MICROBIOTESTS

THAMNOTOXKIT F

TEST PROCEDURE

MICROBIOTESTS

1

PREPARATION OF STANDARD FRESHWATER

- VOLUMETRIC FLASK (1 LITER)
- 5 VIALS WITH SOLUTIONS OF CONCENTRATED SALTS
- DISTILLED (or deionised) WATER

MICROBIOTESTS

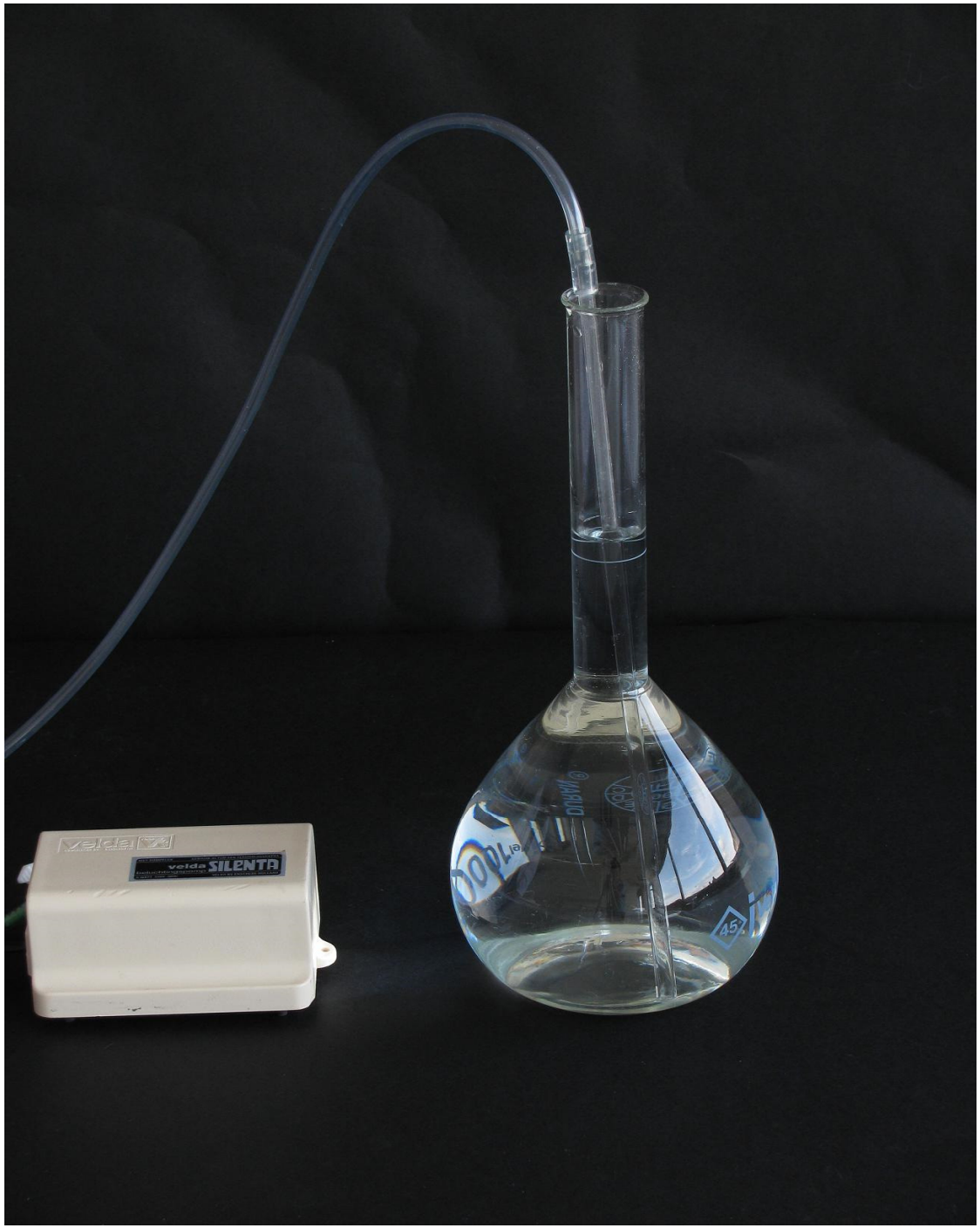
2

POUR THE 5 VIALS

WITH CONCENTRATED SALT SOLUTIONS

IN ± 800 ML DISTILLED WATER,

IN THE 1 LITER VOLUMETRIC FLASK



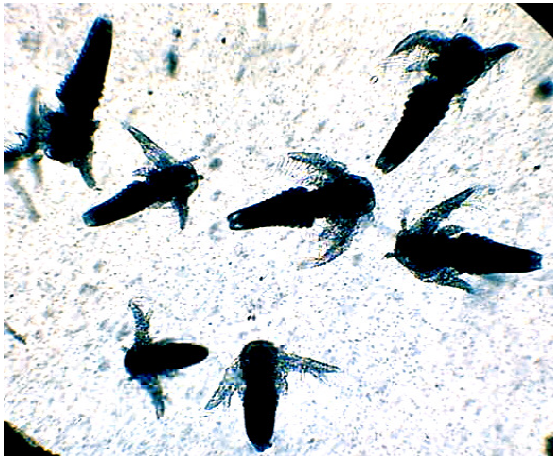
3

- FILL THE FLASK TO THE 1 LITER MARK

- AERATE FOR AT LEAST 15 MINUTES

Tube with
Thamnocephalus platyurus
cysts

Thamnocephalus platyurus cysts

