



## Rapid Testing Methods for Food Pathogens

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### What is foodborne illness?



- Foodborne illness occurs when a person consumes food contaminated with pathogenic bacteria, viruses or parasites.
- This condition is often called “food poisoning”.
- Many cases of foodborne illness go unreported because their symptoms often resemble flu symptoms.
- The most common symptoms of foodborne illness may include stomach cramps, nausea, vomiting, diarrhea and fever.

## Rapid Testing

### Food Safety



**Number of recalls of foods + cosmetics in the US (10/93 - 9/98)  
due to microbial contamination:** (Wong et al. J. Food Prot. 63, 2000)

**Total: 1,330 cases**

Product type:	# of incidents:	Microorganism detected:	# of cases:
Milk	304	<i>L. monocytogenes</i>	813
Seafoods	157	<i>Salmonella spp.</i>	143
Bakery	147	<i>Staphylococcus aureus</i>	69
Salad	126	<i>C. botulinum</i>	38
Sandwich	124	Coliforms (not <i>E. coli</i> O157)	36
Dip/Sauce	108	<i>E. coli</i> O157:H7	16
Beverage	94	Pseudomonas (in cosmetics)	25
Vegetable	81	Viruses (Hep.A, Norwalk-like)	94
Cosmetics	56	Mold/Yeast/ Fungi	96

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## Rapid Testing

### Food Pathogens



**Estimated Numbers of Infections Caused by Food Pathogens in USA**

	Total No. Cases	No. Deaths
Campylobacter	2500.000	99
Salmonella	1400.000	553
Shigella	450.000	14
<i>Clostridium perfringens</i>	250.000	7
<i>Staphylococcus aureus</i>	185.000	2
<i>E. coli</i> O157:H7	96.000	52
<i>Yersinia enterocolitica</i>	73.000	2
<i>Bacillus cereus</i>	51.000	0
Vibrio	8.000	31
<i>Listeria monocytogenes</i>	2.500	499

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# Rapid Testing

## Food Pathogens



### 1. Screening of organisms (especially pathogens)

- Culture Methods (non selective, selective broths/agars; chrom. broths/agars)
- Immunoassays (ELISA, Lateral Flows, Dot Blots)
- Molecular methods (PCR, Hybridization methods [GENE TRAK])

***Positive findings are presumptive and must be confirmed in general***

### 2. Confirmation of organisms

- Culture, biochemical test (substrate use, API)
- Immunoassays (Latex, ELISA, Lateral Flows)
- Molecular methods (PCR, Ribotyping, Pulsefield-Gel-Electrophoreses, Hybridization methods [Accuprobe])

# Rapid Testing



- Singlepath<sup>®</sup> Salmonella
- Singlepath<sup>®</sup> E. coli O157
- Duopath<sup>®</sup> Verotoxins
- Singlepath<sup>®</sup> Campylobacter

## Rapid Testing



- Singlepath<sup>®</sup> Listeria
- Singlepath L'mono
- Duopath<sup>®</sup> Cereus Enterotoxins
- Duopath<sup>®</sup> Legionella

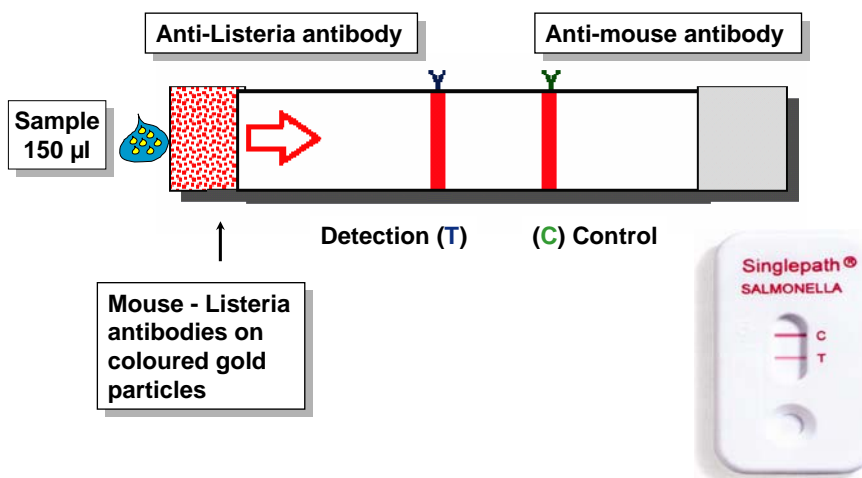
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## Rapid Testing

### Principle of Lateral Flow Tests



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# Rapid Testing

## Pre-Conditions



- All rapid testing methods are fully dependent on the quality of the corresponding non/selective enrichment media
- If an enrichment medium does not allow growth of a minimum of  $10^5$  cfu / ml a false negative result will occur !



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# General Application of Lateral Flow Tests



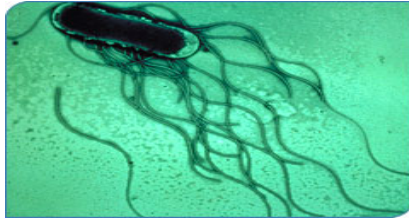
<b>1 ADD MEDIUM</b>	<b>2 ADD SAMPLE</b>	<b>3 BLENDING</b>	<b>4 INCUBATE</b>	<b>5 PIPETTE COMPLETE</b>
<b>6 PLACE</b>	<b>Test device</b>	<b>NEGATIVE</b>	<b>POSITIVE</b>	
				Read after 20 min

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## Rapid Testing Singlepath® Salmonella



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## Established Pathogen: *Salmonella*



- Leading cause of foodborne bacterial diseases in many countries
- ~2500 Serovars, divided in 2 species:
  - S. enterica* - non-typhoid serovars
  - S. bongori* - typhoid serovars
- high environmental resistance (can survive 73 days at 2°C or at pH 3.5)
- Infections result from contaminated meat (poultry!!!), eggs, milk products (cheese, cream, ice cream, etc.), spices, etc.

### Major outbreaks of Salmonellosis:

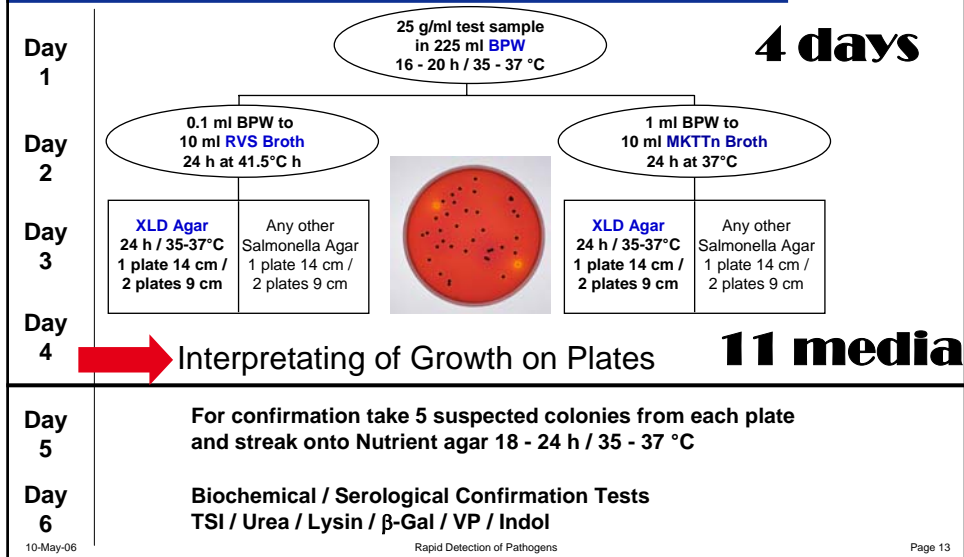
Year	Country	Food	Serovar	No. Infection	No. Deaths
1984	Canada	Cheddar Cheese	<i>S. typhimurium</i>	2,700	0
1985	USA	Pasteur. Milk	<i>S. typhimurium</i>	16,284	11
1988	Japan	Cooked eggs	<i>Salmonella spp.</i>	10,476	N.S.
1993	Germany	Paprika Chips	<i>S. saint-paul</i>	1,000	0

