

# PATH-CHEK

## SUMMARY OF TECHNICAL DATA TO DATE



# Section 1

This section deals with the ability of the media to detect the target organism in pure culture and in the presence of potentially interfering organisms.

## 1.0 Initial Sensitivity Test

Each target bacteria was inoculated into PATH-CHEK units at varying dilutions and compared to standard plating technique (on BBL Tryptose agar) and contact plate.

### 1.0.1 Coliforms

	$3 \times 10^5$	$3 \times 10^4$	$3 \times 10^3$	$3 \times 10^2$	$3 \times 10^1$	$3 \times 10^0$
PATH-CHEK	+	+	+	+	-	-
	(yellow)	(yellow)	(yellow)	(yellow)	(green)	(green)
Plating	+	+	+	+	-	-
Contact Plate	+	+	+	+	-	-

### 1.0.2 Salmonella

	$3 \times 10^5$	$3 \times 10^4$	$3 \times 10^3$	$3 \times 10^2$	$3 \times 10^1$	$3 \times 10^0$
PATH-CHEK	+	+	+	+	-	-
	(grey/black ppt)	(grey/black ppt)	(grey/black ppt)	(grey/black ppt)	(blue)	(blue)
Plating	+	+	+	+	-	-
Contact Plate	+	+	+	+	-	-

### 1.0.3 *Staphylococcus aureus*

	$3 \times 10^5$	$3 \times 10^4$	$3 \times 10^3$	$3 \times 10^2$	$3 \times 10^1$	$3 \times 10^0$
PATH-CHEK	+	+	+	+	-	-
	(yellow)	(yellow)	(yellow)	(yellow)	(red)	(red)
Plating	+	+	+	+	-	-
Contact Plate	+	+	+	+	-	-

### 1.0.4 *Vibrio parahaemolyticus*

	$3 \times 10^5$	$3 \times 10^4$	$3 \times 10^3$	$3 \times 10^2$	$3 \times 10^1$	$3 \times 10^0$
PATH-CHEK	+	+	+	+	-	-
	(yellow)	(yellow)	(yellow)	(yellow)	(purple)	(purple)
Plating	+	+	+	+	-	-
Contact Plate	+	+	+	+	-	-

This data shows that the PATH-CHEK unit will grow out the target bacteria to at least the same level as conventional techniques.

## 1.1 Initial Specificity Test

Each PATH-CHEK unit was inoculated with different bacteria to determine the specificity of the culture medium.

	<i>S. aureus</i>	Salmonella	<i>V. parahaemolyticus</i>	Coliforms
<i>S. aureus</i> (MRSA)	+	-	-	-
<i>S. aureus</i> (MSSA)	+	-	-	-
<i>S. epidermidis</i>	-	-	-	-
<i>S. asaprophyticus</i>	-	-	-	-
<i>S. haemolyticus</i>	-	-	-	-
<i>Enterococcus spp.</i>	-	-	+	-
<i>Bacillus spp.</i>	-	-	-	-
<i>S. typhimurium</i>	-	+	-	-
<i>S. typhi</i>	-	-	-	-
<i>P. vulgaris</i>	-	-	-	-
<i>P. mirabilis</i>	-	-	-	-
<i>C. freundii</i>	-	-	-	+
<i>C. diversus</i>	-	-	-	-
<i>M. morgani</i>	-	-	-	-
<i>Klebsiella spp.</i>	-	-	-	+
<i>H. alvei</i>	-	-	-	-
<i>Serratia spp.</i>	-	-	+	-
<i>Enterobacter spp.</i>	-	-	-	+
<i>Pseudomonas spp.</i>	-	-	-	-
<i>Acinetobacter spp.</i>	-	-	-	-
<i>Flavobacterium spp.</i>	-	-	-	-
<i>Aeromonas spp.</i>	-	-	-	-
<i>P. shigelloides</i>	-	-	-	-
<i>V. parahaemolyticus</i>	-	-	+	-
<i>V. fluviialis</i>	-	-	-	-
<i>V. vulnificus</i>	-	-	-	-
<i>E. coli</i> (0157 EHEC)	-	-	-	+
<i>E. coli</i> (0157 EPEC)	-	-	-	+
<i>E. coli</i> (0124 EIEC)	-	-	-	+
<i>E. coli</i> (025 ETEC)	-	-	-	+

1. *Citrobacter* species, *Klebsiella* species, *Enterobacter* species, *Serratia* species and *E. coli* are all classified as coliforms.
2. The false positive results obtained with *Enterococcus* species and *Serratia* species with the Path Chek Vibrio was a result of the high dose of organisms used in these initial tests. Lower numbers of bacteria will not produce these false positive tests (See 1.1.4.)

The initial specificity test was further developed to show at what level different bacteria could be expected to show a positive result in each of the four media.

