OSTRACODTOXKIT F

Test procedure

1

PREPARATION OF

STANDARD FRESHWATER

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

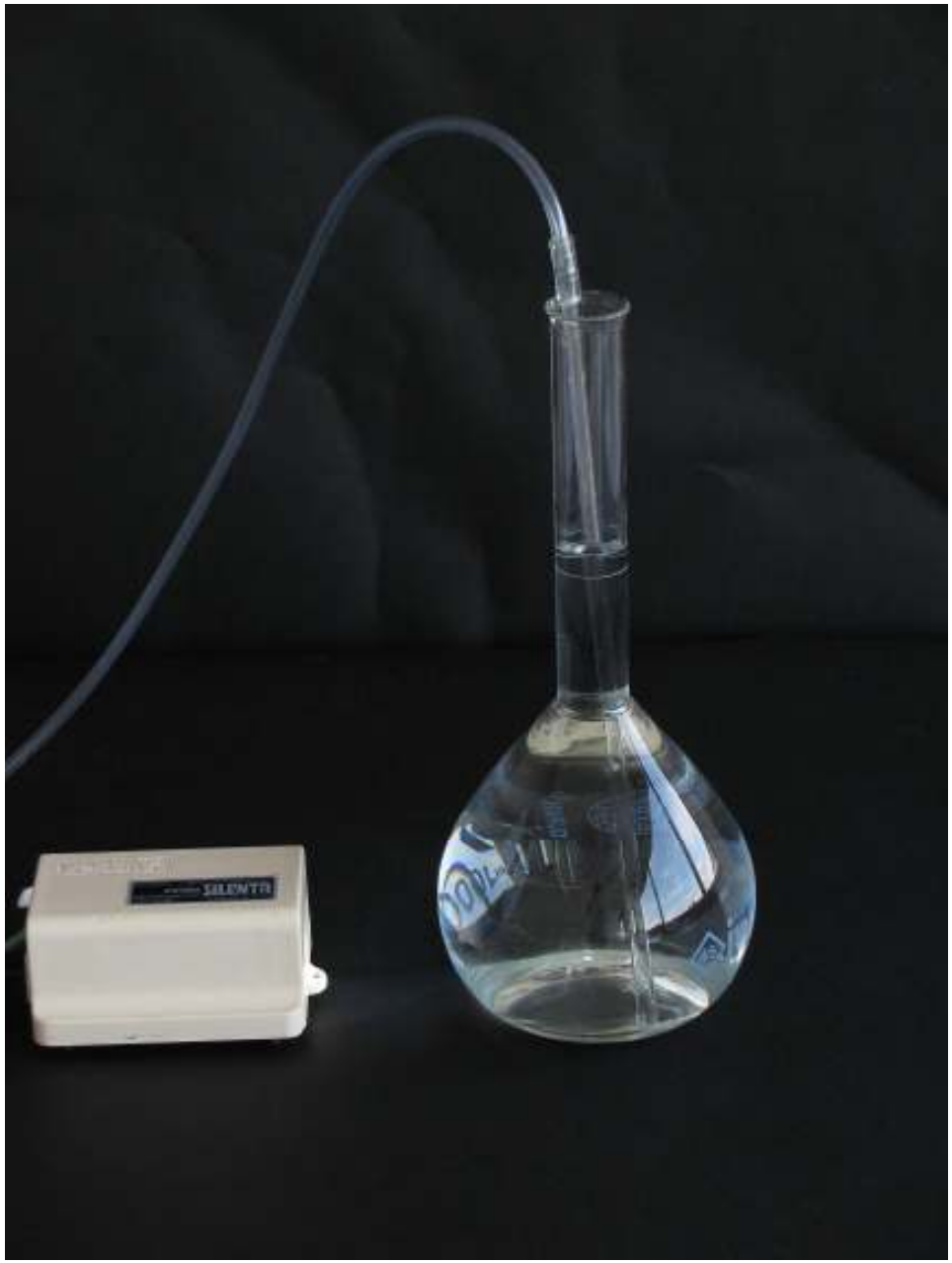
2

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

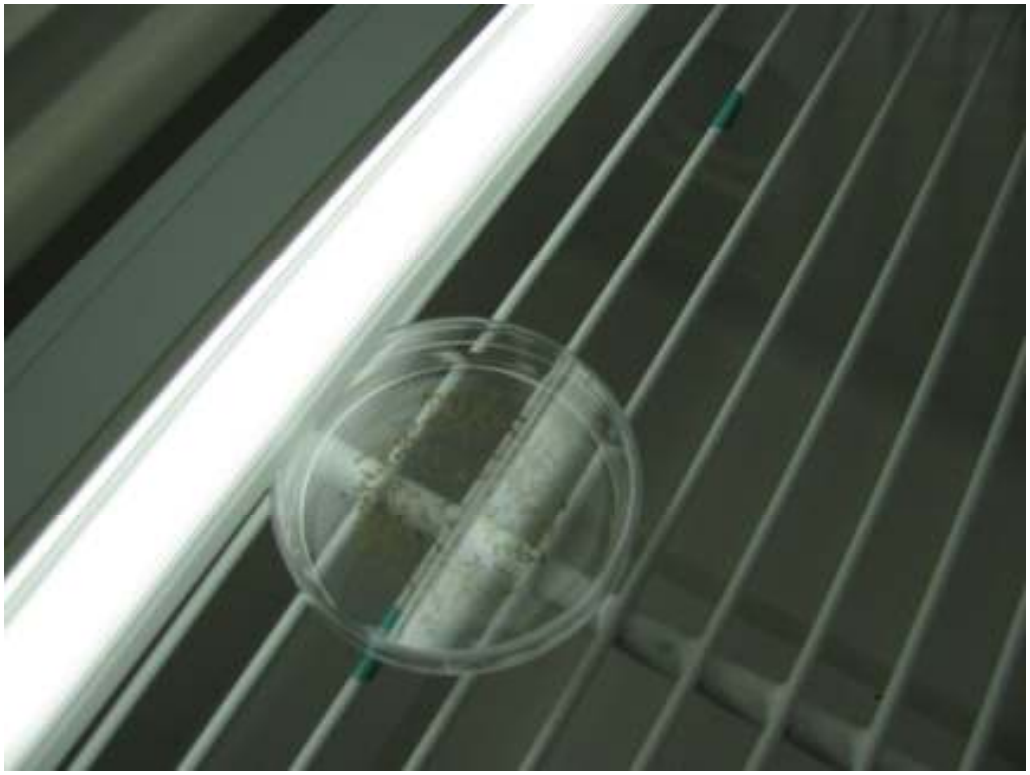
4

HATCHING OF OSTRACOD CYSTS

POUR THE CONTENTS OF ONE VIAL WITH CYSTS IN THE PETRI DISH

5

TO ENSURE THE TRANSFER OF ALL THE CYSTS, THE VIAL SHOULD BE RINSED TWICE WITH 1 mL STANDARD FRESHWATER



