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TITLE (QPS 2009-024)

An ELISA method for rapid and quantitative determination of Gelsolin in human plasma

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ABSTRACT

Purpose: Plasma Gelsolin depletion is associated with poor outcomes in seriously ill patients. To evaluate the effect of repletion of Gelsolin on improving clinical outcome, plasma Gelsolin from ICU patients needs to be evaluated quickly and quantitatively.

Methods: Functional kinetic assay and multiple ELISA assays were evaluated. A sandwich ELISA was selected and further developed. The developed assay used monoclonal anti-Gelsolin antibody (clone 2C4) to capture the Gelsolin in plasma. The bound Gelsolin was then detected by a polyclonal rabbit anti-Gelsolin antibody specific to plasma Gelsolin. The complex was bound with anti-rabbit IgG-HRP conjugate. The bound HRP conjugate was detected by TMB color reagent. The color development was stopped by hydrochloric acid and the plate was measured for optical density at 450 nm (correction wavelength 650 nm) on a Molecular devices SpectramaxPLUS384 plate reader. To reduce assay time, the antigen-antibody bindings were carried out in one step by combining anti-Gelsolin polyclonal antibody, HRP conjugate and Gelsolin standards, QCs and samples, and incubated together in microplate wells coated with the monoclonal anti-Gelsolin antibody for 30 min at room temperature. The subsequent color development time took about 10~30 min. The assay results could be generated in about an hour from the beginning of sample incubation.

Results: The established assay range was 90-1500 ng/mL. Assay inter-batch precision and accuracy from assay QCs were 6.8~11.9% and -1.8~-0.6%, respectively. Plasma samples showed linearity of measurement between 100 and 4000-fold dilution. Plasma samples measured at a default 200x dilution are all within assay range, with inter-assay precisions ranging from 13.8 to 20.6%. Gelsolin spiked into plasma recovered 113.2 +/- 5.4% with this assay.

Conclusions: An ELISA assay was developed to accurately and rapidly generate results for critical care use.