CEDIA® Heroin Metabolite (6-AM) Application for the Ortho Clinical Diagnostics VITROS® 4600 Chemistry System and VITROS® 5600 Integrated System

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Abstract

Introduction: Heroin (3, 6-diacetylmorphine) is a Schedule 1 substance and commonly abused opioid within the United States and in the rest of the world. It is generally synthesized through chemical modification of morphine, a naturally occurring alkaloid. Heroin can be administered by intravenous and subcutaneous injection or by nasal insufflation. It is rapidly metabolized (half life of 9 minutes) to Monoacetylmorphine (6-AM) by various esterases in the blood; then it is converted to morphine within the liver through hydrolysis. 6-AM is a distinctive metabolic marker for heroin use because it cannot be formed by acetylation of morphine within the body. Use and possession of heroin is illegal and is associated with a number of adverse effects including lung complications, kidney disease, and bacterial infection of blood vessels. The ability to detect heroin within overdose patients and drug offenders proves vital for health practitioners and the members of the criminal justice system.

Method: The CEDIA 6-AM Assay utilizes the enzyme β-galactosidase, which has been genetically engineered into two inactive fragments (EA and ED). The heroin metabolite present within human urine samples and the 6-AM conjugated to an inactive enzyme fragment (ED-LC) compete for antibody binding sites. Because 6-AM inhibits the binding of ED-LC to the antibody, its presence allows the two inactive enzyme fragments to better re-associate into an active enzyme. The concentration of 6-AM within the sample will affect the complementation of the enzymes fragments. Enzyme activity results in an absorbance change that is directly proportional to the concentration of 6-AM in the sample; this change can be measured spectrophotometrically. The VITROS 4600 Chemistry and VITROS 5600 Integrated Systems are new applications for the CEDIA 6-AM Assay for the qualitative detection of heroin metabolite in human urine with a cutoff of 10 ng/mL. Analyzer performance was determined for precision and accuracy. Correlation studies using the two instruments were conducted in comparison to liquid chromatography–mass spectrometry (LC-MS) values.

Results: All studies were evaluated using CLSI guidelines. Three levels of 6-AM controls were used in the studies. The within-run precision ranged from 0.3 to 0.4% CV and the total precision, 1.6 to 2.2% CV. Accuracy was measured using patient correlation against LC-MS values. The VITROS 4600 Chemistry System yielded 92.7% Positive Agreement, 97.8% Negative Agreement, and 95.0% Total Agreement ($n_{negative}$=45, $n_{positive}$=55, n_{total}=100). The VITROS 5600 Integrated System yielded 92.7% Positive Agreement, 97.8% Negative Agreement, and 95.0% Total Agreement ($n_{negative}$=45, $n_{positive}$=55, n_{total}=100).

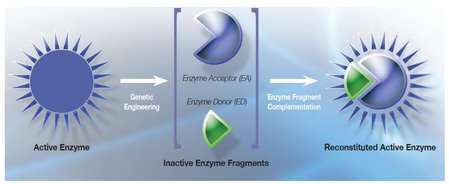
Conclusions: All measured studies demonstrated acceptable performance, validating use of the CEDIA Heroin Metabolite Assay on the Ortho Clinical Diagnostics VITROS 4600 Chemistry and VITROS 5600 Integrated Systems. The assay will provide an effective detection system to screen individuals who use heroin in its various forms.

Introduction

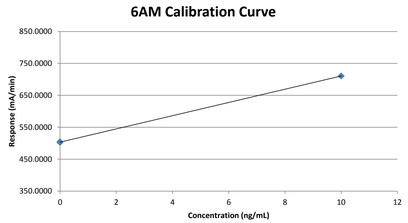
The CEDIA Heroin Metabolite Assay is a homogeneous enzyme immunoassay. The assay utilizes a specific antibody that can detect Heroin metabolites. The CEDIA Heroin Metabolite Assay is intended for the qualitative detection and estimation of Heroin and its metabolites in human urine at a cutoff of 10 ng/mL.

