

ThermoFisher
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Culture media for *Cronobacter* species

Patrick Druggan

Pathogen Detection

- Pre-enrichment
 - Recovery of injured cells
- Enrichment
 - Increase in proportion of pathogen relative to competitive microflora
- Isolation
 - Presumptive identification of pathogen on an agar
- Identification
 - Biochemical confirmation of presumptive identification of pathogen
- Confirmation
 - Confirmation of presence of pathogen serologically or using molecular methods

Sensitivity & Specificity

		Gold Standard	
		Positive	Negative
New Test	Positive	True Positive (TP)	False Positive (FP)
	Negative	False Negative (FN)	True Negative (TN)

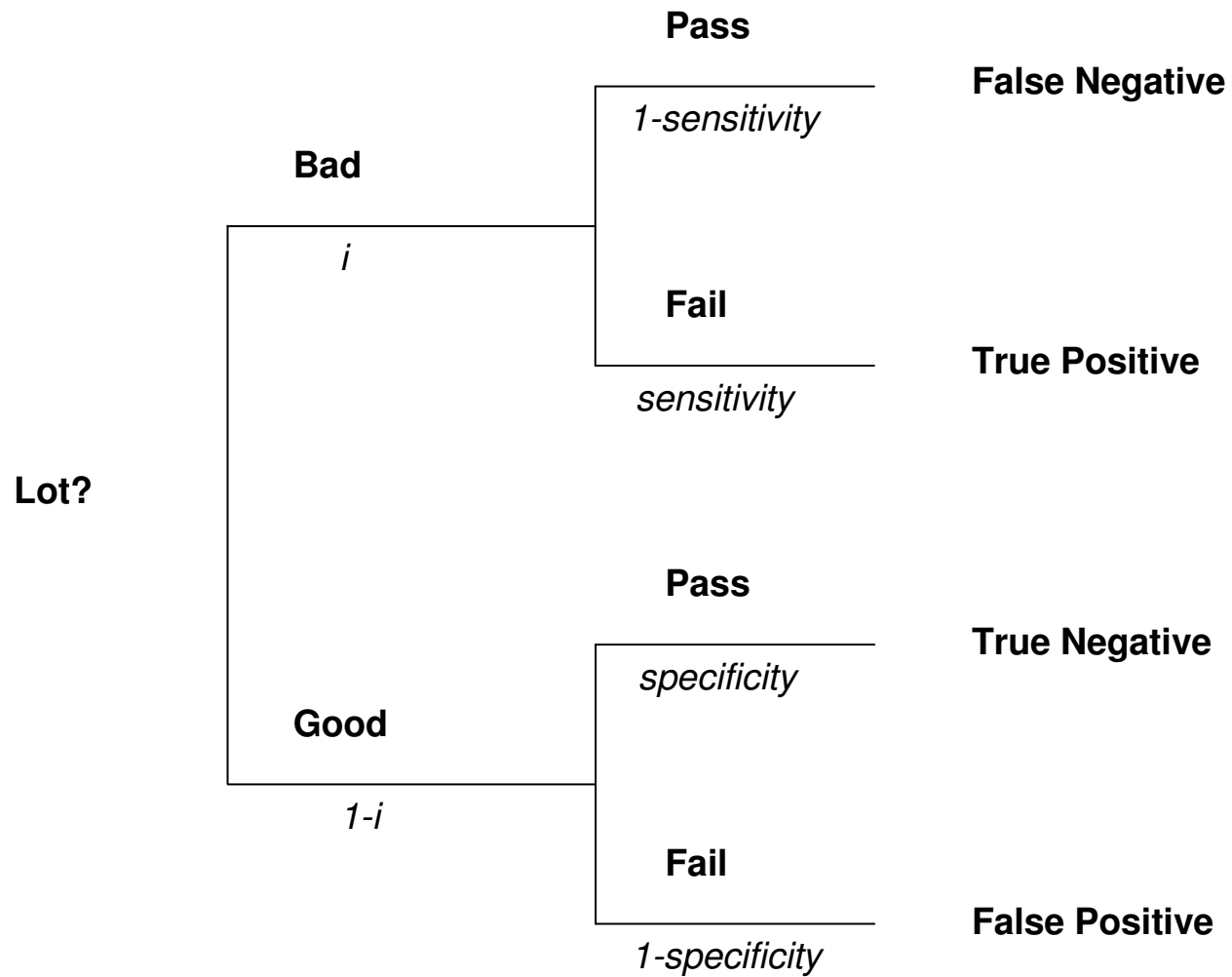
$$\text{Sensitivity} = \frac{TP}{TP+FN}$$

