Call-Off Order 9

HPC1 characterization

WP-101.3

Characterization of staggered cultures

TN 101.4

Test Protocol for staggered culture experiments in the HPC1

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Date	Issue	Reason of the change	Modified paragraphs
15/01/2011	(0)	Creation	
15/09/2011	(1)	Update as per ESA comments	See comments in Section 7

TABLE OF CONTENT

1. Scope

The present protocol describes the main steps to follow in order to operate the HPC1 compartment during staggered culture of lettuce. This document and its associated operational protocols will be used in a routine basis in the future operation of the MPP.

2. Reference and applicable documents

2.1 Applicable documents

AD1	MPP-QA-07-0001	MPP Quality Manual
AD2	MPP-QA-07-0003	MPP rules for good lab practices
AD3	MPP-OFR-10-4101(0)	MPP Proposal for Call-off Order 9: HPC1 characterization phase in the MELiSSA Pilot Plant
AD4	TN 96.3, Section 6.5	Test protocol for lettuce cultivation, 6.5 Solution Preparation
AD5	TN 96.4, Section 4.4b (except for ethylene sampling)	Protocols for sampling and analysis, 4.4b Harvest and sampling protocol
AD6	MPP-OP-11-4101	Lettuce Germination and Transfer Procedure
AD7	MPP-OP-11-4102	Cleaning Operating Procedure
AD8	MPP-OP-11-4103	Gas Leak Test Procedure
AD9	MPP-OP-11-4104	Microbial Monitoring Procedure
AD10	MPP-OP-10-4101	Procedure for rockwool safe manipulation
AD11	MPP-PID-10-4101-A6	HPC1 P&ID
AD12	MPP-UM-11-4101	MPP Internal User manual for HPC1
AD13	TN 95.1	Sherpa HPC Control requirement and software description

2.2 Reference documents

RD1	TN 85.71	HPC1 User Manual

3. Acronyms/Definitions

HPC1	Higher Plant Compartment 1
MPP	MELiSSA Pilot Plant
EC	Electrical Conductivity
MSDS	Material Safety Data Sheet
ppm	Part per Million
UAB	Universitat Autònoma de Barcelona
UoG	University of Guelph
PID	Piping and Instrumentation Diagram
C / NC	Compliant / Non-Compliant

4. Test items

4.1 Description (PID, technical drawings, user manual)

- Higher Plant Compartment (HPC1) is described in document RD1 and AD12
- Maintenance and calibration of HPC1 components are described in document AD12
- PID and Sherpa control are describe in documents AD11 and AD13 respectively
- The main MPP operating procedures applicable for this protocol are described in documents AD4, AD5, AD6, AD7, AD8, and AD9
- Instruction on safe utilization of MPP material and equipment can be found in document AD10, and corresponding MSDS

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

- Mechanical hazard (pump, blower)
  - Hazards are mitigated through the use of closed access panels. Removal shall only be performed by qualified personnel.
- Pressure hazards (compressed gases mixtures in K-size tanks at 200barg, and N_2 and O_2 building supplies at 6 bars)
  - Gas pressure regulators reduce gas cylinder and building supply tubing pressure to 2 barg

- Chemical hazards (use of acid, base)
  - Labcoats, gloves and goggles ensure protection of personnel from corrosive reagents
- Handling of rockwool
  - Labcoats, gloves, goggles and dust masks permit safe handling of rockwool material ; if needed, an extraction hood is also available (see AD10)
- Microbial hazards
  - Labcoats and fresh disposable gloves will be worn when transferring seedlings in order to mitigate microbial contamination
  - For microbial sampling activities, the use of masks is as well needed.

4.3 Instructions for operation

- See user manual and operating procedures (AD4, AD5, AD6, AD7, AD8, AD9, and AD12).

4.4 Instructions for maintenance

4.4.1 Hydroponic System

- Make sure that while running the hydroponic system, no leaks are observed from:
  - the HPC1 plant trays
  - the nutrient collector, particularly at the middle junction (VSSL_4111_01)
  - the nutrient tanks (VSSL_4106_01, LSL_4110_01, LSH_4110_01, HV_4106_09)
  - the acid, base and concentrate nutrient reservoirs (VSSL_4107_01, SV_4107_01, HV_4107_01, LSL_4107_01, VSSL_4107_02, SV_4107_02, HV_4107_02, LSL_4107_02, VSSL_4108_01, SV_4108_01, HV_4108_01, LSL_4108_01, MP_4108_01, VSSL_4108_02, SV_4108_02, HV_4108_02, LSL_4108_02 and MP_4108_02)
  - the condensate reservoir (VSSL_4110_01, LSH_4110_02, LSL_4110_02, CP_4110_01, and HV_4110_02)
  - any pumps and valves (HV_4106_01, HV_4106_02, HV_4106_03, HV_4106_04, HV_4106_05, HV_4106_06, HV_4106_07, HV_4106_08, HV_4106_10, HV_4106_11, HV_4106_12, HV_4106_13, HV_4106_14, GP_4106_01, FT_4106_01 and HV_4110_01)
- if any leak is detected from the above componentscorrective measure should be taken, such as:
  - retighten leaky junctions
  - replace defective components (tubing, tees, adapters, ferrules, etc.)
  - for the nutrient collector tray (VSSL_4111_01)apply MS-tech sealant (by Ceys SA) to the middle section if leaky and wait for the sealant to cure before restarting the hydroponic loop

4.4.2 Gas and Air Handling System

- Perform a leak test according to AD8. If the calculated CO_2 daily leakage rate is higher than 7% per day, identify the leak source using the leak identification procedures outlined in AD8 and repeat until the CO_2 leak rate is less than 7% per day.

5. Recall of test sequence

Below is a summary of the test sequence and its corresponding flow chart (Figure 1).

- Phase-1: Phase-1 consists of HPC1 preparation for the staggered culture experiment and evaluating the microbial population present before and after cleaning. The required steps are as follows:
  - Pre-culture microbiological sampling before cleaning
  - Preparation of HPC1 compartment including cleaning of the chamber, the air handling unit and the liquid loop
  - Pre-culture microbiological sampling after cleaning
  - Preparation of hydroponic solutions and liquid loop
  - Verification of equipment calibration
  - HPC1 Leak tests

- Phase-2: Phase-2 is repeated weekly, except for the change of hydroponic solution which is performed every two weeks. During Phase-2 new seedlings are started every week and transferred to the chamber after 9 days. It will take 4 weeks to reach a full staggered culture profile (i.e. simultaneous presence of plants of 4 different ages). The required steps are as follows:
  - Preparation of lettuce seedlings in the nursery
  - Transfer of seedlings into HPC1 plant trays
  - Transfer of the trays into the HPC1 compartment (20 trays the first week, and 5 trays per week thereafter)
  - Harvest of lettuce (5 trays per week and all the trays on the last harvest at week-10) and preparation of samples for subsequent analytical procedures
  - Replacement of hydroponic solution is performed every two weeks

- Phase-3: Phase-3 consists of verifying the status of the HPC1 and of a post experiment microbial population evaluation of the chamber. The microbial population will be tested before and after final growth chamber cleaning.
- The steps are defined as follows:
  - HPC1 Leak tests (take note that these may be inconsistent if there is a high residual microbial population)
  - Verification of equipment calibration
  - Post-culture microbiological sampling before cleaning
  - Cleaning of the chamber, the air handling unit and the liquid loop

- Post-culture microbiological sampling after cleaning

- After Phase 3, the chamber is clean and ready to be used for the next experiment. If no experiment is planned, the system should be conditioned for long term shut down according to the AD12 "MPP-UM-4101 HPC Internal User Manual". Some of the actions during long term shut down include:
  - Storage of pH and EC probes in electrolyte solutions
  - Drying off the hydroponic loop (Tank, reservoirs, tubing, HVAC area, etc)
  - Emptying stock solutions, acid and base

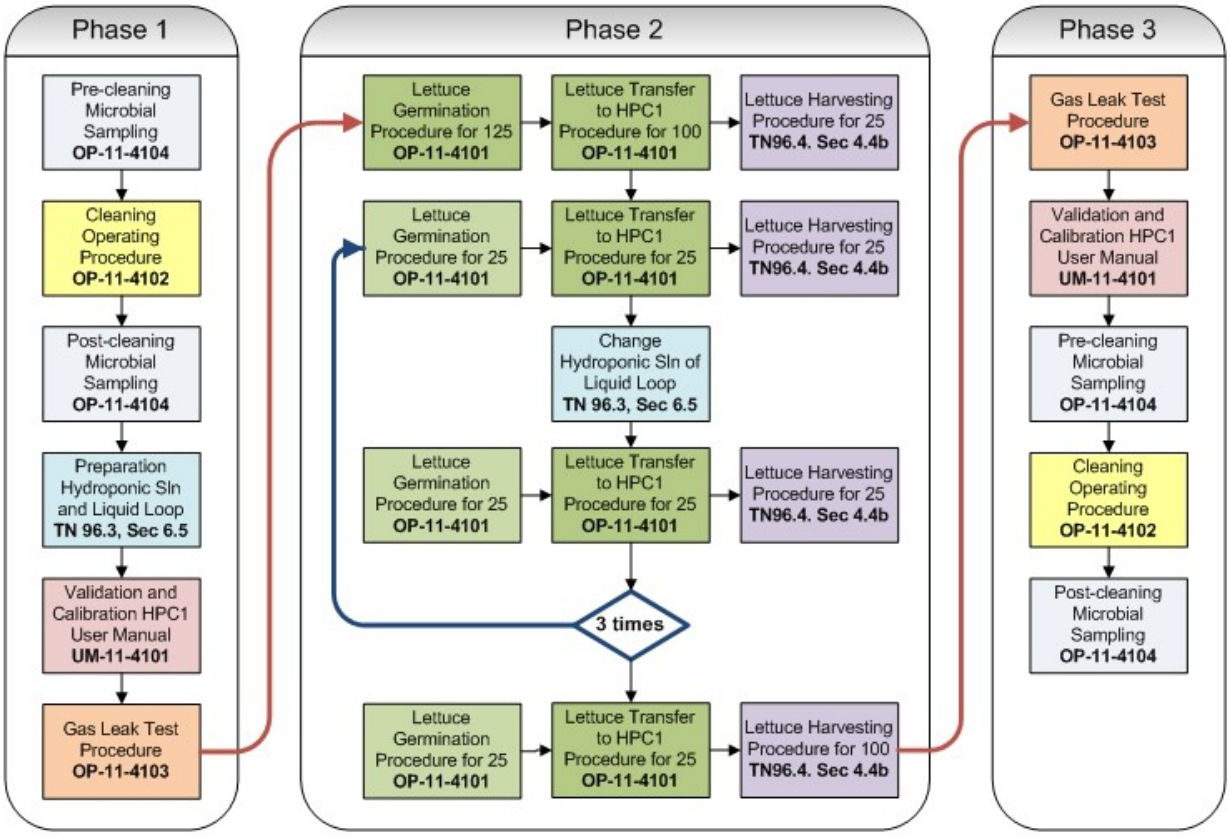

Figure 1 Staggered Culture Flow Chart

6. Test protocol

6.1 Requirements addressed by the test

Taking into account all the previous available results in batch conditions, a subsequent series of experiments has been designed to observe staggered culture of lettuce in the HPC1, with the aim to characterize HPC1's performances under this mode of operation and, on a longer term, to optimize

experimental variables. Staggered culture should provide a steady production of O_2, potable water and edible biomass as well as uptake of CO_2.

Phase-2 which defines the experimental phase of this protocol is comprised of two parts (see Figure 2). The first part starts by filling the whole chamber with 9-days old plants (20 trays of 5 plants each). Then, each week, 5 new trays containing 9-days old plants are introduce to the chamber while 5 of the oldest trays are removed for harvest and analysis, resulting in the following profiles:

Week2: 100 plants from Crop 1
Week3: 25 plants of Crop 2 and 75 plants of Crop1
Week4: 25 plants of Crop 3, 25 plants of Crop 2 and 50 plants of Crop1

Part 2 starts on Week 5, when the 4 stages of plant development are present simultaneously in the chamber and define the beginning of the staggered culture profile (Weeks 5, 6,7,8, and 9: 25 plants of Crop 4, 25 plants of Crop 3, 25 plants of Crop 2 and 25 plants of Crop 1).

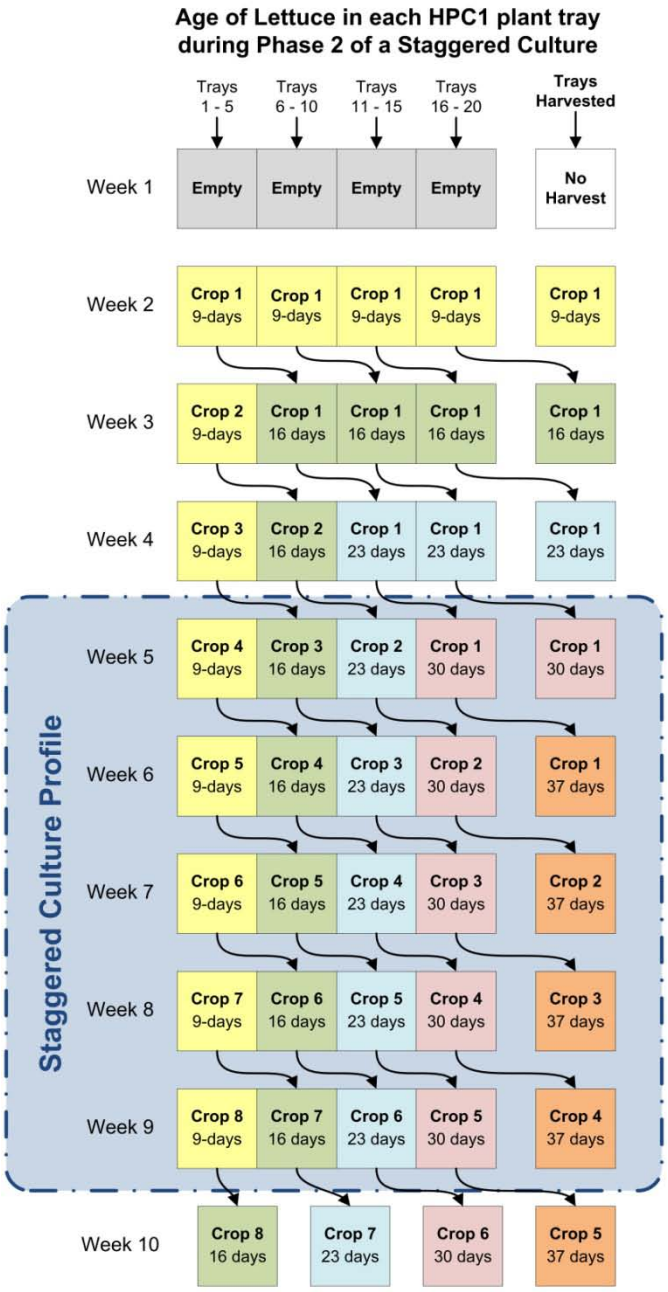

Figure 2 Age of lettuce for each plant trays during the course of the experiment

6.2 Features to be tested: functions, hardware, software

Staggered culture of lettuce will be evaluated in terms of production aspects, providing the data necessary for the characterization of the HPC1 in the scope of the MELiSSA loop closure.

Monitoring of physiological parameters that would normally increase on a daily basis (carbon assimilation, O2 accumulation, water accumulation, nutrient consumption) should be continuously performed by qualified personnel (minimally daily) and the HMI to ensure plant health and proper growing conditions are not compromised. Also assessment of situations that cause poor growth and development provides a wealth of information to be used in the study, management and mitigation of system failure scenarios.

At the conclusion of data analysis (TN 101.5), recommendations for daily monitoring requirements will be made to ensure the system evolves into one that provides consistent results on a continuous basis. The method of sample collection and data analysis for specific parameters is indicated below. Details on data collected from the HMI can be found in section 6.5.