6

INCUBATION OF THE CYSTS

INCUBATE THE PETRI DISH
FOR 48 HOURS AT 25 °C
UNDER CONTINOUS ILLUMINATION
OF MIN. 3 000 – 4 000 LUX

7

PRE-FEEDING

OF THE TEST ORGANISMS

TAKE ONE VIAL

WITH SPIRULINA POWDER

AND FILL IT

WITH STANDARD FRESHWATER

8

- SHAKE THE VIAL WITH THE SPIRULINA SUSPENSION

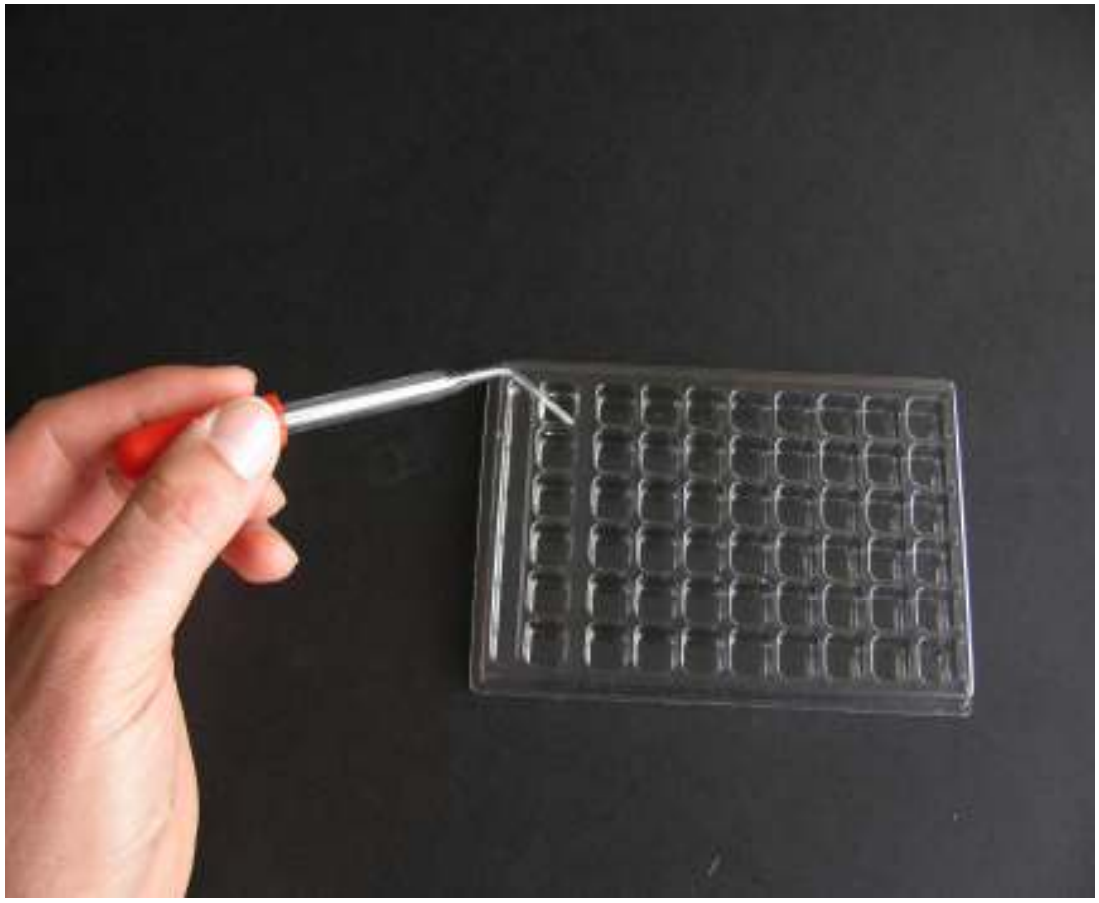
- POUR THE CONTENTS IN THE PETRI DISH CONTAINING THE HATCHED OSTRACODS AND SWIRL THE PETRI DISH GENTLY

