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## APPENDICES

### CHEMICAL REAGENTS.

charcoal	WHO RIA Matched Reagent Program.
(1,2,6,7,- <sup>3</sup> H)cortisol	Amersham International PLC, England.
cortisol antiserum	WHO RIA Matched Reagent Program.
cortisol standard	WHO RIA Matched Reagent Program.
(2,4,6,7,- <sup>3</sup> H)estradiol	Amersham International PLC, England.
dextran	WHO RIA Matched Reagent Program.
diethyl ether	Merck Ltd., Darmstadt, Germany.
2,5,-diphenyloxazol(PPO)	Merck Ltd., Darmstadt, Germany.
disodium hydrogen phosphate	Merck Ltd., Darmstadt, Germany.
17 $\beta$ -estradiol antiserum	WHO RIA Matched Reagent Program.
17 $\beta$ -estradiol standard	WHO RIA Matched Reagent Program.
absolute ethanol	Merck Ltd., Darmstadt, Germany.
gelatin	WHO RIA Matched Reagent Program.
heparin	Leo Pharmaceutical Products, Balelrb, Denmark.
hydrochloric acid	Merck Ltd., Darmstadt, Germany.
ketamine	Parke Davis PTV, Ltd., Sydney, Australia.
(1,- <sup>3</sup> H)morphine	Amersham International PLC, England.
phenyl-oxazolyphenyl- oxazolyphenyl	Sigma Chemical Company, St.Louis, USA.
(1,2,6,7,- <sup>3</sup> H)progesterone	Amersham International PLC, England.

progesterone antiserum	WHO RIA Matched Reagent Program
progesterone standard	WHO RIA Matched Reagent Program
$^{125}\text{I}$ -prolactin kit	Diagnostic Products Corporation (DPC), USA.
sodium bicarbonate	Merck Ltd., Darmstadt, Germany.
sodium chloride	Merck Ltd., Darmstadt, Germany.
thiomersal	Sigma Chemical Company, St.Louis, USA.
toluene	Merck Ltd., Darmstadt, Germany.
 <b>INSTRUMENTS</b>	
autoclave	Forma Scientific Mariette, Ohio, USA.
balance	W.M.Ainsworth and Sons Inc., Benver, Colo, USA.
centrifugator	Cay Adams, Decton, Dickinson and Company, Parsipany, USA.
magnetic stirrer	S-18521 Thermolyne Corporation, Jowa, USA.
mixer	Thermolyne Corporation, subsi- diary of Sybom Corporation, Dubugelowa, USA.
pH-meter	5985 Cole Parmer Instrument Company, Illinois, USA.
pipettegun	Clay Adam, USA.
refrigerated centrifuge	International Equipment Company Mass, USA.

Ultrasonic cleaner	W.M.Ainsworth and Sons Inc., Benver, Colo, USA.
millipore size 0.22 m	Corp Bedford Mass, USA.
tri-block heater	Thecam Incorporated, Princeton, N.J., USA.
$\beta$ -liquid scintillation counter	Packard Instrument Co.,USA.
micropipet	Pipetteman M.81, Gilson, France. Eppendorf 3130, Germany.

#### REAGENT PREPARATION

##### 1. Morphine Solution.

One hundred milligram of morphine hydrochloride and eighty-three milligram of sodium chloride were dissolved completely in 10 ml tri-distilled water to final morphine concentration 10 mg/ml. The solution was sterilized by filtering through 0.22 mm millipore filter and stored in 4 °C for up to one month.

##### 2. Assay Buffer (Buffer S,BS)

Dissolved gelatin 1.0 gm in warm distilled water 300 ml completely, then added the chemicals as below;

sodium dihydrogenophosphate-1-hydrate( $\text{NaHPO}_4 \cdot 2\text{H}_2\text{O}$ )	3.1 gm
disodium hydrogenophosphate( $\text{Na}_2\text{HPO}_4$ )	11.6 gm
sodium chloride( $\text{NaCl}$ )	8.8 gm
thiomersol(merthiolate)	0.1 gm

Added up the solution to 1 liter with distilled water, mixed until the chemicals dissolution completely. Adjusted the pH to the range 7.2-7.2 and stored at 4°C. This solution is used as a diluent for all the reagents in steroid assays and should be stable for up to 1 month at 4°C.

### 3. Charcoal Suspension

Dissolved detran 0.0625 gm in 100 ml of assay buffer completely, then added activated charcoal 0.625 gm and shaken vigorously by magnetic stirrer for 30 seconds. Stored at 4 °C. The charcoal suspension should be stable for up to 1 month and need to be thoroughly mixed during usage.

### 4. Counting Solution

Mixed 2,5-diphenyloxazol(PPO) 5 gm, phenyl-oxazolyphenyl-oxazolyphenyl(POPOP) 0.3 gm, toluene 1 litre and dioxane 200 ml homogenously and stored in brown bottles.

### 5. Steriod Antiserum

Cortisol, estradiol and progesterone antiserum were raised in intact rabbit against cortisol-21-hemisuccinate-BSA, estradiol-6CMO-BSA and progesterone-3CMO-BSA, respectively. Each was obtained from WHO RIA Reagent Program and bottled in lyophilized form. Immediately before use, the contents should be reconstituted with 10 ml of assay buffer.

### 6. Steriod Tracers

1,2,6,7-<sup>3</sup>H-cortisol, 1,2,6,7-<sup>3</sup>H-estradiol and 2,4,6,7-<sup>3</sup>H-progesterone each was filled in vial with concentration 25 Ci and was diluted up to 5 ml with analytical grade of toluene: ethanol; 9:1 v/v and stored at 20 °C. The working tracer was prepared by evaporating the tracer solution and diluted in assay in buffer to 100 nCi/ml in assay.

## 7. Steroid Standards

Cortisol, estradiol and progesterone standards were provided in ethanolic solution at a concentration of 6.0 mol/l, 150 nmol/l and 250 nmol/l, respectively, in ampoule. Reconstituted the residue from 100  $\mu$ l of the ethanolic solution with 10 ml of assay buffer. Heated to 40 °C for 30 minutes. Mixed vigorously and allowed as stock standards to cool to 4 °C before use. These stock standard solutions contained cortisol, estradiol and progesterone at concentration of 60 nmol/l, 1.5 nmol/l and 2.5 nmol/l, respectively.

Prepared seven serial dilution (1:1, stock:assay buffer) of each stock standard solution shortly before use with serial cortisol concentration of 6,000, 3,000, 1,500, 750, 375, 188 and 94 fmol/100  $\mu$ l respectively, serial estradiol concentration of 750, 375, 188, 94, 47, 24 and 12 fmol/500  $\mu$ l respectively and progesterone concentration of 1,250, 625, 313, 156, 78, 39 and 20 fmol/500  $\mu$ l respectively.

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## BIOGRAPH

Miss Wannapa Settheetham was born on November 22, 1962 at Khonkaen. She received Bachelor Degree of Science (nursing, second honor class ) from Khonkaen University in 1984, and Master Degree of Science (Pharmacy), Mahidol University in 1987. At present she is the instructor of Department of Physiology, Faculty of Medicine, Srinakharinwirot University.



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