MICROBIOTESTS

DAPHTOXKIT F

TEST PROCEDURE

MICROBIOTESTS

1

PREPARATION OF STANDARD FRESHWATER

- VOLUMETRIC FLASK (2 liter)
- VIALS WITH SOLUTIONS OF CONCENTRATED SALTS
- DISTILLED (or deionised) WATER

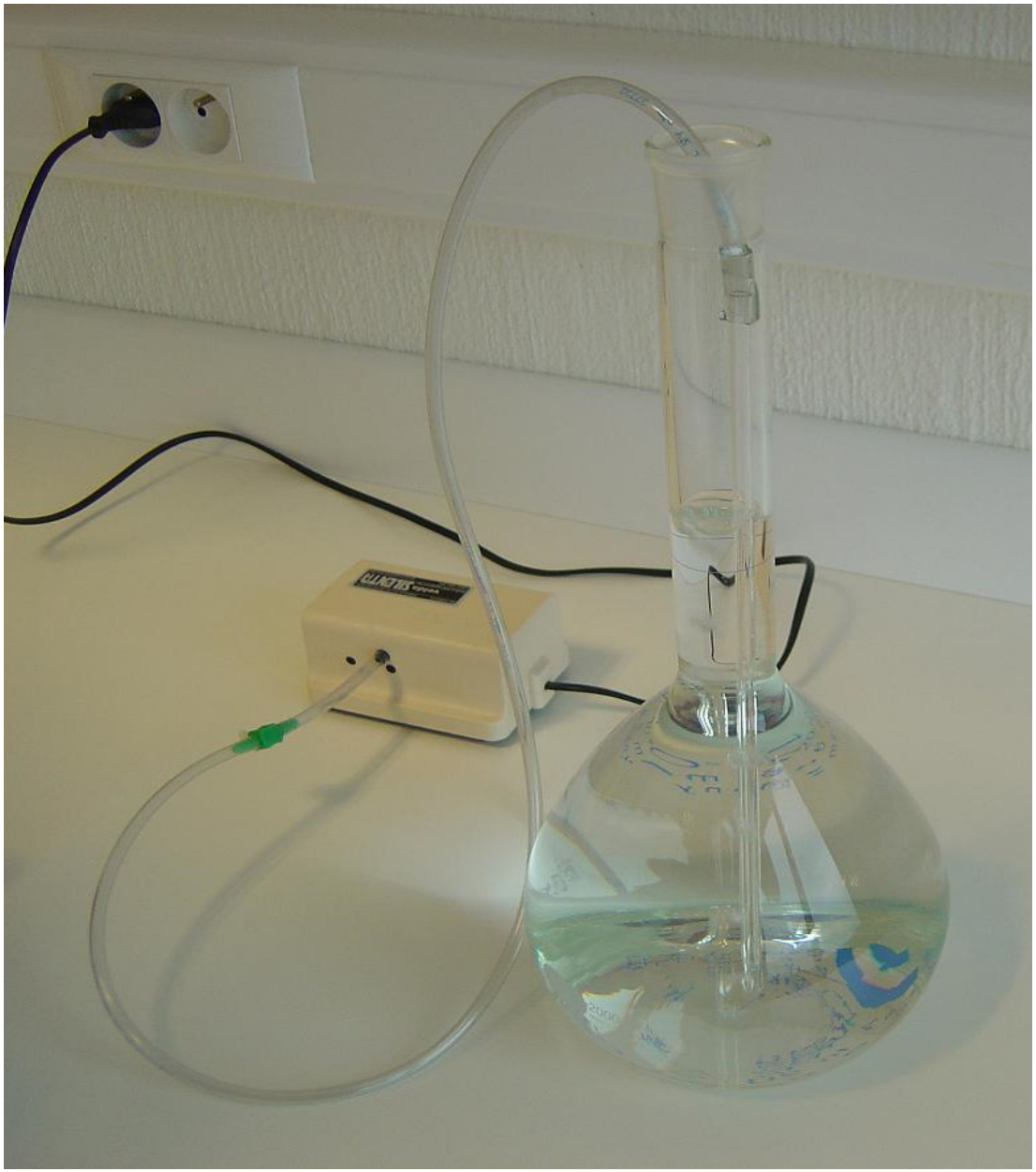
2

POUR THE 4 VIALS

WITH CONCENTRATED SALT SOLUTIONS

IN ± 1 LITER DISTILLED WATER,

IN THE 2 LITER VOLUMETRIC FLASK



3

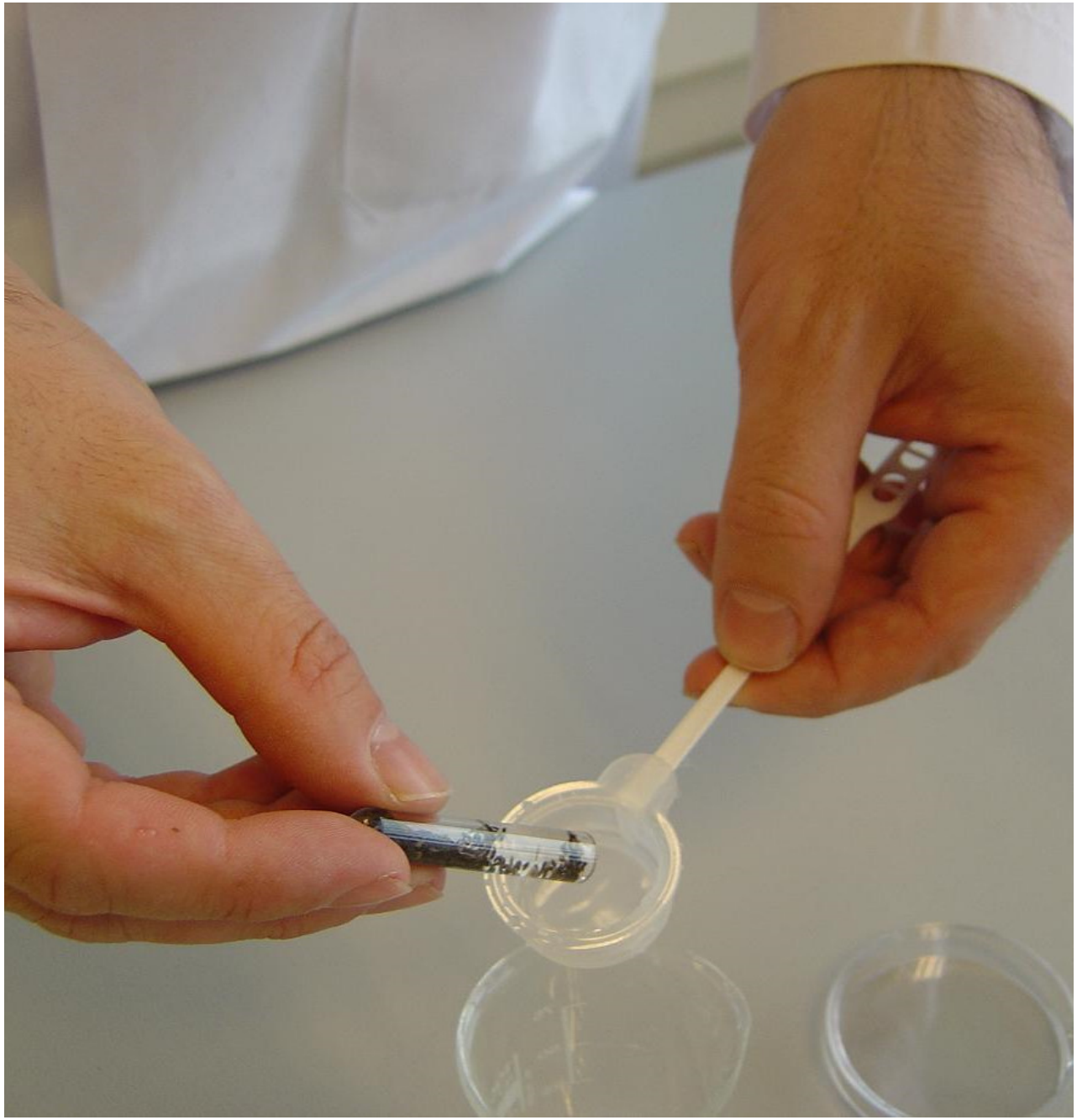
FILL THE FLASK TO THE 2 LITER MARK
AND AERATE FOR AT LEAST 15 MINUTES

MICROBIOTESTS

4

HATCHING OF THE EPHIPPIA

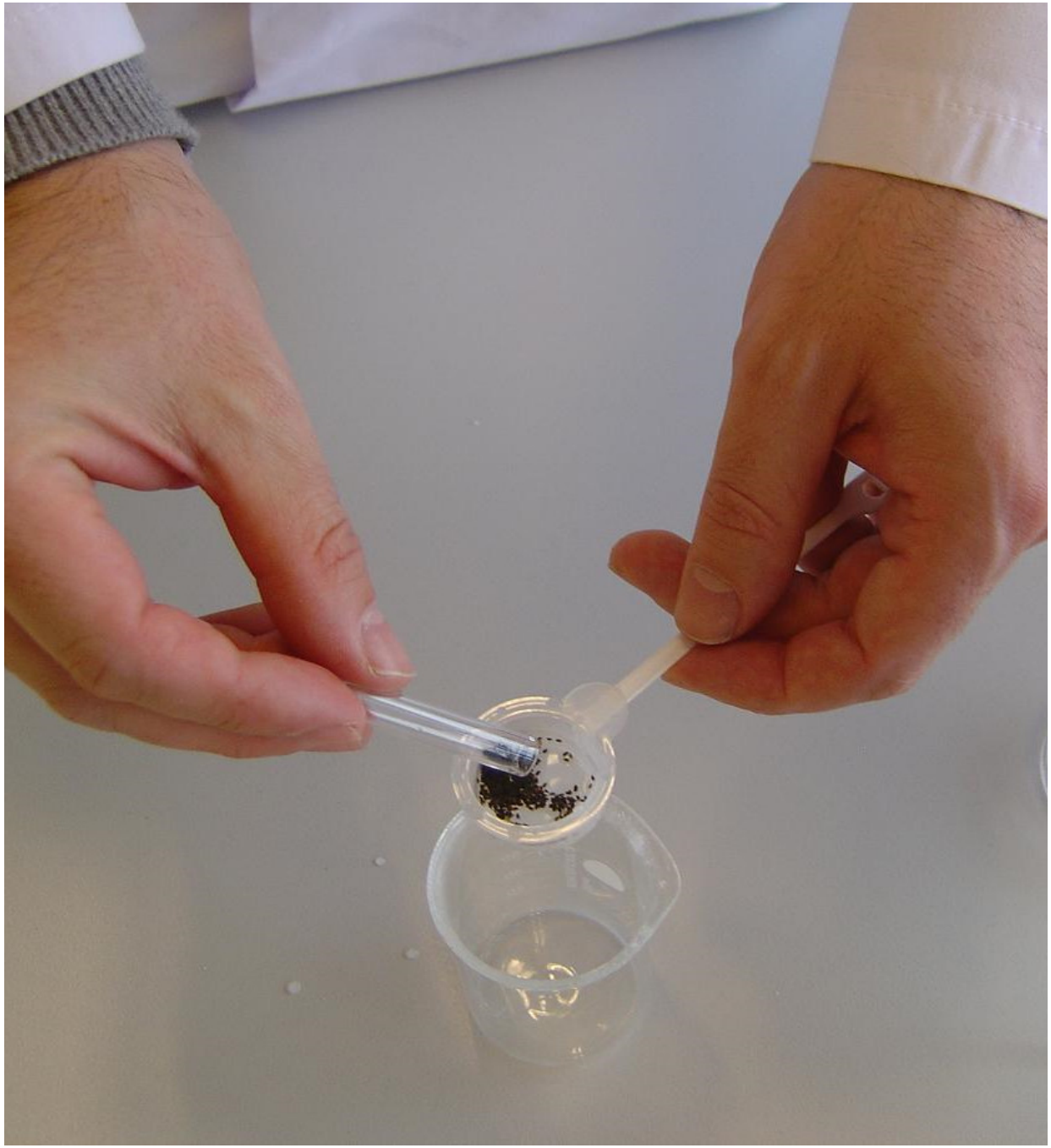
REMOVE THE ALUMINIUM FOIL FROM A TUBE WITH DAPHNIA EPHIPPIA



