Manufactured By: HYPHEN BioMed

Evaluation of a new Collagen Binding Assay

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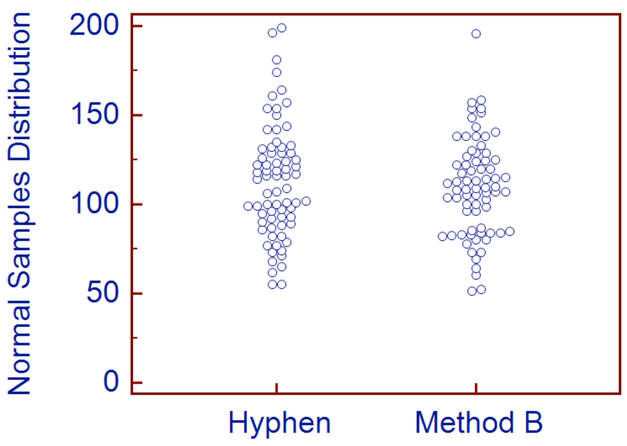
Introduction:

The von Willebrand Factor (VWF) plays a key role in primary haemostasis. One of its functions is supporting the platelets to bind at sub-endothelial collagen of a lesioned vessel wall. The Collagen Binding Assay (CBA) serves as a determination of the VWF-binding-capacities to collagen. We evaluated the new CBA (Zymutest vWF:CBA, Hyphen BioMed, France, Method A) and compared this to the Technozym vWF:CBA ELISA (Technoclone GmbH, Vienna, Austria, Method B). The VWF-Antigen and the Ristocetin CoFactor were determinated on the Behring Coagulation System BCS XP with commercial methods of Siemens.

Material and Methods:

All CBA were performed on the Behring ELISA Processor 2000 (BEP 2000). Method A uses equine collagen types I and III and method B human collagen III. We determined the coefficient of variation by measuring a normal and a pathological control plasma once daily for 13 days for method A and for 17 days for methods B.
The limits of the reference ranges were defined by the 5th and the 95th percentile of the results of 71 obviously healthy donors. Methods were compared by Passing Bablok regression.
Sensitivity and specificity were calculated by measuring 71 normal donors and 20 patients suffering from type 1 of the von Willebrand Disease. We used the Ristocetin-CoFactor Assay as a gold standard for detecting a type 1 VWD.

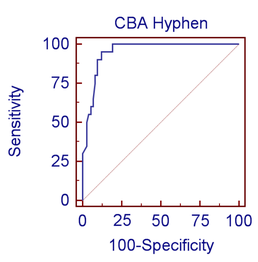
Reference Ranges:

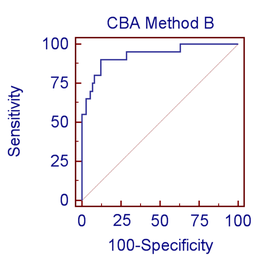


	Hyphen	Method B
calculated	67 - 169%	67 - 154 %
manufacturer	50 - 160%	40 - 250 %

The reference ranges of both methods seem to be very similar, but the lower limit given by the manufacture are much lower then the 5th percentile of our reference group.

Sensitivity and Specificity:





cutoff	Hyphen		Method B	
	Sens	Spec	Sens	Spec
67 %	61.9 %	95 %	66.7 %	95 %
50 %	25 %	100 %		
40 %			5 %	100 %

For evaluation we used mostly mild cases of the type 1 von Willebrand Disease, which were presented in the Ristocetin CoFactor Assay, but not in the CBA. Not using the reference ranges determinated in our own lab, would made the tests very insensitive with a little loss of specificity.

Impression and BIAS:

	Hyphen		Method B	
Numbers of determinations	13	13	17	17
target value	56.5	93.0	14.0	66.0
mean	59.3	93.5	20.0	77.3
BIAS / [%]	5.0	0.5	42.7	17.1
CV / [%]	5.5	7.1	21.9	40.5
Numbers of results out of target range	0	1	7	3

The lot of method B used in this evaluation offered many problems with the results of the internal controls. It was the second one used in our lab and has many more outliers than the previous one. That was the main reason for us to change. The Zymutest vWF:CBA of Hyphen produced in 26 determinations only one outside the target range and seems to be very stable.

