Plant Pathogenic Bacteria
A Basic Guide to Isolation

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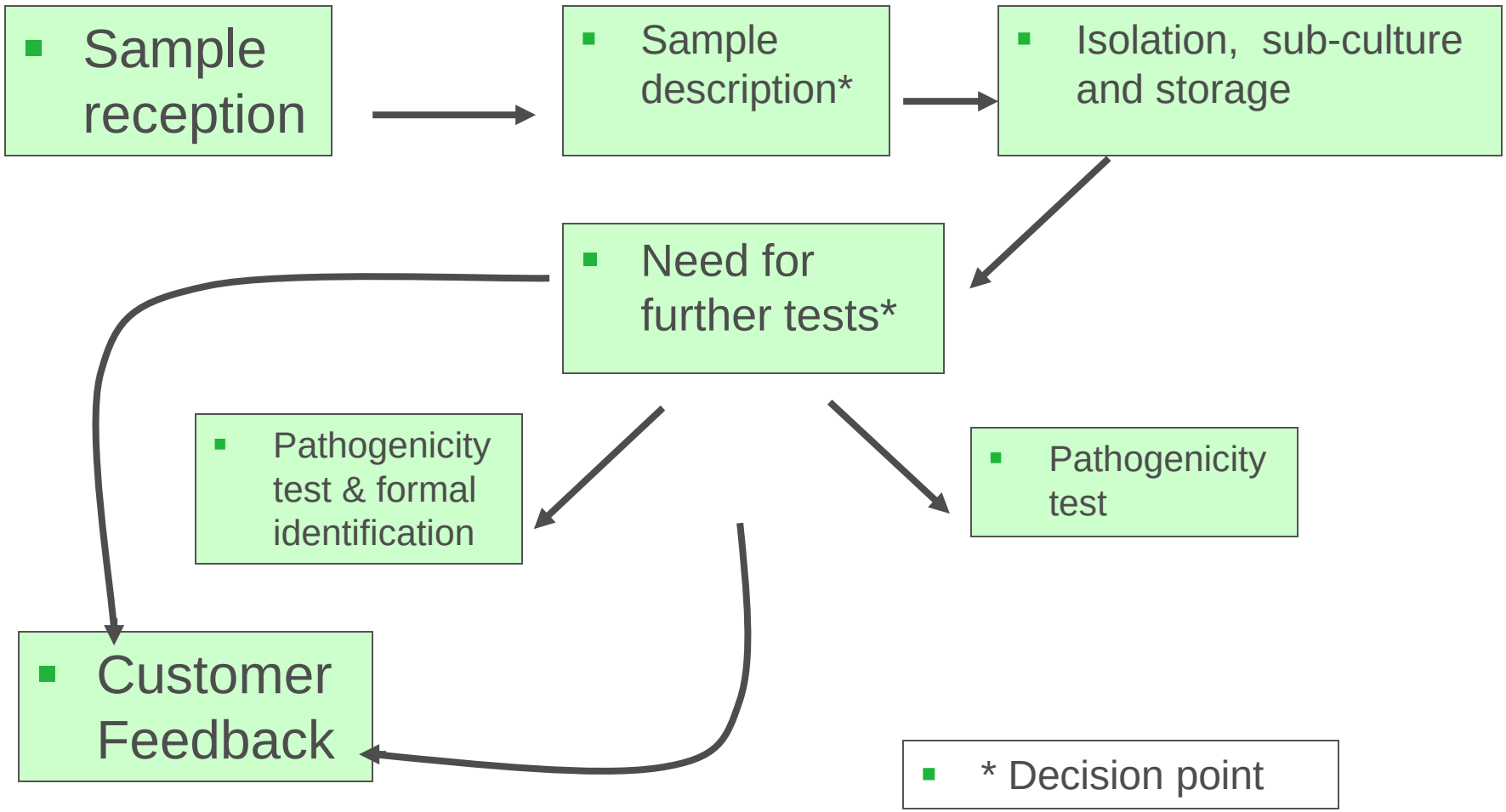
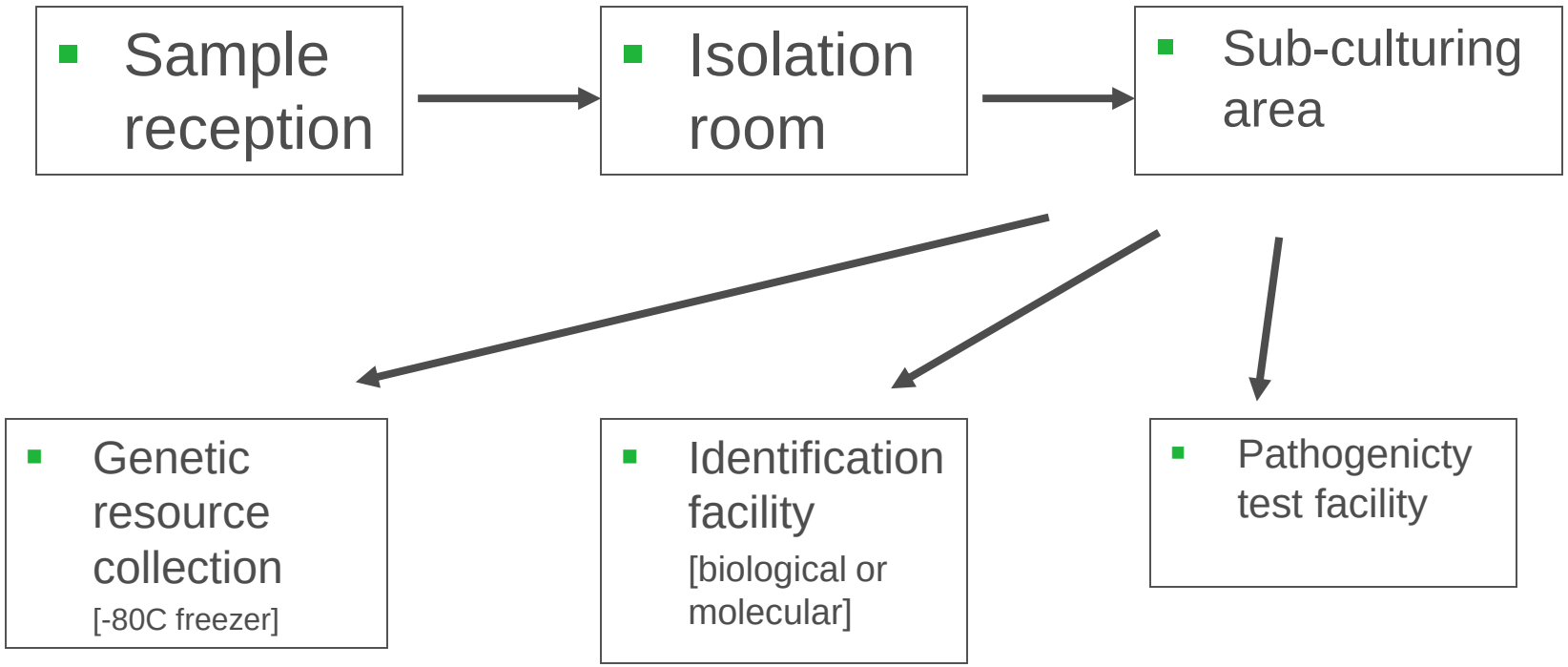
22nd September,
35th IVTC
Module 1