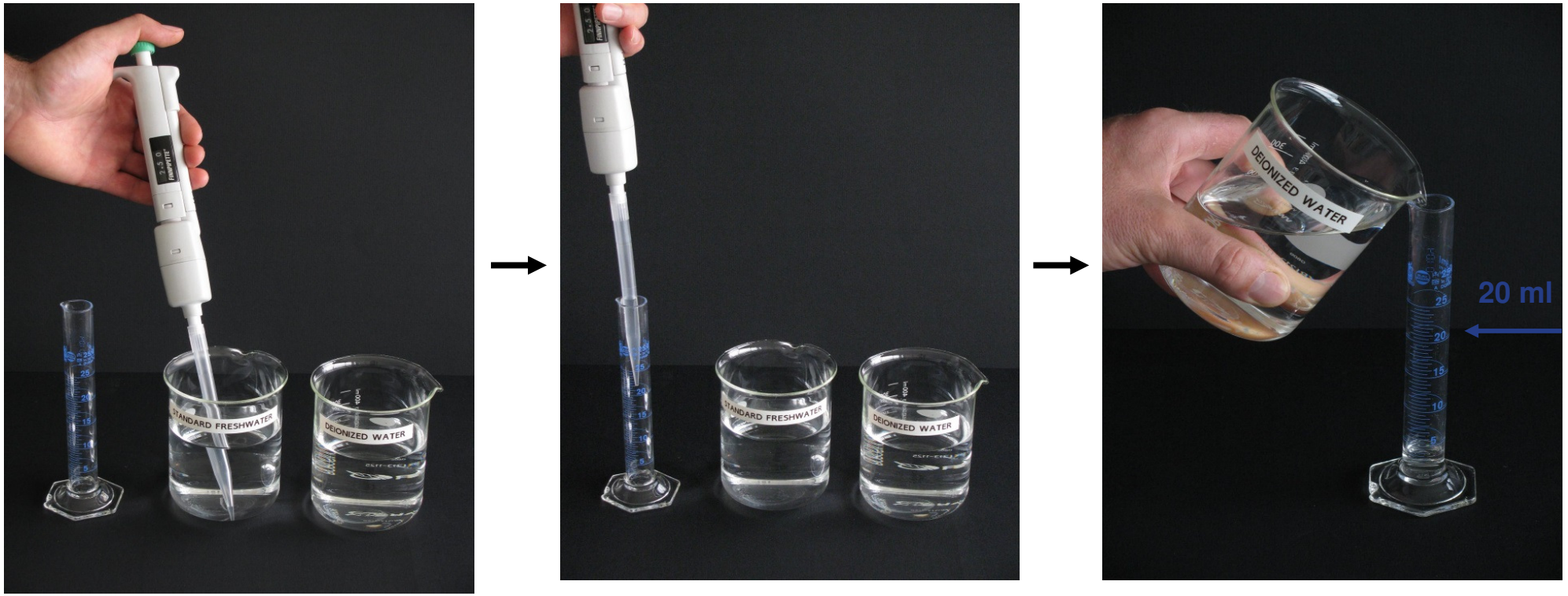


Thamnocephalus platyurus larvae

4

HATCHING OF THE CYSTS

CYST HATCHING SHOULD BE INITIATED 20-22 HOURS PRIOR TO THE START OFTHE TOXICITY TEST



5

1. PREHYDRATION OF THE CYSTS

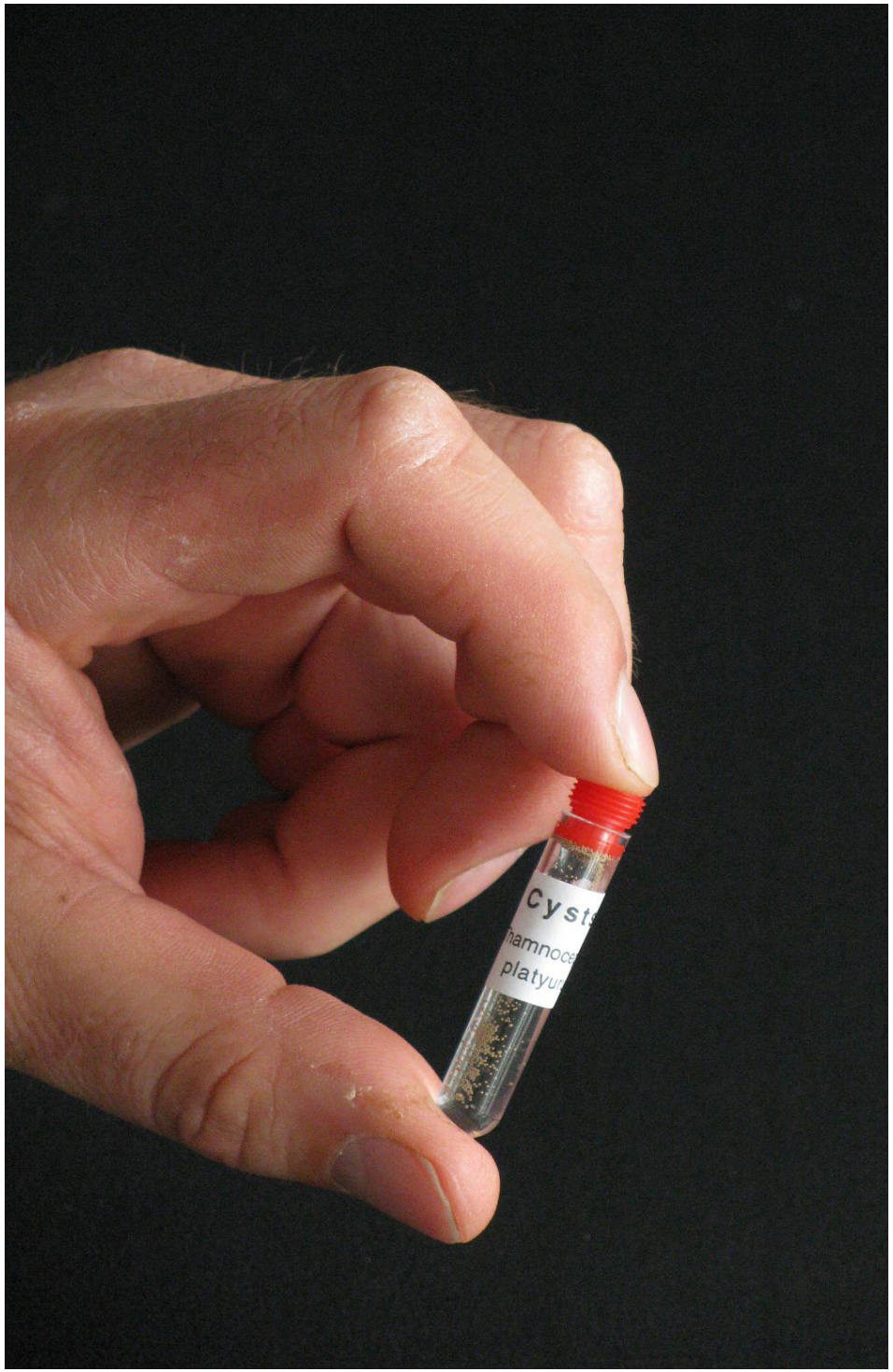
PREPARE 20 ML “HATCHING MEDIUM” (=DILUTED STANDARD FRESHWATER)
BY PUTTING 2,5 ML STANDARD FRESHWATER IN A GRADUATED 25 ML CYLINDER
AND ADDING DEIONISED WATER TO THE 20 ML MARK

MICROBIOTESTS

MICROBIOTESTS

6

OPEN A TUBE WITH CYSTS AND
FILL IT WITH HATCHING MEDIUM
(approximately 1 ml)



