



Automated Rapid and Accurate Enumeration of Viable Microorganisms in Pure Cultures Using the RBD 3000

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ABSTRACT

The microbial fermentation industry holds significant interest in real-time viability monitoring, which allows for optimal harvest times and increased product yield. Advanced Analytical's automated system, the RBD 3000, accurately enumerates and determines the viability of microorganisms within 20 minutes of sampling. Pure cultures of *Bacillus atrophaeus*, *Candida albicans*, *Clostridium perfringens* Type A, *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Streptococcus bovis* and *Mycoplasma bovis* were serially diluted in phosphate buffer (PB) from 1 to 6 log₁₀ cfu/mL. Diluted cultures were placed on the RBD 3000 for enumeration and viable cell count results were obtained using Advanced Analytical's FASTEST TVO Kit or *Mycoplasma* Kit. Additionally, each diluted culture was spread-plated and incubated for one to seven days. The RBD 3000 viable counts of pure cultures (n ≥ 12 per microorganism) correlated well, R² ≥ 0.9832, with the traditional plate count method.

MATERIALS

Bacterial Cultures and Media: *Bacillus atrophaeus* (formerly *B. subtilis* var. *niger*) ATCC No. 9372, *Candida albicans* ATCC No. 10231, *Escherichia coli* ATCC No. 25922, *Salmonella typhimurium* ATCC No. 14028, *Staphylococcus aureus* ATCC No. 6538, *Streptococcus bovis* ATCC No. 33317, and *Mycoplasma bovis* ATCC No. 25025 (American Type Culture Collection, Manassas, VA), *Clostridium perfringens* Type A (Iowa State University Veterinary Services Laboratory, Ames, IA). *C. albicans* was cultured aerobically using Yeast Extract and Malt Extract (YM) broth and agar. *C. perfringens* was cultured anaerobically using Thioglycollate Fluid Medium and Tryptic Soy Agar (TSA) II w/ 5% Sheep Blood (Difco, Sparks, MD). *M. bovis* was cultured using PPLO broth and agar containing *Mycoplasma* supplement (Difco, Sparks, MD). All other microorganisms were cultured aerobically using Tryptic Soy broth (TSB) and TSA (Difco, Sparks, MD).

Enumeration: FASTEST Total Viable Organism (TVO) Kit, *Mycoplasma* Kit, and fully automated RBD 3000 (Advanced Analytical Technologies, Inc., Ames, IA).

METHODS

All cultures were serially diluted 10-fold in PB to yield approximately 10¹ to 10⁶ cfu/mL. Three milliliters of the diluted samples were dispensed into 5 mL sample tubes and loaded onto the RBD 3000. The RBD 3000 automatically adds the appropriate reagents from each kit, mixes the sample, and enumerates the labeled microorganisms in each sample. All samples were analyzed using the FASTEST Total Viable Organism (TVO) Kit, except those containing *M. bovis* which were analyzed using the *Mycoplasma* Kit. The FASTEST TVO kit enumerates the total number of viable microorganisms using Nucleic Acid Dye and BRAG3. The Nucleic Acid Dye is a membrane permeable nucleic acid stain that labels all live and dead cells (biomass) in the sample. The BRAG3 acts as a counter-stain to the Nucleic Acid Dye allowing enumeration of viable cells only. The *Mycoplasma* kit contains Nucleic Acid Dye and Dead Cell Dye. Two separate *M. bovis* samples were analyzed, one using the Nucleic Acid Dye and the other using the Dead Cell Dye. The Dead Cell Dye is a non-membrane permeable nucleic acid stain that labels dead cells only. The total number of viable *M. bovis* in each sample was determined by subtracting the Dead Cell RBD count result from the corresponding Biomass RBD count result, which was calculated within the RBD 3000 software. Additionally, prior to RBD 3000 enumeration, each sample was spread-plated for plate count comparison.

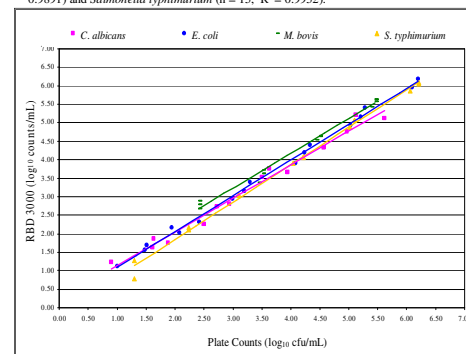
CONCLUSIONS

- The RBD 3000 viable count results (n ≥ 12 per microorganism) correlated exceptionally well, R² ≥ 0.9832, with the traditional plate count method (Graph 1 and Graph 2). The RBD 3000 yielded results within 20 minutes of sampling versus one to seven days of total test time required for the traditional plate count method.

- Advanced Analytical's fully automated RBD 3000, in conjunction with the FASTEST TVO and *Mycoplasma* Kits, provide rapid and accurate enumeration of a wide variety of microorganisms (e.g., Gram positive bacilli and cocci, Gram negative bacilli, anaerobes, spore-formers, yeast, and mycoplasmas) across a dynamic range of 10¹ to 10⁶ cfu/mL.

RESULTS

Graph 1: Correlation of RBD 3000 Counts vs. Plate Counts of *Candida albicans* (n = 14, R² = 0.9982), *Escherichia coli* (n = 17, R² = 0.9959), *Mycoplasma bovis* (n = 15, R² = 0.9891) and *Salmonella typhimurium* (n = 15, R² = 0.9952).



Graph 2: Correlation of RBD 3000 Counts vs. Plate Counts of *Bacillus atrophaeus* (n = 15, R² = 0.9839), *Clostridium perfringens* Type A (n = 12, R² = 0.9981), *Staphylococcus aureus* (n = 18, R² = 0.9857) and *Streptococcus bovis* (n = 13, R² = 0.9832).

