



DUCKWEED TOXKIT F

TEST PROCEDURE

1

PREPARATION OF DUCKWEED GROWTH AND TEST DILUTION MEDIUM

- VOLUMETRIC FLASK (500 ml)
- VIALS WITH CONCENTRATED
STEINBERG MEDIUM SOLUTIONS
- PURE WATER (deionised or distilled)



2



TRANSFER 10 ml FROM “STOCK SOLUTIONS A, B AND C” WITH A PIPET IN +/- 300 ml PURE WATER IN THE 500 ml VOLUMETRIC FLASK

3

TAKE “NUTRIENT STOCK” VIALS
D AND E” AND TRANSFER 0,5 ml
OF EACH INTO THE FLASK



4

FILL THE FLASK UP TO THE
500 ml MARK WITH PURE WATER
STOPPER THE FLASK AND SHAKE
THOROUGHLY TO HOMOGENIZE THE
MEDIUM



5

GERMINATION OF THE *SPIRODELA* *POLYRHIZA* TURIONS

TAKE A TUBE WITH *SPIRODELA*
TURIONS AND SHAKE IT SLIGHTLY
TO RESUSPEND THE TURIONS



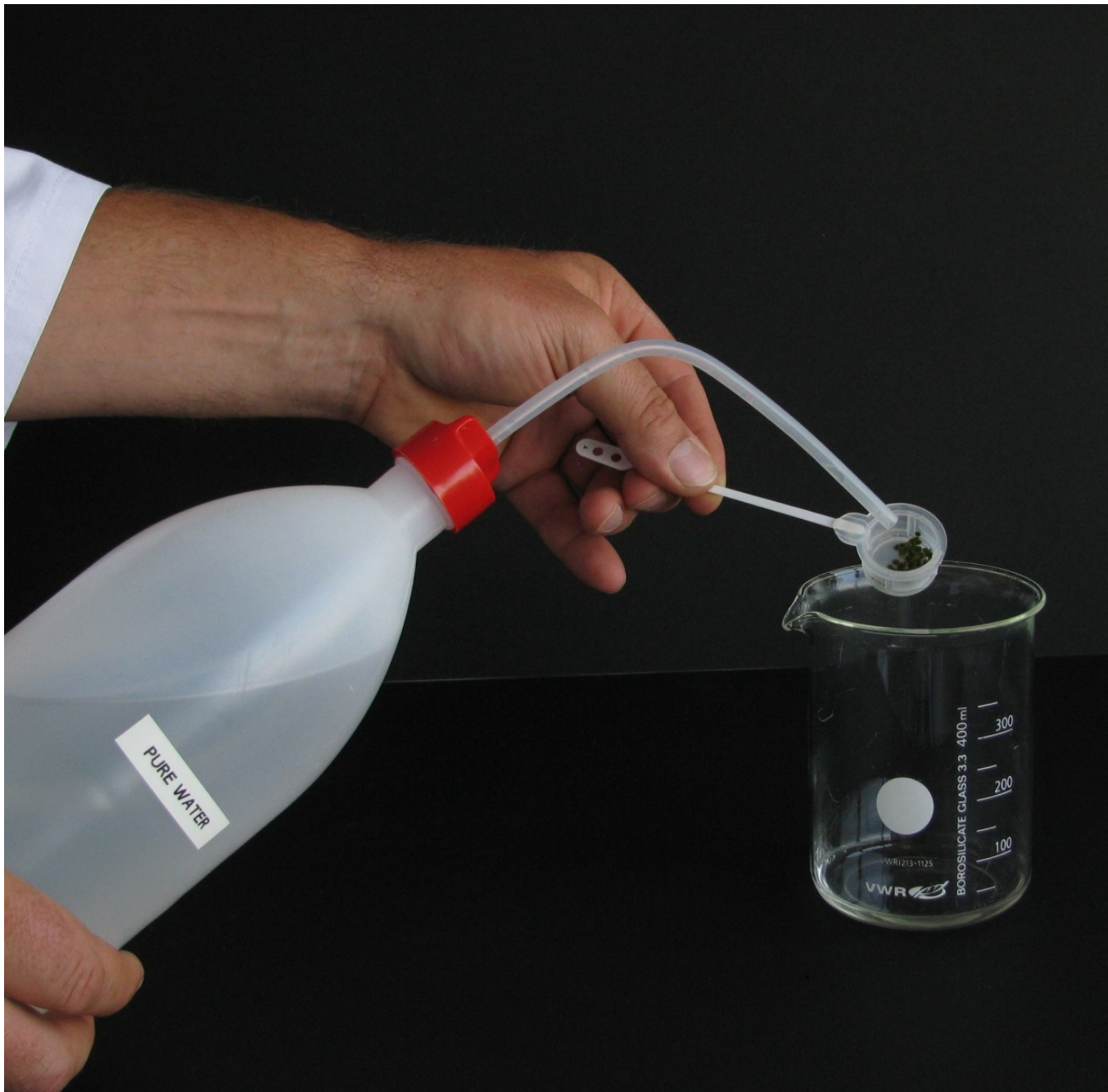
6



- POUR THE CONTENTS OF THE TUBE WITH TURIONS IN THE MICROSIEVE
- MAKE SURE THAT ALL THE TURIONS ARE TRANSFERRED

7

**RINSE THE TURIONS
THOROUGHLY
WITH PURE WATER**



8



**TURN THE SIEVE UPSIDE DOWN
AND FLUSH THE TURIONS
