Universitat Autònoma de Barcelona

TN 101.2

Protocol for lettuce batch culture experiments in the HPC1 of the MPP

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0	0	Final	01/03/10
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Date	Issue	Reason of the change	Modified paragraphs
01/03/2010	(0)	Creation	
02/09/2011	(1)	Update as per ESA comments and deviations encountered during batch tests (documented in TN-101.3)	See comments in Section 9

INDEX OF THE DOCUMENT

1. Scope

The present protocol describes the main steps to follow in order to perform the batch culture experiments with lettuce in the HPC1 compartment.

2. Reference and applicable documents

2.1 Applicable documents

AD1	19071/05/NL/CP	Memorandum of Understanding between MELiSSA Partners
AD2	MPP-QA-07-0001	MPP Quality Manual
AD3	MPP-QAP-08-0002	Quality Assurance Procedure for the control of non conformities
AD4	MPP-PID-10-4101-A6	HPC1 P&ID
AD5	TN96.3	Test protocol for lettuce cultivation
AD6	TN96.4	Protocols for sampling and analysis
AD7	MPP-OP-10-41010	Procedure for rockwool safe manipulation

2.2 Reference documents

RD1	TN 96.6 and 96.7	Functional Test Plan and Test Protocols with Schneider Controller
RD2	TN85.71	HPC1 User Manual

3. Definitions

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MELiSSA	Micro Ecological Life Support System Alternative
HPC	Higher Plant Chamber

4. Test items

4.1 Description (PID, technical drawings, user manual)

- Higher Plants Compartment HPC1
- Document MPP : reference MPP-PID-4100-01
- User manual TN85.71

4.2 Hazards induced by test item and safety measures to be taken

- Mechanical hazard (pump)

- Pressure hazard (compressed mixtures: 6barg)
- Chemical hazards (use of acid, base)

4.3 Instructions for operation

See User Manual

4.4 Instructions for maintenance

- See User manual; additionally:
  - Check that gaskets and valve membranes are not damaged
  - During water circulation, no leaks have to be observed below the trays nor the collector. If there are leaks, stop the test and retighten the junctions.

5. Recall of test sequence

- Phase 1 : Preparation of the chamber, including: cleaning of the chamber, air handling unit and liquid loop, and preparation of the culture campaign
- Phase 2 : Seedlings phase from planting to 8 days growth
- Phase 3 : Maturity phase from day 8 till day 28 and harvesting

6. Test protocol for phase 1

6.1 Features to be tested: functions, hardware, software

- Cleanability
- Leak tightness for liquid loop
- Leak tightness for gas loop

6.2 Success/failure criteria

- Clean aspect
- Absence of leaks
- For liquid loop: Absence of leak upon visual inspection.
- For gas loop : leak test

6.3 Resources for the test

6.3.1 Personnel: staff qualification and training needs

- MPP Technician trained to HPC1 operation
- MPP Higher Plant Scientist educated to HPC1 operation and results analysis

The table in Appendix 1 should be filled and attached to the Record sheet.

6.3.2 Personnel Protective Equipments

- Safety shoes
- Laboratory coat
- Dust mask
- Gloves and goggles
- Sun goggles if working in presence of full lighting inside the HPC1
- Shoe covers when working inside the chamber

6.3.3 Hardware: instruments, specific part, hardware for software operation, calibration certificates

- Millwright work (screwdriver, pipe-wrench, …)
- No specific tools are needed
- All sensors are calibrated with certificates

The table in Appendix 2 should be filled and attached to the Record sheet.

6.3.4 Software: verification of software, backup needs

- All acquisitions have been validated
- PLC is connected to the acquisition server

6.3.5 Test conditions

There is no testing in this phase ; the chamber is prepared for the culture, mainly at atmospheric pressure and temperature.

6.4 Measurement and data sampling

6.4.1 Data logfile

HPC1 28 days test – phase 1-01032010.dat

The acquired parameters are at least the following ones :

Tag Updated 05/05/09	Inputs	Outputs	Description
ZS_4100_01	Y		Upper Exterior Air Lock Door Contact - Side A
ZS_4100_02	Y		Lower Exterior Air Lock Door Contact - Side A
FAN_4105_01_MV		Y	Operation of Light Loft Fan A
ZS_4101_01	Y		Upper Exterior Air Lock Door Contact - Side C

ZS_4101_02	Y		Lower Exterior Air Lock Door Contact - Side C (NC)
FAN_4105_02_MV		Y	Operation of Light Loft Fan B
FAN_4105_03_MV		Y	Operation of Light Loft Fan C
GP_4110_01_MV		Y	Condensate pump relay
PT_4102_01	Y		Pressure sensor for airlock A
FSL_4105_01	Y		Flow/Noflow of Light Loft Fan A
SV_4102_01_MV		Y	Solenoid Valve for injection of pressurized air into airlock A
SV_4102_02_MV		Y	Airlock A ventilation Solenoid Valve
PT_4103_01	Y		Pressure sensor for airlock C --> Reaffected to External Pressure
FSL_4105_02	Y		Flow/Noflow of Light Loft Fan B
SV_4103_01_MV		Y	Solenoid Valve for injection of pressurized air into airlock C
SV_4103_02_MV		Y	Airlock C ventilation Solenoid Valve
IY_4104_01_MV		Y	Turn On/Off lamps - A
RT_4104_01	Y		PAR Sensor - A
RT_4104_02	Y		PAR Sensor - B
RT_4104_03	Y		PAR Sensor - C
FSL_4105_03	Y		Flow/Noflow of Light Loft Fan C
PS_4102_01	Y		Airlock A pressure switch
PS_4103_01	Y		Airlock C pressure switch
IY_4104_02_MV		Y	Turn On/Off lamps - B
IY_4104_03_MV		Y	Turn On/Off lamps - C
GP_4106_01_MV		Y	Main irrigation Pump P2001
TT_4105_01	Y		Light Loft Temperature sensor A
TT_4105_02	Y		Light Loft Temperature sensor B
TT_4105_03	Y		Light Loft Temperature sensor C
SV_4107_01_MV		Y	Acid Tank Valve
AT_4112_01	Y		Humidity A1 associated with temp A1
AT_4107_01	Y		pH sensor