MICROBIOTESTS

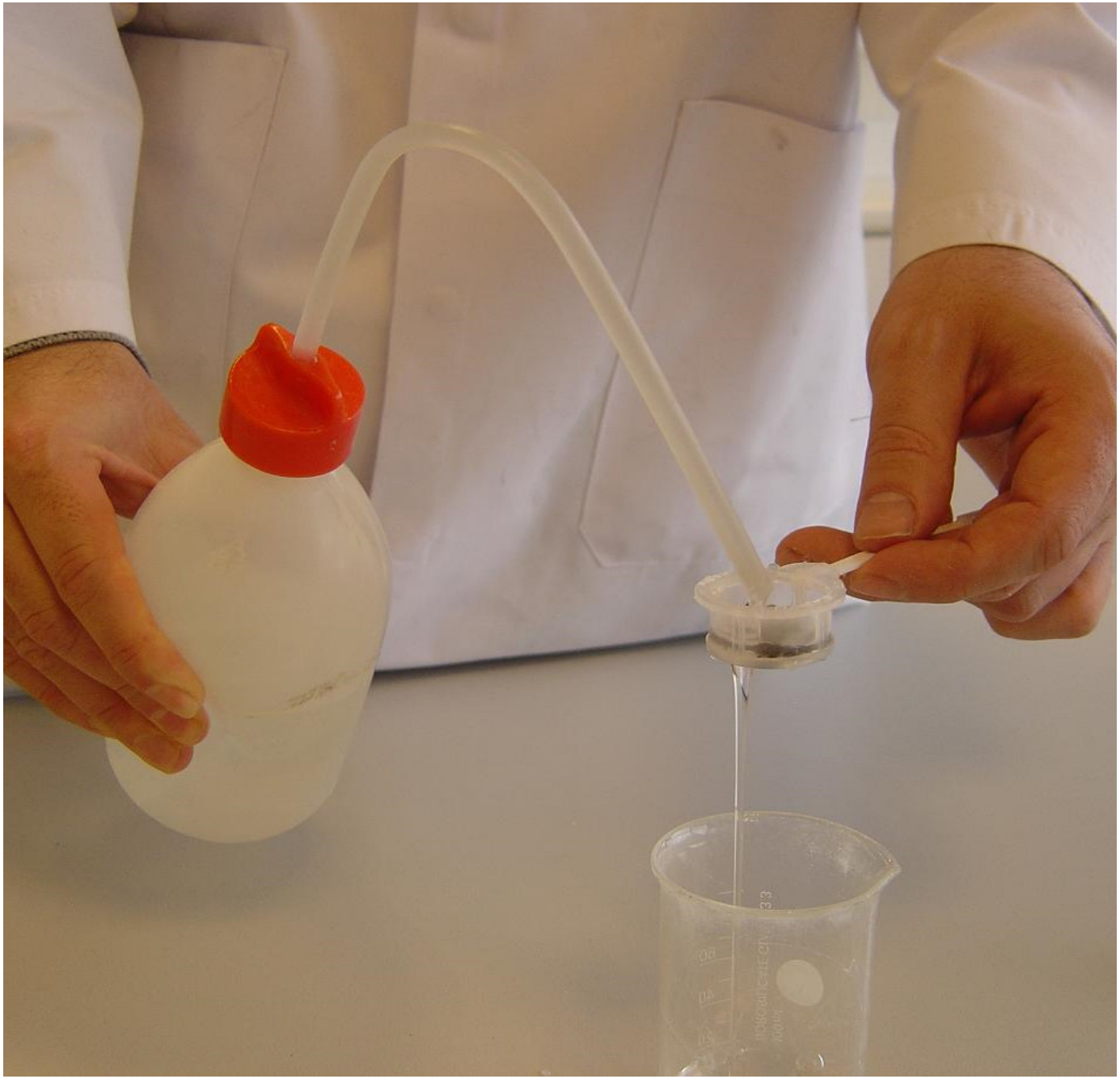
5

POUR THE CONTENTS
OF THE TUBE WITH EPHIPPIA
IN THE MICROSIEVE



6

MAKE SURE THAT
ALL THE EPHIPPIA
ARE TRANSFERRED
INTO THE MICROSIEVE



7

RINSE THE EPHIPPIA
THOROUGHLY
WITH TAP WATER

MICROBIOTESTS

8

TRANSFER THE EPHIPPIA
INTO THE
HATCHING PETRI DISH
IN STANDARD FRESHWATER

MICROBIOTESTS

9

INCUBATION OF THE EPHIPPIA

INCUBATE THE PETRI DISH

FOR 72h AT 20-22 °C

UNDER CONTINUOUS ILLUMINATION

OF 6 000 LUX

MICROBIOTESTS

10

PREPARATION OF THE TOXICANT DILUTIONS

For example :

TEST ON A EFFLUENT

IN 5 DILUTIONS (C1-C5)

