

Varroa mite – alcohol wash sampling guide

To protect South Australia's honey bee and honey bee pollination dependant industries, the Department of Primary Industries and Regions (PIRSA) is requiring all apiarists to regularly sample their hives for Varroa mites.

Tables 1a – 3 of this fact sheet provide information on alcohol wash sampling procedures to be undertaken by all apiarists with 1 -19 hives and registered in and/or keeping honey bees in South Australia.

Sampling forms need to be filled by all apiarists registered in and/or keeping honey bees in South Australia. These are available at www.pir.sa.gov.au/varroa

Table 1a. Services and equipment - PIRSA provided (at no cost)

Services:

- delivery of sampling equipment to individual apiarists, via either:
 - delivery to a PIRSA Varroa sampling workshop or nominated nearest PIRSA office/ Australian Clinical Labs collection centre
 - postage to apiarist residential address
- pick up of samples from apiarist from a PIRSA office or Australian Clinical Labs collection centre
- testing and reporting of appropriately samples (turnaround time for samples, from lab receipt to result reporting is about 12-18 working days, in peak periods there may be additional delays).

Sampling equipment:

- 120 ml sample containers, sample bags and sample labels (1 per colony sample)
- apiary bags (1 per 10-20 sample containers)
- Varroa mite sampling form
- PIRSA Varroa mite - alcohol wash sampling guide (this document) and sampling form
- Gribbles VETLAB delivery labels
- bulk sample container(s) (buckets/boxes).

Table 1b. Services and equipment - Apiarist provided

Services:

- pick up sampling equipment from a designated collection point within 1-2 weeks
- collect samples (as per Table 2) and submit samples with sampling forms within the required timeframes (samples must be submitted within 1-2 weeks of sampling)
- return samples to the designated collection point within 1-2 weeks of collection

Sampling equipment:

- hive inspection equipment and personal protection equipment (as required)
- decontamination equipment to minimise disease spread between colonies (e.g. bucket of water and detergent, and rags to dry funnel/ optional equipment between colonies - as required)
- PIRSA provided sampling equipment
- additional sampling equipment including:
 - permanent markers, pens, clipboard
 - sample container holders (as required)
 - large funnel with outlet trimmed to fit into top 10 mm of a 120 ml container
 - 30 ml of 70% ethanol per sample (e.g. methylated spirits), diluted by adding 300 ml of water to 700 ml of methylated spirits. Solution is flammable - keep away from smoker and open flames
 - optional: measuring cup, queen excluder/queen cage, spare super, drone comb uncapper.

Table 2. Sampling procedures

Sampling rate, frequency and targets:

- sampling rate:
 - 100% of the total number of colonies (hives, nucs and swarms) in each apiary, rounded up
 - all apiaries
- sampling frequency:
 - Round 1 - submit samples by January 31 2023
 - Round 2 - submit samples by April 28 2023
- sampling targets:
 - nurse bees in each colony
 - colonies that had a queen introduced within the last 16 weeks.

Note, where sampling is required under an Order/ under Chief Inspector of Stock Permission (e.g. where introducing hives/queens into SA), sampling rates, frequencies and targets must be as directed.

Equipment preparation:

- decontaminate and dry common equipment used between colonies (including funnel/ measuring cup, queen excluder/queen cage, spare super, drone comb uncapper used to assist sampling), between each colony sampled and between apiaries, to prevent cross contamination of samples. Additionally, keep bee-proof sample containers not being used in the apiary being sampled

- ensure sampling equipment is handy, container lids are loosened, and containers are not going to get knocked over.

Table 2. Sampling procedures (continued)

Equipment preparation (continued):

- for each apiary:
 - identify colonies to be sampled with the required identification details of apiary ID and colony ID and whether it has been requeened
 - e.g. for 2 hives at home no requeening
Hm – H1 where Hm = home Apiary and H1 = hive 1
 - e.g. for 2 hives at home and requeened within the last 16 weeks
Hm – H1 – Q where Hm = home Apiary, H2 = hive 1 and Q = requeened
 - pre-label sample container lids with colony ID to ensure traceability
 - for each apiary, do not add ethanol until bees have been added to each sample container and stopped flying, but within approximately ½ hour of collection, and where possible, to groups of samples at the same time
 - label sample container and bag at the apiary after ethanol has been added, lids secured tightly and container exterior dried.

Sampling:

1. Open colony to access brood nest and to ensure nurse bees are sampled.
2. Select two frames with large areas of worker brood (preferably also with drone brood).
3. Inspect frames to ensure queen is not present (if seen, either select another brood frame or remove queen to another frame).
4. Remove lid from a sample container and insert into funnel outlet.
5. Shake/ scoop nurse bees from frames into funnel and in sample container. Add additional bees as necessary to fill sample container (fill to within approximately 10 mm from top, around 300 bees), then quickly remove funnel and attach lid.
6. Close colony, clean hands, and decontaminate common equipment used between colonies.
7. Check that colony and sample container are pre-identified as above (if not, label), then proceed to sample the next colony.

Labelling and packing:

1. Within approximately ½ hour of collection, add 30 ml of diluted ethanol per sample container, then secure lid(s) tightly and dry outer surfaces of the sample container(s) (as necessary),
2. Use a permanent marker to:
 - label colony(s) (if not already completed), sample container(s), sample bag(s),
 - complete sampling form with the required codes (e.g. beekeeper brand, apiary code I.D, colony I.D and Q – if colony had queen introduced within the last 16 weeks)
 - label apiary bag(s) with apiary code I.D. (e.g. **Hm** - for Home Apiary)
3. Place collected sample(s) into corresponding sample bag(s), expel air, seal bag(s), and place upright into corresponding apiary bag(s),
4. Place sample containers upright into bulk sample container, limiting maximum number of sample containers to 2 layers of 50 containers each, with a maximum weight of 16 kg).

Table 3. Sample submission and delivery

- Checklist:
 - Sampling form is complete and is placed into a separate sealable plastic bag
 - sample containers are not leaking (re-secure/ re-bag/ re-label as necessary) and are upright
 - no honey bees (other than those in sample containers) are present
 - bulk sample containers are secure (tape if necessary) with a maximum weight of 16 kg
 - bulk sample containers are labelled with a completed Gribbles VETLAB delivery label
 - collection centre opening hours.

- Confirm opening hours and deliver to the closest collection centre, either a:
 - PIRSA office - visit https://www.pir.sa.gov.au/contact_us for locations of the Clare, Glenside, Kingscote, Loxton, Mount Gambier, Murray Bridge, Nuriootpa, Port Lincoln, and Struan offices
 - Australian Clinical Labs collection centre - visit www.clinicallabs.com.au/location for collection centre locations.