Sample Preservation Methods

Collection of plant samples in the field and shipping to laboratory for virus diagnostics is an important component of virus disease surveillance programs. Samples collected includes intact leaves, bits of leaf tissue, portions of stems, roots, fruits, tubers, etc., depending on the crop, virus and purpose of testing. Lag time between sample collection to shipping to laboratory varies from few hours to several days. Longer lag time often results in deterioration (chemical, physical or biological) of sample, making it unsuitable for diagnostic testing. Therefore, preservation methods are required to maintain the sample integrity from the time of collection to analysis.

Numerous methods are available for sample preservation for users to select the most appropriate method depending on the plant type, sample type, sample size, lag time from collection to appropriate preservation / end use, length of storage, sensitivity of virus detection, facilities for preservation, etc.

- Type of tissue: leaf, root, stem, flower, fruit, etc.
- Labile nature of tissue / virus: rapid or slow deterioration
- Lag time: Few hours to days
- Preservation facilities: Most sophisticated (electric coolers), readymade tissue preserving media (silica gel, RNAlater, glycerol) to rudimentary (plastic bags), herbarium press, etc.

Users are advice to select best method for the specific purpose. We recommend reviewing literature to find appropriate methods and also interact with other research teams, if possible, for experience sharing and advice on best method. Ultimately, users are advised to test and fine tune preservation method that is most appropriate and feasible under your conditions. Sample preservation is the most critical step for reliable detection of viruses in the field collected samples. We strongly advice users to validate suitability to your purpose before adopting. Also note that one procedure may not work for all crops/conditions.

Methods commonly used for preservation of samples of IITA mandate crops (banana, cassava, cowpea, maize, soybean and yam) at Virology and Molecular Diagnostics Unit of IITA, Ibadan, are presented in this protocol.

Some preservation methods and suitability for crops				
	Glycerol	Silica gel	Aluminum foil	Plastic bag
Banana	+++	+++	+++	+++
Cassava	+++	+++	+++	+++
Cowpea	+++	+++	+++	+++
Maize	+++	+++	+++	+++
Soybean	+++	+++	+++	+++
Yam	+++	++	++	++
+++ = most suitable; ++ = moderately suitable; = + least suitable				
Comment	*Room temperature*	*Room temperature*	*Few hours to couple of days. Advice preservation in cool box during transit from field to lab*	*Few hours to couple of days. Advice preservation in cool box during transit from field to lab*

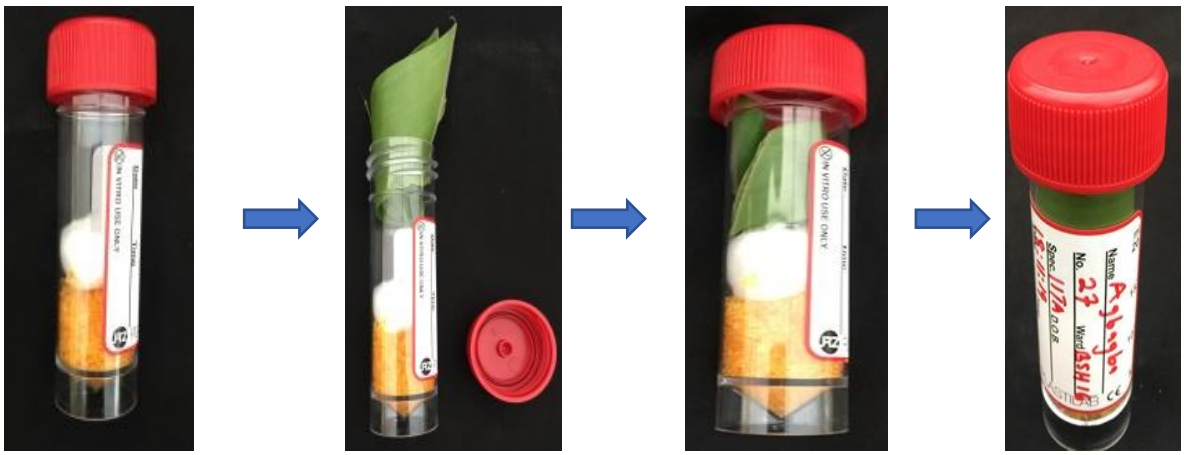
Sample preservation on silica gel

Materials:

