Bacterial Identification and Characterization

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Campylobacter disease (C. jejuni & C. coli)

Leading cause world wide of bacterial diarrheal disease

1,300,000 cases annually in the United States*

Incidence of Campylobacteriosis in the US is increasing while most other foodborne diseases are decreasing.

Sporadic cases associated with consuming or handling raw or undercooked poultry

Outbreaks are typically associated with unpasteurized milk or contaminated water

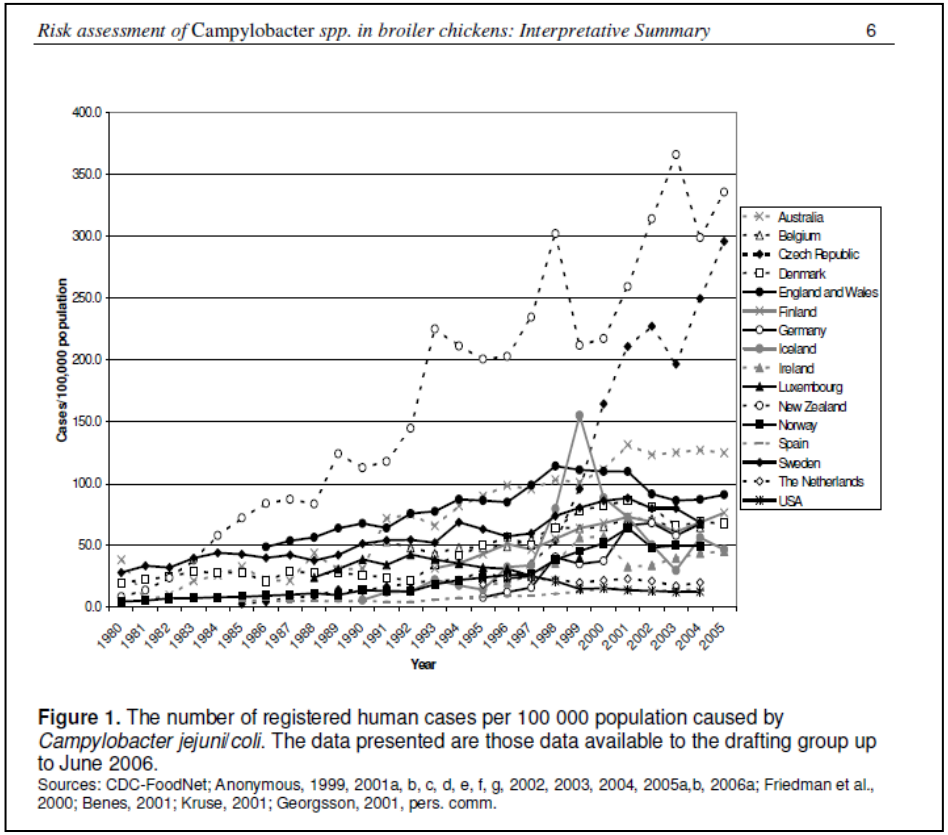

Figure 1. The number of registered human cases per 100 000 population caused by *Campylobacter jejuni/coli*. The data presented are those data available to the drafting group up to June 2006.
Sources: CDC-FoodNet; Anonymous, 1999, 2001a, b, c, d, e, f, g, 2002, 2003, 2004, 2005a,b, 2006a; Friedman et al., 2000; Benes, 2001; Kruse, 2001; Georgsson, 2001, pers. comm.

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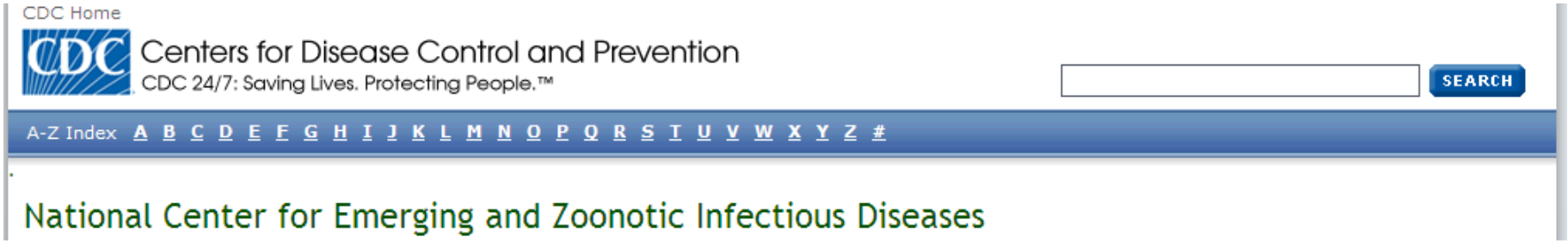


How does food or water get contaminated with *Campylobacter*?

