K13 Laboratory Analysis of Remotely Collected Oral Fluid Specimens for Ethanol by Enzymatic Assay

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Currently, the drugs of abuse that are available from Orasure Technologies, Inc. for testing with the microplate ELISA assays do not include ethanol, which is one of the most abused drugs. This poster describes an enzymatic assay for ethanol that can be performed in a 96 well microplate which can be analyzed using the same equipment used for the analysis of the other drugs of abuse by ELISA.

This should enable laboratories to analyze ethanol in a 96 well plate format along with the other Intercept microplate assays to improve throughput and decrease sample handling, thereby decreasing chances of error.

The performance characteristics of this method for detecting ethanol in oral fluid specimens using a qualitative, enzymatic assay were examined. The assay uses oral fluid which is obtained using the Intercept® DOA Oral Specimen Collection device. The Intercept® DOA Oral Specimen Collection device utilizes a collection pad which collects approximately 0.4mL of oral fluid. The collection pad is then placed in a vial containing 0.8mL of a buffered preservative solution, which is then shipped to the laboratory for analysis. The laboratory then centrifuges the device to collect the diluted oral fluid mixture. Sample from the device is added to a blank 96 well plate and the plate is read at 340nm for a background reading. This background is performed before the addition of the ethanol reagent since other prescription medications absorb at the same wavelength, which could interfere with the assay. Ethanol reagent, which consists of NAD and ADH, is then added to the plate and the plate is read again at 340nm. The resultant reaction causes a change in absorbance, which is directly proportional to the amount of alcohol present.

Both positive and negative specimens were collected from 560 patients in a Methadone treatment clinic and tested in the ethanol microplate assay using a cutoff of 2.5mg/dL. Ethanol analysis was then performed by Headspace GC/FID (Gas Chromatography/Flame Ionization Detection) using a cutoff of 2.5mg/dL.

The enzymatic assay was tested for precision, stability of the sample, stability of the ethanol reagent and the effects of potential crossreactants. The total precision for 5 consecutive days of testing yielded CV's less than 10%. The assay was tested and compared against the following possible cross-reacting alcohols: n-butanol, isopropanol, methanol, ethylene glycol, and acetone. The following adulterants were also tested for interference with the assay: sugar water, toothpaste, antacid, antiseptic, cola, and cranberry juice.

Of the 560 patients tested, 19 were positive by both the enzymatic assay and GC/FID. The range of positive results was 2.9 to 150mg/dL of diluted oral fluid sample. The results would need to be multiplied by three to correct for the dilution of the oral fluid in the collection device. The results yielded 99.3% agreement between the enzymatic assay and GC/FID using a cutoff of 2.5mg/dL of diluted oral fluid for both methods.

Oral Fluids, Ethanol, Intercept

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