بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Assessment of Von Willebrand Factor Antigen Level in Sudanese Patients with Aortic Stenosis in Khartoum State

تقييم مستوى عامل فون ويلبراند لدي مستضد المرضى السودانيين الذين يعانون ضيق الأبهر في ولاية الخرطوم

A thesis submitted in partial fulfillment for degree of the requirements for the award of the degree of (M.SC) in medical laboratory science

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الآية

بسم الله الرحمن الرحيم

قال تعالى:

(يا أَيُّهَا النَّاسُ إِنْ كُنْتُمْ فِي رَيْبٍ مِنَ الْبَعْثِ فَإِنَّا خَلَقْنَاكُمْ مِنْ تُرَابٍ ثُمَّ مِنْ نُطْفَةٍ ثُمَّ مِنْ عَلَقَةٍ ثُمَّ مِنْ مُضْغَةٍ مُخَلَّقَةٍ وَغَيْرِ مُخَلَّقَةٍ لِنُبَيِّنَ لَكُمْ ۚ وَنُقِرُّ فِي الْأَرْحَامِ مَا نَشَاءُ إِلَىٰ أَجَلٍ مُسَمًّى ثُمَّ نُخْرِجُكُمْ طِفْلًا ثُمَّ لِتَبْلُغُوا أَشُدَّكُمْ ۖ وَمِنْكُمْ مَنْ يُتَوَفَّىٰ وَمِنْكُمْ مَنْ يُرَدُّ إِلَىٰ أَرْذَلِ الْعُمُرِ لِكَيْلَا يَعْلَمَ مِنْ بَعْدِ عِلْمٍ شَيْئًا وَتَرَى الْأَرْضَ هَامِدَةً فَإِذَا أَنْزَلْنَا عَلَيْهَا الْمَاءَ اهْتَزَّتْ وَرَبَتْ وَأَنْبَتَتْ مِنْ كُلِّ زَوْجٍ بَهِيجٍ).(٥)

(صدق الله العظيم)

سورةالحج الآية(٥)

Dedication

To my family ….

To my husband….

To my sweet daughter and son(Taleen&Mohammed Almustafa)…

To all my teachers and friends….

Acknowledgments

Firstly I thank Allah for blessing my life, and helped me to start This work and supported me strength to complete it this humanity Work.

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Last but not the least I would like to extend thanks to my brothers ,sistersmy teachers, friends and all people whom support me and believe on me.

Abstract

This Descriptive analytical Case control study, carried out to study the assessment of von willebrand factor antigen level in aortic stenosis Sudanese patients conducted at Ahmed Gasim hospital during the period from January 2017 to May 2017 at Khartoum state. the study include eighty samples forty as control and forty were patients already diagnosed as Aortic stenosis 27 (68%)of them were male and 13(33%) of them were female . 4.5 ml of venous blood was with drown from each patient, placed in tri sodium citrate container then we separate the sample by centrifuge and extract plasma to measure von willebrand factor antigen level by using Enzyme Linked Immuno Sorbent Assay. the results was analyzed by statistical package for social science computer program (SPSS) version 16.

The result showed that the mean VWF:Ag level was insignificant difference between case study and control (p. value> 0.05).the mean of von willebrand factor antigen level within case (0.735 ± 0.0174) and in control (0.713 ± 0.0212) .

While There was no statistically significant difference between the vWF: Ag concentration and severity of disease (p.value>0.05) and there was no statistically significant difference between the plasma vWF: Ag concentration and gender (p.value>0.05).

مستخلص البحث

هذه دراسه تحليليه حاله وحاله ضابطه ،أجريت لتقييم مستوى مستضد الفون ويلبراند في تضيق الأبهر لدي المرضي السودانيين في مستشفى أحمد قاسيم خلال الفترة من يناير 2017 إلى مايو 2017 في ولايةالخرطوم. وقد شملت الدراسة ثمانين عينة أربعين كعينات ضابطه وأربعين تم تشخيصهم بالفعل بمرض تضييق الابهر 27 (68٪) من الذكور و 13 (33٪) من الإناث. كان 4.5 مل من الدم الوريدي اخذ من كل مريض،وتم وضعه في وعاء يحتوي على مانع تجلط ثلاثي سترات الصوديوم ثم فصل العينة عن طريق أجهزة الطرد المركزي واستخراج المصل الدموي لقياس مستوى عامل مستضد فون ويلبراند باستخدام(ELISA).

تم تحليل النتائج من خلال حزمة إحصائية لبرنامج علوم العلوم الاجتماعيةالإصدار 16.

