A Guide to Environmental Microbiological Testing for the Food Industry

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INTRODUCTION

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The Food Safety Pyramid (Figure 1.) provides food manufacturers and processors at all levels with a simple structure to enable the protection of both the products manufactured by the company from contamination by microorganisms (which have the potential to cause food spoilage and food poisoning), and the customers who may later consume these products.

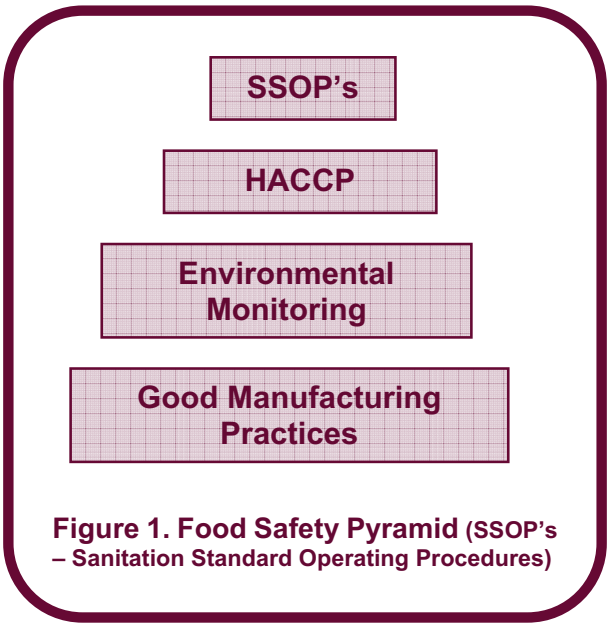


Figure 1. Food Safety Pyramid (SSOP's – Sanitation Standard Operating Procedures)

Microorganisms are always present in food handling environments. These microorganisms can be characterised as belonging to 2 distinct groups: transient and resident. Transient microorganisms are usually introduced into the food environment through raw materials, water and employees. Normally the routine application of good sanitation practises are able to kill these organisms. However, if contamination levels are high or sanitation procedures are inadequate, transient microorganisms may be able to establish themselves, multiply and become resident. Organisms such as Coliforms and *Salmonella* spp. and *Listeria* spp. have a well established history of becoming residents in food handling environments.

Environmental Monitoring

Food processors should employ environmental sampling programs to monitor for general levels of hygiene (the efficacy of general cleaning and sanitation for the removal of transient microorganisms) or indicator testing may be achieved through a variety of methods including visual inspection, ATP monitoring or the detection of surface protein residues. In addition, pathogen specific environmental sampling should be undertaken to monitor for the presence of specific pathogens that may be present as transient or resident microorganisms. The detection of specific pathogens serves two important roles. Firstly it highlights the presence of important food pathogens which may have been introduced into a food handling environment but may not have been eliminated by routine sanitation practises and therefore may be passed onto other food materials being processed. Secondly, it assists in determining sources of these important pathogens that may be resident.

Environmental Sampling Programs

Introduction

All food handling companies and establishments should employ an environmental sampling program to monitor for food spoilage microorganisms and food poisoning pathogens. Such a program, if well designed will enable the detection of unacceptable microbial contamination in a timely manner. Over the last decade environmental monitoring has changed from essentially random sampling, employing imaginary grids over a production area and testing points within each grid, to current methods that are focussed on risk assessment to determine the most appropriate methods for monitoring. Sampling programs should include the collection of samples during production on a regular basis from work surfaces in a randomised manner which will reflect the differing working conditions. In addition, samples should be taken from these sites after sanitising and from sites which may serve as harbours of resident organisms.

Sampling should not only be conducted on food contact surfaces, but the evaluation of non-food contact surfaces such as conveyor belts, rollers, walls, drains and air is equally as important as there are many ways (aerosols and human intervention) in which microorganisms can migrate from non-food contact surfaces to food.

The results of these samples should be tabulated as soon as available and in such a way that they can be compared with previous results in order to highlight trends.

Determining the Frequency of Monitoring

The development of an effective environmental monitoring program should reflect a balance between employing the available resources efficiently and monitoring at sufficient intervals so as to ensure that a meaningful picture of the levels and nature of bacterial contamination can be obtained.

When establishing an environmental monitoring program, the frequency of monitoring different areas may be determined based on “Criticality Indexes” relevant to each specific processing area or environment, or by using a Zones of Risk System.

Criticality Indexes

The development of a criticality program on which monitoring frequencies can be based should be focussed on the targeting of the critical steps in the manufacturing process. Therefore, the final manufactured product should receive more monitoring than early manufacturing steps i.e. end product testing of product.

The use of “criticality indexes” provides a means whereby the frequency of monitoring can be assigned to each designated critical area. The assessment of risk should be based on the potential impact any risk may have on the final quality or safety of the products being manufactured e.g. exposure to low temperatures would constitute a low risk whilst the presence of water or warmer temperatures would constitute a high risk.

The scheme employed by each food handling or manufacturing environment will necessarily be unique to the processes and types of food being handled in that environment. An example of how such a scheme can be constructed is shown in Table 1.

In the development of such a scheme, all manufacturing areas should be evaluated against a series of guiding questions which may include:

Higher Weighting should be given to:

- Dirtier activities.
- Areas where dirty activities are performed in close relative proximity to clean areas.
- Areas which are often wet.
- Areas with open drains.
- Areas with high levels of staff activity.

Higher Monitoring Frequencies should be assigned to:

- Warm or ambient handling areas as apposed to cold rooms.
- Areas with sinks, drains or ongoing wetness as opposed to dry areas.
- Areas where unprocessed raw foods are handled.
- Product filling.
- Packaging

Criticality Index	Frequency of Monitoring
1	Daily or Each Batch
2	Weekly
3	Fortnightly
4	Monthly
5	Three Monthly
6	Six Monthly

Table 1. Criticality Indexes and Monitoring Frequency

Once the critical factors have been established a final Monitoring Schedule can be developed (Table 2.).