### 1.1.1 Coliform Unit

Test Organism Name	ATCC Number	Concentration (cfu/ml)										
		10 <sup>8</sup>	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>1</sup>	10 <sup>0</sup>	Neg	
<i>A. hydrophila</i>	7966	-	-	-	-	-	-	-	-	-	-	-
<i>Bacillus cereus</i>	11778	-	-	-	-	-	-	-	-	-	-	-
<i>Bacillus subtilis</i>	6633	-	-	-	-	-	-	-	-	-	-	-
<i>C. freundii</i>	8090	+	+	+	+	+	+	+	+	-	-	-
<i>E. aerogenes</i>	13048	+	+	+	+	+	+	-	-	-	-	-
<i>E. cloacae</i>	13047	+	+	+	+	+	+	+	+	-	-	-
<i>E. faecalis</i>	19433	+	+	+	+	-	-	-	-	-	-	-
<i>E. faecalis</i>	11700	-	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	25922	+	+	+	+	+	+	+	+	-	-	-
<i>E. coli</i>	11775	+	+	+	+	+	+	+	+	-	-	-
<i>E. coli</i>	8739	+	+	+	+	+	+	+	+	-	-	-
<i>E. coli</i>	35150	+	+	+	+	+	+	+	+	-	-	-
<i>K. pneumoniae</i>	13883	+	+	+	+	+	+	+	+	-	-	-
<i>Micrococcus luteus</i>	10240	-	-	-	-	-	-	-	-	-	-	-
<i>M. morganii</i>	25830	-	-	-	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	29906	-	-	-	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	12453	-	-	-	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	14273	-	-	-	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	13315	-	-	-	-	-	-	-	-	-	-	-
<i>P. vulgaris</i>	27853	-	-	-	-	-	-	-	-	-	-	-
<i>Ps. aeruginosa</i>	14028	-	-	-	-	-	-	-	-	-	-	-
<i>S. typhimurium</i>	14756	-	-	-	-	-	-	-	-	-	-	-
<i>S. marcescens</i>	12022	-	-	-	-	-	-	-	-	-	-	-
<i>S. flexneri</i>	11060	-	-	-	-	-	-	-	-	-	-	-
<i>S. sonnei</i>	25923	-	-	-	-	-	-	-	-	-	-	-
<i>S. aureus</i>	6538	-	-	-	-	-	-	-	-	-	-	-
<i>S. aureus subsp aureus</i>	6538P	-	-	-	-	-	-	-	-	-	-	-
<i>S. epidermidis</i>	12228	+	+	+	+	-	-	-	-	-	-	-
<i>S. epidermidis</i>	14990	+	+	+	-	-	-	-	-	-	-	-
<i>V. alginolyticus</i>	17749	-	-	-	-	-	-	-	-	-	-	-
<i>V. parahaemolyticus</i>	17802	-	-	-	-	-	-	-	-	-	-	-
<i>Y. enterocolitica</i>	23715	-	-	-	-	-	-	-	-	-	-	-
<i>Y. enterocolitica</i>	9610	-	-	-	-	-	-	-	-	-	-	-

The results show that the media will indicate the growth of Coliforms (*E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp. and *Serratia* spp.) down to 10<sup>1</sup> cfu/ml after 24 hours and at varying levels for non target organisms, but in all cases, the growth of the non target organisms only occurred when they were present in at least 10,000 x the numbers of the various Coliform organisms tested.

### 1.1.2 Salmonella Unit

Test Organism Name	ATCC Number	Concentration (cfu/ml)										
		10 <sup>8</sup>	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>1</sup>	10 <sup>0</sup>	Neg	
<i>A. hydrophila</i>	7966	-	-	-	-	-	-	-	-	-	-	-
<i>Bacillus cereus</i>	11778	-	-	-	-	-	-	-	-	-	-	-
<i>Bacillus subtilis</i>	6633	-	-	-	-	-	-	-	-	-	-	-
<i>C. freundii</i>	8090	-	-	-	-	-	-	-	-	-	-	-
<i>E. aerogenes</i>	13048	-	-	-	-	-	-	-	-	-	-	-
<i>E. cloacae</i>	13047	-	-	-	-	-	-	-	-	-	-	-
<i>E. faecalis</i>	19433	-	-	-	-	-	-	-	-	-	-	-
<i>E. faecalis</i>	11700	-	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	25922	-	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	11775	-	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	8739	-	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	35150	-	-	-	-	-	-	-	-	-	-	-
<i>K. pneumoniae</i>	13883	-	-	-	-	-	-	-	-	-	-	-
<i>Micrococcus luteus</i>	10240	-	-	-	-	-	-	-	-	-	-	-
<i>M. morgani</i>	25830	-	-	-	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	29906	-	-	-	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	12453	-	-	-	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	14273	-	-	-	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	13315	-	-	-	-	-	-	-	-	-	-	-
<i>P. vulgaris</i>	27853	-	-	-	-	-	-	-	-	-	-	-
<i>Ps. aeruginosa</i>	14028	-	-	-	-	-	-	-	-	-	-	-
<i>S. typhimurium</i>	14756	+	+	+	+	+	+	+	+	-	-	-
<i>S. marcescens</i>	12022	-	-	-	-	-	-	-	-	-	-	-
<i>S. flexneri</i>	11060	-	-	-	-	-	-	-	-	-	-	-
<i>S. sonnei</i>	25923	-	-	-	-	-	-	-	-	-	-	-
<i>S. aureus</i>	6538	-	-	-	-	-	-	-	-	-	-	-
<i>S. aureus subsp aureus</i>	6538P	-	-	-	-	-	-	-	-	-	-	-
<i>S. epidermidis</i>	12228	-	-	-	-	-	-	-	-	-	-	-
<i>S. epidermidis</i>	14990	-	-	-	-	-	-	-	-	-	-	-
<i>V. alginolyticus</i>	17749	-	-	-	-	-	-	-	-	-	-	-
<i>V. parahaemolyticus</i>	17802	-	-	-	-	-	-	-	-	-	-	-
<i>Y. enterocolitica</i>	23715	-	-	-	-	-	-	-	-	-	-	-
<i>Y. enterocolitica</i>	9610	-	-	-	-	-	-	-	-	-	-	-

The results indicated that growth of Salmonella could be detected at levels as low as 10cfu/ml. No cross reaction due to non target organisms was detected.

