

Food Stamp

Introduction

The surface wiping method is commonly used for the hygienic control of bacteria in/on foodstuffs and environments. However only well-trained microbiologic technicians obtain the reliable results, as the method requires a variety of apparatus and techniques. In addition, the test cannot be conducted on site, and the results show remarkable individual difference depending on the skill of the technician. Nevertheless daily sanitary tests are indispensable for the hygienic control at food production plants and warehouses. Desired is a simple bacteriologic test method that indicates the presence of bacteria and can be conducted anywhere and by anyone.

A stamp method utilizing an agar "sausage" was developed by Cate (1963, 1965) in response to the needs, and has been widely used in Great Britain.

In Japan, The Ministry of Health and Welfare and The Food Sanitation Association also conducted research by organizing a special research team to simplify foodstuff test method. The stamp method was verified to be satisfactory for practical use as an indicator of the degree of bacterial contamination of foodstuffs and environment, based on the results of reliability study of the stamp method, stability of the medium used, and practical applicability of the method when used by inexperienced persons, etc.

Features

1. Ten (10) kinds of Food Stamp are available according to the sample or bacteria to be tested.
2. The presence of bacteria in foodstuffs or environment can be easily detected by anyone.

Testing Procedures Specimen Selection

1. Specimens suitable for Food Stamp testing

- Kitchen apparatus (chopping board, kitchen knife, saucepan and dishcloth etc.)
- Kitchen tables
- Hands and fingers of peoples working in cooking places.
- Other materials on which Food Stamp can be stamped (including foodstuffs)

2. Specimens not suitable for Food Stamp testing

- Liquid materials.
- Materials with rough surface.
- Materials with much fat or oil, while soupcon of fat or oil do not disturb the tests.

Selection of Food Stamp

Select the test from following 10 kinds of Food Stamp:

Food Stamp	Ident No.	Purpose	Shelf life
Standard Method Agar (SMA)	1000029 (100 plates)	Viable bacterial count	See box label
	1000030 (30 plates)		
Desoxycholate Agar (DESO)	1000025 (100plates)	Coliform group	See box label
	1000026 (30 plates)		
X-GAL Agar (XGAL)	1000041 (100 plates)	Coliform group and E. coli	See box label
	1000042 (30 plates)		
XM-G Agar (XMG)	1000043 (100 plates)	Vibrios	See box label
	1000044 (30 plates)		
TCBS Agar (TCBS)	1000031 (100plates)	Staphylococcus aureus	See box label
	1000032 (30 plates)		
TGSE Agar (TGSE)	1000033 (100 plates)	Salmonella	See box label
	1000034 (30 plates)		
MLCB Agar (MLCB)	1000039 (100 plates)	Bacillus cereus	See box label
	1000040 (30 plates)		
Cereus Agar (CERE)	1000035 (100 plates)	Fungi	See box label
	1000036 (30 plates)		
Sabouraud Agar (SABO)	1000027 (100 plates)	Food borne fungi	See box label
	1000028 (30 plates)		
Potato dextrose Agar with chloramphenicol (PDA)	1000037 (100 plates)		
	1000038 (30 plates)		

Preparation of Testing

1. One clean film bag contains 5 Food Stamps connected in series.
2. Cut off a side of bag, and take out Food Stamp from the bag.
3. Hold the cap of Food Stamp and cut off each of them by bending. Take out only Food Stamps needed for the tests.
4. Put extra Food Stamps back into the bag, and store at 4 – 10°C in dark cool place.

Test Method

1. Take off the cap of Food Stamp and gently press the medium against the surface of specimen. The surface of medium/agar is elastic enough to be pressed firmly against the specimen. Press against the different parts of the specimen when several kinds of Food Stamps are tested simultaneously. Put the cap again immediately after pressing.

Precaution

- Food Stamp should not be used for the specimen soiled with oozing water on its surface, while some moisture does not affect the testing. Nevertheless some moisture may help to detect bacteria on the palm.
 - Do not press too hard or scrub.
 - If there were any water drops inside the cap, shake down the drops from the container just before the testing.
 - Do not touch medium surface.
2. With oil felt pen or pencil for glass, write down on the cap the date of test and the name of specimen. All other information should be also recorded in the separate recording sheet. Recording of all key information at this point of testing is critically important. Without written records all test results may be mixed and confused later.
 3. Put the Food Stamp into the incubator. In case of the incubator was not available, it may be possible to keep them at room temperature for longer time (about 1.5 – 2.0 times).

4. Incubate for 2 – 5 days for Sabouraud and PDA Agars, 1 - 2 days for all other Food Stamps except XM-G Agar that should strictly observe 35°C, 20 ± 2 hours.

