DETECTION OF ABRIN IN FOODS USING ELISA AND ELECTROCHEMILUMINESCENCE (ECL) TECHNOLOGIES

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ABSTRACT

Abrin is a potent ribosome inactivating protein (RIP-2) present in beans of *Abrus precatorius*, commonly known as rosary pea, jequirite bean, and crab's eyes. The possibility that abrin may adulterate food has made the development of assays for the detection of abrin a priority for the FDA. Rabbit derived polyclonal antibodies and mouse monoclonal antibodies were prepared against a mixture of the abrin isozymes. The specificity / cross-reactivity of the antibodies were evaluated against a challenge library consisting of 40 different grains, nuts, legumes, and foods at concentrations of 2, 0.2 and 0.02 % (w/v). ELISA and ECL-based assays were assembled and optimized, the later employing the 96-well format, Sector PR^{TM} 100 ECL detector manufactured by Meso Scale Diagnostics (MSD). Polyclonal (capture) / polyclonal (detection) ELISA, polyclonal / monoclonal ELISA, and polyclonal / monoclonal ECL assays displayed limits of detection (LOD) of 0.1 to 0.5 ng/mL with purified Abrin C and various abrin extracts in buffer. The LOD for abrin dissolved into juices, dairy products, soda, chocolate drink, and condiments analyzed using the ECL assay ranged from 0.1 – 0.5 ng/mL. In contrast, the LODs for the ELISA assays were usually between 1 - 4 ng/mL (up to 20 ng/mL), depending on the assay configuration. In all cases, the LODs were considerably less than the concentration at which abrin may pose a health concern in food.

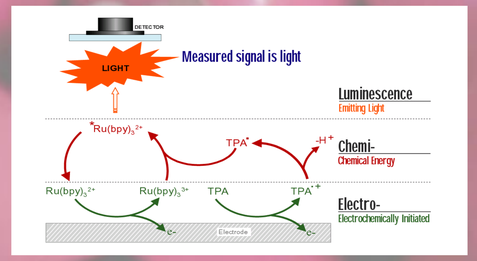
MATERIALS & METHODS

Abrin C was obtained from Sigma Chemical Company. Fractions of mixtures of abrin isozymes (I, II, III), and *Abrus precatorius* agglutinin were prepared under contract for the FDA. *Abrus precatorius* extract was the gift of Robert L. Bull, Ph.D. (BDRD, NMRC). Rabitt polyclonal and mouse monoclonal antibodies against a mixture of abrin isozymes were prepared under contract to the FDA by Tetracore, Inc. All food samples were prepared by spiking with either Abrin C or Fraction II dissolved in PBS and allowed to absorb into the food. Food samples were diluted 5-fold with 200 mM sodium phosphate, pH 6.8 (NaPi) prior to analysis.

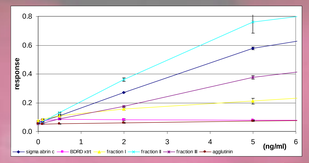
ELISA Assays Sandwich polyclonal-capture / biotinylated-polyclonal-detection (poly/poly) and polyclonal-capture / monoclonal-detection (poly/mono) ELISAs were developed. The poly/poly assay gave optimum results when the plates were coated with 2.5 μg/mL of the polyclonal capture antibody, detected with 10 μg/mL biotinylated polyclonal antibody, and signal generated using 200 ng/mL streptavidin conjugated HRP. The poly/mono ELISA gave optimum results with 20 μg/mL of the polyclonal capture antibody, 2.5 μg/mL detector monoclonal antibody, and 80 ng/mL anti-mouse conjugated HRP. Plates were coated for 16 h at 4°C followed by blocking with PBS/0.1% Tween-20/5% non-fat skim milk (PBSTM) at 37°C for 1 h. Sample, detector, and conjugate incubations were conducted at 37°C for 1 h. The plates were read at 405 nm after incubating the substrate for 30 min at 37°C.

ECL Assays All assays, entailed preparation/coating of 96-well streptavidin coated multi-assay plates with integrated screen-printed electrodes with biotinylated capture antibody (either monoclonal or polyclonal) for 16 h at 22°C. Sample and ruthenium-labeled detector antibody (either monoclonal or polyclonal) incubation steps were for 1 h at 22°C, with shaking for the initial 27 min. Electrochemiluminescence was measured using MSDT^{TM} tripropylamine buffer. The simultaneous incubation of sample and detector antibody entailed diluting the sample 2.5-fold with NaPi followed by mixing the sample 1:1 with the detector antibody dissolved in the diluent buffer; a net dilution of 5-fold, comparable to the sequential procedure. ECL was measured using an MSD Sector PR 100 Multi-Array^{TM} plate reader, read time 2 min/plate.

