

A Practical ELISA for Azaspiracids in Shellfish via Development of a New Plate-Coating Antigen

Ingunn A. Samdal, Kjersti E. Løvberg, Anja B. Kristoffersen, Lyn R. Briggs, Jane Kilcoyne,

Craig J. Forsyth, and Christopher O. Miles

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Table S1. Mean molar cross-reactivities of AZA analogs in the new ELISA

Compound	Origin	n	assay range ^a (pg/mL)			% CR ^b	Intra-assay CV (%) ^c
			<i>I</i> ₂₀	<i>I</i> ₈₀	<i>I</i> ₅₀		
AZA-1 ^d	NRC	4	295	4089	1093	100	13
AZA-2 ^d	NRC	3	416	7895	1771	63	7
AZA-3 ^d	NRC	3	368	3678	1156	93	8
AZA-4 ^e	MI	3	345	4320	1219	90	9
AZA-5 ^e	MI	3	455	4850	1468	75	22
AZA-6 ^e	MI	4	346	3323	1057	103	18
AZA-7 ^e	MI	3	550	7662	2047	54	17
AZA-8 ^e	MI	4	389	5681	1483	75	10
AZA-9 ^e	MI	4	468	4285	1409	79	11
AZA-10 ^e	MI	2	345	4537	1250	89	1
AZA-33 ^e	MI	5	463	7129	1803	52	19
AZA-34 ^e	MI	5	284	4557	1136	93	11
37- <i>epi</i> -AZA-1 ^e	MI	3	380	6518	1515	72	10

^a*I*₂₀, *I*₅₀, *I*₈₀ are the concentrations of analog giving 20, 50 and 80% inhibition, respectively, of binding of antibody to the coating antigen (OVA-cdiAZA1).

^bMolar cross-reactivity; % CR = 100 × (*I*₅₀ AZA-1 CRM)/(*I*₅₀ analog).

^cIntra-assay variation; CV was 1–22% for *I*₅₀-values based on 2–6 competition curves, calculated for each analog as follows; CV (%) = 100 × (standard deviation of *I*₅₀)/(mean of *I*₅₀).

^dCRM.

^eRM

Table S2. Impurities in the AZA-standards

Standard	Origin	Component	% (total purity)	Concentration (µg/mL)	
				Std	Total AZAs
AZA-1 ^a Lot 20060719	NRC	AZA-1	94.6	1.2	1.3
		AZA-3	0.4		
		AZA-1 isomer	0.4		
		AZA-1 isomer	1.1		
		AZA-25	0.7		
		AZA-1 isomer	2.8		
AZA-2 ^a Lot 20081211	NRC	AZA-2	99.8	1.3	1.3
		AZA-1	0.05		
		AZA-1 isomer	0.06		
		AZA-1 isomer	0.07		
AZA-3 ^a Lot 20081210	NRC	AZA-3	93.8	1.0	1.1
		AZA-3 isomer	0.6		
		AZA-3 isomer	1.7		
		AZA-3 isomer	2.9		
		AZA-4 isomer	0.5		
		AZA-3 methyl ester	0.5		
AZA-4	MI	AZA-4	98.5	1.1	1.1
		37- <i>epi</i> -AZA-4	1.5		
AZA-5	MI	AZA-5	94.5	1.1	1.2
		AZA-48	1.7		
		AZA-7	1.6		
		37- <i>epi</i> -AZA-5	2.2		
AZA-6	MI	AZA-6	96.5	1.2	1.4
		37- <i>epi</i> -AZA-6	3.5		
AZA-7	MI	AZA-7	63.0	1.1	1.5
		AZA-5	13.6		
		AZA-48	20.4		
		37- <i>epi</i> -AZA-7	3.0		
AZA-8	MI	AZA-8	86.0	1.1	1.3
		AZA-48	5.6		
		37- <i>epi</i> -AZA-8	8.6		
AZA-9	MI	AZA-9	86.2	1.8	2.1
		AZA-26	11.5		
		37- <i>epi</i> -AZA-9	2.3		
AZA-10	MI	AZA-10	95.2	0.8	0.8
		AZA-11	4.8		
		37- <i>epi</i> -AZA-10	?		
AZA-33	MI	AZA-33	92.8	1.1	1.2
		AZA-1	0.9		
		37- <i>epi</i> -AZA-33	6.3		
AZA-34	MI	AZA-34	96.6	1.1	1.1
		37- <i>epi</i> -AZA-34	3.4		
37- <i>epi</i> -AZA-1	MI	37- <i>epi</i> -AZA-1	96.0	1.0	1.0
		AZA-1	4.0		

^aCRM's from NRC; see the certificates for further information

Table S3. The regression of the cross-reactivity data including the outlier for AZA-33.

	Estimate	Std. Err.	Pr(> t)
(Intercept)	101.2	5.8	< 0.001
AZA-2	-39.2	8.8	< 0.001
AZA-3	-6.2	8.8	0.490
AZA-4	-11.0	8.8	0.221
AZA-5	-23.9	8.8	0.010
AZA-6	4.6	8.2	0.580
AZA-7	-46.6	8.8	< 0.001
AZA-8	-26.9	8.2	0.002
AZA-9	-22.9	8.2	0.008
AZA-10	-13.7	10.0	0.178
AZA-33	-38.4	7.7	< 0.001
AZA-34	-3.9	7.7	0.615
37- <i>epi</i> -AZA-1	-28.6	8.8	0.003

The regression in table S3 had an adjusted R^2 of 0.63.

AZA-2, -5, -7, -8, -9, -33 and -37 have $p < 0.05$ and their cross-reaction are considered to be significantly different from AZA-1.

Table S4. The regression of the cross-reactivity data without the outlier for AZA-33.

	Estimate	Std. Err.	Pr(> t)
(Intercept)	101.2	5.3	< 0.001
AZA-2	-39.2	8.2	< 0.001
AZA-3	-6.2	8.2	0.456
AZA-4	-11.0	8.2	0.188
AZA-5	-23.9	8.2	0.006
AZA-6	4.6	7.6	0.552
AZA-7	-46.6	8.2	< 0.001
AZA-8	-26.9	7.6	0.001
AZA-9	-22.9	7.6	0.005
AZA-10	-13.7	9.3	0.148
AZA-33	-44.4	7.6	< 0.001
AZA-34	-3.9	7.2	0.588
37- <i>epi</i> -AZA-1	-28.6	8.2	0.001

The regression in table S4 exclude one outlier and the adjusted R^2 increased to 0.68. The resulting estimates changed little for all AZAs except AZA-33, and AZA-2, -5, -7, -8, -9, -33 and -37 have still $p < 0.05$ and are considered significantly different from AZA-1. The residuals were visually examined and found to contain no systematic errors.