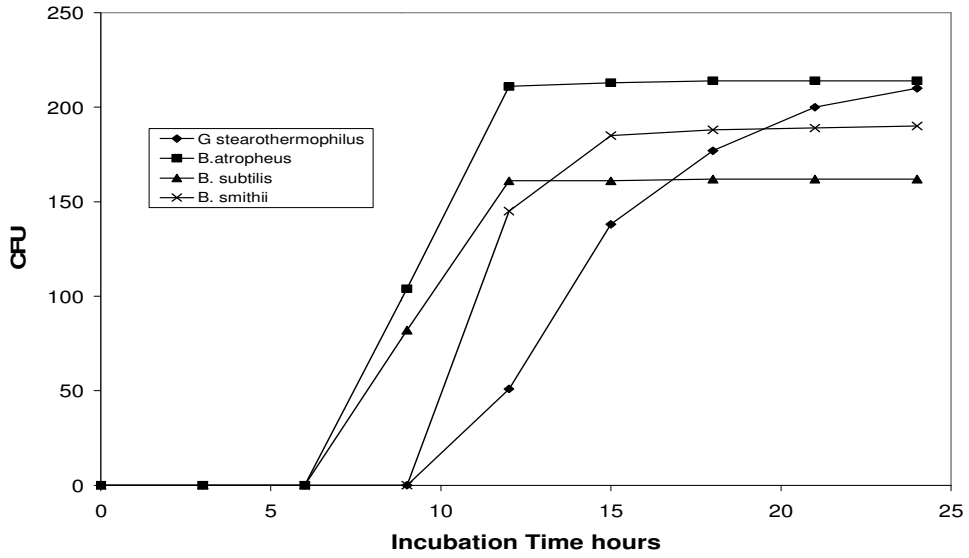
Rapid Enumeration of Biological Indicators Using the Growth Direct™ System

A biological indicator (BI) is a characterized preparation of a microorganism that has a stable and known resistance to a specific sterilization process. Biological Indicators are used to perform qualification of sterilizing equipment, materials, packaging components, and to ensure that finished product is sterilized to the appropriate assurance level. There are multiple types of sterilization processes steam, dry heat, ionizing radiation, vapor phase hydrogen peroxide and ethylene oxide. Three types of BIs are used in these various sterilization processes. The first consists of spores added to a packaged carrier such as paper, glass, plastic or other material. The second type is a spore suspension which is directly inoculated into product or a representative form that would go through the sterilization process. Lastly, the third form of BI consists of a self-contained ampule containing spores and an indicator media.

The microbiological testing of a BI varies from 2-7 days. On reception, a new BI lot will be tested to verify its spore content, and to establish a positive or negative result following its use in a validation or process. During release testing using BIs, product, equipment, materials and pack-ing components will be quarantined with impact on production and product release schedules.

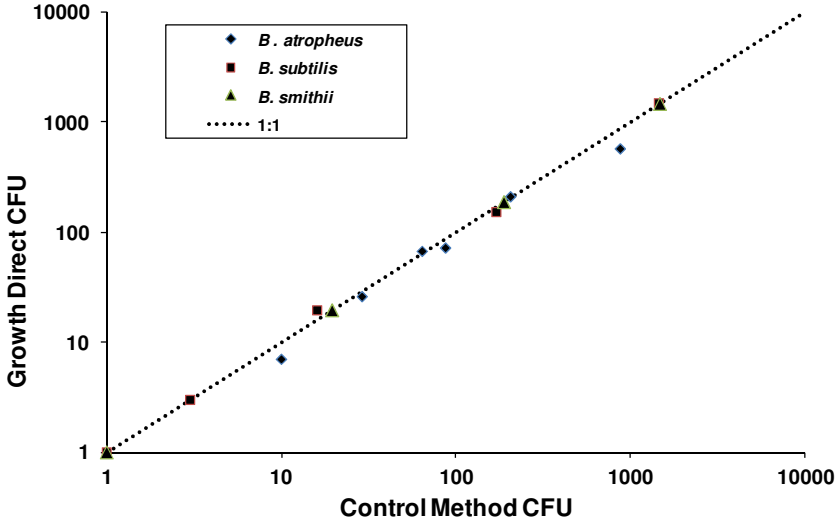
Use of the Growth Direct™ System for BI enumeration speeds time to result through the system's ability to accurately count micro-colonies before they become visible to the naked eye, thereby saving up to 50% of the time required to perform a test using a standard culture method. Figure 1 shows the growth curves on the Growth Direct™ for each BI tested: *B. atropheus* from a spore strip preparation, ampuled *G.stearothermophilus* spores intended for testing steam sterilization, and *B subtilis*, and *B. smithii* preparations used for direct inoculation testing. In all cases quantitative results are obtained within 24 h compared to current culture methods that normally require up to three days or more.