$$\text{Specificity} = \frac{TN}{TN+FP}$$

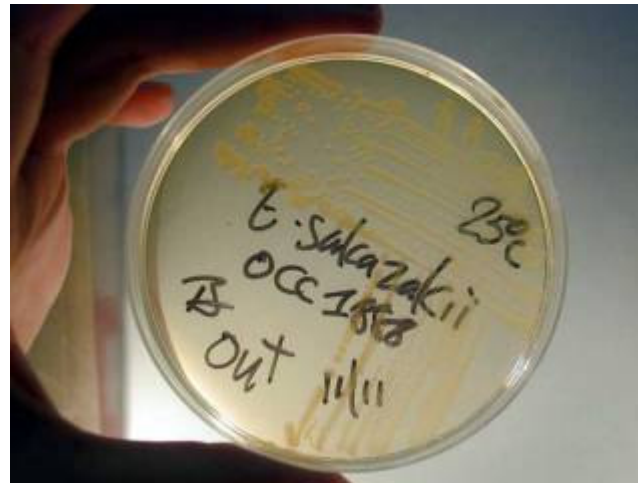
Why Specificity is Important?

		Incidence	Diagnostic Test	
			Fail	Pass
Batch	Good	$(1 - i)$	(1-Sensitivity)	Specificity
	Bad	i	Sensitivity	(1-Specificity)

