

## ABSTRACT

Influenza viral vaccine production using the traditional method of viral expansion in Specific Pathogen-Free (SPF) fertile chicken eggs has an inherent potential for microbial contamination. Eggs with microbial bioburden may escape detection, which could result in rejection of the in-process bulk, and subsequently the entire lot of vaccine material. To minimize the risk of rejecting the entire in-process bulk, allantoic fluids from the eggs are harvested into multiple 1 liter sub-lots which are then screened for the presence of bioburden. Highly contaminated sub-lots are discarded prior to downstream processing, which subsequently removes all remaining bioburden. This process warrants the use of a rapid bioburden detection method that provides a quick assessment of bioburden levels to facilitate straight-through processing, without having to quarantine the in-process bulk. The ability of the *Micro PRO™* flow cytometer (Advanced Analytical, Ames, IA) to address this issue was demonstrated. Sub-lots of vaccine material from a mock production run were analyzed on the *Micro PRO™* system using Advanced Analytical's *FASTEST* Total Viable Organisms detection kit. Prior to testing samples on the *Micro PRO™* detection system, samples were pre-treated using a novel method comprising of filter clarification using 0.45µm cellulose acetate membrane filters and a detergent wash to reduce the background noise contributed by cellular debris and other components of allantoic fluid. All samples were plated in parallel on Tryptic Soy Agar plates for count comparisons. Ten percent of the sub-lots were positive for bioburden with counts ranging from 10<sup>4</sup> to 10<sup>5</sup> cfu/ml. The *Micro PRO™* system provided accurate detection of relative contamination levels in each of the contaminated sub-lots of vaccine material. This rapid bioburden technique provides a faster time to result than the conventional bioburden method and eliminates the need to quarantine the in-process bulk.

## MATERIALS

**Test Samples:** Sixty-three sub-lots of in-process bulk influenza vaccine material.

**Solutions & Diluents:** 0.45µm cellulose acetate filters (NalgeneNunc International, Rochester, NY ) were used to concentrate microbes. Wetting solution contained 0.1% (v/v) Tween 20 (VWR International, West Chester, PA ) and 1.0% (w/v) Peptone (Difco, Sparks, MD). Lysis solution contained 0.01% (v/v) surfactant and was pre-warmed to 50°C. Phosphate Buffer was used as the sample diluent. Tryptic Soy Agar (Difco, Sparks, MD) was used for plating.

**Detection:** *FASTEST* Total Viable Organisms (TVO) kit and *Micro PRO™* Detection System (Advanced Analytical, Ames, IA).

## METHODS

**Sample Collection:** A 2 ml sample aliquot from each sub-lot was obtained from a mock production lot. Samples were maintained at refrigeration temperature until the testing was performed

**Sample Processing:** Filter membranes were pre-wetted with Wetting solution to block irreversible adsorption of microbes. Sample were passed through pre-wetted membranes and washed once with Lysis solution. Microbes captured on the membrane surfaces were eluted in 20 ml phosphate buffer by mechanical agitation in sterile sample cups.

**Detection:** A 3 ml aliquot of the elution volume corresponding to each sample was loaded on to the auto-sampler of *MicroPRO™* Detection System. Bioburden levels were assessed using *FASTEST (TVO)* kit and a pre-defined method.

## RESULTS

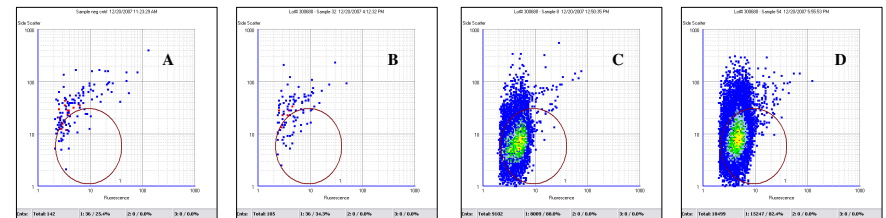
**Table 1.** Overall results of the sub-lot samples analyzed on the *Micro PRO™* Detection System and the agar plate method.

| Sample           | <i>Micro PRO™</i> | Agar Plate |
|------------------|-------------------|------------|
| Positive Samples | 6                 | 6          |
| Negative Samples | 57                | 57         |
| False Positives  | 0                 | 0          |
| False Negatives  | 0                 | 0          |

**Table 2.** Results of select sub-lots analyzed on the *Micro PRO™* Detection System and the corresponding agar plate results.

| Sample           | <i>Micro PRO™</i> counts/mL | Plate counts cfu/mL |
|------------------|-----------------------------|---------------------|
| Negative Control | 383                         | 0                   |
| Sub-lot #32      | 383                         | 0                   |
| Sub-lot #8       | 85,429                      | 240,000             |
| Sub-lot #54      | 162,634                     | 560,000             |

**Figure 1.** Intensity plots of Negative Control (A), Negative in-process sub-lot #32 (B) and Positive in-process sub-lots #8 (C) and #54 (D).



## DISCUSSION/CONCLUSIONS

- The *Micro PRO™* Detection System provided accurate detection of relative contamination levels in each of the contaminated sub-lots of vaccine material.
- This innovative bioburden technique provides a rapid time to result in comparison to the conventional bioburden test method and eliminates the need to quarantine the in-process bulk.
- This method is currently being simplified and optimized to yield lower negative control counts as well as providing better correlations to the plate count method.