Many chicken flocks are infected with *Campylobacter* but show no signs of illness. *Campylobacter* can be easily spread from bird to bird through a common water source or through contact with infected feces. When an infected bird is slaughtered, *Campylobacter* organisms can be transferred from the intestines to the meat.

Unpasteurized milk can become contaminated if the cow has an infection with *Campylobacter* in her udder or if the milk is contaminated with manure. Surface water and mountain streams can become contaminated from infected feces from cows or wild birds.

Campylobacter is common in the developing world, and travelers to foreign countries are at risk for becoming infected with *Campylobacter*. Approximately one-fifth (19%) of *Campylobacter* cases identified in FoodNet are associated with international travel.

In 2011, *Campylobacter* was found on 47% of raw chicken samples bought in grocery stores and tested through the National Antimicrobial Resistance Monitoring System (NARMS). *Campylobacter* can also be present in the giblets, especially the liver.

DRUG-RESISTANT CAMPYLOBACTER

310,000 DRUG-RESISTANT *CAMPYLOBACTER* INFECTIONS PER YEAR

1,300,000 *CAMPYLOBACTER* INFECTIONS PER YEAR

13,000 HOSPITALIZATIONS

120 DEATHS

THREAT LEVEL
SERIOUS

This bacteria is a serious concern and requires prompt and sustained action to ensure the problem does not grow.

Campylobacter usually causes diarrhea (often bloody), fever, and abdominal cramps, and sometimes causes serious complications such as temporary paralysis.

RESISTANCE OF CONCERN

Physicians rely on drugs like ciprofloxacin and azithromycin for treating patients with severe disease. Resistant infections sometimes last longer. *Campylobacter* is showing resistance to:

- ciprofloxacin
- azithromycin

PUBLIC HEALTH THREAT

Campylobacter is estimated to cause approximately 1.3 million infections, 13,000 hospitalizations, and 120 deaths each year in the United States. CDC is seeing resistance to ciprofloxacin in almost 25% of *Campylobacter* tested and resistance to azithromycin in about 2%. Costs are expected to be higher for resistant infections because antibiotic-resistant *Campylobacter* infections sometimes last longer.

	Percentage of all *Campylobacter**	Estimated number of illnesses per year	Estimated illnesses per 100,000 U.S. population	Estimated number of deaths per year
Resistance to ciprofloxacin	23%	310,000	102.3	28
Resistance to azithromycin	2%	22,000	7.4	<5
Resistance to azithromycin or ciprofloxacin	24%	310,000	103.9	28

Campylobacter drug resistance increased from 13% in 1997 to almost 25% in 2011.

Increasing Resistance to Ciprofloxacin in *Campylobacter*, 1989–2011

*3-year average (2009–2011)

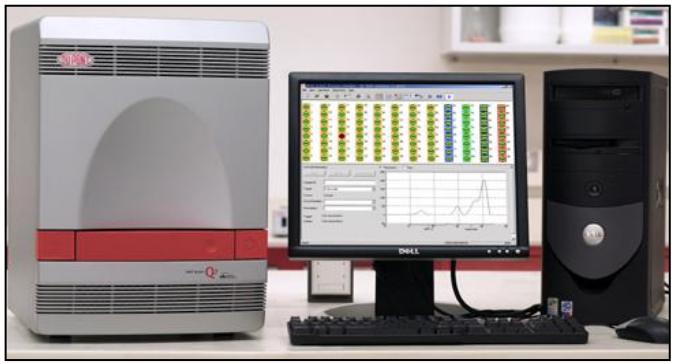
*Data for 1989–1990 were from a sentinel county survey. Annual testing began in 1997.

For more information about data methods and references, please see appendix.

U.S. Department of Health and Human Services
Centers for Disease

BAX® Screening System

Pathogen Detection by PCR



Is a specific bacterial pathogen present in a food matrix?

RiboPrinter® System

Automated Southern Blotting for Identification & Characterization

Which species is my unknown?
Which strain of that species is it?
Where is it coming from?
Have we isolated this strain before?

BAX® System Pioneered Tableted PCR Reagents for Food Safety

All the reagents needed for PCR

- Taq polymerase
- Oligonucleotide primers and probes
- Nucleotides
- Internal Positive Control
- Fluorescent dyes

Closed tube system reduces potential for amplicon contamination

Built-in process control

Reduces the risk of operator error

DuPont Nutrition & Health Facilitates Food Exports

DuPont N&H (Qualicon) and USDA

- Since 2002, the BAX system has been used by the United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) to detect Listeria monocytogenes in the nation's meat and poultry supply
- Since 2003, the BAX system has been used by the USDA-FSIS to detect Salmonella in ready-to-eat food, meat, poultry supply and pasteurized eggs.
- Since 2005, the BAX system has been used by the USDA-FSIS to detect E. coli O157:H7 in ground beef and trim.
- In 2008, DuPont Qualicon and USDA Agricultural Research Service (ARS) agreed to collaborate on the development of a new real-time test for E. coli O157:H7
- In 2010, Qualicon and USDA-ARS collaborate on six non-E. coli O157 Shiga toxin-producing E. coli (STEC).