Most food manufacturing processes involve one or more steps that effectively kill pathogenic bacteria, although manufacturing involving the production of fresh (salads etc), frozen products (vegetables, meats, poultry and fish) or some dairy products may not. In manufacturing in which processes designed to kill bacteria are employed, the challenge is to prevent the processed food from becoming recontaminated. In these situations, food handling surfaces and possibly equipment become contaminated by bacteria travelling through the food processing environment through a series of steps before finally coming into contact with the food that has been processed. *Listeria* species for example can multiply rapidly to high numbers on wet areas such as floors and drains and then be transferred to conveyor belts and benches through human intervention or the use of high pressure water hose that both can result in the production aerosols . Any processed food that subsequently touches these surfaces may the become recontaminated.

In the development of any monitoring program, post-processing environmental monitoring should always be considered as likely areas where pathogens may reappear and contaminate food post-processing. Generally, these post processing environments should be relatively free of bacteria when production commences. After periods of production, it should be expected that the level of bacterial

contamination of these areas should increase. However, the presence of microorganisms normally present in the pre-processed foods should not be expected.

Criticality Index	Likelihood of Impact on Finished Product	Definition	Frequency of Monitoring
1	Highly Likely	Mixing and Filling Machines work places are sanitised daily	Daily or Each Batch
2	Likely	Packaging areas or areas in which final handling is performed	Weekly
3	Moderately Likely	Areas where process food is exposed to the environment	Fortnightly
4	Unlikely	Cold areas where little or no processing is performed	Monthly
5	Very Unlikely	Areas in which indirect exposure to prepared and packaged product is unlikely	Three Monthly
6	Highly Unlikely	Any are that is uncontrolled or where microbial contamination is very unlikely such as freezers.	Six Monthly

Table 2. Monitoring Schedule Based on the Determination of Criticality Factors.

Zones of Risk System Approach

A more simplistic approach that can be adopted is to employ a program based around zones that have different levels of risk.

A typical three zone plan for example would divide a production facility into zones that cover low, medium and high risk areas within the production facility, with high risk zones being those in which their is direct contact between food products and surfaces (International Commission on Microbiological Specifications of Foods. 2002**.** *Microorganisms in Foods. 7. Microbiological Testing in Food Safety Management.* Blackwell Scientific. London.). These high risk areas would be those most stringently monitored. (See Table 3.)

Zone	Food Contact Surfaces
1	After dryer Pipes Conveyor belts Silos
2	Lids, Covers External Surfaces of Silos
3	Floors Walls Pipes

Table 3. Zones of Risk Analysis – Milk Powder Manufacturer

What to Monitor

Organisations involved in food handling should employ environmental monitoring as a means of :

1. Monitoring the general levels of hygiene within the environment in question. The monitoring of the general level of hygiene provides an overall impression of the level of cleanliness within the test environment – it measures the efficiency of the general cleaning and sanitation procedures in place and their ability to remove food residues and transient microorganisms. A variety of methods are available to achieve this task, including general physical inspections, ATP Monitoring Systems and the detection of the presence of food residues (generally protein).

2. Environmental microbiological monitoring for the presence of specific pathogens within the processing environment. The detection of specific pathogens serves two important roles:

    a. It highlights the presence of important food pathogens which may have been introduced into the food handling environment generally through human contact or from raw ingredients, but which may not have been eliminated by routine cleaning and sanitation procedures.
    b. Secondly, it highlights the sources of these pathogens that may be resident in the environments being tested.

Microbiological environmental monitoring should be used to indicate either unacceptable conditions or practises which in turn should aid in controlling pathogenic bacteria such as Salmonella and Listeria. The presence of Coliforms may also be valuable as they will provide an indication of the general levels of microbiological cleanliness within test environments.

How to Collect Swab Samples for Microbiological Environmental Testing

At this point in time no detailed standard methods (AOAC, USDA FSIS, USFDA etc) exist for the performance of microbiological environmental monitoring. ISO 18593:2004(E) Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs does however provide a general platform for the critical steps that should be considered in the development of testing procedures.

The key elements of this standard that should be taken into consideration include:

1. Moistened swabs should be employed for all sampling of surfaces. (Item 4.1.)
2. The solution used to moisten swabs should neutralise any detergents and sanitisers employed. (Item 6.1.)
3. Swab moisturizer solution must preserves the integrity of the sample i.e. bacterial numbers should remain constant until the sample collected onto the swab can be evaluated. (Item 6.1. and 7.)
4. Wherever possible the size of the area sampled should be greater than 100cm^2. (Item 8.2.)
5. The analysis of the samples for specific pathogens is achieved by transferring the swabs into an appropriate enrichment broth. (Item 8.2)
6. After enrichment transfer a sample to an appropriate agar plate medium for the target organism being sought. (Item 8.3.4.)
7. Report the target microorganism as present or absent. (Item 9.2.5.)
8. The contact plate method (including dipslides, RODAC plates and 3M Petrifilm™) shall not be used for the specific detection of pathogenic microorganisms. (Item 8.1.)

Detection of Target Microorganisms

As discussed above, the examination of environmental swabs for specific food pathogens is not described in any specific standard methods, however the general principle that the analysis of the samples for specific pathogens can be achieved by transferring swabs into an appropriate enrichment broth can be applied to any specific pathogen being sought.

In such instances, methods such as AOAC and USDA FSIS methods can be applied to any specific pathogen. In doing so however, it must be recognized that different methods employ different culture media and brands (and hence recipes) of different culture media will vary in terms of their selectivity, sensitivity and specificity. On this basis, although methods such as these may be applied, results may vary.

Therefore, it should be standard practice that prior to the adoption of any method it should be thoroughly evaluated to ensure that it is capable of recovering low numbers (<10 cfu) of the target organism whilst inhibiting high numbers (>10^3 cfu) of potentially competing organisms. Any system should also be evaluated to ensure that it is capable of recovering damaged organisms in low numbers.

The recovery of *Listeria monocytogenese* for example may be achieved using one of the following combinations employed in various standard methods. (Table 4.)

Method	24 Hours	48 Hours
USDA FSIS	UVM Broth	Fraser Broth
Health Canada (HFLP-38, 2002)	PALCAM Broth	UVM2 Broth
USFDA (BAM) AOAC/FDA	LEB	LEB

Table 4. Culture media suitable for the selective enrichment of *Listeria* spp.

