Use of snake venoms in the coagulation laboratory

Dr Gary Moore

viapath

Guy's and St Thomas' NHS
NHS Foundation Trust

SNAKES

Phylum:	Chordata
Class:	Reptilia
Order:	Squamata
Suborder:	Serpentes

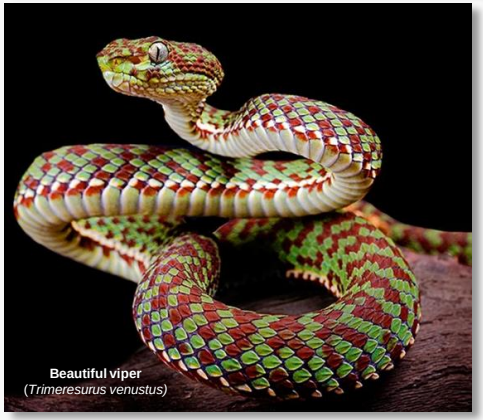
Most recently evolved reptiles

Probably evolved from burrowing lizards

130 million years ago	first known snakes
15 million years ago	rear-fanged snakes elapids
10 million years ago	vipers

FAMILY	Common names	No. species
Acrochordidae	Wart snakes	3
Aniliidae	False coral snake	1
Anomalepidae	Primitive blind snakes	15
Anomochilidae	Dwarf pipe snakes	2
Atractaspididae	Stiletto snakes	64
Boidae	Boas	43
Bolyeridae	Splitjaw snakes	2
Colubrids	Typical snakes	1938
Cylindrophiidae	Asian pipe snakes	8
Elapids	Elapids	235
Leptotyphlopidae	Slender blind snakes	87
Loxocemidae	Mexican burrowing snake	1
Pythonidae	Pythons	26
Tropidophidae	Dwarf boas	22
Typhlopidae	Typical blind snakes	203
Uropeltidae	Shield-tailed snakes	47
Vipers	Vipers and pit vipers	224
Xenopeltidae	Sunbeam snakes	2


Boomslang
(*Dispholidus typus*)


Beautiful viper
(*Trimeresurus venustus*)


Black Mamba
(*Dendroaspis polylepis*)

Snake venom toxins

Snake venoms are modified saliva/digestive juices

Venomous snakes produce a cocktail of proteins, enzymes & toxins

Hyaluronidase accelerates absorption into tissues

Toxicity	Mechanism	Effect
Neurotoxic	destroy or block acetylcholine	tetany, paralysis, respiratory arrest
Cyotoxic	rupture cell membranes	destroy RBC, muscles, blood vessels
Cardiotoxic	irregular beat or stop beating	heart failure
Haemotoxic	procoagulants	DIC

Effects of snake venom toxins on haemostasis

Metalloproteinases

Degrade blood vessel extracellular matrix

Venom proteins acting on coagulation factors/inhibitors

Activators of FII, FV, FX & protein C

Snake venom thrombin like enzymes (SVTLE)

Inhibitors of thrombin, FIX & FX

Inactivation of antithrombin

Fibrinolytic enzymes

Direct degradation of fibrinogen and fibrin

Direct plasminogen activators

Phospholipase A_2 (PLA_2)

Destroy phospholipid

Compete for phospholipid binding sites

Inhibit prothrombinase activity by directly binding FXa

King Cobra

(*Ophiophagus hannah*)

PLA_2 that disrupts platelet cytoskeleton

Venom components affecting primary haemostasis

Cleavage of VWF

Platelet glycoproteins: activation
blockage
disintegrins

SVLTEs activate platelets

viapath

PNAS

A central role for venom in predation by *Varanus komodoensis* (Komodo Dragon) and the extinct giant *Varanus* (*Megalania*) *priscus*

Bryan G. Fry[a,b,1], Stephen Wroe[c], Wouter Teeuwisse[d], Matthias J. P. van Osch[d], Karen Moreno[c,e], Janette Ingle[f], Colin McHenry[f], Toni Ferrara[c], Phillip Clausen[f], Holger Scheib[g], Kelly L. Winter[h], Laura Greisman[a,b,h], Kim Roelants[i], Louise van der Weerd[d,j], Christofer J. Clemente[k], Eleni Giannakis[l], Wayne C. Hodgson[h], Sonja Luz[m], Paolo Martelli[n], Karthiyani Krishnasamy[o], Elazar Kochva[p], Hang Fai Kwok[q,2], Denis Scanlon[b], John Karas[b], Diane M. Citron[r], Ellie J. C. Goldstein[r], Judith E. Mcnaughtan[s], and Janette A. Norman[a,b,t]

Table 1. Molecular biodiversity of toxin types detected in *V. komodoensis* venom

Toxin type	Previously characterized bioactivities (refs. 6, 9, and 13)
AVIT	Potent constriction of intestinal smooth muscle, resulting in painful cramping, and induction of hyperalgesia.
CRISP	Basal toxic activity of paralysis of peripheral smooth muscle and induction of hypothermia via blockage of L-type Ca^{2+}- and BK_{Ca} K^+-channels. Derived activities include blockage of cyclic nucleotide gated calcium channels.
Kallikrein	Basal toxic activity of increasing vascular permeability and production of hypotension in addition to stimulation of inflammation. Derivations affect the blood through the cleavage of fibrinogen.
Natriuretic	Basal activity potent induction of hypotension leading to loss of consciousness. Derived activities include cardiovascular effects independent of the GC-A receptor and antiplatelet activities evolved for emergent domains upstream of the natriuretic peptide domain.
PLA_2 (T-III)	Anticoagulation via platelet inhibition.

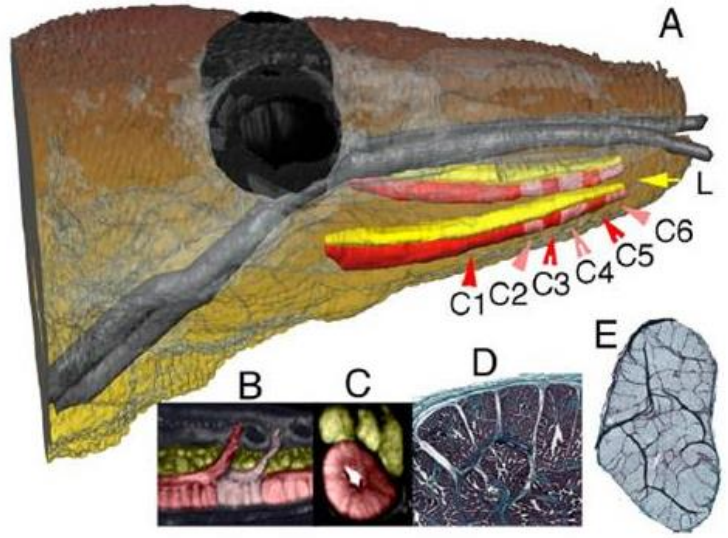


Fig. 2. Anatomical investigation of the *Varanus komodoensis* venom system. (*A*) Magnetic resonance imaging of the *V. komodoensis* head showing the protein-secreting mandibular venom gland, with the 6 compartments colored in alternating red and pink (C1–C6), and the mucus-secreting infralabial gland in yellow (L). (*B*) Longitudinal MRI section showing the large duct emerging separately from each compartment of the mandibular venom gland and threading between the mucus lobes of the infralabial gland to terminate between successive teeth (black oval areas). (*C*) Transverse MRI section showing the large central lumen of the mandibular venom gland and individual lobes of the labial gland. (*D*) Transverse histology of Masson's Trichrome-stained section showing the intratubular lumina of the mandibular venom gland that feed into the large central lumen. (*E*) Transverse histology of Masson's Trichrome-stained section of a mucus infralabial gland showing numerous tightly packed internal lobules (note that the ~6 large dark folds are histology artifacts).


Australia
Zoo

Fer-de-lance
(*Bothrops atrox*)

Reptilase time

Direct conversion of fibrinogen to fibrin by SVLTE

Venom releases FPA but not FPB

Sensitive to:
- reduced fibrinogen concentration
- dysfibrinogenemias
- elevated fibrin(ogen) degradation products
- paraproteins interfering with fibrin polymerisatio
- amyloidosis

Insensitive to:
- Heparins
- Direct thrombin inhibitors

Dysfibrinogenemias

PT & APTT are often normal

Primary screening test is the thrombin time

Dysfibrinogens prolong TT by: inhibiting release of FPA &/or FPB
inhibiting polymer formation

Fibrinogens Oslo I & Valhalla have short TT but elevated reptilase time

If TT &/or RT elevated: fibrinogen activity – antigen ratio

Assay activity & antigen levels on same sample; fibrinogen levels can fluctuate

Reduced activity-antigen ratio indicative of dysfibrinogenemia

Malayan Pit Viper
(*Callesolasma rhodostoma*)


Russell’s viper venom

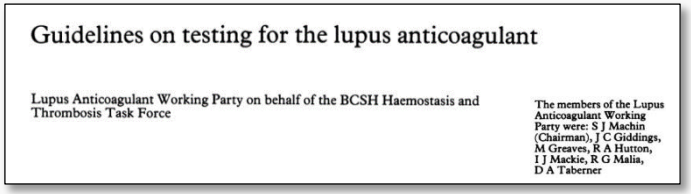
Thrombosis and Haemostasis © F.K. Schattauer Verlagsgesellschaft mbH (Stuttgart) 65 (3) 320–322 (1991)

Scientific and Standardization Committee Communications

Guidelines for Testing and Revised Criteria for Lupus Anticoagulants

SSC Subcommittee for the Standardization of Lupus Anticoagulants

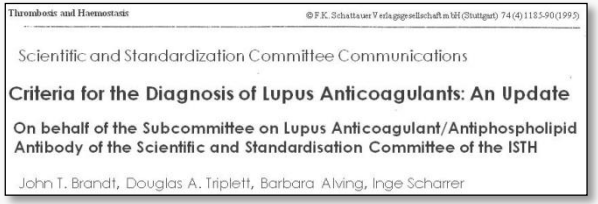
Thomas Exner[1], Douglas A. Triplett[2], David Taberner[3], and Samuel J. Machin[4]



Guidelines on testing for the lupus anticoagulant

Lupus Anticoagulant Working Party on behalf of the BCSH Haemostasis and Thrombosis Task Force

