Advance Journal of Food Science and Technology 7(11): 872-874, 2015
DOI:10.19026/ajfst.7.2524
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: November 3, 2014 Accepted: December 18, 2014 Published: April 10, 2015

Research Article

Validation of Method in Microbial Limit Tests for Two Types of Health Foods

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Abstract: In order to discuss that whether the methods of total aerobic microbial count and absence bacterial test were appropriate in microbial limit tests for health foods hygiene. As the requirement of microorganism limits test and validation on the Chinese Pharmacopoeia 2010, the microbial examination method of two kinds of health foods has been validated. It is found that vitamin B was antibacterial for *Bacillus subtilis* and *Aspergillus niger*. The results indicated the method in foods microbial limit test GB/T4789-2010 were not suitable for the microbial limit tests of health foods. It is suggested that setting more reasonable method by validation as well as referring to the Chinese Pharmacopoeia 2010.

Keywords: Health foods, microbiological examination, validation

INTRODUCTION

With the development of economy and society, consumer demands in the field of food production have changed considerably. Consumers more and more believe that foods contribute directly to their health. Today foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers (Jones and Jew, 2007). Generally, a health (functional) food can be defined as in addition to their basic nutritive value and natural being, will contain the proper balance of ingredients which will help us to function better and more effectively in many aspects of our lives, including helping us directly in the prevention and treatment of illness and disease (Goldberg, 1994). In this regard, functional foods play an outstanding role. The increasing demand on such foods can be explained by the increasing cost of healthcare, the steady increase in life expectancy and the desire of older people for improved quality of their later years (Siro *et al*., 2008). Microbiology testing is an important issue for their security of functional food. Nowadays, the food microbiology test method is mainly in accordance with GB4789-2010 (GB, 2010). However, the Chinese Pharmacopoeia 2010 is considered the validation of this microbial limit test method, which is different of GB4789-2010 (Committee, 2010). In this study, several types of health food were used to investigate the necessity and feasibility of the health food microbiological testing methods based on the validation of Chinese Pharmacopoeia 2010 method.

MATERIALS AND METHODS

Media and reagents: Lactose bile enrichment broth, sodium tellurite broth enrichment medium, rose red sodium agar, nutrient agar, nutrient broth, improved Martin agar, improved Martin agar broth, blood nutrient agar, eosin methylene blue agar, 4-Methylumbelliferyl-Glucosidase (MUG), Salmonella agar and Shigella agar were offered by the China pharmaceutical and biological products.

Samples: Protein powder and vitamin B were purchased from the local supermarket.

Strain: Escherichia coli [CMCC B44102], *Bacillus subtilis* [CMCC63501], *Staphylococcus aureus* [CMCC 26003], *Streptococcus hemolytic*-β [CMCC32210], *Aspergillus niger* [CMCC98003], *Shigella flexneri* [CMCC51572] and *Salmonella* [CMCC50094] were from Guangdong provincial institute of microbial culture collection center.

Preparation of bacteria for bactericidal assay: A 1-mL sample of a broth culture of a strain of *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*,

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Streptococcus hemolytic-β, *Aspergillus niger*, *Shigella flexneri* and *Salmonella* was added to a 1.9-mL microfuge tube and the bacteria were pelleted by centrifugation in a microfuge at 12, 000 rpm for 30s. After the removal of the supernatant, 1 mL of sterile PBS was added to the pellet and the pellet was resuspended by gentle aspiration in and out of a transfer pipette. Then, 10-fold dilutions by PBS buffer to final 30-200 CFU/mL dilution grade (Friedman *et al*., 2002).

Validation of method: The microbial limit test method referring to the Chinese Pharmacopoeia 2010 were performed three times independently in parallel experiments. The experimental based on GB4789-2010 method.

RESULTS AND DISCUSSION

Conventional method: *B. subtilis* and *A. niger* on behalf of gram-positive bacteria and fungus respectively, were tested by the method GB4789-2010 for recoveries for each bacteria. As shown in Table 1, the protein powder plate method using *B. subtilis* and *A. niger* recoveries were up more than 70%. According to the Chinese Pharmacopoeia 2010 not less than 70% of the provisions of the above, protein powder can be considered there is no significant inhibitory effect for gram-positive bacteria and fungus. However, the recovery of vitamin B was obvious less than 70%, it meant that it was necessary to eliminate or reduce the antibacterial activity.

