FOOD BIOLOGICAL CONTAMINANTS

Detection of *Salmonella* in Fresh Cheese, Poultry Products, and Dried Egg Products by the ISO 6579 *Salmonella* Culture Procedure and the AOAC Official Method: Collaborative Study

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Three food types were analyzed for the presence of *Salmonella* by the AOAC culture method and by the International Organization for Standardization (ISO 6579:2002) culture method. Paired test portions of each food type were simultaneously analyzed by both methods. A total of 21 laboratories representing federal government agencies and private industry, in the United States and Europe, participated in this interlaboratory study. Foods were artificially contaminated with *Salmonella* and competing microflora if naturally contaminated sources were not available. No statistical differences ($p < 0.05$) were observed between the AOAC and ISO culture methods for fresh cheese and dried egg products. A statistically significant difference was observed for one of the 2 lots of poultry from the first trial. The poultry meat used in this run was radiation sterilized, artificially contaminated with *Salmonella* and competitive flora, and then lyophilized. A second trial was conducted with 2 separate lots of raw ground chicken that were naturally contaminated. The results from the second trial showed no statistical difference between the 2 culture methods. A third trial involving 4 laboratories was conducted on 2 separate lots of naturally contaminated raw poultry. Again, no statistically significant differences occurred. It is recommended that ISO 6579:2002 culture method for *Salmonella* be adopted Official First Action for the analysis of fresh cheese, fresh chilled and frozen poultry, and dried egg products.

Submitted for publication December 2002.
The recommendation was approved by the Methods Committee on Microbiology and Extraneous Materials as First Action. *See* "Official Methods Program Actions," (2003) *Inside Laboratory Management*, March/April issue.
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Over several decades, standardized methods for detection of *Salmonella* in food and food ingredients have been independently developed in both the United States and Europe. Although the basic procedures are similar, differences exist in the specified media and incubation conditions. The rapidly increasing development of international commerce and the critical need for worldwide cooperation and exchange of data, particularly during the occurrence of a food contamination outbreak, necessitates harmonized testing standards worldwide for the detection of *Salmonella*.

A multilaboratory international collaborative validation study was conducted to demonstrate the equivalence of the

Table 1. *Salmonella* growth levels[a] and colony description after ISO 6579 enrichment and isolation onto various agar plates

	PCA[b]			XLD[c]			HE[d]			BSA[e]			Rambach[f]			BGA[g]			XLT4[h]			SS[i]		
Salmonella species	CFU/mL after preenrichment with BPW[j]	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]
Aba	4	2	3	T	2	3	T	2	2	T	2	3	T	2	3	T	2	3	T	2	3	T	2	4
Abaetetuba	4	1	1	T	2	2	T	1	1	T	2	2	T	2	0.5	T	2	1	T	1	2	T	2	2
Aberdeen	4	1	2	T	1	2	T	1	2	T	1	3	T	1	2	T	1	2	T	0.8	2	T	1	2
Adelaide	6	1	3	T	1	3	T	2	3	T	2	3	T	1	3	T	2	2	T	1	3	T	1	3
Adjame	5	2	2	T	2	2	T	1	2	T	2	2	T	1	2	T	2	2	T	2	2	T	1	2
Agona	4	2	2	T	1	2	T	1	3	T	1	3	T	1	2	T	2	2	T	1	2	T	1	3
Ajiobo	4	2	2	T	0.6	2	T	0.3	1	T	0.8	2	T	0.7	2	T	0.7	2	T	0.6	2	T	0.4	2
Albany	4	3	3	T	3	2	T	0.6	1	T	2	2	T	2	3	T	2	2	T	3	3	T	1	2
Altona	3	2	2	T	2	2	T	1	2	T	2	2	T	2	2	T	2	2	T	2	2	T	2	2
Amina	5	1	1	T	1	2	T	0.9	0.6	T	0.8	1	T	1	2	T	1	1	T	0.8	2	T	1	1
Anatum	3	2	2	T	1	2	T	1	1	T	2	2	T	1	1	T	1	2	T	2	0.7	T	NA[m]	2
Antonio	3	0.7	0.5	A3	0.4	0.7	A4	0.4	0.4	T	0.8	0.6	T	0.6	0.6	T	0.9	0.6	A3	0.3	0.8	T	0.5	0.5
Apapa	0.7	0.4	0.3	T	0.2	0.9	T	0.3	0.1	T	0.002	NA[m]	T	0.2	0.2	T	0.4	0.3	T	0.2	0.9	T	0.1	0.1
Aqua	5	2	3	T	2	2	T	2	2	T	2	3	A4	2	2	T	3	3	T	2	2	T	1	3
Aschersleben	10	2	0.6	A2	2	0.5	A2	0.6	0.3	T	2	3	T	2	0.6	A2	2	0.4	A2	1	0.9	A2	1	0.5
Babelsberg	3	2	3	T	2	3	T	2	3	T	2	3	T	1	3	T	1	2	T	2	3	T	NA[m]	3
Baildon	5	2	2	T	2	2	T	1	2	T	2	2	T	1	2	T	2	3	T	3	3	T	2	2
Bergen	4	1	1	T	1	2	T	1	1	T	2	1	T	1	1	T	1	1	T	2	0.6	T	0.9	1
Bergues	6	2	1	T	2	2	T	2	1	T	2	2	T	2	1	T	2	NA[m]	T	2	1	T	2	2
Berlin	4	2	0.9	T	2	0.8	T	3	0.9	T	2	1	A2	2	2	T	2	2	T	2	0.8	T	2	0.8
Berta	4	2	3	T	2	3	T	1	2	T	2	2	T	1	2	T	2	2	T	1	2	T	1	2
Blijdorp	4	1	2	T	0.6	2	T	0.3	1	T	0.2	2	T	0.9	2	T	0.6	3	T	0.3	2	T	0.3	2
Blockley	5	0.9	1	T	2	1	T	0.9	1	T	1	2	T	1	1	T	1	2	T	1	2	T	1	2
Bovismorbificans	3	1	2	T	0.9	0.5	T	1	3	T	2	3	T	1	2	T	1	3	T	3	0.8	T	0.9	2
Braenderup	4	0.9	3	T	1	3	T	0.9	3	T	1	3	T	2	3	T	1	2	T	2	2	T	1	3
Brandenberg	4	1	3	T	2	2	T	2	0.4	T	2	2	T	2	2	T	1	2	T	2	2	T	1	2
Bredeney	6	2	2	T	2	1	T	1	0.6	T	2	2	T	2	1	T	2	2	T	2	1	T	2	2
Broughton	4	2	3	T	2	3	T	1	3	T	2	2	T	1	2	T	2	3	T	2	2	T	1	2
Caen	5	2	3	T	2	3	T	0.7	2	T	2	2	T	2	3	T	2	3	T	2	3	T	0.8	2
Cannbio	4	2	2	T	1	2	T	1	2	T	2	3	T	1	2	T	2	3	A2	2	2	T	1	2
Cannstatt	4	0.7	2	T	1	2	T	1	2	T	2	3	T	1	2	T	0.9	2	T	0.8	2	T	2	3
Carmel	4	0.5	3	A2	0.4	3	A2	0.3	3	T	0.7	3	T	2	3	T	0.8	2	T	0.4	2	T	0.5	3
Cerro	4	1	2	T	1	2	T	1	2	T	1	2	T	2	2	T	1	2	T	1	3	T	1	2
Chandans	4	2	2	T	1	2	T	1	2	T	2	2	T	2	2	T	0.7	2	T	2	2	T	2	2
Chicago	5	0.4	2	A2	0.4	2	T	0.3	2	T	0.5	2	T	0.3	2	T	0.4	2	T	0.4	2	T	0.4	2
Chingola	4	1	2	T	1	2	T	1	NA[m]	T	1	2	T	1	2	T	1	2	T	2	NA[m]	T	0.3	2
Choleraesuis	5	0.5	0.2	A3	1	0.2	T	0.4	0.2	A2	0.6	0.3	A2	0.8	0.2	T	1	0.3	A3	0.6	0.3	A3	0.5	0.2
Coein	4	1	3	T	2	2	T	1	2	T	2	2	T	2	2	T	2	3	T	2	2	T	1	2
Corvalis	3	2	2	T	1	3	T	0.9	1	T	2	3	T	1	2	T	1	2	T	2	2	T	1	2
Cubana	5	1	3	T	2	3	T	1	3	T	1	3	T	1	2	T	1	3	T	2	2	T	1	3

Table 1. (*continued*)

