The 2010 Stago User Group Meeting was held at Crowne Plaza Coogee Beach, NSW in May. It wasn’t quite hot enough to hit the beach but over 120 customers attended each of the two days and participated in the conference that included 20 scientific presentations.

Our presenters included Prof Paul Monagle (Royal Children’s Hospital), Associate Professor Chris Ward (Royal North Shore Hospital), Dr Erwan Choblet (Noumea Regional Hospital), Roslyn Bonar (RCPA), Stago colleagues from France and Australia, and a number of our esteemed Australia and New Zealand customers.

During the conference we were pleased to present a selection of recent Stago innovations including STA Satellite, STA Coag Connect (data manager), Qualiris (Stago’s world-wide Quality Assurance Program), STA Liquid Anti-Xa assay, and Stago’s new line of Direct Thrombin Inhibitor assays – the Ecarin Chromogenic Assays.

The continued success of this event showcases the depth of knowledge of the participants and the group dynamics provided by the interaction of attendees. This learning forum is also pivotal for creating networking opportunities for our customers.

If you attended the Stago User Group Meeting, you should have received the CD featuring these presentations.

If your laboratory hasn’t received the 2010 Stago UGM CD or you would like more information, please contact info@stago.com
Direct Thrombin Inhibitors

Because of its pivotal role in haemostasis, thrombin is a key therapeutic target in the treatment and prevention of thromboembolic disorders.

Conventional anticoagulant therapies, such as warfarin, unfractionated heparin, and low-molecular-weight heparin exert their pharmacologic action, more or less, by indirect thrombin inhibition.

Although these agents are effective, each has limitations, prompting a search for more effective, specific, better-tolerated, and convenient anticoagulants.

The efficacy and safety of factor Xa inhibitors are being investigated. Furthermore, the development of DTIs such as recombinant hirudin (lepirudin), bivalirudin, and argatroban continues.

Challenges in the development of DTIs include establishing a binding affinity for thrombin that is not associated with excessive bleeding, attaining high thrombin specificity, achieving inhibition of both unbound and clot-bound thrombin, and producing an effective, fixed-dose oral anticoagulant to improve the practicality of anticoagulation therapy.

Significant progress has been made in developing DTIs. The recent emergence of orally administered DTIs may simplify the prevention and treatment of thrombosis.

Stago is pleased to provide a means to monitor these new DTIs - the Ecarin Chromogenic Assay (ECA).

The ECA is based on the cleavage of prothrombin by ecarin, a snake venom metalloprotease from Echis carinatus. The generated activation products (mainly meizothrombin) enzymatically cleave a chromogenic substrate thereby p-nitroaniline is released. The cleavage is concentration-dependently inhibited according to the amount of DTI, present in the sample, bound to the activation products.

ECA is independent of plasma prothrombin level because prothrombin is a component of the ECA prothrombin buffer and therefore it is available in sufficient amounts in the assay. Both oral anticoagulants as well as heparin/antithrombin III do not interfere with ECA, because PIVKA (prothrombin induced by vitamin K antagonists) is also cleaved by ecarin and the enzymatic activity of the generated activation products is not inhibited by heparin/antithrombin III.

The ECA is not a coagulation assay therefore it is independent of plasma fibrinogen level.

The Stago ECA kits, calibrators and controls are available now.

INR’s – The importance of knowing your reagent

There are two main groups of Thromboplastin reagent (used to measure the Prothrombin Time and INR) – the Tissue Factor based reagents (either human or animal) and the Recombinants.

An International Sensitivity Index (ISI) is created for each reagent from the secondary standards created and provided by the WHO.

Sometimes an ISI is provided by the manufacturer of the Thromboplastin (as is the case with Stago Neoplastine-CI+ and Neoplastine-R), whilst with other reagents you may need to develop your own ISI.

The ISI is then used in conjunction with the laboratory-derived MNPT to allow us to calculate the INR – a value that should be standardized no matter what reagent or analyser you are using.