The members of the Lupus Anticoagulant Working Party were: S J Machin (Chairman), J C Giddings, M Greaves, R A Hutton, I J Mackie, R G Malia, D A Taberner



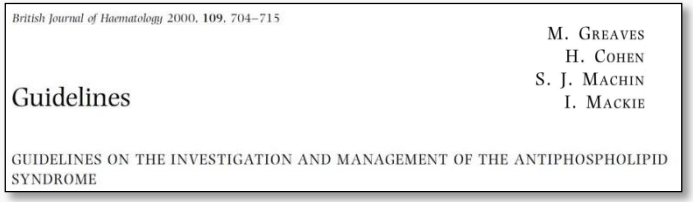
Thrombosis and Haemostasis © F.K. Schattauer Verlagsgesellschaft mbH (Stuttgart) 74 (4) 1185-90 (1995)

Scientific and Standardization Committee Communications

Criteria for the Diagnosis of Lupus Anticoagulants: An Update

On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH

John T. Brandt, Douglas A. Triplett, Barbara Alving, Inge Scharrer

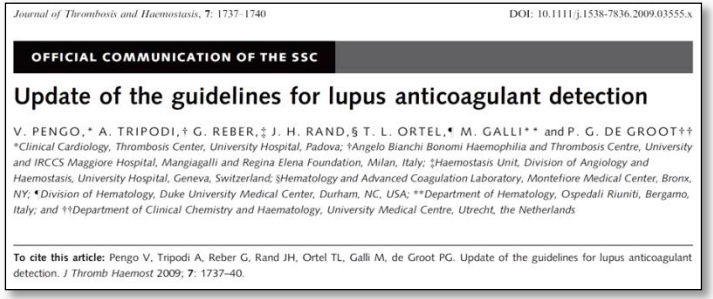


British Journal of Haematology 2000, **109**, 704–715

M. Greaves
H. Cohen
S. J. Machin
I. Mackie

Guidelines

GUIDELINES ON THE INVESTIGATION AND MANAGEMENT OF THE ANTIPHOSPHOLIPID SYNDROME



Journal of Thrombosis and Haemostasis, **7**: 1737–1740 DOI: 10.1111/j.1538-7836.2009.03555.x

OFFICIAL COMMUNICATION OF THE SSC

Update of the guidelines for lupus anticoagulant detection

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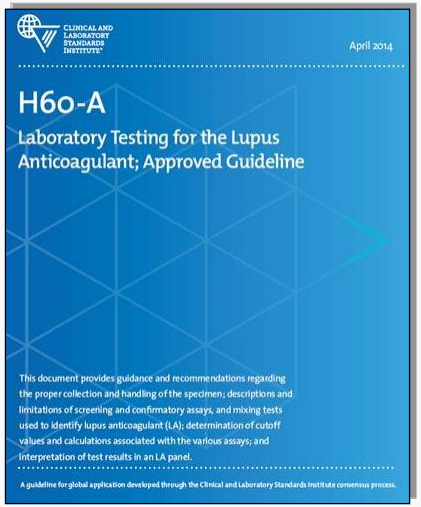


CLINICAL AND LABORATORY STANDARDS INSTITUTE®

April 2014

H60-A

Laboratory Testing for the Lupus Anticoagulant; Approved Guideline

This document provides guidance and recommendations regarding the proper collection and handling of the specimen; descriptions and limitations of screening and confirmatory assays, and mixing tests used to identify lupus anticoagulant (LA); determination of cutoff values and calculations associated with the various assays; and interpretation of test results in an LA panel.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

bjh British Journal of Haematology

Guidelines on the investigation and management of antiphospholipid syndrome

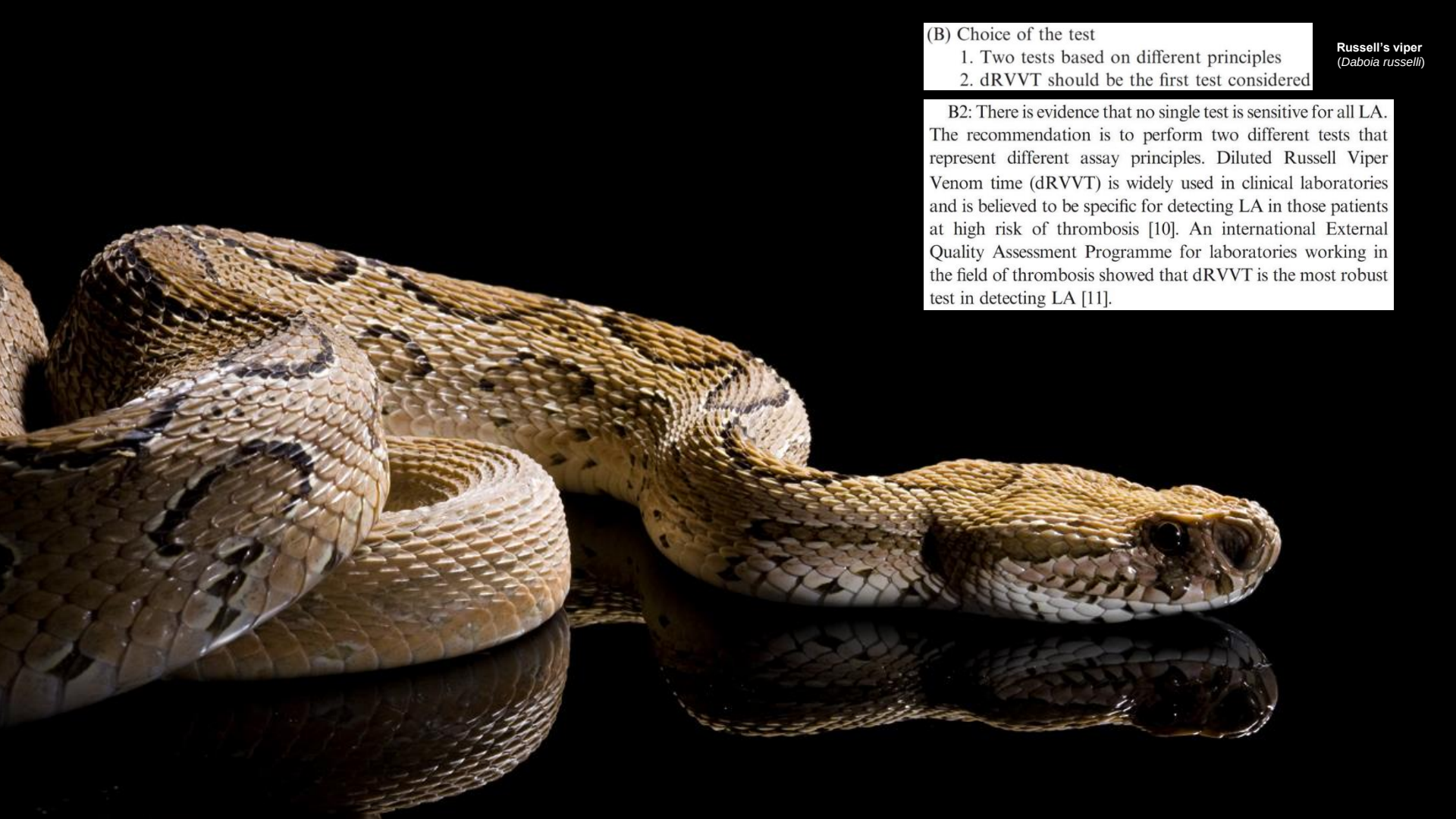
David Keeling,[1] Ian Mackie,[2] Gary W. Moore,[3] Ian A. Greer,[4] Michael Greaves[5] and British Committee for Standards in Haematology

[1]Oxford Haemophilia and Thrombosis Centre, Churchill Hospital, Oxford, UK, [2]Haemostasis Research Unit, Haematology Department, University College London, London, UK, [3]Centre for Haemostasis and Thrombosis, GSTS Pathology, Guy's & St. Thomas' Hospitals, London, UK, [4]University of Liverpool, Liverpool, UK and [5]School of Medicine & Dentistry, University of Aberdeen, Aberdeen, UK

British Journal of Haematology, 2012, **157,** 47–58

(B) Choice of the test
1. Two tests based on different principles
2. dRVVT should be the first test considered

B2: There is evidence that no single test is sensitive for all LA. The recommendation is to perform two different tests that represent different assay principles. Diluted Russell Viper Venom time (dRVVT) is widely used in clinical laboratories and is believed to be specific for detecting LA in those patients at high risk of thrombosis [10]. An international External Quality Assessment Programme for laboratories working in the field of thrombosis showed that dRVVT is the most robust test in detecting LA [11].



Russell's viper
(*Daboia russelli*)

dRVVT analytical variation

Jennings I, Kitchen S, Woods TA, Preston FE, Greaves M. Potentially clinically important inaccuracies in testing for the lupus anticoagulant: an analysis of results from three surveys of the UK national external quality control scheme (NEQAS) for blood coagulation. *Thromb Haemost* 1997; 77: 934-937

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Lawrie AS, Mackie IJ, Purdy G, Machin SJ. The sensitivity and specificity of commercial reagents for the detection of lupus anticoagulant show marked differences in performance between photo-optical and mechanical coagulometers. *Thromb Haemost* 1999; 81: 758-762

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Moore GW, Savidge GF, Smith MP. Improved detection of lupus anticoagulants by the dilute Russell's Viper venom time. *Blood Coagul Fibrinolysis* 2000; 11: 767-774

Triplett DA. Use of the dilute Russell's viper venom time (DRVVT): its importance and pitfalls. *J Autoimm* 2000; 15: 173-178

Jennings I, Greaves M, Mackie IJ, Kitchen S, Woods TA, Preston FE. UKNEQAS for Blood Coagulation. Lupus anticoagulant testing: improvements in performance in a UK NEQAS proficiency testing exercise after dissemination of national guidelines on laboratory methods. *Br J Haematol* 2002; 119: 364-369

Tripodi A, Biasiolo A, Chantarangkul V, Pengo V. Lupus anticoagulant (LA) testing: performance of clinical laboratories assessed by a national survey using lyophilised affinity-purified immunoglobulin with LA activity. *Clin Chem* 2003; 49: 1608 -1614

Moore GW & Savidge GF. Heterogeneity of Russell's viper venom affects the sensitivity of the dilute Russell's viper venom time to lupus anticoagulants. *Blood Coagul Fibrinolysis* 2004; 15: 279-282