وأظهرت النتائج انه لاتوجد فروقات ذات دلاله احصائيه بين دراسة الحالة والحاله الضابطه في متوسط مستوى مستضد الفون ويلبراند .متوسط مستوى مستضد فون ويلبراند في الحالة (0.735 ± 0.0174) وفي الحاله الضابطه(0.713 ± 0.0212

في حين لم يكن هناك فروق ذات دلالة إحصائية بين تركيزمستضد فون ويلبراند وشدة المرض (p.value> 0.05) ولم يكن هناك فرق ذو دلالة إحصائية بين تركيزمستضد فون ويلبراند والجنسين (p.value> 0.05)

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List of Abbreviation

Abbreviation	Full text
APTT	Activated partial thromboplastin time
ARIC	Atherosclerosis Risk in Communities
AS	Aortic stenosis
CAD	Coronary artery disease
CBC	Complete blood count
cDNA	Complementary Deoxyribonucleic Acid
ELISA	Enzyme-linked immunosorbent assay
HMW	High-molecular-weight
PT	Prothrombin time
TTP	Thrombotic thrombocytopenic purpura
VWD	Von Willebrand disease
VWF	Von Willebrand Factor

CHAPTER ONE

1. Introduction & literature review

1.1 Introduction

Haemostasis is a pivotal process and requires the combined action of blood platelets, vascular- and plasmatic factors. It is divided into two steps: (i) the primary haemostasis, associated with cellular mechanisms (thrombocytes), and (ii) the secondary haemostasis, mediated by a complex system of extrinsic and intrinsic signaling cascades involving coagulation factors. In flowing blood, platelet adhesion to sites of vascular injury is mediatedby the von Willebrand Factor (VWF), being the critical determinant of thrombus formation at high arterial shear rate conditions. Von Willebrand factor (VWF) is an adhesive and multimeric glycoprotein that found its historical origin in 1924, when the Finnish physician Erik von Willebrand first reported a family with serious hereditary bleeding affecting consanguineous families(von Willebrand, 1926).VWF also associates with the procoagulant factor VIII (FVIII), forming a complex that regulates FVIII secretion inside cells and prevents its rapid clearance in the circulation (Nogami K *et al.,* 2002).The congenital deficiency of VWF function results in a bleeding tendency known as von Willebrand disease,(Castaman *et al.,* 2003), which occasionally may present as an acquired condition(Federici *et al.,* 2004). Comprehensive reviews on VWF have been published (Ruggeri *et al.,* 2003 &Budde *et al.,* 2001).

1.2 Literature review

1.2.1 Molecular biology of von Willebrand factor:-

The advent of modern molecular techniques led tothe cloning of the VWF gene in 1985 using endothelial cell cDNA libraries 6. The gene is located on the shortarm of chromosome 12 at the locus 12p13.37 and spans178 kilobases. The human VWF gene contains 52 exonsand the exon 28 is the largest, its length is 1.4 kb8. TheVWF gene is transcribed into a 9 kb mRNA which istranslated into a protein of 2813 amino acids with an estimated of 310,000 daltons (Romijn *et al*., 2001).

1.2.2 Biosynthesis and structure of VWF:

Mature VWF is a multimeric protein; molecules are composed of a variable number of identical subunits, each comprising 2,050 amino acid residues and up to 22 carbohydrate side chains.Subunits are disulfide-bonded into dimers of approximately 500 kp, which in turn are disulfide-linked into multimers of increasing size that may exceed 10,000 kp. The largest VWF molecules have been visualized by electron microscopy as elongated or coiled filaments with lengths up to 1,300 nm (about the diameter of a platelet) or cross sections of 200 to 300 nm, respectively.VWF is an adhesive plasma glycoprotein which performs its haemostatic functions through binding to FVIII, to platelets surface glycoprotein, and toConstituents of connective tissue. VWF acts as a stabilizer of FVIII in the circulation. This is obtained by the formation of a non-covalently bound VWF-FVIII complex that protects FVIII from degradation by activated protein C, and localizes FVIII to sites of platelet plug and subsequent clot Formation .VWF has a central role in primary haemostasis where it mediates platelet adhesion to damaged vascular sub endothelium and subsequently platelet aggregation. Following

avascular injury, VWF binds specifically to fibrillar collagen type I and III (Romijn *et al*., 2001).

1.2.3 Pathophysiology of VWF:-

The pathophysiologic importance of VWF is not limited to the phenotypes of VWD and TTP. In fact, VWF level also correlates with thrombosis risk and inversely with bleeding risk within the apparently healthy population Furthermore, bleeding risk and thrombosis risk appear to vary continuously and reciprocally across the normal range of VWF levels, and there is no clear boundary between a normal and a pathological level of risk for these adverse events (Jurk ,*et al*., 2003).

1.2.4 Funcution of VWF:-

Initial steps in thrombogenesis ,Platelet attachment to thrombogenic surfaces depends on the velocity of flowing blood. Above a limiting shear rate of approximately(1500s)1 the process has been considered to be absolutely dependent on VWF and its GPIb a receptor. the platelet adhesion to thrombospondin at shear rates of up to (4000 s) still awaits confirmation even a substrate such as collagen, for which platelets exhibit several receptors, requires prior binding of plasma VWF to allow platelet adhesion (Jurk ,*et al*., 2003).