However the various Thromboplastins have different sensitivities to Factor levels, and these sensitivities aren’t necessarily linear. Significant variations occur between the Recombinant and Tissue Factor reagents.

When tested with both a Recombinant and a Tissue Factor reagent the difference in the INR at the ‘normal’ and ‘low therapeautic’ (INR <3.0) levels are usually minimal and not clinically significant. However once INR’s start getting above the therapeutic level (>3.5) the results can differ significantly.

This is of concern because the result will affect treatment options for patients who are over-warfarinised.

In particular, the difference in Factor VII sensitivity can
INR’s – The importance of knowing your reagent (continued)

cause a divergence in results. While Recombinant reagents are very sensitive to changes in Factor VII levels (and moderately sensitive to changes in other Factors), Tissue Factor reagents are only moderately sensitive to changes in FVII (and also moderately sensitive to changes in other Factors). This is particularly significant when a patient is first put on warfarin as their Factor VII levels tend to decrease fairly fast.

A recent study performed by Sullivan Nicolaides Pathology in Brisbane showed the different Thromboplastins and their sensitivity to Factor VII. The following graph shows, for example, how a sample with low Factor VII can produce PT results varying anywhere from 27 seconds to 41 seconds. The ISI and MNPT will correct for this to a certain amount, but there will still be a significant difference in INR results. While there is no ‘solution’ to this issue, it is something for all Haemostasis scientists to be aware of, particularly if your laboratory tests patients who may also go to other laboratories where different reagents are used.

The STA Satellite is now officially launched in Australia and New Zealand.

It is ideally suited to small laboratories looking for full automation or as a backup to the STA Compact.

The STA Satellite features a small footprint and a wide menu including PT, APTT, Fib, ATIII, D-Dimer and anti-Xa. Viscosity based clot detection ensures accurate results on icteric, lipaemic and haemolysed samples. Imagine never having to reflex test to the water-bath again?

The same reagents can be used across the entire Stago range of analysers helping your laboratory network achieve the standardisation it desires. Excellent on-board stability of reagents is also assured.

The STA Satellite facilitates fully random access testing.

Please contact your Stago sales representative or info@stago.com for more information.
Deleting old lot numbers from the STAR software can dramatically improve the performance of your analyzer.

Keep in mind that when creating and entering the ID during loading of a new reagent or control, do NOT use either an apostrophe OR a space in the identifier name. If these are inadvertently used, they will cause software problems.

In order to ensure efficient use of the analyzer, the number of expired lot numbers should be kept to a minimum. If they are not deleted periodically, then there will be a slowing in performance once the file has built up.

To delete these old kit lots firstly make a note of the current lot numbers for all the reagents and QCs. Now go into Maintenance then into the User Menu.

From the User Menu select “Delete Lot”.

You will now see the following screen.

Using the list of current Lot numbers, scroll down the IDs and click on the line you wish to delete.

You can only delete one line at a time so it does take time to delete a large number of lot numbers.

This means that it is better to carry out the cleaning on a regular basis.

So, select the line you wish to delete then click on the “Delete this lot”.

The box asking you to confirm deletion then appears. Click on OK.

In order to delete another lot number repeat this procedure.
Case Study: A 1 year old patient with severe Haemophilia A (FVIII:C <1%) was commenced on weekly FVIII infusions to prevent the development of FVIII antibodies (inhibitor), given his family history of a sibling with Haemophilia A and a high responder. This was regardless of an unremarkable first year with negligible bleeding episodes.

In August 2009, at 16 months of age, the patient presented in the emergency department of the RCH, with persistent oozing from the gums following a prior injury with minimal response to FVIII concentrate. As a consequence of this, coagulation studies were commenced, identifying FVIII:C of <1% and an inhibitor titre, using the Bethesda assay, of >100 BU/mL. Residual factor activity was 2% at a dilution of 1:100. A subsequent sample confirmed the inhibitor to be >100 BU/mL. Due to sample size limitations, as is often the case in a paediatric setting, no further dilutions or testing were able to be performed.