Evaluation of the Results from Environmental Microbiological Testing

Following sampling and microbiological analysis a series of results will be available that provide an indication of the overall levels of hygiene in the processing environments evaluated. This body of information provides a valuable tool for maintaining and improving the quality and safety of products. In addition, the detection of specific pathogens such as Salmonella and Listeria is critical in ensuring food safety for the consumer.

It is not uncommon for food manufacturers only to react to unacceptable results when these pathogens appear when final food products are evaluated however, it is important (particularly in high risk products) that on-going environmental sampling practices are implemented and performed. The evaluation of samples and sampling plans and the test data generated over extended periods should lead to changes in test sample frequency and location, which in turn should lead to improvements in cleaning and sanitising practises.

Path-Chek Hygiene Pathogen Detection

The Path-Chek Hygiene Pathogen Detection product range is a range of microbiological environmental monitoring products that combine all of the requirements of the ISO Standard, ISO 18593:2004(E) Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs and the pathogen isolation, and detection methods employed in pathogen isolation and detection methods such as those described in AOAC, USDA FSIS, USFDA methods into a single convenient package.

Features

The following is a list of the key features of the Path-Chek Hygiene Pathogen Systems (Table 5.):

PRODUCT FEATURE	BENEFITS TO USERS
Pre-Moistened Swabs	• Meets the requirements of **ISO 18593:2004(E) Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.** • This improves the recovery of organisms from both wet and dry surfaces, increasing the sensitivity of the test.
Swab Moisturiser Neutralises Detergents and Sanitisers	The wetting agent neutralises the effects of residual detergents and sanitisers remaining on surfaces after cleaning, this maintains integrity of the sample if such residues are present.
Swab Moisturizer Preserves the Integrity of the Sample	The wetting agent ensures that the samples introduced into the various Pathogen Detection Broths are representative of the sample taken. This is especially important if there are delays in transferring the sample swabs to the testing laboratory.
Swab sticks have special "break point"	The shaft of the swabs used have a special "break point" to simplify the transfer of samples into the Pathogen Detection Broths.
A Range of Individual Growth Media	Three different individual Pathogen Detection Broths are currently available: • Coliforms – generally used as an indicator of overall hygiene conditions. • Salmonella – an important cause of food poisoning. • Listeria – a very important foodborne organism that causes a range of diseases with potentially high mortality rates.
High Sensitivity and Specificity	• Sensitivity – able to detect <1 organism per $10cm^2$ of surface tested. • Specificity – high levels of specificity minimize problems with false positive tests.
Detection Media and Confirmation Methods Compliant with International Standards	The Specific Growth Media, the Methods of Use and the Recommended Confirmation Methods all comply with recognised Food Standards such as USDA/ FSIS, US FDA etc. **Meets laboratory accreditation requirements.**

Table 5. Key Features of Path-Chek Hygiene Pathogen Systems

Kit Description

Path-Chek Hygiene Pathogen Systems are a range of screening tests intended for use in food handling and manufacturing environments and on food contact surfaces for the detection of Coliforms, *Salmonella* ssp. *Listeria* species. Path-Chek Hygiene Pathogen Systems should be considered as a fundamental component of Good Manufacturing Practice and an integral component of any HAACP (Hazard Analysis Critical Control Points) plan, providing a highly sensitive and specific indication of the presence of the foodborne pathogen being tested for.

Kit Contents

PC-010	Path-Chek Hygiene Coliform Detection Broth (3ml).	100 x 3ml
PC-020	Path-Chek Hygiene Salmonella Detection Broth (3ml).	100 x 3ml
PC-080	Path-Chek Hygiene Listeria Detection Broth (3ml).	100 x 3ml
PCS-100	Pre-moistened Path-Chek Hygiene swabs.	100 swabs

Instructions for use

Additional Materials

Incubator set to appropriate temperature
Rack to hold tubes during incubation
Template 10 x 10cm to assist with sampling

Note: The Path-Chek Hygiene Detection Broths (3ml) and Pre-moistened Path-Chek Hygiene swabs, (CODE: PCS-100 per 100 swabs) are purchased separately and combined to create the appropriate Path-Chek system.

Storage and Shelf Life

The Path-Chek Hygiene Detection Broths should be stored at 2 - 8°C when not in use. The pre-moistened sample swabs should be stored at 4 - 25°C. Both components should not be used after the expiry date printed on the carton label.

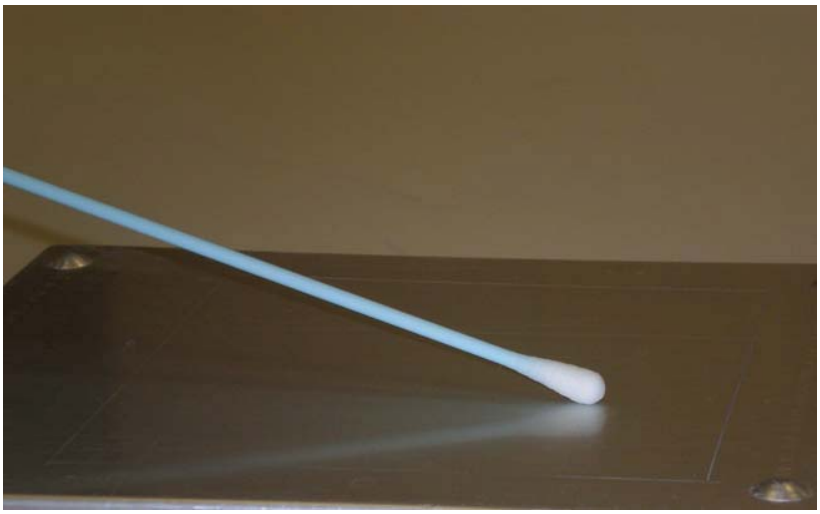
Procedure

Step 1

Carefully remove the cap from the pre-moistened Path-Chek Hygiene swab.

Step 2

Thoroughly swab a standard sample area (10 x 10cm), rotating the swab as the sample is being collected. If sample areas are irregular develop a standard sampling procedure which is documented and used consistently.

