

## Inclusivity and Exclusivity Study for *Cronobacter* Detection by PCR

### Introduction

In June 2008, the organism *Enterobacter sakazakii* was re-classified in the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM) into a new species called *Cronobacter*. An internal validation study was performed to demonstrate the performance of the BAX® System PCR Assay for *Cronobacter* (previously the BAX System PCR Assay for *E. sakazakii*) when testing strains now classified as *Cronobacter*. Results showed that the BAX System assay accurately detects *Cronobacter* species.

### Methodology

#### Equipment, Reagents and Supplies

- BAX System PCR Assay for *Cronobacter* (KIT2001) – previously BAX System PCR assay for *E. sakazakii* (D17720657)
- BAX System Q7 instrument and peripherals
- Standard BAX System equipment and supplies
- Buffered Peptone Water (BPW)
- Brain Heart Infusion (BHI) Broth

#### Strain Selection and Enrichment

For inclusivity testing, 53 isolates of *Cronobacter* were selected from 10 culture collections to include a variety of typical and atypical strains (see Table 1). Each strain was grown in Buffered Peptone Water (BPW) for 20 - 24 hours at 37 °C, then diluted with additional BPW to reach 10<sup>6</sup> cfu/mL, an appropriate level for inclusivity testing with the BAX System assay. For exclusivity testing, 37 non-*Cronobacter* species were selected from a variety of genera. Strains were grown in Brain Heart Infusion (BHI) broth for 24 hours at 35 °C, then diluted to appropriate levels for testing with the BAX System assay.

**Table 1: Source information for selected *Cronobacter* strains**

Abbreviation	Name	Location
ATCC	American Type Culture Collection	Manassas, VA USA
NCTC	National Collection of Type Cultures	London, UK
CDC	Centers for Disease Control and Prevention	Atlanta, GA, USA
ILSI	Ivy, Food Safety Lab, Cornell University	Ithaca, NY, USA
FDA	US Food and Drug Administration-Center for Safety and Applied Nutrition	College Park, MD, USA
HCSC	Health Products and Food Branch, Health Canada	Ottawa, Ontario, Canada
UCD	Centre for Food Safety, University College Dublin	Belfield, Dublin 4, Ireland
UZH	Institute for Food Safety, University of Zurich	Winterthurerstrasse 272, CH-8057, Switzerland
NRC	Nestlé Research Centre	Vers-chez-les-Blanc, Lausanne, CH-1000, Switzerland
RF	R&F Laboratories	Downers Grove, IL, USA

### **BAX System Method**

Isolates were prepared according to the protocol described in the BAX System User Guide. Lysis reagent was prepared by adding 150 µL protease to one 12 mL bottle of lysis buffer. Culture dilutions were added in 5 µL aliquots to 200 µL lysis reagent in cluster tubes. Cluster tubes were heated at 37 °C for 20 minutes and 95 °C for 10 minutes, then cooled at 2 - 8 °C for 5 minutes in a cooling block. Samples were transferred in 50 µL aliquots to PCR tubes with tablets, and a full process was run in the BAX System Q7 instrument.

### **Results**

The results of inclusivity testing are summarized in Table 2. Of the 53 strains tested, 51 returned positive results. Three of the isolates (indicated with an asterisk) returned negative results at the initial density but were positive when re-run at 10<sup>7</sup> cfu/mL.

For the two strains reporting negative results, further extensive phenotypic taxonomic characterization identified one as *Pantoea agglomerans* and the other as an atypical *Enterobacter*. This suggests that these strains may have been mis-identified as *Cronobacter* before testing.

Exclusivity results are summarized in Table 3. All 37 strains of non-*Cronobacter* species returned negative results.

### **Conclusions**

This study, with the exception of two unconfirmed samples, demonstrates that the current BAX System PCR Assay for *Cronobacter* can reliably detect *Cronobacter* species.