### 1.1.3 Staph Unit

Test Organism Name	ATCC Number	Concentration (cfu/ml)									
		10 <sup>8</sup>	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>1</sup>	10 <sup>0</sup>	Neg
<i>A. hydrophila</i>	7966	-	-	-	-	-	-	-	-	-	-
<i>Bacillus cereus</i>	11778	-	-	-	-	-	-	-	-	-	-
<i>Bacillus subtilis</i>	6633	-	-	-	-	-	-	-	-	-	-
<i>C. freundii</i>	8090	-	-	-	-	-	-	-	-	-	-
<i>E. aerogenes</i>	13048	+	-	-	-	-	-	-	-	-	-
<i>E. cloacae</i>	13047	-	-	-	-	-	-	-	-	-	-
<i>E. faecalis</i>	19433	+	-	-	-	-	-	-	-	-	-
<i>E. faecalis</i>	11700	+	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	25922	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	11775	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	8739	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	35150	+	-	-	-	-	-	-	-	-	-
<i>K. pneumoniae</i>	13883	+	-	-	-	-	-	-	-	-	-
<i>Micrococcus luteus</i>	10240	-	-	-	-	-	-	-	-	-	-
<i>M. morgani</i>	25830	-	-	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	29906	+	+	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	12453	+	+	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	14273	+	+	+	-	-	-	-	-	-	-
<i>P. mirabilis</i>	13315	-	-	-	-	-	-	-	-	-	-
<i>P. vulgaris</i>	27853	-	-	-	-	-	-	-	-	-	-
<i>Ps. aeruginosa</i>	14028	-	-	-	-	-	-	-	-	-	-
<i>S. typhimurium</i>	14756	-	-	-	-	-	-	-	-	-	-
<i>S. marcescens</i>	12022	-	-	-	-	-	-	-	-	-	-
<i>S. flexneri</i>	11060	+	+	-	-	-	-	-	-	-	-
<i>S. sonnei</i>	25923	+	+	-	-	-	-	-	-	-	-
<i>S. aureus</i>	6538	+	+	+	+	+	+	+	-	-	-
<i>S. aureus subsp aureus</i>	6538P	+	+	+	+	-	-	-	-	-	-
<i>S. epidermidis</i>	12228	+	+	+	-	-	-	-	-	-	-
<i>S. epidermidis</i>	14990	+	+	+	+	-	-	-	-	-	-
<i>V. alginolyticus</i>	17749	+	+	+	+	-	-	-	-	-	-
<i>V. parahaemolyticus</i>	17802	-	-	-	-	-	-	-	-	-	-
<i>Y. enterocolitica</i>	23715	-	-	-	-	-	-	-	-	-	-
<i>Y. enterocolitica</i>	9610	-	-	-	-	-	-	-	-	-	-

The results indicated that *Staph. aureus* could be detected in numbers as low as 10<sup>2</sup>. In all cases, the growth of the non target organisms only occurred when they were present in at least 1000x higher numbers than *Staph. aureus*.

### 1.1.4 Vibrio Unit

Test Organism Name	ATCC Number	Concentration (cfu/ml)									
		10 <sup>8</sup>	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>1</sup>	10 <sup>0</sup>	Neg
<i>A. hydrophila</i>	7966	-	-	-	-	-	-	-	-	-	-
<i>Bacillus cereus</i>	11778	-	-	-	-	-	-	-	-	-	-
<i>Bacillus subtilis</i>	6633	-	-	-	-	-	-	-	-	-	-
<i>C. freundii</i>	8090	-	-	-	-	-	-	-	-	-	-
<i>E. aerogenes</i>	13048	+	-	-	-	-	-	-	-	-	-
<i>E. cloacae</i>	13047	-	-	-	-	-	-	-	-	-	-
<i>E. faecalis</i>	19433	+	+	-	-	-	-	-	-	-	-
<i>E. faecalis</i>	11700	+	+	+	+	+	+	-	-	-	-
<i>E. coli</i>	25922	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	11775	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	8739	-	-	-	-	-	-	-	-	-	-
<i>E. coli</i>	35150	-	-	-	-	-	-	-	-	-	-
<i>K. pneumoniae</i>	13883	+	+	-	-	-	-	-	-	-	-
<i>Micrococcus luteus</i>	10240	-	-	-	-	-	-	-	-	-	-
<i>M. morgani</i>	25830	-	-	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	29906	-	-	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	12453	-	-	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	14273	-	-	-	-	-	-	-	-	-	-
<i>P. mirabilis</i>	13315	-	-	-	-	-	-	-	-	-	-
<i>P. vulgaris</i>	27853	-	-	-	-	-	-	-	-	-	-
<i>Ps. aeruginosa</i>	14028	-	-	-	-	-	-	-	-	-	-
<i>S. typhimurium</i>	14756	-	-	-	-	-	-	-	-	-	-
<i>S. marcescens</i>	12022	-	-	-	-	-	-	-	-	-	-
<i>S. flexneri</i>	11060	-	-	-	-	-	-	-	-	-	-
<i>S. sonnei</i>	25923	-	-	-	-	-	-	-	-	-	-
<i>S. aureus</i>	6538	-	-	-	-	-	-	-	-	-	-
<i>S. aureus subsp aureus</i>	6538P	+	+	-	-	-	-	-	-	-	-
<i>S. epidermidis</i>	12228	-	-	-	-	-	-	-	-	-	-
<i>S. epidermidis</i>	14990	-	-	-	-	-	-	-	-	-	-
<i>V. alginolyticus</i>	17749	+	+	+	+	+	+	-	-	-	-
<i>V. parahaemolyticus</i>	17802	+	+	+	+	+	+	-	-	-	-
<i>Y. enterocolitica</i>	23715	-	-	-	-	-	-	-	-	-	-
<i>Y. enterocolitica</i>	9610	-	-	-	-	-	-	-	-	-	-

