The Clotting Times

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Editorial

This is the second issue of The Clotting Times in 2010. Again an issue with interesting information about laboratory- and quality control-related issues in the fields of thrombosis and haemostasis.

In November we organized our 7th ECAT Participants' Meeting. In this issue you will find report about this meeting. A special report is included on the organised pre-symposium wet workshop on platelet function testing (see the contribution of Dr. C.M. Hackeng). In this issue you can also find an abstract of the publication "External Quality Assessment for Thrombin Generation Tests: An Exploration", recently published in Seminars of Thrombosis and Hemostasis.

The editorial board wishes you a healthy 2011 and a good educational time by reading this newsletter.

7th ECAT Participants' Meeting

On 10 – 12 November 2010 the 7th ECAT Participants' Meeting was held in Leiden, The Netherlands.

On Wednesday November 10 we started with a wet workshop on Platelet Function testing. Twenty participants took part in this workshop. Three companies (Kordia Life Sciences, Multiplate and Siemens) kindly supported the workshop and provided four devices: the Chronolog whole blood aggregometer, Accumetrics VerifyNow, PFA-100 and the Multiplate. It was a challenge to organise such a workshop using fresh whole blood from 8 different patients.

Platelet workshop

We wish to thank Dr. Eikenboom from the Leiden University Medical Center for his wonderful contribution to this workshop by recruiting interesting patients and performing the blood collection just before the start of the workshop. Together with Dr Hackeng from the St. Antonius Hospital in Nieuwegein, he providedexcellent support during the workshop. During the symposium Dr. Hackeng gave a comprehensive report on the outcome of the workshop. A summary of this presentation is included in this issue of The Clotting Times on page 5.

Dr. Hackeng and Dr. Eikenboom

The next morning two separate courses were organised. The first course, case studies in Thrombosis and Hemostasis, was given by Dr Devreese and Dr Schouwers from Ghent, Belgium. They presented in total 8 different cases with interesting challenges for the laboratory.

Course

Using an audiovisual response system, participants in this course were able to actively participate in the presentation.

The second course was on the interpretation of external quality control results and trouble-shooting based on these results, given by Dr. Meijer, the director of the ECAT Foundation.

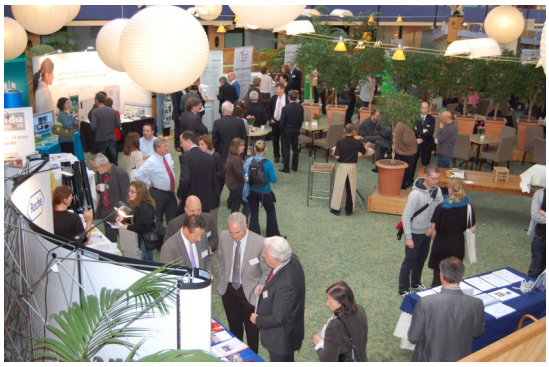
On Thursday afternoon November 11 and Friday November 12, the 7th ECAT symposium was held. In total 190 participants took part in this symposium. A variety of different topics were discussed, such as Fibrinolysis, Lupus Anticoagulant Testing, Von Willebrand Disease, Anticoagulation Testing, Case studies in Thrombosis and Bleeding Disorders, Factor VIII Inhibitor Testing, the use of biological variation in quality control, interpretation of quality control data and many others.

Symposium

A highlight of the meeting was the Haverkate Lecture, this time given by Professor James Westgard, entitled: "Quality Planning in the Hemostasis Laboratory". This presentation describes a plan for analytical Quality Management that integrates many critical steps such as the definition of quality requirements, selection of measurement procedures to assure tracebility, validation of measurement procedures to assure performance, design of statistical QC procedures to verify the attainment of the intended quality of test results and development of an Analytical QC Plan on the basis of a risk analysis of the laboratory testing process.

Dr. Haverkate and Dr. Westgard

In January 2011 most of the lectures will become available in the educational part of the CLOT-ED section on the ECAT website.



Exhibition area

Questionnaire on Courses and Workshops

The ECAT Foundation is a so-called educational external quality assessment programme. This means that one of the goals of ECAT is to assist laboratories in their duties for quality improvement. One of the manners to give substance to this is to organise courses and workshop. Up to now we organised several courses and workshop in conjunction with our biennial symposium. These courses and workshops are very well received by the participants. Therefore we would like to investigate whether participants are interested in further extension of our programme on courses and workshops.

We have conducted a questionnaire to examine your opinion on this topic. Completion of this questionnaire takes only 5 minutes. You can find this questionnaire in the member section at our website (www.ecat.nl). Select in the member section the option questionnaires followed by the questionnaire Courses and Workshops.

We highly appreciate if you can complete this questionnaire before February 28th, 2011.

Thank you very much for your co-operation.

