

PP 07 - A novel and accurate tool for quantification of proteins in micro-volume applications

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Introduction: Protein quantification is often an essential step in any research field that involves proteins. Although the standard Lowry assay and its modifications are most abundantly used in protein quantification, existing methods are rigid or often demonstrate non-linearity between protein concentration and colour intensity. A method for fast and accurate qualitative and/or quantitative determination of total soluble/insoluble proteins or micro-well plate immobilized proteins isolated from *Leishmania* parasites in micro-volumes is described.

Methods: This is a simplification to standard Lowry protein assay. Concentrations of unknown samples were calculated using a standard curve prepared using a standard series of bovine serum albumin (BSA). Method validation was carried out according to guidelines for bioanalytical method validation distributed by Food and Drug Administration (FDA), USA. Cost per sample was calculated according to standard approved guidelines.

Results: The optimized reagents were 2N NaOH (sodium hydroxide), 2% Na₂CO₃ (sodium carbonate), 1% CuSO₄ (copper sulfate), 2% KNaC₄H₄O₆ (potassium sodium tartrate) and 2N Folin and Ciocalteu's phenol. This new protein assay was sensitive for quantifying *Leishmania* proteins in a total crude extract or soluble fraction within the approximate range of 10-500 µg/ml (1-50 µg/assay) and showed a linearity between colour intensity and concentration of the protein. Also, the described method was 100% repeatable, and showed a high accuracy for quantifying proteins. Cost per sample is approximately 2.8 USD by considering expenses for consumables, chemicals and equipment.

Conclusions: The new methodology avoids the requirement of purchasing commercial protein quantification kits. This is an easier, fast and accurate method for quantifying proteins with micro-volumes in a cost-effective manner for routine use in research laboratories in resource limited settings.

Keywords: Protein quantification, *Leishmania*, Micro-plate, Cost-effective

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