Moore GW, Tugnait S, Savidge GF. Evaluation of a new generation dilute Russell's viper venom time assay system for lupus anticoagulant detection utilising frozen reagents and controls. *Br J Biomed Sci* 2005;62:127 -131

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McGlasson DL & Fritsma GA. Comparison of six dilute Russell Viper venom time lupus anticoagulant screen/confirm assay kits. *Semin Thromb Hemost* 2013; 39: 315-319

Tripodi A, Chantarangkul V, Cini M, Devreese K, Dlott JS, Giacomello R, Gray E, Legnani C, Martinuzzo ME, Pradella P, Siegemund A, Subramanian S, Suchon P, Testa S. Variability of cut-off values for the detection of lupus anticoagulants: results of an international multicenter multiplatform study. *J Thromb Haemost* 2017; 15: 1180-1190

Moore GW, Peyrafitte M, Dunois C, Amiral J. Newly developed dilute Russell's viper venom reagents for lupus anticoagulant detection with improved specificity. *Lupus* 2017; 27: 95-104

Depreter B, Devreese KM. Dilute Russell's viper venom time reagents in lupus anticoagulant testing: a well-considered choice. *Clin Chem Lab Med* 2017; 55: 91-101

Lupus Anticoagulant Testing

Performance and Practices by North American Clinical Laboratories

Francine R. Dembitzer, MD,[1] Marlies R. Ledford Kraemer, MBA, BS, MT(ASCP)SH,[2] Piet Meijer, PhD,[3] and Ellinor I.B. Peerschke, PhD[1]

Am J Clin Pathol 2010;134:764-773

Table 3
Performance of Major LAC Screening Assays*

Assay	2008-1 High-Titer LAC Plasma Pool	2008-2 Medium-Titer LAC Plasma Sample	2008-3 Low-Titer LAC Plasma Pool	2008-4 Medium-Titer LAC Plasma Sample (Diluted)	2009-2 Normal Plasma Pool
	False-Negative (%)	False-Negative (%)	False-Negative (%)	False-Negative (%)	False-Positive (%)
All	0	0	5.9	9.6	6.6
APTT (combined)	0	0	4.5	2.4	5.4
APTT (LAC sensitive)	0	0	3.0	3.2	7.4
APTT (LAC moderate sensitivity)	0	0	7.1	0	0
dRVVT	0	0	7.5	17	7.8

APTT, activated partial thromboplastin time; dRVVT, dilute Russell viper venom time; LAC, lupus anticoagulant.
* Performance of APTT and dRVVT screening assays in LAC testing challenges was examined by determining false-positive and false-negative rates for each assay type based on participant response of normal (negative) or abnormal or borderline (positive).

Table 7
Performance of Confirmatory Assays for LAC Identification*

Assay	2008-1 High-Titer LAC Plasma Pool	2008-2 Intermediate-Titer LAC Plasma Sample	2008-3 Low-Titer LAC Plasma Pool	2008-4 Intermediate-Titer LAC Plasma Sample (Diluted)	2009-2 Normal Plasma Pool
	False-Negative (%)	False-Negative (%)	False-Negative (%)	False-Negative (%)	False-Positive (%)
Integrated	0	82	5	89	31
dRVVT	3	29	26	62	5
PNP	0	92	33	83	11

dRVVT, dilute Russell viper venom time; LAC, lupus anticoagulant; PNP, platelet neutralization procedure.
* The false-negative and false-positive rates for assay types were calculated based on confirmatory test results shown in Table 6.

Journal of Thrombosis and Haemostasis 2004; **2**: 2178–2184

ORIGINAL ARTICLE

Lupus anticoagulant testing using plasma spiked with monoclonal antibodies: performance in the UK NEQAS proficiency testing programme

I. JENNINGS,* I. MACKIE,† J. ARNOUT‡ and F. E. PRESTON* ON BEHALF OF THE UK NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME FOR BLOOD COAGULATION

Table 4 Median DRVVT results obtained with different kits

Sample	Method	Median result *n*	Test/ Normal	Confirm/ Normal	Test/ confirm	Normalized test/confirm	% correction of ratio
Anti-β2GP1 S01/05	American Diagnostica	54	1.56	1.06	1.72	1.48	32.4
	Dade-Behring	17	1.93	1.06	2.12	1.75	42.6
	Gradipore	35	1.80	1.03	1.92	1.71	43.7
	Institute Laboratory	49	1.95	1.04	2.04	1.88	46.2
	In house	19	1.68	1.24	1.34	1.35	25.8
	Manchester	33	1.48	1.05	1.44	1.45	90.5*
	Unicorn/Technoclone	8	1.41	1.28	0.77	1.09	7.8
Anti-prothrombin S01/06	American Diagnostica	54	2.06	1.67	1.43	1.29	21.6
	Dade-Behring	17	2.02	1.17	2.02	1.71	40.3
	Gradipore	35	2.00	1.10	1.91	1.80	45.2
	Institute Laboratory	49	2.09	1.14	2.03	1.86	46.1
	In house	19	1.84	1.33	1.28	1.28	21.5
	Manchester	33	1.81	1.12	1.62	1.62	84.0*
	Unicorn/Technoclone	8	1.56	1.56	0.65	1.01	1.4
Anti- β2GP1 + prothrombin S01/07	American Diagnostica	54	2.10	1.52	1.61	1.41	29.0
	Dade-Behring	17	2.28	1.15	2.30	1.92	47.6
	Gradipore	35	2.18	1.08	2.20	2.00	50.9
	Institute Laboratory	49	2.30	1.12	2.30	2.12	52.4
	In house	19	1.77	1.38	1.34	1.26	20.7
	Manchester	33	1.82	1.12	1.64	1.63	87.0*
	Unicorn/Technoclone	8	1.53	1.48	0.70	1.05	4.5
Normal pool S01/08	American Diagnostica	54	1.02	1.03	1.16	1.01	5.0
	Dade-Behring	17	1.09	1.04	1.21	1.02	1.9
	Gradipore	35	1.10	1.02	1.15	1.08	6.9
	Institute Laboratory	49	1.15	1.03	1.26	1.15	13.7
	In house	19	1.05	1.06	1.19	1.01	1.9
	Manchester	33	0.98	1.06	0.94	0.94	55.7*
	Unicorn/Technoclone	8	1.11	1.07	0.80	1.02	1.8
LA positive patient S01/09	American Diagnostica	54	2.53	1.51	1.86	1.66	39.6
	Dade-Behring	17	2.19	1.22	2.10	1.80	44.5
	Gradipore	35	2.26	1.15	2.06	1.98	49.7
	Institute Laboratory	49	2.45	1.16	2.33	2.10	52.2
	In house	19	1.83	1.25	1.47	1.38	27.6
	Manchester	33	1.80	1.09	1.61	1.66	91.0*
	Unicorn/Technoclone	8	1.80	1.39	0.86	1.27	21.0

*Manchester results recorded as percentage correction of clotting time. The manufacturers report a cut-off value of 65% for LA positivity. Data are calculated from information provided by participants; different commercial kit suppliers recommend different algorithms. For example, the Unicorn kit does not recommend the use of test/confirm ratios as the clotting times with the screen and confirm reagents are not matched. Ratios against normal plasma and percentage correction of ratio are recommended.

JAI

Use of the Dilute Russell Viper Venom Time (dRVVT): Its Importance and Pitfalls

Douglas A. Triplett

Journal of Autoimmunity (2000) 15, 173–178

Table 3. *Commercial dRVVT test systems*

Kit	Origin of phospholipid	RVV-form	Reagent: combined or multi-reagent	Heparin neutralizing reagent	Integrated system
DVV test® (ADI)	Plant	RVV-X	Combined	Present	Yes
Bioclot LA (Biopool)	Rabbit brain	RVV-X	Combined	Not present	No
LAC-screen (IL)	Vegetable	Native	Combined	Present	Yes
Viperquik (OT)	Rabbit brain	RVV-X	Combined	Not present	No
LA-screen (Gradipore)	Not disclosed	Native	Combined	Present	Yes
Manchester	Rabbit brain	Native	Multi-reagent	Not present	Yes[a]
Unicorn (UDL)	Bovine brain (Bell and Alton)	Native	Multi-reagent	Not present	No X[a]

Heterogeneity of Russell's viper venom affects the sensitivity of the dilute Russell's viper venom time to lupus anticoagulants

Gary W. Moore and Geoffrey F. Savidge

Blood Coagulation and Fibrinolysis 2004, 15:279–282

Table 3. Detection rates of alternative Russell's viper venom (RVV) reagents with 86 lupus anticoagulant (LA)-positive samples

RVV reagent	Number of LAs detected (percentage)	Number of LAs detected in combination with Sigma RVV (percentage)
Sigma	48 (55.8)	– –
Diagen	39 (45.3)	67 (79.0)
Manchester	37 (43.0)	67 (78.0)
Diagnostica Stago	35 (40.7)	64 (74.4)
American Diagnostica	49 (57.0)	73 (84.9)

Phospholipid

Low concentration in screen to accentuate effects of LA

High concentration in confirm to swamp/overwhelm LA

Source and composition affect sensitivity

Insensitive composition remains insensitive when diluted

Venom

Can be native or fractionated RVV-X

Venom composition can vary between sub-species

Environmental conditions of individual snake

BRIEF REPORT

WILEY rpth | ISTH

Application of different lupus anticoagulant diagnostic algorithms to the same assay data leads to interpretive discrepancies in some samples

