Development of Reference ELISA Assays For Urinary Oestrone-3 α-Glucuronide and Pregnanediol-3 α-Glucuronide Using Timed Urine Specimens

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Abstract

Enzyme-linked immunosorbent assays (ELISA) have been developed which measure oestrone glucuronide (E1-3G) and pregnanediol glucuronide (PdG) in timed, diluted urine samples. Measurement of these urinary metabolites allows information to be collected, non-invasively, on the hormonal interplay between the ovaries and the hypothalamic-pituitary axis, which determines or helps to make predictions about the potentially infertile and fertile phases of the human menstrual cycle.

Immunoglobulin Class G (IgG) antibodies raised in sheep against the analyte of interest (E1-3G and PdG) were adsorbed onto polystyrene microtitre wells. The enzyme conjugate tracer was horseradish peroxidase (HRP), and was prepared by conjugation with either E1-3G or PdG using the active ester coupling procedure. A direct competitive immunoassay configuration in which both analyte and tracer were added to the wells simultaneously allowed a direct competition between them for the immobilised antibody sites. A chromogenic detection system involving o-phenylenediamine (OPD) was used for the measurement of the amount of bound tracer (HRP conjugate) which could be related to the amount of analyte in a urine sample.

The sensitivity of the E1-3G assay was 3.4 nmoles/ 24 h, and for the PdG the sensitivity was 0.5 µmoles/ 24 h. Both assays were reliable, and were successfully validated against World Health Organisation (WHO) assays performed on the same urine samples in a multicentre study of the Ovarian Monitor (project #90905).

The E1-3G and PdG reference assays developed in the present study are acceptable for use in the laboratory and can be used to validate new non-instrumental colour tests, or other home fertility kit assays currently being developed.

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Abbreviations

Aromatase	Oestrogen synthetase
A_{278}	Absorbance at 278 nm
A_{280}	Absorbance at 280 nm
A_{404}	Absorbance at 404 nm
A_{490}	Absorbance at 490 nm
B/B_0	Absorbance value of the steroid standard divided by the absorbance value of the zero standard
CAb	Capture antibody
CV	Coefficient of Variance
DCC	Dicyclohexylcarbodiimide
DMF	Dimethylformamide
`E'	Mean absorbance reading for the steroid glucuronide standard
`E_0'	Mean absorbance reading of the zero standard
ED_{50}	At the midpoint of a normalised standard curve
ED_{20}	At a point 20% from the bottom of a normalised standard curve
EIA	Enzyme Immunoassay
ELISA	Enyme Linked Immunosorbent Assay
E1-3G	Oestrone-3 α-Glucuronide
E1-3G-HRP	Oestrone-3 α-Glucuronide-Horseradish Peroxidase Conjugate
Fab	Antigen-binding fragment of antibody
Fc	Constant fragment of antibody
FSH	Follicle Stimulating Hormone
HEWL	Hen Egg White Lysozyme
HRP	Horseradish Peroxidase
IgG	Immunoglobulin Class G
LH	Luteinising Hormone
NC	Nitrocellulose paper
NHS	N-hydroxysuccinimide
NSB	Non specific binding
OPD	O-phenylenediamine
PBS	Phosphate buffered saline

Pd	Pregnanediol
PdG	Pregnanediol-3 α-Glucuronide
PdG-HRP	Pregnanediol-3 α-Glucuronide-Horseradish Peroxidase Conjugate
RZ	reinheitzahl
RIA	Radioimmunoassay
SD	Standard Deviation
SEM	Standard Error of the Mean
SPI	Solid Phase Immunoassay
Tween(20)	Polyoxyethylene (20)-sorbitan monolaurate
ΔT	Change in Transmission
T_0	Transmission at time zero
T_{20}	Transmission time of 20 minutes
WHO	World Health Organisation