		PCA[b]		XLD[c]			HE[d]			BSA[e]			Rambach[f]			BGA[g]			XLT4[h]			SS[i]		
Salmonella species	CFU/mL after preenrichment with BPW[j]	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]
Derby	3	1	2	T	1	2	T	1	2	T	1	2	T	1	1	T	1	3	T	1	3	T	1	2
Djakarta	3	0.07	1	T	0.01	2	T	0.002	1	A3	0.05	1	T	0.03	1	T	0.04	2	T	0.03	2	T	0.008	2
Doom	4	2	3	T	2	3	T	2	2	T	2	3	T	2	4	T	2	3	T	2	3	T	1	3
Dublin	5	0.5	2	T	0.7	2	T	0.5	2	T	0.6	2	T	0.5	2	T	0.7	2	T	1	0.2	T	0.5	2
Durban	4	1	1	T	1	2	T	1	1	T	1	2	T	1	2	T	1	2	T	1	2	T	1	2
Ealing	4	2	3	T	2	3	T	1	3	T	2	3	T	2	3	T	2	3	T	2	3	T	2	4
Egusitoo	3	2	0.8	T	2	0.1	T	1	0.7	T	2	0.8	T	1	0.9	T	1	1	T	2	0.9	T	1	0.9
Enteritidis	6	1	1	T	2	1	T	0.8	0.9	T	2	2	T	2	1	T	2	2	T	2	2	T	1	1
Epinay	4	2	2	T	2	2	T	2	2	T	2	2	T	2	3	T	2	2	T	2	2	T	2	2
Fass	2	0.1	2	T	0.1	0.1	T	0.1	0.2	T	0.1	2	T	0.1	2	T	0.1	2	T	0.8	1	T	0.9	2
Give	3	1	2	T	2	2	T	1	2	T	2	2	T	1	2	T	1	2	T	1	2	T	1	3
Goldcoast	5	2	1	T	2	1	T	0.8	1	T	2	2	T	2	2	T	2	2	T	2	3	T	1	2
Hadar	4	1	3	T	1	2	T	0.7	1	T	1	3	T	1	3	T	2	3	T	1	3	T	1	3
Havana	4	2	3	T	2	2	T	0.8	2	T	2	3	T	2	2	T	2	3	T	2	3	T	0.7	2
Heidelberg	5	1	NA[m]	A2	1	1	A2	1	2	A2	1	2	T	0.9	0.8	T	1	2	A2	2	0.7	A2	2	1
Infantis	5	0.9	2	T	1	1	T	1	2	T	1	2	T	1	2	T	1	2	T	2	2	T	0.5	2
Kedougou	5	0.8	0.5	T	0.5	0.4	T	0.3	0.5	T	1	0.8	T	0.7	0.5	T	1	0.7	T	0.4	0.3	T	0.2	0.4
Kottbus	5	2	2	T	0.6	2	T	0.1	0.6	T	1	2	T	1	2	T	1	3	T	2	2	T	0.1	1
Leda	4	2	3	T	2	3	T	1	3	T	2	4	T	1	2	T	2	3	T	2	3	T	2	3
Liverpool	3	2	4	A4	2	3	A3	2	3	T	1	3	T	2	3	A2	2	3	A2	3	3	T	2	3
Livingstone	5	2	2	T	2	2	T	0.8	2	T	2	2	T	1	2	T	2	3	T	2	2	T	1	2
Llandoff	4	1	4	T	2	3	T	0.7	3	T	1	3	A3	1	3	T	1	3	T	1	0.5	T	1	3
London	4	0.8	3	T	0.9	3	T	0.5	2	T	1	2	T	0.8	2	T	1	3	T	1	3	T	0.6	2
Mbandaka	6	0.8	3	T	0.9	2	T	0.9	2	T	0.9	2	T	0.7	2	T	2	4	T	2	2	T	0.7	3
Meleagridis	4	1	2	T	1	2	T	0.7	1	T	2	2	T	0.6	2	T	1	2	T	0.9	1	T	0.8	2
Montevideo	9	2	2	T	1	2	T	2	2	T	2	2	T	2	2	T	2	3	A2	3	2	T	1	2
Montevideo H_2S negative	4	0.7	1	A2	0.6	2	A2	NA[m]	1	T	0.9	0.3	T	0.8	1	T	NA[m]	2	A2	0.7	0.8	A2	1	2
Muenchen	3	1	2	T	2	2	T	0.8	1	T	2	2	T	1	1	T	1	2	T	1	2	T	1	2
Muenster	2	2	2	T	2	2	T	1	2	T	2	2	T	2	2	T	2	2	T	3	2	T	2	NA[m]
Newport	4	1	3	T	1	3	T	1	2	T	2	2	T	1	2	T	1	2	T	1	3	T	2	3
Nima	3	1	3	T	1	2	T	1	2	T	1	3	T	1	2	T	2	3	T	1	3	T	1	3
Orion	2	0.8	3	T	1	3	T	0.6	2	T	1	2	T	0.7	2	T	1	3	T	0.6	3	T	0.9	2
Ouakam	4	2	2	T	2	2	T	1	1	T	2	2	T	2	2	T	3	3	T	2	2	T	NA[m]	2
Panama	3	2	2	T	2	2	T	2	1	T	2	2	T	2	2	T	2	2	T	2	2	T	2	2
Panama LDC neg.	4	1	2	T	1	1	T+A3	0.6	1	T	2	1	T	1	1	T	2	1	T	0.9	0.9	T	0.9	1
Panama saccharose+	4	1	2	A4	1	2	A3	1	2	T	1	1	T	1	2	A2	2	2	A2	2	2	T	1	2
Panama saccharose+	3	1	2	A4	1	0.7	T+A3	0.5	0.9	T	1	1	T	0.9	1	T+A2	1	1	T	0.5	0.8	T+A4	0.6	1
Paratyphi B	5	1	2	T	1	3	T	1	2	T	1	3	T	2	3	T	2	3	T	2	3	T	1	3
Paratyphi C	4	0.0002	<0.0001	NG[n]	NG[n]	NG[n]	NG[n]	NG[n]	NG[n]	NG[n]	NG[n]	NG[n]	NG[n]	NG[n]	NG[n]	T+A3	ND[o]	ND[n]	NG[n]	NG[n]	NG[n]	NG[n]	NG[n]	NG[n]
Perth	3	0.03	1	A2	0.03	3	A2	0.02	2	T	0.03	1	T	0.02	1	T	0.05	1	A2	0.02	0.7	A2	0.02	0.9

Table 1. (*continued*)

Salmonella species	PCA[b]			XLD[c]			HE[d]			BSA[e]			Rambach[f]			BGA[g]			XLT4[h]			SS[i]		
	CFU/mL after preenrichment with BPW[j]	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]
Quentin	3	2	2	A2	2	2	A2	0.6	0.8	T	2	2	T	2	2	T	2	2	A2	2	2	A2	0.7	0.9
Reading	4	0.7	2	T	0.6	2	T	0.5	1	T	0.9	2	T	0.9	2	T	0.9	2	T	0.9	2	T	0.8	2
Regent	3	1	1	A4	1	2	A3	0.8	1	T	1	1	T	0.5	1	A2	0.9	1	A2	0.6	1	T	0.8	0.9
Rissen	5	2	2	T	3	2	T	2	1	T	3	2	T	1	2	T	2	2	T	2	1	T	2	2
Ruiru	4	2	2	T	2	3	T	NA[m]	2	T	2	3	T	1	3	T	2	2	T	1	3	T	1	3
Saintemarie	6	0.4	0.9	T	0.3	0.7	T	0.2	0.5	T	2	0.7	T	2	0.7	T	0.3	0.7	T	0.3	0.8	T	0.2	0.6
Saintpaul	6	2	2	T	1	1	T	0.6	2	T	2	3	T	1	2	T	2	3	T	3	2	T	1	2
Saphra	5	2	2	A2	0.5	2	A2	1	2	T	1	2	T	2	2	T	1	2	A2	2	2	A2	1	2
Schwarzengrund	5	2	3	T	2	3	T	1	2	T	2	2	T	2	3	T	3	0.7	T	1	3	T	2	3
Sculcoates	4	1	2	T	1	1	T	1	2	T	2	2	T	1	2	T	1	2	T	1	2	T	1	2
Senftenberg	4	1	2	T	1	2	T	0.9	1	T	2	2	T	1	2	T	1	3	T	1	1	T	0.8	2
Senftenberg lactose+; H_2S negative	4	0.6	2	A4	0.5	2	A3	0.2	1	T	0.5	1	A4	0.3	2	A2	0.6	1	A2	0.3	2	A4	0.2	1
Senftenberg saccharose+; LDC neg.	3	1	1	T	0.7	1	T	NA[m]	1	T	1	2	T	1	2	T	NA[m]	1	T	0.6	1	T	0.9	2
Shamba	5	0.5	2	T	0.3	2	T	0.3	2	T	0.6	2	T	0.4	1	T	0.6	1	T	0.3	1	T	0.3	2
Sofia malonate+	4	2	2	A2	1	NA[m]	A2	0.9	1	A4	1	1	T	1	NA[m]	A3	2	1	A2	1	NA[m]	A2	1	NA[m]
Thompson	6	2	0.7	T	2	2	T	0.9	2	T	2	2	T	1	1	T	1	3	T	2	2	T	0.5	1
Typhi MB255	3	0.002	0.06	A4	ND[o]	0.09	A4	ND[o]	0.09	T	ND[o]	0.2	T+A2	ND[o]	0.09	T	ND[o]	0.2	A2	ND[o]	0.02	A4	ND[o]	0.02
Typhi MB257	4	0.007	0.07	A4	ND[o]	0.06	A4	ND[o]	0.07	T	ND[o]	0.08	T+A2	ND[o]	0.07	T	ND[o]	0.1	A2	ND[o]	0.04	A4	ND[o]	0.05
Typhi MB37	3	0.005	0.1	A3	ND[o]	0.02	A4	ND[o]	<0.001	T	ND[o]	0.03	T+A2	ND[o]	0.03	T	ND[o]	0.04	A3	ND[o]	<0.001	A4	ND[o]	<0.001
Typhi MB38	3	0.04	0.05	A4	ND[o]	0.05	A4	ND[o]	0.04	T	ND[o]	0.09	T+A2	ND[o]	0.001	T	ND[o]	<0.001	A3	ND[o]	0.005	A4	ND[o]	0.003
Typhi MB39	2	0.01	0.06	A3	ND[o]	0.06	A4	ND[o]	0.05	T	ND[o]	0.05	T+A2	ND[o]	0.03	T	ND[o]	0.06	A3	ND[o]	0.002	A4	ND[o]	0.02
Typhi MB40	4	0.007	0.1	A3	ND[o]	0.09	A4	ND[o]	0.07	T	ND[o]	0.1	T+A2	ND[o]	0.07	T	ND[o]	0.1	A3	ND[o]	0.02	A4	ND[o]	0.03
Typhi MB41	3	0.009	0.06	A4	ND[o]	0.06	A4	ND[o]	0.04	T	ND[o]	0.1	T+A2	ND[o]	0.04	T	ND[o]	0.1	A3	ND[o]	0.002	A4	ND[o]	0.01
Typhi MB42	4	0.005	0.2	A4	ND[o]	0.03	A4	ND[o]	0.04	T	ND[o]	0.06	T+A2	ND[o]	0.05	T	ND[o]	0.04	A2	ND[o]	0.01	A4	ND[o]	0.01
Typhimurium	6	2	2	T	2	2	T	2	1	T	2	1	T	2	1	T	2	2	T	2	2	T	2	1
Veneziana	7	0.6	2	T	0.6	2	T	0.4	1	T	0.8	2	T	0.9	1	T	0.9	2	T	0.5	1	T	0.4	2
Virchow	8	0.5	3	T	0.9	3	T	0.4	2	T	0.9	4	T	0.8	3	T	0.8	4	R	0.5	3	T	0.4	2
Weltevreden	3	0.6	2	T	2	2	T	0.4	2	T	0.6	2	T	0.4	2	T	0.4	2	T	0.4	2	T	0.7	3
Wentworth	5	2	3	T	2	3	T	1	3	T	2	2	T	2	3	T	2	2	T	2	2	T	1	2
(II) (6),14:k:e,n,x	5	0.6	2	A4	0.6	2	T	0.6	2	A4	0.6	2	T	0.5	2	T	1	2	T	0.5	2	T	0.4	2
(II)1,9,12,46,27:a:z6	4	0.9	2	T	0.9	3	T	0.5	2	T	0.1	2	A4	0.5	2	T	1	2	T	0.9	2	T	0.9	3
(II)11:z:e,n,x	5	NG[n]	4	T	NG[n]	3	T	NG[n]	0.2	T	NG[n]	4	T	NG[n]	3	T	NG[n]	3	T	NG[n]	3	T	NG[n]	4
(II)13,22:z:-	5	1	2	T	1	3	T	0.9	3	T	1	3	T	1	3	T	2	3	T	1	2	T	1	3
(II)16:b:e,n,x	3	0.4	2	T	1	2	T	0.2	3	A4	0.3	2	T	0.3	2	T	0.4	3	T	0.09	2	T	0.2	1
(II)3,10:a:l,v	3	0.7	2	T	1	2	T	2	2	T	1	3	T	1	2	T	1	2	T	1	2	T	1	3
(II)4,5:-	4	1	2	T	1	2	T	1	1	T	1	2	T	1	2	T	0.4	2	T	1	2	T	1	2
(II)50:z:e,n,x	20	0.2	0.6	A2	0.03	0.5	A4	0.002	0.3	T	0.1	0.5	T	0.05	0.4	A3	0.2	0.9	A2	0.07	0.2	T	0.004	0.4
(II)6,7:z42:1,5,7	4	2	0.5	T	0.4	0.5	T	0.2	0.2	T	0.4	0.5	T	0.3	0.6	T	0.5	0.7	T	0.3	0.5	T	0.4	0.5
(II)9,12:l,v:e,n,x	5	1	3	T	1	2	T	2	3	T	1	2	T	1	3	T	0.8	2	T	0.9	3	T	0.9	3
(II)1,13,23:g,m,s,t:-	3	0.6	3	T	0.8	3	T	0.4	2	T	0.4	2	T	0.6	2	T	0.6	3	T	0.8	3	T	0.4	2