Medium dilution method: The per dish was added trail solution 0.2 mL, inoculated *B. subtilis* and *A. niger*, then added medium 15-20 mL for incubation 18-24 h, finally counts observation and measured recoveries. As shown in Table 2, the recovery of these two positive bacteria were able to more than 70% by using the medium dilution method (per dish 0.2 mL), this results indicated that the use of the medium dilution method can effectively eliminate its antibacterial activity and increase the accuracy on total number of colonies test.

Method validation of control bacterias: When developing a microbiological test for foods or drugs, it should find a way to validate the methods used to check the foods or drug suitable for the control of bacteria.

From the Table 3, the trail team detected each bacterial growing normally and the negative control detected none growing. It indicated that there was no inhibitory effect on the control bacterial using the medium dilution method for vitamin B. It was specific and could meet the microbiological testing requirements for verification purpose.

It should be noted that successful validation of any analytical method is very difficult without thorough and systematic planning and preparation. According to the guidance to U.S. Environmental Protection Agency (EPA) personnel, a written study plan should be prepared for each step of the validation process and subjected to appropriate review prior to implementation. In addition, it is expected that all laboratories involved in each step of a validation process will have a quality system or Quality Assurance (QA) program in place to ensure standardization of laboratory operations, as well as adequate Quality Control (QC) activities (Parshionikar *et al*., 2009).

Table 1: Microbial limit tests for recovery (conventional method %)

Sample	Test times	*B. subtilis*	*A. niger*
Protein powder	1	82.4	88.00
	2	73.0	105.9
	3	79.0	86.40
Vitamin B	1	0.00	0.000
	2	0.00	0.000
	3	0.00	0.000

Table 2: Recovery of dilution method (0.2 mL per plate, %)

Sample	Test times	*B. subtilis*	*A. niger*
Vitamin B	1	78.5	100.0
	2	84.1	88.20
	3	71.8	98.20

In this study, two different types of health food were investigated for inhibitory effect. The results showed that vitamin B had the strong inhibitory effect on gram-positive bacteria and fungus. Therefore, it can conclude that vitamin B is a major cause of these health food ingredients antibacterial. The protein powder showed no significant inhibitory effect. The health foods usually contain extracts or chemical additives, the experimental results showed these ingredients themselves may affect microbial growth, according to the national standard GB4789-2010 microbiological examination of food hygiene methods for the health food limitations. As this reason, it is very necessary to eliminate or reduce the antibacterial activity by using medium dilution method or membrane filtration method and other methods, in order to make the test results more scientific, reasonable and reliable.

Validation is a process that determines the fitness of an assay, which has been properly developed, optimized and standardized, for an intended purpose. All diagnostic assays (laboratory and on-site assays) should be validated for the species in which they will be used (Jacobson, 1998). Method validation is the process of determining if a method is suitable for its intended purpose. Based on the issue of EPA guidance, validation can be classified as primary validation and secondary validation according to its purpose. Primary validation is an exploratory process for establishing the operational limits and performance characteristics of a new, modified or otherwise inadequately characterized method. Secondary validation on the other hand, is the process of gathering evidence that a laboratory can meet the specifications established in primary validation (Parshionikar *et al*., 2009). In this experiment, two types of health food of bacteria, mold and yeast counting method, coliform bacteria and

Table 3: Results of absence bacteria test

	E. coli	*Salmonella*	*Shigella flexneri*	*S. aureus*	Streptococcus hemolytic-β
Vitamin B	+	+	+	+	+
Negative control	-	-	-	-	-

"+": Indicated growing normally; "-": Indicated growing none

testing methods were methodological study established. This study may provide a reference as primary validation for subsequent microbiological testing and validation of health food.

ACKNOWLEDGMENT

This study was supported by a collegial research start-up grant (contract/grant number 2013QDJZR04 to Jian Li), a key discipline construction project (contract/grant number 2014XKJSXJ04 to Guangyu Zhang) and a basic research exploration project (contract/grant number 2014XKJSTS01 to Jian Li) funded by the Hubei University of Medicine.

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