

## Inoculation of fruit and leaves of Apple Tree with *Erwinia amylovora*

Note: For this protocol, it is necessary to obtain fruits and leaves of the best quality, in order to avoid misleading results.

### Materials

Scalpel  
Ziploc bags  
Atomizer  
Plastic containers  
Distilled water  
Cotton balls  
Petri plates  
20% Ethanol  
1% Sodium hypochlorite

### Protocol

1. Inside the biosafety cabin, disinfect the apple and the leaves by leaving them in 1% sodium hypochlorite for 2 minutes, following for 2 minutes in ethanol. Rinse with distilled water and let the samples dry.
2. Inoculation by spraying: Place the vegetal tissue into the ziploc bag, spray the inoculated media over the sample. Take the tissue out of the bag and place it either in a plastic container for the apple cuts or Petri plate for the leaves.
3. Inoculation by wound: Soak up the scalpel in the inoculated media, and cut the tissue in x-shaped wounds along the surface of the sample. Place it either in a plastic container for the apple cuts or Petri plate for the leaves.
4. To maintain a humid environment, place a beaker with distilled water in the containers and wet cotton balls in the Petri plates.
5. Place in the incubator at 28 °C.
6. Keep under observation for 7 days.