INTO THE PETRI DISH WITH
STEINBERG MEDIUM**

**N.B. The final volume of Steinberg
medium in the petri dish shall be 30 ml**

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A



INCUBATE THE PETRI DISH FOR 72h AT 25 °C
UNDER CONTINUOUS “TOP” ILLUMINATION
OF 6 000 LUX

B



A : an incubator provided with illumination

B : an incubator with a LED Illumination Unit

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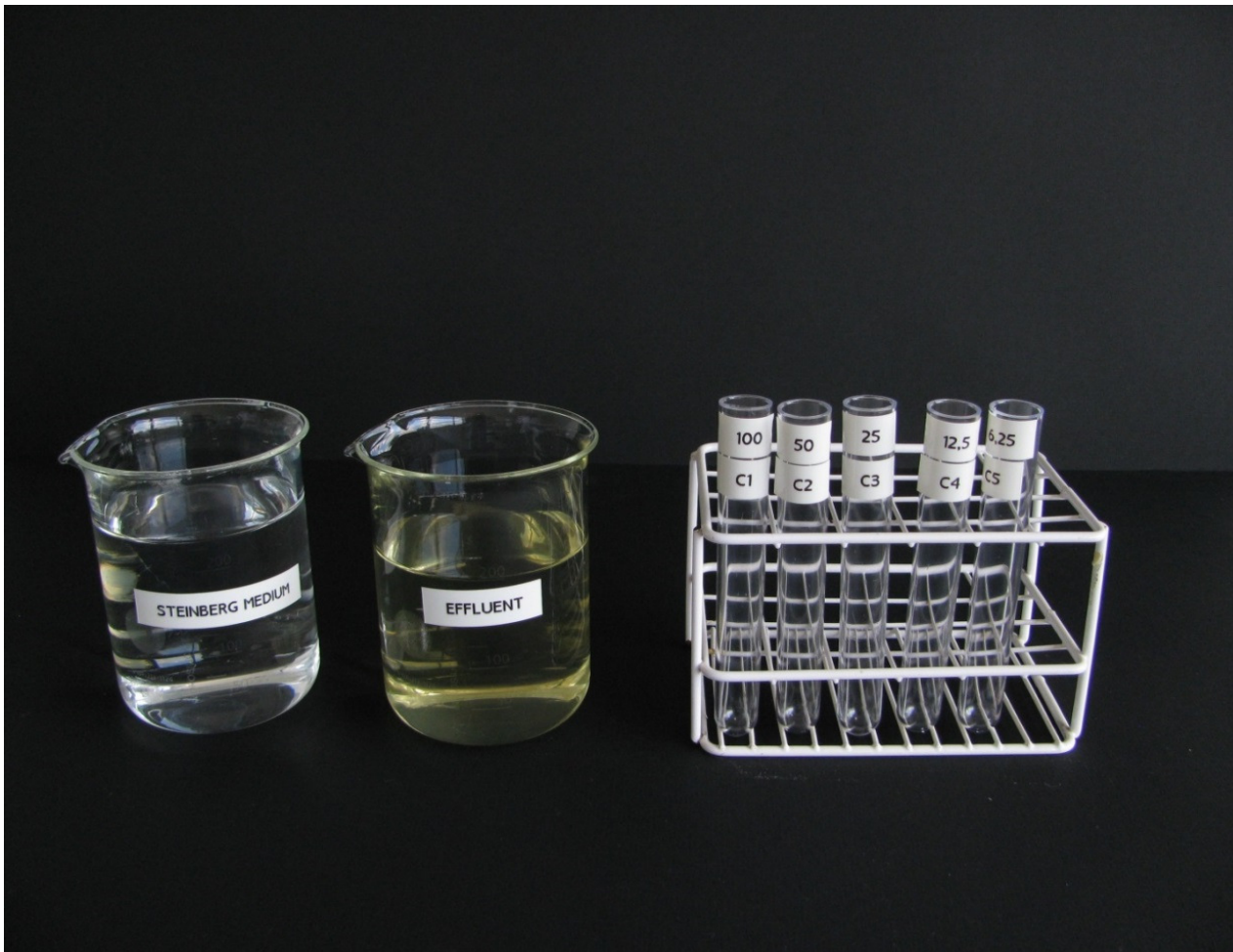
PREPARATION OF THE TOXICANT DILUTIONS

For example :

TEST ON A EFFLUENT

IN 5 DILUTIONS (C1-C5)

100% - 50% - 25% - 12.5% - 6.25%



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ADDITION OF CONCENTRATED STEINBERG MEDIUM SOLUTIONS TO THE EFFLUENT

- TRANSFER ABOUT 80 ml EFFLUENT IN A 100 ml VOLUMETRIC FLASK
- ADD 2 ml NUTRIENT STOCK SOLUTION OF VIALS A, B , C



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ADD 100 μ l OF NUTRIENT STOCK
SOLUTIONS D AND E
TO THE VOLUMETRIC FLASK