- Silica gel with indicator (orange) (Merck-Supelco, Cat. No. 1019695000)
- Plastic vials (size depends on the sample volume; recommend 25 ml plastic tubes)
- Parafilm
- Permanent marker pen (Sharpie)
- Self-adhesive labels (optional; for labelling)
- Cardboard box (optional, to store plastic vials)
- Non-absorbent cotton wool

Method:

1. Prior to field visit/sample collection: In a laboratory or a convenient room, fill plastic tubes with silica gel to about 60% volume, place small amount of non-absorbent cotton wool and firmly cap the tube and arrange them in cardboard box or other material convenient for carrying to field.
2. Sample collection and preservation in the field:
    a. Detach leaf or plant tissue of choice (if necessary, shred into small bits) from the plant and insert into vials loaded with silica gel.
    b. Firmly cap the tube.
    c. Label the tube with marker pen and transfer sample to lab.
    d. Seal tubes with parafilm.
3. Store sample at room temperature.
4. Silica gel turns blue with absorption of moisture from the tissue sample.



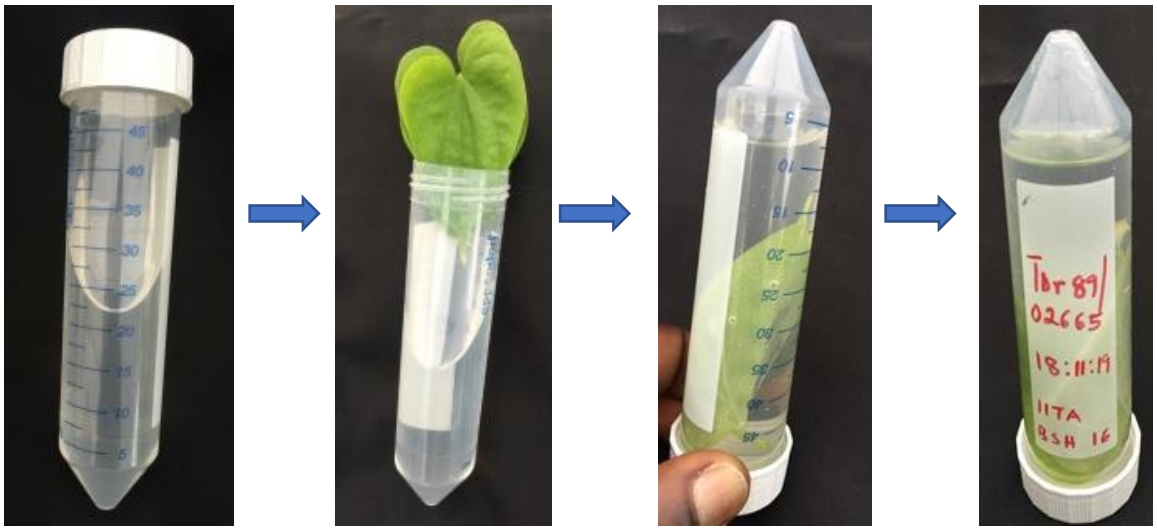
Sample preservation in Glycerol

Materials:

- Glycerol (analytical grade; Sigma-Merck Cat. No. G7757)
- Plastic vials (size depends on sample volume; recommend 50 ml falcon tubes for preserving intact leaf tissue or tissue bits of interest; 2 ml microfuge tubes for small volumes of leaf/tissue tissues)
- Parafilm
- Permanent marker pen (Sharpie)
- Self-adhesive labels (optional; for labelling)
- Cardboard box (optional, to store plastic vials)

Method:

