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# MALDI-TOF MS preparation for identification of mosquitoes V.2

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**We use this protocol and it's working**

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## Abstract

MALDI-TOF MS specimen preparation for rapid identification of Australian mosquitoes

## Guidelines

There are 3 main steps involved with preparing and launching a mosquito sample for MALDI-TOF analysis:

- Preparing the matrix

The matrix solution ionises the proteins in the sample and is applied to the plate after the sample. The matrix must be very acidic.

- Dissecting and crushing the sample
- Preparing and launching the plate

## Materials

MATERIALS

- 1.5 mL Eppendorf tubes
- Acetonitrile **Bio Basic Inc.** **Catalog #**AC1400.SIZE.1L
- Trifluoroacetic acid (TFA) **Bio Basic Inc.** **Catalog #**TC8960.SIZE.100mL
- Water, uHPLC grade
- α-cyano-4-hydroxycinnamic acid **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**C8982 SIGMA
- Formic acid, 70%
- Glass beads, acid washed ≤106µm **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**G4649-500G

## Troubleshooting

1 Preparing the matrix

2 1. Using a small spatula, put approx. 50µl worth of matrix powder (α-cyano-4-hydroxycinnamic acid) in the Eppendorf tube and return the stock powder to the fridge immediately.

3 2. Add 500µl of 100% ACN (acetonitrile), 475µl of HPLC grade water and 25µl of 100% trifluoroacetic acid to the Eppendorf tube.

4 3. Put Eppendorf tube with solution in an ultrasonic bath for 10 minutes to homogenise the solution (yellow matrix powder will still be visible); then centrifuge the solution for 10 minutes at 13 000 rpm/ 20 784 x g.

5 4. Retrieve the supernatant using a pipette, taking care to avoid taking up any powder. Discard the powder. The matrix can be kept at 4 °C in the dark for up to 2-3 days when creating high quality spectra for reference database. It can be kept for up to a week when using it for simple identification when high quality spectra are not vital.

6 Preparing the sample

7 1. If using live mosquitoes, put the mosquitoes in the freezer (-20) for at least 1 minute prior to dissection.

8 2. Using two sets of forceps, carefully remove the legs from the mosquito, using one pair to steady the specimen and the other to remove the legs. Place the legs in a labelled and sterile Eppendorf tube. The legs will stick to the sides of the tube due to static so try to put the legs at the bottom of the tube.

9 3. If more than one species are being processed, decontaminate the forceps with 70% ethanol between species.

10 4. Spin down the legs for 1 minute at 13 000 rpm/ 20 784 x g.

11 5. Add 15µl of 50% CAN and 15µl of 70% formic acid to each tube then add glass beads (<106um, acid washed). Estimate the volume, making the amount of glass beads just less than half of the volume of liquid.

12 6. Place tubes in the TissueLyser II (Qiagen) canisters, making sure each side is balanced. NB: Both the tubes and the canisters need to be balanced. Make sure the top

and bottom lids correlate and that the heavy sides are both either facing inward or both outward.

13 7. Crush the legs using a cycle of 3 × 1 minute at 30 Hz, letting the sample rest for approx. 15 seconds between each minute. NB: the legs will not be fully crushed in the tube; too much protein will inhibit the reaction as in PCR.

14 Preparing the plate

15 1. Load 1µl of each sample on the plate in quadruplicate keeping within the spots.

16 2. Allow the sample to evaporate then load 1µl of matrix solution over the sample, allow to dry completely.

17 3. Place the plate in a cool, dark (photosensitive matrix) place until it's time to launch it. NB: the prepared plates can be left for max. 2-3 days in a dark place at room temperature before affecting the spectra. If using for simple identification – it can be kept for a longer period.

18 4. Plate is ready to be launched in MALDI-TOF MS machine.