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

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## Abstract

**Introduction:** Hydrocodone is a commonly used semi-synthetic opioid derivative. It is synthesized from codeine, a naturally occurring alkaloid. Due to its ability to relieve moderate and severe pain, hydrocodone has grown in usage, particularly within the United States. The analgesic effect of the drug begins within 20-30 minutes of taking it[1] and can last up to 8 hours.[2] Furthermore, an increase in the abuse of hydrocodone in its various forms has been observed in recent years. Thus, an effective monitoring system is necessary for clinicians and law enforcement to determine drug levels within addicts and criminals.

**Method:** The DRI Hydrocodone Assay utilizes a drug-labeled variant of glucose-6-phosphate dehydrogenase (G6PDH) and the effects of competitive inhibition. When in the presence of select antibodies, G6PDH competes with free drug present within a sample for antibody binding sites. When the drug-labeled enzyme binds to the antibodies, enzyme activity is decreased as a result. Thus, drug concentration and enzyme activity are directly proportional. This relationship can be determined by monitoring the conversion of NAD to NADH, which is measured spectrophotometrically at 340 nm. The VITROS 4600 Chemistry System and VITROS 5600 Integrated System are new applications for the DRI Hydrocodone Assay for the qualitative and semiquantitative determination of hydrocodone in human urine at a cutoff of 300 ng/mL. The analyzers were subjected to precision and accuracy studies.

**Results:** All studies were evaluated in adherence to CLSI guidelines. Total precision was conducted over the span of 20 days. In this timeframe, the low and high control values were compared to the cutoff calibrator in both qualitative and semiquantitative methods. Within-run precision results ranged from 0.1% to 0.3% CV qualitatively and 1.1% to 1.9% CV semiquantitatively between the two instruments. Total precision results ranged from 0.2% to 0.5% CV qualitatively and 1.9% to 3.2% CV semiquantitatively between the two instruments. Additionally, linearity was evaluated by comparing calibrator blends to their nominal values. The VITROS 4600 Chemistry System yielded 95.2% to 102.0% recovery, and the VITROS 5600 Integrated System yielded 94.5% to 101.7% recovery.

**Conclusions:** All studies aforementioned demonstrate acceptable performance of the DRI Hydrocodone Assay on the Ortho Clinical Diagnostics VITROS 4600 Chemistry System and VITROS 5600 Integrated System.

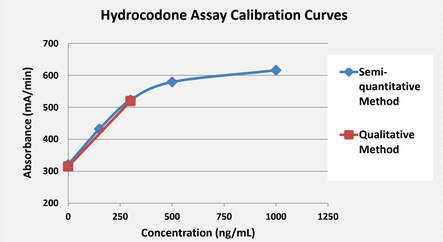
## Introduction

The DRI Hydrocodone Assay is supplied as a liquid ready-to-use homogeneous enzyme immunoassay. The assay uses specific antibody that can detect Hydrocodone and its metabolites. The DRI Hydrocodone Assay is intended for the qualitative and semi-quantitative detection and estimation of Hydrocodone and its metabolites in human urine at a cutoff of 300 ng/mL. The semi-quantitative mode allows laboratories to determine an appropriate dilution of specimen for confirmation by a confirmatory method such as LC-MS/MS or GC-MS. The qualitative mode determines if one is Hydrocodone positive or negative at the cutoff.

## 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 Hydrocodone Assay was applied to the VITROS 4600 Chemistry System and VITROS 5600 Integrated System. The performance data of the DRI Hydrocodone 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 | Within-Run SD | Within-Run %CV | Total-Run SD | Total-Run %CV |
|---|---|---|---|---|---|
| VITROS 4600 - Qualitative | | | | | |
| Ctrl Low | 577.9 mA/min | 1.36 mA/min | 0.2 % | 2.7 mA/min | 0.5 % |
| Cutoff | 606.9 mA/min | 1.6 mA/min | 0.3 % | 2.6 mA/min | 0.4 % |
| Ctrl High | 628.6 mA/min | 1.1 mA/min | 0.2 % | 2.7 mA/min | 0.4 % |
| VITROS 4600 – Semiquantitative | | | | | |
| Ctrl Low | 227.0 ng/mL | 3.1 ng/mL | 1.4 % | 6.0 ng/mL | 2.7 % |
| Cutoff | 304.6 ng/mL | 5.2 ng/mL | 1.7 % | 8.5 ng/mL | 2.8 % |
| Ctrl High | 385.8 ng/mL | 6.4 ng/mL | 1.7 % | 12.4 ng/mL | 3.2 % |
| VITROS 5600 - Qualitative | | | | | |
| Ctrl Low | 579.8 mA/min | 1.45 mA/min | 0.3 % | 1.57 mA/min | 0.3 % |
| Cutoff | 608.8 mA/min | 1.83 mA/min | 0.3 % | 1.89 mA/min | 0.3 % |
| Ctrl High | 630.5 mA/min | 0.93 mA/min | 0.1 % | 1.42 mA/min | 0.2 % |
| VITROS 5600 – Semiquantitative | | | | | |
| Ctrl Low | 252.2 ng/mL | 3.25 ng/mL | 1.4 % | 4.48 ng/mL | 2.0 % |
| Cutoff | 302.3 ng/mL | 5.83 ng/mL | 1.9 % | 7.07 ng/mL | 2.3 % |
| Ctrl High | 382.9 ng/mL | 4.11 ng/mL | 1.1 % | 7.37 ng/mL | 1.9 % |

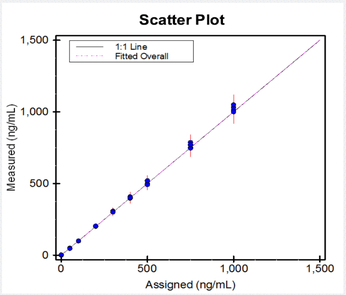
### Linearity

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.

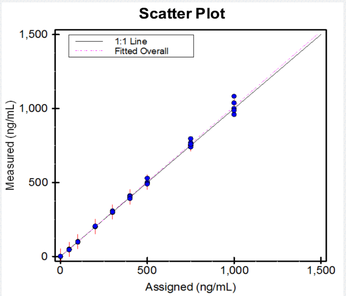
The linearity ranges of each analyzer are summarized in the table below.

| Instrument | Minimum % Recovery | Maximum % Recovery |
|---|---|---|
| VITROS 4600 | 95.2% | 102.0% |
| VITROS 5600 | 94.5% | 101.7% |

***Linearity – Hydrocodone on VITROS 4600***




***Linearity – Hydrocodone on VITROS 5600***




### Method Comparison

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

***Method Comparison Data Summary***

***Qualitative***

| Analyzer | N | Positive Agreement | Negative Agreement | Total Agreement |
|---|---|---|---|---|
| VITROS 4600 | 114 | 96.4% | 100.0% | 98.2% |
| VITROS 5600 | 114 | 96.4% | 100.0% | 98.2% |

***Semiquantitative***

| Analyzer | N | Positive Agreement | Negative Agreement | Total Agreement |
|---|---|---|---|---|
| VITROS 4600 | 114 | 96.4% | 100.0% | 98.2% |
| VITROS 5600 | 113 | 96.4% | 100.0% | 98.2% |

## Conclusion

The results of the DRI Hydrocodone 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.

## References

1. Brucker, M.C., King, T.L. Pharmacology for Women's Health. Jones & Bartlett Publishers. 2010. pp. 322–. ISBN 978-1-284-05748-5.
2. Elliott, J.A., Smith, H.S. *Handbook of Acute Pain Management*. CRC Press. 2016. pp. 79–. ISBN 978-1-4665-9635-1.

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