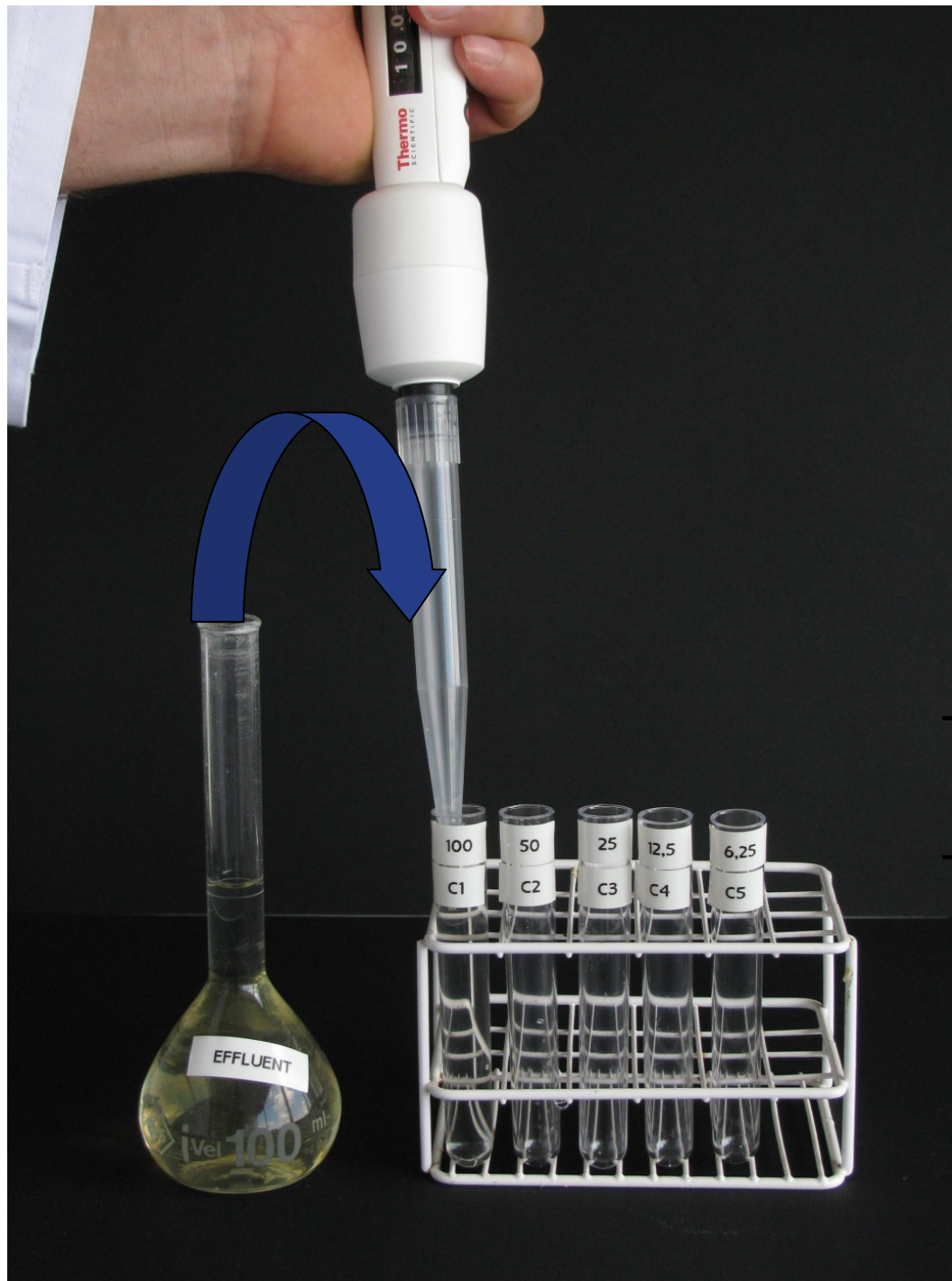
13



- FILL THE FLASK UP TO THE 100 ml MARK WITH EFFLUENT
- STOPPER THE FLASK AND SHAKE TO MIX THE CONTENTS

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PREPARATION OF THE EFFLUENT DILUTIONS