Figure 1: 24 hour detection of BIs on Growth Direct™ system



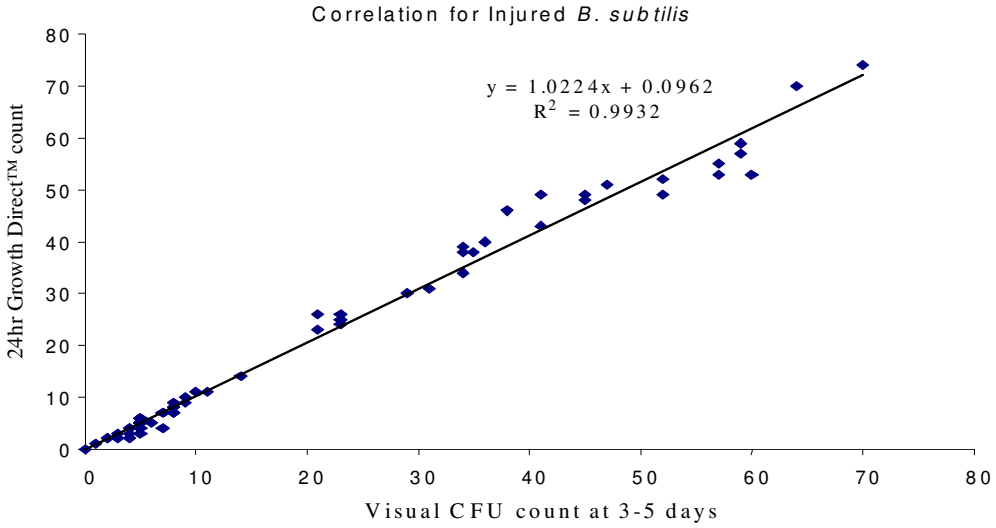
Concentrations of microbes in a BI can be as high as 10^8 CFU/ml, to obtain an accurate count of organisms (<250 CFU) in the BI, multiple dilutions of the sample must be performed resulting in extensive labor and material costs as well as experimental variability due to handling errors leading to confounded results. Additional human error during data collection and recording can introduce even more inconsistencies into the final result. The Growth Direct™ System with automated handling, results reporting, and higher dynamic detection range (1500 CFU) requires fewer dilutions, and produces more accurate data. Figure 2 presents enumeration accuracy versus the standard visual 3 day plate count for *B. atropheus, B subtilis*, and *B. smithii* preparations described previously demonstrating one-to-one correlation between Growth Direct™ enumeration and control spread plate counts

Figure 2: Equivalent accuracy of: Growth Direct™ at 24 hours compared to 3 day Petri plate counts.



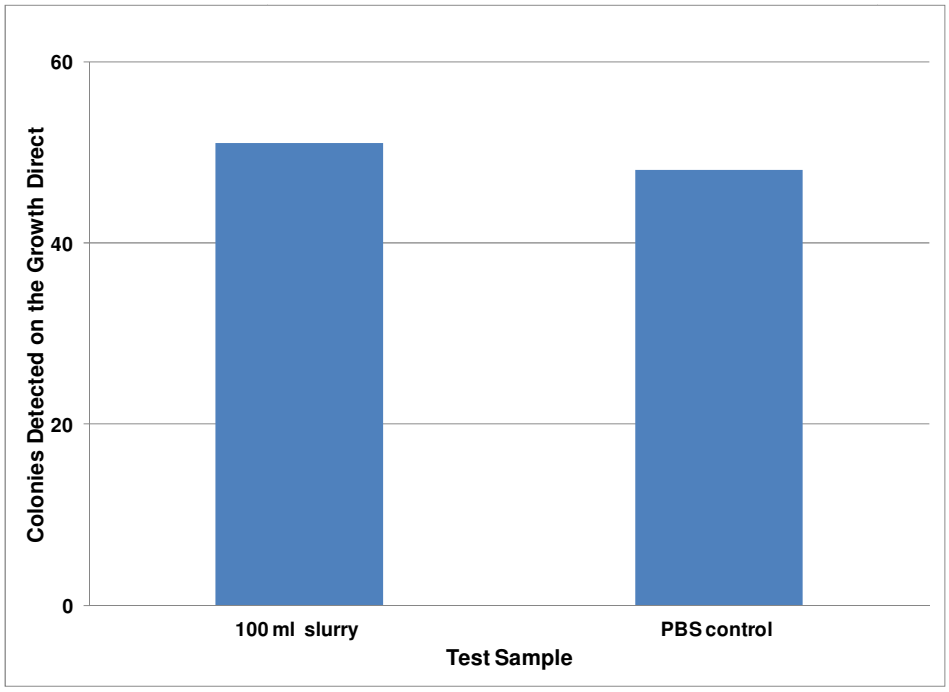
To verify the effect of stress on BI detection, a Bier vessel was used to perform a sub-lethal kill. Populations of *B. subtilis* spores were subjected to moist -heat sterilization for 3.1 and 3.3 minutes prior to analysis. The resulting populations were analyzed on the Growth Direct™ for 24 hours and compared to the results obtained at 3 days on the traditional Petri Plate (Figure 3).

Figure 3: Equivalent recovery of stressed B subtilis spores by the Growth Direct™ system and traditional spread plate culture on Petri plates



All the test samples except the *B. atropheus* spore strips came as easily filtered liquid suspensions, to liberate spores from the paper matrix for analysis, several strips were shredded in water. The slurry of paper fibers would then be pre-filtered to separate the cellulosic fibers from the spores. To show that spores present in the slurry could be enumerated in such a sample on the Growth Direct™, 100 ml of slurry prepared from three sterilized strips was spiked with *B atropheus* spores, pre-filtered to remove the fibers and subsequently filtered and analyzed on the Growth Direct™. The results presented in Figure 4 demonstrate that *B. atropheus* spores in up to 100 ml of slurry from spore strips could separated from the fibers, and then accurately enumerated on the Growth Direct™ system

Figure 4: Accurate detection of B. atropheus spores in 100 ml spore strip suspensions



Conclusion:

Equivalent numbers of BI colonies can be detected by the Growth Direct™ system within 24 hours as are counted after 3 days with the standard method, a time savings of 2 full days. Samples from spore strips These BIs came from samples normally used for testing sterilization and include ampoule and spore strip BIs. Such time savings combined with the lower labor costs and improved quality due to system automation and data reporting and fewer sample dilutions allow more efficient QC release of spore BI preparations and test results for validation studies.