

STANDARD PROTOCOL FOR SHIPBOARD ROTOXKIT M TESTING DURING MC252 OIL SPILL RESPONSE

22 June 2010

NOTE: As of the date of this revision, inclusion of a reference toxicant has not been added to the protocol. As soon as SDS solutions are prepared in an analytical laboratory, the solutions will be sent to each vessel and this protocol will be updated to include use of the reference toxicant.

PREPARATION OF STANDARD SEAWATER (35 ppt) AND HATCHING MEDIUM

1. Add 800mL Deionized (DI) water to a 1000mL volumetric flask or graduated cylinder.
2. Add the contents of vial #1 (NaCl) and shake to dissolve.
3. Add the contents of salt media solutions in vials #2 through #7 (in sequential order) located in the Rotoxkit M in numerical order.
4. Add DI water so the total volume equals 1000mL and shake to homogenize the solution.
5. To prepare the Hatching Medium, dilute the standard seawater solution to 20ppt by mixing 5.7mL of standard seawater to 4.3mL DI water for a total of 10mL hatching medium. If large volume testing is required, increase the scale to make 100mL at a time.

HATCHING THE ROTIFERS

6. Label the multi-well test plate with date of cyst initiation and appropriate ID number.
7. Empty One (1) rotifer cyst vial¹ into hatching well.
8. Add 2.5 mL of Hatching Medium (20ppt seawater) to the hatching well, using a pipette to rinse cysts away from edges. If using hatching medium to rinse the cyst vial, add 2.0 mL hatching medium to the hatching well.
9. Cover plate with parafilm, replace lid and place in incubator (25°C) for 28 hours under continuous illumination. If an insufficient number of rotifers have hatched, allow rotifers to incubate for a longer time period. Continue to check on rotifers each hour until optimal hatch rate has occurred. Do not exceed 30 hours incubation.

PERFORMING TOXICITY TESTING

10. Check hatching success to ensure adequate numbers of rotifers are available for testing.
11. Add 0.7mL of standard seawater (35 ppt) to the rinsing trough of the first row and use the microscope and pipette to transfer approximately 50 rotifers to the rinsing trough of row X of the test plate. This is the control.

¹ Up to two vials of cysts may be used to ensure optimal hatching rate.

12. For rows 1-5, transfer 0.7 ml of the water sample taken at the first depth of the station to the rinsing trough for row 1. The 0.7 ml of the sample taken at the second depth should be added to row 2, and so on. Each row contains a sample taken at a specific depth. Rows 1 through 5 will be used to test up to five water samples collected from the rosette sampler. The chief scientist will determine which samples will be tested, by using fluorometry readings accompanying the CTD cast to identify the samples with highest likely oil concentrations. Samples will not be diluted during initial screening. Use 1.0 mL pipette to transfer 0.3 mL of the sample water to each of six wells in a given treatment row.
13. Allow the rotifers to acclimate in the rinsing trough for 1-2 hours. While this acclimation is occurring, proceed with steps 14-17 below.
14. Use a 1.0 mL pipette to transfer 0.3mL of standard seawater (35 ppt) to each of six wells in the first row (Row X). Row X will serve as the control and each well will serve as one replicate of n=5 rotifers.
15. This same procedure is followed to add each toxicant sample to the respective row (1-5).
16. All water samples used in the Rotoxkit M will be archived in a refrigerator until the test has concluded. In the event that mortality exceeds 50% in any given treatment, the test will be repeated by warming the archived sample to room temperature, and repeating the testing using the prescribed dilution series.
17. Record the treatment sample numbers assigned to each row.
18. Once the 1-2 hour acclimation (Step 13 above) is complete, transfer exactly 5 rotifers to each well. Once transfers are complete, recount each well in that row to confirm a count of 5 rotifers.
19. Once rotifers have been added to all wells, remove and dispose of all excess rotifers in hatching and rinsing troughs by pipette to avoid spill over during the exposure period.
20. Cover the plate with the parafilm, replace lid, and wrap in aluminum foil to shield from light since the exposure is conducted in darkness.
21. Place in incubator at 25°C for 24 hours.

READING AND RECORDING RESULTS

22. At T=24 hour exposure, remove the test plate from the incubator, remove aluminum foil and place under dissection scope for counting.
23. Count the number of live rotifers in each well. If rotifer is not moving, gently prod the organism with a micropipette to determine if it is alive. A rotifer is considered dead if it does not exhibit any signs of movement for 5 seconds after gentle prodding.
24. Record number of dead and alive rotifers in each well.
Tally up each row and add together to get a row total, which should be 30 (5 rotifers x 6 wells). Mortality data is reported on a per well basis and on a cumulative (total row) basis.
25. Data findings are reported to the Chief Scientist.