+ ONE CONTROL

MICROBIOTESTS

11

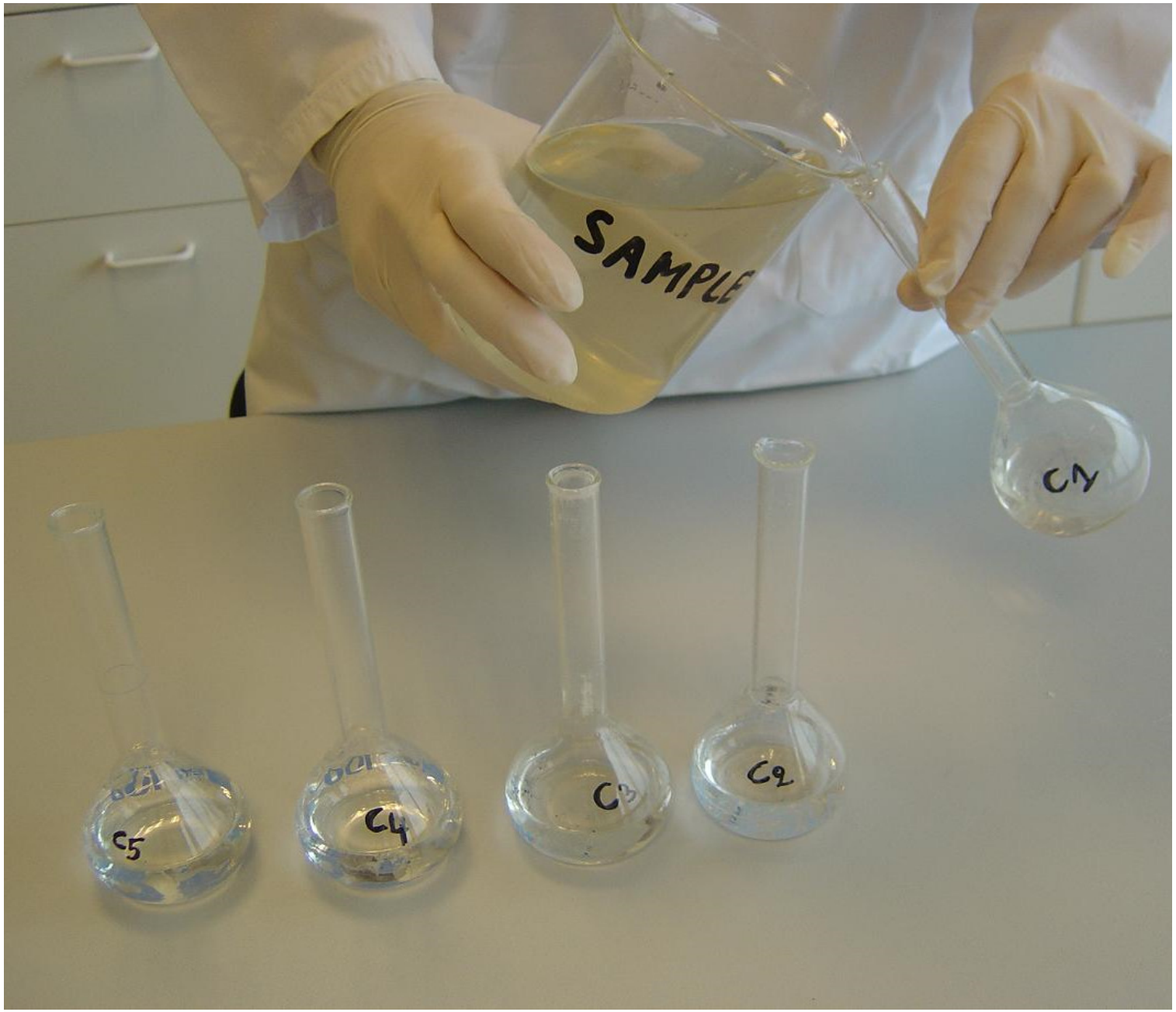
TRANSFER 50 ML

STANDARD FRESHWATER

INTO FLASKS

C2, C3, C4 AND C5

MICROBIOTESTS



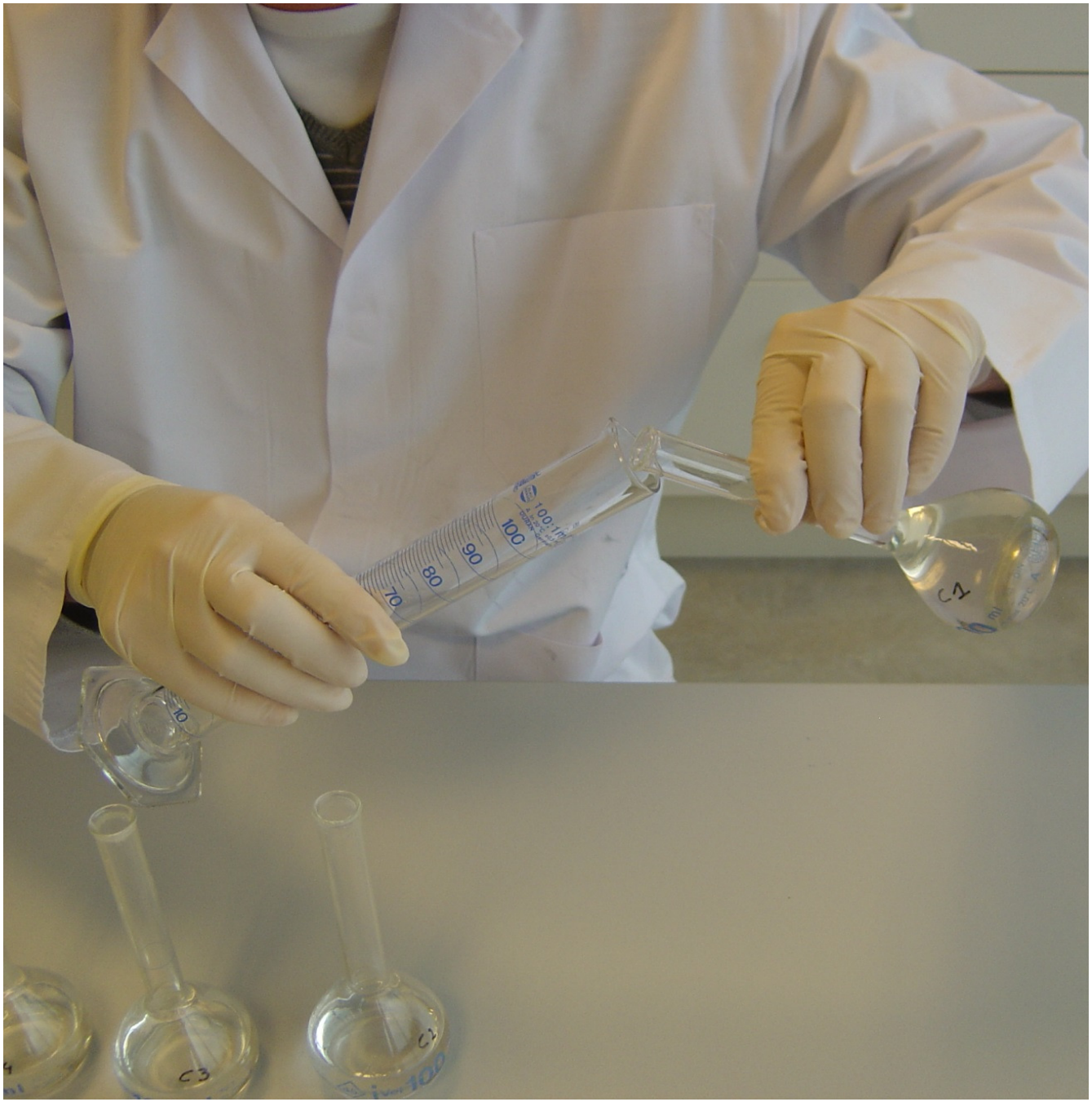
12

FILL FLASK C1

TO THE 100 ML MARK

WITH EFFLUENT

MICROBIOTESTS

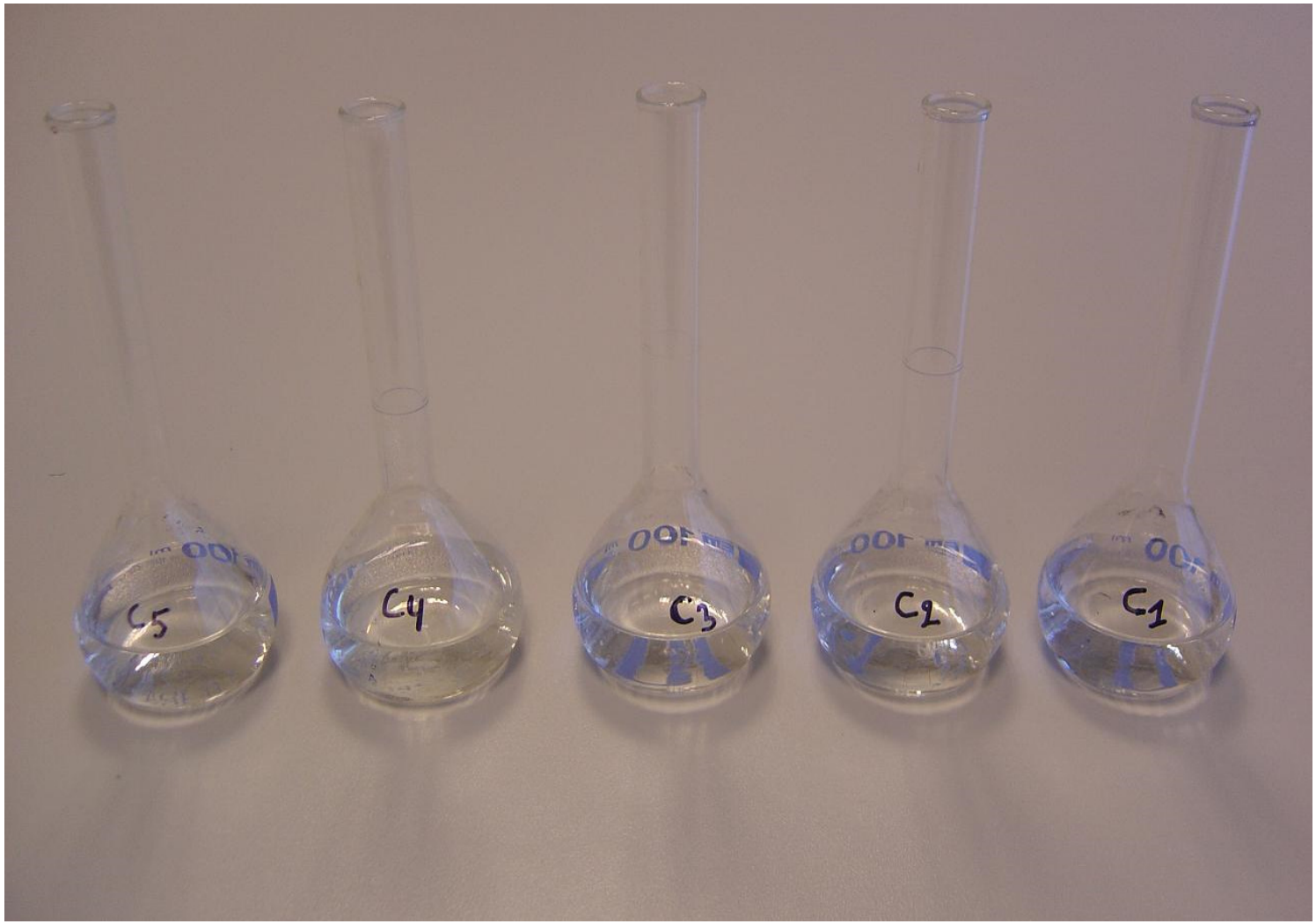


13

TRANSFER 50 ML EFFLUENT
FROM FLASK C1
INTO A GRADUATED CYLINDER.