- ALLOW THE OSTRACODS TO PRE- FEED FOR 4 HOURS

9

LENGTH MEASUREMENT OF
FRESHLY HATCHED OSTRACODS

PICK UP 10 OSTRACODS FROM THE
HATCHING PETRI DISH
WITH A GLASS MICROPIPETTE

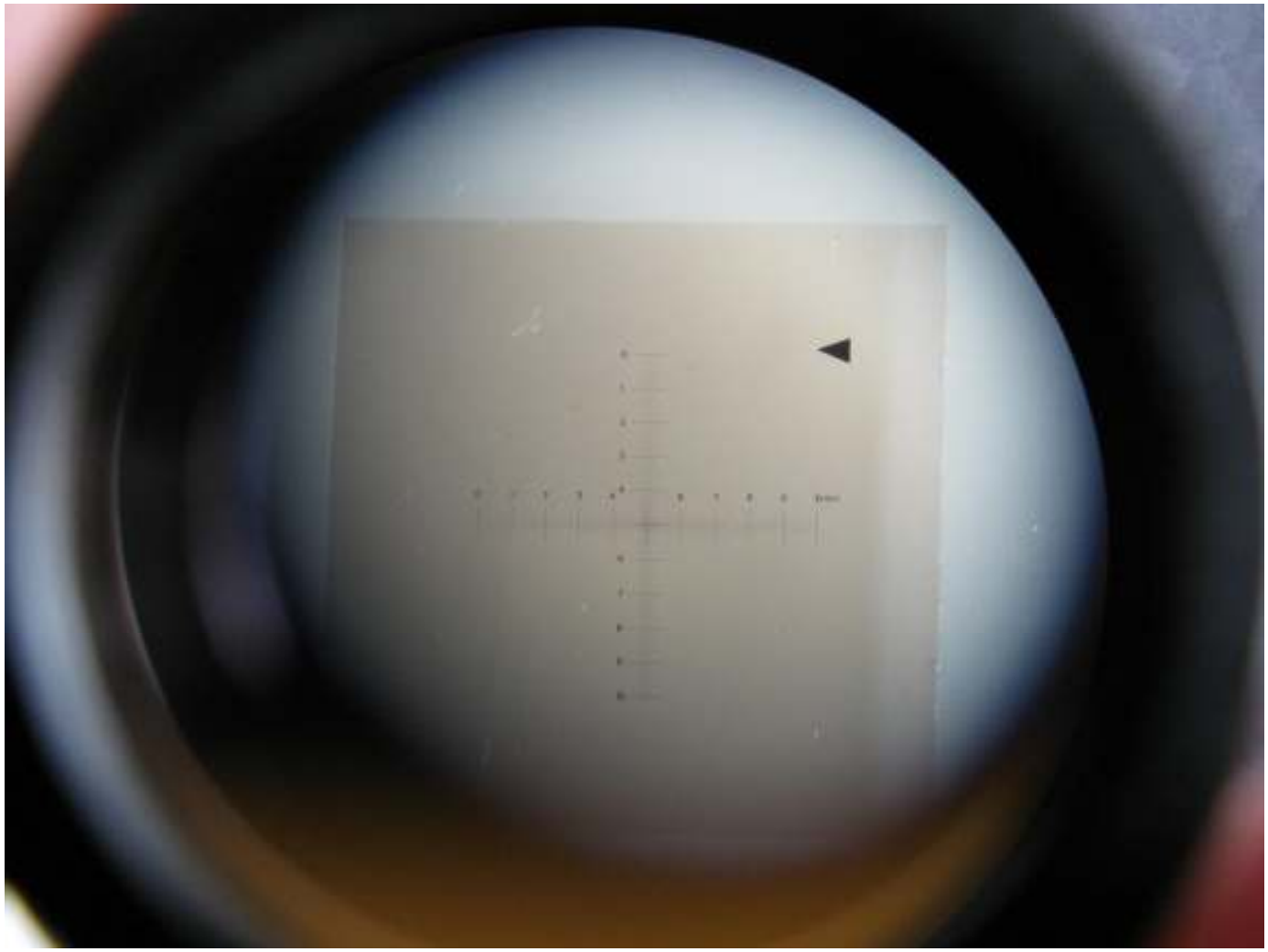


10

TRANSFER THE OSTRACODS
INTO ONE CUP
OF THE MULTIWELL
FOR “LENGTH MEASUREMENTS”