MICROBIOTESTS

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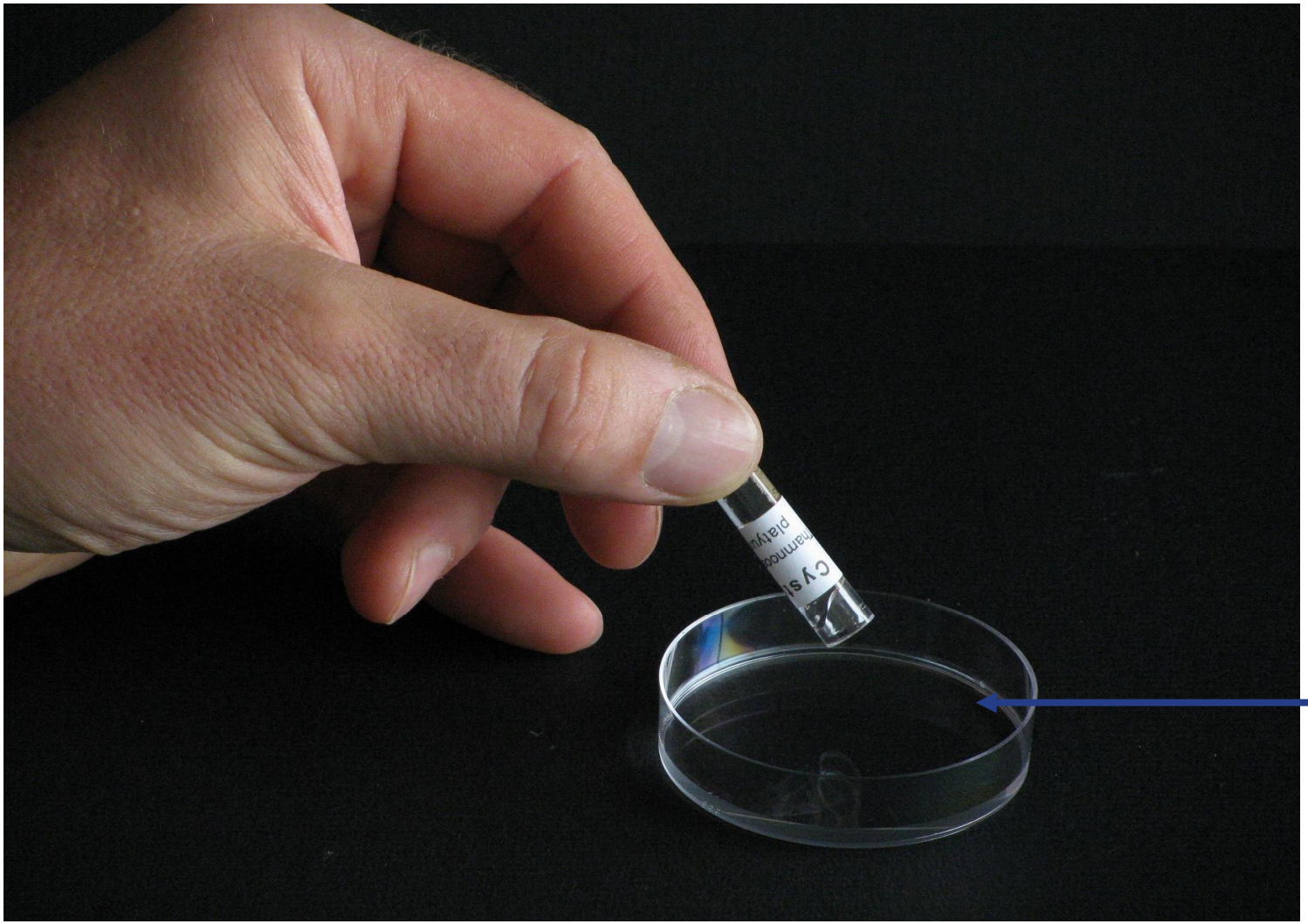
- CLOSE THE TUBE WITH THE STOPPER

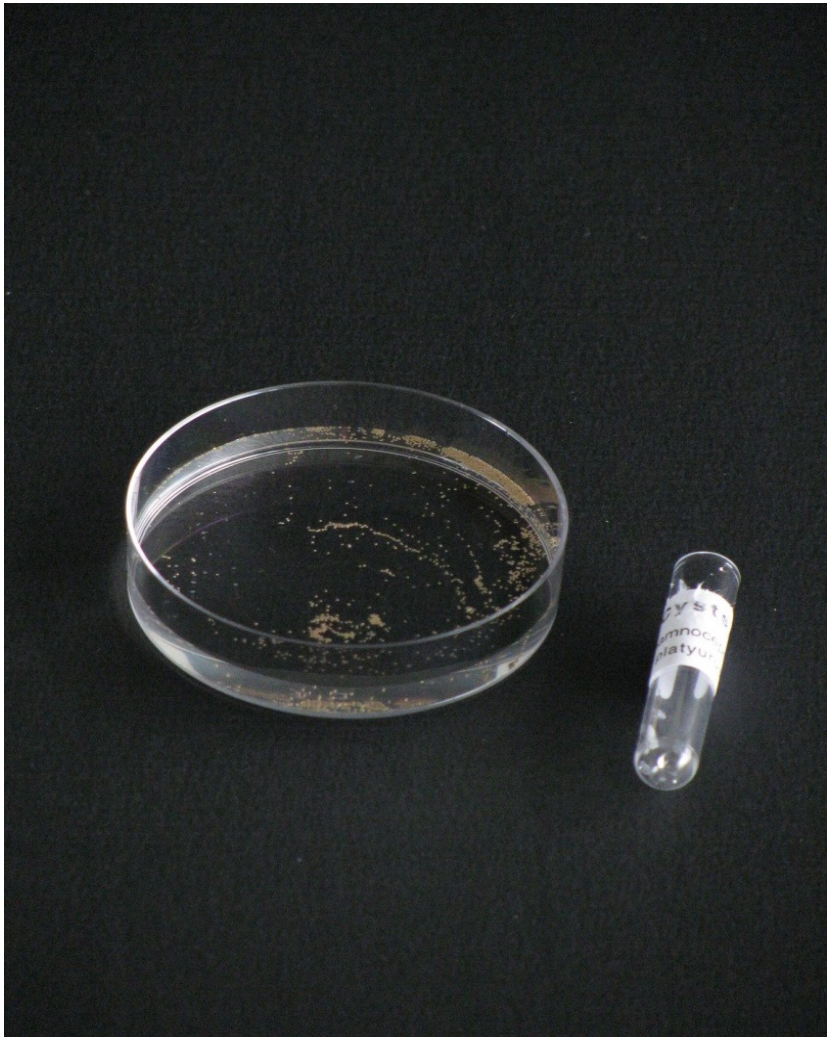
- SHAKE THE TUBE AT REGULAR INTERVALS DURING A 30 MINUTES PERIOD



8

2. TRANSFER OF THE PREHYDRATED CYSTS INTO THE HATCHING PETRI DISH

EMPTY THE CONTENTS OF THE VIAL WITH PREHYDRATED CYSTS INTO A PETRI DISH



9

- MAKE SURE THAT ALL THE CYSTS ARE TRANSFERRED BY RINSING THE TUBE WITH HATCHING MEDIUM
- ADD 10 ML HATCHING MEDIUM TO THE PETRI DISH AND SWIRL GENTLY TO DISTRIBUTE THE CYSTS EVENLY

MICROBIOTESTS

MICROBIOTESTS

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INCUBATION OF THE CYSTS

INCUBATE THE PETRI DISH
FOR 20-22 HOURS AT 25 °C
UNDER CONTINOUS ILLUMINATION
OF MIN. 3 000 – 4 000 LUX

PREPARATION OF THE TOXICANT DILUTIONS (e.g. a test on an effluent)

- TAKE 5 TUBES OF 10-15 ML CONTENTS AND LABEL THEM

C1 (100), **C2** (50), **C3** (25), **C4** (12.5), AND **C5** (6.25)

