Automated liquid-liquid extraction of 25-Hydroxy Vitamin D From Serum and preparation for LC/MS analysis using the Hamilton Robotics Microlab® STARlet Workstation

Application Note

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Hamilton Instrument: Microlab STARlet

Abstract

Vitamin D is essential for the growth of healthy bone and its deficiency is often associated with several cardiovascular and autoimmune disorders in addition to depression, osteoarthritis, osteoporosis, cancer and many other diseases. There are several methods available to measure Vitamin D from blood serum samples, of which LC/MS is the most widely accepted in clinical labs. This application note describes the steps involved for automated extraction of 25-Hydroxy Vitamin D from blood serum and sample preparation forLC/MS analysis. An automated approach to this application eliminates tedious manual processes, resulting in an estimated 600% increase in processing and analysis efficiency.

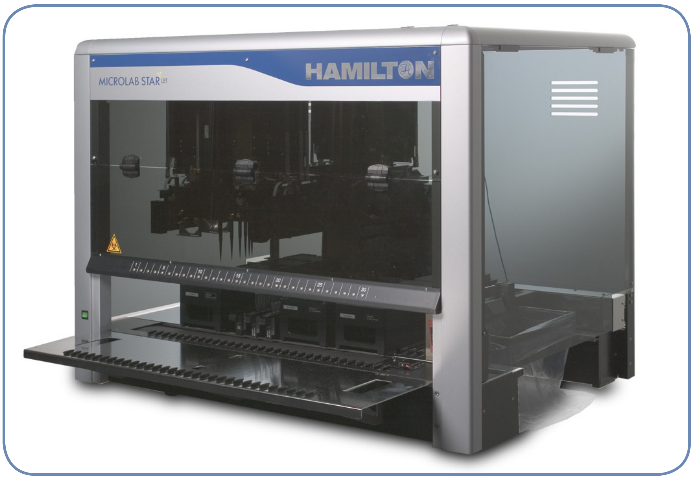

Figure 1: The Hamilton Robotics Microlab STARlet Automated Pipetting Workstation

Introduction

Vitamin D is essential for the growth of healthy bone. Recent studies have shown that Vitamin D deficiency is more prevalent than previously thought[1]. As a result, test orders for Vitamin D quantification have increased significantly. However, due to the short half life (6 hrs) of Vitamin D in blood, rapid sample preparation and processing methods have become increasingly important. Also, due to significant growth in the number of diagnostic tests, there is a need for high throughput sample preparation and processing for the LC/MS methodology, which has become the most widely accepted technology to measure Vitamin D levels in blood serum. Research has also indicated that liquid-liquid extraction is the most cost effective approach for isolating 25-Hydroxy Vitamin D from blood serum.

The Hamilton Company provides world-class automation solutions for a variety of applications, offering increased robustness, flexibility, precision and accuracy using its unique CO-RE technology. This application note demonstrates the automation capabilities of Hamilton Robotics' Microlab STARlet and how it can be used to prepare clinical serum samples for Vitamin D analysis quickly, reliably and cost effectively.

Hamilton Robotics Microlab STARlet Workstation Automated Liquid Handling Workstation

The Microlab STARlet is a small and flexible robotic pipetting platform that can be adapted to perform a variety of applications. Here, we demonstrate its ability to perform liquid-liquid extraction from starting specimen to a final, analysis-ready sample.

With this workstation, it is possible to integrate on- or off-deck peripheral devices such as microplate heating/cooling modules, shakers, and vacuum systems as needed by the application. The STARlet platform uses air displacement technology that offers increased pipetting accuracy and reproducibility while eliminating sample contamination or dilution effects commonly associated with fluid-based systems.

The STARlet workstation can be configured with multiple arms, each arm housing multiple pipetting channels or labware gripping options. Pipetting channels and labware grippers move independently for increased efficiency, and support the use of a wide range of labware. An autoload option provides real-time barcode tracking of samples, labware, racks and carriers as they are loaded onto the deck.

