

New, Microbiological Automated System for Nutraceutical and Dietary Supplement Makers Simplifies Microbial Testing

Abstract

New U.S. Food and Drug Administration regulations for current good manufacturing practices (cGMP) enacted in June 2007 require more microbiological testing by nutraceutical and dietary supplement manufacturers. Producers will require faster and more cost-effective methods of testing to remain competitive while meeting the new regulations. The BioLumix system has been specifically designed to meet this challenge.

To reduce costs and testing times, the BioLumix system provides automated microbiological assays needed by the Nutraceuticals and Dietary Supplements industries. It is simple enough to be operated by non-microbiologists, cutting staffing costs, providing accurate data for raw materials, environmental samples, and finished products.

The BioLumix system uses ready-to-use vials with a CO₂ sensor to detect bacteria, and yeast and molds. Color and fluorescent sensors are available to detect coliform and E. coli in a single vial, gram negative bacteria, Enterobacteriaceae, Pseudomonas spp. and Staphylococcus spp.

Introduction

Until a decade ago, taking vitamin or mineral supplements - especially herbs - was considered to be at the fringe of respectability by mainstream medicine. But by 2005 the nutraceutical and dietary supplement industry had become a \$182 billion global market. Sales of all dietary supplements, including vitamins, minerals, herbs, and similar compounds grew 2.6 % annually from \$8.8 billion in 1994 to \$20.3 billion by 2005. With a growing number of ingredients in these products, it has become increasingly important to ensure they are free of potentially harmful microbial contaminants at the manufacturing level.

The U.S. Food and Drug Administration announced in June of 2007 a regulation requiring compliance with cGMP. Manufacturers are required to evaluate the microbial quality of raw materials and finished products. If dietary supplements contain contaminants, the FDA

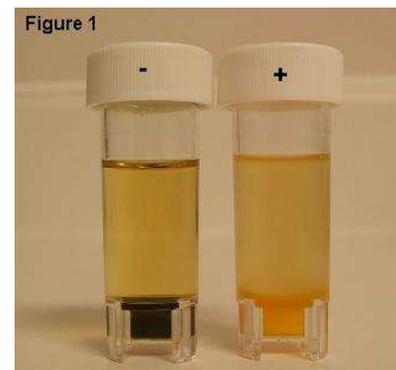
considers those products adulterated or misbranded. Companies with more than 500 employees must comply by June 2008, companies with less than 500 employees by June 2009, and companies with fewer than 20 employees by June 2010. Consequently, faster, simpler and more cost-effective microbiological tests are required by the industry to meet the new challenges.

Material & Methods

Technology

Carbon dioxide (CO₂) is a universal metabolite produced by all microorganisms. Technology was developed to detect this released CO₂. A disposable test vial contains a transparent, solid sensor that changes color whenever CO₂ diffuses into the sensor at the bottom of the vial. Only gases can penetrate the sensor, blocking 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.

Figure 1 shows a sterile vial on the left and a positive vial on the right, with a yellow sensor, indicating CO₂ production and metabolic growth.



Instrumentation

The **BioLumix instrument** (Figure 2) has a capacity of 32 test vials with one incubating temperature (capable of both heating and cooling). Multiple instruments can be used to accommodate variable incubation temperatures. A single personal computer controls up to 32 instruments for an ability to monitor over 1,000 samples simultaneously. The interlocking, front-loading design allows for safe stacking of multiple instruments to save valuable counter space.

Figure 2



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

Disposable Vials

Vials are available to perform multiple assays. CO₂ sensors provide for total aerobic count and for yeast and mold detection. Color or fluorescent vials are available to test for coliform and E. coli, gram negative bacteria, Enterobacteriaceae, Pseudomonas and Staphylococcus. All come with sterile ready-to-use media and certificates of analysis.

Products Tested

Total Aerobic Count:

Finished products: Fiber tablets, Flaxseed Oil capsules, Psyllium Husk capsules, Spirulina tablets, Alfalfa tablets, Wheat Germ Oil capsules, Iron tablets, Garlic Parsley tablets, and Hoodia Gordonii capsules

Raw materials: Empty gelatin capsules (red and blue), Citrus Bioflavanoid, Devil's Claw powder, Echinacea Purpurea powder, Ginkgo Bilboa powder, Grape Skin and Blueberry powder, and Golden Seal Root.

Yeast and Mold

Finished products: Psyllium Husk capsules, Spirulina tablets, Alfalfa tablets, Wheat Germ Oil capsules, Flaxseed Oil capsules.

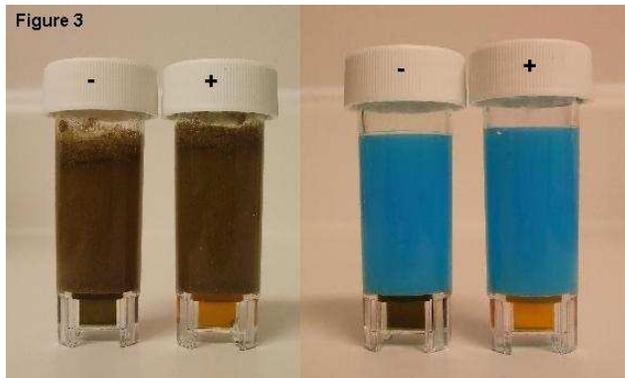
Raw materials: Corn Protein, Soybean Extract for animal feed, Echinacea Purpur powder, and Rice Meal.