1.2.5 Epidemiology of von Willebrand disease:-

VWD is the most common inherited bleeding disorder in humans. The disorder shows a worldwide distribution, and it is also common in other animal species including dogs and pigs. Its prevalence in the human population varies depending upon the approach undertaken to define the diagnosis. In at least two large prospective epidemiologic studies, up to 1% of a predominantly pediatric population has been found to manifest symptoms, and laboratory signs, of VWD In contrast, the prevalence of severe, type 3 VW has been estimated in several

different countries to be between 1 and 3 per million, while the number of symptomatic cases of VWD referred to tertiary care medical centers, is approximately 10 to 30 per million population (Sadler *et al*., 2006).

1.2.6 Classification of von Willebrand Disease :-

VWD is classified on the basis of criteria developedby the VWF Subcommittee of the ISTH, firstpublished in 1994 and revised in 2006.the classification was intended to be clinicallyrelevant to the treatment of VWD (Sadler *et al*., 2006).

1.2.7 Diagnosis of von Willebrand Disease:-

The diagnosis of VWD requires attention tothree clinical and laboratory components: apersonal history of excessive mucocutaneous bleeding, a family history of excessive bleeding, and, a laboratory evaluation that isConsistent with a quantitative and/or qualitative defect in VWF. (Favaloro *et al.*, 2003).

1.2.8 Laboratory testing for von Willebrand disease:-

In the hemostasis laboratory, the criticalcomponents of VWD diagnosis involvequantitative and qualitative measurements of VWF and FVIII (Favaloro *et al*., 2003).

- Evaluation of bleeding symptoms and bleeding risk by history and physical examination.
- Evaluation by laboratory testing:-

A. complete blood count (CBC including platelet count), prothrombin time (PT), activated partial thromboplastin time (PTT), and optionally either thrombin time or fibrinogen level.

B. Initial tests for diagnosing or excluding VWD include the following three tests:

- VWF:RCo
- VWF:Ag (presence of vwf in the plasma)VWF antigen (VWF:Ag) was measured using a home-made enzyme-linked immunosorbent assay (ELISA).
- Factor VIII activity

C. If any one of the above test results is abnormally low, a discussion with or a referral to a hemostasis expert is appropriate. In addition to repeating the initial three tests (in most cases)

- VWF multimer study.
- Ristocetin-induced platelet aggregation
- VWF collagen binding activity.(Favaloro *et al*., 2004).

1.2.9 Associated conditions :-

- lymphoproliferative (48%)
- cardiovascular disease (21%)
- auto immune disordere (2%)
- thyroid disorders (2%)
- myeloproliferative (15%)
- other neoplasic (5%)
- drug effect. (Favaloro *et al*., 2003).

1.2.9 .1 Association with cardiovascular disorders:-

Increased association with cardiovascular disorders, suggesting increasing awareness of cardiologist and surgeons. mechanism thought to be secondary to high stress leading to increased proteolysis of VWF multimers (Favaloro *et al.,* 2004).

1.2.9.2 Characteristics:-

- Decreased HMW multimers .
- Antigen activity and collagen binding may be normal or increased (Favaloro *et al*., 2004).

1.2.9.3 Aortic stenosis :-

Aortic valve stenosis affects 3 percentof persons older than 65 years and leads to greater morbidity and mortality than other cardiacvalve diseases. The pathology of aorticstenosis includes processes similar to thosein atherosclerosis, including lipid accumulation,inflammation, and calcification. Development of significant aortic stenosistends occur to earlier in those with congenital bicuspid aortic valves.This hemorrhagic syndromeis associated with acquired type 2A von Willebrand syndrome, which is characterized by loss of the largest multimers of von Willebrand factor .Proteolysis of von Willebrand factor as it passes through the stenotic valve is one of the proposed causes of the bleeding. the local expression of vWF has been detected in porcine aortic valves, which is consistent with the findings in human aortic valvesof patients undergoing valve replacement due to severe AS .Moreover, histamine stimulated porcine aorticvalve endothelial cells released vWF protein into the culture medium, and VWF significantly increased valvular interstitial cell nodule formation and calcification (Balaoing,*et al*., 2014).

1.3 Rationale

Aortic-valve stenosis can be complicated by bleeding that is associated with von Willebrand syndrome. However, the prevalence and cause of the hemostatic abnormality in aortic stenosis are unknown. Patients with severeAS who were deficient in high molecular weight multimers of VWF are characterized by enhanced thrombin formation and platelet activation. Phenomenon may result from the ambivalent effect of high shear stress in a stenosed aortic valve on the hemostatic system and might help explaintwo aspects of AS, namely, Heyde syndrome and an increased tendency for thromboembolic episodes. In Heyde syndrome, a tendency for bleeding ,mostly mucocutaneous and gastrointestinal episodes, is observed in up to 20% of patients with severe AS(Warkentin*et al.*,2010) ·Also when we search in the literature we didn`t find any published report in Sudan about this article so our study will be done to asses of VWF in Sudanese people with aortic stenosis.

1.4 Objectives

1.4.1 General objective

To assess the VWF:Ag level in Sudanese patients with aortic stenosis compare with normal subjects.