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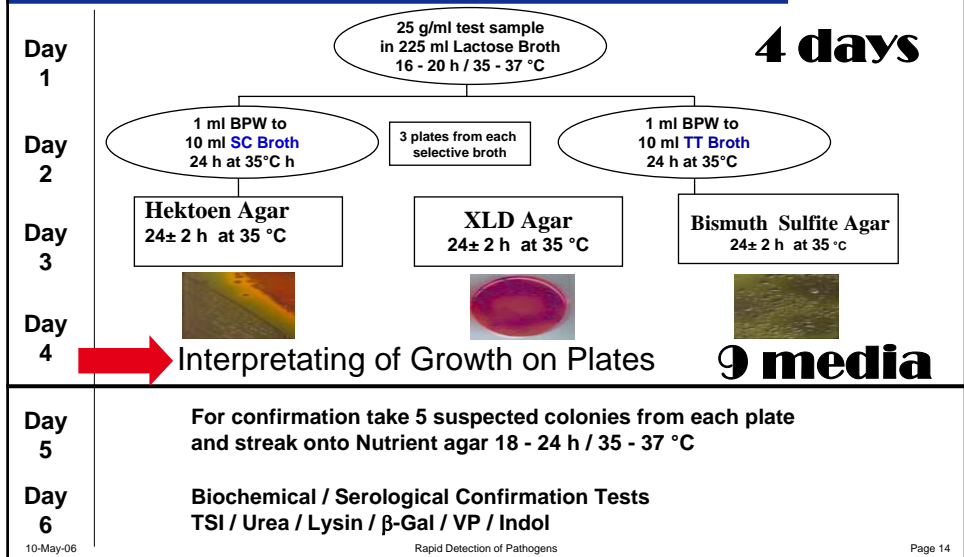
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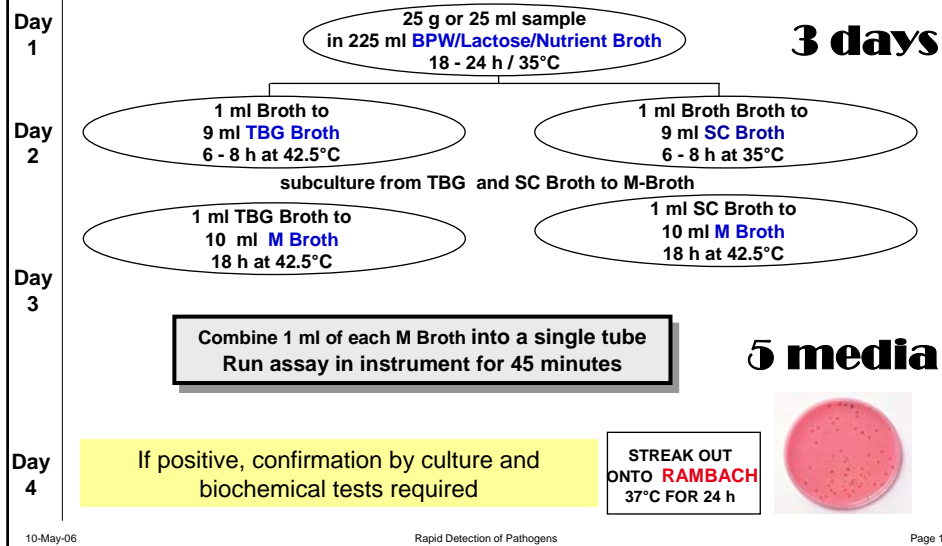
# ISO Standard 6579 for Detection of Salmonella



# FDA-BAM Standard for Detection of Salmonella



## Automated Salmonella Testing Protocol for Processed Foods



## Rapid Testing Singlepath® Salmonella



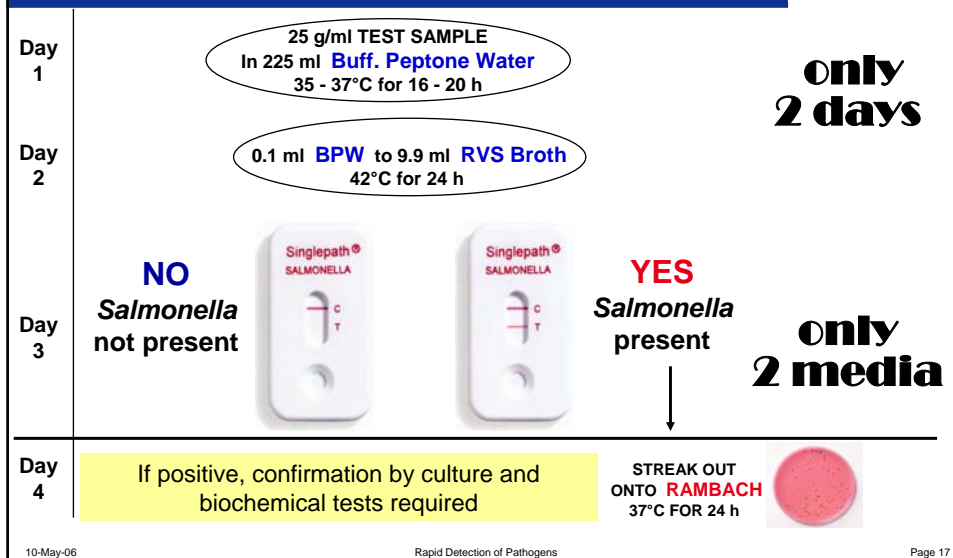
### Singlepath® Salmonella

- For detection of *Salmonella* spp. in foods directly after 2-step enrichment
- No post-enrichment step needed:  
**sample directly taken from RVS**  
**+ 15 min heating,**  
**transfer to sample port**  
**read results within 20 min**
- Advantage over competitor products:  
**Detection of multiple *Salmonella* serological groups**  
 Lower detection limit: **10<sup>5</sup> cfu / ml**



## Rapid Testing

### Singlepath® Salmonella - Screening



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## Rapid Testing

### Singlepath® Salmonella



#### Evaluation of Singlepath® Salmonella:

- Evaluated with 105 *Salmonella* and 58 non-*Salmonella* species
  - skimmed milk powder
  - black pepper
  - pet food
  - seafood
  - ground beef
  - ground poultry
- Sensitivity and Specificity each >98%
- AOAC approval granted October 2004



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## Rapid Testing

### Singlepath® Salmonella



#### Evaluation at University of Giessen: Comparison with CELSIS Path Stik *Salmonella*

- Tested with spiked + native ground meat, poultry, milk powder
- Both tests have 98 % Sensitivity and Specificity (n = 50 food samples)
- Significant stronger bands than with PATHSTIK