14

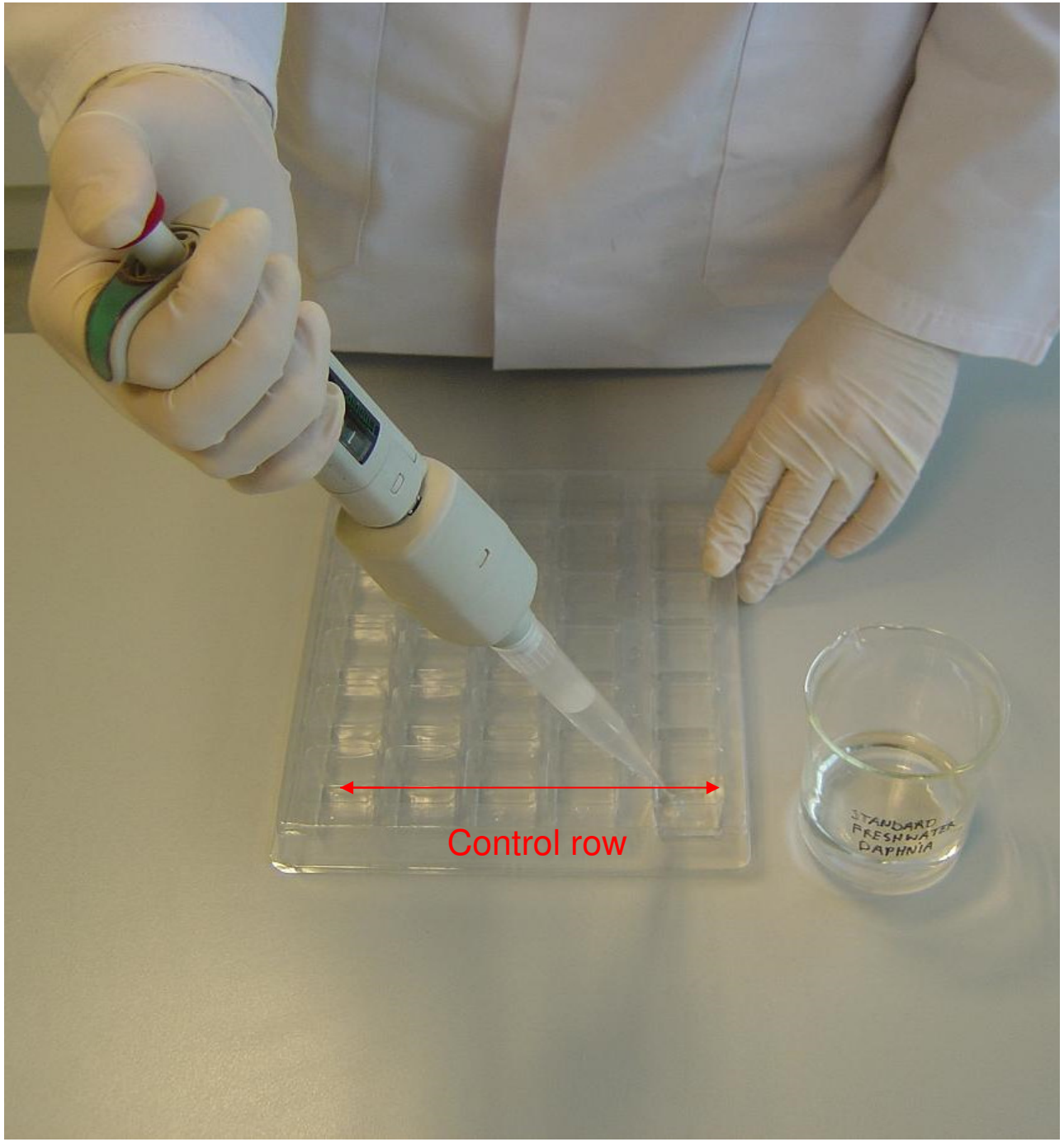
TRANSFER THE 50 ML EFFLUENT FROM THE GRADUATED CYLINDER TO FLASK C2 AND SHAKE THOROUGHLY



15

REPEAT THE FORMER DILUTION PROCEDURE FOR THE OTHER FLASKS (i.e. 50 ml from C2 to C3, etc).

MICROBIOTESTS



16

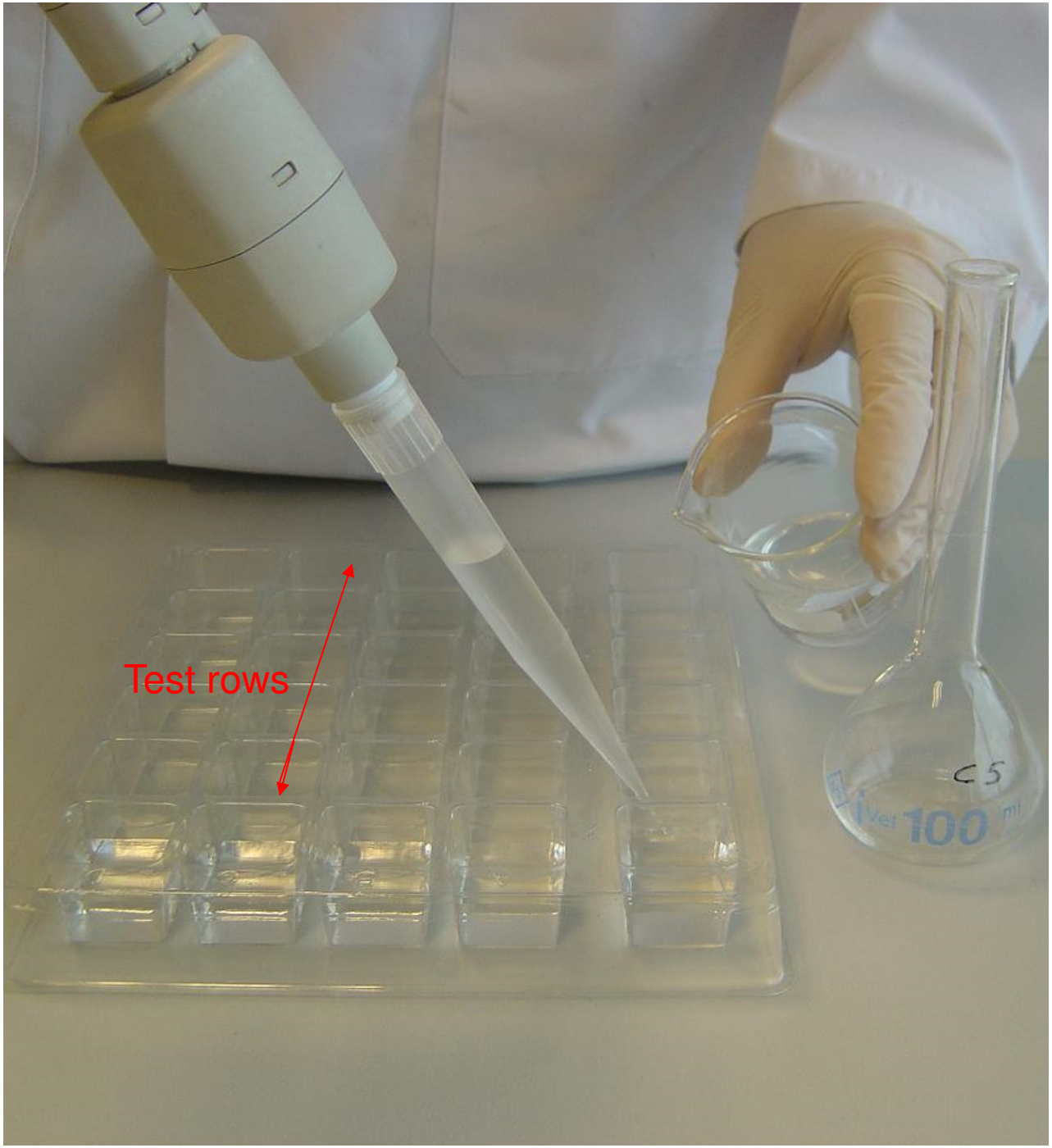
FILLING OF THE TEST PLATE :

