Protocol for the Analysis of Ballast Water to determine the Concentration of Vibrio Cholerae using the Fluorescence in situ Hybridisation Method

1 PREPARATION OF SOLUTIONS NECESSARY FOR THE ANALYSIS (ONCE PER TEST KIT)

1. "Solution B3": Addition of 4 ml of pure ethanol to "Solution B3", mixing and marking bottle
2. "Washing buffer": 2 ml of washing buffer are required per test. For this 10-fold concentrated "Solution D5B" is diluted with distilled water. Washing buffer is filled in "Washing bottle" and preheated to 46 °C for at least 30 min.
3. "Aqua Bottle": 2 ml of distilled water are required per test. For easier application distilled water is added to "Aqua bottle" and stored at room temperature.

2 ANALYSIS : FILTRATION AND CULTIVATION

1. Filtration of Ballast Water sample (100 ml) on 25 mm membrane filter with filtration funnel
2. Transfer of membrane filter to TCBS agar plate by using sterile tweezers
3. Incubation of TCBS agar with membrane filter aerobically at 37 °C for 8 h

3 ANALYSIS : PRE-HYBRIDIZATION

1. Filtration of Ballast Water sample (100 ml) on 25 mm membrane filter with filtration funnel
2. Transfer of membrane filter to TCBS agar plate by using sterile tweezers
3. Incubation of TCBS agar with membrane filter aerobically at 37 °C for 8 h
4. Transfer of cultivated membrane filter from TCBS agar into an empty petri dish
5. Application of 3 drops of "Solution B3" to the membrane filter. Drying at room temperature (RT), 15 min.
6. Application of 2 drops of "Pre-VIT5" to the membrane filter. Drying at RT, 10 min.
7. Compilation of "ScanVIT-Reactor" with caps and placing the base component of the "ScanVIT-Reactor" on a suitable support stand
8. Place one "Pad" on the base component
9. Application of 6 drops of "Pre-VIT5" onto "Pad"
10. Place pre-hybridized membrane filter on top of the humid "Pad" by using tweezers

4 ANALYSIS : HYBRIDIZATION - WASHING

1. Application of 3 drops of gene probe solution "VIT (BwV)" onto the membrane filter
2. Closing "ScanVIT-Reactor" with upper component and placing the "ScanVIT-Reactor" in stable straight position

3. Incubation: 46 °C, 90 min.
4. Removal of upper component of “ScanVIT-Reactor“
5. Placing filled Water bottle on top of “ScanVIT-Reactor“
6. Filling “ScanVIT-Reactor“ with 2 ml of Washing buffer from “Washing bottle”
7. Replacing upper cap and incubation “ScanVIT-Reactor“ at 46 °C for 15 min.
8. Removal of both caps from the “ScanVIT-Reactor“
9. Pressing “ScanVIT-Reactor“ onto the rubber stopper of the filter funnel, and removal of washing buffer by turning on the vacuum
10. Filling “ScanVIT-Reactor“ with 2 ml of distilled water from “Aqua bottle”
11. Pressing “ScanVIT-Reactor“ onto the rubber stopper of the filter funnel, and removal of washing buffer by turning on the vacuum
12. Removal of membrane filter from “ScanVIT-Reactor“ by using tweezers to either empty petri dish (longer storage) or “slide” (direct microscopy)

5 ANALYSIS : FLUORESCENCE MICROSCOPY

1. Placing membrane filter onto “slide”
2. Application of 3 drops of “Finisher S” to membrane filter
3. Microscopy: Using a 100-fold magnification analysis of the complete membrane filters for green shining micro-colonies (= *Vibrio cholerae*)