No Measurement		Y	Led Indicator when door is open - Side A (connected to PLC Cabinet)
SV_4107_02_MV		Y	Base Tank Valve
LSL_4107_01	Y		Acid Tank Level
LSL_4107_02	Y		Base Tank Valve
AT_4108_01	Y		Electrical Conductivity of nutrient
Suppressed		Y	Nutrient cooling line valve
No Measurement		Y	Led Indicator when door is open - Side C (connected to PLC Cabinet)
LSL_4108_01	Y		Level sensor Stock A
LSL_4108_02	Y		Level sensor Stock B
TT_4109_01	Y		Temperature sensor for solution reservoir
SV_4108_01_MV		Y	Stock A inject Valve
LSH_4110_01	Y		High Level sensor for reservoir tank
LSL_4110_01	Y		Low Level sensor for reservoir tank
LSH_4110_02	Y		High Level sensor for condensate tank
LSL_4110_02	Y		Low Level sensor for condensate tank
SV_4108_02_MV		Y	Stock B inject Valve
MVFD_4111_01_MV		Y	Air circulation fan with VFD / not connected
TT_4112_01	Y		Temperature A1 associated with humidity
BLWR_4111_01_MV		Y	Blower
GP_4112_02_MV (NEW)		Y	Hot water Circulation pump
GP_4112_01_MV (NEW)		Y	Chilled water Circulation pump
FT_4111_01	Y		Air velocity sensor
TT_4112_04	Y		Temperature A2
TT_4112_05	Y		Temperature A3
TT_4112_06	Y		Temperature A4 --> Reaffected to External T
TT_4112_02	Y		Temperature B1 associated with humidity
TT_4112_07	Y		Temperature B2

TT_4112_08	Y		Temperature B3
TT_4112_09	Y		Temperature B4
TT_4112_03	Y		Temperature C1 associated with humidity
TT_4112_10	Y		Temperature C2
TT_4112_11	Y		Temperature C3
TT_4112_16	Y		Heating coil surface temperature
TT_4112_20 (NEW)	Y		Outlet Air (TO BE CONFIRMED), hot exchanger
TT_4112_13	Y		Temperature for facility chilled water
TT_4112_14	Y		Temperature for facility hot water line
TT_4112_17	Y		Chilled Exit temperature
TT_4112_18	Y		Hot Exit temperature
TT_4112_19 (NEW)	Y		Outlet Air (TO BE CONFIRMED), chilled exchanger
TT_4112_12	Y		Temperature C4 --> Reaffected to External T
TT_4112_15	Y		Chilled coil surface temperature
TT_4112_21 (NEW)	Y		Inlet water Chilled Exchanger
TT_4112_22 (NEW)	Y		Inlet water Hot Exchanger
AT_4112_02	Y		Humidity B1 associated with temp B1
AT_4112_03	Y		Humidity C1 associated with temp C1
AT_4113_01	Y		CO2 Analyser
S3CV_4112_01_MV		Y	Chilled Water Control Valve
S3CV_4112_02_MV		Y	Hot Water Control Valve
AT_4113_02	Y		O2 Analyser
FC_4113_01_SP		Y	CO2 Mass Flow set point
PT_4114_01	Y		Growing Area Pressure
FC_4113_01	Y		CO2 Mass Flow
SV_4113_01_MV		Y	CO2 injection line. Solenoid
FS_4114_01 (NEW)	Y		Flowswitch

FT_4106_01	Y		Outlet nutrient flow sensor
PS_4114_01 (NEW)			Vent Detect
TT_4115_01	Y		Ambient temperature : now assured by TT_4112_06 and 4112_12
PT_4115_01	Y		Ambient pressure (NOT CONNECTED)

PT sensors from mapping 01 to 06

6.4.2 Special requirements if any (frequency, duration, synchronization)

Every minute for all instrumentation except for manual phases.

6.5 Reporting of status for a test

The test sequence is performed by MPP personnel, under the expertise and advice of MPP Engineer. The final status of the test (passed/fail) is decided at the end of the test in agreement between MPP personnel and MPP management.

6.6 Deviations and non conformances

In case the test sequence cannot be performed as planned or some results are out of their expected range, a deviation is opened and appended to the test record (see Appendix 3). The process to fill out the deviation form is identical to the one to fill out the NCR as per the Quality Assurance Procedure for the control of non conformities MPP-QAP-08-0002.

This deviation is discussed among MPP, and together with ESA for high criticality deviations, in order to decide how to address it. If necessary, on the basis of a given deviation, MPP can decide to open a NCR as planned by the Quality Manual and the Quality Assurance Procedure for the control of non conformities MPP-QAP-08-0002.

The discussion of all deviations is made before the final decision of the status for the test.

6.7 Record for the test procedure with the various steps

The test procedure associated to the present protocol is : MPP-REC-10-4101 (0).

It has to be printed and filled out every time the present protocol is executed.

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TEST RECORD SHEET

Type	Reference	Chrono	Page :
MPP-REC	**10 -4101(0)**	**_ _**	**/ 8**

Compartment : CIVb **Test Phase : 1**

Test title : Preparation of the chamber

Objectives: To perform the cleaning of the chamber and to demonstrate the leak tightness before the batch culture campaign

Applicable test plan and test protocols TN101.2 Test Protocol for Batch Culture in the HPC1

Hardware: HPC1 compartment and control system

Person responsible for the test :

Test prerequisites :

- Chamber ready for cleaning: maintenance operations finished

Step No.	Action description	Expected results / Nominal behaviour	Date / Hour	Observed results / calculated / remarks - ref. of Deviation	C/ NC	Initials
1	In case the natural light is not sufficient, switching on 1 MH lamp per module for manipulation inside the chamber	Lamps are switched on and environmental conditions in HPC1 allow to perform manipulations inside the chamber				
2	Putting on Personal Protective Equipment: goggles, lab coat, safety shoes, gloves, shoe covers	Personal Protective Equipment is put on operator is ready to perform cleaning manipulations inside the chamber				
3	Removal of air balancing panels out of HPC1	Aluminium air balancing panels are removed in order to enter inside the chamber for free manipulation				

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TEST RECORD SHEET	Type	Reference	Chrono	Page :
	MPP-REC	**10 -4101(0)**	**_ _**	**/ 8**

Compartment : CIVb Test Phase : 1

4	1. Demounting of plastic tips of spigots for further cleaning ; 2. pictures are taken	1. Tips are removed and can be cleaned separately 2. Pictures available				
5	Cleaning of plastic tips of spigots with soap and deionized water and subsequent rinsing with deionized water	Plastic tips of spigots are cleaned and rinsed well				
6	Cleaning of the bottom inside the chamber with vacuum cleaner	No dust can be observed inside the chamber				
7	Preparation of 1,5 L of 70% ethanol, filling of disinfected bottles with sprayers for disinfection of surfaces in HPC1	There is no problem with supply of ethanol in sufficient quantity from department, tools are ready for disinfection				
8	Preparation of the chamber inside for disinfection: taking trays out of the chamber	Chamber is ready for entering inside and disinfection of the surfaces				
9	Putting on mask against vapours and disinfection of all accessible surfaces inside the chamber with ethanol	All accessible surfaces in the chamber are treated with ethanol				
10	Disinfection of HVAC surfaces	All accessible surfaces in HVAC of HPC1 are disinfected with ethanol				
11	Disinfection of aluminium air balancing panels with ethanol outside of the chamber	Panels are disinfected and can be placed back into the chamber				

