*FAST*est® BEE AFB

Test-kit for the qualitative detection of *Paenibacillus larvae* genotypes ERIC I and ERIC II (AFB) in bee colonies

***In vitro* diagnosticum**

INSTRUCTIONS FOR USE

1. INTRODUCTION

Honeybees (*Apis mellifera*) are among the top three agricultural livestock worldwide by pollinating a wide range of crops and ornamental plants. This important ecosystem service is essential for sustainable, productive agriculture and for the maintenance of the non-agricultural ecosystem. Therefore, monitoring the health and vitality of honeybee colonies plays a crucial role.

American Foulbrood (AFB) is a serious brood disease in honey bees and is caused by the spore-forming, rod-shaped, Gram-positive bacterium *Paenibacillus larvae*. Only young larvae are susceptible to infection through the ingestion of spores. Adult bees are immune but act as carriers. If dead larvae are not removed quickly enough by nurse bees, their tissue dries out inside the capped brood cell to form a black scab containing up to 2.5 billion spores, which are highly infectious to other larvae. As a result, the infection can spread very rapidly to the entire brood of a bee colony, leading to a massive weakening or the death of the entire colony.

Paenibacillus larvae occurs primarily in two virulent genotypes, ERIC I and ERIC II. These genotypes differ in the time course of the infection (ERIC I approx. 12 days, ERIC II approx. 7 days to 100 % mortality). Due to its high virulence and rapid lethality, AFB is classified as a notifiable animal disease in the European Union (Regulation 2016/429 and Implementing Regulation (EU) 2018/1882). Even a suspected case of AFB must be reported!

With the ***FAST*est® BEE AFB**, beekeepers can detect *Paenibacillus larvae* easily, cost-effectively and directly at the hive.

2. TEST-KIT COMPONENTS

1 test-kit ***FAST*est® BEE AFB** contains:

- 2*, 5** or 25*** test cassettes, coated with monoclonal antibodies against *Paenibacillus larvae*
- *2, *5 or ***25 sample extraction tubes **A** with 2.0 ml buffer diluent and stainless steel beads each
- *2, *5 or ***25 disposable plastic pipettes
- *2, *5 or ***25 wooden sticks
- 1 instructions for use

3. STABILITY AND STORAGE


15–25 °C

Store at 15–25 °C

Expiry date – see label

In vitro diagnosticum

LOT Lot number

Follow instructions for use precisely

Do not use test-kit components from different kits, lot numbers or beyond stated expiry date.

4. LIABILITY

The entire risk due to the performance of this product is assumed by the purchaser. The manufacturer shall not be liable for indirect, special or consequential damages of any kind resulting from the use of this product.

The duty to report lies with the person conducting the test. Even a suspected case must be reported, regardless of whether the test result is negative or positive.

5. TEST PRINCIPLE

The ***FAST*est® BEE AFB** is based on an immunochromatographic "sandwich principle".

The antigens of *Paenibacillus larvae* or ERIC II, respectively, will react with specific, mobile antibodies which are conjugated to colloidal gold particles in the conjugate pad. These antigen-antibody complexes are migrating ("lateral flow", **LF**) along the nitrocellulose membrane and bind to fixed, monoclonal antibodies forming a blue-purple TEST line (**T**).

A correct test procedure will be indicated by a second, blue-purple CONTROL line (**C**).

6. PRECAUTIONS

- For hygienical reasons, we recommend to wear disposable gloves and other personal protective equipment (protective clothing, possibly a face mask). Wash your hands and disinfect them properly after completing the test
- Label sample material, sample extraction tubes and associated test cassettes to ensure a precise assignment.
- Use a new sample extraction tube and a new test cassette for each test and for each sample a new wooden stick.
- The buffer diluent contains low concentrations of Bronidox as preservative, therefore avoid skin/eye contact and/or ingestion.
- The sample material must be seen as potentially infectious and disposed of accordingly and bee-safe after testing, together with the used test-kit components.

7. SAMPLE MATERIAL

a. Keep in mind that the sample material, as well as all used test-kit components, should have reached **room temperature (15–25 °C)** at the time of application.

b. Suitable sample material: larva (from a cell where development has stalled, with a sunken cell lid), scab, stringy mass

c. The sample material must be collected using the wooden stick provided and placed into the sample extraction tube.

d. Thoroughly crush the sample material before use and ensure uniform mixing (see also 8.2 – Sample preparation).

