Tissue Culture standard for the movement of *Actinidia* plant material into Exclusion and Containment Regions

Version 2: Updated June 2021

1 Purpose

To provide a pathway for kiwifruit growing regions classified as Exclusion and Containment under the National Psa-V Pest Management Plan (NPMP) to access *Actinidia* plant material while minimising the risk of introducing *Pseudomonas syringae* pv. *actinidiae* (Psa biovar 3 referred to here as Psa).

2 Background

The kiwifruit industry currently has movement controls in place to prevent the spread of Psa into regions where this pathogen is not present or has a limited distribution. For growers in these regions these movement controls are an effective means of protection that has contributed to their orchards remaining free of Psa, however they have also had the unintended consequence of restricting access to new kiwifruit cultivars (as these have been developed primarily in Recovery regions where Psa is widespread).

It is currently prohibited to move plant material from a Recovery region to a Containment or Exclusion region as this presents an unacceptable risk. This paper proposes a pathway to manage this risk to a negligible level to enable growers to access these new kiwifruit cultivars.

2.1 Benefits from the movement of *Actinidia* plant material

Plant breeders are actively working to develop improved *Actinidia* cultivars that offer additional commercial benefits for growers. Allowing the movement of these new cultivars would provide growers in Exclusion and Containment regions with the opportunity for the same commercial advantage from new cultivars that is available to growers in other regions.

2.2 Importance of restricting plant movement

Actinidia propagation material is considered the main pathway for long-distance spread of Psa, between countries, but also between growing regions within New Zealand. Restricting the movement of *Actinidia* plant material into Exclusion and Containment regions is one of the key control factors that has helped slow the spread of the pathogen into these regions, and why we still have regions without Psa today, more than ten years after it was first detected in New Zealand.

2.3 Legal basis for controls

Kiwifruit Vine Health Incorporated (KVH) is the management agency responsible for implementing the National Psa-V Pest Management Plan (NPMP). Section 131 of the Biosecurity Act 1993 enables KVH to institute movement controls to:

- limit the spread;
- limit damage caused; and
- protect any area from the incursion of Psa-V.

KVH has declared areas of New Zealand to be controlled areas, enabled by s.131(2) of the Act; and Movement Control Notices, enabled by s. 131(3) of the Act.

The movement of risk goods (such as any kiwifruit plant material) into, within or from any Controlled Area is restricted (or regulated or prohibited) subject to the conditions of the Controlled Area and Movement Control Notices. The risk goods within the controlled area may also be subject to treatment and procedures specified in the Notice.

Section 134(1)(b) of the Act states: No person shall move, or direct or arrange the movement of, any organism, organic material, risk goods, or other goods in contravention of a notice under section 131 (3), unless permitted by an inspector or authorised person.

Accordingly, a KVH authorised person may issue a permission to move risk goods (i.e. *Actinidia* plant material) out of a controlled area. This document outlines the proposed requirements that must be met in order to possibly allow the movement of *Actinidia* plant material from a controlled area to another area of New Zealand with negligible increase in risk.

2.4 Summary of proposed measures

The proposed pathway incorporates elements from the Import Health Standard *Actinidia* Plants for Planting (2018) and does not rely on a single mitigation measure to manage risk. Proposed risk management measures are summarised in more detail in the following pages and outlined below.

a) Non-destructive screening for Psa *in vitro*

Psa can be present at low levels in both asymptomatic mother plants and derived tissue culture explants on standard media, making detection of contaminating organisms difficult. Experiments in Europe suggest Psa is able to remain present asymptomatically on micropropagated kiwifruit plantlets three years after pathogen inoculation (Minardi *et al.* 2015).

Therefore, to increase the likelihood of detecting Psa when present at low levels, the proposed pathway utilises the findings of Psa screening experiments specifically developed for *in vitro* plants, as described in Tyson et al. (2017), which incorporates peptone in the growing media to promote Psa growth and thereby provides a rapid and non-destructive visual indicator of Psa presence. This screening technique can be repeated multiple times to achieve a high level of confidence. By increasing the bacteria levels present, this method will also increase the reliability of detection with molecular techniques.