- TAKE FIVE 20 ml TUBES AND LABEL THEM C1 TO C5
- ADD 20 ml OF THE TREATED EFFLUENT TO TEST TUBE C1

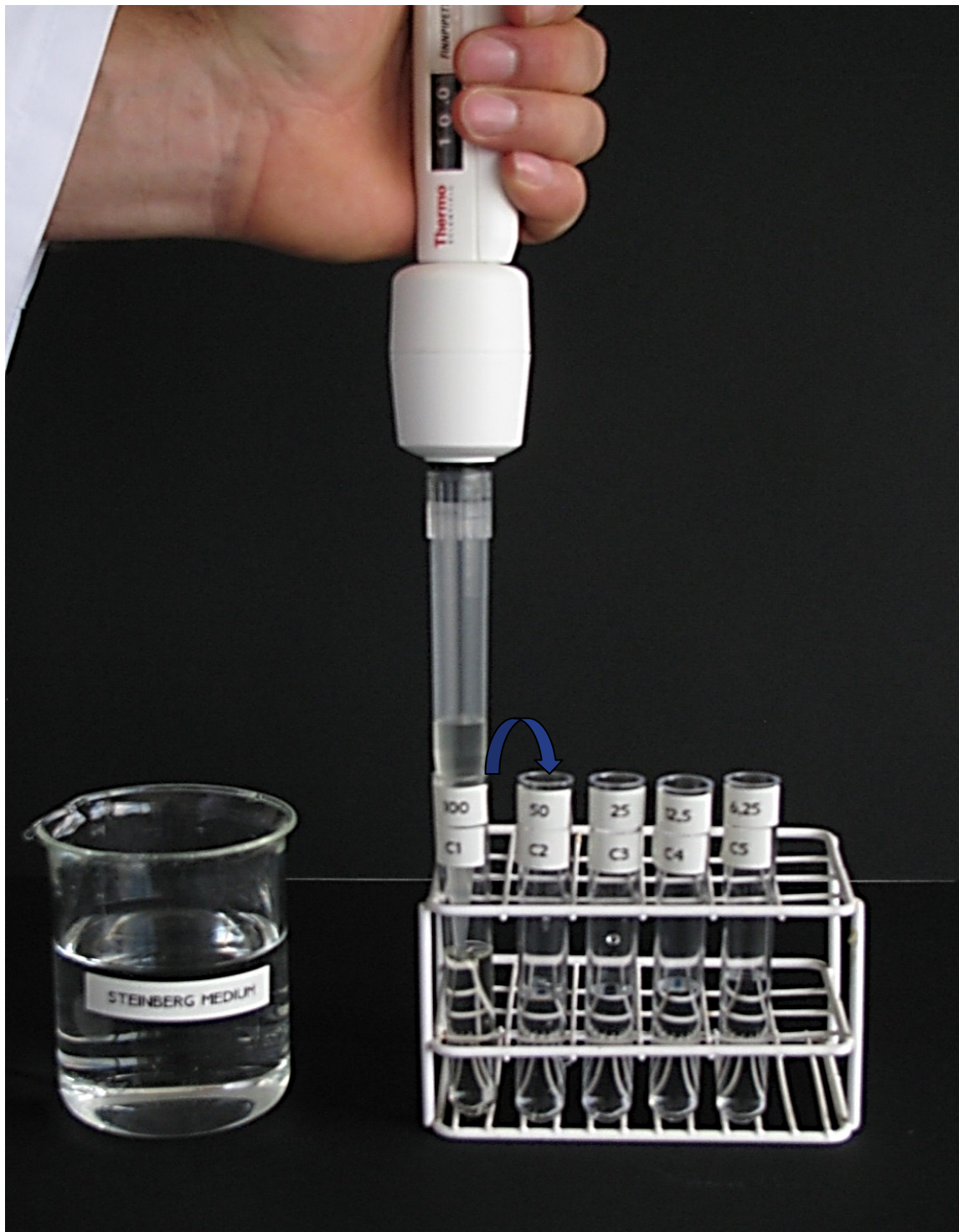
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**ADD 10 ml STEINBERG MEDIUM TO THE
TEST TUBES C2, C3, C4 AND C5**



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- TRANSFER 10 ml EFFLUENT FROM TUBE C1 TO TUBE C2
- CAP AND SHAKE THE TEST TUBE



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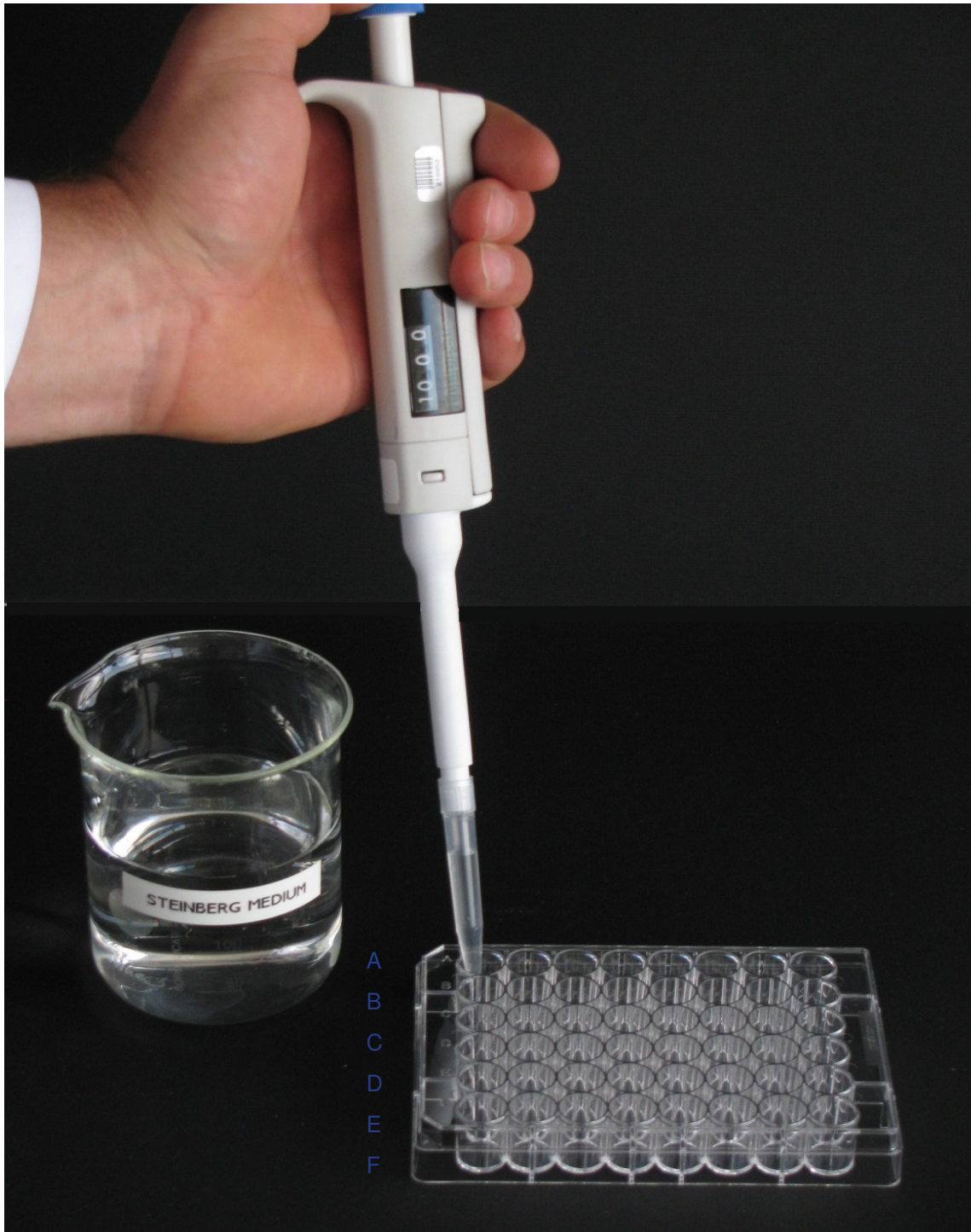
- TRANSFER 10 ml TEST DILUTION FROM TUBE C2 TO C3
- CAP AND SHAKE THE TEST TUBE.
- REPEAT THIS PROCEDURE FOR THE NEXT DILUTIONS