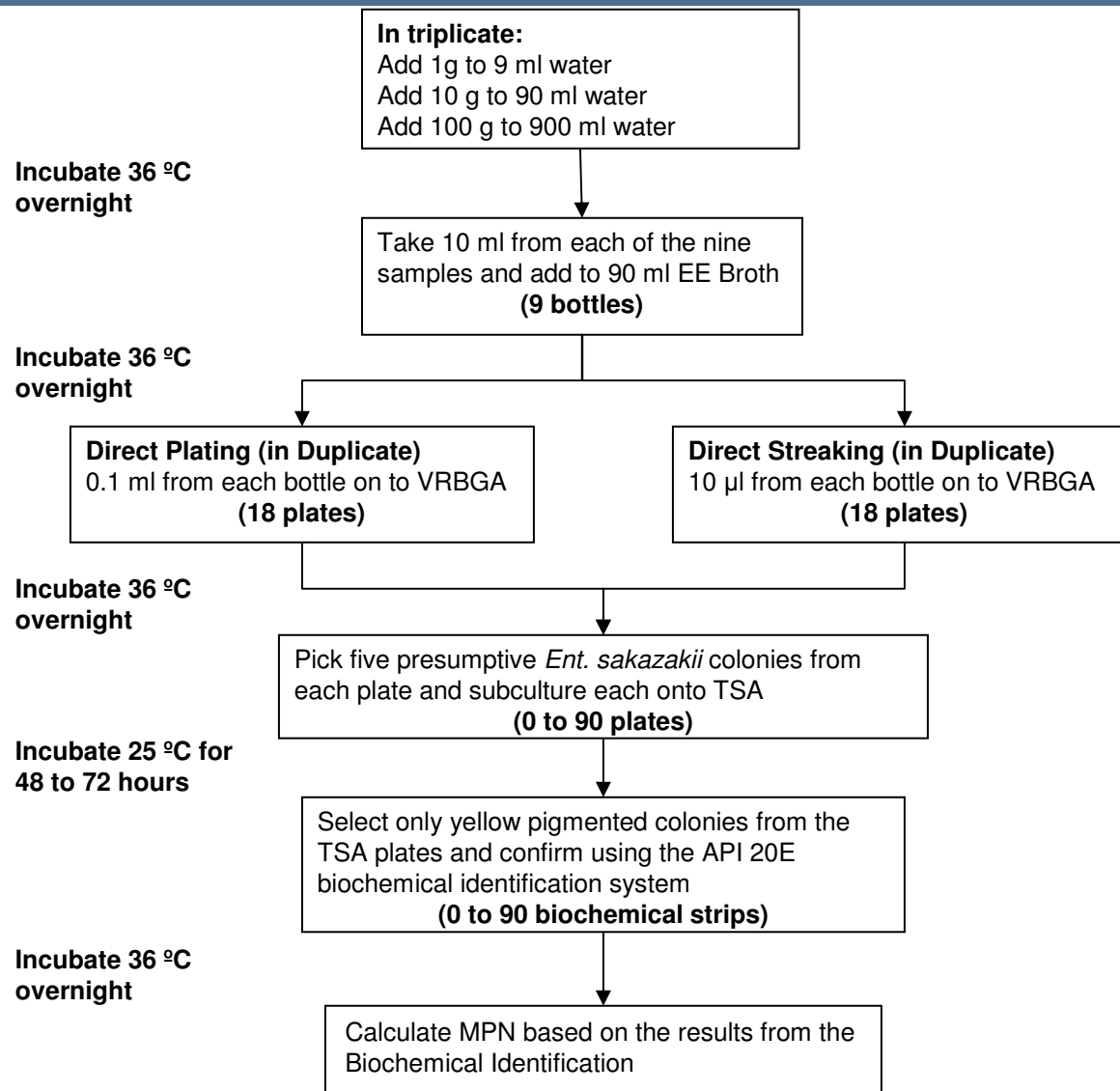
Decision Tree for a Diagnostic Test



FDA method for *Enterobacter sakazakii* (*Cronobacter* spp.)



Isolation and Enumeration of *Enterobacter sakazakii* (*Cronobacter* spp.) from Infant Formula (FDA Aug 2002)



FDA Culture Media

- EE Broth
 - Bile salts and Brilliant Green
- VRBGA
 - Bile salts and Crystal Violet
- TSA
 - Between 5 % and 25 % of strains do not express yellow pigment