6.2.1 Biomass production and harvest index

Fresh weight of individual shoots will be taken at harvest as indicated in Figure 2. Plant shoots will be bagged individually and dried in ventilated drying oven at 60-70 degrees Celsius in order to obtain the shoot dry weight. The lower part of the plant, which is comprised of the roots and pre-weighted rockwool cubes, will also be dried in ventilated drying oven at 60-70 degree Celsius. Roots emerging from the rockwool cubes will be grouped on a per tray basis since they would be impossible to separate from each other at this stage. They will then be dried in ventilated oven (60-70°C). Data will be used to determine biomass production and harvest index for each stage of growth (9-days, 16-days, 23-days, 30-days and 37-days) and to estimate the contribution that each stage of growth has on the chamber mass balance (see sections below) .

1. **Root dry weight (g)**

lower plant part including rockwool cubes (g) – pre-weighted rockwool cubes (g) + emerging roots (g)

2. **Biomass production**

total plant dry weight (g) / time (day)

3. **Harvest index**

edible* part dry weight (g) / total plant dry weight (g)

** In the case of lettuce the edible portion of the plant is the entire shoot*

6.2.2 Oxygen production

Concentration of oxygen is continuously monitored by the HMI system. From these data the weekly and total oxygen production for the entire experiment will be calculated.

1. **Weekly Increase in O_2 concentration (%)**

O_2 conc after weekly transfer (%) – O_2 conc before the following weekly transfer (%) – (7 x Daily O_2 leak (%))

2. **Weekly O_2 production (mol.week^{-1}.m^{-2})**

$$\frac{\text{Weekly increase in } O_2 \text{ concentration (\%) x HPC1 volume (L) x HPC1 pressure (Pascal)}}{100 \times 8.314472\ J.K^{-1}.mol^{-1} \times \text{HPC1 temperature (K) x HPC1 area } (m^2)}$$

3. **Total O_2 production (mol.m^{-2})**

$$\sum \text{Weekly } O_2 \text{ production } (mol.m^{-2})$$

6.2.3 Carbon mass balance

Concentration of CO_2 in the chamber is maintained constant at 1000 ppm. The injection of CO_2 is monitored continuously by the HMI system and this data is used in subsequent calculations of CO_2 assimilation, net carbon exchange rate (NCER), and night time respiration. Carbon balance is calculated from the total carbon obtained from the plant material, divided by the total carbon dioxide injected (weekly calculated), minus losses.

Preliminary calculations:

a) Total carbon accumulated for gas injection per one week period

Estimated C accumulation (g) calculated from CO_2 injection data recorded in the HMI

b) Total carbon removed from hydroponic solution per 2 weeks period

C analyzed in sln after 2 weeks

c) Total carbon accumulation in plant tissues per one week period

C in plant* (g. g^{-1} dry weight) x total plant dry weight (g)

Three stage calculation for carbon balance:

1. Total gross C balance

$$\frac{\text{C in plant (g. } g^{-1} \text{ dry weight) x total plant dry weight (g)}}{\text{C accumulation from gas injection (g) - C discarded with nutrient solution (weekly estimate)}}$$

2. Total C balance corrected for chamber weekly leakage

$$\frac{\text{C in plant (g. } g^{-1} \text{ dry weight) x total plant dry weight (g)}}{\text{[C accum. gas injection (g) x (1 - (\%leak per week / 100))] - C discarded with nutrient sol. (weekly estimate)}}$$

3. Total C balance corrected for weekly chamber leakage and air lock losses

$$\frac{\text{C in plant (g. } g^{-1} \text{ dry weight) x total plant dry weight (g)}}{\text{[C accum. gas injection (g) x (1 - (\%leak per week / 100))]− C from airlocks (g)** - C discarded nutrient sol. (weekly estimate)}}$$

The ratio of measured carbon injected to actual carbon from analysis **should equal 1** for complete system closure and carbon accountability, however there will be unaccounted gains/losses due to microbial respiration, algae photosynthesis, inaccuracies in root mass quantification, inaccuracies in the mass flow quantification at the sensor level (sensor accuracy).

* For actual carbon from analysis, see harvest data (TN96.4 and related records)
** Carbon from airlock was determined by a series of separate experiments (according to AD8)

6.2.4 Water production

The production of clean water via the process of evapotranspiration is monitored by HMI by calculating the accumulated condensate water returning to the hydroponic loop after being condensate in the HVAC compartment of the HPC1. The value is usable directly as is since it is reported in volume unit (L).

1. **Total water production for the experiment**

Total accumulated water from evapotranspiration (condensate volume) (L)

2. **Rate of water production corrected**

Total accumulated water from evapotranspiration (L) − total water lost or gained during plant transfer (L)

3. **Water use efficiency**

$$\frac{\text{Total edible biomass (g)}}{\text{Total water production (L)}}$$

6.2.5 Nitrogen mass balance

In order to calculate nitrogen mass balance the following parameters are required:

- Volume (recorded by the HMI) and concentration (from nutrient recipe) of acid injected,
- Volume (recorded by HMI) and elemental composition (from nutrient recipe) of nutrient solution A and B injected
- Volume (from nutrient recipe) and elemental analysis (submitted to laboratory) of fresh nutrient solution transferred to the main nutrient tank
- Total volume of nutrient solution in the hydroponic loop including rockwool holding capacity (calculated value recorded in MPP-REC-11-4107)
- Volume (measured and recorded in MPP-REC-11-4107) and mineral and elemental analysis (submitted to laboratory) of hydroponic solution prior to replacement every 2 weeks
- Total nitrogen content of shoot and root at harvest and at the time of transfer into the HPC1 (submitted to laboratory)
- Dry weights of plants at harvest and seedlings at time of introduction in the chamber (recorded in MPP-REC-11-4108 and MPP-REC-11-4106 respectively)

The above parameters will permit the calculation of the nitrogen mass balance.

1. **Total nitrogen removed from hydroponic solution per 2 weeks period**

   (N in fresh sln + N injected as nutrient + N injected as acid) –(N in sln after 2 weeks + N in sln Sampling)

2. **Total nitrogen accumulation in plant tissues per two week period**

   N in plant (g. g^{-1} dry weight) x total plant dry weight (g)

3. **Nitrogen mass balance**

$$\frac{\sum \text{nitrogen accumulation in plant tissue (g)}}{\text{(N in fresh sln + N injected as nutrient + N injected as acid) –(N in sln end of cycle + N in sln Sampling)}}$$

The ratio of nitrogen accumulation in plant tissue to nitrogen added to the hydroponic solution **should equal 1** for complete system closure and nitrogen accountability, however there will be unaccounted gains/losses due to microbial nutrition, inaccuracies in root mass quantification, inaccuracies in quantification method (analysis accuracy). In order to account for the different stages of development and plant size in the chamber, the calculation for the Total nitrogen accumulated in plant tissues will be:

Nitrogen in harvest tissues (37 days) + Estimated Nitrogen in younger plant tissues (16, 23, 30 days)

Estimated Nitrogen in Young tissues (16,23,30 days) is estimated by using data harvest prior to the beginning of the staggered culture (Weeks 3,4,5) and at the end of the last harvest (week 10)

6.2.6 Homogeneity of the different generations

The homogeneity of the different generations will be calculated on a dry weight basis. Plants will be compared on a per tray basis for each harvest and their standard deviations calculated. Each harvest will also be compared with harvests of similar age and their standard deviations calculated. The different generations will be considered homogeneous if the standard deviation observed between generation of the same age is similar (± 10%) to the standard deviation observed between trays of the same generation.

6.2.7 HPC1 internal volume estimation

The internal HPC1 volume can be calculated by measuring the volume of all components comprising the internal chamber space. However the volume of the expansion bags needs to be determinate by the following procedure:

- At time of HPC1 closure, after leak test has been completed and passed, chamber has been cleaned, microbial samples have been taken, hydroponic system has been cleaned, tested and filled with fresh solution, that all reservoirs have been filled, that all equipment have been calibrated i.e. when Phase 1 has been completed, the bags are emptied by applying gentle pressure on them until emptied,
- The chamber is then sealed and temperature and atmospheric pressure measured.

- At this point an air pump is connected to a flow meter which is itself connected to one of the inlet port located behind the chamber and given direct access to the HVAC compartment
- 300 L of air is then pumped into the chamber. Theoretically the chamber will compensate with this additional pressure by filling the bags with 300 L of air.

Volume of air injected in pressure compensation bags (L)

duration of air injection (min) x flow rate (L per min)

HPC1 total internal volume (L)

Volume airlock A (L) + volume module B (L) + volume airlock C (L) + volume plenum (L) + volume air HVAC compartment (L) + volume air injected in pressure compensation bags (L)

Quantity of gases in HPC1 at start-up (mol)

$$\frac{\text{HPC1 total internal volume (L)} \times \text{HPC1 pressure (Pascal)}}{8.314472\ J.K^{-1}.mol^{-1} \times \text{HPC1 temperature (K)}}$$

The volume of gas in the chamber is only an approximation since the volume occupied by the chambers components has not been taken into account.

6.2.8 Nutrient sampling and analysis

Nutrient solution samples will be taken weekly and consists of:

- Starting solution prior to HPC1 transfer
- One week sample taken from HPC1 sampling port
- Two week sample prior to removal from HPC1

Samples will be contained within 1L food grade containers and, if necessary, subdivided and submitted to an external laboratory for analysis. The following parameters will be analyzed:

Table 1. List of parameters to be analyzed from hydroponic samples taken from HPC1 during the course of the staggered crop experiment.

Ammonium	Calcium
Nitrite	Magnesium
Nitrate	Potassium
Carbon	Sodium
Sulphate	Copper
Chloride	Manganese
Phosphate	Boron
Thiosulphate	Zinc
Molybdenum	Iron

6.2.9 Monitoring of microbial population

The monitoring of microbial population will be performed before and after each cleaning processes, as well as before and after the experiment (Stage 2). Details instruction for the microbial sampling is described in *MPP-OP-11-4104.*

6.3 Success/failure criteria

In order to determine if plant growth and development is progressing as expected, three physiological parameters, carbon assimilation, oxygen production and nutrient uptake, will be assessed on a weekly basis (water production could be seen as a potential parameter as well, but it is a combination of evaporation and transpiration, so not a direct measurement of plant activity):

Carbon assimilation: As the lettuce crop grows it will consume carbon dioxide. The CO_2 analyzer monitors [ppmCO_2] within the chamber on a continuous basis, so as the carbon dioxide is depleted by the plants, the HMI will compensate by injecting pure CO_2 in order to maintain the experimental set point of 1000 ppm. As the crop increases in biomass, more CO_2 will be consumed and injection of CO_2 to compensate for photosynthesis will follow that of a typical plant growth curve (Figure 3). The HMI compensation results in the record of accumulation (HMI tag CL4113_CO2) measured in total litres accumulated. This increase will continue as long as the crop remains viable, and is used to assess the status of growth. If CO_2 ceases to inject based on demand, it will not accumulate in the HMI record, and the cause should be determined. Causes can include plant mortality, a lack of CO2 available to be injected (no pressure at MFC injection point), CO2 analyzer failure, or injection valve failure. It is this accumulation value that will be monitored on a weekly basis.

Once the mature crop is established (after 5 weeks), the net weekly accumulation should be approximately equal from week to week, considering 10% variation as acceptable.. This assessment should be used to initiate further system evaluation by qualified personnel with knowledge of plant growth and systems function.

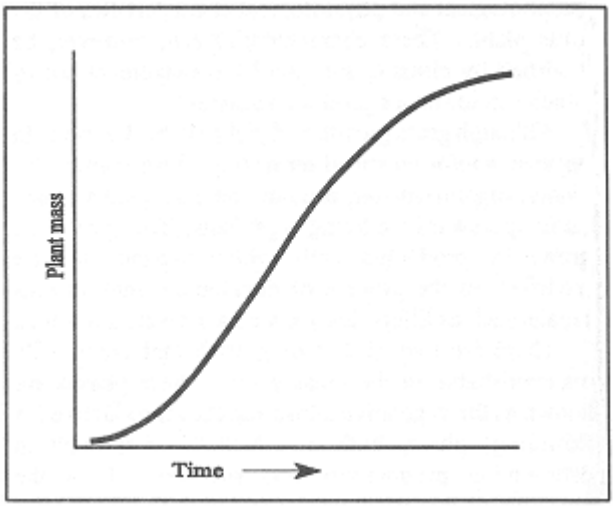

Figure 3. Typical plant growth curve.

Oxygen production: Oxygen production is dependent upon a number of factors, the primary being a suitable rate of photosynthesis. In general, in a sealed environment, oxygen levels will continue to increase from one day to the next and will follow the same plant growth profile shown in Figure 3. However, there are a number of factors which can impact O_2 production, the primary being O_2 consumption by non-target organisms (bacteria) within the system. In the HPC1, oxygen is continuously monitored by the HMI. On a weekly basis, the [%O_2] will be compared to the [%O_2] from the previous week. The experiment will be considered as progressing normally if the [%O_2] is larger than the [%O_2] of the previous week.

Again, once the mature crop is established (after 5 weeks), the net weekly accumulation should be approximately equal from week to week, considering 10% variation as acceptable. If [%O_2] does not evolve as per the described behaviour, further system evaluation should be made by a qualified plant scientist who is familiar with all aspects of plant growth and development within a sealed environment.

Nutrient uptake: As with CO_2 and O_2, plant demand for nutrients will follow a typical plant growth curve (Figure 3). In a sealed hydroponics system, plant demand for nutrients will cause the depletion of ions from the nutrient solution. Ion concentration is monitored by an electrical conductivity probe (EC) and when the ion concentration falls below a specified set point, the HMI will inject concentrated stock solutions to maintain the proper concentration. Stock solution injection is recorded in litres by tag HMI CL4108_EC_TIME and as the experiment progresses, this value will increase as nutrients are injected to compensate for plant consumption. The experiment will be considered as progressing normally if the actual value of accumulated nutrient (litres) increases from one week to the next.

Once the mature crop is established (after 5 weeks), the net weekly accumulation should be approximately equal from week to week, considering 10% variation as acceptable. An inequality

assessment should initiate further system evaluation which should be made by a qualified plant scientist who is familiar with all aspects of plant growth and development within a sealed environment.

6.4 Requirements to execute the test sequence

6.4.1 Personnel: staff qualification and training needs

- MPP technicians trained in HPC1 operation
- MPP personnel with expertise in controlled environment operation, plant physiology and results analysis

6.4.2 Personnel Protective Equipment

- 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

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

- Millwright work (screwdriver, pipe-wrench, …)
- HPC1 nursery as described in AD7
- All sensors are calibrated with certificates (or within validity period of the previous calibration)
- Gas analyzer calibration

6.4.4 Software: verification of software, backup needs

- PLC is connected to the acquisition server
- All acquisitions have been validated
- No back-up acquisition system is needed

6.4.5 Test conditions

The temperature of the HPC1 laboratory should be maintained at 20.0 ± 2.5 degree Celsius and not fluctuate between day and night in order to avoid variation in gas analysis reading as well as large fluctuation of temperature surrounding the Teflon pressure compensation bags (FRT_4114_01, FRT_4114_02 and FRT_4114_03).

Maintenance of hydroponics: the experiment shall continue unless the required maintenance interferes with long-term operation of the hydroponics delivery system. The growing system

provides a suitable buffering capacity that will allow short duration system shut downs to allow repair. MPP engineer/technician in charge of the test should be consulted before proceeding.

6.5 Measurement and data sampling

6.5.1 Recall applicable data collection plan and sampling plan

- PLC is connected to the acquisition server and all HPC1 parameters are being monitored at a frequency of once per second
- Sampling of plant material is performed according to the operating procedure described in TN96.4 (AD6)
- Schematic drawing in Figure 2 shows a dynamic representation of plant age for each tray position during the course of the staggered experiment, as well as the age of the plant being harvested each week.