Flow diagram for routine diagnosis [1]



Flow diagram for routine diagnosis [2]



- Consider the locations of these areas and the quarantine needs associated for each, extending to transportation of material in-between
- All unidentified pest material should be considered of quarantine status
- Quarantine and non-quarantine processes need to be separated

Generalised isolation procedure from plant material

- Examine the plant material carefully, and describe plant symptoms
- Dissect out a small portion [1 x 1 x 3mm] of infected plant material and place on a clean, flamed slide
- Drop 1 – 2 drops of sterile water over the plant part
- Allow to stand for 5 – 30mins
- Examine slide under microscope at 40x magnification for microbial presence – fungal or bacterial
- If bacterial, inoculate appropriate medium with 1-2 loops and streak out
- Retain the plant sample for further analysis if required
- Incubate at 27-30°C for 2 – 5 days
- Sub-culture from a single well-separated colony onto richer media, ensure culture is pure; check sub-culture for purity and sub-culture again if necessary
- From confluent growth of a pure culture make bacterial slant [store in fridge at 5C] and freeze culture [at –80C] for storage purposes

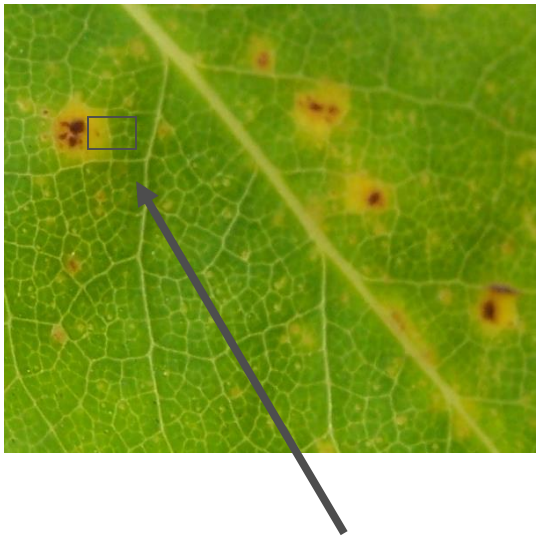
General isolation – some principles [1]

