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# SCIENTIFIC REVIEW

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## Laboratory Testing for Heparin-Induced Thrombocytopenia (HITS) at Royal Perth Hospital

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In July 1991, an article in Today's Life Science: Platelet Aggregation Testing for Heparin Induced Thrombocytopenia: Caveat Emptor by CC Chooi and AS Gallus, highlighted the pitfalls and difficulties associated with laboratory diagnosis. There was a lack of standardisation between laboratories in testing protocols and reports of poor specificity and sensitivity. It confirmed what most laboratories already knew – that HIT testing was far from definitive and the diagnosis was very dependent on the clinical situation.

So have we moved on in the last decade? Certainly our understanding of the pathogenesis has grown considerably. We now know that the antigen is most often, but not always, heparin/PF4 and that antibodies produced in response to this, form antigen/antibody complexes that activate platelets via the Fc $\gamma$ RIIs. This means that only IgG antibodies will cause aggregation in vitro. We understand that the number of copies of Fc $\gamma$ R2 per platelet is important and suspect that Fc $\gamma$ R2 polymorphisms may also play a role, either in sensitivity to the antibody or in susceptibility to developing the antibody.

### **But certain testing dilemmas remain for laboratories**

One of the major ones is which test to use. The 14C serotonin release assay is generally seen as the most reliable but, because of the expertise and time required to obtain results, it is not really suitable as a test for routine diagnostic use. Several other techniques have been suggested, such as platelet microvesicle formation and annexin V expression, which utilise flow cytometry, have the potential to become rapid routine methods but they have not yet gained wide acceptance.

So the front line test is still platelet aggregometry – an imperfect technique to diagnose an important condition. Treatment of HIT is fundamentally cessation of heparin and the consequences of not doing so in a patient with a HIT antibody can be devastating. Sometimes it is difficult to stop heparin and it is understandable that medical staff are reluctant to do so without reasonable suspicion of HIT in a patient who could be thrombocytopenic due co-existing conditions. However to report negative result when method sensitivity is low, may lead to a false sense of security and appropriate cautions should be given with negative results.

### **Has aggregometry improved since 1991?**

The quality of donor platelets is central to obtaining reliable results. Some platelets are more responsive than others and often labs have favourite staff members who are called upon when a sample is received. However HIT is a syndrome that seems to be more prevalent at weekends, especially long weekends, when it is hard to find any donors, far less well characterised ones. Other steps which can be taken to compensate for platelet variability are to use at least two sets of platelets, to test with appropriate controls including a weak positive HIT antibody if available, or even washing the platelets to remove interfering plasma factors such as soluble Fc receptor.

At Royal Perth Hospital we have an anti-Fc $\gamma$ R2 antibody which binds to the Fc $\gamma$ R2 at a region distant to the Fc binding site. Pre-incubation of donor platelets with F(ab) fragments of this reagent increases their reactivity with HIT antibody and, in addition to increasing the sensitivity of the method, helps to standardise donor platelet response.

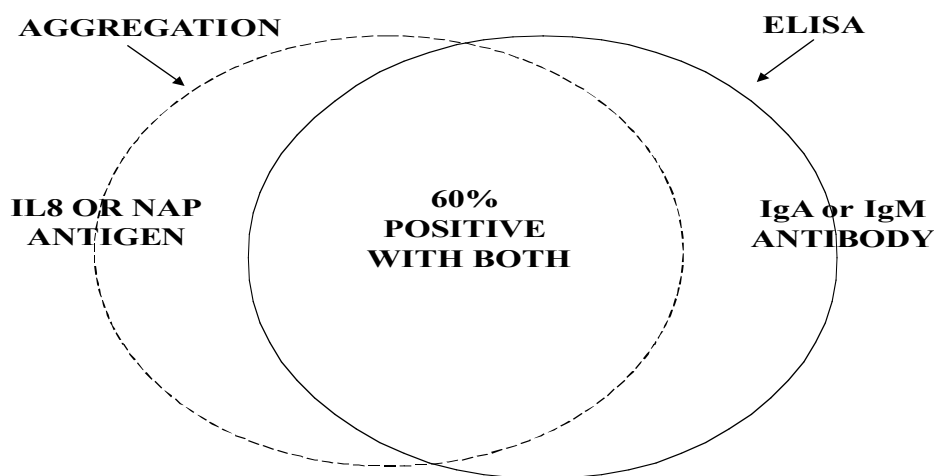
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It is important to be able to differentiate between a HIT antibody and other causes of positive aggregometry. Most laboratories are aware of the importance of two point heparin testing – a lower concentration of 0.1 – 0.5 units/ml and a higher of 100 units/ml. A truly heparin dependent Fc $\gamma$ RII mediated reaction will usually be inhibited or retarded by the higher concentration. Sometimes it may be necessary to remove heparin from the patient plasma in order to demonstrate heparin dependent aggregation. Positive aggregation caused by other circulating immune complexes will be unaffected by heparin concentration.

Another technique is to pre-incubate the test platelets with an Fc $\gamma$ RII inhibitory antibody such as IV.3 which completely blocks the receptor and can be used to distinguish immune mediated aggregation from other causes.

Using modifications such as these, aggregometry appears to have a reasonable sensitivity and specificity but will still be unable to detect IgA or IgM antibodies. These have been reported into up to 30% of cases of HIT and although not reactive in the laboratory aggregation test, such antibodies may still cause thrombocytopenia or thrombosis in vivo.

The relatively new ELISA test is a simple, if somewhat expensive, technique, which will detect all IgG subclasses of heparin/PF4 antibodies. The clinically significant antibodies that will not aggregate donor platelets justify the use of this technique as an adjunct to aggregometry. Unfortunately neither will current commercial ELISA kits detect all cases of HIT because HIT antibodies occasionally develop against other antigens such as NAP or IL8.



At Royal Perth Hospital our practice is to test using platelet rich plasma from at least two sets of normal donor platelets. We run a two point assay (0.5 and 100 units/ml) and another cuvette enhanced with 8.2 F(ab) fragments. We run controls on each donor to check platelet viability and if aggregation occurs we rule out a direct heparin effect. We simultaneously run an ELISA for heparin/PF4 antibodies.

It is wonderfully re-assuring when both tests concur, but there is disagreement in a significant number of cases and the clinical picture remains of paramount importance.