Table 1. (*continued*)

		PCA[b]		XLD[c]			HE[d]			BSA[e]			Rambach[f]			BGA[g]			XLT4[h]			SS[i]		
Salmonella species	CFU/mL after preenrichment with BPW[j]	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]	Colony descrip.	CFU/mL after RVS enrichment[k]	CFU/mL after MKTT+n enrichment[l]
(IIIb)61:i:z53	4	0.1	2	T+A2	0.05	3	T_A3	0.01	NA[m]	T	0.1	3	A4	0.03	2	T+A2	0.1	3	T	0.03	2	T+A4	0.01	3
(IV)51:a:-	2	0.7	0.3	A2	0.5	0.2	A2	0.4	0.2	T	0.5	0.3	T	0.3	0.2	NG[n]	NG[n]	NG[n]	A2	0.3	0.02	A2	0.4	0.2
(IV)1,42:g,z51:-	6	2	2	T+A2	2	2	T+A3	1	2	T	2	2	A2	2	3	T	2	2	T	2	2	T	2	3
(IV)17:z29:-	3	2	2	T+A2	2	1	T+A3	1	2	T	2	1	A3	1	1	T	2	2	T	1	1	T	1	1
(IV)44:a:-	5	0.8	0.9	A2	1	1	A2	1	0.8	T	1	0.8	A2	1	0.8	T	1	0.5	T	1	0.7	A2	0.9	0.7

[a] All populations are reported as log 10^8 CFU/mL of enrichment broth.

[b] PCA = Plate count agar.

[c] XLD = Xylose lysine desoxycholate agar. Colony description: typical = pink colonies with large black centers (sometimes completely black colonies); A2 = yellow/white colonies with small black centers; A3 = pink colonies without black centers; A4 = yellow/white colonies without black centers.

[d] HE = Hektoen enteric agar. Colony description: typical = blue-green colonies with large black centers (sometimes completely black colonies); A2 = yellow/green colonies without black centers; A3 = orange colonies with small black centers; A4 = yellow/green colonies with black centers; A5 = transparent colorless colonies; T+A3 = a mixture of typical colonies and orange colonies with black centers.

[e] BSA = Bismuth sulfite agar. Colony description: typical = brown, gray, or black colonies; sometimes with a metallic sheen. Surrounding medium is usually brown at first, but may turn black in time with increased incubation, producing the halo effect; A2 = green colonies with darkening of the edge, no darkening of surrounding medium; A3 = small colorless colonies; A4 = green colonies with dark centers.

[f] Rambach agar. Colony description: typical = fushia to crimson colonies; A2 = salmon pink colonies; A3 = colorless colonies, sometimes with dark pink centers; A4 = green colonies and dark pink/purple colonies.

[g] BGA = Brilliant green-phenol red agar. Colony description: typical = colonies are lightly transparent, reddish color due to the indicator change; A2 = colonies are lightly transparent, yellow color due to the indicator change; A3 = small colonies that are lightly transparent, yellow color due to the indicator change.

[h] XLT4 = Xylose lysine Tergitol 4 agar. Colony description: typical = colorless colonies with black centers; A2 = white colonies (no black centers); A3 = small lightly transparent colonies (no black centers).

[i] SS = *Salmonella-Shigella* agar. Colony description: typical = lightly transparent/colorless colonies with large black centers; A2 = colorless with or without small black center; A3 = colorless colonies with dark centers; A4 = light pink colonies.

[j] BPW = Buffered peptone water.

[k] RVS = Rappaport-Vassiliadis soya broth.

[l] MKTT+n = Muller-Kauffmann + novobiocin broth.

[m] NA = Not available, instrument malfunction.

[n] NG = No growth on agar plate.

[o] ND = Growth levels not determined for this culture.

Table 2. Non-*Salmonella* growth levels[a] and colony description after ISO 6579 enrichment and isolation onto various agar plates

	PCA[b]			XLD[c]		BSA[d]		Rambach[e]		BGA[f]		XLT4[g]		SS[h]	
Microorganism tested	CFU/mL after preenrich-ment with BPW[i]	CFU/mL after RVS enrichment[j]	CFU/mL with MKTT+n enrichment[k]	Colony morphology after RVS enrichment[j]	Colony morphology after MKTT+n enrichment[k]	Colony morphology after RVS enrichment[j]	Colony morphology after MKTT+n enrichment[k]	Colony morphology after RVS enrichment[j]	Colony morphology after MKTT+n enrichment[k]	Colony morphology after RVS enrichment[j]	Colony morphology after MKTT+n enrichment[k]	Colony morphology after RVS enrichment[j]	Colony morphology after MKTT+n enrichment[k]	Colony morphology after RVS enrichment[j]	Colony morphology after MKTT+n enrichment[k]
Escherichia coli	2	0.2	ND[l]	A	A	A	NG	A	A	A	NG	A	A	A	A
Escherichia coli	3	0.2	ND[l]	A	A	NG	NG	A	A	A	A	A	A	A	A
Escherichia coli	5	2	0.1	A	A	A	A	A	A	A	A	A	A	A	NG
Citrobacter freundii	3	0.003	ND[l]	A	A	A	A	A	A	A	A	A	A	A4	A4
Citrobacter freundii	7	0.03	0.8	A	A	A	A	A	A	A	A	A	A	A	A
Citrobacter freundii	4	0.0002	2	A	A	A	A	A	A	A	A	A	A	A	A
Citrobacter sp.	2	1	ND[l]	A	A	A	A	A	A	T	T	A	A	A	A
Citrobacter koseri	6	0.0002	ND[l]	NG	NG	NG	A	NG	A	A3	A3	A	A	NG	A
Citrobacter amalonaticus	2	0.9	ND[l]	A	A	A	A	A	A	A3	A3	A	A	A	A
Citrobacter koseri/farmeri	6	0.00004	1	A	A	NG	A	A	A	A	A	A	A	A	A
Citrobacter sedlakii	9	3	0.9	A	A	A	A	A	A	T	T	A	A	A	A
Citrobacter amalonaticus/farmeri	10	0.1	3	A	A	A	A	A	A	T	T	A	A	A	A
Proteus mirabilis	6	0.003	ND[l]	A4	A4	A	A	A	A	A3	A3	NG	NG	A2	A2
Proteus mirabilis	7	0.0008	ND[l]	A2	A2	A	A	NG	A2	A3	NG	NG	NG	A2	A2
Proteus vulgaris	4	<[m]	ND[l]	A	A	NG	NG	NG	A	NG	NG	NG	NG	NG	A3
Providencia stuartii	3	0.0001	ND[l]	NG	A	NG	A	A	A2	NG	NG	NG	A3	NG	A
Morganella morganii	10	0.0001	0.8	A3	A3	A	A	A	A	NG	A3	NG	A3	A	A
Yersinia enterocolitica	0.7	0.001	ND[l]	A	A	NG	NG	A	A	A3	NG	NG	NG	A	A4
Serratia marcescens	5	0.000002	ND[l]	NG	NG	NG	NG	A	A	NG	A3	NG	NG	NG	A4
Klebsiella pneumoniae	3	0.00003	ND[l]	A	A	A	A	A	A	A	A	A	A	A4	A4
Klebsiella oxytoca	2	0.000009	ND[l]	NG	A	NG	A	A	A	A	A	NG	A	NG	A4
Klebsiella oxytoca	6	ND[l]	0.2	A	A	T	T	A	A	A	A	A	A	A	A
Enterobacter cloacae	5	0.3	0.6	A	A	A	A	A	A	A	A	A	A	NG	A
Hafnia alvei	5	0.2	0.6	A	A	A	A	A	A	A3	A3	A	A	A	A
Hafnia alvei	9	0.2	2	A	A	A	A	A	A	A	A	A	A	A	A
Hafnia alvei	8	ND[l]	0.4	A3	A3	A	A	A	A	A	A	A	A	A	A
Hafnia alvei	10	0.001	ND[l]	A	A	A	A	A	A	NG	A	A3	A	A	A
Hafnia alvei	10	0.0003	ND[l]	A	A	A	A	A	A2	NG	A3	A	A	A	A
Pseudomonas stutzeri	1	<[m]	0.1	NG	A3	NG	NG	NG	A	NG	A3	NG	A3	NG	A4
Kocuria kristinae	<0.0004	<[m]	<[m]	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Staphylococcus aureus	2	<[m]	<[m]	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Staphylococcus aureus	4	<[m]	<[m]	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Staphylococcus aureus	3	<[m]	ND[l]	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Staphylococcus chromogenes	1	<[m]	<[m]	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Enterococcus faecalis	0.2	0.004	0.000002	A	A	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

[a] All populations are reported as log 10^8 CFU/mL of enrichment broth (i.e., 5×10^8 is reported as 5).

[b] PCA = Plate count agar.

[c] XLD = Xylose lysine desoxycholate agar. Colony description: A = atypical, different characteristics from *Salmonella* colonies; not likely to be misinterpreted; A2 = yellow/white colonies with small black centers; A3 = pink colonies without black centers; A4 = yellow/white colonies without black centers; NG = no growth on agar plate.

Table 2. (*continued*)

Microorganism tested	PCA[b]		XLD[c]		BSA[d]	Rambach[e]	BGA[f]	XLT4[g]	SS[h]
	CFU/mL after preenrichment with BPW[i]	CFU/mL after RVS enrichment[j]	CFU/mL with MKTT+n enrichment[k]	Colony morphology after RVS enrichment[j]	Colony morphology after MKTT+n enrichment[k]	Colony morphology after RVS enrichment[j]	Colony morphology after MKTT+n enrichment[k]	Colony morphology after RVS enrichment[j]	Colony morphology after MKTT+n enrichment[k]	Colony morphology after RVS enrichment[j]	Colony morphology after MKTT+n enrichment[k]	Colony morphology after RVS enrichment[j]	Colony morphology after MKTT+n enrichment[k]	Colony morphology after RVS enrichment[j]	Colony morphology after MKTT+n enrichment[k]

[a] BSA = Bismuth sulfite agar. Colony description: typical = brown, gray, or black colonies; sometimes with a metallic sheen. Surrounding medium is usually brown at first, but may turn black in time with increased incubation, producing the halo effect; A = atypical different characteristics from *Salmonella* colonies; not likely to be misinterpreted; NG = no growth on agar plate.

