One-Step Enrichment Broth for the Simultaneous Recovery of *Salmonella enterica* and *Cronobacter sakazakii* in Powdered Infant Formula

Afia Boumail[1], Anne Helmer[1], Marie-Goreth Nicizanye[1], Anna Yattara[1], Michael Giuffre[2] and Sergiy Olishevskyy[1]

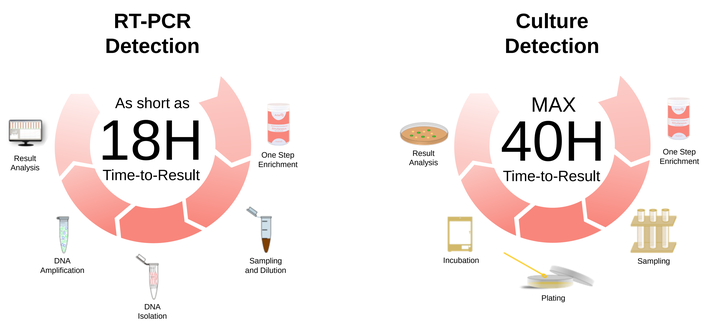
[1]FoodChek Laboratories Inc., Ste-Julie, Quebec, Canada [2]FoodChek Systems Inc., Calgary, Alberta, Canada

Introduction

Salmonella enterica and *Cronobacter sakazakii* are categorized as dangerous contaminants of powdered infant formula that represent a serious health risk for newborn infants. Conventional culture methods are time consuming and not user-friendly for detecting both pathogens. Therefore, the development and implantation of rapid and cost-effective methods allowing for the simultaneous detection of these pathogens remain important.

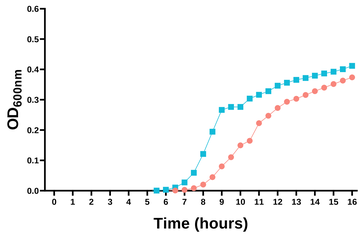
The objective of this study was to develop a novel enrichment broth, Actero Simultaneous Recovery Broth (ASRB), that allows the one-step simultaneous recovery of *S. enterica* and *C. sakazakii* followed by both RT-PCR and culture detection.

Method Principle



In vitro Culture Studies

Fig. 1. Growth of *Salmonella* Agona and *Cronobacter sakazakii* in Actero Simultaneous Recovery Broth



Salmonella
Cronobacter

Culture Conditions:
- Initial Inoculum – 20 CFU/well
- Medium Volume – 200 µL
- Temperature – 35°C
- Time – 16 hours

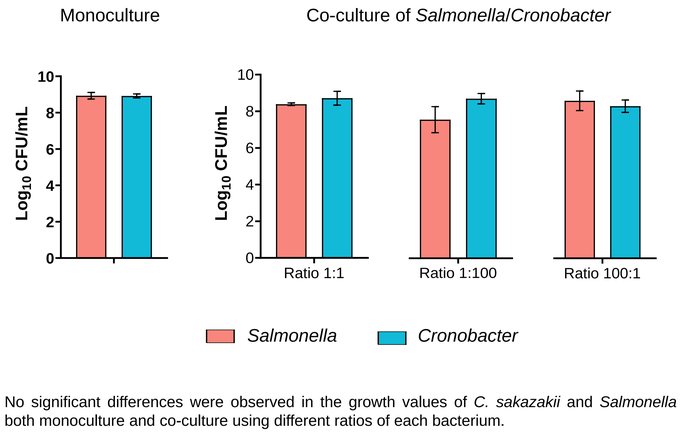
Table 1. Growth Kinetics Data

Strain	Kinetics Parameter	ASRB
***S.* Agona**	Lag Phase Duration	5.912 ± 0.469
	Growth Rate	0.195 ± 0.002
C. sakazakii	Lag Phase Duration	4.985 ± 0.609
	Growth Rate	0.298 ± 0.013

Both strains showed short lag-phases and comparable growth rates in ASRB.