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- ADD 5 ML STANDARD FRESHWATER TO TUBES C2, C3, C4 AND C5

MICROBIOTESTS

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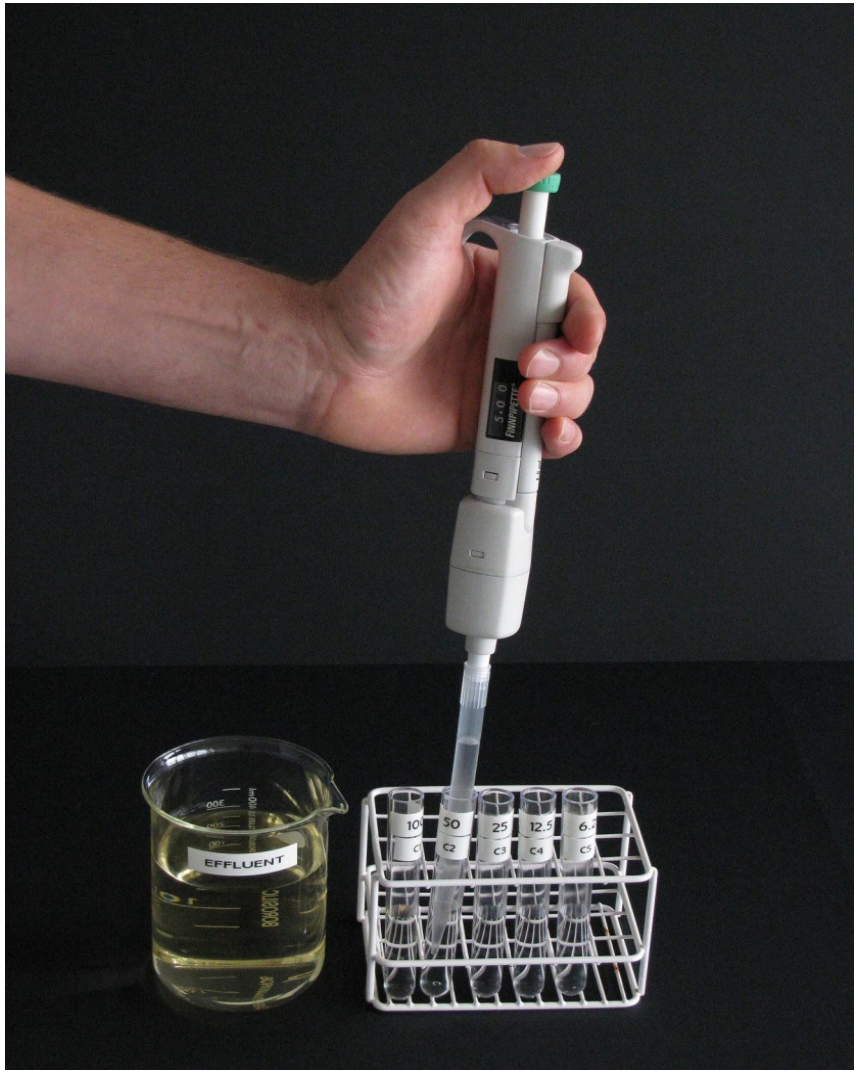
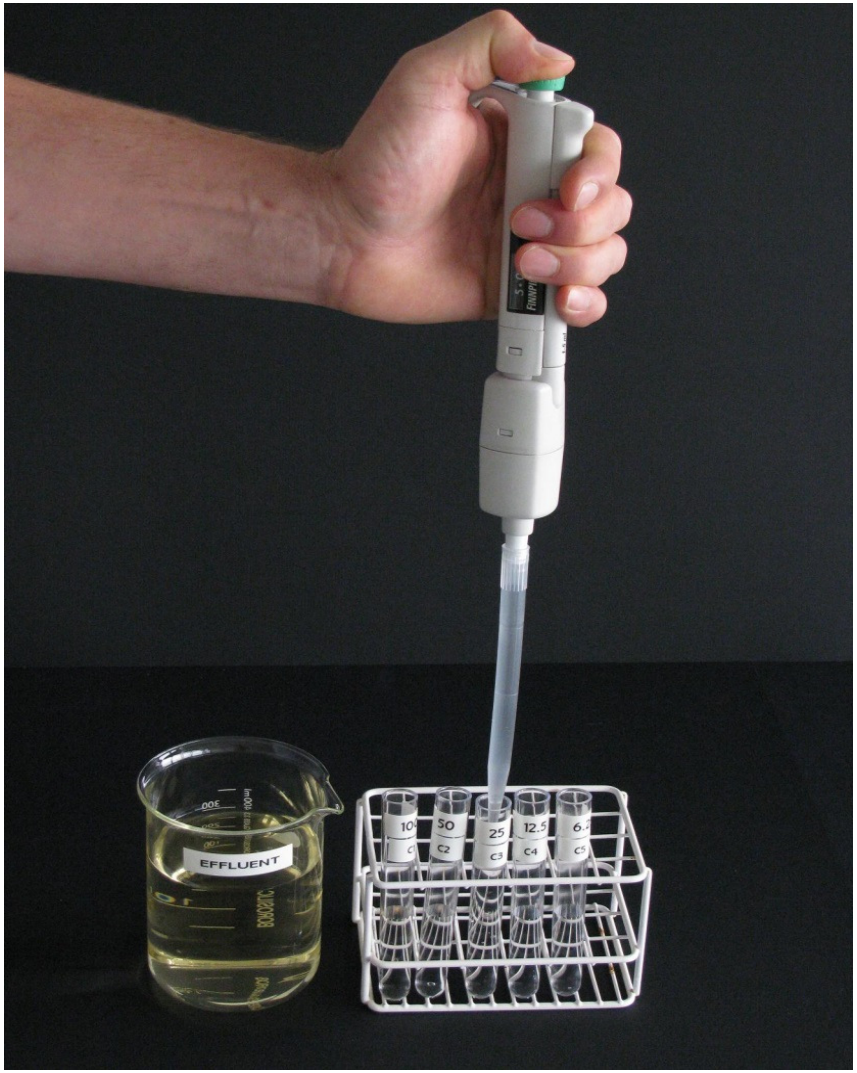
ADD 5 ML EFFLUENT SAMPLE TO TUBE C1 (= 100% sample)

