

# Total Viable Organism Detection in Purified Water Systems in 18 Minutes

Julio C. Petersen, Ph.D., Steven J. Lasky, Ph.D, Kelley A. Molitor  
Advanced Analytical Technologies, Inc., Ames, IA 50010

2901 South Loop Drive Suite 3300  
Ames, IA 50010  
(515)296-6600 phone  
(515)296-6789 fax  
www.aatl-us.com

## ABSTRACT

In many industries, purified water systems are one of the most important pieces of the final product equation. However, when using traditional plate count methods, possible contamination levels are not known for 5-10 days. Alternatively, using the RBD 3000 and the Total Viable Organism (TVO) Kit, it is possible to obtain accurate and consistent purified water counts within 18 minutes of sampling. Test samples were collected from two different faucets (A and B) of a de-ionized water system and analyzed in duplicate on the RBD 3000 periodically for 12 weeks. Background criteria were established by analyzing filter sterilized de-ionized water (n=96) samples. All samples were also plated in parallel on R2A agar in duplicate, incubated at room temperature, and counts were recorded after 5 and 10 days of growth. Seventeen sets of RBD 3000 counts for each faucet A and B were plotted against the plate counts. The RBD counts trended with the standard plate count method. Three of the 34 total samples analyzed from faucets A and B resulted in both RBD 3000 and R2A agar counts  $\geq 50$ cfu/mL, which would trigger an alert. The RBD 3000's 18-minute time to result allows for a proactive solution as opposed to reactive, because potential contamination would be detected 5-10 days sooner than by the standard plate count method.

## MATERIALS

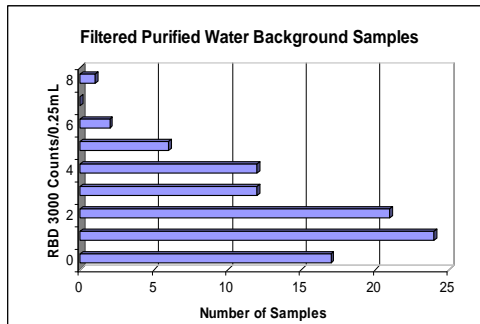
**Water System:** 18mega-ohm de-ionized water system with a 0.2 $\mu$ m final filter. Two separate faucets (A and B) were tested. **Media:** R2A agar plates (Difco, Sparks, MD). **Filters:** 0.2 $\mu$ m PES membrane SteriFlip filter units (Millipore, Billerica, MA) **Detection:** *FasTest* Total Viable Organism (TVO) Kit and fully automated RBD 3000 (Advanced Analytical Technologies, Inc. Ames, IA).

## METHODS

**Background Determination:** Water from faucets A and B were filtered using a 0.2 $\mu$ m SteriFlip filter unit. Three-mL aliquots of the filtered water was dispensed into 96 5mL sample tubes and analyzed on the RBD 3000 using the Total Viable Organism (TVO) kit. This was done to determine normal background contribution from filtered water. **Sample Processing:** Purified water samples were collected from 2 faucets (A and B) over a 12 week period, for a total of 17 sets of samples. Three-mL aliquots from each faucet were analyzed in duplicate on the RBD 3000 using the TVO kit. One-mL of each sample was also plated in duplicate on R2A agar and incubated at 22 $\pm$ 2 $^{\circ}$ C. Plates were counted at 5 and 10 days.

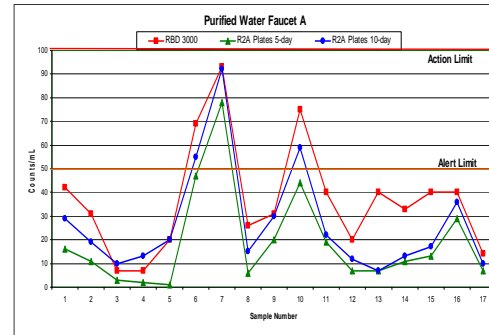
## RESULTS

**Figure 1.** RBD 3000 Counts vs. Number of Samples for Background Determination of Filtered Purified Water

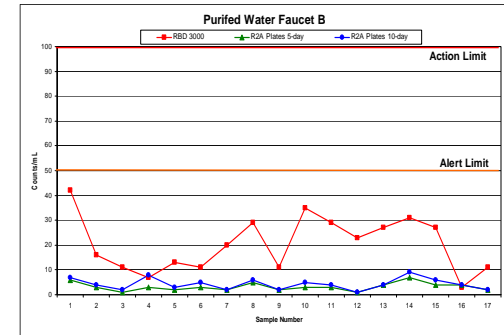


## RESULTS CONTINUED

**Figure 2.** RBD 3000 Counts vs. R2A Plate Counts for Faucet A



**Figure 3.** RBD 3000 Counts vs. R2A Plate Counts for Faucet B



## DISCUSSION/CONCLUSIONS

- The RBD 3000 counts trended well with the R2A plate counts.
- 97% of the filtered purified water samples had RBD 3000 counts  $\leq 5/0.25$ mL.
- R2A plates were allowed to incubate for a total of 10 days, because additional growth was observed following the initial 5 day count result.
- Three of the 34 test samples analyzed resulted in both RBD 3000 and R2A agar counts  $\geq 50$ cfu/mL, which was the alert limit. These results show an alert would have been triggered the same day the sample was collected.
- The RBD 3000's 18-minute time to result allows for a proactive solution as opposed to reactive, because potential contamination is detected 5-10 days sooner than by the standard plate count method.

