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Automation of chromatographic peak review and order to result data transfer in a clinical mass spectrometry laboratory

Faye B. Vicente[a], David C. Lin[a,b], Shannon Haymond[a,b,*]

[a] Ann & Robert H. Lurie Children's Hospital of Chicago, USA
[b] Northwestern Feinberg School of Medicine, Department of Pathology, USA

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ABSTRACT

Introduction: Mass spectrometry-based assays have increasingly been implemented in clinical laboratories for their multiplexing capacity and high specificity and sensitivity. However, these methods are often associated with labor-intensive and error-prone data-related workflows, due to the volume of data generated that is often manually reviewed and resulted. We aimed to establish a system within our clinical mass spectrometry laboratory to facilitate data 'flow' from electronic medical record order to result and to automate processes for chromatogram peak review. The processes and validation are described for a 25-hydroxyvitamin D assay.

Methods: Automating chromatogram review and order to result data transfer required flat file interfacing, file transfers of standardized data formats, barcode scanning, and software for peak processing and review. Validation of the automated workflow involved (1) correlation of quantified results generated by two chromatogram analysis methods: Waters TargetLynx and Indigo Bioautomation ASCENT, (2) manual verification of quality assurance flags applied in ASCENT, and (3) testing data flow and integrity across all the systems from order to result. Efficiency and quality improvements were assessed through calculation of batch review times and rates for autoverification and manual manipulations.

Results: The correlation of TargetLynx and ASCENT quantitation methods for 25-hydroxyvitamin D2 in patient samples yielded slope of 0.99 (95% CI: 0.989 to 0.996), intercept of 0.46 (95% CI: 0.363 to 0.565), with $r = 0.999$. The correlation for the D3 fraction showed Deming regression slope of 0.98 (95% CI: 0.969 to 0.989), intercept of 0.06 (95% CI: −0.115 to 0.313), and $r = 0.995$. Results from both quantitation approaches were also compared to the assigned value in CDC reference samples. The mean bias relative to the CDC was 4.6% for ASCENT and 2.5% for TargetLynx. The median time for chromatogram review of a full 96-well plate of vitamin D results is reduced from approximately 2 h to 14 min and 80% of batches were reviewed within 30 min. Instead of 100% peak review, technologists review only the peaks that have been flagged by the system based on applied rules. Analysis of full plate batches showed that 2–20% of peaks per batch were flagged for manual review. Manipulations made by technologists during chromatogram review were reduced by 75% when using the automated versus manual system.

Conclusions: We describe a system to facilitate data 'flow' from electronic order to result and to automate chromatogram peak review in a clinical liquid chromatography mass spectrometry assay for 25-hydroxyvitamin D. This eliminated manual result entry, repetitive transcription, and unnecessary review of high quality data while enabling systematic evaluation of data quality indicators. The new processes were accurate, improved the data review and processing times, and helped to reduce manual manipulations during chromatogram review.

1. Introduction

Mass spectrometry-based assays have increasingly been implemented in clinical laboratories for their multiplexing capacity and high specificity and sensitivity. However, these methods are often associated with labor-intensive and possibly error-prone data-related workflows, due to the volume of data generated that has largely been manually reviewed and resulted. These manual data workflows are a travesty that undermines the improved quality designed into the analytical method.

For example, a 96-well plate mass spectrometry-based assay for 25-hydroxy vitamin D2 (25OHD2) and D3 (25OHD3) results in nearly 600

* Corresponding author at: Ann & Robert H. Lurie Children's Hospital of Chicago, USA.
E-mail address: shaymond@luriechildrens.org (S. Haymond).