As a follow-up investigation in September 2009, extended dilutions were performed and titred the FVIII inhibitor level to 440 BU/mL. Bleeding was controlled successfully with the administration of recombinant FVIIia (rFVIIa) with further doses being administered as required. Monitoring over the next few months showed a decrease in the inhibitor level, although it never decreased below 100 BU/mL. Tolerisation was commenced with regular doses of FVIII. However by December 2009, the patient began suffering from a number of bleeding episodes into his thigh and elbow, complicated by staphylococcus sepsis presumably originating from his infusion port. He subsequently underwent removal of his port and reinsertion of a new port. Once again, bleeding was controlled by recombinant FVIIia. A daily dose of rFVIIa was continued to be administered as prophylaxis in conjunction with daily FVIII infusions for further tolerisation. In January 2010, a slight increase in his inhibitor level was noted. By February 2010, his inhibitor level had surged and peaked at 1850 BU/mL. The inhibitor level continued to be monitored, displaying a gradual decrease over the subsequent few months. The patient’s most recent visit was in May 2010 where his inhibitor level had decreased to 140 BU/mL. To date, this patient has received a total of 312 vials of rFVIIa.

Discussion
Approximately 30% of Haemophilia A patients may develop inhibitors as a result of treatment with FVIII concentrates. Development of an inhibitor poses a major clinical challenge that complicates the treatment of these patients, as these antibodies inhibit FVIII, therefore resulting in a bleeding tendency and a low recovery and half-life of infused FVIII. From a laboratory perspective, the accurate identification and quantification of an inhibitor to Factor VIII is crucial to the management of these patients. However, inhibitor testing in a paediatric setting is faced with a limited sample size of variable quality given the difficulty of collecting a venous sample from a distressed child. A number of clot-based assays are available to identify inhibitors with the Bethesda assay currently being the most common method employed to quantify FVIII inhibitors. An increasing number of laboratories, however, are slowly adopting the Neijmegen modification of the Bethesda assay, which involves buffering the normal pooled plasma and replacing the diluent Imidazole buffer with FVIII deficient plasma. This modification improves the sensitivity and specificity to low titre FVIII inhibitors.

Our laboratory currently uses the Bethesda assay to quantify FVIII inhibitors. The procedure for a newly suspected inhibitor involves testing the patient plasma NEAT and diluted up to 1:100. According to the Bethesda method, the dilution that yields a residual FVIII activity of 50% of the control mixture is determined as the inhibitor titre. The initial inhibitor testing in the case study patient showed a residual activity of only 2% at 1:100 dilution. A repeat test, and with pre-analytical error excluded, showed similar results. Due to the small sample volume on both occasions, no further dilutions were able to be performed. Since this was the first ever case of such a strong inhibitor seen in our laboratory, there were initial discussions regarding the clinical significance and utility of performing dilutions beyond 1:100. It was decided, however, that the quantification of the inhibitor was critical to the clinical management of this patient. With this in mind, the laboratory requested a larger sample volume to be collected. The larger volume on all subsequent samples allowed extended dilutions to be performed with determination of an end-point titre. The inhibitor assays were often performed in two stages: first by using a number of widely varying dilutions ranging from 1:100 – 1:4000 to gauge the approximate strength of the inhibitor and then a second set of precise dilutions narrowed to within the indicated range. During the course of treatment of this particular patient, it was observed that on no single occasion has the inhibitor strength dropped below 100 BU/mL, yet we have been able to observe a trend of the inhibitor strength with its peak at 1850 BU/mL following tolerisation. This has allowed improved clinical management by tailoring the patient’s treatment according to inhibitor titre levels. It is concluded that extended dilutions and determination of an end-point titre is crucial in all future testing of cases with extremely strong factor inhibitors.

Note: Initial testing not included in this graph as result was reported as >100BU/mL.
Category of Inhibitor Strength: <5 BU/mL WEAK; >5 BU/mL STRONG
Using the correct stir bar is important!

