

# Recommendations of the SPDN Infrastructure Committee

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## Abstract

The ad hoc SPDN Lab Infrastructure Committee was formed during the 2005 SPDN Annual Meeting. This committee compiled the infrastructure components required to meet SPDN minimum standards (First Tier Infrastructure Guidelines) and developed Second Tier Infrastructure Guidelines (STIG) for diagnostic laboratories aiming for a very high standard in diagnostic capabilities. The STIG allow for a broad range of diagnostic capabilities, ranging from traditional taxonomic identification of plant pathogens, which remains a critical component of plant diagnostics, to current serological, chemical and molecular diagnostic methods. The STIG also include recommendations on the locations of equipment and activities within the laboratory that address the need to segregate sensitive molecular assays from potential contaminants. Other recommendations applicable to both tiers are included, such as documentation of standard operating practices and access to literature. The committee recognizes that different labs face different constraints and that strict adherence to the guidelines is not always possible. In many cases, labs can adhere to the intent of the STIG through various mechanisms, while not strictly following the STIG. The intent of this poster is to promote dialogue toward development of official guidelines for lab infrastructure requirements within the SPDN.

## First Tier Infrastructure Guidelines (FTIG)

FTIG outline SPDN's minimum infrastructure standards. FTIG labs are equipped for traditional taxonomic identification of plant pathogens, certain serological and chemical diagnostic techniques and are able to participate in critical aspects of the NPND.

- compound microscope with reticule
- stereoscope
- digital imaging capacity
- culturing capability
- laminar flow hood
- equipment for bacterial identification (e.g. carbon utilization system, gas chromatography)
- capability to perform ELISA assays (e.g. using kits)
- plate reader with appropriate filters
- autoclave
- sufficient trained personnel to provide timely and accurate diagnosis
- capability to upload diagnostic data to regional center
- participation in NPND training and meetings



## Second Tier Infrastructure Guidelines (STIG)

STIG are appropriate for labs aiming for a very high standard in diagnostic capability. Labs achieving STIG are equipped to perform APHIS-approved molecular diagnostic protocols and positioned to participate in the APHIS provisional lab approval program for diagnosis of certain pathogens of regulatory concern, such as *Phytophthora ramorum*.



## Final Notes

### Other General Recommendations:

- compliance with safety standards
- equipment receives maintenance checks/certification
- lab documentation (standard operating practices, records, etc.)
- access to a broad range of literature and current publications



### Handout

More detailed recommendations are listed on the handout provided.

### Feedback

The intent of this poster is to promote dialogue toward development of official guidelines for lab infrastructure requirements within the SPDN.

### Contact info

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### Equipment Alternatives

Ready and efficient access to equipment housed within other laboratories may be an acceptable substitute for purchasing your own equipment.



### The Bottom Line

The final decision on where to house equipment or perform assays is a judgment that should be based on an understanding of both the risk factors associated with sensitive diagnostic procedures and modifications that can be made to overcome risks. Decisions will take into account specific individual lab constraints and available modifications that can overcome them. Therefore, it is stressed that these are general recommendations. (Refer to considerations below.)



## Considerations for STIG

Plant diagnostic labs that perform highly sensitive diagnostic tests, such as PCR, must ensure that the lab infrastructure supports the segregation of potential contaminants from areas where activities and equipment sensitive to contamination are located. For example, plant samples and cultures must be located in a separate room ('dirty' lab) from areas where sensitive assays are performed ('clean' lab).

Segregation is necessary because of the high sensitivity of these methods and the ease of introduction of contaminating DNA, which can result in inaccurate diagnostic results. However, even when basic segregation between the 'dirty' and 'clean' lab is in place, risks of cross-contamination remain. Therefore careful consideration is necessary to identify the best location for all equipment and activities.

## Considerations for Equipment in the 'Clean' Lab

### 1) Equipment that harbors potential contaminants

should be physically separated from highly sensitive equipment and activities. Examples of such equipment:

- DNA extraction equipment (grinding apparatus, vortex, microcentrifuge, etc.)
- gel electrophoresis equipment
- spectrophotometer
- freeze drier

### 2) Equipment used for sensitive assays

should be housed in an area of the lab that is at low risk of contamination. Examples of such equipment:

- multicycler
- real-time PCR machine

### 3) Refrigerators/freezers

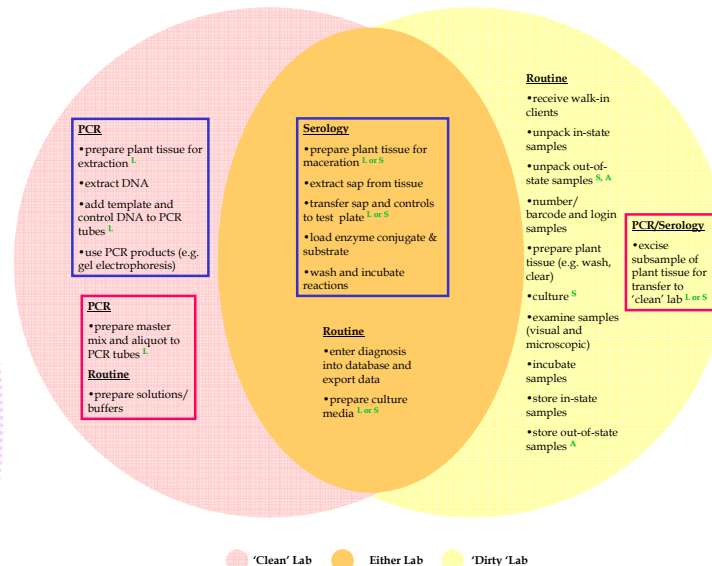
should be dedicated to storage of 'clean' items OR to storage of DNA/tissue samples. Examples:

- enzyme freezer (-20°C)—for primers, polymerase and other PCR reagents
- 20°C freezer—for storage of DNA/tissue samples
- refrigerator—for DNA-free solutions, buffers, reagents

## Alternative Approaches

Labs limited in physical separation options may be able to compensate by temporally separating activities, preparing sensitive reactions in an ultraviolet light-disinfested laminar flow hood, or other modifications.

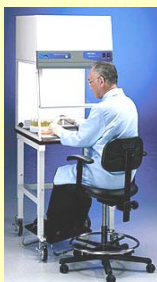
## STIG Consideration: Segregation of Activities



Activity results in potential contaminants and should be segregated from areas where sensitive tests are performed.

Activity is highly sensitive to contamination and care should be taken to avoid potential contaminants.

Superscript Code: L=laminar flow hood recommended ♦ S=biological safety cabinet recommended ♦ A=subject to APHIS 52b permit



A laminar flow hood equipped with an ultraviolet (UV) light is not standard equipment for preparing PCR reactions; however, its use is highly recommended. The sterile environment inside the hood and the ability to UV-disinfect the hood between activities can maximize use of available lab space.