Rapid Enumeration of Bioburden in Fiber Wipes using the Growth Direct™ System

Background:

Bioburden testing involves the analysis of a wide variety of substances, many of them composed of solid, insoluble matrices containing anti-microbial agents. For such materials, a first step in analysis is to suspend and agitate the material in a liquid buffer to release associated microbial contaminants for enumeration using membrane filtration or other methods. Treatment of fibrous materials used in any number of medical and consumer products may also release particulates as well as microbial contaminants during this procedure. These materials could then disrupt accurate detection of microbial contaminants by preventing discrete, countable colonies from forming. Thus, such methods as membrane filtration, or even spread plating could be ineffective unless an additional step is employed to remove any fibers from the rinse fluid.

The Growth Direct™ System for automated rapid microbial enumeration addresses all the QC applications required inclusive of raw material bioburden testing. Sample preparation is the same as with compendial membrane filtration methods, and like the compendia it uses growth-based detection to enumerate sample contaminants, unlike many other rapid testing platforms. The Growth Direct™ fully automates incubation, sample handling, analysis, and results reporting; only sample preparation is performed by the user. Finally, it is a non-destructive test; this attribute allows subsequent microbial ID, a necessity for root cause investigation and contamination prevention.

To demonstrate that fibrous materials can be effectively tested on the Growth Direct™ System, woven cloth wipes containing preservatives were obtained, agitated by stomaching in a rinse buffer in a sieved bag to capture any released fibers that may interfere with analysis, and the rinse fluid tested on the system. Test organisms were spiked into the preparations to ascertain the accuracy of the system in detecting contaminants, and the time savings over a standard raw material bioburden test determined.

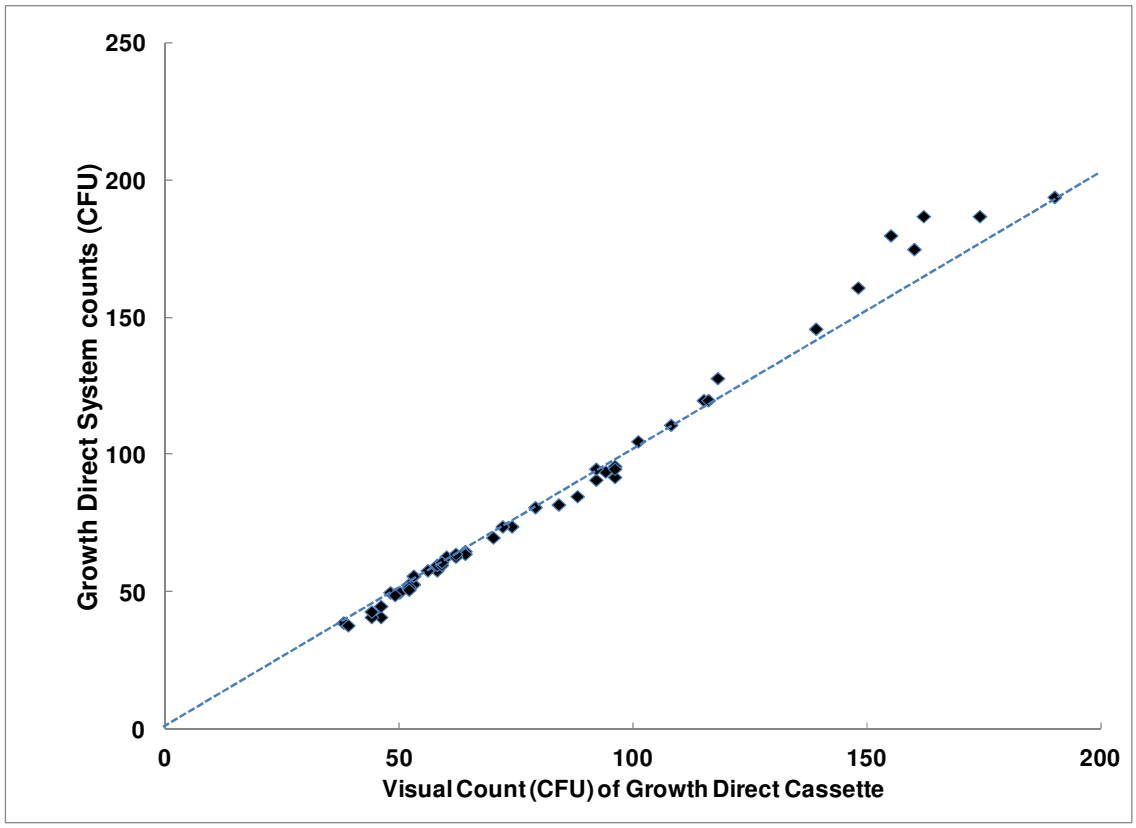
Analysis of test matrices:

Analysis was performed thus: a 10% (w/v) suspension of cloth wipes with antimicrobial components was prepared in neutralizing buffer, and then agitated by stomaching. The stomacher bag contained an interior sieve of a pore size that captured particulates that could disrupt colony development and subsequent analysis, while allowing microbial contaminants to pass through with the cleared wash liquid. Following stomaching 10 ml aliquots were removed, filtered using the Growth Direct™ Filtration Kit, and subsequently analyzed on the Growth Direct™ System using TSA media. Test organisms were spiked into the wash preparations in order to test the accuracy the system in detecting contaminants in this sample matrix. Filterability of the samples, disruption of Growth Direct™ enumeration by components in the sample matrix captured on the membrane filter were examined. Also the accuracy of Growth Direct™ detection was determined versus a spread plate control, and the time savings of the Growth Direct™ System detection over that of the standard test method was also established.

Filterability and analysis of the sieved wash fluid from the cloth materials was not an issue. Samples were easily filtered in no more time than is required for PBS- less than 20 seconds for 10 ml of sample. Disruption of Growth Direct™ testing by materials in the sample would have resulted in the termination of the analysis. All the samples successfully completed their analytical runs.

Accuracy of the Growth Direct™ was determined by comparing the Growth Direct™ counts with spread plate titer controls of the test organisms. The organisms tested were *P. aeruginosa, B. subtilis, C. albicans, E. coli,* and *S. aureus*. Figure 1 presents this analysis demonstrating that the Growth Direct™ detection of the spiked counts was comparable to the visual counts on the growth cassettes at the end of the assay. These data show that the preparation method effectively removed interfering materials that could have interfered with detection colonies by the Growth Direct™ System.

Figure 1: Comparability of Growth Direct™ system counts with Growth Cassette visual counts



The accuracy of the Growth Direct™ count in detection of spiked test organisms was evaluated versus the standard method titer control grown on spread plates is shown in Figure 2 for each of the five strains tested. This plot demonstrates that the Growth Direct™ detects the same numbers as the control method. The Growth Direct™ result was obtained after 24 hrs of analysis compared to a three day incubation for the control providing an equivalent result in nearly one-third the time of the standard method

Conclusion:

These results demonstrate a method for detecting and enumerating microbial contaminants in wash fluid obtained from fibrous wipes. Equivalent numbers of colonies were detected by the Growth Direct™ in 24 hours as were counted via the culture method after 72 hrs: a 60-70% times savings. These results show that the Growth Direct™ System's versatility ranges beyond the testing of "clean", easily filtered samples, and encompasses test materials that one would not necessarily think can be accurately analyzed on the Growth Direct™ System.

Figure 2. Mean counts of spiked test organisms in rinse fluids from wipe suspension is comparable to spread plate titer controls