*FAST*est® BEE 3T

Test-kit for the qualitative detection of deformed wing virus (DWV), acute bee paralysis virus (ABPV) and sacbrood virus (SBV) in the bee / bee brood

***In vitro* diagnosticum**

INSTRUCTIONS FOR USE

1. INTRODUCTION

Honeybees (*Apis mellifera*) are among the top 3 agricultural livestock world-wide 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. Besides numerous parasites and fungi (e.g. *Varroa destructor* and *Nosema* spp.), viruses (e.g., deformed wing virus DWV, acute bee paralysis virus ABPV, sacbrood virus SBV) pose a great threat to the health and welfare of honeybees.

DWV, triggered by stress (heavy infestation with *V. destructor*, lack of food, incorrect colony management), can cause characteristic disease symptoms (shrunken, flightless wings, reduced body size, discolouration in adult bees).

ABPV multiplies mainly in the pupae. After activation (by mite infestation, bacterial infections, environmental pollution, chemicals, insecticides, etc.), the infection is characterized by rapidly progressing paralysis, trembling, inactivity to fly, gradual darkening and loss of hair from the chest and abdomen and rapid death in adult bees.

SBV causes significant morphological changes of the brood (no pupation, sac-like accumulation of ecdysial fluid, discolouration from pearly white to pale yellow, desiccation after death in the form of a dark brown, ship-shaped scale). Adult bees develop an infection without visible disease signs, characterized only by a reduced lifespan.

Numerous studies indicate that there is a mutual relationship between the infestation level of *V. destructor* (parasitic mite Varroa) and certain viral diseases (especially DWV, ABPV, SBV). The Varroa mite serves as a virus reservoir and carrier of these viruses. This fatal combination is considered the main cause of winter losses! DWV shows the best correlation between the level of Varroa infestation and the virus load.

For the assessment and containment of these winter losses, the direct virus detection on-site using ***FAST*est® BEE 3T** is a quick and easy diagnostic tool for the beekeeper. In the positive case, regardless of which virus test is positive, the Varroa control strategy (Varroa status) of the hive should be reviewed according to the guidelines of the respective country.

2. TEST-KIT COMPONENTS

1 test-kit ***FAST*est® BEE 3T** contains:

- 2*, 10**, 25*** or 50****×3 dipsticks, coated with monoclonal antibodies against DWV, ABPV or SBV
- *2, **10, ***25 or ****50 dropper bottles **A** with 1.5 ml buffer diluent each
- *2, **10, ***25 or ****50 sample tubes with squeezer
- 1 tweezer
- 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.

5. TEST PRINCIPLE

The ***FAST*est® BEE 3T** is based on an immunochromatographic "sandwich principle".

The virus antigens of DWV, ABPV and SBV present in the bee will react in the conjugate pad with specific, mobile antibodies, which are conjugated to colloidal gold particles. These antigen-antibody complexes are migrating ("lateral flow", **LF**) along the nitrocellulose membrane and bind to fixed, monoclonal anti-virus antibodies forming a pink-purple TEST line (**TL**).

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

6. PRECAUTIONS

- For hygienical reasons, we recommend to wear disposable gloves and other personal protective equipment (protective clothing, possibly a face mask). Wash and disinfect hands after completing the test.
- Label sample material, sample tubes and associated dipsticks to ensure a precise assignment.
- Use a new sample tube and new dipsticks for each sample.
- The buffer diluent contains low concentrations of toxic sodium azide as a 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. 5 bees or bee brood (maggots, pupae, at least three days after capping) can be used for testing, they can be picked up with the enclosed tweezers and placed in the sample tube.

c. Crush the bees / bee brood well with the squeezer before use and ensure uniform mixing.

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Art. No. 301010BG1 (10's)
301024BG1 (12×2's)
301025BG1 (25's)
301050BG1 (50's)

8. TEST PREPARATION

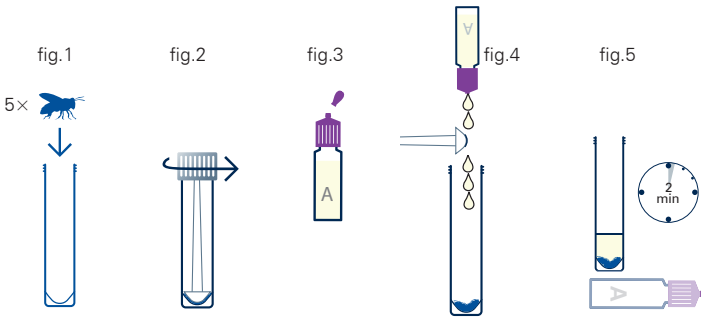
1. **Read instructions for use carefully before starting the test.**
2. **For the reliability of the results, it is necessary to follow the instructions for use exactly. Carry out the test exactly step by step.**

8.1. GENERAL

- Remove the dipsticks from their foil pouch shortly before use.
- Mix the buffer diluent well immediately before use (swirl the dropper bottle **A** without foaming).
- Once used, dipsticks must not be reused.
- Damaged test kit components must not be used.

