700 g. It has 3 simple steps in testing. An ATP-free swab is moistened with swabbing solution and then the test area is swabbed. The swab is dipped into a tube containing enzyme reagent and rotated for about 10 seconds. A cap is placed on the tube and the tube is inserted into the chamber of the System Sure. Results in RLU (pass/fail) are read instantly.

OTHERS: There are several other instruments available for equipment hygiene monitoring. **Luminator-K** is an ATP based instrument from American company Charm. It is a lightweight, portable system which uses the **Pocketswab**, a single-use swab with built-in reagents that are stable at room temperature for upto one month and gives result in 30 seconds. Results are given as pass/fail and stored as RLUs.

The **Hy-Lite** ATP monitor is from German Company Merck. This comprises a sampling pen, again a self-contained one-shot device. Results are read as pass/caution/fail, and the instrument has a memory for 1000 results.

Another instrument is **Bioprobe** from Hughes Whitlock Company. It is a light weight (2 kg) portable ATP based instrument. It is claimed that levels of bacteria as low as 10 cells per sq. cm.

can be detected using this system in 1 to 100 seconds.

Japanese Konica's simple, rapid and easy-to-use system is **Swab 'N' Check**. This is based on protein residues as a measure of the hygiene status of work surfaces. The test is a colorimetric swab test which gives a colour change from green to darkish purple in the presence of peptide bonds. The results are obtained in about 10 minutes.

Another similar instrument is **Check Pro** from Diversey Lever.

Lightning (Fig-5) from US Company Idexx is an ATP based hand-held instrument with a one-shot swab.



Figure 5: Lightning from Idexx

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