14

- ADD 5 ML EFFLUENT TO TUBE C2

- MIX THE CONTENTS OF TUBE C2 (= 50% dilution) WITH THE AID OF THE PIPET

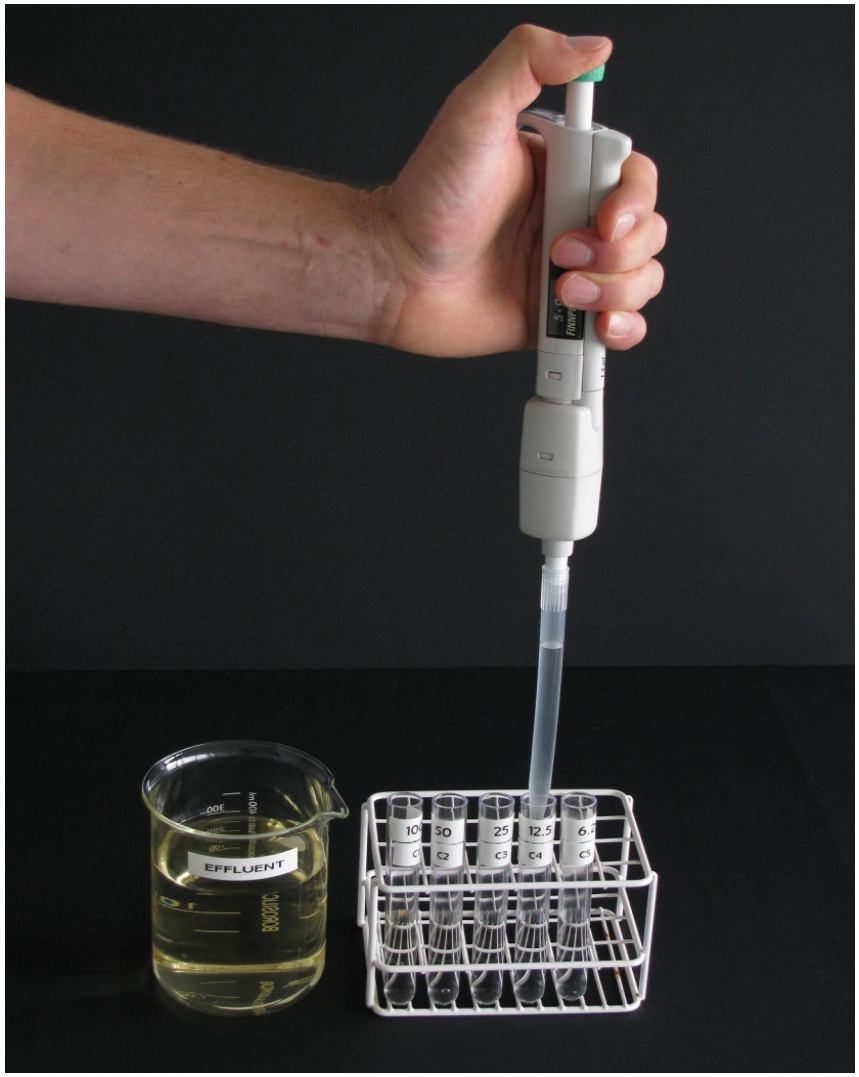


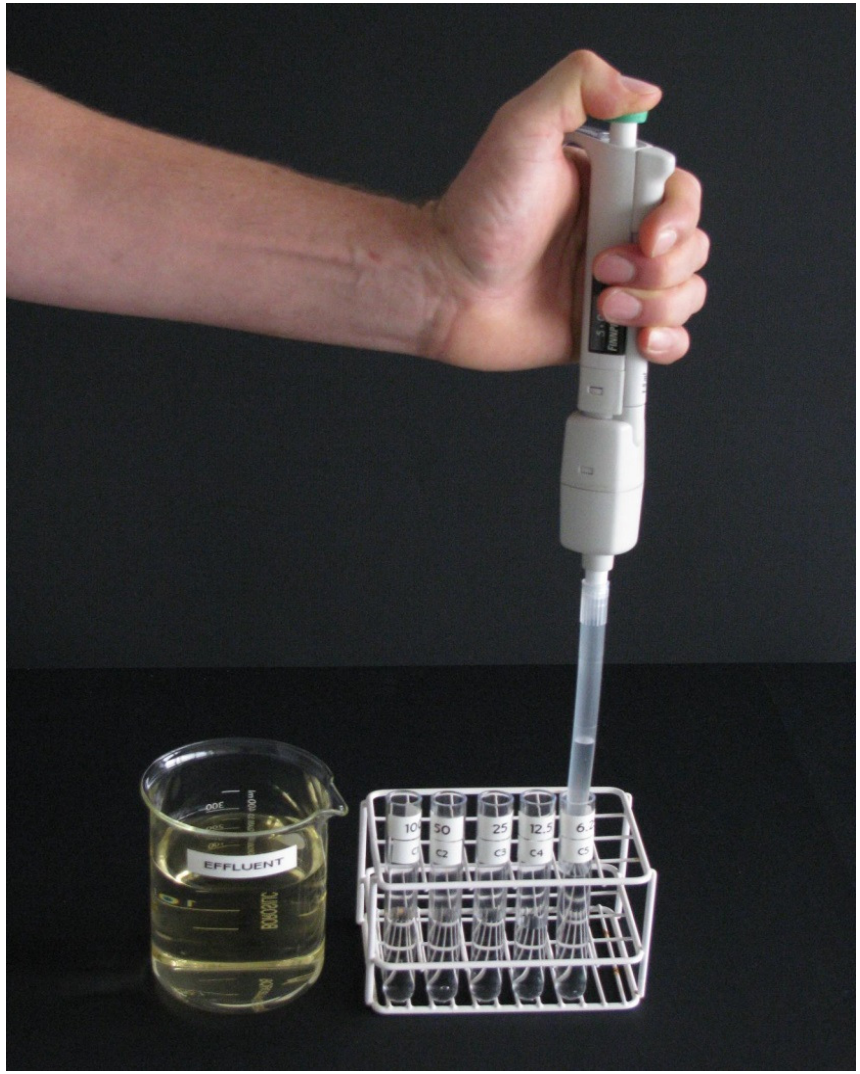


15

- TRANSFER 5 ML FROM TUBE C2 TO TUBE C3
- MIX THE CONTENTS OF TUBE C3 (= 25% dilution) WITH THE AID OF THE PIPET

MICROBIOTESTS



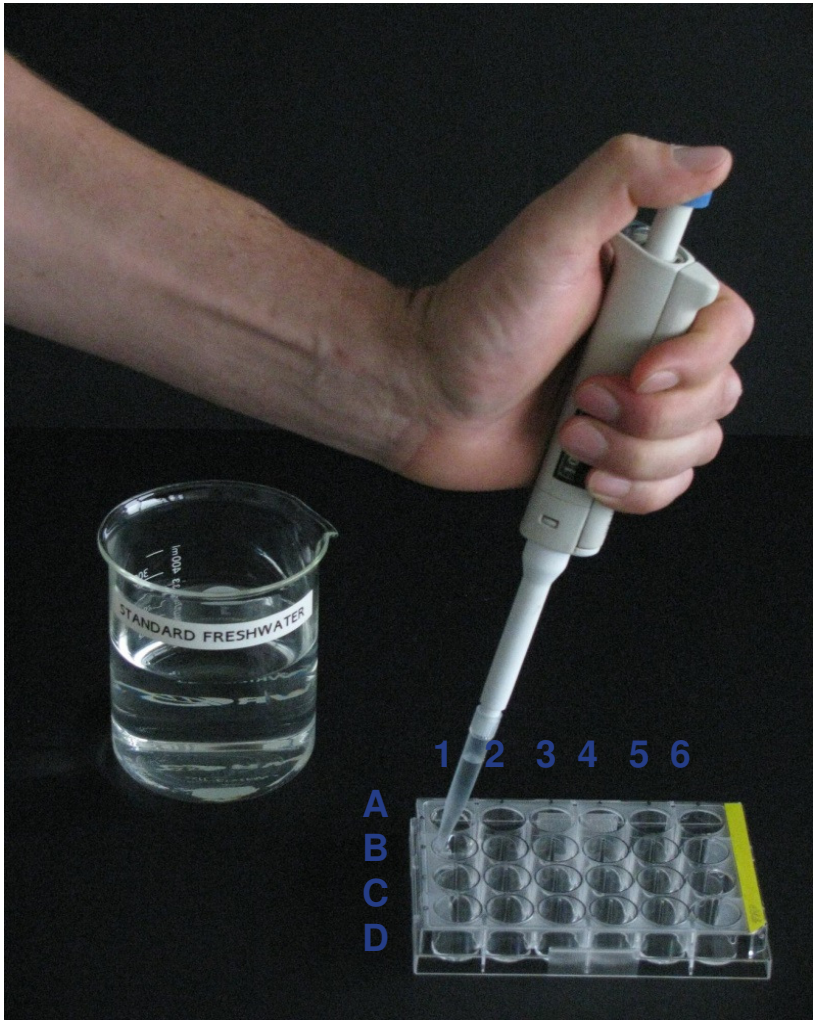


16

REPEAT THE SAME PROCEDURE FOR THE NEXT DILUTIONS :