6.5.2 Data log files

Data extracted from the MPP acquisition server will used the following naming convention:

- The file containing the data related to gas analysis will be named:
  - SCL O2CO2 *yyyy-mm-dd_yyyy-mm-dd*.dat
  - The acquired parameters associated with this file are at least the following ones:

MPP Tags	***Description***
AT_4113_01	CO_2 Analyzer
AT_4113_02	O_2 Analyzer
TT_4112_avg	Chamber Average Temperature
AT_4112_avg	Chamber Average Humidity
FC_4113_01	CO_2 Mass Flow Rate
FC_4113_01_SP	CO_2 Mass Flow Rate set point
TT_4112_06	Laboratory Ambient Temperature
TT_4112_12	Laboratory Ambient Temperature
CL4113_CO2_QUANTITY_INJECTED	CO_2 Volume Injected (L) use set point
CL4113_CO2_QUANTITY_INJECTED2	CO_2 Volume Injected (L) use mass flow reading
CL4113_CO2_injected_in_mol	CO_2 Injected (mole)
PT_4114_01	Chamber Pressure (mbar)
PT_4114_02	Chamber Pressure (mbar)

PT_4114_03	Chamber Pressure (mbar)
PT_4114_04	Chamber Pressure (mbar)
PT_4102_01	Airlock “A” Pressure (mbar)
PT_4103_01	Airlock “C” Pressure (mbar)
RT_4104_01	Light Sensor (µE)
RT_4104_02	Light Sensor (µE)
RT_4104_03	Light Sensor (µE)

- The file containing the data related to hydroponic nutrient loop will be name:
  - SCL NUTRIENT *yyyy-mm-dd_yyyy-mm-dd*.dat
  - The acquired parameters associated with this file are at least the following ones:

MPP Tags	***Description***
FT_4106_01	Hydroponic Solution Flow Rate (L/min)
CL_41_CONDENSATE_TOTALVOLUME	Condensate cumulated (L)
CP_4110_01	Condensate cumulated (number of occurrence)
AT_4107_01	pH (pH)
AT_4108_01	EC (mS)
TT_4109_01	Temperature in Hydroponic Solution
CL4107_Acid_calibration	Acid Calibration Factor
CL4107_Base_calibration	Base Calibration Factor
CL4107_Acid_Opening_Time	Acid valve opening time (s)
CL4107_Base_Opening_Time	Base valve opening time (s)
CL4107_Acid_Injection	Acid Injected volume(ml)
CL4107_Base_Injection	Base Injected volume (ml)
CL4108_SolA_calibration	Nutrient Stock “A” Calibration Factor
CL4108_SolB_calibration	Nutrient Stock “B” Calibration Factor
CL4108_SolA_OP_Time	Nutrient Stock “A” Injected (s)
CL4108_SolB_OP_Time	Nutrient Stock “B” Injected (s)
CL4108_SolA_injection	Nutrient Stock “A” Injected (ml)

CL4108_SolB_injection	Nutrient Stock "B" Injected (ml)

- The file containing the all data related to one staggered experiment be named:
  - SCL ALL *yyyy-mm-dd_yyyy-mm-dd*.dat
  - All acquired parameters will be included in this file
- Samples collected from plant harvest will be named following the convention indicated in Figure 3 (Note: In plant part, S=shoot, R=root, L=Lower part which include roots and rockwool cubes)

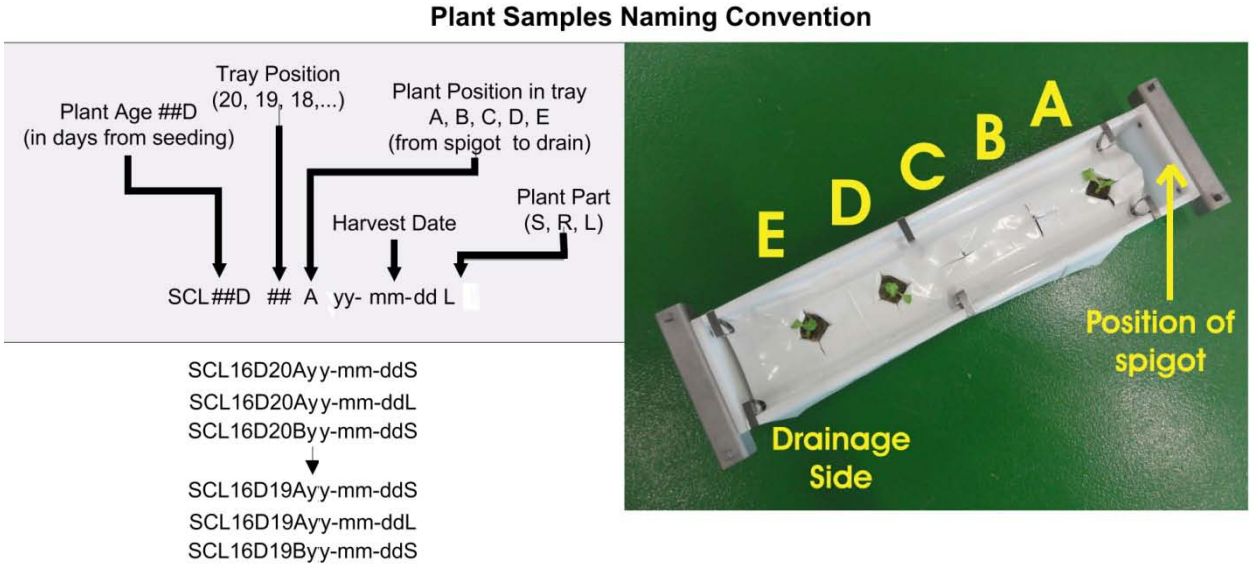

Figure 4. Naming convention for plant samples

6.5.3 Harvest data

- Weight data are recorded in the following records:
  - *MPP-REC-11-4108* for rockwool cubes
  - *MPP-REC-11-4109* for plant material
- All data files including harvest data received from external analytical laboratories are recorded electronically in the files "Tissues CHN analysis" and "Tissues mineral analysis" and stored on the MPP server under the *"COO9- HPC1 Data"* folder and used the sample naming convention indicated in 4.

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

- HMI data are collected every second for all instrumentation.
- The *"yyyy-mm-dd_yyyy-mm-dd"* in a filename stand for the starting and end date of a period covered by the data file (*yyyy*: 4-digits year, *mm*: 2-digits month, and *dd*: 2-digits day)

6.6 Reporting of status for a test (recall of the test plan)

The test sequence is performed by MPP personnel

The final status of the test (passed/fail) is decided at the end of the test in agreement between MPP personnel and MPP management.

6.7 Deviations and non conformances (recall of the test plan)

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".

6.8 Record for the test procedure with the various steps

The test procedure associated to the present protocol is: "MPP-REC-11-4106". A specimen of this record is shown below.

Other records are also used and referred to in this Test Protocol:

- *MPP-REC-11-4101*
- *MPP-REC-11-4102*
- *MPP-REC-11-4103*
- *MPP-REC-11-4104*
- *MPP-REC-11-4106*
- *MPP-REC-11-4107*
- *MPP-REC-11-4108*
- *MPP-REC-11-4109*
- *MPP-REC-11-4110*
- *MPP-REC-11-4111*

The main record "MPP-REC-11-4106" has to be printed and filled out every time the present protocol is executed. Associated records need to be printed and filled out as required.

Compartment : CIVb	
Test procedure title :	Test Record for Lettuce Staggered Culture
Objectives:	This test record is the core record for the lettuce staggered culture in HPC1, it referred to other records as required since each record template may be used multiple times.
Applicable test plan and test protocols	TN 101.4 Test Protocol for staggered culture experiments in HPC1, MPP-OP-11-4101, MPP-OP-11-4102, TN 96.3, Section 6.5, MPP-UM-11-4101, MPP-OP-11-4103, TN 96.4, Section 4.4 and MPP-OP-11-4104
Applicable records	MPP-REC-11-4101, MPP-REC-11-4102 , MPP-REC-11-4103, MPP-REC-11-4104, MPP-REC-11-4107 , MPP-REC-11-4108, MPP-REC-11-4109, MPP-REC-11-4110 and MPP-REC-11-4111
Hardware:	HPC1, HPC1 nursery
Person responsible for the test :	
Test prerequisites:	

Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
		PHASE 1					
1.		Prepare nutrient and stock solutions according to **TN 96.3, Section 6.5.** Solutions must be kept in a dark location in order to prevent algae growth	Should have: 10 L stock A 10 L stock B 10 L acid and base 20 L seedling sln 160 L nutrient sln				
2.		Perform pre-cleaning microbial sampling procedure according to **MPP-OP-11-4104** and fill associated record			MPP-REC-11-4110 ( )____ MPP-REC-11-4111 ( )____		
3.		Perform Cleaning Procedure according to **MPP-OP-11-4102** and fill associated record	Chamber clean with all components in place ready to be tested or used		MPP-REC-11-4102 ( )____		
4.		Perform post-cleaning microbial sampling procedure according to **MPP-OP-11-4104** and fill associated record			MPP-REC-11-4110 ( )____ MPP-REC-11-4111 ( )____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
5.		Prepare nutrient solution loop according to **TN 96.3, Section 6.5**. Complete record **MPP-REC-11-4107(0)**	Nutrient in HPC1 reservoir and hydroponic loop ready to be used		MPP-REC-11-4107( ) ___		
6.		Place the 20 empty HPC1 plant trays inside the chamber if there are not there already	All trays in place, hydroponic system ready to be started				
7.		Activate irrigation system from HMI in auto mode, set point: EC 1.9 mS/cm, and pH 5.9	Irrigation system is activated, water flow is 10-15 L/min		FT_4106_01 : _____ L/min		
8.		Examine every components of the hydroponic loop to see if there is any leak. Place some sheets of papers under the middle junction of the main solution collector to help visualized leakage. If leaks are found interrupt system and repair leak before to restart	no leaks are observed				
9.		Let nutrient solution pH and EC reach their set points, it should take approximately 30 minutes, and then turn irrigation system off	Reading of: AT_4107_01 : EC 1.9 mS/cm AT_4108_01 : pH 5.9 Irrigation system off		pH : _____ EC : _____		
10.		Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take a 100 ml sample in a plastic cup, close valve immediately, and store this sample in the fridge.	Samples of the solution are taken, labelled and place in fridge		Sample ID: Store location: Volumes discard and sampled recorded in: MPP-REC-11-4107(__)___		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
11.		Close nutrient reservoir putting its lid carefully in place and tighten with screws. Make sure it is airtight	Nutrient reservoir is closed and ready to be used				
12.		Calibrate Gas Analyser according to **MPP-UM-11-4101**	Analysis of calibration gas within acceptable error range ± 10 ppm CO_2, reported in appendix 2		Std 999: _____ ppm CO_2 Std 2999: _____ ppm CO_2 Std 21.9%: _____ % O_2		
13.		Verify Nutrient Injection Pumps (MP_4108_01, MP_4108_02) flow rates according to **MPP-UM-11-4101**, enter flow rates into HMI	Flow rate measure and enter into HMI, reference reported in appendix 2		Stock A, MP_4108_01: _____ ml/sec Stock B, MP_4108_02: _____ ml/sec		
14.		Verify acid and base flow rates according to **MPP-UM-11-4101**, enter flow rates into HMI	Flow rate measure and enter into HMI, reference reported in appendix 2		Acid: _____ ml/sec Base: _____ ml/sec		
15.		Verify condensate volume of each event according to **MPP-UM-11-4101**, enter volume into HMI	Volume measure and enter into HMI, reference reported in appendix 2		Flow: _____ ml/min		
16.		Verify nutrient solution flow rate(FT_4106_01) according to **MPP-UM-11-4101**, compare with HMI reading, notify Sherpa if different in order to make the correction	Volume measure same than HMI reading, reference reported in appendix 2		FT_4106_01 : _____ L/min		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
17.		Verify EC probe (AT_4108_01) according to **MPP-UM-11-4101,** if last calibration record is more than three months calibrate	Test verified, reference reported in appendix 2		Std 0.147: _____ mS/cm Std 1.413: _____ mS/cm Std 12.88: _____ mS/cm		
18.		Verify pH probe (AT_4107_01) according to **MPP-UM-11-4101,** if last calibration record is more than three months calibrate	Test verified, reference reported in appendix 2		Std pH 4.01: _____ Std pH 7.00: _____ Std pH 9.21: _____		
19.		Perform Gas Leak Test Procedure **MPP-OP-11-4103**			MPP-REC-11-4103 ( ) ____ CO_2 Leak rate: _____ % / day		
20.		System ready to operate, status off until crop ready to be transferred					
21.		Remove all trays from HPC1 chamber. They will be used to transplant seedlings in the following step. Cover with black plastic film to protect if not used immediately.					
		PHASE 2					
22.	0	**Crop # 1** Germinate 200 seeds according to Lettuce Germination Procedure **MPP-OP-11-4101.** Adapt protocol to produce 100 good seedlings (x4), you will need to germinate at least 200 seeds	At least 200 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( ) ____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
23.	1	**Crop # 1** Label and weight 200 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
24.	1	**Crop # 1** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
25.	7	**Crop # 2** Germinate seeds according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
26.	8	**Crop # 2** Label and weight 50 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
27.	8	**Crop # 2** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
28.	8	**Crop # 1** Label and weight 100 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
29.	8	**Crop # 1** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
30.	9	**Crop # 2** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
31.	9	Activate all components of the HPC1 (except irrigation) with the following parameter: • Light mode auto: 1 MH and 2 HPS lamps per module • Fan mode auto • Temperature and Humidity mode auto: 26/20 °C, 50%/70% RH and 16hrs/8hrs day/night regime • PAR: 270-300 µE • CO_2 Inject auto: 1000 ppm					
32.	9	**Crop # 1** Transfer seedlings to 20 HPC1 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.** The protocol is written for 5 trays, you have to adapt it for 20 trays (x4)	20 trays with 5 seedlings each ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
33.	9	Turn off CO_2 injection					

