PROVIDER OF CE-CERTIFIED LC-MS/MS DIAGNOSTIC KITS

MASSDETECT™ PETH

INSTRUCTIONS FOR USE
FOR THE IN VITRO DETERMINATION OF PETH 16:0/18:1 IN BLOOD

CE

CE-IVD label according to European Directive 98/79/EC

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1. PHOSPHATIDYLETHANOL, MASSDETECT PETH LC-MS/MS KIT

Art. No. 50-2002, MassDetect™ PEth 200 analyzes including column.

Art. No. 50-2001, MassDetect™ PEth 200 analyzes, replacement kit.

Risk class 1 according to IVDD, and risk class A according to IVDR

Global Trade Item Number (GTIN) 07350143680007

US Pat. 9499572, 9784701

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2. INTENDED USE

The described LC-MS/MS application is intended for the quantitative determination of phosphatidylethanol (PEth) 16:0/18:1 in blood.

The method is for monitoring long term alcohol intake to total abstinence.

The method is intended to be performed by a laboratory professional in a clinical laboratory.

For in vitro diagnostic use only.

3. INTRODUCTION

Phosphatidylethanol (PEth) in blood is a biomarker for previous alcohol consumption (1). Alcohol consumption can give rise to many different symptoms (2). An analysis of PEth concentrations in the blood of a patient could thus be a start for an investigation of a disease.

PEth is a phospholipid formed only in the presence of ethanol, giving a theoretical diagnostic specificity of 100% as a biomarker for alcohol consumption.

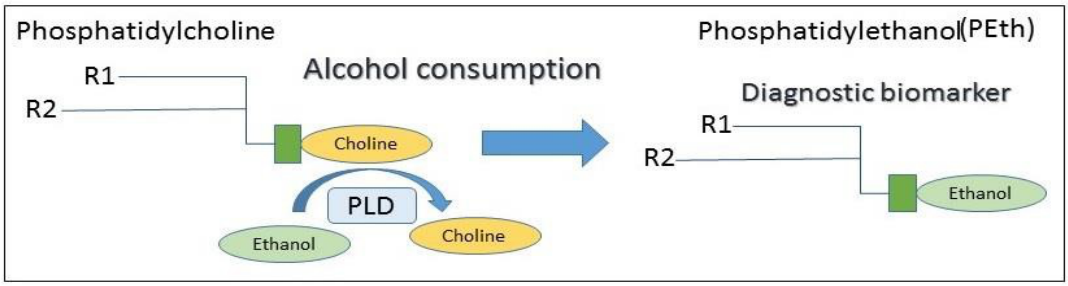
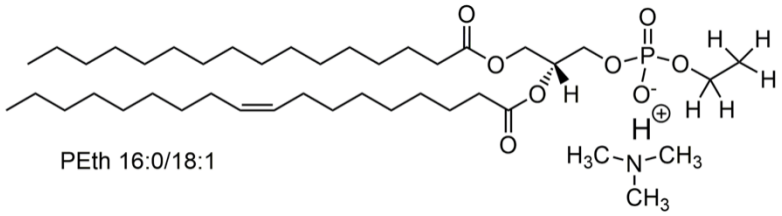


Figure 1. The principle for in-vivo generation of PEth depends on rearrangement of phosphatidylcholine in presence of phospholipase D.



PEth-16:0/18:1 is the most abundant individual form of PEth-homologues (3) but there are inter individual variations (4). PEth-16:0/18:1 has been accepted as the best form for use in quantitative LC-MS/MS analysis to estimate the level of alcohol consumption in patients. It has been shown that there is a direct correlation between alcohol consumption and the levels of PEth in blood (5, 6).

PEth is the only biomarker that gives a direct correlation to alcohol consumption, and it has been shown to be a more sensitive indicator of alcohol consumption than other markers (7, 8). The half-life of PEth in circulation is three to eight days, which means that PEth can be detected up to 3 weeks after ethanol has been cleared from the body.

The PEth levels in blood have been shown to be very useful in areas where alcohol consumption is difficult to assess (9).

The test developed by redhot diagnostics is a quantitative test which is robust with high specificity and sensitivity. The method is developed as a manually performed test.