The results indicated that *Vibrio* spp. could be detected at levels as low as 10<sup>2-3</sup>. With the exception of *E. faecalis* the growth of non target organisms only occurred when they were present in at least 10,000 x the number of *Vibrio* spp.

## 1.2 Performance in the Presence of Mixed Bacterial Populations

A series of studies was set up to determine how the PATH-CHEK media performed when presented with the target organism and a mixture of potential contaminating organisms to show inhibition (or enhancement) of growth.

The organisms were subcultured from BHI plates into 10ml of BHI broth and the resulting broth cultures used to prepare dilutions containing 10, 100 and 1000 target organisms and 10<sup>6</sup> cfu/ml contaminant. All incubations were carried out at 37°C for a maximum of 24 hours.

### 1.2.1 PATH-CHEK Coliforms

Tube	Target (cfu)	Contaminant	24 hr. Colour	Result
1	<i>E. coli</i> (10)	-	Yellow	POSITIVE
2	<i>E. coli</i> (100)	-	Yellow	POSITIVE
3	<i>E. coli</i> (1000)	-	Yellow	POSITIVE
4	<i>E. coli</i> (10)	10 <sup>6</sup> <i>P. mirabilis</i>	Yellow	POSITIVE
5	<i>E. coli</i> (100)	10 <sup>6</sup> <i>P. mirabilis</i>	Yellow	POSITIVE
6	<i>E. coli</i> (1000)	10 <sup>6</sup> <i>P. mirabilis</i>	Yellow	POSITIVE
7	<i>E. coli</i> (10)	10 <sup>6</sup> <i>Ps. aeruginosa</i>	Yellow	POSITIVE
8	<i>E. coli</i> (100)	10 <sup>6</sup> <i>Ps. aeruginosa</i>	Yellow	POSITIVE
9	<i>E. coli</i> (1000)	10 <sup>6</sup> <i>Ps. aeruginosa</i>	Yellow	POSITIVE
10	<i>E. coli</i> (10)	10 <sup>6</sup> <i>S. typhimurium</i>	Yellow	POSITIVE
11	<i>E. coli</i> (100)	10 <sup>6</sup> <i>S. typhimurium</i>	Yellow	POSITIVE
12	<i>E. coli</i> (1000)	10 <sup>6</sup> <i>S. typhimurium</i>	Yellow	POSITIVE
13	<i>E. coli</i> (10)	10 <sup>6</sup> each all 3	Yellow	POSITIVE
14	<i>E. coli</i> (100)	10 <sup>6</sup> each all 3	Yellow	POSITIVE
15	<i>E. coli</i> (1000)	10 <sup>6</sup> each all 3	Yellow	POSITIVE
16	-	10 <sup>6</sup> <i>Pr. mirabilis</i>	Green	NEGATIVE
17	-	10 <sup>6</sup> <i>Ps. aeruginosa</i>	Green	NEGATIVE
18	-	10 <sup>6</sup> <i>S. typhimurium</i>	Green	NEGATIVE
19	-	-	Green	NEGATIVE

Comments:

The PATH-CHEK Coliform units have detected *E. coli* down to a starting dose of ~10cfu/ml without any false positives seen with the contaminating organisms, or false negatives due to inhibition.

1.2.1.1 At this stage the ability of the unit to detect *E. coli* 0157 was confirmed as was the ability of the decontamination fluid to kill the strain.

Dilution	<i>E. coli</i> 0157			<i>E. coli</i>		
	Cfu/ml	Colour 6 hr.	Colour 18 hr.	Cfu/ml	Colour 6 hr.	Colour 18 hr.
Neat	NP	Yellow	yellow	NP	Yellow	Yellow
10 <sup>-1</sup>	NP	Yellow	Yellow	NP	Yellow	Yellow
10 <sup>-2</sup>	NP	Yellow	Yellow	NP	Yellow	Yellow
10 <sup>-3</sup>	NP	Yellow	Yellow	NP	Yellow	Yellow
10 <sup>-5</sup>	NP	Green	Yellow	NP	Green	Yellow
10 <sup>-6</sup>	Confluent	Green	Yellow	Confluent	Green	Yellow
10 <sup>-6</sup>	~400	Green	Yellow	~800	Green	Yellow
10 <sup>-7</sup>	48	Green	Yellow	95	Green	Yellow
10 <sup>-8</sup>	NP	Green	Yellow	NP	Green	Yellow
10 <sup>-9</sup>	NP	Green	Green	NP	Green	Green

NP is Not Performed. The results show that the units will support the growth of *E. coli* 0157. BHI plates incubated after the organisms had been exposed to decontamination fluid showed no growth after 18 hours indicating that they were no longer viable.