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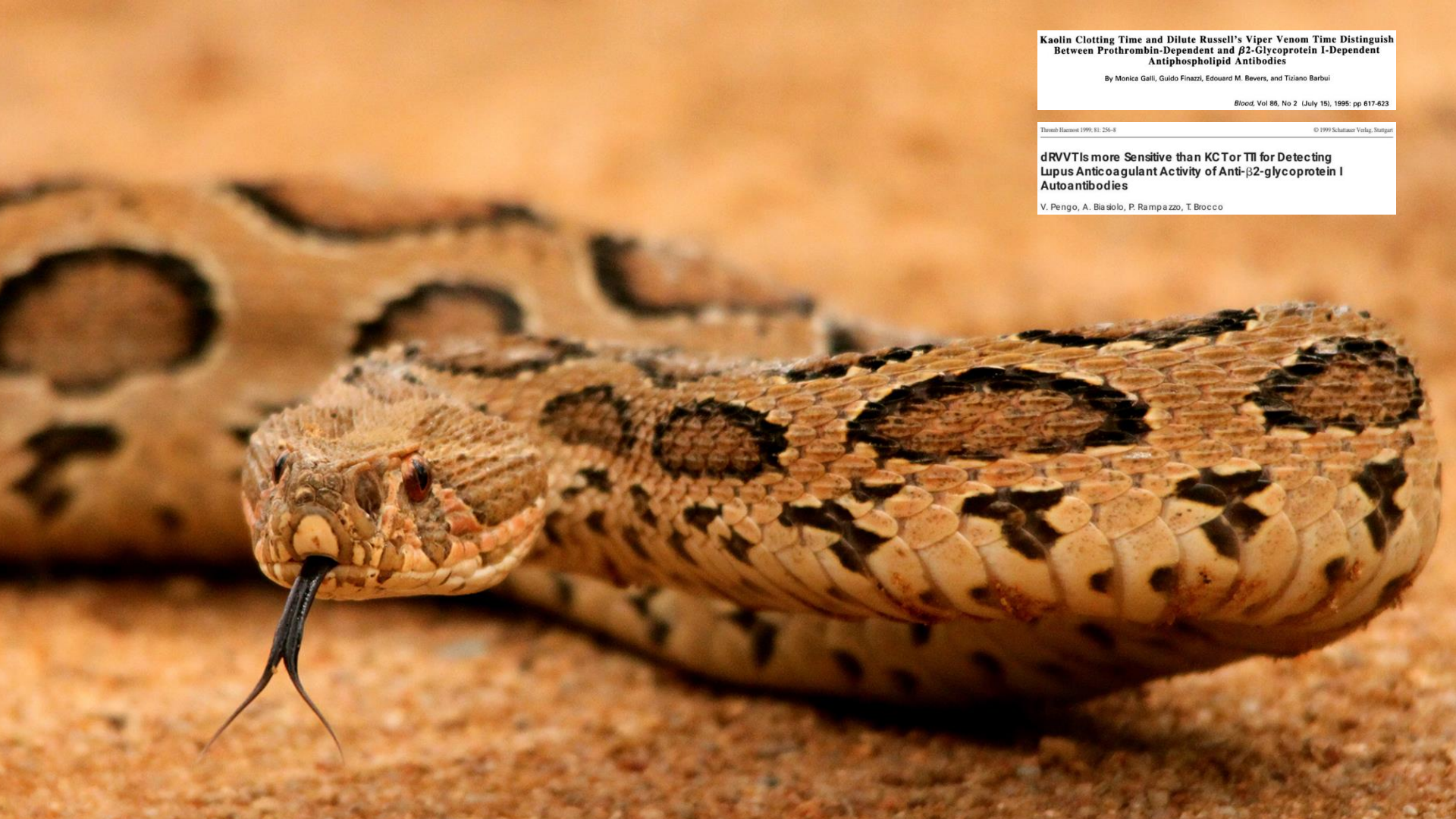
Res Pract Thromb Haemost. 2017;1:62–68.

viapath



Kaolin Clotting Time and Dilute Russell's Viper Venom Time Distinguish Between Prothrombin-Dependent and β2-Glycoprotein I-Dependent Antiphospholipid Antibodies

By Monica Galli, Guido Finazzi, Edouard M. Bevers, and Tiziano Barbui

Blood, Vol 86, No 2 (July 15), 1995: pp 617-623

Thromb Haemost 1999; 81: 256-8

dRVVTIs more Sensitive than KCTor TTI for Detecting Lupus Anticoagulant Activity of Anti-β2-glycoprotein I Autoantibodies

V. Pengo, A. Biasiolo, P. Rampazzo, T. Brocco

Protein S activity assay

FX

FXa

APC

Ca^{++}

Initiates coagulation when supplied with PL & Ca^{++}

FVi

APC

FVa

PS

Anticoagulation

Clotting time prolongation proportional to PS level

Interferences: coagulopathies, LA, other inhibitors
therapeutic anticoagulation
APCR

Activated protein C resistance assay

FX

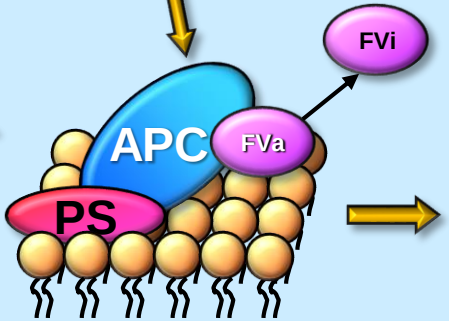
FXa

PC

APC

Ca^{++}

Initiates coagulation when supplied with PL & Ca^{++}



APC-R ratio

RVVT + pre-incubation with *A. c. contortrix* venom (s)
RVVT (s)

High PL concentration reduces LA interference

Heparin neutraliser

Dilution in FVDP overcomes factor deficiencies, VKA effects, PC & PS deficiencies

DOAC interferences

Activated protein C resistance assay

FV

APC

FVa

FVa

FVi

Residual

Noscarin

FII activator

FV-dependent

Ca^{++} & PL independent

FII

FIIa

APC-R ratio

If FVa inactivated by APC, clotting time prolonged

$$\frac{\text{Clotting time + APC (s)}}{\text{Clotting time - APC (s)}}$$

No PL – No LA interference

Heparin neutraliser

Dilution in FVDP overcomes factor deficiencies, VKA effects; patient PC & PS do not take part

Direct FXa inhibitors do not interfere

Tiger snake
(*Notechis scutatus scutatus*)

Prothrombinase-induced clotting time

International Journal of Laboratory Hematology
The Official journal of the International Society for Laboratory Hematology

Preincubation in the Prothrombinase-induced Clotting Time test (PiCT) is necessary for *in vitro* evaluation of fondaparinux and to be avoided for the reversible, direct factor Xa inhibitor, rivaroxaban

C. KLUFT*†, P. MEIJER†, R. KRET†, J. BURGGRAAF*

Int. Jnl. Lab. Hem. 2013, **35**, 379–384

Monitoring direct FXa-inhibitors and fondaparinux by Prothrombinase-induced Clotting Time (PiCT): Relation to FXa-activity and influence of assay modifications

Sebastian Harder *, Jeannine Parisius, Bettina Picard-Willems

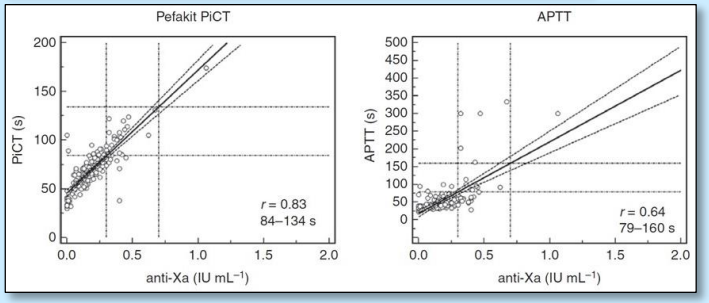
Thrombosis Research (2008) **123**, 396–403

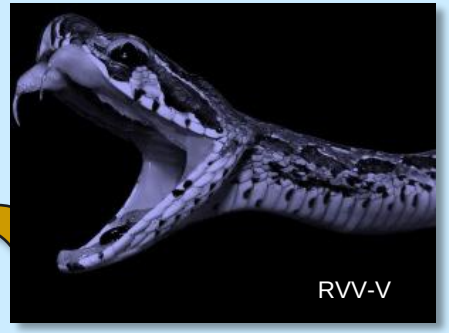
Journal of Thrombosis and Haemostasis 2016; **14**: 2187-2193 DOI: 10.1111/jth.13489

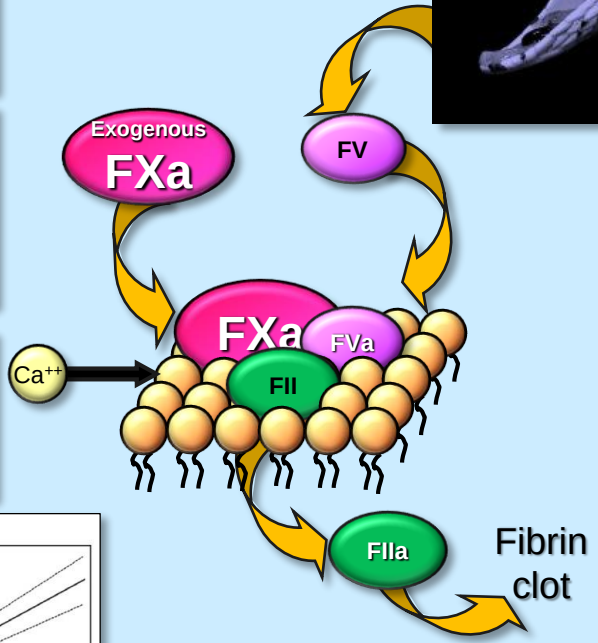
BRIEF REPORT

The PiCT® test is a reliable alternative to the activated partial thromboplastin time in unfractionated heparin therapy management: results from a multicenter study

A. C. BRISSET,* A. FERRÁNDEZ,* M. KRAUSE,† S. RATHBUN,‡ R. MARLAR‡ and W. KORTE§







Population Pharmacokinetics and Pharmacodynamics of Rivaroxaban in Patients with Non-valvular Atrial Fibrillation: Results from ROCKET AF

The Journal of Clinical Pharmacology
54(8) 917-927
© 2014, The American College of Clinical Pharmacology
DOI: 10.1002/jcph.288

I.G. Girgis, PhD[1], M.R. Patel, MD[2], G.R. Peters, MD[1], K.T. Moore, MS[3], K.W. Mahaffey, MD[2], C.C. Nessel, MD[1], J.L. Halperin, MD[4], R.M. Califf, MD[5], K.A.A. Fox, MB, BS[6], and R.C. Becker, MD[2]

Comparison of the aPTT With Alternative Tests for Monitoring Direct Thrombin Inhibitors in Patient Samples

Stuart E. Lind, MD, Mary Ellen Boyle, MBA, MT(ASCP), Sheila Fisher, MT(ASCP), Jan Ishimoto, MT(ASCP), Toby C. Trujillo, PharmD, and Tyree H. Kiser, PharmD

Am J Clin Pathol May 2014;141:665-674

DE GRUYTER Clin Chem Lab Med 2015; 53(8): 1237–1247

Shanshan Du, Christel Weiss, Giese Christina, Sandra Krämer, Martin Wehling, Roland Krämer and Job Harenberg*

Determination of dabigatran in plasma, serum, and urine samples: comparison of six methods

Prothrombinase-Induced Clotting Time Assay for Determination of the Anticoagulant Effects of Unfractionated and Low-Molecular-Weight Heparins, Fondaparinux, and Thrombin Inhibitors

Andreas Calatzis, MD,[1] Dirk Peetz, MD,[2] Sylvia Haas, MD,[3] Michael Spannagl, MD,[1] Karin Rudin,[4] and Marianne Wilmer, PhD[4]

Am J Clin Pathol 2008;130:446-454

RUSVEN

Russell's Viper Venom
NOT FOR INJECTION

The properties of Rusven

Indications

Method of application

RUSVEN

is supplied as follows:

0·1 mg. in rubber-stoppered vials, together with an ampoule of sterile distilled water containing 0·5% phenol, for use as a solvent.
0·5 mg. in rubber-stoppered vials, together with an ampoule of solvent.