[b] Rambach agar. Colony description: A = atypical, different characteristics from *Salmonella* colonies; not likely to be misinterpreted; A2 = salmon pink colonies; NG = no growth on agar plate.

[c] BGA = Brilliant green-phenol red agar. Colony description: typical = colonies are lightly transparent, reddish color due to the indicator change; A3 = small colonies that are lightly transparent, yellow color due to the indicator change; NG = no growth on agar plate.

[d] XLT4 = Xylose lysine Tergitol 4 agar. Colony description: A = atypical, different characteristics from *Salmonella* colonies; not likely to be misinterpreted; A3 = small lightly transparent colonies (no black centers); NG = no growth on agar plate.

[e] SS = *Salmonella-Shigella* agar. Colony description: A = atypical, different characteristics from *Salmonella* colonies; not likely to be misinterpreted; A2 = colorless with or without small black center; A3 = colorless colonies with dark centers; A4 = light pink colonies. NG = no growth on agar plate.

[f] BPW = Buffered peptone water.

[g] RVS = Rappaport-Vassiliadis soya broth.

[h] MKTT+n = Muller-Kauffmann + novobiocin broth.

[i] ND = No data, technician error.

[j] < = Growth is <0.000002 CFU/mL.

AOAC culture method (1) to the draft Standard prEN ISO/DIS 6579:2000 culture method (2) for the detection of *Salmonella* in fresh cheese, dried egg products, and poultry products.

Collaborative Study

Design of Study

This interlaboratory study was conducted in 2 parts. In the first phase of the study, specificity data were generated for the isolation agars used in the ISO 6579 enrichment protocol (2). A total of 125 *Salmonella* strains and 35 non-*Salmonella* strains were examined. All strains were initially pre-enriched in buffered peptone water (BPW). Growth in the overnight BPW enrichment was enumerated on plate count agar (PCA). A 0.1 mL aliquot of the pre-enrichment broth was transferred to a 10 mL tube of Rappaport-Vassiliadis soya peptone broth (RVS) and incubated overnight. A 1.0 mL aliquot of pre-enrichment broth was also transferred to a 10 mL tube of Muller-Kauffmann tetrathionate broth + novobiocin (MKTT+n) for overnight incubation. The relative recovery of organisms (both *Salmonella* and non-*Salmonella*) in each of the selective broths was compared by counting populations grown in broth cultures on a nonselective agar (e.g., PCA). Cultures grown in selective broths were also spiral plated onto several *Salmonella* isolation agars and counted to compare the selectivity of the agars. Colony morphology was noted for each organism isolated on each selective agar. The data for the *Salmonella* strains are presented in Table 1. Data for non-*Salmonella* strains are presented in Table 2. All specificity data were produced at Agence Française de Sécurité Sanitaire des Aliments (AFSSA; Ploufragan, France).

Three food types were tested for the second part of this interlaboratory study: fresh cheese, dried egg product, and diced poultry. Raw ground poultry was also analyzed in 2 independent runs after the initial evaluation of diced poultry. If a naturally contaminated source was not obtained for a selected food group, the product was artificially contaminated with a species of *Salmonella* and an excess of competitive microflora (Table 3). The contaminating microflora was at least 100 times higher than the *Salmonella* culture used to inoculate the food. Foods were artificially contaminated with *Salmonella* at 2 levels: a high level, where predominantly positive results were expected, and a low level, where fractional recovery was anticipated. Recovery was fractional when at least one of the methods being compared yielded a partial number of positive determinations at one contamination level.

Five test portions were tested for each contamination level in the initial and second trials of dried egg product. For the second (poultry II) and third (poultry III) trials of raw poultry, 6 test portions were analyzed for each contamination level to comply with revisions to the AOAC sample requirements for collaborative studies evaluating qualitative methods (3).

Inoculation of Foods

Laboratories in Europe prepared all foods, except poultry II and III. Fresh cheese test portions were prepared by Cen-

Table 3. Foods types analyzed

Food type	*Salmonella* strains used	O group	Competing microflora
Fresh cheese	*S. montevideo* (lactose positive strain)	C1	*Lactococcus lactis lactis, Enterococcus faecalis, Lactobacillus paracasei, Lactobacillus plantarum, Escherichia coli, Klebsiella oxytoca, Citrobacter freundii, Pseudomonas aeruginosa*
Dried egg I	*S. panama*	D1	*Enterococcus faecium*
Dried egg II	*S. panama*	D1	*Enterococcus faecium*
Poultry I	*S. typhimurium*	B	*Lactobacillus plantarum, Micrococcus luteus, Pseudomonas aeruginosa, Citrobacter freundii*
Poultry II	*Salmonella* spp.[a]		Naturally contaminated
Poultry III	*Salmonella* spp.[b]		Naturally contaminated

[a] *Salmonella* species with somatic (O) groups B, C_1, and C_2.
[b] *Salmonella* species with somatic (O) group C_2.

tre d'Étude et de Controle des Analyses en Industrie Laitière (CECA LAIT; Poligny, France); dried egg product test portions were prepared by Rijks Instituut voor Volksgezondheid en Milieu–Microbiological Laboratory for Health Protection (RIVM; Bilthoven, The Netherlands) for both trials (dried egg product I and II). The artificially contaminated diced poultry (poultry I) was prepared by the Ministry of Agriculture, Fisheries and Food [Central Science Laboratory (CSL), York, UK]. Poultry trials II and III test portions were prepared by BioControl Systems (BCS; Bellevue, WA). The *Salmonella* species and other competing organisms used to contaminate each food are listed in Table 3. The fresh cheese test portions were inoculated with a lactose-positive, atypical strain of *S. montevideo*, provided by the U.S. Food and Drug Administration (FDA).

All foods, except poultry II and III, were artificially inoculated to achieve fractional recovery for at least one contamination level on the day of analysis. Fresh cheese, dried egg product, and poultry I test portions were maintained in a chilled condition during shipment; poultry II and III test portions were shipped frozen. Most probable number (MPN) procedures were conducted on the day of initiation of analyses and were used to estimate the number of *Salmonella* per gram for each food. Three replicates of 100, 10, 1, and 0.1 g test portions were evaluated as stated in the AOAC Official Method for poultry and dried egg product. Fresh cheese was enriched in accordance with the FDA *Bacteriological Analytical Manual* (BAM; 4).

Test Portion Distribution

Test portions of each product were prepared and distributed to appropriate collaborators. Food types prepared in Europe, which were to be analyzed in the United States, were shipped by express mail under chilled conditions (one shipment per food) to BCS. The appropriate test portions were then shipped overnight to North American participants. The poultry II test portions, prepared by the BCS laboratory, were shipped on dry ice to AFSSA and subsequently delivered overnight to the European collaborators. Poultry III test portions were shipped overnight on dry ice from the BCS laboratory to the participating collaborators in North America.

Collaborators received paired sets of 15 test portions of fresh cheese, dried egg product I, and poultry I, each containing 25 g test material per portion. For each inoculated food type, 5 of the 15 test portions were uninoculated controls. For the second trial of dried egg product (dried egg product II), collaborators received paired sets of 10 test portions containing 25 g test material in each portion. For poultry II and III, collaborators received paired sets of 12 test portions containing 25 g test material in each portion.

Analysis of Foods

Collaborators were instructed to analyze paired test portions by both the AOAC (1) and ISO 6579:2002 (2) culture methods. The enrichment procedures for AOAC and ISO methods are given in Figures 1 and 2, respectively. Enrichments from all test portions were analyzed to determine if *Salmonella* were present according to ISO and AOAC methods. Raw data were recorded on the appropriate worksheets and submitted to AFSSA (for the European laboratories) and BCS (for U.S. laboratories) for review and tabulation of results.

Statistical Analysis

A pair-wise statistical analysis of the methods was performed for each food type and inoculation level by using the method of McNemar (5). A Chi square value of 3.84 was indicative of a significant difference at the 5% level. Data analysis included sensitivity and specificity rates, and percent agreement for each food type, according to the method of McClure (6).

AOAC Official Method 2002.10
Salmonella Detection in Fresh Cheese, Dried Egg Products, and Fresh Chilled and Frozen Poultry

ISO 6579:2002
First Action 2002

(Applicable for the detection of *Salmonella* in fresh cheese, dried egg products, and fresh chilled and frozen poultry.)

See Table **2002.10** for the results of the interlaboratory study supporting acceptance of the method.

Note: Selective enrichment combination Muller-Kauffmann tetrathionate broth/Rappaport-Vassiliadis soya broth may not be effective for the recovery of *S. typhi* and *S. paratyphi* from foods.

A. Principle

Salmonella are resuscitated under nonselective conditions and then propagated through the use of selective enrichment broths to levels that can be successfully recovered when isolated on selective agars.

B. Apparatus

(**a**) *Masticator*.—IUL Instruments (Cincinnati, OH) stomacher (masticator), or equivalent, for homogenizing test portions.

(**b**) *Masticator bags, sterile*.—Appropriate capacity to accommodate test portions and masticator used.

(**c**) *Top loading balance*.—Capacity of 2000 g with sensitivity of 0.1 g.

(**d**) *Incubator*.—Maintaining 35–37°C.

(**e**) *Water baths*.—Maintaining 41.5 ± 1.0°C.

(**f**) *Sterile culture tubes with rack*.—16 × 150 mm tubes.

(**g**) *Syringe with filter*.—10 mL sterile plastic syringe with 0.2 μm filter.

(**h**) *Pipets*.—Sterile glass or plastic pipets, 1 mL with 0.01 mL graduations; 5 and 10 mL with 0.1 mL graduations.