**Table 2: Inclusivity results for *E. sakazakii* (*Cronobacter* species)**

Strain ID	<i>Cronobacter</i> species (biogroup)	Source	Result	Strain ID	<i>Cronobacter</i> species (biogroup)	Source	Result
ATCC 29544	<i>C. sakazakii</i> (1)	Human (throat)	POS	NCTC 9238	<i>C. sakazakii</i> (1)	Human (abdominal pus)	POS
ATCC 12868	<i>C. sakazakii</i> (1)	Unknown	POS	NCTC 9529	<i>C. genomospecies I</i> (16)	Water	POS
ATCC 51329	<i>C. muytjensii</i> (15)	Unknown	POS	UCD CFS112	<i>C. sakazakii</i> (1)	Milk powder	POS
ATCC BAA893	<i>C. sakazakii</i> (2)	Tennessee strain	POS	UCD CFS349N	<i>C. sakazakii</i> (1)	Milk powder	POS
ATCC BAA894	<i>C. sakazakii</i> (2)	Tennessee strain	POS	UCD CFS1001	<i>C. sakazakii</i> (2)	Milk powder	POS
CDC 996-77	<i>C. sakazakii</i> (3)	Human (spinal fluid)	POS	UCD CFS352N	<i>C. sakazakii</i> (2)	Milk powder	POS
CDC 407-77	<i>C. sakazakii</i> (4)	Human (sputum)	POS	UCD CFS237	<i>C. dublinensis subsp. Dublinensis</i> (12)	Milk powder	NEG
CDC 5960-70	<i>C. dublinensis subsp. Lactaridi</i> (6)	Human (blood)	POS*	UCD E632	<i>C. sakazakii</i> (4/2)	Food	POS
CDC 9369-75	<i>C. sakazakii</i> (7)	Unknown	POS	UCD E265	<i>C. malonaticus</i> (5)	Milk powder	POS
CDC 1058-77	<i>C. malonaticus</i> (9)	Human (breast abscess)	POS	UCD E767	<i>C. sakazakii</i> (1)	Milk powder	POS
CDC 3128-77	<i>C. sakazakii</i> (11)	Human (sputum)	POS	UCD E773	<i>C. sakazakii</i> (1)	Milk powder	POS
CDC 3523-75	<i>C. muytjensii</i> (15)	Human (bone marrow)	POS	UCD E786	<i>C. sakazakii</i> (1)	Neonate	POS
CDC 289-81	<i>C. sakazakii</i> (1)	Clinical	POS	UCD E770	<i>C. sakazakii</i> (2)	Milk powder	POS
CDC 1716-77	<i>C. malonaticus</i> (9)	Human (blood)	POS	UCD E784	<i>C. sakazakii</i> (2)	Neonate	POS
FDA 607	(not classified)	Unknown	POS	UCD E772	<i>C. malonaticus</i> (5)	Milk powder	POS
HCSC HPB2873	<i>C. sakazakii</i> (4)	Clinical	POS	UCD E766	<i>C. malonaticus/sakazakii</i> (5/7)	Milk powder	POS
HCSC HPB2784	<i>C. sakazakii</i> (4/3)	Clinical	POS	UCD E755	<i>C. sakazakii</i> (7/2/3)	Unknown	POS
HCSC HPB2848	<i>C. sakazakii</i> (8/2)	Clinical	POS	UCD E775	<i>C. sakazakii</i> (13)	Milk powder	POS
HCSC SK90	<i>C. sakazakii</i> (1)	Clinical	POS	UCD E464	<i>C. dublinensis subsp. Lactaridi</i> (6)	Milk powder	POS*
HCSC SK81	<i>C. sakazakii</i> (1)	Human	POS	UCD E515	<i>C. dublinensis subsp. Lausannensis</i> (10)	Water	POS*
ILSI F6-036	<i>C. sakazakii</i> (1)	Milk powder	POS	UZH 1084	<i>C. sakazakii</i> (2)	Fruit powder	NEG
ILSI F6-040	<i>C. sakazakii</i> (1)	Milk powder	POS	UZH 3032	<i>C. genomospecies I</i> (16)	Neonate (meningitis)	POS
ILSI F6-034	<i>C. sakazakii</i> (2)	Clinical	POS	CDC 9363-75	<i>C. sakazakii</i> (13)	Stool	POS
ILSI F6-038	<i>C. sakazakii</i> (3)	Milk powder	POS	CDC 4963-71	<i>C. sakazakii/malonaticus</i> (2/14)	Stool	POS
ILSI F6-043	<i>C. sakazakii</i> (8)	Clinical	POS	CDC 1895-73	<i>C. malonaticus</i> (14)	Human (faeces)	POS
ILSI F6-029	<i>C. sakazakii</i> (1)	Neonate	POS	RF Es626	<i>C. sakazakii</i> (8)	Rice flour	POS
ILSI F6-032	<i>C. sakazakii</i> (2/3)	Milk powder	POS				