Bacterial isolations from plant material show that if Psa is present at extremely low levels in the mother plant and happened to give a false negative result initially, the rate at which Psa multiplies within *in vitro* plant material over time would result in subsequent tests returning positive results (Table 1, Tyson *et al.* (2017)). Once the inoculum level rises above c. 30 cfu / plant sample, the probability of getting a false negative result is close to zero (Tyson *et al.* 2017).

Table 1. Concentration of Psa within plant tissue following Psa inoculation (reproduced from Tyson *et al.* 2017).

inoculum conc. (cfu/mL)	plant section	*A. chinensis* var. *chinensis* 'Hort16A' cfu/0.1 mL (means of 5 plants) day 0	day 2	day 7	day 14	*A. deliciosa* 'Hayward' cfu/0.1 mL (means of 5 plants) day 0	day 2	day 7	day 14	*A. polygama* cfu/0.1 mL (means of 5 plants) day 0	day 2	day 7	day 14
BS control	Top	-	0	0	0	-	0	0	0	-	0	0	0
	Middle	-	0	0	0	-	0	0	0	-	0	0	0
	Base	0	0	0	0	0	0	0	0	0	0	0	0
10^2	Top	-	11	3201	1830	-	4	2141	800	-	0	0	3
	Middle	-	3	3200	2401	-	0	2438	800	-	0	0	343
	Base	0	3	3200	2403	0	1	1777	800	0	0	64	576
10^4	Top	-	802	4000	4000	-	1	1673	3200	-	0	911	1349
	Middle	-	179	4000	4000	-	186	1849	3200	-	3	1077	1984
	Base	35	2083	4000	4000	13	125	2430	4000	1	800	3423	4000
10^6	Top	-	1343	4000	4000	-	1607	585	3832	-	2	14	346
	Middle	-	2351	4000	4000	-	2834	2070	3994	-	6	857	2128
	Base	1172	4000	4000	4000	494	2814	4000	4000	213	1759	4000	4000
10^8	Top	-	4000	4000	4000	-	2104	4000	4000	-	68	2664	364
	Middle	-	4000	4000	4000	-	4000	4000	4000	-	355	3312	2610
	Base	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000

The protocol described has been developed using plants inoculated with Psa in the lab rather than field infested plants as these are difficult to establish in culture. There is research underway to try and establish *in vitro* kiwifruit cultures with Psa directly from field plants infected with Psa.

Peptone screening of *in vitro* cultures has been used to screen *in vitro* cultures of multiple shipments of plants initiated from Psa regions in New Zealand that cleared PEQ in the European Union (EU) and are now being grown in trials in the EU.

Repeat screening with this protocol will provide a high level of confidence that material is Psa free, however it is proposed that additional measures are included in this pathway to provide even greater confidence. Once *in vitro* screening is complete, plants are held in a greenhouse for further inspection and PCR testing over a growing season, then grown in isolation in the field before the final round of testing and release.

3 Specific Requirements

The proposed pathway for the movement of *Actinidia* plant material into an Exclusion or Containment region is outlined below and summarised in the diagram in Appendix 1.

3.1 Qualifying plant material

Actinidia plant material eligible for movement must meet the following criteria;

- KVH must be notified of the intention to use this pathway to move plant material and will provide any necessary permissions associated with the movement of plant material under the NPMP.
- Plants eligible for movement must be stage 3 (rooting) *in vitro* cultures ready to be deflasked and have an *in vitro* Psa-free status. The stages of TC plants are as follows; Stage 1 (initiation) explant taken directly from *in vivo* mother plant, Stage 2 (multiplication) plants in tissue culture being multiplied to rapidly increase plant numbers, Stage 3 (rooted) plants being prepared for deflasking including rooting and hardening off of plants.
- Psa free **mother plants** can only be identified from shoots of established stage 2 *in vitro* cultures growing in a PC2 containment facility. Potential **mother plants** for propagation of tentative Psa-free status progeny will be transferred as individual shoots to culture vessels (one shoot per culture vessel) and labelled in such a way that each shoot and its derived shoots can be traced to their origin. The basal 5-10 mm of the labelled shoot (the oldest part of the plant and considered most likely to harbour any bacteria) will be excised, labelled so it can be traced back to its originating shoot, transferred individually to a culture vessel with peptone supplemented medium (3g/L) and incubated for at least seven days. After visual screening for bacterial growth at seven days, the bases of shoots showing no visible contamination will be

sent to a KVH approved facility for PCR testing (KPCS PCR nursery testing using Rees George primers to detect all strains of Psa). The shoots from shoot bases that remained visibly clean and return a non-detected PCR test for Psa will be classified as **mother plants**. Shoots and culture vessels with visual contamination or positive PCR test results, will be destroyed by autoclaving.

