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WS

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

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**Test methods of fly resistance to insecticides-A molecular
method for detecting insensitive ace allele in the *Musca
domestica***

(English Translation)

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Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

This standard is mainly drafted by. Institute of Zoology, Chinese Academy of Sciences, Beijing Municipal Center for Disease Control and Prevention, Yangzhou University, China Disease Pre-Prevention and Control Center of Infectious Disease Prevention and Control Center, China Agricultural University, Military Medical Research Institute of the Chinese People's Liberation Army Academy of Military Sciences Institute of Diseases, Shanghai Municipal Center for Disease Control and Prevention.

1 Scope

This standard specifies the detection method of the housefly insensitive acetylcholinesterase allele.

This standard applies to the detection of the 342 amino acid locus genotype of *Musca domestica* acetylcholinesterase.

2 Terms and definitions

The following terms and definitions apply to this document.

Acetylcholinesterase acetyl cholinesterase A hydrolase present in an organism, encoded by the acetylcholinesterase gene *ace*.

Allele

Different forms of the same gene present in the same species are referred to as alleles.

342G, 342V and 342A

342G, 342V and 342A respectively indicate that the amino acid at the 342th amino acid residue position of *Musca domestica* acetylcholinesterase is glycine (Gly), Proline (Val) and alanine (Ala).

3 Sample collection

The housefly adults were collected from the scene by trapping or net trapping, and immersed in 100% ethanol solution and brought back to the laboratory. Soaking the sample can be Store at 4 °C for a short period of time (within 6 months) and store at -20 °C for a long time.

4 Primers

The sequence of primer F is CGGTGCATTTGGGTTTCTAC, and the sequence of primer R is CGTAACCGCAGAGTCGCGCTG. Primer design reference Record A. Prepare at a concentration of 10 µmol/L and store in a freezer at -20 °C.

5 Instruments

5.1

PCR (polymerase chain reaction) instrument.

5.2

Agarose gel electrophoresis instrument.

5.3

Gel Imager.

5.4

Constant temperature water bath.

6 Main reagents

6.1

DNA polymerase (Taq enzyme).

6.2

Restriction enzyme Bsp1286I.

6.3

Restriction enzyme Eae I.

7 Operating steps

7.1

Add 10x PCR buffer (containing 100 mmol/L Tris-HCl, 500 mmol/L KCl, 15 mmol/L MgCl₂) to the PCR tube.

2.5 µL, dNTP (2.5 mmol/L) 2.0 µL, primer F 0.5 µL, primer R 0.5 µL, genomic DNA of *Musca domestica* 1 µL (100 ng ~ 300 ng, extraction method can be found in Appendix B, can also be extracted with commercially available DNA extraction kits or other similar methods) and Taq Enzyme (2 to 5 units), add up to 25 µL with sterile water, and mix.

7.2

Put the PCR tube into the PCR machine and set the PCR reaction procedure to. 94 °C for 3 min; 94 °C for 30 s, 50 °C for 30 s, 72 °C.

30 s, 35 cycles; 72 °C for 10 min. Run the program.

7.3

After the end of the PCR run, 5 µL of the PCR product was electrophoresed on a 1.5% agarose gel, and the voltage was set at 160 V.

After 20 min, it was detected under ultraviolet light, and a PCR product with a clear single 609 bp band was used for the following digestion reaction.

7.4

Two PCR reactions were performed on the PCR product of the qualified sample (that is, the electrophoresis product was 609 bp), and the reaction A was treated with Bsp1286I.

Reaction B is digested with the Eae I enzyme, and the enzyme digestion reaction system is set according to the instructions for use of the enzyme product (see Appendix C).

7.5

The digested product was loaded onto a 1.5% agarose gel at a voltage of 160 V, electrophoresed for 20 min and examined under UV light.

Measurement.

8 Genotype determination

The genotype of acetylcholinesterase in *Musca domestica* was determined according to the electrophoresis results of the digested products (see Appendix D), as shown in Table 1.

Table 1 Genotypic determination of acetylcholinesterase in *Musca domestica*

Reaction I (with Bsp1286I digestion)	Reaction II (with Eae I digestion)	Acetylcholinesterase
342 Amino acid site genotype	361 bp, 248 bp	609 bp sensitive homozygote (G/G)
	609 bp	

609bp insensitive type I homozygous (V/V) 609bp 361bp, 248bp insensitive type II homozygote (A/A) 609bp, 361bp, 248bp 609bp insensitive type I/sensitive heterozygote (G/V) 609bp, 361bp, 248bp 609bp, 361bp, 248bp insensitive type II/sensitive heterozygote (G/A) 609bp 609bp, 361bp, 248bp insensitive type I/insensitive type II heterozygote (V/A)
 Note. G indicates that the amino acid residue is Gly (glycine); V indicates that the amino acid residue is Val (valine); A indicates that the amino acid residue is Aal (C Amino acid).

Appendix A

(informative appendix) Primer design reference sequence 342V

CGGTGCATTTGGGTTTCTACATCTTTCACCGTTATGCCAGGTTTTGAAGAAGAAGCTCC
 342A

CGGTGCATTTGGGTTTCTACATCTTTCACCGTTATGCCAGGTTTTGAAGAAGAAGCTCC
 342G

CGGTGCATTTGGGTTTCTACATCTTTCACCGTTATGCCAGGTTTTGAAGAAGAAGCTCC
 342V

CGGCAACGTGGGTCTTTGGGATCAGGCCTTGGCCTTGC GTTGGCTGAAGGAGAATGC
 CCG 342A

CGGCAACGTGGGCCTCTGGGATCAGGCCTTGGCCTTGC GTTGGCTTAAGGAGAATGC
 CCG 342G

CGGCAACGTGGGCCTTTGGGATCAGGCCTTGGCCTTGC GTTGGCTGAAGGAGAATGC
 CCG 342V

TGCATTTGGCGGCAATCCGGAATGGATGACGCTGTTTGGTGAATCGGCTGGTTCGAGT
 TC 342A

TGCATTTGGCGGCAATCCGGAATGGATGACGCTGTTTGGTGAATCGGCTGGTTCGAGT
 TC 342G

TGCATTTGGCGGCGATCCCGAATGGATGACGCTGTTTGGTGAATCGGCTGGTTCGAGT
 TC 342V

CGTGAATGCTCAACTGATGTCGCCGGTAACGCGTGGCCTGGTCAAACGTGGTATGATG
 CA 342A

CGTGAATGCTCAACTGATGTCGCCGGTAACGCGTGGCCTGGTCAAACGTGGCATGATG
 CA 342G

CGTGAATGCTCAACTGATGTCGCCGGTAACGCGTGGCCTGTCAAACGTGGCATGATG
 CA 342V

GTCGGTCACAATGAATGCTCCCTGGAGCCACATGACATCAGAGAAGGCGGTTGAAATTG
 G 342A

GTCGGCCACAATGAATGCTCCCTGGAGCCACATGACATCAGAGAAGGCGGTTGAAATT
 GG 342G

GTCGGGCACAATGAATGCTCCCTGGAGCCACATGACATCAGAGAAGGCGGTTGAAATT
 GG 342V

TAAAGCTTTGGTAAATGACTGTAAGTGAATGCCTCATTGTTACCGGTAAGCAGTGGAGT
 342A