From time to time we get calls from or get called out to laboratory sites for a variety of issues that end up being caused by a seemingly insignificant factor, not related to a problem with the analyser.

The problems range from needle is bent error messages, washing issues, result errors, reagent changing colour etc etc.

The cause of quite a few of these is the use of non Stago stir bars. Stago stir bars are necessarily small to minimise the potential of a needle strike when reagent volumes are getting low. They are also formulated to work well in the reagents that we use within coagulation.

Use of an oversize non Stago stir bar can result in a range of different issues, because they are larger than they should be.

Dead volumes for the various vial sizes that can be loaded onto Stago analysers are calculated on the volumes above which a reagent needle can reliably detect and pipette the required reagent volume.

Stago stir bars will operate happily below this liquid level. If a stir bar is oversize there is the potential that the needle can strike the stir bar causing damage to the needle, cause pipetting inaccuracy and thus result inaccuracy by blocking the end of the needle during aspiration. Needles can pick up the stir bar and push it into the wash well and cause needle is bent and washing errors.

The seriously oversized stir bars can also reduce the efficiency of the stirring process leading to variability in results and reagent performance.

The problems can also be further complicated by the use of non Stago reagent bottles that don’t have flat bases.

We have even had situations where the quality of the stir bars was so poor that they reacted with the reagents on board causing colour change, result errors, and on board stability issues.

Stir bars are a fairly simple but vital component of coagulation testing and the consequences of using the incorrect size stirrer can be considerable. If you are currently using or are considering using non Stago stir bars let us know and we can adjust the dead volumes on your reagents to allow for the larger size.

This unfortunately will lead to fewer tests per vial. The use of reagent chimneys and ensuring the stirrer is outside it can also keep the stir bar away from the path of the needle.

Please call your Stago Hotline if you need more information.
1800 984 422 (Australia)
0508 984 422 (New Zealand)
Stago Continuing Education

In 2010 we introduced a set of Educational CD’s. This is a set of 9 CD’s which feature presentations from prominent Pathologists and Scientists in the USA.

Accompanying these CD’s is access to a website to participate in a quiz and PACE points can be printed.

Also available are educational files on Heparin and D-Dimers. These are recent articles and scientific information.

If you are interested please email enquiries to info@stago.com

AIMS NSW & ACT Haemostasis Workshops

Workshop Title: “Problems and solutions in coagulation and haemostasis”

Date: Friday 3rd of December, 2010

Location: Westmead Hospital at Westmead, NSW 2145, Level 2, Education Block, Lecture Theatre 2

Workshop Outline: A full-day dry-workshop covering various issues in laboratory testing for routine coagulation tests as well as specialised haemostasis tests.

Diagnostica Stago is the Gold Sponsor of this event

If you would like to know more please contact:
Ashraf Mina - Phone: +612 9845 7197; Email: Ashraf.Mina@swahs.health.nsw.gov.au
Emmanuel Favaloro - Phone: +612 9845 6618; Email: efavaloro@optusnet.com.au

Upcoming Conferences

New Zealand Institute of Medical Laboratory Science (NZIMLS) Conference:
23 - 27 August 2010; Bay of Islands, New Zealand

APSTH (56th Congress of the Asia Pacific Society on Thrombosis and Haemostasis:
13 – 16 October 2010; Bali - Indonesia

Haematology Association of Australasia (HAA) Annual Scientific Meeting:
(ASTH Workshop 16 October) 17 - 20 October 2010; Auckland, New Zealand

Australasian Association of Clinical Biochemists (AACC) and Australian Institute of Medical Scientists (AIMS) Combined Scientific Meeting: 25 - 29 October 2010; Perth, Australia

AIMS NSW & ACT Haemostasis Workshop: 3 December 2010, Westmead Hospital, NSW, Australia

RCPA Pathology Update: 4 - 6 March 2011; Melbourne, Australia

ISTH 2011, XXIII Congress of the International Society on Thrombosis and Haemostasis:
23 – 28 July, 2011; Kyoto, Japan