

ANALYSES 2024

Sensitivity test for tau-fluvalinate and amitraze on *V. destructor*

1 – ANALYSES PRICING

To make an analysis request, please complete the order form on our website (in french) and contact us beforehand to schedule the tests.

Analyses	Unit price € HT
Fluvalinate sensitivity	280
Amitraze sensitivity	280
Fluvalinate AND Amitraze sensitivity	350



Warning: If the number of live varroas is too low (phoretic varroas < 5% or bad transport conditions), the test is not possible. The support of the sample will be charged 35 € HT, corresponding to the time of search for varroas in the brood.

2 – SAMPLING PROTOCOL AND COLLECT BROOD

Please follow this protocol carefully for sampling and brood sending steps to allow for proper testing.

Sensitivity tests require a large number of varroas: about **150 LIVE varroas mite** to each molecule tested (amitraze / tau-fluvalinate). Varroa mites are collected from pieces of brood sampled from several hives of the same apiary.

2.1) Minimum infestation rate

Colony infestation monitoring is necessary to ensure sufficient infestation before brood collection.

Within the same apiary, infestation rates can be very heterogeneous. It is often necessary **to test several colonies** to find one that meets **the minimum infestation criteria**:

- Phoretic varroas % count : Minimum 5% phoretic varroas (per 100 bees). When the phoretic varroa rate is greater than 10%, the number of varroas to perform the test is more than enough.
- Count the number of varroas in natural fall / day : > 5 varroas /day

If the colonies are not sufficiently infested, it is useless to make a sample because the tests will not be feasible and the time of search for varroas will be charged.

2.2) COLLECT BROOD

For a good representativeness of the sensitivity of the apiary, **the collection of broods must be carried out on 3 different colonies** (of the same apiary). However, depending on the infestation rate, 1 or 2 colonies may be sufficient.



It is important to collect broods from the **EMERGING BEE NYMPHAL** step. This promotes survival during transport and facilitates the collection of varroa mites. Conversely, the larval stage should be avoided because the larvae are too fragile.

Ideally, **BUMBLEBEE brood** is more suitable for testing as it is generally more infested. However, worker brood is very suitable when the colony is sufficiently infested.

The quantities of brood to be collected are given in the following table :

<u>Collection from 1 hive of an apiary</u>	<u>Collection from 2 to 3 hives of an apiary</u>
<ul style="list-style-type: none">• 1 piece of brood 25cmx15cm on a single frame• 2-3 pieces of 15x15cm on different frames	<ul style="list-style-type: none">• 1 piece 15x15cm brood apiary
CAUTION: Do not take pieces less than 15cmx15cm (tendency to crash)	

2.3) Sample form

Fill in the sampling sheet with the following information:

- Collect Date
- Sedentary / transhumant apiary
- Varroa mite treatment history
- Apiary postal code or GPS point
- Number of beehives collected

Please complete **1 sample form per shipment**, each sample consists of 1 to 3 hives of the same apiary. This form must be sent by email with the tracking number of the package (contact@apinov.com) + printed and sent with broods.

Download the form



2.4) Brood Preparation

- Broods should be carefully wrapped, individually, **in paper towels or newspaper (no plastic bag that would prevent broods from breathing)**.
- **Use a transport carton and secure the brood pieces** to prevent them from crashing during transport.
- Attach the sample sheet (form) previously completed with.
- If several samples in the same shipment: identify the samples with a label indicating the postal code of the apiary on the packaging of each piece of brood.

2.4) Sending of broods

Please contact us before sending to confirm our availability to perform the tests.

To ensure the survival of varroa mites, transport must be as fast as possible. Also, it is imperative to send the couvains in express transport type Chronopost, DHL, FEDEX or other private carrier with a delivery for the next day (**never shipping in Colissimo**). Avoid sending broods at temperatures below 15°C or above 35°C. Since the tests require 2 days, the broods must arrive at APINOV no later than Thursday morning. In order to anticipate possible delivery delays, broods must therefore be shipped at the beginning of the week, **on Monday or Tuesday**.



Delivery address: APINOV – 10 rue Henri Bessemer – 17140 LAGORD - FRANCE
Contact and phone/ email for tracking the package:
Myriam LAURIE – +33 6 24 85 21 77 contact@apinov.com



*Thank you to systematically send us **the tracking numbers of the packages by email.***

3 – PROCEDURES FOR CARRYING OUT TESTS

The tests are carried out as soon as the samples are received, and the results are sent to the customer no later than the following week.