Countries who have an "equivalent" testing program are subjected to much less testing of their product imported to the US than those who don't.

- FSIS Office of International Affairs -

BAX® System Approvals, Adoptions and Certifications

AOAC International Official Method

Salmonella #2003.09; L. monocytogenes #2003.12

AOAC-RI Performance Tested Method

Real-time E. coli O157:H7 #031002; Salmonella #100201; L. monocytogenes #070202; L. monocytogenes 24E #090801; E. coli O157:H7 #010401; E. coli O157:H7 MP #050501; Genus Listeria #030502; Genus Listeria 24E #050903; Campylobacter jejuni/coli/lari #040702; Staphylococcus aureus #120701; Reverse-Transcriptase PCR Listeria species #030801; Yeast and Mold #010902; Vibrio cholerae/parahaemolyticus/vulnificus #050902

USDA-FSIS Adoption

Salmonella #MLG 4C.00; Listeria monocytogenes #MLG 8A.03; E. coli O157:H7 and E. coli O157:NM #MLG 5A.00 – MP assay

USDA-APHIS NPIP Approval

Salmonella

Health Canada Certification

Salmonella #MFLP-29; Listeria monocytogenes #MFLP-28; Genus Listeria #MFLP-15e; E. coli O157:H7 #MFLP-30; E. coli O157:H7 MP – Supp #2 to MFLP-30; Enterobacter sakazakii #MFLP-27

AFNOR Approval

Salmonella #QUA 18/3-11/02; E. coli O157:H7 MP #QUA 18/04-03/08; Listeria monocytogenes 24E #QUA 18/05-07/08; Genus Listeria 24E #QUA 18/06-07/08

Danish Veterinary and Food Administration

Salmonella

NordVal

Salmonella #30

Brazil MAPA Official Reference Method

Salmonella MLG-4C.01; Listeria MLG-8A.01

Japanese Ministry of Health, Labour and Welfare

Listeria; Listeria monocytogenes

People's Republic of China AQSIQ

Standard SN/T1869-2007 – includes *Salmonella; E. coli O157:H7 MP; Listeria monocytogenes; Campylobacter jejuni/coli/lari; Enterobacter sakazakii*

Russian Federal Consumer Rights and Human Health Control Service (Rospotrebnadzor)

Standard #02.036-208 – includes *Salmonella; Campylobacter jejuni/coli/lari; Genus Listeria; Listeria monocytogenes; E. coli O157:H7*

Campylobacter jejuni/coli/lari Real-Time Multiplex Assay

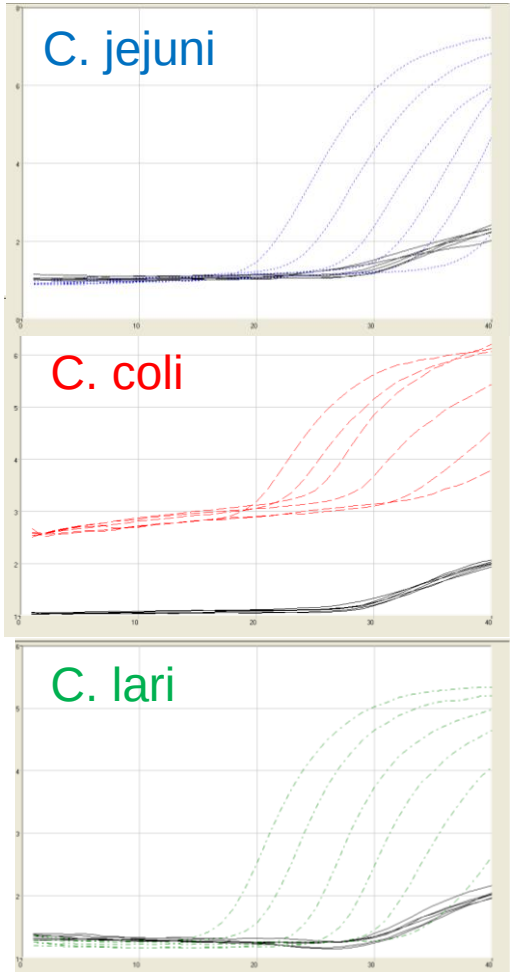
Three pathogenic Campylobacter species independently detected in one assay