**Singlepath showed better Detection Limit ( $10^5$  CFU / ml)**

#### Evaluation at Agriquality (NZ): Comparison with TECRA ELISA *Salmonella*

- Tested with roasted chicken, grated cheddar cheese, whole milk powder, non-fat milk powder, environmental swabs (n = 279 food samples)
- Both assays give 100 % identical results
- Both assays with >99 % Sensitivity / Specificity

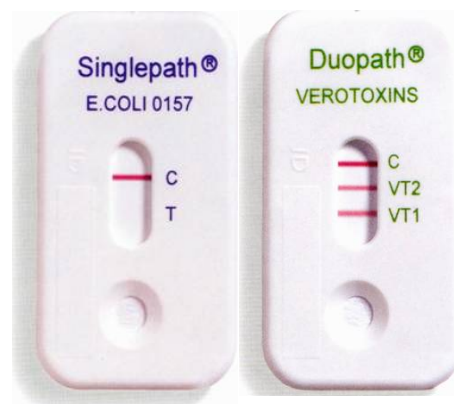
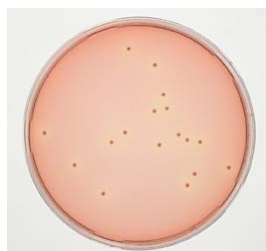
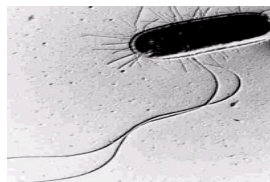
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## Rapid Testing

### Singlepath® E. coli O157 / Duopath® Verotoxins



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## Rapid Testing

### Singlepath® *E. coli* O157 / Duopath® Verotoxins



#### *E. coli* O157 characteristics

- There are a variety of *Escherichia coli* bacteria present in nature. They are usually found in the intestines of healthy humans and healthy animals. Even though these bacteria offer beneficial properties, there are some variations, or strains, that are pathogenic (have the ability to cause disease).
- *Escherichia coli* O157:H7 is one particular strain that is "... an emerging cause of foodborne illness ..." Symptoms such as bloody diarrhea and abdominal cramps may be observed or no such symptoms may appear
- The elderly and children under five years old are highly susceptible to Hemolytic Uremic Syndrome, a disease in which red blood cells are destroyed and kidneys fail (Kendrick & Wrobel-Woerner, 1997).

## Rapid Testing

### Singlepath® *E. coli* O157 / Duopath® Verotoxins



#### Outbreaks and implicated foods

- The most frequently known outbreaks of *E. coli* O157:H7 are associated with fecal contaminated meats in slaughterhouses. However, it can also be associated with:
- water
- raw milk
- unpasteurized apple juice/cider
- sandwiches
- lettuce
- dry cured salami
- produce from manure-fertilized gardens
- handling potatoes
- radish sprouts, alfalfa sprouts
- yogurt
- undercooked beef, poultry, venison, lamb (Buchanan & Doyle, 1997)



## ISO and FDA-BAM Standard for testing of *E. coli* O157 in Food

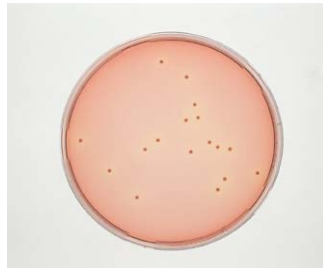


Day 1

25 g/ml TEST SAMPLE  
225 ml m-EC or m-TSB / EEB  
37°C for 24 h

Day 2

Day 3



CT - SMAC Agar

Sorbitol (+)  
are non  
pathogenic  
*E. coli*



37°C / 24 h Fluorocult *E. coli* 0157 Agar

Day 4

Confirmation by culture and biochemical tests required

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## Rapid Testing

### Singlepath® *E. coli* O157



### Singlepath *E. coli* O157

- For the detection of *E. coli* O157 in foods as well as confirmation.
- Singlepath detects *E. coli* O157:H7 + *E. coli* O157:non-H7.
- Low detection limit: 10<sup>5</sup> cfu / ml.




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## Rapid Testing

### Singlepath® E. coli O157 - Screening




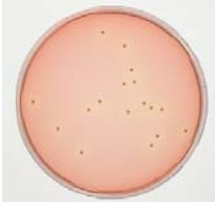

Day 1	<p>25 g/ml TEST SAMPLE in 225 ml mEC+N or mTSB+N 37- 42°C for 24 h</p>		<b>only 1 day</b>
Day 2	<p><b>NO</b> <i>E. coli</i> O157 not present</p>		<p><b>YES</b> <i>E. coli</i> O157 present</p> <p><b>only 1 medium</b></p>
Day 3	<p>If positive, confirmation by culture and biochemical tests required</p>		<p><b>STREAK OUT ON CT-SMAC Agar 37°C FOR 24 h</b></p> 

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## Rapid Testing

### Singlepath® E. coli O157 - Confirmation



Day 1	<p>Suspend 1-5 presumptive colonies in 250 µl PBS, pipette 150 µl into Singlepath®</p>		 <p>SMAC, CT-SMAC Agar</p>
	<p><b>NO</b> <i>E. coli</i> O157 not present</p>		<p><b>YES</b> <i>E. coli</i> O157 present</p>

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## Rapid Testing

### Singlepath® *E. coli* O157 - Evaluation



- **Prof. Bülte / University Giessen, Germany**

	[in %]	Ground Beef (n = 158)		Raw Milk (n = 30)	
		Sens.	Spec.	Sens.	Spec.
Merck		96	98	100	100
Competitor		97	88	100	100

Singlepath® shows 10% less false-positives than competitor  
Singlepath® is well suited for routine testing for *E. coli* O157

- **AOAC certification (granted 06/ 2004)**

Test with 55 *E. coli* O157 and 30 non-*E. coli* O157 strains  
Test with ground beef and whole milk samples  
Stability- and test for ruggedness, batch variations

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## Rapid Testing

### Duopath® Verotoxins



### Verotoxins

- Amongst the *E. coli* human pathogens, verotoxin (Shiga like toxin) - forming strains (VTEC) have risen in importance in recent years.
- The group of enterohaemorrhagic *E. coli* (EHEC) with its highly pathogenic serovars O157:H7, O26, O103, O111, O145, and other strains are particularly interesting in this respect.
- **Production of verotoxins is the most common criteria for the detection of this group of bacteria.**
- Verotoxins can be classified in the main category verotoxin 1 (VT1, SLT1, Stx1) and 2 (VT2, SLT2, Stx2).
- EHEC strains are able to produce either VT1 or VT2 only or even both VT1 and VT2 simultaneously.

