ROTOXKIT F CHRONIC

Test procedure

1

PREPARATION OF

STANDARD FRESHWATER

- VOLUMETRIC FLASK (1 liter)
- VIALS WITH SOLUTIONS OF CONCENTRATED SALTS
- DISTILLED (or deionized) WATER

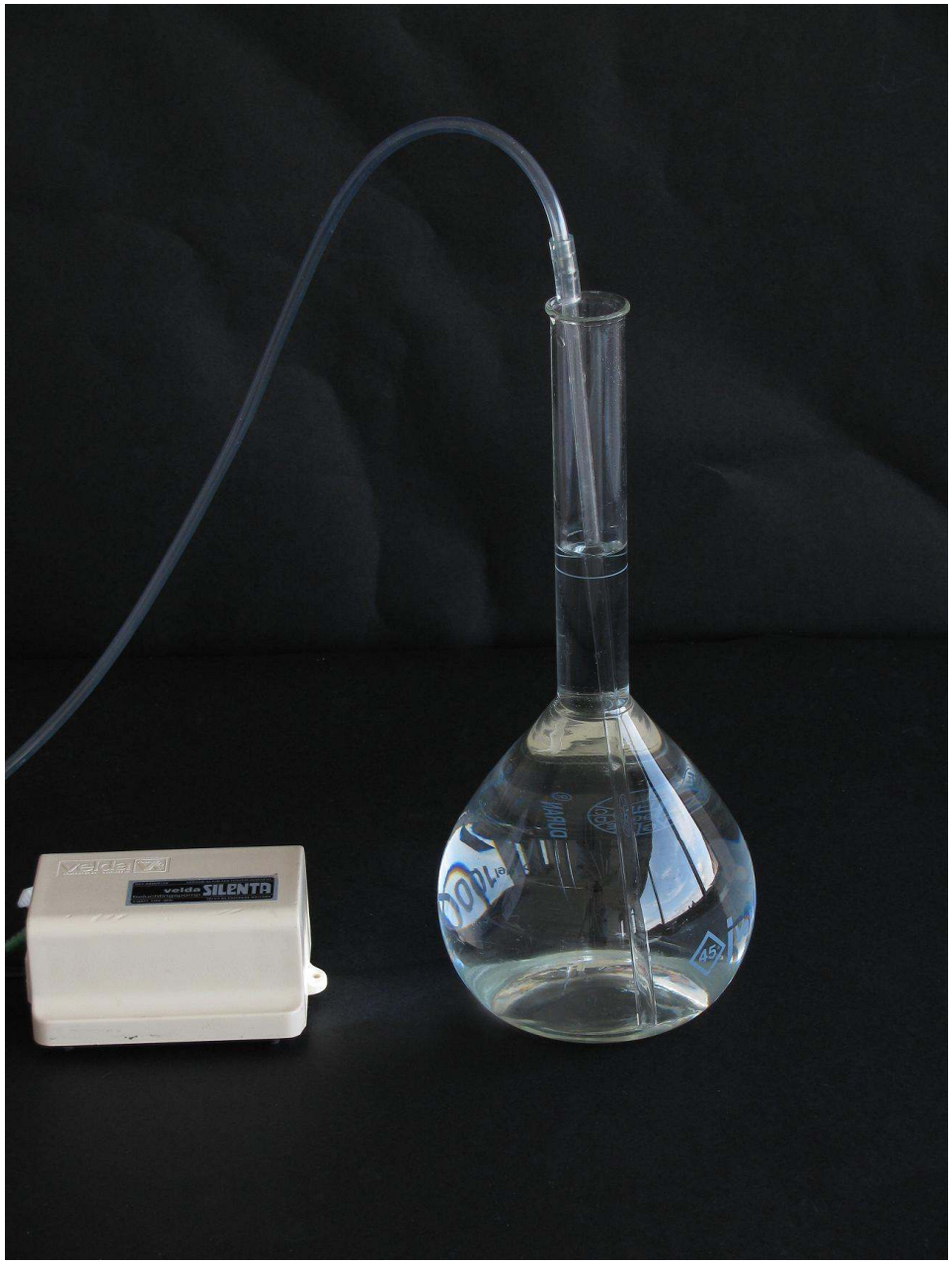
2

POUR THE 5 VIALS

WITH CONCENTRATED SALT SOLUTIONS

IN ± 800 ML DISTILLED WATER,

IN THE 1 LITER VOLUMETRIC FLASK



3

- FILL THE FLASK TO THE 1 LITER MARK
- AERATE FOR AT LEAST 15 MINUTES

4

HATCHING OF THE ROTIFER CYSTS

CYST HATCHING SHOULD BE INITIATED 16-18 HOURS PRIOR

TO THE START OF THE TOXICITY TEST

5

PUT 9 ML STANDARD FRESHWATER

IN A 5 CM PETRI DISH

6

EMPTY THE CONTENTS OF ONE VIAL
WITH CYSTS INTO THE HATCHING
PETRI DISH
MAKE SURE MOST OF THE CYSTS ARE
CARRIED OVER DURING THE TRANSFER

7

TO SECURE COMPLETE TRANSFER OF THE CYSTS, THE VIAL SHOULD BE RINSED WITH 2 X 0,5 ML STANDARD FRESHWATER

8

INCUBATION OF THE CYSTS

INCUBATE THE PETRI DISH

FOR 16-18 HOURS* AT 25 °C

UNDER CONTINOUS ILLUMINATION

OF MIN. 3 000 – 4 000 LUX

* The hatching time for different cyst batches may vary slightly, and is indicated on the specification sheet

9

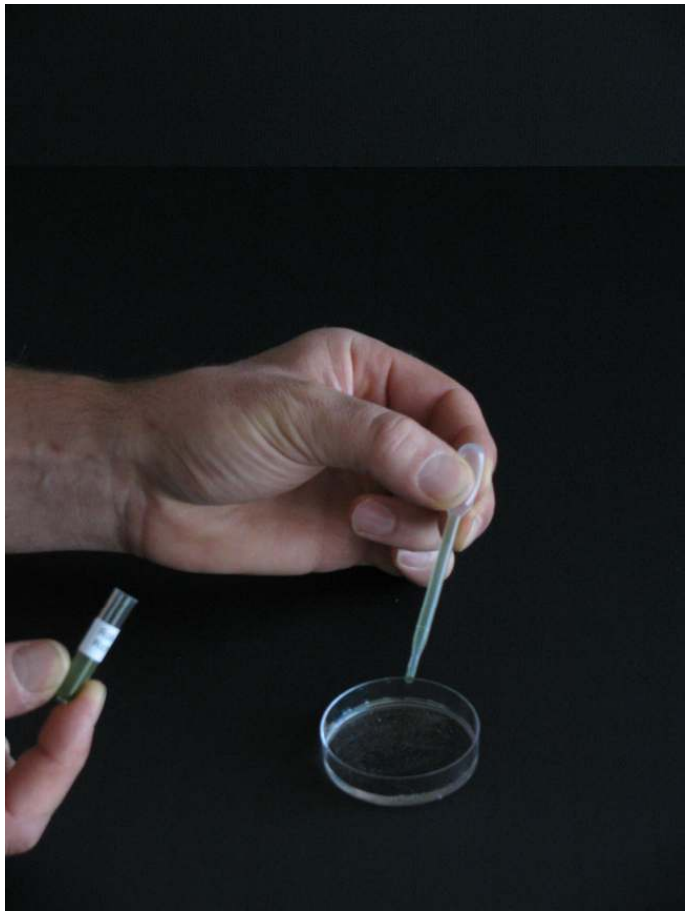
PRE-FEEDING OF FRESHLY HATCHED ROTIFERS

Freshly hatched rotifers are “pre-fed”
for 2 hours with a specific inert food (RotiRich),
prior to the start of the toxicity test