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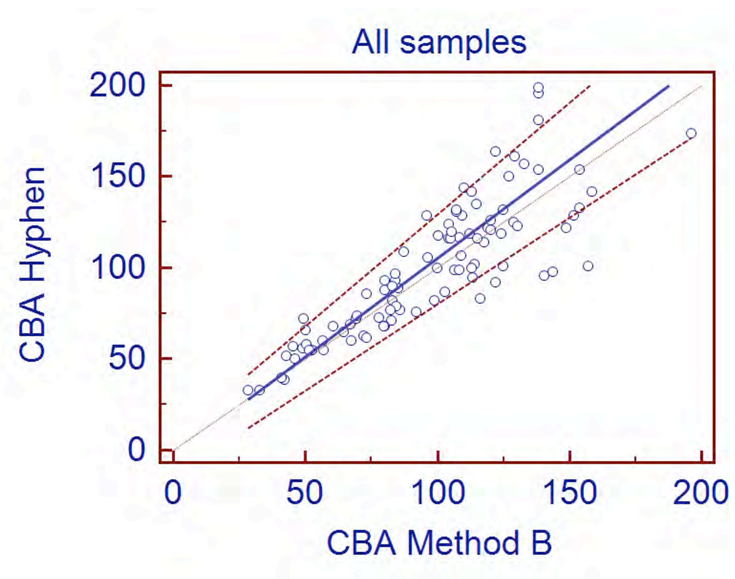
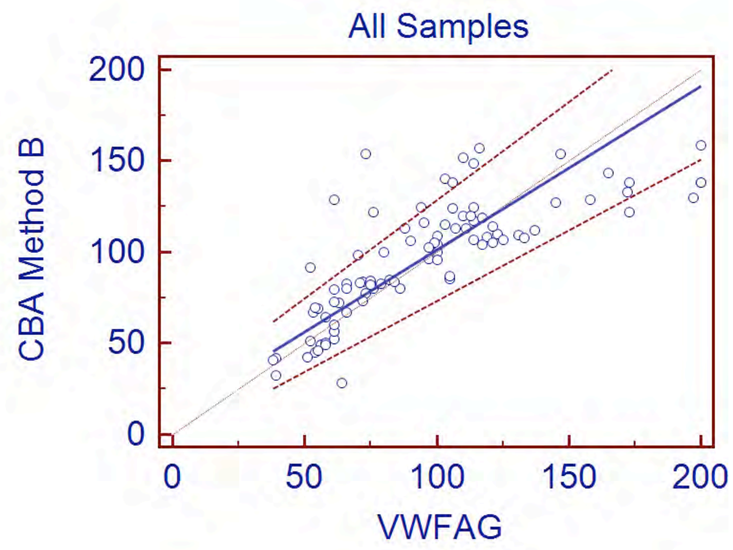
Method Comparison:



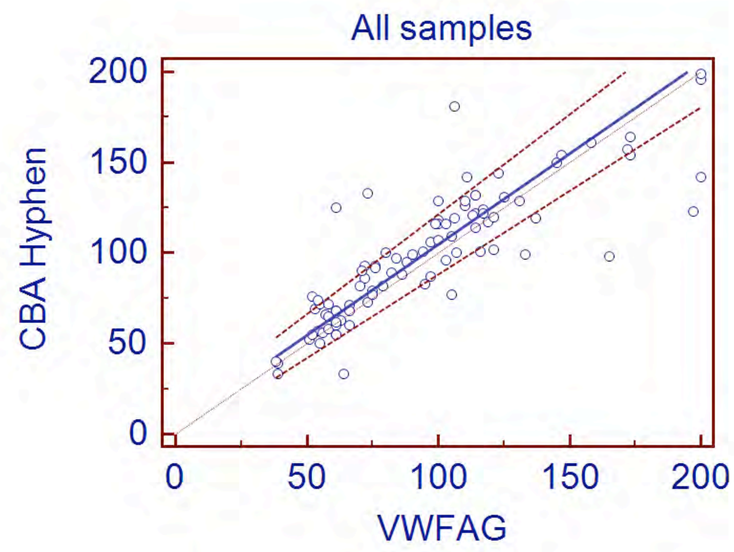
y = -2.851459 + 1.080541 x	
Correlation coefficient	
Pearson	0.8442
95% CI	0.7735 to 0.8941
Systematic differences	
Intercept A	-2.8515
95% CI	-14.7207 to 6.5972
Proportional differences	
Slope B	1.0805
95% CI	0.9479 to 1.2275
Linear model validity	
Cusum test for linearity	No significant deviation from linearity (P=0.33)

There was no significant difference between both methods in intercept or slope. But the result of the correlation coefficient is pure.

Comparison to antigen concentration:



y = = 11,188672 + 0,898919 x	
Correlation coefficient	
Pearson	0.7767
95% CI	0.6794 to 0.8471
Systematic differences	
Intercept A	11,1887
95% CI	-4.2000 to 21.1000
Proportional differences	
Slope B	0.8989
95% CI	0.7753 to 1.0750
Linear model validity	
Cusum test for linearity	Significant deviation from linearity (P=0.01)



y = 5.000000 + 1.000000 x	
Correlation coefficient	
Pearson	845
95% CI	0.7738 to 0.8952
Systematic differences	
Intercept A	5,0000
95% CI	-3.9000 to 11.6923
Proportional differences	
Slope B	1.0000
95% CI	0.9231 to 1.1000
Linear model validity	
Cusum test for linearity	Significant deviation from linearity (P=0.05)

The Hyphen Assay seems to be closer to the antigen concentration when measuring samples of healthy donors or of patients with a quantitative defect of the vWF.

Conclusion:

The Zymutest vWF:CBA of Hyphen presents good sensitivity and precision. This test is comparable to well-established methods and is running very stable on our platform BEP 2000. The only disadvantage that we could see is its time consumption due to a first incubation step of 2 hours. In our next step, we are going to evaluate this assay with sample of patients suffering from different subtypes of the type 2 VWD.

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