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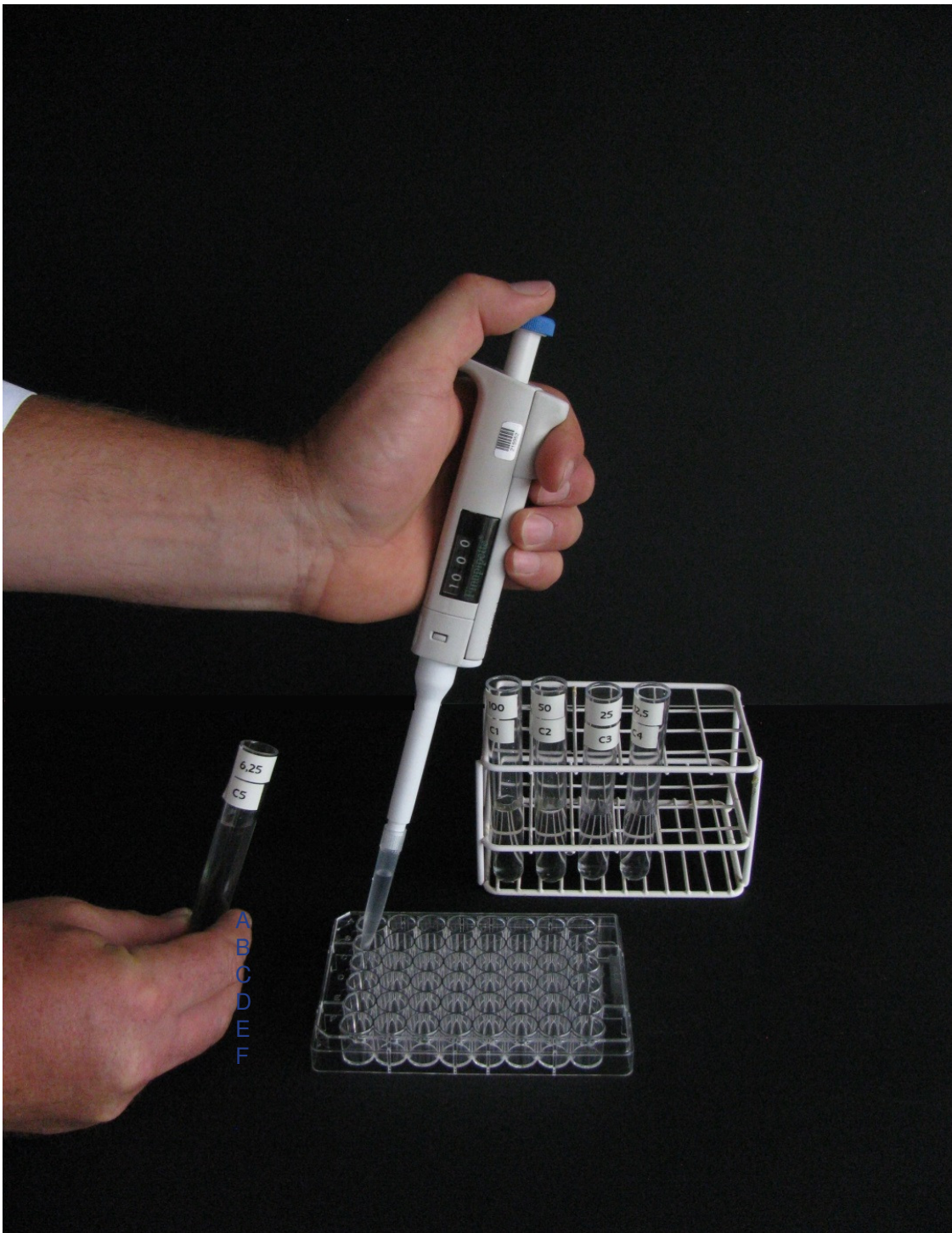
FILLING OF THE TEST PLATE WITH THE TOXICANT DILUTIONS