TAKE ONE TUBE WITH ROTI RICH AND
ADD 1 ML STANDARD FRESHWATER

10

CLOSE THE TUBE AND MIX THE CONTENTS THOROUGHLY TO OBTAIN A HOMOGENOUS FOOD SUSPENSION



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- SUCK UP A SMALL VOLUME OF ROTI RICH FOOD SUSPENSION WITH A MICROPIPETTE
- ADD 5 DROPS TO THE PETRI DISH WITH THE FRESHLY HATCHED ROTIFERS
- SWIRL THE PETRI DISH GENTLY
- ALLOW THE ROTIFERS TO FEED FOR TWO HOURS BEFORE THE START OF THE TEST

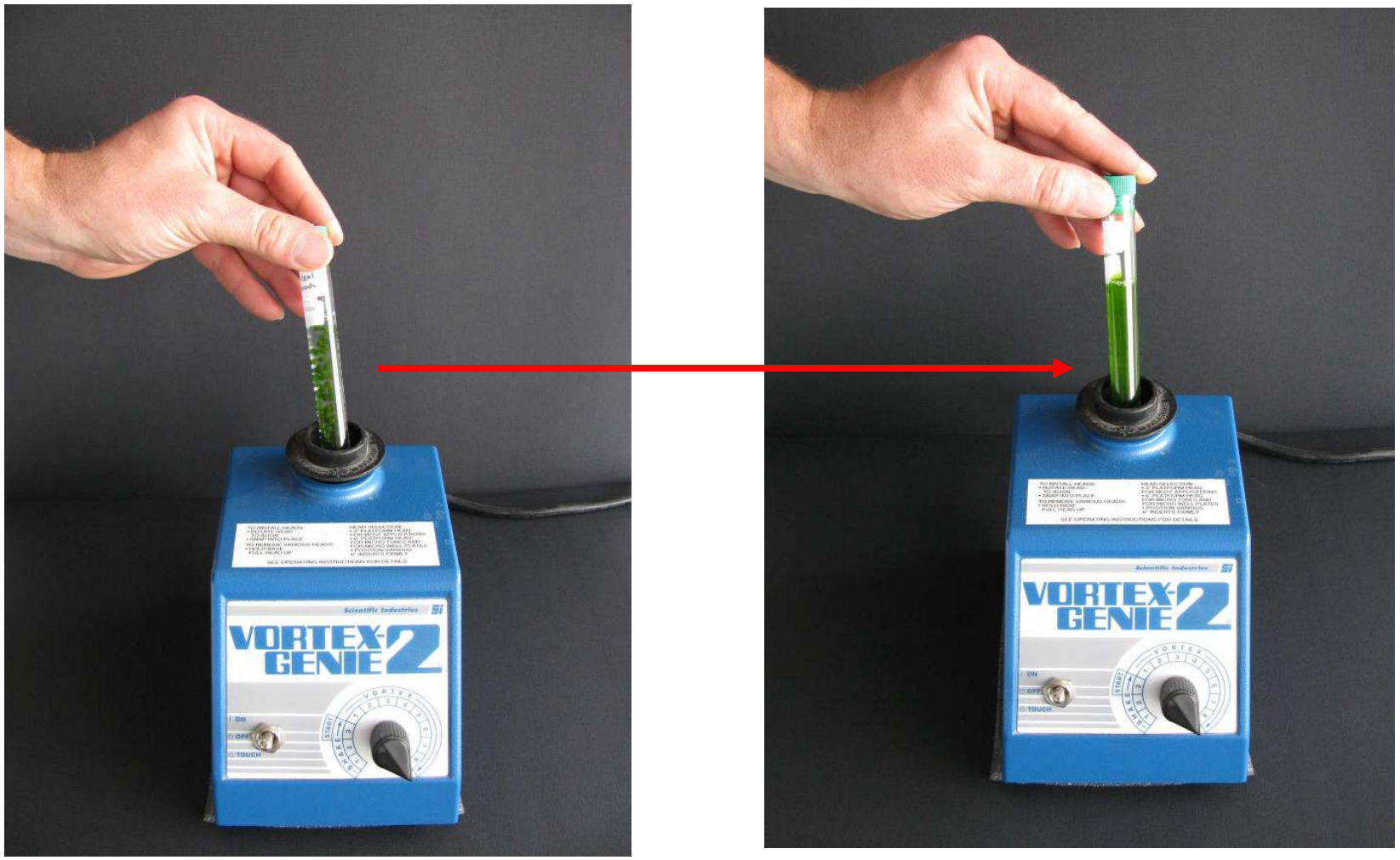
12

PREPARATION OF THE ALGAL FOOD SUSPENSION

TAKE ONE TUBE WITH ALGAL BEADS AND POUR OUT THE STORAGE MEDIUM

13

ADD 4 ML MATRIX DISSOLVING MEDIUM TO THE TUBE WITH THE ALGAL BEADS AND CAP THE TUBE



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SHAKE THE TUBE ON A VORTEX UNTIL THE MATRIX SURROUNDING THE ALGAE HAS FULLY DISSOLVED AND THE MICROALGAE ARE TOTALLY SET FREE