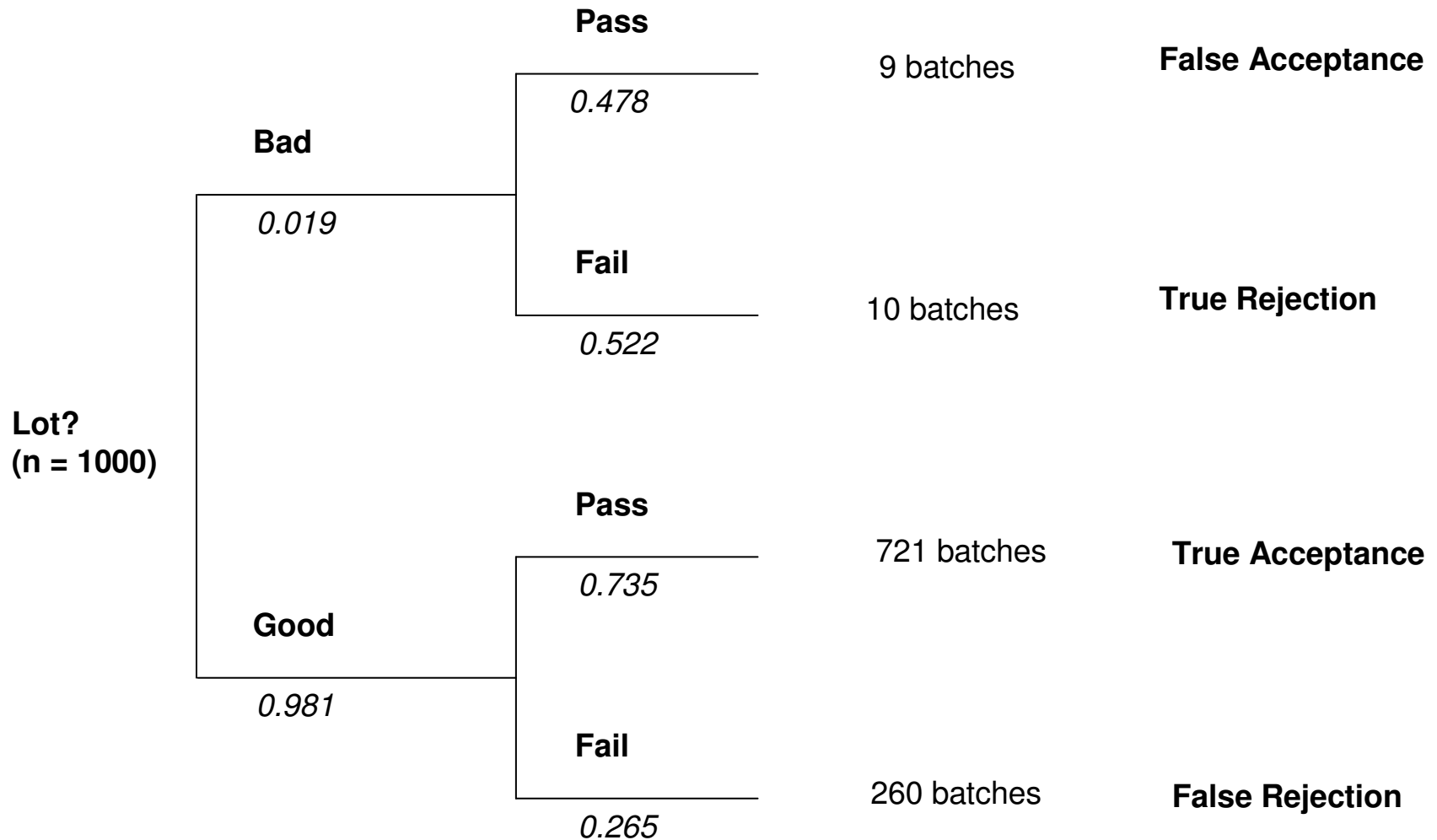
FDA method for *Enterobacter sakazakii*

		Incidence	Binary Test	
			Fail	Pass
Batch	Good	0.981	0.478	0.735
	Bad	0.019	0.522	0.265