HAMILTON

All workstation functions and integrated third-party devices can be controlled by Hamilton Robotics' VENUS one software. The STARlet system offers several unique features such as Anti-Droplet Control (ADC), Total Aspirate and Dispense Monitoring (TADM) technology, CO-RE technology, capacitative and pressure-based liquid level sensing, etc. Some of these features are described below.

Anti-Droplet Control (ADC)

The STARlet is equipped with ADC for reliable pipetting of volatile compounds. The combination of air-tight tip seals and air displacement pipetting with pressure monitoring allows Hamilton Robotics to offer ADC. Liquids with extremely low viscosities and high vapor pressures like Acetone, Ether, Acetyl Nitrile and Methanol can be pipetted with confidence knowing that there will be no loss of liquid and no contamination of other samples because of dripping tips.

ADC works by continuously monitoring the pressure in the channel. The STARlet channels can sense the small increase in the vapor pressure inside the barrel. When the pressure reaches the threshold where a drip is likely to occur, the plunger is withdrawn a step at a time, as needed, to keep the liquid inside the tip and prevent dripping.

Sample Identification and Chain of Custody Controls for Clinical Laboratory Applications

The STARlet liquid handling workstation supports chain of custody in every aspect of pipetting, labware handling and sample tracking.

Barcode Tracking

When the autoload option is used, the barcodes of all tips, labware, and carriers can be read and recorded as they are loaded. This confirms the correct items are loaded into the correct location on the instrument deck. Barcode data sets can be compared to worklists from LIMS, exported as data files, or printed.

Labware Sensing

The STARlet can monitor and record the hysteresis on the z-drive (up/down) of the pipetting channels. This information can be used to determine if labware is present or not prior to pipetting, further confirming that the method executed correctly.

Liquid Level Detection

The STARlet pipetting channels are equipped with capacitive and pressure-based level sensing and recording functions. These techniques can be used to confirm liquid levels in tubes or microplate wells before or after pipetting.

Sample Tracking

The VENUS one software offers seamless sample tracking over single runs, multiple steps or even multiple runs.

Descriptions and Part Numbers

Description	Part Number
Microlab STARlet autoloading liquid handling workstation with 8 independent channels	Hamilton 173000-028
DWP carrier (3 SBS positions)	Hamilton 182065
VENUS one Software	Hamilton 911004-09
Sample carriers (32 positions)	Hamilton 173410
Tip carrier (5X96 Tips)	Hamilton 182025
Reagent carrier (3 positions: 120 mL reagent troughs)	Hamilton 185290

Labware Required	Part Number
300 uL CO-RE disposable filter tips (black conductive)	Hamilton 235903
1000 uL CO-RE disposable filter tips (black conductive)	Hamilton 235905
120-mL Reagent troughs	Hamilton 182703

User Supplied Materials
Serum samples in glass tubes (12X100)
96-well plate vortex mixer
Centrifuge with 96-well deep well plate adapter
Internal standard: Hexa Deuterated 25-Hydroxy Vitamin D3
Methanol: solvent/diluent

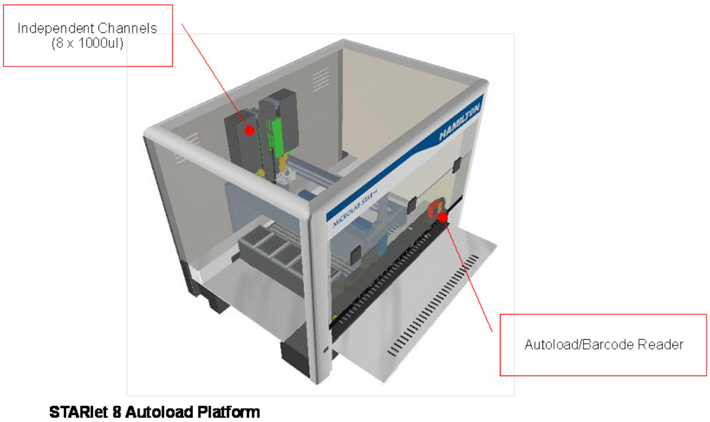

Figure 2: 3-D rendering of deck layout of the Microlab STARlet

Method Overview

The Microlab STARlet automated workstation processes the liquid-liquid extraction of Vitamin D using the deck layout shown in Figure 3.