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TEST RECORD SHEET	Type	Reference	Chrono	Page :
	MPP-REC	**10 -4101(0)**	**_ _**	**/ 8**

Compartment : CIVb Test Phase : 1

12	Placement of aluminium air balancing panels on the bottom of the chamber to their nominal position preliminary putting on disposable shoes covers	Aluminium air balancing panels are placed in their nominal position				
13	Connecting of spigots plastic tips	Tips are connected to spigot at their nominal position				
14	Placement of trays, preliminary cleaned with soap, inside the chamber	Trays are placed in the chamber in their nominal position				
15	Cleaning of 120-L external tank with soap and rinsing with decalcified water	Tank is clean and ready for further manipulation				
16	Filling of external tank with decalcified water and 2 % NaOH, mixing in external tank	Tank is filled and solution is mixed, left for 1 hour pH of water measured				
17	Taking of 3 samples of solution for pH, measurements of pH	pH is measured, data is considered as experimental point 1				
18	Disinfection of external pump and flexible tubing, to be used for main nutrient tank filling, with solution of NaOH from external tank	Pump and flexible tubing are disinfected				
19	Empting of the tank, rinsing with decalcified water, measurement of pH of water until it's equal to pH	Tank is rinsed well and pH of last sample from the tank is equal to pH				

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Universitat Autònoma de Barcelona

TEST RECORD SHEET

Type	Reference	Chrono	Page :
MPP-REC	**10 -4101(0)**	**_ _**	**/ 8**

Compartment : CIVb **Test Phase : 1**

	of decalcified water (control point)	of decalcified water				
20	Rinsing of external pump and tubing with decalcified water until pH of water, going out of the pump and tubing is equal to pH of decalcified water	Pump is rinsed well and pH of last sample from the tank is equal to pH of decalcified water				
21	Rinsing of external tank, tubing and pump with deionized water	Tank, tubing and pump are rinsed and ready for further use				
22	Emptying stock A, stock B, Acid and Base tanks by opening electro valves from HMI in manual mode for 4 minutes	Indicator of low level of liquid for all tanks can be seen from HMI				
23	When level of water in the tanks is low, emptying of the rest of water manually with plastic cup and after with pipette	No liquid can be observed on the bottom of the tanks				
24	Disinfection of stock A, stock B, Acid and Base tanks with ethanol and drying with paper towel	Tanks are disinfected and no ethanol can be observed in the tanks				
25	Cleaning of main nutrient tank with soap and rinse several times with decalcified water	Nutrient tank is cleaned with soap and ready for further manipulation				

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TEST RECORD SHEET	Type	Reference	Chrono	Page :
	MPP-REC	**10 -4101(0)**	**_ _**	**/ 8**

Compartment : CIVb Test Phase : 1

26	Filling of nutrient tank with decalcified water until top of the tank	Approximately 200 L				
27	Taking of 3 samples of water from nutrient tank for pH and EC measurements, pH and EC measuring	pH and EC are measured and will be a control point for further measurements				
28	Closing of sampling loop of HPC1 liquid loop before following manipulations	Sampling loop is closed, values of EC and pH are not correct				
29	Preparation of KOH solution using previously put on Personal Protective Equipment	4 kg of KOH for 200 L of water in nutrient tank				
30	Using external pump mixing and dissolving of KOH in the nutrient tank	KOH is completely dissolved in the nutrient tank, no pallet can be noticed in the nutrient tank				
31	Taking of 3 samples of solution from nutrient tank for pH measurement	pH is measured and data will be used as first experimental point				
32	Closing of nutrient tank with nutrient tank lid	Nutrient tank is closed with screws				

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TEST RECORD SHEET

Type	Reference	Chrono	Page :
MPP-REC	**10 -4101(0)**	**_ _**	**/ 8**

Compartment : CIVb Test Phase : 1

33	Enabling of irrigation in manual mode, water flow is 15 L/min	Irrigation enabled flow =15L/min Duration :1hour				
34	Disabling of irrigation mode	Irrigation mode is off				
35	Taking of 3 samples of solution from nutrient tank for pH and EC measurements	pH and EC are measured and data will be the second experimental point				
36	Empting of nutrient tank using drain valve	Nutrient tank is almost empty but some liquid is observed on the bottom of the tank due to design of the tank				
37	Rinsing of nutrient tank several times with decalcified water and pH measurements after each rinse until pH value is equal to control point (see step No 27).	pH of last sample taken from nutrient tank is equal to control point so tank is rinsed well				
38	Filling of nutrient tank with decalcified water and enabling of irrigation mode for 15 minutes	Rinsing of liquid loop from residues of KOH				
39	Taking of liquid samples from 3 extreme spigots of module A and module C (6 in total) and from nutrient tank drain valve for pH measurements	pH is measured and data will be considered as 3d, 4^{th} and 5^{th} experimental points				

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Universitat Autònoma de Barcelona

TEST RECORD SHEET	Type	Reference	Chrono	Page :
	MPP-REC	**10 -4101(0)**	**_ _**	**/ 8**

Compartment : CIVb Test Phase : 1

40	Disabling of irrigation mode and emptying of nutrient tank	Irrigation mode is off, tank is almost empty				
41	Repetition of steps No 38-40 until pH of the solution is equal to control value	pH of solution is equal to control value, so liquid loop is rinsed well from KOH residues				
42	Taking of 3 samples of deionized water and pH measurement	pH is measured and data will be the 2d control point				
43	Filling of nutrient tank with deionized water and enabling of irrigation mode for 15 minutes	Final rinsing of liquid loop				
44	Taking of liquid samples from 3 extreme spigots of module A and module C (6 in total) and from nutrient tank drain valve for pH measurements	pH is measured and data will be considered as experimental points				
45	Disabling of irrigation mode and emptying of nutrient tank	Irrigation mode is off, tank is almost empty				
46	In case pH of solution is equal to pH of deionized water, rinsing of liquid loop is finished. If it's higher than 2d control point, repetition of steps No 43-45 until pH value is equal to control one.	pH of water is equal to 2d control point, rinsing of liquid loop is finished				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET

Type	Reference	Chrono	Page :
MPP-REC	**10 -4101(0)**	**_ _**	**/ 8**

Compartment : CIVb **Test Phase : 1**

47	Rinsing of external pump with decalcified water until pH of water, going out of the pump is equal to pH of decalcified water	Pump is rinsed well and pH of last sample from the tank is equal to pH of decalcified water				
48	Rinsing of external tank and pump with deionized water, pH of water, going out of the pump is equal to pH of deionized water	Tank and pump are rinsed and pH of last sample from the pump is equal to pH of deionized water				
49	Cleaning of pH and EC probes from sampling loop with deionized water and paper towel	pH and EC probes are rinsed and dried				
50	Chamber shell Integrity Leakage test: CO_2 is injected into the chamber in a closed configuration (all sub-systems off, main centrifugal blower excepted) to a set-point of 1500 ppm. Allowing the system to equilibrate at 1500 ppm for 2 hours to allow time for equilibration with the passive air pressure compensation bags. CO_2 is allowed to passively decay through the chamber shell over a 24 hour period. The rate of leackage is calculated as the slope of a tangent to a 24 hour CO_2 curve, expressed as % Leackage of CO_2 (relative to initial value) per day.					

