GUIDE TO MANUKA NECTAR SAMPLING

Preparation before collecting a nectar sample

- Identify the Manuka plants to be sampled with a tag or some other form of semi-permanent identification. You might even consider taking a photo or recording a GPS co-ordinate for future records. Use this identification as a sample name, to make it easy to return to the plant at a later point.
- Cover the selected plants, or some of their branches, with a fine mesh bag and tie the bag tightly at the base using tape to seal it to prevent insects from feeding on the nectar (ants and other small insects may still be able to get in). A plastic bag could be used, but may cause the branch to sweat.
- Cover the plant before noon on day one.
- Collect the sample from the plant before noon the next day - say about 10 - 11am.
- If a branch is removed for sampling, the nectar may dry up or rub off if they are left too long before sampling.

Selecting good flowers

- Select flowers that have a soft center (not dried), and have not started to set seed.
- Ensure that the flowers chosen for sampling **have visible nectar droplets.** If there is no nectar on some flowers the sample may not be adequate for the laboratory to analyse.
- You can cut the branch or flower from the bush if that is more convenient, but ensure sampling occurs immediately to prevent nectar drying up.

Mature flower with nectar

Seed capsules

There are three options for nectar sampling:

1. **Wash Method** – Flowers in a tube
2. **Direct Pipette Method** – Nectar taken directly from the flower
3. **Pipette Rinse Method** – Nectar rinsed and taken directly from the flower

These methods are described in more detail on the following pages.

Nectar Collection Option 1 – **Wash Method**

List of items required:

Supplied by Analytica in kit

- Disposable plastic pipettes
- 2 mL plastic tubes
- 10 mL plastic tubes
- Tweezers
- Containers to courier tubes back to lab
- Polystyrene chilly bin

Other items required

- Fine mesh or plastic bags
- Tape
- Scissors
- Fresh clean water
- Permanent marker
- Sticky labels (optional)
- Ice / slicker pad

1. Refer to Preparation before collecting a nectar sample (*Page 1)* to prepare plant for sampling (day before sampling)
2. Select 10 flowers from one plant. Ensure that the flowers have **visible nectar** (refer to Selecting good flowers – *Page 1*).
3. Remove **flower 1** from the plant.
4. Use tweezers to remove and discard any leaves from the flower base.
5. Use tweezers to remove the flower petals (optional).
6. Place the flower base in a 10mL plastic tube.
7. Repeat the process (steps 2 to 5) for **flowers 2 - 10**, adding the flower bases to the same 10 mL plastic tube.
8. Add 1.5mL of clean water to the 10 mL tube using the disposable plastic pipette. Cap and rotate the tube to maximize contact of the water with the flower heads. Either:
   - Allow to stand for 20 minutes then repeat the agitation of the tube.
   - *OR,* gently shake tube for ~1 minute to maximise water content with flowers.
9. Clearly label a 2 mL tube with the sample ID (use a fine tipped permanent marker or sticky label and permanent marker), and record this sample identification on the Analysis Request form.
10. Pour the washing from the 10mL tube into the 2 mL tube.
11. Repeat this process (steps 1 to 10) of harvesting the nectar from 10 flowers from each plant you wish to sample.
12. Place samples in the container provided, and store them on ice in a chilly bin immediately after collection. After sampling, store samples in the freezer until they are ready to be couriered.
    - If samples are warm they will ferment, and this will affect the test results.
13. Send the samples and completed Analysis Request form to Analytica Laboratories, making sure that the samples are on ice so that they remain chilled until they arrive at the laboratory for testing.