### 1.2.2 PATH-CHEK Salmonella

Tube	Target (cfu)	Contaminant (10 <sup>6</sup> )	24 hr. Colour	Result
1	<i>S. typhimurium</i> (10)	-	Black ppt	POSITIVE
2	<i>S. typhimurium</i> (100)	-	Black ppt	POSITIVE
3	<i>S. typhimurium</i> (1000)	-	Black ppt	POSITIVE
4	<i>S. typhimurium</i> (10)	<i>P. mirabilis</i>	Black ppt	POSITIVE
5	<i>S. typhimurium</i> (100)	<i>P. mirabilis</i>	Black ppt	POSITIVE
6	<i>S. typhimurium</i> (1000)	<i>P. mirabilis</i>	Black ppt	POSITIVE
7	<i>S. typhimurium</i> (10)	<i>Ps. aeruginosa</i>	Black ppt	POSITIVE
8	<i>S. typhimurium</i> (100)	<i>Ps. aeruginosa</i>	Black ppt	POSITIVE
9	<i>S. typhimurium</i> (1000)	<i>Ps. aeruginosa</i>	Black ppt	POSITIVE
10	<i>S. typhimurium</i> (10)	<i>E. coli</i>	Black ppt	POSITIVE
11	<i>S. typhimurium</i> (100)	<i>E. coli</i>	Black ppt	POSITIVE
12	<i>S. typhimurium</i> (1000)	<i>E. coli</i>	Black ppt	POSITIVE
13	<i>S. typhimurium</i> (10)	Each all 3	Black ppt	POSITIVE
14	<i>S. typhimurium</i> (100)	Each all 3	Black ppt	POSITIVE
15	<i>S. typhimurium</i> (1000)	Each all 3	Black ppt	POSITIVE
16	-	<i>P. mirabilis</i>	Blue Liquid	NEGATIVE
17	-	<i>Ps. aeruginosa</i>	Blue Liquid	NEGATIVE
18	-	<i>E. coli</i>	Blue Liquid	NEGATIVE
19	-	-	Blue Liquid	NEGATIVE

PATH-CHEK Salmonella detected *S. typhimurium* down to a starting dose of ~10 cfu/ml without any false positives seen from the contaminating organisms, or false negatives due to inhibition.

1.2.2.1 At this stage the ability to detect other common Salmonella strains was confirmed.

Tube	Target	24 hr. Colour	Result
1	~ 100 <i>S. typhimurium</i> RD390	Black ppt	POSITIVE
2	~ 100 <i>S. enteritidis</i> RD392	Black ppt	POSITIVE
3	~100 <i>S. dublin</i> C445	Black ppt	POSITIVE
4	~ 100 <i>S. anatum</i> C443	Black ppt	POSITIVE
5	~ 100 <i>S. nottingham</i> C231	Black ppt	POSITIVE
6	~ 100 <i>S. poona</i> C461	Black ppt	POSITIVE
7	~ 100 <i>S. norton</i> C446	Black ppt	POSITIVE
8	~ 100 <i>S. typhimurium</i> C444	Black ppt	POSITIVE
9	-	Blue Liquid	NEGATIVE

PATH-CHEK Salmonella detected 100 cfu/ml of the eight isolates after 24 hrs at 37°C.

This work was further expanded in the next section with a new panel of organisms.

## 1.2.2.2

Confirmation of reactivity of a range of *Salmonella* spp in PATH-CHEK Salmonella

Microgen Code	Organism	Group	PATH-CHEK Salmonella	Biochem ID	Sal TEC Salmonella EIA	PATH-CHEK Coliform
C787	<i>Salmonella californica</i> Group B	B	Positive			
C788	<i>Salmonella coeln</i> Group B	B	Positive			
C790	<i>Salmonella derby</i> Group B	B	Positive			Negative
C809	<i>Salmonella heidelberg</i> Group B	B	Positive			
C810	<i>Salmonella heidelberg</i> Group B	B	Positive			
C815	<i>Salmonella kingston</i> Group B	B	Positive			
C848	<i>Salmonella kubacha</i> Group B	B	Positive			
C861	<i>Salmonella typhimurium</i> Group B	B	Positive			Negative
C863	<i>Salmonella typhimurium</i> Group B	B	Positive			
C864	<i>Salmonella typhimurium</i> Group B NCIMB 13034	B	Positive			
C780	<i>Salmonella albany</i> Group C	C	Positive			
C849	<i>Salmonella lille/rumforal</i> Group C1	C1	Positive			
C853	<i>Salmonella ohio</i> Group C1	C1	Positive			
C858	<i>Salmonella tennessee</i> Group C1	C1	Positive			
C859	<i>Salmonella thomson</i> Group C1	C1	Positive			
C865	<i>Salmonella virchow</i> Group C1	C1	Positive			
C806	<i>Salmonella glostrup</i> Group C2	C2	Positive			
C807	<i>Salmonella hadar</i> Group C2	C2	Positive			
C808	<i>Salmonella hadar</i> Group C2 NCIMB13033	C2	Positive			Negative
C797	<i>Salmonella dublin</i> Group D	D	Positive			
C792	<i>Salmonella dublin</i> Group D1	D1	Positive			
C793	<i>Salmonella dublin</i> Biotype C Group D1	D1	Positive			
C794	<i>Salmonella dublin</i> Biotype D Group D1	D1	Positive			
C795	<i>Salmonella dublin</i> Group D1	D1	Black Swab	<i>Salmonella</i> spp	Positive	Negative
C796	<i>Salmonella dublin</i> Group D1	D1	Positive			
C798	<i>Salmonella dublin</i> Biotype E Group D1	D1	Positive			