3.1) Sensibility tests

The method used is that of Almecija et al 2020¹, from Maggi *and al*, 2008² and Milani (1995)³ and Lindberg *and al* (2000)⁴. The Milani method is the method referenced in the Beebook (Dietemann et al, 2013)⁵.

Varroa mites are collected directly in the brood and then put in contact in Petri dishes contaminated with the active ingredient (here amitraze or fluvalinate).

They are left in contact with the active substance for 1 hour and then moved into uncontaminated Petri dishes, with nymphs. The mortality rate is observed at 24 hours.

3.2) Controls and corrected mortality

For optimal validation of the results, the mortality rate of the controls should not exceed 30%. According to the mortality of the controls, a corrected mortality (Mc) is calculated by the formul:

$$Mc = (M-Mt)/(1-Mt)$$

with Mt: Control mortality and M: Varroa mortality at test concentration

If the control mortality is less than 5%, no correction is required⁶.

¹ Almecija G, Poirot B, Cochard P, Suppo C. Inventory of Varroa destructor susceptibility to amitraz and tau-fluvalinate in France. *Exp Appl Acarol.* 2020 Sep;82(1):1-16. doi: 10.1007/s10493-020-00535-w. Epub 2020 Aug 18. PMID: 32809187.

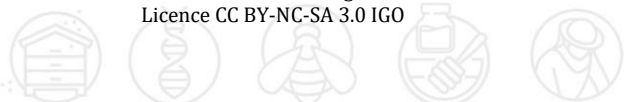
² Maggi, M. D., Ruffinengo, S. R., Gende, L. B., Eguaras, M. J., & Sardella, N. H. (2008). LC50 baseline levels of amitraz, coumaphos, fluvalinate and flumethrin in populations of Varroa destructor from Buenos Aires Province, Argentina. *Journal of apicultural research*, 47(4), 292-295.

³ Milani, N. (1995). The resistance of Varroa jacobsoni Oud to pyrethroids: a laboratory assay. *Apidologie*, 26(5), 415-429.

⁴ Lindberg, C. M., Melathopoulos, A. P., & Winston, M. L. (2000). Laboratory evaluation of miticides to control Varroa jacobsoni (Acari: Varroidae), a honey bee (Hymenoptera: Apidae) parasite. *Journal of economic entomology*, 93(2), 189-198.

⁵ Dietemann, V., Nazzi, F., Martin, S. J., Anderson, D. L., Locke, B., Delaplane, K. S., ... & Rosenkranz, P. (2013). Standard methods for varroa research. *Journal of Apicultural Research*, 52(1), 1-54.

⁶ Procedures for testing insecticide resistance in malaria vectormosquitoes_ second edition. Geneva: World Health Organization; 2017. Licence CC BY-NC-SA 3.0 IGO



3.3) 90% Lethal Concentration (CL90)

The 90% lethal concentration (CL90) corresponds to the reference concentration for which a mortality of 90% of the varroas is observed for a sensitive population. It is used as a discriminating concentration. It varies by active substance. This LC90 is obtained from susceptible varroa populations that exhibit an optimal sensitivity reaction to the active substance. These sensitive populations come from sedentary apiaries untreated for more than 5 years with tau-fluvalinate and amitraz

The LC90 for amitraz was defined as 0.4 µg/mL from 8 populations considered sensitive (n=8). The LC90 for tau-fluvalinate was defined as 20µg/mL from 6 populations considered sensitive (n=6).

From of reference populations, varroa mite populations may be phenotyped into different sensitivity classes:

Varroa phenotyping	Mortality at LC90 (M)
Sensitive	M>75%
Moderate resistance	40%<M<75%
Strong resistance	M<40%

4 – EXEMPLE OF RESULTS

Sample Réf	Mortality	
	Control (hexane)	AMITRAZE
X1	8%	73±3%
X2		45±1%

- Control mortality rate: 8%
- Corrected mortality : 73±3% for X1 and 45±1% for X2
- Conclusion of **amitraz** sensitivity test: **Moderately Resistant for X1 and X2**

