

## Introduction

Historically, detection of Sarin exposure has relied on the fact that substantial amounts of Isopropyl Methylphosphonic Acid (IMPA) can be detected in urine and blood plasma shortly after exposure. In order to confirm an exposure it is necessary to utilize multiple methods to verify the presence of Sarin. In the event of an attack there may be severe limitations on the ability to collect timely samples in a sufficient quantity for analysis. The Multi-Method approach presented here offers the ability to verify exposures from initial exposure for up to 28 days utilizing 100 microliters of plasma in the process.

## Experimental Method

An analysis scheme for determining three separate biomarkers of Sarin (GB) exposure from a single aliquot of blood has been developed. The detection of butyrylcholinesterase (BChE)-bound Sarin, Albumin-bound Sarin, and the primary hydrolysis product Isopropyl Methylphosphonic Acid (IMPA) have been demonstrated utilizing a series of sample preparation steps (Figure 1). Studies are being conducted to determine diagnostic tests that will confidently detect multiple biomarkers of exposure in order to yield a definitive confirmation of an exposure with minimal sample volume required.

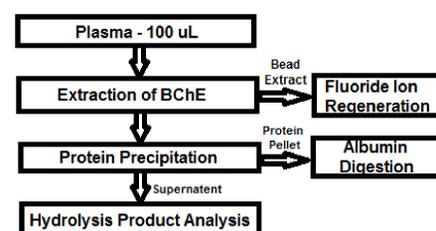


Figure 1. Sample preparation sequence used for Multi-Method analysis.

## Immunomagnetic BChE Extraction

Immunomagnetic beads are prepared by coating the surface of the magnetic bead with antibutrylcholinesterase and stored at 4°C until use. An aliquot of 100 uL of the prepared bead mixture is placed into a centrifuge tube and the storage solution removed. The BChE extraction step was optimized by reviewing the volume of plasma extracted, the time allowed for the extraction, and the method of mixing that was utilized. The efficiency was evaluated by using the Ellman Activity assay to determine the percentage of BChE activity that remained in solution following extraction. The optimal conditions were determined to be 100 uL of plasma shaken using a TomTec shaker nest for 60 minutes. The extraction efficiency versus time is shown in Figure 2.

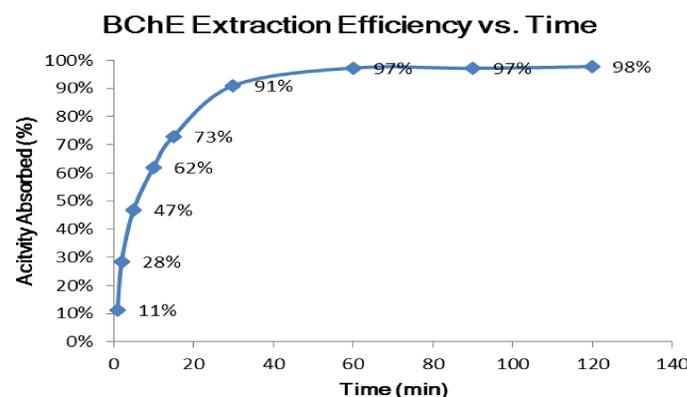


Figure 2. Activity analysis results for different mixing times using 100 uL of plasma and a Tomtec Shaker nest plate to mix the samples.

## Method Validation

After determining a set of parameters that result in a quantitative extraction (>95%) of BChE from the plasma, a validation study was conducted to incorporate all three analysis studies. A four-day set of experiments were carried out to both validate the process and determine a mass balance for plasma samples spiked at Low (1 ng/mL), Medium (5 ng/mL), and High (20 ng/mL) levels. Activity measurements for the spiked plasma samples used for the validation study are shown in Table 2.

Table 2. Activity measurements for spiked plasma samples used for the validation study.

	Activity (U/mL)	%BChE Inhibited
Control	5924	--
Low (1 ng/mL)	6009	-1%
Medium (5 ng/mL)	2848	52%
High (20 ng/mL)	56	99%

The results of the mass balance shown in Table 1 are in close agreement and with samples that were analyzed by each method independently (data not shown) with the exception of the Medium concentration for IMPA. The sensitivity of the instrument was not able to determine a reliable concentration at that level over the course of the validation study. The validation demonstrates the ability to recover more than 85% of GB spiked as low as 1 ng/mL utilizing a small volume of plasma sample with a relative standard deviation of less than 15%. This represents levels of exposure that are not distinguishable by the Ellman activity analysis and would be asymptomatic to the victim.

Table 1. Mass balance from the average of three days (n=9) including an analysis of the residual plasma after the BChE was removed. Results normalized to the equivalent quantity of GB recovered.

	Extracted BChE Fluoride Ion Regeneration (ng/mL GB)	Residual Plasma (n=3) Fluoride Ion Regeneration (ng/mL GB)	Albumin (ng/mL GB)	Hydrolysis Product (ng/mL GB)	Total (ng/mL GB)	% GB Recovery
Control	--	--	--	--	--	--
Low (1 ng/mL)	0.77 +/- 0.08	--	0.09 +/- 0.01	--	0.86	86%
Medium (5 ng/mL)	3.38 +/- 0.27	--	0.41 +/- 0.03	--	3.79	76%
High (20 ng/mL)	5.44 +/- 0.47	0.60 +/- 0.02	4.97 +/- 0.25	8.23 +/- 0.96	19.25	96%

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