- Details of all plants subsequently propagated from the mother plant are to be recorded such that every individual shoot can be traced back to its mother plant. If a mother plant is identified as Psa-free at this step, all of its progeny are assigned a tentative Psa-free status.
- Tentative Psa-free plants derived from the **mother plants** will be propagated *in vitro* for at least another two 4–6-week culture cycles (cycle length may be genotype dependent), with peptone screening (one week) of shoot bases at the end of each cycle or a minimum of two culture cycles. The entire lineage from a **mother plant** of any plants found to be contaminated with Psa will be discarded.
- Following the completion of the third peptone screening test in which ALL plants from a given **mother plant** are found free of Psa these plants will be assigned an "*in vitro* Psa-free status" and may proceed to Stage 3 (rooting) TC in preparation for deflasking.
- Application will be made to KVH and MPI for approval to transfer these plants to a Psa quarantine greenhouse (see definition and requirements in Section 3.2 below).

3.1.1 Inspection of qualifying material

In vitro cultures and plant material must be inspected for any visual signs of Psa bacteria at each stage of the pathway process and a record of each inspection maintained for auditing by KVH or its representative.

3.1.2 Laboratory Standard

The laboratory used for the preparation of the qualifying plant material as outlined above must operate to a standard equivalent to a Level 3 tissue culture facility as per the MPI Facility Standard: Post Entry Quarantine for Plants, by meeting the requirements listed in Appendix 2.
Evidence that the laboratory meets these requirements must be presented to KVH in advance, or KVH will undertake an audit of the facility at the operator's expense.

3.2 Psa Quarantine

3.2.1 Movement to Psa quarantine greenhouse

Once KVH is satisfied that requirements have been met and has provided authorisation, qualifying tissue culture plant material may be moved from the tissue culture lab to an approved Psa quarantine greenhouse. The green house must operate to a standard equivalent to the operational requirements of a level 3A facility, and the structural requirements of a PEQ Level 2 greenhouse as per the MPI Facility Standard: Post Entry Quarantine for Plants. These requirements are listed in Appendix 3.

Furthermore, the quarantine greenhouse must be located outside of kiwifruit growing regions (as defined by KVH www.kvh.org.nz/maps_stats).

The combination of these structural and operational measures are sufficient to manage the low levels of residual risk present at this point in the pathway.

3.2.2 Quarantine period

- The quarantine period will commence once the tissue culture material has been deflasked and started active growth.

- Plant material must be held in the PEQ Level 2 quarantine greenhouse for a minimum of six months active growth.
- Multiplication of plants is not permitted during the indoor quarantine period.

3.2.3 Monitoring

Plants must be monitored for Psa symptoms by a KVH approved person, or photographs of individual plants sent to KVH for verification they are free of symptoms 14 days after deflasking and then weekly by the facility operator during the quarantine period. Details of each monitoring round must be recorded and retained for auditing.

Any symptomatic plant material must immediately (within 24 hours) be reported to KVH. KVH will arrange sampling and testing for Psa.

3.2.4 Spraying and pruning

The application of sprays or pruning is not permitted during the quarantine period without KVH authorisation as this may mask disease symptoms.

3.2.5 Sample collection

Leaf samples are to be collected from at least three positions on each plant for PCR testing in the last month of active growth in the quarantine facility, including:

(a) A young fully extended leaf at the top of the stem
(b) An older leaf from a midway position for testing
(c) Any leaf showing any form of disease symptom.

3.2.6 Testing

The leaf material must be tested for Psa using the KVH authorised test method and laboratory (KPCS PCR nursery testing using Rees George primers to detect all strains of Psa) at a KVH approved testing laboratory.

A positive Psa test result will require the immediate destruction of all *Actinidia* plant material held in the Psa quarantine facility, which would be conducted in accordance with KVH guidelines.