15

CENTRIFUGE THE TUBE FOR 10 MINUTES AT 3000 RPM IN A CONVENTIONAL LAB CENTRIFUGE

16

POUR OUT THE SUPERNATANT FROM THE TUBE

17

ADD 10 ML DISTILLED WATER
TO THE TUBE WITH THE ALGAL CLOTH

CAP AND SHAKE THE TUBE
TO RESUSPEND THE ALGAE

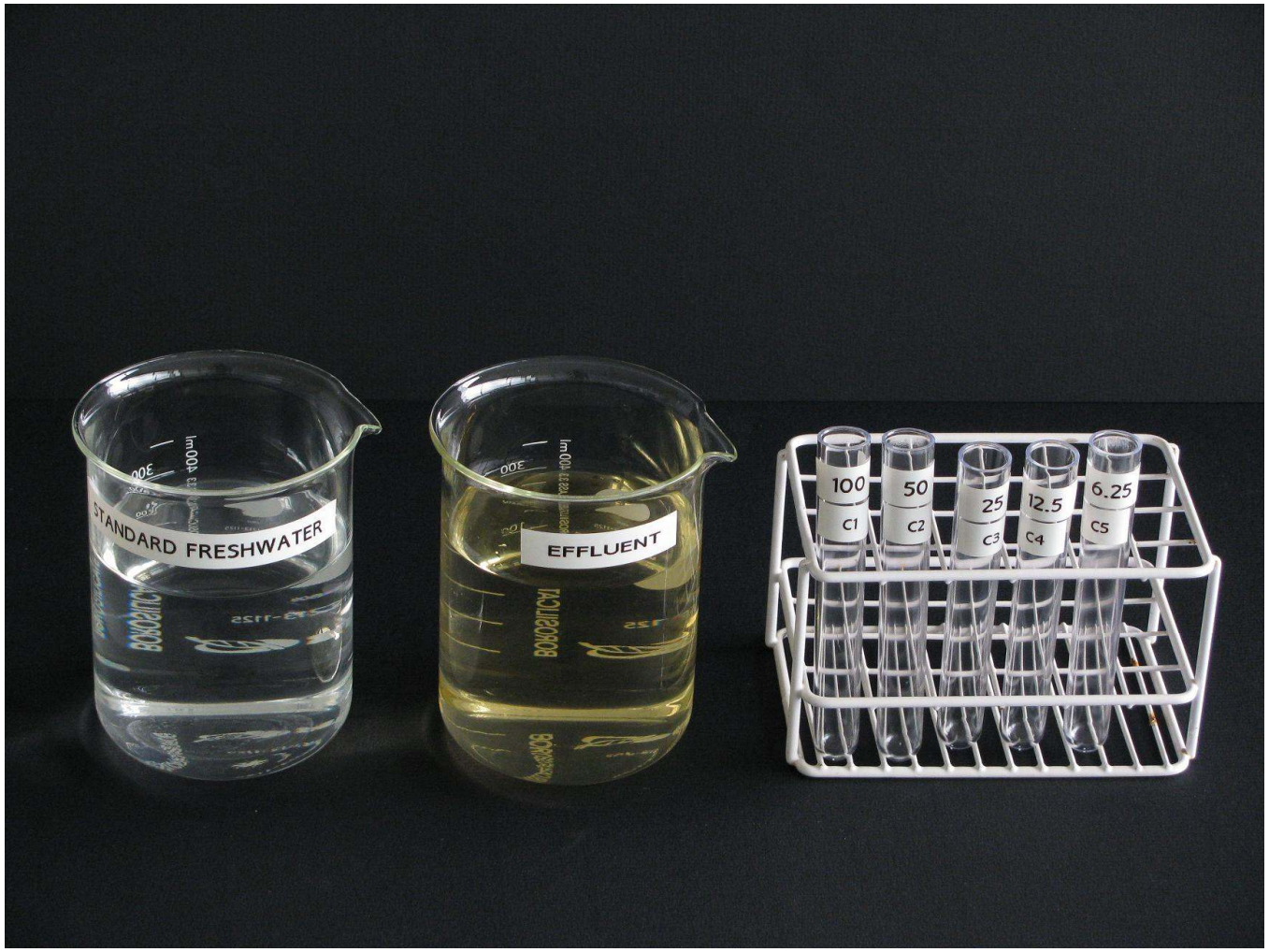
18

CENTRIFUGE THE TUBE AGAIN AT 3000 RPM FOR 10 MINUTES

AND POUR OUT THE RINSING WATER

19

- ADD 1,8 ML STANDARD FRESHWATER TO THE TUBE WITH THE ALGAL CLOTH
- CAP THE TUBE AND SHAKE THOROUGHLY TO RESUSPEND THE ALGAE



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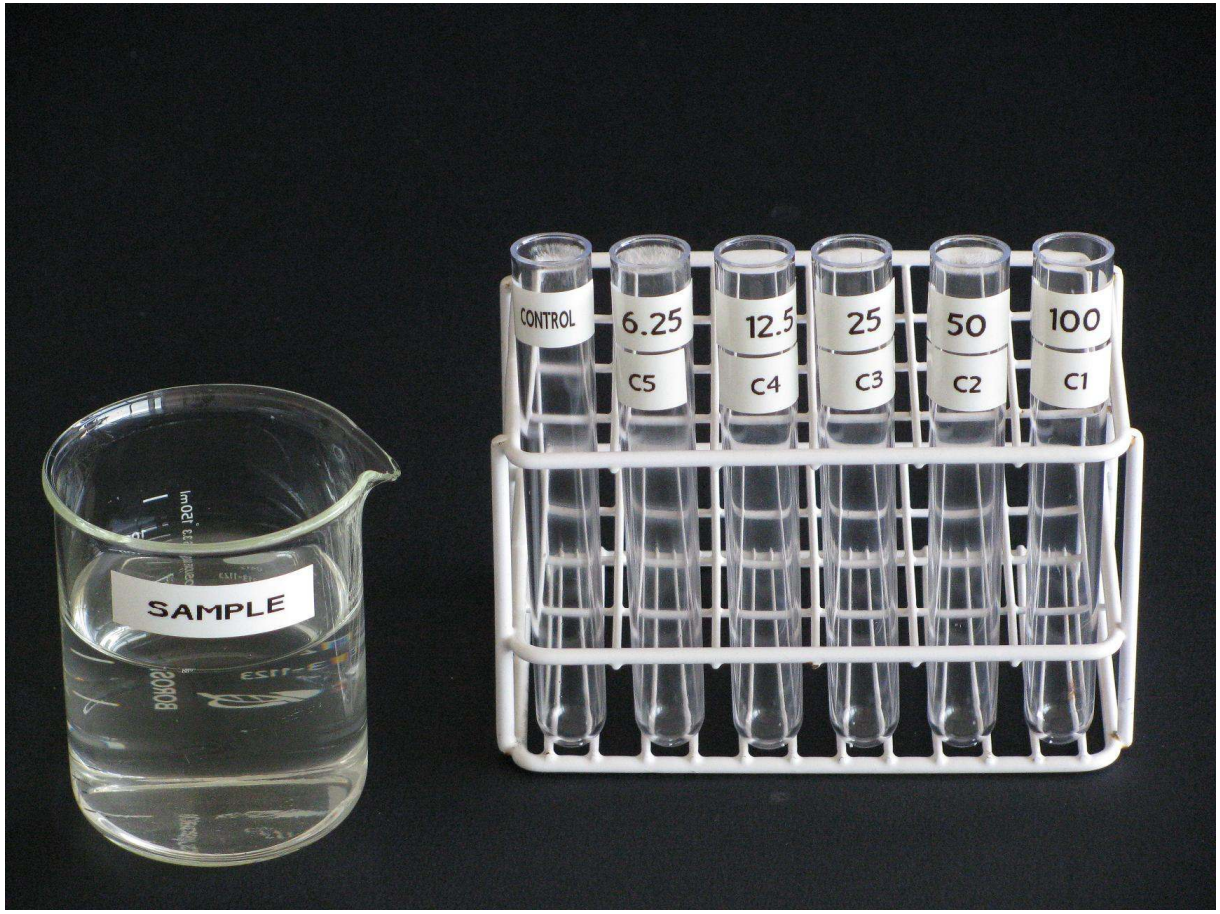
PREPARATION ON THE TOXICANT DILUTIONS

e.g. A TEST ON A EFFLUENT SAMPLE

1. SAMPLE PREPARATION

To avoid interference by particulate matter and/or organisms which may be present in the effluent sample it is recoomended to clean the sample prior to testing.

Preference is given to centrifugation with use of the supernatant for testing. Alternatively, filtration on a glass fibre filter with low porosity can be performed.