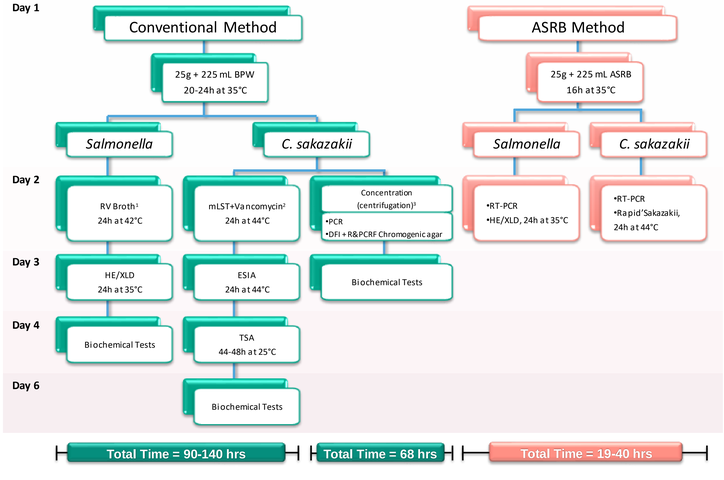
In vitro Simultaneous Culture Studies

Fig. 2. Growth of *Cronobacter sakazakii* and *Salmonella* Agona Co-cultured in Actero Simultaneous Recovery Broth for 16 hours at 35°C



No significant differences were observed in the growth values of *C. sakazakii* and *Salmonella* in both monoculture and co-culture using different ratios of each bacterium.

Matrix Study Design



Notes:
1. ISO6579-1:2017
2. ISO/TS 22964 (2006)
3. BAM Ch. 29

BPW – Buffered Peptone Water

Infant Formula Samples Studies

A total of 170 artificially contaminated infant formula samples were examined to evaluate performance of the ASRB alternative method in comparison with the conventional method using culture detection.

Table 2. Experimental Design

Matrix	Powdered Infant Formula (25 g)	
Strain	*S.* Agona	*C. sakazakii*
Stress	Drying	
Mortality	90%	85%
Inoculum Level (CFU/sample)	5.5	2.7

Table 3. Recovery Rates of *S.* Agona and *C. sakazakii* in Powdered Infant Formula

Detection Method	N	*S.* Agona	*C. sakazakii*	*S.* Agona *C. sakazakii*	No Recovery
ASRB	16	35/170	45/170	62/170	28/170
Conventional	16	34/170	34/170	63/170	39/170

Notes : N – Number of experiments.

The simultaneous recovery of *Cronobacter* and *Salmonella* was comparable between the two methods.

Table 4. Performance Parameters for Culture Detection in Powdered Infant Formula Using ASRB

Strain	Time (h)	Total Tested	P	N	FP	FN	Relative Sensitivity, %	Relative Specificity, %	FP Rate, %	FN Rate, %	Test Efficacy, %
***S.* Agona**	16	170	97	73	0	0	100	100	0.0	0.0	100
C. sakazakii	16	170	107	63	0	0	100	100	0.0	0.0	100

Notes: P – positive, N – negative, FP – false positive, FN – false negative.

Among 170 tested samples, no false positives or false negatives were detected by direct plating using the alternative method.

The performance of RT-PCR detection method following the enrichment with ASRB was assessed using 30 artificially contaminated powdered infant formula samples.

Table 5. Performance Parameters for RT-PCR Detection in Powdered Infant Formula Using ASRB

Strain	Time (h)	Total Tested	P	N	FP	FN	Relative Sensitivity, %	Relative Specificity, %	FP Rate, %	FN Rate, %	Test Efficacy, %
***S.* Agona**	16	30	25	5	0	0	100	100	0.0	0.0	100
C. sakazakii	16	30	21	9	0	0	100	100	0.0	0.0	100

Notes: P – positive, N – negative, FP – false positive, FN – false negative.

Detection by RT-PCR showed no false positives or false negatives using the alternative method.

Conclusions

Actero Simultaneous Recovery Broth has a strong ability to provide an ideal growing environment for low numbers of sublethally injured *Salmonella enterica* and *C. sakazakii* in powdered infant formula.

Single-step enrichment with Actero Simultaneous Recovery Broth reduces presumptive reporting time to as short as 18 hours without the loss of sensitivity and reliability of methods used for detection of *Salmonella enterica* and *C. sakazakii* in powdered infant formula.