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Aspartate aminotransferase (EC2.6.1.1) or Aspartate transaminase, known as AST/AspAT/ASAT/AAT or serum glutamic oxaloacetic transaminase (SGOT), which exists in two isoenzymic forms-mitochondrial (m-AST) and cytosolic (c-AST), is a pyridoxal phosphate (PLP)-dependent transaminase enzyme. AST is present in most organs, and the highest concentrations are found in the liver, heart, skeletal muscle, kidneys, brain and red blood cells. Because of its wide tissue distribution, elevated AST levels have low specificity for any single disease. AST activities in liver are 7000 times higher than that in serum. Historically, AST has been used clinically to diagnose hepatitis, myocardial infarction and skeletal muscle disease. Serum m-AST, serum AST and their ratio (m-AST/AST) are measured clinically as biomarkers for liver diseases and the damage of mitochondria associated with hepatocellular damage, such as acute and chronic viral hepatitis, fulminant hepatitis, chronic active hepatitis and cirrhosis.

AST levels of myocardial infarction increase after 3 to 8 hours of onset of attack and return to normal in 4 to 6 weeks. The duration and variability of AST are proportional to the severity of attack. The change in levels over a period of time is useful to the physician in evaluating myocardial infarction, following chronic heart disease or resolving hepatitis.

The activity of m-AST was investigated with immunoinhibition assay, in which the anti-c-AST antibodies can inhibit the activity of c-AST in the specimen. The system monitored the rate of the change of m-AST activities at absorbance 340 nm over a fixed-time interval. The differences of m-AST activities were observed in samples before and after inhibition on biochemistry platform.

Anti-c-AST monoclonal antibody

A new generation of anti-c-AST monoclonal antibodies, which was recently produced by CUSAg, makes possible the development of immunoinhibition assay. Anti-c-AST monoclonal antibodies can be used for a full-range m-AST assay, which is a simple, rapid, sensitive and homogeneous assay that can be performed using automated systems for high-throughput immunoassays.

Properties	Specification
Target species	Human
Host animal	Mice Balb/c
Cell line used for fusion	Sp2/0
Immunogen	Human Aspartate aminotransferase isoenzymes (c-AST) protein
Purification method, Purity	Protein G affinity chromatography, >95%
Presentation	MAb solution in NaCl with 15 mM NaN_3 (pH 7.2)
Application	Immunoinhibition assay, IHC etc.
Catalog Number	CSB-DA222BmN①

c-AST
c-AST
c-AST

1 Predicted Linear Correlation

Different concentrations of m-AST samples were prepared by means of mixing m-AST protein with c-AST protein in different proportions. The m-AST activities of samples were tested with CUSAg m-AST reagent on our CUSAg biochemistry platform. Fig.1 shows that the predicted m-AST concentrations were increasing gradually along with the measured m-AST values of compound samples.

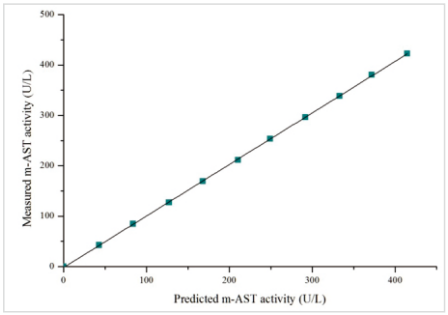

Fig.1 Linear correlation of predicted m-AST activity and measured m-AST activity

2 Accuracy

One level of m-AST control was analyzed in replicates of three at two separated time using our two lots of reagents on the CUSAg biochemistry platform. Datas from this study are summarized in the below table.1, the relative bias was <3%.

Table.1 Accuracy of m-AST reagent

Reagent Lot	Control Target value (U/L)	Determined Con.(U/L)			Mean Con. (U/L)	Bias (%)
1	24.29	23.9	23.9	23.4	23.7	-2.3
2	24.29	24.0	24.1	23.9	24.0	-1.2

3 Thermal Stability

◆Our anti-c-AST monoclonal antibodies presented in CUSAg m-AST reagent were stored at 4℃ and 37℃ for 14 days, respectively. During this period, m-AST activity of calibrators was determined on our biochemistry platform. Fig.2 shows that both m-AST activities determined by heated reagent and non-heated reagent are parallel, representing that our CUSAg m-AST reagent was stable from 4℃ to 37℃.

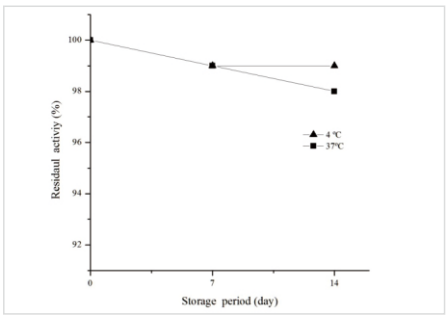

Fig.2 Thermal stability of m-AST reagent

◆Our anti-c-AST monoclonal antibodies were respectively stored at 4℃ and 37℃ for 14 days, which were prepared into our CUSAg in-house m-AST reagent to test the m-AST control with interval of 7 days. Fig.3 shows our c-AST antibodies were stable no matter at 4℃ or 37℃ stored without any stabilizer.

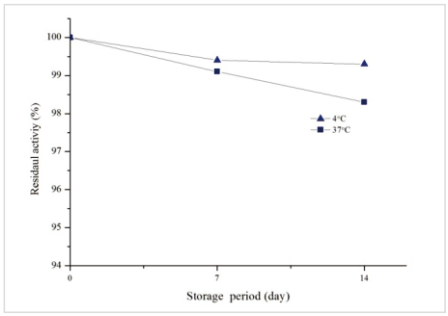

Fig.3 Thermal stability of anti-c-AST monoclonal antibodies

4 Precision

Four members of serum samples based panel were assayed, using a single lot of reagents, in replicates of ten on the CUSAg biochemistry platform (from 8 to 18 U/L as normal). As shown in table.2, the system shows excellent precision with CV≤3%.

Table.2 Precision profile of m-AST reagent

Panel Member	n	Mean Conc.(U/L)	SD	%CV
1	10	5.52	0.08	1.43
2	10	6.06	0.11	1.77
3	10	8.00	0.08	1.02
4	10	9.44	0.21	2.19

5 Clinical Comparison

Anti-c-AST monoclonal antibodies were also evaluated in medium-scale clinical trials with random blood samples from donations (n=50). Fig.4 shows that the correlation coefficient (r) is as high as 0.99 between in-house m-AST assay and commercial immunoinhibition assay. These results show good agreement between the two systems.

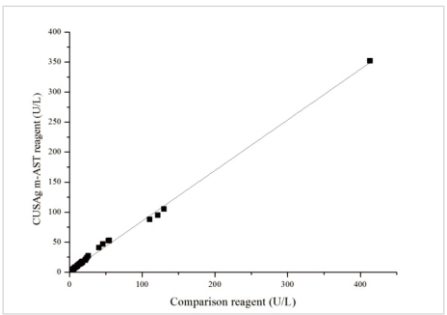

Fig.4 Clinical comparison of in-house m-AST reagent and commercial kit

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