# Workflow Analysis Comparing Manual and Automated Specimen Processing for Mass Spectrometry–Based Vitamin D Testing

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Lab Med Fall 2014;45:357-363

DOI: 10.1309/LMZL47EN6KDODMXJ

### ABSTRACT

**Objective:** To quantify the benefits of automating specimen extraction in terms of specimen-preparation times and labor usage.

**Methods:** We used workflow modeling and time-motion studies to compare manual and automated solid-phase extraction methods to prepare specimens for a mass spectrometry-based vitamin D assay. We processed 20 batches, that included 5 to 90 specimens each, with both methods in parallel and randomly over a 4-week period. Technologist discomfort/fatigue was subjectively measured.

Results: Batch preparation time, per-specimen processing time, and

Laboratory automation has several advantages over manual processes when testing large numbers of specimens,<sup>1</sup> including reduced variability, fewer errors, and decreased amount of manual work. Clinical laboratories that perform testing via liquid chromatography–tandem mass spectrometry (LC–MS/MS) must decide whether to implement automated methods to prepare specimens for analysis on these instruments.

Manual methods used to prepare specimens for LC–MS/ MS analysis can be time consuming and technically

#### Abbreviations

LC–MS/MS, liquid chromatography–tandem mass spectrometry; SOPs, standard operating procedures; QC, quality-control; FTE, full-time equivalent; Man, manual extraction; Auto, automated extraction; NA, not applicable; ZnSO4, zinc sulfate; IPA, isopropanol

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\*To whom correspondence may be addressed. dtacker@hsc.wvu.edu labor requirements were significantly lower for all batch sizes on the Tecan Freedom EVO 150 robotic liquid-handling system (EVO). Technologist fatigue was significant when batch sizes reached 60 specimens. Cycle times were more uniform on the EVO. Automation provided as many as 85 minutes of useable technologist idle time for the 90-specimen batch.

**Conclusions:** Automated specimen preparation should be considered when batch sizes reach 35 to 40 specimens per day.

**Keywords:** automation, specimen extraction, mass spectrometry, workflow, cycle time, full-time equivalent, labor usage

demanding for the technologist; increasing workflow can quickly overwhelm staff. Automated specimen preparation methods require capital investment but are likely to improve specimen-processing consistency.<sup>2</sup> Although the benefits of automation are evident when testing large numbers (eg, hundreds to thousands) of specimens per day,<sup>3</sup> the advantages of automating specimen preparation for laboratories with smaller workflow are less clear.<sup>4</sup>

Workflow mapping and analysis studies can be used to determine whether a laboratory should consider automated specimen preparation to handle its testing volume. The example process we used for this study (25-hydroxyvitamin D) involves solid-phase extraction in a 96-well plate format, whether performed manually or with automation. We implemented vitamin D testing using LC–MS/MS starting in 2011 largely because immunoassay-based reagents were not available on our laboratory instruments and because our primary reference laboratory was performing this testing via LC–MS/MS. We conducted a workflow analysis as part of method development, first for the manual version of the extraction method, which is a key component of our justification for automation, and later for the automated version.

Our first objective in conducting this study was to determine the workflow volume that justifies the use of automated specimen-preparation equipment for a smaller LC–MS/MS laboratory with limited technical resources based on technologist fatigue and specimen-processing times. Our second objective was to quantify the benefits of automation (eg, more useable technologist time and improved process consistency).

## **Materials and Methods**

### **Workflow Analysis**

We processed 20 batches of 5 to 90 specimens each to compare the manual and automated processes for preparing specimens for LC–MS/MS analysis. We adapted the in-use manual process from the semiautomated solid-phase extraction method described in an application note by the Waters Corporation (Milford, MA; application note 720003139EN, July 2009). The new automated process used the same method described in the Waters application note and a Freedom EVO 150 robotic liquid handling system (Tecan Group, Ltd., Männedorf, Switzerland; hereafter, EVO). All reagents and solid-phase extraction components were identical between manual and automated methods. The program that controls the extraction method on the EVO was written by Tecan in cooperation with the Waters Corporation.