* 5 ML FROM TUBE C3 TO TUBE C4 (= 12,5% dilution)
* 5 ML FROM TUBE C4 TO TUBE C5 (= 6,25% dilution)

MICROBIOTESTS



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FILLING OF THE TEST PLATE

CONTROLS

ADD 1 ML STANDARD FRESHWATER TO EACH WELL OF COLUMN 1 (WELLS A1, B1, C1, D1)

MICROBIOTESTS

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TOXICANT DILUTIONS

TRANSFER 1 ML OF TEST TUBE 5 TO EACH WELL IN COLUMN 2 (WELLS A2, B2, C2, D2)

MICROBIOTESTS

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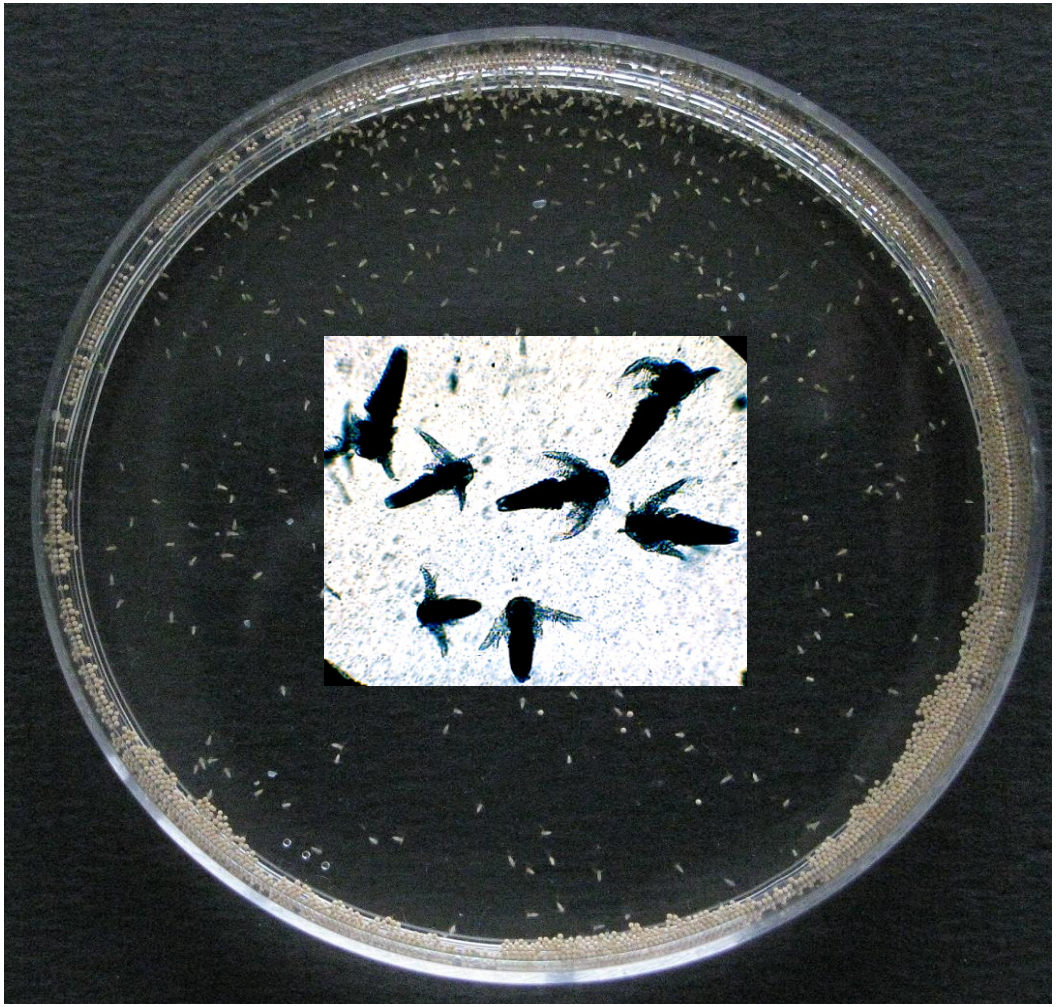
REPEAT THIS PROCEDURE WITH TEST TUBES 4, 3, 2 AND 1 TO FILL THE WELLS OF COLUMNS 3, 4, 5 AND 6 RESPECTIVELY