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chromatograms that must undergo multi-level quality review. At a minimum, the four peaks of each sample (i.e., 25OHD2 qualifier, 25OHD2 quantifier, 25OHD3 qualifier, and 25OHD3 quantifier) are evaluated for multiple parameters, including retention time, shape, and signal intensity. In each sample, further criteria are applied for 25OHD2 and 25OHD3 to assess the quality and area of their respective internal standard peaks and the ratios of the qualifier to quantifier peak areas. The calibration and quality control samples for each compound within each batch are also reviewed for acceptance. In a manual workflow, a technologist may be expected to make > 1000 decisions when reviewing a single batch of vitamin D measurements. It is unlikely that any human applies quality rules to the multiple levels in a batch as reproducibly or as thoroughly as expected. In addition, much of human review is often of peaks that meet all acceptance criteria. In high volume clinical chemistry laboratories, autoverification systems are routinely implemented to reduce the cognitive load on technologists and increase the quality and efficiency of result review and approval [1]. These systems employ rules-based engines that enable result review by exception. In contrast, there are few software products available that perform similarly for chromatographic peak review.

Data transfer steps in clinical mass spectrometry workflows are often manual and disconnected from hospital informatics systems or file networks. In these cases, the connection is made through manual entry. Manual entry creates risk for errors and requires more technologist time. Automated data transfer in clinical mass spectrometry labs or workflows are increasingly being pursued [2,3]. A basic format uses a unidirectional interface where results are uploaded from the mass spectrometry software to the laboratory information system (LIS) (and then to the electronic medical record (EMR)). This may or may not include data transfer from an automated liquid handler. Though this eliminates manual entry of results into the LIS (and EMR), there are still plenty of opportunities for manual entry errors and delays upstream in the workflow when using unidirectional interfacing.

We aimed to establish a system in our clinical mass spectrometry laboratory to facilitate data 'flow' from EMR order to result and to automate chromatogram peak review. This required custom-built interface drivers and coordination of several instruments and software programs, unlike the relatively routine processes to interface automated analyzers in clinical chemistry laboratories. Most mass spectrometry workflows include sample preparation, analysis, and data processing performed by individual systems that must be connected to each other and the LIS (Fig. 1). There is currently limited availability for single vendor LC–MS/MS systems that are fully integrated from sample to result [4]. The data format output from each system is not standardized. To achieve order to result data flow, there must be collaboration among all involved vendors to design processes facilitating data transfer.

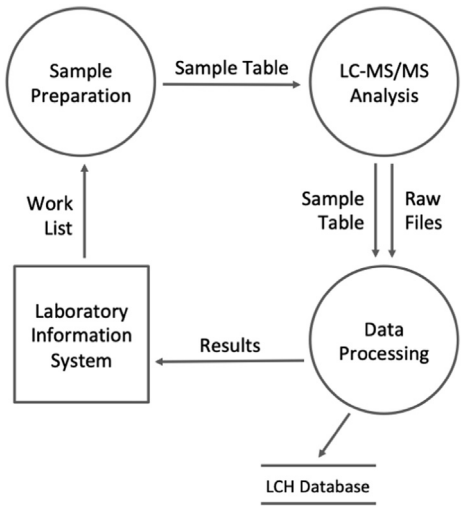

Fig. 1. Data Flow Diagram.

We further wanted to improve the data processing step where each chromatogram was manually reviewed by technologists for quality assessment. While seemingly a robust process with specified criteria, manual peak review actually results in staff subjectively examining 'good' data, creating an opportunity for optimization through automation. Mass spectrometry software programs are beginning to offer tools to aid laboratories with more efficient and effective chromatogram review. This enables a process similar to auto-verification commonly used in clinical chemistry laboratories. Quality criteria are systematically applied using a software-based rules engine and technologists are able to focus their attention on investigating results that violate those rules and may need corrective action. One example by Dickerson et al., described the design and implementation of a software application that performs quality control calculations for a mass spectrometry-based opioid assay [5]. Skyline, a freely available, vendor-neutral software package has also been used to quantitate 25-hydroxy vitamin D [6] and enables application of quantitative review parameters. To our knowledge, there is a single commercial product available for chromatographic peak review, ASCENT from Indigo Bioautomation.