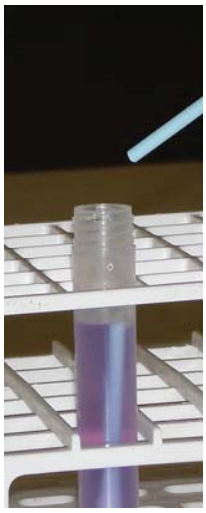


Step 3

After swabbing the test area, aseptically remove the cap from the Path-Chek Hygiene Detection Broth and carefully place the swab into the tube. If the swab cannot be transferred immediately into the Path-Chek Hygiene Detection Broth, return it to it's holding tube and store in a cool place. Label the Path-Chek Hygiene Detection Broth or the holding tube for the swab.

NOTES:

1. Swabs should be placed into the Path-Chek Hygiene Detection Broth at an angle of 45° with the tip of the swab against the side of the tube. Press down on the shaft of the swab. The shaft of the swab will break at breakpoint of the swab, 45mm from the swab tip.



2. If the swab cannot be transferred immediately into the Path-Chek Hygiene Detection Broth, the swab should be returned it to it's holding tube and store in a cool place. Swabs may be held at a maximum temperature of 20°C for up to 24 hours.

Step 4

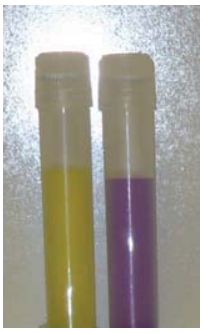
Place inoculated tubes into a suitable rack and incubate at 35-37°C, for 18-24 hours for Coliforms and Salmonella, and at 28-30°C, for 24-48 hours, for Listeria.

Note: If Path-Chek Hygiene Listeria is incubated at 35-37°C there will, in certain circumstances, be an increased risk of false positives.

Step 5

Observe for colour changes and record the results. A positive result may be interpreted as early as 18 hours however, results must not be considered as negative until the Path-Chek Hygiene Detection Broth has been incubated for up to 24 hours for the Coliform and *Salmonella* spp. systems and 48 hours for the *Listeria* spp. system.

Listeria **Coliforms** **Salmonella**

(+)  (-) (+)  (-) (+)  (-)

Step 6

Interpretation:

System	Positive	Negative
Coliform	Yellow	Purple
Salmonella	Black	Purple/ Yellow
Listeria	Black	Straw Colour

Table 6. Interpretation Criteria for Path-Chek Hygiene Detection Broths.

Step 7

OPTIONAL CONFIRMATION PROCEDURES

Presumptive positive tests may be confirmed by sub-culturing a drop of the growth medium onto an appropriate selective agar plate medium for the organism being tested.

The use of the following media with provide compliance with standard testing methods such as BAM and USDA/ FSIS etc.

System	Media
Coliform/ E.coli	mENDO VRBA
Salmonella	XLD Bismuth Sulphite
Listeria	Oxford Palcam ALOA

Table 7. Culture media for Confirmation of Positive Detection Broths

After incubation at 35 - 37°C for 24 – 48 hours, plates should be examined for colonies resembling the targets being sought.

Any suspect colonies should be further identified using more definitive tests such as microscopy and biochemical tests such as the Microgen® GNA ID (MID-64) and GNB ID (MID-65) and Listeria ID (MID-67).

Performance Characteristics

Pre-Moistened Swabs

Preservative Efficiency of Neutralizing Buffer

Eight species of commonly encountered environmental bacteria and food pathogens bacteria were grown overnight on Tryptone Soya Agar plates and suspended in 10ml Ringers solution to an approximate turbidity of Browns Opacity Standard No 1. 0. 1ml of these dilutions were transferred into the 100ml neutraliser. Six sponges were inoculated with 5ml neutraliser for each type of bacteria. The sponges were incubated at 22^{o}C for the duration of the test.

A semi-quantitative total viable counting method was used to test the number of bacteria surviving in the neutraliser. Bacterial levels were tested at 0, 24, 48, 72 and 168 hours.

RESULTS

Organism	0 hrs	24 hrs	48 hrs	72 hrs	168 hrs
S. enteritidis (NCIMB 50073)	100%	100%	70%	70%	70%
B. cereus (ATCC 11778)	100%	70%	70%	50%	30%
S. typhimurium (ATCC 14028)	100%	100%	100%	70%	50%
S. aureus (NCTC 6571)	100%	100%	50%	25%	0%
E. coli (NCIMB 11943)	100%	100%	100%	70%	70%
L. innocua (NCTC 11288)	100%	100%	100%	100%	100%
L. monocytogenes (NCTC 11994)	100%	100%	100%	70%	70%
L. monocytogenes (ATCC 7645)	100%	100%	70%	70%	70%

DISCUSSION

All of the organisms in this test maintained constant numbers up to 24 hours when stored at 22ºC. Beyond 24 hours a gradual decrease in the numbers of organisms recovered occurred with all species tested, however *S. aureus* was the only organism to suffer significant reductions in numbers.

The results show that the swab neutralising buffer is able to preserve the viability of the bacteria used in the test whilst also preventing over growth.

Path-Chek Hygiene Coliform Detection Broth

Sensitivity

Organism	Dilution	cfu/swab	Results
E.coli MBCC 69	10^4	TNC	Positive
	10^3	230	Positive
	10^2	30	Positive
	10^1	2	Positive
	10^0	0	Negative
E.coli MBCC 70	10^4	TNC	Positive
	10^3	290	Positive
	10^2	50	Positive
	10^1	3	Positive
	10^0	0	Negative
E.coli MBCC 71	10^4	TNC	Positive
	10^3	356	Positive
	10^2	35	Positive
	10^1	5	Positive
	10^0	0	Negative
E.coli MBCC 72	10^4	TNC	Positive
	10^3	430	Positive
	10^2	20	Positive
	10^1	1	Positive
	10^0	0	Negative
E.coli MBCC 73	10^4	TNC	Positive
	10^3	190	Positive
	10^2	10	Positive
	10^1	0	Positive
	10^0	0	Positive
K. oxytoca MBCC 46	10^4	500	Positive
	10^3	60	Positive
	10^2	6	Positive
	10^1	<1	Positive
K. pnuemoniae MBCC 47	10^4	TNC	Positive
	10^3	150	Positive
	10^2	15	Positive
	10^1	1	Positive
K. pnuemoniae MBCC 161	10^4	TNC	Positive
	10^3	100	Positive
	10^2	12	Positive (weak)
	10^1	1	Purple

Pre-moistened Path-Chek swabs were inoculated with a range of coliform species at dilutions down to zero cfu. The swabs were transferred into Path-Chek Hygiene Coliform Detection Broth and the broths incubated at 35 - 37ºC for 24 hours and then observed for any colour changes.