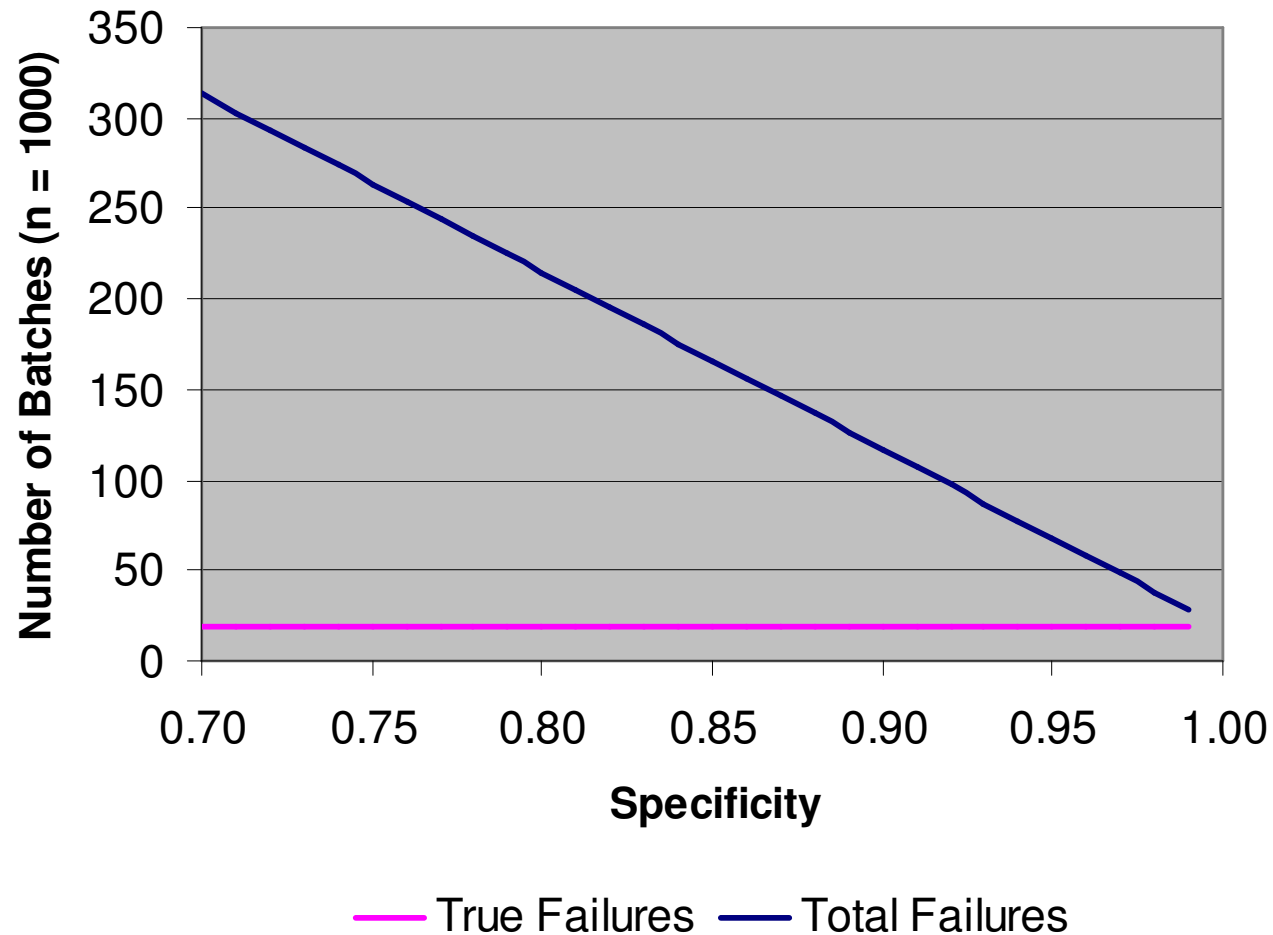
Appl Environ Microbiol. (2008) 74:2550-3

Int J Food Microbiol. (2008) 127:129-38

FDA method for *Enterobacter sakazakii*



Specificity – what is the cost to you? (incidence 1.9 %)