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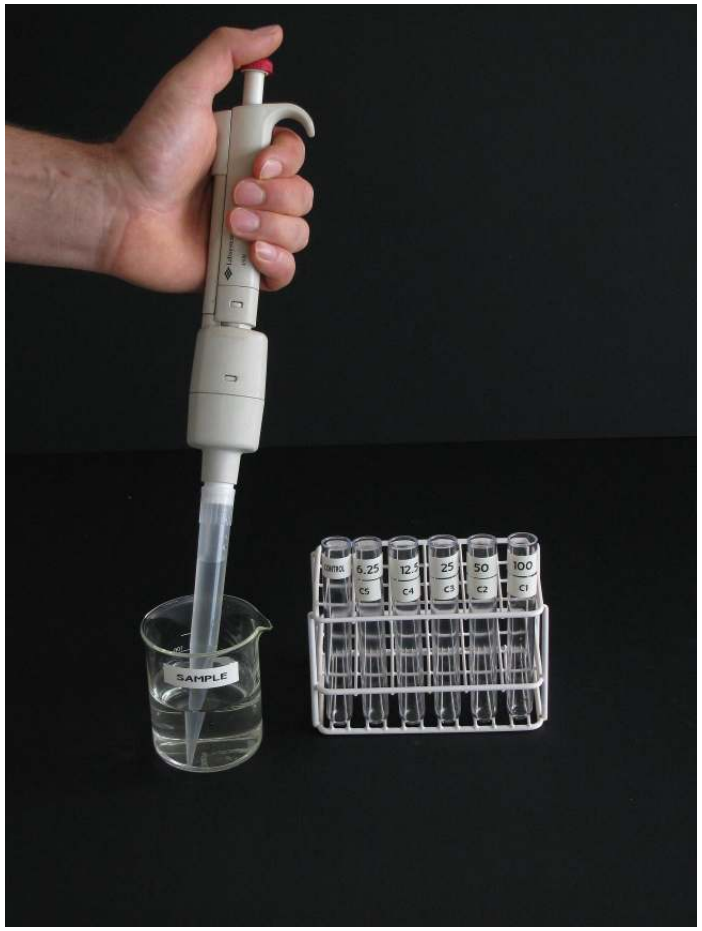
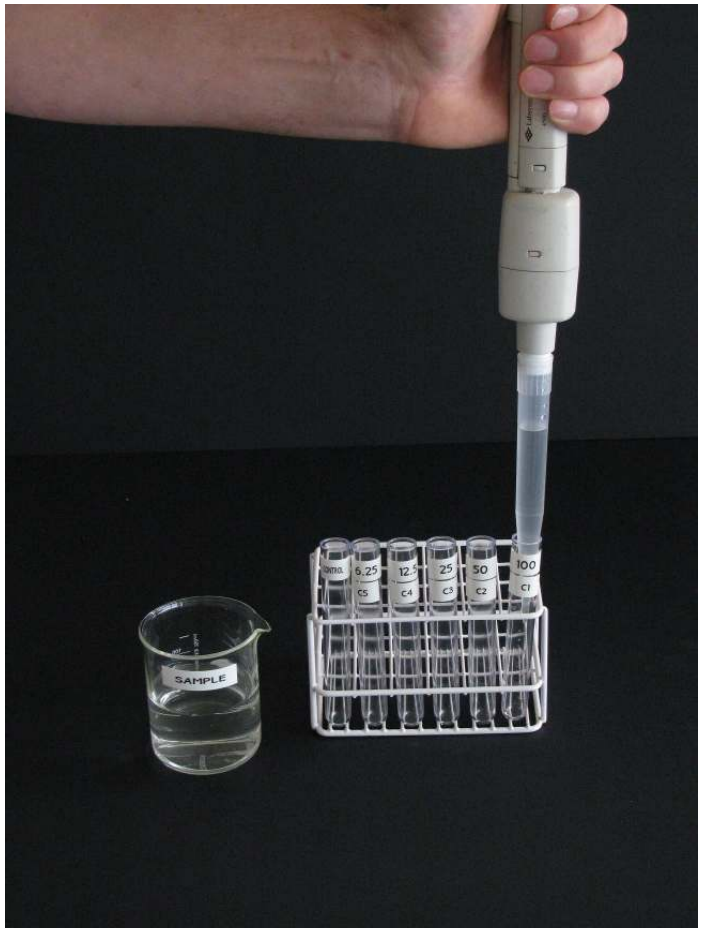
2. <u>PREPARATION OF THE TOXICANT DILUTIONS</u>

(100% - 50% - 25% - 12,5% - 6,25% effluent)

TAKE SIX 20 ML TEST TUBES AND LABEL THEM FROM C1 TO C5 AND A CONTROL





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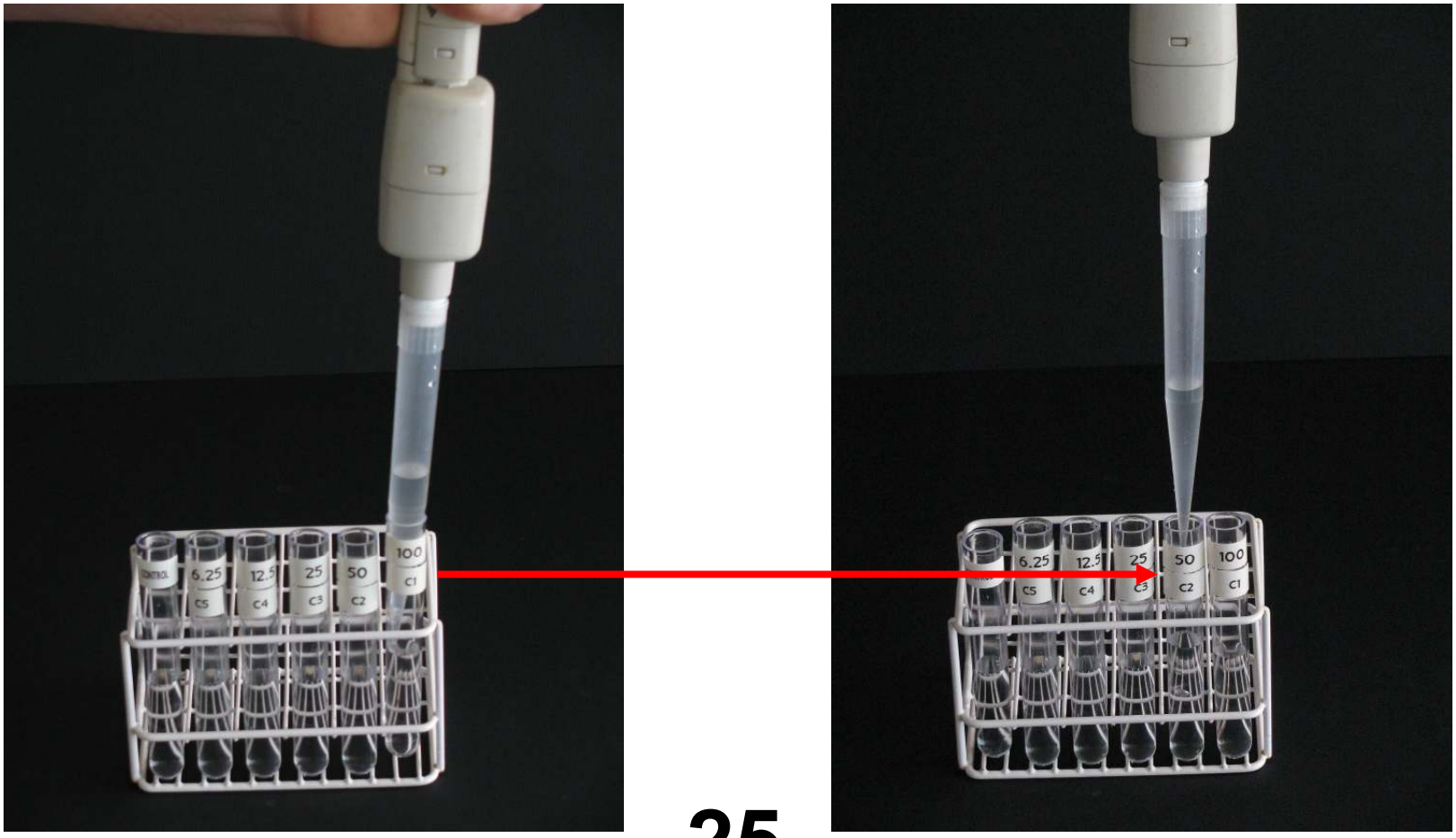
PUT 20 ML OF THE TREATED SAMPLE IN TUBE C1 (= 100% effluent)