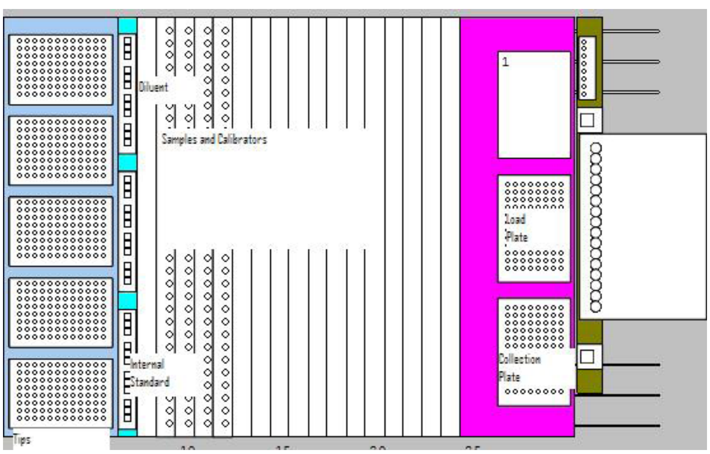

Figure 3: Deck layout on the Microlab STARlet workstation

Figure 4 illustrates an overview of the method employed. Below is the list of steps involved in the process:

Step 1: Load the carriers with samples and reagents: VENUS one software auto-calculates the number of samples and reads the associated barcodes. Barcode IDs are auto-assigned to samples, reagent reservoirs and internal controls.

Step 2: An optional serial dilution of the calibrator is performed.

Step 3: 800 uL of Methanol-based solvent is pipetted into 6 tubes. A 6-step serial dilution of the internal standard is performed with ADC turned on.

Step 4: 100 uL of each calibrator and sample are aspirated from the appropriate source tubes and added to the collection plate with ADC turned off.

Step 5: 350 uL of internal control is added to each well of the collection plate, with ADC turned on.

Step 6: The software pauses for user interaction. The collection plate is removed by the operator and vortexed for 3 minutes, followed by centrifugation at 3000 rpm for 20 minutes, at room temperature (Note: It is possible to automate this step with an optionally integrated vortexer and centrifuge).

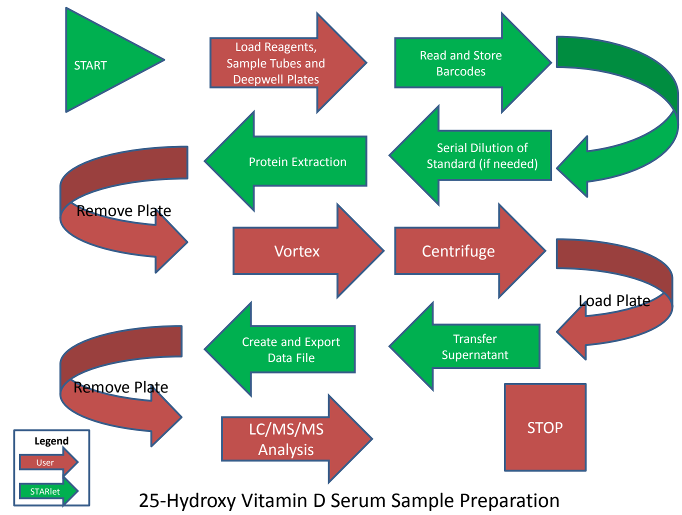

Figure 4: Steps involved in automated protocol for liquid-liquid extraction of vitamin D from clinical serum samples.

Step 7: The operator returns the collection plate to the STARlet pipetting deck for automated transfer of samples to the final load plate. The operator presses 'OK' to confirm that the deep well plate has been vortexed and centrifuged, and the method continues.