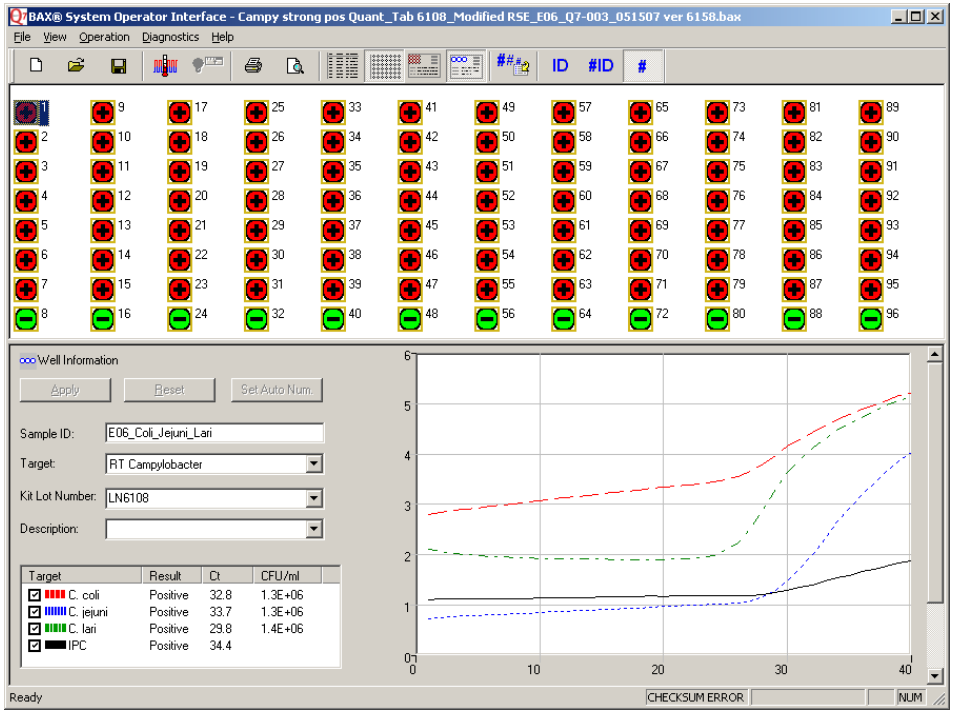
C. jejuni
gyrB (gyraseB)
VIC®

C. coli
rnpB (RNAse P)
FAM™

C. lari
gyrB (gyraseB)
LIZ®

Internal Control
NED®





Next-day results for enriched samples - at least 3 days faster than culture methods

Sensitivity : E09 to E04 cfu/ml

BAX® System Assays for Food Safety and Quality

Salmonella

Listeria genus

Listeria monocytogenes

E. coli O157:H7

E. coli STEC suite

(O26, O46, O103, O111, O121, O145, O157)

Enterobacter sakazakii (Cronobacter species)