1.4.2 Specific objectives

- To measure VWF Ag level in Aortic Stenosis patients by using Enzyme Linked Immuno Sorbent Assay technique.
- To compare mean of vWF:Ag level between cases and control group.
- To study association between vWF:Ag level and severity of disease.
- To correlate vWF:Ag level with patient`s gender and age.

Materials and methods

2.1 Study design:

This was an observational analytical case control study.

2.2 Study area:

Study was conducted at Ahmed Gasim hospital in Khartoum state

2.3 Study population:

Sudanese patients with Aortic stenosis.

2.4 Study Duration:

The study was conducted during the period From January to May 2017.

2.5 Sample size:

40 samples from patients with Aortic stenosis and 40 from healthy individual as control group.

2.6 Sampling technique:

Its non probability sampling technique known as convenience sample collected according to certain criteria.

2.7 Selection criteria:

2.7.1 Inclusion criteria:

Diagnosed Aortic Stenosis patients .

Non Aortic Stenosis individuals as control group for comparing.

2.7.2 Exclusion criteria

Patients with aortic stenosis transfused with blood to them specially plasma and

Aortic stenosis patients after valve replacement.

2.8 Data collection:

Data was collected questionnaire to obtain information that helped in study.

2.9 Method of blood sample collection:

Venous blood collected using sterile disposable plastic syringe after cleaning the vein puncture area with 70% ethanol ,the blood 4.5ml was placed into container containing 0.5ml of 3.2%Ttrisodium citrate and gently mixed.The sample was centrifuged at 5000 rpm for 15min to obtain platelet poor plasma then stored at -20ċ until analysis.

2.10 Estimation of vWF:Ag:

Estimation of vWF level was done by using Enzyme Linked Immunosorbent Assay kit (Technoclone. Austria).

2.10.1 Procedure:

VWF (Ag) was measured for each sample According to manufacturer instructions (TECNOZYM, Austria)

2.11 Ethical consideration:

Ethical approval for conducting the research was obtained from the College of Laboratory Medial Science. The participants were provided with information about the study and assured that all the obtained information will be kept highly confidential and will not be used for any other purpose than for this study.

2.12 Data analysis:

By using statistical package for social science computer program (SPSS). The level of significance was set at $p = 0.05$.

CHAPTER THREE

Chapter Three

Results

Demographic data:

Study included 80 sample 40 of them were cases and 40 of them were control. The results showed that 27/40 (68%) of cases were males and 13/40 (33%) were females and 13(33%),the severity of disease varies from moderate which showed most abundant compared with mild 17(43%) moderate and 10(25%) sever .

Table 3.1 showed the Frequencies of Sample,gender and severity of disease

		Frequency	Percent%
Sample	Case	40	50
	control	40	50
Gender	Male	27	68
	female	13	33
Severity of disease	Mild	13	33
	Moderate	17	43
	Sever	10	25

Table 3.2 vWF:Ag level among case study and control subjects:

The results showed that the mean of vWF:Ag among cases was 0.735±0.0174 and compared with control subjects was 0.713±0.0212 with insignificant difference between case study and control (p.value 0.415).

Table 3.3 Association of vWF:Ag level and severity of disease

The results showed that the mean of vWF:Ag among mild was 0.785±0.21, among moderat was 0.729±0.19 and among sever was 0680±0.17 with insignificant difference of mean of vWF: Ag level among severity of disease (p.value 0.071).

	Sample	**Mean**	**p.value**
vWF:Ag	**Case**	0.735±0.0174	0.415
	Control	0.713±0.0212	

	Severity of disease	**Mean**	**p.value**
vWF:Ag	Mild	0.785±0.21	0.071
	moderate	0.729±0.19	
	Sever	0680±0.17	

Table 3.4 Association between vWF:Ag level and gender

The results showed that the mean of vWF:Ag among male was 0.735 and among female was 0.713 with insignificant difference of mean of vWF: Ag level and gender (p.value 0.415).

	Gender	**Mean±SD**	**p.value**
vWf:Ag	Male	0.735 ±0.1099	0.145
	Female	0.713±0.1343	

Table 3.5 To correlate vWF:Ag level with Age

Table 3.5 showed no correlation between vWF: Ag and Age (p.value insignificant)

		Age
vWF:Ag	**Pearson correlation**	-0.280
	p.value	0.081

CHAPTER FOUR

4.1 Discussion

This isDescriptive analytical Case control study,carried out to study the assessment of von willebrand factor antigen level in aortic stenosis Sudanese patients was conducted in Ahmed Gasim hospital during the period from January 2017 to May 2017at Khartoum state. the study include eighty samples fourty as control and fourty were patients already diagnosed as Aortic stenosis were selected 27 (68%) male and 13(33%) female

FVIII vWF Ag is part of the blood-clotting factor VIII. The physiological control of FVIII vWF Ag synthesis and release by human endothelial cells has been extensively studied. Early work using immunofluorescent probes identified FVIII vWF Ag in the intima of all human blood vessels

In the view of data analysis presented in the previous chapter the following are finding of the research, the patients have low FWF Ag in case and control, reported a relationship and low VWF:Ag, **(Rauch *et al.*,2002)** did not find any association between bleeding episodes and the von Willebrand deficiency in patients That agree with our results .We also could not find any relationship between low VWF:Ag levels and bleeding, the patients have in significant difference when compare with age ,gender and severity of disease.

