Innovative High Sensitive Method for Quantification of Angiotensin Converting Enzyme (ACE) on Several Clinical Chemistry Analyzers

DiaSys
Diagnostic Systems

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Background-Aim

Angiotensin-converting enzyme (ACE) is a membrane-bound glycoprotein, mainly found on the surface of endothelial cells of pulmonary capillaries. As part of the renin-angiotensin-system, ACE plays a major role in the regulation of blood pressure. During fibrosis of the lung, the bound ACE is released into blood and therefore is used as a biomarker for monitoring of pulmonary sarcoidosis. The currently established method to monitor ACE serum levels uses the hydrolyzation of FAPGG (N-[3-(2-furyl)-acryloyl]-L-phenyl-alanylglycylglycine). This photometric method yields very low signals, resulting in poor sensitivity and is prone to interferences.
Here we present the first two component, ready to use ACE reagent, which utilizes the method of Kasahara and Ashihara [1]. This innovative method of ACE quantification produces approx. four times the signal by using half of the sample-reagent ratio, compared to the current FAPGG-based assays and can be performed on any clinical chemistry analyzer.

Methods

All data were measured with the new ACE assay and a FAPGG-based competitor reagent on a BioMajesty 6010c clinical chemistry analyzer.
Method comparison: 50 serum and 50 plasma leftover routine samples were measured. In a separate comparison, 25 serum und 25 plasma samples were selected, considering the acceptance criteria of triglyceride and bilirubin as indicated by the IFU of the used ACE assays.
Interference: Serum samples were spiked with the indicated interferents, and the ACE levels were measured, subsequently.
Precision data: The ACE levels of spiked buffer solutions were measured by 20x repetition.

Results

Interference: Triglyceride 1500 mg/dL (Figure 4) (competitor: <200 mg/dL (Figure 5)), bilirubin 50 mg/dL (Figure 6) (20 mg/dL (Figure 7)), hemolysis 600 mg/dL (120 mg/dL), N-acetylcysteine (NAC) 600 mg/L (100 mg/dL). NAC is frequently used as an expectorant in the respiratory system, therefore a high insensitivity to NAC is of high importance in ACE quantification.
Analytical sensitivity was measured with a concentration of 10 U/L and resulted in a precision of 1.3 % (competitor: 14%).

ACE-based hydrolyzation of the substrate p-hydroxybenzoyl-Gly-His-Leu and its subsequent hippuricase-based conversion to p-hydroxybenzoic-acid. This intermediate reacts in a trinder-like reaction and is measured at 505 nm. Production of the required oxidizing agent is initiated *in situ* by an oxidase upon mixing of both reagents.

