QualiChem (Sea) Sdn. Bhd. (863673-M)
No. 6, Jalan Bayu Tinggi 2/KS6, Taman Bayu Tinggi, 41200 Klang, Selangor Darul Ehsan, Malaysia.
Tel: +603-3325 5525 Fax: +603-3325 5535 Email: qualichem.info@gmail.com Website: www.qualichemsea.com

MATRIX METALLOPROTEINASE-3 (MMP-3)

REAGENT KIT

Cat. No.: QMP-3001/1 20 ml/kit
QMP-3001/2 40 ml/kit
QMP-3001/3 60 ml/kit

Reagent kit for the quantitative determination of Matrix metalloproteinase-3 (MMP-3) concentration in serum, immunoturbidimetry method.

MMP-3 (Matrix Metalloproteinase-3) is one of the proteolytic enzymes what is produced with Rheumatoid Arthritis (RA) progression , activates joint destruction and MMP-3 bleeds into the blood.

Concentration of MMP-3 in blood is correlated with the activity of RA.

Rheumatoid Arthritis (RA) is a symmetric, inflammatory, peripheral polyarthritis of unknown etiology. It typically leads to deformity through the stretching of tendons and ligaments and destruction of joints through the erosion of cartilage and bone. If it is untreated or unresponsive to therapy, inflammation and joint destruction lead to loss of physical function, inability to carry out daily tasks of living, and difficulties in maintaining employment. Uncontrolled inflammation may have other health risks including higher rates of cardiovascular disease and osteoporosis.

Early recognition and treatment with disease-modifying anti-rheumatic drugs (DMARDs) is important in achieving control of disease and prevention of joint injury and disability.

Principle

MMP-3 (Matrix Metalloproteinase-3) is one of the proteolytic enzymes what is produced with Rheumatoid Arthritis (RA) progression , activates joint destruction and MMP-3 bleeds into the blood.

The MMP-3 in the sample reacts with the Latex coated anti-human MMP-3 monoclonal antibody and forms aggregates. The concentration of the amount of the aggregates can be detected by spectrophotometry and the change of absorbance is proportional with concentration of MMP-3 in the sample. The concentration of MMP-3 in the serum is calculated from the comparison of change in absorbance in the calibration curve that obtain from change in absorbance of the known concentrations of MMP-3.

Clinical relevance

The quantification of serum MMP-3 levels by immunoturbidimetric method provides useful new information for the clinician to monitor the activity of rheumatoid arthritis disease, together with the usual diagnostic parameters (ACPA, RF, CRP, radiological diagnostics), and thus to monitor the efficacy of the therapy used.

Reference values

Male : **36.9 ~ 121 ng/mL**
Female : **17.3 ~ 59.7 ng/mL**

It is recommended that each laboratory should assign its own normal range.

Content of the reagent kits

1. Reagent (R1)
TRIS buffer, pH=7.2 0 0.1 mol/l
Sodium chloride 1.8 g/l
2. Reagent (R2)
Latex coated with anti-human MMP-3 mouse monoclonal antibody 0.5 g/l
3. Control low
4. Control high
5. Calibrator 5 level: 100 ng/ml, 200 ng/ml, 400 ng/ml, 800 ng/ml, 1600 ng/ml

Samples

Serum free of haemolysis.

Stability

without opening: till the expiry date indicated on the label
after opening: 30 days
calibration frequency: 7 days
onboard stability: 30 days
Stability data are valid only when using new system bottle!

PROCEDURE

The reagents are ready to use

Assay conditions

BECKMAN COULTER AU480

[Specific TestParameters]

Test Name:		*1			Type:	Serum	Operation Yes
Sample Volume:		4.0 ul	Dilution	0.0 ul	Pre-dilution Rate:	1	
Reagents Volume:	R1(R1-1)	120 ul	Dilution	0 ul	OD Limit:		
	R2(R2-1)	30 ul	Dilution	0 ul	Min OD	-2,0000	Max. OD
Wavelength:	Pri.	700	Sec.		Reagent OD limit:		
Method:		FIXED			First Low	-2,0000	High
Reaction slope:					Last Low	-2,0000	High
Measuring Point 1:	First	13	Last	27	Dynamic Range:		
Measuring Point 2:	First		Last		Low	-99999	High
Linearity:		%			Correlation Factor:		
No Lag Time:		NO			A	1,00000	
					Onboad Stability		

[Calibration Specific]

Test Name: *1 Type: Serum
Calibration Type: 6AB Formula: Spline Counts Process CONC