24

PUT 10 ML STANDARD FRESHWATER IN THE CONTROL TUBE AND THE TUBES C5 TO C2



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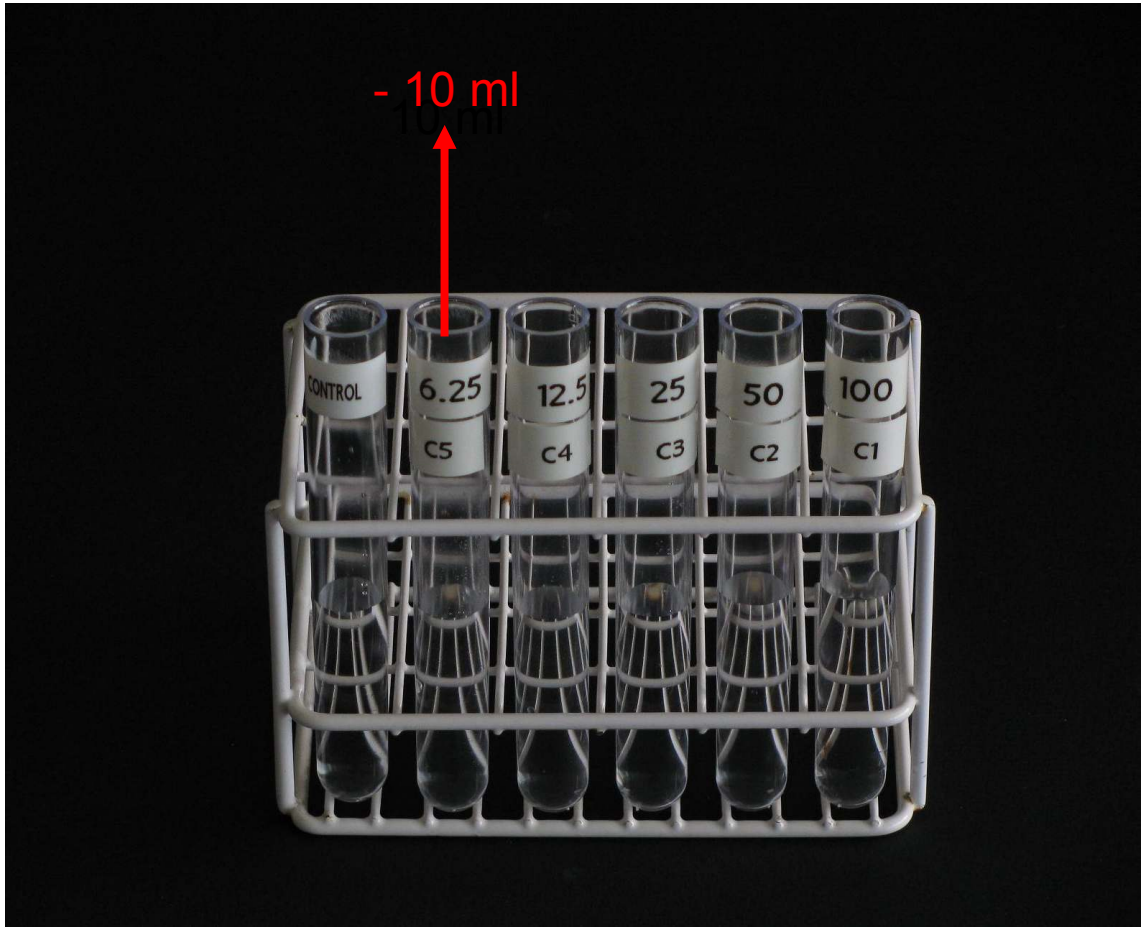
- TRANSFER 10 ML SAMPLE FROM C1 TO C2 AND MIX (= 50% effluent)
- REPEAT THIS 10 ML TRANSFER OPERATION FOR C2 TO C3 (= 25% effluent), C3 TO C4 (= 12.5% effluent) AND C4 TO C5 (= 6.25% effluent)



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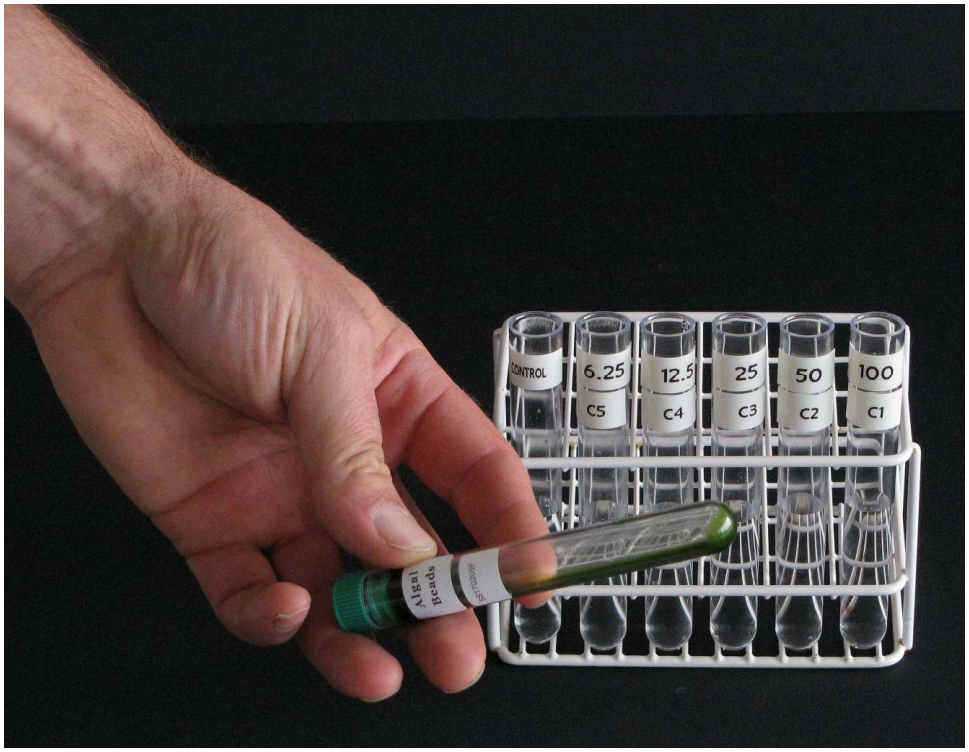
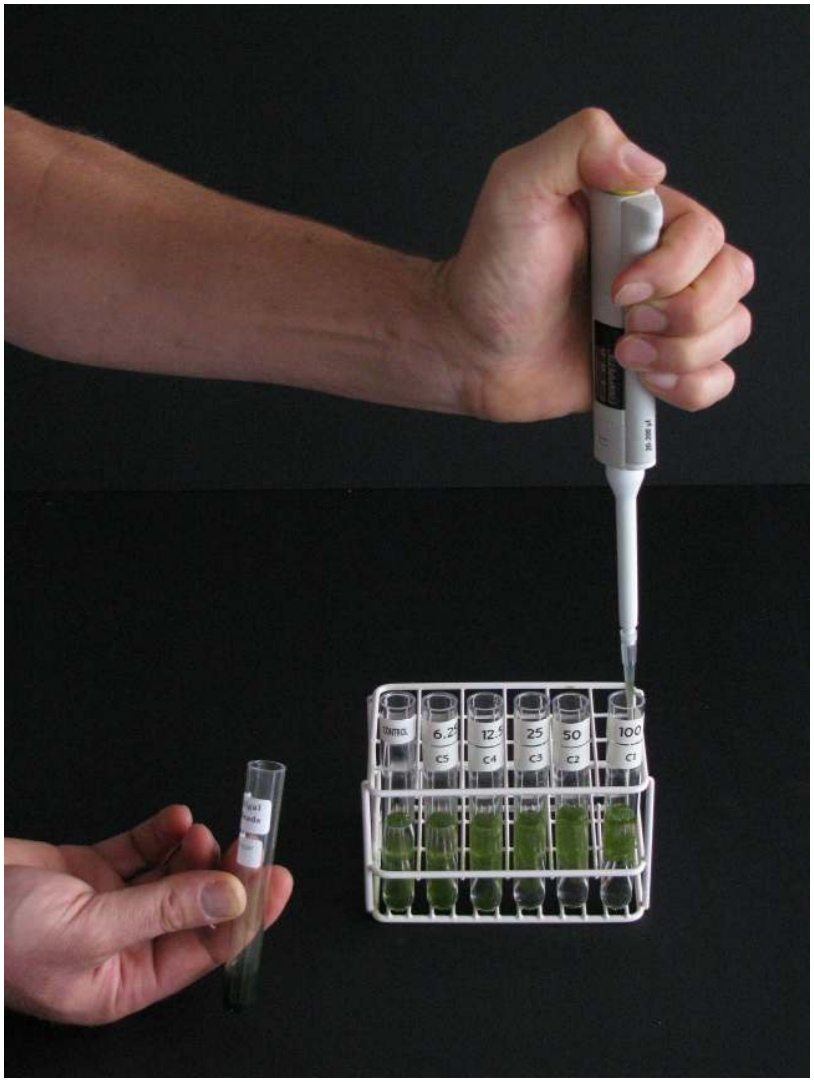
REMOVE AND DISCARD 10 ML FROM TUBE C5





