Novel System for the Detection of Indicator Organisms in Swabs and UHT Products

Abstract

Introduction: The presence of most indicator organisms such as total aerobic count (TC), yeast and mold (YM), coliform and E. coli (EC) can be determined by the time consuming plate count methodology. In recent years the application of rapid automated methods, as alternatives to the plate count method, has become increasingly significant. BioLumix has developed a new optical system for rapid and automated detection of indicator microorganisms.

Purpose: To evaluate the new system and technology for the detection of TC, YM, coliform and EC, and show its applicability to environmental and UHT products testing.

Methods: The BioLumix system detects optical changes, due to microbial growth in liquid growth medium containing optically sensitive reagents (color or fluorescence). An embedded optical sensor for the detection of CO_2, was used for TC and YM. The simultaneous detection of coliform and E. coli was achieved with a combination of color and fluorescent dyes. Stainless steel coupons inoculated with various types of bacteria, yeast and mold were swabbed. The inoculated swabs were tested for TC, YM or a combination of coliform/EC. UHT products were evaluated for the presence/absence of microorganisms using the BioLumix system.

Results: The surface of stainless steel coupons was inoculated with 83 different strains of bacteria, 14 molds and 12 yeasts. All detected by the CO_2 sensor, while none of the un-inoculated samples were detected. The coliform/EC vial was capable of distinguishing between coliform and E. coli in environmental samples. There were 37 store-bought UHT products tested including: milk, half & half, shakes, and whipped cream. Additionally, 71 products were inoculated with low numbers of bacteria or yeast. The system clearly distinguished between clean and contaminated samples.

Significance: The data suggests that the new method is useful for determining total

aerobic count, yeast and mold as well as coliform and E. coli. More assays are currently under development.

Methods

Vials - CO_2 Sensor: Carbon dioxide is a universal metabolite produced by all microorganisms. A disposable test vial contains a transparent solid sensor located at the bottom of the vial that changes color whenever CO_2 diffuses due to microorganism growth. Only gases can penetrate the sensor that blocks liquids, microorganisms and particulate matter. The carbon dioxide generated by microbial metabolism diffuses into the sensor and reacts with a reagent to provide indication of the presence of the gas. This type of vial is used for sterility testing and for yeast & mold

Coliform - E. coli Combination: In addition to the optical CO_2 sensor, the BioLumix system can also be used to determine the presence of coliform and E. coli in samples. The production of the yellow *ortho*-Nitrophenyl-β-galactoside (ONPG) by coliform is monitored with a blue LED, while 4-methylumbelliferyl-β-D-glucuronide (MUG) utilization is detected by a fluorescence sensor. The Indole reaction can be utilized at the end of the assay to verify the presence of E. coli

Instrumentation

The **BioLumix instrument** (Figure 1 above) has a capacity of 32 test vials with one incubating temperature (capable of both heating and cooling). Multiple instruments can be used to accommodate several incubation temperatures. A single personal computer controls up to 32 instruments enabling over 1,000 simultaneous tests. The interlocking, front-loading design allows safe stacking of multiple instruments to save valuable counter space.

Rapid, real-time results allow for quick corrective action. Using the software on the PC computer, the BioLumix system displays assay results as soon as detections occur. No operator involvement is needed. Out of specification samples are indicated in red, borderline samples in yellow and acceptable samples in green. Results can be communicated rapidly quickly across any standard company network. The software is GMP (Good Manufacturing Practice) compliant, providing an audit trail, generating trend analysis, and providing multiple customizable reports formats.

Figure 1

Tested Samples

Products tested
Kroger Break free eggs
C. F. Burger Creamery Half & Half
Kroger Break free real egg
Nesquick Strawberry milk UHT
Nesquick Chocolate milk UHT
Nesquick Strawberry milkshake
Nesquick Chocolate milkshake
International delight Irish Cream coffee creamer
International delight Amaretto coffee creamer
Instant whip Arizona Half & Half
Land O'Lakes Half & Half
Kroger Heavy Whipping Cream
Land O'Lakes Fat Free Half & Half
Kroger Whipping Cream
Land O'Lakes Heavy Whipping Cream
Land O'Lakes Traditional Half & Half
Kroger 2% reduced fat Milk
C. F. Burger Creamery Half & Half
Nesquick Strawberry milk UHT
Nesquick Chocolate milkshake
International Delight Irish Cream coffee creamer
International Delight Amaretto coffee creamer
Instantwhip-Arizona Half & Half
Land O'Lakes Half & Half
Kroger Heavy Whipping Cream
Land O'Lakes Fat Free Half & Half

Kroger Whipping Cream
Land O'Lakes Heavy Whipping Cream
Land O'Lakes Traditional Half & Half

Stainless steel coupons were inoculated with bacteria (83 strains), yeast (12 strains), and mold (14 strains). The coupons were let to dry for several hours, cotton swabs were used to remove the bacteria from the coupons and the swab was inserted directly into the vial. Some of the coupons were used to test for coliform and E. coli.

Results

UHT-Sterile products:

None of the store bought products had any organisms and all generated flat curves. Two non-UHT naturally contaminated products (eggnog and pasteurized milk) contained bacteria and did detect in the system.