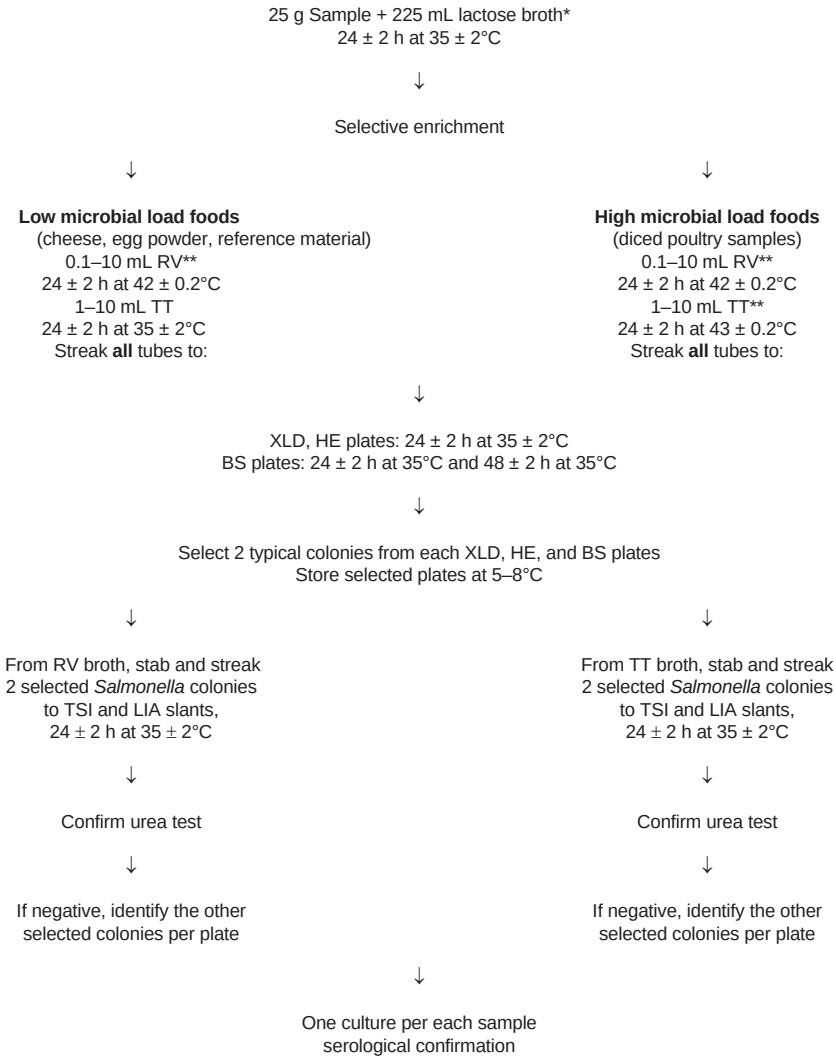

Figure 1. AOAC enrichment procedure for recovery of *Salmonella* from foods with a low (Method 2000.06) and high microbial load (Method 995.20). * = For this study, lactose broth is the appropriate pre-enrichment broth for these food types. ** = Incubation of RV and TT at elevated temperatures should be conducted in circulating, thermostatically controlled waterbath.

(**i**) *Vortex mixer*.—For mixing tube contents.

(**j**) *Sterile inoculating loops*.—Ca 3 mm id or 10 μL, nichrome, platinum-iridium, or sterile plastic.

C. Media and Reagents

(**a**) *Buffered peptone water (BPW)*.—Suspend 10 g peptone, 5.0 g NaCl, 9.0 g $Na_2HPO_4 \cdot 12H_2O$, and 1.5 g KH_2PO_4 in 1 L water, and mix thoroughly. Dispense 225 mL aliquots in 500 mL containers. Autoclave for 15 min at 121°C. Final pH should be 7.0 ± 0.2.

(**b**) *Rappaport-Vassiliadis soya peptone broth (RVS)*.—Medium may be made from individual ingredients or from ISO-compliant commercial formulation. Prepare the following solutions: *Solution A*.—Dissolve 5.0 g soya peptone, 8.0 g NaCl, 1.4 g KH_2PO_4, and 0.2 g K_2HPO_4 in l L water. Heat to ca 70°C to completely dissolve medium. Prepare solution A on the day that complete medium is to be made. *Solution B*.—Dissolve 400 g $MgCl_2 \cdot 6H_2O$ in 1 L water. Solution B can be stored at room temperature in a dark bottle up to 1 year. *Solution C*.—Dissolve 0.4 g malachite green oxalate in 100 mL water. Solution C can be stored at room temperature

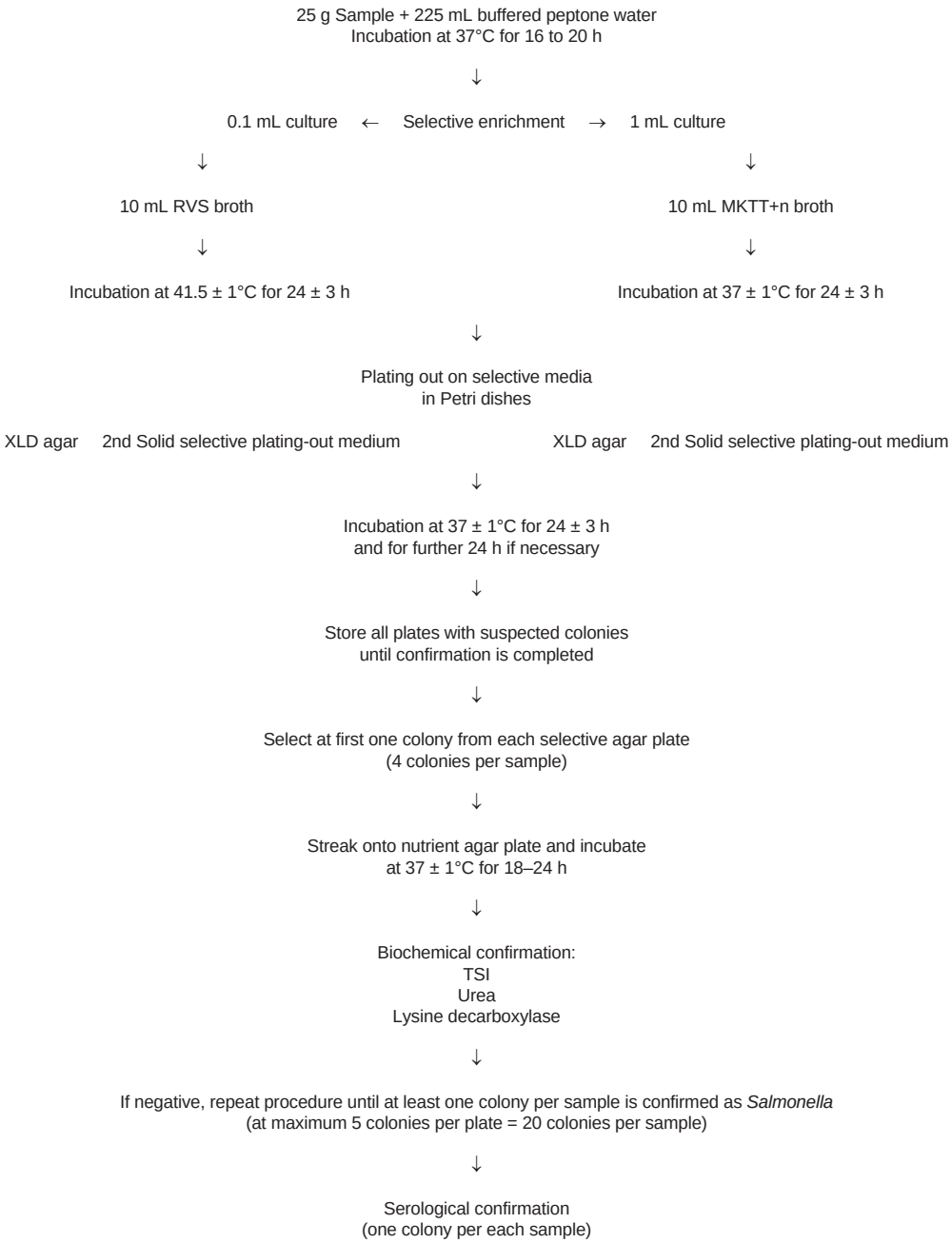

Figure 2. ISO 6579 (draft Standard prEN ISO/DIS 6579:2000) enrichment procedure for the recovery of *Salmonella* from all foods.

Table 2002.10. Interlaboratory study results for detection of *Salmonella* in foods by ISO 6579 and AOAC 2000.06 and 995.20 culture methods

Food matrix	Level	MPN/g	No. of labs	No. test portions	Test positive[a]		Chi square[b]	Sensitivity rate[c]		Incidence of false negatives among total positive test portions, %[d]		Specificity rate[e]		Incidence of false positives among total negative test portions, %[f]		Agreement between ISO and AOAC methods, %[g]
					ISO	AOAC		ISO	AOAC	ISO	AOAC	ISO	AOAC	ISO	AOAC	
Cheese	Low	0.028	15	75	57	65	3.06	82.6	94.2	17.4	5.8	100.0	100.0	0.0	0.0	78.7
	High	1.49	15	75	66	70	1.50	93.0	98.6	7.0	1.4	100.0	100.0	0.0	0.0	92.0
	Control	NA[h]	15	75	0	0	—	—	—	—	—	—	—	—	—	—
Egg powder I	Low	0.385	15	75	73	75	0.50	97.3	100.0	2.7	0.0	100.0	100.0	0.0	0.0	97.3
	High	4.62	15	74	73	74	0.00	98.6	100.0	1.4	0.0	100.0	100.0	0.0	0.0	98.6
	Control	NA[h]	15	74	0	0	—	—	—	—	—	—	—	—	—	—
Egg powder II	Low	0.028	8	40	13	19	1.56	54.2	79.2	45.8	20.8	100.0	100.0	0.0	0.0	60.0
	Control	NA[h]	8	40	0	0	—	—	—	—	—	—	—	—	—	—
Poultry I	Low	0.147	15	74	72	39	29.26	98.6	53.4	1.4	46.6	100.0	100.0	0.0	0.0	52.7
	High	0.231	15	75	75	70	3.20	100	93.3	0.0	6.7	100.0	100.0	0.0	0.0	93.3
	Control	NA[h]	15	75	0	0	—	—	—	—	—	—	—	—	—	—
Poultry II	Lot 1	0.009	13	78	15	14	0.00	57.7	53.8	42.3	46.2	100.0	100.0	0.0	0.0	70.5
	Lot 2	0.042	13	78	14	24	3.12	43.8	75.0	56.3	25.0	100.0	100.0	0.0	0.0	66.7
Poultry III	Lot 1	0.023	4	24	13	16	0.31	61.9	76.2	38.1	23.8	100.0	100.0	0.0	0.0	45.8
	Lot 2	0.042	4	24	17	20	0.44	73.9	87.0	26.1	13.0	100.0	100.0	0.0	0.0	62.5

[a] Test positive = culturally confirmed data by ISO or AOAC method.

[b] Chi square, as defined by McNemar is $(|a - b| - 1)^2 / (a + b)$ where a = number of samples positive by ISO and negative by AOAC and b = number of samples negative by ISO and positive by AOAC. A Chi square value ≥ 3.84 indicates significance at $p \leq 0.05$.

[c] The sensitivity rate is the ratio of the number of test positives to the number of known positive test portions. A known positive test portion is defined as a test portion that was positive from either the ISO or AOAC methods.

[d] Incidence of false negatives is 100 – sensitivity rate. Low number of total confirmed positives will result in high false negative data.

