###### Customer sampling form

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| ***Section to be filled by the EU RL:***  |
| Date of sample receipt:  | Registration date: |
| Person in charge of the file:  | LIMS registration n°: |
| Form completed:  yes  no |
| Request for additional information filed on: by:  telephone  e-mail  other |

**Information**

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| **Laboratory** |  | **Contact person** |
|  |  |  |
| Name:       |  | Last name:       |
| Address:       |  | First name:       |
| Zip code:        | City:        |  | Tel:        |
| Country:       |  | Email:       |
| Tel:       | Fax:       |  |  |

**Samples**

**Please note: section to be filled in by the laboratory. For sampling procedure, please follow the protocols described below.**

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| **List of samples**  | ***Section to be filled in by the*** ***EU RL*** |
| **Identification** *= indicate reference ID on sample (ex: hive, apiary, date, etc.)* | **Sample type\*\*****Mandatory field** | **Analyses requested***(indicate the number corresponding to the analyses requested)* | LIMS reference n° for sample |
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**\*\*** Please specify: bees (dead bees, living internal bees or living external bees), brood larvae, pollen, honey, beebread, beeswax, microscope slides, etc.

**Analyses requested**

***Screening for diseases and biological pathogens***

**American and European foulbrood:**

1. Screening for American foulbrood through macroscopic and microscopic examination**\***[[1]](#footnote-1)*/ brood, larvae*
2. Isolation and culture of the American foulbrood bacterium (*Paenibacillus larvae*) / *brood, larvae, honey, wax*
3. Identification of *P. larvae* by PCR **\*** */ brood\*, larvae****\*****, bacterial culture*
4. Screening for European foulbrood through macroscopic and microscopic examination**\*** */ brood, larvae*
5. Isolation and culture of the European foulbrood bacterium (*Melissococcus plutonius*) / *brood, larvae*
6. Identification of *M. plutonius* by PCR */ brood\*, larvae\*, bacterial culture*

**Mycosis:**

1. Screening for brood mycosis through macroscopic and microscopic examination */ brood*

**Varroosis:**

1. Screening for varroosis through macroscopic examination**\*** */ bees and/or brood*

**Nosemosis:**

1. Diagnosis of nosemosis through microscopic examination (10 symptomatic honeybees)\* / bees
2. Screening for nosemosis through microscopic examination (60 honeybees) */ bees*
3. PCR identification of the *Nosema* species (*N. apis / N. ceranae*)**\*** */ bees*

**Tracheal acariosis:**

1. Screening for tracheal acariosis through microscopic investigation */ bees*
2. Detection and identification of *Acarapis woodi* by PCR */ bees*

**Viral disease / bee virus:**

1. Acute bee paralysis virus (ABPV): PCR detection and quantification of the viral load */ bees and/or brood*
2. Black queen cell virus (BQCV): PCR detection and quantification of the viral load */ bees and/or brood*
3. Chronic bee paralysis virus (CBPV): PCR detection and quantification of the viral load*/ bees*
4. Deformed wing virus (DWV-A and DWV-B): PCR detection and quantification of the viral load */ bees and/or brood*
5. Sacbrood virus (SBV): PCR detection and quantification of the viral load */ bees and/or brood*
6. Israeli acute paralysis virus (IAPV): PCR detection */ bees and/or brood*
7. Kashmir bee virus (KBV): PCR detection */ bees and/or brood*

**Small hive beetle (*Aethina tumida*):**

1. Identification by morphological examination**\*** */ insects, larvae*
2. Identification by PCR\* */ insects, larvae, eggs*

**Asian hornet (*Vespa velutina*):**

1. Identification by morphological examination */ insect*

***Tropilaelaps* spp.:**

1. Identification by morphological examination**\*** */ mite*
2. Identification by PCR / *mite*

**Other:**

1. Please, specify:

***Pesticide residue analyses***

**Neonicotinoids by LC-MS/MS in:**

1. Honey**\***[[2]](#footnote-2) 28. Bees**\*** 29. Pollen**\*** 30. Beebread**\***
2. Nectar 32. Feed syrup 33. Bee larvae

**Neonicotinoids and sulfoxaflor by LC-MS/MS in:**

34. Bees 35. Pollen

**Other** **pesticides** (organochlorines, organophosphorus, pyrethroids, boscalid, vinclozolin, iprodione and bromopropylate) **by GC-MS/MS in**:

* Honey**\***: *36. Multi-residue or 37. Identified pesticide: ………………………………………………..*
* Bees: *38. Multi-residue or 39. Identified pesticide: ………………………………………………..*
* Pollen: *40. Multi-residue or 41. Identified pesticide: ………………………………………………..*
* Beebread: *42. Multi-residue or 43. Identified pesticide: ………………………………………………..*

**Sampling protocol**

***Screening for diseases and biological pathogens***

**Type and quantity of samples to be taken:**

* **Bees**:

**Living "symptomatic" specimens should be collected first**, *i.e.* samples of living bees presenting clinical signs or abnormal behaviour justifying your request for analysis.

Dead bees can be sampled. Be careful when choosing specimens: dried or rotten bees cannot be used for analyses. Choose **recently** deceased bees (maximum 1 week) and avoid rainy periods.

Internal bee sample is less reliable than the others when screening for adult bee diseases. When clinical signs are present, internal bee sample should be collected with "symptomatic" bees from the same colony.