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
34.	9	Open doors and airlock curtains of both sides of the HPC1 chamber, and sequentially place the 20 trays containing 5 seedlings of lettuce each in the HPC1 according to Lettuce Germination Procedure **MPP-OP-11-4101.**	20 trays containing 5 seedlings each place in chamber		MPP-REC-11-4101 ( ) ____		
35.	9	Turn on irrigation system, and verify that the flow of nutrient solution is distributed evenly in each trays and that no leak are present in the system	All trays in place with proper irrigation				
36.	9	Take pictures of the trays from both end of the chamber	Pictures taken				
37.	9	Close doors A and C, leave airlock curtains open for the moment					
38.	9	Turn on CO_2 Injection to auto mode with a set point of 1000 ppm	Injection of CO_2 until set point, and irrigation system running under nominal conditions				
39.	9	Wait for stable reading of CO_2 and then closed both airlock curtains					
40.	14	**Crop # 3** Germinate seeds according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( ) ____		
41.	15	**Crop # 3** Label and weight 50 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( ) ____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
42.	15	**Crop # 3** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( ) ____		
43.	15	**Crop # 2** Label and weight 25 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( ) ____		
44.	15	**Crop # 2** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( ) ____		
45.	15	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
46.	16	**Crop # 3** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( ) ____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
47.	16	**Crop # 2** Transfer 25 seedlings to 5 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.** Introduce through door A and collect through door C	5 plant trays ready to transfer into HPC1		MPP-REC-11-4101 ( ) ____		
48.	16	**Crop # 1** (16-days old) Harvest trays 16-20 according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
49.	21	**Crop # 4** Germinate according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( ) ____		
50.	22	**Crop # 4** Label and weight 60 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( ) ____		
51.	22	**Crop # 4** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( ) ____		
52.	22	**Crop # 3** Label and weight 25 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( ) ____		
53.	22	**Crop # 3** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( ) ____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
54.	22	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken BEFORE change of solution, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
55.	22	Change hydroponic solution according to **TN 96.4, Section 4.4.** Record solution change in record MPP_REC_11_4107			MPP_REC_11_4107(__)___		
56.	22	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken AFTER change of solution, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
57.	30	**Crop # 4** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
58.	30	**Crop # 3** Transfer 25 seedlings to 5 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.**	5 plant trays ready to transfer into HPC1		MPP-REC-11-4101 ( )____		
59.	23	**Crop # 1** (23-days old) Harvest trays 16-20 according to TN 96.4, Section 4.4.	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( )____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
60.	28	**Crop # 5** Germinate according to Lettuce Germination Procedure MPP-OP-11-4101.	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
61.	29	**Crop # 5** Label and weight 60 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
62.	29	**Crop # 5** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
63.	29	**Crop # 4** Label and weight 25 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
64.	29	**Crop # 4** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
65.	29	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
66.	30	**Crop # 5** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( ) ____		
67.	30	**Crop # 4** Transfer 25 seedlings to 5 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.**	5 plant trays ready to transfer into HPC1		MPP-REC-11-4101 ( ) ____		
68.	30	**Crop # 1** (30-days old) Harvest trays 16-20 according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
69.	35	**Crop # 6** Germinate according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( ) ____		
70.	36	**Crop # 6** Label and weight 60 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( ) ____		
71.	36	**Crop # 6** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( ) ____		
72.	36	**Crop # 5** Label and weight 25 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( ) ____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
73.	36	**Crop # 5** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( ) ____		
74.	36	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken BEFORE the change of solution, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
75.	36	Change hydroponic solution according to **TN 96.4, Section 4.4.** Record solution change in record MPP_REC_11_4107			MPP_REC_11_4107(__)___		
76.	36	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken AFTER the change of solution, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
77.	44	**Crop # 6** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( ) ____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
78.	44	**Crop # 5** Transfer 25 seedlings to 5 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.**	5 plant trays ready to transfer into HPC1		MPP-REC-11-4101 ( ) ____		
79.	44	**Crop # 1** (37-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
80.	42	**Crop # 7** Germinate according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( ) ____		
81.	43	**Crop # 7** Label and weight 60 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( ) ____		
82.	43	**Crop # 7** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( ) ____		
83.	43	**Crop # 6** Label and weight 25 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( ) ____		
84.	43	**Crop # 6** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( ) ____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
85.	43	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
86.	44	**Crop # 7** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( ) ____		
87.	44	**Crop # 6** Transfer 25 seedlings to 5 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.**	5 plant trays ready to transfer into HPC1		MPP-REC-11-4101 ( ) ____		
88.	44	**Crop # 2** (37-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
89.	49	**Crop # 8** Germinate according to Perform Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( ) ____		
90.	50	**Crop # 8** Label and weight 60 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( ) ____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
91.	50	**Crop # 8** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( ) ____		
92.	50	**Crop # 7** Label and weight 25 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.** Then autoclave them	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( ) ____		
93.	50	**Crop # 7** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( ) ____		
94.	50	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken BEFORE the change of solution, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
95.	50	Change hydroponic solution according to **TN 96.4, Section 4.4.** Record solution change in record MPP_REC_11_4107			MPP_REC_11_4107(__)___		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
96.	50	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken AFTER the change of solution, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
97.	51	**Crop # 8** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( ) ____		
98.	51	**Crop # 7** Transfer 25 seedlings to 5 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.**	5 plant trays ready to transfer into HPC1		MPP-REC-11-4101 ( ) ____		
99.	51	**Crop # 3** (37-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
100.	56	**Crop # 9** Germinate according to Perform Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( ) ____		
101.	57	**Crop # 9** Label and weight 60 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( ) ____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
102.	57	**Crop # 9** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( ) ____		
103.	57	**Crop # 8** Label and weight 25 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( ) ____		
104.	57	**Crop # 8** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( ) ____		
105.	57	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
106.	58	**Crop # 9** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( ) ____		
107.	58	**Crop # 8** Transfer 25 seedlings to 5 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.**	5 plant trays ready to transfer into HPC1		MPP-REC-11-4101 ( ) ____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
108.	58	**Crop # 4** (37-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
109.	64	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
110.	65	**Crop # 5** (37-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
111.	65	**Crop # 6** (30-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
112.	65	**Crop # 7** (23-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
113.	65	**Crop # 8** (16-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
		PHASE 3					
114.		Calibrate Gas Analyser according to **MPP-UM-11-4101**	Analysis of calibration gas within acceptable error range ± 10 ppm CO_2, reported in appendix 2		Std 999: _____ ppm CO_2 Std 2999: _____ ppm CO_2 Std 21.9%: _____ % O_2		
115.		Perform Gas Leak Procedure **MPP-OP-11-4103**			MPP-REC-11-4103 ( ) ____ CO_2 Leak rate: _____ % / day		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
116.		Perform pre-cleaning microbial sampling procedure according to **MPP-OP-11-4104** and fill associated record			MPP-REC-11-4110( ) ____ MPP-REC-11-4111 ( ) ____		
117.		Verify Nutrient Injection Pumps (MP_4108_01, MP_4108_02) flow rates according to **MPP-UM-11-4101**, enter flow rates into HMI	Flow rate measure and enter into HMI, reference reported in appendix 2		Stock A, MP_4108_01: _____ ml/sec Stock B, MP_4108_02: _____ ml/sec		
118.		Verify acid and base flow rates according to **MPP-UM-11-4101**, enter flow rates into HMI	Flow rate measure and enter into HMI, reference reported in appendix 2		Acid: _____ ml/sec Base: _____ ml/sec		
119.		Verify condensate volume of each event according to **MPP-UM-11-4101**, enter volume into HMI	Volume measure and enter into HMI, reference reported in appendix 2		Flow: _____ ml/min		
120.		Verify nutrient solution flow rate(FT_4106_01) according to **MPP-UM-11-4101**, compare with HMI reading, notify Sherpa if different in order to make the correction	Volume measure same than HMI reading, reference reported in appendix 2		FT_4106_01 : _____ L/min		
121.		Verify EC probe (AT_4108_01) according to **MPP-UM-11-4101**, if last calibration record is more than three months calibrate	Test verified, reference reported in appendix 2		Std 0.147: _____ mS/cm Std 1.413: _____ mS/cm Std 12.88: _____ mS/cm		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
122.		Verify pH probe (AT_4107_01) according to **MPP-UM-11-4101,** if last calibration record is more than three months calibrate	Test verified, reference reported in appendix 2		Std pH 4.01: _____ Std pH 7.00: _____ Std pH 9.21: _____		
123.		Perform Cleaning Procedure according to **MPP-OP-11-4102** and fill associated record	Chamber clean with all components in place ready to be tested or used		MPP-REC-11-4102 ( ) ____		
124.		Perform post-cleaning microbial sampling procedure according to **MPP-OP-11-4104** and fill associated record			MPP-REC-11-4110( ) ____ MPP-REC-11-4111 ( ) ____		
125.		If chamber is not going to be used shortly, a HPC1 long term shut down procedure should be performed according to **MPP-UM-11-4101**					

Conclusion for the Test (as provided in the test plan)	*Name*	*Signature*	*Date*
☐ Passed ☐ Failed			
Comments **Add deviation record including decision description**			
Checked **MPP-UAB**	*Name*	*Signature*	*Date*

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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	Signature

Appendix 3 – Deviations

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

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7. Comments

general comments		MPP answers
1	The logic to put some of these documents as RDs only (i.e. and not AD) remains not clear to us. This is especially true for RD3 and RD5.To be updated along the doc	Amended: RD3 and RD5 included as AD

detailed comments				MPP answers
nber	page	section	comment	
1	5	2,1	name of OP -11-4103 to be updated	Section 2.1 modified
2	6	4,2	A bullet about microbial hazards should be included	Bullet added in Section 4.2: Microbial hazards
3	8	5 phase1	for the verification of equipment calibration, we have to clarify what belongs to the TRR, where hardware status should go through verification (corresponding to be given as justification), and what belongs to this phase 1	We understand the activities included in this phase are performed inside the testing campaign, so after the TRR has been performed successfully
4	8	5 after phase3	Difficult to review without the details of the user manual	Draft version of the manual included with this package
5	11	6,2	There is no clear distinction between parameters followed regularly, authorizing the continuation of the experiment, and global parameters to be assessed over a full staggered culture campaign. To be clarified all along 6.2. This comment remains valid; for the sake of clarity, it would be better to identify the weekly followed parameters and the ones evaluated over the whole culture. The main reason is that you do not calculate them the same way, sometimes the success/failure is not evaluated the same way	The parameters to be followed regularly for authorizing the continuation of the experiment are in the updated section 6.3. All parameters discussed in section 6.2 are intended for final analysis on a weekly or total experimental periods and not for authorizing continuation of exp.
6	11	6.2.2	What about the total oxygen production over the whole test?	This is the 3th equation in the table which is the sum of each week.

7	12	6.2.3	Carbon balance is targeted here. Therefore carbon evolution in the gas phase, in the solid phase (plant and possibly rockwool) and in the liquid phase is to be looked at. In the calculations provided below, a certain number of assumptions are made (there seems to be no carbon transfer to the hydroponic system, carbon loss from chamber leakage and airlock opening seem to be constant,…) and they should be clearly stated and justified. In addition the actual carbon from analysis (harvest data) is referred to but not described elsewhere in the document. The global carbon balance should be clarified. Is there no interest to look at the carbon content for the different development stages and plant size as done for the nitrogen?	Carbon in the gas phase: - Carbon lost from leakage is in equation 2 - Carbon lost from airlock is in equation 3 and is a constant that was demonstrated in previous experiments - Carbon in hydroponic should be constant since the volume of water, pH and Ec, and the pressure of CO2 maintain in the system, we can measure the dissolved CO2 and add it to gain in precision - The harvest was described in TN96.4 and is now referred in section 6.5.3
8	12	6.2.3	Where are recorded the carbon analyses from plant harvest?	In the corresponding tissues analysis files. Reference to these files have been added in section 6.5.3
9		6.2.3	Do you mean daily or weekly CO_2 up-take?	Both the daily and weekly are all evaluated and part of the SLC gases file, see Section 6.5.2
10	12	6.2.3	Several comments on the table: - the daily (or weekly) or global approaches should follow different calculations, as for the N related calculations; - the losses linked to the nutrient loop, as described in the corresponding OP are not visible here. - Wording consistency issues between this TN and the OP - In the OP, losses/leaks are evaluated as % per event, here you have g of C, please clarify	Calculations reformulated as for the N calculations C losses in the hydroponics described, even if consider minor Percent C is converted to g C as this is the unit of mass.
11	13	6.2.5	It would be interesting, during the weeks of staggered culture, to analyze total N, and nitrate and ammonium as well	This is described in Section 6.2.8. The data will be part of the SLC nutrient file, section 6.5.2
12	13	6.2.5 bullet4	Where are those analyses recorded?	This is described in Section 6.2.8. The data will be part of the SLC nutrient file, section 6.5.2
13	13	6.2.5 bullet5	Where are those data recorded?	In the corresponding tissues analysis files. Reference to these files have been added in section 6.5.3

14	15	6,3	3 physiological parameters: From previous discussions we understood oxygen and water would also be a physiological parameters CP: agreed for the water issue. To avoid confusion, this explanation should be reported in the text. In addition, weekly records should be kept and provided for info	Oxygen could be relevant but water is a combination of evaporation and transpiration and is therefore not a direct measurement of plant activity, to our opinion won't be a good indicator of that the plants are growing normally The weekly screen print out of these parameters are sufficient to evaluate the continuation or not of the experiment Records are kept by the control system and in the HPC1 log book. A daily parameter checklist should be employed for future experiments to ensure proper system functioning. This will be described in detail in TN 101.5 Explanation regarding water reported in the text
15	15	6.3 C assimil.	These successful criteria up to week 5. From previous discussions, I understood that from week 5, the carbon injection profile would be compared from one week to the other and that reproducibility of the carbon injection profile would be the successful criteria. Idem for the oxygen accumulation profile and condensed water accumulation profile.	Agreed. Periods before 5 weeks and after that time have been distinguished in the text. For the steady period after 5 weeks, an acceptance range of +/- 10% is proposed.
16	15	6.3 C assimil.	Larger is a very vague statement	Considered valid enough for the initial period before 5 weeks, as the steady periodf is considered the critical one.
17	16	6.3 nutrient up	same comments as for CO2 injection, O2 and condensed water accumulation	See previous comments
18	17	6,5	This section does not mention the hydroponic solution sampling and analyses (mineral, microbial, etc.). Please clarify.	Section 6.5 was completed
19	19	figure3	From this naming convention, how do you intent to trace the crop number when performing a staggered test?	The number attributed to each tray is unique since it is assigned sequentially from tray 1 up to the last tray, each batch being attributed the next set of number (i.e if the last tray was 35, the next batch will be 36, 37,38, 39 40). Section 6.5.3 was corrected accordingly.
20	19	6.5.3	Naming convention for plant samples should be used in the record	It is now used in all records

21	20	6,8	Titles of the records would help, or reference to protocol and procedures diagram as a minimum	Titles were added to section 6.8
22	20	6.8 REC-11-4107	time of addition, replacement is of importance and should be the ones of the control/acquisition system (synchronization)	The "Time" has been added to the "Date" column of the RECORD, The HMI is collecting data every 10 sec, so I am not sure that there is any synchronization issue
23	21		Please indicate in the header of the record the versions of the documents mentioned	Versions can be added at the beginning of a campaign, space has been reserved for this purpose in the header

ANNEXES

ANNEX 1: Commented Draft

Call-Off Order 9

HPC1 characterization

WP-101.3

Characterization of staggered cultures

TN 101.4

Test Protocol for staggered culture experiments in the HPC1

Prepared by/Préparé par Côté, R. and Peiro, E.

Reference/Réference MELiSSA Pilot Plant Frame Contract 19445/05/NL/CP
Issue/Edition 1
Revision/Révision 0
Date of issue/Date d'édition 15/09/11
Status/Statut Draft

APPROVAL

Title *Titre*	TN101.4 Test Protocol for staggered culture experiments in the HPC1	Issue *Edition* 1	Revision *Révision* 0

Prepared by *Auteur*	Côté, R. and Peiro, E.	Date *Date*	15/09/11
Checked by *Verifié par*	E. Peiro and A. Fossen	Date *Date*	
Approved by *Approuvé par*	Gòdia, F.	Date *Date*	
Approved by customer *Approuvé par le client*	Lamaze, B. and Paillé, C.	Date *Date*	

CHANGE LOG

Issue/*Edition*	Revision/*Révision*	Status/*Statut*	Date/*Date*
0	0	Final	15/01/11
1	0	Draft	15/09/11

Distribution List

Name/*Nom*	Company/*Société*	Quantity/*Quantité*
Brigitte LAMAZE	ESA	2 hardcopies + electronic version
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Overall Manager	MPP	1 copy in QC 1119
Quality Manager	MPP	the original signed copy in QC1004

Change log:

Date	Issue	Reason of the change	Modified paragraphs
15/01/2011	(0)	Creation	
15/09/2011	(1)	Update as per ESA comments	See comments in Section 7

TABLE OF CONTENT

1. Scope

The present protocol describes the main steps to follow in order to operate the HPC1 compartment during staggered culture of lettuce. This document and its associated operational protocols will be used in a routine basis in the future operation of the MPP.