	Calibrator	OD	Conc	Factor Range Low	Factor Range High
Point1:	*2		*3 (0)	-2,0000	2,5000
Point2:	*2		*3 (100)	-2,0000	2,5000
Point3:	*2		*3 (200)	-2,0000	2,5000
Point4:	*2		*3 (400)	-2,0000	2,5000
Point5:	*2		*3 (800)	-2,0000	2,5000
Point6:	*2		*3 (1600)	-2,0000	2,5000
Point7:					
1-Point Calibration Points					
MB Type Factor:			Calibration stability		

*1 User defined
*2 Please input calibrator position
*3 Please in ut calibrator concentration

Calibration: Five level calibrator set included in the kit

Calibration frequency
- after reagent lot change,
- as required following quality control procedures.

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Recommended controls: „Contol low" and „Control high" included in the kit

PERFORMANCE DATA

The following data were obtained using the Olympus 480 analyzer.

Linearity
The method is linear 10-1600 ng/ml

Sensitivity
It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used.
Under manual conditions however, a change of 0.001 Abs units/min is equivalent to 3.00 U/l (0,05µkat/l) Alkaline-phosphatase activity at 405 nm.

Precision

	Reproducibility		
Sample	Average concentration (ng/mL)	SD	CV%
Sample I.	100	1.28	1.3
Sample II.	302	1.6	0.5

	Repeatability		
Sample	Average concentration (ng/mL)	SD	CV%
Sample I.	100	1.56	1.58
Sample II.	303	6.17	0.96

Correlation

Reagent	Sample species	Regression equation	Correlation coefficient
Y company	serum	Y=0.95x+6.09	r=0.994 (n=14
X company	serum	Y=1.06x+0.43	r=0.999 (n=80

(x= other commercial reagent, y= own reagent).

Specificity
Bilirubin C: up to 20 mg/dl, Bilirubin F: up to 20 mg/dl, lipids 2000 FTU don't interfere with the assay up to the given levels.

Correlation between DAS28 levels and serum MMP-3 concentrations

DAS28 levels were associated with MMP3 levels in patients with Rheumatoid Arthritis. Patients with low disease activity had significantly lower MMP-3 levels compared to patients with intermediate and high disease activity (44.00 (12.06-231.827) vs 57.77 (9.0-829.817) p=0.02 and 44.00 (12.06-231.827) vs 125.190 (38.13-496.907) p=0.000) (Figure 5)

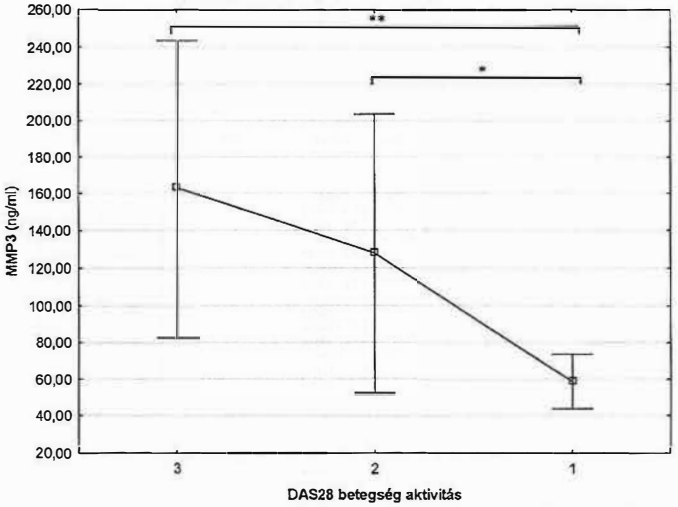

Figure 5. DAS28 levels as a function of MMP3 (mean, mean ± 0.95 confidence interval)
*: p<0,05 Based on DAS28 value moderate disease activity vs remission
**: p<0,01 Based on DAS28 value high disease activity vs remission
DAS28 values 3= high disease activity DAS28>5,1
2= moderate disease activity 3,2<DAS28≤5,1
1= low disease activity DAS28≤3,2

Note

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For *in vitro* diagnostic use only.