4. PRINCIPLES OF THE PROCEDURE

Phosphatidylethanol is extracted from blood by addition of 20 µL of blood to 150 µL of precipitation solution, containing an internal standard (deuterated phosphatidylethanol). After thorough mixing, the tube is centrifuged, and an aliquot of the supernatant is injected in the LC-MS/MS system. The analytes are separated on an LC-column (art.no. 52015) using a binary gradient. The effluent is monitored with electrospray ionization mass spectrometry using multiple reaction monitoring (MRM) to follow the respective characteristic transitions for PEth and the internal standard. The ratio between the chromatographic peak areas for PEth and the internal standard are used to quantify the concentration of PEth in the samples.

5. WARNING AND PRECAUTIONS

Materials included in this kit should not be used past the expiration date on the kit label.

Solutions included in this kit should not be mixed or substituted with solutions from other kits.

Precautions should be taken when the extraction solution and mobile phases are handled.

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6. HEALTH AND SAFETY PRECAUTIONS

Wear personal protective equipment such as safety glasses, gloves, laboratory coats when blood samples are handled.

Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to discard residues in accordance with local laboratory regulations.

7. KIT CONTENTS

Art. no. 50-2001, 200 determinations, including column.

Art. no. 50-2002, 200 determinations, replacement kit.

Label	Component	Quantity
CAL 1 - 7	Calibrators in blood* 0, 0.02, 0.05, 0.1, 0.2, 0.5, and 1.0 µM	7 x 0.2 mL
IS	D_5-PEth 16:0/18:1, 4.5 nmol	0.5 mL
EXT	Extraction solution	44.5 mL
TUN	Tuning solution (PEth and D_5-PEth) (0.5 µM of each))	0.5 mL
COL	LC-column	1 pcs

*The concentrations are guideline values, certificate with exact values is added to each kit

8. STORAGE CONDITIONS

The reagents should be stored at +2-8°C.
Long-time storage: Calibrator and internals standard should be stored at -20°C.
Short time storage: Room temperature max 8h and refrigerator 2 days.
In use stability has been described by Isaksson et al. (11)

9. MATERIALS REQUIRED BUT NOT SUPPLIED

1. LC-MS/MS-Equipment
2. Mobile phase A
3. Mobile phase B
4. Vortex
5. Pipettes
6. Centrifuge
7. Vials
8. Appendix 1 lists products that are available for the PEth method.

10. PREPARATION OF MOBILE PHASES

Mobile phase A

5 mM ammonium acetate in water	Preparation of 1 000 mL
Ammonium acetate (5 M)	1 mL
Milli-Q water	999 mL

Mobile phase B

10% 2-propanol in 90% methanol	Preparation of 1 000 mL
Methanol	900 mL
2-propanol	100 mL

11. MS/MS METHOD

Listed as an example for Sciex API5500

Instrument	Sciex API5500
Ionization	Electrospray
Scan Type	MRM
Polarity	ESI-
Curtain Gas	10
Collision Gas	8
Ion Spray Voltage (kV)	-4500
Temperature	500
Ion Source Gas 1	40
Ion Source Gas 2	40
DP (declustering potential)	150 – 220
CE (collision energy)	38 - 40

12. TRANSITION

Molecule	Transitions
PEth 16:0/18:1	701.5 > 281.2
D_5-PEth 16:0/18:1	706.5 > 281.2

Fragment 281 is used for quantification of PEth.

13. LC-GRADIENT

Flow rate: 0.4 mL/min

Analysis time: 3.5 min

Time [min]	Mobile phase A [%]	Mobile phase B [%]
0-0.2	30	70
0.2-1.5	0	100
1.5-2.5	0	100
2.5-2.6	30	70

If the back pressure is high for the LC-system, it is recommended to use a column oven at 40 – 50°C.

14. WASH SOLUTION

Due to the sticky properties of PEth we recommend that the autosampler is washed regularly (in between injections). A recommendation is to use two wash solutions, strong and weak wash.

Strong wash: Methanol/IPA 4/1 (start with)

Weak wash: 70 % B-phase and 30 % A-phase.

15. STARTUP – OPTIMIZATION OF PARAMETERS FOR THE ANALYTES

Use the included PEth tuning solution to find the exact transitions for the MRM traces and the other

parameters when setting up the kit for the first time.