Nectar Collection Option 1 – **Wash Method**

Supplied equipment *(chilly bin not shown)*:

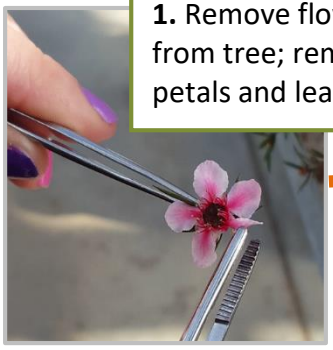
Tweezers *(plastic)*

Disposable pipette

Container for dispatch of tubes to lab

10mL plastic tube

2mL plastic tube



1. Remove flower from tree; remove petals and leaves

2. Put flower in 10mL tube

3. Repeat steps 1 and 2 for 10 flowers

4. Add 1.5mL of water to tube. *(Volumes are on side of pipette)*

5. Soak for 20mins or agitate by shaking/rotating tube

6. Pour washing from 10mL tube into 2mL tube

7. Label 2mL tube, chill and send to Analytica with any other nectar samples

Nectar Collection Option 2 – **Direct Pipette Method**

List of items required:

Supplied by Analytica in kit

- Disposable plastic pipette
- Yellow 200µL pipette tips
- 2mL plastic tubes
- Containers to courier tubes back to lab
- Polystyrene chilly bin

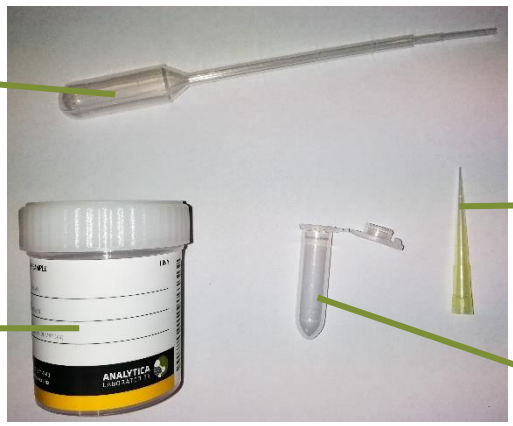
Other items required

- Fine mesh or plastic bags
- Tape
- Permanent marker
- Sticky labels (optional)
- Ice / slicker pad

1. Refer to Preparation before collecting a nectar sample (*Page 1)* to prepare plant for sampling (day before sampling)
2. Select 10 flowers from one plant. Ensure that the flowers have **visible nectar** (refer to Selecting good flowers – *Page 1*). You will be combining the nectar from these flowers into a 2mL plastic tube that Analytica will supply to you.
3. Clearly label the 2mL tube with the sample identification (use a fine tipped permanent marker or sticky label and permanent marker), and record this sample identification on the Analysis Request form.
4. ***Flower 1:*** Press a yellow pipette tip firmly onto the end of the plastic pipette (make sure it creates a seal.) Use the pipette to take up the nectar off the disk of the flower, then squeeze the nectar into a 2mL plastic tube.
5. **Flowers 2 – 9:** Repeat step 4
6. Place samples in the container provided, and store them on ice in a chilly bin immediately after collection. After sampling, store samples in the freezer until they are ready to be couriered.
   - If samples are warm they will ferment, and this will affect the test results.
7. Send the samples and completed Analysis Request form to Analytica Laboratories, making sure that the samples are on ice so that they remain chilled until they arrive at the laboratory for testing.

Nectar Collection Option 2 – **Direct Pipette Method**

Supplied equipment *(chilly bin not shown)*:



1. Label 2mL tube with Sample ID

2. Firmly attach pipette tip to end of pipette

3. Remove nectar from disk of flower using pipette

4. Dispense nectar into 2mL tube

5. Repeat steps 3 and 4 for 10 flowers *(dispense nectar from 10 x flowers into <u>one</u> 2mL tube)*

6. Chill and send to Analytica with any other nectar samples

Nectar Collection Option 3 –**Pipette Rinse Method**

List of items required:

Supplied by Analytica in kit

- Disposable plastic pipette
- Yellow 200µL pipette tips
- 2 mL plastic tubes
- Containers to courier tubes back to lab
- Polystyrene chilly bin