Procedure

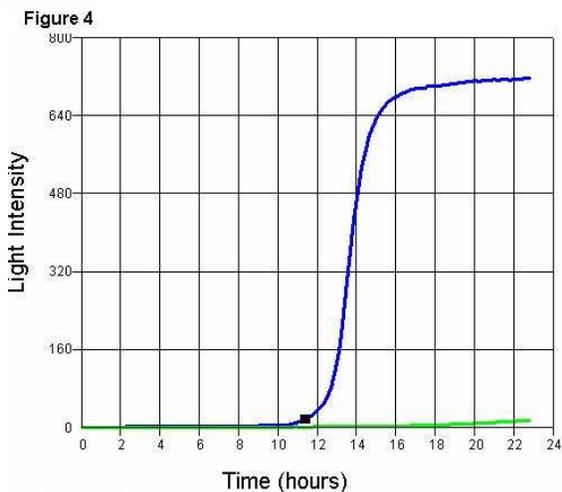
A 1:10 dilution of each product was made by adding 11 grams of product to 99 ml of Butterfield's Phosphate Buffer. The product was thoroughly mixed with the diluent and 1.0 ml was transferred to the appropriate vial. The vial was inserted into the instrument and monitored by the system. Vials for total aerobic count were incubated at 35°C while the vials for yeast and molds were incubated at 28°C.

Results

Figure 3 demonstrates that the product does not interfere with the microbiological



assays. The set of vials on the left contains 1.0 ml of the 1:10 dilution of Ginkgo Bilboa powder. The set on the right contains the same dilution of the blue empty capsules. In the vial top, the product totally masks the reactions. But the sensor window shows a clear difference between vials with no growth (dark green color) and vials that had growth (yellow).



Bacteria, even in very low numbers (2-10 cfu/vial), were detected within 18 hours. Figure 4 (see above) shows typical curves obtained from a naturally contaminated sample (dark blue) and a clean sample (green). Based upon the user's specifications, the instrument automatically determines if the product is clean or contaminated.

Bacteria-Natural Products (un-inoculated)

Product	Detection Time (hr)	Plate Count (cfu/g)
Empty Gelatin capsules-Red Capsules	ND	<10
Empty Gelatin capsules-Blue Capsules	ND	<10
Alfalfa Tablets	13.5	250
Flaxseed Oil Capsules	ND	<10
Psyllium Husk Capsules	9.6	85,000
Golden Seal Root	ND	<10
Spirulina Tablets	10.9	91,000
Wheat Germ Oil Capsules	ND	<10
Grape Skin and Blueberry Powder	14.5	150
Citrus Bioflavanoid	ND	<10
Devil's Claw Powder	17.6	50
Echinacea Purpurea Powder	12.6	310
Ginkgo Bilboa Powder	ND	<10
Garlic Parsley Tablets	9	~8,000
Hoodia Gordonii Capsules	14.7	10
Iron Tablets	ND	<10
Fiber Tablets	ND	<10

ND indicated no detection

Bacteria-Inoculated Products

Products inoculated with various bacteria are shown in the table below:

Product	Organism	Level	DT (hr)
Red Capsules	Escherichia coli ATCC 25922	~1,000	11.5
Blue Capsules	Enterobacter aerogenes	~1,000	11.2
Alfalfa Tablets	Klebsiella pneumoniae ATCC 13883	~500,000	3.3
Flaxseed Oil Capsules	Enterobacter aerogenes	~500,000	2.5
Psyllium Husk Capsules	Micrococcus luteus	~10,000	7.4
Golden Seal Root	Bacillus cereus ATCC 13061	~50,000	10.9
Wheat Germ Oil Capsules	Escherichia coli ATCC8739	~500,000	2.7
Grape Skin and Blueberry	Proteus vulgaris	~50,000	5.1
Citrus Bioflavanoid	Staphylococcus aureus ATCC4330	~10,000	6.9
Devil's Claw Powder	Pseudomonas aeruginosa ATCC 27853	~500	13.3

Echinacea Purpurea Powder	Staphylococcus warneri	~10,000	8
Ginkgo Bilboa Powder	Salmonella enteritidis ATCC 13076	~500,000	3.2
Hoodia Gordonii Capsules	Streptococcus bovis	~500	14.7

Yeast and Molds

Products naturally contaminated and inoculated with various yeast and molds are shown in the table below:

Product	Inoculation	DT (hr)
Corn Protein	Natural	13.1
Wheat Germ Oil	Alternaria spp.	8.9
Corn Distiller	Natural	25.7
Expeller Meal	Natural	16.3
Rice Meal	Natural	30.1
Psyllium Husk	Natural	29.9
Spirulina	Natural	37.3
Alfalfa Pills	Penicillium spp.	7.7
Echinacea Purpurea Powder	Aspergillus spp.	10.5
Flaxseed Oil	A. niger ATCC 16404	9.7
Citrus Bioflavanoid	Candida albicans	12.6
Fiber Tablets	Saccharomyces cerevisiae	11.9

When testing for yeast and mold, none of the finished products tested contained molds and none were detected in the system. Raw materials, products naturally contaminated with mold, and

samples inoculated with molds isolated from products were tested. All contaminants were detected properly in the system.

Conclusions

The BioLumix system was capable of quickly distinguishing clean samples from samples containing bacteria, yeast or molds with a high degree of accuracy. The system enables users to perform their microbial testing operation in-house rather than sending their samples to outside laboratories. Companies are able to greatly reduce the amount of time and money required to test their samples, allowing earlier release of raw materials and finished products. The BioLumix system can help reduce quarantine time from 5 - 6 days to approximately 35 - 48 hours for microbiology testing, and the system is fully automated. Microbial tests that once required days and weekend-long operator intervention can now be performed without supervision to eliminate the need for costly overtime operations.



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