



Benzodiazepines in oral fluid: Effect of screening cut-off concentrations

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Abstract

• The concentration of benzodiazepines in oral fluid is considerably lower than the concentration in corresponding blood specimens. In an effort to eliminate false negative results in oral fluid screening, reduced cut-off values were studied.

Objectives

* To determine how many additional oral fluid specimens will test positively for benzodiazepines using a cut-off level of 5ng/mL and how many positive specimens will be missed (test negative) using a 20ng/mL cut-off.

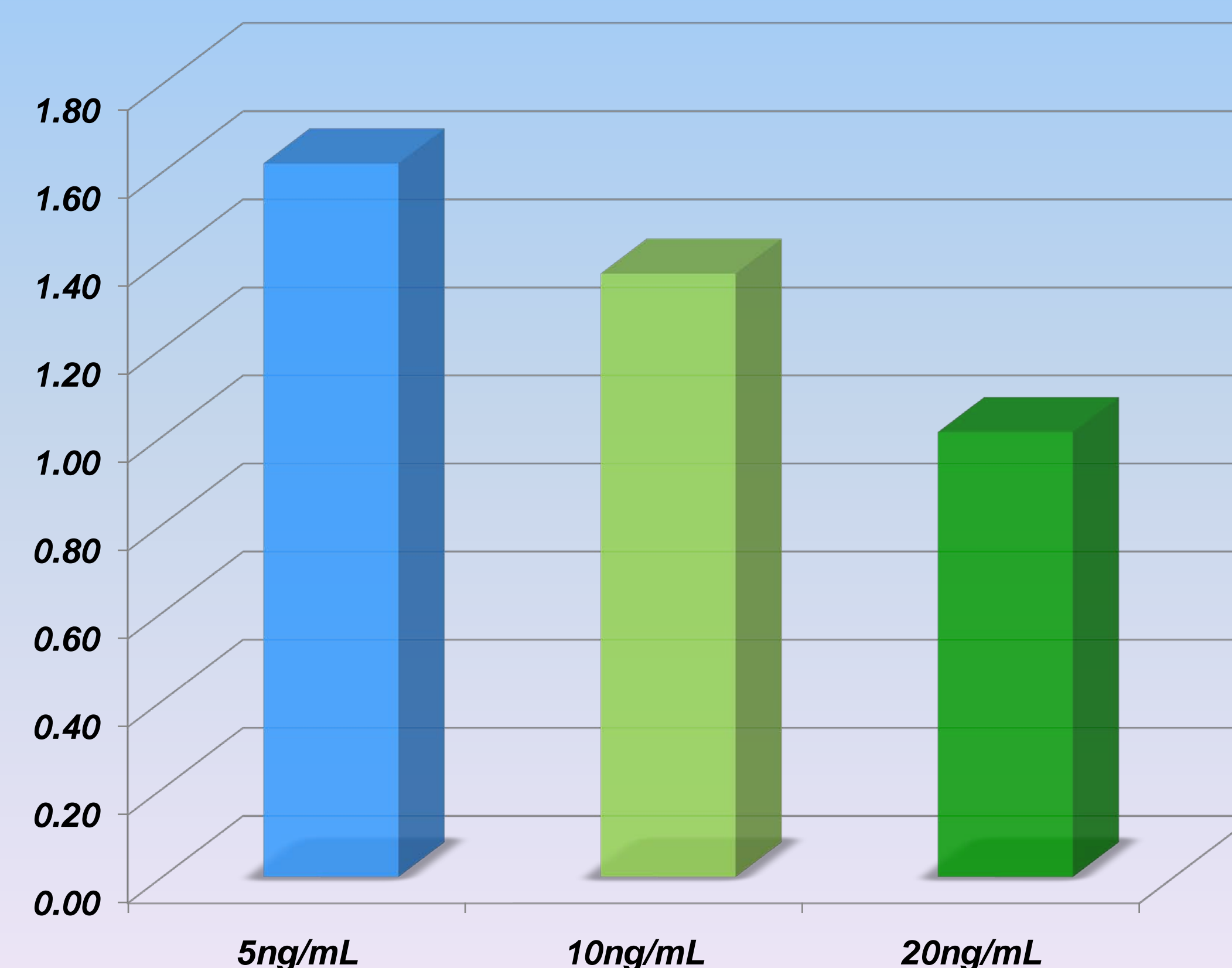
Methods

- Oral fluid specimens received into our laboratory during routine testing for drugs of abuse were analyzed with an ELISA screen for benzodiazepines.
- Oxazepam was used as the calibration standard with a cut-off concentration of 10ng/mL.
- For confirmation using LC-MS/MS, a limit of quantitation of 0.5ng/mL was used on a fully validated method.