Other items required

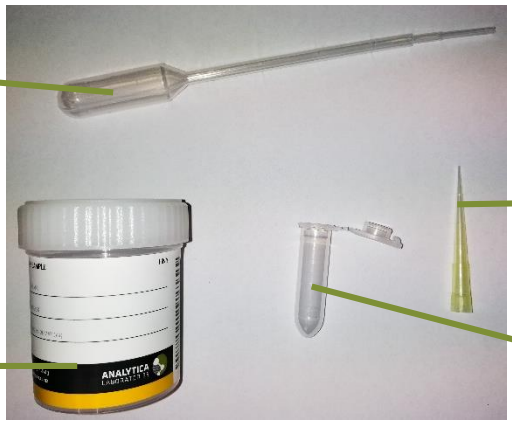
- Fine mesh or plastic bags
- Tape
- Fresh clean water
- Permanent marker
- Sticky labels (optional)
- Ice / slicker pad

1. Refer to Preparation before collecting a nectar sample (*Page 1)* to prepare plant for sampling (day before sampling)
2. Select 10 flowers from one plant. Ensure that the flowers have **visible nectar** (refer to Selecting good flowers – *Page 1*).
3. Clearly label the 2mL tube with the sample identification (use a fine tipped permanent marker or sticky label and permanent marker), and record this sample identification on the sample submission form.
4. ***Flower 1:***
   - Press a yellow pipette tip firmly onto the end of the plastic pipette (make sure it creates a seal.)
   - Use the pipette to transfer approx. 10µL of clean water to the soft centre of the first of the 10 flowers.
     - *Note: 10µL is a volume of liquid that reaches the first line on the pipette tip*
   - Rinse the nectar on the disk of the flower by sucking the nectar off the flower with the 10µL of water up and down with the pipette, being careful not to scrape the flower.
     - *Note: Try not to suck the sample too far up the pipette tip as it will be difficult to dispense back into the sample tube.*
   - Transfer the nectar/water solution from the pipette into the 2mL plastic tube. Suck and repeat the transfer until all the dissolved nectar is removed from the flower.
5. ***Flowers 2 – 10:***
   - Take 10 µL of fresh water and repeat step 4 for each of the remaining 9 flowers, adding the washings to the same plastic tube.
   - You should have at least 100µL of nectar washings in the receiving plastic tube after harvesting the 10 flowers. **Cap the tube tightly**
6. Repeat steps 2 to 5) of harvesting the nectar from 10 flowers for each plant you wish to sample. Use a new pipette tip for each new plant *(not each new flower.)*
7. Place samples in the container provided, and store them on ice in a chilly bin immediately after collection. After sampling, store samples in the freezer until they are ready to be couriered.
   - If samples are warm they will ferment, and this will affect the test results.
8. Send the samples and completed Analysis Request form to Analytica Laboratories, making sure that the samples are on ice so that they remain chilled until they arrive at the laboratory for testing.

Nectar Collection Option 3 – **Pipette Rinse Method**

Supplied equipment *(chilly bin not shown)*:

Disposable pipette



200µL pipette tip

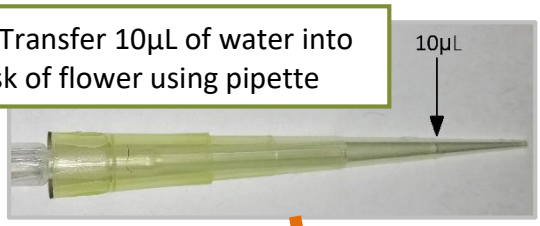
Container for dispatch of tubes to lab

2mL plastic tube

1. Label 2mL tube with Sample ID

2. Firmly attach pipette tip to end of pipette

3. Transfer 10µL of water into disk of flower using pipette



4. Rinse nectar with water by sucking liquid up and down in the pipette tip.

5. Remove nectar washings from disk of flower and dispense into 2mL tube

6. Repeat steps 3 to 5 for 10 flowers *(dispense nectar washings from 10 x flowers into <u>one</u> 2mL tube)*

7. Chill and send to Analytica with any other nectar samples