



The Clotting Times

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Editorial

Welcome to the first issue of The Clotting Times since the educational internet resource. CLOT-ED became a part of The ECAT Foundation. The Clotting Times is our regular newsletter and will be published twice a year and include news from the ECAT EQA programme, information about the CLOT-ED website and ongoing projects at ECAT (in this issue see the contribution by Dr. Verbruggen).

Our intention is to provide to you, through The Clotting Times newsletter, interesting and relevant topics/ideas about laboratory-related issues in the fields of thrombosis and haemostasis. The editorial board will always be open to your comments and suggestions. And now enjoy this new newsletter and your learning!

CLOT-ED A Tree (of Knowledge) Grows Up

Marlies Ledford-Kraemer, MBA, Former Owner www.CLOT-ED.com

CLOT-ED, as all ideas, began many years ago as I “learned” the joy of teaching. The seed was sown early in my career when I taught medical laboratory technology students. The years 1989-2001 saw an idea grow into a seedling as I, in my role at the University of Miami, sponsored thirteen annual educational symposia. Arising from these meetings was a sapling called *The Clotting Times*. It was a quarterly newsletter, first published in 2000, to educate laboratorians and clinicians about the advances made, from the laboratory perspective, in the fields of hemostasis and thrombosis. After leaving the University in 2002, this newsletter sapling became, in 2003, a young tree called CLOT-ED. CLOT, is an acronym (Coagulation, (fibrino)Lysis, Or Thrombosis) and of course ED means education. The logo depicts coagulation as an amplification process (coagulation enzymes represented by gold ovals) that results in thrombin generation and fibrin formation (formed fibrin clot represented by magenta oval). Those of you who know me well, have interpreted the logo as a cat’s paw (yes, Ms Bingo helped to write many articles in the late night hours with me)! My vision for the website was that it be an

educational resource devoted to providing concise and current information in the fields of haemostasis and thrombosis and to do so with excellence and creativity. I believe, for the most part, that bar was reached and especially so when articles by many wonderful contributors are added to the equation. This level of quality was appreciated by many educational institutions, which were using the website to teach laboratory medicine students, as well as residents and fellows in Hematology or Pathology. As CLOT-ED began to branch out over the years, it became obvious to me that one individual could no longer nurture this now maturing tree. Therefore I began a search for the right persons/organization who would share my vision for this tree of knowledge. I firmly believe that The ECAT Foundation, with its resources and network, will be able to allow this tree to continue branching out, flourish, and provide a wonderful canopy of information. I am delighted that they have taken this educational challenge. To all of you that have been loyal followers and readers of the CLOT-ED website, I encourage you to enjoy your learning experience at the tree’s new home!





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The growth of the CLOT-ED “tree of knowledge” can be compared to any tree as it matures but I am fond of the lignum vitae tree, which is a trade wood, also called guayacan (in Europe known as pockenholz). Other names for lignum vitae include palo santo (Spanish for “holy wood”) and greenheart. The wood from this tree was once very important for applications requiring a material with its extraordinary combination of strength, toughness and density. The wood is obtained chiefly from *Guaiacum officinale* and *Guaiacum sanctum* (pictured at left in the Florida

Keys), both small, slow growing trees. All species of the genus *Guaiacum* are now listed in Appendix II of CITES as potentially endangered species. “Lignum vitae” is Latin for “wood of life”, and derives from its medicinal uses; lignum vitae resin has been used to treat a variety of medical conditions from coughs to arthritis, and chips of the wood can also be used to brew a tea. Lignum vitae is also one of the numerous hard, dense woods referred to as ironwood. It is the densest wood traded and it will easily sink in water. Accessed from http://en.wikipedia.org/wiki/Lignum_vitae.

CLOT-ED, The Educational Resource Arm of ECAT

On January 1 2010 the ECAT Foundation took over ownership of the educational website CLOT-ED. Since the beginning of this year we have worked on incorporating the CLOT-ED website into the ECAT website (www.ecat.nl). CLOT-ED can be found easily in the main task bar at the left side of the ECAT website. The “new” CLOT-ED consists of two parts: an open-access area and a password-protected section. Any visitor to the ECAT website can view pages such as

Calendar, Corporate Corner, Links and Terminology. The password-protected part, can be found under “Education” and is only accessible to ECAT subscribers. Therein one can find extended educational information related to the field of thrombosis and haemostasis. Here you can view pages such as previous issues of The Clotting Times, Educational Topics, Focus Articles and Lab Pointers. We invite you to visit this new educational addition to the ECAT website.