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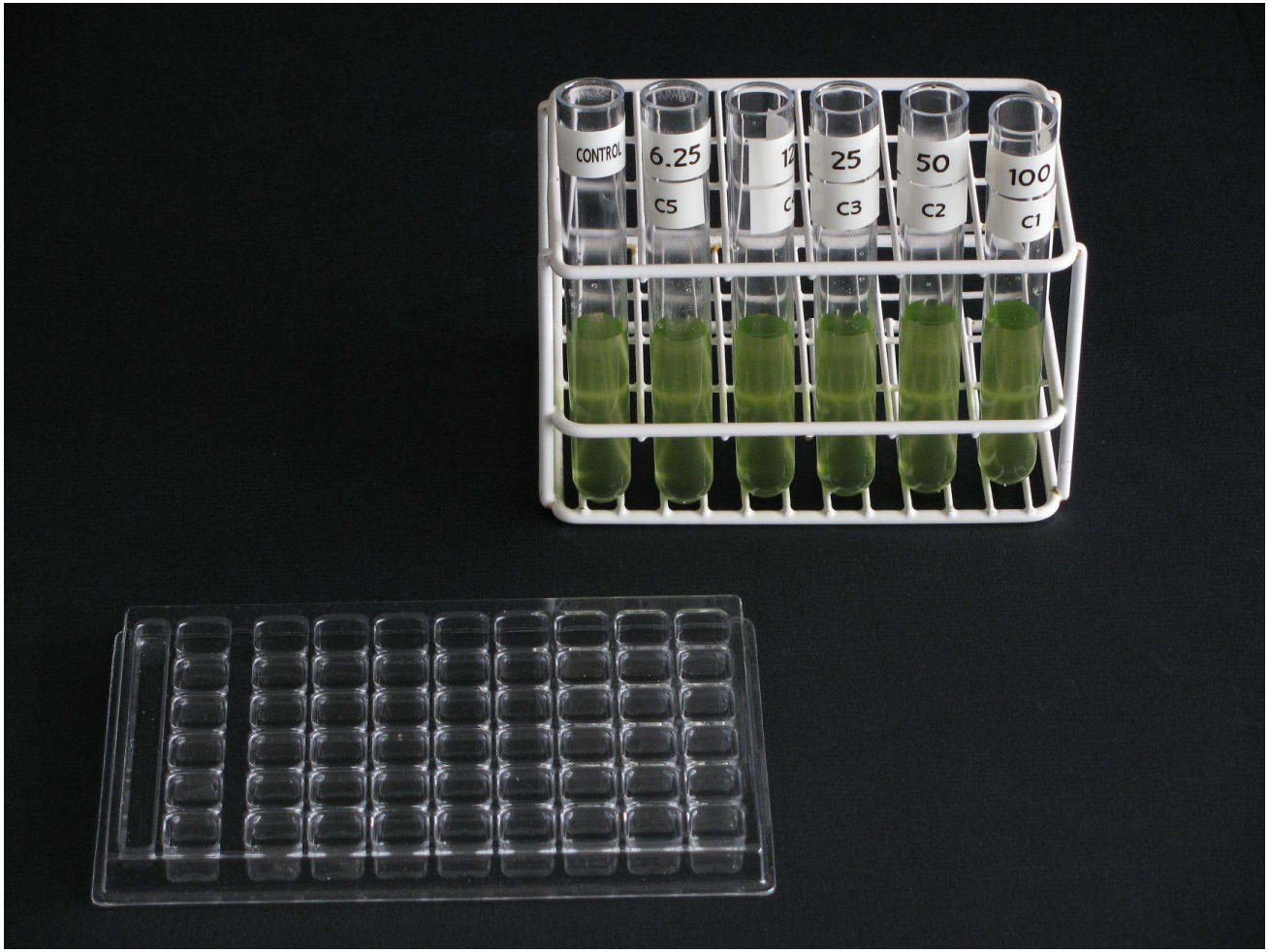
3. <u>ADDITION OF ALGAL FOOD TO THE TOXICANT DILUTIONS AND CONTROL</u>

- SHAKE THE TUBE WITH CONCENTRATED ALGAL FOOD THOROUGHLY
- ADD 100 µl TO ALL THE TEST TUBES



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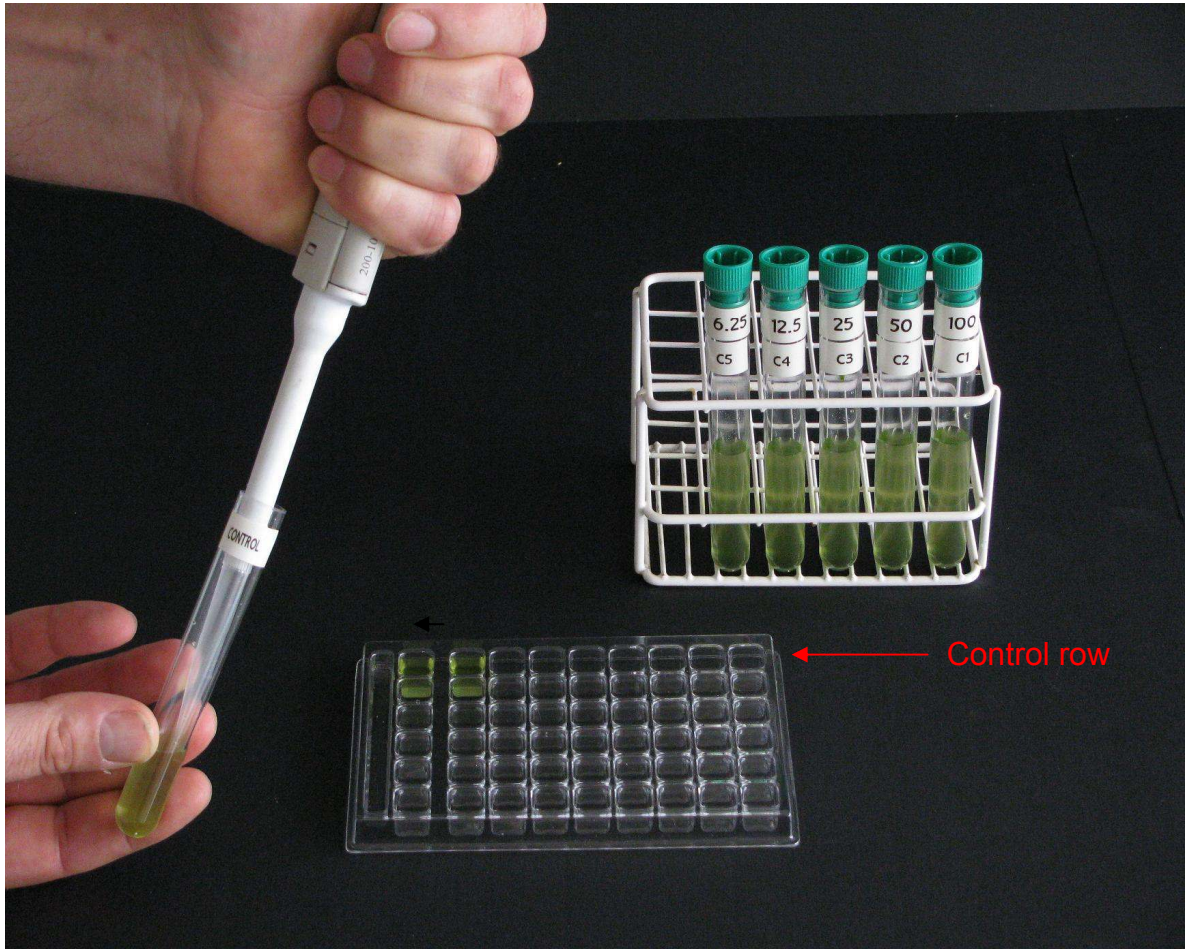
FILLING OF THE TEST PLATE

