

**P013** Demonstration of biological activity of heparin immobilised on microplates

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Immobilisation of heparin on a solid substrate facilitates study of its interaction with heparin-binding molecules like cytokines. Previous studies have shown that heparin can be immobilised on primed microtitre plates (Plaso Technology Ltd). To determine whether the heparin retains biological activity, we have used a well-characterised anticoagulant assay model system. The assay is based on heparin enhancement of antithrombin inactivation of Factor IIa. Incubation of a range of unfractionated heparin (5<sup>th</sup> IS, code 97/578) concentrations in the plates was followed by extensive washing to remove unbound heparin. Bound heparin was assayed by measuring anti-IIa activity. Heparin binding was dose-dependent and saturable. By contrast, no anti-IIa activity was detected on unprimed control plates. Comparison of bound heparin and solution phase heparin permits quantitation of the activity. We have also demonstrated a heparin-dose dependent anti-Xa activity. In conclusion, the immobilised heparin remains biologically active, continuing to associate with two relatively large biological molecules (antithrombin and Factor IIa/Xa). As anti-IIa and anti-Xa activities differ in their dependence on structural elements of heparin, comparison of these may assist in further characterising the heparin binding to the plates.