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# Rapid Testing

## Duopath® Verotoxins



### Duopath® Verotoxins

- Only available Lateral Flow Test for Verotoxins
- Confirmation test for the detection of Verotoxins (Shiga-like Toxins) 1 and 2 from pathogenic *E.coli* isolates (VTEC's or STEC's)
- 6 h enrichment in CAYE Broth required
- No centrifugation step
- No boiling
- 10 min. treatment with Polymyxin recommended to free cell-associated Verotoxins
- Low detection limit for toxins:  $10^7$ - $10^8$  cfu/ml
- Approvals: AOAC-RI, CE, 510(k), IVD (Japan)



# Rapid Testing

## Duopath® Verotoxins - Screening



DAY  
1

25 g/ml Sample  
in 225 ml mEC+N or mTSB+N  
42°C for 18 - 24 h



DAY  
2

Transfer 50 µl in  
2 ml CAYE Broth + Carbadox  
37°C for 6 h

**NO**  
VTEC  
not detected



**YES**  
VTEC detected

**No more agar plating required!**

# Rapid Testing

## Duopath® Verotoxins - Confirmation



**Day 1**

CT / SMAC Agar

Suspend 1 – 5 colonies into CAYE Broth + Carbadox at 37°C for 6 h, Polymyxin 10 min

**or**

**Day 2**

Streak out on BHI Agar + Carbadox at 37°C for 24 h, suspend in 250µl PBS, Polymyxin 10 min

**NO**  
Verotoxin not present

**YES**  
Verotoxins detected and presence of VTEC confirmed !

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# Rapid Testing

## Duopath® Verotoxins - Evaluation



- **Prof. Bülte / University of Giessen, Germany**

Used 26 VT producing *E. coli* O157 as pure culture PLUS minced beef- + raw milk samples inoculated with 4 different *E. coli* O157 [1-192 cfu]

[in %]	VT 1 (n=139)		VT 2 (n= 114)	
	Sens.	Spec.	Sens.	Spec.
Duopath®	100	98,1	98,8	96,8

**Duopath® Verotoxins is very well suited as a simple routine test for the confirmation of VTEC's**

- **AOAC-RI Performance Test (granted 06/ 2004)**

50 VTEC's and 30 non-VTEC's tested for Verotoxin 1 and/or 2 production  
Spiked minced beef and milk samples tested

## Rapid Testing

### Duopath® Verotoxins - Comparison



	<u>Duopath®</u>	<u>RPLA Verotoxin</u>
Application:	confirmation of VTEC	confirmation of VTEC
Bacteria taken from:	SMAC, CT-SMAC Agar	SMAC, CT-SMAC Agar
Enrichment:	in CAYE for 6 h	in CAYE for 24 h
Availability of CAYE:	yes	no
Polymixin step:	yes (for 10 min)	no
Centrifugation steps:	none	yes
Reaction time of test:	20 min	24 h

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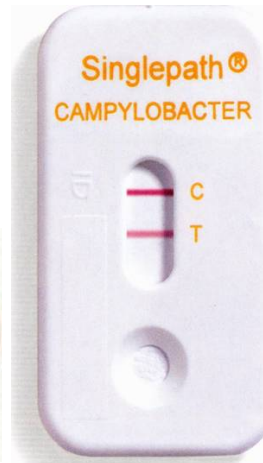
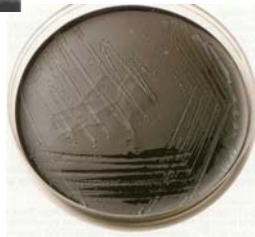
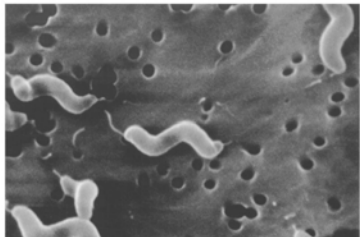
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**Immunological Rapid Method for Campylobacter**

## Rapid Testing Singlepath® Campylobacter



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## Rapid Testing Singlepath® Campylobacter



### ***Campylobacter spp. characteristics***

- Found in birds, wild and domestic animals
- One of the most common bacterial cause of food-borne diseases
- Grows under microaerophilic conditions (difficult to culture in a lab)

Problem: Many food labs are not able to detect *Campylobacter* in food samples because of a lack of knowledge of handling of these pathogens

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# Rapid Testing

## Singlepath® Campylobacter



### *Campylobacter spp. disease and implicated foods*

- Thermophilic *C. jejuni* and *C. coli* cause fever and gastroenteritis  
Duration: 2 - 4 days; incubation period: 1 - 7 days
- 1/1000 patients develop Guilian Barré Syndrome  
(Paralysis for weeks - months)
- Infections result from consumption of contaminated poultry, raw milk products

## Detection of Campylobacter in Food acc. to FDA-BAM and ISO



Day 1

**BOLTON Broth & Supplement**

25g/ml Test Sample in 225 ml BoltonBroth  
37°C for 4 h  
41,5°C for 44 h

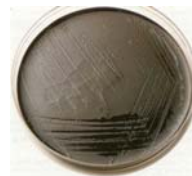


Day 2-3

Day 4-5

**CCDA Agar & Supplement**

Streak out onto CCDA, 37°C for 48 h



Day 6

If positive, confirmation required by culture and biochemical tests

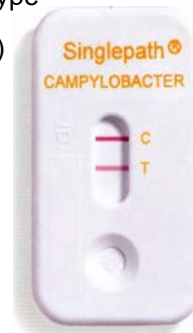
# Rapid Testing

## Singlepath® Campylobacter



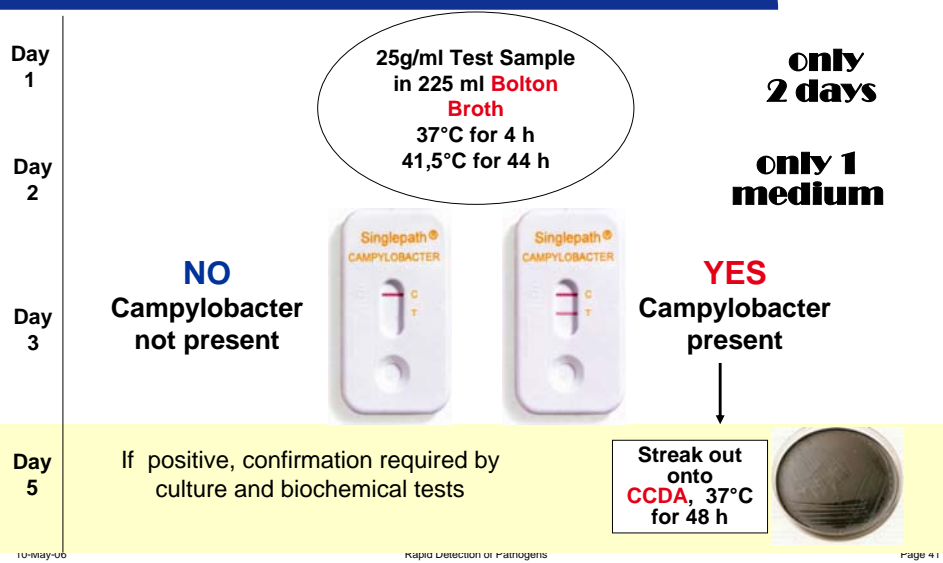
### Singelpath® Campylobacter

- Only Lateral Flow Test available worldwide
- Test detects thermophilic *Campylobacter jejuni* / *coli* from food
- Low detection limit: 10<sup>5</sup>-10<sup>6</sup> cfu/ml, depending on serotype
- Ready to use after enrichment in Bolton Broth (+ blood)
- Procedure after enrichment:
  - heat 1 ml of sample
  - transfer 150 µl
  - result within 20 min



# Rapid Testing

## Singlepath® Campylobacter



## Rapid Testing

### Singlepath® Campylobacter - Evaluation



- **INLAB, Dortmund, Germany**

Reference method: - Bolton Broth; comparison to VIDAS' Preston Broth  
- Culture / biochemical methods; VIDAS Campy

>60 native foods (poultry, intestins, raw milk) tested

55% of all tested poultry samples were positive for *Campylobacter jejuni* / coli

**Sensitivity: 98% Specificity: 100% Comparison to VIDAS: 100%**

**Test is well suited for the routine testing of *Campylobacter jejuni/coli***

- **AOAC-RI Performance Test (granted 12/ 2004)**

Testing with 50 *Campylobacter* and 30 non-*Campylobacter* strains

Testing with native minced beef from poultry and dairy samples

Stability- and ruggedness tests, batch variations

## Detection Methods for Listeria and *L. monocytogenes*



## Rapid Testing Singlepath® L' mono



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## Rapid Testing Singlepath® L' mono



### Listeria characteristics



- Genus *Listeria* comprises 6 different species
- Only *L. monocytogenes* can be pathogenic for humans
- All *Listeria* species serve as indicators of improper hygiene conditions

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## Listeria disease - Listeriosis

- Listeriosis is an invasive infection caused when contaminated food is ingested by susceptible individuals.
- Symptoms vary, ranging from flu-like symptoms to meningitis and encephalitis.
- Mortality rate for Listeriosis is 23%.
- The infection usually occurs sporadically.