RESULTS

Based on these studies, the Path-Chek Hygiene Coliform Detection Broth is capable of detecting levels as low as 1 coliform organism per swab.

Specificity

A total of 31 isolates of *E.coli* were inoculated onto pre-moistened swabs at a level of 10 < cfu. Inoculated swabs were then transferred into Path-Chek Hygiene Coliform Detection Broth and the broths incubated at 35 - 37ºC for 24 hours and then observed for any colour changes.

Target Bacteria	Number	Positive	Negative	Comment
E.coli	31	30	1	
Klebsiella sp.	9	9	0	
Enterobacter sp.	9	7	2	2 x non lactose fermenting strains
Citrobacter sp.	13	8	5	5 x non lactose fermenting strains
Total Coliform	**62**	**54**	**1**	

The Path-Chek Hygiene Coliform Detection Broth detected 30/31 (97%) of the *E.coli* examined after 24 hours incubation at initial levels of < 10 cfu per swab. The one isolate that was not detected was or clinical origins and may have developed unique resistance patterns. In addition, a range of other "Coliform" organisms were examined, some of which were non lactose fermenting and failed to be detected by the indicator system employed in the Path-Chek Hygiene Coliform Detection Broth

In a second study, 49 non Colifo*rm species* comprising both Gram negative and Gram positive organisms were inoculated onto pre-moistened swabs at a level of 10^2 cfu. Inoculated swabs were then transferred into Path-Chek Hygiene Coliform Detection Broth and the broths incubated at 35 - 37ºC for 24 hours and then observed for any colour changes.

Non-Target Bacteria	Number	Positive	Negative	Comment
Salmonella spp.	5	0	5	
Listeria spp.	4	0	4	
Enterococcus spp.	4	0	4	
Staphylococcus spp.	5	0	5	
Streptococcus spp.	3	0	3	
Bacillus spp.	6	0	6	
Pseudomonas spp.	3	0	3	
Burkholderia spp.	8	0	8	
Morganella spp.	3	0	3	
Serratia spp.	2	0	2	
Providencia spp.	2	0	2	
Acinetobacter spp.	1	0	1	
Aeromonas spp.	1	0	1	
Proteus spp.	1	0	1	
Micrococcus spp.	1	0	1	
Total Non-Coliform	**49**	**0**	**49**	

Competitor Analysis

The Path-Chek Coliform and the Medical Wire® Coliform Environmental swab system were compared to determine both their efficiency in the recovery of Coliform organisms and their specificity.

Individual swabs of each system were challenged with <10 cfu of a total 28 different species of Coliform, or 100 cfu of a range of non-Coliform species. Both tests were read after 24 hours incubation.

Sensitivity Comparison

Organism	Total	Path- Chek Hygiene Coliform	Medical Wire® Coliform
E. coli	14	14	14
K. pneumoniae	6	6	6
K. oxytoca	1	1	1
C. youngii	1	1	1
C. freundii	2	2	2
E. cloacae	4	4	4
Total	**28**	**28**	**28**

Both the the Path-Chek Hygiene Coliform and the Medical Wire® Coliform were able to detect 28/28 (100%) of the isolates tested after 24 hours incubaction.

Specificity Comparison

Organism	Path-Chek Coliform	Medical Wire®	Comments
L. monocytogenes	Negative	Positive	
L. innocua	Negative	Negative	
L. grayi	Negative	Weak Positive	
Micrococcus sp.	Negative	Negative	
B. cereus	Negative	Negative	
B. licheniformis	Negative	Negative	
E. avium	Negative	Negative	
E. faecalis	Negative	Positive	
E. faecium	Negative	Positive	
E. gallenarum	Negative	Negative	
Bacillus sp.	Negative	Negative	
Staphylococcus sp.	Negative	Positive	
M. morganii	Negative	Positive	
S. maltophilia	Negative	Negative	
A. baumannii	Negative	Negative	
S. seftenberg	Negative	Positive	
P. mirabilis	Negative	Positive	
P. stuartii	Negative	Positive	
P. aeruginosa	Negative	Negative	
S. typhimurium	Negative	Positive	
S. marcesens	Negative	Positive	
Total	**21**	**10**	

A total of 21 species of both Gram negative and Gram positive organisms were examined. The Path-Chek Coliform inhibited the growth of all species tested i.e. 100% specificity.

The Medical Wire® Coliform failed to inhibit the growth of 11 species which were also able to produce positive reactions similar to Coliforms. “False positive” results were caused by a range of both Gram negative and Gram positive organisms. On the basis of this trial, the specificity of the Pat-Chek Coliform was 94% and the Medical Wire® Coliform was 48%.

Path-Chek Hygiene Salmonella Detection Broth

Sensitivity

Pre-moistened Path-Chek swabs were inoculated with a range of *Salmonella* species at dilutions down to zero cfu. The swabs were transferred into Path-Chek Hygiene Salmonella Detection Broth and the broths incubated at 35 - 37ºC for 24 hours and then observed for any colour changes.

RESULTS

Organisms	Dilution	cfu/swab	Results
Salmonella typhimuruim MBCC 215	10^4	TNC	Black
	10^3	TNC	Black
	10^2	120	Black
	10^1	10	Black
	10^0	1	Black
Salmonella bispeberg MBCC 274	10^4	TNC	Black
	10^3	250	Black
	10^2	20	Black
	10^1	2	Black
	10^0	0	Purple
Salmonella java MBCC 275	10^4	TNC	Black
	10^3	180	Black
	10^2	20	Black
	10^1	1	Black
	10^0	0	Purple
Salmonella virginia MBCC 277	10^4	TNC	Black
	10^3	420	Black
	10^2	30	Black
	10^1	2	Black
	10^0	0	Purple
Salmonella saint-paul MBCC 278	10^4	500	Black
	10^3	40	Black
	10^2	10	Black
	10^1	1	Black
	10^0	0	Purple
Salmonella derby MBCC 281	10^4	TNC	Black
	10^3	270	Black
	10^2	30	Black
	10^1	1	Black
	10^0	0	Purple
Salmonella senftenberg MBCC 282	10^4	TNC	Black
	10^3	290	Black
	10^2	50	Black
	10^1	3	Black
	10^0	0	Purple