PEth ionizes and fragments easily depending on instrument and parameters, the MS response might be too high for the electron multiplicator. If so, decrease the injection volume and/or dilute the samples.

Check the accuracy of the mass scales after annual preventive maintenance of the mass spectrometer, and after all other manipulations which can affect the accuracy of the mass scales.

16. SAMPLES – STORAGE AND TRANSPORTATION CONDITIONS

Analysis is performed in blood: Use only EDTA tubes. NOTE! Do not centrifuge! Mix the sample in a blood cradle or by turning it upside down ten times immediately after sampling to prevent clots in the tube.

If transport to the laboratory cannot be arranged immediately, the sample must be stored in a refrigerator. Shelf life in the fridge: 1 week. Transport takes place without refrigeration.

For longer time storage -20^{0}C is recommended.

Extracted samples are stored at +2-8^{o}C with a shelf-life of one weeks.

Allow samples and reagents to reach room temperature before use.

17. PREPARATION OF EXTRACTION SOLUTION

Centrifuge the ampoule containing internal standard (IS) (2000 RCF, 2 min), open the ampoule and add 0.5 ml extraction solution (EXT), transfer the solution in its entirety to the extraction bottle, repeat 2 times to quantitatively transfer the internal standard to the EXT bottle. The final concentration of D_5-PEth 16:0/18:1 internal standard is 0.1 µM. Stored at +2-8^{o}C, before use the solution should be at room temperature.

18. SAMPLE PREPARATION

1. To 150 µL EXT add 20 µL blood, vortex thoroughly.
2. Centrifuge the tubes at approx. 16400 RCF for 10 min.
3. Transfer 120 µL of the supernatant to an autosampler vial, the vial in the autosampler, inject 5 µL of the sample into the instrument.

19. CALIBRATOR CURVE

The calibrator curve in blood is ready-to-use. See sample preparation pkt.18

20. DETERMINATION OF PETH CONCENTRATIONS

For each calibrator concentration, the peak area of the analyte is divided by the corresponding peak area of the internal standard. These ratios are plotted against the calibrator concentrations to calculate the calibrator curve equation, which is used to determine the PEth concentration of the sample. First order linear regression weighted by 1/x is preferred.

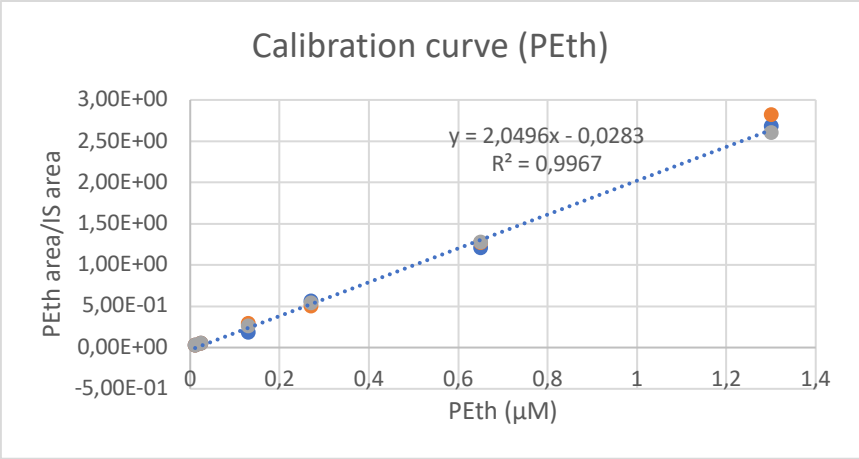

Figure 2. Example of a calibration curve based on chromatograms from 701.5 -> 281.2.