C799	Salmonella enteritidis Group D1	D1	Positive			Negative
C800	Salmonella enteritidis Group D1	D1	Positive			
C803	Salmonella enteritidis Group D1	D1	Positive			Negative
C804	Salmonella enteritidis Group D1	D1	Positive			
C850	Salmonella miami (Hope Hosp.) Group D1	D1	Positive			
C851	Salmonella ndolo Group D1	D1	Positive			
C854	Salmonella panama Group D1	D1	Positive			
C860	Salmonella tranoroa Group D1	D1	Positive	Salmonella spp	Positive	Negative
C782	Salmonella anatum Group E	E	Positive			
C783	Salmonella assinie Group E	E	Positive			
C786	Salmonella bolombo Group E1	E1	Positive			
C855	Salmonella rutgers Group E1	E1	Positive			Negative
C852	Salmonella newbrunswick Group E2	E2	Black Swab	Salmonella spp	Negative	Negative
C856	Salmonella senftenburg Group E4	E4	Positive			Negative
C857	Salmonella senftenburg Group E4	E4	Positive			
C789	Salmonella cubana Group G2	G2	Positive			
C805	Salmonella gaminara Group I	I	Positive	Salmonella spp	Negative	Negative
C811	Salmonella hvittingfoss Group I	I	Positive			
C781	Salmonella alandale Group R	R	Positive			
C785	Salmonella berkeley Group U	U	Positive			
C791	Salmonella deversoir Group W	W	Positive			
C784	Salmonella bergen Group X	X	Positive			
C812	Salmonella indiana Group B1????	B1	No Change	Citrobacter spp	Negative	Negative
C814	Salmonella karamoja Group R ?????	R	No Change	E. coli spp	Negative	Confirmed

From this study it was evident that some strains will produce a black precipitate on the swab with little or no colour change in the medium. Organisms exhibiting this reaction in PATH-CHEK all identified as Salmonella in a biochemical identification system and were negative in PATH-CHEK Coliform. Two of these isolates were also negative in the EIA but this is more likely to be a function of the EIA as the isolates were confirmed as Salmonella species. In the light of this finding the results colour chart will be changed to include this observation.

### 1.2.3 PATH-CHEK Staph

Tube	Target (cfu)	Contaminant (10 <sup>6</sup> )	24 hr. Colour	Result
1	<i>S. aureus</i> (10)	-	Yellow	POSITIVE
2	<i>S. aureus</i> (100)	-	Yellow	POSITIVE
3	<i>S. aureus</i> (1000)	-	Yellow	POSITIVE
4	<i>S. aureus</i> (10)	<i>S. epidermidis</i>	Yellow	POSITIVE
5	<i>S. aureus</i> (100)	<i>S. epidermidis</i>	Yellow	POSITIVE
6	<i>S. aureus</i> (1000)	<i>S. epidermidis</i>	Yellow	POSITIVE
7	<i>S. aureus</i> (10)	<i>B. subtilis</i>	Yellow	POSITIVE
8	<i>S. aureus</i> (100)	<i>B. subtilis</i>	Yellow	POSITIVE
9	<i>S. aureus</i> (1000)	<i>B. subtilis</i>	Yellow	POSITIVE
10	<i>S. aureus</i> (10)	<i>E. coli</i>	Yellow	POSITIVE
11	<i>S. aureus</i> (100)	<i>E. coli</i>	Yellow	POSITIVE
12	<i>S. aureus</i> (1000)	<i>E. coli</i>	Yellow	POSITIVE
13	<i>S. aureus</i> (10)	<i>Ps. aeruginosa</i>	Yellow	POSITIVE
14	<i>S. aureus</i> (100)	<i>Ps. aeruginosa</i>	Yellow	POSITIVE
15	<i>S. aureus</i> (1000)	<i>Ps. aeruginosa</i>	Yellow	POSITIVE
16	<i>S. aureus</i> (10)	Bac, Ps, <i>E. coli</i>	Yellow	POSITIVE
17	<i>S. aureus</i> (100)	Bac, Ps, <i>E. coli</i>	Yellow	POSITIVE
18	<i>S. aureus</i> (1000)	Bac, Ps, <i>E. coli</i>	Yellow	POSITIVE
19		<i>E. coli</i>	Red	NEGATIVE
20		<i>Ps. aeruginosa</i>	Red	NEGATIVE
21		<i>B. subtilis</i>	Yellow	POSITIVE
22		<i>S. epidermidis</i>	Yellow	POSITIVE
23		-	Red	NEGATIVE

The PATH-CHEK Staph units have detected *Staph. aureus* at 10 cfu/ml and also the *Staph. epidermidis*. The *B. subtilis* has given a positive result at 10<sup>6</sup> cfu/ml but the other two contaminant organisms gave negative results.