29

SHAKE THE TUBE WITH THE CONTROL MEDIUM



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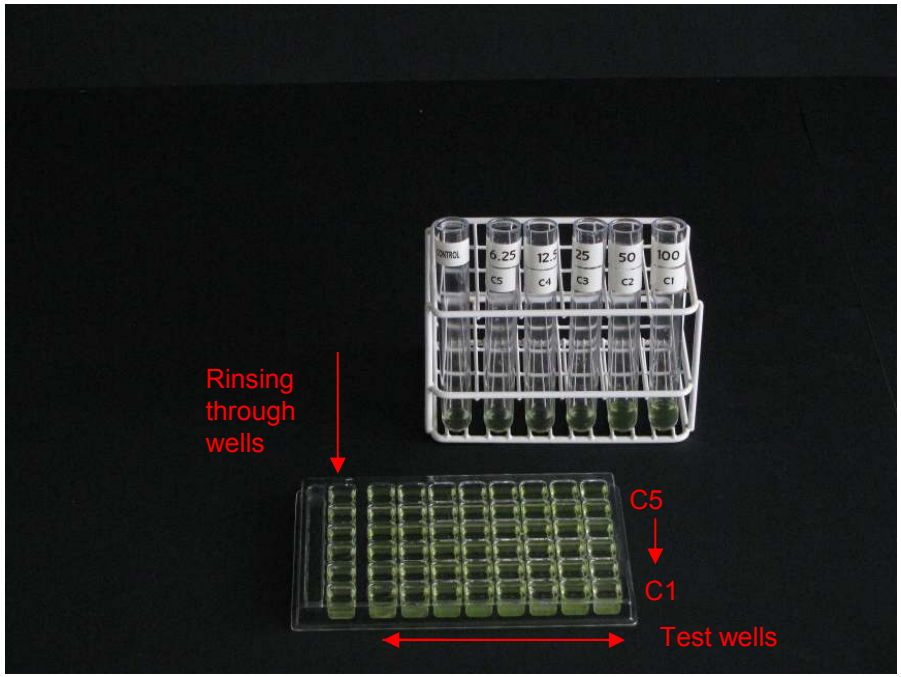
TRANSFER 1ML INTO EACH WELL OF THE CONTROL ROW



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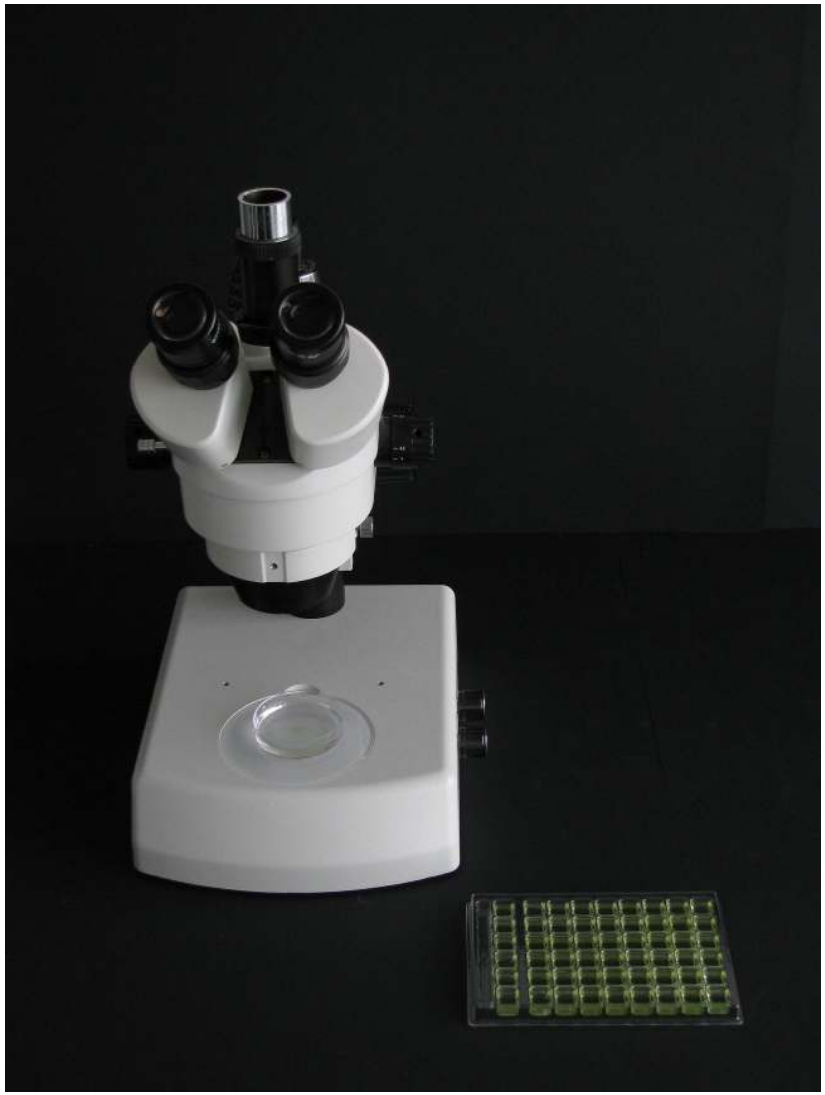
- SHAKE THE TUBES WITH THE TOXICANT CONCENTRATIONS
- TRANSFER 1 ML OF EACH TOXICANT CONCENTRATION INTO THE RINSING TRHOUGH WELL AND THE 8 TEST WELLS OF EACH ROW,
- PERFORM THESE TRANSFERS IN THE SEQUENCE OF INCREASING TOXICANT CONCENTRATION, (i.e. from the top row to the bottom row of the multiwell plate, so from C5 to C1)



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TRANSFER OF THE ROITFERS
TO THE TEST WELLS

Because of their small size, the transfer of the rotifers into the test wells has to be carried out under a dissection microscope at a mganification 10-12 X

