*FAST*est® BEE BQCV Strip

Test-kit for the qualitative detection of black queen cell virus (BQCV) in the bee maggot / bee pupa

***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. One of the greatest threats to honey bees is viral infections, as these can be significantly linked to colony losses. In particular, the sacbrood virus SBV, the deformed wing virus DWV, the black queen cell virus BQCV and the acute bee paralysis virus ABPV are associated with the death of individual bees and the collapse of individual colonies. Early detection of virus outbreaks could help reduce colony losses and prevent the spread of disease. The availability of specific rapid tests can greatly simplify and speed up virus diagnosis.

The black queen cell virus (BQCV) is an RNA virus (order picornavirales). Despite its widespread distribution, symptoms are rarely reported. It can therefore be assumed that this virus primarily occurs as a hidden infection without symptoms. If infection occurs, it mainly affects queen maggots and pupae, which die and decompose as a result of the infection. During this process, the queen cell turns black, hence the name. Interestingly, adult bees can also become ill and die if they are artificially injected with BQCV. This is taken as an indication that BQCV can also be dangerous to adult bees if it is transmitted to other bees.

BQCV diagnosis in honey bees is currently only possible using reverse transcriptase polymerase chain reaction (RT-PCR) and real-time PCR, both of which are expensive and time-consuming laboratory procedures.

With the ***FAST*est® BEE BQCV** Strip, beekeepers can now detect BQCV easily, cost-effectively and directly at the hive.

2. TEST-KIT COMPONENTS

1 test-kit ***FAST*est® BEE BQCV** Strip contains:

- 2*, 10**, 25*** or 50**** dipsticks, coated with monoclonal antibodies against BQCV
- *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 BQCV** Strip is based on an immunochromatographic "sandwich principle".

The virus antigens of BQCV present in the maggot 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, monocolonal 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 recommended 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 dipstick to ensure a precise assignment.
- Use a new sample tube and a new dipstick 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. **1–3 maggots / 1 pupa (e. g., 1 large maggot or pupa (ca. 15 mm) or 2–3 smaller maggots (ca. 7–10 mm)** can be used for testing. They can be picked up with the enclosed tweezers and placed in the sample tube.

c. Crush the maggots well with the squeezer before use and ensure uniform mixing.

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Art. No. 305010BG1 (10's)
305024BG1 (12×2's)
305025BG1 (25's)
305050BG1 (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.**

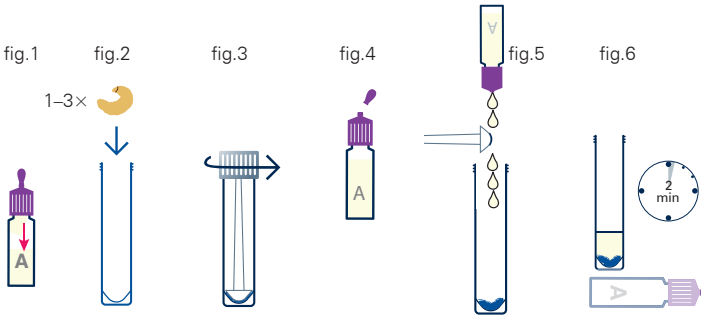
8.1. GENERAL

- Remove the dipstick from its 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. Before opening the dropper bottle **A**, shake the entire buffer diluent down to the bottom of the bottle (fig.1).
2. Open the sample tube and add 1–3 maggots (pupae) into its deepening (fig.2, please see also point 7b. Sample material).
3. Squeeze the maggot material by closing the blue cap with the attached squeezer tightly onto the sample tube for several times (fig.3).
4. Open the sample tube again. Take one dropper bottle **A**, break the tip (fig.4) and add the **whole buffer diluent (1.5 ml)** into the sample tube with the maggots. In case of any maggot remnants on the squeezer, let some drops flow over the squeezer into the sample tube (fig.5).
4. Mix the squeezed maggot material homogeneously with the buffer diluent by twisting and untwisting the blue cap for several times (see fig.3).
5. Remove the squeezer from the cap. Screw the cap onto the tube and leave the maggot buffer mixture (MBM) on a flat surface for at least two minutes (fig.6) to allow the coarse maggot particles to settle (sedimentation time).

fig.1 fig.2 fig.3 fig.4 fig.5 fig.6



9. TEST PROCEDURE

1. Remove the dipstick from its foil pouch shortly before use.
2. Place the dipstick carefully vertically and in the direction of the arrows into the sample tube. The liquid level (meniscus!) must not exceed the blue arrowheads (fig.7). The incubation time of 15 minutes starts now.
3. Remove the dipstick from the sample tube when the pink-purple CONTROL line (CL) becomes visible (see figs.8/9). If the CL will not appear after 2 minutes, a new MBM with new maggots must be prepared and sedimented for at least 5 minutes. The dipstick must be held only in the supernatant until the LF has reached the CL.
4. Place the dipstick on a flat and horizontal surface for incubation.

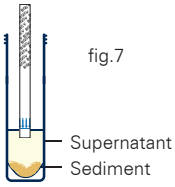


fig.7

10. READING OF THE TEST RESULTS

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

POSITIVE TEST RESULT (fig.8)
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.8

NEGATIVE TEST RESULT (fig.9)
Only a pink-purple CONTROL line (CL) appears. This line indicates, irrespective of its intensity, that the test has been performed properly.

fig.9

INVALID TEST RESULT (fig.10)
No CONTROL line visible. The test should be repeated using new dipsticks.

fig.10

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 15 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 (MBM) can be used for confirmation by PCR testing (sending into the laboratory).

***FAST*test® BEE BQCV** Strip is only to be used as an additional diagnostic tool for "Nosema 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 (1 ng/ml BQCV antigen)
– the taken sample does not have to be 100 % free of Nosema infestation

Note on a positive rapid test result
– after a single positive test and subsequent beekeeping required measures, the ***FAST*test® BEE BQCV** Strip can continue to be positive for some time after retesting (virus quantity still above the detection limit)
– in the positive case, the Nosema control strategy of the hive should be checked

Important:
All colonies that died of unknown cause can be examined with the ***FAST*test® BEE BQCV** Strip. In this way, the actual cause of death can be deduced. If the test is strongly positive, this is an indication of too high a Nosema infestation. In this case, the colony has most probably perished from Nosema + BQCV. Therefore, all beekeeping measures should be taken in the next season to minimise/prevent nosema infestation.

ABBREVIATIONS

BQCV	Black queen cell virus
CL	CONTROL line
LF	Lateral flow
MBM	Maggot buffer mixture
TL	TEST line