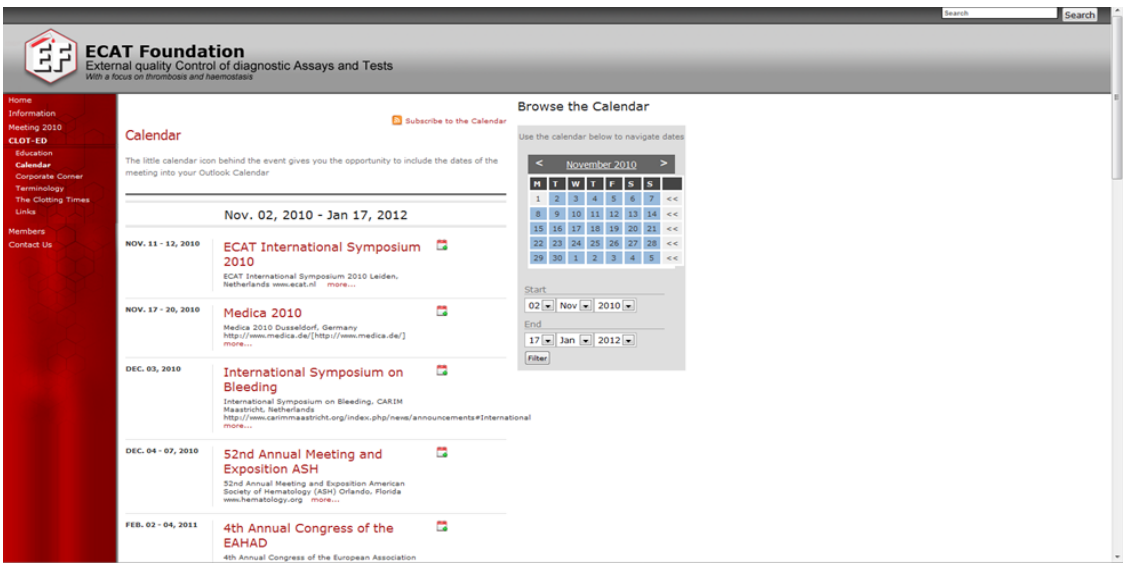
CLOT-ED™
Coagulation Lysis Or Thrombosis

Corporate Corner page of CLOT-ED Website

In issue 1 we explained the portal function of the Corporate Corner. In summary, the Corporate Corner provides you with easy access to the web-sites of diagnostic companies in the field of thrombosis and haemostasis. Currently several companies already contribute to the Corporate Corner. Please take a look at the website and experience the convenience of having a range of companies in one overview (http://www.ecat.nl/corporate-corner-2/). The following companies are available in the Corporate Corner:

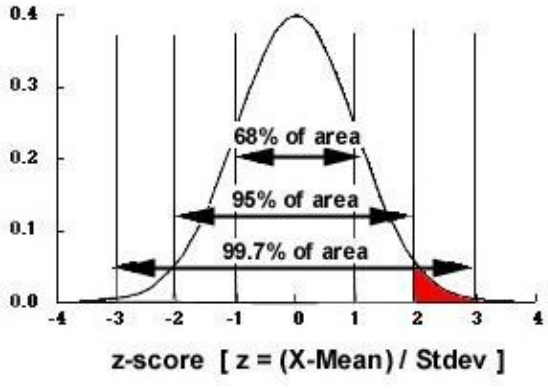
Calendar

The Calendar section of CLOT-ED became available on April 1, 2010. In the Calendar you can find International Meetings and Congresses concerning Thrombosis and Haemostasis. Each event has a direct link to the related website. This Calendar is useful for accessing a comprehensive overview of upcoming International Congresses and Meetings and details of the event-related websites. It is therefore an easy way to get general information, and to see the programme and register. The calendar will be updated on a monthly basis. The goal is to give laboratory professionals an easy tool for surveying upcoming International events. If you would like to have a meeting included in the Calendar, please contact the editor-in chief for further information. When on the ECAT site, be sure to take some time to investigate the features of this new Calendar.



Interpretation of the Z-score

The task of an external quality assessment programme is to assess a participant's performance for a particular method. The main focus here is to determine the deviation of the participant's result from the "true value". In the field of thrombosis and haemostasis one is not able to assess true value because there are no reference methods and primary calibrators. Therefore the "true value" for a particular parameter in a particular survey is considered to be the mean value of all the results, after the exclusion of outliers. This is the so-called consensus value. The deviation of a participant's results from this consensus value can be expressed by the Z-score. The Z-score is an individual performance indicator and represents the distance between the consensus value and the participant's result expressed as a multiplier of the standard deviation. For instance, if the consensus value is 100 U/dL, the standard deviation is 7.5 U/dL and the participant's result is 110 U/dL, the Z-score being (110-100)/7.5 = 1.33. The Z-score can either be positive or negative depending on whether the participant's result is higher or lower than the consensus value. The major question is which Z-score represents an acceptable performance and which Z-score does not.