The Corporate Corner page of the CLOT-ED Website

The Corporate Corner section of CLOT-ED became available June 1, 2010. On the Corporate Corner page you can find information about diagnostic companies and other companies with products and services related to the fields of thrombosis and haemostasis. For each company there is a direct link to their website. In the near future several companies will present their latest news and links to informative articles concerning their new products or methods. The Corporate Corner page will allow visitors to obtain more information about a company’s products, developments, studies and relevant literature. The advantage for the web visitor is that one can access

and have available a comprehensive overview of many companies on one web page. This will permit direct access to a particular company’s website without needing to know its URL address. Moreover one can compare and contrast the latest information from many different companies at this one easy location. The Corporate Corner will continue to expand and be updated regularly. The goal is to give laboratory professionals a portal for obtaining up-to-date and complete overviews of companies and their products which are provided by the companies themselves. When on the ECAT site, be sure to take some time to investigate the features of this new Corporate Corner.





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Frits Haverkate, pioneer of ECAT has retired

Not many of you will know the history of ECAT Foundation in detail.

Original Study

ECAT originally was the acronym for **E**uropean **C**oncerted **A**ction on **T**hrombosis. This Action was a cooperation between research Institutes within the European member states, financially supported by the EU in Brussels. Normally, a Concerted Action took only a few years, but the ECAT lasted approximately 12 years, from 1981 – 1993, thanks to repeated attempts of the ECAT Project Leader (Dr F. Haverkate, Gaubius Institute, Leiden, NL) to finalize the epidemiological studies. The objectives of the studies were to trace haemostasis risk factors of both arterial and venous thrombosis. To reach this goal, three large multicentred, epidemiological studies were performed, i.e. Angina pectoris Study, PTCA Study and DVT Study.

Within the framework of the ECAT a quality control on the performance of 10 haemostasis assays was necessary in 20 laboratories, as each of these laboratories had to perform the assays on the blood of patients recruited in the ECAT studies. To that end samples were sent to the laboratories each half year during 2.5 year. To harmonize the procedures, technicians from each laboratory were trained in Leiden in advance.

To a Quality Control accessible to more laboratories.

The need existed for a quality control in more laboratories, i.e. outside those participating in the Angina pectoris study. This idea came up for the first time in 1988, when John Davidson and Isobel Walker from the Royal Infirmary in Glasgow, UK, Cas Kortmann from

Kordia, Leiden, NL and Frits Haverkate, from TNO, Leiden discussed the topic during the International Fibrinolysis Congress in Amsterdam. It was decided to focus on thrombophilia assays, to perform the work in Glasgow, to support the project financially by the ECAT. The latter had to be approved by the CEC in Brussels. The president of the ECAT Advisory Committee did not see the link between quality control and the objectives of the ECAT, but after some correspondence and crosstalk, the ECAT Project Leader began the extensive European Quality Control. The first exercise was on 1 October 1990, and included 4 thrombophilia assays i.e. Protein C, Protein S, Antithrombin and APC Resistance. The number of participating laboratories in- and outside the Angina pectoris Study was 36. Isobel Walker and Jim Conkie in Glasgow managed the project. An advisory board consisting of RM Bertina, AMPH van den Besselaar, JF Davidson, J Jespersen, C. Kortmann, SG Thompson and F. Haverkate met regularly in Glasgow.

End of the ECAT in Brussels.

At the end of 1993 the Commission of the European Communities in Brussels decided to terminate financing of the ECAT. It implicated that all activities came to an end. However, it was decided to continue the quality control of haemostasis assays under the following conditions:

The name ECAT remains for the quality control of laboratory assays.

The ECAT will continue as a Foundation without making profit.

The participating laboratories pay an annual contribution to cover the costs.





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The management remained in Glasgow and the Board of the Foundation existed of F. Haverkate, A. de Oude and I. Walker. A. de Oude helped the ECAT at its first independent steps considerably both with administration and a loan as first capital. The amount could be reimbursed within 3 years.

To an independent ECAT

In January 1996 the daily activities were moved from Glasgow to Leiden, NL, as the organisation of the work in 2 countries was too complicated. Moreover, the ECAT became independent of the industry, i.e. of Kordia (C. Kortmann) and of Nodia (A. de Oude). It has to be mentioned that the number of participating laboratories during the "Glasgow period" (1990 – 1995) grew from 36 to 65.