Food Stamp	Temperature (°C)	Incubation
SABO, PDA	20 - 25	2 – 5 days
Others	35 - 37	1 – 2 days
XM-G	35	20 ± 2 hours

How to read colonies

Colony means an aggregate of bacteria, which grow big enough being observed by the naked eye.

Viable Bacterial Count

- Medium: Standard Agar (clear light yellow medium)
- Colony reading:
 - Count all colonies grown on the surface
- Assessment: Refer to the section of Interpretation of the Results.

Coliform Group

- Medium: Desoxycholate Agar (clear reddish-orange medium)
- Colony reading:
 - Coliform group develop pink/red colonies grown on the surface with diameter of 1 – 3 mm. In case of heavy contamination, red color may not be so distinctive due to too many colonies on the surface.
 - Usually many colonies grow on the surface without red-color, and one to several red colonies are observed among them if coliform are present.
 - Coliform group: Whole colony is pink – red, or center of colony shows pink – red color
 - Non Coliform group: Clear, white or yellow colony is not coliform.
 - It is recommended to test again with X-GAL AGAR when if only part of colonies shows pink color, which makes determination difficult.
- Medium: X-GAL Agar (clear light yellow medium)
- Colony reading:
 - Coliform group develop blue/blue-green colonies grown on the surface with diameter of 1 – 3 mm.
 - Usually many colonies grow on the surface without blue-color, and blue colonies are easily distinguished among them if coliform are present.
 - Coliform group: Whole colony is blue – blue green, or center of colony shows blue – blue green color
 - Non Coliform group:

Clear, white or yellow colony is not coliform.

- Medium: XM-G Agar (clear light yellow medium)
- Colony reading:
 - E.coli develops colony of blue (blue – blue purple) color on the surface.
 - Coliform group develops colony of red (pink – red) color on the surface.
 - Combined number of both colonies is the total number of coliform group.
 - So long as an incubation time is strictly observed, almost all other bacteria do not grow on this medium. Even if some of them grew, they develop white colonies and do not develop any colored colonies.
 - Observe strictly the specified time (35°C, 20 ± 2 hours), since an over-time incubation may foster growth of microorganism other than E. coli and Coliform group.
- Assessment: Since an existence of coliform group indicates a possible contamination with feces, no coliform group should be detected as an ultimate goal of sanitation management. Soil may contain certain coliform, and vegetables should be well washed out before cooking.

Vibrio parahaemolyticus

- Medium: TCBS Agar (clear greenish-blue medium)
- Colony reading:
 - Vibrio parahaemolyticus develops green colonies grown on the surface with diameter of 1 – 3 mm.
 - For the meanwhile, Vibrio alginolyticus develops yellow colonies on the surface, and changes whole medium to yellow color when a great many V. alginolyticus grew. Though V. alginolyticus is not pathogenic, their existence, if too many, implies a risk of contamination by V. parahaemolyticus.
 - Usually many yellow colonies grow on the surface, and one to several green colonies is observed among them when V. parahaemolyticus are present.
 - V. parahaemolyticus:
Whole colony is green.
 - Non V. parahaemolyticus:
Yellow or white colonies are not V. parahaemolyticus. Whole medium becomes yellow in case of many such colonies grew.

- Assessment: Contamination of *V. parahaemolyticus* comes mainly from fishery products. Check thoroughly the process of disinfection for cocking tools with at most care for mutual contamination.

Staphylococcus aureus

- Medium: TGSE Agar (clear light yellow medium)
- Colony reading:
 - *Staphylococcus aureus* develops black colony with an opaque ring around grown on the surface with diameter of 1 – 3 mm.
 - Usually many black colonies without any opaque ring grow on the surface, and one to several black colonies with an opaque ring are observed among them when *S. aureus* are present.
 - Staphylococcus aureus:
Whole colony is black with opaque ring and milky surrounding (positive egg yolk reaction).
 - Non Staphylococcus aureus:
Those colonies of black color are not *Staphylococcus aureus* unless they have opaque ring around them. Those colonies that have a black center and clear white ring around them are not *Staphylococcus aureus* either. Black colony with negative egg yolk reaction is not *S. aureus* neither.
- Assessment: Contamination of *Staphylococcus aureus* comes mainly from person (their fingers, hairs, saliva etc.) Repeat the inspection of their working dresses and washing procedures until no *Staphylococcus aureus* are detected any more.

Salmonella

- Medium: MLCB Agar (clear purple medium)
- Colony reading:
 - *Salmonella* develops black colonies and/or center black colonies with diameter of 1 – 3 mm.
 - Usually many purple colonies without black color grows on the surface and one to several black colonies are observed among them when *Salmonella* are present.
 - Salmonella:
Whole of the colony is black and/or center is black.
 - Non Salmonella:
Some clear purple colonies may turn to center black after long incubation, but they are not *Salmonella*.
 - Purple colonies grown on MLCB Agar are not *Salmonella*. *Citrobacter* may also develop black colony just like *Salmonella*. It is recommended that certain identification tests be performed simultaneously.
In general, only few numbers of *Salmonella* contaminate the foodstuffs, it is still recommended that the conventional method test would be regularly performed even though no *Salmonella* were detected by the Stamp method.

