High-throughput automated Evosep-MRM for glycated albumin quantification to facilitate the diagnosis of Diabetes mellitus

Bharath Kumar Raghuraman[1,2], Dorte B. Bekker-Jensen[1], Ole Vorm[1], Nicolai Bache[1], Martin Overgaard[2]

[1]Evosep Biosystems, Denmark, [2]Odense University Hospital, Denmark

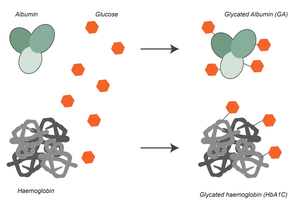
Highlights

- Development of a high-throughput end-to-end automated workflow with high intra-day and inter-day precision.
- Assay displays higher correlation to classical %HbA1C measurements and is two times faster than existing assays.
- Potential for remote monitoring of short term glycaemic status.

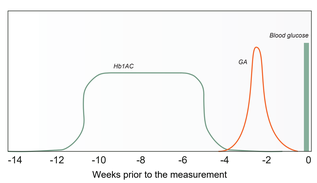
Looking beyond HbA1C in diagnosis of diabetes

Introduction

Glycated haemoglobulin (HbA1C) is considered the golden standard for assessing the glycaemic status, but it is susceptible to high variation in conditions like diabetic nephrophathy, anaemia and pregnancy. Haemoglobin also exhibit longer turnover times (8-12 weeks), which renders it ineffective for short term glycaemic status monitoring. Glycated albumin (GA) has gained attention due to its shorter turnaround times (2-3 weeks) and higher affinity to glycation. Here, we present a fully automated Evosep-MRM workflow for the quantification of GA.



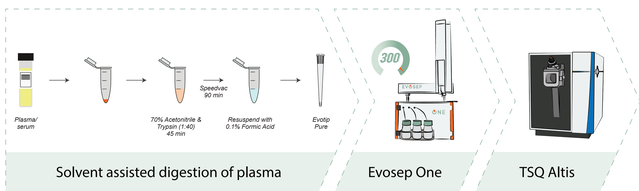
Formation of glycated Haemoglobulin (HbA1C) and glycated albumin (GA).



Turnaround times of HbA1C (8-12 weeks), GA (3 weeks) and blood glucose.

Evosep-MRM for glycated albumin quantification

We designed an Evosep-MRM assay targeting two glycated peptides (*kQTALVELVK, AEFAEVSk*LVTDLTK), two unglycated peptides (KQTALVELVK, AEFAEVSKLVTDLTK) and one QC peptide (QTALVELVK). Three transitions per peptide were analyzed. *marks site of glycation.

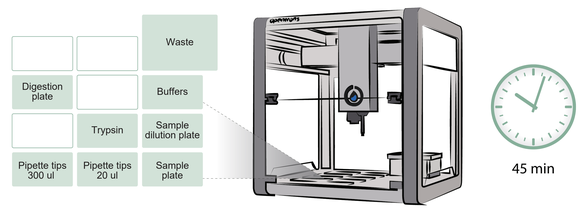


Workflow for solvent assisted digestion of plasma

End-to-end workflow with Opentrons

Automation of workflow

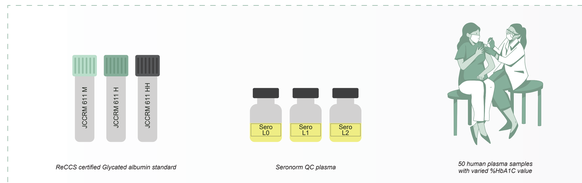
The workflow for solvent assisted digestion of plasma was transferred and optimized for full automation using an Opentrons. The assay is designed for high throughput with 45 minutes needed to process 96 samples, which are subsequently measured with the 300 SPD Evosep One method.



Layout of Opentrons deck for automation of solvent assisted digestion of plasma.

Further validation of assay

ReCCS (Japan) certified GA standard (JCCRM 611-1) with 3 glycation levels; M, H, HH and Seronorm plasma were used to test repeatability and inter-day precision of the 'Evosep-MRM Glycated albumin assay'. 50 patient samples with known HbA1C levels were used for this proof-of-concept study.



Overview of samples used to validate the assay.

Quantification of glycated albumin level

The assay measures the ratio between a glycosylated and an un-glycosylated peptide within a sample. This facilitates robust CVs and doesn't require additional use of an internal standard for quantification.

$$\%GA_{*kQTALVELVK} = \frac{{}^{\text{best transition}}XIC_{*kQTALVELVK}}{({}^{\text{best transition}}XIC_{*kQTALVELVK} + {}^{\text{best transition}}XIC_{KQTALVELVK})}$$

$$\%GA_{AEFAEVSk*LVTDLTK} = \frac{{}^{\text{best transition}}XIC_{AEFAEVSk*LVTDLTK}}{({}^{\text{best transition}}XIC_{AEFAEVSk*LVTDLTK} + {}^{\text{best transition}}XIC_{AEFAEVSKLVTDLTK})}$$

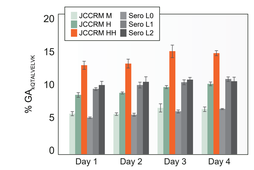
Formulas used to calculate glycated albumin.

Proof-of-concept study with 50 patient samples

High intra-day precision (Repeatability)

The ReCCS JCCRM 611-1 standard and Seronorm QC samples were analyzed for four consecutive days. The repeatability was measured from n=8 per sample per day. The 'Evosep-MRM Glycated albumin assay' exhibited high repeatability with coefficient of variation (CV) between 2-6%.

	Day 1	Day 2	Day 3	Day 4
JCCRM M	5.1%	3.3%	5.9%	5.1%
JCCRM H	3.8%	1.7%	2.3%	2.6%
JCCRM HH	4.5%	4.9%	6.0%	2.5%
Sero L0	2.2%	3.8%	3.6%	1.3%
Sero L1	2.1%	6.1%	3.4%	3.0%
Sero L2	5.2%	4.2%	3.2%	5.7%

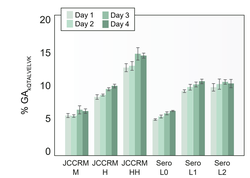


Intra-day precision of GA levels in glycated albumin standards and Seronorm QC plasma across 4 days.

Low inter-day variation (Intermediate-precision)

The %GA of the JCCRM 611-1 (M, H, HH) standard and three Seronorm QC plasma samples were monitored over four days (n=32). The inter-day variation of the 'Evosep-MRM Glycated albumin assay' was less than 10%.

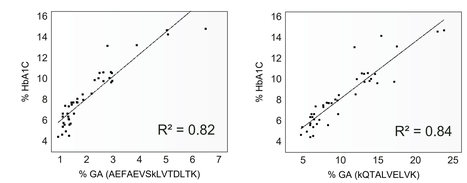
	CV (4 days)
JCCRM M	6.3%
JCCRM H	6.9%
JCCRM HH	6.4%
Sero L0	7.9%
Sero L1	5.1%
Sero L2	2.7%



Inter-day variation of GA levels in glycated albumin standards and Seronorm QC plasma

High correlation between level of GA and HbA1C

In order to establish if our 'Evosep-MRM Glycated albumin assay' can faithfully correlate to %HbA1C, we analyzed 50 patient plasma samples with known HbA1C levels. %GA calculated from both peptides exhibited the same trend when compared with %HbA1C.



Correlation of GA and HbA1C levels in patient plasma samples.

We would like to thank Dr. Martin Overgaard and Dr. Lars Melholt Rasmussen from Odense University Hospital for providing access to the human plasma samples.