- Invest time in observing the plant sample, noting possible causes of symptoms seen
- If possible, take digital images of the symptoms seen; these can serve as a useful reference and mailed to colleagues whom may help in the diagnosis
- Undertake a literature search for the main pests associated for the host and the symptoms seen; do not restrict your analysis to your area of expertise, be inclusive of all pests!
- Proceed with the isolation as soon as practicable; isolation will be more successful from fresh material
- Isolate from leading edge of infection; not necrotic zones or zones with excessive wet rot; such areas are likely to be dominated by saprophytes
- For isolations from twigs or fruit look to isolate from below the plant bark / epidermis as such areas will give rise to purer cultures

General isolation – some principles [2]

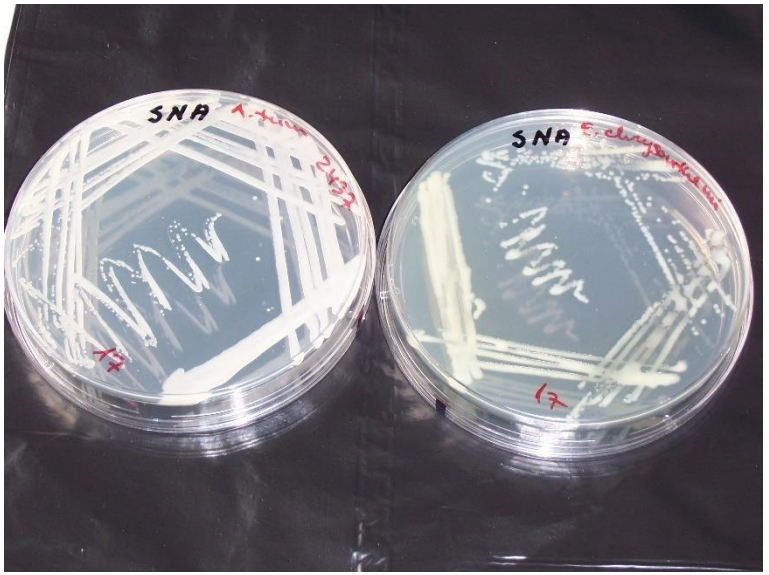
- The length of soak of the plant material on the slide in water is proportional to the woody nature of the plant sample; the tougher the material the longer the soak needed
- Isolations attempted from small pieces of material yield better result – less is more!
- Always use agar plates that are dry [2-3 days old]
- Saprophytes tend to grow faster on isolation media than the pathogens
- But the pathogen colony number will outnumber the saprophytes
- Observe colony formation at 24hrs and note those colonies that appear subsequently; these slower growing colonies are more likely to be your pathogen

General isolation



- Plant dissection from leading edge of infection zone

Isolation



- Microscopic study & 5-point streaking onto medium for single colonies

Some decision to make!

- Do you need to surface sterilize?
  - Generally it is not necessary to do so, and in cases where the plant material is dry it is advised not to.
  - However, where the material is robust and / or the risk of saprophytes is high then surface sterilisation will help
  - Various levels of sterilising stringency are possible. Employ common sense as to what is appropriate; do not over do the sterilisation process!

Some decision to make!

- What isolation media do I use?
  - Isolation and routine culturing [sub-culturing] are 2 different things and one media may not be ideal to both
  - Various selective and semi-selective media exist, but for effective use these require a strong indication as to the pathogens identity
  - Media low is sugar will slow the growth of bacterial saprophytes, delaying overgrowth of the slower growing bacteria; such media frequently present the best media for isolation
  - By example, NA and KB works well for most bacteria
  - Likewise for fungi, isolation is often optimal on TWA

Some decision to make!

- How do I know which bacterium to select from the isolation plate for further study
  - When isolating from an active lesion caused by a bacterial infection this decision should not be difficult
  - A strong numerical dominance of a single colony morphology type should be evident on the isolation plate, notably within the latter sectials
  - Never make the decision as to the colony to sub-culture on for identification at 24hrs, always allow time for slower-growing organisms to become evident [48 – 72hrs, or more!]
  - If this is not evident and a variety of colony types are present then it is best to attempt another isolation

Some exceptions

- Working from dried, woody lesions and galls may need some maceration and an extended time in water to release the bacteria
- Growth on NA and KB can be variable
  - *Erwinia* grow better on NA supplemented by 1% [w/v] glucose
  - *Xanthomonas albilineans & X. populi* with sucrose or glucose and peptone
  - *Leifsonia xylie* requires highly specialised media
  - Some bacterial pathogens remain unculturable on media
- Whilst virtually all bacteria will be visible as discrete colonies with 48 – 72hrs, some take much longer to grow