Development and Validation of a Homogeneous Immunoassay for the Detection of Ethyl Glucuronide in Urine

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Introduction and Objective

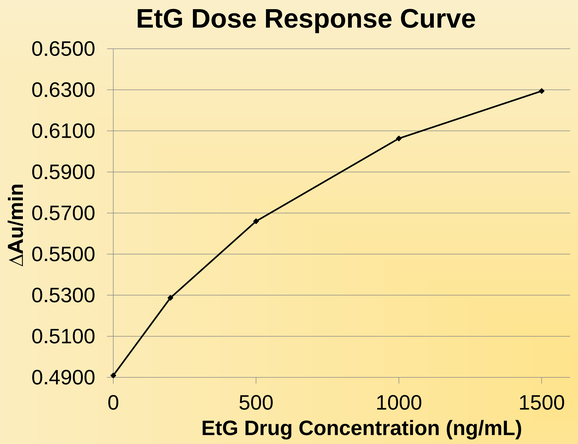
The objective of this project was to develop and validate a high throughput homogeneous enzyme immunoassay (HEIA) for the rapid detection of ethyl glucuronide in human urine.

Ethyl Glucuronide (EtG) is a minor metabolite of ethanol. Ethanol is primarily used as a social drug; however it can be found in mouthwashes, medical liquids, and manufactured solvent and gasoline additives, therefore it is not a ideal biomarker for alcohol consumption. Ethanol metabolizes about 95% of its dose and the remaining 5% is unchanged in breath, urine, sweat and feces. EtG is used as a biomarker for ethanol intake over its major metabolite, acetaldehyde due to its slow elimination in urine and specificity for ethanol. EtG can be detected up to 130 hours at a cutoff of 500 ng/mL(1). For this reason, it is valuable to develop and validate a homogeneous immunoassay (HEIA) to detect EtG in urine.

Advantages

1. Ready to use reagents suitable for high throughput instruments
2. Assay working range : 0 to 1500ng/ml with duel cutoff concentrations at 500ng/ml and 1000ng/ml
3. Specific assay with accuracy >95% based on 54 urine specimens

Results and Discussion



Precision: Daily Calibration Required

The precision was determined by assaying calibrators and controls in synthetic urine for 4 days, 2 runs per day in replicates of 10 (N=80). The results are summarized below.

Conc. ng/mL	Mean Conc. (ng/ml)	C.V.%
Intra-day Precision (n = 20)		
375 ng/mL (Control LOW)	550.1	0.7
500 ng/mL Calibrator	569.2	0.5
625 ng/mL (Control HIGH)	592.4	0.5
Inter-day Precision (n = 80)		
375 ng/mL (Control LOW)	554.3	0.8
500 ng/mL Calibrator	573.8	0.7
625 ng/mL (Control HIGH)	597.1	0.8

Cross Reactivity:

Structurally related compounds that are potentially found in urine were tested using the 500 ng/mL cutoff calibrator. All these compounds produced a negative value at the concentration listed on table below.

Analytes	Analyte Conc. (ng/mL)
Morphine-3-Glucuronide	1,000,000
Morphine-6-Glucuronide	100,000
Norbuprenorphine-Glucuronide	100,000
Oxazepam Glucuronide	100,000
Glucuronic Acid	1,000,000
Ethanol	1,000,000

Authentic specimens:

• 54 urine specimens previously confirmed by an outside laboratory by LC-MS/MS were analyzed with this Immunalysis EtG EIA assay
• Cutoff concentration: 500 ng/mL for both EIA and confirmation method
• 23 specimens were negative by both methods
• 30 specimens were positive by both methods

• The sensitivity, specificity and accuracy were 97%, 100%, and 98%, respectively

		LC-MS/MS +	LC-MS/MS -
HEIA	+	30	0
	-	1*	23

•Negative result: absorbance rate reading just below cutoff
•Confirmation = EtG 663 ng/mL, EtS 339 ng/mL.

Summary

A high throughput HEIA has been developed for the detection of ethyl glucuronide in human urine which correlates well with LC-MS/MS.

References

1. G.Reisfield, B.Goldberger, B.Crews, A. Pesce, G. Wilson, S. Teitelbaum, and R. Bertholf. Ethyl glucuronide, ethyl sulfate, and ethanol in urine after sustained exposure to an ethanol-based hand sanitizer. *J. Anal. Toxicol.* **35:** 85-91 (2011)

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