Conclusion for the Test	*Name*	*Signature*	*Date*
☐ Passed ☐ Failed			
- **Number of deviations attached to the document :** - **All deviations have been justified or corrected ? YES / NO**			
Comments			
Checked by **MELiSSA Pilot Plant**	*Name*	*Signature*	*Date*

Appendix 1 – record of implied personnel

Name	ORGANIZATION	Function	Initials

Appendix 2 – record of calibration certificates for the test instruments

Instrument description	Inv. Number	Calibration record reference	Date of calibration	Calibration valid until

Appendix 3 – deviations list

DEV. FORM #	Deviation:	Criticality Low Medium High

	Corrective action:		**Resp.**	**Due date**
	Corrective action performed and checked: **Ref. of retests:**	**Checked / approved by**	**Closing Date**	

DEV. FORM #	**Deviation:**		**Criticality** Low Medium High	
	Corrective action:		**Resp.**	**Due date**
	Corrective action performed and checked: **Ref. of retests:**	**Checked / approved by**	**Closing Date**	

DEV. FORM #	**Deviation:**		**Criticality** Low Medium High	
	Corrective action:		**Resp.**	**Due date**
	Corrective action performed and checked: **Ref. of retests:**	**Checked / approved by**	**Closing Date**	

7. Test protocol for phase 2

7.1 Features to be tested: functions, hardware, software

- capability to grow lettuce seedlings under controlled conditions

7.2 Success/failure criteria

- good germination of seeds
- Good visual aspect of seedlings (similar to those shown in Figure 1)

Figure 1. Seedlings of lettuce in rockwool cubes in the end of the germination

7.3 Resources for the test

7.3.1 Personnel: staff qualification and training needs

- MPP Technician trained to HPC1 operation
- MPP Engineer educated to HPC1 operation and results analysis

7.3.2 Personnel Protective Equipments

- Safety shoes
- Laboratory coat and trousers
- Hair net
- Dust mask
- Gloves and goggles
- Sun goggles if working in presence of full lighting inside the HPC1

- Shoe covers when working inside the chamber

7.3.3 Hardware: instruments, specific part, hardware for software operation, calibration certificates

- Millwright work (screwdriver, pipe-wrench, …)
- No specific tools are needed
- All sensors are calibrated with certificates

7.3.4 Software: verification of software, backup needs

- All acquisitions have been validated
- PLC is connected to the acquisition server

7.3.5 Test conditions

Lighting cycle
Temperature
Pressure
Humidity

7.4 Measurement and data sampling

7.4.1 Data logfile

HPC1 28 days test – phase2-01032010.dat

The acquired parameters are the same as for phase 1

7.4.2 *Special requirements if any* (frequency, duration, synchronization)

Every minute for all instrumentation.

7.5 Reporting of status for a test

The test sequence is performed by MPP personnel, under the expertise and advice of MPP Engineer. The final status of the test (passed/fail) is decided at the end of the test in agreement between MPP personnel and MPP management.

7.6 Deviations and non conformances

In case the test sequence cannot be performed as planned or some results are out of their expected range, a deviation is opened and appended to the test record. The process to fill out the deviation form is identical to the one to fill out the NCR as per the Quality Assurance Procedure for the control of non conformities MPP-QAP-08-0002.

This deviation is discussed among MPP, and together with ESA for high criticality deviations, in order to decide how to address it. If necessary, on the basis of a given deviation, MPP can decide to

open a NCR as planned by the Quality Manual and the Quality Assurance Procedure for the control of non conformities MPP-QAP-08-0002.

The discussion of all deviations is made before the final decision of the status for the test.

7.7 Record for the test procedure with the various steps

The test procedure associated to the present protocol – phase 2 is: MPP-REC-10-4102 (0).

It has to be printed and filled out every time the present protocol phase 2 is executed.

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TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	**10 -4102(0)**	**01**	/

Compartment : CIVb **Test Phase : 2**

Test title : Seedlings phase

Objectives: To grow lettuce seedlings in good conditions to be used for batch culture in the HPC1

Applicable test plan and test protocols TN101.2 Test Protocol for Batch Culture in the HPC1

Hardware: HPC1 and HPC1 control system

Person responsible for the test :

Test prerequisites :

- **Cleaning operation of HPC1 finished**
- **Leak test finished**

Step No.	Day No.	Action description	Expected results / Nominal behaviour	Date / Hour	Observed results / calculated / remarks - ref. of Deviation	C/NC	Initials
1	0	Sterilization of 2 flats of rockwool small cubes - Grodan AO 36/40 6/15W using following procedure for Rockwool safe manipulation: OP-10-4101 (0)	Rockwool flats are sterilised and ready for seeds sowing				
2	0	Sterilization of 1 litre of deionized water in autoclave	30 minutes at 120°C				
3	0	Sterilization of 10-litres tanks for stock A and stock B (2), 20-litres tank for seedlings solution in autoclave	30 minutes at 120°C				
4	0	Preparation of 10 L of stock A and 10 L of stock B solutions and 10 L of seedling solution	Solutions must be kept in a dark place in order to prevent algae growth				

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Universitat Autònoma de Barcelona

TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	**10 -4102(0)**	**01**	/

Compartment : CIVb **Test Phase : 2**

		according to TN 96.3.					
5	**0**	Taking a bag with lettuce seeds from the fridge and placement about 200 seeds into a glass. Addition of 5% hypochlorite (bleach) to the glass with the seeds (until bleach covers all the seeds)	Sterilization during 15 minutes		Seeds Supplier: _____ Seeds Lot nº: _____		
6	**0**	Placement of the seeds into a sieve and rinse with 1 L of deionized sterile water	Seeds are sterilized but some lost of colour can be observed due to treatment with bleach				
7	**0**	Sterilization 2 seed germination trays with 70% ethanol and paper towel	No ethanol can be observed on the trays				
8	**0**	Placement of 2 flats of rockwool small cubes - Grodan AO 36/40 6/15W into 2 seed germination trays	Each tray must contain 98 cubes				
9	**0**	Using tweezers, placement a single lettuce seed in each hole of Rockwool cubes	Attention should be paid during process of seeds sowing in order not to sow more than 1 seed per cube and not miss any cube				
10	**0**	Measurement of pH of irrigation solution for seedlings, in case it´s 5.8-5.9, watering of Rockwool cubes with this solution. If pH is higher	pH of seedlings solution is 5.8-5.9, Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET	Type	Reference	Chrono :	Page :
	MPP-REC	**10 -4102(0)**	**01**	/