TRANSFER 10 ML

STANDARD FRESHWATER

INTO EACH WELL

OF THE CONTROL ROW



17

TRANSFER 10 ML OF THE RESPECTIVE TOXICANT CONCENTRATIONS INTO EACH WELL OF THE CORRESPONDING ROWS FROM C5 TO C1

MICROBIOTESTS

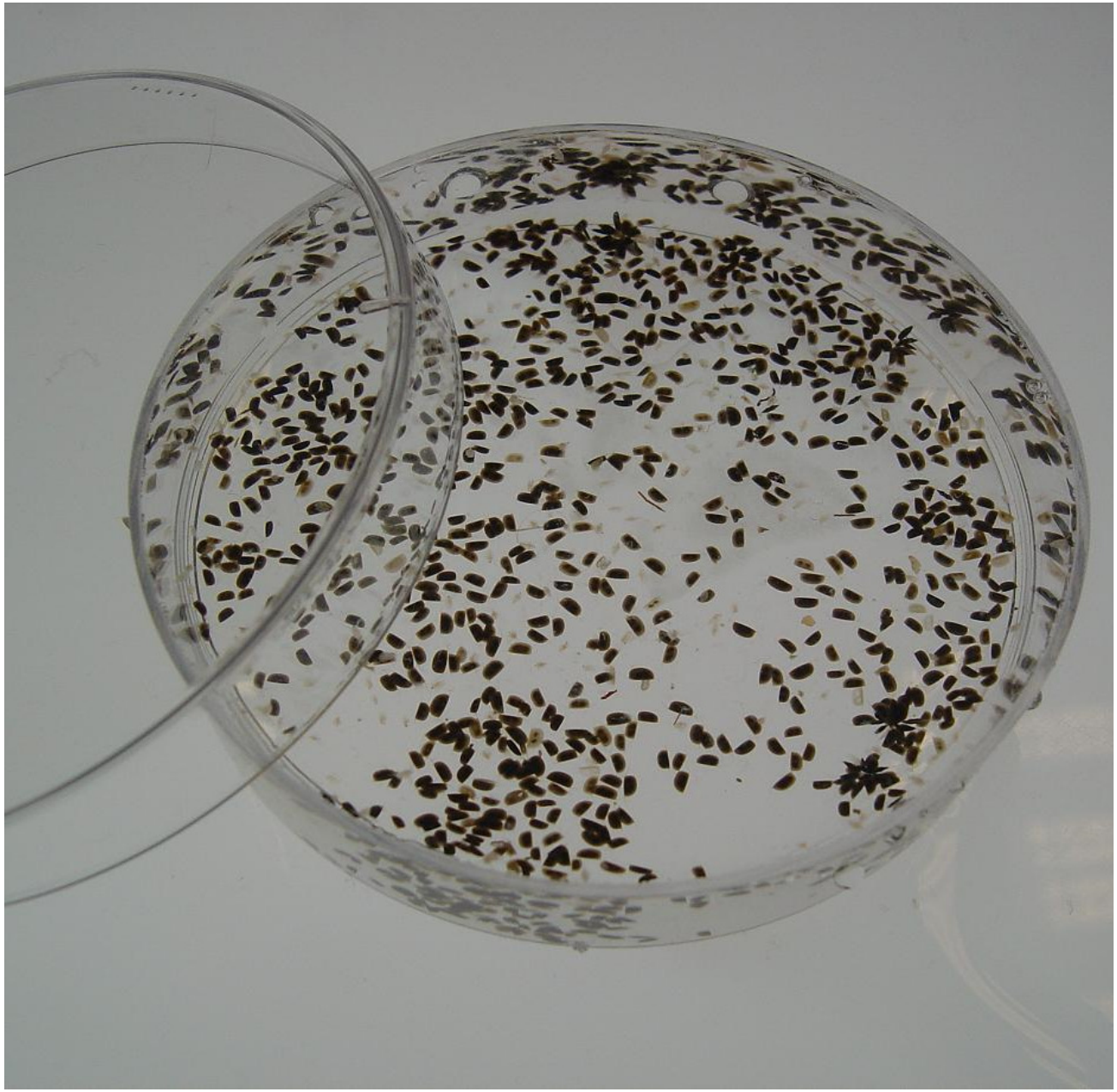
18

AFTER 72h TO 80h

INCUBATION

VERIFY THE HATCHING

OF THE DAPHNIA NEONATES



19

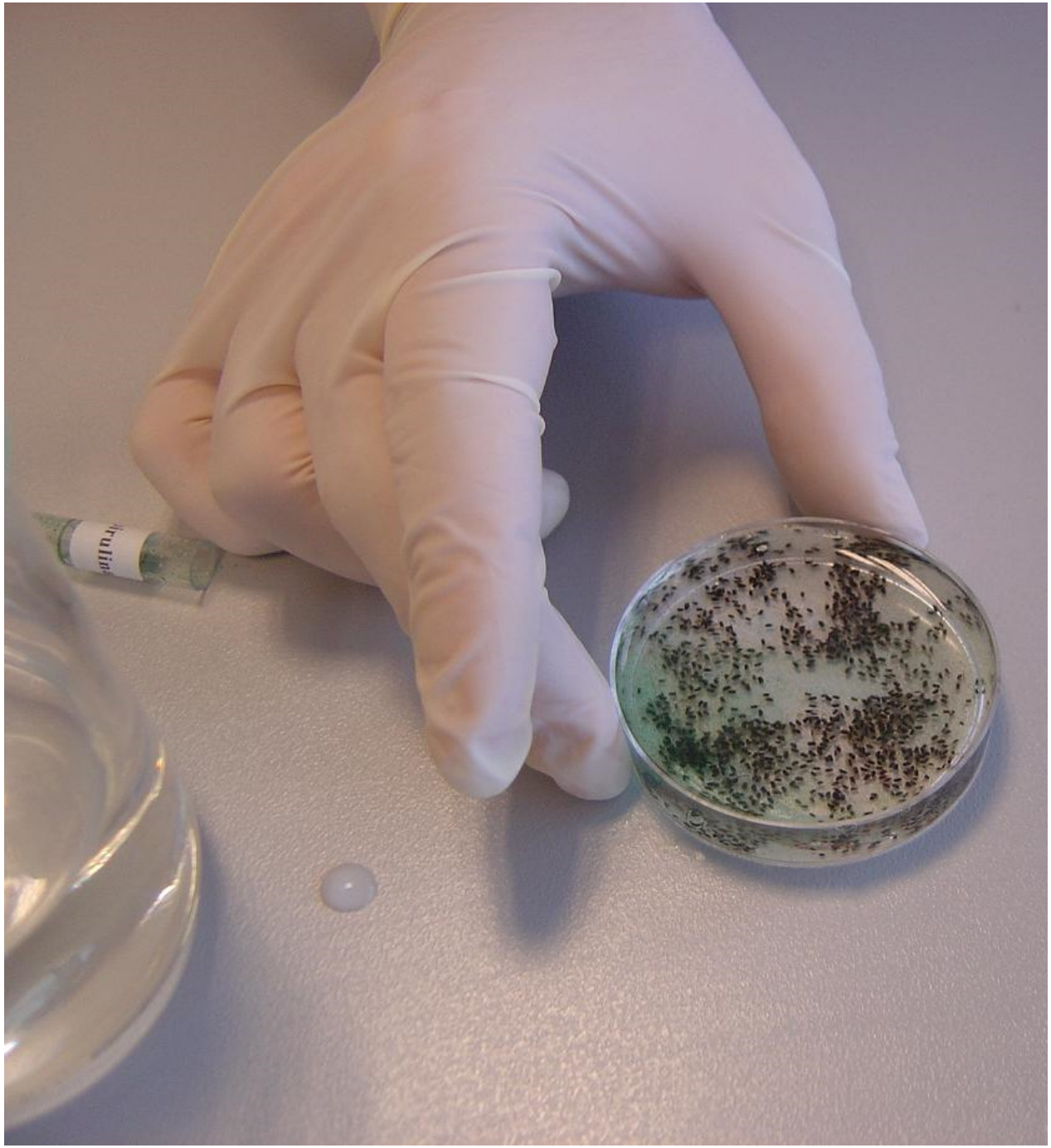
A MINIMUM OF 120 NEONATES ARE NEEDED TO PERFORM ONE TEST AND THE NEONATES SHOULD NOT BE OLDER THAN 24H