3.2.7 Release from Psa Quarantine

KVH authorisation for release from the PEQ Level 2 quarantine facility will only be granted if all the requirements outlined above have been met to KVH satisfaction.

Release will only be granted for movement to a preapproved outdoor containment location.

3.3 Outdoor containment

At this point plants may be grown and propagated outdoors, in a containment location that is preapproved by KVH.

3.3.1 Outdoor containment facility requirements

The outdoor containment facility must be located outside of current kiwifruit growing regions and at least 20km from any known kiwifruit orchards and operate to a standard equivalent to PEQ Level 1 (L1) open field facility as defined by the MPI Facility Standard: Post Entry Quarantine for Plants by meeting the requirements listed in Appendix 4;

The application of sprays or pruning is not permitted during the quarantine period without KVH authorisation as this may mask disease symptoms.

Any tools or machinery must be cleaned before moving offsite. Movement of any tools from this site to a kiwifruit growing region requires KVH authorisation.

Plants must be held in the L1 containment site for a minimum period of eight months that includes at least three months of spring growth, when symptoms are most likely to be evident.

Plants must be monitored for Psa symptoms by the facility operator on a fortnightly basis during the containment period. Details of each monitoring round must be recorded and retained for auditing.

Any symptomatic plant material must immediately (within 24 hours) be reported to KVH. KVH will arrange sampling and testing for Psa.

A verified positive Psa test result will require the immediate destruction of all Actinidia plant material held in the containment location.

3.3.2 Sample collection

Leaf samples are to be collected within the last 21 days of the active growing period in the outdoor containment location before release for Psa testing with PCR. Sampling will follow the Kiwifruit Plant Certification Scheme protocol, which involves collecting six samples of up to 100 leaves from across all plants. If there are 600 plants or more, then six samples of 100 leaves must be collected, for less than 600 plants at least one leaf is to be collected from each plant.

This provides the final layer of mitigation measures to reduce risk to negligible levels.

3.3.3 Testing

The leaf material is to be tested for Psa using the KVH authorised test method (KPCS PCR nursery testing using Rees George primers to detect all strains of Psa) at a KVH approved testing laboratory.

A positive Psa test result will require the immediate destruction of all Actinidia plant material held on the containment site.

3.4 Release from outdoor containment to Exclusion regions

For permission to be granted by KVH for the *Actinidia* plant material to be released from the containment location for distribution into an Exclusion or Containment region the above pathway steps must be followed with all audits passed and all Psa test results confirmed negative.

References

Ministry for Primary Industries 2018. Facility Standard: Post Entry Quarantine for Plants. MPI Facility Standard: Post Entry Quarantine for Plants