11

ADD ONE DROP OF LUGOL FIXATIVE
TO THE CUP CONTAINING THE OSTRACODS
AND WAIT UNTIL THE ORGANISMS ARE
COMPLETELY IMMOBILE



12

PUT THE MICROMETER SLIP ON THE GLASS STAGE OF THE DISSECTION MICROSCOPE, IN THE CENTRE OF THE VISUAL FIELD

13

PUT THE MULTIWELL

FOR LENGTH MEASUREMENTS

ON THE STAGE OF THE DISSECTION

MICROSCOPE, AND MEASURE

THE LENGTH OF THE OSTRACODS

N.B : the smallest subdivisions of the micrometer slip are 50 µm

FRESHLY HATCHED OSTRACODS HAVE

A LENGTH OF ABOUT 200 µm

14

SCORE THE LENGTH

OF THE OSTRACODS

ON THE “RESULTS SHEET”

IN COLUMN “Day O”

15

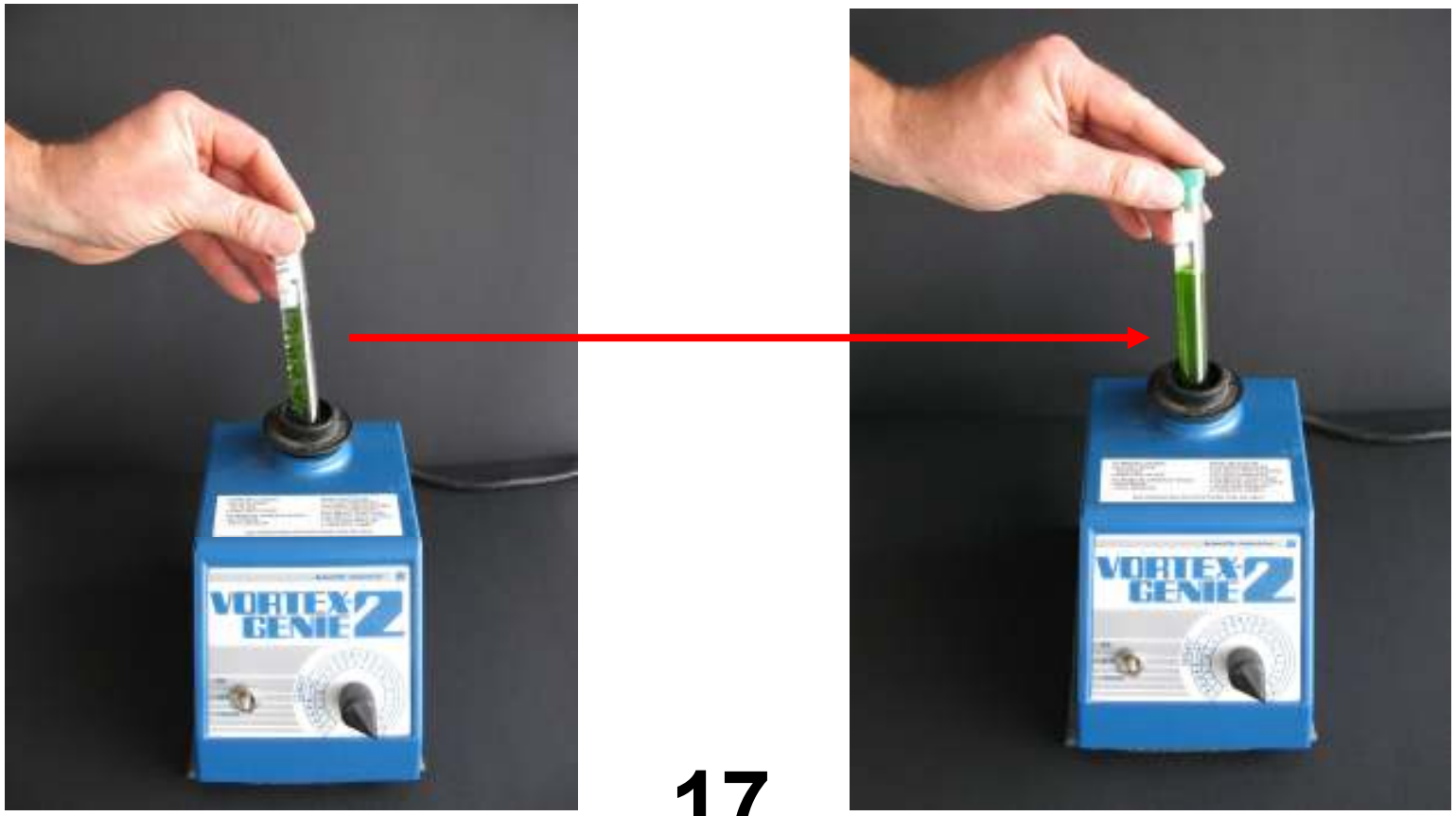
PREPARATION OF THE
ALGAL FOOD SUSPENSION