#### 1.2.4 PATH-CHEK Vibrio

Tube	Target (cfu)	Contaminant (10 <sup>6</sup> )	24 hr. Colour	Result
1	<i>V. parahaemolyticus</i> (10)	-	Purple	NEGATIVE
2	<i>V. parahaemolyticus</i> (100)	-	Yellow	POSITIVE
3	<i>V. parahaemolyticus</i> (1000)	-	Yellow	POSITIVE
4	<i>V. parahaemolyticus</i> (10)	<i>E. coli</i>	Yellow	POSITIVE
5	<i>V. parahaemolyticus</i> (100)	<i>E. coli</i>	Yellow	POSITIVE
6	<i>V. parahaemolyticus</i> (1000)	<i>E. coli</i>	Yellow	POSITIVE
7	<i>V. parahaemolyticus</i> (10)	<i>S. typhimurium</i>	Purple	NEGATIVE
8	<i>V. parahaemolyticus</i> (100)	<i>S. typhimurium</i>	Yellow	POSITIVE
9	<i>V. parahaemolyticus</i> (1000)	<i>S. typhimurium</i>	Yellow	POSITIVE
10	<i>V. parahaemolyticus</i> (10)	<i>Ps. aeruginosa</i>	Purple	NEGATIVE
11	<i>V. parahaemolyticus</i> (100)	<i>Ps. aeruginosa</i>	Yellow	POSITIVE
12	<i>V. parahaemolyticus</i> (1000)	<i>Ps. aeruginosa</i>	Yellow	POSITIVE
13	<i>V. parahaemolyticus</i> (10)	Each all 3	Yellow	POSITIVE
14	<i>V. parahaemolyticus</i> (100)	Each all 3	Yellow	POSITIVE
15	<i>V. parahaemolyticus</i> (1000)	Each all 3	Yellow	POSITIVE
16	-	<i>E. coli</i>	Purple	NEGATIVE
17	-	<i>S. typhimurium</i>	Purple	NEGATIVE
18	-	<i>Ps. aeruginosa</i>	Purple	NEGATIVE
19	-	-	Purple	NEGATIVE

PATH-CHEK Vibrio units detected 100 cfu/ml *V. parahaemolyticus* without any false positives seen due to the contaminating organisms, or false negatives due to inhibition.

The foregoing results indicated that the units were capable of detecting the target organisms in pure culture and in the presence of interfering organisms with acceptable sensitivity and specificity.

## Section 2

The ability of the decontamination fluid contained within the PATH-CHEK units was investigated for its ability to inactivate the target organism and three other common bacteria although they have been shown not to grow in any of the PATH-CHEK units.

### 2.0 Decontamination Study

PATH-CHEK units were inoculated with a number of different bacteria, incubated for 24 hours and the fluid released into the unit. At various times the tubes were sampled and plated onto BHI plates and inoculated into BHI broth.

#### 2.1 BHI Plates

	Time in contact with Decontamination Liquid				
	0 min	5 min	1 hr.	3 hr.	6 hr
<i>E. coli</i>	2 x 10 <sup>8</sup>	0	0	0	0
<i>S. typhimurium</i>	3.2 x 10 <sup>8</sup>	0	0	0	0
<i>S. aureus</i>	1.8 x 10 <sup>8</sup>	0	0	0	0
<i>V. parahaemolyticus</i>	1.2 x 10 <sup>8</sup>	0	0	0	0
<i>P. mirabilis</i>	2.4 x 10 <sup>8</sup>	0	0	0	0
<i>Ps. aeruginosa</i>	3.25 x 10 <sup>8</sup>	0	0	0	0
<i>B. subtilis</i>	1 x 10 <sup>8</sup>	+	0	0	0

#### 2.2 BHI Broth

	Time in contact with Decontamination Liquid				
	0 min	5 min	1hr.	3 hr.	6 hr
<i>E. coli</i>	Growth	No Growth	No Growth	No Growth	No Growth
<i>S. typhimurium</i>	Growth	No Growth	No Growth	No Growth	No Growth
<i>S. aureus</i>	Growth	No Growth	No Growth	No Growth	No Growth
<i>V. parahaemolyticus</i>	Growth	No Growth	No Growth	No Growth	No Growth
<i>P. mirabilis</i>	Growth	No Growth	No Growth	No Growth	No Growth
<i>Ps. aeruginosa</i>	Growth	No Growth	No Growth	No Growth	No Growth
<i>B. subtilis</i>	Growth	No Growth	No Growth	No Growth	No Growth

The results show that all organisms with the exception of *B. subtilis* are prevented from growing after 5 minutes in contact with the decontamination fluid. *B. subtilis* failed to grow after 1 hour contact. The BHI broths showed no growth for any of the organisms after 5 minutes exposure.

## Section 3

This section deals with the ability of the units to detect the target bacteria on a standard stainless steel surface.

