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THE DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY  
FOR BOVINE LACTOFERRIN

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## ABSTRACT

Methods were established for the estimation of bovine lactoferrin by enzyme-linked immunosorbent assay (ELISA) on microtitre plates and nitrocellulose (dot assay).

Affinity-purified antibodies to bovine lactoferrin were prepared and conjugated to horseradish peroxidase by the periodate method. Conjugates with enzymatic and immunological activity had an apparent molecular weight of 65 or 95 kd.

Four methods of ELISA on microtitre plates and three dot assays were developed. Differences between the seven assays could be attributed to the absorption capacity of the solid phase and the types of conjugates and substrates used. The range of the two successful quantitative assays were 3-1000ng lactoferrin/ml (sandwich) and 60-8000ng lactoferrin/ml (competitive), while the qualitative dot assays had a range of 1-100 $\mu$ g lactoferrin/ml. More replicates would be required to reduce variability.

Results from these assays generally corresponded to results from ROCKET electrophoresis. Dot assay on nitrocellulose has a greater potential for reproducible and quantitative assays than assay on microtitre plates, because of the greater adsorption capacity of the nitrocellulose. In addition, the dot assays are faster and lend themselves to more applications than either ROCKET electrophoresis or ELISA on microtitre plates.

The ELISA developed in this project appear to be the first alternatives to radial immunodiffusion and ROCKET electrophoresis for the measurement of bovine lactoferrin.

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## ABBREVIATIONS

ALPase	alkaline phosphatase
BSA	bovine serum albumin
CDI	carbonyl diimidazole
cDNA	copy deoxyribonucleic acid
CMC	1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho- <i>p</i> -toluene sulphonate
DEA	diethanolamine buffer
DMF	dimethylformamide
ELISA	enzyme-linked immunosorbent assay
FCS	foetal calf serum
HRPase	horseradish peroxidase
IgG	immunoglobulin G
LF	lactoferrin
mRNA	messenger ribonucleic acid
pNPP	p-nitrophenyl phosphate
PB	phosphate buffer
PBS	phosphate buffered-saline
PBS-S	phosphate buffered-saline with foetal calf serum
PBS-T	phosphate buffered-saline with Tween 20
SDS	sodium dodecyl sulphate
TCA	trichoroacetic acid
TEMED	N, N, N', N'-tetramethylethylene diamine
Tween 20	polyoxyethylene (20)-sorbitan monolaurate

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