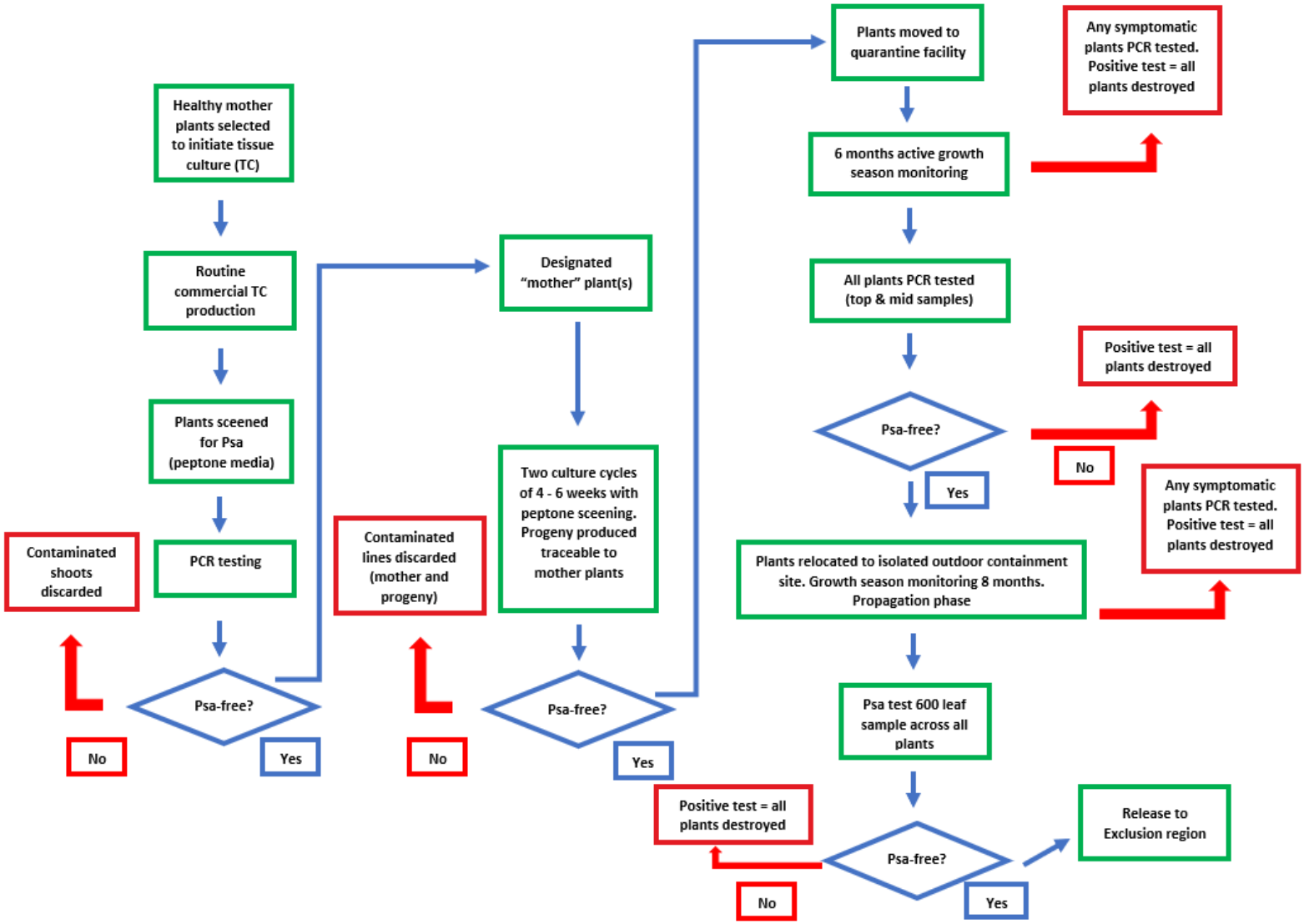
Ministry for Primary Industries 2018. Import Health Standard *Actinidia* Plants for Planting https://www.mpi.govt.nz/dmsdocument/27879/loggedIn

Ministry for Primary Industries 2018. Import risk analysis: *Actinidia* plants for planting (plants *in vitro*). Draft for external review.

Tyson, JL, Vergara MJ, Butler RC, Seelye JF, Morgan ER 2017. Survival, growth, and detection of *Pseudomonas syringae* pv. *Actinidiae* in *Actinidia* in vitro cultures. New Zealand Journal of Crop and Horticultural Science, DOI: 10.1080/01140671.2017.1414064

Minardi P, Ardizzi S, Lucchese C, Bertaccini A 2015. Latent infection by *Pseudomonas syringae* pv. *actinidiae* in *Actinidia chinensis* cv. Hort16A asymptomatic plants: five years of survival and colonization of a mutant virulent strain. II International Psa Symposium. Pp. 19.

Appendix 1. Proposed process for moving *Actinidia* plant material from Recovery to Exclusion or Containment regions



Appendix 2. Laboratory requirements

As stated in Section 3.1.2, the laboratory used for the preparation of the qualifying plant material must operate to a standard equivalent to a Level 3 tissue culture facility as per the MPI Facility Standard: Post Entry Quarantine for Plants, by meeting the requirements listed below.