BOOTS PURE DRUG COMPANY LIMITED
NOTTINGHAM ENGLAND

Acknowledgement
Catriona Haggart
Edinburgh Royal Infirmary

Southern copperhead
(*Agkistrodon contortrix contortrix*)

Protein C activity assays

A. c. contortrix **protein C activator**

Protein C

APC

Anticoagulate clotting test

APTT

APC FVa FVIIIa

APC FVa

Cleave chromogenic substrate

Chromogenic assay

Reliable assays

Not affected by other coagulopathies

Detects abnormalities of:
- protein C activation
- enzymatic active site

Clotting assay

Interferences:
- coagulopathies, LA & high FVIII
- therapeutic anticoagulation
- APCR

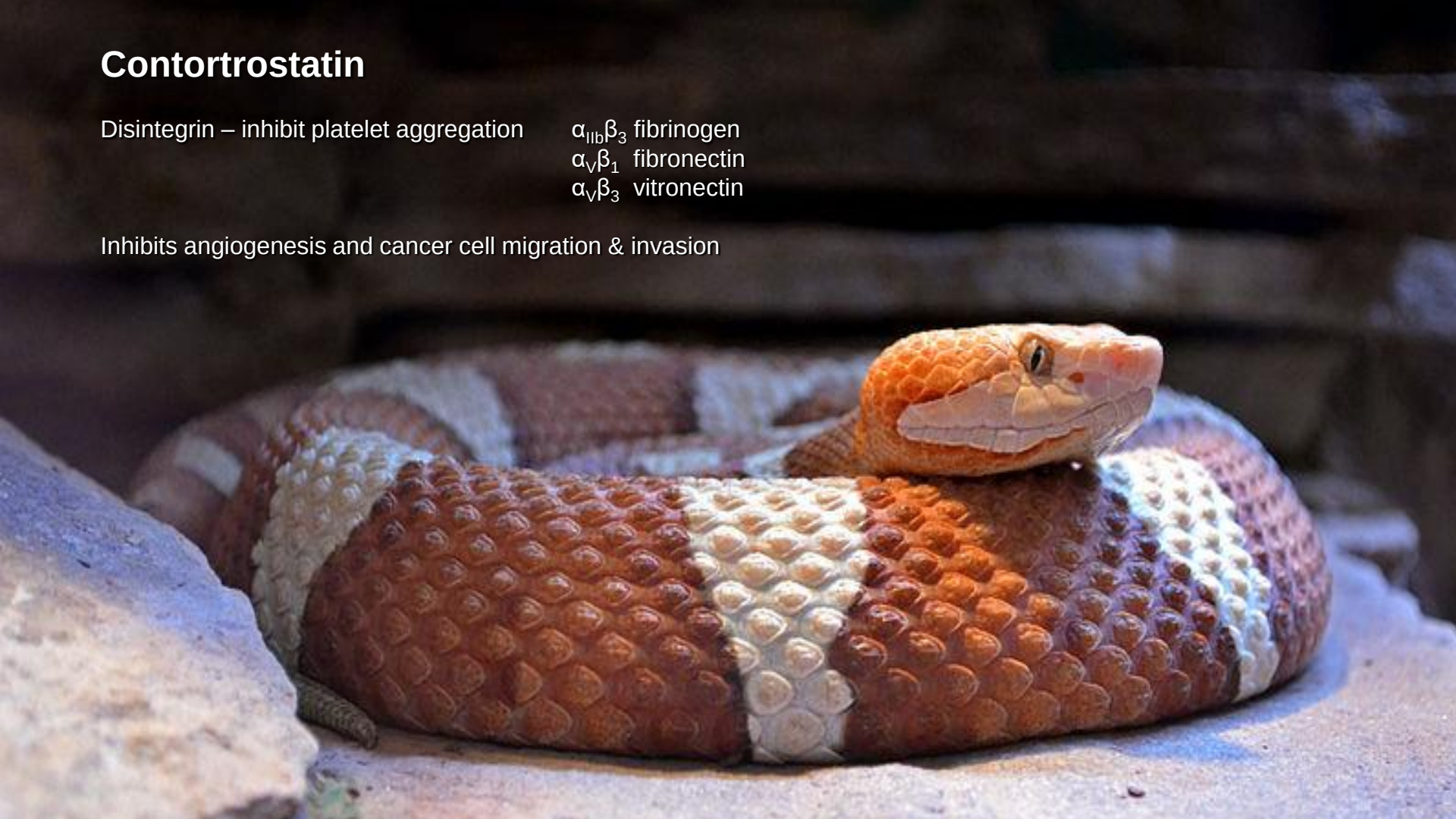
Detects abnormalities of:
- protein C activation
- enzymatic active site
- substrate binding
- protein S binding
- phospholipid binding

Contortrostatin

Disintegrin – inhibit platelet aggregation

$\alpha_{IIb}\beta_3$ fibrinogen
$\alpha_V\beta_1$ fibronectin
$\alpha_V\beta_3$ vitronectin

Inhibits angiogenesis and cancer cell migration & invasion



Australian Eastern Brown Snake
(*Pseudonaja textilis*)

Group D Prothrombin Activator

Co-factor requirements: FV
phospholipid
Ca^{2+}

Coastal Taipan
(*Oxyuranus scutellatus*)

Group C Prothrombin Activator

Co-factor requirements: phospholipid

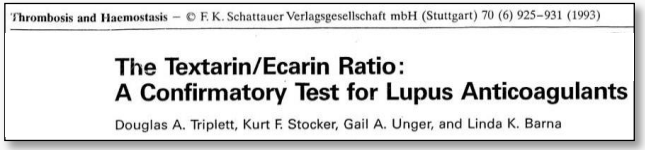
Ca^{2+}

Saw-scaled Viper
(*Echis carinatus*)

Group A Prothrombin Activator

Co-factor requirements: None

Brief history of snake venom FII-activators in LA detection



Thrombosis and Haemostasis – © F. K. Schattauer Verlagsgesellschaft mbH (Stuttgart) 70 (6) 925–931 (1993)

The Textarin/Ecarin Ratio: A Confirmatory Test for Lupus Anticoagulants

Douglas A. Triplett, Kurt F. Stocker, Gail A. Unger, and Linda K. Barna

Activate undercarboxylated FII to meizothrombin – insensitive to VKAs

Direct FII activation - improved specificity over dRVVT & APTT

J Clin Pathol 1994;47:497–501

The Taipan snake venom time: a new test for lupus anticoagulant

A M Rooney, T McNally, I J Mackie, S J Machin

TSVT with high phospholipid confirmatory test

The Ecarin time is an improved confirmatory test for the Taipan snake venom time in warfarinized patients with lupus anticoagulants

Gary W. Moore, Mark P. Smith and Geoffrey F. Savidge

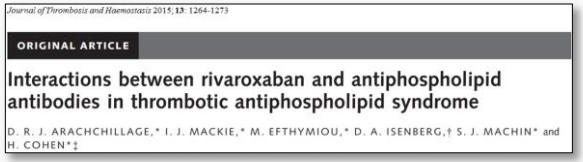
Blood Coagulation and Fibrinolysis 2003, **14**:307–312

First description of TSVT with ecarin time as confirmatory test

Journal of Thrombosis and Haemostasis 2011; **9**: 1657-1659

Detection of lupus anticoagulant in the presence of rivaroxaban using Taipan snake venom time

G. M. A. VAN OS,*† B. DE LAAT,*‡§ P. W. KAMPHUISEN,¶ J. C. M. MEIJERS†¶ and PH. G. DE GROOT*



Journal of Thrombosis and Haemostasis 2015; **13**: 1264-1273

ORIGINAL ARTICLE

Interactions between rivaroxaban and antiphospholipid antibodies in thrombotic antiphospholipid syndrome

D. R. J. ARACHCHILLAGE,* I. J. MACKIE,* M. EFTHYMIOU,* D. A. ISENBERG,† S. J. MACHIN* and H. COHEN*‡

Direct FII activation bypasses direct FXa inhibitors

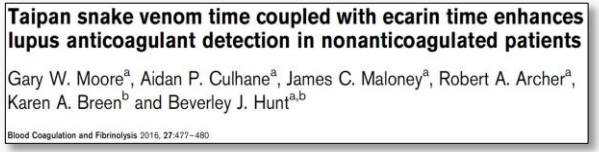
LETTER TO THE EDITOR

WILEY ISLH International Journal of Laboratory Hematology

The Taipan snake venom time can be used to detect lupus anticoagulant in patients treated by rivaroxaban

C. Pouplard, C. Vayne, C. Berthomet, E.A. Guery, B. Delahousse, Y. Gruel

International Journal of Laboratory Hematology 2017, 39: e60-e63



Taipan snake venom time coupled with ecarin time enhances lupus anticoagulant detection in nonanticoagulated patients

Gary W. Moore[a], Aidan P. Culhane[a], James C. Maloney[a], Robert A. Archer[a], Karen A. Breen[b] and Beverley J. Hunt[a,b]

Blood Coagulation and Fibrinolysis 2016, **27**:477–480

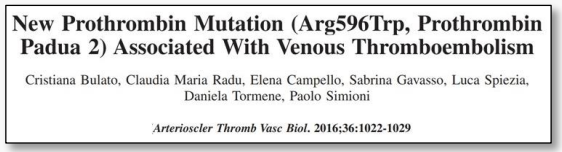
The NEW ENGLAND JOURNAL of MEDICINE

N Engl J Med 2012;366:2390-6

BRIEF REPORT

Thrombosis from a Prothrombin Mutation Conveying Antithrombin Resistance

Yuhri Miyawaki, M.Sc., Atsuo Suzuki, M.Sc., Junko Fujita, B.Sc., Asuka Maki, B.Sc., Eriko Okuyama, B.Sc., Moe Murata, B.Sc., Akira Takagi, Ph.D., Takashi Murate, M.D., Ph.D., Shinji Kunishima, Ph.D., Michio Sakai, M.D., Kohji Okamoto, M.D., Ph.D., Tadashi Matsushita, M.D., Ph.D., Tomoki Naoe, M.D., Ph.D., Hidehiko Saito, M.D., Ph.D., and Tetsuhito Kojima, M.D., Ph.D.