Assay Method

The foundation for the CEDIA immunoassay is the use of two polypeptides that are created by separating the bacterial enzyme ß-galactosidase into two inactive fragments: the enzyme acceptor (EA) and the enzyme donor (ED). These two fragments can spontaneously recombine to create an active enzyme. To detect an analyte in a patient sample, the ED fragment is first chemically coupled to the target analyte. The labeled ED fragment is referred to as an ED-ligand conjugate (ED-LC).The labeling of the ED fragment does not restrict its ability to recombine (complement) with the EA fragment and create an active enzyme. When the reagents and the sample fluid are brought together, the labeled fragments (ED-LC) and free analyte in the sample fluid (if present) compete in binding to a limited number of analyte-specific antibody binding sites. If the analyte is not present in the sample, the ED-LC will bind to the antibody, allowing fewer active enzymes to form. However, if the analyte is present in these analyte molecules, it will successfully compete for analyte binding sites, resulting in greater complementation of ED-LC and EA fragments.



Standard Curve



Performance Studies

The CEDIA Heroin Metabolite Assay was applied to the VITROS 4600 Chemistry System and VITROS 5600 Integrated System. The performance data of the CEDIA Heroin Metabolite Assay on the VITROS chemistry analyzers is presented in the following sections. The results obtained from different analyzer or laboratory may differ from these data.

Precision

Each level of control was assayed in forty runs over a period of twenty days in replicates of two per run, following CLSI protocol. Each of the runs per day was separated by at least two hours. The means, within-run and total-run SDs, and within-run and total-run %CVs are summarized in the following table.

Samples	Mean (mA/min)	Within-Run SD (mA/min)	Within-Run %CV	Total-Run SD (mA/min)	Total-Run %CV
VITROS 4600 - Qualitative					
Low Control	627.2	1.95	0.3	10.11	1.6
Cutoff	672.1	2.26	0.3	12.81	1.9
High Control	706.3	2.30	0.3	13.68	1.9
VITROS 5600 - Qualitative					
Low Control	627.0	2.38	0.4	13.04	2.1
Cutoff	671.2	2.53	0.4	14.32	2.1
High Control	706.4	2.17	0.3	15.41	2.2

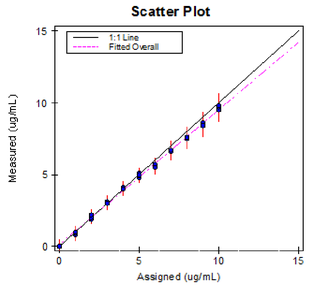
Linearity

Eleven levels of manufacturing calibrator blends spanning the range of the assay were tested under a single calibration curve. Each sample was tested in replicates of four, with the mean of measured concentration compared to its expected concentration for the % recovery. The linear relation between measured and expected is described using the % Expected Recovery of the sample.

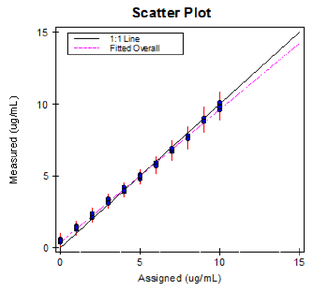
The linearity ranges of each analyzer are summarized in the table below.

Instrument	Minimum % Recovery	Maximum % Recovery
VITROS 4600	92.5%	103.8%
VITROS 5600	95.6%	137.5%

Linearity – Heroin Metabolite on the VITROS 4600



Linearity – Heroin Metabolite on the VITROS 5600



Method Comparison

Clinical urine samples were obtained from patients and tested with the CEDIA Heroin Metabolite Assay in multiple runs over multiple calibration curves. The results were compared to the LC-MS/MS method. Data analysis was done on the sample sets, and is shown in the table below.

Method Comparison Data Summary

Instrument	N	Positive Agreement	Negative Agreement	Total Agreement
VITROS 4600	100	92.7%	97.8%	95.0%
VITROS 5600	100	92.7%	97.8%	95.0%

Conclusion

The results of the CEDIA Heroin Metabolite Assay Application demonstrates acceptable performance on the VITROS 4600 Chemistry System and VITROS 5600 Integrated System. The validation results obtained for assay sensitivity, precision, linearity, stability, and method comparison will ensure appropriate results and repeatability throughout the assay range, which is extremely important in the clinical setting. Proper monitoring of heroin metabolite is essential for health professionals and law enforcement. The application validation of CEDIA 6AM on VITROS 4600 and VITROS 5600 systems brings an effective monitoring system for clinical laboratories and their customers.

References

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