We performed workflow mapping by first listing the steps in each process as detailed in the standard operating procedures (SOPs). Each step was verified through direct observation of specimen extractions and interviews with technologists. We examined manual processing in greater detail by separating the steps into 5 major phases that required individual specimen handling. Those steps are as follows: phase I, assembly of specimens, reagents, and materials; phase II, adding internal standards and precipitating proteins; phase III, centrifuging specimens and conditioning solid-phase extraction columns; phase IV, performing solid-phase extraction and eluting extracts; and phase V, sealing and swirling extract-collection plates. We aligned automated preparation steps to these phases to allow for more detailed comparison of cycle times.

We then performed time and motion studies for both methods to measure the time required to perform individual phases of the extraction protocols. The timing studies were performed using varying specimen sizes over several weeks. Each specimen batch included 4 calibrators and 2 levels of quality control (QC) to simulate real-world conditions. The series was repeated 4 times by testing 1 batch per day according to a randomized schedule of varying batch sizes. Batch sizes for the automated process were designed in multiples of 4 because the EVO has 4 pipettors that work simultaneously. We used cycle-time data to verify the manufacturer claims for turnaround time on the automated instrument and to further refine the current state workflow models were created in the iGrafx Process 2011 (iGrafx LLC, Tualatin, OR) for Six Sigma software (Web X.0 Media, Bainbridge Island, WA).

### **Statistical Analysis**

Performance calculations included total time to complete batch preparation using manual and automated methods, time to complete each major phase of manual specimen preparation per batch, labor requirements per batch size for manual and automated methods, per-specimen times for completing specimen batches, and a qualitative assessment of technologist discomfort for manual specimen preparation (eg, none, mild, moderate, or persistent). Cycle time for the processes began when the technologist assembled specimens, materials, and reagents and ended when the 96-well extract collection plates were ready for transport to the mass spectrometer. Intervals included active work and technologist idle times.

We based labor (by technologist) usage calculations on our assumption that a single technologist was available to perform the testing from start to finish. For labor usage estimates, we assumed that a single technologist working 8 hours per day (1 full-time equivalent [FTE]) was available for LC-MS/MS testing. Thus, for estimating labor usage per batch, we calculated FTE use via the following equation:

(time to prepare the batch in minutes/60 minutes per hour)/8 hours

Similarly, for hands-on time, we used the following equation:

(hands-on preparation time in minutes/60 minutes per hour)/8 hours

Both calculations yield a unitless ratio that can be converted to a percentage. We did not include labor costs as part of the assessment.



Flow diagram of steps used to prepare mass-spectrometry specimens using manual or automated methods. The yellow box indicates steps omitted by automation; blue box, automatable steps; pink highlighted box, transcriptional step. *QC indicates quality control; VitD, vitamin D, MS, mass spectrometer; ZnSO4, zinc sulfate; IPA, isopropanol; Tecan, Tecan Group, Ltd. (Männedorf, Switzerland); TargetLynx, TargetLynx Application Manager (Waters Corporation, Milford, MA).* 



Specimen-preparation times by batch size: time required to prepare batches for mass-spectrometry analysis via automated or manual process. We calculated linear regression equations using Microsoft Excel, version 2010 (Microsoft Corporation, Redmond, WA) from plotted data. EVO indicates the Freedom EVO 150 robotic liquid handling system (Tecan Group, Ltd., Männedorf, Switzerland).

We calculated per-specimen processing times by dividing median batch time by the number of patient specimens in the batch, excluding calibrators and controls. The per-specimen processing times per batch size were graphed in Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA). Batch preparation times were compared by linear regression analysis and power functions to fit the per-specimen processing time data points. Statistical significance was assessed using the Student's paired, 2-tailed *t*-test in Microsoft Excel 2010. We considered *P* values of less than .05 to be significant.

# Results

Workflow mapping detailed each of the steps used to prepare samples for LC–MS/MS analysis by manual and automated methods (**Figure 1**). We identified a total of 45 steps in the entire specimen-testing process; **Figure 1** shows those steps in a compressed format for readability. Of the 45 steps in the total process, 4 steps (shown in yellow) were omitted by automating the extraction process; 19 steps (shown in blue) could be automated. The time needed to process specimens increased proportionally with increasing batch size when either method was used (**Figure 2**). However, time demands for batch processing increased greater than 2-fold for the manual process compared with the automated process. Batch-processing times were significantly lower for the automated process at all batch sizes (**Figure 2**, **Table 1**).

