



# A Simple, Rapid, Automated Solution for Microbiological Testing

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**BioLumix**<sup>®</sup>

## Abstract

To meet the challenges of the new FDA regulations requires simpler, faster, and more streamlined microbiological tests. The BioLumix System enables users to perform the entire microbial testing operation in-house rather than sending their samples to outside laboratories for testing. Accordingly, the system allows companies to greatly reduce the amount of time and money required to have their samples tested by independent outside laboratories. Consequently, it enables 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. In addition, the system is fully automated. The methodology developed is described.

The new FDA regulations regarding cGMP (current Good Manufacturing Practices) will require the Nutraceutical and Dietary Supplement Industries to streamline and develop faster and more effective microbiological assays. Currently, many manufacturers send their samples to outside testing laboratories. This is expensive and takes many days to obtain results. Internal laboratories are a better option but many manufacturers do not have the infrastructure or the personnel to internalize microbiological testing. The BioLumix System offers a fully automated microbiological system that can

deliver much faster results, without the requirement of a full microbiological laboratory. The BioLumix System (Figure 1) is comprised of an instrument with a capacity for testing 32 individual assay vials, software, and disposable vials.



**Figure 1 – The System**

Each instrument serves as an incubator. Up to 32 instruments can be connected to one computer. The software enables rapid, real-time results to be transferred to where they are needed most, without any operator involvement. The disposable vials are available for use in required microbiological assays including: total aerobic count (TAC), Yeast and Mold (YM), coliform (CM), E. coli (EC), Pseudomonas (PSE), Staphylococcus (SA), and Probiotics. All assay vials are provided with a ready to use sterile media and a certificate of analysis.



## Technology

The technology provides simultaneous detection of changes in either color or fluorescence as a result of microbial growth and metabolism. Figure 2 Shows the BioLumix Technology.

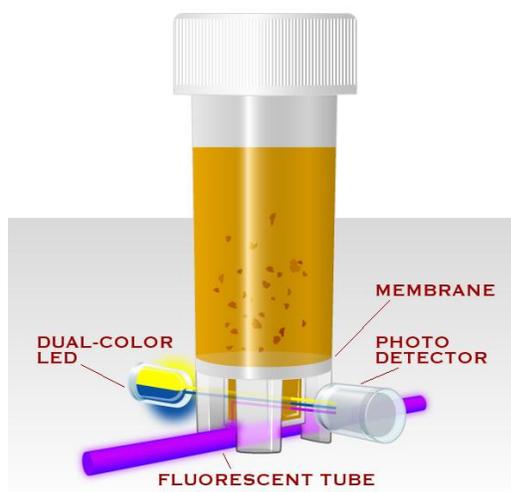


Figure 2- Optical system

## Methods

**Samples Used** - The 29 products listed below were tested for total aerobic count, yeast and mold, Pseudomonas, E. coli, and Staphylococcus.

Alfalfa Tablets	Hoodia Gordonii
Apricot Powder	Iron Tablets
Carragenan Powder	Joint Advanced
Citrus Bioflavanoid	Joint Shield Advanced
Devil's Claw Powder	Lemon Bioflavanoid
Echinacea Purpurea Powder	Multi Vitamin for Woman
Empty Gelatin Blue Capsules	Psyllium Husk Capsules
Empty Gelatin Red Capsules	Strawberry Powder
Fiber Tablets	Rauwolfia Vomitoria
Flaxseed Oil Capsules	Spirulina Tablets
Flex Tablets	Vitamin: One-A-Day Men
Garlic Parsley Tablets	Wheat Germ Oil Capsules
Ginko Bilboa Powder	Whey Powder
Golden Seal	Zinc Gluconate
Grape Skin and Blueberry	

## Vials

**Total Aerobic Count (TAC):** These vials contain a CO<sub>2</sub> sensor at the bottom of the vial and nutrient growth medium above. The carbon dioxide generated by the bacterial metabolism diffuses into the sensor and reacts with the reagents in the sensor, providing an indication of their presence. Only gases can penetrate the sensor, blocking liquids, microorganisms and particulate matter. Results are obtained after overnight (18-22 hours) incubation.

**Yeast and Molds:** These vials contain the CO<sub>2</sub> sensor at the bottom of the vial and have selective medium above for the detection of only yeast and molds. Results are obtained after 48 hours of incubation.

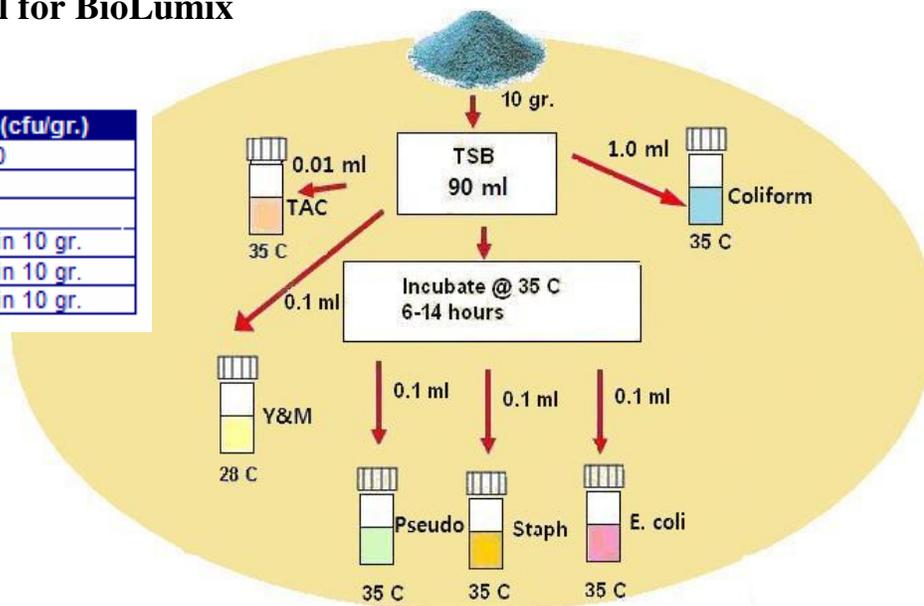
**Pseudomonas:** These vials contain the CO<sub>2</sub> sensor at the bottom of the vial and have selective medium above for the detection of Pseudomonas strains. The results are available after overnight (18-22 hours) incubation. Positive results can be verified with the oxidase reaction.