TAKE ONE TUBE WITH ALGAL BEADS
AND POUR OUT THE STORAGE MEDIUM
TAKING CARE NOT TO LOSE ANY BEAD
DURING THE OPERATION

16

ADD 7 mL MATRIX DISSOLVING MEDIUM TO THE TUBE WITH ALGAL BEADS

AND CLOSE THE TUBE WITH THE CAP



17

DE-IMMOBILISATION OF THE ALGAE

SHAKE THE TUBE ON A VORTEX UNTIL THE MATRIX IN WHICH THE ALGAE ARE IMMOBILISED IS TOTALLY DISSOLVED AND THE ALGAE ARE SET FREE

18

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

CAREFULLY POUR OUT THE SUPERNATANT FROM THE TUBE

19

ADD 10 mL DISTILLED WATER
TO THE TUBE WITH THE ALGAL PELLET

CAP AND SHAKE THE TUBE
TO RESUSPEND THE ALGAE

20

CENTRIFUGE THE TUBE AGAIN AT 3000 rpm FOR 10 MINUTES

AND POUR OUT THE SUPERNATANT

21

ADD 10 mL STANDARD FRESHWATER
TO THE TUBE WITH THE ALGAL PELLET

CAP AND SHAKE THE TUBE
TO RESUSPEND THE ALGAE

22

- POUR THE CONCENTRATED ALGAL SUSPENSION INTO A 25 mL VOLUMETRIC FLASK
- ADD STANDARD FRESHWATER TO THE 25 mL MARK
- CAP THE FLASK AND SHAKE TO OBTAIN A HOMOGENOUS ALGAL SUSPENSION

23

ADDITION OF SEDIMENT, ALGAL FOOD AND OSTRACODS TO THE TEST PLATES

PUT 2 mL STANDARD FRESHWATER INTO EACH WELL OF TWO TEST PLATES (multiwell for reference sediment and multiwell for test sediment)

24

TEST PLATE FOR REFERENCE SEDIMENT

- FILL THE SPOON WITH REFERENCE SEDIMENT AND STRIKE OFF THE EXCESS SEDIMENT WITH THE PLASTIC SPATULA (the filled spoon then contains 500 µl sediment)
- PUT 2 SPOONS (= 1000 µl) REFERENCE SEDIMENT INTO EACH WELL OF THE TEST PLATE

25

TEST PLATE WITH TEST SEDIMENT

- FILL THE SPOON WITH TEST SEDIMENT AND STRIKE OFF THE EXCESS SEDIMENT WITH THE PLASTIC SPATULA (the filled spoon then contains 500 µl sediment)
- PUT 2 SPOONS (= 1000 µl) OF TEST SEDIMENT INTO EACH WELL OF THE TEST PLATE (use the tip of the spatula to perform the transfer)

26

- POUR THE ALGAL FOOD SUSPENSION FROM THE 25 mL FLASK INTO A BEAKER
- SHAKE THE BEAKER TO DISTRIBUTE THE ALGAE EVENLY
- PIPET 2 mL ALGAL SUSPENSION INTO EACH WELL OF THE TWO TEST PLATES

27

FILL THE LID OF THE HATCHING PETRI DISH WITH 10 mL STANDARD FRESHWATER

28

TRANSFER WITH THE GLASS MICROPIPETTE A NUMBER OF OSTRACOD NEONATES FROM THE HATCHING PETRI DISH INTO THE LID OF THIS DISH

29

TRANSFER 10 OSTRACODS FROM THE PETRI DISH LID INTO EACH WELL
OF THE TWO TEST PLATES

30

INCUBATION OF THE TEST PLATES

- COVER THE TWO TEST PLATES WITH A SHEET OF PARAFILM AND PUT THE LID ON TOP
- INCUBATE THE TEST PLATES AT 25 °C, IN DARKNESS, FOR 6 DAYS

31

TEST SCORING – 1. TRANSFER OF THE OSTRACODS INTO A PETRI DISH

WITH THE AID OF THE “LARGE MOUTH” MICROPIPET, SUCK UP PART OF THE SEDIMENT SUSPENSION FROM ONE CUP OF THE TEST PLATE AND TRANSFER IT INTO THE MICROSIEVE

32

- GENTLY RINSE THE CONTENTS OF THE MICROSIEVE WITH TAPWATER
  TO WASH OUT THE FINE SEDIMENT
- PROCEED FURTHER WITH THE STEPWISE TRANSFER OF THE SEDIMENT SUSPENSION
  TO THE MICROSIEVE AND RINSE EACH TIME THE CONTENTS OF THE MICROSIEVE

33

- ADD A FEW mL STANDARD FRESHWATER TO THE CUP

- MIX THE WATER WITH THE REMAINING SEDIMENT AND TRANSFER IT TO THE MICROSIEVE FOR RINSING.