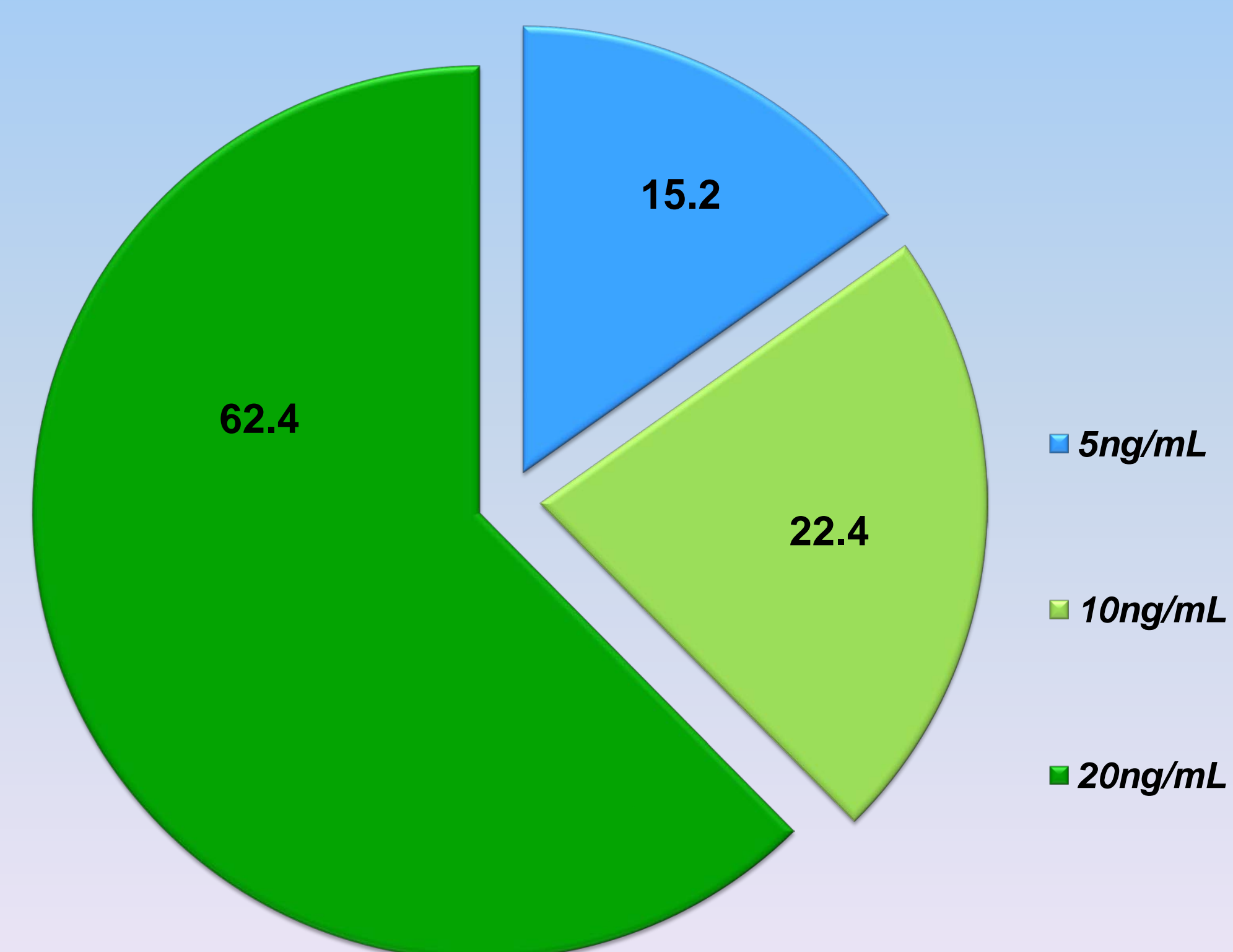
Results

- Data was extracted from oral fluid batches received over the last year.
- A total of 125 specimens screened above 5ng/mL.
- Of the 125 specimens, 62.4% (n=78) screened above 20ng/mL, 22.4% (n=28) were between 10ng/mL and 20ng/mL, and 15.2% (n=19) were between 5ng/mL and 10ng/mL.
- Of the 19 specimens screening between 5 and 10ng/mL, 8 were present in sufficient amounts for confirmation.
- All 8 samples confirmed positively for benzodiazepines:
- Alprazolam (n = 4); lorazepam (n=1); diazepam (n = 1); nordiazepam and diazepam (n= 2)

% of Total Specimens Received



% of Total Positives (n=125)



Data Summary

ID	5ng/mL B/Bo	10ng/mL B/Bo	SMP B/Bo	KIT X-R	Confirmation (ng/mL)
1	33.3	21.9	29.4	180%	1.5 alprazolam
2	32.6	22	29.1	70% / 50%	1.0 diazepam/nordiazepam
3	41.7	29.4	29.5	180%	1.7 alprazolam
4	36.8	24.2	27.5	180%	1.8 alprazolam
5	35.2	26.1	29.3	70% / 50%	0.59 diazepam/nordiazepam
6	39.3	27.6	36.6	70%	1.5 diazepam
7	36.8	26.9	31.1	180%	1.8 alprazolam
8	34.7	22.9	28.7	90%	13 lorazepam

B/Bo = Absorbance of sample (SMP)/Absorbance of negative x 100%

Conclusions

- An immunoassay screening cut-off concentration of 5ng/mL for benzodiazepines in oral fluid is recommended
- For each doubling of the cut-off level, 15-20% of positive specimens would not be subjected to confirmation analysis
- Several specimens confirmed at concentrations lower than the screening cut-off of 5ng/mL indicating the synergistic effect of immunoassays and importance of cross-reactivity

