

Varroa mite – alcohol wash sampling guidelines

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 1-3 of in these guidelines provide information on alcohol wash sampling procedures.

Sampling forms 1a-1b need to accompany the samples. These are available at www.pir.sa.gov.au/varroa

Table 1a. Services and equipment - PIRSA provides (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 or 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 collected samples (turnaround time for Round 1 and Round 2 samples, from lab receipt to result reporting may be 10-12 working days; in peak periods there may be additional delays).

Sampling equipment:

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

Table 1b. Services and equipment - Apiarist provides**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 1a-1b 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 frequency:
 - Round 1 - submit samples by Jan 31, 2023
 - Round 2 - submit samples by April 28, 2023
- sampling rate:
 - sample each apiary at the rate of 10% of the total number of colonies (hives, nucs and swarms) in each apiary, rounded up. See example below:

| Total No. of colonies in total No. of apiaries | Total No. samples/ apiary at 10% rate | Total No. samples Round 1 | Total No samples Round 2 |
|---|--|----------------------------------|---------------------------------|
| 20 hives in 2 apiaries | 2 samples/apiary | 4 samples | 4 samples |
| 84 hives in 4 apiaries | 9 samples/apiary | 36 samples | 36 samples |
| 96 hives in 3 apiaries | 10 samples/apiary | 30 samples | 30 samples |
| 100 hives in 1 apiary | 10 samples | 10 samples | 10 samples |
| 106 hives in 12 apiaries | 11 samples/apiary | 132 samples | 132 samples |

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

Table 2. Sampling procedures (continued)

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 with sampling), between each colony sampled and between apiaries, to prevent cross contamination of samples. Additionally, keep bee-proof the sample containers not being used in the apiary being sampled.
- ensure sampling equipment is handy and container lids are loosened
- for each apiary:
 - use a permanent marker to label colonies to be sampled with the required identification details (e.g. apiary code/ I.D. [e.g. **Gr** - for Green Apiary], colony I.D [e.g. **H1** - for hive 1], and if colony had queen introduced within the last 16 weeks, queen producer I.D. [e.g. **Q1** - for queen producer 1, as identified in sampling form 1a])
 - use a permanent marker to pre-label sample containers to be used with the colony I.D [e.g. **H1** - for hive 1]
 - only add ethanol to each sample container after bees have been collected and have ceased activity in the containers (e.g. within approximately ½ hour after collection)
- finalise labelling sample containers and sample bags at the apiary after ethanol has been added, lids have been 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 ½ 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), and sample bag(s),
 - complete Forms 1a - 1b with the required codes (e.g. brand [e.g. **AAA**], apiary code I.D. [e.g. **Gr** - for Green Apiary], colony I.D [e.g. **H1** - for hive 1], and if colony had queen introduced within the last 16 weeks [e.g. **Q1** - for queen producer 1, as identified in Form 1a])
 - label apiary bag(s) with apiary code I.D. (e.g. **Gr** - for Green 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 forms 1a-1b are complete and are 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.

- 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.