The ISO Guide 13528 (*Statistical methods for use in proficiency testing by inter-laboratory comparisons*) indicates that a Z-score < -3 or > 3 means an unacceptable result and should be seen as an action signal. A Z-score between -3 and -2 or 2 and 3 means a warning signal. An action or warning signal in two successive rounds should be taken as evidence that an anomaly has occurred that requires thorough investigation and where possible corrective action should be undertaken.

We hope this information helps you with the interpretation of the Z-scores represented in reports from the ECAT external quality control programme.

External Quality Asessment for Thrombin Generation Tests: An Exploration

Since 2007, the ECAT Foundation provides external quality control surveys for thrombin generation.

Recently remarkable observations from these surveys were summarised in a publication in Seminars in Thrombosis and Hemostasis. Here the summary of the publication is given.

External Quality Control of Diagnostic Assays and Tests (ECAT) surveys on thrombin generation tests (TGT) have shown that various assays have more than 30-fold difference in time to peak (TTP). The survey included pooled normal plasmas, microparticle (MP)-depleted plasmas, and factor (F)XII-deficient patient plasma. Between 4 and 11 laboratories participated per test; analyzed were a time (TTP) and a quantity variable, the area-under-the-curve (AUC) of the thrombin generation test. MP depletion of plasma showed a progressive increase in TTP, up to 29%, with a decreasing amounts of tissue factor, the trigger of the TGT via the extrinsic pathway. The same was found for the AUC with the largest decrease of 38%. It was observed that MP depletion showed large individual differences. The FXII-deficient plasma showed no effect on TTP for rapid tests (a TGT with a high tissue factor concentration), but for slow tests (a TGT with a low tissue factor concentration) it increased from 248 to 331%. The AUC declined gradually the slower the test, reaching a decline of 85%.

The effects of FXII deficiency were not mimicked by the addition of corn trypsin inhibitor but were confirmed by inhibiting activated FXI. Interlaboratory variability was between 11% and 57% for all methods, showing differences mainly related to the use of normal pooled plasma. The different sensitivities of TGTs to MPs and contact activation predict that they will associate differently with clinical situations, according to which of these aspects are important. Future ECAT surveys should include samples with variation in MPs and contact activation to match with features of the TGT variants.

For further information, please read the complete publication: Kluft C, Meijer P. External quality assessment for thrombin generation tests: an exploration. Semin Thromb Hemost 2010;36:791-6.

Wet Workshop on Platelet Function Testing

C.M. Hackeng, Department of Clinical Chemistry, and St Antonius Center for Platelet Function Research, St Antonius Hospital, Nieuwegein, the Netherlands.

Preceding the biennial ECAT symposium, a workshop on platelet function testing was held on November 10, 2010. The Workshop was open to a maximum of twenty ECAT participants and they came from Belgium, Denmark, France, Germany, Norway, Poland, the Netherlands, Sweden, Turkey and the United Kingdom. Organizers Dr. P. Meijer and Dr. H. Verbruggen arranged that Dutch representatives of Siemens, Chronolog (Kordia Life Sciences), VerifyNow (Kordia Life Sciences) and Multiplate (Nodia) provide the following whole blood platelet function analysers:

-Point-of-care: PFA 100 using collagen/ADP (COL/ADP) and , collagen/epinephrine (COL/EPI) cartridges and the P2Y12 specific Innovance P2Y* for clopidogrel monitoring
-Point-of-care: VerifyNow (P2Y12 and Aspirin)
-Whole blood aggrometer (WBA): Chronolog aggregometer, determining aggregation by impedance and ATP release via luminescence (using various agonists)
-Whole blood aggregometer (WBA): Multiplate aggregometer (using various agonists)

Dr. J.M. Eikenboom (Leiden Universital Medical Center) asked some of his patients to donate blood one hour before the workshop. The participants, divided into 5 groups, were asked to diagnose these patients on basis of a short anamnesis and results from the platelet function tests. The following patients and controls were selected:

-Two related patients with von Willebrand Disease type 2A
-A patient with von Willebrand Disease type 2B
-A patient with Glanzmann's Thrombasthenia (GT) type II (qualitative disorder)
-A patient with Essential Thrombocythemia (ET) (500 x 10^9/L) taking aspirin
-A normal control (screening for a fictitious kidney biopsy)
-A normal individual taking aspirin
-A normal sample spiked with the P2Y12 antagonist Cangrelor (an ADP receptor antagonist) at a suboptimal concentration.