2. Reference and applicable documents

2.1 Applicable documents

AD1	MPP-QA-07-0001	MPP Quality Manual
AD2	MPP-QA-07-0003	MPP rules for good lab practices
AD3	MPP-OFR-10-4101(0)	MPP Proposal for Call-off Order 9: HPC1 characterization phase in the MELiSSA Pilot Plant
AD4	TN 96.3, Section 6.5	Test protocol for lettuce cultivation, 6.5 Solution Preparation
AD5	TN 96.4, Section 4.4b (except for ethylene sampling)	Protocols for sampling and analysis, 4.4b Harvest and sampling protocol
AD6	MPP-OP-11-4101	Lettuce Germination and Transfer Procedure
AD7	MPP-OP-11-4102	Cleaning Operating Procedure
AD8	MPP-OP-11-4103	Gas Leak Test Procedure
AD9	MPP-OP-11-4104	Microbial Monitoring Procedure
AD10	MPP-OP-10-4101	Procedure for rockwool safe manipulation
AD11	MPP-PID-10-4101-A6	HPC1 P&ID
AD12	MPP-UM-11-4101	MPP Internal User manual for HPC1
AD13	TN 95.1	Sherpa HPC Control requirement and software description

2.2 Reference documents

RD1	TN 85.71	HPC1 User Manual

Comentario [RC1]: The issue number is identify in the record, which is a representation of what is actually done for each test

BL: agreed, but not always handy to follow (e.g. from REC 11-4106, you have to go to e.g. REC -11-4102 to see which version of OP -11-4102 is applicable in REC-11-4106), should be valid for other information, see comments in various REC

Comentario [bl2]: The latest version changed name! then we face wording consistency issues all along the TN

MAS: Names change as documents evolve. Consistency in the TN has been reviewed.

Comentario [bl3]: The logic to put some of these documents as RDs only (i.e. and not AD) remains not clear to us. This is especially true for RD3 and RD5. See later in the docs. Can be addressed AFTER the audit, for the TRR

MAS: How about just a list of “reference documents”? ‘Applicable’ and ‘reference’ differ little in meaning.

MPP: we keep considering RD and AD to be different meaning; groups have been updated accordingly

3. Acronyms/Definitions

HPC1	Higher Plant Compartment 1
MPP	MELiSSA Pilot Plant
EC	Electrical Conductivity
MSDS	Material Safety Data Sheet
ppm	Part per Million
UAB	Universitat Autònoma de Barcelona
UoG	University of Guelph
PID	Piping and Instrumentation Diagram
C / NC	Compliant / Non-Compliant

4. Test items

4.1 Description (PID, technical drawings, user manual)

- Higher Plant Compartment (HPC1) is described in document RD1 and AD12
- Maintenance and calibration of HPC1 components are described in document AD12
- PID and Sherpa control are describe in documents AD11 and AD13 respectively
- The main MPP operating procedures applicable for this protocol are described in documents AD4, AD5, AD6, AD7, AD8, and AD9
- Instruction on safe utilization of MPP material and equipment can be found in document AD10, and corresponding MSDS

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

- Mechanical hazard (pump, blower)
  - Hazards are mitigated through the use of closed access panels. Removal shall only be performed by qualified personnel.
- Pressure hazards (compressed gases mixtures in K-size tanks at 200barg, and N_2 and O_2 building supplies at 6 bars)
  - Gas pressure regulators reduce gas cylinder and building supply tubing pressure to 2 barg

- Chemical hazards (use of acid, base)
  - Labcoats, gloves and goggles ensure protection of personnel from corrosive reagents
- Handling of rockwool
  - Labcoats, gloves, goggles and dust masks permit safe handling of rockwool material ; if needed, an extraction hood is also available (see AD10)
- Microbial hazards
  - Labcoats and fresh disposable gloves will be worn when transferring seedlings in order to mitigate microbial contamination
  - For microbial sampling activities, the use of masks is as well needed.

4.3 Instructions for operation

- See user manual and operating procedures (AD4, AD5, AD6, AD7, AD8, AD9, and AD12).

4.4 Instructions for maintenance

4.4.1 Hydroponic System

- Make sure that while running the hydroponic system, no leaks are observed from:
  - the HPC1 plant trays
  - the nutrient collector, particularly at the middle junction (VSSL_4111_01)
  - the nutrient tanks (VSSL_4106_01, LSL_4110_01, LSH_4110_01, HV_4106_09)
  - the acid, base and concentrate nutrient reservoirs (VSSL_4107_01, SV_4107_01, HV_4107_01, LSL_4107_01, VSSL_4107_02, SV_4107_02, HV_4107_02, LSL_4107_02, VSSL_4108_01, SV_4108_01, HV_4108_01, LSL_4108_01, MP_4108_01, VSSL_4108_02, SV_4108_02, HV_4108_02, LSL_4108_02 and MP_4108_02)
  - the condensate reservoir (VSSL_4110_01, LSH_4110_02, LSL_4110_02, CP_4110_01, and HV_4110_02)
  - any pumps and valves (HV_4106_01, HV_4106_02, HV_4106_03, HV_4106_04, HV_4106_05, HV_4106_06, HV_4106_07, HV_4106_08, HV_4106_10, HV_4106_11, HV_4106_12, HV_4106_13, HV_4106_14, GP_4106_01, FT_4106_01 and HV_4110_01)
- if any leak is detected from the above componentscorrective measure should be taken, such as:
  - retighten leaky junctions
  - replace defective components (tubing, tees, adapters, ferrules, etc.)
  - for the nutrient collector tray (VSSL_4111_01)apply MS-tech sealant (by Ceys SA) to the middle section if leaky and wait for the sealant to cure before restarting the hydroponic loop

Comentario [RC4]: Tag not existing, not necessary to have tags, but each tray should be IDed (Enrique will check with CIFA for punch labelling)

Comentario [m5]: All of this makes things far too complicated and overwhelming. Just look for leaks – if they are found then follow up with repair.

Comentario [AF6]: Is there any special requirement to prevent interaction of sealant with hydroponic solution? ? ACCORDING TO THE MANUFACTURER THE SEALANT SHOULD BE INERT AFTER CURED

4.4.2 Gas and Air Handling System

- Perform a leak test according to AD8. If the calculated CO_2 daily leakage rate is higher than 7% per day, identify the leak source using the leak identification procedures outlined in AD8 and repeat until the CO_2 leak rate is less than 7% per day.

5. Recall of test sequence

Below is a summary of the test sequence and its corresponding flow chart (Figure 1).

- Phase-1: Phase-1 consists of HPC1 preparation for the staggered culture experiment and evaluating the microbial population present before and after cleaning. The required steps are as follows:
  - Pre-culture microbiological sampling before cleaning
  - Preparation of HPC1 compartment including cleaning of the chamber, the air handling unit and the liquid loop
  - Pre-culture microbiological sampling after cleaning
  - Preparation of hydroponic solutions and liquid loop
  - Verification of equipment calibration
  - HPC1 Leak tests

Comentario [bl7]: We have to clarify what belongs to the TRR, where hardware status should go through verification (corresponding to be given as justification), and what belongs to this phase 1

- Phase-2: Phase-2 is repeated weekly, except for the change of hydroponic solution which is performed every two weeks. During Phase-2 new seedlings are started every week and transferred to the chamber after 9 days. It will take 4 weeks to reach a full staggered culture profile (i.e. simultaneous presence of plants of 4 different ages). The required steps are as follows:
  - Preparation of lettuce seedlings in the nursery
  - Transfer of seedlings into HPC1 plant trays
  - Transfer of the trays into the HPC1 compartment (20 trays the first week, and 5 trays per week thereafter)
  - Harvest of lettuce (5 trays per week and all the trays on the last harvest at week-10) and preparation of samples for subsequent analytical procedures
  - Replacement of hydroponic solution is performed every two weeks

- Phase-3: Phase-3 consists of verifying the status of the HPC1 and of a post experiment microbial population evaluation of the chamber. The microbial population will be tested before and after final growth chamber cleaning.
- The steps are defined as follows:
  - HPC1 Leak tests (take note that these may be inconsistent if there is a high residual microbial population)
  - Verification of equipment calibration
  - Post-culture microbiological sampling before cleaning
  - Cleaning of the chamber, the air handling unit and the liquid loop

Comentario [bl8]: In between two cultures, most probably, a post-conditioning of the chamber should be performed which may vary with the time lag between one campaign and the next one. To be described here or in another document.

BL:OK

- Post-culture microbiological sampling after cleaning

- After Phase 3, the chamber is clean and ready to be used for the next experiment. If no experiment is planned, the system should be conditioned for long term shut down according to the AD12 "MPP-UM-4101 HPC Internal User Manual". Some of the actions during long term shut down include:
  - Storage of pH and EC probes in electrolyte solutions
  - Drying off the hydroponic loop (Tank, reservoirs, tubing, HVAC area, etc)
  - Emptying stock solutions, acid and base

Comentario [bl9]: See previous comment; this doc is an AD not only an RD.

fixed

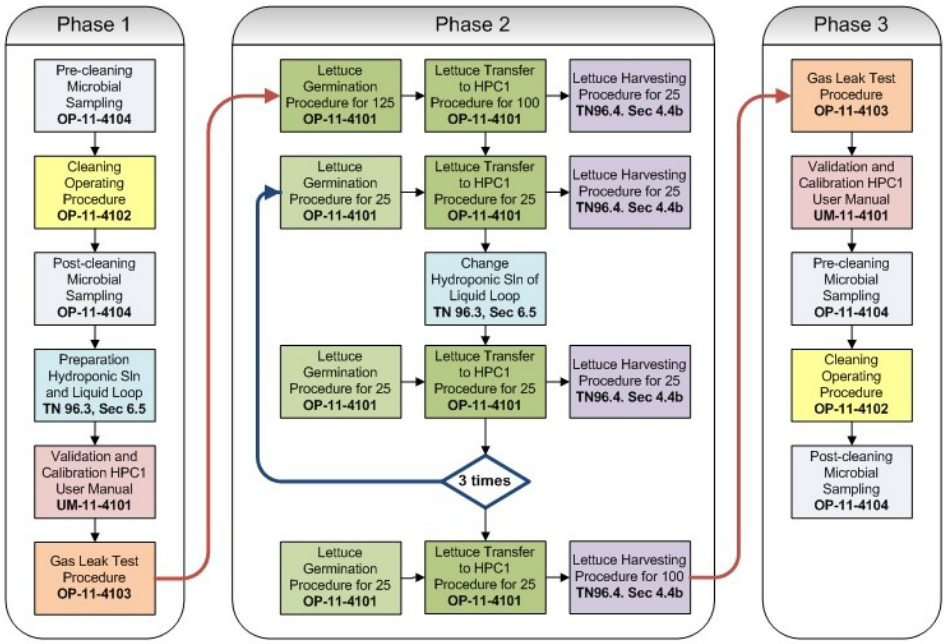

Figure 1 Staggered Culture Flow Chart

6. Test protocol

6.1 Requirements addressed by the test

Taking into account all the previous available results in batch conditions, a subsequent series of experiments has been designed to observe staggered culture of lettuce in the HPC1, with the aim to characterize HPC1's performances under this mode of operation and, on a longer term, to optimize

experimental variables. Staggered culture should provide a steady production of O_2, potable water and edible biomass as well as uptake of CO_2.

Phase-2 which defines the experimental phase of this protocol is comprised of two parts (see Figure 2). The first part starts by filling the whole chamber with 9-days old plants (20 trays of 5 plants each). Then, each week, 5 new trays containing 9-days old plants are introduce to the chamber while 5 of the oldest trays are removed for harvest and analysis, resulting in the following profiles:

Week2: 100 plants from Crop 1
Week3: 25 plants of Crop 2 and 75 plants of Crop1
Week4: 25 plants of Crop 3, 25 plants of Crop 2 and 50 plants of Crop1

Part 2 starts on Week 5, when the 4 stages of plant development are present simultaneously in the chamber and define the beginning of the staggered culture profile (Weeks 5, 6,7,8, and 9: 25 plants of Crop 4, 25 plants of Crop 3, 25 plants of Crop 2 and 25 plants of Crop 1).

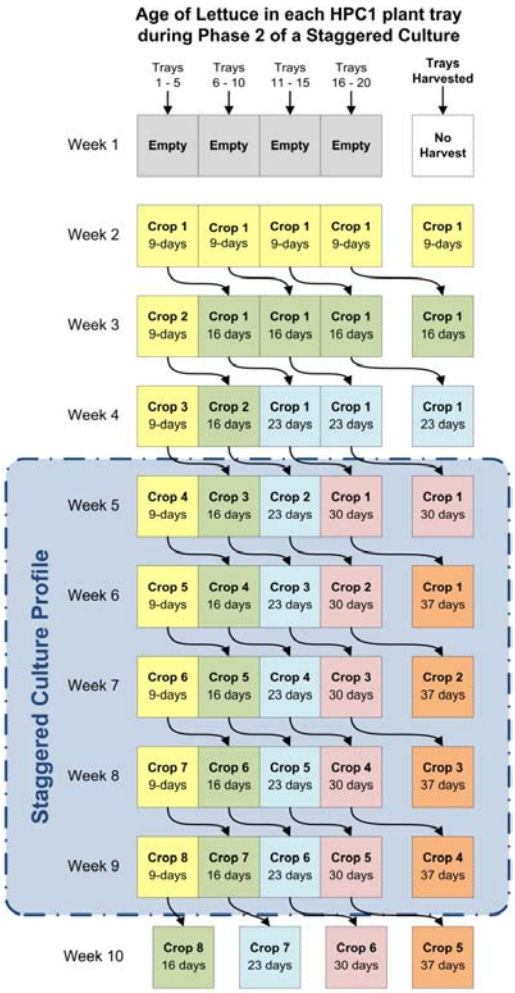

Figure 2 Age of lettuce for each plant trays during the course of the experiment

6.2 Features to be tested: functions, hardware, software

Staggered culture of lettuce will be evaluated in terms of production aspects, providing the data necessary for the characterization of the HPC1 in the scope of the MELiSSA loop closure.

Monitoring of physiological parameters that would normally increase on a daily basis (carbon assimilation, O2 accumulation, water accumulation, nutrient consumption) should be continuously performed by qualified personnel (minimally daily) and the HMI to ensure plant health and proper growing conditions are not compromised. Also assessment of situations that cause poor growth and development provides a wealth of information to be used in the study, management and mitigation of system failure scenarios.

At the conclusion of data analysis (TN 101.5), recommendations for daily monitoring requirements will be made to ensure the system evolves into one that provides consistent results on a continuous basis. The method of sample collection and data analysis for specific parameters is indicated below. Details on data collected from the HMI can be found in section 6.5.

6.2.1 Biomass production and harvest index

Fresh weight of individual shoots will be taken at harvest as indicated in Figure 2. Plant shoots will be bagged individually and dried in ventilated drying oven at 60-70 degrees Celsius in order to obtain the shoot dry weight. The lower part of the plant, which is comprised of the roots and pre-weighted rockwool cubes, will also be dried in ventilated drying oven at 60-70 degree Celsius. Roots emerging from the rockwool cubes will be grouped on a per tray basis since they would be impossible to separate from each other at this stage. They will then be dried in ventilated oven (60-70°C). Data will be used to determine biomass production and harvest index for each stage of growth (9-days, 16-days, 23-days, 30-days and 37-days) and to estimate the contribution that each stage of growth has on the chamber mass balance (see sections below) .

1. **Root dry weight (g)**

lower plant part including rockwool cubes (g) – pre-weighted rockwool cubes (g) + emerging roots (g)

2. **Biomass production**

total plant dry weight (g) / time (day)

3. **Harvest index**

edible* part dry weight (g) / total plant dry weight (g)

** In the case of lettuce the edible portion of the plant is the entire shoot*

6.2.2 Oxygen production

Concentration of oxygen is continuously monitored by the HMI system. From these data the weekly and total oxygen production for the entire experiment will be calculated.

Comentario [bl10]: There is no clear distinction between parameters followed regularly, authorizing the continuation of the experiment, and global parameters to be assessed over a full staggered culture campaign. To be clarified all along 6.2

MAS: This is a single replicate experiment. The parameters assessed are based on 'can we grow lettuce in a continuous production scenario'. The primary result is biweekly yield. We are also assessing 'does the chamber work'. The basic yield data will tell us that. The rest of the information gathered is mostly fodder for modelling this concept into the MELiSSA system.