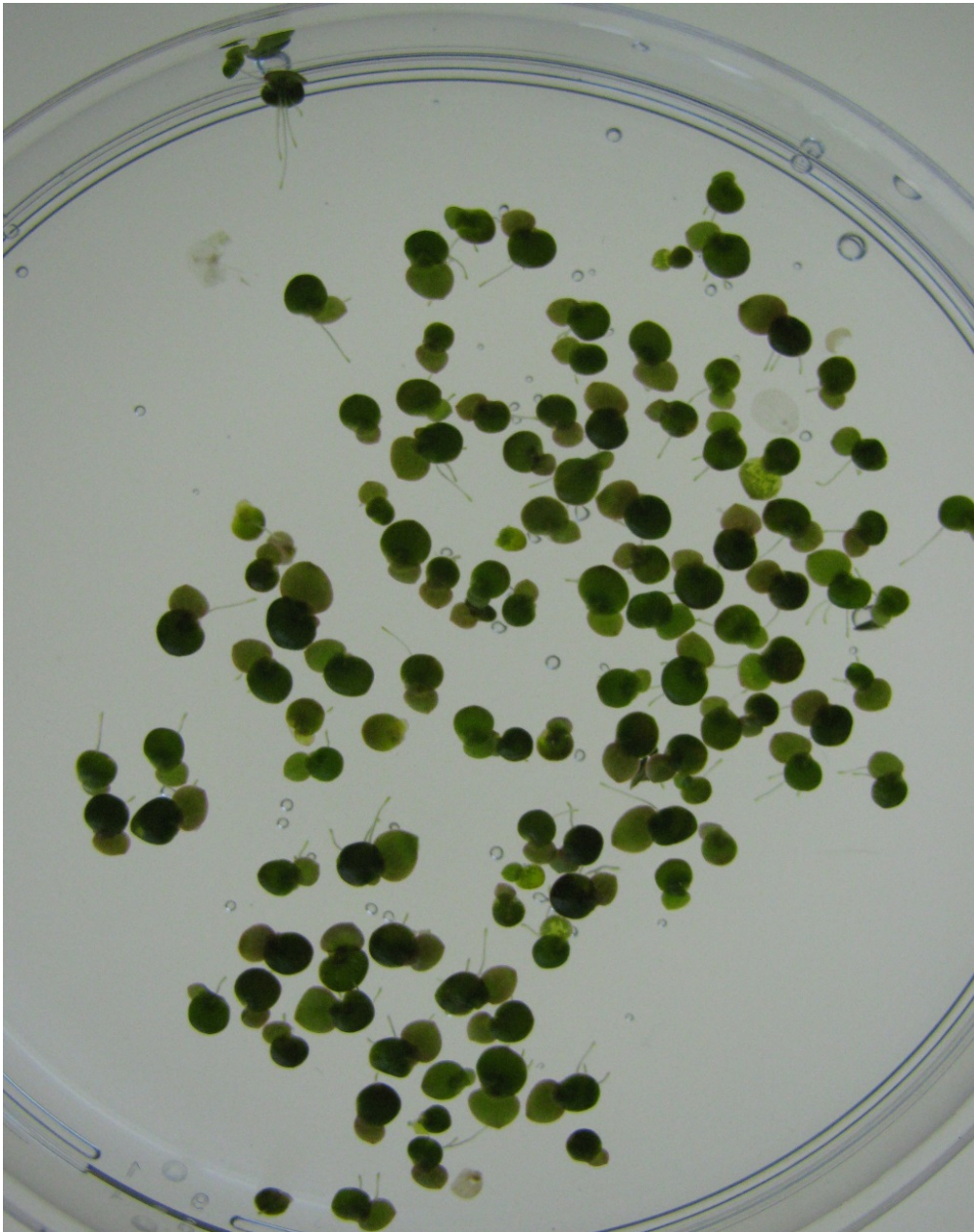
PUT 1 mL STEINBERG MEDIUM IN THE
8 CUPS OF ROW A (= Control row)