New Prothrombin Mutation (Arg596Trp, Prothrombin Padua 2) Associated With Venous Thromboembolism

Cristiana Bulato, Claudia Maria Radu, Elena Campello, Sabrina Gavasso, Luca Spiezia, Daniela Tormene, Paolo Simioni

Arterioscler Thromb Vasc Biol. 2016;36:1022-1029

Journal of Thrombosis and Haemostasis 2017; 15: 670 - 677 DOI: 10.1111/jth.13618

ORIGINAL ARTICLE

Clinical and biochemical characterization of the prothrombin Belgrade mutation in a large Serbian pedigree: new insights into the antithrombin resistance mechanism

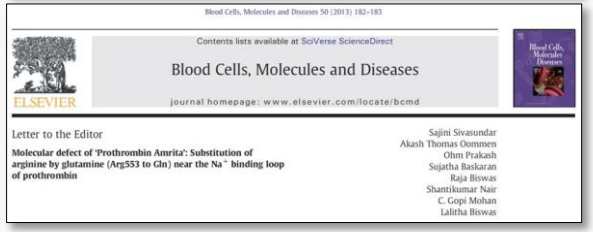
P. MILJIC,*† M. GVOZDENOV,‡ Y. TAKAGI,§ A. TAKAGI,§ I. PRUNER,‡ M. DRAGOJEVIC,‡ B. TOMIC,‡ J. BODROZIC,† T. KOJIMA,§ D. RADOJKOVIC‡ and V. DJORDJEVIC‡

A novel prothrombin mutation in two families with prominent thrombophilia – the first cases of antithrombin resistance in a Caucasian population

V. DJORDJEVIC,* M. KOVAC,†‡ P. MILJIC,†§ M. MURATA,¶ A. TAKAGI,¶ I. PRUNER,* D. FRANCUSKI,* T. KOJIMA¶ and D. RADOJKOVIC*

*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade; †Faculty of Medicine, University of Belgrade; ‡Hemostasis Department, Blood Transfusion Institute of Serbia; §Clinic of Hematology, University Clinical Center, Belgrade, Serbia; and ¶Department of Pathophysiological Laboratory Sciences, Nagoya University Graduate School of Medicine, Higashi-ku, Nagoya, Japan

To cite this article: Djordjevic V, Kovac M, Miljic P, Murata M, Takagi A, Pruner I, Francuski D, Kojima T, Radojkovic D. A novel prothrombin mutation in two families with prominent thrombophilia – the first cases of antithrombin resistance in a Caucasian population. *J Thromb Haemost* 2013; **11**: 1936-9.



Blood Cells, Molecules and Diseases 50 (2013) 182–183

Contents lists available at SciVerse ScienceDirect

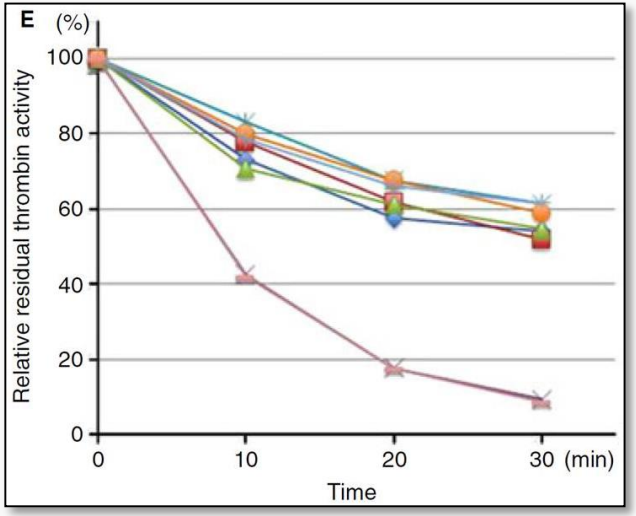
Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Letter to the Editor

Molecular defect of 'Prothrombin Amrita': Substitution of arginine by glutamine (Arg553 to Gln) near the Na+ binding loop of prothrombin

Sajini Sivasundar
Akash Thomas Oommen
Ohm Prakash
Sujatha Baskaran
Raja Biswas
Shantikumar Nair
C. Gopi Mohan
Lalitha Biswas



Antithrombin resistance assay

- Activate FII with Taipan venom + PL + Ca^{++}
- Can use ecarin in place of Taipan venom
- Incubate with excess AT over time
- Add chromogenic substrate for thrombin (S-2238)
- Measure absorbance change per minute
- Relative residual thrombin activity calculated as % of data at 0 min

viapath

Saw-scaled viper

(*Echis carinatus*)

Echistatin

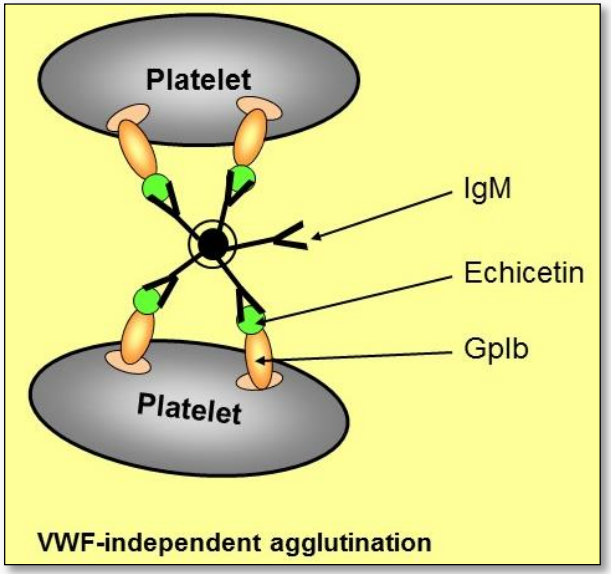
Strong competitive inhibitor of GPIIbIIIa binding to fibrinogen

Inhibits tumour cell adhesion

Echicetin

Agglutinates platelets via GPIb and IgM and induces signalling

- One IgM molecule can bind up to 5 molecules of echistatin
- Binding several molecules causes GPIb clustering
- Binding of echicetin to platelet surface causes VWF-independent agglutination



Brazilian Pit Viper or Jararaca

(*Bothrops jararaca)*

Botrocetin-induced platelet aggregation

VWF-dependent but only partly GPIb-dependent

Botrocetin partially aggregates some BSS platelets

can be used to differentiate from severe VWD

VWF:Botrocetin co-factor assay similar to VWF:RCo

Botrocetin & Echicetin in platelet aggregometry

Agonist	Organism	Platelet aggregation
Ristocetin		**VWF & GPIb dependent** Reduced/absent in VWD & BSS
Botrocetin		**VWF dependent** Reduced in VWD but some aggregation in BSS
Echicetin		**GPIb dependent** Reduced only in BSS

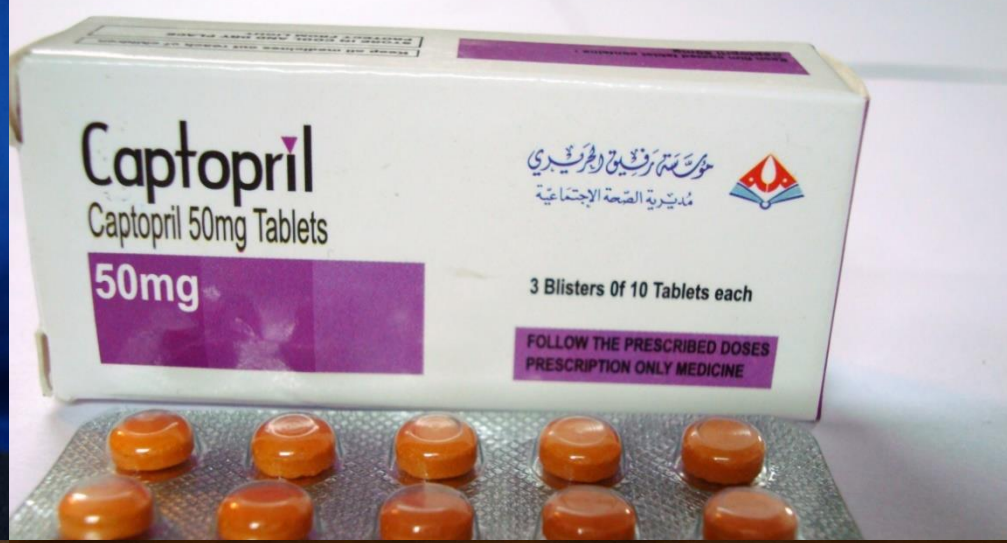
FITC-labelled echicetin can measure GPIb expression by flow cytometry

viapath


Alimentação
Feeding
Reprodução Vivípara
Reproduction Viviparous
Dentição Solenóglifa
Dentition Solenoglyphous
Hábito Diurno e Noturn
Diurnal and Noct

Synthetic version of an *E. carinatus* disintegrin

Non-peptide GPIIbIIIa inhibitor



Angiotensin-converting enzyme (ACE) inhibitor for treating hypertension

Developed from peptide in Jararaca venom (bradykinin potentiating factor)

Hindawi Publishing Corporation
Thrombosis
Volume 2010, Article ID 461238, 11 pages
doi:10.1155/2010/461238

Review Article

Porcine and Canine von Willebrand Factor and von Willebrand Disease: Hemostasis, Thrombosis, and Atherosclerosis Studies