Patients with vascular diseases have been shown to have normal levels of plasma vWF Ag this agree with our finding results. Also other done by(**Joseph et al., in feberuary 2013)** and(**Alessandra Casonato et al., 2011)** Other findind that similar and agreement our result done by That found no significant correlation between vWF Ag levels and the duration of disease. Additionally, there was no significant difference in the vWF Ag levels, but significant positive correlation

between vWF Ag levels this not similar to our result that p.value 0.07 considerd in significant.

4.2 Conclusions

We conclude that von willebrand factor antigen level is within normal range and this reflect that vWF:Ag level not affected by Aortic Stenosis .

4.3 Recommendations

Further studies is needed to study the assessments of VWF Activity in AS patients with more subjects to be included.

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Appendices

Sudan University of Science &Technology

College of Medical Laboratories Science

Assessment of Von willebrand factor antigen level in Sudanese patients with Aortic stenosis in Khartoum State.

Questionnaire

-Name: …… ID: ……

-Age:…….

-Sex : M …… F …….

-severity of disease ……

-Investigation :

vWF:Ag

technoclone

TECHNOZYM® vWF:Ag ELISA
Calibrators and Controls LOT RA64B00

3015988-058

Table • Tabelle • tabella • tabla • tabela • tabell

DIL 0.5mL AQUA

REF CALIBRATORS	LOT	⌛	Sollwert/ Nominal value	Kalibrierung durch®, nach Calibration by®, acc. to
vWF CAL 1 TH10001ND	1ND61B0	2018-04-30	0.87 IU/mL	IS 07/316
vWF CAL 2 TH10002ND	2ND61B0	2018-04-30	0.46 IU/mL	IS 07/316
vWF CAL 3 TH10003ND	3ND61B0	2018-05-31	0.22 IU/mL	IS 07/316
vWF CAL 4 TH10004ND	4ND61B0	2018-04-30	0.12 IU/mL	IS 07/316
vWF CAL 5 TH10005ND	5ND61B0	2018-04-30	0.01 IU/mL	IS 07/316

REF CONTROLS	LOT	⌛	Sollwert/ Nominal value	Vertrauensbereich Confidence range	Kalibrierung durch®, nach Calibration by®, acc. to
vWF CONT L TH1000BND	BND61B0	2018-05-31	0.16 IU/mL	0.10 – 0.22 IU/mL	IS 07/316
vWF CONT H TH1000AND	AND61B0	2018-05-31	0.59 IU/mL	0.44 – 0.74 IU/mL	IS 07/316

09/2016

Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH
Brunner Str. 67 - 1230 Vienna, Austria

3015983-014 03/2015

technoclone
TC

TECHNOZYM® vWF:Ag ELISA

CE (GB) (DE)

REF	5450201	TECHNOZYM® vWF:Ag ELISA	Σ 96
REF	5450210	TECHNOZYM® vWF:Ag Calibrator Set	5 x 0.5 mL
REF	5450212	TECHNOZYM® vWF:Ag Control Set	2 x 0.5 mL

symbols key / Symbolschlüssel / interpretazione dei simboli / explicación de simbolos / explicação dos símbolos / clé des symboles / Symbolnyckel / symbolforklaring / Tegnforklaring / Κλειδί συμβόλων / Използвани символи / символы / Klíčova slova / Značenje simbola

