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Method Article

A sandwich enzyme-linked immunosorbent assay for the quantitation of human plasma ferritin

Andie V. Bleicher[a], Holger W. Unger[b], Stephen J. Rogerson[a], Elizabeth H. Aitken[a,*]

[a] Department of Medicine at the Doherty Institute, University of Melbourne, Melbourne, Australia
[b] Department of Obstetrics and Gynaecology, Royal Infirmary of Edinburgh, Edinburgh, UK

A B S T R A C T

There is a lack of published enzyme linked immunosorbent assay (ELISA) protocols which use commercially available reagents for the measurement of ferritin in human plasma for research purposes. ELISA kits are often expensive and do not always provide detailed information about reagents included. A commercially available antibody pair was used to develop an in-house ELISA to measure ferritin in small (25 μl) volumes of human plasma. ELISA results were compared to ferritin levels measured using a commercial immune-assay system. The sensitivity, intra and inter assay variation of the ELISA assay were also determined. ELISA measurements of plasma ferritin ranged between 3.2–232 ng/mL and were comparable to those measured by a commercial immunoassay system (Pearson correlation $r = 0.93$ $P < 0.0001$). Ferritin levels as low as 0.5 ng/mL were detectable and samples with both low and normal ferritin levels showed low inter and intra-assay variation. This ELISA protocol allows the accurate, reliable and cost-effective quantitative determination of plasma ferritin levels from small volumes of human plasma.

- No published protocols detail how to measure ferritin by ELISA using commercially available antibodies.
- ELISA kits are expensive and information on antibodies included are often lacking.
- We have identified a commercially available antibody pair to measure plasma ferritin using a cost-effective ELISA.

A R T I C L E I N F O

Method name: Measurement of ferritin by ELISA
Keywords: ELISA, Ferritin, Anemia, Iron deficiency, Plasma
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* Corresponding author.
E-mail addresses: andie.bleicher@unimelb.edu.au (A.V. Bleicher), hwunger@doctors.org.uk (H.W. Unger), sroger@unimelb.edu.au (S.J. Rogerson), elizabeth.aitken@unimelb.edu.au (E.H. Aitken).

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Specifications Table

Subject area	*Select one of the following subject areas*: • *Medicine and Dentistry*
More specific subject area	*Iron deficiency*
Method name	*Measurement of ferritin by ELISA*
Name and reference of original method	**Name:** Serum ferritin by a rapid and inexpensive ELISA method. **Reference:** Anderson MG, Kelly AM. Serum ferritin by a rapid and inexpensive ELISA method. Clin Chim Acta. 1981 Nov 11;116(3):405-8.
Resource availability	

Method details

Background

Plasma ferritin is a marker of iron stores and along with haemoglobin concentration, it can be used to identify iron deficiency anemia [1,2], a condition with a large health burden, contributing to maternal and perinatal mortality as well as maternal cognitive impairment and reduced fitness and productivity [3]. The Global Burden of Disease 2000 project estimated that iron deficiency anemia accounts for 841 000 deaths and 35 million disability-adjusted life years [3].

There are published protocols for in-house enzyme linked immunosorbent assays (ELISAs) to measure whole ferritin in plasma [4,5], but the antibody pair described in these protocols is no longer commercially available. With this in mind we have identified a new antibody pair which we then used to develop a cost-effective ELISA to measure plasma ferritin for research purposes.

Protocol

To do the ELISA Nunc MaxiSorp® flat-bottom 96 well plates (ThermoFisher Scientific 442404) were coated with 100 μl/well goat anti-ferritin polyclonal antibody (Abcam ab33574) diluted to 367 ng/mL in phosphate buffered saline (PBS), overnight at 4 °C. The following day, the plates were washed with wash buffer (0.05% Tween20® in PBS) and then blocked with 200 μl/well of reagent diluent (1% Bovine Serum Albumin (Sigma A7906), 0.05% Tween20® in PBS) for 1 h at room temperature. After further washing, 100 μl/well of samples (human plasma diluted 1 in 8 in reagent diluent) and standards (Liquichek Immunology Control L3 (BIORAD 596) diluted 1 in 4 in reagent diluent followed by a 1 in 2 serial dilution over 10 points) were aliquoted in duplicate and incubated for 2 h at room temperature. After further washing, 100 μl of biotinylated rabbit anti-ferritin polyclonal antibody (Abcam ab7333) diluted to 125 ng/ml in reagent diluent was added to each well for 1 h at room temperature, plates were washed, and 100 μl of streptavidin-horseradish peroxidase (Abcam ab7403) diluted to 67 ng/mL in reagent diluent was added to each well for 1 h at room temperature. Plates were washed and 100 μl of 3,3′,5,5′-tetramethylbenzidine substrate (BD 555214) was added to each well. After 30 min at room temperature 50 μl/well of stop solution (1 M H_2SO_4) was added. The optical density at 450 nm was measured using a FLUOstar Omega BMG LABTECH microplate reader. A standard curve was generated and values for unknown samples were extrapolated.

Development and validation methods

Working concentrations of capture and detection antibodies were determined by a series of checkerboard dilutions using a pool of plasma as a sample (capture and detection antibodies were tested at concentrations ranging from 0.2 to 11 and 0.125–5 μg/mL respectively). Selected concentrations chosen based on results from checkerboard dilutions were further tested using a ferritin standard curve (Liquichek Immunology Control L3 (BIORAD 596)) and three test plasma samples of known ferritin concentrations (13 ng/mL, 27 ng/mL and