UHT-Inoculated Samples

Seventy one UHT samples were inoculated with 38 different strains of bacteria at low levels (10-100 cfu/container) and allowed to incubate at 30^0 C for 48 hours. Thereafter, 1 ml of sample was added to the vials containing the CO_2 sensor. All the vials which contained organisms turned the sensor to yellow/orange (vial on right in Figure 2) indicating CO_2 production and metabolic growth., while in the clean samples the sensor remained dark green (vial on the left in Figure 2).

Figure 2

Typical curves obtained with the various bacteria are shown in Figure 3. Figure 4 shows the yeast curves, while Figure 5 shows the curves obtained with molds.

Figure 3: Bacteria Curves

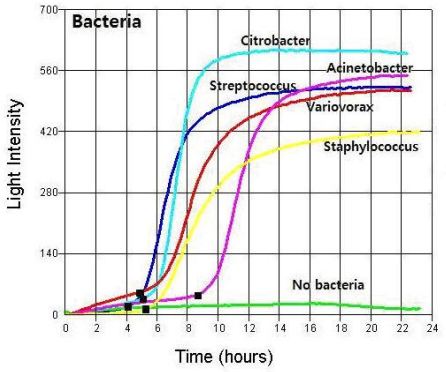


Figure 4: Yeast Curves

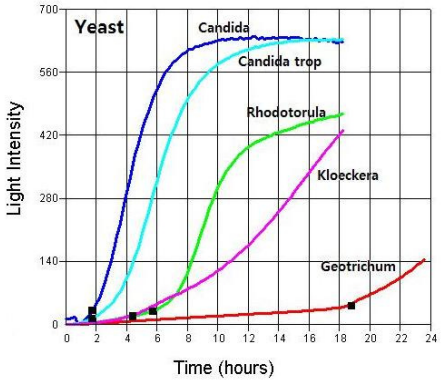
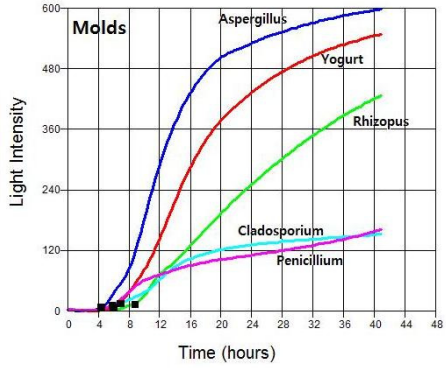


Figure 5: Mold Curves



Coliform / E. coli Combination

The coliform/E. coli vial contains a selective and differential medium capable of simultaneously detecting coliform and E. coli. The method is based on the detection of two enzymes, ß-glucuronidase, characteristic of E. coli, and ß-galactosidase, characteristic of the coliform group. Coliform bacteria using the

chromogenic substrate ortho-nitrophenyl-ß-D-galactopyranoside (ONPG), to turn the vial color from colorless to yellow. For detection of E. coli, the system utilizes the fluorogenic enzyme substrate 4-methylumbelliferyl-ß-D-glucuronide (MUG), and an increase in fluorescence is seen.

Figure 6 shows the results of the simultaneous curves of ONPG (blue) and MUG (green) for E. coli. It shows both ONPG and MUG utilization.

Figure 6: E. coli Curves in Combo Vial

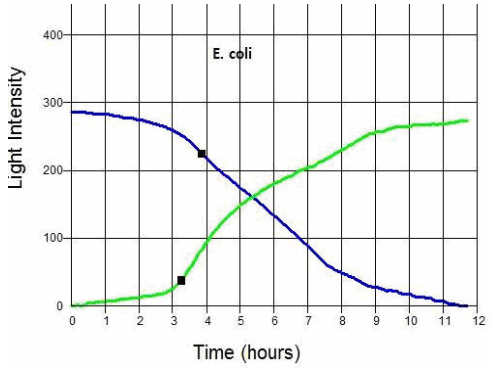


Figure 7: Citrobacter Curves in Combo Vial

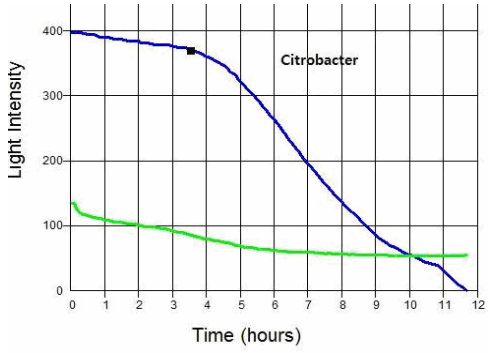


Figure 7 shows the results for Citrobacter, with ONPG utilization and lack of MUG consumption.

The BioLumix system **simplifies, expedites, and reduces the cost** of indicator organisms testing. Prepared, ready to use vials with growth media are available to meet the testing needs. To complete an assay, simply place the sample (or 1:10 dilution of a sample) to be tested in a vial and load it into the BioLumix instrument. Over a period of hours (rather than days), the instrument obtains color and fluorescence readings from the vials with an optical detector, and analyzes the results to determine the level of contaminants. Results are easy to interpret, and are automatically communicated across the intranet to where they are needed, such as the warehouse, to approve shipping.

Conclusion

The BioLumix system is capable of rapidly distinguishing clean UHT samples from samples containing bacteria, yeast or molds with a high degree of accuracy. The system is also capable of detecting microorganisms on surfaces, by inserting the swab directly into the vial. Due to the system's ability to simultaneously monitor color and fluorescence changes in a single vial, the system is also capable of simultaneous detection of coliform with color reaction and E. coli with fluorescence reaction.

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