Organisms	Dilution	cfu/swab	Results
Salmonella derby MBCC 281	10^4	TNC	Black
	10^3	270	Black
	10^2	30	Black
	10^1	1	Black
	10^0	0	Purple
Salmonella senftenberg MBCC 282	10^4	TNC	Black
	10^3	290	Black
	10^2	50	Black
	10^1	3	Black
	10^0	0	Purple
Salmonella senftenberg MBCC 282	10^4	TNC	Black
	10^3	250	Black
	10^2	30	Black
	10^1	2	Black
	10^0	0	Purple
Salmonella rostock MBCC 283	10^4	TNC	Black
	10^3	500	Black
	10^2	70	Black
	10^1	10	Black
	10^0	1	Black

This study demonstrated that the Path-Chek Hygiene Salmonella Detection Broth is capable of detecting levels as low as 1 *Salmonella* spp. organism per swab.

Specificity

A total of 97 different serotypes of *Salmonella* spp. were inoculated onto pre-moistened swabs at a level of 10 < cfu. Inoculated swabs were then transferred into Path-Chek Hygiene Salmonella Detection Broth and the broths incubated at 35 - 37ºC for 24 hours and then observed for any colour changes.

Target Bacteria	Number	Positive	Negative	Comment
S. enteritidis	5	5	0	
S. typhimurium	8	8	0	
S. dublin	7	7	0	
Salmonella spp.	77	76	1	1 Salmonella spp. H_2S Negative
Total Salmonella	**97**	**96**	**1**	

The Path-Chek Hygiene Salmonella Detection Broth detected 99% of the *Salmonella* spp. examined after 24 hours incubation at initial levels of < 10 cfu per swab. The one isolate that was not detected was found to be H_2S negative.

In a second study, 88 non *Salmonella* spp., comprising both Gram negative and Gram positive organisms were inoculated onto pre-moistened swabs at a level of 10^2 cfu. Inoculated swabs were then transferred into Path-Chek Hygiene Salmonella Detection Broth and the broths incubated at 35 - 37ºC for 24 hours and then observed for any colour changes.

Non-Target Bacteria	Number	Positive	Negative	Comment
E. coli	15	0	15	
Klebsiella spp.	5	0	5	
Enterobacter spp.	6	0	6	
Citrobacter spp.	14	11	3	
Listeria spp.	9	0	9	
Enterococcus spp.	4	0	4	
Staphylococcus spp.	5	0	5	
Streptococcus spp.	3	0	3	
Bacillus spp.	6	0	6	
Pseudomonas spp.	3	0	3	
Burkholderia spp.	6	0	6	
Morganii spp.	3	0	3	
Maltophilia spp.	2	0	2	
Serratia spp.	2	0	2	
Providencia spp.	2	0	2	
Acinetobacter spp.	1	0	1	
Aeromonas spp.	1	0	1	
Proteus spp.	1	0	1	
Micrococcus spp.	1	0	1	
Total Non-Salmonella	**88**	**11**	**77**	

The Path-Chek Hygiene Salmonella Detection Broth demonstrated a high degree of selectivity for the Gram positive isolates tested and most of the Gram negative isolates. Some isolates of *Citrobacter* spp. were not inhibited by the selective agents incorporated into the Path-Chek Salmonella Detection Broth, resulting in false positive results.

The occurrence of false positive results due to detection of *Citrobacter* spp. should still be considered as a significant result. The detection of significant levels of *Citrobacter* spp. from surfaces is an important indication of faecal contamination and/ or poor cleaning and sanitising and should therefore be investigated.

Recovery from Surfaces

A study was performed using the method of G. Moore and C. Griffith (A Comparison of Surface Sampling Methods for Detecting Coliforms on Food Contact Surfaces. Food Microbiology. 2002: **19**, - 73), to determine the efficiency of the swabbing process combined with the Path-Chek Salmonella Detection Broth when used to sample both wet and dry surfaces.

Organism	Wet Surface		Dry Surface	
	Results	cfu	Results	cfu
S. tranora MBCC 171	Positive	306	Positive	520
	Positive	40	Positive	52
	Positive	10	Positive	4
	Negative	2	Negative	0

The combination of the pre-moistened swabs and the Path-Chek Salmonella Detection Broth was successful in detecting < 10 cfu recovered from both wet and dry sample surface areas of 100 cm^2.

Competitor Analysis

No competitor products are available for comparison purposes.

Path-Chek Hygiene Listeria Detection Broth

Sensitivity

Pre-moistened Path-Chek swabs were inoculated with a range of *Listeria* species at dilutions down to zero cfu. The swabs were transferred into Path-Chek Hygiene Listeria Detection Broth and the broths incubated at 35 - 37ºC for up to 48 hours and then observed for any colour changes.
Note: If Path-Chek Hygiene Listeria is incubated at 35-37 ºC, there will, in certain circumstances, be an increased risk of false positives, compared to incubation at 28-30°C.

RESULTS

Organism	Dilution	cfu/ Swab	24 Hours	48 Hours
L. monocytogenes MBCC 97	10^4	TNC	Positive	Positive
	10^3	800	Positive	Positive
	10^2	110	Positive	Positive
	10^1	10	Negative	Positive
	10^0	0	Negative	Negative
L. monocytogenes MBCC 98	10^4	TNC	Positive	Positive
	10^3	250	Positive	Positive
	10^2	20	Positive	Positive
	10^1	2	Negative	Positive
	10^0	0	Negative	Positive
L. monocytogenes MBCC 99	10^4	TNC	Positive	Positive
	10^3	560	Positive	Positive
	10^2	60	Positive	Positive
	10^1	2	Negative	Positive
	10^0	0	Negative	Positive
L. innocua MBCC 93	10^4	TNC	Positive	Positive
	10^3	700	Positive	Positive
	10^2	45	Positive	Positive
	10^1	5	Positive	Positive
	10^0	0	Positive	Negative
L. innocua MBCC 94	10^4	TNC	Positive	Positive
	10^3	440	Positive	Positive
	10^2	20	Positive	Positive
	10^1	3	Positive	Positive
	10^0	0	Negative	Negative
L. innocua MBCC 95	10^4	TNC	Positive	Positive
	10^3	450	Positive	Positive
	10^2	30	Positive	Positive
	10^1	2	Positive	Positive
	10^0	0	Negative	Negative
L.. seeligeri MBCC 110	10^4	TNC	Negative	Positive
	10^3	400	Negative	Positive
	10^2	60	Negative	Positive
	10^1	0	Negative	Negative
	10^0	0	Negative	Negative

