R A P I D T O X K I T

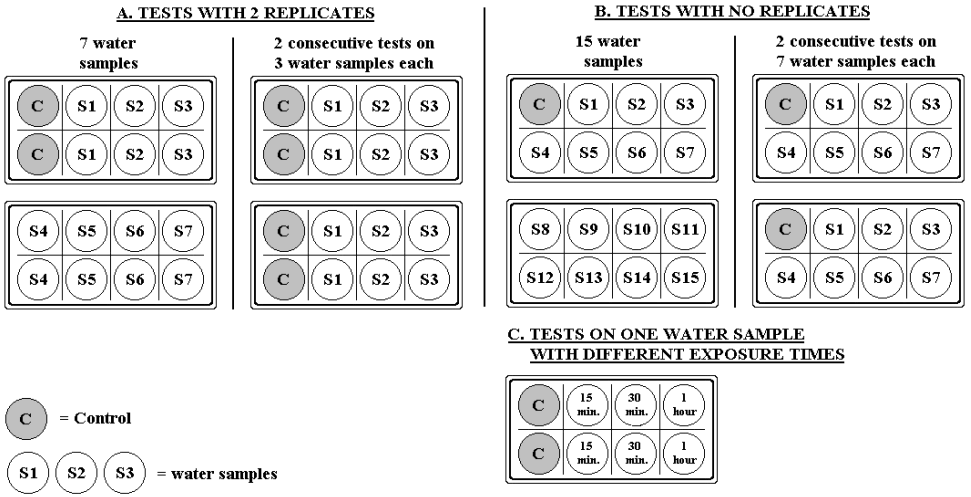
RAPID TOXICITY SCREENING MICROBIOTEST

BENCH PROTOCOL

1. Principle

A rapid bioassay is performed in disposable test tubes using larval crustaceans (*Thamnocephalus platyurus*) hatched from cysts. The test organisms are exposed for a very short period of time (15 minutes to one hour) to the suspected water samples, after which a suspension of red microspheres is added. In the control (a standard freshwater) the organisms ingest the microspheres resulting in a deep red colour of the digestive tract. Stressed (intoxicated) organisms either seize to take up particles altogether or ingest at a much lower rate. The presence or the absence of coloured microspheres in the digestive tract of the larval crustaceans is observed 15 minutes later under a stereomicroscope. Each Rapidtoxkit provides for 3 distinct series of assays on 3 to 15 water samples depending on whether the tests are performed separately or concurrently in singulate or duplicate.

Figure 1 illustrates the number of test waters that can be analysed with the organisms hatched from a single tube of cysts. The number of assays that can be performed depends on the number of replicates used in one single test or in 2 consecutive tests. The table also shows the procedure for analysis of a suspected water sample with exposure time of 15 minutes, 30 minutes or 1 hour.



For reasons of simplicity the procedure described below is for a test on 3 water samples with 2 replicates and 1 hour exposure.

2. Hatching of the cysts

Prehydrate the cysts for one hour by adding 1 ml standard freshwater to a tube with cysts and transfer the cyst suspension into the hatching vessel. Rinse the cyst tube with 1 ml standard freshwater to carry over all the cysts into the hatching vessel. Add 8 ml standard freshwater to the hatching vessel and incubate for 30h (minimum) to 45h (maximum) at 25°C with continuous illumination (4000 lux).

3. Filling of the test tubes with control water and test water(s)

Fill the first 2 tubes adjacent to the sub-sampling tube to the horizontal mark with standard freshwater, and the next pairs of tubes with the 3 test waters respectively.

4. Transfer of organisms into the sub-sampling tube

Pour the contents of the hatching vessel into the sub-sampling tube.

5. Transfer of the test organisms into the test tubes and incubation.

Set the 1 ml micropipette to 0.5 ml and lower the tip into the sub-sampling tube containing the larvae, to approximately half the depth of the liquid. Distribute the larvae evenly in the water column by repeated aspiration and dispensation of the 'water + larvae' from the micropipette. Then collect 0.5 ml larval suspension and transfer it into the first test tube. Repeat this operation until all the test tubes have received 0.5 ml larval suspension. Incubate the the test tubes for one hour at 25°C.

