Evaluation of the Roche Benzodiazepines II Immunoassay for Urine Drug Testing in Clinical Specimens

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Introduction

Benzodiazepines are one of the most commonly prescribed medications in the United States and are frequently linked to instances of abuse and overdose. Historically, FDA-cleared benzodiazepine urine immunoassays cross-react poorly with glucuronidated metabolites excreted in urine. False negative results are especially prevalent with lorazepam which is almost exclusively excreted at lorazepam-glucuronide. Some clinical laboratories have addressed this problem with the addition of beta-glucuronidase to enhance assay sensitivity as a laboratory developed test (LDT). Roche Diagnostics recently received FDA clearance to offer a benzodiazepine immunoassay that includes beta-glucuronidase.

Methods

Performance characteristics of two FDA-cleared benzodiazepine urine immunoassays (Benzodiazepines Plus, no glucuronidase and Benzodiazepines II, with glucuronidase; Roche Diagnostics) and a benzodiazepine immunoassay LDT (with glucuronidase) were evaluated using 258 urine specimens. These immunoassays were directly compared to an LC-MS/MS benzodiazepine LDT to determine clinical sensitivity and specificity. Cross-reactivity of all three immunoassays were compared and evaluated based on the measured benzodiazepine concentrations determined by the LC-MS/MS LDT. The cutoff for a positive result on all three immunoassays is 200 ng/mL based on a nordiazepam calibrator. Cross-reactivity for 7-aminoclonazepam, lorazepam, and lorazepam-glucuronide were assessed using drug-free urine spiked with reference materials (Cerilliant) at concentrations ranging from 100 to 1000 ng/mL.

Flow Diagram

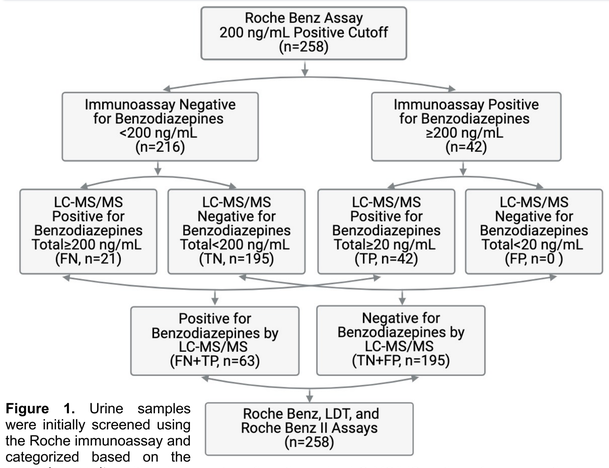

Figure 1. Urine samples were initially screened using the Roche immunoassay and categorized based on the screening results, with subsequent confirmation using LC-MS/MS as the reference method.

Clinical Sensitivity And Specificity

Assays (Total=258)	Negative	Positive	FN	FP	TN	TP	Sensitivity	Specificity
Roche Benz	216	42	21	0	195	42	66.7%	100.0%
LDT	198	60	3	0	195	60	95.2%	100.0%
Roche Benz II	195	63	0	0	195	63	100.0%	100.0%
LC-MS/MS*	195	63	* Reference method					

Table 1. Clinical sensitivity and specificity of the three assays. LC-MS/MS analysis was used as the reference method. FN: false negative; FP: false positive; TN: true negatives; TP: true positives.

Comparison of Benzodiazepine Immunoassays

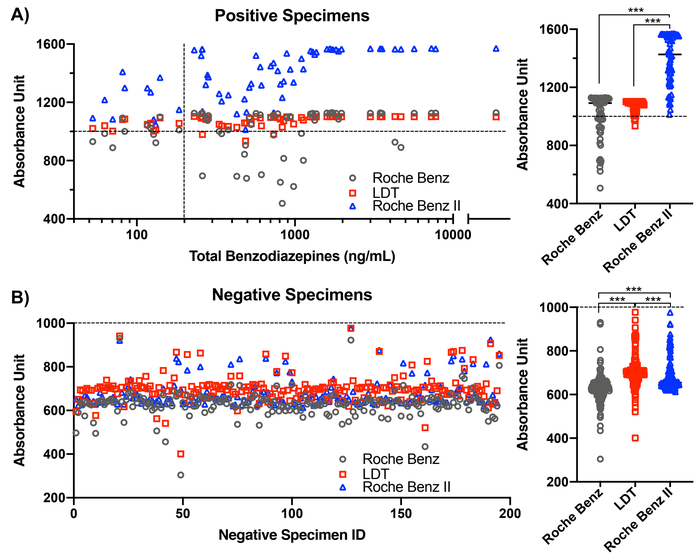

Figure 2. Comparison of absorbance values in benzodiazepine immunoassays. The cutoff for a positive result (200 ng/mL of benzodiazepines, vertical line) is indicated by a horizontal line at 1000 abs units.