- Assessment: It is serious if Salmonella was detected by Food Stamp. It is recommended that the conventional method test should be performed concurrently with stamp method, as Salmonella contamination of foodstuff contains just few Salmonella bacteria only.

Bacillus cereus

- Medium: Cereus Agar (opaque orange)
- Colony reading:
 - B. cereus develops white, slightly thick colonies with an irregular rim grown on the surface with diameter of around 5mm. The colonies form a opaque zone around, and the color of medium around the colonies changes to red.
 - Usually small colonies grow without forming any opaque zone around them. Among them B. cereus develops white, slightly thick colonies with an irregular rim, and forms opaque zone around, while the color of medium around them changes to red.
 - B. cereus:
White colonies with irregular rim. Medium around the colonies forms opaque zone, and turns medium red.
 - Non B. cereus:
Bacteria other than B. cereus hardly grow on this agar. Small colonies with negative egg yolk reaction may grow, but they are not B. cereus.
- Assessment: Many B. cereus exist in natural world and all of them are not necessarily source of food poisoning. Nevertheless sanitary condition should be carefully reviewed once B. cereus were detected.

Fungi

- Medium: Sabouraud Agar (clear light yellow)
Medium: Potato Dextrose Agar with Chloramphenicol (clear light white)
- Colony reading:
 - Fungi develop characteristic fluffy colonies on the surface. All colonies should be counted for evaluation.
 - Some bacteria may grow on Sabouraud Agar, and they are discriminated by Gram's stain.
- Assessment: Refer to the section of Interpretation of the Results.

Interpretation of the Results

1. A piece of Food Stamp has a medium surface of 10 cm². After incubation, the colonies grown on the surface should be counted. When there are great many colonies grown on the surface, it may be recommended to use the squares imprinted on the back of the container. Each square of 4 squares in center has 1 cm².
2. Following is a criterion to evaluate the degree of contamination based on the number

of colonies detected, which applies for the test of

- Standard Method Agar (SMA) for viable bacterial count
- Sabouraud Agar (SABO) for fungi
- Potato Dextrose Agar with Chloramphenicol (PDA) for food-borne fungi.

Number of Colony per plate	Evaluation	Mark	Comments
0	Not contaminated	-	Well kept clean. Maintain these conditions
1 - 9	Barely contaminated	±	
10 - 29	Slightly contaminated	+	Contamination is detected. More hygiene controls are requested.
30 - 99	Moderately contaminated	++	
More than 100	Heavily contaminated	+++	

- Criteria is based on J. Appl. Bact., 28, 221-223, 1965
3. Following is a criterion to evaluate the degree of contamination based on the number of colonies detected, which applies for the test of
- Desoxycholate Agar (DESO) for Coliform
 - X-GAL Agar (XGAL) for Coliform
 - TCBS Agar (TCBS) for *Vibrio parahaemolyticus*
 - TGSE Agar (TGSE) for *Staphylococcus aureus*
 - MLCB Agar (MLCB) for *Salmonella*
 - Cereus Agar (CERE) for *Bacillus cereus*
 - XM-G Agar for *E. coli* and Coliform

Number of Colony per plate	Evaluation	Mark	Comments
0	Not contaminated	-	Well kept clean. Maintain these conditions
>1	Contaminated	+	Contamination is detected. More sever hygiene controls are requested.

Note

1. The number of colonies on Food Stamp does not represent an absolute number of contaminating bacteria. The number of contaminating bacteria calculated, therefore, does not necessarily agree with that of the wiping method.
2. Food Stamp indicates merely the degree of cleanliness of a surface of specimen, and by no means indicates the whole pictures of the specimen out-side and also inside inclusive. Limitation of Food Stamp should be always taken into account when food specimen is handled.

Disposal of Food Stamp

1. Do not dispose the Food Stamp direct into garbage box after testing, as microorganism grown on the Food Stamp does alive and may be pathogenic even for those which grew on Standard Method Agar.
2. Boil them up well in boiling water before dispose, or destroy them by fire.
3. It is highly recommended to dispose them after autoclaving.

Storage

Store at 4 – 10°C. Do not freeze.

Precaution for Use

1. Read and observe the instruction described in the package insert.
2. Do not use the product after expiry date.
3. Carefully check the product before the test to ensure that there are no breakage, contamination, change in color, foreign body and drying.
4. Do not freeze the product to keep the quality.
5. After open the bag, Food Stamp unused should be back into the bag, and store dark cool place to avoid drying.
6. If any of medium were put into mouth or eyes, wash thoroughly with water and consult doctor.