MICROBIOTESTS

20

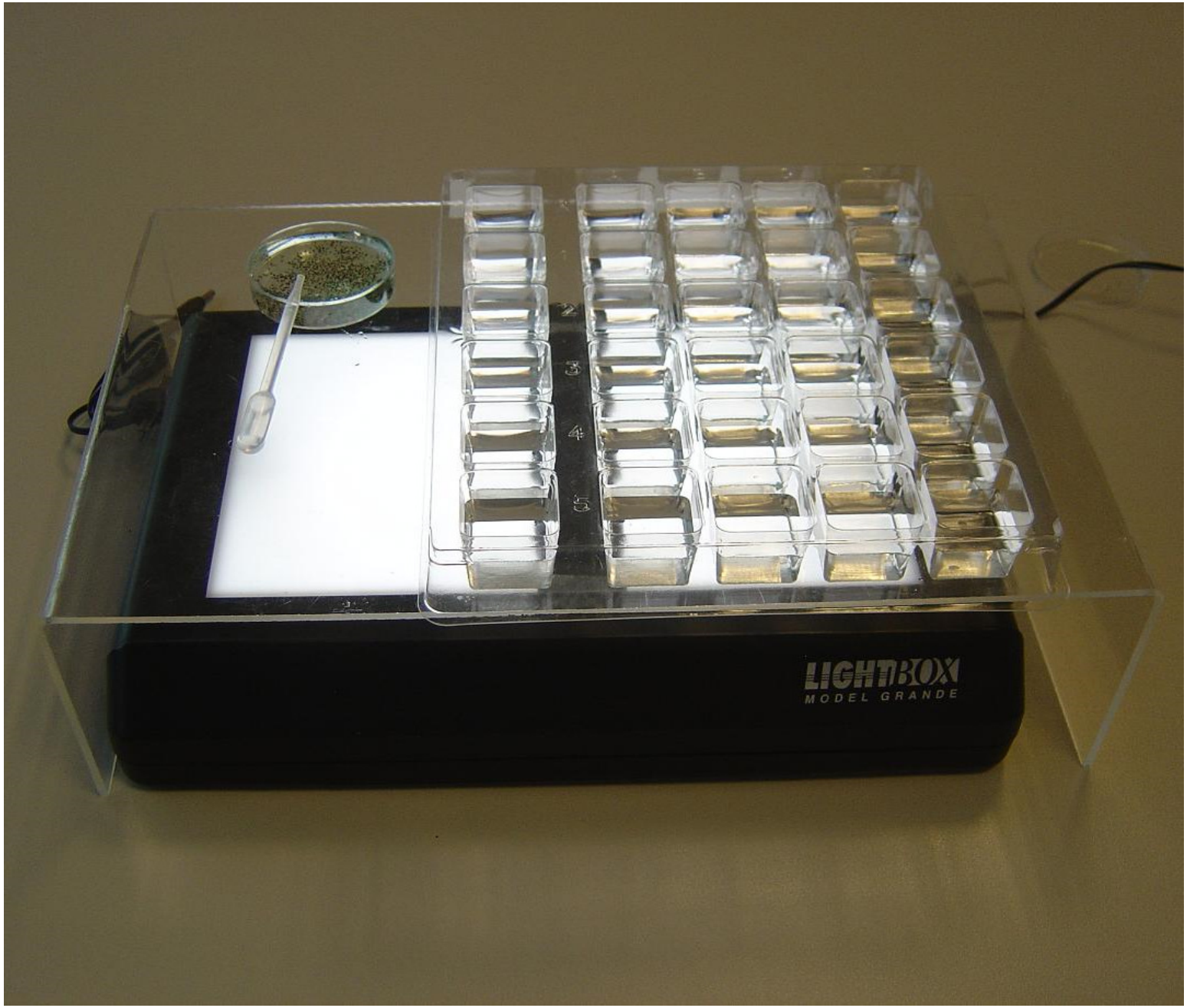
2h PRE-FEEDING
OF THE TEST ORGANISMS

TAKE ONE VIAL
WITH SPIRULINA POWDER
AND FILL IT
WITH STANDARD FRESHWATER



21

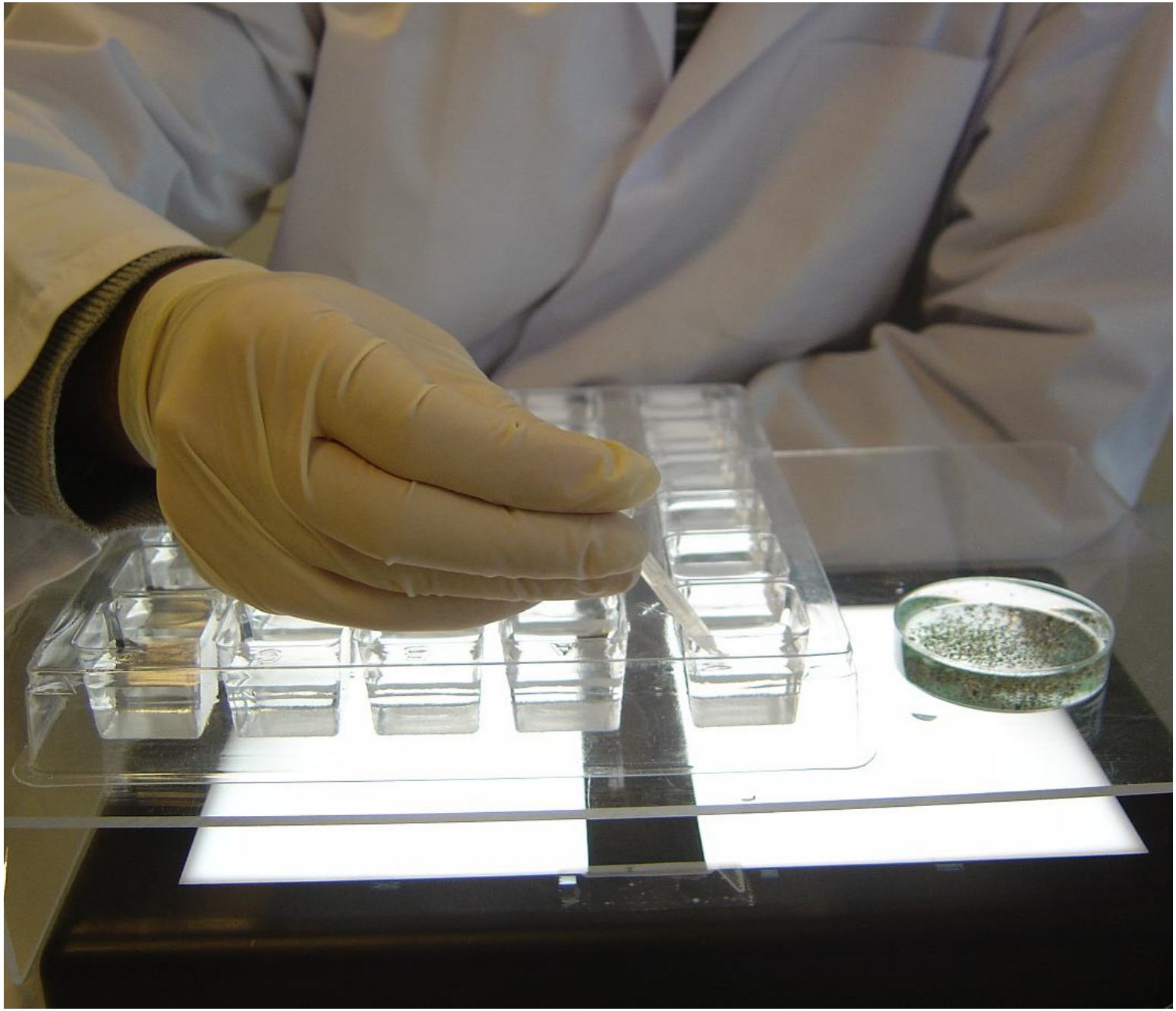
SHAKE THE VIAL
WITH THE SPIRULINA SUSPENSION,
POUR IT IN THE PETRI DISH
WITH THE DAPHNIA NEONATES
AND SWIRL THE PETRI DISH GENTLY



22

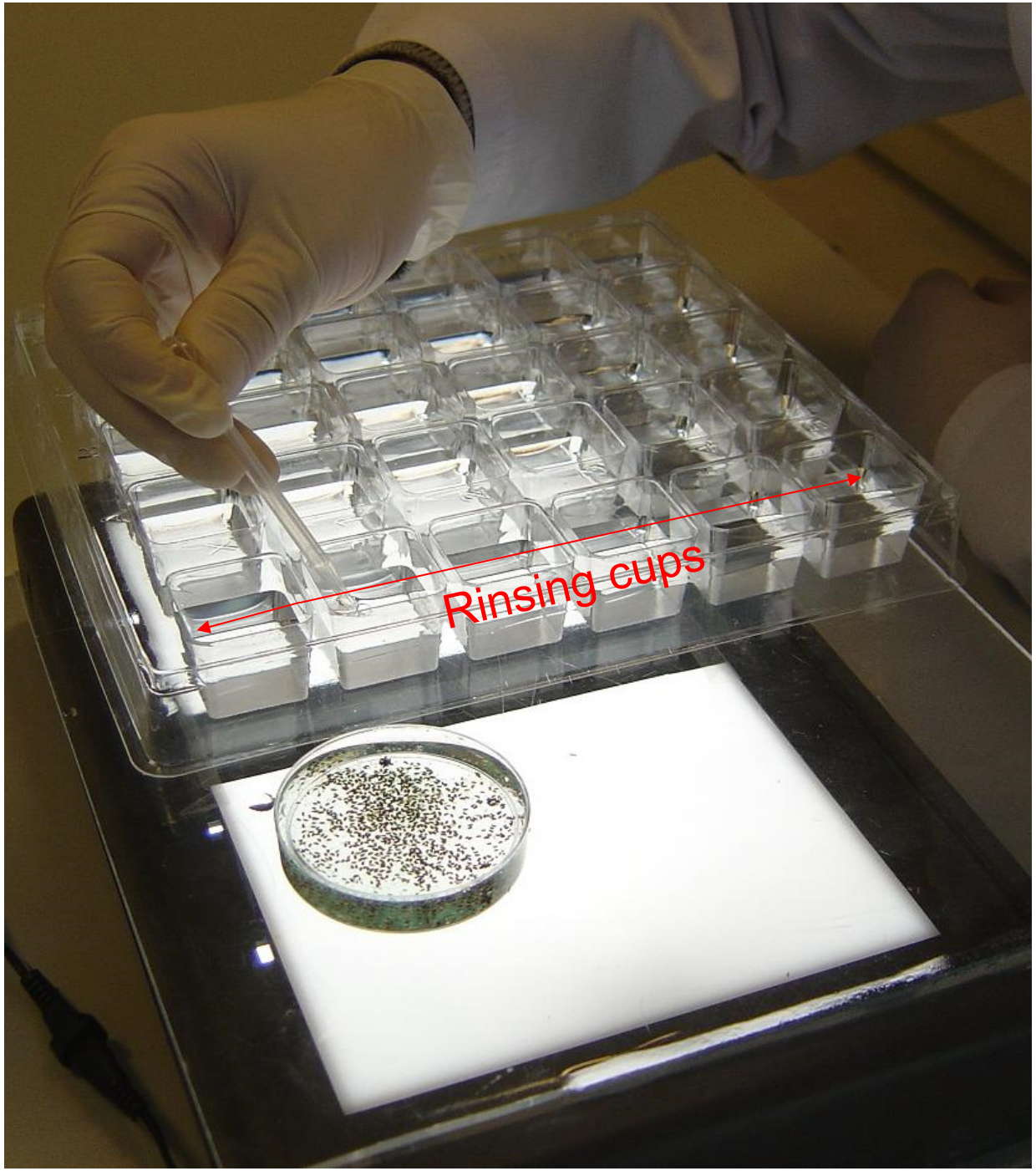
SET UP
OF THE TRANSFER
OF THE DAPHNIAS
TO THE TEST WELLS

- MULTIWELL PLATE
- LIGHT BOX WITH
  TRANSPARENT STAGE
- MICROPIPETTE



23

TRANSFER AT LEAST 20
(actively swimming)
DAPHNIAS INTO
THE RINSING CUP
OF THE CONTROL ROW,

