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**Development of Reference ELISA Assays  
For Urinary Oestrone-3  $\alpha$ -Glucuronide  
and Pregnanediol-3  $\alpha$ -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  $\mu$ moles/ 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 <sub>0</sub> '	Mean absorbance reading of the zero standard
ED <sub>50</sub>	At the midpoint of a normalised standard curve
ED <sub>20</sub>	At a point 20% from the bottom of a normalised standard curve
EIA	Enzyme Immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
E1-3G	Oestrone-3 $\alpha$ -Glucuronide
E1-3G-HRP	Oestrone-3 $\alpha$ -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 $\alpha$ -Glucuronide
PdG-HRP	Pregnanediol-3 $\alpha$ -Glucuronide-Horseradish Peroxidase Conjugate
RZ	reinheitszahl
RIA	Radioimmunoassay
SD	Standard Deviation
SEM	Standard Error of the Mean
SPI	Solid Phase Immunoassay
Tween(20)	Polyoxyethylene (20)-sorbitan monolaurate
$\Delta T$	Change in Transmission
$T_0$	Transmission at time zero
$T_{20}$	Transmission time of 20 minutes
WHO	World Health Organisation