**Table 3: Exclusivity results for *E. sakazakii* (*Cronobacter* species)**

Strain ID	<i>Cronobacter</i> species (biogroup)	Source	Result	Strain ID	<i>Cronobacter</i> species (biogroup)	Source	Result
ATCC 13047	<i>Enterobacter cloacae</i>	Spinal fluid	NEG	ATCC 33650	<i>Escherichia hermanii</i>	Human toe	NEG
ATCC 13048	<i>Enterobacter aerogenes</i>	Sputum	NEG	ATCC 15246	<i>Alcaligenes faecalis</i>	Unknown	NEG
ATCC 13182	<i>Klebsiella oxytoca</i>	Pharyngeal tonsil	NEG	ATCC 33832	<i>Escherichia vulneris</i>	Blood (human)	NEG
ATCC 13880	<i>Serratia marcescens</i>	Pond water	NEG	ATCC 29212	<i>Enterococcus faecalis</i>	Urine	NEG
ATCC 14485	<i>Streptococcus thermophilus</i>	Unknown	NEG	ATCC 10054	<i>Micrococcus luteus</i>	Unknown	NEG
ATCC 14807	<i>Bacillus subtilis</i>	Soil	NEG	ATCC 51713	<i>Buttiauxella noakiae</i>	Snail	NEG
ATCC 15469	<i>Edwardsiella tarda</i>	Faeces	NEG	ATCC 25741	<i>Pediococcus acidilactici</i>	Plants	NEG
ATCC 23055	<i>Acinetobacter calcoaceticus</i>	Unknown	NEG	ATCC 8090	<i>Citrobacter freundii</i>	Unknown	NEG
ATCC 23216	<i>Leclercia adecarboxylata</i>	Drinking water	NEG	ATCC 9789	<i>Bacillus licheniformis</i>	Milk	NEG
ATCC 25830	<i>Morganella morganii</i>	Human (faeces)	NEG	UZH E440	<i>Enterobacter helveticus sp. nov</i>	Milk powder	NEG
ATCC 25922	<i>Escherichia coli</i>	Clinical isolate	NEG	UZH E441	<i>Enterobacter novel species</i>	Milk powder	NEG
ATCC 27028	<i>Citrobacter koseri</i>	Blood culture	NEG	UZH E644	<i>Enterobacter cloacae</i>	Human (faeces)	NEG
ATCC 27982	<i>Pantoea agglomerans</i>	IV fluid	NEG	UZH E904	<i>Enterobacter homaechei</i>	Milk powder	NEG
ATCC 29013	<i>Klebsiella pneumoniae</i>	Blood	NEG	ILSI E883	<i>Enterobacter asburiae</i>	Environment	NEG
ATCC 29944	<i>Providencia rettgeri</i>	Unknown	NEG	ILSI E890	<i>Enterobacter hormaechei</i>	Milk powder	NEG
ATCC 27853	<i>Pseudomonas aeruginosa</i>	Blood culture	NEG	LMG E910	<i>Enterobacter turicensis, sp. nov</i>	Fruit powder	NEG
ATCC 13472	<i>Bacillus cereus</i>	Unknown	NEG	LMG E912	<i>Enterobacter helveticus, sp. nov</i>	Fruit powder	NEG
ATCC 33105	<i>Serratia ficaria</i>	Calimyrna fig	NEG	UZH E908	<i>Enterobacter novel species</i>	Fruit powder	NEG
ATCC 33420	<i>Proteus vulgaris</i>	Clinical isolate	NEG				