As mentioned elsewhere, the only thing that should prevent continuation of the experiment is complete plant mortality. Failure analysis is a very important part of this research. There are a number of parameters that should be assessed on a daily basis to ensure proper system operation, but they are for taking remedial action and not for premature conclusion of an experiment.

Comentario [bl11]: This comment remains valid; for the sake of clarity, it would be better to identify the weekly followed parameters and the ones evaluated over the whole culture. The main reason is that you do not calculate them the same way, sometimes the success/failure is not evaluated the same way.

Comentario [EP12]: So individual weighing is not needed for the rockwool cubes? Otherwise what is the meaning of this?

RC: INDIVIDUAL WEIGHTING IS STILL REQUIRED' THE REASON FOR COMBINING THE ROOT MATERIAL FROM ONE TRAY IS THAT AT THIS STAGE THE ROOTS FROM THE DIFFERENT BLOCS SHOULD BE ALL ENTANGLED AND IMPOSSIBLE TO SEPARATE, HOWEVER THE UPPER PARTS CAN BE EASILY BE SEPARATED

Comentario [C. P.13]: What about the total oxygen production over the whole test?

MAS: 'total oxygen production' = the whole test

1. **Weekly Increase in O_2 concentration (%)**

O_2 conc after weekly transfer (%) – O_2 conc before the following weekly transfer (%) – (7 x Daily O_2 leak (%))

2. **Weekly O_2 production (mol.week^{-1}.m^{-2})**

$$\frac{\text{Weekly increase in } O_2 \text{ concentration (\%) x HPC1 volume (L) x HPC1 pressure (Pascal)}}{100 \times 8.314472\ J.K^{-1}.mol^{-1} \times \text{HPC1 temperature (K) x HPC1 area } (m^2)}$$

3. **Total O_2 production (mol.m^{-2})**

$$\sum \text{Weekly } O_2 \text{ production } (mol.m^{-2})$$

6.2.3 Carbon mass balance

Concentration of CO_2 in the chamber is maintained constant at 1000 ppm. The injection of CO_2 is monitored continuously by the HMI system and this data is used in subsequent calculations of CO_2 assimilation, net carbon exchange rate (NCER), and night time respiration. Carbon balance is calculated from the total carbon obtained from the plant material, divided by the total carbon dioxide injected (weekly calculated), minus losses.

Comentario [EP14]: The term "carbon from airlocks" should be better defined, shouldn't it? CARBON FROM AIRLOCK IS A VALUE OBTAINED FROM PREVIOUS EXPERIMENT AND IS CONSTANT VALUE PER OPENING MPP-TN-10-4103_ Leak and Diffusion Tests

Preliminary calculations:

a) Total carbon accumulated for gas injection per one week period

Estimated C accumulation (g) calculated from CO_2 injection data recorded in the HMI

b) Total carbon removed from hydroponic solution per 2 weeks period

C analyzed in sln after 2 weeks

c) Total carbon accumulation in plant tissues per one week period

C in plant* (g. g^{-1} dry weight) x total plant dry weight (g)

Three stage calculation for carbon balance:

1. Total gross C balance

$$\frac{\text{C in plant (g. g}^{-1}\text{ dry weight) x total plant dry weight (g)}}{\text{C accumulation from gas injection (g) - C discarded with nutrient solution (weekly estimate)}}$$

2. Total C balance corrected for chamber weekly leakage

$$\frac{\text{C in plant (g. g}^{-1}\text{ dry weight) x total plant dry weight (g)}}{\text{[C accum. gas injection (g) x (1 - (\%leak per week / 100))] - C discarded with nutrient sol. (weekly estimate)}}$$

3. Total C balance corrected for weekly chamber leakage and air lock losses

$$\frac{\text{C in plant (g. g}^{-1}\text{ dry weight) x total plant dry weight (g)}}{\text{[C accum. gas injection (g) x (1 - (\%leak per week / 100))]− C from airlocks (g)** - C discarded nutrient sol. (weekly estimate)}}$$

The ratio of measured carbon injected to actual carbon from analysis **should equal 1** for complete system closure and carbon accountability, however there will be unaccounted gains/losses due to microbial respiration, algae photosynthesis, inaccuracies in root mass quantification, inaccuracies in the mass flow quantification at the sensor level (sensor accuracy).

* For actual carbon from analysis, see harvest data (TN96.4 and related records)
** Carbon from airlock was determined by a series of separate experiments (according to AD8)

Comentario [bl15]: Several comments on the following table:
-the daily (or weekly) or global approaches should follow different calculations, as for the N related calculations;
-the losses linked to the nutrient loop, as described in the corresponding OP are not visible here.
-Wording consistency issues between this TN and the OP
-In the OP, losses/leaks are evaluated as % per event, here you have g of C, please clarify

RC: Percent C is converted to g C as this is the unit of mass.

6.2.4 Water production

The production of clean water via the process of evapotranspiration is monitored by HMI by calculating the accumulated condensate water returning to the hydroponic loop after being condensate in the HVAC compartment of the HPC1. The value is usable directly as is since it is reported in volume unit (L).

Comentario [C. P.16]: For the water use efficiency: you mean total carbon accumulation in the plant? In addition, the time basis should be clarified otherwise using 1. to calculate 2. does not make sense. (1. and 2. should be calculated for the same time period).

Should be total edible biomass, not carbon

1. **Total water production for the experiment**

   Total accumulated water from evapotranspiration (condensate volume) (L)

2. **Rate of water production corrected**

   Total accumulated water from evapotranspiration (L) − total water lost or gained during plant transfer (L)

3. **Water use efficiency**

$$\frac{\text{Total edible biomass (g)}}{\text{Total water production (L)}}$$

6.2.5 Nitrogen mass balance

In order to calculate nitrogen mass balance the following parameters are required:

- Volume (recorded by the HMI) and concentration (from nutrient recipe) of acid injected,
- Volume (recorded by HMI) and elemental composition (from nutrient recipe) of nutrient solution A and B injected
- Volume (from nutrient recipe) and elemental analysis (submitted to laboratory) of fresh nutrient solution transferred to the main nutrient tank
- Total volume of nutrient solution in the hydroponic loop including rockwool holding capacity (calculated value recorded in MPP-REC-11-4107)
- Volume (measured and recorded in MPP-REC-11-4107) and mineral and elemental analysis (submitted to laboratory) of hydroponic solution prior to replacement every 2 weeks
- Total nitrogen content of shoot and root at harvest and at the time of transfer into the HPC1 (submitted to laboratory)
- Dry weights of plants at harvest and seedlings at time of introduction in the chamber (recorded in MPP-REC-11-4108 and MPP-REC-11-4106 respectively)

The above parameters will permit the calculation of the nitrogen mass balance.

Comentario [bl17]: It would be interesting, during the weeks of staggered culture, to analyse total N, and nitrate and ammonium as well

Comentario [m18]: The total dry mass of 9-day-old seedlings would be minimal. Should there be a discrepancy in the calculated values after data analysis, revised methodology for this calculation will be suggested.

1. **Total nitrogen removed from hydroponic solution per 2 weeks period**

   (N in fresh sln + N injected as nutrient + N injected as acid) –(N in sln after 2 weeks + N in sln Sampling)

2. **Total nitrogen accumulation in plant tissues per two week period**

   N in plant (g. g^{-1} dry weight) x total plant dry weight (g)

3. **Nitrogen mass balance**

$$\frac{\sum \text{nitrogen accumulation in plant tissue (g)}}{\text{(N in fresh sln + N injected as nutrient + N injected as acid) –(N in sln end of cycle + N in sln Sampling)}}$$

The ratio of nitrogen accumulation in plant tissue to nitrogen added to the hydroponic solution **should equal 1** for complete system closure and nitrogen accountability, however there will be unaccounted gains/losses due to microbial nutrition, inaccuracies in root mass quantification, inaccuracies in quantification method (analysis accuracy). In order to account for the different stages of development and plant size in the chamber, the calculation for the Total nitrogen accumulated in plant tissues will be:

Nitrogen in harvest tissues (37 days) + Estimated Nitrogen in younger plant tissues (16, 23, 30 days)

Estimated Nitrogen in Young tissues (16,23,30 days) is estimated by using data harvest prior to the beginning of the staggered culture (Weeks 3,4,5) and at the end of the last harvest (week 10)

6.2.6 Homogeneity of the different generations

The homogeneity of the different generations will be calculated on a dry weight basis. Plants will be compared on a per tray basis for each harvest and their standard deviations calculated. Each harvest will also be compared with harvests of similar age and their standard deviations calculated. The different generations will be considered homogeneous if the standard deviation observed between generation of the same age is similar (± 10%) to the standard deviation observed between trays of the same generation.

6.2.7 HPC1 internal volume estimation

The internal HPC1 volume can be calculated by measuring the volume of all components comprising the internal chamber space. However the volume of the expansion bags needs to be determinate by the following procedure:

- At time of HPC1 closure, after leak test has been completed and passed, chamber has been cleaned, microbial samples have been taken, hydroponic system has been cleaned, tested and filled with fresh solution, that all reservoirs have been filled, that all equipment have been calibrated i.e. when Phase 1 has been completed, the bags are emptied by applying gentle pressure on them until emptied,
- The chamber is then sealed and temperature and atmospheric pressure measured.

Comentario [C. P.19]: Please confirm this is done after the hydroponic system has been filled in with solution.

MAS: confirmed – it says this in the text.

- At this point an air pump is connected to a flow meter which is itself connected to one of the inlet port located behind the chamber and given direct access to the HVAC compartment
- 300 L of air is then pumped into the chamber. Theoretically the chamber will compensate with this additional pressure by filling the bags with 300 L of air.

Volume of air injected in pressure compensation bags (L)

duration of air injection (min) x flow rate (L per min)

HPC1 total internal volume (L)

Volume airlock A (L) + volume module B (L) + volume airlock C (L) + volume plenum (L) + volume air HVAC compartment (L) + volume air injected in pressure compensation bags (L)

Quantity of gases in HPC1 at start-up (mol)

$$\frac{\text{HPC1 total internal volume (L) x HPC1 pressure (Pascal)}}{8.314472\ J.K^{-1}.mol^{-1} \text{ x HPC1 temperature (K)}}$$

The volume of gas in the chamber is only an approximation since the volume occupied by the chambers components has not been taken into account.

6.2.8 Nutrient sampling and analysis

Nutrient solution samples will be taken weekly and consists of:

- Starting solution prior to HPC1 transfer
- One week sample taken from HPC1 sampling port
- Two week sample prior to removal from HPC1

Samples will be contained within 1L food grade containers and, if necessary, subdivided and submitted to an external laboratory for analysis. The following parameters will be analyzed:

Table 1. List of parameters to be analyzed from hydroponic samples taken from HPC1 during the course of the staggered crop experiment.

Ammonium	Calcium
Nitrite	Magnesium
Nitrate	Potassium
Carbon	Sodium
Sulphate	Copper
Chloride	Manganese
Phosphate	Boron
Thiosulphate	Zinc
Molybdenum	Iron

Comentario [EP20]: Thiosulphate could not be analysed in our staggered exp

6.2.9 Monitoring of microbial population

The monitoring of microbial population will be performed before and after each cleaning processes, as well as before and after the experiment (Stage 2). Details instruction for the microbial sampling is described in *MPP-OP-11-4104*.

6.3 Success/failure criteria

In order to determine if plant growth and development is progressing as expected, three physiological parameters, carbon assimilation, oxygen production and nutrient uptake, will be assessed on a weekly basis (water production could be seen as a potential parameter as well, but it is a combination of evaporation and transpiration, so not a direct measurement of plant activity):

Carbon assimilation: As the lettuce crop grows it will consume carbon dioxide. The CO_2 analyzer monitors [ppmCO_2] within the chamber on a continuous basis, so as the carbon dioxide is depleted by the plants, the HMI will compensate by injecting pure CO_2 in order to maintain the experimental set point of 1000 ppm. As the crop increases in biomass, more CO_2 will be consumed and injection of CO_2 to compensate for photosynthesis will follow that of a typical plant growth curve (Figure 3). The HMI compensation results in the record of accumulation (HMI tag CL4113_CO2) measured in total litres accumulated. This increase will continue as long as the crop remains viable, and is used to assess the status of growth. If CO_2 ceases to inject based on demand, it will not accumulate in the HMI record, and the cause should be determined. Causes can include plant mortality, a lack of CO2 available to be injected (no pressure at MFC injection point), CO2 analyzer failure, or injection valve failure. It is this accumulation value that will be monitored on a weekly basis.

Once the mature crop is established (after 5 weeks), the net weekly accumulation should be approximately equal from week to week, considering 10% variation as acceptable.. This assessment should be used to initiate further system evaluation by qualified personnel with knowledge of plant growth and systems function.

Comentario [C. P.21]: From previous discussions I understood oxygen and water would also be a physiological parameters OXYGEN COULD BE RELEVENT' BUT WATER IS A COMBINAISON OF EVAPORATION AND TRANSPIRATION AND IS THEREFORE NOT A DIRECT MEASUREMENT OF PLANT ACTIVITY, TO MY OPOINION WON'T BE A GOOD INDICATOR OF THAT THE PLANTS ARE GROWING NORMALLY

BI: agreed, however, weekly records should be kept for info

MAS: Records are kept by the control system and in the HPC1 log book. A daily parameter checklist should be employed for future experiments to ensure proper system functioning. This will be describe in detail in TN 101.5

Comentario [m22]: As the experiment has concluded, this will have to remain. Monitoring recommendations will be made in TN 101.5

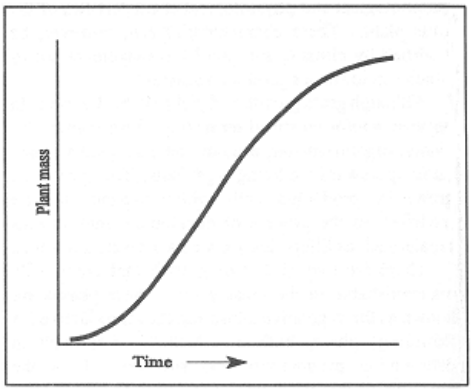

Figure 3. Typical plant growth curve.

Oxygen production: Oxygen production is dependent upon a number of factors, the primary being a suitable rate of photosynthesis. In general, in a sealed environment, oxygen levels will continue to increase from one day to the next and will follow the same plant growth profile shown in Figure 3. However, there are a number of factors which can impact O_2 production, the primary being O_2 consumption by non-target organisms (bacteria) within the system. In the HPC1, oxygen is continuously monitored by the HMI. On a weekly basis, the [%O_2] will be compared to the [%O_2] from the previous week. The experiment will be considered as progressing normally if the [%O_2] is larger than the [%O_2] of the previous week.

Again, once the mature crop is established (after 5 weeks), the net weekly accumulation should be approximately equal from week to week, considering 10% variation as acceptable. If [%O_2] does not evolve as per the described behaviour, further system evaluation should be made by a qualified plant scientist who is familiar with all aspects of plant growth and development within a sealed environment.

Nutrient uptake: As with CO_2 and O_2, plant demand for nutrients will follow a typical plant growth curve (Figure 3). In a sealed hydroponics system, plant demand for nutrients will cause the depletion of ions from the nutrient solution. Ion concentration is monitored by an electrical conductivity probe (EC) and when the ion concentration falls below a specified set point, the HMI will inject concentrated stock solutions to maintain the proper concentration. Stock solution injection is recorded in litres by tag HMI CL4108_EC_TIME and as the experiment progresses, this value will increase as nutrients are injected to compensate for plant consumption. The experiment will be considered as progressing normally if the actual value of accumulated nutrient (litres) increases from one week to the next.