1. Prior to field visit/sample collection: In a laboratory or a convenient room, fill plastic tubes with glycerol to about 70% volume, firmly cap the tube and arrange them in cardboard box or other material convenient for carrying to field.
2. Sample collection and preservation in the field:
    a. Detach leaf or plant tissue of choice (if necessary, shred into small bits) from the plant and insert into vials loaded with glycerol.
    b. Firmly cap the tube, and gently invert the tube to immerse leaf sample fully under glycerol.
    c. Label the tube with marker pen and transfer sample to lab.
    d. If necessary, top up with glycerol to fill the void space to ensure that sample is fully immersed in glycerol.
    e. Seal tubes with parafilm to avoid leakage.
3. Store sample at room temperature. Refrigeration not required but avoid direct exposure to sunlight or heat.
4. Use forceps to full the sample out of glycerol, slice amount of tissue required for DNA or RNA extraction. Retain unused portion in glycerol for further use.
5. Don't reuse glycerol or vial. For safe disposal, take out leaf or plant tissue sample from glycerol and put in autoclavable bag for destruction by autoclaving.



Sample preservation in aluminum foil paper

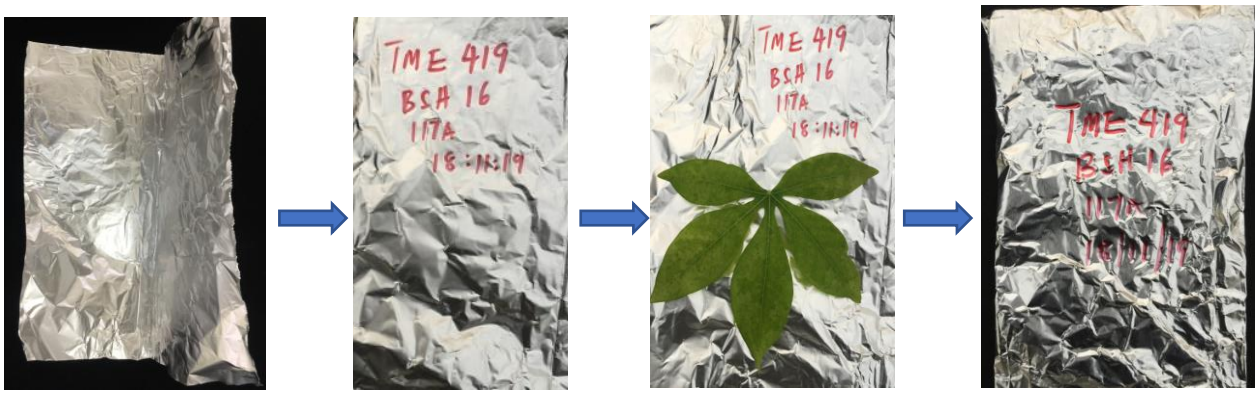
Materials:

- Aluminum foil (Recommend Fisherbrand™ Aluminum Foil, Standard-Gauge Roll, Cat. No. 01-213-100; aluminum foil roll available in local markets can also be used)
- Permanent marker pen (Sharpie)

Method:

1. Cut aluminum foil to size necessary to wrap leaf sample
2. Place sample on foil and wrap around to fully cover the tissue
3. Label sample and store in plastic bag.
4. Store at room temperature (away from direct sunlight or any heating source) for short term (1 to 2 days; depending on the sample type and use purpose) or in a cool box (electric cooler or filled with ice).
5. After reaching lab, store samples at 4°C, -20°C or -80°C according to the requirement. Samples can also be transferred onto silica gel or glycerol vials for long term preservation.

Note: This method is best suitable for preserving intact leaf tissues. Not suitable for tissues with lot of moisture or water content.



Standard cool box.

Sample preservation in aluminum foil paper

Materials:

- Sealable plastic bags (size depends on the sample volume)
- Permanent marker pen (Sharpie)

Method:

1. Collect tissue sample and place it in the sample bag
2. Label sample and store in plastic bag
3. Store at room temperature (away from direct sunlight or any heating source) for short term (1 to 2 days; depending on the sample type and use purpose) or in a cool box (electric cooler or filled with ice).

After reaching lab, store samples at 4°C, -20°C or -80°C according to the requirement. Samples can also be transferred onto silica gel or glycerol vials for long term preservation.

Standard cool box.