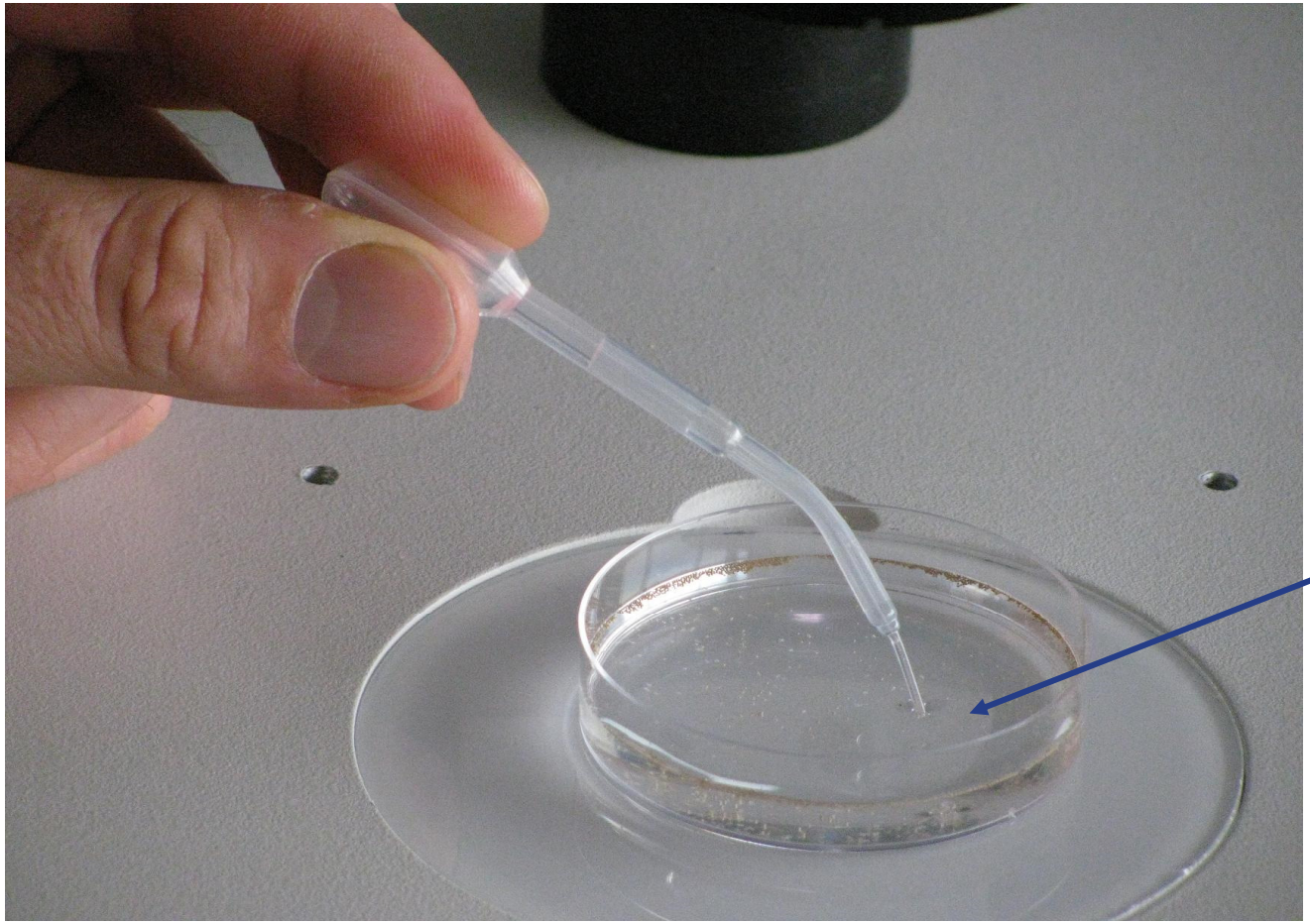
MICROBIOTESTS

MICROBIOTESTS



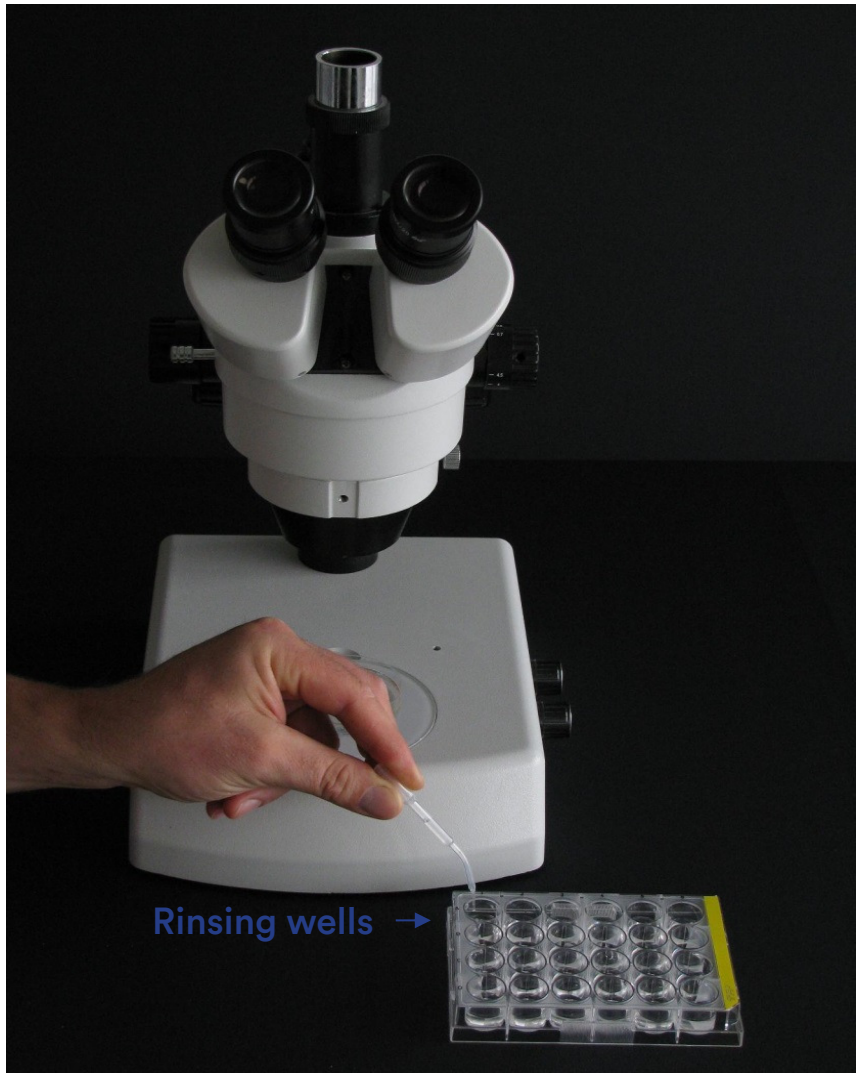
20

TRANSFER OF THE LARVAE FROM THE HATCHING PETRI DISH TO THE TEST WELLS



21

- PUT THE HATCHING PETRI DISH ON THE STAGE OF THE DISSECTION MICROSCOPE
- TAKE THE MICROPIPETTE LIKE A PENCIL WITH THE INDEX FINGER AND THE THUMB TO EXERT CONTROLLED PRESSURE ON THE BULB.
- SQUEEZE THE BULB GENTLY TO PROVIDE ADEQUATE SUCTION FOR PICKING UP LARVAE.



22

TRANSFER APPROXIMATELY 50 LARVAE FROM THE **PETRI DISH** TO EACH **RINSING WELL** IN THE FOLLOWING SEQUENCE: A1 (control), A2, A3, A4, A5 AND A6 (= increasing concentrations of toxicant)