[e] The specificity rate is the ratio of the number of test negatives to the number of known negative test portions. A known negative test portion is defined as a test portion that was negative from either the ISO or AOAC methods.

[f] Incidence of false positives is 100 – specificity rate.

[g] Rate reflects number of confirmed determinations that were equivalent between ISO and AOAC.

[h] NA = Not applicable. Samples were not inoculated with *Salmonella.*

in the dark for up to 6 months. To prepare complete RVS broth, combine 1000 mL Solution A, 100 mL Solution B, and 10 mL Solution C. Dispense complete medium in 10 mL aliquots into 16 × 150 mm tubes and autoclave 15 min at 115°C. Final pH should be 5.2 ± 0.2.

(**c**) *Muller-Kauffmann tetrathionate broth + novobiocin (MKTT+n).*—Suspend 4.23 g meat extract, 8.45 g tryptone, 2.54 g NaCl, 38.04 g $CaCO_3$, 30.27 g $Na_2S_2O_3$ (anhydrous), 4.75 g ox bile, and 9.5 mg brilliant green in 1 L water. Boil gently for 1 min. Cool below 45°C and store at 5–8°C. The base solution should be pH 7.0 ± 0.2. Prepare I–KI solution by dissolving 25 g KI in 25 mL water, adding 20 g resublimed I, dissolving, and diluting to 100 mL with sterile water. Prepare novobiocin solution by dissolving 0.04 g novobiocin sodium salt in 5 mL water. Filter-sterilize through 0.2 μm filter. On the day the medium is used, add 19 mL I–KI solution, 9.5 mL brilliant green solution, and 5.0 mL novobiocin per 1 L basal broth. Resuspend precipitate by gentle agitation, and aseptically dispense 10 mL portions into 16 × 150 mm sterile test tubes. Do not heat medium after addition of I–KI and novobiocin solutions.

(**d**) *Xylose lysine desoxycholate (XLD) agar.*—*See* **967.25A**(**d**).

(**e**) *Second selective agar*.—The second agar used is at the discretion of the analyst. The agar used should be complementary to XLD and appropriate for isolation of lactose-positive strains of *Salmonella*, *S. typhi*, and *S. paratyphi*.

(**f**) *Nutrient agar*.—Suspend 3.0 g meat extract, 5.0 g peptone, and 15 g agar in 1 L water, and mix thoroughly. Heat to boiling to dissolve completely. Autoclave at 121°C for 15 min. Cool in water bath, and pour 20 mL portions into 15 × 100 mm Petri dishes. Let agar cool and dry before use. Final pH should be 7.0 ± 0.2.

(**g**) *Triple sugar iron agar (TSI).*—Suspend 3.0 g meat extract, 3.0 g yeast extract, 20.0 g enzymatic digest of casein, 5.0 g NaCl, 10.0 g lactose, 10.0 g sucrose, 1.0 g glucose, 0.3 g iron(III) citrate, 0.3 g $Na_2S_2O_3$, 0.024 g phenol red, and 13 g agar in 1 L water, and mix thoroughly. Heat to boiling to dissolve completely. Dispense medium into 16 × 150 mm tubes, 1/3 full, and cap or plug to maintain aerobic conditions during use. Autoclave tubes for 15 min at 121°C. Before the medium solidifies, place tubes in a slanted position to form deep butts (ca 3 cm) and adequate slants (ca 5 cm) on solidification. The final pH should be 7.4 ± 0.2.

(**h**) *Urea agar*.—Suspend 1.0 g peptone, 1.0 g glucose, 5.0 g NaCl, 2.0 g KH_2PO_4, 0.012 g phenol red, and 15 g agar in 1 L water. Heat to boiling to dissolve completely. Autoclave for 15 min at 121°C. Cool to 50–55°C. Prepare urea solution by dissolving 400 g urea in water and dilute to a final volume of 1 L. Sterilize by filtration through 0.2 μm filter. To prepare complete medium, aseptically add 50 mL urea solution to 950 mL cooled urea agar base. Mix thoroughly. *Note*: Do not heat the complete medium. Dispense complete medium in 10 mL quantities into sterile tubes. Let tubes set in sloping position. The final pH should be 6.8 ± 0.2.

(**i**) *L-lysine decarboxylation medium (Falkow).*—*See* **967.25A**(**m**)(2).

(**j**) *Bromocresol purple solution*.—0.2%. Dissolve 0.2 g in sterile water, and dilute to 100 mL.

(**k**) *Sterile physiological saline solution.*—*See* **940.36B**(**c**).

(**l**) *Salmonella polyvalent somatic (O) antiserum.*—Antiserum A-I and Vi, Difco Laboratories (Becton Dickenson Sciences, Sparks, MD), or equivalent.

(**m**) *Salmonella polyvalent flagellar (H) antiserum*.—Poly A–Z (Difco) or equivalent.

D. Preparation of Test Suspensions

(**a**) *Fresh cheese*.—Aseptically weigh 25 g test portion into stomacher bag. Add 225 mL prewarmed (35°C) BPW, **C**(**a**), and homogenize in stomacher for 1–3 min. Incubate the test suspension for 16–20 h at 35–37°C.

(**b**) *Dried egg products*.—Aseptically weigh 25 g test portion into stomacher bag. Add 225 mL BPW, **C**(**a**), and homogenize in stomacher for 1–3 min. Incubate test suspension for 16–20 h at 35–37°C.

(**c**) *Poultry products*.—Aseptically weigh 25 g test portion into stomacher bag. Add 225 mL BPW, **C**(**a**), and homogenize in stomacher for 1–3 min. Incubate test suspension for 16–20 h at 35–37°C.

E. Isolation

(**a**) *Growth in selective broth*.—Gently shake incubated test suspension, **D**, and transfer 0.1 mL into 10 mL RVS medium, **C**(**b**), and an additional 1.0 mL into 10 mL MKTT+n broth, **C**(**c**). Incubate RVS medium at 41.5 ± 1°C for 24 ± 3 h. Incubate MKTT+n broth at 35–37°C for 24 ± 3 h. Mix on a Vortex mixer all selective tubes. Streak loopful of incubated RVS medium onto selective enrichment plates of XLD agar, **C**(**d**), and the second agar selected (*see* **C**(**e**) for details). Repeat isolation with 3 mm loopful of MKTT+n test broth. Incubate plates 24 ± 3 h at 35–37°C. If growth is slight or if no typical colonies of *Salmonella* are present, reincubate at 35–37°C for additional 24 h. Re-examine plates for typical colonies of *Salmonella*.

(**b**) *Appearance of typical Salmonella colonies on XLD*.—Pink colonies with or without black centers. Many *Salmonella* may have large, glossy black centers or may appear as almost completely black colonies. Atypically, a few *Salmonella* cultures produce yellow colonies with or without black centers.

F. Treatment of Colonies

(**a**) *Inoculation of TSI*.—Pick with a sterile needle 2 or more typical or suspicious colonies, if present, from each XLD plate and second agar plate. Inoculate TSI slant, **C**(**g**), by streaking agar slant and then stabbing the butt. Store picked selective plates at 5–8°C. Incubate slants at 35–37°C for 24 ± 2 h. Cap tubes loosely to maintain aerobic conditions while incubating slants to prevent excessive H_2S production. *Salmonella* cultures typically have alkaline (red) slant and acid (yellow) butt, with or without H_2S (blackening of agar) in TSI.

(**b**) *Inoculation of L-lysine decarboxylation medium*.—Using a sterile needle, transfer a portion of TSI culture

to L-lysine decarboxylation medium, **C(i)**. Close tube caps tightly after inoculation and incubate at 35–37°C for 24 ± 2 h. *Salmonella* spp. give purple color of alkaline reaction throughout broth (final color is slightly darker than original purple color of medium). Sometimes tubes that are yellow after 8–12 h of incubation change to purple later. Negative test is permanently yellow throughout broth. If medium appears to be discolored (neither purple nor yellow), add a few drops of 0.2% bromocresol purple dye, **C(j)**, and reread the reaction.

(**c**) *Selection for identification*.—Retain all presumptive positive *Salmonella* cultures on TSI (alkaline slant and acid butt) agar for biochemical and serological test whether or not corresponding lysine decarboxylation reaction is positive (alkaline) or negative (acid). Do not exclude a TSI culture that appears to be non-*Salmonella* if the reaction in L-lysine decarboxylation broth is typical for *Salmonella*. Treat these cultures as presumptive positive and submit them to further examination. Lysine decarboxylation medium is useful in detection of *S. arizonae* and atypical *Salmonella* strains that utilize lactose and/or sucrose. Discard only apparent non-*Salmonella* TSI cultures (acid slant and acid butt) if corresponding lysine decarboxylation broth is not typical (acid) for *Salmonella*. Test retained TSI cultures as directed in **F(d)** to determine if they are *Salmonella* spp., **967.27D(e)**(*1*), or *S. arizonae* organisms, **967.27D(e)**(*2*). If TSI slants fail to give typical *Salmonella* reactions, pick additional suspicious colonies from selective medium plate not giving any presumptive positive cultures, and inoculate TSI and lysine decarboxylation broth as in **F(a)** and (**b**).

(**d**) *Identification*.—Apply biochemical and serological identification tests to 3 presumptive positive TSI cultures picked from selective agar plates streaked from RVS medium and to 3 presumptive positive TSI cultures picked from selective agar plates streaked from MKTT+n broth as directed in **967.27** and **967.28**. Examine minimum of 6 TSI and 6 lysine decarboxylation broth cultures for each 25 g test portion tested. Any AOAC-approved *Salmonella* biochemical identification test kit may be used instead of performing the individual biochemical tests presented in this method.

Ref.: *J. AOAC Int.* **83**, 283–287(2003)

Results

Specificity Study

In the first phase of the interlaboratory study, specificity data were generated for *Salmonella* and non-*Salmonella* strains using the ISO 6579 enrichment protocol with isolation agars used in this interlaboratory study. PCA enumeration of all *Salmonella* strains were performed after pre-enrichment and after selective enrichment to compare growth levels in nonselective and selective broths. In general, after overnight incubation in BPW, most *Salmonella* levels were approximately 10^8 colony forming units/mL (CFU/mL; Table 1). Overall, growth levels of *Salmonella* strains in MKTT+n or RVS, as determined by plating on PCA, were equal to or as great as 1 log lower than those of the same strain in BPW. Twelve strains showed sensitivity (2 logs lower in growth) to RVS broth as evidenced by the decreased populations in RVS compared to BPW. Also, there was an increased frequency of *Salmonella* strains with lower populations in RVS compared to MKTT+n. One *Salmonella* strain, 11:z:e,n,x, did not grow in RVS medium at all, but was recovered after MKTT+n enrichment (Table 1).