	manufacturer / Hersteller / fabbricante / fabricante / fabricante / fabricant / Tillverkaren / Fabrikanten / Produsent / Κατασκευαστής / Производител / Производитель / výrobce / Proizvođač		expiry date / Verfallsdatum / data di scadenza / fecha de caducidad / data de validade / date d'expiration / utgångsdatum / udløbsdato / Utløpsdato / Ημερομηνία λήξης / срок на годност / datum expirace/ срок годности / datum exspirace / Rok trajanja
	storage temperature / Lagertemperatur / temperatura di conservazione / temperatura de conservación / temperatura de conservação / température de stockage / lagringstemperatur / opbevaringstemperatur / Oppbevaringstemperatur / Θερμοκρασία αποθήκευσης / съхранение на / teplota skladování / температура хранения / teplota skladování / Temperatura lagerovanja		consult instructions for use / Gebrauchsanweisung beachten / consultare le istruzioni per l'uso / consulte las instrucciones de uso / consultar o manual de instruções / instruction d'utilisation / se användarinstruktioner / følg brugsvejledning / Følg bruksanvisningen / συμβουλευθείτε τις οδηγίες για τη χρήση / прочетете инструкциите за работа / potřeba řídit se instrukcemi / перед использованием читайте инструкцию / sleduj̈te návod k použití / Pročitaj upustvo pre upotrebe
CE	CE-mark / CE-Kennzeichnung / marchio CE / marca de CE / Simbolo da CE / marquage CE / CE-märkning / CE-mærket / CE-merke / CE-σήμανση / CE марка / CE-označení / маркировка CE / značka CE / CE-marka	Σ	determinations / Bestimmungen / determinazioni / determinaciones / determinações / déterminations / bestämningar / bestemmelser / Bestemmelser / προσδιορισμοί / брой тестове / stanovení / определений / počet stanovení / Definicija
AQUA	distilled water / destilliertes Wasser / acqua distillata / agua destilada / agua destilada / eau distillée / destillerat vatten / destilleret vand / Destillert vann / απεσταγμένο νερό / дестилирана вода / destilovaná voda / дистиллированная вода / destilovaná voda / Serija	LOT	lot / Charge / lotto / lote / lote / lot / sats / serie / Parti / παρτίδα / партида номер / šarže / лот / šarže / in vitro dijagnostika
BUF	Reaction buffer / Reaktionspuffer / tampone di reazione / tampón de reacción / Tampão de reacção / tampon de réaction / Reaktionsbuffert / Reaktionsbuffer / Reaksjonsbuffer / διάλυμα αντίδρασης / Реакционен буфер / Рабочий буферный раствор / Reakční pufr / Reakcioni pufer	MTP	microtiter plate / Mikrotiterplatte / piaca microtiter / microplaca / microplaca / microplaques sensibilisées / Mikrotiterplatta / Mikrotiterplade / mikrotiterplate / πλάκα μικροτιτλοδότησης / Микротитърна плака / Микропланшет / Mikrotitrační destička / Mikrotitracione ploče
CAL	Calibrator / Kalibrator / Calibratore / calibrador / calibrador / calibrateur / Kalibrator / Kalibrator / Kalibrator / Βαθμονομητής / Калибратор / калибратор / kalibrátor / Kalibrator	REF	catalogue number / Katalognummer / numero di catalogo / número de catálogo / número de referência / réf. de catalogue / katalognummer / Katalognummer / αριθμός καταλόγων / каталожен номер / katalogové číslo / каталожный номер / katalogové číslo / Kataloški broj
CONJ	Conjugate / Konjugat / Coniugato / conjugado / conjugado / conjugaté / Konjugerad / Konjugat / Konjugat / συνδετικό / Конюгат / Конъюгат / Konjugát / Konjugat	RTU	ready to use / gebrauchsfertig / pronto all'uso / listo para usar / pronto a usar / prêt à l'emploi / färdig att användas / færdig til brug / klar til bruk/ έτοιμο προς χρήση / Готов за употреба / готов к использованию / k přímému použití / Razrediti ili rastvoriti
CONT	Control / Kontrolle / controllo / control / control / contrôle / Kontroll / Kontrol / Kontroll / διάλυμα ελέγχου / Контрола / Контрольный образец / Kontrola / Kontrola	STOP	stop solution / Stopplösung / Soluzione di arresto / solución de parada / solução de paragem / solution d'arrêt / Stopplösning / Stop-opløsning / Stoppløsning / διάλυμα παύσης / Стоп разтвор / Стоп-раствор / Zastavovací roztok / Stop solucije
DIL	dilute or disolve in / verdünnen oder lösen in / diluire o dissolvere in / diluir o disolver / diluir ou dissolver em / diluer ou dissoudre dans / späd eller upplös i / fortyndes eller opløses i / Fortyndes eller oppløses i / αραίωση ή διάλυση σε / разтворете или разредете с / zředit anebo rozpustit v / разбавить или растворить в / nařeďte nebo rozpusťte v / razrediti ili rastvoriti u	SUB	substrate / Substrat / substrato / substrato / substrato / substrat / Substrat / Substrat / Substrat / υπόστρωμα / Субстрат / Субстрат / Substrát / Substrat
INC	incubation buffer / Inkubationspuffer / tampone di incubazione / tampón de incubación / tampão de incubação / tampon d'incubation / Inkubationsbuffert/ Inkubationsbuffer/ Vaskebufferkonsentrat / διάλυμα επώασης / Инкубационен буфер / Буфер для инкубации / Inkubační pufr / Inkubacioni pufer	WASH	washing solution concentrate / Waschlösungskonzentrat / concentrado de solución de lavado / solución de lavado concentrada / tampão de lavagem concentrado / Tampon de lavage concentré / Vattenlösningskoncentrat / Vaskeopløsningskoncentrat / vaskeløsningskonsentrat / συμπυκνωμένο διάλυμα πλύσης / Концентриран миещ разтвор / Концентрат промывочного раствора / Koncentrát promývacího roztoku / Koncetrat solucije za ispiranje
IVD	in vitro diagnostic use / in vitro Diagnostikum / diagnostico in vitro / diagnóstico en vitro / diagnóstico in vitro / diagnostic in vitro / för in vitro diagnostik / in vitro diagnostik / in vitro diagnostisk bruk / χρήση διαγνωστικής εντός σωλήνα / за ин витро диагностика / pro in vitro diagnostiku / использовать для диагностики in vutro / diagnostický prostředek in vitro / Destilisana Voda		

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TECHNOZYM® vWF:Ag ELISA

GB

PRODUCT DESCRIPTION

INTENDED USE

The von Willebrand Factor (vWF) is a large, multifunctional glycoprotein, occupying a key position in primary haemostasis. It has a multiple structure with several functions:

- It is the carrier protein for Factor VIII in plasma; it forms a complex and thus protects Factor VIII from early proteolytic decomposition.
- It acts as a mediator for platelet aggregation by attaching itself to platelet membrane receptors (GP Ib and GP IIb/IIIa) following previous platelet activation.
- It plays a part in primary haemostasis by acting as a mediator between adhesioned platelets and the subendothelium (lesioned vascular wall).