Staphylococcus aureus

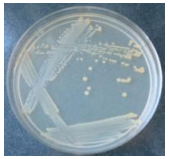
Shigella species

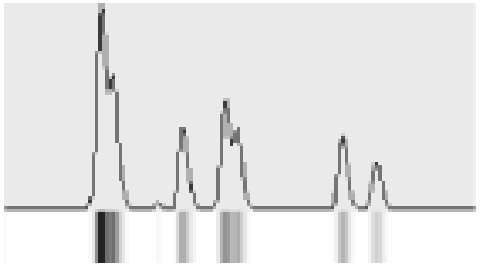
Campylobacter jejuni/coli/lari

Vibrio cholerae/parahaemolyticus/vulnificus

Yeasts/molds

Automated DNA fingerprinting of bacteria





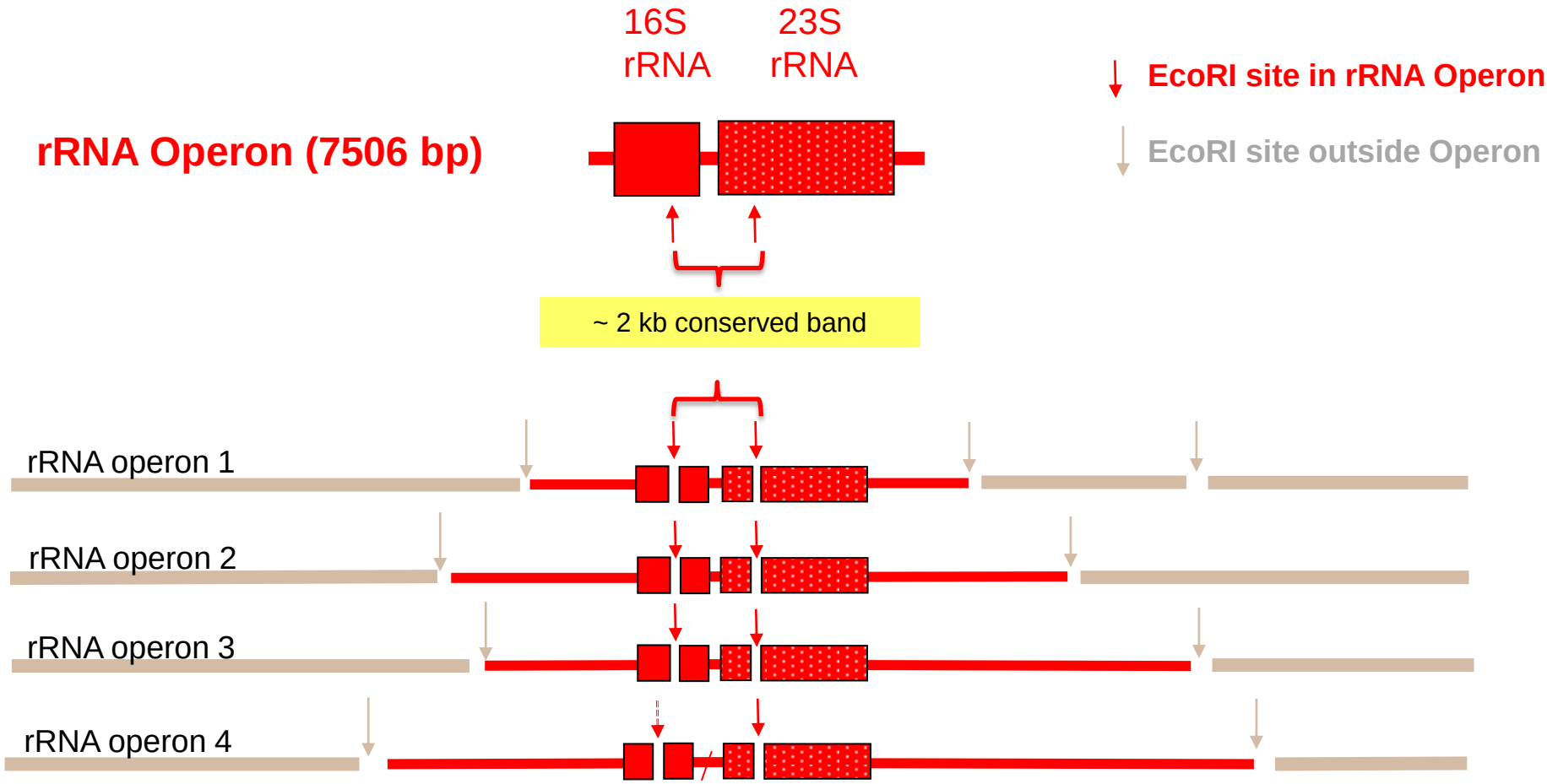
- Taxonomic Identification of unknowns below Genus level
  - species, subspecies, serotype, strain
  - Reference database of patterns for vetted strains
- Characterization (strain/source tracking)
  - Novel fingerprint
- Fully automated results in 8 hours
- Automated data analysis & data sharing

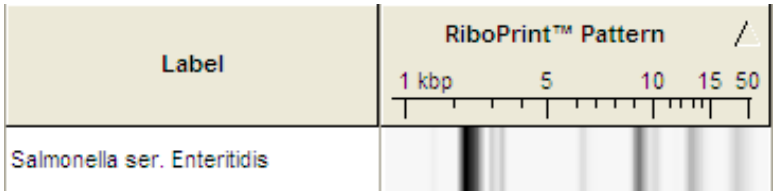
Manual ribotyping reagents

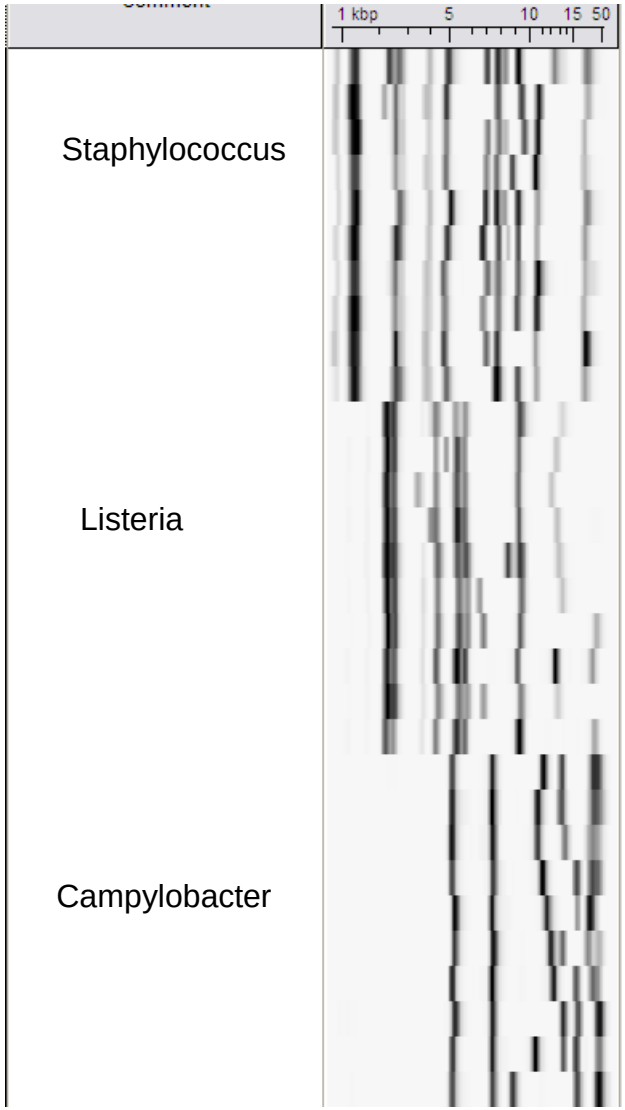
Adapt and develop methods through flexibility of modular design and packaging of reagents.

