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National Food Safety Standard – General Rules for Verification of Microbial Test Methods

食品安全国家标准 微生物检验方法验证通则

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National Food Safety Standard – General Rules for Verification of Microbial Test Methods

1 Scope

This Standard specifies the general requirements for the verification of microbial test methods for national food safety standards.

This Standard is applicable to the verification process during the development and revision procedure microbial test methods for national food safety standard.

2 Terms and Definitions

2.1 Method verification

Test activities that provide objective and valid evidence to confirm that the performance parameters of an analytical method meet the intended use of the method.

2.2 Qualitative methods

A method that tests a sample for the presence of a target microorganism.

2.3 Quantitative methods

A method that determines the number or concentration of target microorganisms in a sample.

2.4 Reference methods

The traditional culture methods of microorganisms that are recognized or widely accepted.

2.5 Sensitivity

The ability of the qualitative method to be validated to detect target microorganisms from a sample.

2.6 Inclusiveness

The detection ability of the method to be verified for target microorganisms.

2.7 Exclusivity

The anti-interference ability of the method to be verified against non-target microorganisms.

2.8 Accuracy

The degree of consistency between the test results of the quantitative method and the true value of a sample.

2.9 Specimen

The part that is derived from a sample, and is used for testing and representative of its characteristics.

2.10 Partial detection level

When a qualitative method is used to test a set of parallel samples, the contamination level of the sample is when the detection probability is between 25% and 75%.

2.11 50% limit of detection (LOD_{50})

The concentration of target microorganisms in the sample when the detection probability of the qualitative method is 50%.

2.12 Relative limit of detection (RLOD)

If there is a reference method for verification, the ratio of the 50% detection limit of the method to be verified and the reference method.

2.13 Paired/unpaired analysis

When two qualitative methods test a sample at the same time, if the bacterial enrichment method in the first step is the same, the same specimen of such sample can be used for analysis; this analysis is called "paired analysis". If the bacterial enrichment method in the first step is different, then using different specimens of such sample for analysis; this analysis is called "unpaired analysis".

3 General Requirements for Verification of Microbiological Test Methods

3.1 Selection of performance parameters

According to the type of method to be verified, select appropriate performance parameters for verification according to Table 1.

different food subcategories are isolated from the corresponding food subcategories as much as possible. Strains isolated from other laboratories or purchased from strain preservation institutions can also be used. All isolated strains shall be accurately identified and their sources shall be known and traceable.

For the verification of methods with confirmatory procedures, only one target microorganism needs to be selected for each food subcategory. For the verification of quantitative methods without confirmatory procedures, different types of representative microorganisms shall be selected for each food subcategory. For example, the method verification samples for total bacterial count can choose Staphylococcus aureus (representing Gram-positive bacteria), large intestine Escherichia (representing Gram-negative bacteria) and Bacillus subtilis (representing spore-containing bacteria).

Cultivate the target microbial strain under appropriate conditions. If there are multiple target microorganisms, they shall be cultured separately first and then mixed in equal amounts.

If the sample does not contain natural background flora, background flora can be added if necessary. The contamination level of the background flora shall be at least 10 times higher than the target microbial concentration; and appropriate counting methods can be used to confirm that the contamination level meets the requirements.

3.3.2.2 Artificial contamination of samples

The culture is diluted with diluent to the appropriate concentration required to contaminate the sample. If dilution does not effectively remove the medium from the culture, rinse with the diluent and centrifuge 2 to 3 times to remove the culture medium before diluting.

When culture medium diluent is added to a sample, the volume of the diluent must not affect the state of the sample. When the volume of the diluent may affect the sample state, the diluent of appropriate concentration shall be made into freeze-dried powder. When the target microorganism concentration in the freeze-dried powder cannot reach the concentration level required to prepare low-contamination level samples, the freeze-dried powder is mixed evenly with other freeze-dried powder without culture medium to dilute to the appropriate concentration of the contaminated sample.

