

# Grange Research Centre

## GRANGE LABORATORIES: TECHNIQUE AND DEVELOPMENT

### **Fibrinogen: investigation of alternative method of analysis**

Fibrinogen is a soluble precursor of insoluble fibrin, the major component of a blood clot. Elevated levels of fibrinogen are associated with inflammation, trauma, surgery, and malignancy. It is therefore a useful “acute phase protein” (ACP) indicator of stress and/or trauma in animals. The measurement of fibrinogen has been carried out up to 2004 using a kinetic method from Roche (Fibrinogen Kinetic, Cat no. 524484). The method performed well on a number of analysers (Hitachi 705 originally, Space analyser 1999-2003). Unfortunately, Roche discontinued the kit in 2003 resulting in a search for a suitable alternative.

Most laboratories doing coagulation tests (including fibrinogen) use dedicated coagulation instruments. The technology used however does not transfer to clinical analysers, which produce results from colour/turbidity changes. An extensive trawl of the internet resulted in two possible replacements; Zymutest Fibrinogen and Kamiya Biomedical. Zymutest is based on an Elisa platform. Large serial dilutions are required to bring samples into the working range of the test; it is expensive to buy, and therefore considered unsuitable for our needs. A trial kit was obtained from Kamiya for evaluation purposes. Calibration was performed successfully and all controls read within range. However, all controls and calibrators are of human origin; the method uses an antiserum against purified human fibrinogen. When bovine samples are tested against the calibration, only trace levels of fibrinogen were detected. The antiserum used in the kit is a goat polyclonal antibody specific to the human protein; hence it is inappropriate for animal use.

In an attempt to provide some continuity in the test, it was decided to source the original method on which the Roche method was based (Becker et al, *Thrombos Research* 1984; 35:475-484). It has the disadvantage of apparent activity differences in vials of Atroxin™ (ie, batroxobin enzyme), requiring standardisation/validation of each vial purchased. However, the precision of this reformulated method compares very favourably with the original Roche method at different levels of fibrinogen, as follows;

<u>“New” method</u>	<u>Roche method</u>
<u>Inter-assay;</u> Mean = 264mg/dl (Precichrom™, Std. dev. = 24.3 commercial control) CV% = 9.2 n=31	Mean = 271 Std.dev. = 22.0 CV% = 8.0 n=84
<u>Intra-assay;</u> (random samples) Mean = 680mg/dl Std. dev. = 28.47 CV% = 4.19 n = 20	Mean = 430 Std. dev. = 43.9 CV% = 10.21 n = 10

In addition, a commercially available bovine standard Hyphen Biomed™ (not available when the Roche method was in place) with a nominal value of 1000mg/dl fibrinogen was analysed for quality control purposes. This gave an inter- assay mean of 1015, Std. dev. =64.3, CV%= 6.33 (n=17).

The data indicates that this new formulated method is an adequate replacement for the original Roche method, and will successfully monitor animals for fibrinogen levels.

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