For this project, we had four goals related to saving technologist time and reducing errors: (1) Eliminate manual entry of results; (2) Eliminate repetitive manual transcription throughout workflow; (3) Eliminate the unnecessary review of 'good' peaks and valid results; and (4) Systematically apply data quality indicators. Our approach included interfacing, file transfers, and barcode scanning to eliminate manual and repetitive entries and we implemented a commercially available software program for the automated peak review and result verification steps. We describe our methods and results when implementing this approach for one of our highest volume and most labor-intensive assays, 25-hydroxy vitamin D. Once the underlying infrastructure is in place, we have found that it is relatively straightforward to apply this workflow to additional assays. Automating data flow also facilitates the collection of metadata that is useful for quality monitoring of system performance, as recommended in available standards for clinical mass spectrometry operations [7].

2. Methods

2.1. Data flow

Interfacing results for 25-hydroxy vitamin D involved the integration of processes and associated software in our laboratory (Fig. 1):

1) LIS (Sunquest, 7.1),
2) Software for controlling the automated liquid handler (Hamilton MicroLab NIMBUS, VENUS 4.3.0.7270),
3) Software for controlling LC–MS/MS instrument method settings (Waters, MassLynx V4.1),
4) Software for processing the chromatogram data generated by the LC–MS/MS instrument (Waters, TargetLynx V4.1), and
5) Software for chromatogram processing and review (IndigoBioAutomation, ASCENT 3.6.1).

Each data transfer step also includes a parallel path for test and archive folder locations.

A list of samples to be analyzed must be created and maintained throughout the process, ultimately linking those samples with results and transmitting this information from the LIS back to the EMR. A critical first step needed to facilitate file transfers was to get each of the involved workstations on the institution's computing network. Another key aspect was formatting the file outputs to be compatible across the different instruments.

The process begins with an order from the electronic medical record (Epic) that is transmitted to the LIS. A worklist including all samples to be analyzed is generated from the LIS using function LL (loadlist). This

is exported as a comma-separated values (CSV) file to a specified network folder location. The file contains limited data: a unique container identification (CID) number for each specimen and the accession number. Barcoded samples in serum aliquot tubes are randomly loaded into racks for the automated liquid handler. Quality controls and calibrators are loaded into designated racks on the system.

The barcode reader of the automated liquid handler scans the samples and NIMBUS software compares each barcode ID to those included in the selected worklist. The software alerts technologists to missing samples (i.e., expected from the worklist) and extra samples (i.e., scanned but not on the worklist). Once samples are reconciled, the processing method is started. During operation, the NIMBUS software generates a plate-mapping file using CID and the corresponding accession number. The mapping file contains sample information (e.g., sample IDs, type, dilution factor, calibrator and QC assignments) and plate location, including ancillary information required for LC–MS/MS analysis (e.g., injection volume, and method file names). The file is saved in csv format to a specified folder location. This file is imported into the MassLynx software where it becomes the sample list table that provides the sequence for analysis. This step prevents the need for manual entry into the sample list table. The LC–MS/MS analysis is started.

A technologist accessions the run into the ASCENT software, assigning samples to a batch. The ASCENT software automatically queries a designated network folder for new files from the mass spectrometer. Once the batch run is completed, ASCENT uploads the raw data files and the sample list table to the cloud-based service, performs a required data conversion, and analyses the chromatograms to produce quantitative results for compounds. Generally, within five minutes, results for the batch are available in the ASCENT web browser for technologist review, approval, and certification.

The system is set up for technologist review by exception. Technologists first review calibrators and quality controls for any deviations that were flagged for violating quality rules (e.g., concentration, ion ratio, fit quality), taking appropriate corrective action as needed. They next review quality flags on individual samples and take corrective actions. Once all quality flags have been reviewed and resolved, the batch is certified. Upon certification, a portable document format (PDF) summary report and a results file (in csv format) are generated for each processed batch and saved to a network folder. Additional data about the calibration, batch, chromatographic peaks, and quality flags are stored in tables of a cloud-hosted relational database (LCH database in Fig. 1) that is specific for our laboratory.

The approved results in the csv file are automatically transmitted to Sunquest through a Samba file share and a custom-built flat file interface. If the results fall within the defined reportable range, the results are automatically released to LIS without additional intervention. Results outside the clinical reportable range are flagged in Sunquest for review, and once accepted, are converted to the designated limits for reporting (i.e., < 4 ng/mL).

