GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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National Food Safety Standard - Determination of neurotoxic shellfish poisoning in shellfish

食品安全国家标准

贝类中神经性贝类毒素的测定

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Table of Contents

National Food Safety Standard - Determination of neurotoxic shellfish poisoning in shellfish

1 Scope

This Standard specifies the method of mouse biology for the determination of neurotoxic shellfish poisoning (NSP) in shellfish.

This Standard is applicable to the determination of neurotoxic shellfish poisoning (NSP) in shellfish.

2 Principle

Use ether to extract the neurotoxic shellfish poisoning in shellfish. After the extract is pressure-reduced and evaporated to dryness, use 1%Tween-60 saline as dispersion medium to prepare NSP-1%Tween-60 saline suspension. Inject this suspension into abdominal cavity. Observe the survival of the mouse. Calculate its virulence.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are analytically pure; the water is grade one water specified in GB/T 6682.

3.1 Reagents

3.1.1 Hydrochloric acid (HCl).

3.1.2 Anhydrous ether ($C_4H_{10}O$).

3.1.3 Tween-60 ($C_{64}H_{126}O_{26}$).

3.1.4 Sodium chloride (NaCl).

3.1.5 Sodium hypochlorite (NaClO).

3.2 Reagent preparation

3.2.1 Sodium chloride solution (0.85%): weigh 0.85 g of NaCl, add water to dissolve and set volume to 100 mL.

3.2.2 1%Tween-60: weigh 1.0 g of Tween-60, use 0.85% sodium chloride

sample, it shall be opened to remove moisture and then sent for inspection.

5.1.2 Specimen preparation

5.1.2.1 Fresh shell sample

Use clean water to wash the shell surface thoroughly. Cut off the muscle. Open shell. Use water to rinse the interior so as to remove sediment and other foreign objects. Separate the occipital muscle from the tissue attached to the gluing. Take out the shellfish. Never cut the flesh. Do not heat or use anesthetic before opening the shell. Collect 200g of shellfish and drain it on a metal sieve with a pore size of about 2mm for 5min. Pick up broken shells and other debris. Homogenize the shellfish, for use.

5.1.2.2 Frozen sample

At room temperature, make the frozen sample semi-frozen. For shell frozen sample, according to the method in 5.1.2.1, clean, open shell, rinse and take the meat. Remove the borneol attached to the outside of the shellfish, wipe off the water, and then relax at room temperature. Collect 200g of shellfish and drain it on a metal sieve with a pore size of about 2mm for 5min. Homogenize the shellfish, for use.

5.1.2.3 Canned shellfish

Drain the contents of the can. Pour into the homogenizer for full homogenization, for use.

5.1.2.4 Dried shellfish product

Weigh 100g of dried product into sufficient water, soaking 24h ~ 48h (refrigerated at 4°C). Drain, homogenize, for use.

5.1.2.5 Salted product

Use clean water to rinse. Use running water to desalt. Drain, homogenize, for use.

5.2 Specimen extraction

Take 100g of specimen into a 500mL beaker. Add 5g of sodium chloride and 1mL of concentrated hydrochloric acid. Mix them well. While stirring, heat the mixture to it is boiling. Boil it on a slow fire for 5min. Cool to room temperature.

Transfer the mixture to a 500mL centrifuge tube. Use 50mL of ether to rinse the beaker. Move the rinse together into the centrifuge tube. Add 100mL of ether into the centrifuge tube. Cap a plug. Shake thoroughly. Centrifuge at 6000 r/min for 15min.

The NSP virulence in the sample is calculated according to formula (1):

$$X = \frac{CMU \times 10}{100} \times DF \qquad \cdots\cdots(1)$$

where,

X - NSP virulence in the sample, in mouse unit per gram (MU/g);

CMU - Median correction mouse unit of the testing group of the mice of testing sample, in mouse unit per milliliter (MU/mL);

DF - Dilution multiple.

NOTE: 10 - the unit is milliliter; 100 - the unit is gram.

6.2.2 Expression of results

In the case where the blank control mice are normal, the following judgments and expressions are made.

If the death time of the mouse is equal to 360min, the NSP virulence of the testing sample is equivalent to 0.2 MU/g.

If the median death time of the experimental group is less than 120min, the sample extract shall be diluted. Select another 3 mice for the test till the median death time is 120min ~ 360min. Calculate the mouse unit virulence of the sample based on the final dilution test results. Report the NSP virulence of this sample as: XXX MU/g.

If the median death time of the experimental group is greater than 360min, directly calculate and determine the virulence of the sample mouse. Report the NSP virulence of this sample as: XXX MU/g.

If all the mice in the experimental group do not die within 930min of observation, the NSP virulence of the sample shall be also reported as less than 0.1 MU/g.

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