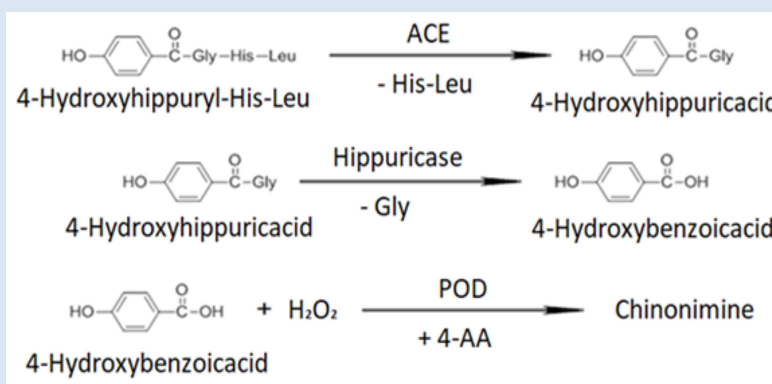


Figure 1: DiaSys ACE FS assay principle

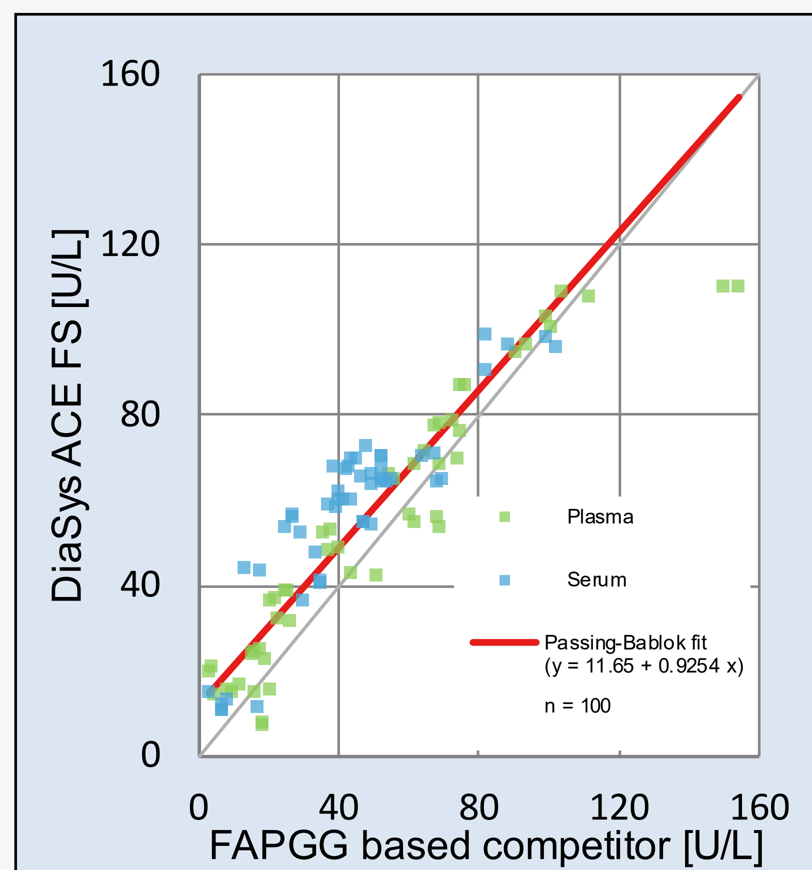


Figure 2: Method comparison DiaSys ACE FS vs. FAPGG-based competitor with **random** samples