2.2. Validation

The validation involved (1) correlation of quantified results generated by two chromatogram analysis methods: TargetLynx and ASCENT, (2) verification of quality assurance (QA) flags in ASCENT, and (3) testing data flow and integrity across all the systems from order to result.

2.2.1. Correlation of quantified results

We compared results processed using TargetLynx and ASCENT software from 19 consecutive batches analyzed from May through November 2016 using two LC–MS/MS software systems. Comparisons included 1531 patients with measurable 25-hydroxy vitamin D3 and, of those, 249 patients with measurable 25-hydroxy vitamin D2. Deming regression was used to determine the correlation between methods.

2.2.2. Comparison of Centers for Disease Control and Prevention (CDC) reference samples

Both methods were used to quantitate total 25-hydroxy vitamin D in 40 single-donor serum samples received as a single shipment from the CDC Vitamin D Standardization Certification Program. The CDC's Vitamin D Reference Laboratory assigned the vitamin D concentrations in these samples [8]. Deming regression was performed for the comparison. The mean percent bias relative to the CDC values was calculated for each quantitation method.

2.2.3. Verification of quality assurance flags

Each QA rule was validated independently in ASCENT through either manual preparation of samples designed to violate a given rule or reprocessing of previously analyzed samples expected to violate a given rule. The full process for automated peak review was validated by performing complete manual peak review in TargetLynx and in ASCENT for the 19 batches used for result correlations.

2.2.4. Data flow testing

Manual review of information that crosses platforms (e.g., accession numbers and CIDs in the Sunquest worklist transferred to the NIMBUS plate mapping file) was performed to verify data was transmitted accurately. File transfer steps were manually confirmed by ensuring the correct files were deposited to the desired locations. File contents were manually inspected for correct formatting and completeness. Results displayed in Sunquest and Epic were compared to those certified in ASCENT. In addition to each step being verified, tests were conducted to validate the complete data flow from order to result.

2.3. Post-implementation monitoring

To assess the impact of the ASCENT software workflow on batch review time and application of quality flags for vitamin D analysis, 138 batches of 96-injection runs (i.e., 'full plate runs') analyzed between February 2017 and October 2018 were investigated. Batch review times were determined as the difference in system timestamps between when the batch first becomes under review and when it is marked as reviewed.

2.4. Statistical methods

The data analyses and visualizations were conducted using the R programming language [9], and specifically packages, readxl [10], mcr [11], dplyr [12], tidyr [13], ggplot2 [14], and gridExtra [15].

2.5. Analyte quantitation

Calibration curves were generated using a 5-point weighted (1/x) linear regression curve. Allowable calibrator concentration deviation was less than or equal to 10% of the expected concentration. Analyte peaks were quantitated by normalizing the peak area of the quantifier ion of each compound to that of the internal standard, and concentration was calculated from the calibration curve.

3. Results and discussion

3.1. Comparison of quantitation methods

Results generated using TargetLynx and ASCENT software in parallel show a very good agreement between the two data processing methods. The linear regression analysis of the 1531 paired 25-hydroxy vitamin D3 results from TargetLynx and ASCENT is shown in Fig. 2. A summary of the values by method is shown in Table 1. The Deming regression slope is 0.98 (95% CI: 0.969 to 0.989), and intercept of 0.06 (95% CI: −0.115 to 0.313), with $r = 0.995$ and RMSE = 0.938. Restricting the analysis to the 1528 paired samples with 25-hydroxy