Compartment : CIVb **Test Phase : 2**

		than 6.0 or lower than 5.8 addition of about 1 mL of 0.5M HNO_3 or 1mL of 0.5M KOH (depending on pH if it's low or high) to the 10L of the solution, mixing well and checking of pH again. Repetition if necessary until pH is about 5.8-5.9.					
11	**0**	Taking photos of the trays.	Day_0 of crop test (Rockwool sheets without seedlings)				
12	**0**	Addition of a plastic tray cover to each tray	Trays are covered in order to maintain humidity inside the tray and enhance seeds germination				
13	**0**	Activation of Schneider controller of HPC1. Light mode: auto, 1 MH and 1 HPS lamps per module on. Day time – 16 hours, night time – 8 hours. Fan Mode: auto. Temperature and humidity mode: auto. T day and night set points = 20°C, day Rh =50%, night Rh=70%. Air Blower Mode: auto.	Schneider controller is activated with environmental conditions set points used for seeds germination and seedlings growth				
14	**0**	Placement of the germination trays into HPC1 and closing of the doors from both sides of HPC1.	Chamber is closed and all conditions in the chamber correspond to conditions for seeds germination				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	**10 -4102(0)**	**01**	/

Compartment : CIVb **Test Phase : 2**

15	**1**	Opening of the chamber, taking photos of the trays with plastic cover and after without the cover.	Day_1 of crop test				
16	**1**	In case not more than 60% of seedlings in each tray can be observed , addition of plastic cover and closing the chamber	Check of seedlings germination, repeat in the afternoon				
17	**1**	In the afternoon checking of the seeds germination again. If more than 60% of seedlings are observed opening of the trays. If not the trays are left covered until next day	Check of seedlings germination				
18	**2**	Opening of the chamber, taking photos of the trays with plastic cover and after without the cover.	Day_2 of crop test				
19	**2**	In case not more than 60% of seedlings in each tray can be observed , addition of plastic cover and closing the chamber	Check of seedlings germination, repeat in the afternoon				
20	**2**	In the afternoon checking of the seeds germination again. If more than 60% of seedlings are observed opening of the trays. If not the trays are left covered until next day	Check of seedlings germination				
21	**3**	Checking the seeds germination and if more than 60% of seedlings are	By this day usually germination percentage is more				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	**10 -4102(0)**	**01**	/

Compartment : CIVb Test Phase : 2

		observed in each tray opening of the trays (taking out the plastic covers).	than 60% per tray and plastic covers can be removed from the trays				
22	**3**	Taking photos of both trays	Day_3 of crop test				
23	**3**	Checking of pH of the seedlings nutrient solution and in case it´s 5.8-5.9, watering Rockwool cubes with this solution, approximately 900 mL per each tray. Regulation of pH with acid or base in case it is higher than 6.0 or lower than 5.7 (see step No.10)	pH of seedlings solution is 5.8-5.9, Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)				
24	**4**	Taking photos of both trays	Day_4 of crop test				
25	**4**	In the afternoon watering of the plants with the solution if necessary, approximately 500 mL per tray.	Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)				
26	**5**	Taking photos of both trays. Watering of the plants with the solution if necessary	Day_5 of crop test. Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)				
27	**6**	Cutting of polypropylene film for trays covering against algae growth at maturity phase	100 stripes using template				
28	**6**	Taking photos of both trays. Watering of the plants with the solution if necessary	Day_6 of crop test. Rockwool cubes are wet, but not overwatered (no liquid				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	**10 -4102(0)**	**01**	/

Compartment : CIVb **Test Phase : 2**

			beneath Rockwool)				
29	**7**	Checking of pH of the seedlings nutrient solution and in case it´s 5.8-5.9, watering Rockwool cubes with this solution, approximately 900 mL per each tray. Regulation of pH with acid or base in case it is higher than 6.0 or lower than 5.7 (see step No.10)	pH of seedlings solution is 5.8-5.9, Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)				
30	**7**	Taking photos of both trays.	Day_7 of crop test.				
31	**7**	Addition of stock A, stock B, acid and base solutions into the appropriate tanks until a mark on each tank	Tank are filled for maturity test start up				
32	**7**	Addition of 1.3 litres of stock A and 1.3 litres of stock B into the 120L external tank, mixing thoroughly solution in the 120L external tank using a pump	Solution is mixed and ready for transfer into HPC1 nutrient tank				
33	**7**	Transfer of 120 liters of the nutrient solution from external tank into HPC1 nutrient tank using a pump	Solution is transferred without any leak				
34	**7**	Addition of 40 liters of deionized water into 120L external tank and transfer of 40 liters of deionized water from external tank into HPC1	Water is transferred without any leak				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	**10 -4102(0)**	**01**	/

Compartment : CIVb **Test Phase : 2**

		nutrient tank using a pump					
35	**7**	Taking the trays with seedlings out of the chamber	During 1 hour the trays are in HPC room under ambient conditions				
36	**7**	Taking 20 trays out of the chamber and wiping 20 trays with paper towel wetted with ethanol	Sterilization of the trays				
37	**7**	Placement of 20 trays into the chamber	Trays are inside the chamber in their nominal position				
38	**7**	Activation of irrigation system from HMI in auto mode in order to adjust pH and EC of the nutrient solution	Irrigation system is activated, water flow is 10-11 L/min				
39	**7**	Performance of leakage test of the main nutrient solution collector in the chamber	No leaks				
40	**7**	When pH is equal to 5.9 and EC to 1.9, disabling of irrigation system	Irrigation system is off, no liquid circulation in the HPC1				
41	**7**	Taking of 6 samples of nutrient solution from nutrient tank: a. Irrigation system is off. b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this solution but empty it into sewerage. c. After taking 3 samples	Samples of the solution are taken according to the written procedure, nutrient tank is tightly closed				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	**10 -4102(0)**	**01**	/

Compartment : CIVb Test Phase : 2

		continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge. d. Taking 3 samples manually from the top of the nutrient tank and placement them into the fridge f. Closing nutrient tank with plastic cover and screws, making sure it is airtight					
42	**8**		Day_8 of crop test.				