## OUTBREAKS AND IMPLICATED FOODS

**Milk and milk products**

Soft cheese, raw milk,  
pasteurized milk,  
ice cream, fresh cream

**Meat and meat products**

Pate, turkey, frankfurters,  
raw/undercooked beef,  
chicken, hot dogs, pork sausages,  
tongues, rillettes

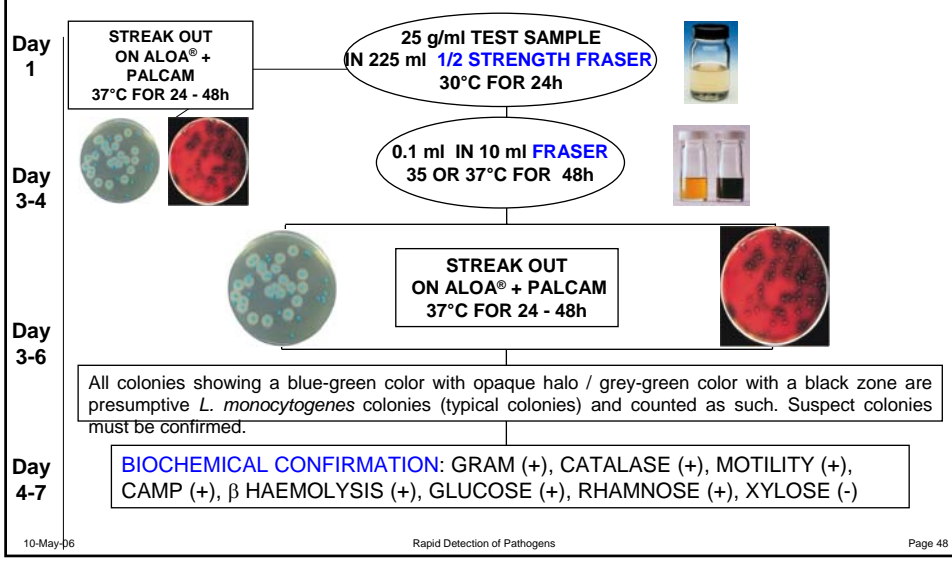
**Fish and fish products**

Fish and shell fish, shrimps,  
smoked cod roe

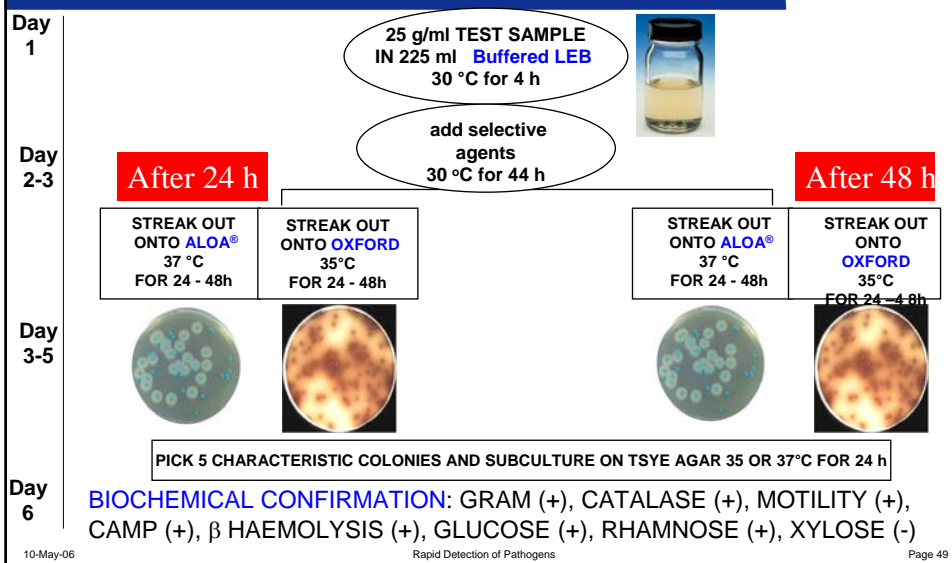
**Vegetable and  
vegetable products**

Vegetable rennet, coleslaw, vegetables,  
alfalfa, salted mushrooms

# ISO Standard 11290-2004 for testing of Listeria in Food and Animal feeds



# FDA-BAM Standard for the detection of Listeria



## IDF-FIL Standard for testing of Listeria in DAIRY / MILK Products

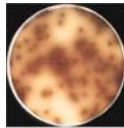


Day 1

25 g/ml TEST SAMPLE  
IN 225 ml **LEB**  
30 °C FOR 48h



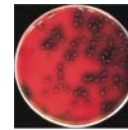
Day 2



STREAK OUT  
ONTO  
**OXFORD**  
30, 35 or 37 °C  
FOR 24 - 48h

OR

STREAK OUT  
ONTO  
**PALCAM**  
30, 35 or 37 °C  
FOR 24 - 48h



Day 3-5

PICK OFF 5 CHARACTERISTIC COLONIES AND SUBCULTURE ON TSYE AGAR 35 OR 37°C FOR 24 h

Day 6

**BIOCHEMICAL CONFIRMATION:** GRAM (+), CATALASE (+), MOTILITY (+), CAMP (+),  $\beta$  HAEMOLYSIS (+), GLUCOSE (+), RHAMNOSE (+), XYLOSE (-)

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## USDA Standard for testing of Listeria in Red Meat, Poultry, Eggs, Environmentals