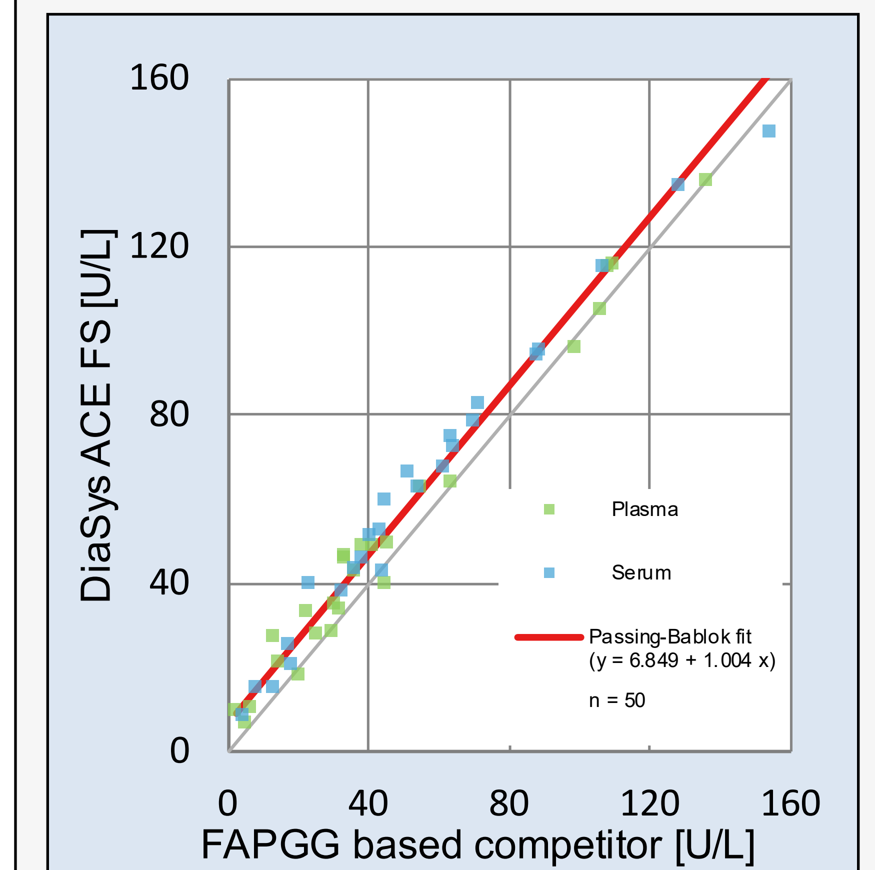


Figure 3: Method comparison DiaSys ACE FS vs. FAPGG-based competitor with **selected** samples