From 1 January 1996 Piet Meijer became the executive Director of the ECAT, the daily work including the exercises and the statistical elaboration of the results were prepared in the Gaubius Laboratory, TNO, Leiden, NL. A new Board was nominated i.e. C.Kluft, chairman, F. Haverkate, vice-chairman, M. de Maat, secretary, A van den Besselaar, J. Hoffmann and H.C. van Houwelingen.

ECAT in 2010

The ECAT has in 2010 more than 1000 participants, 80 % in Europe. One third of the participants are individual participants, the others form part of national organisations in Austria, Czech Republic, Denmark, Germany, Norway, Spain, Sweden, USA, Australia and other countries; the participants remain however, independent.

From the brief history above, it is clear that Frits Haverkate was the instigator and also the successful leader of it all. He remained active in the board of ECAT up till recently in 2010, watching

closely that his baby grew up prosperous and he acted/corrected when he felt it necessary. He decided this year that he was fully confident that the ECAT was grown up and could stand on its own feet. As a good parent he left us alone now in daily practice, and retired, but we know that he remains close by.

We are very much indebted to him, and wish him very many more years in good health.

In his honour we created four years ago the "Haverkate Lecture" to be issued during each biennial participant meeting.

The Board in 2010:

C. Kluft, chairman
M.P.M. de Maat, vice-chairman/secretary
D. van Cuilenburg, treasurer
H.W. Verbruggen
R. Niessen
F.J.M. Haas
O Paauwe-Insinger

Director
P. Meijer



Dr. Frits Haverkate (2010)



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ECAT EQA Programme

The ECAT Foundation, an **E**xternal quality **C**ontrol programme for **A**ssays and **T**ests with a focus on thrombosis and haemostasis, began in 1994. The first module included only thrombophilia markers: Antithrombin, Protein C, Protein S and APC Resistance. Today there are 23 modules covering a wide range of haemostasis parameters.

The programme started in Western Europe with about 40 participants. At present more than 1050 different laboratories from 29 different countries around the world take part in the external quality assessment (EQA) programme of the ECAT Foundation. Figure 1 shows the increase in number of participants since the ECAT programme was established.

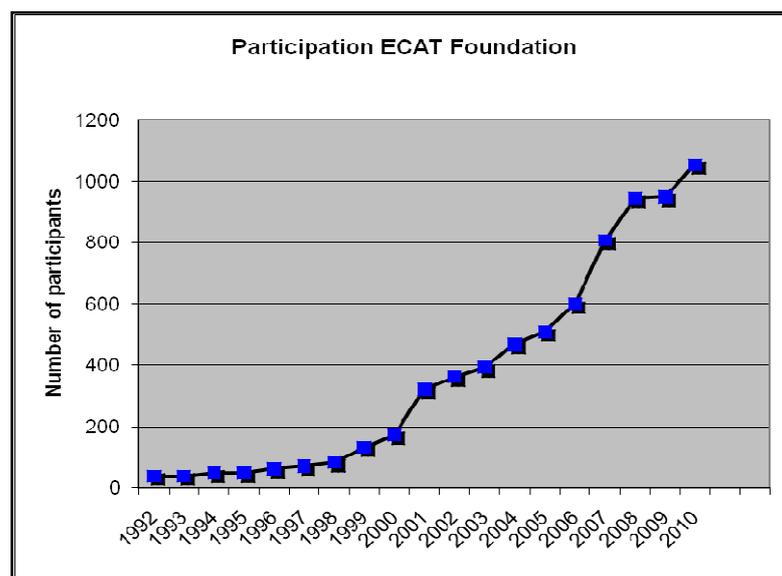


Fig. 1: The growth in the number of participants since 1992.

The ECAT external quality assessment programme focuses mainly on more specialised haemostasis assays.

The table that follows shows the current programme, including the number of participants per module.

The Molecular Biology modules are a cooperation with the German EQA organisation DGKL.

The Post-Analytical Platelet surveys are a cooperation with the North-American Specialized Coagulation Laboratory Association (NASCOLA).