RiboPrinter® Reagents

Southern Blotting with Conserved rRNA Gene Hybridization Probes







RiboPrinter Identification Database:

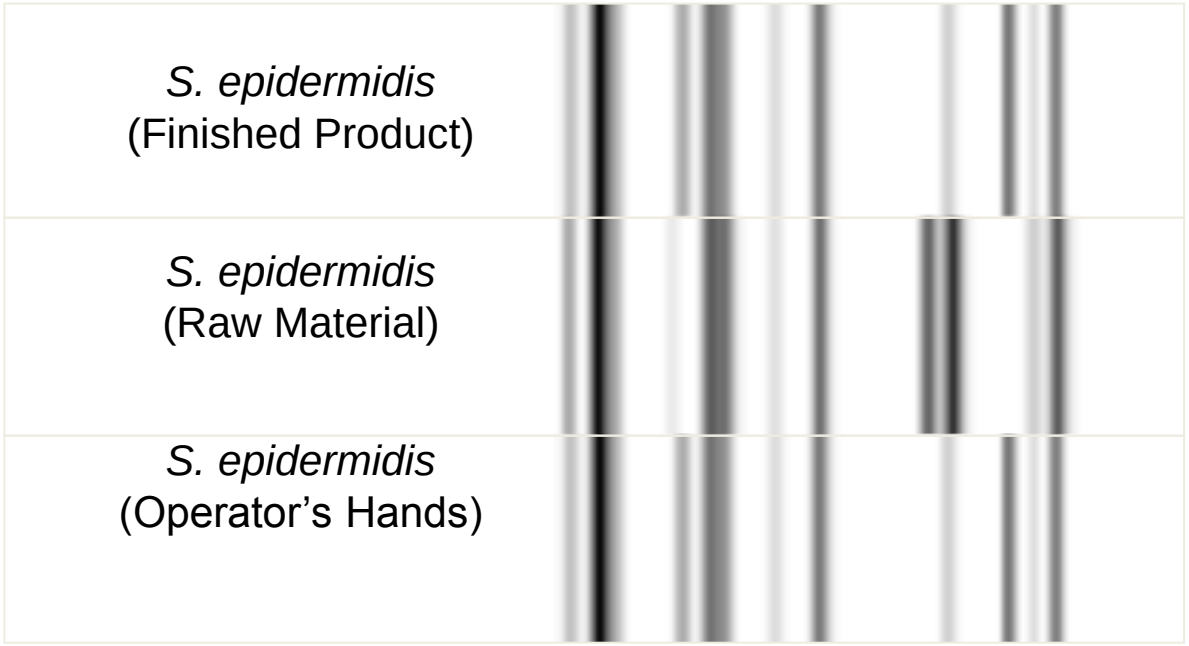
8528 Reference Patterns from
296 Bacterial genera
1740 Species & serotypes

(199 patterns for Campylobacter species)

RiboPrinter® Data Enables Contamination Source Tracking

How the RiboPrinter provides value:

- An unknown bacteria is found in the finished product. RiboPrinter identifies it as Staphylococcus epidermidis. But where did the contamination come from?
- By sampling various points along the production process, the RiboPrint® pattern reveals that the S. epidermidis found in the finished product came from the hands of a worker, and not from the raw material.