Conclusion for the Test	*Name*	*Signature*	*Date*
☐ Passed ☐ Failed			
- **Number of deviations attached to the document :** - **All deviations have been justified or corrected ? YES / NO**			
Comments			
Checked by **MELiSSA Pilot Plant**	*Name*	*Signature*	*Date*

Appendix 1 – record of implied personnel

Name	ORGANIZATION	Function	Initials

Appendix 2 – record of calibration certificates for the test instruments

Instrument description	Inv. Number	Calibration record reference	Date of calibration	Calibration valid until

Appendix 3 – deviations list

DEV. FORM #	Deviation:		Criticality Low Medium High	
	Corrective action:		Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date	

DEV. FORM #	Deviation:		Criticality Low Medium High	
	Corrective action:		Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date	

DEV. FORM #	Deviation:		Criticality Low Medium High	
	Corrective action:		Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date	

8. Test protocol for phase 3

8.1 Features to be tested: functions, hardware, software

- control of required culture conditions
- Capability to grow adequately biomass in the HPC1

8.2 Success/failure criteria

- weight of biomass
- Visual aspect of biomass
- Homogeneity of biomass inside the chamber
- Absence of leaks

8.3 Resources for the test

8.3.1 Personnel: staff qualification and training needs

- MPP Technician trained to HPC1 operation
- MPP Plant Scientist educated to HPC1 operation and results analysis

8.3.2 Personnel Protective Equipments

- Safety shoes
- Laboratory coat and trousers
- Hair net
- Dust mask
- Gloves and goggles
- Sun goggles if working in presence of full lighting inside the HPC1
- Shoe covers when working inside the chamber

8.3.3 Hardware: instruments, specific part, hardware for software operation, calibration certificates

- Millwright work (screwdriver, pipe-wrench, …)
- No specific tools are needed
- All sensors are calibrated with certificates

8.3.4 Software: verification of software, backup needs

- All acquisitions have been validated
- PLC is connected to the acquisition server

8.3.5 Test conditions

Lighting cycle
Temperature

Pressure
Humidity
Flow of nutrient solution
EC, pH of nutrient solution
CO2 and O2 control

8.4 Measurement and data sampling

8.4.1 Data logfile

HPC1 28 days test – phase3-01032010.dat

The acquired parameters are the same as for phase 1

8.4.2 *Special requirements if any* (frequency, duration, synchronization)

Every minute for all instrumentation.

8.5 Reporting of status for a test

The test sequence is performed by MPP personnel, under the expertise and advice of Higher Plant Scientist.
The final status of the test (passed/fail) is decided at the end of the test in agreement between MPP personnel and MPP management.

8.6 Deviations and non conformances

In case the test sequence cannot be performed as planned or some results are out of their expected range, a deviation is opened and appended to the test record. The process to fill out the deviation form is identical to the one to fill out the NCR as per the Quality Assurance Procedure for the control of non conformities MPP-QAP-08-0002.

This deviation is discussed among MPP, and together with ESA for high criticality deviations, in order to decide how to address it. If necessary, on the basis of a given deviation, MPP can decide to open a NCR as planned by the Quality Manual and the Quality Assurance Procedure for the control of non conformities MPP-QAP-08-0002.

The discussion of all deviations is made before the final decision of the status for the test.

8.7 Record for the test procedure with the various steps

The test procedure associated to the present protocol – phase 3 is : MPP-REC-10-4103 (0).

It has to be printed and filled out every time the present protocol phase 3 is executed.

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	**10 -4103(1)**	**01**	/

Compartment : CIVb **Test Phase : 3**

Test title : Maturity phase

Objectives: Batch growth of lettuce in batch to obtain adequate and homogeneous biomass production in absence of leaks

Applicable test plan and test protocols TN101.2 Test Protocol for Batrch Culture in the HPC1

Hardware: HPC1 and HPC1 control system

Person responsible for the test :

Test prerequisites :

- **Seedlings cultivation finished**

Step No.	Day No.	Action description	Expected results / Nominal behaviour	Date / Hour	Observed results / calculated / remarks - ref. of Deviation	C/NC	Initials
1	8	Use of procedure for Rockwool safe manipulation: OP-10-4101 (0) in order to prepare rockwool large cubes – Grodan Delta 4G 42/40 for seedlings planting	Rockwool large cubes – Grodan Delta 4G 42/40 are dampened and can be used for the following planting				
2	8	Taking trays with seedlings out of the chamber. Taking photos of the seedlings before starting transfer to bigger rockwool cubes.	t_8 of crop test				
3	8	Selection of 100 small rockwool cubes with seedlings that look similar (height of the	Selected plants are practically the same in their morphology and will be used for				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET	Type	Reference	Chrono :	Page :
	MPP-REC	**10 -4103(1)**	**01**	/

Compartment : CIVb **Test Phase : 3**

		plants, size and quantity of leaves). Taking photos of these plants.	transfer to bigger rockwool cubes				
4	**8**	Taking 20 trays out of the chamber	Trays are prepared for planting				
5	**8**	**Placement of 5 big Rockwool cubes into each tray,** dampen with deionized water if necessary	Cubes are prepared for planting				
6	**8**	Covering of each tray with polypropylene nutrient film. Placement of small rockwool cubes with seedlings into each hole of big rockwool cubes	Attention should be paid during transfer process. Seedlings are not to be under the nutrient film. Size of the holes in the nutrient film can be increased if necessary.				
7	**8**	Taking of photos of several trays with transferred seedlings. Placement of 20 trays with transferred seedlings into HPC1	Beginning of maturity phase of crop test				
8	**8**	Activation of control system: Light Mode: auto, fan mode: auto, all lamps on, day/night=16hours/8hours Temp. and Hum.mode: auto, air blower mode: auto, day average temperature =26ºC, night average	All activated loops are running				

		temperature=20ºC, day average humidity=50%, night average humidity=70%. Irrigation mode: auto, pH mode: auto, EC mode: auto, pH set point=5.9, EC set point=1.9 mS/cm, Condensate level: auto.					
9	**8**	When all lamps are on taking photos of all trays with seedlings inside the chamber before closing the chamber	View of the plants inside the chamber before tightening of the system				
10	**8**	Closing of the inner airlock doors (curtains) and latching of the outer doors. Activating of CO_2 mode: auto, CO_2 setpoint= 1000 ppm	Chamber is tightly closed and crop test is started				
11	**8**	Addition of stock A, stock B, acid and base solutions into the appropriate tanks until a mark on each tank	Tanks are filled until initial volume (2.5 L)				
12	**9**	Disabling of CO_2 control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO_2 control	Use of peristaltic pump for taking a sample				
13	**9**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	**10 -4103(1)**	**01**	/