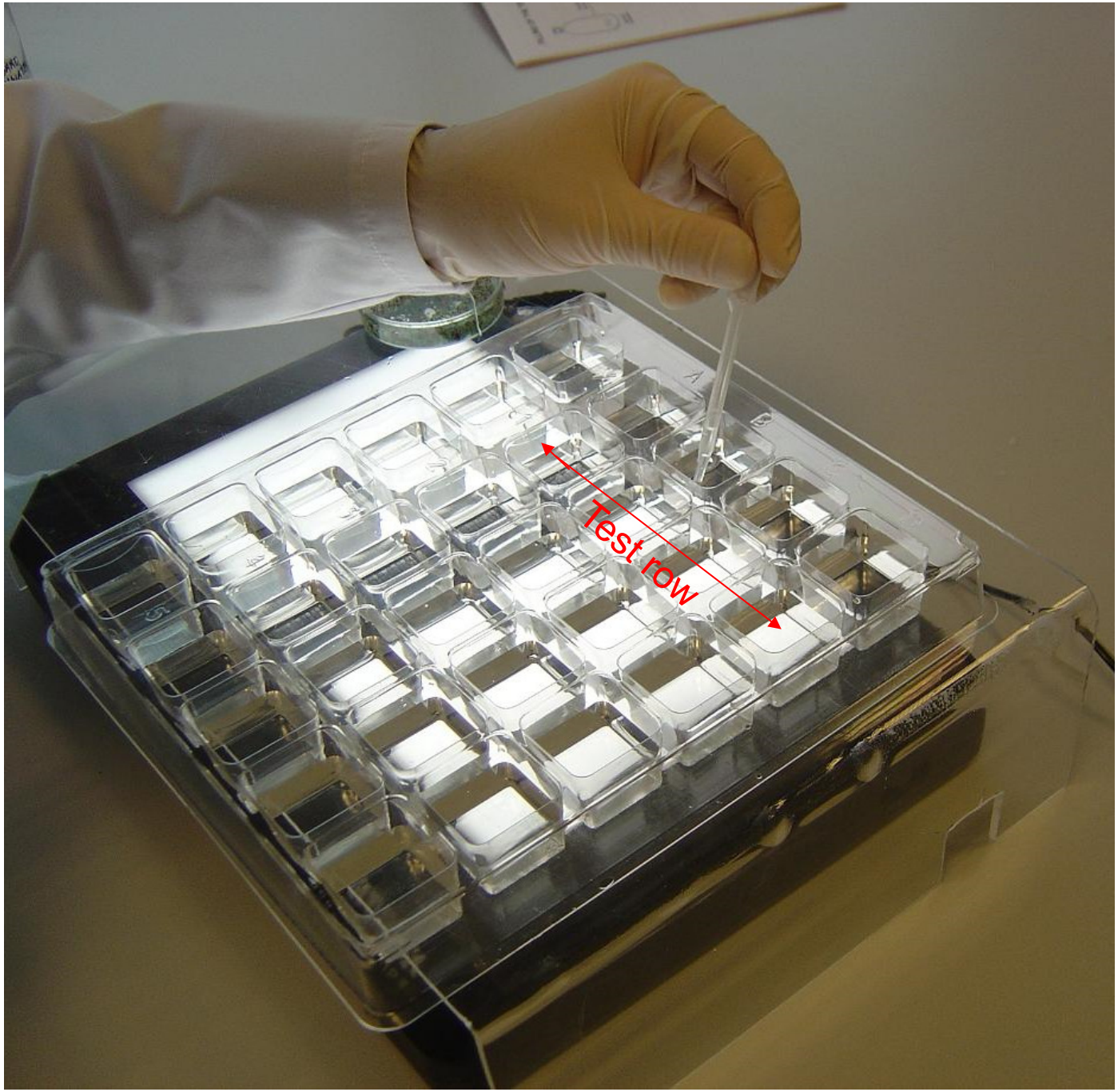


MICROBIOTESTS

24

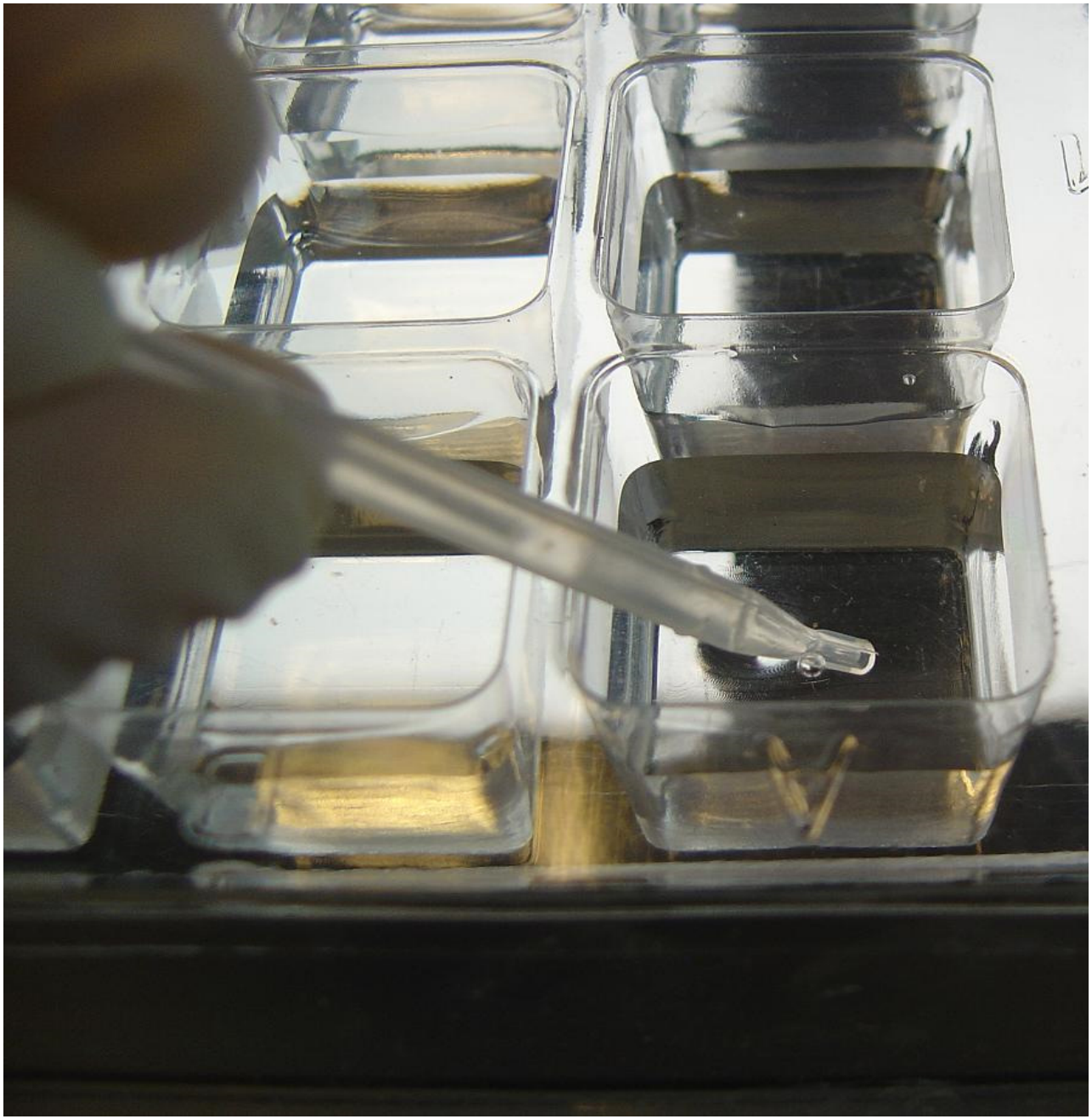
TRANSFER 20 DAPHNIAS (minimum)
TO ALL THE OTHER RINSING CUPS,
IN ORDER OF INCREASING
CONCENTRATIONS OF TOXICANT

MICROBIOTESTS



25

TRANSFER EXACTLY 5 DAPHNIAS FROM EACH RINSING WELL INTO THE 4 WELLS OF THE CORRESPONDING ROW

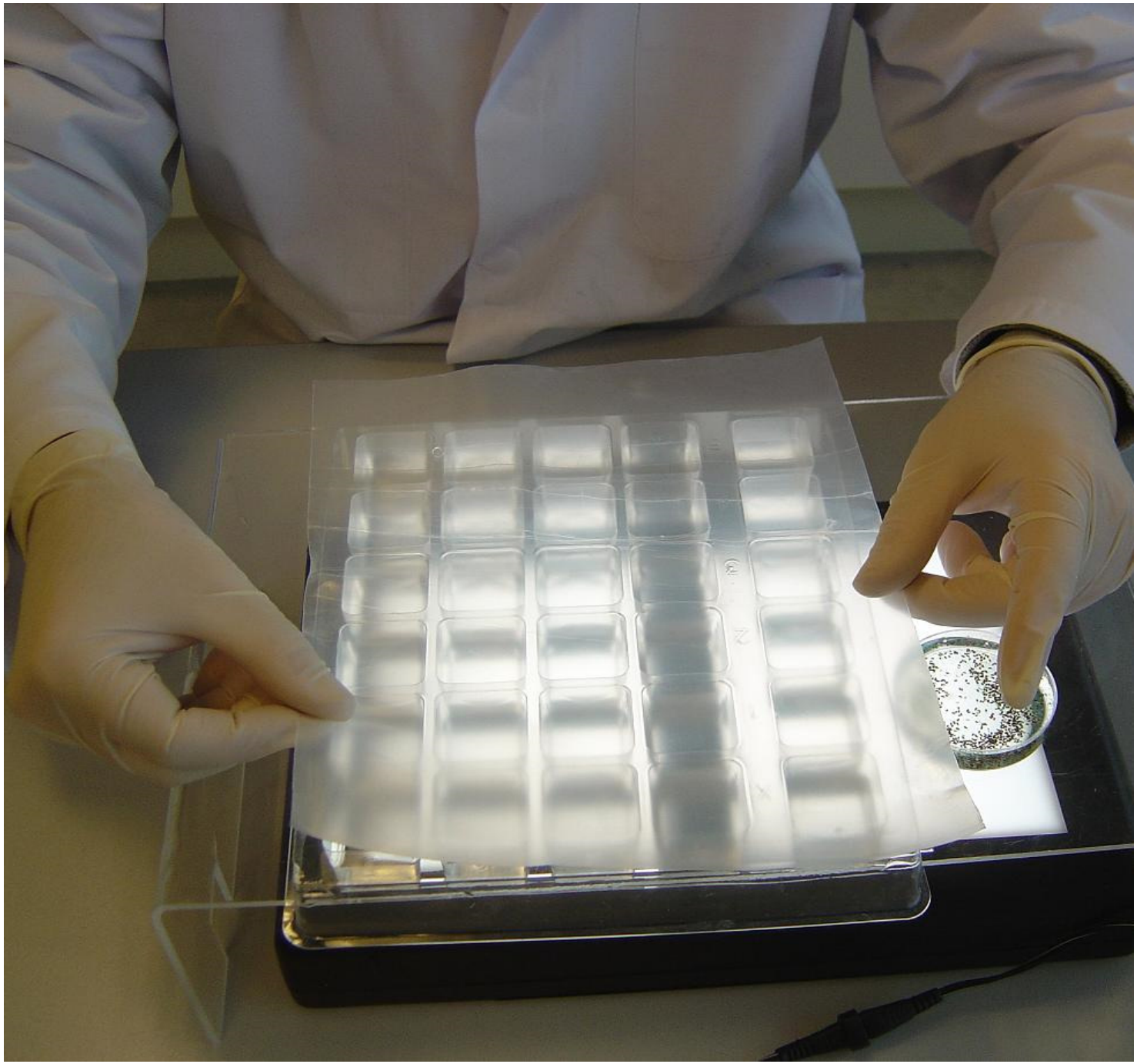


MICROBIOTESTS

26

TO AVOID SURFACE FLOATING
OF THE DAPHNIAS
DURING THE TRANSFER,
PUT THE TIP OF THE
MICROPIPETTE IN THE MEDIUM,
AND DO NOT DROP THE ORGANISMS
AT THE SURFACE OF THE MEDIUM