- REPEAT THIS OPERATION UNTIL ALL THE SEDIMENT AND OSTRACODS HAVE BEEN TRANSFERRED INTO THE MICROSIEVE

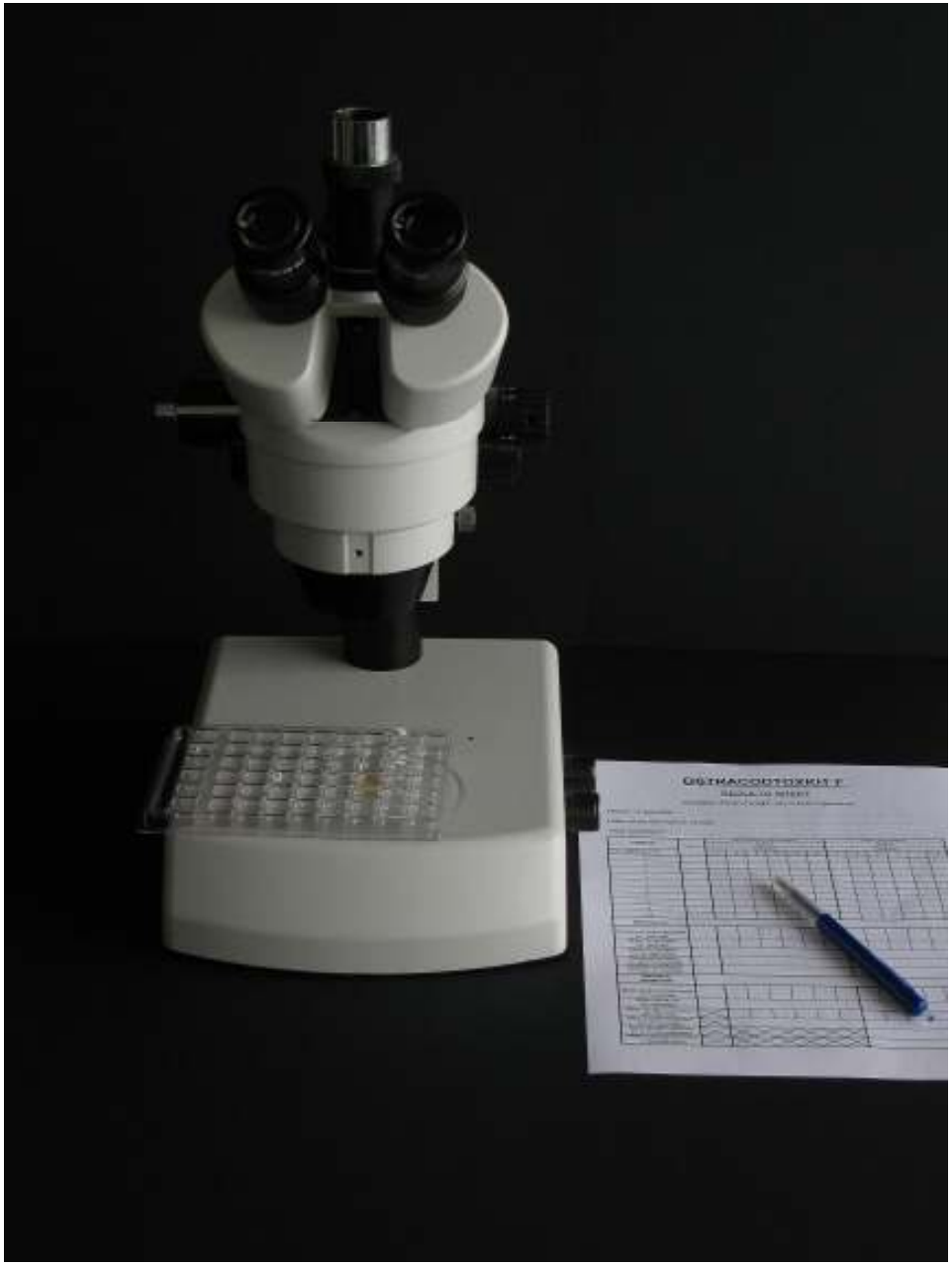
34

- TURN THE MICROSIEVE UPSIDE DOWN AND WASH THE CONTENTS INTO A PETRI DISH, WITH THE AID OF A WASH BOTTLE CONTAINING TAPWATER
- REPEAT THE SEDIMENT TRANSFER AND RINSING OPERATIONS FOR ALL THE CUPS OF THE TWO TEST PLATES

35

TEST SCORING – <u>2. MORTALITY SCORING</u>

PICK UP ALL THE LIVE OSTRACODS FROM THE PETRI DISH WITH A GLASS MICROPIPETTE AND TRANSFER THEM INTO ONE CUP OF THE “LENGTH MEASUREMENTS” MULTIWELL