Timothy C. Nichols,[1,2] Dwight A. Bellinger,[2,3] Elizabeth P. Merricks,[2] Robin A. Raymer,[2] Mark T. Kloos,[2] Natalie DeFriess,[2] Margaret V. Ragni,[4,5] and Thomas R. Griggs[1,2]

Ristocetin does not support agglutination of porcine/canine platelets by their own VWF

Botrocetin supports agglutination of porcine/canine platelets by their own VWF & by human VWF

Many veterinary laboratories only perform VWF:Ag

Type 1 VWD	Airedale, Akita, Bernese Mountain Dog, Dachshund, Doberman Pinscher, German Shepherd, Golden Retriever, Greyhound, Irish Wolfhound, Manchester Terrier, Schnauzer, Pembroke Welsh Corgi, Poodle, Shetland Sheepdog
Type 2 VWD	German Shorthaired Pointer, German Wirehaired Pointer
Type 3 VWD	Chesapeake Bay Retriever, Dutch Kooiker, Scottish Terrier, Shetland Sheepdog

Single form of VWD predominates in each breed

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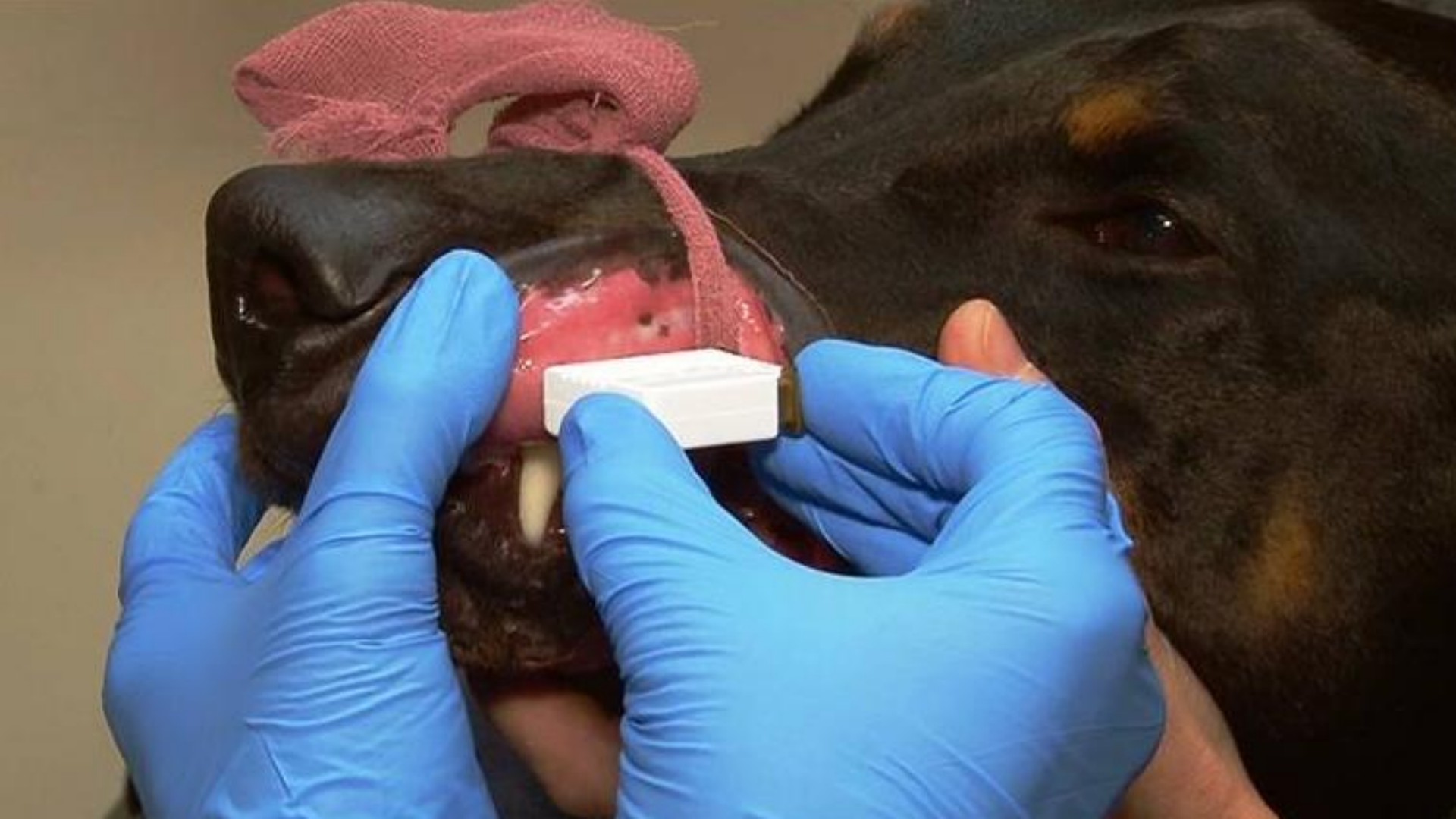


South American Rattlesnake or Cascavel
(*Crotalus durissus terrificus*)

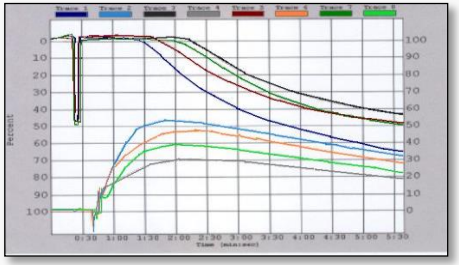
Convulxin

C-type lectin binds and clusters GPVI receptors

Induces aggregation & platelet prothrombinase activity

Also binds weakly to GPIbα

Convulxin in the laboratory

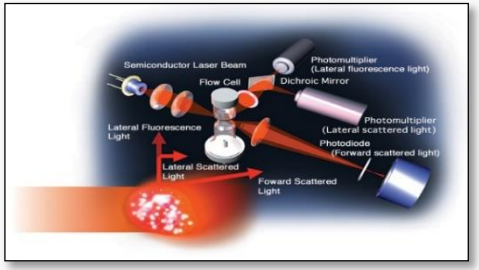


Platelet aggregometry

If reduced aggregation with collagen:
CRP & convulxin as agonists

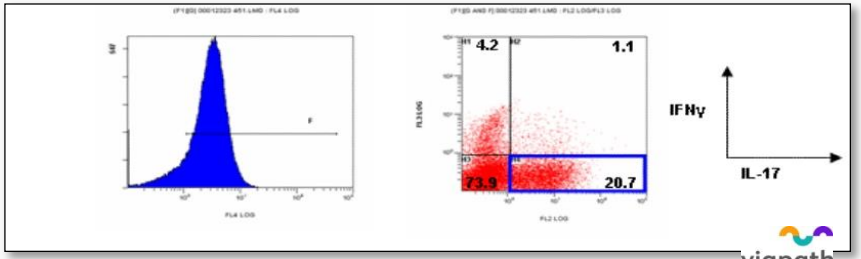
Reduced aggregation with CRP only: GPIa

Reduced with CRP & convulxin: GPVI



Flow cytometry

GPVI quantitation by flow cytometry
FITC labelled convulxin



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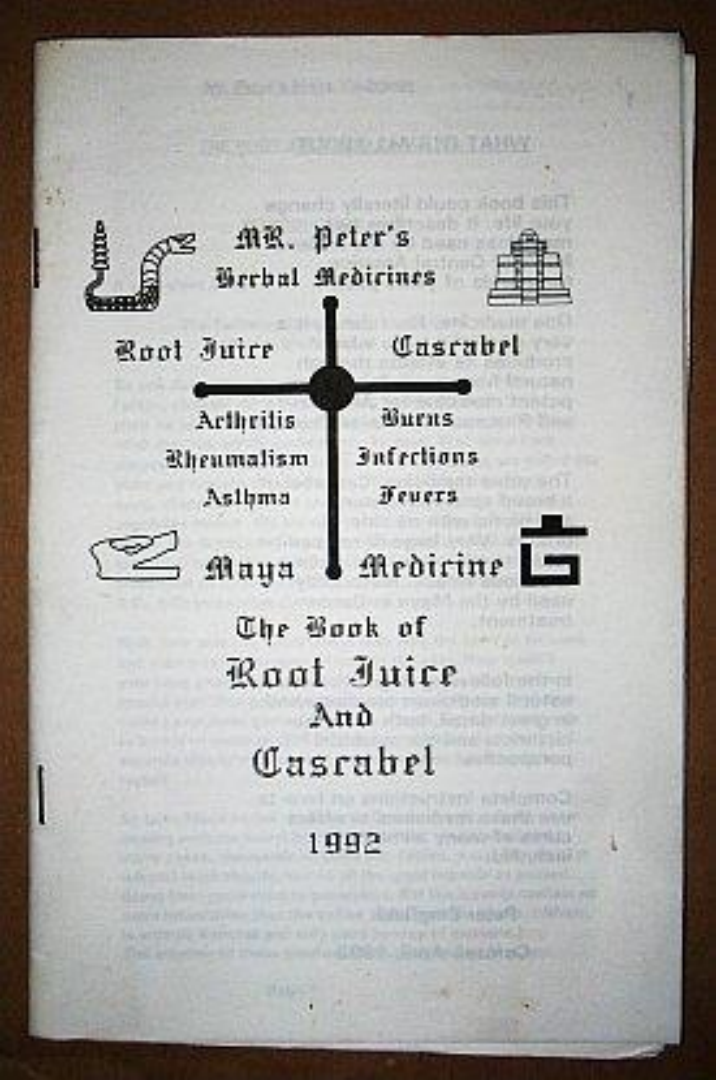


Cascabel medicine

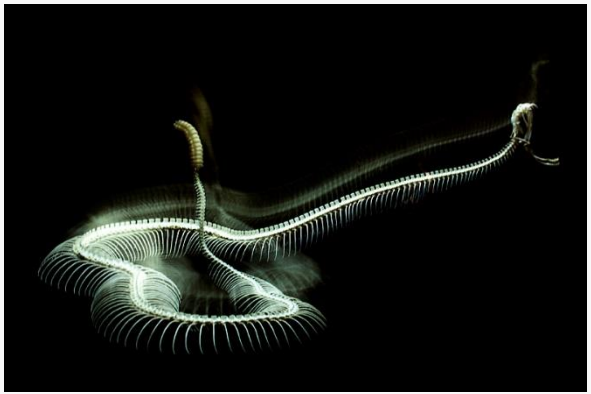
Made from the roasted bones of the snake