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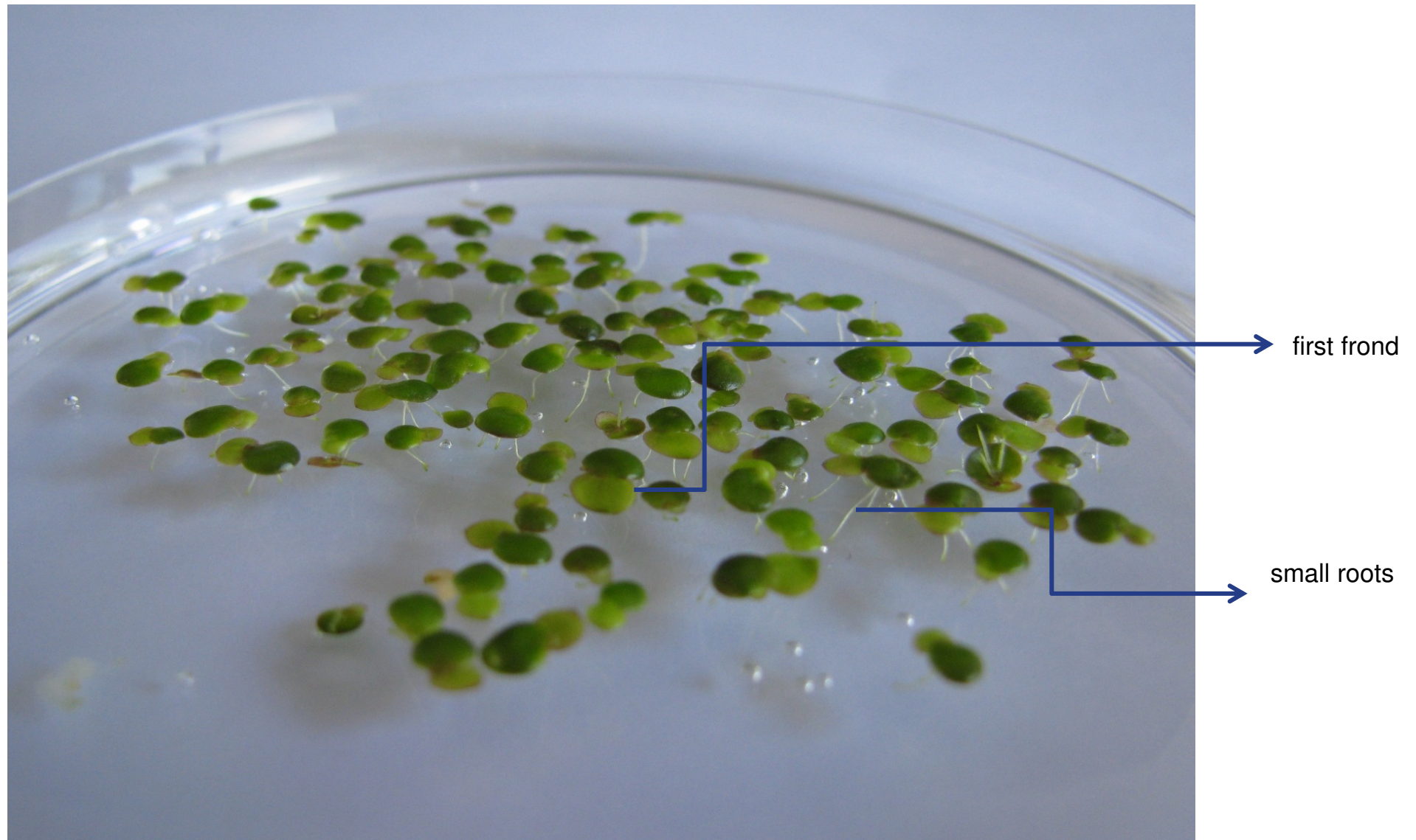
- PUT 1 ML OF THE TUBE C5 IN THE 8 CUPS OF ROW B.
- REPEAT THIS PROCEDURE WITH THE TUBES C4, C3, C2 AND C1 FOR THE 8 CUPS IN THE ROWS C, D, E AND F RESPECTIVELY