Organism	Dilution	cfu/ Swab	24 Hours	48 Hours
L.. ivanovii MBCC 111	**10^4**	TNC	Positive	Positive
	10^3	560	Negative	Positive
	10^2	40	Negative	Positive
	10^1	3	Negative	Positive
	10^0	0	Negative	Negative
L. welshmeri MBCC 114	**10^4**	TNC	Positive	Positive
	10^3	880	Positive	Positive
	10^2	100	Positive	Positive
	10^1	10	Positive	Positive
	10^0	1	Positive	Positive

This study demonstrated that the Path-Chek Hygiene Listeria Detection Broth is capable of detecting levels as low as 1 *Listeria* spp. organism per swab.

Specificity

A total of 97 different species of Listeria were inoculated onto pre-moistened swabs at a level of 10 < cfu. Inoculated swabs were then transferred into Path-Chek Hygiene Listeria Detection Broth and the broths incubated at 35 - 37ºC for up to 48 hours and then observed for any colour changes.

Target Bacteria	Number	Positive	Negative	Comment
L. monocytogenes	42	42	0	
L. innocua	24	24	0	
L. seeligeri	12	12	0	
L. ivanovii	8	8	0	
L. welshimeri	11	11	0	
Total Listeria	**97**	**97**	**0**	

This investigation demonstrated that 100% of the Listeria spp. examined could be detected after 48 hours incubation at initial levels < 10cfu per swab.

In a second study, 76 non *Listeria* spp. comprising both Gram negative and Gram positive organisms were inoculated onto pre-moistened swabs at a level of > 5 x 10^2 cfu. Inoculated swabs were then transferred into Path-Chek Hygiene Listeria Detection Broth and the broths incubated at 35 - 37ºC for 48 hours and then observed for any colour changes.

Non-Target Bacteria	Number	Positive	Negative	Comment
E. coli	4	0	4	
Klebsiella spp.	10	4	6	
Enterobacter spp.	5	0	5	
Citrobacter spp.	3	0	3	
Salmonella spp.	9	0	9	
Enterococcus spp.	6	4	2	6/6 Negative when challenged with < 5 x 10^2
Staphylococcus spp.	5	0	5	
Streptococcus spp.	3	0	3	
Bacillus spp.	5	1	4	5/5 Negative when challenged with < 1 x 10^2
Pseudomonas spp.	4	0	4	
Burkholderia spp.	7	0	7	
Lactobacillus spp.	3	0	3	
Carnobacterium spp.	2	0	2	
Cornebacterium spp.	1	0	1	
Kurthia spp.	1	0	1	
Acinetobacter spp.	1	0	1	
Achromobacter spp.	1	0	1	
Proteus spp.	2	0	2	
Rhodococcus equi	1	0	1	
Micrococcus spp.	1	0	1	
Morganella spp.	1	0	1	
Stenotrophomonas spp.	1	0	1	
Total Non-Listerias	**76**	**9**	**67**	

Path-Chek Hygiene Listeria Detection Broth demonstrated a high degree of selectivity for the all of the Gram positive isolates tested with the exception of 2/6 *Enterococcus* spp. and 1/4 *Bacillus* spp. which produced positive reactions at levels > 5 x 10^2 but failed to grow at lower levels (< 1 x 10^2) after 48 hours incubation.

With the exception of *Klebsiella* spp, all other Gram negative isolates either failed to grow or produced negative results after 48 hours incubation. The Path-Chek Listeria Detection Broth, resulting in false positive results.

The occurrence of false positive results due to detection of *Enterococcus* spp. should still be considered as a significant result. The detection of significant levels of *Enterococcus* spp. from surfaces is an important indication of faecal contamination and/ or poor cleaning and sanitising and should therefore be investigated.

Recovery from Surfaces

A study was performed using the method of G. Moore and C. Griffith (A Comparison of Surface Sampling Methods for Detecting Coliforms on Food Contact Surfaces. Food Microbiology. 2002: **19**, - 73), to determine the efficiency of the swabbing process combined with the Path -Chek Salmonella Detection Broth when used to sample both wet and dry surfaces.

Organism	Wet Surface		Dry Surface	
	Results	cfu	Results	cfu
L. monocytogenes MBCC 305	Positive	300	Positive	100
	Positive	30	Positive	10
	Positive	6	Negative	1
	Positive (weak)	2	Negative	0

The combination of the pre-moistened swabs and the Path-Chek Listeria Detection Broth was successful in detecting < 10 cfu recovered from both wet surfaces and 10 cfu from dry sample surface areas of 100 cm^2.

Competitor Analysis

The Path-Chek Listeria and the Medical Wire® Listeria Environmental swab system were compared to determine both their efficiency in the recovery of *Listeria* spp. and their specificity.

Individual swabs of each system were challenged with <10 cfu of a total 62 different species of *Listeria* species, or 100 cfu of a range of non-Listeria species. Both tests were read after 24 hours and 48 hours incubation.

Sensitivity Comparison

Organism	Total	Path- Chek Hygiene Listeria		Medical Wire® Listeria	
		24 Hours	48 hours	24 Hours	48 hours
L. monocytogenes	30	29	30	20	30
L. innocua	18	18	18	16	18
L. ivanovii	4	3	4	0	4
L. seeligeri	4	4	4	0	4
L. welshimeri	6	6	6	0	6
Total	**62**	**60**	**62**	**36**	**62**

The Path-Chek Hygiene Listeria detected 60 (97%) of the Listeria isolates tested after 24 hours incubation and 62/62 (100%) of the isolates after 48 hours incubation. The Medical Wire® Listeria was able to detect 36/62 (58%) of the isolates tested after 24 hours incubaction and 62/62 (100%) after 48 hours incubation. The Medical Wire® was only able to detect *L. monocytogenes* and *L. innocua* species after 24 hours incubaction.