1) **Sites, buildings, and structures:** Facilities must be constructed in accordance with Physical Containment Level 2 (PC2) requirements as specified in section 5.3.3 of the current edition of Australia/New Zealand Standard (AS/NZS) 2243.3:2010, Safety in Laboratories Part 3: Microbiological safety and containment, excluding the requirements for emergency drench showers.
2) **Operation of facilities:** Records must be held and made available to KVH on request detailing;
    a) the number and species of plants currently held in the facility;
    b) traceability information relating to the source of all plant material;
    c) the status of all plants in the facility (for example under treatment, awaiting biosecurity clearance etc.);
    d) which plants have been removed from the facility (i.e. given clearance, transferred to another facility or destroyed).
3) **Keeping track of plant material**
    a) A unique code must be assigned to every consignment when it arrives at a facility, or to each lot if a consignment consists of more than one lot.
    b) The consignment (or lot) must retain the same code until it is given a biosecurity clearance.
    c) All culture containers must be directly labelled with the unique code.
4) **Growing medium** must not contain fungicides or antibiotics.
5) **Facility Hygiene**
    a) Tools and other equipment must be labelled and must not be removed from the facility unless they are:
        a. decontaminated before removal using an approved method that is documented in the manual; or
        b. disposed of according to the requirements of section 3.5 of the PEQ Facility Standard (for example when single-use or disposable implements are used).
    b) All tools must be decontaminated (or discarded) between use on every plant.
    c) Culture containers must be wiped or sprayed with sanitiser solution before being opened.
    d) Any contamination of cultures must be reported to KVH.
    e) All people entering the facility must wear protective clothing.
    f) Protective clothing must be labelled and retained within the facility except when being cleaned:
        a. clothing to be cleaned must be bagged and sealed and delivered to a commercial laundry or an onsite washing machine;
        b. clothing which is no longer required must be handled as set out in PEQ section 3.5.
    g) Sticky mats or absorbent foot mats must be placed inside the door on the floor at the entrance to the facility and:
        a. must be used by all persons when entering and leaving the facility;
        b. must be replaced, or have disinfectant replaced as required to maintain efficiency;
        c. must have records retained of replacement of disinfectant;
        d. disinfectant must be stored in accordance with label recommendations.

h) All staff and visitors must wash their hands with soap and water and dry them thoroughly when leaving the facility.
i) Documented procedures must be put in place to prevent cross-contamination of quarantine material and non-quarantine material.
j) The procedures must be included in the manual.
k) Work surfaces must be decontaminated at least daily and immediately after all work involving consignments of imported material.

6) Managing waste

a) Records must be kept of any plants that are destroyed, including:
   a. the reason for destruction;
   b. the permit number;
   c. the consignment number and lot number (if applicable);
   d. the date and method of destruction.
b) Requirements of PEQ section 3.5 must be complied with.

7) Plant inspections by the operator

a) Plants must be inspected for signs and symptoms of pests and disease at least once per week as described in PEQ section 3.6.1.
b) A record is to be kept of weekly inspections.

Appendix 3. Greenhouse requirements

As stated in Section 3.2.1, the greenhouse must operate to a standard equivalent to the structural requirements of a Level 2 greenhouse facility, and operational requirements of a Level 3 facility as per the MPI Facility Standard: Post Entry Quarantine for Plants, by meeting the requirements listed below.

Structural requirements

(Level 2 equivalence by meeting the following)

1. **Site, buildings, and structures**

1.1. Construction

1) The facility must have a concrete floor with a drain that is connected through a gully or soil trap to sewerage, a septic tank, or a suitable rubble drain.

2) Locks must be fitted to all external doors and windows.

3) Facilities must be constructed using one of the following types of cladding, or a combination of these types of cladding (excluding entry/exit and ventilation requirements):

a) glass, polycarbonate or other rigid material;

b) twin skin polyethylene (polyfilm) or equivalent provided that:

i) polyethylene is at least 200 microns thick;
ii) integrity of both skins (i.e. no holes) is maintained at all times;
iii) polyethylene is replaced at regular intervals, as directed by the MPI Inspector.

{Note: Depending on the facility design and location, and the biological risk associated with material to be held in a facility, if a facility is constructed of a combination of types of cladding, a twin skin design may not be mandatory}.

4) The roof must be constructed of a continuous weather-proof material (excluding ventilation requirements), or the facility must be contained within a building with a weather-proof roof.

5) Benches must be constructed of dressed and treated timber, metal, or similar inert material and must be able to be easily cleaned and decontaminated.

6) Chairs or seats must be made of smooth material that is impervious to liquids and can be easily cleaned and decontaminated.

