Performance Summary

RAPID'*Sakazakii* Method

Introduction

RAPID'*Sakazakii* Agar is a chromogenic medium for the detection of *Cronobacter* spp. after food and environmental samples are enriched in Buffered Peptone Water (BPW) or supplemented BPW. The RAPID'*Sakazakii* method allows the presumptive identification of *Cronobacter* spp. based on the detection of α-glucuronidase enzymatic activity. Under its action, the chromogenic substrate 5-bromo-4-chloro-3-indolyl α-D-glucopyranoside is hydrolyzed, producing a blue to blue-green color in *Cronobacter* spp. colonies. The RAPID'*Sakazakii* method has been rigorously tested and validated by an internationally recognized validation agency (Table 1).

Table 1. Validations for the RAPID'*Sakazakii* method.

Validation	Certificate Number
NF Validation	BRD 07/22–05/12

Inclusivity/Exclusivity Testing

Inclusivity testing is performed to verify that the method can detect *Cronobacter* spp., while exclusivity studies test non-*Cronobacter* strains to ensure there is no cross-reactivity. Exclusivity strains were enriched in nonselective broth for 20 hr at 37 ± 1°C and were tested at high levels. A target of 10–100 colony forming units (CFU) of each *Cronobacter* inclusivity strain (listed below) was cultured in BPW and BPW + PIF Supplement for 16–18 hr at 37 ± 1°C and diluted to a low level (~10^3) before testing. All colonies were confirmed regardless of morphology. Results are shown in Table 2.

The following strains were tested in the inclusivity study:

C. dublinensis
C. dublinensis lactaridii
C. dublinensis lausannensis
C. malonaticus
C. muytjensii
C. sakazakii
C. turicensis

Table 2. Results of inclusivity/exclusivity testing.

Strains Tested	Positives Detected	Results
52 *Cronobacter* strains tested	52/52	100% inclusivity
31 non-*Cronobacter* strains tested	0/31	100% exclusivity

Limit of Detection

Limit of detection (LOD_{50}) is an estimation of the contamination level required to achieve positive detection in 50% of cases. This is measured by inoculating food matrices with *Cronobacter* strains and carrying out the validated enrichment and detection protocols (Table 3).

The average LOD_{50} of the RAPID'*Sakazakii* method was determined to be 1.2 (range: 0.9–1.7).

Table 3. LOD_{50} for the RAPID'*Sakazakii* method.

Matrix/Strain Pair	LOD_{50}, CFU/sample size (range)
Infant cereal with probiotics/C. *sakazakii*	1.3 (0.7–2.5)
Infant formula with probiotics/C. *sakazakii*	0.9 (0.5–1.6)

Method Comparison/Matrix Studies

Matrix testing is critical to demonstrating the performance of a method compared to the reference method with real-world food samples. The RAPID'*Sakazakii* method has been verified with external and internal testing on a wide variety of foods. No significant difference was found between the reference method and alternative method for all matrices tested (Table 4).

Table 4. Matrices tested with the RAPID'*Sakazakii* method.

Category	Matrices
Infant formula, cereals, and ingredients	Infant formula with and without probiotics (stage 1, 2, and 3), sensitive-infant formula with probiotics, infant formula with iron, non-GMO infant formula, soy-based infant formula, organic infant formula with lactose, infant formula with probiotics for fat malabsorption, infant cereal with and without probiotics (stage 1, 2 and 3), organic infant cereal, multigrain banana infant cereal, single-grain infant cereal, single-grain raisin infant cereal, oats and quinoa infant cereal with probiotics, vanilla infant cereal without probiotics, rice/apple/banana infant cereal with probiotics, organic DHA infant cereal with probiotics, single-grain rice infant cereal with probiotics, oatmeal, oatmeal with probiotics, organic oatmeal with choline and probiotics, organic strawberry-banana oatmeal, old fashioned oats, whole wheat flour, brown rice flour, quinoa flour, all-purpose flour, coconut oil, organic soy oil, organic sunflower oil, instant milk powder, skim milk powder, organic goat milk powder, sodium citrate, folic acid, manganese sulfate, zinc sulfate, biotin, maltodextrin, whey powders, nonfat dry milk, whey protein

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