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Staphylococcal Enterotoxin C: Solid-Phase Radioimmunoassay

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A solid-phase radioimmunoassay test employing ^{125}I-labeled enterotoxin C and polystyrene tubes coated with specific antibody was used for the detection and quantitation of enterotoxin C in condensed milk, cheddar cheese, custard, and ham salad. The assay was sensitive to 1 to 10 ng of toxin per g of food; nonspecific inhibitions were 16% or less.

A solid-phase radioimmunoassay test employing radiolabeled enterotoxins A and B and polystyrene tubes coated with specific antibody was used for the detection and quantitation of these enterotoxins in various food samples (4). We report here the application of this immunoassay procedure to the detection and quantitation of enterotoxin C in condensed milk, cheddar cheese, custard, and ham salad.

Purified enterotoxin C, containing less than 5% impurities (1), was obtained from M. S. Bergdoll, University of Wisconsin, Madison. The toxin was labeled with ^{125}I by the chloramine-T procedure (2, 3), modified as previously described (5). Labeled enterotoxin C contained approximately 4 μCi of activity per μg of protein (6).

The antiserum was sodium sulfate precipitated as previously described (5). One-milliliter amounts of sodium sulfate-precipitated antiserum containing approximately 2 μg of protein per ml were used to coat the polystyrene tubes.

Various concentrations of enterotoxin C were added to 10-g amounts of condensed milk, custard, cheddar cheese, and ham salad. The extracts were then prepared as described previously (4). The food extracts were added in 1-ml amounts to antibody-coated tubes, and the test was carried out as described elsewhere (4). All determinations were carried out in duplicate, and each sample was counted for 10 min in a Packard Auto Gamma Counter. ^{125}I-labeled enterotoxin C in the absence of inhibitors gave an average 10-min count of 66,415, with a corresponding uptake of 54.4%.

Table 1 presents data on the inhibition of binding of ^{125}I-labeled enterotoxin C to anti-enterotoxin C-coated tubes by extracts of enterotoxin C obtained from condensed milk, cheddar cheese, custard, and ham salad. Nonspecific inhibition ranged from −0.8% (ham salad) to 16.1% (cheddar cheese). Inhibition at 0.001 μg/g of food ranged from 10.9% (ham salad) to 30.3% (custard); inhibition at 0.0025 μg/g of food ranged from 16.4% (ham salad) to 49.3% (milk).

The data indicate that the solid-phase radioimmunoassay procedure is suitable for the sensitive and specific detection of enterotoxin C in various foods. The results compare favorably with the data (4) on the detection and quantitation of enterotoxins A and B in various foods. No cross-reactivity occurred when enterotoxin

TABLE 1. *Inhibition of binding of ^{125}I-labeled enterotoxin C to anti-enterotoxin C-coated tubes by food extracts*

Food	Inhibitor concn. (μg added/g of food)	Inhibition (%)	
		Duplicates	Avg ± SE[a]
Milk	0.0	8.9, 8.6	8.8 ± 0.212
	0.001	26.2, 22.8	24.5 ± 2.40
	0.0025	46.4, 52.2	49.3 ± 4.10
	0.005	55.5, 53.6	54.5 ± 1.34
	0.01	66.8, 63.3	65.1 ± 2.47
Cheddar cheese	0.0	19.6, 12.6	16.1 ± 4.95
	0.001	17.1, 21.3	19.7 ± 2.97
	0.0025	41.5, 34.7	42.1 ± 1.56
	0.005	62.8, 59.2	61.0 ± 2.55
	0.01	68.2, 69.1	68.3 ± 0.64
Custard	0.0	15.4, 10.0	12.7 ± 3.82
	0.001	30.3, 30.3	30.3 ± 0.0
	0.0025	36.5, 45.6	41.1 ± 6.43
	0.005	69.3, 69.2	69.2 ± 0.07
	0.01	75.3, 74.3	74.8 ± 0.71
Ham salad	0.0	9.2, −10.8	−0.8 ± 14.14
	0.001	11.7, 10.1	10.9 ± 1.13
	0.0025	15.4, 17.5	16.4 ± 1.48
	0.005	35.6, 45.6	40.5 ± 7.07
	0.01	60.5, 58.9	59.7 ± 1.13

[a] SE, Standard error.

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C extracts were tested in enterotoxin A and B systems. The solid-phase radioimmunoassay, then, has been successfully applied to the detection and quantitation of enterotoxin C in food.

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