Version 06/2026

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Art. No. 306005BG1 (5's)
306025BG1 (25's)
306024BG1 (12×2's)

8. TEST PREPARATION

1. **Read instructions for use carefully before starting the test.**
2. **For the reliability of the results, it is necessary to follow the instructions for use exactly. Carry out the test exactly step by step.**

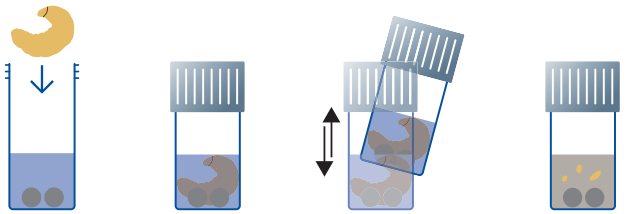
8.1. GENERAL

- Remove the test cassette from its foil pouch shortly before use.
- Mix the buffer diluent well immediately before use (swirl the sample extraction tube **A** without foaming).
- Once used, test cassettes must not be reused.
- Damaged test-kit components must not be used.

8.2. SAMPLE PREPARATION

1. Open the sample extraction tube. Collect the sample using the wooden stick (fig.1) and transfer it as completely as possible into the sample extraction tube (fig.2).
2. Close the sample extraction tube and break up the sample material by shaking the tube vigorously for approx. 30 seconds (fig.3). The stainless steel beads and buffer components contained within break down the sample and release the spores it contains. The resulting sample-buffer mixture (SBM) may turn slightly brown during extraction (fig.4).

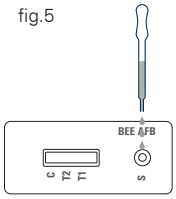
fig.1 fig.2 fig.3 fig.4



9. TEST PROCEDURE

1. Remove the test cassette from its foil pouch shortly before use. Place it on a smooth, flat surface.
2. Suck up the extracted SBM using the disposable plastic pipette and add **3–4 drops** into the sample window of the test cassette (hold pipette vertically, fig.5).
3. The 10-minute incubation time starts now.
4. If there is no beginning blue-purple LF visible within 1 minute after adding the SBM, add 1 additional drop of SBM into the sample window.

fig.5



10. READING OF THE TEST RESULTS

Read the test result after the incubation time of **10 minutes**. Beyond this time, test results most not be interpreted.

POSITIVE TEST RESULT
A blue-purple TEST line (T) **of any intensity** (varying from very weak to strongly intensive) and a blue-purple CONTROL line (C) of any intensity appear.

If only a TEST line (T2) appears in the middle region of the rectangular window, *Paenibacillus larvae* is detected (fig.6).

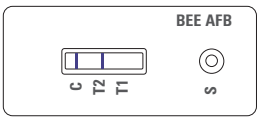


fig.6

If two TEST lines (T1, T2) appear, this indicates the specific detection of *Paenibacillus larvae* genotype ERIC II (fig.7).

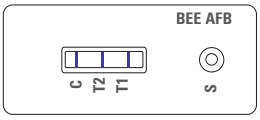


fig.7

NEGATIVE TEST RESULT (fig.8)
Only a blue-purple CONTROL line (C) appears. This line indicates, irrespective of its intensity, that the test has been performed properly.

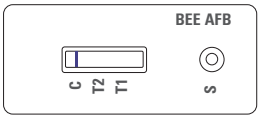


fig.8

INVALID TEST RESULT (fig.9)
No CONTROL line visible. The test should be repeated using a new test cassette.

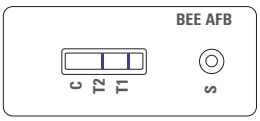


fig.9

11. INFORMATION FOR THE INTERPRETATION

- The interpretation of the test result should always be based on previous history, visible signs of disease, therapy and prophylaxis possibilities.
- Any non-described colour or contour variation of T1, T2 and C (e.g., greyish, shadow-like lines) has to be considered as unspecific reactions and therefore as negative test result.
- Positive test results may be observed within 10 minutes.
- T1 and T2 can vary both in intensity and in width. Therefore, any blue-purple line which appears within the required incubation time has to be interpreted as a positive test result.
- If the wrong sample material is selected, the test may also come back negative, i. e. produce false-negative results.

11.1 PROCEDURE IN THE EVENT OF SUSPETED AMERICAN FOULBROOD

In accordance with animal health regulations (Regulation 2016/429 and Implementing Regulation (EU) 2018/1882), any well-founded suspicion of American Foulbrood must, as a matter of principle, be reported to the competent authority (veterinary office or district administrative authority). Both clinical symptoms and a positive pathogen detection (spores of *Paenibacillus larvae*) constitute grounds for suspicion. **The reporting obligation applies to both negative and positive *FAST*est® BEE AFB test results**. The authority will initiate further steps such as colony inspection and diagnostic investigation via a reference laboratory.

Further information is also available, for example, from

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Austria
AGES
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1220 Wien
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On-site *FAST*est® BEE AFB testing

a. Negative ***FAST*est® BEE AFB**
→ Repeat the test if clinical suspicion persists!

b. Positive ***FAST*est® BEE AFB**
→ Suspicion of American Fouldbrood confirmed

ABBREVIATIONS

AFB	American Foulbrood
C	CONTROL line
ERIC	Enterobacterial Repetitive Intergenic Consensus
LF	Lateral flow
SBM	Sample-buffer mixture
T	TEST line