Specificity Comparison

Organism	Path-Chek Listeria		Medical Wire	
	24 Hour	48 Hour	24 Hour	48 Hour
C. diversus	Negative	Negative	Negative	Negative
S. enteritidis	Negative	Negative	Negative	Negative
S. enteritidis	Negative	Negative	Negative	Negative
S. hadar	Negative	Negative	Negative	Negative
S. indiana	Negative	Negative	Negative	Negative
S. infantis	Negative	Negative	Negative	Negative
S. typhimurium	Negative	Negative	Negative	Negative
P. mirabilis	Negative	Negative	Negative	Negative
P. stuartii	Negative	Negative	Negative	Negative
P. aeruginosa	Negative	Negative	Negative	Negative
E. aerogenes	Negative	Negative	Negative	Negative
E. coli	Negative	Negative	Negative	Negative
E. coli	Negative	Negative	Negative	Negative
K. oxytoca	Negative	Negative	Negative	Negative
K. oxytoca	Negative	Negative	Negative	Negative
K. pneumoniae	Negative	Negative	Negative	Negative
K. pneumoniae	Negative	Negative	Negative	Negative
K. pneumoniae	Negative	Negative	Negative	Negative
K. pneumoniae	Positive	Positive	Positive	Positive
K. pneumoniae	Negative	Positive	Negative	Positive
K. pneumoniae	Positive	Positive	Negative	Negative
K. pneumoniae	Negative	Negative	Negative	Negative
K. pneumoniae	Positive	Positive	Negative	Negative
E. cloacae	Negative	Negative	Negative	Negative
E. cloacae	Negative	Negative	Negative	Negative
E. cloacae	Negative	Negative	Negative	Negative
E. cloacae	Negative	Negative	Negative	Negative
Carnobacterium divergens	Negative	Negative	Positive	Positive
Carnobacterium piscicola	Negative	Negative	Negative	Negative
Lactobacillus casei	Negative	Negative	Negative	Negative
Lactobacillus lactis	Negative	Negative	Negative	Negative
Lactobacillus plantarum	Negative	Negative	Negative	Negative
Bacillus mycoides	Negative	Negative	Negative	Negative
Kurthia zopfii	Negative	Negative	Negative	Negative
Micrococcus spp.	Negative	Negative	Negative	Negative
Rhodococcus equi	Negative	Negative	Negative	Negative
Enterococcus durans	Negative	Negative	Negative	Positive
Pseudomonas fluorescens	Negative	Negative	Negative	Negative
Corynebacterium renale	Negative	Negative	Negative	Negative
Bacillus cereus	Negative	Negative	Negative	Negative
Bacillus licheniformis	Negative	Negative	Negative	Negative
Enterococcus avium	Negative	Negative	Positive	Positive
Enterococcus faecalis	Negative	Negative	Positive	Positive
Enterococcus faecium	Negative	Negative	Positive	Positive
Enterococcus gallinarum	Negative	Negative	Negative	Positive

Organism	Path-Chek Listeria		Medical Wire	
	24 Hour	48 Hour	24 Hour	48 Hour
Streptococcus spp.	Negative	Negative	Negative	Negative
Streptococcus spp.	Negative	Negative	Negative	Negative
Streptococcus spp.	Negative	Negative	Negative	Negative
Bacillus spp	Negative	Negative	Negative	Negative
Bacillus spp	Negative	Negative	Negative	Negative
Bacillus spp	Negative	Negative	Negative	Negative
Staphylococcus spp.	Negative	Negative	Negative	Negative
S. saprophyticus.	Negative	Negative	Negative	Negative
S. aureus	Negative	Negative	Negative	Negative
S. aureus	Negative	Negative	Negative	Negative
S. hyicus ss hyicus	Negative	Negative	Negative	Negative
B. stabilis	Negative	Negative	Negative	Negative
B. cenocepacia	Negative	Negative	Negative	Negative
B. multivorans	Negative	Negative	Negative	Negative
B. cenocepacia	Negative	Negative	Negative	Negative
A. xylosoxidans	Negative	Negative	Negative	Negative
B. cepacia	Negative	Negative	Negative	Negative
R. mannitolilytica	Negative	Negative	Negative	Negative
B. cenocepacia	Negative	Negative	Negative	Negative
P. aeruginosa	Negative	Negative	Negative	Negative
P. putida	Negative	Negative	Negative	Negative
B. vietnamiensis	Negative	Negative	Negative	Negative
Total	**58**	**58**	**54**	**54**

A total of 62 species of both Gram negative and Gram positive organisms were examined. The Path-Chek Listeria failed to inhibit the growth of 4 species which were also able to produce positive reactions similar to *Listeria* spp.. These “false positive” results were caused By *K. pneuomoniae* (3) and *Enterococcus* spp.(1).

The Medical Wire® Listeria failed to inhibit the growth of 8 species which were also able to produce positive reactions similar to *Listeria* spp.. “Falso positive” results were caused by *K. pneuomoniae* (1), *Enterococcus* spp.(6) and *C. divergens* (1).

On the basis of this trial, the specificity of the Pat-Chek Listeria was 94% and the Medical Wire® Listeria was 87%.

POSITIVE RESULTS – WHAT DO THEY MEAN?

With any of the Path-Chek Hygiene Pathogen systems, a positive result for a specific pathogen can mean one of two things:

1. The presence of a specific pathogen such as Salmonella or Listeria at levels as low as 1 – 2 cfu per sampled area which should be at least 100cm^2, OR
2. The presence in high numbers, usually > 10^2 of potentially cross reacting organisms

Path-Chek Detection System	Most Probable Cause of "False Positive Tests
Coliform	Nil
Salmonella	*Citrobacter* spp.
Listeria	*K. pneumoniae* *Enterococcus spp.*

Table 8. Most Probable Cause of "False Positive Tests

In each of these cases, the potential causes of "False Positive" tests are organisms of faecal origin.

As such, ALL Positive Tests should be confirmed by subculture and identification of suspicious colonies. If a specific target pathogen cannot be isolated, the results should still be considered as significant as they indicate the presence of significant levels of organisms of faecal origin on the test surfaces. These organisms should not be present if adequate cleaning and sanitising procedures are in place and being performed correctly.