The von Willebrand Syndrome (vWS) is the most frequently occurring hemorrhagic disease; it may be hereditary as well as acquired, caused by quantitative or qualitative defects of the vWF. Determining the vWF antigen is an essential part of the diagnosis.
The vWF: Ag ELISA allows a differential diagnosis between hemophilia A and vWS and additionnal diagnostics in case of hepatic and vascular diseases.

COMPOSITION

1. ELISA test stripe (12) with 8 wells each, coated with polyclonal anti-vWF; the drying agent is supplied in an aluminium bag.
2. Washing buffer concentrate: (PBS; pH 7.3); containing detergent; 0.01% merthiolate; 1 bottle, 80 ml.
3. Incubation buffer: (PBS; pH 7.3); contains stabiliser protein; 0.05% proclin; dye, 1 bottle, 90 ml; ready for use.
4. Calibrators (Standards) numbered; lyophilised; 1 bottle each.
   Concentrations are lot-dependent; consult label on the vial.
5. Control plasmas "low level" and "high level" for checking purposes, lyophilised; 1 bottle each. **Concentrations are lot-dependent; consult the label on the vial.**
6. Conjugate polyclonal Anti-vWF-POX; dyed blue; 1 bottle, 0.3 ml.
7. Chromogen TMB (tetramethylbenzidine); 1 bottle, 12 ml; ready to use.
8. Stopping solution: sulphuric acid 0.45 mol/l; 1 bottle 12ml; ready for use.
9. Adhesive film: for ELISA test strips (2)

MATERIAL REQUIRED (but not supplied with the kit)

1. Distilled water
2. Test tubes for diluting standard and samples
3. Measuring cylinder (1000 ml)
4. Precision pipettes (10, 100 and 1000 µl)
5. Variable pipette (1000 µl)
6. Multichannel and/or dispensing pipettes (100 and 200 µl)
7. ELISA washer or multichannel pipette
8. ELISA reader with 450 nm filter, with a 620 nm reference filter if available
9. Incubator (37 °C)

WARNING AND PRECAUTIONS

- All human blood or plasma products as well as samples must be considered as potentially infectious. They have to be handled with appropriate care and in strict observance of safety regulations. The rules pertaining to disposal are the same as applied to disposing hospital waste.
- Calibrators and control plasmas are made from human blood and any individual plasma involved in the procedure is HBsAg, HIV 1/2 Ab and HCV-Ab-negative (see labels on kit and/or bottles).
- Stopping solution (sulphuric acid) may irritate the skin. Should acid get into your eyes, wash out immediately with water and consult a doctor.
- The reagents sometimes contain preserving agents (merthiolate). Beware of swallowing! Avoid contact with skin or mucous membranes.

STABILITY AND STORAGE

All components contained in the kit may be used until the expiry date as indicated. The bench stability of the components after opening, reconstitution and/or dilution may be inferred from the table below:
When necessary the samples, controls and calibrators can be frozen/thawed up to 5 times. But making aliquots is recommended.

Material/Reagent	State	Storage	Stability
Calibrators, controls plasmas	after reconstitution	-20 °C room temperature	6 months 8 hours
ELISA test strip	after opening	2 ... 8 °C with adhesive film in plastic bag with	expiry date
Washing buffer concentrate	after opening	2 ...8°C	6 months
Washing buffer	1+11.5 dilution of concentrate	2 ... 8 °C	3 weeks
Incubation buffer	after opening	2 ... 8 °C	2 months
Conjugate	after opening	2 ... 8 °C	6 months
	working solution	room temperature	60 minutes
Chromogen TMB	after opening	2 ... 8 °C	expiry date

TEST PROCEDURE

PREPARATION OF SAMPLES

Material: plasma
Obtaining plasma: mix 9 parts venous blood with 1 part sodium citrate solution (0.11 mol/l) and centrifuge for 15 minutes at a minimum of 2500g (DIN 58905). The plasma sample may be stored for 3 hours at room temperature; otherwise the sample ought to be frozen immediately after centrifugation. Stable at -20°C for 6 months.