Electrochemiluminescence

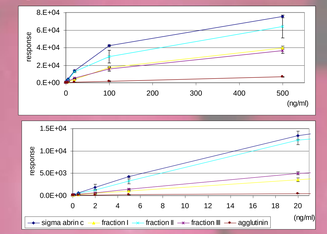


Abrin Poly / Mono ELISA



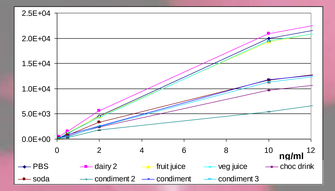
capture rabbit polyclonal @ 20ug/ml
detector mouse monoclonal 5F6 @ 5 ug/ml
conjugate goat anti - mouse IgG @ 80ng/ml

Poly / Mono ECL Detection of Abrin

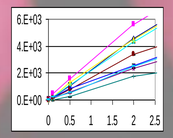


Simultaneous incubation of sample with detector antibody
LOD for Abrin C < 0.5 ng/mL (S/N 2x BKGD)

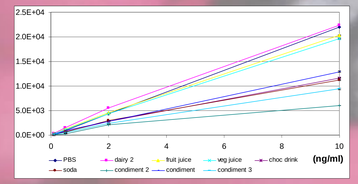
ECL Sequential Incubations



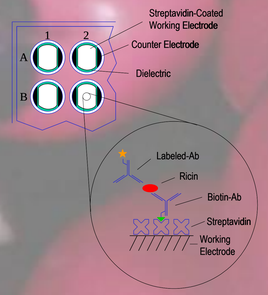
capture: 2.5 μg/mL biotinylated rabbit polyclonal antibody
detector: 5 μg/mL mouse monoclonal 5F6 antibody
background (subtracted) varied from 140 - 300 dependent on the food.



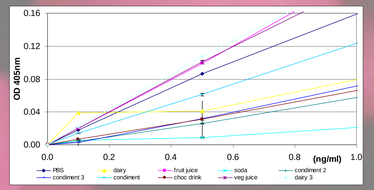
ECL Simultaneous Incubation



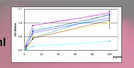
capture: 2.5ug/mL biotinylated rabbit polyclonal antibody
detector: 5 ug/mL mouse monoclonal 5F6 antibody
LOD 0.1 – 0.5 ng/mL



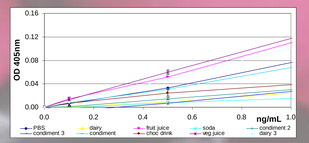
Abrin Poly / Mono ELISA



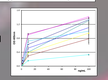
capture with rabbit polyclonal @ 20 ug/ml
detector used MAb @ 2.5 ug/ml
conjugate: Goat anti - mouse IgG at 80 ng/ml
LOD usually 1 – 4 ng/mL (max 20 ng/mL)



Abrin Poly / Poly ELISA



capture: 2.5 ug/mLrabbit polyclonal
detector: 10 ug/ml biotinylated rabbit polyclonal
conjugate: 200 ng/mL HRP-streptavidin



CROSS - REACTIVITY / SPECIFICITY [a]

	Poly / Mono ELISA 2% (w/v)	0.20% (w/v)	0.02% (w/v)	Poly / Poly ELISA 2% (w/v)	0.20% (w/v)	0.02% (w/v)		Poly / Mono ELISA 2% (w/v)	0.20% (w/v)	0.02% (w/v)	Poly / Poly ELISA 2% (w/v)	0.20% (w/v)	0.02% (w/v)
amaranth (flour)	< [b]	<	<	<	<	<	**mung beans**	<	<	<	<	<	<
barley	<	<	<	<	<	<	**pinto beans**	<	<	<	<	<	<
buckwheat (flour)	<	<	<	4	2	<	**soy (flour)**	<	<	<	<	<	<
celiac (flour)	<	<	3	2	2	3	**poppy seeds**	<	<	<	<	<	<
corn (flour)	<	<	<	8	3	2	**pumpkin seeds**	<	<	<	<	2	<
kamut	<	<	<	<	<	<	**sesame seeds**	<	<	<	2	<	2
millet (flour)	<	<	<	<	<	<	**sunflower seeds**	<	<	<	4	2	<
oat	<	<	<	<	<	<	**almond nuts**	<	<	<	<	<	<
rice (flour)	<	<	<	<	<	<	**brazil nuts**	<	<	<	<	<	<
rye (flour)	<	<	<	2	<	<	**cashew nuts**	<	<	<	<	<	<
spelt (flour)	<	<	<	<	<	<	**chestnuts**	<	<	<	<	<	<
teff	<	<	<	<	<	<	**hazelnuts**	<	<	<	3	<	7
wheat, whole (flour)	<	<	<	<	<	<	**madacamia nuts**	<	<	<	<	<	<
adzuki beans	<	<	<	<	2	2	**peanuts**	<	<	<	<	<	<
black-eyed peas	<	<	<	4	2	2	**pecan**	<	<	<	<	<	<
green beans (boiled)	<	<	<	7	<	<	**pistachio nuts**	<	<	<	<	<	<
green beans	<	<	<	<	<	<	**walnuts**	<	<	<	6	2	<
green peas	<	<	<	<	<	<	**cocoa**	<	<	<	9	8	6
lentils	<	<	<	<	<	<	**arrowroot**	<	<	<	2	<	<
lima beans	<	<	<	<	<	<	**gluten free cereal**	<	<	<	2	<	<

[a] response after subtracting background calculated in ng/mL of Abrin based on standard curves
polymono ELISA negative control antibody (NCA) ranged from 0.06-0.1 OD (<3ng/mL), polypoly ELISA NCA typically equiv. to <4ng/mL, one sample was equiv. to 6 ng/mL.
[b] < indicates response was less than the lowest abrin standard of 2 ng/mL

CONCLUSIONS

ELISA (Poly/Poly and Poly/Mono) and ECL assays for the detection of abrin were developed and demonstrated capable of detecting abrin.

The ELISA and ECL assays were able to detect abrin in food at concentrations orders of magnitude less than that associated with abrin posing a health concern.

The antibodies used for the assays showed no significant cross-reactivity with components of a challenge library at concentrations of 2, 0.2, and 0.02 % (w/v).

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FDA

Department of Health and Human Services