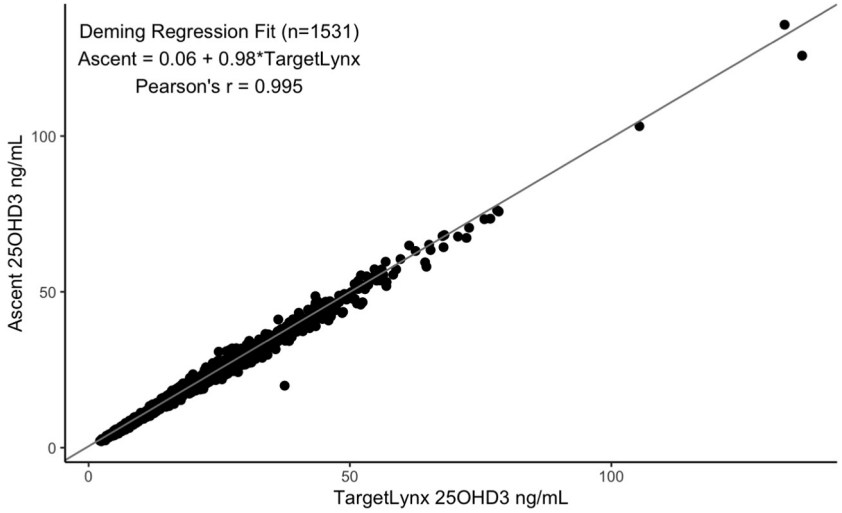

Fig. 2. Linear Regression of 25-OH Vitamin D3 Concentrations.

Table 1
Summary of 25-OH Vitamin D3 Concentrations (ng/mL).

	Low	Mean	High
ASCENT	2.1	23.1	135.7
TargetLynx	2.2	23.5	136.5

Table 2
Summary of 25-OH Vitamin D2 Concentrations (ng/mL).

	Low	Mean	High
ASCENT	4.1	17.1	82.1
TargetLynx	3.7	16.8	82.3

vitamin D3 concentrations < 80 ng/mL yielded Deming regression slope of 0.98 (95% CI: 0.975 to 0.988), and intercept of 0.03 (95% CI: −0.099 to 0.156), with $r = 0.994$ and RMSE = 0.922. The specimen with a difference of −17.6 showed a fit quality failure flag in ASCENT.

The Deming regression plot in Fig. 3 shows results processed by TargetLynx versus ASCENT in the 249 patients with measurable D2 (summarized in Table 2). The correlation yields slope of 0.99 (95% CI: 0.989 to 0.996), and intercept of 0.46 (95% CI: 0.363 to 0.565) with $r = 0.999$ and RMSE = 0.394.

3.2. Quantitation of CDC reference samples

The 40 reference samples had total vitamin D ranging from 9.6 to 79.3 ng/mL (Table 3). Results from both quantitation approaches were compared to the assigned value from CDC. The mean percent bias relative to the CDC was 4.6% for ASCENT and 2.5% for TargetLynx. The goal for the CDC's standardization program is to achieve mean bias less than or equal to 5%. Systematic errors calculated for each quantitation approach at relevant medical decision limits are listed in Table 3. These values are highest at the low end, but are well within the total allowable error specification for total vitamin D (e.g., New York State Department of Health: ± 3 ng/mL or 25%, whichever is greater).

Table 3
Summary of Total Vitamin D Concentrations (ng/mL) and Error in CDC Samples.

	Low	Mean	High	Mean % Bias	Sys Error (10 ng/mL)	Sys Error (20 ng/mL)
CDC	9.6	29.9	79.3	–	–	–
ASCENT	10.9	31.0	78.0	4.6	1.66	1.36
TargetLynx	10.0	30.5	76.2	2.5	1.25	0.92