Following selective enrichment in either MKTT+n or RVS, *Salmonella* strains were spread-plated onto 7 different selective agars commonly used for *Salmonella* isolation. These selective agar counts were compared against those on PCA plated from the same broth tube. Most strains produced similar levels on all selective agars, except XLT4, when compared with PCA. A higher incidence of decreased growth ($\leq 10^6$ CFU/mL) was seen on XLT4 compared to the other 7 agars, regardless of which selective enrichment was used.

A concern was raised about the recovery of *S. typhi* and *S. paratyphi* in MKTT+n selective broth. Two strains of *S. paratyphi* and 8 strains of *S. typhi* were tested with the ISO enrichment protocol. All 10 strains survived enrichment in MKTT+n except *S. paratyphi* C. This strain, however, survived the enrichment in RVS (Table 1).

For the non-*Salmonella* strains tested, RVS appeared to be equal or more productive than MKTT+n at minimizing competitor growth (Table 2). The colony morphology of surviving microorganisms was different from that of typical *Salmonella* for the 6 selective agars evaluated, thus facilitating *Salmonella* isolation and recovery.

Collaborative Study

Twenty-one laboratories participated in this study (Table 4). There were 6 interlaboratory test runs: one of cheese, 3 of poultry, and 2 of dried egg product. Two laboratories participated in all 6 runs, 6 in 5 test runs, 5 in 4 runs, and another 5 in 3 runs. Two laboratories participated in 2 food runs and one participated in only one food run. The first run for each food type had 15 paired test portions, representing 5 portions each of high level, low level, and uninoculated samples, as well as a positive culture and negative media control. Ten paired test portions, representing 5 inoculated and 5 control samples, were analyzed in dried egg run II. Collaborators participating in poultry runs II and III analyzed 12 paired test portions, representing 6 samples from each of 2 lots of naturally contaminated poultry. The change in the number of test portions analyzed for poultry II and III were made to comply with AOAC guidelines that increased the sample size to 6 per level and reduced the minimum number of laboratories to 10 (3).

At the end of the study, valid data were submitted from 956 paired test portions that included 204 naturally contaminated, 488 inoculated, and 264 control test portions. Of the 956 total reported test portions, 411 were confirmed positive and 393 were negative by both the AOAC and ISO culture methods. Seventy-five test portions were confirmed positive by the AOAC method but negative by the ISO culture method; 77 test portions were negative by AOAC and positive by the ISO method. Tables 5–10 present individual collaborator results. Table **2002.10** summarizes the test results for AOAC and ISO, as well as sensitivity rates for each food type and inoculation

level. For each inoculation level or lot of naturally contaminated food, the actual population of *Salmonella* was quantified by MPN determination on the day of analysis for each food type. These results are discussed under individual food types.

Fresh Cheese

Seventeen laboratories from Europe and North America agreed to participate in the analysis of fresh cheese. Laboratory 22 reported an uninoculated control as positive for the inoculated microorganism. Laboratory 27 did not complete test portion confirmations. Data from these laboratories were excluded from the analysis. The remaining 15 laboratories followed study instructions (Table 5). Test portions inoculated at the low level contained 0.028 CFU/g. Fifty-three test portions were confirmed positive by both AOAC and ISO methods; 6 test portions were confirmed negative by both methods. Four test portions were negative by the AOAC method, but confirmed positive by the ISO method. Twelve test portions were positive by the AOAC method, but negative by ISO. The high inoculation level test portions contained 1.49 CFU/g. Sixty-five test portions were confirmed positive and 4 were confirmed negative by both methods. One test portion was negative by the AOAC method but confirmed positive by ISO, and 5 test portions were positive by AOAC, but negative by the ISO method. All uninoculated control test portions were negative by AOAC and ISO.

Analysts on both continents reported similar results by both culture methods, indicating that no bias in the results could be attributed to either group of participants lacking familiarity with the other procedure (i.e., ISO or AOAC). On a qualitative basis, the productivity of the AOAC method appeared to be higher than that of the ISO method. Apparent differences between the 2 methods were magnified, however, when different primary enrichment broths were used. Statistically, Chi square analysis for fresh cheese at the low level

Table 4. Collaborator participation by food type[a]

Laboratory	Poultry I	Poultry II	Poultry III	Cheese	Dried egg I	Dried egg II
1	Y	Y	N	Y	Y	Y
2	Y[b]	Y	N	Y	Y	N
4	Y[b]	Y	N	Y	Y[b]	Y
5	Y	Y	N	Y	Y	Y
11	Y	Y	N	Y	Y	Y
12	Y[b]	N	N	N	N	N
13	Y	N	N	Y	Y	N
14	Y	N	N	Y	Y	N
16	Y	N	N	N	Y	N
17	Y	N	N	Y	Y	N
18	Y	Y	N	N	Y	Y
19	Y	Y	N	Y	Y	N
20	Y[b]	Y	N	Y	Y	N
21	Y	Y	N	Y	Y	Y
22	Y	Y	Y	Y[b]	Y[b]	N
23	Y	N	N	Y	Y	N
24	Y	Y	Y	Y	Y	Y
25	Y	Y	Y	Y	Y[b]	Y
26	Y	N	Y	Y	Y	N
27	Y[c]	N	N	Y[d]	Y[b]	N
28	N	Y	N	N	N	Y[b]
Total[e]	20	13	4	17	19	9

[a] Y = Collaborator analyzed this food type; N = collaborator did not analyze this food type.

[b] Uninoculated control samples were confirmed as *Salmonella*. Results were not included in the statistical analysis for the designated food types.

[c] Laboratory did not follow study instructions. Results were not included in the statistical analysis for the designated food types.

[d] Incomplete/incorrect sample confirmation. Results were not included in the statistical analysis for the designated food types.

[e] Total number of laboratories providing data.

was 3.1 and 1.5 for the high level, indicating comparable results between the 2 methods.

Dried Egg Powder

Dried egg powder was prepared 2 times. In the first interlaboratory run, 19 laboratories from Europe and North America agreed to participate. Laboratories 4, 22, 25, and 27 reported uninoculated control test portions as positive for the inoculated microorganism. Data from these laboratories were excluded from the analysis. Laboratory 18 reported 2 leaky enrichment bags. These paired samples were removed from data analysis. The remaining 15 laboratories reported all uninoculated control test portions as negative and reported valid data as indicated by summary worksheets (Table 6). Test portions inoculated at the low level contained 0.385 CFU/g. Seventy-three test portions were confirmed positive by both AOAC and ISO methods. Two test portions were confirmed positive by AOAC, but negative by the ISO method. Test portions inoculated at the high level contained 4.62 CFU/g. Seventy-three test portions were confirmed positive by both AOAC and ISO methods. One test portion was confirmed positive by AOAC, but negative by the ISO method. Chi

Table 5. Analysis of fresh cheese by individual collaborators[a]

Lab	High level portions						Low level portions				Uninoculated portions				
							ISO method								
1	+	+	+	+	+	+	–	+	+	+	–	–	–	–	–
2	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–
4	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
5	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
13	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
14	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
17	+	+	+	+	+	–	+	+	+	+	–	–	–	–	–
19	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
20	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–
21	+	+	+	+	+	+	–	+	+	–	–	–	–	–	–
23	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
24	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
25	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
26	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
							AOAC method								
1	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
2	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–
4	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
5	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
13	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
14	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
17	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
19	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
20	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
21	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
23	–	–	–	+	+	–	–	+	+	+	–	–	–	–	–
24	+	+	+	+	+	–	+	–	+	–	–	–	–	–	–
25	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
26	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–

[a] European laboratories = 1–17; North American laboratories = 19–26.

Table 6. Analysis of dried egg product I by individual collaborators[a]

Lab	High level portions					Low level portions					Uninoculated portions				
	ISO method														
1	+	+	+	+	+	–	–	+	+	+	–	–	–	–	–
2	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
5	+	+	+	+	–	+	+	+	+	+	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
13	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
14	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
16	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
17	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
18	+	+	+	NT[b]	+	+	+	+	+	+	–	–	NT	–	–
19	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
20	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
21	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
23	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
24	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
26	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
	AOAC method														
1	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
2	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
5	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
13	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
14	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
16	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
17	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
18	+	+	+	NT	+	+	+	+	+	+	–	–	NT	–	–
19	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
20	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
21	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
23	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
24	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
26	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–

[a] European laboratories = 1–17; North American laboratories = 18–26.
[b] NT = Not tested due to sample bag leakage.

square analysis for dried egg product was 0.5 at the low level and 0.0 at the high level, indicating that the 2 methods are comparable. The recovery rate of the dried egg product was higher than expected. Therefore, a second trial, involving fewer laboratories, was conducted with a lower inoculation level of *Salmonella* to achieve fractional recovery.

In the second run, 9 laboratories agreed to participate. Laboratory 28 reported an uninoculated control test portion as positive for the inoculated microorganism. Data from this laboratory were excluded from the analysis. All other laboratories submitted valid data (Table 7). Only a low contamination level and uninoculated controls were analyzed in the second run. The MPN of the low level was 0.028 CFU/g. Eight test portions were confirmed positive by both AOAC and ISO methods; 16 test portions were negative by both methods. Eleven test portions were confirmed positive by AOAC, but negative by the ISO method; 5 test portions were positive by the ISO method, but negative by AOAC. All uninoculated

Table 7. Analysis of dried egg product II by individual collaborators[a]

Lab	Low level portions					Uninoculated portions				
					ISO method					
1	–	+	–	+	+	–	–	–	–	–
4	–	–	+	–	+	–	–	–	–	–
5	+	–	–	–	–	–	–	–	–	–
11	–	+	–	–	–	–	–	–	–	–
18	–	–	–	–	–	–	–	–	–	–
21	–	–	–	–	+	–	–	–	–	–
24	+	+	+	+	+	–	–	–	–	–
25	–	–	–	–	–	–	–	–	–	–
					AOAC method					
1	+	+	–	+	+	–	–	–	–	–
4	+	–	+	+	+	–	–	–	–	–
5	–	–	–	+	–	–	–	–	–	–
11	+	+	–	–	+	–	–	–	–	–
18	–	+	+	+	+	–	–	–	–	–
21	–	–	–	–	+	–	–	–	–	–
24	–	–	–	–	+	–	–	–	–	–
25	–	–	–	–	+	–	–	–	–	–

[a] European laboratories = 1–11; North American laboratories = 18–25.

controls were negative. Chi square analysis for the low level was 1.6, indicating that the methods are comparable.