Comentario [C. P.23]: Same comment as for CO2 injection and O2 and condensed water accumulation

Once the mature crop is established (after 5 weeks), the net weekly accumulation should be approximately equal from week to week, considering 10% variation as acceptable. An inequality

assessment should initiate further system evaluation which should be made by a qualified plant scientist who is familiar with all aspects of plant growth and development within a sealed environment.

6.4 Requirements to execute the test sequence

6.4.1 Personnel: staff qualification and training needs

- MPP technicians trained in HPC1 operation
- MPP personnel with expertise in controlled environment operation, plant physiology and results analysis

6.4.2 Personnel Protective Equipment

- 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

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

- Millwright work (screwdriver, pipe-wrench, …)
- HPC1 nursery as described in AD7
- All sensors are calibrated with certificates (or within validity period of the previous calibration)
- Gas analyzer calibration

6.4.4 Software: verification of software, backup needs

- PLC is connected to the acquisition server
- All acquisitions have been validated
- No back-up acquisition system is needed

6.4.5 Test conditions

The temperature of the HPC1 laboratory should be maintained at 20.0 ± 2.5 degree Celsius and not fluctuate between day and night in order to avoid variation in gas analysis reading as well as large fluctuation of temperature surrounding the Teflon pressure compensation bags (FRT_4114_01, FRT_4114_02 and FRT_4114_03).

Maintenance of hydroponics: the experiment shall continue unless the required maintenance interferes with long-term operation of the hydroponics delivery system. The growing system

provides a suitable buffering capacity that will allow short duration system shut downs to allow repair. MPP engineer/technician in charge of the test should be consulted before proceeding.

6.5 Measurement and data sampling

6.5.1 Recall applicable data collection plan and sampling plan

- PLC is connected to the acquisition server and all HPC1 parameters are being monitored at a frequency of once per second
- Sampling of plant material is performed according to the operating procedure described in TN96.4 (AD6)
- Schematic drawing in Figure 2 shows a dynamic representation of plant age for each tray position during the course of the staggered experiment, as well as the age of the plant being harvested each week.

Comentario [AF24]: Is it strictly required or can we get enough information with every 10seconds for example ? 10 sec IS ENOUGH BUT THE SYSTEM IS SET TO TAKE DATA EVERY SECOND BY DEFAULT

6.5.2 Data log files

Data extracted from the MPP acquisition server will used the following naming convention:

- The file containing the data related to gas analysis will be named:
  - SCL O2CO2 *yyyy-mm-dd_yyyy-mm-dd*.dat
  - The acquired parameters associated with this file are at least the following ones:

MPP Tags	***Description***
AT_4113_01	CO_2 Analyzer
AT_4113_02	O_2 Analyzer
TT_4112_avg	Chamber Average Temperature
AT_4112_avg	Chamber Average Humidity
FC_4113_01	CO_2 Mass Flow Rate
FC_4113_01_SP	CO_2 Mass Flow Rate set point
TT_4112_06	Laboratory Ambient Temperature
TT_4112_12	Laboratory Ambient Temperature
CL4113_CO2_QUANTITY_INJECTED	CO_2 Volume Injected (L) use set point
CL4113_CO2_QUANTITY_INJECTED2	CO_2 Volume Injected (L) use mass flow reading
CL4113_CO2_injected_in_mol	CO_2 Injected (mole)
PT_4114_01	Chamber Pressure (mbar)
PT_4114_02	Chamber Pressure (mbar)

PT_4114_03	Chamber Pressure (mbar)
PT_4114_04	Chamber Pressure (mbar)
PT_4102_01	Airlock “A” Pressure (mbar)
PT_4103_01	Airlock “C” Pressure (mbar)
RT_4104_01	Light Sensor (µE)
RT_4104_02	Light Sensor (µE)
RT_4104_03	Light Sensor (µE)

- The file containing the data related to hydroponic nutrient loop will be name:
  - SCL NUTRIENT *yyyy-mm-dd_yyyy-mm-dd*.dat
  - The acquired parameters associated with this file are at least the following ones:

MPP Tags	***Description***
FT_4106_01	Hydroponic Solution Flow Rate (L/min)
CL_41_CONDENSATE_TOTALVOLUME	Condensate cumulated (L)
CP_4110_01	Condensate cumulated (number of occurrence)
AT_4107_01	pH (pH)
AT_4108_01	EC (mS)
TT_4109_01	Temperature in Hydroponic Solution
CL4107_Acid_calibration	Acid Calibration Factor
CL4107_Base_calibration	Base Calibration Factor
CL4107_Acid_Opening_Time	Acid valve opening time (s)
CL4107_Base_Opening_Time	Base valve opening time (s)
CL4107_Acid_Injection	Acid Injected volume(ml)
CL4107_Base_Injection	Base Injected volume (ml)
CL4108_SolA_calibration	Nutrient Stock “A” Calibration Factor
CL4108_SolB_calibration	Nutrient Stock “B” Calibration Factor
CL4108_SolA_OP_Time	Nutrient Stock “A” Injected (s)
CL4108_SolB_OP_Time	Nutrient Stock “B” Injected (s)
CL4108_SolA_injection	Nutrient Stock “A” Injected (ml)

CL4108_SolB_injection	Nutrient Stock "B" Injected (ml)

- The file containing the all data related to one staggered experiment be named:
  - SCL ALL *yyyy-mm-dd_yyyy-mm-dd*.dat
  - All acquired parameters will be included in this file
- Samples collected from plant harvest will be named following the convention indicated in Figure 3 (Note: In plant part, S=shoot, R=root, L=Lower part which include roots and rockwool cubes)

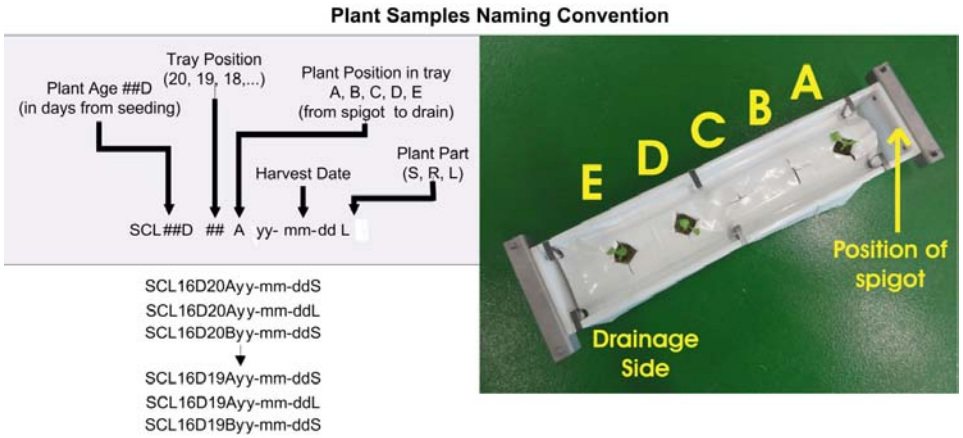

Figure 4. Naming convention for plant samples

6.5.3 Harvest data

- Weight data are recorded in the following records:
  - *MPP-REC-11-4108* for rockwool cubes
  - *MPP-REC-11-4109* for plant material
- All data files including harvest data received from external analytical laboratories are recorded electronically in the files "Tissues CHN analysis" and "Tissues mineral analysis" and stored on the MPP server under the "*COO9- HPC1 Data"* folder and used the sample naming convention indicated in 4.

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

- HMI data are collected every second for all instrumentation.
- The *"yyyy-mm-dd_yyyy-mm-dd"* in a filename stand for the starting and end date of a period covered by the data file (*yyyy*: 4-digits year, *mm*: 2-digits month, and *dd*: 2-digits day)

6.6 Reporting of status for a test (recall of the test plan)

The test sequence is performed by MPP personnel

The final status of the test (passed/fail) is decided at the end of the test in agreement between MPP personnel and MPP management.

6.7 Deviations and non conformances (recall of the test plan)

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".

6.8 Record for the test procedure with the various steps

The test procedure associated to the present protocol is: "MPP-REC-11-4106". A specimen of this record is shown below.

Other records are also used and referred to in this Test Protocol:

- *MPP-REC-11-4101*
- *MPP-REC-11-4102*
- *MPP-REC-11-4103*
- *MPP-REC-11-4104*
- *MPP-REC-11-4106*
- *MPP-REC-11-4107*
- *MPP-REC-11-4108*
- *MPP-REC-11-4109*
- *MPP-REC-11-4110*
- *MPP-REC-11-4111*

The main record "MPP-REC-11-4106" has to be printed and filled out every time the present protocol is executed. Associated records need to be printed and filled out as required.

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Document Identification : Test Record Sheet for Lettuce Staggered Culture	Type	Ref (Issue)	Chrono	Page
	MPP-REC	11-4106(0)		1 of 23

Compartment : CIVb	
Test procedure title :	Test Record for Lettuce Staggered Culture
Objectives:	This test record is the core record for the lettuce staggered culture in HPC1, it referred to other records as required since each record template may be used multiple times.
Applicable test plan and test protocols	TN 101.4 Test Protocol for staggered culture experiments in HPC1, MPP-OP-11-4101, MPP-OP-11-4102, TN 96.3, Section 6.5, MPP-UM-11-4101, MPP-OP-11-4103, TN 96.4, Section 4.4 and MPP-OP-11-4104
Applicable records	MPP-REC-11-4101, MPP-REC-11-4102 , MPP-REC-11-4103, MPP-REC-11-4104, MPP-REC-11-4107 , MPP-REC-11-4108, MPP-REC-11-4109, MPP-REC-11-4110 and MPP-REC-11-4111
Hardware:	HPC1, HPC1 nursery
Person responsible for the test :	
Test prerequisites:	

Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
		PHASE 1					
1.		Prepare nutrient and stock solutions according to **TN 96.3, Section 6.5.** Solutions must be kept in a dark location in order to prevent algae growth	Should have: 10 L stock A 10 L stock B 10 L acid and base 20 L seedling sln 160 L nutrient sln				
2.		Perform pre-cleaning microbial sampling procedure according to **MPP-OP-11-4104** and fill associated record			MPP-REC-11-4110 ( )____ MPP-REC-11-4111 ( )____		
3.		Perform Cleaning Procedure according to **MPP-OP-11-4102** and fill associated record	Chamber clean with all components in place ready to be tested or used		MPP-REC-11-4102 ( )____		
4.		Perform post-cleaning microbial sampling procedure according to **MPP-OP-11-4104** and fill associated record			MPP-REC-11-4110 ( )____ MPP-REC-11-4111 ( )____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
5.		Prepare nutrient solution loop according to **TN 96.3, Section 6.5**. Complete record **MPP-REC-11-4107(0)**	Nutrient in HPC1 reservoir and hydroponic loop ready to be used		MPP-REC-11-4107( ) ___		
6.		Place the 20 empty HPC1 plant trays inside the chamber if there are not there already	All trays in place, hydroponic system ready to be started				
7.		Activate irrigation system from HMI in auto mode, set point: EC 1.9 mS/cm, and pH 5.9	Irrigation system is activated, water flow is 10-15 L/min		FT_4106_01 : _____ L/min		
8.		Examine every components of the hydroponic loop to see if there is any leak. Place some sheets of papers under the middle junction of the main solution collector to help visualized leakage. If leaks are found interrupt system and repair leak before to restart	no leaks are observed				
9.		Let nutrient solution pH and EC reach their set points, it should take approximately 30 minutes, and then turn irrigation system off	Reading of: AT_4107_01 : EC 1.9 mS/cm AT_4108_01 : pH 5.9 Irrigation system off		pH : _____ EC : _____		
10.		Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take a 100 ml sample in a plastic cup, close valve immediately, and store this sample in the fridge.	Samples of the solution are taken, labelled and place in fridge		Sample ID: Store location: Volumes discard and sampled recorded in: MPP-REC-11-4107(__)___		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
11.		Close nutrient reservoir putting its lid carefully in place and tighten with screws. Make sure it is airtight	Nutrient reservoir is closed and ready to be used				
12.		Calibrate Gas Analyser according to **MPP-UM-11-4101**	Analysis of calibration gas within acceptable error range ± 10 ppm CO_2, reported in appendix 2		Std 999: _____ ppm CO_2 Std 2999: _____ ppm CO_2 Std 21.9%: _____ % O_2		
13.		Verify Nutrient Injection Pumps (MP_4108_01, MP_4108_02) flow rates according to **MPP-UM-11-4101**, enter flow rates into HMI	Flow rate measure and enter into HMI, reference reported in appendix 2		Stock A, MP_4108_01: _____ ml/sec Stock B, MP_4108_02: _____ ml/sec		
14.		Verify acid and base flow rates according to **MPP-UM-11-4101**, enter flow rates into HMI	Flow rate measure and enter into HMI, reference reported in appendix 2		Acid: _____ ml/sec Base: _____ ml/sec		
15.		Verify condensate volume of each event according to **MPP-UM-11-4101**, enter volume into HMI	Volume measure and enter into HMI, reference reported in appendix 2		Flow: _____ ml/min		
16.		Verify nutrient solution flow rate(FT_4106_01) according to **MPP-UM-11-4101**, compare with HMI reading, notify Sherpa if different in order to make the correction	Volume measure same than HMI reading, reference reported in appendix 2		FT_4106_01 : _____ L/min		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
17.		Verify EC probe (AT_4108_01) according to **MPP-UM-11-4101**, if last calibration record is more than three months calibrate	Test verified, reference reported in appendix 2		Std 0.147: _____ mS/cm Std 1.413: _____ mS/cm Std 12.88: _____ mS/cm		
18.		Verify pH probe (AT_4107_01) according to **MPP-UM-11-4101**, if last calibration record is more than three months calibrate	Test verified, reference reported in appendix 2		Std pH 4.01: _____ Std pH 7.00: _____ Std pH 9.21: _____		
19.		Perform Gas Leak Test Procedure **MPP-OP-11-4103**			MPP-REC-11-4103 ( )____ CO_2 Leak rate: ____ % / day		
20.		System ready to operate, status off until crop ready to be transferred					
21.		Remove all trays from HPC1 chamber. They will be used to transplant seedlings in the following step. Cover with black plastic film to protect if not used immediately.					
		PHASE 2					
22.	0	**Crop # 1** Germinate 200 seeds according to Lettuce Germination Procedure **MPP-OP-11-4101**. Adapt protocol to produce 100 good seedlings (x4), you will need to germinate at least 200 seeds	At least 200 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		

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Document Identification : Test Record Sheet for Lettuce Staggered Culture	Type	Ref (Issue)	Chrono	Page
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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
23.	1	**Crop # 1** Label and weight 200 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
24.	1	**Crop # 1** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
25.	7	**Crop # 2** Germinate seeds according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
26.	8	**Crop # 2** Label and weight 50 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
27.	8	**Crop # 2** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
28.	8	**Crop # 1** Label and weight 100 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
29.	8	**Crop # 1** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
30.	9	**Crop # 2** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
31.	9	Activate all components of the HPC1 (except irrigation) with the following parameter: • Light mode auto: 1 MH and 2 HPS lamps per module • Fan mode auto • Temperature and Humidity mode auto: 26/20 °C, 50%/70% RH and 16hrs/8hrs day/night regime • PAR: 270-300 µE • CO_2 inject auto: 1000 ppm					
32.	9	**Crop # 1** Transfer seedlings to 20 HPC1 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.** The protocol is written for 5 trays, you have to adapt it for 20 trays (x4)	20 trays with 5 seedlings each ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
33.	9	Turn off CO_2 injection					