The following symbols can be used on the labels

IVD	In vitro diagnostic device	LOT	Batch code
	Manufacturer	REF	Catalogue number
CE	CE-marking		This way up
	Temperature limitations		Biological risk
	Use by (year/month)		

Bibliography

- *MMPs that degrade the matrix of articular cartilage leading to bone resorption* in RA- Matrix metalloproteinases (MMPs) are implicated in the degradation of extracellular matrix (ECM). Serum MMP-3 also showed modest yet significant correlation with acute phase reactants, DAS28 score and synovial histology

(Humby F, et al. Ann Rheum Dis 2019;78:761–772. doi:10.1136/annrheumdis-2018-214539)"

- „In our cohort with onset of RA symptoms < 2-years, multivariate analysis identified anti-CCP status and baseline MMP-3 as the strongest independent predictors of radiographic disease outcome at 8.2-years. This finding suggests determination of baseline MMP-3, in conjunction with traditional serologic markers, may provide additional prognostic information for patients with RA.

(Houseman et al. Arthritis Research & Therapy 2012, 14:R30)

- „Serum MMP-3 and the US7 scores could both effectively reflect disease activity and therapeutic responses in patients with moderate to severe RA."

(Zhou et al. Arthritis Research & Therapy (2017) 19:250 DOI 10.1186/s13075-017-1449-z)

- „Our data showed that continuously elevated serum MMP-3 for 3 ~ 6 months predicted one-year radiographic progression which implied that monitoring of dynamic serum MMP-3 combined with core disease activity indicators may be more helpful for predicting radiographic progression and treatment decision in RA".

(Ma et al. Arthritis Research & Therapy (2015) 17:289 DOI 10.1186/s13075-015-0803-2)

- „The percentage of lining MMP3+ cells was significantly higher in RA patients especially with high grade synovitis and it was significantly correlated with Krenn's synovitis score ($r = 0.574$, $P < 0.001$) and sublining inflammatory cells. Multivariate stepwise linear regression analysis revealed that the association of the percentage of lining MMP3+ cells with activation of synovial stroma, sublining CD68+ macrophages, and CD15+ neutrophils was stronger than other histological indicators. The percentage of lining MMP3+ cells was significantly correlated with serum MMP-3 in RA ($r = 0.656$, $P < 0.001$). Serum MMP-3 was higher in RA patients with high grade synovitis than that of low grade synovitis and significantly correlated with synovitis score and activation of synovial stroma subscore (all $P < 0.05$). Conclusion. Serum MMP-3 may be an alternative noninvasive biomarker of histological synovitis and RA diagnosis."

(Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2014, Article ID 179284, 10 pages
http://dx.doi.org/10.1155/2014/179284)

„Half of rheumatoid patients treated with MTX monotherapy for 3 years exhibited structural remission, and this outcome can be predicted at the outset by lower serum MMP-3."
(Shiozawa et al. Arthritis Research & Therapy (2016) 18:55
DOI 10.1186/s13075-016-0948-7)

- „After 2 years of follow-up, US assessment showed a higher number of new bone erosions in MMP-3-positive compared to MMP-3-negative patients with early RA and no visible initial radiographic changes. High baseline levels of MMP-3 predict significantly higher structural damage progression at the level of feet, but not at the level of hand joints."

(Med Princ Pract 2018;27:378–386 Prodanovic et al.
DOI: 10.1159/000490350)

- „MMP-3 levei was shown to be useful as a disease activity marker in RA patients. In addition, serial measurement of MMP-3 may be helpful to evaluate the effect of treatments with MTX and IFX. [Original]"

(Yuko UEMURA*1,HidetoshiHAYASHI*2, Toshio TAKAHASH/*3, Toshiharu SAITH0*4, Ryousuke UMEDA, MD*5, Yoshihide JCHISE, MD*6, Sho SENDO, MD*7, Goh TSUJI, MD, PhD*8 and Shunichi KUMAGAI, MD, PhD*9
Rinsho Byori 63: 1357~1364, 2015])

- „MMP-3 can be a useful marker for prediction of joint destruction"

(AKIRA MAMEHARA1,2, TAKESHI SUGIMOTO1, DAISUKE SUGIYAMA1, SAHOKO MORINOBU1, GOH TSUJI1, SEIJI KAWANO1, AKIO MORINOBU1, and SHUNICHI KUMAGAI1,*
Kobe J. Med. Sci., Vol. 56, No. 3, pp. E98-E107, 2010)

- „These results indicate that serum MMP-3 levels may be used as an indicator for structural damage such as erosions in the early stages of the disease, and to monitor disease activity."

(Turkan Tuncer1,A–C, Arzu Kaya1,A–C, Arif Gulkesen1,A,B, Gul Ayden Kal2,B, Dilara Kaman3,C, Gurkan Akgol1,A,B,D
Adv Clin Exp Med. 2019;28(5)