Recommendations of the Southern Plant Diagnostic Network (SPDN) Infrastructure Committee (poster handout), NPDN National Meeting, Orlando, January 28-31, 2007. E. A. Bush and M. A. Hansen, Virginia Tech (Contact: E. A. Bush, ebush@vt.edu)

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The following guidelines are designed to serve as a planning resource for plant diagnostic laboratories. First Tier Infrastructure Guidelines (FTIG) outline SPDN's minimum infrastructure standards. FTIG labs are equipped for traditional taxonomic identification of plant pathogens, certain serological and chemical diagnostic techniques, uploading diagnostic data to the regional center, and are able to participate in SPDN/National Diagnostic Network meeting and training opportunities. The SPDN Second Tier Infrastructure Guidelines (STIG) are appropriate for laboratories aiming for a very high standard in diagnostic capabilities. The STIG allow diagnostic labs a broad range of diagnostic capabilities, ranging from traditional taxonomic identification of plant pathogens, which remains a critical component of plant diagnostics, to current methods in molecular diagnostic methods. Labs that implement STIG are equipped to perform APHIS-approved molecular diagnostic protocols and positioned to participate in the APHIS provisional lab approval program for diagnosis of pathogens of regulatory concern, such as *Phytophthora ramorum.*

For plant diagnostic laboratories that choose to include molecular diagnostic methods as a component of their diagnostic repertoire, it is critical that the physical infrastructure support the separation of plant material, associated airborne debris and cultures from areas where molecular diagnostic assays, such as polymerase chain reaction (PCR), are performed. Separation is necessary because of the high sensitivity of these methods and the ease of introduction of contaminating DNA. Therefore, activities and equipment listed in the STIG are coded (Table 1) to reflect a strong recommendation on where the item/activity should be housed/performed. Labs with limited options for physical separation of activities/equipment may be able to compensate by separating activities temporally, preparing sensitive reactions in an ultraviolet light-disinfested laminar flow hood, or other modifications. 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.

Ready and efficient access to equipment housed within other laboratories is an acceptable substitute for purchasing equipment. This situation is most applicable to equipment that is typically shared by laboratories within a building, such as autoclave, liquid nitrogen, ice machine, etc. However, laboratories may make the most efficient use of funds by seeking access to equipment, such as gas chromatography equipment, plate readers, etc. housed in other laboratories.

All labs must comply with applicable health and safety standards. Certain equipment, such as balances and biological safety cabinets, must be certified and receive regularly scheduled maintenance checks. Documentation of standard operating practices for receiving samples, performing assays, preparing media and solutions, is critical to ensure standardization. Additionally, labs should have a broad range of diagnostic literature resources and access to current publications.

SPDN First Tier Infrastructure Guidelines:

1. autoclave
2. capability to perform routine ELISA assays (e.g. using Agdia™, Envirologix™ kits)
3. capability to upload diagnostic data to regional center (i.e. database, computer)
4. compound microscope with reticule
5. culturing capability
6. digital imaging capacity
7. equipment for bacterial identification (e.g. carbon utilization system, gas chromatography, fingerprinting analysis)
8. laminar flow hood
9. participation in NPDN training and meetings
10. plate reader with appropriate filters
11. stereoscope
12. sufficient trained personnel to provide timely and accurate diagnosis

SPDN Second Tier Infrastructure Guidelines:

1. activities
    a. routine
        i. culture [D,L or S]
        ii. enter diagnosis into database and export data to regional center [O]
        iii. examine samples (visual and microscopic)[D]
        iv. incubate samples [D]
        v. number/barcode and login samples [D]
        vi. prepare bacterial cultures and test plates for identification [D, L or S]
        vii. prepare culture media [O, L or S]
        viii. prepare solutions/buffers [C]
        ix. prepare tissue for examination/culturing (e.g. wash off debris, clear tissue, etc.)[D]—Note: A soil trap is necessary in situations where quarantine weed seeds may be released into waste water.
        x. receive walk-in clients [D]
        xi. unpack in-state samples [D]
        xii. unpack out-of-state samples [D,S,A]
    b. serological assays (ELISA)
        i. excise subsample of plant tissue for transfer to ‘clean’ lab [D, P, L or S]
        ii. prepare tissue for maceration [O,L or S,X]
        iii. extract sap from tissue [O,X]
        iv. transfer sample sap and controls to test plate [O,L or S,X]
        v. wash and incubate reactions [O, X]
        vi. load enzyme conjugate & substrate [O,X]
    c. molecular assays (e.g. PCR, real-time PCR)
        i. excise subsample of plant tissue for transfer to ‘clean’ lab [D,P, L or S]
        ii. prepare tissue for extraction [C,X, L]
        iii. extract DNA [C,X]
        iv. prepare PCR master mix (i.e. enzyme, buffer, primers, chemicals) and aliquot to PCR tubes [C,P,L]
        v. add DNA template and controls to PCR tubes [C,X,L]
        vi. perform downstream activities using PCR products (e.g. gel electrophoresis)[C,X]
2. equipment
    a. apparatus for macerating tissue for ELISA[O,X], many options, for example:
        i. drill press with Agdia™ grinding apparatus (use with Agdia™ mesh sample bags)
        ii. KLECO™ (grinds hard and fibrous tissue)