PREPARATION OF REAGENT

1. Before starting the test, all the required components are to be brought to room temperature.
2. Preparing the washing buffer: Dilute 1 part by volume washing buffer concentrate with 11.5 parts by volume distilled water (1+11.5). Mix well! (Diluted washing buffer concentrate = washing buffer). There may be crystalline precipitations which will dissolve at 37°C within 10 minutes.
3. Reconstituting calibrators and control plasmas:
   Calibrators and control plasmas are reconstituted with 500 µl distilled water and mixed for 10 seconds after a reconstitution time of 15 minutes (vortex mixer). Reconstituted components are clear to slightly turbid.
4. Diluting calibrators, control plasmas and samples (1+25):
   Dilute 10 µl samples, 10 µl calibrators and 10 µl controls with 250 µl each of incubation buffer. Mix for 10 seconds!
5. Preparing the conjugate working solution (1+50):
   Dilute 1 part by volume conjugate with 50 parts by volume incubation buffer.

For 8 test wells: Mix 20 µl conjugate with 1000 µl incubation buffer.

PERFORMANCE OF THE TEST

SAMPLE AND CONJUGATE CO-INCUBATION (reference 1, 2)	**diluted calibrators diluted control plasmas diluted samples** pipette into test wells	50 µl
	pipette conjugate working solution into wells Cover test strips with film	50 µl
	incubate at 37°C	**45 minutes**
WASHING (reference 1,3,4)	**washing buffer**	3 x 200 µl
SUBSTRATE REACTION (reference 1,2)	pipette **substrate** solution into test wells cover test strips with film	100 µl
	incubate at room **temperature**	**15 minutes**
STOP SOLUTION (reference 1,2)	pipette **stopping solution** into wells	100 µl
MEASURING (reference 5)	ELISA-Reader, **450 nm**	Shake 10 sec., Measure within 10 minutes

Room temperature is 20 ... 25°C

References
1. Reagents of different lots must not be combined
2. Precision and performance, among others, essentially depend on the following factors:
   - Thorough mixing of all substances used for dilution
   - Test calibrators, controls and samples in duplicates.
   - Incubation to be done at correct temperatures
   - Strict observance of the order of pipetting and of the time element as indicated:
   - The time for sample incubation, conjugate and substrate reaction as indicated starts after pipetting the last sample. Incubation times should not vary by more than ±10%.
   - During sample incubation and conjugate reaction, the time for pipetting the diluted calibrators/samples/control plasmas and/or conjugate solutions must not exceed 60 seconds per ELISA test strip (8 wells).
   - During substrate reaction and at stopping, the time needed for pipetting the substrate and/or the stopping solution must not exceed 10 seconds per ELISA test strip. Short pipetting times may be secured by using multichannel- and dispensing pipettes.
3. Label/number strips with a water resistant pen in case the strips accidentally fall out of the frame during testing.
4. After the last washing, wells must be aspirated thoroughly, turned upside down and positioned on a blotting paper; by gentle tapping, the last remnants must be removed.
5. Measuring the difference in wave lengths at 450 and 620 nm or at 450 and 690 nm, the precision of the test is increased.

LIMITATION OF THE TEST

Reduced levels of vWF:Ag are associated with blood group 0.
vWF:Ag is also affected by physical exercise, pregnancy, use of contraceptive pill, ethnic group and the antigen increases with age.

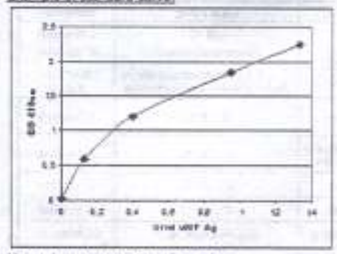
ANALYSIS RESULTS

CALCULATION OF THE RESULTS

Setting up a reference curve: X axis: Concentration vWF-Ag U/ml (1U/ml = 100%)
Y axis: Extinction
Graph plot is linear-linear with a linear or point to point fit

Assessment of reference curve
- The extinction coefficient of the highest calibrator should be between 1.0 and 2.5.
- The validity of the test may be checked on the basis of the calculated control values.

Example of standard curve.



Measuring concentration of samples
- Read off the concentration from the reference curve.
- If there are samples with extinction coefficients higher than that of the highest point on the curve, they have to be prediluted with incubation buffer (1+1). The measured concentration then has to be multiplied with the dilution factor 2.

REFERENCE RANGE

Normal range for vWF:Ag is between 0.5 – 1.5 U/ml (50 – 150%). It is recommended that individual laboratories establish their own normal range.

STANDARDIZATION

The calibration material used is the WHO International standard for Blood coagulation Factor VIII and von Willebrand factor in plasma (human)

PERFORMANCE CHARACTERISTICS

Performance data are given below. Results obtained in individual laboratories may differ.

PRECISION

Reproducibility was determined with different samples (in series and day to day). The following results were obtained.

	Intra assay variation		Inter assay variation	
Sample	Sample 1	Sample 2	Sample 3	Sample 4
N	96	10	192	10
Mean (U/mL)	1.435	0.867	1.40	0.35
SD	0.08	0.04	0.08	0.02
CV (%)	5.56	5.00	5.95	4.36

ASSAY RANGE

0.025 – 1.50 U/mL

DETECTION LIMIT

0.01U/mL

LITERATURE

1) Blood 69, 1691 – 1695, 1987. The effect of ABO blood group on the diagnosis of vWD. Gill et al.