Together with the diagnostic companies, the different groups performed platelet function tests, were trained in the different devices, and were tutored about the principles of the technique. Using all test results, attempts were made to identify the various samples and explain any discrepancies from the actual diagnoses. The discussion was intense and the temperature in the room rose to high levels! The setup of the workshop had some flaws, as the VerifyNow assay and the PFA-100 Innovance P2Y* were developed to measure platelet inhibition, and would normally not be used to screen patients with a bleeding diathesis. The same is true for a patient on antiplatelet therapy, in which a ristocetin induced platelet aggregation test might be considered obsolete.

For the normal control all results were as expected, with only a slightly prolonged PFA-100 result. One group attributed this to a uremic thrombopathy, as the patient was (hypothetically) to be screened for a kidney biopsy.

The two sisters with VWD 2A gave the expected results for all devices: Multiplate and Chronolog gave normal results for all agonists except the high concentration ristocetin, which was low. All PFA-100 results, including the Innovance P2Y*,

gave prolonged closure times due to the vWF dependent shear induced platelet activation. VerifyNow, well known to be independent of vWF, gave normal results.

The patient with VWD2B had a thrombocytopenia (30 x 10^9/L) as a complicating factor. Surprisingly, both whole blood aggregometers still gave interpretable results. Although reponses to all agonist were low, which is expected due to the low platelet count, the response to low dose ristocetin (absent in normal individuals) pointed to a VWD 2B for both the Chronolog and Multiplate. As might be expected, PFA100 (due to low platelet count and/or low vWF activity) and VerifyNow (low platelet count) gave abnormal or non interpretable results.

The patient with Glanzmann's Thrombasthenia has a qualitative disorder of the platelet glycoprotein IIb-IIIa (GPIIb/IIIa) receptor. Although the aggregation pattern when using light transmittance aggregometry was characteristic for GT, the Multiplate (TRAP agonist) and Chronolog (collagen agonist) devices still showed some residual aggregation. Due to GP IIb/IIIa mediated adhesion to the electrodes, ristocetin induced aggregation was also impaired severely. PFA-100 showed "no-closure" for all tests, as would be expected. Both VerifyNow tests showed decreased aggregation responses.

The normal individual taking aspirin gave results as might be expected. Multiplate showed normal results with all agonists except arachidonic acid, which was impaired. Chronolog corporation claims that arachidonic acid is too sensitive for aspirin detection therefore they prefer to use low and high concentrations of collagen to determine aspirin response. The low concentration (1 μg/ml) gave a good response, however, its relationship to the high concentration (5 μg/ml) suggested only a partial aspirin response. The PFA-100 showed normal closure times for COL/ADP and Innovance P2Y*, and "no closure" for COL/EPI, in agreement with a good response to aspirin. The VerifyNow Aspirin test pointed to a good response to aspirin, with a "normal" value for the P2Y12 test.

The patient with Essential Thrombocythemia on aspirin showed a high reponse to all agonists with the Multiplate and Chronolog whole blood aggregometers, including reagents for aspirin testing (arachidonic acid in Multiplate, low collagen 1 μg/mL for Chronolog). This can be explained by the thrombocytosis and the known lesser response to aspirin in these patients. The VerifyNow Aspirin test reported a value that should be interpreted as aspirin resistance, and only the PFA100 (COL/EPI) gave prolonged closure times, indicating a good response to aspirin. The conclusion for this patient was that Thromboxane B2 measurements should point out whether this patient responds to aspirin.

The normal sample spiked with Cangrelor was meant to mimic a patient with P2Y12 receptor blockade without the use of aspirin. The concentration used (0.1 μmol/L) was considered suboptimal, as the therapeutic concentration is 0.5 μmol/L. The Multiplate device showed low responses to all agonists, including arachidonic acid. Also the Chronolog device pointed to aspirin use (by the low response to collagen 1 μg/mL) in addition to an expected low response to ADP. This incorrect clue to aspirin use was not observed with the point-of-care tests: PFA-100 showed a non-closure for Innovance P2Y* and a normal closure time for COL/EPI. As it is well known that COL/ADP is not sufficiently sensitive for P2Y12 blockade, the results for this test were inconclusive. The VerifyNow device showed a strong P2Y12 inhibition with a normal result in the VerifyNow Aspirin.

Overall results from this workshop showed that all groups reached the correct diagnoses for the patients that were tested, with some variation because participants were unable to perform further testing. Results from this workshop showed that patients with a bleeding diathesis can be screened properly by the PFA-100, and diagnosed correctly with whole blood impedance aggregometry (Chronolog and Multiplate). The point-of-care instruments for monitoring antiplatelet therapy appear to be sufficiently sensitive in their detection of the agents used, although some discrepancies between the different tests were observed. However, our exercise was limited in scope and laboratories should consult the literature and instrument manufacturers before drawing a final conclusion as to which devices should be used for screening patients with bleeding symptoms and monitoring those receiving antiplatelet therapy.