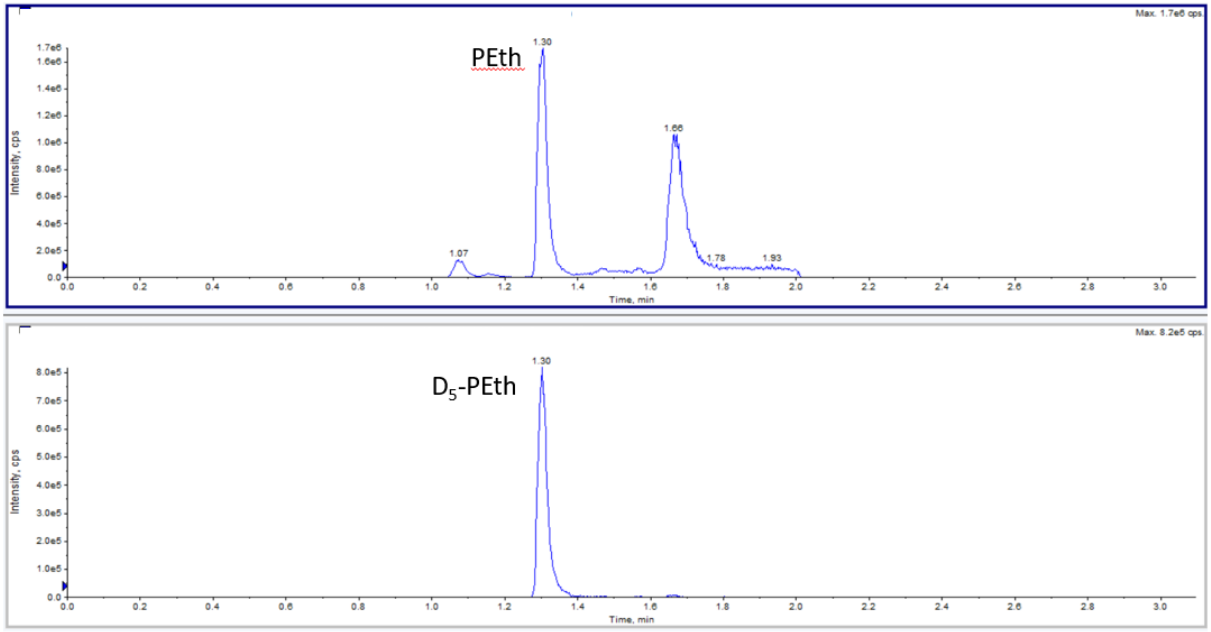

Figure 3 Example of calibration sample at 0.5 µM

21. QUALITY CONTROL PROCEDURES

Control samples must be analyzed together with each batch of samples. Results generated from the analysis of control samples should be evaluated by statistical methods to ensure that the method shows accurate results. It is recommended to monitor the peak areas of the internal standard for all batches. The peak area must be consistent, and any inconsistency or systematic decrease of the internal standard can indicate interference, carryover effects and/or hardware related issues, such as contaminated LC-column or ion source. Individual outliers can indicate issues with the sample or the preparation of the sample.

Control samples can be ordered from redhot diagnostics, Appendix 1

22. PERFORMANCE CHARACTERISTICS

Performance characteristics may vary depending on the instrument used.

Measuring range

0.02 – 1.0 µM

LOQ (limit of quantification)

0.02 µM (S/N 13)

Reproducibility

PEth [µmol/L]	Intra Assay CV (%)
0.032	7.7 (n=6)
0.27	10 (n=10)
0.82	6.1 (n=6)

23. REFERENCE INTERVAL FOR PETH

According to Swedish Transport Agency (10)

PEth [µmol/L]	PEth [µg/L]	Comments
< 0.03	<20	None or low
0.03 – 0.3	20 – 200	Moderate intake
> 0.3	>200	Large intake

24. REFERENCES

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25. APPENDIX 1

50–1011	PEth Calibrator curve in blood	Ready in blood 0, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0 µM, 0.2 mL The concentrations are guideline values, certificate with exact values is added to each kit
50-1021	PEth-d5 16:0/18:1 int.std. 10 nmol	10 nmol
50-1023	PEth-d5 16:0/18:1 int.std. 100 nmol	100 nmol
51-1008	QC sample high PEth	5x 0,5 mL
51-1009	QC sample low PEth	5x 0,5 mL
51-1017	QC sample medium PEth	5x 0,5 mL
50–2003	PEth LC-MS/MS kit 500 analyzes	Incl Column
50–2004	PEth LC-MS/MS kit - 500 analyzes	Replacement kit
50–2005	PEth LC-MS/MS kit - 1 000 analyzes	Incl Column
50–2006	PEth LC-MS/MS kit 1 000 analyzes	Replacement kit
Products for blood sampling		
10-5001	PEth - rhelise	1 pcs sampling kit
10-5010	PEth - rhelise	10 pcs sampling kit
10-5050	PEth - rhelise	50 pcs sampling kit