iii. tissue homogenizer
iv. mortar and pestle

b. autoclave with a sufficient capacity to fulfill autoclave requirements associated with APHIS 526 permit [H,A]
c. bacterial identification equipment [C,X]--Some examples:
    i. carbon utilization identification system (Biolog™)
    ii. fatty acid analysis equipment
    iii. fingerprinting analysis equipment
d. balances
    i. analytic balance (suggested range: ~ 0.1 mg to 100 g)[C, X]—for solution/buffer prep and weighing tissue samples during PCR/ELISA tissue prep
    ii. top-loading balance (suggested range: ~ 0.001 g to 400 g)[D]
e. biological safety cabinet, Class II, Type A or equivalent [D,A]-- equipped with an ultraviolet light for disinfestation purposes is highly recommended (**Note*:*** a biological safety cabinet that also functions as a laminar flow hood can eliminate the need for both pieces of equipment in the dirty lab. [See information listed with laminar flow hood.])
f. cold storage for plant samples
    i. cold storage of plant samples not associated with APHIS permit 526[D]
    ii. cold storage of plant samples associated with APHIS permit 526[D, A]—(optional) The capability to secure samples associated with APHIS permit 526 is necessary, since this permit specifies that access to samples must be restricted. Storing these samples in a locking cabinet within a cold room, a locked refrigerator, or a locked lab are options that will fulfill this requirement. Alternatively, samples associated with the permit can be stored with routine samples, but this will require that lab access be restricted to authorized personnel (e.g. no walk-in clients) and that the lab must be locked.
g. compound microscope with reticule [D]
h. database, computer—to store and upload diagnostic data to regional center[C and D]
i. equipment for spinning down PCR/real-time PCR reaction components into PCR tube [C, X]--This will maximize reaction efficiency and detection. (The particular type of equipment will vary depending on the PCR platform being used.) Examples: For Cepheid™ machines, a special centrifuge is used; for platforms that use strip tubes or 96-well plates a bench top centrifuge with appropriate rotors and accessories is required.
j. freeze-drier/lyophilizer [C, X] (This is a requirement for *P. ramorum* PCR assay when using bark samples, but can also be used for other applications in the molecular biology lab.)
k. freezers/refrigerators
    i. enzyme freezer [C] (optional)—These are typically small (about 5.6 cubic feet). A manual defrost freezer is necessary for enzymes to avoid degradation associated with thaw/freeze cycles. Inclusion of a freezer dedicated to storage of enzymes and other reagents used in PCR master mix preparation reduces the risk of introducing contaminants into these sensitive reagents.
    ii. -20 C° freezer [C]--(A manual defrost freezer is necessary for DNA, primers, enzymes to avoid degradation associated with thaw/freeze cycles.) A freezer dedicated to storage of template DNA/tissue samples is recommended, but can also be used to store enzymes with adequate segregation.
    iii. -80 C freezer [C, H] (optional) -- Useful for long-term storage of primers, probes, archiving samples, etc.
    iv. refrigerator for storing reagents/chemicals used in PCR and ELISA assays [C]
    v. refrigerator/freezer [D]—for storing reagents and culture media needed in ‘dirty lab’
l. fume hood [C, H-- This is necessary for lab health and safety when using certain chemicals.
m. gel electrophoresis and visualization equipment (e.g. power supply box, trays, electrophoresis buffer/tray box, UV box and camera or other gel imaging equipment)[C,X]

n. grinding equipment for grinding tissue for DNA extraction [C,X]—many options, such as:
    i. BeadBeater™
    ii. tissue homogenizer
    iii. TissueRuptor™
    iv. mortar and pestle
o. high temperature incubator [D]
p. ice machine [H]
q. laminar flow hood, preferably with ultraviolet light for disinfestation
    i. laminar flow hood [D]--preferably equipped with an ultraviolet light for disinfestation purposes (**Note**: a biological safety cabinet that also functions as a laminar flow hood can eliminate the need for a laminar flow hood in the dirty lab.[See information listed under biological safety cabinet.])
    ii. (optional in clean lab)[C]— While this is not standard equipment for preparing PCR reactions, for example, its inclusion in the 'clean' lab is highly recommended. The sterile environment inside the hood and the ability to UV-disinfest the hood between activities can maximize use of available lab space.
r. liquid nitrogen [H]
s. low temperature incubator [D] (optional—could use room temperature in some cases)
t. microcentrifuge and rotor with lid for aerosol containment [C,X] (recommended: capable of 14,000 rpm or 20,000 *x* g)
u. microwave for preparing agarose gels [C]
v. PCR multi-cycler (preferably with a temperature gradient to optimize reactions) **and/or** real-time PCR machine[C,P]--(**Note:** It is highly recommended that labs purchase the model of machine with which APHIS-approved protocols/NPDN protocols have been ring-tested, since not all machines have undergone ring-testing and performance may vary.)
w. plate reader with necessary filters for reading ELISA plates [C,X]
x. spectrophotometer [C, X] (optional)—for DNA quantification and quality assessment. This is necessary for standardizing molecular assays and very useful when troubleshooting.
y. stereoscope [D]
z. vortex [C,X]—primary used during DNA extraction
aa. water bath or heating block [C]

3. Permit
    a. APHIS 526 permit—This permit allows the lab to receive out-of-state samples for diagnosis. See the APHIS website (http://www.aphis.usda.gov/permits/learn_epermits.shtml) and the SPDN website (http://spdn.ifas.ufl.edu/Forms-n-Things.htm) for more information.

Table 1. Activity and equipment codes

Code	Recommended Location of Activity/Equipment
C	'clean' lab
D	'dirty' lab
O	may be housed in 'clean' or 'dirty'
P	highly sensitive to contamination and care should be taken to avoid potential contaminants
X	creates potential contaminants and should be segregated from areas where sensitive tests are performed.
L	use of a laminar flow hood is recommended for this activity
S	use of a biological safety cabinet is recommended for this activity
A	certain requirements associated with APHIS 526 permit
H	likely to be housed within the department