Figure 4: Triglyceride Interference DiaSys ACE FS

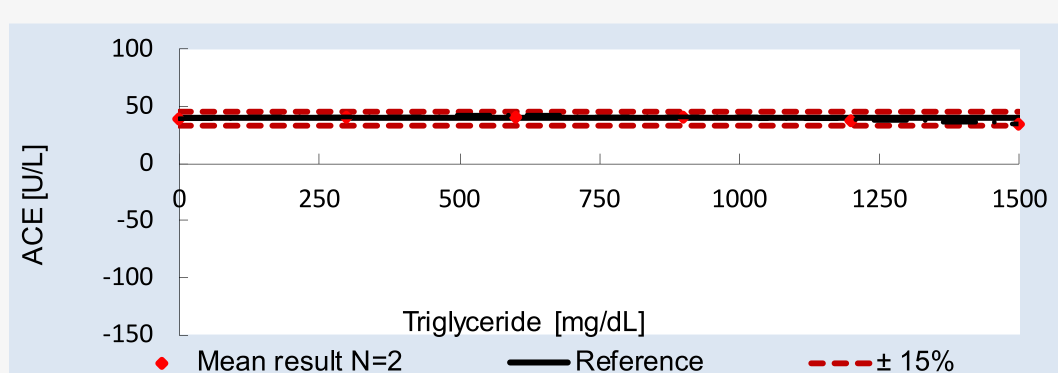


Figure 5: Triglyceride Interference FAPGG-based competitor

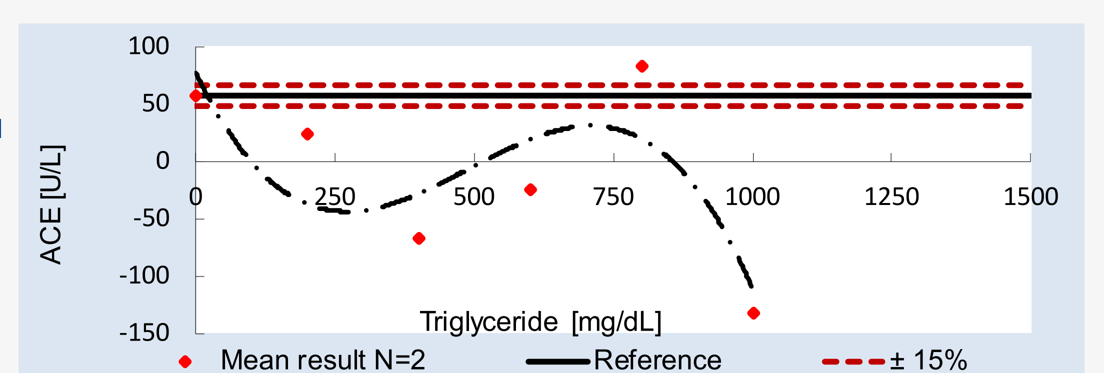


Figure 6: Billirubin Interference DiaSys ACE FS

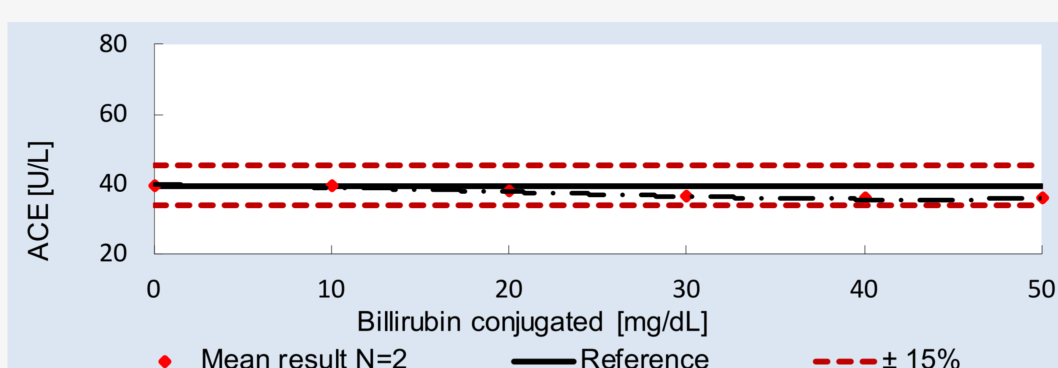
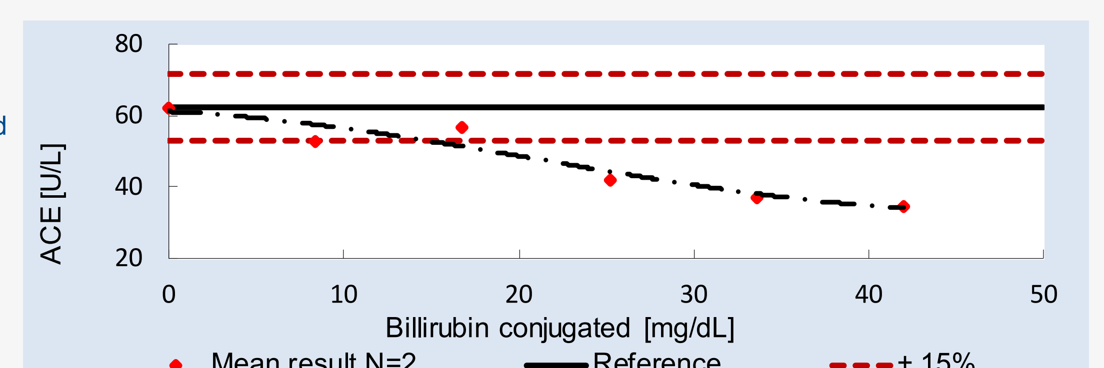


Figure 7: Billirubin Interference FAPGG-based competitor



	Sample 1 [U/L]	Sample 2 [U/L]	Sample 3 [U/L]	Sample 4 [U/L]
Mean (n=20)	9.87	14.3	49.0	90.8
SD	0.130	0.292	0.640	0.354
CV [%]	**1.32**	**2.04**	**1.30**	**0.390**

Figure 8: Precision data DiaSys ACE FS

	Sample 1 [U/L]	Sample 2 [U/L]	Sample 3 [U/L]	Sample 4 [U/L]
Mean (n=20)	17.2	26.1	76.0	160
SD	2.43	2.28	3.76	3.32
CV [%]	**14.1**	**8.74**	**4.96**	**2.07**

Figure 9: Precision data FAPGG-based competitor

Conclusion

The increase in signal, the used low sample volume and the utilized wavelength significantly improves interferences, compared to the established FAPGG-method. Beside the more robust and precise routine quantification, this innovative method may allow for new applications of ACE measurement e.g. in cerebrospinal fluid to diagnose Alzheimer's disease or the improved development of antihypertensives, which are often ACE inhibitors.

References

1. Kasahara, Y. and Ashihara, Y. (1981) Colorimetry of Angiotensin-I Converting Enzyme Activity in Serum. Clinical Chemistry, 27, 1922-1925