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
34.	9	Open doors and airlock curtains of both sides of the HPC1 chamber, and sequentially place the 20 trays containing 5 seedlings of lettuce each in the HPC1 according to Lettuce Germination Procedure **MPP-OP-11-4101.**	20 trays containing 5 seedlings each place in chamber		MPP-REC-11-4101 ( )____		
35.	9	Turn on irrigation system, and verify that the flow of nutrient solution is distributed evenly in each trays and that no leak are present in the system	All trays in place with proper irrigation				
36.	9	Take pictures of the trays from both end of the chamber	Pictures taken				
37.	9	Close doors A and C, leave airlock curtains open for the moment					
38.	9	Turn on CO_2 injection to auto mode with a set point of 1000 ppm	Injection of CO_2 until set point, and irrigation system running under nominal conditions				
39.	9	Wait for stable reading of CO_2 and then closed both airlock curtains					
40.	14	**Crop # 3** Germinate seeds according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
41.	15	**Crop # 3** Label and weight 50 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
42.	15	**Crop # 3** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
43.	15	**Crop # 2** Label and weight 25 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
44.	15	**Crop # 2** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
45.	15	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
46.	16	**Crop # 3** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
47.	16	**Crop # 2** Transfer 25 seedlings to 5 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.** Introduce through door A and collect through door C	5 plant trays ready to transfer into HPC1		MPP-REC-11-4101 ( )____		
48.	16	**Crop # 1** (16-days old) Harvest trays 16-20 according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( )____		
49.	21	**Crop # 4** Germinate according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
50.	22	**Crop # 4** Label and weight 60 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
51.	22	**Crop # 4** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
52.	22	**Crop # 3** Label and weight 25 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
53.	22	**Crop # 3** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		

Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
54.	22	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken BEFORE change of solution, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
55.	22	Change hydroponic solution according to **TN 96.4, Section 4.4**. Record solution change in record MPP_REC_11_4107			MPP_REC_11_4107(__)___		
56.	22	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken AFTER change of solution, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
57.	30	**Crop # 4** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
58.	30	**Crop # 3** Transfer 25 seedlings to 5 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.**	5 plant trays ready to transfer into HPC1		MPP-REC-11-4101 ( )____		
59.	23	**Crop # 1** (23-days old) Harvest trays 16-20 according to TN 96.4, Section 4.4.	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( )____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
60.	28	**Crop # 5** Germinate according to Lettuce Germination Procedure MPP-OP-11-4101.	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
61.	29	**Crop # 5** Label and weight 60 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
62.	29	**Crop # 5** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
63.	29	**Crop # 4** Label and weight 25 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
64.	29	**Crop # 4** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
65.	29	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
66.	30	**Crop # 5** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
67.	30	**Crop # 4** Transfer 25 seedlings to 5 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.**	5 plant trays ready to transfer into HPC1		MPP-REC-11-4101 ( )____		
68.	30	**Crop # 1** (30-days old) Harvest trays 16-20 according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( )____		
69.	35	**Crop # 6** Germinate according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
70.	36	**Crop # 6** Label and weight 60 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
71.	36	**Crop # 6** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
72.	36	**Crop # 5** Label and weight 25 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
73.	36	**Crop # 5** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
74.	36	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken BEFORE the change of solution, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
75.	36	Change hydroponic solution according to **TN 96.4, Section 4.4**. Record solution change in record MPP_REC_11_4107			MPP_REC_11_4107(__)___		
76.	36	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken AFTER the change of solution, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
77.	44	**Crop # 6** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
78.	44	**Crop # 5** Transfer 25 seedlings to 5 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.**	5 plant trays ready to transfer into HPC1		MPP-REC-11-4101 ( )____		
79.	44	**Crop # 1** (37-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( )____		
80.	42	**Crop # 7** Germinate according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
81.	43	**Crop # 7** Label and weight 60 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
82.	43	**Crop # 7** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
83.	43	**Crop # 6** Label and weight 25 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
84.	43	**Crop # 6** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
85.	43	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
86.	44	**Crop # 7** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )___		
87.	44	**Crop # 6** Transfer 25 seedlings to 5 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.**	5 plant trays ready to transfer into HPC1		MPP-REC-11-4101 ( )___		
88.	44	**Crop # 2** (37-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( )___		
89.	49	**Crop # 8** Germinate according to Perform Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )___		
90.	50	**Crop # 8** Label and weight 60 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )___		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
91.	50	**Crop # 8** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
92.	50	**Crop # 7** Label and weight 25 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.** Then autoclave them	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
93.	50	**Crop # 7** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
94.	50	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken BEFORE the change of solution, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
95.	50	Change hydroponic solution according to **TN 96.4, Section 4.4.** Record solution change in record MPP_REC_11_4107			MPP_REC_11_4107(__)___		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
96.	50	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken AFTER the change of solution, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
97.	51	**Crop # 8** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )___		
98.	51	**Crop # 7** Transfer 25 seedlings to 5 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.**	5 plant trays ready to transfer into HPC1		MPP-REC-11-4101 ( )___		
99.	51	**Crop # 3** (37-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( )___		
100.	56	**Crop # 9** Germinate according to Perform Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )___		
101.	57	**Crop # 9** Label and weight 60 small dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )___		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
102.	57	**Crop # 9** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
103.	57	**Crop # 8** Label and weight 25 large dried rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes weighted and labelled		MPP-REC-11-4109 ( )____		
104.	57	**Crop # 8** Moist rockwool cubes with nutrient solution and autoclave according to Lettuce Germination Procedure **MPP-OP-11-4101.**	Rockwool cubes are sterile and ready to be used		MPP-REC-11-4101 ( )____		
105.	57	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
106.	58	**Crop # 9** Transfer seedlings to small rockwool cubes according to Lettuce Germination Procedure **MPP-OP-11-4101.**	At least 50 seedlings growing until ready to be transferred into HPC1		MPP-REC-11-4101 ( )____		
107.	58	**Crop # 8** Transfer 25 seedlings to 5 plant trays according to Lettuce Germination Procedure **MPP-OP-11-4101.**	5 plant trays ready to transfer into HPC1		MPP-REC-11-4101 ( )____		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
108.	58	**Crop # 4** (37-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
109.	64	Discard 200 ml of the nutrient solution from the nutrient tank by opening the drain valve HV_4106_09 and then take one 100 ml samples in a plastic cup for mineral analysis and one 100 ml sample for microbial analysis, close valve immediately, and store samples according to **MPP-OP-11-4104 and TN96.3**	Samples of the solution are taken, labelled and stored		Mineral (ID and location): Microbial (ID and location): Volumes discard and sampled recorded in: MPP_REC_11_4107(__)___		
110.	65	**Crop # 5** (37-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
111.	65	**Crop # 6** (30-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
112.	65	**Crop # 7** (23-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
113.	65	**Crop # 8** (16-days old) Harvest according to **TN 96.4, Section 4.4.**	5 trays (25 plants) have been harvested		MPP-REC-11-4108 ( ) ____		
		PHASE 3					
114.		Calibrate Gas Analyser according to **MPP-UM-11-4101**	Analysis of calibration gas within acceptable error range ± 10 ppm CO_2, reported in appendix 2		Std 999: _____ ppm CO_2 Std 2999: _____ ppm CO_2 Std 21.9%: _____ % O_2		
115.		Perform Gas Leak Procedure **MPP-OP-11-4103**			MPP-REC-11-4103 ( ) ____ CO_2 Leak rate: ____ % / day		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
116.		Perform pre-cleaning microbial sampling procedure according to **MPP-OP-11-4104** and fill associated record			MPP-REC-11-4110( ) ____ MPP-REC-11-4111 ( ) ____		
117.		Verify Nutrient Injection Pumps (MP_4108_01, MP_4108_02) flow rates according to **MPP-UM-11-4101**, enter flow rates into HMI	Flow rate measure and enter into HMI, reference reported in appendix 2		Stock A, MP_4108_01: _____ ml/sec Stock B, MP_4108_02: _____ ml/sec		
118.		Verify acid and base flow rates according to **MPP-UM-11-4101**, enter flow rates into HMI	Flow rate measure and enter into HMI, reference reported in appendix 2		Acid: _____ ml/sec Base: _____ ml/sec		
119.		Verify condensate volume of each event according to **MPP-UM-11-4101**, enter volume into HMI	Volume measure and enter into HMI, reference reported in appendix 2		Flow: _____ ml/min		
120.		Verify nutrient solution flow rate(FT_4106_01) according to **MPP-UM-11-4101**, compare with HMI reading, notify Sherpa if different in order to make the correction	Volume measure same than HMI reading, reference reported in appendix 2		FT_4106_01 : _____ L/min		
121.		Verify EC probe (AT_4108_01) according to **MPP-UM-11-4101**, if last calibration record is more than three months calibrate	Test verified, reference reported in appendix 2		Std 0.147: _____ mS/cm Std 1.413: _____ mS/cm Std 12.88: _____ mS/cm		

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Step No.	Day	Action description	Expected results / Nominal behaviour	Date Hour	Observed results / calculated	C / NC	Initials
122.		Verify pH probe (AT_4107_01) according to **MPP-UM-11-4101**, if last calibration record is more than three months calibrate	Test verified, reference reported in appendix 2		Std pH 4.01: _____ Std pH 7.00: _____ Std pH 9.21: _____		
123.		Perform Cleaning Procedure according to **MPP-OP-11-4102** and fill associated record	Chamber clean with all components in place ready to be tested or used		MPP-REC-11-4102 ( )____		
124.		Perform post-cleaning microbial sampling procedure according to **MPP-OP-11-4104** and fill associated record			MPP-REC-11-4110( )____ MPP-REC-11-4111 ( )____		
125.		If chamber is not going to be used shortly, a HPC1 long term shut down procedure should be performed according to **MPP-UM-11-4101**					

Conclusion for the Test (as provided in the test plan)	*Name*	*Signature*	*Date*
☐ Passed ☐ Failed			
Comments **Add deviation record including decision description**			
Checked **MPP-UAB**	*Name*	*Signature*	*Date*

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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	Signature

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Appendix 3 – Deviations

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	

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7. Comments

general comments		MPP answers
1	The logic to put some of these documents as RDs only (i.e. and not AD) remains not clear to us. This is especially true for RD3 and RD5.To be updated along the doc	Amended: RD3 and RD5 included as AD

detailed comments				MPP answers
nber	page	section	comment	
1	5	2,1	name of OP -11-4103 to be updated	Section 2.1 modified
2	6	4,2	A bullet about microbial hazards should be included	Bullet added in Section 4.2: Microbial hazards
3	8	5 phase1	for the verification of equipment calibration, we have to clarify what belongs to the TRR, where hardware status should go through verification (corresponding to be given as justification), and what belongs to this phase 1	We understand the activities included in this phase are performed inside the testing campaign, so after the TRR has been performed successfully
4	8	5 after phase3	Difficult to review without the details of the user manual	Draft version of the manual included with this package
5	11	6,2	There is no clear distinction between parameters followed regularly, authorizing the continuation of the experiment, and global parameters to be assessed over a full staggered culture campaign. To be clarified all along 6.2. This comment remains valid; for the sake of clarity, it would be better to identify the weekly followed parameters and the ones evaluated over the whole culture. The main reason is that you do not calculate them the same way, sometimes the success/failure is not evaluated the same way	The parameters to be followed regularly for authorizing the continuation of the experiment are in the updated section 6.3. All parameters discussed in section 6.2 are intended for final analysis on a weekly or total experimental periods and not for authorizing continuation of exp.
6	11	6.2.2	What about the total oxygen production over the whole test?	This is the 3th equation in the table which is the sum of each week.

7	12	6.2.3	Carbon balance is targeted here. Therefore carbon evolution in the gas phase, in the solid phase (plant and possibly rockwool) and in the liquid phase is to be looked at. In the calculations provided below, a certain number of assumptions are made (there seems to be no carbon transfer to the hydroponic system, carbon loss from chamber leakage and airlock opening seem to be constant,…) and they should be clearly stated and justified. In addition the actual carbon from analysis (harvest data) is referred to but not described elsewhere in the document. The global carbon balance should be clarified. Is there no interest to look at the carbon content for the different development stages and plant size as done for the nitrogen?	Carbon in the gas phase: - Carbon lost from leakage is in equation 2 - Carbon lost from airlock is in equation 3 and is a constant that was demonstrated in previous experiments - Carbon in hydroponic should be constant since the volume of water, pH and Ec, and the pressure of CO2 maintain in the system, we can measure the dissolved CO2 and add it to gain in precision - The harvest was described in TN96.4 and is now referred in section 6.5.3
8	12	6.2.3	Where are recorded the carbon analyses from plant harvest?	In the corresponding tissues analysis files. Reference to these files have been added in section 6.5.3
9		6.2.3	Do you mean daily or weekly CO_2 up-take?	Both the daily and weekly are all evaluated and part of the SLC gases file, see Section 6.5.2
10	12	6.2.3	Several comments on the table: - the daily (or weekly) or global approaches should follow different calculations, as for the N related calculations; - the losses linked to the nutrient loop, as described in the corresponding OP are not visible here. - Wording consistency issues between this TN and the OP - In the OP, losses/leaks are evaluated as % per event, here you have g of C, please clarify	Calculations reformulated as for the N calculations C losses in the hydroponics described, even if consider minor Percent C is converted to g C as this is the unit of mass.
11	13	6.2.5	It would be interesting, during the weeks of staggered culture, to analyze total N, and nitrate and ammonium as well	This is described in Section 6.2.8. The data will be part of the SLC nutrient file, section 6.5.2
12	13	6.2.5 bullet4	Where are those analyses recorded?	This is described in Section 6.2.8. The data will be part of the SLC nutrient file, section 6.5.2
13	13	6.2.5 bullet5	Where are those data recorded?	In the corresponding tissues analysis files. Reference to these files have been added in section 6.5.3

14	15	6,3	3 physiological parameters: From previous discussions we understood oxygen and water would also be a physiological parameters CP: agreed for the water issue. To avoid confusion, this explanation should be reported in the text. In addition, weekly records should be kept and provided for info	Oxygen could be relevant but water is a combination of evaporation and transpiration and is therefore not a direct measurement of plant activity, to our opinion won't be a good indicator of that the plants are growing normally The weekly screen print out of these parameters are sufficient to evaluate the continuation or not of the experiment Records are kept by the control system and in the HPC1 log book. A daily parameter checklist should be employed for future experiments to ensure proper system functioning. This will be described in detail in TN 101.5 Explanation regarding water reported in the text
15	15	6.3 C assimil.	These successful criteria up to week 5. From previous discussions, I understood that from week 5, the carbon injection profile would be compared from one week to the other and that reproducibility of the carbon injection profile would be the successful criteria. Idem for the oxygen accumulation profile and condensed water accumulation profile.	Agreed. Periods before 5 weeks and after that time have been distinguished in the text. For the steady period after 5 weeks, an acceptance range of +/- 10% is proposed.
16	15	6.3 C assimil.	Larger is a very vague statement	Considered valid enough for the initial period before 5 weeks, as the steady periodf is considered the critical one.
17	16	6.3 nutrient up	same comments as for CO2 injection, O2 and condensed water accumulation	See previous comments
18	17	6,5	This section does not mention the hydroponic solution sampling and analyses (mineral, microbial, etc.). Please clarify.	Section 6.5 was completed
19	19	figure3	From this naming convention, how do you intent to trace the crop number when performing a staggered test?	The number attributed to each tray is unique since it is assigned sequentially from tray 1 up to the last tray, each batch being attributed the next set of number (i.e if the last tray was 35, the next batch will be 36, 37,38, 39 40). Section 6.5.3 was corrected accordingly.
20	19	6.5.3	Naming convention for plant samples should be used in the record	It is now used in all records

21	20	6,8	Titles of the records would help, or reference to protocol and procedures diagram as a minimum	Titles were added to section 6.8
22	20	6.8 REC-11-4107	time of addition, replacement is of importance and should be the ones of the control/acquisition system (synchronization)	The "Time" has been added to the "Date" column of the RECORD, The HMI is collecting data every 10 sec, so I am not sure that there is any synchronization issue
23	21		Please indicate in the header of the record the versions of the documents mentioned	Versions can be added at the beginning of a campaign, space has been reserved for this purpose in the header