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**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

SN/T 5283-2020

Technical Specification for Bumblebee Nosema quarantine

熊蜂微孢子虫检疫技术规范

(English Translation)

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Foreword

This document is drafted in accordance with the rules given in GB/T 1.1-2020.

This document was proposed and managed by the General Administration of Customs of the People's Republic of China.

1 Scope

This document specifies the optical phase contrast microscopy, polymerase chain reaction and real-time fluorescent polymerase chain Reaction detection technology.

This document is applicable to the quarantine of Bumblebee Nosema.

2 Normative references

The contents of the following documents constitute indispensable clauses of the documents through normative references in the text. Among them, dated quotations Only the version corresponding to that date is applicable to this document; for undated references, the latest version (including all amendments) is applicable Used in this document.

GB/T 6682 Analytical laboratory water specifications and test methods

4.11 Electronic balance (sensitivity 0.01 g)

4.12 Refrigerator (2 °C ~ 8 °C and -20 °C).

5 Reagents

5.1

Water. Comply with the first grade water specification in GB/T 6682.

5.2

Phenol/chloroform/isoamyl alcohol mixture, the preparation method is shown in Appendix A.1.

5.3

Trichloromethane/isoamyl alcohol mixture, the preparation method is shown in Appendix A.2.

5.4

TE buffer.

5.5 10xPCR buffer.

5.6

dNTPs (including dCTP, dGTP, dATP, dTTP).

5.7

Taq DNA polymerase.

5.8 0.5xTBE buffer (see Appendix A.3 for the preparation method).

5.9

Loading buffer (see Appendix A.4 for the preparation method).

5.10

EB (10 mg/mL), see Appendix A.5 for the preparation method.

5.11

DNA relative molecular mass standard Marker.

5.12

DNA purification and recovery kit.

6.1 Sampling ratio

6.1.1

When the number of bee colonies is less than or equal to 10 colonies, all bee colonies are sampled.

6.1.2

When the number is between 11 clusters and 100 clusters, based on the sampling volume of 10 clusters, each additional 10 clusters will increase the sampling volume by 1 cluster.

6.1.3

When the number of bees is from 101 groups to 200 groups, 15% of the total number of bee groups will be sampled.

6.1.4

When the number of bee colonies > 200 colonies, based on the sampling amount of 200 colonies, each additional 20 colonies will increase the sampling quantity by 1 colony.

6.2 Sampling method

Take 30 bumblebees with weaker vigor from each selected bee colony, put them into the beaker and cover it, and record the colony number. Use live bees before inspection Put them to death or place them in the freezer of the refrigerator for 10 min.

7.1.1 Sample processing

For bee samples after sacrifice or fresh bee samples after death, take the intestines, add physiological saline according to 1.10, and fill with a masher Divide and mash (for dried dead bee samples, first cut off the head and chest, take the abdomen, soak for 12 hours and then mash). Yarn for homogenate Filter through a net (50 mesh to 100 mesh) and collect the filtrate. Differential centrifugation of the filtrate (500 r/min, centrifugation for 1 min, discard the precipitate, and then supernatant Centrifuge at 5 000 r/min for 10 min,

discard the supernatant) 2 to 3 times. Suspend the sample with 1 to 10 times the volume of normal saline, as Microsporidian liquid to be tested.

7.1.2 Microscopic examination

Take out 50 μL of 2 test solution samples from the test solution prepared in 7.1.1, drop them on a glass slide, cover with a cover glass, and use 40x15 (or

10) Double optical phase-contrast microscopes are checked by 2 people, first look at the middle, adjust the fine-tuning knob, and slowly move around to ensure that there are many samples for each in 20 fields of view.

7.1.3 Judgment of results

Under the optical phase contrast microscope, it can be seen that the length is about 5 μm ~7 μm and the width is about 3 μm ~4 μm . The external shape is short and wide, with blue refraction.

Oval particles with dark edges are microsporidia and are judged as positive (see Appendix B).

7.2.1 Primer

Designed according to the nucleic acid sequence of the 16S rRNA gene of *Nosema bombycis*, the amplified length is about 213 bp, and it is dissolved with ddH₂O to After 10 $\mu\text{mol/L}$, store at -20 °C for later use.

7.2.2 Setting up a comparison

Blank control, negative control and positive control should be set for each reaction. The blank control can replace the sample with ddH₂O; the negative control is Abdominal tissues of healthy bumblebees that were not infected with *Nosema bombyma*; the positive control was the abdomen tissues of bumblebees infected with *Nosema bomby*.

7.2.3 DNA extraction

7.2.3.1

Take 0.5 mL of the 7.1.1 test solution and transfer to a 1.5 mL centrifuge tube, add 0.5 mL of 0.2 mol/L KOH, and fully Mix, induce at 27 °C for 1 h, centrifuge at 5,000 r/min for 5 min, and discard the supernatant.

7.2.3.2

Resuspend with 0.5 mL of normal saline and place at 25°C for 30 min. Add 0.5 mL saturated phenol to each tube and mix thoroughly for 5 min by inverting.

Centrifuge for 10 min at 12 000 r/min at 4 °C.