Linear relationships were evident between batch volume and the time needed to complete each major extraction step (**Figure 3**). The regression slopes show that phase

# Table 1. Total Batch Preparation Times forManual and Automated Processes

Batch Size, No. of Specimens	Туре	Process Time, Min.	P Value <sup>a</sup>
5	Man	58	.006
4	Auto	45	
15	Man	75	<.001
16	Auto	51	
30	Man	91	.002
32	Auto	60	
60	Man	145	.001
	Auto	76	
90	Man	184	.01
	Auto	97	

Man, manual extraction; Auto, automated extraction.

<sup>a</sup>P <.05 was statistically significant via the Student's t-test (2-tailed, paired).

processing times are proportional to batch size for phases that require individual specimen handling or manipulation, including phases II, III, and IV for the manual process (**Figure 3A**) and phases II and IV for the automated process (**Figure 3B**). Phases that included steps in which specimens were simultaneously manipulated were less dependent on batch size; these included phases I and V for the manual process (**Figure 3A**) and phases I, III, and V for the automated process (**Figure 3B**).

The time required to prepare a single specimen (ie, perspecimen processing time) using the manual or automated processes decreased exponentially with increasing batch sizes (**Figure 4**). Overall batch processing times were significantly shorter for the automated process compared with the manual process at all batch sizes ( $P \le .01$ ; **Table 1**). Similarly, the amount of labor required to process a batch (ie, FTE hands-on time) was significantly lower for the au-

Batch Size, No. of Specimens	Batch Type	FTE Hands-On Time, Min.	FTE Usage, %	Useable Idle Time, Min.	Fatigue Score
5	Man	58	12.0	0	None
4	Auto	12	2.4	33	NA
15	Man	75	15.7	0	None
16	Auto	12	2.4	39	NA
30	Man	91	18.9	0	Mild
32	Auto	12	2.4	48	NA
60	Man	145	30.3	0	Moderate
	Auto	12	2.4	64	NA
90	Man	184	38.3	0	Persistent
	Auto	12	2.4	85	NA

FTE, full-time equivalent; Man, manual extraction; Auto, automated extraction;

NA, not applicable.

 $^{a}P$  <.001 was statistically significant via t-testing for all batch sizes, manual versus automated workflow.

tomated process at all batch sizes (P < .001; **Table 2**). As batch size increased, labor requirements rapidly increased for the manual process but remained constant for the automated process. Although the automated extraction method required 45 to 97 minutes to complete 1 batch (**Table 2**), the method required only 12 minutes of technologist handson activity (0.03 FTE/batch) regardless of batch size. In comparison, manual extraction required between 58 and 184 minutes per batch, nearly all of which involved direct technologist activity (0.12 to 0.38 FTE/batch; **Table 2**).

During manual extractions, technologists reported moderate and persistent discomfort in the hands, neck, and shoulders when batch sizes reached 60 and 90 specimens (**Table 2**); this was particularly true during specimen aliquoting and supernatant-transfer steps. Technologists reported no discomfort in processing batches of 5, 15, or 30 specimens. The steps involving the most technologist discomfort were automatable (**Figure 1**, blue boxes) or rendered unnecessary by automation of other steps (**Figure 1**, yellow boxes), thereby eliminating a potential upper limit of batch size for manual processing based on technologist discomfort.

# Discussion

Specimen extraction is typically the most time- and laborintensive component of the workflow in mass-spectrometry laboratories. We launched this study shortly after implementing our first in-house mass-spectrometry assay based on manual specimen extraction using a solid-phase extraction method in a 96-well plate format. We anticipated the potential need to automate the manual-extraction steps of the same specimen-preparation method based on the rapidly increasing volume of requests for vitamin D testing. Our initial evaluation was based on estimates from the manufacturer of the time needed to process a full 96-well plate of specimens on the EVO platform, not on the results of formal timing studies. The results of this limited evaluation suggested that automated specimen preparation could provide benefit based on our specimen volume and staffing levels, which provided sufficient justification for platform purchase (Waters Application Note 720003139EN, March 2014).

During the validation and implementation of the EVO platform, we again performed workflow assessment and timing studies to more accurately determine the effects of automation at different batch sizes. The results of these studies confirmed that efficiency is achieved when certain manually intensive stages in the specimen-preparation process are automated (**Figure 1**, yellow and blue boxes). Our data demonstrate linear relationships between cycle time and batch size for manual and automated processes (**Figure 2**).

