Automated Sample Preparation for PEth Analysis in Whole Blood

ARCHImedline PEth from whole blood (RUO) | Hamilton Microlab Prep

Introduction

Phosphatidylethanol (PEth) is a direct biomarker of alcohol consumption formed in red blood cell membranes. The ARCHImedline “PEth from whole blood” kit enables quantitative determination of PEth 16:0/18:1 and PEth 16:0/18:2 from human whole blood using LC-MS/MS. The kit is designed for professional users in clinical and forensic laboratories and supports integration into existing LC-MS/MS workflows.

Routine PEth analysis requires consistent handling of whole blood, reliable addition of precipitation solution with internal standard and reproducible separation of the processed sample from precipitated matrix components. An automated sample preparation workflow reduces manual handling, supports standardization between runs and improves the scalability of routine LC-MS/MS analysis.

This application note describes the automation of the ARCHImedline PEth from whole blood kit on the Hamilton Microlab Prep. The automated workflow is compared with the established manual sample preparation procedure with focus on analytical agreement, precision, potential carryover and workflow efficiency.

System description

The automated sample preparation workflow was implemented on the Hamilton Microlab Prep. The platform is intended to automate all liquid handling steps required, including reagent transfer, sample transfer, mixing steps and preparation of processed samples for subsequent LC-MS/MS analysis. The exact deck layout and consumable configuration should be adapted to the laboratory-specific method file and verified during installation.

Element	Planned configuration / content
Automation platform	Hamilton Microlab Prep
Application	Automated sample preparation for PEth 16:0/18:1 and PEth 16:0/18:2 from whole blood
Kit	ARCHImedline PEth from whole blood (RUO), A371
Matrix	Human whole blood
Analytical measurement	LC-MS/MS with standard ESI source
Critical liquid handling step	Whole blood sample transfer, addition of precipitation solution containing internal standard, and transfer of the supernatant after mixing and precipitation
Output	Processed samples ready for LC-MS/MS analysis

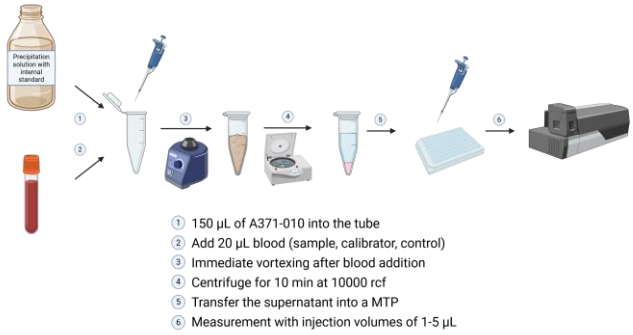
Kit description

The ARCHImedline PEth from whole blood kit (RUO) is designed for quantitative LC-MS/MS determination of PEth 16:0/18:1 as the main analyte and PEth 16:0/18:2 as an additional analyte. The routine kit size is 330 assays including a 7-point calibrator set and a 3-point control set. Optional extended calibrators and controls are available to support extended calibration ranges up to 4000 ng/mL.

Parameter	PEth 16:0/18:1	PEth 16:0/18:2
Matrix	Human whole blood	Human whole blood
Measurement principle	LC-MS/MS	LC-MS/MS
Linearity	4.9-6373.8 ng/mL	4.1-6109.4 ng/mL
LOD	2.1 ng/mL	1.4 ng/mL
LOQ	4.9 ng/mL	4.1 ng/mL
Precision data on product page	CV 8%, 5%, 5% across three levels; n=160	CV 6%, 5%, 5% across three levels; n=160

Automated workflow

The automated workflow follows the same analytical path as the manual protocol. Whole blood samples, calibrators and controls are processed by protein precipitation using precipitation solution with internal standard (A371-010). The processed samples are subsequently transferred for LC-MS/MS analysis. The automated method is designed to maintain equivalence to the manual workflow while reducing repetitive pipetting and improving standardization of reagent and sample transfer.