**E. coli:** These vials contain a membrane filter to separate the incubation zone where the media, sample and microorganism are present, from the measuring zone in the bottom of the vial where the detector reads the results. This design allows for optically testing of opaque samples. The E. coli assay is based upon the detection of the microbial cleavage of 4-methylumbelliferyl-β-D-glucuronide (MUG) using the fluorescence sensor. The results are available after overnight (18-22 hours) incubation. At the end of the assay, the indole reaction can be utilized to verify the presence of E. coli in approximately 10 minutes.

**Staphylococcus:** These vials contain the membrane filter and selective and differential growth medium above containing mannitol and the pH indicator (Phenol Red). Growth of Staphylococcus results in a color change from red to yellow. The results are available after overnight (18-22 hours) incubation. The coagulase reaction can be used to verify the presence of *S. aureus*.

## Schematic Protocol for BioLumix

Organism	Level (cfu/gr.)
TAC	<1,000
Y&M	<100
Coliform	<10
E. coli	None in 10 gr.
Ps. aeruginosa	None in 10 gr.
Staph. aureus	None in 10 gr.



**The Dilute-to-Specification** protocol was used, requiring dilution of the sample to the specification limit required for product action or release. If there is microbial growth above specification limit, the sample fails; if there is no microbial growth or growth below the specification limit, the sample passes.

### Results

**Total Aerobic Count:** Five of the 29 products tested contained counts above 1,000 cfu/gr. and all detected by the instrument within 22 hours. Twenty vials were inoculated with a variety of bacteria including *Acinetobacter*, *Bacillus*, *Citrobacter*, *Enterobacter*, *Proteus*, *Pseudomonas*, *Micrococcus*, *Staphylococcus*, *Streptococcus* and *Yersinia* at a level of 5,000-20,000 cfu/gr. All species were detected in the instrument within 18 hours.

**Yeast and Molds:** Six samples had molds above the specified level of 100 cfu/gr. and all detected in the instrument within 40 hours. Twenty vials were inoculated with a variety of yeast and molds including the yeasts *Candida albicans*, *Candida tropicalis*, *Geotrichum candidum*, *Rhodotorula mucilaginosa*, and *Zygosaccharomyces* and molds *Aspergillus niger*, *Aspergillus oryzae*, *Rhizopus stolonifer* and a variety of *Penicillium* species at a level of 500 - 4,000 cfu/gr. All species were detected in the instrument within 35 hours.

**Pseudomonas:** Seven of the 21 samples tested detected using the *Pseudomonas* vials and *Pseudomonas* was isolated from each. Most of the samples were detected very quickly in the System (2-6 hours). Two samples were positive for *Pseudomonas* at a level >1 cfu in 10 gr. of product and were detected using the instrument within 16 hours following an overnight incubation in TSB. There was no detection in the samples that did not contain *Pseudomonas*. Ten samples were inoculated with 4 different strains of *Pseudomonas aeruginosa*, *P. fluorescens*, and *P. putida* at a level of 10-100 cfu/10 gr. of product. After 16 hours of pre-incubation all species were detected in the system within 12 hours.

**E. coli:** Only one of the 21 samples tested was naturally contaminated with *E. coli* at a level of <1 in 10 grams of product. All clean samples did not detect in the system. Ten different samples were inoculated with *E. coli* at a level of 1-20 cfu/10 gr. All were detected in the system in 18 hours after an overnight incubation in either TSB or Lactose broth. Ten different strains of *E. coli* were separately inoculated into 10 vials at a level of 10-100 cfu/10 gr. of product. After 16 hours of pre-incubation all species were detected in the system within 14 hours.



**Staphylococcus:** Three samples were positive for Staphylococcus at a level of  $>1$  in 10 grams of product. They were detected within 20 hours in the system after an overnight incubation in TSB. Eight vials were inoculated using 8 different strains of *S. aureus* at a level of 10-100 cfu/10 gr. of product. After 16 hours of pre-incubation all were detected in the system within 14 hours.

### Conclusion

Twenty nine Nutraceutical products were selected for this study. They were analyzed for microbial quality using the BioLumix instrument and the standard methodology. Samples were diluted so that only samples with counts above the specified level would detect in the system. The results showed that the BioLumix System

was capable of quickly distinguishing samples with counts above the allowed levels from clean (below spec.) samples. All samples that contained microorganisms above the specified level did detect in the BioLumix System, showing a high correlation between the instrument results and the standard methodology. The BioLumix instrument offers a significant reduction in time to obtain results and reduces hands-on labor due to its automation and simplicity of use.

The BioLumix System can be used to internalize the microbiological testing without the requirement of a microbiologist. It simplifies testing, expedites time to results, reduces testing costs and accelerates product release.



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