Step 8: 375 uL of supernatant is aspirated from each collection plate well and dispensed into a corresponding well on the load plate with ADC turned off.

Step 9: A file is generated, containing all sample IDs and their final locations, along with the status (error type or no error) of all pipetting steps. The sample and deep well carriers are unloaded by the operator.

Step 10: The load plate is removed for sample analysis via liquid chromatography, tandem mass spectrometry (LC/MS/MS)

Note: Steps 6 through 10 can be completely automated by having the vortex mixer, centrifuge and LC/MS in close proximity to the STARlet platform. However, even with operator intervention during these steps, a 600% increase in throughput efficiency can be realized.

Results

A validation study was performed to compare results achieved by manual and automated methods. Approximately 120 patient samples with known results were run for the validation study. Resulting data showed a correlation coefficient of 0.9911. The scatter plot (Figure 5) demonstrates good correlation between the manual extraction method and the automated method. Figure 6 illustrates the regression analysis between the two methods. Figures 7 and 8 demonstrate the medical point analysis, supporting statistics and experimental details.

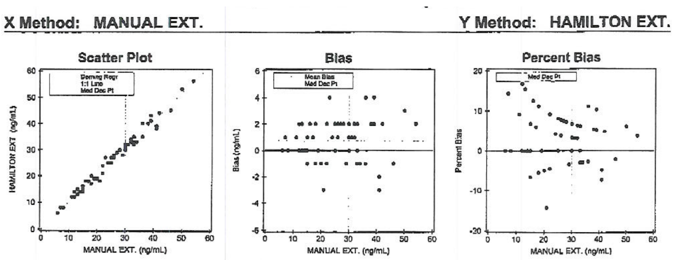

Figure 5 : Alternate Method Comparison

	Deming	Regular
Slope:	1.030 (0.994 to 1.067)	1.021 (0.985 to 1.057)
Intercept:	0.0 (-1.0 to 1.0)	0.2 (-0.8 to 1.2)
Std Err Est:	1.5	1.5

95% Confidence Intervals are shown in parentheses

Figure 6: Regression Analysis

Calculated by Deming Regression (R>=0.9)

X Method MDP	Y Method Pred. MDP	95% Conf. Limits Low	High
30	30.9	30.4	31.3

Figure 7: Medical Decision Point Analysis

Supporting Statistics

Corr Coef (R):	0.9911	Y Mean ± SD:	25.9 ± 11.3	Points (Plotted/Total):	60/60
Bias:	0.7	Std Dev Diffs:	1.5	Outliers:	Not Tested
X Mean ± SD:	25.2 ± 11.0	SubRange Bounds:	None	Scatter Plot Bounds:	None

Experiment Description

	X Method	Y Method
Expt Date:	31 Oct 2008	31 Oct 2008
Rep SD:	1	1
Result Ranges:	6 to 54	6 to 56
Units:	ng/mL	ng/mL

Figure 8: Medical Decision Point Analysis

Conclusion

Automation-enabled 25-Hydroxy Vitamin D extraction was shown to be a successful alternative to the laborious manual approach. Hamilton Robotics provides a highly customizable and flexible automation platform that can enable a variety of clinical and research applications resulting in high reproducibility, robustness, throughput, precision and accuracy.

Current Status

Laboratories have achieved a 600% average increase in processing throughput upon implementation of the Microlab STARlet automated solution. A new LIS/LIMS interface via VENUS instrument control software is currently under development.

References:

1. Jonathan M. Mansbach, MD, Adit A. Ginde, MD, MPH, Carlos A. Camargo, Jr, MD, DrPH. 2009. Serum 25-Hydroxyvitamin D Levels Among US Children Aged 1 to 11 Years: Do Children Need More Vitamin D? PEDIATRICS Vol. 124 No. 5 November 2009, pp. 1404-1410 (doi:10.1542/peds.2008-2041).

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