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Table 1: The ECAT quality assessment programme in 2010

Programme Modules	No. of Participants
Thrombophilia module Antithrombin (activity and antigen), Protein C (activity [chromogenic and clotting] and antigen), Protein S activity, Protein S antigen (total and free), APC Resistance	323
Protein C Pathway Test	16
Lupus Anticoagulant / Antiphospholipid Antibodies	428
D-Dimer	630
Coagulation Factor module I (Factor VIII, IX, XI and XII)	216
Coagulation Factor module II (Factor II, V, VII and X)	186
Von Willebrand Factor module (antigen, activity, collagen binding, multimers, Factor VIII)	236
Factor VIII inhibitor	217
Thrombin Generation Test	45
HIT	205
Homocysteine	73
Factor XIII	90
Fibrinolysis parameters (Plasminogen, Antiplasmin, t-PA, PAI-1)	50
Post Analytical Platelet Function EQA (electronic survey)	80
Molecular Biology Set A FV-Leiden, Prothrombin, MTHFR (C677T, A1298C), PAI-1 4g5g	72
Molecular Biology Set B FXIII V34L, GPIIIa, βFib g-455a, VKORC1 (g-1639a/c1173t), FXII c46t, FV H1299R	9
Molecular Biology Set C a1 PI, Apo E, Apo B100, ACE, CETP	24
Molecular Biology Set D TPMT, Cyp2D6, Cyp2C8 (K399R), Cyp2C9 *2/*3, UGT1a1 (*28), DPD Exon 14 skipping, BCHE A/K	11
Molecular Biology Set E HFE (H63D, C282Y, S65C), LCT c-13910t, NOD2 (R702W, G908R, L1007fins C)	24
Molecular Biology Set F M. Wilson ATP7B-C3207 A, FSAP (Marburg-I), ITGA2 Gplalla C807T	0
Molecular Biology Set G K-Ras: Codon 12/13/61	5
DNA Sequencing (Sequencing and diagnostic interpretation)	6
DNA Isolation (DNA isolation and FV genotyping)	16

In 2010 a pilot study will be undertaken on heparin monitoring (unfractionated heparin [UFH] and low molecular weight heparin [LMWH]) as

well as the measurement of new anticoagulant drugs. These parameters will be added as new modules for the EQA programme in 2011.



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ECAT Participants' Meeting 2010

The ECAT Foundation will organise the 7th ECAT Participants' Meeting on 11 and 12 November 2010.

The programme will include the following topics:

- FVIII Inhibitor Testing
- Lupus Anticoagulant Testing
- Fibrinolysis
- Anticoagulation Testing
- Von Willebrand Disease
- Platelet Function Testing
- Case Studies
- Quality Management

The Haverkate Lecture will be given by **Dr. James Westgard** and is entitled: **Quality Planning in the Haemostasis Laboratory**.

In conjunction with the symposium a workshop on platelet function testing will be organised. The scope of this workshop is to investigate the applicability of different platelet function tests for the screening of bleeding disorders and platelet function disorders. This workshop will be held on 10 November 2010.

Two additional courses will be organised: one on case studies in haemostasis and the other on EQA result evaluation and troubleshooting. The courses will be given on 11 November 2010.

The venue is the Holiday Inn Hotel in Leiden.

More information will be available soon at the ECAT website.

Results of a Workshop on the Inter-laboratory Variability of Analysis of Type 1 Factor VIII Inhibitors

*Bert Verbruggen, PhD
Piet Meijer, PhD*

Introduction

Factor VIII (FVIII) inhibitors are immunoglobulin's that recognise functional epitopes on the FVIII molecule, thereby inhibiting the functionality of the protein. The origin of the inhibitors may be autologous or allogenic.

All FVIII inhibitor assays are based on a universal principle of measuring the decrease of FVIII activity in a mixture of an exogenous source of FVIII (e.g. normal pooled plasma) and the inhibitor containing plasma relative to a reference measurement with the same method substituting the patient

plasma by a control plasma sample that does not contain FVIII inhibitors. Residual FVIII activities in the assay mixtures are measured by either one-stage-based clotting assays (mostly aPTT) or chromogenic assays.

The inter-laboratory coefficient of variation for the FVIII inhibitor assays in successive ECAT surveys appears to be more than 40 % without a tendency of improvement. However, there is an urgent need for an improvement as reliable inhibitor measurements, especially in the lower range, are needed for early detection of inhibitors (related to better outcome of therapy), monitoring of treatment, detection of eradication of inhibitors and for epidemiologic studies.





