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中华人民共和国出入境检验检疫行业标准

SN/T 1773—2006

进出口贝类中麻痹性贝类毒素检验方法 酶联免疫吸附试验法

Inspection of paralytic shellfish poison in shellfish for import and export—ELISA method

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relatively dry meat, add 140mL 0.1 mol/L HCl solution in the same coefficient of dilution, boil and mix them for 5 min; decenter 3 500 g such sample at 4℃ for 10 min, and then control pH value; use 5 mol/L HCl solution to adjust to be lower than 4.0; take 100 μL clear liquid and dilute it with buffer solution in the dilution ratio of 1∶10 (1+9), and then take 50 μL for ELISA according to 5.5. The extension rate this time is 80. For high-concentration sample, if beyond the range of standard curve, furthre dilute it with buffer solution until its concentration is within the range.

5.5 Determination of ELISA

Insert adequate ELISA plates in the micropore frame (make multiple pores respectively for titer and sample solution), and record the positions of titer and sample solution; add 6 portions of appropriate paralytic shellfish poison titer and sample solution into every micropore (50 μL in each one), add 50 μL paralytic shellfish poison enzyme-labeled substances to every micropore and then mix them gently; afterwards, add 50 μL paralytic shellfish poison antibody into the mixed solution and mix them fully; seal the micropore with viscose paper for fear of volatilization of solution, and incubate it in a dark place at 20℃～25℃ for 15min; bottom up micropore frame above absorbent paper and flap it several times so as to absolutely eliminate solution in micropores; fill every micropore with heavy distilled for rinsing and then flap the frame until no solution remained; repeat the above processes 5 times; add 100 μL stroma/chromogenic reagent in every micropore, lightly flap it to make it well mixed and then incubate it in a dark place at 20℃～25℃ for 15 min; add 100 μL reaction stop solution in every micropore, lightly flap it to make it well mixed, set empty tube to zero, measure and record the absorption value of solution (450 nm wavelength) in every micropore.

5.6 Calculation and formulation of results

5.6.1 Absorption percentage

Calculate the average absorption values of titer and sample solution of paralytic shellfish poison, and work out the percent absorption value of each portion of titer and sample solution of paralytic shellfish poison according to formula (1):

$$A=\frac{S}{S_0}\times 100\% \quad \cdots\cdots (1)$$

Where

A——percent absorption value;

S——average absorption value of 6 portions of appropriate paralytic shellfish poison titer and sample solution;

S_0——average absorption value of 0 μg/kg paralytic shellfish poison titer.

5.6.2 Plotting calibration curve

Percent absorption values (arithmetic degree) as Y coordinate, concentration (μg/kg) (logarithm degree) of paralytic shellfish poison solution as X coordinate, plot the calibration curve for the percent absorption value of paralytic shellfish poison titer and the concentration of paralytic shellfish

前　言

本标准由国家认证认可监督管理委员会提出并归口。

本标准起草单位:中华人民共和国辽宁出入境检验检疫局、中华人民共和国山东出入境检验检疫局。

本标准主要起草人:曹际娟、于兵、王刚、赵昕、于灵、郑秋月、马惠蕊、李丽、张艺兵。

本标准系首次发布的出入境检验检疫行业标准。

4.5.2 Scallop

Inspect the edible part. The processes of draining and homogenization are as same as specified in 4.5.1.

4.5.3 Canned shellfish

Put all the substances (meat and liquid) in the container into homogenizer for full homogenization. If the container is big, weigh the drained excessive water and meat respectively, and mix the solid and liquid in proportion (1∶1) and homogenize them fully.

4.5.4 Storing meat in acid

Drain acid liquor, store meat and acid liquor separately, and fully homogenize meat without excessive water.

4.5.5 Freezing shellfish

At room temperature, make the frozen samples (with or without shells) in semi-frozen state, and then open the shells, clean, take out meat and homogenize it in the processes of 4.5.1.

4.6 Preserving samples

If the homogenized samples in 4.5 cannot be inspected in time, store 100 g homogenized meat in 100 mL HCl (0.1 mol/L) at 4℃ (inspect it as soon as possible).

5 Determination method

5.1 Principle

This determination method is based on competing ELISA reaction. Free paralytic shellfish poison competes with paralytic shellfish poison enzyme-labeled substances for paralytic shellfish poison antibody, and at the same time paralytic shellfish poison antibody is linked with capture antibody. The enzyme-labeled substances that are not linked with others are removed in the process of washing. The linked enzyme-labeled substances will convert colorless chromogenic reagent into blue product. After adding reaction stop solution, the blue turns yellow. Use lab-systems dragon in 450nm wavelength to measure the absorption value of solution in wells, the paralytic shellfish poison solution in sample is inversely proportional to the absorption value and calculate according to the drawn calibration curve.

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1 范围

本标准规定了进出口贝类及其制品中麻痹性贝类毒素检验的抽样、制样和酶联免疫吸附试验检验方法。

本标准适用于进出口双壳类贝肉、贝柱和其他可食用部分的麻痹性贝类毒素的筛选检测。

2 规范性引用文件

下列文件中的条款通过本标准的引用而成为本标准的条款。凡是注日期的引用文件，其随后所有的修改单(不包括勘误的内容)或修订版均不适用于本标准，然而，鼓励根据本标准达成协议的各方研究是否可使用这些文件的最新版本。凡是不注日期的引用文件，其最新版本适用于本标准。

GB/T 6682 分析实验室用水规格和实验方法

3 术语和定义

下列术语和定义适用于本标准。

3.1

麻痹性贝类毒素 Paralytic shellfish poisoning，PSP

化学结构以石房蛤毒素(Saxitoxin)为代表的，摄食后可产生麻痹作用的存在于贝类体内的海洋生物毒性物质的总称。

4 抽样和制样

4.1 检验批

以不超过 100 t 为一检验批，同一检验批的商品应具有相同的特征，如包装、标准、产地、季节和规格等。

4.2 抽样数量

按各检验批的数量，依表 1 抽取样品。

表 1

检验批的数量/t	抽样点(件)数	混合样品数
10 以下	10	2
11～50	15	3
51～100	20	3

如为散装的应均匀抽样。

4.3 抽样方法

按 4.2 规定的抽样点(件)数，随机抽取样品。每 5 点(件)、抽取的原始样品组成一个混合样(即检验样品)。其质量不少于 2 kg，装入清洁容器内，加封标记后，及时送交实验室检验。

4.4 分析样品的采集

分析样品要有充分的代表性，依受检贝类品种决定样品的采集量。取样个数不少于 12 个贝类个