MICROBIOTESTS



27

PUT A PIECE OF PARAFILM
ON THE MULTIWELL PLATE
AND PUT THE COVER
ON TIGHTLY

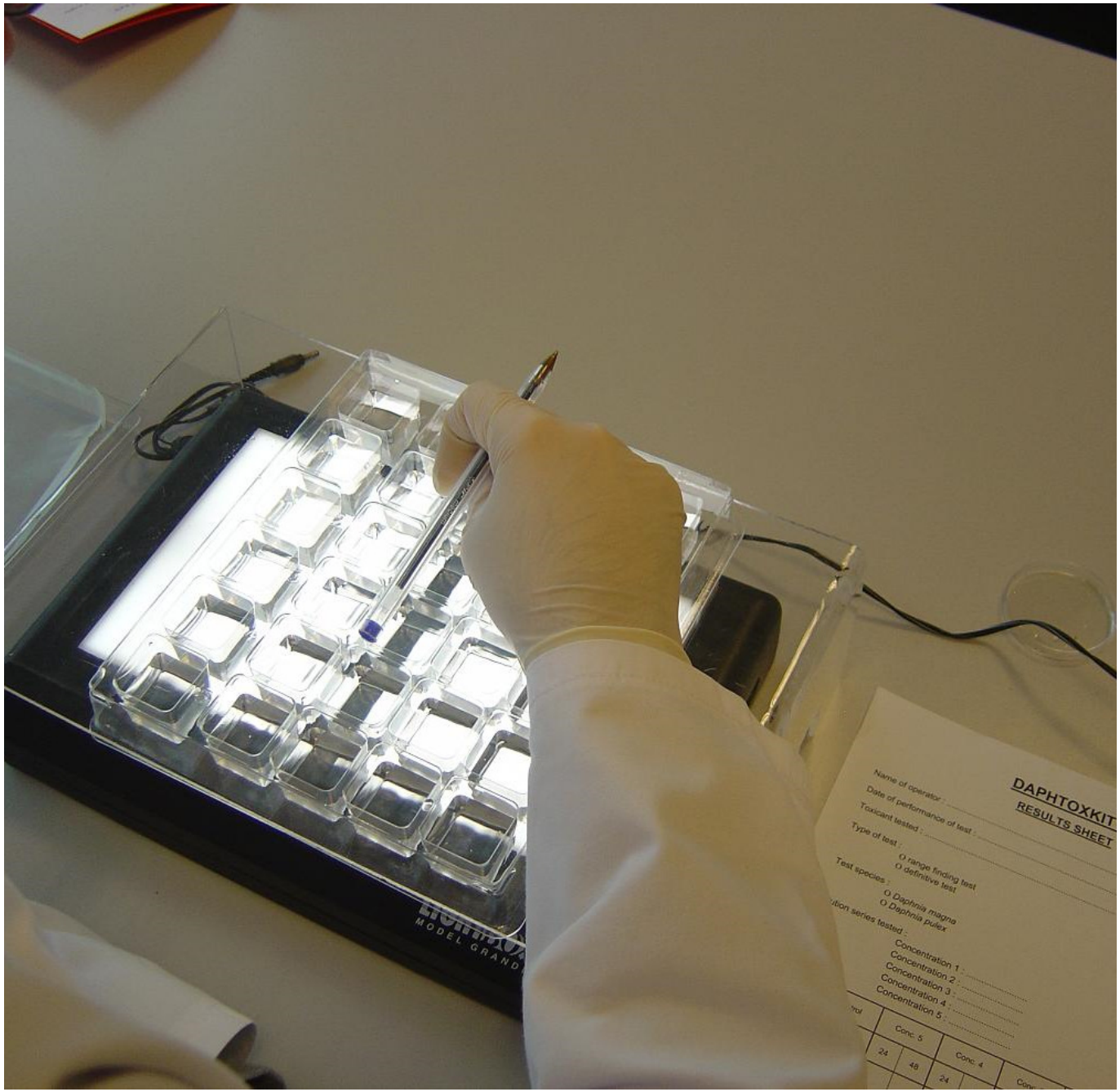
MICROBIOTESTS

28

INCUBATION OF THE TEST PLATE

INCUBATE THE MULTIWELL

AT 20 ± 2 ° C IN DARKNESS

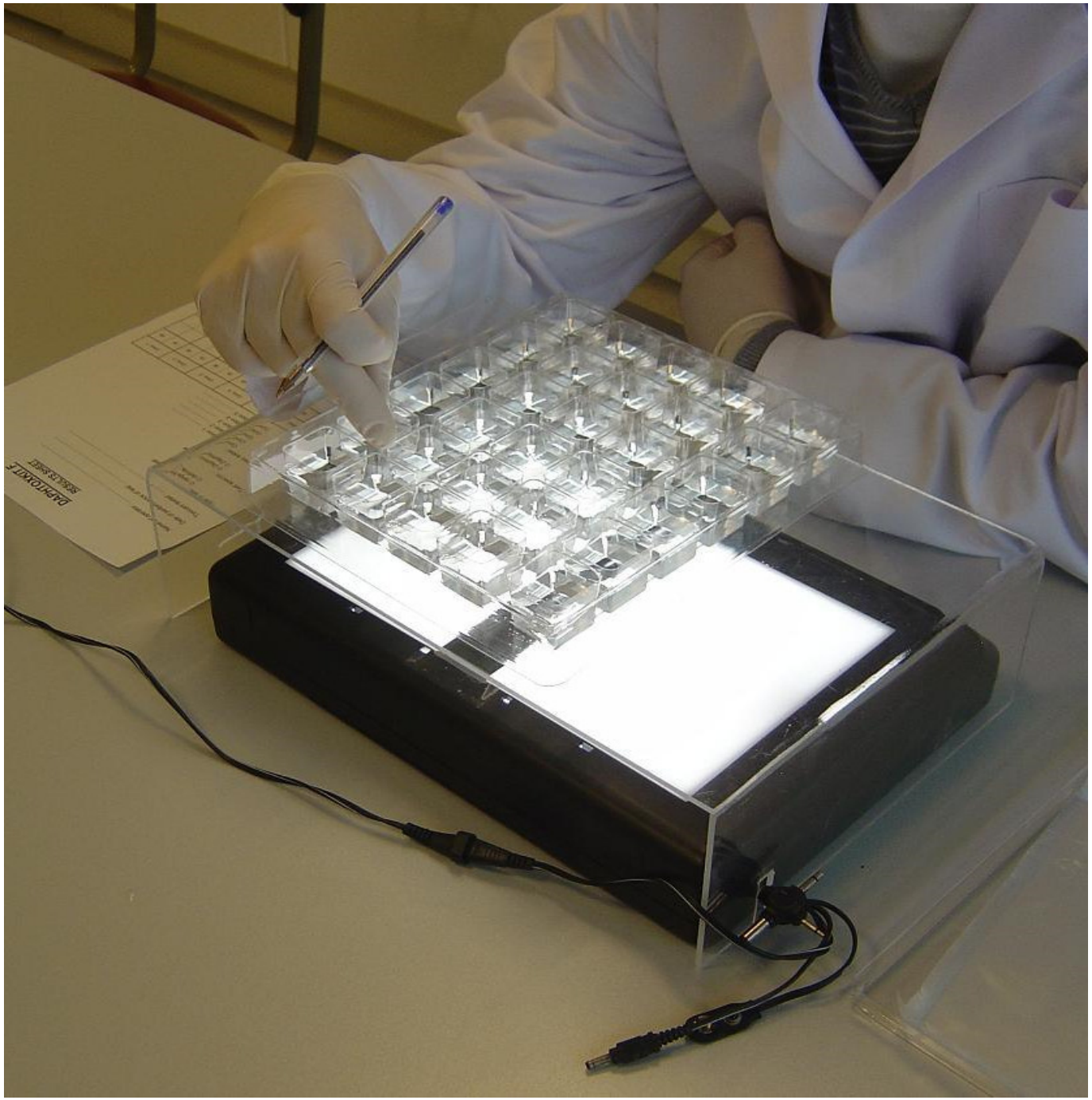


MICROBIOTESTS

29

SCORING OF THE RESULTS

AFTER 24h AND 48h INCUBATION
PUT THE MULTIWELL PLATE
ON THE LIGHT TABLE
AND RECORD THE NUMBER
OF DEAD AND
IMMOBILISED DAPHNIAS



30

DAPHNIAS WHICH ARE NOT
ABLE TO SWIM
AFTER GENTLE AGITATION
OF THE LIQUID FOR 15 SECONDS
SHALL BE CONSIDERED
AS IMMOBILISED
(even if they can still
move their antennae)

MICROBIOTESTS

31

- SCORE THE FIGURES ON THE RESULTS SHEET.

- CALCULATE THE TOTAL NUMBER OF DEAD AND IMMOBILE DAPHNIAS FOR EACH TOXICANT CONCENTRATION

- CALCULATE THE MEAN EFFECT AND THE PERCENTAGE EFFECT