1.2. Anteroom

1) An anteroom must be installed at each entrance/exit to the facility (excluding emergency exits). The anteroom must:
   a) comply with the construction requirements above;
   b) be insect proof and free from recesses which may conceal insects or other pests;
   c) be large enough to allow one door to remain closed at all times (including when moving plants and equipment in or out of the facility);
   d) contain an area for storing protective clothing to be worn when in the facility;
   e) contain hand washing facilities, paper hand towels and soap.
   {Note: Hand washing facilities may also be located in an enclosed room immediately adjacent (and connected to) the anteroom};
   f) contain a waste container as described in PEQ section 3.5.
2) As well as the above, an anteroom may also be used to store items such as visitor log books and paper records, as specified in the manual.

3) Equipment (other than items listed in sub-section (1)) must not be stored in the anteroom unless approval has been given by the MPI Inspector. Any such items must be listed in the manual.

1.3. Area surrounding the facility.

1) A buffer strip a minimum of 1 metre wide must be present on all sides of the facility. The buffer strip must either be covered to prevent the growth of plants, or must be closely mowed lawn, or must be regularly treated with herbicide to prevent plant growth.

1.4. Facilities for plant inspection

1) The operator must ensure that facilities and staff are available to enable the following requirements to be met:
    a) sufficient lighting for inspection (minimum 1000 lux) must be provided upon request from the Inspector;
    b) benches for inspection must be provided upon request from the Inspector;
    c) if requested, the operator must also provide staff to assist the Inspector (for example with lifting plants, etc.) during plant inspections.

Operational requirements

(L3 equivalence by meeting the following)

1.5. Records

In addition to requirements set out in PEQ Section 3.2, the following records must also be retained:

1) The operator must provide a quarterly report to the MPI Inspector summarising the following:
    a) the number and species of plants currently held in the facility;
    b) whether any material has been imported, and if any plants have been propagated from any imported material since the last report;
    c) the status of all plant material in the facility (for example under treatment, awaiting biosecurity clearance etc.);
    d) which plants have been removed from the facility (i.e. given a biosecurity clearance, transferred to another facility, or destroyed) since the previous report.

1.6. Receiving material

Plant material must be held and opened within designated places that are identified in the manual.

1.7. Growing medium

1) Plants must be grown in pasteurised or inert medium.
2) The type of growing medium, along with where and how it will be stored must be recorded in the manual.

1.8. Water

1) Only potable water may be used (for example treated, mains supply, roof-collected or deep borehole water).
2) Any water that is collected for re-use must be disinfected before reuse to ensure that it is free from pathogens.

1.9. Keeping track of plant material

1) A unique code must be assigned to every plant in a facility.
2) Where material is grafted onto rootstocks in PEQ, each rootstock must only be grafted with buds derived from a single budstick. Each grafted rootstock is considered as a single daughter plant and must have a unique code assigned to it.

1.10. Facility hygiene

1) Tools and other equipment must be labelled and must not be removed from the facility unless they are:
    a) decontaminated before removal using an approved method that is documented in the manual; or
    b) disposed of according to the requirements of PEQ section 3.5 (for example when single-use or disposable implements are used).
2) All tools must be decontaminated (or discarded) between use on each plant.
3) Disposable gloves must be worn whenever handling plant material.
4) All people entering the facility must wear protective clothing. Where plants are grown on low benches, overalls or leggings must be worn.
5) Protective clothing must be labelled and retained within the facility except when being cleaned;
    a) clothing to be cleaned must be bagged and sealed and delivered to a commercial laundry, or must be autoclaved prior to being sent to a commercial laundry;
    b) clothing which is no longer required must be handled as set out in PEQ section 3.5.
6) All plants must be grown on raised benches, or trolleys, with adequate drainage.
7) The facility must be kept clean and as far as practicable must be retained free from algae, lichen, moss, weeds, and live pests such as arthropods (insects and spiders) and molluscs (slugs and snails).
8) A footbath filled to a minimum depth of 10 mm, or an absorbent foot mat containing disinfectant, must be placed at the main entrance to the facility (inside the anteroom) and:
    a) must be used by all persons when entering and leaving the facility;
    b) must have disinfectant replaced as required to maintain efficiency;
    c) the facility must have records retained of replacement of disinfectant;
    d) disinfectant must be stored in accordance with label recommendations.