8.2. SAMPLE PREPARATION

1. Open the sample tube and add five bees / bee brood (maggots, pupae) into its deepening (fig.1).
2. Squeeze the bee material by closing the blue cap with the attached squeezer tightly onto the sample tube for several times (fig.2).
3. Open the sample tube again. Take one dropper bottle **A**, break the tip (fig.3) and add the **whole buffer diluent (1.5 ml)** into the sample tube with the bees / bee brood. In case of any bee remnants on the squeezer, let some drops flow over the squeezer into the sample tube (fig.4).
4. Mix the squeezed bee material homogeneously with the buffer diluent by twisting and untwisting the blue cap for several times (see fig.2).
5. Remove the squeezer from the cap. Screw the cap onto the tube and leave the bee buffer mixture (BBM) on a flat surface for at least two minutes (fig.5) to allow the coarse bee particles to settle (sedimentation time).



9. TEST PROCEDURE

1. Remove the dipsticks from their foil pouch shortly before use.
2. Place the three dipsticks carefully parallel, vertically and in the direction of the arrows into the sample tube. The front sides of the dipsticks must not touch each other. The liquid level (meniscus!) must not exceed the blue arrowheads (fig.6). The incubation time of 10–15 minutes starts now.

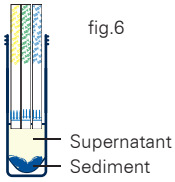


fig.6

3. Remove the dipsticks from the sample tube when the pink-purple CONTROL line (CL) becomes visible (see figs.7/8). If the CL will not appear after 2 minutes, a new BBM with new bees / bee brood must be prepared and sedimented for at least 5 minutes. The dipsticks must be held only in the supernatant until the LF has reached the CL.
4. Place the dipsticks on a flat and horizontal surface for incubation.

10. READING OF THE TEST RESULTS

Read the test result after the incubation time of 10–15 minutes. Beyond this time, test results must not be interpreted.

POSITIVE TEST RESULT (fig.7)
A pink-purple TEST line (TL) **of any intensity** (varying from very weak to strongly intensive) and a pink-purple CONTROL line (CL) appear.

fig.7

NEGATIVE TEST RESULT (fig.8)
Only a pink-purple CONTROL line (CL) 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 new dipsticks.

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 TL and CL (e.g., greyish, shadow-like links) has to be considered as unspecific reactions and therefore as negative test result.
- Positive test results may be observed within 10 minutes, depending on the concentration of antigen in the sample.
- TL can vary both in intensity and in width. Therefore, any pink-purple line which appears within the required incubation time has to be interpreted as a positive test result.
- In the case of doubtful, very weakly positive pink-purple coloured TEST lines and clearly recognisable clinical symptoms, a confirmatory test in the laboratory is advisable.
- The rest volume (BBM) can be used for confirmation by PCR testing (sending into the laboratory).

	DWV	ABPV	SBV	Interpretation	Virus status
Spring	negative	negative	negative	Colony "virus free" or below the detection limit of the test	Ideal
Summer	negative	negative	negative	Colony "virus free" or below the detection limit of the test	Ideal
Autumn	negative	negative	negative	Colony "virus free" or below the detection limit of the test	Ideal – very good management strategy
	at least 1 or more of the dipsticks slightly positive			Colony with rising virus load	Review management strategy
	at least 1 or more of the dipsticks clearly positive			Colony with increasingly rising virus load	Urgently review management strategy

***FAST*test® BEE 3T** is only to be used as an additional diagnostic tool for Varroa monitoring

Note on a negative rapid test result
– the taken sample is 100 % virus-free or the concentration of viruses may be below the detection limit of the test
– the taken sample does not have to be 100 % free of Varroa mite infestation

Note on a positive rapid test result
– after a single positive test and subsequent measures required by beekeepers, the ***FAST*test® BEE 3T** can continue to be positive for some time after re-testing (virus quantity still above the detection limit)
– in the positive case, regardless of which virus test is positive, the Varroa control strategy of the hive should be checked according to the guidelines of the respective country

Important:
All colonies that died of unknown cause can be examined with the ***FAST*test® BEE 3T**. In this way, the actual cause of death can be deduced. If the test is strongly positive (especially DWV), this is an indication of too high a mite infestation. In this case, the colony has most probably perished from varroosis + DWV and a possibly insufficient summer treatment against the varroa mite. Therefore, the varroa infestation should be checked in the next season.

ABBREVIATIONS

ABPV Acute bee paralysis virus
BBM Bee-buffer mixture
CL CONTROL line
DWV Deformed wing virus
LF Lateral flow
SBV Sacbrood virus
TL TEST line