Although the EVO processes all 96 wells of the extraction plate regardless of the number of specimens, automated cycle times do not remain constant with batch size. We largely attribute this finding to the logic used by the pipetting system on the EVO, designed to reduce the extra motions that occur during some of the phases. For example, during specimen pretreatment and pipetting steps, only the wells of the plate containing the specimen receive the reagent. However, during the solid-phase extraction steps, the system processes the entire plate to ensure uniform application of vacuum pressure to the extraction columns during elution. Despite the unnecessary motion that occurs with the EVO platform during smaller batch runs in the extraction phase, cycle times remained shorter than manual extraction (**Figure 3**).



Specimen processing times by phase. **A**, Manual method. **B**, Automated method (using the Freedom EVO 150 robotic liquid handling system [Tecan Group, Ltd., Männedorf, Switzerland]). Diamond indicates phase I (assembly); square, phase II (precipitation); triangle, phase III (preparation for extraction); circle, phase IV (extraction and elution); asterisk, phase V (extract plate sealing and mixing). We calculated linear regression equations using Microsoft Excel, version 2010 (Microsoft Corporation, Redmond, WA) from plotted data.

Also, per-specimen processing times dramatically increased at smaller batch sizes because of the relatively greater impact of the time required to coprocess calibrators and control materials (**Figure 4**). For example, to prepare 5 patient specimens using automated or manual methods, the technologist or platform must extract a total of 11 specimens. For a 90-patient specimen run, however, the batch size increases to only 96. Thus, the time required to process the extra 6 control specimens is more evident at smaller batch sizes.

Batch completion times appeared less variable using the automated extraction method based on the uniformity of the phase-regression plots (**Figure 3A** and **3B**). This finding appears related to the elimination of variations in motion that occur during the manual process. For example, the manual process requires the technologist to move between the laboratory bench and a chemical hood that is located in another room. In contrast, all steps of the automated process except extraction-plate centrifugation occur on the EVO deck, and the centrifuge is located less than 1 meter from the EVO deck.

The uniformity of the automated platform has also improved efficiency and specimen consistency. Since we have implemented automated specimen preparation in

our laboratory, the number of patient specimens that require repeat extraction and/or analysis has decreased from approximately 5% to less than 2% per day (data not shown). Also, internal standard recovery appears to be more consistent with the automated process, which has improved postanalytical review. In the first year of manual processing, we attributed 3 batch failures to manualextraction problems. Based on batch volumes of 70 to 80 patient specimens, these failures generate an additional 2.5 to 3.0 hours (0.31 to 0.38 FTE) of work per batch repeated. No batch failures attributed to the extraction process have occurred over the 12 months that we have used the automated platform. Because both specimenextraction approaches (manual and automated) use the same reagents, extraction plates, and solid-phase extraction technology, we were able to focus specifically on the aspect of labor in this study.

Another clear labor benefit that we have realized since automating vitamin D specimen extraction has been an increase in the usability of technologist idle time. For the largest batch sizes, automation provided as many as 85 minutes (0.18 FTE) of idle time per batch that technologists could use to perform other tasks, including instrument maintenance and quality-assurance activities. The resulting net gain of nearly 0.2 FTE per day has allowed



Per-specimen processing times for manual and automated processes. Diamond indicates manual process; square, automated process (using the Freedom EVO 150 robotic liquid-handling system [Tecan Group, Ltd., Männedorf, Switzerland]). We calculated power regression equations using Microsoft Excel, version 2010 (Microsoft Corporation, Redmond, WA) from plotted data.

technologists in our facility to develop 3 new mass-spectrometry assays during the past year without increasing staffing. However, laboratories that routinely use robotic specimen preparation systems must be prepared if the automated system becomes inoperative. We have had no equipment failures since implementing the EVO platform in 2012 but have devised contingency plans in the event of unexpected downtime.

We conclude that automated LC–MS/MS specimen-preparation equipment should be strongly considered when daily batch sizes exceed 35 to 40 specimens per day due to technologist fatigue and the increased labor requirements that occur when manually processing these batch sizes. Further, the amount of technologist time required to prepare manual batches limits the ability to expand vitamin D testing without additional staff. The workflow mapping studies we conducted helped us compare resource usage and efficiency in mass spectrometry–based vitamin D testing. We expect that many of our findings are consistent with the experience of others who have automated areas of the chemistry laboratory.<sup>1,3,4</sup> However, our study demonstrated that labor savings are evident even at modest batch sizes. Finally, workflow studies can help justify process automation in laboratories with a limited ability to expand technical staffing. LM

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