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| **Disease** | **Minimum quantity** |
| Varroosis (*Varroa destructor*) | >30 bees |
| Nosemosis (*Nosema apis, Nosema ceranae*) | >10 bees |
| Tracheal acariosis (*Acarapis woodi*) | >20 bees |
| Viral disease | >10 bees |

**In the absence of clinical signs, and in order to determine the infectious status of the colony (screening): collect live asymptomatic bees inside or at the entrance of the hive.**

For the *Nosema* and virus research, carry out the sampling on border frames, in the honey super or at the entrance of the hive to favour the sampling of the bee foragers.

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| **Pathogen agents** | **Minimum quantity** |
| Virus | >60 bees |
| Etiological agents of nosemosis (*Nosema apis, Nosema ceranae*) | >60 bees |
| Etiological agents of tracheal acariosis (*Acarapis woodi*) | >200 bees |

* **Brood:**

Cut off a 10x10 cm piece of brood containing at least 10 larvae and/or pupae with abnormal appearance justifying the request for analysis. It is also possible to send an entire frame.

* **Suspicious parasites** (*e.g*. mites, insects):

Take samples of several individuals of each species, at various development stages of development (*e.g*. eggs, larvae, pupae, adults) if possible. Indeed, sometimes it is essential to examine several individuals to identify the parasite.

* **Hive products for diagnosis of American foulbrood by culture and PCR**

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| --- | --- |
| Honey: > 40 g | Wax: > 5g |

**Packaging:**

* Place the samples in **clean, closed packages displaying the number or name of the samples.**

*E*.*g*. cardboard (*e.g*. matchboxes) or paper packaging (*e.g*. brown or Manila envelopes – special care should be taken to avoid crushing samples), queen cages (for transporting live bees).

**Please note: do not use plastic bags (risk of maceration).**

* **Suspicious parasites** (*e.g.* mites, insects)

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| Parasites must be sent **dead** in tightly-sealed receptacles in order to avoid risks of dissemination during transport. Specimens can be killed preferably by freezing (-20°C over night) or by placing them in ethanol.In case of exotic parasitosis suspicion, and due to the urgency of the analysis, warn the EU RL that the samples have been sent.  |

Send the dead specimens preferably kept dry. They can also be sent in a 70% ethanol solution.

**Please note: Only ethylic alcohol (= ethanol) must be used. Do not use methylated spirits or denatured alcohol which contains other chemical compounds which could spoil the sample.**

**Sample conservation:**

* **If the samples are sent within a few hours from sampling, on the same day, if possible**:

Store the samples in the refrigerator (make sure they remain dry) and send them at room temperature.

* **If the samples cannot be sent immediately after sampling:**

Freeze the samples (at -20°C) and ensure that the cold chain remains unbroken until the arrival at the laboratory. Use a specialized transport carrier for transport.

* **Specimens in ethanol** can be sent at room temperature.

***Pesticide residue analyses***

**Quantity of samples to be taken:**

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| --- | --- |
| **Matrix** | **Minimum quantity to collect \*** |
| Honey | 30 g |
| Bees | 30 g |
| Pollen | 30 g |
| Beebread (without comb) | 20 g |
| **Matrix** | **Minimum quantity to collect \*** |
| Nectar | 20 µL |
| Bee larvae | 10 g |
| Feed syrup | 10 g |

\* Minimum quantity to collect for performing all the research proposed in the selected matrix.

**Packaging and Sample conservation:**

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| --- | --- |
| **Packaging** | **Sample conservation** |
| **Honey and feed syrup** |
| A propylene jar, tightly-sealed and placed in a plastic bag. | In the refrigerator at about +5°C |
| **Nectar** |
| Microcapillaries containing nectar samples in clean tightly-sealed tubes placed in a plastic bag or preferably, nectar sample collected in a Eppendorf tube. | In the refrigerator at about +5°C |
| **Wax** |
| A propylene jar, tightly-sealed or a clean cardboard and placed in a plastic bag. | In the refrigerator at about +5°C |
| **Pollen, beebread and bees** |
| A clean cardboard or paper recipient. Avoid using plastic as well as crushing the samples.  | In the freezer at about -20°C |
| **Bee larvae** |
| A propylene jar, tightly-sealed and placed in a plastic bag. | In the freezer at about -20°C |

Label samples and join the information sheet in the corresponding package.

**Dispatching samples**

* **Avoid sending samples at the end of the week** (after Wednesday), since the samples can be blocked during the weekend. The analysis reliability will be compromised.
* Samples must be sent to the laboratory in **rigid, triple packaging** in order to prevent **crushing** them.
* Ensure that the cold chain remains unbroken until the arrival at the laboratory.
* Biological materials infectious for honey bee diseases are classified UN 3373. Therefore, packages containing UN3373 materials must be marked with the proper shipping name of "Biological substance, Category B".
* The **information sheet** must be filled out and enclosed with the samples. Do not place the information sheet in direct contact with samples.

**Indicate the following information on the package:**

|  |
| --- |
| **EU RL address:**ANSESHoney bee pathology unitLes Templiers, 105 Route des ChappesCS 20111F-06410 BiotFRANCETel: +33 4 92 94 37 00 - Fax: +33 4 92 94 37 01Email: eurl.bee@anses.fr |

The laboratory guarantees that the samples will be kept for two months

following the transmission of the results to the petitioner.

1. **\*** The analyses with an asterisk are accredited by the French accreditation body / Cofrac (accreditation number: 1-2229). Scope available on www.cofrac.fr. [↑](#footnote-ref-1)
2. **\*** The analyses with an asterisk are accredited by the French accreditation body, Cofrac (accreditation number: 1-2229). Scope available on [www.cofrac.fr](http://www.cofrac.fr). [↑](#footnote-ref-2)