<table>
<tr><th>Step</th><th>Manual sample preparation</th><th colspan="2">Step</th><th>Automated sample preparation</th></tr>
<tr><td colspan="2"> 
</td><td colspan="3"> 
</td></tr>
<tr><td>0. Loading</td><td>Operator prepares tubes, reagents, calibrators, controls and consumables manually.</td><td colspan="2">1. Loading</td><td>Operator loads samples, calibrators, controls, reagents, tips and collection consumables according to the deck layout.</td></tr>
<tr><td>1 Reagent addition</td><td>Precipitation solution with internal standard is pipetted manually.</td><td rowspan="4">2. Automated workflow</td><td>2.1. Sample transfer</td><td>Whole blood aliquots are transferred according to the automated pipetting routine.</td></tr>
<tr><td>2. Sample transfer</td><td>Whole blood aliquots are pipetted manually after appropriate mixing.</td><td>2.2. Reagent addition</td><td>Precipitation solution with internal standard is dispensed by the automated method.</td></tr>
<tr><td>3. Mixing / precipitation</td><td>Manual vortexing or equivalent mixing step.</td><td>2.3. Mixing</td><td>The plate is shaken using the device's automated mixing routine.</td></tr>
<tr><td>4&5. Separation / Transfer</td><td>Centrifugation according to manual protocol followed by sample transfer onto the measurement plate</td><td>2.4. Transfer</td><td>The liquid is automatically transferred to the measurement plate.</td></tr>
<tr><td>6. LC-MS/MS analysis</td><td>Samples are injected into LC-MS/MS.</td><td colspan="2">3 LC-MS/MS analysis</td><td>Samples are injected into LC-MS/MS.</td></tr>
</table>

Technology and workflow control

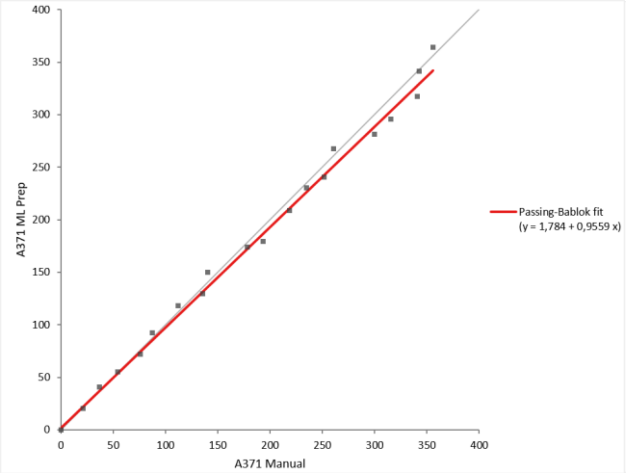
Automation of whole blood sample preparation requires robust handling of a complex biological matrix while maintaining reproducible sample processing. The automated workflow implemented on the Hamilton Microlab Prep was designed to provide standardized and traceable sample preparation with minimal operator intervention. By automating critical handling steps, the workflow supports consistent processing of larger sample batches while reducing hands-on time compared to manual preparation. The resulting extracts were analyzed using the ARCHImedline PEth LC-MS/MS assay and compared to the established manual workflow.

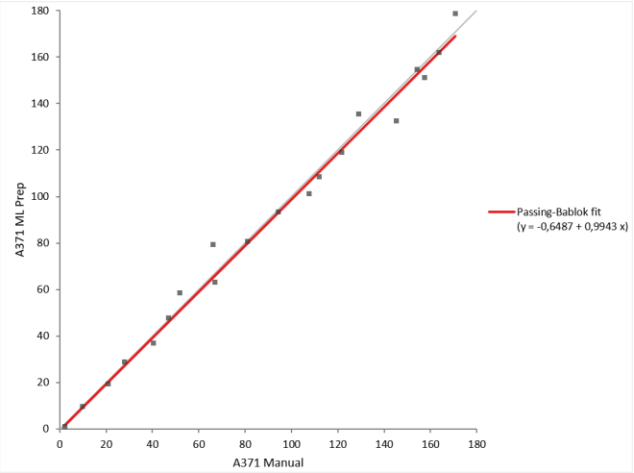
Results

Method comparison: manual vs. automated sample preparation

A set of whole blood samples covering the relevant measuring range was prepared manually and with the automated Hamilton Microlab Prep workflow. PEth 16:0/18:1 and PEth 16:0/18:2 concentrations obtained after automated preparation were compared with the corresponding manually prepared samples using regression analysis and bias evaluation.