### 3.1 Coliform Unit

Test Condition	Organism	Detection Level
Direct Swab Inoculation	<i>E. coli</i> ATCC 25922	<1 cfu/μl
	<i>E. coli</i> (environmental)	<1 cfu/μl
	<i>Enterobacter cloacae</i>	<1 cfu/μl
Wet Surface	<i>E. coli</i> ATCC 25922	<1 cfu/cm <sup>2</sup>
	<i>E. coli</i> (environmental)	<1 cfu/cm <sup>2</sup>
	<i>Enterobacter cloacae</i>	<1 cfu/cm <sup>2</sup>
Dry Surface	<i>E. coli</i> ATCC 25922	<130 cfu/cm <sup>2</sup>
	<i>E. coli</i> (environmental)	13 cfu/cm <sup>2</sup>
	<i>Enterobacter cloacae</i>	13 cfu/cm <sup>2</sup>

The minimum detection limit is generally better than, or as good as, current minimum limits for general-purpose hygiene swabbing.

From wet surfaces, PATH-CHEK is easily capable of detecting the residual level of Coliforms following disinfection i.e. <2.5 cfu/cm<sup>2</sup> can be readily detected in less than 24 hours.

It is advisable to use a wetting/neutralising agent to wet the swab before sampling a surface.

### 3.2 Salmonella Unit

Limits of Detection

	Direct Swab inoculation	Recovery from Inoculated Wet Surfaces	Recovery from Inoculated Dry Surfaces
<i>S. enteritidis</i> (culti-loop)	>10 cfu/μl 10 cfu/μl	21 cfu/cm <sup>2</sup> <1 cfu/cm <sup>2</sup>	>21 x 10 <sup>5</sup> cfu/cm <sup>2</sup> >21 x 10 <sup>5</sup> cfu/cm <sup>2</sup>
<i>S. typhimurium</i> (culti-loop)	<1 cfu/μl <1 cfu/μl	2 cfu/cm <sup>2</sup> <1 cfu/cm <sup>2</sup>	2.0 x 10 <sup>4</sup> cfu/cm <sup>2</sup> 2.0 x 10 <sup>3</sup> cfu/cm <sup>2</sup>
<i>Salmonella</i> (66) Environmental isolate	2 cfu/μl <1 cfu/μl	<1 cfu/cm <sup>2</sup> <1 cfu/cm <sup>2</sup>	1.4 x 10 <sup>5</sup> cfu/cm <sup>2</sup> 1.4 x 10 <sup>5</sup> cfu/cm <sup>2</sup>
<i>Salmonella</i> (121) Environmental isolate	10 cfu/μl <1 cfu/μl	<1 cfu/cm <sup>2</sup> <1 cfu/cm <sup>2</sup>	2.3 x 10 <sup>4</sup> cfu/cm <sup>2</sup> 2.3 x 10 <sup>3</sup> cfu/cm <sup>2</sup>
<i>E. coli</i> Environmental isolate	>3 x 10 <sup>6</sup> cfu/μl >3 x 10 <sup>6</sup> cfu/μl	>8 x 10 <sup>4</sup> cfu/cm <sup>2</sup> >8 x 10 <sup>4</sup> cfu/cm <sup>2</sup>	>8 x 10 <sup>4</sup> cfu/cm <sup>2</sup> >8 x 10 <sup>4</sup> cfu/cm <sup>2</sup>

**18 hours incubation**

24 hours incubation

Pick up from Surfaces as a Percentage of Viable Remaining Organisms

	% Pick Up	
	Wet Swab	Dry Swab
Wet Surface	<b>72%, 45%</b> 66%, 59%	<b>85%, 78%</b> 72%, 62%
Dry Surface	<b>95%, 84%</b> 77%, 81%	N/A

- 1) The limits of detection varied with incubation time. Incubating for the maximum recommended 24 hours rather than 18 hours gave improved limits of detection. Nevertheless an indication of heavier Salmonella surface contamination was obtained within 18 hours. Repeatability of tests was good.
- 2) Limits of detection varied with strain of Salmonella with *S. enteritidis* being the most difficult to recover. Whilst results were repeatable with the strain used there are many strains of *S. enteritidis* and it is not known if the results are consistent for other strains. It must also be remembered that the strain used was a type culture rather than an environmental isolate. The latter typically would be easier to recover as a result of stress response.
- 3) Included as a control, no surface inoculation of the *E. coli* environmental isolate gave a positive result. This is indicated in the table as detection limits greater than the heaviest inoculation used in the particular experiment.
- 4) Compared to the number of viable organisms in the original inoculation, recovery was greatest from a wet surface.
- 5) Recovery was statistically significantly greater from a wet surface with a heavy inoculation if the swab was dry, compared to a moistened swab. For a lower inoculation the difference was not significant. Moistening agent was essential if the surface to be swabbed was dry.
- 6) No surface specifications for Salmonella are available although any salmonella on a cleaned surface would be regarded as undesirable and a food safety threat. Specifications for general microbial levels on cleaned surfaces recommend >2.5 cfu/cm<sup>2</sup> and the Salmonella PATH-CHEK was capable of determining this for all Salmonella from wet surfaces at 24 hours. It was not capable of detecting this level on dry surfaces but Salmonella recovered from dry surfaces grew well in the PATH-CHEK culture medium..
- 7) The indications are that the minimum detection limits are generally as good as conventional Salmonella surface swab testing using enrichment techniques requiring 5 days.

The above Salmonella data was extracted from an independent report commissioned from the University of Wales Institute, Cardiff.