Compartment : CIVb Test Phase : 3

14	**9**	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics					
15	**10**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol					
16	**11**	Disabling of CO_2 control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO_2 control	Use of peristaltic pump for taking a sample					
17	**11**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol					
18	**11**	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics					
19	**11**	Taking of 3 samples of nutrient solution from nutrient tank: a. Irrigation system is off. b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this solution but empty it into sewerage.	Samples of the solution are taken according to the written procedure, nutrient tank is tightly closed					

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET	Type	Reference	Chrono :	Page :
	MPP-REC	**10 -4103(1)**	**01**	/

Compartment : CIVb **Test Phase : 3**

		c. After taking 3 samples continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge. d. Enabling of irrigation system in auto mode					
20	**12**	Printing history graphs for all loops for the period of 24 hours.Taking photos of the plants	Results are studied and kept together with test protocol				
21	**13**	Printing history graphs for all loops for the period of 24 hours.Taking photos of the plants	Results are studied and kept together with test protocol				
22	**14**	Disabling of CO_2 control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO_2 control	Use of peristaltic pump for taking a sample				
23	**14**	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics				
24	**14**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				
25	**14**	Changeover of the nutrient solution: disabling of irrigation mode, closing with manual valves inlet and outlet of the liquid in the chamber. Opening	Solution is changed and samples of solution before and after changeover are taken.				

		nutrient tank and taking 3 samples of nutrient solution from the top of the tank (manually) and from the bottom using drain valve (after outpouring first 3 samples into sewerage). Emptying of nutrient tank using drain valve. Rinsing of nutrient tank with deionized water. Preparation of nutrient solution in external tank (see TN 96.3) and transfer into nutrient tank. Taking 6 samples of nutrient solution: 3 from the top and 3 from the bottom. Sealing of nutrient tank. Opening of inlet and outlet of liquid in the liquid loop. Enabling of CO_2 control.					
26	**15**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				
27	**16**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				
28	**17**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	**10 -4103(1)**	**01**	/

Compartment : CIVb **Test Phase : 3**

29	**18**	Disabling of CO_2 control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO_2 control	Use of peristaltic pump for taking a sample				
30	**18**	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics				
31	**18**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				
32	**18**	Taking of 3 samples of nutrient solution from nutrient tank: a. Irrigation system is off. b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this solution but empty it into sewerage. c. After taking 3 samples continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge. d. Enabling of irrigation system in auto mode	Samples of the solution are taken according to the written procedure, nutrient tank is tightly closed				
33	**19**	Printing history graphs for all loops for the period of 24 hours.	Results are studied and kept together with test protocol				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	**10 -4103(1)**	**01**	/

Compartment : CIVb **Test Phase : 3**

		Taking photos of the plants					
34	**20**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				
35	**21**	Disabling of CO_2 control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO_2 control	Use of peristaltic pump for taking a sample				
36	**21**	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics				
37	**21**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				
38	**21**	Changeover of the nutrient solution: disabling of irrigation mode, closing with manual valves inlet and outlet of the liquid in the chamber. Opening nutrient tank and taking 3 samples of nutrient solution from the top of the tank (manually) and from the bottom using drain valve (after outpouring first 3 samples into sewerage).	Solution is changed and samples of solution before and after changeover are taken.				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET	Type	Reference	Chrono :	Page :
	MPP-REC	**10 -4103(1)**	**01**	/

Compartment : CIVb Test Phase : 3

		Emptying of nutrient tank using drain valve. Rinsing of nutrient tank with deionized water. Preparation of nutrient solution in external tank (see TN 96.3) and transfer into nutrient tank. Taking 6 samples of nutrient solution: 3 from the top and 3 from the bottom. Sealing of nutrient tank. Opening of inlet and outlet of liquid in the liquid loop. Enabling of CO_2 control.					
39	**22**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				
40	**23**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				
41	**24**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				
42	**25**	Disabling of CO_2 control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO_2 control	Use of peristaltic pump for taking a sample				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	**10 -4103(1)**	**01**	/

Compartment : CIVb **Test Phase : 3**

43	**25**	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics				
44	**25**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				
45	**25**	Taking of 3 samples of nutrient solution from nutrient tank: a. Irrigation system is off. b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this solution but empty it into sewerage. c. After taking 3 samples continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge. d. Enabling of irrigation system in auto mode	Samples of the solution are taken according to the written procedure, nutrient tank is tightly closed				
46	**26**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				
47	**27**	Printing history graphs for all loops for the period of 24 hours. Taking photos of the	Results are studied and kept together with test protocol				

		plants					
48	**28**	Disabling of CO_2 control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO_2 control	Use of peristaltic pump for taking a sample				
49	**28**	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics				
50	**28**	Disabling of all control loops. Opening the nutrient tank and taking 6 samples of nutrient solution: 3 from the top and 3 from the bottom of the tank. Opening the chamber and taking pictures of the plants. Harvesting of the plants using procedure for rockwool safe manipulation. Taking pictures of plants in each tray. Writing down fresh weight of lettuce shoots (TN 96.4). Putting of lettuce shoots into preliminary weighed labeled paper bags. Weighing of paper bags for roots drying.	Plants are harvested, control system is disabled				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET	Type	Reference	Chrono :	Page :
	MPP-REC	**10 -4103(1)**	**01**	/

Compartment : CIVb Test Phase : 3

51	**28**	Delivery of paper bags with lettuce shoots into Veterinary School and plants drying in the oven at 70ºC with preliminary fixation at 103ºC during 10 minutes. Rockwool cubes are stored in a cold cabinet at 4 ºC.	Beginning of lettuce shoots drying				
52	**29**	Delivery of rockwool cubes with roots inside into Veterinary School and roots separation from rockwool using Procedure for Rockwool safe manipulation	Roots are separated and put into the oven for drying at 70ºC				
53	**30**	Cleaning of the trays with soap and water	Trays are clean and stored in the chamber				
54	**32**	Checking of shoots and roots dry weight in Veterinary School	Weight of 3 samples of shoots and 3 samples of roots from each level in the oven, it total 24 samples				
55	**35**	Checking of shoots and roots dry weight in Veterinary School	Weight of 3 samples of shoots and 3 samples of roots from each level in the oven, it total 24 samples				
56	**36**	Checking of shoots and roots dry weight in Veterinary School. In case weights of the samples don't change, final records of dry biomass should be done next day.	Weight of 3 samples of shoots and 3 samples of roots from each level in the oven, it total 24 samples				

MELiSSA Pilot Plant

Universitat Autònoma de Barcelona

TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	**10 -4103(1)**	**01**	/

Compartment : CIVb **Test Phase : 3**

57	**37**	Recording of dry weight of lettuce shoots and roots	Dry weights are recorded, samples are taken back to MPP				
58	**38**	Mixing of dry samples of lettuce shoots in order to have 1 average sample per tray, filling of plastic cups with samples	Samples are prepared for further milling				
59	**39**	Mixing of dry samples of lettuce roots in the extraction cabinet of the Department in order to have 1 average sample per tray, filling of plastic cups with samples	Samples are prepared for storage				
60	**40**	Delivery of shoots samples to Veterinary School for milling					