36

- COUNT THE NUMBER OF LIVE OSTRACODS IN THE CUP

- SUBSTRACT THIS NUMBER FROM 10 (i.e. from the original number of ostracods put in the cup)

-SCORE THE OUTCOME (i.e. the number of dead ostracods) ON THE RESULTS SHEET

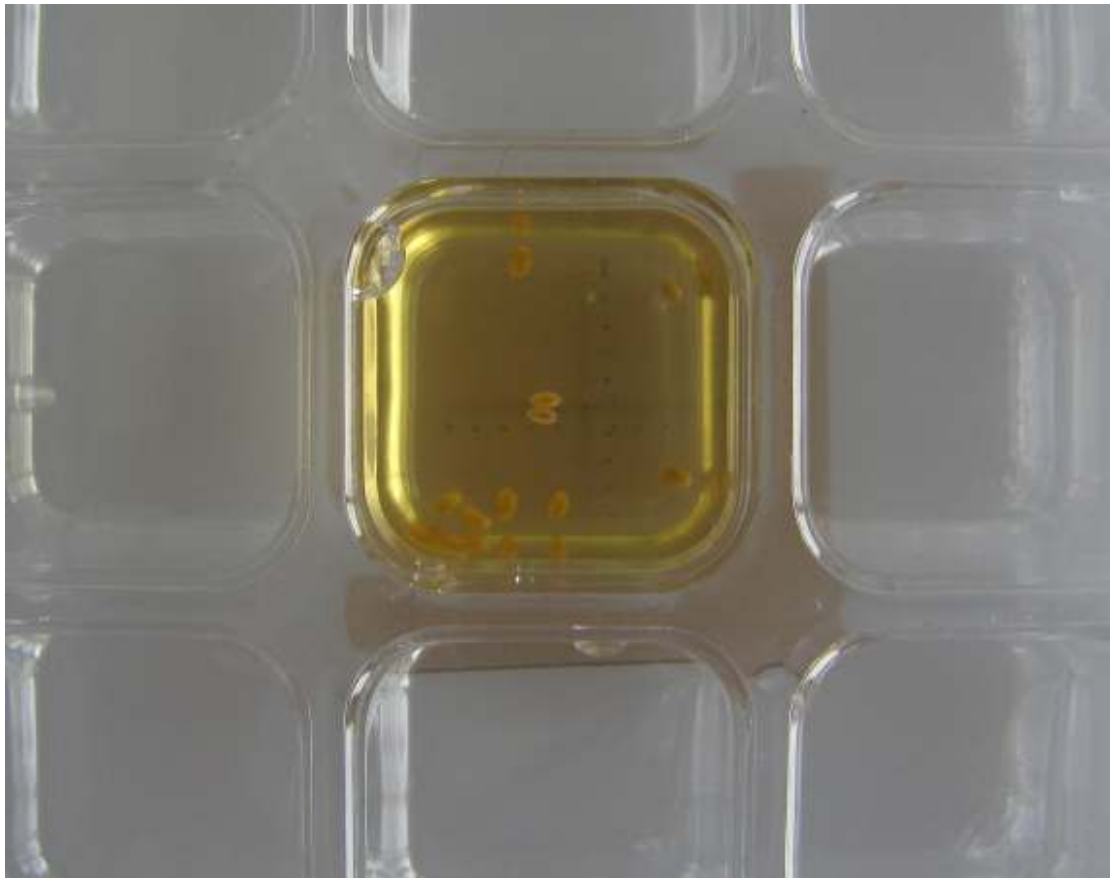
- REPEAT THIS OPERATION FOR ALL THE CUPS OF THE TWO TEST PLATES

- CALCULATE AND SCORE THE MEAN % OSTRACOD MORTALITY FOR ALL THE CUPS

TEST SCORING – 3. LENGTH MEASUREMENT

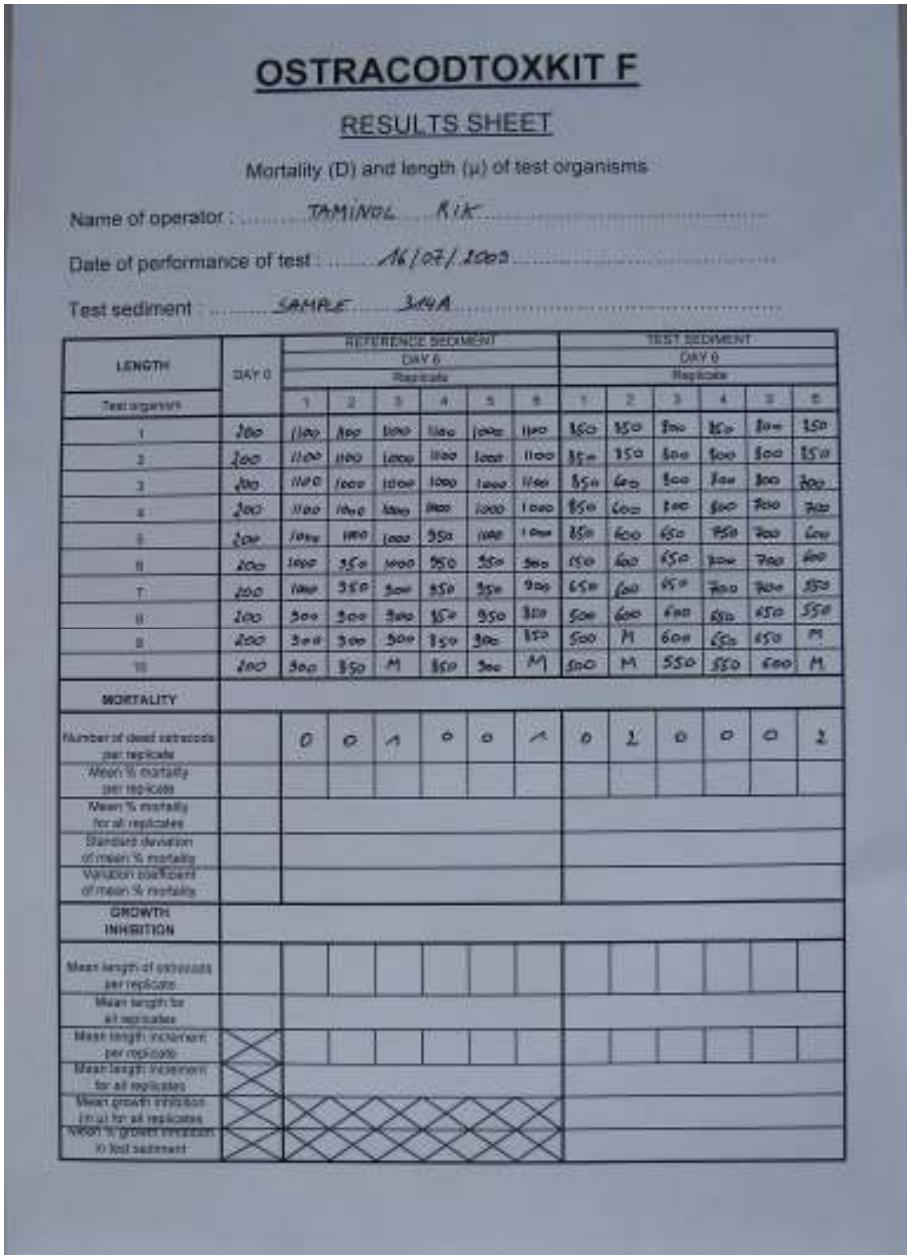
NB : only to be performed if
the percentage mortality is < 30%

- ADD ONE DROP OF LUGOL FIXATIVE TO EACH CUP OF THE LENGTH MEASUREMENTS MULTIWELL WHICH CONTAIN THE LIVE OSTRACODS FROM THE TWO TEST PLATES



38

- WAIT UNTIL THE OSTRACODS ARE IMMOBILE
- MEASURE THE LENGTH OF EACH OSTRACOD FOLLWING THE PROCEDURE INDICATED IN STEPS 13 & 14
- SCORE THE RESULTS IN THE CORRESPONDING “LENGTH” BOXES OF THE RESULTS SHEET



OSTRACODTOXKIT F

RESULTS SHEET

Mortality (D) and length (μ) of test organisms

Name of operator : TAMINOL RIK

Date of performance of test : 16/07/2003

Test sediment : SAMPLE 34A

LENGTH	DAY 0	REFERENCE SEDIMENT DAY 6 Replicate						TEST SEDIMENT DAY 6 Replicate					
Test organism		1	2	3	4	5	6	1	2	3	4	5	6
1	200	1100	800	1000	1100	1000	1100	850	950	900	850	800	950