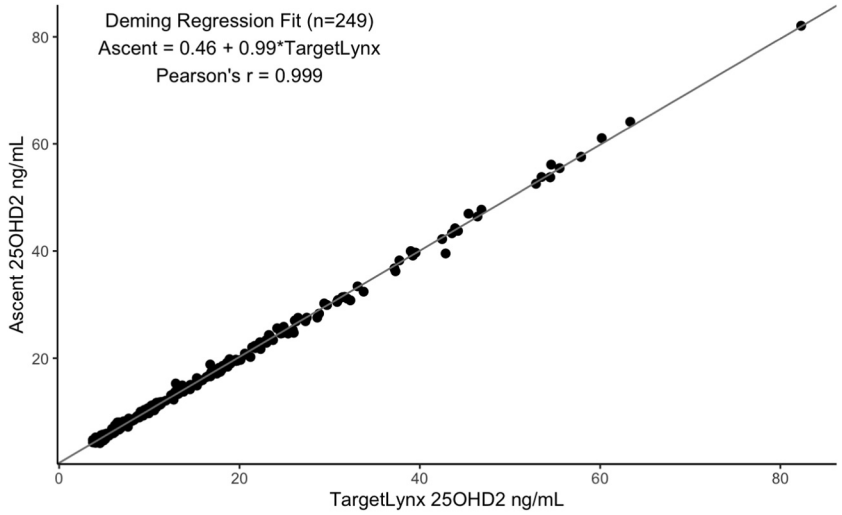

Fig. 3. Linear Regression of 25-OH Vitamin D2 Concentrations.

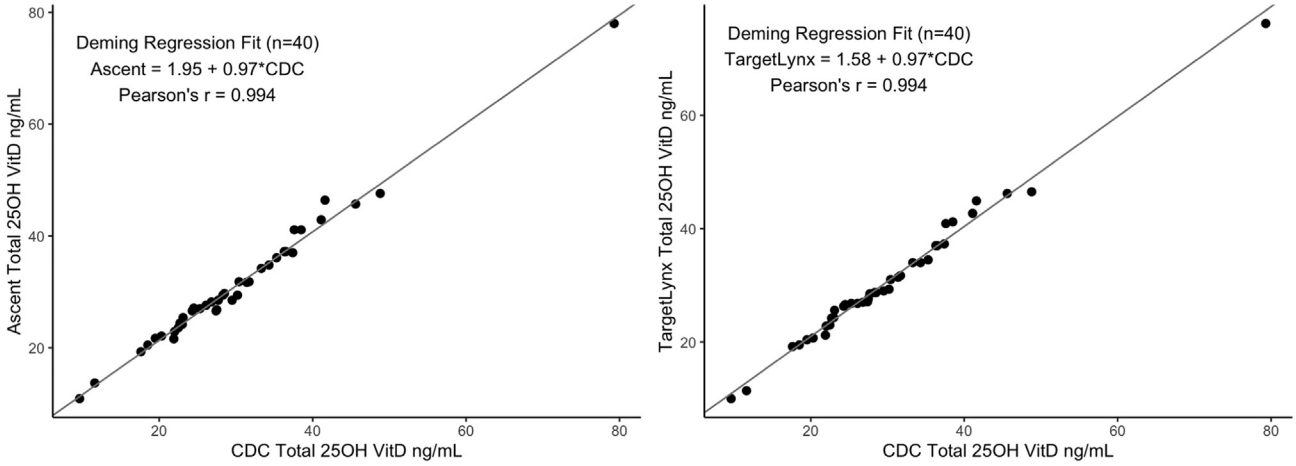

Fig. 4. Linear Regression of Total 25-OH Vitamin D Concentrations.

The Deming regression analysis (Fig. 4) for ASCENT values versus the CDC assigned values resulted in a slope of 0.97 (95% CI: 0.94 to 1.03), and intercept of 1.95 (95% CI: 0.54 to 2.87) with $r = 0.994$ and RMSE = 0.875. The results for the TargetLynx Deming regression versus the CDC assigned values were similar, with slope of 0.97 (95% CI: 0.93 to 1.03), and intercept of 1.58 (95% CI: 0.06 to 2.72) with r = 0.994 and RMSE = 0.858. As observed with the comparison of patient samples, there is strong correlation between TargetLynx and ASCENT quantitation methods for the CDC samples. This is expected since the two peak analysis software methods were applied to the same raw input files from each batch (i.e., there was no deviation in the sample preparation, analysis, or calibration scheme for a given batch). Any errors in the peak integration seem to impact the quantitation methods similarly for the CDC samples and resemble trends observed with patient samples.

3.3. Quality through automated peak review

The quality of the assay is improved in at least three ways by implementing the ASCENT software for peak analysis and review: (1) acceptance criteria covering multiple facets (peak quality, retention time, concentration, peak area, calibration, quality controls, contamination, and clinical 'applicability') are systematically and reproducibly applied to each peak, sample, and batch, as appropriate, (2) there is transparency and version control for the applied rules and associated settings, and (3) there is electronic documentation and peak-level annotation of manual manipulations with user-based permission controls.

