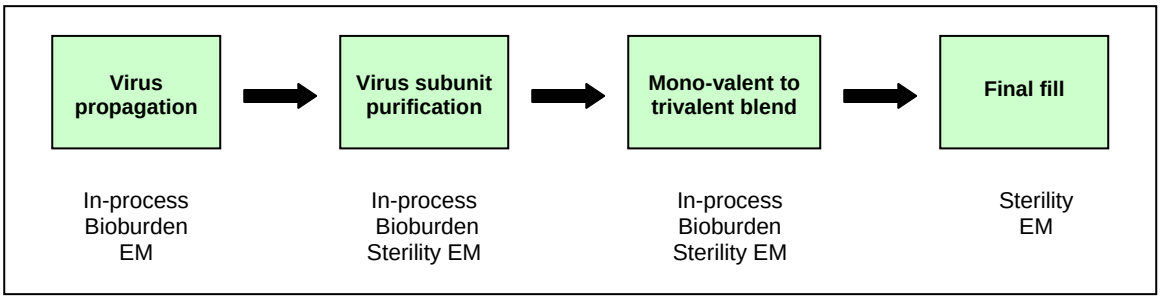
Rapid enumeration of bioburden in a model sample matrix for influenza vaccine using the Growth Direct™ system

Background

The manufacture of influenza vaccine has four major steps: virus propagation, harvest and processing, blending of bulk stocks, and final fill of the finished vaccine (Fig. 1). Influenza virus is traditionally grown using chicken eggs inoculated with seed virus, though cell culture-based methods are now becoming established. Whether egg- or cell culture-based methods are used, propagated virus is then harvested and processed to extract and purify the specific viral subunits required to produce mono-valent bulk stock for vaccine. These stocks are subsequently blended into trivalent bulk which is loaded into the final packaging for release.

Throughout the manufacturing process an assortment of microbial QC assays must be performed to confirm that microbial contaminants are maintained within appropriate limits (e.g. purification steps), or have been completely removed (e.g. final fill). Fig. 1 lists the types of testing performed during each major step of vaccine manufacture. Additionally, environmental monitoring (EM) consisting of air, surface and personnel testing is carried out throughout production to confirm that the manufacturing process is performed under controlled conditions. Microbial QC testing requires from 3 to 14 days to complete, is performed at multiple production steps, and progression to each subsequent step may be contingent upon passing these lengthy tests (e.g. 14 days for sterility); consequently such testing can have significant additive impacts on the production cycle. These procedures also require substantial resources (i.e. labor and materials) to perform. Thus, reducing the turn-around times for microbial QC results could have a significant impact on influenza vaccine production cycle time, costs, and the timing of product distribution to the market.

Fig. 1. Steps of vaccine manufacture and associated microbial testing



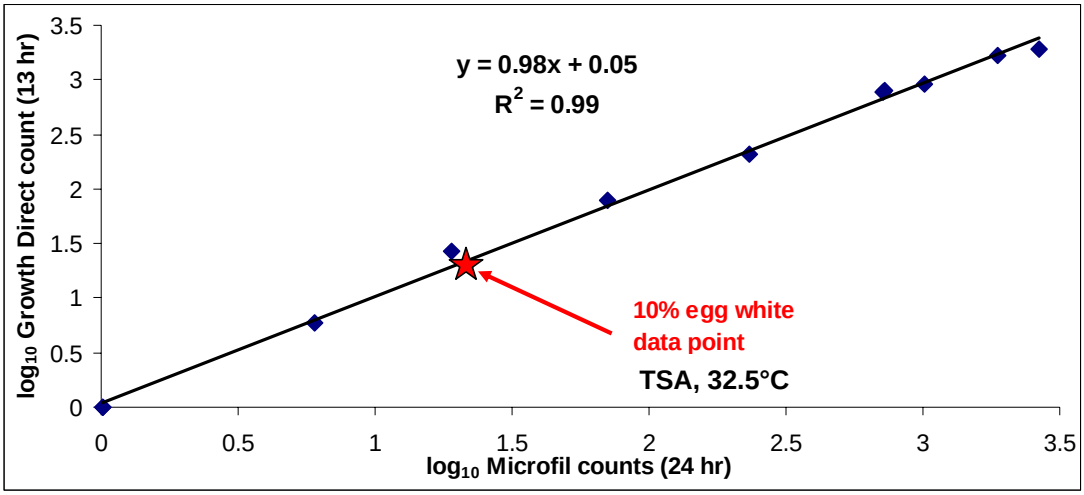
Application

The Growth Direct™ system for rapid microbial enumeration addresses all the applications required for microbial QC testing in vaccine manufacture. It uses the same principles and methods as the compendia, thus avoiding the extensive validation work often found with other rapid testing platforms, while fully automating incubation, sample handling, analysis, and results reporting.

Only sample preparation is performed by the user (i.e. standard membrane filtration method). Finally, it is a non-destructive test; this attribute allows subsequent microbial ID, a necessity for root cause investigation and contamination prevention.

To verify that the Growth Direct system could more rapidly detect and enumerate microbial contamination at the early steps of vaccine manufacture, diluted egg white (1% or 10% in PBS) was inoculated with *Salmonella enterica* and analyzed on the Growth Direct system for a period of 24 hours. Egg white can serve as a model matrix to mimic vaccine raw and in-process materials as well as finished product allantoic fluid. Fig. 2 compares the number of colonies detected by the Growth Direct system after 13 hours of incubation with those counted visually on the standard membrane filtration control method after 24 hours. This result demonstrates that the Growth Direct system detects and enumerates the same number of colonies in one-half the time required to obtain visual counts. This result was observed across the entire range of the assay from <10 to 2000 CFU, an approximately 10-fold higher dynamic range than normally used.

Fig. 2: Correlation between colonies detected by the Growth Direct system and visual counts of *S. enterica* of spiked in egg white.



Conclusion

This study demonstrates that microbial contaminants in egg white, a model matrix for allantoic fluid, can be rapidly analyzed on the Growth Direct system. Equivalent numbers of colonies were detected by the Growth Direct in 13 hours as were counted via the culture method after 24 hrs: a 50% time savings. Such time savings combined with lower labor costs and improved compliance from system automation can:

- significantly lower manufacturing costs by reducing hold times between manufacturing steps and speeding release of product.
- free-up personnel to perform other, higher value tasks.
- reduce product scrap by reducing response times to microbial contamination.
- improve overall QC, QA and manufacturing efficiency.

These benefits can result in a payback on the initial investment of the Growth Direct system of two years or less, due to the resulting substantial improvements in efficiency and productivity, and cost savings realized by the vaccine manufacturer.