Analyte	n	Regression method	Slope	Intercept	Correlation	Interpretation
PEth 16:0/18:1	20	Passing-Bablok	0.956	1.78	0.997	Excellent agreement between manual and automated sample preparation was observed for PEth 16:0/18:1.
PEth 16:0/18:2	20	Passing-Bablok	0.994	-0.649	0.994	Excellent agreement between manual and automated sample preparation was observed for PEth 16:0/18:2.





Precision of automated sample preparation

Precision was evaluated by repeated automated preparation of whole blood samples at low, medium, and high concentration levels. Intra-run and inter-run precision were calculated for both PEth analytes.

Analyte	Level	Concentration [ng/mL]	Intra-run CV [%] (n=5)	Inter-run CV [%] (n=8)
PEth 16:0/18:1	Low	38.88	9	7
PEth 16:0/18:1	Medium	169.88	3	7
PEth 16:0/18:1	High	361.99	6	4
PEth 16:0/18:2	Low	20.99	12	11
PEth 16:0/18:2	Medium	85.54	5	7
PEth 16:0/18:2	High	176.66	6	6

Verification of analytical performance

To further assess the performance of the automated workflow, external quality control material and proficiency testing samples were processed and analyzed alongside routine samples.

Sample	Analyte	Target value [ng/mL]	Measured value [ng/mL] (n=8)	Deviation from target (%)
EQA 1	16:0/18:1	92.20	92.47	0
EQA 2	16:0/18:1	179.00	178.93	0
External QC 1	16:0/18:1	87.70	85.49	-3
External QC 2	16:0/18:1	284.00	267.20	-6
External QC 1	16:0/18:2	56.50	62.24	10

Carryover and contamination assessment

Potential carryover was assessed by processing high-concentration samples followed by low-concentration samples. The observed response in blank and low sample was evaluated against predefined carryover acceptance criteria for both PEth analytes.

Sequence	Purpose	Acceptance criterion	Result
High - Low - Blank	Assessment of analyte carryover after high PEth concentration	Low Level sample and Blank result below LLOQ	No carryover and cross contamination for both analytes

Workflow efficiency

Hands-on time, number of manual pipetting steps, total preparation time, and batch capacity were compared between manual and automated sample preparation. The comparison should be presented as a practical workflow metric rather than an analytical performance claim.

	Manual workflow	Automated workflow
Compared batch size	47	47
Hands-on time [min]	48	4
Total preparation time [min]	58	23
Walkaway time [min]	10	19

Discussion

The results demonstrate that automated sample preparation of whole blood samples using the Hamilton Microlab Prep provides performance comparable to the established manual workflow for the determination of PEth 16:0/18:1 and PEth 16:0/18:2.

Method comparison experiments showed excellent agreement between manual and automated sample preparation for both analytes across the examined concentration range. Passing-Bablok regression analyses resulted in slopes close to unity and correlation coefficients greater than 0.99, indicating a high degree of comparability between both preparation approaches.

The automated workflow also demonstrated robust repeatability and reproducibility. Intra-run and inter-run precision values remained within acceptable ranges across low, medium, and high concentration levels, supporting reliable routine implementation of the automated procedure.

The analytical performance of the assay was further confirmed through the analyses of external quality control material and proficiency testing samples. Measured values showed good agreement with assigned target values, indicating that the automated sample preparation did not adversely affect assay performance.

No carryover or cross-contamination was observed after processing high-concentration samples. This demonstrates the ability for routine batch processing. In addition, automation substantially reduced hands-on time while increasing walkaway time, allowing laboratory personnel to focus on other tasks during sample preparation.

Overall, the results demonstrate that automated sample preparation can support standardized processing of PEth whole blood samples while maintaining analytical performance comparable to the established manual procedure.

Conclusion

The ARCHImedline PEth LC-MS/MS assay was successfully implemented on the Hamilton Microlab Prep platform for automated sample preparation of whole blood samples.

Excellent agreement with the established manual workflow was demonstrated for both PEth analytes. Precision, external quality control performance and proficiency testing results confirmed that automated sample preparation maintained the analytical performance of the assay. No carryover or cross-contamination was observed during workflow evaluation.

In addition to analytical comparability, automation significantly reduced hands-on time and supported standardized sample processing. These results demonstrate that the Hamilton Microlab Prep provides a suitable platform for automated PEth sample preparation and can support efficient implementation of the ARCHImedline PEth workflow in a routine laboratory environment.