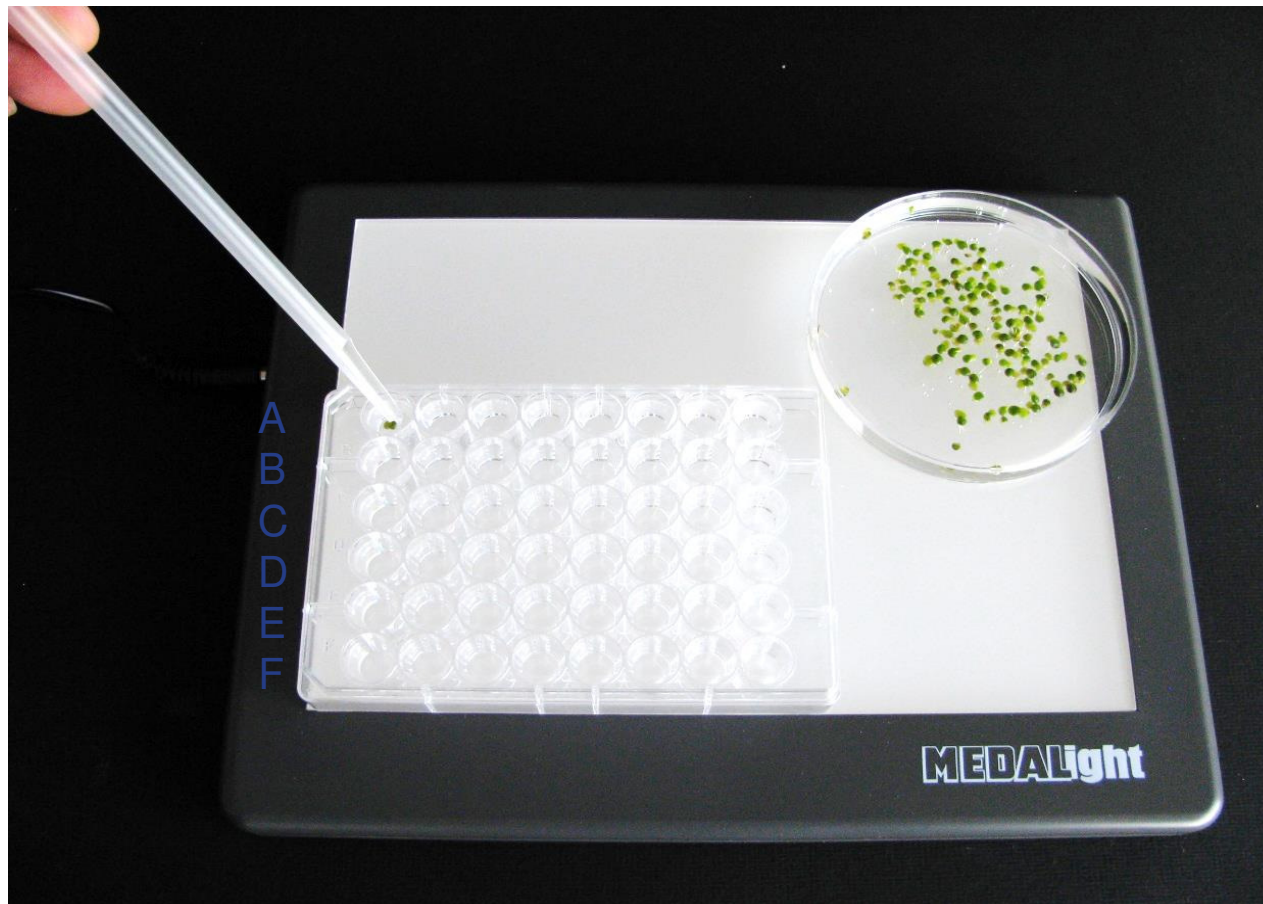


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**TRANSFER OF THE GERMINATED
TURIONS IN THE TEST CUPS**



GERMINATED TURIONS CAN EASILY BE RECOGNIZED BY THE PRESENCE
OF A SMALL FIRST FROND (on one side of the turion), WITH SMALL ROOTS



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- WITH THE AID OF THE SPATULA, TRANSFER “ONE” GERMINATED TURION
- INTO EACH CUP OF THE CONTROL ROW (= ROW A)
- REPEAT THIS PROCEDURE WITH THE OTHER ROWS, STARTING WITH ROW B DOWN TO ROW F

N.B. The transfers must be made “at random”, i.e. not starting with the turions which have the largest first fronds !

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TAKING OF A PHOTO OF THE MULTIWELL AT THE START OF THE TOXICITY TEST

TAKE A PHOTO OF THE MULTIWELL
WITH THE GERMINATED TURIONS
AT THE START OF THE 3 DAYS TEST
(= t₀h), AND TRANSFER THE
PHOTO TO A COMPUTER FILE



N.B. To obtain a photo with the best contrast between the turions and their first frond, it is advised to put the multiwell on a light table



**MULTIWELL PLATE WITH THE GERMINATED TURIONS AND
THEIR SMALL FIRST FRONDS AT t0h**

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INCUBATION OF THE TEST PLATE

- PUT THE COVER ON THE MULTIWELL PLATE.
- INCUBATE THE TEST PLATE FOR 72h AT 25 °C UNDER CONTINUOUS “TOP” ILLUMINATION OF 6 000 LUX

A



B



A : an incubator provided with illumination

B : an incubator with a LED Illumination Unit

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TAKING OF A PHOTO OF THE MULTIWELL AT THE END OF THE TOXICITY TEST

TAKE AGAIN A PHOTO OF THE
MULTIWELL WITH THE GROWN
FRONDS AT THE END OF THE 3 DAYS
TEST, (= t72h) AND TRANSFER THE
PHOTO TO A COMPUTER FILE



N.B. To obtain a photo with the best contrast between the turions and the fronds, it is advised to put the multiwell on a light table



MULTIWELL PLATE WITH THE GROWN FIRST FRONDS
TAKEN AFTER 3 DAYS INCUBATION (t72h)

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MEASUREMENT OF THE AREA OF THE FIRST FRONDS

AREA MEASUREMENTS OF THE SMALL FRONDS OF THE GERMINATED TURIONS ARE MADE IN EACH CUP OF THE MULTIWELL AT THE START OF THE TOXICITY TEST (t_{0h}) AND A SECOND TIME AT THE END OF THE 3 DAYS TOXICITY TEST (t_{72h}) ON THE GROWN FIRST FRONDS.

THE AREA MEASUREMENTS ARE MADE ON THE 2 SAVED PHOTOS OF THE MULTIWELL, WITH THE AID OF AN APPROPRIATE “IMAGE ANALYSIS” PROGRAM (e.g., “IMAGE J”).

N.B. Only the area of the “first frond” (= the largest frond of each turion) shall be measured !

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DATA TREATMENT

THE GROWTH OF THE DUCKWEED IS CALCULATED BY SUBTRACTING THE “INITIAL” SIZE OF THE FIRST FROND (t0h area) FROM THE “FINAL” SIZE OF THIS FROND (t72h area) , IN THE CONTROL AND IN THE DIFFERENT TOXICANT CONCENTRATIONS.

THE PERCENTAGE GROWTH INHIBITION OF THE DUCKWEEDS IN THE RESPECTIVE TOXICANT CONCENTRATIONS CAN THEN BE CALCULATED, FOLLOWED BY THE EVALUATION OF THE 72h EC50.

A data treatment programme has been worked out by Microbiotests Inc. and can be obtained on request.