Information Content of Different Technologies

RiboPrinter® System
16S rRNA sequence
MALDI-TOF
Biochemical

RiboPrinter® System

S. epidermidis (Finished Product)	
S. epidermidis (Raw Material)	
S. epidermidis (Operator's Hands)	

S. epidermidis_One 16S rRNA Sequence vs. 85 RiboPrint® Patterns

	DuPont ID	Label	RiboPrint™ Pattern (1 kbp 5 10 15 50)
1	DUP-4112	Staphylococcus epidermidis	
2	DUP-4113	Staphylococcus epidermidis	
3	DUP-4114	Staphylococcus epidermidis	
4	DUP-4115	Staphylococcus epidermidis	
5	DUP-4116	Staphylococcus epidermidis	
6	DUP-4117	Staphylococcus epidermidis	
7	DUP-4118	Staphylococcus epidermidis	
8	DUP-4119	Staphylococcus epidermidis	
9	DUP-4120	Staphylococcus epidermidis	
10	DUP-4121	Staphylococcus epidermidis	
11	DUP-4122	Staphylococcus epidermidis	
12	DUP-4123	Staphylococcus epidermidis	
13	DUP-4124	Staphylococcus epidermidis	
14	DUP-4125	Staphylococcus epidermidis	
15	DUP-4126	Staphylococcus epidermidis	
16	DUP-14730	Staphylococcus epidermidis	
17	DUP-14740	Staphylococcus epidermidis	
18	DUP-14755	Staphylococcus epidermidis	
19	DUP-14757	Staphylococcus epidermidis	
20	DUP-15235	Staphylococcus epidermidis	
21	DUP-15247	Staphylococcus epidermidis	
22	DUP-16031	Staphylococcus epidermidis	
23	DUP-16034	Staphylococcus epidermidis	
24	DUP-16035	Staphylococcus epidermidis	
25	DUP-16042	Staphylococcus epidermidis	
26	DUP-16046	Staphylococcus epidermidis	
27	DUP-16047	Staphylococcus epidermidis	
28	DUP-16059	Staphylococcus epidermidis	
29	DUP-16066	Staphylococcus epidermidis	
30	DUP-16067	Staphylococcus epidermidis	
31	DUP-16071	Staphylococcus epidermidis	
32	DUP-16080	Staphylococcus epidermidis	
33	DUP-16083	Staphylococcus epidermidis	
34	DUP-16084	Staphylococcus epidermidis	
35	DUP-16086	Staphylococcus epidermidis	
36	DUP-16088	Staphylococcus epidermidis	
37	DUP-16264	Staphylococcus epidermidis	
38	DUP-16298	Staphylococcus epidermidis	
39	DUP-16427	Staphylococcus epidermidis	
40	DUP-16449	Staphylococcus epidermidis	
41	DUP-16455	Staphylococcus epidermidis	

	DuPont ID	Label	RiboPrint™ Pattern (1 kbp 5 10 15 50)
46	DUP-18081	Staphylococcus epidermidis	
47	DUP-18084	Staphylococcus epidermidis	
48	DUP-18093	Staphylococcus epidermidis	
49	DUP-18095	Staphylococcus epidermidis	
50	DUP-18527	Staphylococcus epidermidis	
51	DUP-18551	Staphylococcus epidermidis	
52	DUP-18745	Staphylococcus epidermidis	
53	DUP-18746	Staphylococcus epidermidis	
54	DUP-18751	Staphylococcus epidermidis	
55	DUP-18753	Staphylococcus epidermidis	
56	DUP-18754	Staphylococcus epidermidis	
57	DUP-18782	Staphylococcus epidermidis	
58	DUP-19253	Staphylococcus epidermidis	
59	DUP-19259	Staphylococcus epidermidis	
60	DUP-19289	Staphylococcus epidermidis	
61	DUP-19358	Staphylococcus epidermidis	
62	DUP-19424	Staphylococcus epidermidis	
63	DUP-20388	Staphylococcus epidermidis	
64	DUP-20389	Staphylococcus epidermidis	
65	DUP-20390	Staphylococcus epidermidis	
66	DUP-20391	Staphylococcus epidermidis	
67	DUP-20392	Staphylococcus epidermidis	
68	DUP-20393	Staphylococcus epidermidis	
69	DUP-20394	Staphylococcus epidermidis	
70	DUP-20395	Staphylococcus epidermidis	
71	DUP-20396	Staphylococcus epidermidis	
72	DUP-20397	Staphylococcus epidermidis	
73	DUP-20398	Staphylococcus epidermidis	
74	DUP-20399	Staphylococcus epidermidis	
75	DUP-20400	Staphylococcus epidermidis	
76	DUP-20401	Staphylococcus epidermidis	
77	DUP-20402	Staphylococcus epidermidis	
78	DUP-20403	Staphylococcus epidermidis	
79	DUP-20404	Staphylococcus epidermidis	
80	DUP-20405	Staphylococcus epidermidis	
81	DUP-20406	Staphylococcus epidermidis	
82	DUP-20407	Staphylococcus epidermidis	
83	DUP-20408	Staphylococcus epidermidis	
84	DUP-20409	Staphylococcus epidermidis	
85	DUP-20410	Staphylococcus epidermidis	
86	DUP-20411	Staphylococcus epidermidis	

