DRI™ Methadone Metabolite Applications for the Ortho Clinical Diagnostics VITROS® 4600 Chemistry System and VITROS® 5600 Integrated System

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Abstract

Introduction: Methadone is a synthetic opioid that has been used for decades by clinics and other addiction-treatment facilities to manage opioid dependency. After administration, methadone is metabolized to normethadone by N-demethylation, which is converted by dehydration to the primary metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)[1]. Effective monitoring of methadone and its metabolite is essential for ensuring compliance.

Method: The DRI Methadone Metabolite Assay is a competitive assay between drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) and free drug in the urine sample for a fixed number of antibody binding sites. Antibodies detect EDDP without crossreactivity to the parent drug, methadone. Drug concentration and enzyme activity are directly proportional to the conversion of NAD to NADH, which is measured spectrophotometrically at 340 nm. The VITROS 4600 and 5600 Systems are new applications for the DRI Methadone Metabolite Assay for the qualitative and semiquantitative determination of methadone metabolite in human urine at a cutoff on 1000 ng/mL.

Results: All studies were evaluated using CLSI guidelines. Total precision was evaluated for 20 days in which low and high control levels were compared to the cutoff calibrator level in both qualitative and semiquantitative modes. For the VITROS 4600, qualitative %CV results ranged from 0.3-0.4% and semiquantitative %CV results ranged from 1.4-1.7%. For the VITROS 5600, qualitative %CV results ranged from 0.6-0.9% and semiquantitative %CV results ranged from 1.3-1.7%. One-hundred and ten (110) patient samples were analyzed for positive and negative agreement compared to liquid chromatography-mass spectrometry (LC-MS). Qualitative positive and negative agreement was 100% and 90%, respectively, on both instruments. Semiquantitative positive agreement was 98% on the VITROS 4600 and 100% on the VITROS 5600. Semiquantitative negative agreement was 91.7% on both instruments.

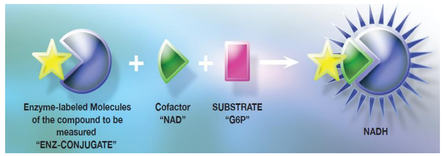
Conclusions: All studies demonstrated acceptable performance, validating the use of the DRI Methadone Metabolite Assay on the Ortho Clinical Diagnostics VITROS 4600 Chemistry System and VITROS 5600 Integrated System.

Introduction

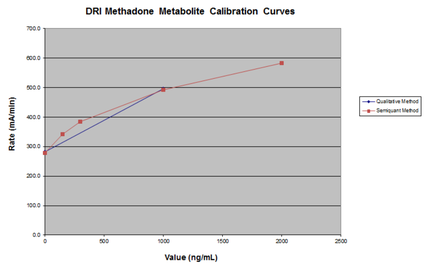
The DRI Methadone Metabolite Assay covers a semiquantitative range of 31-1000 ng/mL. Following the guidelines set by the FDA Replacement Reagent and Instrument Family Policy, the assay and the VITROS 4600 Chemistry System and VITROS 5600 Integrated System have been previously cleared, necessitating the creation of a reagent replacement report to internally document the cleared devices.

Assay Method

The assay is based on competition between a drug labeled with glucose-6- phosphate dehydrogenase (G6PDH), and free drug from the urine sample, for a fixed amount of specific antibody binding sites. In the absence of free drug from the sample, the specific antibody binds the drug labeled with G6PDH and causes a decrease in enzyme activity. In the presence of free drug, the free drug occupies the antibody binding sites, allowing the drug bound G6PDH to interact with the substrate, resulting in enzyme activity. This phenomenon creates a direct relationship between the drug concentration in urine and enzyme activity. The enzyme activity is determined spectrophotometrically at 340 nm by measuring the reduction of nicotinamide adenine dinucleotide (NAD) to NADH.