Snake carcass is prepared by careful cleaning, including elimination of skin, head and tail

Remainder cremated for an extended period

Resulting residue is then ground to a fine powder

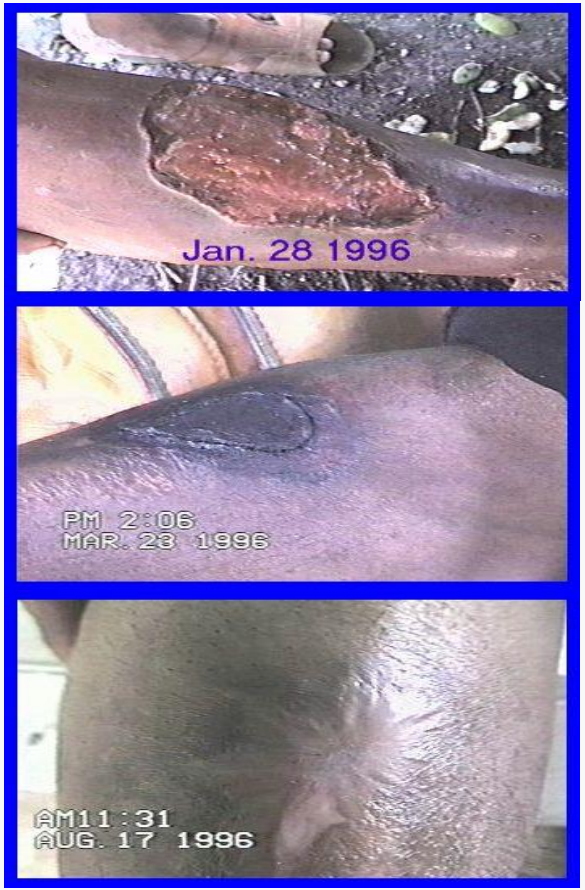
Powder taken orally & said to cure:



- cancer
- burns
- ulcers
- osteomyelitis
- gangrene
- AIDS
- infections
- asthma
- rheumatism
- arthritis

viapath

Diabetic ulcer treated with only oral cascabel over 7 months



Peter Singfield

"Medicine Man"

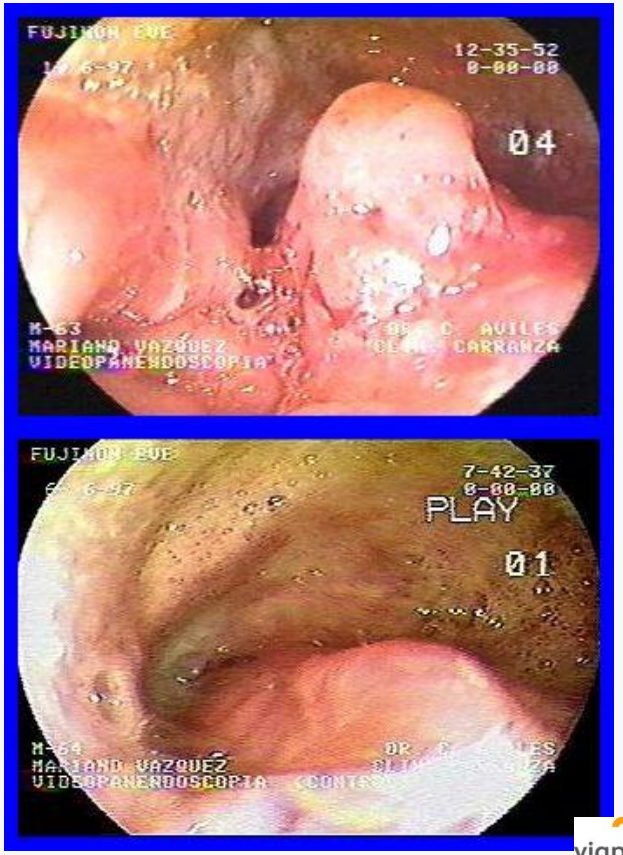
Xaibe Village

Corozal District, Belize, Central America

Tel 501-4-35213

Peter Singfield snkm@btl.net

Incurable stomach tumour treated with only oral cascabel over 4 months



US 20060240117A1

(19) **United States**

(12) **Patent Application Publication**

(10) **Pub. No.: US 2006/0240117 A1**

(43) **Pub. Date: Oct. 26, 2006**

Lecca

(54) **SNAKE POWDER EXTRACT FOR TREATMENT OF CANCER**

(76) Inventor: **Pedro J. Lecca**, Chevy Chase, MD (US)

Correspondence Address:
DELPHINE M. JAMES
2656 SOUTH LOOP WEST #170
HOUSTON, TX 77054 (US)

(21) Appl. No.: **11/336,630**

(22) Filed: **Jan. 20, 2006**

Related U.S. Application Data

(63) Continuation-in-part of application No. 10/306,958, filed on Dec. 2, 2002, now abandoned.

Publication Classification

(51) **Int. Cl.**
A61K 35/58 (2006.01)

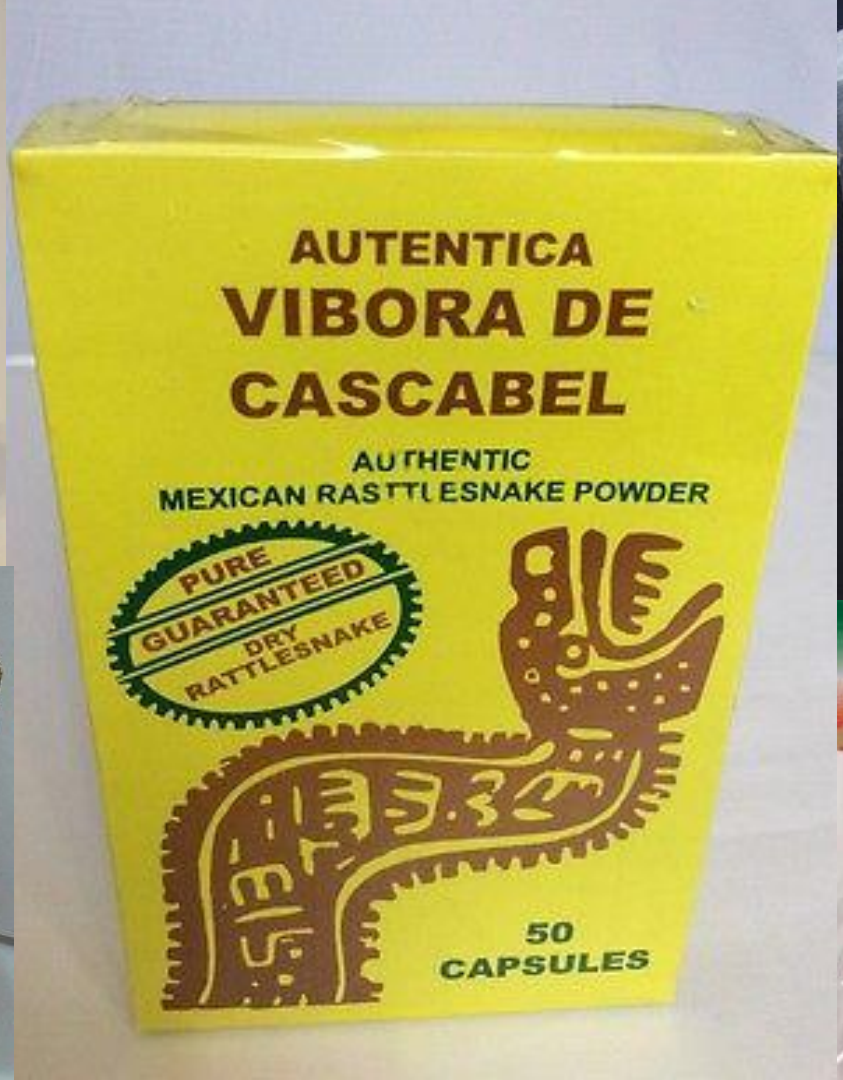
(52) **U.S. Cl.** **424/542**; 435/375

(57) **ABSTRACT**

The present invention relates to a pharmaceutical composition comprising snake powder that is derived from the Tzabcan "Crotalus durissus" rattlesnake. The snake powder is prepared by continuously baking the body of the rattlesnake until it completely dehydrates. Then, the dehydrated body is pulverized into a fine granular powder. The present invention included an in vitro method of inhibiting cancer cell growth utilizing the snake powder exhibited. Accordingly, the snake powder can be applied for the development of drugs which are effective for the treatment of various types of cancers.


SUPLEMENTO ALIMENTICIO
VIBORA DE CASCABEL
Crotalus lepidus
ACNE • CICATRICES • PURIFICA LA SANGRE Y EL HIGADO
CONTENIDO 150 CAPSULAS DE 500 mg c/u
SUPLEMENTO ALIMENTICIO
VIBORA DE CASCABEL
Crotalus lepidus
ACNE • CICATRICES • PURIFICA LA SANGRE Y EL HIGADO
CONTENIDO 150 CAPSULAS DE 500 mg c/u


VIBORA de CASCABEL
Suplemento Alimenticio Coatl
Cont. Neto: 100 cápsulas de 800 mg.
VIBORA de CASCABEL
Suplemento Alimenticio Coatl
Cont. Neto: 100 cápsulas de 800 mg.


AUTENTICA
VIBORA DE CASCABEL
AUTHENTIC
MEXICAN RATTLESNAKE POWDER
PURE
GUARANTEED
DRY
RATTLESNAKE
50
CAPSULES


Cápsulas Víbora de Cascabel
Contenido neto
150 Cápsulas de 400 mg


CHILE CASCABEL
CASCABEL CHILI PODS
Chilis • Snacks • Herbs

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Rattlesnake Soap - Jabon Aceite de Vibora (Pack of 3)

Unknown

4.0 out of 5 stars | 5 customer reviews

Rattlesnake soap aids in the treatment of skin rash, spots, boils, hives and all kinds of varicose ulcers

Combats blackheads, pimples, acne, skin blemishes, itchy sores and mosquito bites

Rattlesnake soap is also known to aid baldness

Extremely popular in Mexico and very difficult to find

This soap does not contain alkaloide, dye or acids, it is made of minerals and herbs

Price: $12.99

In Stock. Sold by KNS-Xpress and Fulfilled by Amazon. Gift-wrap available.

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