Day 1

25 g/ml TEST SAMPLE  
IN 225 ml **UVM 1**  
30 °C FOR 24 h



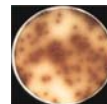
Day 2



0.1 ml to 10 ml to  
**UVM II or FRASER Broth**  
35 °C for 24 h

Day 3-5

STREAK OUT  
ONTO **OXFORD AGAR**  
35 °C FOR 24-48h



PICK OFF 5 -20 CHARACTERISTIC COLONIES AND SUBCULTURE ON HORSE BLOOD AGAR 35°C FOR 24 h

Day 6

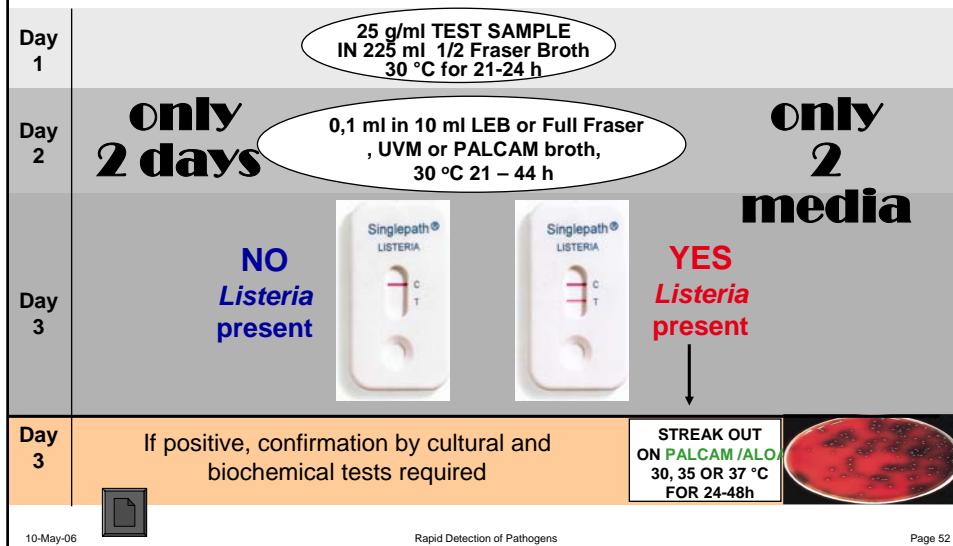
**BIOCHEMICAL CONFIRMATION:** GRAM (+), CATALASE (+), MOTILITY (+), CAMP (+),  $\beta$  HAEMOLYSIS (+), GLUCOSE (+), RHAMNOSE (+), XYLOSE (-)

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## Immunological Rapid Test Method using Singlepath® *Listeria*



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## Rapid Testing Singlepath® L' mono



### Singlepath® L' mono

- Worldwide **first** Lateral Flow Test for the SPECIFIC detection of *Listeria monocytogenes*
- For **screening** of environmental and food samples like milk products, smoked salmon, and liver pastry
- For the **confirmation** of presumptive positive colonies on *Listeria* selective agars like Chromocult® *Listeria* Agar (ALOA®) PALCAM
- Enrichment media used: Fraser, LEB, UVM



**Availability: 3. Quarter 2006**

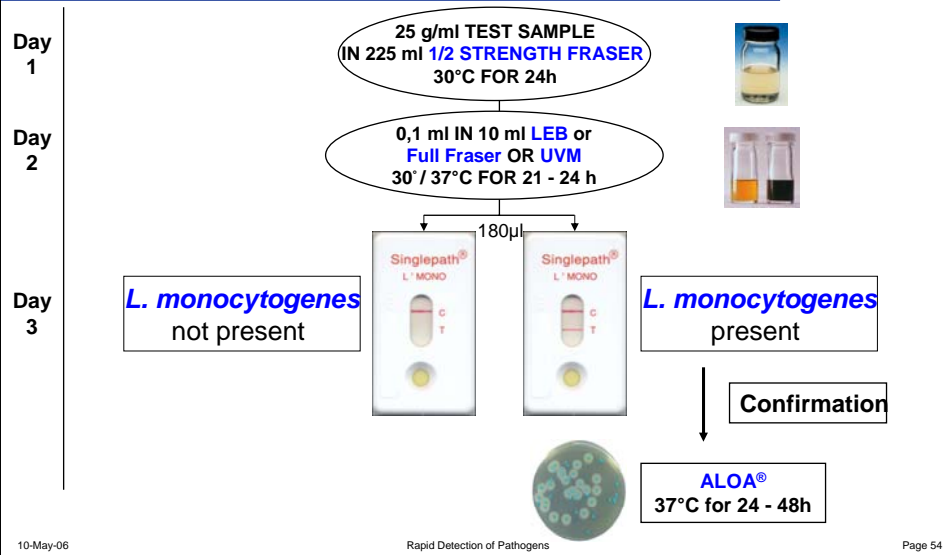
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Rapid Detection of Pathogens

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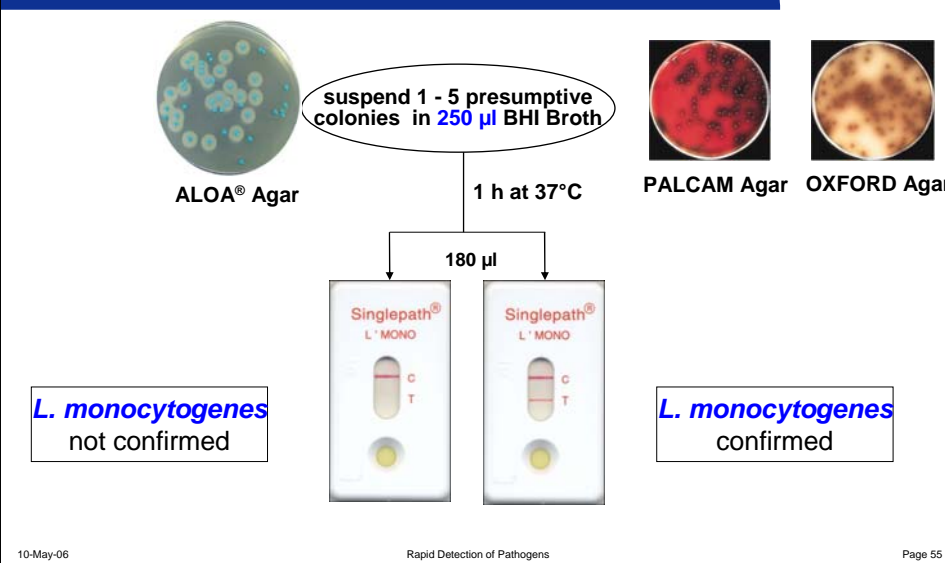
# Rapid Testing

## Singlepath® L' mono - Screening



# Rapid Testing

## Singlepath® L' mono - Confirmation



## Rapid Testing Singlepath® L' mono



### Evaluation of Singlepath® Listeria - Final commer

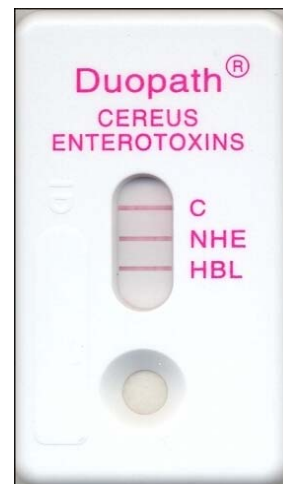
- Singlepath® has broader application spectrum for culture media (Fraser, LEB, UVM, PALCAM) than competitor (LEB only).
- Singlepath® was successfully tested by University of Giessen, Germany, with milk products, smoked salmon, and liver pate.
- **Singlepath® and competitor latex test are not recommended for the screening of minced beef samples (accompanying flora may inhibit growth of Listeria).**
- AOAC approval in progress.