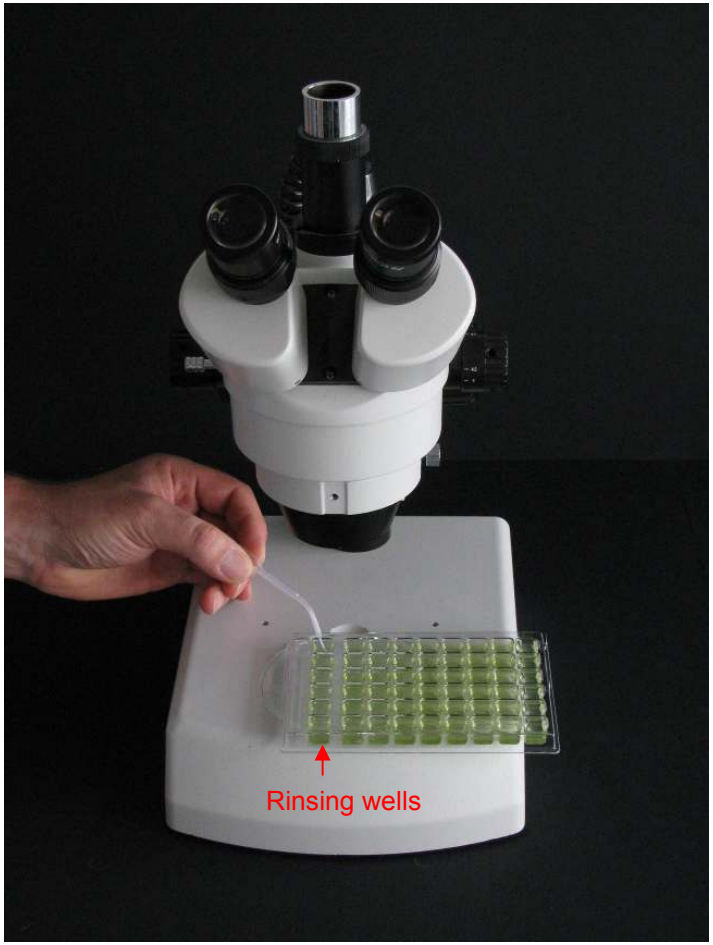
33

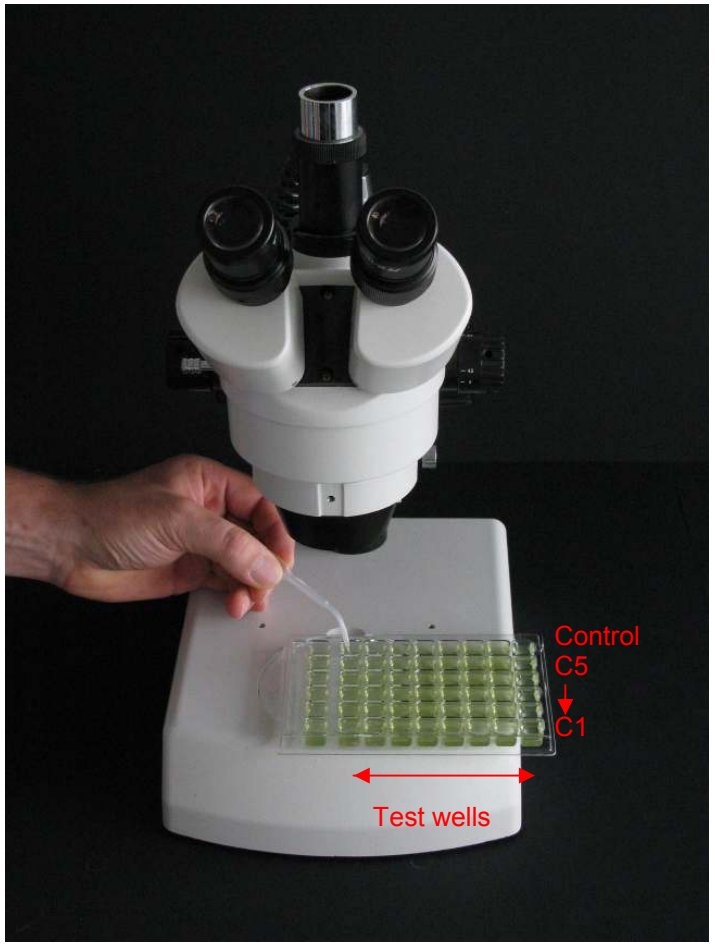
- PUT THE PETRI DISH WITH THE HATCHED AND PRE-FED ROTIFERS ON THE STAGE OF THE DISSECTION MICROSCOPE
- SUCK UP A NUMBER OF ROTIFERS IN THE PLASTIC MICROPIPETTE BY GENTLY SQUEEZING THE BULB TO PROVIDE ADEQUATE SUCTION.

34

TRANSFER 10-15 ROTIFERS FROM THE HATCHING PETRI DISH INTO EACH RINSING WELL IN THE FOLLOWING SEQUENCE: ROW X (Control), ROW C5 – C4 – C3 – C2 – C1

(thus from the top to the bottom of the multiwell, in order of increasing toxicant concentration)

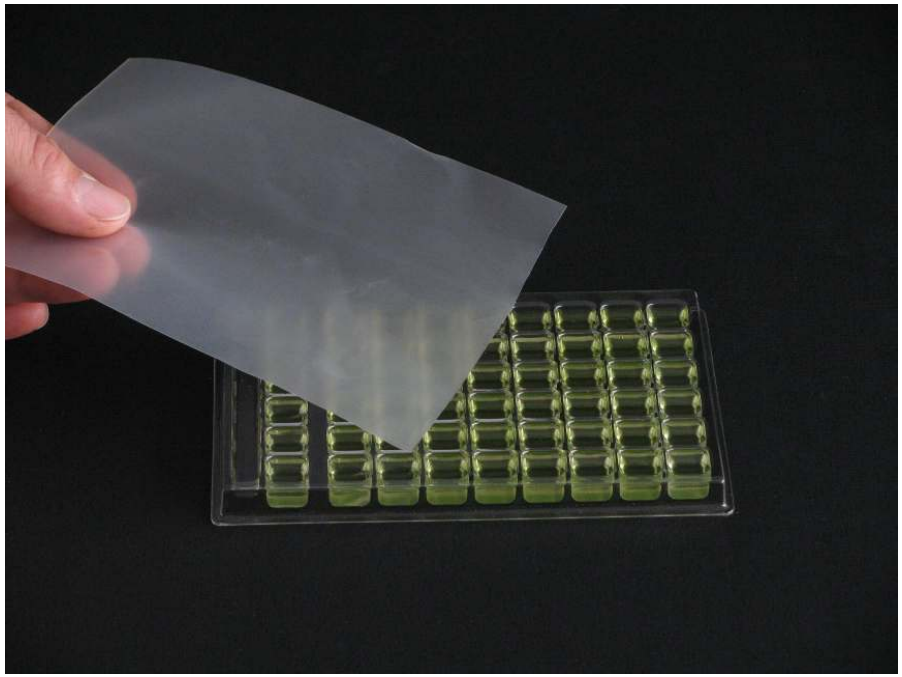




35

TRANSFER **ONE** ROTIFER FROM THE RINSING WELL INTO EACH TEST WELL OF THE SAME ROW.

This transfer shall also be performed in the sequence of increasing test concentrations (i.e. first in the control row and subsequently from row C5 to row C1)



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PUT THE PARAFILM STRIP ON TOP OF THE MULTIWELL PLATE AND PUT THE COVER ON

37

PUT THE MULTIWELL PLATE IN THE INCUBATOR AT 25 °C, IN DARKNESS, FOR 48 HOURS

38

SCORING OF THE RESULTS

AFTER 48H INCUBATION, THE NUMBER OF LIVE ROTIFERS HAS TO BE COUNTED IN ALL THE TEST WELLS AND THE DATA SCORED ON THE RESULTS SHEET