Discordant Samples Across All Three Assays

Sample ID	Roche Benz	LDT	Roche Benz II	7-aminoclonazepam	a-OH-midazolam	a-OH-triazolam	a-OH-alprazolam	2-OH-ethylflurazepam	Lorazepam	Oxazepam	Desalkylflurazepam	Temazepam	Nordiazepam
7	890	**1101**	**1564**							188.2		4474.8	24.3
8	905	934	**1013**	486.9									
22	692	**1028**	**1120**						428.7				
26	651	**1085**	**1324**							69.1		739.9	
27	930	**1020**	**1092**				52.6						
32	986	**1095**	**1500**							223.4		788	89.6
33	975	982	**1129**	733.4									
37	985	**1039**	**1217**				62.6						
42	843	**1058**	**1222**							462.1		10.9	7
43	926	**1102**	**1565**				5.4			353.4		3926.6	7.9
44	678	**1057**	**1132**							39.9		455	
47	815	**1074**	**1344**		54.7				795				
48	922	**1009**	**1046**				63.9			52.7		8.2	7.2
52	984	**1037**	**1208**	177.4					21.6	84.9		29.9	30.4
53	801	**1078**	**1428**	69					1062.5				
58	622	**1051**	**1337**	57.2					923.5				
61	979	**1002**	**1082**		97.5		30.2						
62	506	**1030**	**1232**						832.3				
63	888	**1002**	**1084**				70.4						
64	703	**1038**	**1317**						613.3				
65	695	979	**1138**						259.8				

Table 2. Twenty-one samples exhibited discordance among all three assays. Absorbances exceeding 1000 are bolded. LC-MS/MS was used to test urine specimens for 10 benzodiazepines. Red indicates two urine samples containing 7-aminoclonazepam that only Roche Benz II detects positive.

Cross-reactivity Of The Benzodiazepine Assays

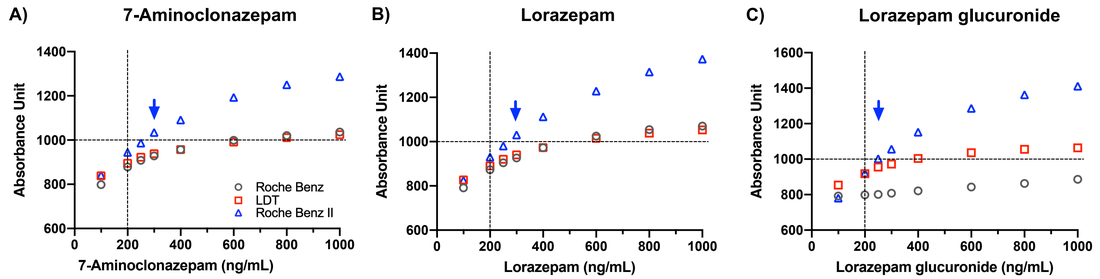

Figure 3. Cross-reactivity of the benzodiazepine assays with 7-aminoclonazepam, lorazepam and lorazepam glucuronide. Drug-free urine samples spiked with A) 7-aminoclonazepam, B) lorazepam, or C) lorazepam glucuronide in the concentration range from 100 to 1000 ng/mL, were tested using three benzodiazepine immunoassays.

Conclusions

A comprehensive evaluation of these three immunoassays demonstrates that the Benzodiazepines II immunoassay has increased clinical and analytical sensitivity compared to the Benzodiazepines Plus and LDT immunoassays. The inclusion of a beta-glucuronidase greatly improved the sensitivity of the Benzodiazepines II and LDT immunoassays for lorazepam, which is primarily excreted as a glucuronide metabolite in urine.