Inspection of documentation for the 19 validation batches showed that technologists made a total of 40 manipulations with the manual review system and 10 total manipulations with the automated system. Only three manipulations were identical between systems. The automated system includes role-based restrictions and tracking of manual modifications during peak review and processing. In the manual process, technologists had less electronic restriction. In both cases, supervisor consultation is required prior to manual manipulations. Examples of modifications requiring supervisor approval include, questionable peak integration, questionable/borderline ion ratio flags, and exclusion of a calibrator. Other role-based restrictions include adjustments to target values for QCs and calibrators, turning on/off specific flag rules, de-certification of batches, adjustments to assay configuration parameters.

We did not include a comparison of multiple reviewers examining the same flagged result for this study. Technologists are trained on acceptable methods to resolve quality flags in ASCENT and their competency is assessed. However, reproducibility across reviewers is an area for further research.

3.4. Review and processing time

Because of the manual peak review of 6 peaks for each of the 96 total injections and the manual entry of the 84 patient results for 25-hydroxy vitamin D2 and 25-hydroxy vitamin D3, a full plate required approximately 2 h of technologist's time to review and result. This was in addition to the time required of another technologist for a secondary review of the results prior to releasing the manually entered results.

Instead of 100% peak review, which is almost 600 chromatograms per assay for each of the quality criteria, technologists review only the peaks that have been flagged by the system based on applied rules. Analysis of 138 full plate batches completed between Feb 2017 and Oct 2018 showed that 2–20% of peaks per batch were flagged. The most frequent flags are those related to concentration and peak quality or retention time rules. Table 4 summarizes the most frequent flag, Fit Quality, and its occurrence for a given concentration interval by compound in unknown sample injections. Natively low 25-hydroxy vitamin D2 concentrations in unknown samples result in the majority of flags for this compound. In these cases, the peak intensity becomes low, decreasing fit quality. The fit quality flag indicates the ability of the ASCENT algorithm to fit a curve under the observed chromatographic peak. Fit quality flags for 25-hydroxy vitamin D3 likely reflect low quality peak shapes with adequate signal, requiring review and corrective action. From our experience, the fit quality flags are triggered more frequently as the column approaches the end of its lifecycle. Further work to optimize the application of this quality rule is underway.

Since fewer peaks are reviewed with the automated system, time for review and resulting has been drastically decreased. The median review time for a full 96-well plate is approximately 14 min and 80% of the 138 full plate batches run between Feb 2017 and Oct 2018 were reviewed within 30 min (Table 5). Outliers are due to discontinuous review workflows that happen infrequently because of competing priorities in the lab. Because results are interfaced, the need for a secondary review to verify manual entry results has been eliminated.

Additional advantages that are difficult to quantify include workflows that are more consistent and predictable. Because steps are more

Table 4
Summary of Fit Quality Flag Counts by Concentration (ng/mL).

	0–4	5–9	10–29	30–119	≥120
25OHD2	1173	32	20	6	0
25OHD3	32	54	497	612	2

Table 5
Summary of Batch Review Times (Minutes) for Full 96-Well Plate Runs.

Minimum	1st Quartile	Median	Mean	3rd Quartile	Maximum
1.2	7.6	13.6	27.5	28.4	477.6

automated, they are easier to scale. Staff may feel more satisfied as they are better utilized for technical versus clerical skills. Finally, data and metadata are now available electronically, facilitating both retrospective and longitudinal analyses and monitoring.

4. Conclusions

We describe our approach for automating chromatogram review and order to result data transfer for a high volume clinical LC–MS/MS assay, 25-hydroxy vitamin D. We implemented flat file interfacing, file transfers of standardized data formats, barcode scanning, and software for peak processing and review. This eliminated manual result entry, repetitive transcription, and unnecessary review of high quality data while enabling systematic evaluation of data quality indicators. The new processes also improved the data review and processing time and helped to reduce manual manipulations during data processing and review.

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