OR

    e) all people entering the facility must use a change of footwear or wear protective shoe covers. Footwear must be kept inside the facility at all times. Protective shoe coverings must be removed and disposed of in a waste bin before exiting the facility.
9) All staff and visitors must wash their hands with soap and water and dry them thoroughly when leaving the facility.

1.11. Managing waste

1) Records must be kept of any plants that are destroyed, including:
    a) the reason for destruction;
    b) the number of plants destroyed;
    c) the permit number;
    d) the unique identification code of the individual plant;
    e) the date and method of destruction.
2) Requirements of PEQ section 3.5 must be complied with.

1.12. Plant inspections by the operator

1) All plants must be inspected either:
    a) as required in the relevant IHS; or
    b) at least twice per week during periods of active growth and once per week during dormancy (unless otherwise specified in the IHS). Where plants are not retained

within a greenhouse chamber during dormancy (for example if plants are bagged and held in cool storage for dormancy) weekly inspections are not required, although plants must be thoroughly inspected when returned to the greenhouse.

1.13. Plant growing conditions.

1) Specific plant requirements for irrigation, nutrition, temperature, and winter chilling must be met.
2) All plants must be grown in individual containers. Surplus containers must be disposed of as set out in PEQ section 3.5, or thoroughly cleaned and disinfected before reuse.
3) Plants must not be allowed to flower unless it is known that there are no pollen transmitted pests or diseases in the species being quarantined, or unless flowering is required to check for flower-specific symptoms.
4) Operational restrictions must be applied to minimise the likelihood of aerial dispersal outside the PEQ facility. In particular, overhead irrigation will be prohibited; this will minimise the chances of Psa escaping the facility as an aerially dispersed bacteria (e.g. by rain splash).

Appendix 4. Outdoor containment facility requirements

In addition to the requirements listed in Section 3.3.1 of this Standard, the outdoor containment facility must operate to a standard equivalent to L1 by meeting the following;

1. Site, buildings, and structures
2. Area surrounding the facility
   (1) The facility site must be clearly delineated on all sides, with boundaries clearly defined by a marker at every corner.
   (2) Signs must be placed at the main entrance and at every corner of each Level 1 PEQ site (see PEQ Part 2.5).
   (3) Both ends of all plots or rows within a quarantine site must be clearly marked with the date of planting and the unique identification code of the consignment, or of each lot (if a consignment consists of more than one lot).
3. Receiving plant material
   (1) Plant material must be held and opened either:
      a) within a designated place in the facility identified as such in the manual; or
      b) in a transitional facility associated with the facility that is approved to MPI-STD-TFGEN and approved for the receipt of plant material.
4. Keeping track of plant material
   (1) A unique code must be assigned to every consignment when it arrives at a facility, or to each lot if a consignment consists of more than one lot.
   (2) The consignment (or lot) must retain the same code until it is given a biosecurity clearance.
5. Facility hygiene
   (1) A weed control programme must be implemented to effectively control weeds within the facility and minimise the risks of the spread of pests and diseases.
   (2) Contingency plans must describe how equipment used at the facility will be decontaminated if a regulated organism is detected within a consignment.
6. Managing waste
   (1) Any material relating to a consignment must not be removed from a facility until a biosecurity clearance is issued for the waste.
   (2) Requirements of PEQ section 3.5 (managing waste) must be complied with.
   (3) Records must be kept of any plants that are destroyed, including:
      a) the reason for destruction;
      b) the number of plants destroyed;
      c) the permit number;
      d) the consignment number and lot number (if applicable);
      e) the date and method of destruction.
7. Pests and diseases
   (1) Psa, or any symptoms detected on plants in L1 facilities must be reported to KVH, with the following exception:
      a) insect pests (or damage or symptoms that are directly attributable to insect pests) do not need to be reported to the MPI Inspector.

{Note: As per sections 44 and 46 of the Biosecurity Act, the presence of what appears to be an organism not normally seen or otherwise detected in New Zealand, or of any notifiable organism, either in a PEQ facility or in the wider environment, must be reported}.

8. Treatments
   The application of sprays or pruning is not permitted during the quarantine period without KVH authorisation as this may mask disease symptoms.
9. Plant inspections by the operator
   (1) All plant material must be inspected for signs and symptoms of pests and disease at least once per week as described in PEQ section 3.6.1
   (2) A record is to be kept of all inspections.