Poultry

Poultry was prepared 3 times. For poultry run I, radiation-sterilized meat was subsequently inoculated with *Salmonella* and an excess of competitive microflora. It was then lyophilized before shipment for analysis. Twenty laboratories from Europe and North America agreed to participate in analyzing poultry. Laboratories 2, 4, 12, and 20 reported uninoculated control test portions as positive for the inoculated microorganism. Laboratory 27 did not follow study instructions. Data from these laboratories were excluded from the analysis. Laboratory 18 reported a leaky enrichment bag. The sample number was removed from data analysis. Fifteen laboratories followed study instructions (Table 8). Test portions inoculated at the low level contained 0.147 CFU/g. Thirty-eight test portions were confirmed positive by both AOAC and ISO methods; one test portion was negative by both methods. Thirty-four test portions were negative by AOAC, but confirmed positive by the ISO method. One test portion was confirmed positive by AOAC, but negative by the ISO method. The high inoculation level test portions contained 0.231 CFU/g. Seventy test portions were confirmed positive by both AOAC and ISO methods. Five test portions were confirmed positive by ISO, but negative by the AOAC method. No test portions were negative by ISO, but positive by AOAC. All uninoculated media controls were negative. Chi square analysis for poultry was 29.3 at the low level and 3.2 at the high level, indicating a significant difference in recovery between the 2 culture methods at the low level. There was no significant statistical difference between the 2 methods at the high level of inoculation, although the ISO method detected more positive samples than did the AOAC method.

The poultry used in the first analysis consisted of radiation-sterilized chicken that was artificially contaminated and then lyophilized at –70°C for 48 h. The lyophilized pellets were stored at 4°C until the day of analysis. Content uniformity studies were conducted at the laboratory that prepared the samples. Overall, the MPN data indicated reasonable uniformity in the samples; however, some of the data points demonstrated nonuniformity at certain test intervals during the uniformity studies. This may have contributed to the differences seen between the 2 culture methods, or it could reflect the variable nature of MPN analysis. Also, MPN data produced at the preparative laboratory (CSL) was compared to MPN data produced in the lead North American laboratory (BCS) on the day that the test portions were analyzed. A significantly lower MPN recovery was measured by the North American laboratory on the test date.

It was decided that a second run should be conducted using raw ground poultry naturally contaminated with *Salmonella.* Two lots of contaminated ground poultry were purchased at the retail level in Seattle, WA. For each lot of poultry, individ-

Table 8. Analysis of poultry I by individual collaborators[a]

	High level portions					Low level portions					Uninoculated portions				
Lab	1	5	7	12	15	2	4	9	11	13	3	6	8	10	14
							ISO method								
1	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
5	+	+	+	+	+	+	+	–	+	–	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
13	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
14	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
16	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
17	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
18	+	+	+	+	+	+	+	NT[b]	+	+	–	–	–	–	–
19	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
21	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
22	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
23	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
24	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
25	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
26	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
							AOAC method								
1	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–
5	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–
11	+	+	+	+	+	–	–	–	–	+	–	–	–	–	–
13	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
14	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–
16	+	–	+	+	+	–	–	–	+	–	–	–	–	–	–
17	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–
18	+	+	+	+	+	+	+	NT	–	+	–	–	–	–	–
19	+	+	+	+	+	+	+	+	–	+	–	–	–	–	–
21	–	–	–	+	+	–	–	–	–	+	–	–	–	–	–
22	+	+	+	+	+	–	+	–	–	–	–	–	–	–	–
23	+	+	+	+	+	–	+	+	+	+	–	–	–	–	–
24	+	+	+	+	+	–	+	+	+	+	–	–	–	–	–
25	+	+	+	+	+	+	–	+	–	–	–	–	–	–	–
26	+	+	+	+	+	+	–	+	–	+	–	–	–	–	–

[a] European laboratories = 1–17; North American laboratories = 18–26.

[b] NT = Not tested due to sample bag leakage.

ual packages were combined and thoroughly mixed, divided into the appropriate number of test portions, and stabilized at –20°C before shipping on dry ice to all collaborators worldwide for analysis.

For poultry run II, 13 laboratories from Europe and North America agreed to participate. All laboratories conducted the study properly (Table 9). Test portions for Lot 1 contained 0.009 CFU/g. Three test portions were confirmed positive by both AOAC and ISO methods; 52 were negative by both methods. Twelve test portions were negative by AOAC culture but confirmed positive by the ISO method. Eleven test portions were confirmed positive by AOAC, but negative by the ISO method.

Poultry test portions from Lot 2 contained 0.042 CFU/g. Six test portions were confirmed positive by both AOAC and ISO methods. Forty-six were negative by both methods. Eight test portions were confirmed positive by ISO, but negative by the AOAC method. Eighteen were confirmed positive by the

Table 9. Analysis of poultry II by individual collaborators[a]

	Lot 1						Lot 2					
Lab	1	2	3	4	5	6	7	8	9	10	11	12
						ISO method						
1	+	–	–	–	–	+	+	–	–	–	–	–
2	–	–	–	–	–	–	+	–	–	–	–	–
4	–	–	–	–	+	–	–	–	–	–	–	–
5	+	–	+	–	–	–	–	–	+	–	–	–
11	–	–	+	–	–	–	+	–	–	–	–	–
18	–	+	–	–	+	–	+	–	–	–	+	–
19	–	–	–	–	–	–	+	–	–	–	–	+
20	+	+	–	+	–	–	–	–	–	+	+	–
21	–	–	–	–	–	–	–	–	–	–	–	–
22	–	–	–	–	–	–	+	–	–	–	–	–
24	–	–	–	–	–	–	+	–	+	+	–	–
25	–	+	–	–	+	–	–	–	–	–	–	–
28	–	–	+	+	–	–	–	–	–	–	–	–
						AOAC method						
1	–	–	–	+	–	–	–	+	–	–	+	+
2	–	–	–	–	+	–	+	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–	+	–	–
11	–	–	–	–	–	–	–	–	–	–	–	–
18	+	–	–	–	–	+	+	–	+	+	–	+
19	–	+	–	–	–	–	–	–	–	–	–	–
20	+	+	+	+	–	–	–	–	+	+	–	–
21	–	–	–	–	–	–	–	–	+	+	–	–
22	–	–	–	+	–	–	+	–	–	+	+	+
24	–	+	–	+	–	–	+	–	+	–	–	–
25	–	–	–	–	–	–	–	–	–	–	+	+
28	+	–	–	–	–	+	–	+	–	+	–	+

[a] European laboratories = 1–11, 28; North American laboratories 18–25.

AOAC method, but confirmed negative by the ISO method. Analysts on both continents reported similar results by both culture methods, indicating no bias in the results that could be attributed to either group of participants lacking familiarity with the reciprocal procedure. Chi square analysis for poultry was 0.0 for Lot 1 and 3.1 for Lot 2, indicating that the methods were comparable, although the AOAC method detected more positives in run II than did the ISO method.

In summary, the ISO method appeared to perform better than the AOAC method in one trial with irradiated diced chicken containing a highly competitive artificial microflora. In another trial using naturally contaminated raw ground chicken, the AOAC method was qualitatively more productive for one lot of poultry analyzed, but not statistically significant. For the second lot of poultry (in run II), the ISO method detected one additional positive, which is not significant.

A concern over the discrepancy in recovery between the AOAC and ISO methods prompted a third evaluation of raw ground chicken. Four laboratories, 3 U.S. regulatory laboratories and the sponsoring laboratory, participated in this analysis (Table 10). Two lots of naturally contaminated raw ground chicken were analyzed. Each collaborator analyzed 6 randomized paired test portions per lot of ground chicken by the AOAC and ISO methods for a total of 48 paired test portions analyzed in the 3rd trial.

Test portions for Lot 1 contained 0.023 CFU/g. Eight test portions were confirmed positive by both AOAC and ISO methods; 3 test portions were negative by both methods. Five

Table 10. Analysis of poultry III by individual collaborators[a]

	Lot 1						Lot 2					
Lab	1	2	3	4	5	6	7	8	9	10	11	12
	ISO method											
22	–	+	+	–	+	+	–	+	–	+	+	+
24	–	+	–	–	–	+	+	–	+	+	+	+
25	–	+	–	–	–	+	–	+	+	+	+	–
26	+	+	+	–	+	+	–	+	+	+	–	+
	AOAC method											
22	+	+	–	–	–	+	–	+	+	+	+	+
24	–	+	+	+	+	+	+	+	–	+	+	+
25	+	–	–	+	+	+	+	+	+	–	+	+
26	–	+	+	+	+	+	+	–	+	+	+	+

[a] North American laboratories = 22–26.

test portions were negative by the AOAC method, but confirmed positive by the ISO method. Eight test portions were confirmed positive by the AOAC method, but negative by the ISO method. Chi square analysis for Lot 1 was 0.31, indicating no significant differences in recovery between the 2 methods.

Test portions for Lot 2 contained 0.042 CFU/g. Fourteen test portions were confirmed positive by both AOAC and ISO methods; one test portion was negative by both methods. Three test portions were negative by AOAC, but confirmed positive by the ISO method. Six test portions were confirmed positive by AOAC, but negative by the ISO method. Chi square analysis for Lot 2 was 0.44, indicating no significant differences in recovery between the 2 methods.

Discussion

The specificity data generated using the ISO 6579:2002 enrichment protocol indicated that 124 of 125 *Salmonella* strains tested were recovered from both selective enrichments, regardless of which isolation agar was used.

The data generated from the fresh cheese and dried egg product runs demonstrated equivalence of AOAC **2000.06** and **967.26** and ISO 6579:2002 culture methods (Table **2002.10**) for these 2 specific food types. No statistically significant differences in recovery, as measured by Chi square, were detected between the methods for any of the inoculation levels of the 2 food types.

The first run for poultry using inoculated, then lyophilized, chicken meat resulted in significantly more *Salmonella* recovered by the ISO method for the low level (Table **2002.10**). There was no statistically significant difference between the AOAC and ISO methods for the high level. A second run using naturally contaminated poultry rather than inoculated and lyophilized chicken was conducted. In the second run, the methods were statistically comparable as measured by Chi square. The third run confirmed that there are no statistical differences in recovery between the 2 methods.

On a qualitative basis, the productivity of the AOAC method generally appeared to be higher than that of the ISO method. Apparent differences between the 2 methods were magnified, however, when different primary enrichment broths were used. However, there were no statistically significant differences between the 2 methods as determined by Chi square analysis. The contamination levels in the study were generally low compared to protocol specified levels. For the low level, actual contamination levels of the foods analyzed ranged from 0.009 to 0.147 CFU/g (excluding egg product I, low level), or 0.23 to 3.7 CFU/25 g test portion compared to the proposed 5–10 CFU/25 g test portion. The low levels of inoculation were combined with 2 separate enrichment protocols. These lower contamination levels provided the benefit of achieving fractional recovery data for end point determination.

Recommendation

Based on the data generated from this international multilaboratory collaborative study, it is recommended that ISO 6579:2002 be adopted Official First Action for the detection of *Salmonella* in fresh cheese, dried egg products, and fresh chilled and frozen poultry products.

Acknowledgments

We thank Cecille Lahellec and Bertrand Lombard (AFSSA, Maisons-Alfort, France) for supporting and organizing the first worldwide collaborative study for harmonization of AOAC and ISO methods in detection of *Salmonella* in selected foods.

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