

# 250 mL Enumeration Protocol Addendum and MPN Table

Follow the procedures in this protocol addendum when using the Pseudalert\* Test for the enumeration of *Pseudomonas aeruginosa* in 250 mL water samples. Refer to the printed package insert for test storage requirements and all other procedures.

## Quanti-Tray enumeration procedure (250 mL samples only)

1. Divide the 250 mL sample into three sterile, transparent vessels with two samples having aliquots of 100 mL and one sample having an aliquot of 50 mL.
2. Add 50 mL of sterile, noninhibitory, oxidant-free water to the 50 mL sample aliquot to bring the volume to 100 mL.
3. Aseptically add the contents of a single 100 mL snap pack of Pseudalert\* reagent to each of the three vessels.
4. Cap vessels and shake until contents are dissolved.
5. Add 2 drops of IDEXX Antifoam Solution to each sample/reagent mixture.  
**Note:** IDEXX 120 mL sample vessels containing antifoam are also available.
6. Pour each sample/reagent mixture into a separate Quanti-Tray\* tray, and then seal with the Quanti-Tray\* Sealer; label the three trays to differentiate between the undiluted and diluted mixtures.
7. Place the sealed trays in an incubator at 38°C ±0.5°C for 24 to 28 hours.
8. Read results according to the result interpretation table below, counting the number of positive wells.
9. Refer to the Pseudalert 250 Quanti-Tray MPN Table on the next page. The most probable number (MPN) is obtained using the sum of the positive counts from the two undiluted Quanti-Trays and the positive count from the diluted Quanti-Tray.

## Result interpretation

Appearance	Result
No blue fluorescence	Negative for <i>Pseudomonas aeruginosa</i>
Blue fluorescence <sup>†</sup>	Positive for <i>Pseudomonas aeruginosa</i>

<sup>†</sup>Greater than the blue fluorescence present in a negative control sample

- Look for blue fluorescence with a 6-watt, 365 nm UV light held within 5 inches of the sample in a dark environment. Face the light away from your eyes and toward the sample.
- Refer to the -/+ fluorescence reading guide on the test box. The colors are as seen under a UV light.
- Pseudalert results are definitive at 24–28 hours.