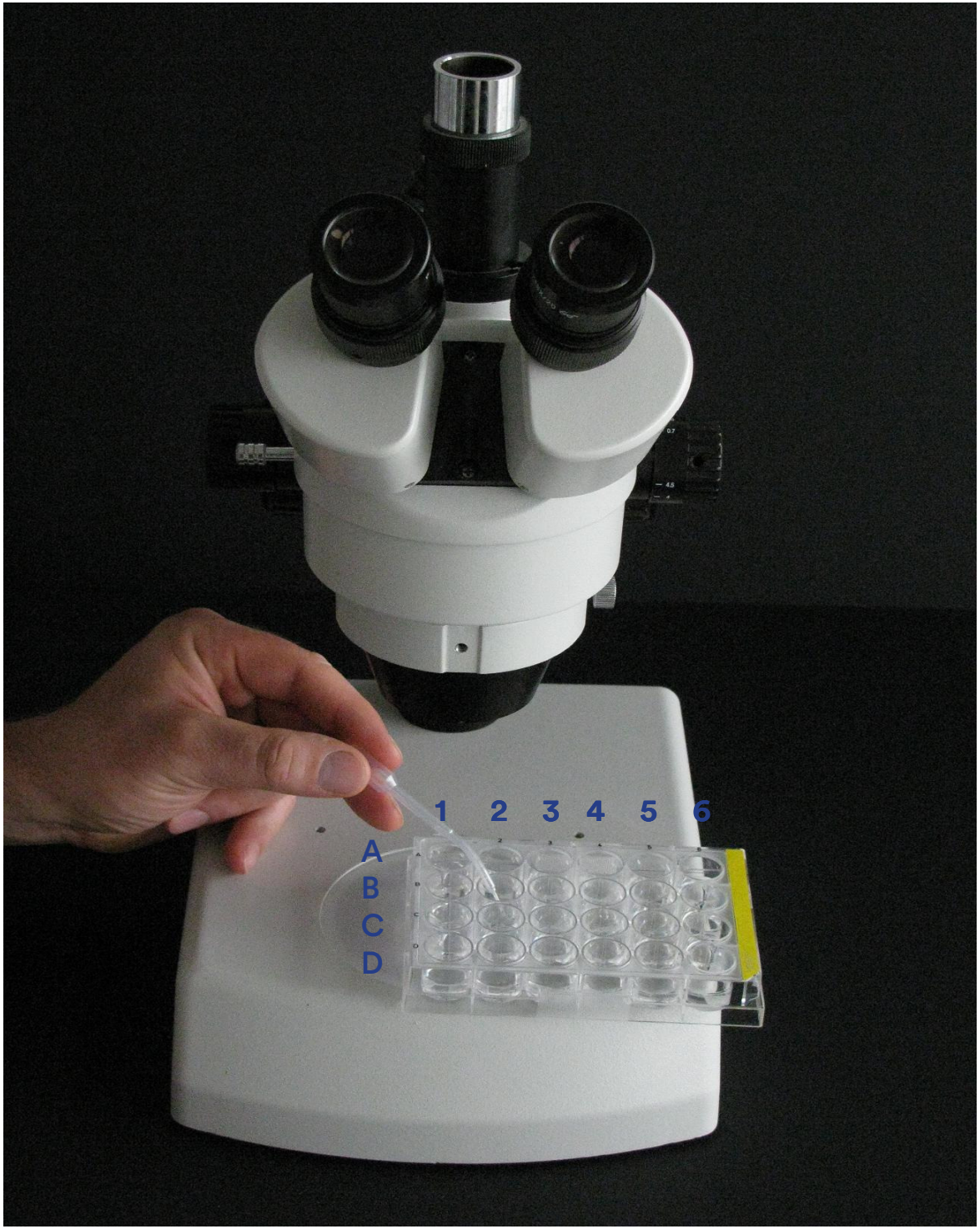
MICROBIOTESTS

MICROBIOTESTS

23

- PUT THE MULTIWELL PLATE ON THE STAGE OF THE DISSECTION MICROSCOPE

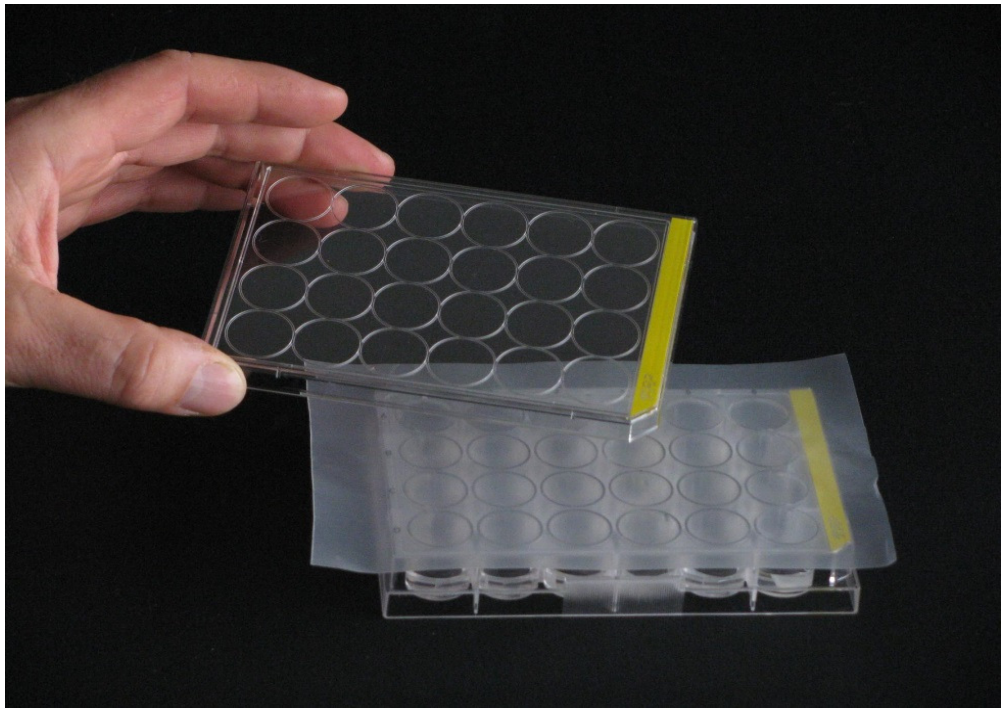
- TRANSFER 10 LARVAE FROM RINSING WELL A1 INTO THE 3 OTHER WELLS OF COLUMN 1 (CUPS B1, C1 AND C1 = controls)

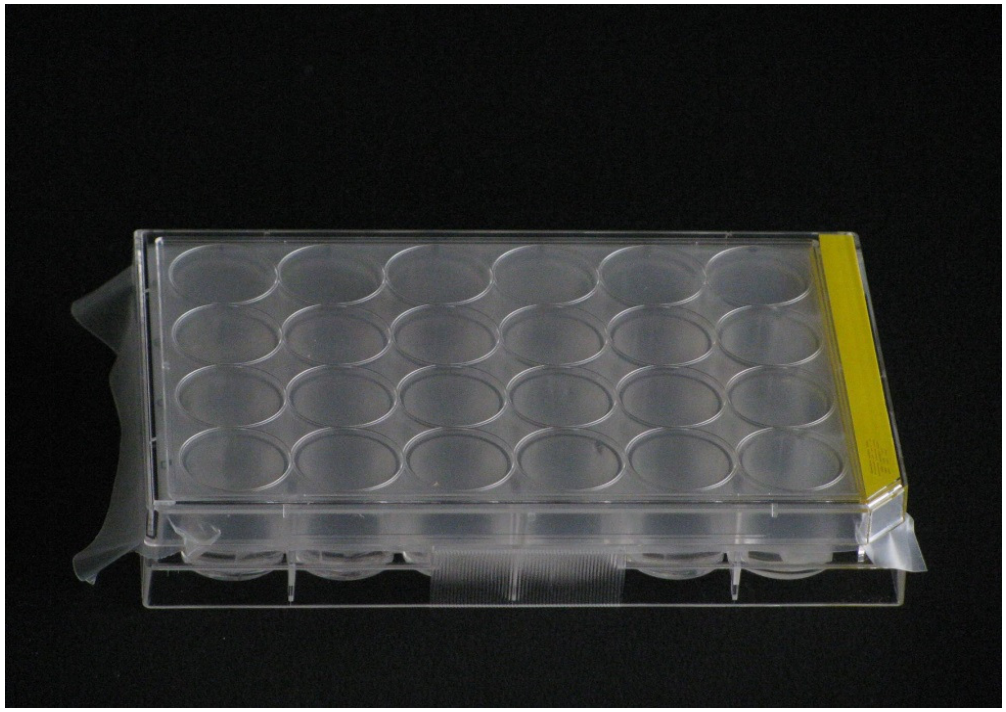


MICROBIOTESTS

24

REPEAT THE SAME TRANSFER OF 10 LARVAE FROM RINSING WELLS A2 TO A6 TO THE 3 WELLS OF COLUMNS 2 TO 6 (in this sequence, i.e. from the lowest to the hightest toxicant concentration)





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

MICROBIOTESTS

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PUT THE MULTIWELL PLATE IN THE INCUBATOR AT 25 °C, IN DARKNESS, FOR 24 HOURS

MICROBIOTESTS

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SCORING OF THE RESULTS

- PUT THE MULTIWELL PLATE ON THE STAGE OF THE DISSECTION MICROSCOPE

- CHECK THE WELLS OF ROWS B, C AND D AND COUNT THE NUMBER OF DEAD LARVAE IN EACH CUP

- SCORE THE MORTALITY DATA ON THE "RESULTS SHEET"

- CALCULATE THE 24h LC50 WITH AN APPROPRIATE PROGRAM