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Therefore the ECAT Foundation, in cooperation with the Radboud University Nijmegen, planned to organise a workshop on Factor VIII inhibitor testing.

Prior to this workshop an external survey with a set of 7 different samples was organised in 51 different laboratories. Also in this survey in all samples a high inter-laboratory variation was observed (35 – 70%), and several participants have measured a positive inhibitor titer in the negative control sample. Based on these results we selected

participants of 15 different laboratories which showed a wide variation in test results as well as variation in test methodology. These participants were invited for the 3-days workshop. The aim of the workshop was to investigate possible causes of the observed high inter-laboratory variation. This workshop was organised from 11 – 13 November 2009.

The samples (see Table 1 for an overview) from the survey were also used in the workshop.

Table 1: Overview of the test samples used, including the inhibitor titer of the samples as established during the preparation of the samples.

SAMPLE	Inhibitor Titre BU/mL	DESCRIPTION
1	1.60	Monoclonal Ab against C2-domain
2	0.81	Monoclonal Ab against A1-domain
3	1.40	Moderate titre patient sample
4	0.74	Low titre 1:1 dilution of sample 3
5	1.94	Moderate titre patient sample
6	15.4	High titre polyclonal inhibitor
7	-	Negative control

Workshop sessions

In addition to the external survey (session 0) four different sessions were performed during the workshop. In session 1 the assays on the 7 samples were repeated using the same reagents, dilutions and test conditions as in the home situation. The only exception was that during the workshop all FVIII measurements were performed on STA analyzers.

In session 2 all participants used buffered normal plasma as source for FVIII and FVIII deficient plasma as control sample. The participants used the same normal plasma that they also used in the survey; it was buffered by the participant unless it was indicated on the insert that it was already buffered by the manufacturer. If participants did already use FVIII deficient plasma in the previous session their own reagents were used.



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Otherwise universal FVIII deficient plasma was provided. The participants used the same sample dilutions as in the previous session and their own reagents were used for FVIII measurement.

In session 3 all participants used universal buffered normal pooled plasma, universal FVIII deficient

plasma, universal sample dilution but their own reagents for FVIII measurement.

In session 4 the same approach as in session 3 was used but with universal reagents for FVIII measurement. An overview is given in Table 2.

Table 2: Summary of the set-up of the different sessions.

SESSION	DESCRIPTION
0	Pre-workshop survey / home method
1	Repeat home method on central location, dilutions as used in survey
2	Buffered (own) NP / FVIII def plasma as control sample / dilutions as used in the pre-workshop survey / own reagents for FVIII measurement
3	Buffered universal NP / universal FVIII def plasma as control sample/ universal dilutions/ own FVIII deficient plasma and reagents for FVIII measurement
4	Nijmegen assay with universal reagents / universal dilutions / universal reagents for FVIII measurement

Results

The mean inhibitor activity of the inhibitor-free sample, sample 7, turned to zero when buffered normal plasma was used by all participants in sessions 2, 3 and 4. However in assays with non-buffered normal pool plasma (sessions 0 and 1) the mean inhibitor activity was 0.2 BU/mL with peak levels up to 1.25 BU/mL. These results clearly show that buffering the normal pool plasma strongly increases the specificity of the assay as was already reported years ago by Giles.

The inter-laboratory coefficient of variation (C.V.) of the different inhibitor-positive samples did not improve in session 2 compared to the survey indicating that buffering of normal pool plasma is not the only determinant for correction of the inter-laboratory variation. In all samples there was a clear correlation between the rate of the used dilution and the inhibitor activity indicating a major effect of the dilution step on the inhibitor assay results.

The C.V. of the inhibitor-containing samples significantly improved to 10-20% in session 3 where further standardisation was reached by the use of universal dilutions in all samples.



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A further decrease of the C.V. to 5-13% was realised by also standardising the reagents for the FVIII assay. This is acceptable for this type of complex methods and rules out a major operator influence on the C.V. of the assay.

The low C.V. in session 4 compared to session 3 may indicate an effect of the FVIII reagents on the inhibitor activity.

Conclusion:

For the measurement of FVIII inhibitors it is essential to:

Use buffered normal pooled plasma as a source for FVIII for optimal specificity.

Use FVIII deficient plasma (or 4% BSA solution) as control sample.

Use the lowest dilution factor as possible to obtain optimal reliable residual FVIII levels (20 – 50%).

Factor VIII assay reagents may influence inhibitor results.