Conclusion for the Test	*Name*	*Signature*	*Date*
☐ Passed ☐ Failed			

- **Number of deviations attached to the document :**
- **All deviations have been justified or corrected ? YES / NO**

Comments			
Checked by **MELiSSA Pilot Plant**	*Name*	*Signature*	*Date*

Appendix 1 – record of implied personnel

Name	ORGANIZATION	Function	Initials

Appendix 2 – record of calibration certificates for the test instruments

Instrument description	Inv. Number	Calibration record reference	Date of calibration	Calibration valid until

Appendix 3 – deviations list

DEV. FORM #	Deviation:		Criticality: Low / Medium / High	
	Corrective action:		Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date	

DEV. FORM #	Deviation:		Criticality: Low / Medium / High	
	Corrective action:		Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date	

DEV. FORM #	Deviation:		Criticality: Low / Medium / High	
	Corrective action:		Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date	

9. Comments

Protocol for lettuce batch culture experiments in the HPC1 of the MPP
Comments

General comments

AD and RD to be updated, and therefore updates needed along the TN; depending on the docs, quality docs are referred to as Ad or RD; maybe consistency to be checked. RD1, RD2 and RD4 look like being AD.

OK, amended. Anyhow, maybe desirable to re-discuss regarding the TNs applicability (especially TN96.3 and 96.4).

ESA agrees to reassess TN96.3 and 96.4 when future activities in the HPC1 will be launched.

Issue, revision number and associated date are not provided in the file; maybe the cover page and change log are missing in the version provided for review.

This data are included in the file sent to you including the cover page and change log.

Reference to lettuce is included in the title of the TN and relevant sections

OK for ESA.

The way of updating this protocol should be discussed, considering the new approach for the staggered: OP for phases 1 and 2; test protocol for phase 3. In principle, we should keep issue 0 of this TN coherent with the already used protocols and as-run procedures, then changes proposed to be implemented in next issue of the document.

Ok for ESA to postpone the change of format to a new edition when new cultures would be prepared

Detailed comments

Page/paragraph	Comment
5/Section 2.1	P&ID should be in the AD OK, included in AD (in principle, we considered to be RD but we see the advantages to include it as AD, so agreed
5/Section 2.1	AD2 reference (isn't it a TN?) is missing Yes, it corresponds to TN96.6 and 7; thought twice, we consider it is more a RD than an AD; changed accordingly.
12/Section 6.7	The test record is titled "preparation of the chamber" and refers to the test phase 1. This should be consistent with the description of the test phase 1 introduced in section 5. The test objective is missing and shall be written in the record in accordance with description in the section 6.1. In addition, the test record mentions "test pre-requisites" which are actually part of the test protocol. The logic followed here is not fully clear and should be reviewed. OK, description of test phase 1 rephrased to be coherent with title of test record. Test objective described in accordance with section 6.1, test pre-requisites amended; also other empty sections in the record (applicable test plan and protocols; hardware) completed. Same amendments made in records for phases 2 and 3.
22/Section 7.2	Can we be a bit more specific for germination and seedlings qualitative features? "Good" is a bit hard to understand here, a picture of germinated seeds or seedlings can be a reference to the "good germination" and "good visual aspects of the seedlings". OK, picture of the seedlings included in section 7.2

24/Section 7.7	The test record is titled "seedlings phase" and refers to the test phase 2. This should be consistent with the description of the test phase 2 introduced in section 5. The test objective is missing and shall be written in the record in accordance with description in the section 7.1. The reference of the seeds lot (date of harvest at least) as well as the origin (field parcel or greenhouse, supplier, country) should be reported in this record. We see the description of test phase 2 in section 5 (*"Phase 2: Seedlings phase from planting to 8 days growth"*) in principle coherent with title of test record (*"Seedlings phase"*). Test objective described in accordance with section 7.1, test pre-requisites amended; also other empty sections in the record (applicable test plan and protocols; hardware) completed. Record of seeds lot nº and supplier included in Step 5
34/Section 8.7	The test record is titled "maturity phase" and refers to the test phase 3. This should be consistent with the description of the test phase 3 introduced in section 5. The test objective is missing and shall be written in the record in accordance with description in the section 8.1. The reference of the seeds lot (date of harvest at least) as well as the origin (field parcel or greenhouse, supplier, country) should be reported in this record. We see the description of test phase 2 in section 5 (*"• Phase 3: Maturity phase from day 8 till day 28 and harvesting"*) in principle coherent with title of test record (*"Maturity phase"*). Test objective described in accordance with section 8.1, test pre-requisites amended; also other empty sections in the record (applicable test plan and protocols; hardware) completed. Regarding the reference to the seeds lot and origin, we don'see necessary to have them as well in this record of phase 3 The seedling record bears the information of the lot; the harvesting records do not bear it but they are traceable to the corresponding seedling records. ESA prefers this point to be reassessed for future campaign.
17/Section 6.7. Step nº16	The cleaning with NaOH is kept in the protocol although it was agreed with ESA for the batch cultures in 2010 (MOM-104101-AF-20100302) not to use NaOH or KOH, only water and soap (Dev. 3 of MPP-REC-10-4101(0)-01) OK to keep traceability including MOM reference, and then in future disinfection method will be validated
19/Section 6.7. Step nº29	The cleaning with KOH is kept in the protocol although it was agreed with ESA for the batch cultures in 2010 (MOM-104101-AF-20100302) not to use NaOH or KOH, only water and soap (Dev. 3 of MPP-REC-10-4101(0)-01) OK to keep traceability including MOM reference, and then in future disinfection method will be validated

31/Section 7.7. Step nº 18	Taking photos of the trays: to be considered if we increase the flexibility of this task, as during the previous cultures sometimes it was not done during week-ends (Dev. 1 of MPP-REC-10-4102(0)-01) ESA agrees with the principle of flexibility when possible for these non critical tasks. In future tests the calendar for the test should be approved ahead, then identifying the criticity of the tasks and matching the appropriate timing for them. The protocol can be kept the way is now written.
29/Section 7.7. Step nº 23	Watering rockwool: To be discussed if we increase the flexibility of this task, as during the previous cultures sometimes it was not done as rockwool was wet (Dev. 2 of MPP-REC-10-4102(0)-01) ESA agrees with the principle of flexibility when possible for these non critical tasks. In future tests the calendar for the test should be approved ahead, then identifying the criticity of the tasks and matching the appropriate timing for them. The protocol can be kept the way is now written.