Standard Curves



Performance Studies

The DRI Methadone Metabolite Assay was applied to the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System. The performance data of the assay on these systems are presented in the following sections. Results obtained from different analyzers or laboratories 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 a 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 tables.

Samples	Mean	Within-Run SD	Within-Run %CV	Total-Run SD	Total-Run %CV
VITROS 4600 – Qualitative					
Low Ctrl	457.0 mA/min	0.59 mA/min	0.1%	1.46 mA/min	0.3%
Cutoff	489.1 mA/min	1.07 mA/min	0.2%	1.61 mA/min	0.3%
High Ctrl	525.4 mA/min	1.39 mA/min	0.3%	2.29 mA/min	0.4%
VITROS 4600 – Semiquantitative					
Low Ctrl	732.5 ng/mL	4.12 ng/mL	0.6%	10.27 ng/mL	1.4%
Cutoff	980.3 ng/mL	9.08 ng/mL	0.9%	13.66 ng/mL	1.4%
High Ctrl	1323.6 ng/mL	14.58 ng/mL	1.1%	23.00 ng/mL	1.7%
VITROS 5600 – Qualitative					
Low Ctrl	459.9 mA/min	0.76 mA/min	0.2%	1.30 mA/min	0.3%
Cutoff	492.7 mA/min	1.02 mA/min	0.2%	1.53 mA/min	0.3%
High Ctrl	529.3 mA/min	0.79 mA/min	0.1%	1.73 mA/min	0.3%
VITROS 5600 – Semiquantitative					
Low Ctrl	727.6 ng/mL	5.28 ng/mL	0.7%	12.73 ng/mL	1.7%
Cutoff	977.5 ng/mL	8.46 ng/mL	0.9%	14.46 ng/mL	1.5%
High Ctrl	1315.3 ng/mL	8.06 ng/mL	0.6%	16.98 ng/mL	1.3%

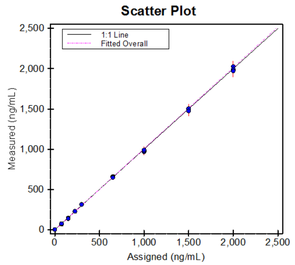
Linearity/Accuracy by Recovery

Seven levels of manufacturing calibrators 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.

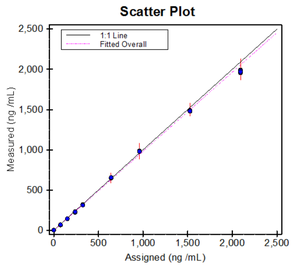
The linearity ranges of each analyzer are summarized in the table below.

Instrument	Minimum % Recovery	Maximum % Recovery
VITROS 4600	**93.2%**	**104.4%**
VITROS 5600	**88.7%**	**102.1%**

Linearity – Methadone Metabolite on the VITROS 4600



Linearity – Methadone Metabolite on VITROS 5600



Method Comparison

Clinical human urine samples were obtained and tested with the DRI Methadone Metabolite Assay in multiple runs and over multiple calibration curves. The results were compared to the same samples analyzed using LC/MS-MS.

Method Comparison Data Summary - Qualitative

Analyzer	N	Positive Agreement	Negative Agreement	Total Agreement
VITROS 4600	**110**	**100%**	**90%**	**94.5%**
VITROS 5600	**110**	**100%**	**90%**	**94.5%**

Method Comparison Data Summary - Semiquantitative

Analyzer	N	Positive Agreement	Negative Agreement	Total Agreement
VITROS 4600	**110**	**98.0%**	**91.7%**	**94.5%**
VITROS 5600	**110**	**100%**	**91.7%**	**95.5%**

Conclusion

The results of the DRI Methadone 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 for the effective monitoring of compliance in the treatment of opioid dependency.

Reference

1. Randall C. Baselt and Robert H. Cravey. Disposition of Toxic Drugs and Chemicals in Man. pp 472-475, 4th Ed. Chemical Toxicology Institute. (1995).

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