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## Rapid Testing Duopath® Cereus Enterotoxins



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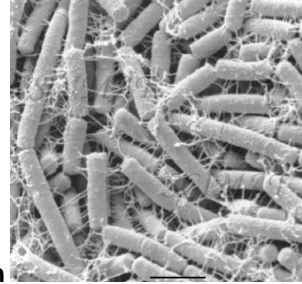
## Rapid Testing

### Duopath® *Cereus* Enterotoxins



#### *Bacillus cereus* Characteristics

- Gram-positive, spore-forming, motile rod
- Large cells 3 - 5 µm in length 1.0 - 1.2 µm
- Common soil saprophyte
- Aerobic, but grows well anaerobically
- The bacilli genus exhibit a wide range of physiological abilities that allow them to live in every natural environment.
- Produce toxins



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## Rapid Testing

### Duopath® *Cereus* Enterotoxins



#### ***B. cereus* group – Similar 16S and 23S rRNA**

- *B. anthracis* – Highly virulent, most distinctive
- *B. cereus* – Indistinguishable from *B. thuringiensis* except plasmids with *cry* genes
- *B. thuringiensis* – Crystalline inclusion protein (Cry protein)
- *B. mycoides*
- *B. pseudomycoides*
- *B. weihenstephanensis*



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## Bacillus cereus: Sources of infections



Meat products, soups, milk & milk products, vegetables, puddings & sauces

– for **diarrhoeal syndrome**



Rice, pasta, noodles, pastry, starchy products

– for **emetic syndrome**



## Rapid Testing

### Duopath<sup>®</sup> Cereus Enterotoxins



#### *Bacillus cereus* diarrhoeal syndrome

- Enterotoxins produced during vegetative growth of *B. cereus* in small intestine
- associated with ingestion of *B. cereus* producing heat-labile toxins (occurs within 8-12 hours)
- abdominal pain, watery diarrhoea, occasional nausea
- very similar to *C. perfringens* diarrhea, but less gas
- infective dose –  $10^5$  to  $10^8$  viable cells or spores
- all individuals susceptible
- recovery in 24 hours

## Rapid Testing

### Duopath® *Cereus* Enterotoxins



#### *Bacillus cereus* diarrhoeal syndrome

#### Implicated foods

- Meat and meat products
- Soups
- Milk and milk products
- Vegetable and vegetable products
- Pudding and sauces



## Rapid Testing

### Duopath® *Cereus* Enterotoxins



#### Enterotoxins produced by *B. cereus*

- Hemolysin BL (Hbl)\*
  - Nonhemolytic enterotoxin (Nhe)\*
  - Cytotoxin K (CytK)\*
- } Major toxins
- Enterotoxin T (BceT)
  - Enterotoxin FM (EntFM)

# Rapid Testing

## Duopath® Cereus Enterotoxins



### Duopath® Cereus Enterotoxins

- Worldwide first Lateral Flow Test for the SPECIFIC detection of *Bacillus cereus* enterotoxins
- For **screening** of foods
- For **confirmation** of enterotoxin-producing presumptive positive *B. cereus* colonies on M.Y.P. Agar
- CGY Broth (+ 1% Glucose) for enhancement of toxin production
- ELISA test: ONLY detects NHE toxin  
RPLA test: ONLY detects HBL toxin
- 90% of *B. cereus* are NHE toxin positive;
- 50% of *B. cereus* are HBL toxin positive



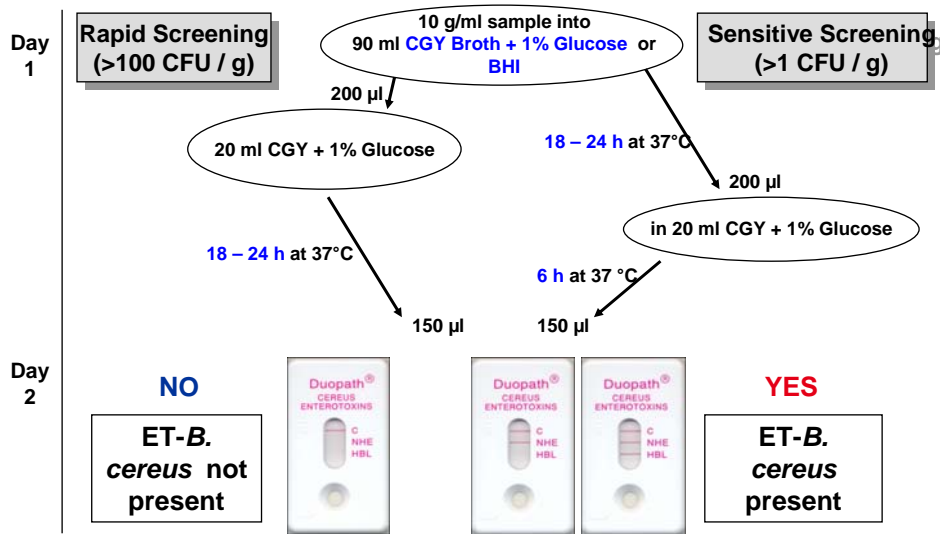
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# Rapid Testing

## Duopath® Cereus Enterotoxins - Screening



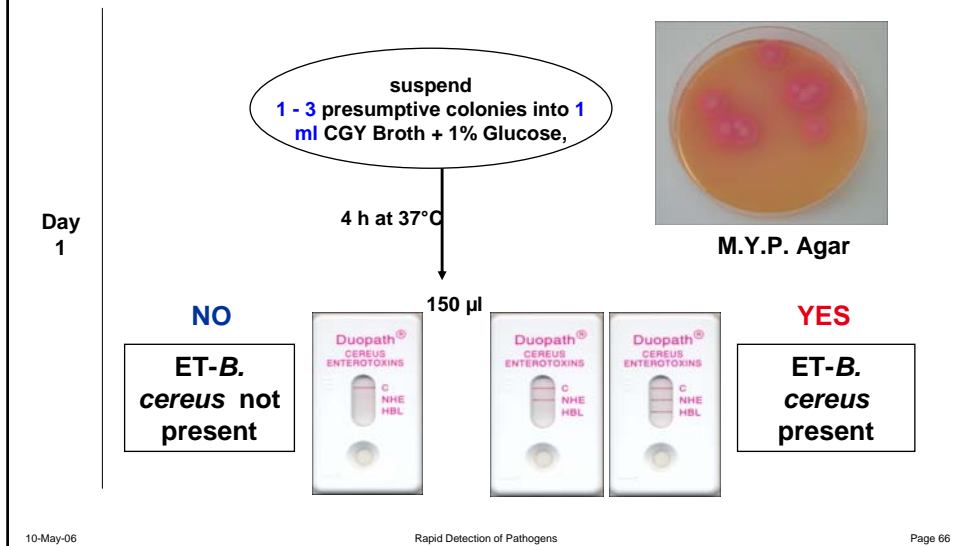
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## Rapid Testing

### Duopath® Cereus Enterotoxins - Confirmation



## Rapid Testing

### Duopath® Cereus Enterotoxins



#### Evaluation of Duopath® Bacillus Cereus

- Participation in ring trial organised by Norwegian accreditation organisation, Norskmatanalyse
- Food samples tested: **Semolina pudding,**  
**Frozen spinach,**  
**Coffee whitener,**  
**Egg powder,**  
**Cocoa powder**



**Duopath® Cereus Enterotoxins always correctly detected  
*B. cereus* in ALL food samples**

## Rapid Testing Duopath® Legionella



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## Rapid Testing Duopath® Legionella



### ***Legionella* spp. characteristics**

48 species and over 70 serogroups.

Approximately half of these species have been implicated in human disease.

*L. pneumophila* is responsible for  $\approx$  90% of infections.

Most cases are caused by *L. pneumophila*, serogroup 1.

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## Rapid Testing

### Duopath® Legionella



### Natural habitat

Legionella are frequently found in aquatic environments and some species have been recovered from soil.

Temperature is a critical determinant for Legionella proliferation.

Colonization of hot water tanks is more likely if tank temperatures are between 40 and 50°C.

## Rapid Testing

### Duopath® Legionella



### Legionnaires' disease

Legionnaires' disease is a lung infection caused by *L. pneumophila*.