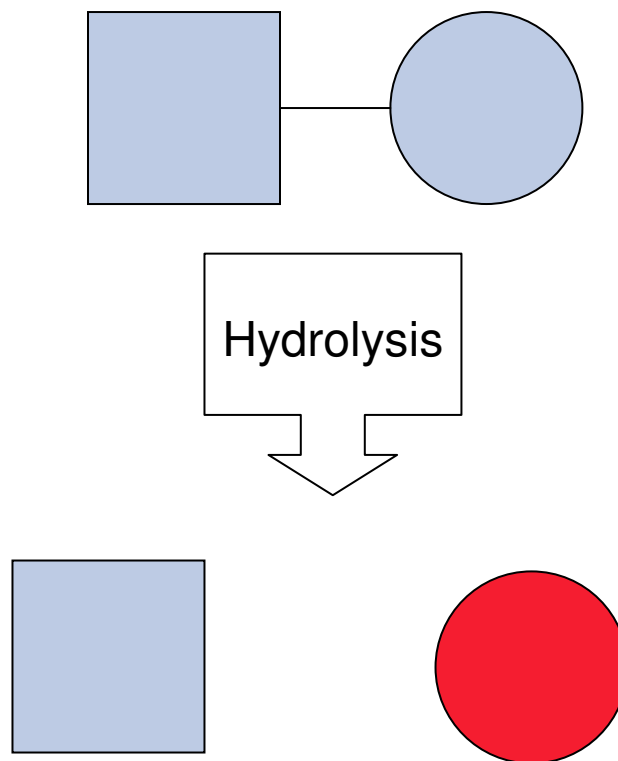
Pre-Enrichment Methods

- Sterile water
 - FDA method
- BPW
 - ISO TS 22964

Enrichment

- mLST
 - ISO TS 22964
- Enterobacter Sakazakii Selective Broth (ESSB)
 - AES Chemunex
- Enterobacter Sakazakii Enrichment (ESE) Broth
 - Appl Environ Microbiol. (2007) 73:48-52.
- Cronobacter Broth
 - Appl Environ Microbiol. (2008) 74:2550-3
- Cronobacter Screening Broth
 - Appl Environ Microbiol. (2008) 74:2550-3.

Chromogenic & Fluorogenic Substrates



Isolation & Identification: 4-methylumbelliferyl- α ,D-glucopyranoside (MU α Glc)

- Oh & Kang (OK) Agar
 - Appl Environ Microbiol. (2004) 70:5692-4
- Leuschner-Bew Agar
 - J AOAC Int. (2004) 87:604-13

Isolation & Identification: 5-bromo-4-chloro-3-indolyl- α ,D-glucopyranoside (X α Glc)

- Enterobacter Sakazakii Isolation Agar™ (ESIA)
 - ISO TS 22964
- Druggan-Forsythe-Iversen (DFI) Agar
 - Int J Food Microbiol. (2004) 96:133-9
- R&F Enterobacter sakazakii chromogenic plating medium (ESPM)
 - J Food Prot (2006) 69:315-22
- modified Druggan-Forsythe-Iversen (mDFI) Agar
 - Appl Environ Microbiol. (2008) 74:2550-3.

Selective Agents (MIC₉₀)

Relative resistance (%) of *Enterobacter* spp. compared to *Cronobacter* spp.

Antibiotic	MW	<i>E. sakazakii</i> (195)	<i>E. cloacae</i> (29)	<i>E. aerogenes</i> (25)	<i>E. agglomerans</i> (27)
Ampicillin	349.4	100	3200	3200	3200
Cefaloridin	415.5	100	800	800	800
Cephalothin	418.4	100	100	100	100
Cefamandole	462.5	100	3200	3200	3200
Cefoperazone	667.7	100	800	6400	6400
Ceforanide	519.6	100	6400	6400	6400
Cefotaxime	477.4	100	6400	400	25600
Cefoxitin	427.4	100	800	800	800
Cefsulodin	533.6	100	400	400	400
Ceftazidime	546.6	100	800	400	1600
Ceftriaxone	554.6	100	6400	400	25600
Cefuroxime	424.4	100	1600	1600	1600
Chloramphenicol	323.1	100	800	800	800
Ciprofloxacin	331.3	100	208	100	1667
Doxycyclin	444.4	100	800	400	3200
Gentamicin	477.6	100	1600	6400	200
Imipenem	299.3	100	400	800	200
Moxalactam		100	800	200	6400
Nalidixic Acid	232.2	100	400	200	1600
Neomycin	614.6	100	50	100	100
Norfloxacin	319.3	100	400	200	1600
Pipemidic acid		100	400	100	1600
Piperacillin	517.6	100	6400	6400	1600
Polymyxin B	1301.6	100	200	200	100
Rifampin	822.9	100	400	800	800
Sulfamethoxazole	253.3	100	100	100	100
Trimethoprim	290.3	100	800	1600	12800
Trimethoprim-Sulfa		100	800	800	3200

Data derived from Antimicrob Agents Chemother. (1986) 29:367-70



Conclusion

- Due to the low prevalence of Cronobacter in IFM and IFM manufacturing facilities a high specificity method is needed that can be used with product and environmental samples

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