It should select samples containing background flora, but ensure that the target microorganisms are not contained in the sample. Inoculate culture medium diluted to the appropriate concentration (diluent or freeze-dried powder) into samples. The samples shall be mixed well and stable. If the samples are not easily mixed evenly, each sample can be prepared separately; the amount of each sample is reduced to the amount of the specimen; and the entire sample is used for testing. If the samples used for unpaired analysis are not easy to mix evenly, different specimens can be prepared separately and regarded as the same sample.

3.3.2.3 Determination of sample acceptance reference value

For verification of non-reference methods, an appropriate counting method can be used to

determine the concentration of the added target microorganisms; and take the concentration converted to the concentration of the target microorganisms in the sample as the sample acceptance reference value.

3.4 Requirements for data processing

The method development laboratory needs to conduct abnormal value inspection and statistical analysis on all laboratory verification data. The method can use statistical methods such as Grubbs inspection or technical analysis methods. The counting results shall be converted into commonly used logarithmic values before subsequent calculations are performed.

4. Verification of Performance Parameters of Microbial Testing Methods

4.1 Sensitivity

4.1.1 Verification method

When performing interlaboratory verification, at least 20 parallel samples shall be tested.

When performing interlaboratory verification, each verification laboratory tests 8 parallel samples.

The sample contamination level shall be a partial detection level. If there is no reference method during verification and the acceptance reference value of the sample is unknown, the sample acceptance reference value must be determined according to the method specified in 3.3.2.3.

If there is no reference method during verification, use the method to be verified for testing. If there is a reference method for verification, respectively use the reference method and the method to be verified for testing. During the test, one blank control and one positive control (the contamination level is more than 10 times the partial detection level) are set up for each food subcategory.

In this Standard, sensitivity is expressed as 50% limit of detection (LOD_{50}) or relative limit of detection (RLOD). If there is no reference method during verification, calculate LOD_{50} according to B.1. If there is a reference method during verification, calculate RLOD according to B.2.

4.1.2 Verification requirements

If there is no reference method during verification, the sensitivity of the method to be verified is expressed as LOD_{50}. The LOD_{50} of the qualitative method has no acceptance limit; and the LOD_{50} acceptance limit of the MPN method is 5 CFU. If the LOD_{50} of the MPN method does not exceed the acceptance limit, then its sensitivity meets the requirements.

If there is a reference method for verification, the sensitivity of the method to be verified is expressed in RLOD. For paired analysis, the RLOD acceptance limit is 1.5; for unpaired analysis, the RLOD acceptance limit is 2.5. If the RLOD does not exceed the acceptance limit, the sensitivity of the method to be verified meets the requirements.

4.2 Inclusiveness and exclusivity

4.2.1 Verification method

The strains selected for inclusiveness and exclusivity tests shall reflect the diversity of phenotypes, genotypes and/or serotypes of target microorganisms and non-target microorganisms. The strains selected for inclusiveness testing shall cover the types of the target microbial taxonomic unit and its lower taxonomic unit (if any). The strains selected for the exclusiveness test shall cover closely related species at the same level outside the target microbial taxonomic unit; and shall also include the dominant species commonly contained in the sample (the number shall not exceed 1/3 of the total number of strains selected for the exclusiveness test).

The inclusiveness test requires the selection of at least 30 strains of target microorganisms. For the inclusiveness test of the Salmonella testing method, at least 50 strains of Salmonella of different serotypes shall be selected, which shall cover the main Salmonella serotypes. Exclusivity testing selects at least 30 strains of non-target microorganisms.

Directly test the pure culture using the method to be verified. The inoculum size of the pure culture in each test shall be 10^2 CFU~10^3 CFU.

4.2.2 Verification requirements

If the number of test results of target microorganisms detected in the inclusiveness test is equal to the number of selected strains, then the inclusiveness of the method to be verified meets the requirements.

If the number of test results of the target microorganism not detected in the exclusivity test is equal to the number of selected strains, the exclusivity of the method to be verified meets the requirements.

4.3 Accuracy

4.3.1 Verification method

For each food subcategory, three concentration levels of low, medium, and high shall be selected. The low level is about 10 times the theoretical detection limit; and the high level is at least 100 times the limit. If there is no limit, the maximum contamination levels of health indicator bacteria and pathogenic bacteria are set to approximately 10^7 CFU/g (mL) and 10^5 CFU/g (mL), respectively.

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