DuPont Offers a Complete Solution for Detection & Tracking

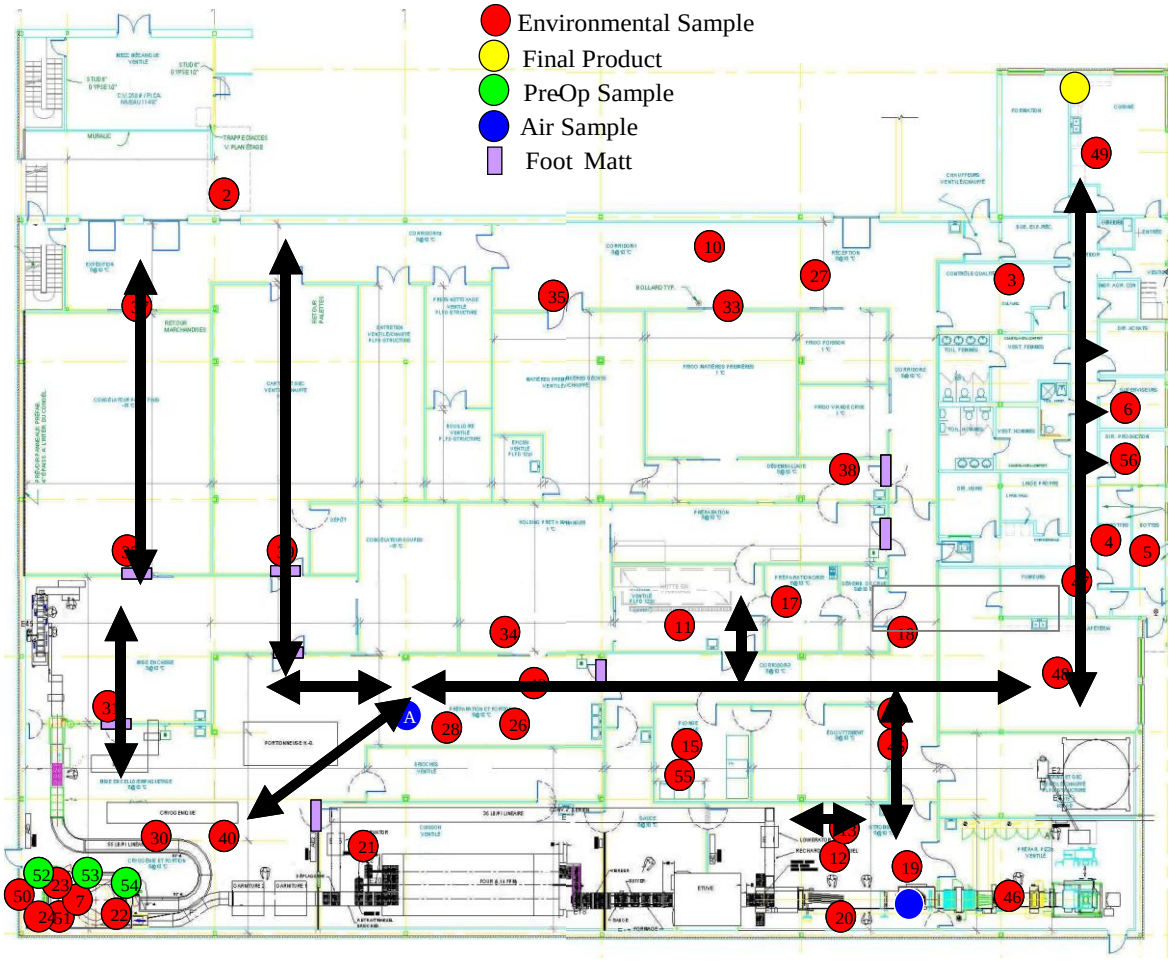
BAX® System

Detects pathogens and quality indicators on:

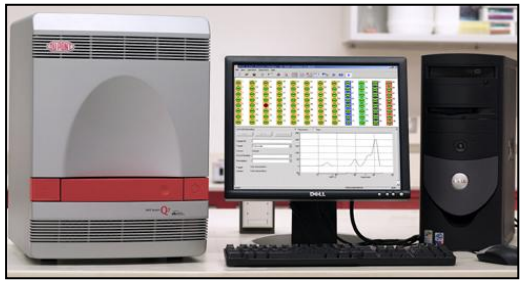
- Raw materials
- Environmental samples
- Final product

RiboPrinter® System

- Identifies species and strain
- Track same strain throughout plant to identify source of contamination
- Implement corrective action quickly



DuPont Nutrition & Health Molecular Diagnostic Products



Food safety technologies & applications to save time, improve detection sensitivity, comply with regulations, identify and solve contamination problems.

The miracles of science™