The first outbreak of the disease was at the 1976 American Legion Convention in Philadelphia – from which *L. pneumophila* gets its name.

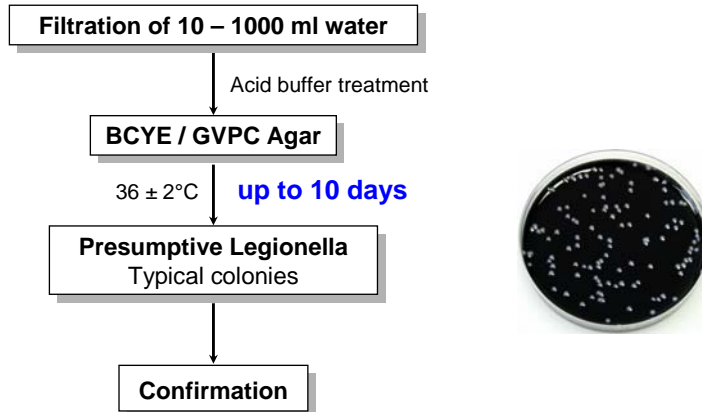
# Rapid Testing

## Duopath® Legionella



### Detection and enumeration of Legionella

#### Direct membrane filtration method (ISO 11731-2)



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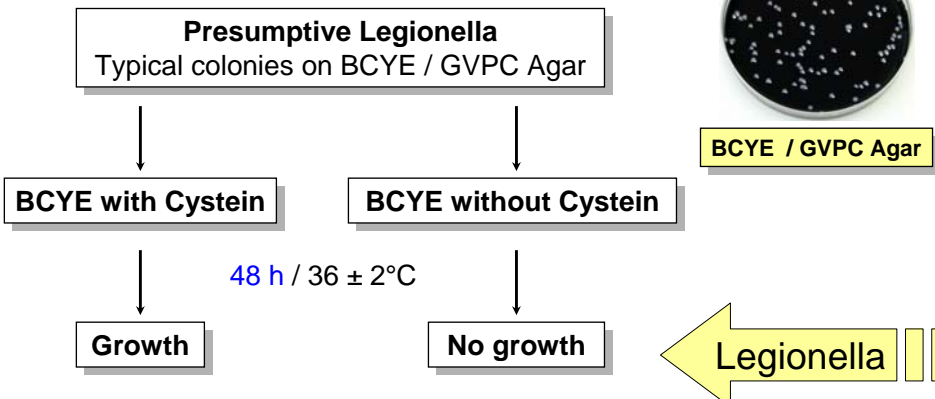
# Rapid Testing

## Duopath® Legionella



### Confirmation of presumptive Legionella colonies

#### (ISO 11731-2)



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## Rapid Testing Duopath® Legionella



### Duopath® Legionella

Worldwide first Lateral Flow Test for the simultaneous specific detection of *Legionella spp.* and *Legionella pneumophila* (all serogroups)

For confirmation of suspect colonies isolated on Legionella Agar (BCYE / GVPC Agar)



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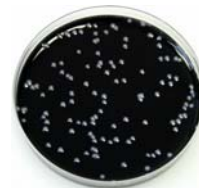
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## Rapid Testing Duopath® Legionella



### Confirmation of presumptive Legionella colonies Duopath® Legionella

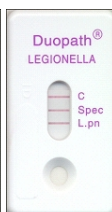
Resuspend 1 suspect colony  
in 160 µl NaCl / Tween / Polymyxin  
2 – 5 min RT, 5 min 95°C  
Pipette 150 µl into device



BCYE /GVPC Agar

30 min.

**Legionella  
not present**



**Legionella spp. or  
*L. pneumophila*  
present**

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## Rapid Testing Duopath® Legionella



### Comparison study: sensitivity of Duopath® versus latex agglutination test

Strains	No.	Duopath® <i>Legionella</i> spp. positive	Duopath® <i>L. pneumophila</i> positive	Latex test <i>Legionella</i> spp. positive	Latex test <i>L. pneumophila</i> positive
<i>L. non-pneumophila</i> Type strains	42	38 (90 %)	0	14 (33 %)	Not tested
<i>L. non-pneumophila</i> Water isolates	45	43 (96 %)	0	13 (29 %)	Not tested
<i>L. pneumophila</i> Type strains	24	24 (100%)	24 (100 %)	Not tested	24 (100 %)
<i>L. pneumophila</i> Human or water isolates	65	65 (100%)	65 (100 %)	Not tested	63 (97 %)

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Rapid Detection of Pathogens

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## Rapid Testing Duopath® Legionella



### Confirmation of presumptive Legionella

ISO	Latex	Duopath®
Results within 2 days	Results within 5 min.	Results within 45 min.
No differentiation of <i>Legionella</i> species and <i>L. pneumophila</i>	Differentiation of <i>Legionella</i> species and <i>L. pneumophila</i>	Differentiation of <i>Legionella</i> species and <i>L. pneumophila</i>
	<b>Detects only 30 % of <i>Legionella</i> species</b>	<b>Detects more than 90 % of <i>Legionella</i> species</b>

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Rapid Detection of Pathogens

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## Merck's Rapid Test Product Portfolio 8 Rapid tests for the most common pathogens



<u>Listeria:</u>	<u>Salmonella:</u>	<u>E. coli O157 / VTEC:</u>	<u>Campylobacter:</u>	<u>Bacillus cereus:</u>	<u>Legionella:</u>
LEB Base	Tetrathionate	mEC + n	Bolton Broth	M.Y.P. Agar	CYE Agar
Fraser Suppl.	Rappaport.-Vas.	mTSB + n	CCD Agar	CGY Broth	BCYE
UVM Selenit Cystin Suppl.		CT-SMAC		BHI	GVPC
PALCAM Plates	Salmosyst	SMAC			CYE-
OXFORD Plates	M Broth	BHI			GVPC-
Chromocult® Listeria Agar	XL <sub>T</sub> , XLD, Anaerocult® C Rambach®	CAYE			



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Rapid Detection of Pathogens

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## Rapid Testing

### Approvals



#### AOAC Approvals for Singlepath®/Duopath® as Food Tests

E. coli O157	- approved May 2005
Verotoxin	- approved June 2005
Salmonella	- approved October 2005
Campylobacter	- approved November 2005
Listeria + L.mono	- expected early 2006

#### Clinical Approvals for Duopath® Verotoxin as IVD Test

CE	approved September 2005
FDA	approved April 2005
Japanese IVD Approval	approved March 2005

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Rapid Detection of Pathogens

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## Advantages of using Rapid Methods



	Singlepath	ISO	FDA-BAM	Automated Systems	Savings Singlepath
Time to Result	<b>2 days</b>	<b>4 days</b>	<b>4 days</b>	<b>3 days</b>	<b>1-2 days</b>
No. of Enrichment Media	<b>2</b>	<b>3</b>	<b>3</b>	<b>5</b>	<b>1-3 Media</b>
No. of Plating Agars	<b>0</b>	<b>8</b>	<b>6</b>	<b>0</b>	<b>6 to 8 Agar media</b>

## Rapid Testing

### Singlepath® / Duopath® - Benefits



#### Important for users daily work

- **PERFECTLY ADAPTED TO MERCK'S HIGH QUALITY CULTURE MEDIA**
- **NO INSTRUMENTS NEEDED**
- **NO SPECIAL TRAINING**
- **FAST ; test results within 20 min.**
- **SIMPLE ; just add 0.16 ml to the sample window**