6. Addition of coloured microspheres to the test tubes

Mix the contents of the bottle with red microspheres (*preferably using a Vortex mixer*) to obtain a homogenous distribution. Add 0.2 ml bead suspension to each test tube, stopper the tubes and shake them gently to homogenize the contents. Incubate the tubes again for 15 to 30 minutes at 25°C.

7. Fixing of the test organisms

Add 3 drops fixative to each test tube, stopper and shake them gently to homogenise the contents. Wait for 5 minutes for the dead larvae to settle to the bottom of the test tubes.

8. Transfer of the test organisms into the wells of the observation plate

Set the 1 ml micropipette to 0.2 ml and put the tip into the first "control" tube, until the aperture is close to the settled test organisms at the bottom of the tube. Collect (*in one rapid movement*) all the dead organisms and transfer them into the first well of the observation plate. Repeat this operation for all the test tubes. Cover the observation plate with its transparent cover.

9. Analysis of the test organisms under the stereomicroscope and scoring of the results

N.B. For the microscopic observations strong incident illumination is needed. Fibre optic cold light or a ring illumination with LEDs is recommended.

Put the observation plate on top of the mirror with the white strip and put the latter on the bottom stage of the stereomicroscope. Centre the first well and select the magnification which allows a complete view of the well surface. Count the total number of larvae in the well and the number of larvae with distinct coloured digestive tract and score the data on the Results Sheet. Repeat this operation for all the wells.

N.B. The mirror gives a very good contrast for counting the number of larvae with or without uptake of red particles. When sliding the white strip under a well, only the coloured digestive tracts of the test organisms remain visible, which makes counting of the latter even easier.

Calculate the mean percentage particle uptake for the "control" tubes and for the "test water" tubes.
Calculate the mean percentage inhibition of particle uptake with the formula :

$$\frac{A - B}{A} \times 100$$

whereby A is the mean percentage particle uptake in the control and B the mean percentage particle uptake in the water sample.

Important remarks for correct application of the rapid test

1. *The larval crustaceans can only take up food (and other) particles after metamorphosis into higher instar stages. For this reason the test organisms should only be collected "at the earliest" 30h from the start of the incubation of the cysts, but not later than 45h (since they then become too weak caused by starvation).*

2. *Hatching of the cysts is not entirely synchronous which means that in the collected population of larvae there will still be some at an early stage of development (smaller, orange in colour and not transparent) which cannot take up the coloured particles. The small (opaque) larvae should be excluded in the scoring.*

3. *All the collected test organisms will not have the same level of vitality, so the uptake of coloured particles will vary from "very pronounced" to "little" and will in some larvae even be nil. Consequently even in the controls the percentage of "coloured organisms" will never be 100% but may vary between 60% and 90%. This will not influence the interpretation since the calculations are based on the percentage of coloured organisms in the test water(s) versus that in the controls.*

4. *In the test water(s) the uptake of coloured particles may be less than in the controls. Faintly coloured larvae also have to be counted as "positive" (in the sense of uptake of particles) but the decrease in intensity of uptake may be taken into consideration in the final interpretation.*

10. Interpretation of the results and evaluation of the toxic hazard of the water sample(s).

The percentage uptake of coloured particles in the test water(s) versus that in the controls can range from 0% to nearly the same and the % inhibition of uptake can hence also range from 0% to 100%. A 30% inhibition of particle uptake is suggested for guidance as a threshold signal for the presence of "unwanted" compounds in the test water(s) which should trigger the need for further attention and action.

11. Interferences

The Rapidtoxkit can be applied to "coloured" waters, but turbid waters have to be filtered on a membrane filter (0.2 µ) to avoid interference in the uptake of coloured particles by particulate material.

12. Test validity

In the control water at least 50% of the organisms should exhibit distinct uptake of the coloured particles as a qualifying validity of the health condition of the test population and the reliability of the assay.