2	200	1100	1100	1000	1100	1000	1100	850	950	800	900	800	950
3	200	1100	1000	1000	1000	1000	1100	850	600	800	800	800	800
4	200	1100	1000	1000	1000	1000	1000	850	600	800	900	700	700
5	200	1000	1000	1000	950	1100	1000	850	600	650	950	800	600
6	200	1000	950	1000	950	950	900	950	600	650	800	700	600
7	200	1000	950	900	950	950	900	650	600	650	700	800	950
8	200	900	900	900	850	950	850	500	600	600	650	650	550
9	200	900	900	900	850	900	850	500	M	600	650	650	M
10	200	900	850	M	850	900	M	500	M	550	550	600	M
MORTALITY													
Number of dead ostracods per replicate		0	0	1	0	0	1	0	1	0	0	0	1
Mean % mortality per replicate													
Mean % mortality for all replicates													
Standard deviation of mean % mortality													
Variation coefficient of mean % mortality													
GROWTH INHIBITION													
Mean length of ostracods per replicate													
Mean length for all replicates													
Mean length increment per replicate													
Mean length increment for all replicates													
Mean growth inhibition (in μ) for all replicates													
Mean % growth inhibition in test sediment													

39

- PERFORM THE DATA TREATMENT OF THE RESULTS WITH AN APPROPRIATE PROGRAMME