



Quantitative measurement of methylglyoxal (MGO), a potent and damaging glycating metabolite, by ELISA

Jan van Groningen, Geurt Schilders, Helma W.H. Rutjes, Jan van Binsbergen

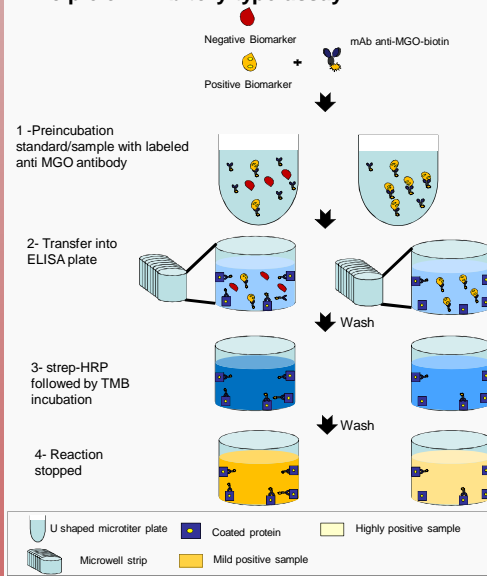
Introduction

Methylglyoxal (MGO) is a highly potent glycating metabolite which is produced during glucose, amino acid as well as fatty acid metabolism. MGO forms covalent adducts with the side chains of lysine-, cysteine- and arginine-residues, which is classified as an early glycation process and occurs in all tissues and body fluids. Subsequent, later-stage reactions result in the formation of advanced glycation end products termed AGEs.

The damaging effect of MGO consist of the covalent cross-linking of proteins, or when the modified amino acid is located in the active site of the enzyme or results in a structural rearrangement of the modified protein. In order to counteract the damaging effect of MGO our body has evolved several non-enzymatic as well as non-enzymatic defenses such as the glyoxalase system. In *C. elegans* silencing of the glyoxalase system resulted in a decreased lifespan by 40%. In human, increased production and accumulation of methylglyoxal (MGO) are hallmarks of aging and a number of pathological conditions such as neurodegenerative disorders and diabetes.

In order to quantitatively measure MGO an ELISA has been developed using MGO-specific antibodies.

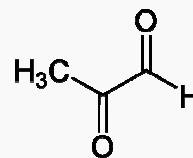
Principle of inhibitory-type assay



Features of the assay

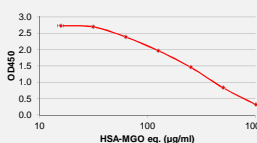
- Assay time: 3.5 hours
- Species independent
- Sample types tested: plasma, serum, urine and faeces
- HIT503 standard consists of MGO labelled HSA (µg/ml)
- Detection limit: 7.8µg/ml HSA-MGO equivalents.
- Dynamic range: 7.8 – 500µg/ml HSA-MGO equivalents.
- Sample volume: 125µl/well
- Non-expensive

Skeletal formula of MGO



HIT503 assay characteristics

Typical standard curve of inhibitory HIT503 assay



Freeze-thaw stability

- Stability of MGO in human citrate plasma and standard after several freeze-thaw cycles (Table 2).
- Stability in plasma is between 80-120%. Stability of standard drops after repeated freeze-thaw cycles.

Table 2. Freeze-thaw stability of standard and plasma samples

sample	1x	2x	3x	4x	5x*
plasma	100%	100%	93%	94%	94%
standard	100%	98%	84%	81%	62%

* Number of freeze-thaw cycles

Bench top stability

- Recovery of MGO in human citrate plasma sample and standard after incubation for 10 min. at RT or 2 or 24hrs at 4°C as compared to freshly prepared samples.
- Stability in plasma sample and standard is between 80-120%.

Table 3. Bench top stability of standard and plasma samples

sample	fresh	10 min RT	2 hrs RT	24 hrs 4°C
plasma	100%	101%	95%	98%
standard	100%	94%	92%	83%

Recovery

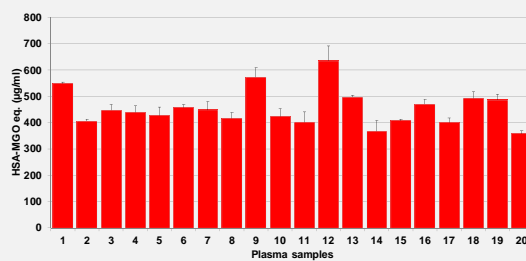
- 100 and 500µg of HSA-MGO eq. were spiked into citrate plasma samples (see Table 1)
- Percentage recovery was calculated as the percentage of measured/expected MGO
- Recovery in both plasma samples is between 80-120%

Table 1. Recovery of CML in human citrate plasma

matrix	spike (µg/ml)	% recovery
Citrate plasma 1	500	107
	100	105
Citrate plasma 2	500	105
	100	109

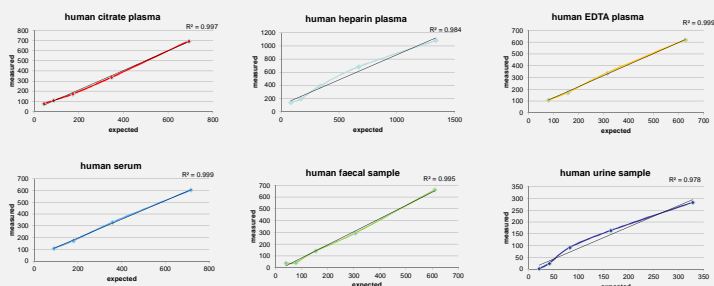
Measurement of MGO in human EDTA plasma

- A panel of 20 plasma samples from randomly selected healthy donors (males and females) was tested for MGO levels
- The average level of MGO was 456µg/ml HSA-MGO equivalents, with a range of 358-636µg/ml HSA-MGO eq.

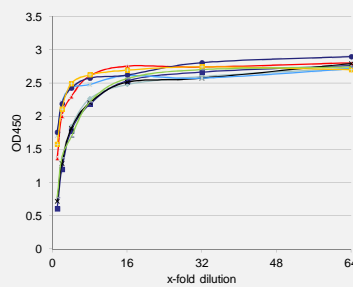


Detection of MGO with HIT503 assay in different matrices

- MGO levels were measured in plasma, urine and faeces samples using the HIT503 assay
- Linearity of dilution was determined by serially diluting samples. The "measured" concentrations are plotted against the "expected" concentrations. A correlation of at least 0.97 was found in the matrices tested.



Detection of MGO with HIT503 assay is species independent



- MGO levels were measured in sera of various species using the HIT503 assay
- The calculated MGO concentrations are depicted in Table 4.

Table 4. Measured MGO concentrations

	HSA-MGO eq (µg/ml)	SD	CV (%)
dog	504	42	8
horse	589	24	4
goat	218	16	8
swine	537	19	3
rat	147	14	10
mouse	145	4	2
rabbit	176	11	6
bovine	533	80	15