

CHAPTER VI

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CHAPTER VII

APPENDICES

Appendix I

1. Chemical Reagents

BSA (RIA grade) : Sigma Chemical Company, USA.

Charcoal Reagent : WHO RIA Reagent Program

Chloramine T (MW 281.69) : E. Merck, Germany.

Dextran : WHO RIA Reagent Program

Decon-90 : BDH Chemical Ltd.

Diethyl Ether : E. Merck, Germany.

Dioxane (GR) : E. Merck, Germany.

Disodium hydrogen phosphate (Na₂HPO₄ .2H₂O: MW 177.99)

: E. Merck, Germany.

EDTA (C_{10} H_{14} N_2 Na_2 O_8 .2 H_2O , MW 372.24)

: E. Merck, Germany.

Ethanol (absolute) : E. Merck, Germany.

Gelatin (GR) : E. Merck, Germany.

Hydrochloric acid : E. Merck, Germany.

Liquidfluor : New England Nuclear, USA.

Sephadex G-100 : Pharmacia, Uppsala, Sweden.

Sodium azide : Sigma Chemical Company, USA.

Soduim cholride (NaCl) : E. Merck, Germany.

Sodium hydroxide (GR) : BDH Chemical Ltd.

Sodium dihydrogen phosphate monohydrate (Na ${
m H}_2{
m PO}_4$. ${
m H}_2{
m O}$, MW

137.99)(GR) : E. Merck, Germany.

Sodium disulfate ($Na_2 S_2 O_5$, MW 190.10)

: E. Merck, Germany.

Tuluene (GR) : E. Merck, Germany.

Thiomersal : Sigma Chemical Company, USA:

2. Radioactive Reagents

Iodine-125 (in NaOH) 5 mCi, IMS 300, batch no. 138AA

: Amersham International plc, England.

125 I- Cyn LH : Purified Cyn LH from NIH, USA.

125- I-TSH (Lot no. 0323) : Baxter Travenol, USA.

125 I-T4 (Lot no. 5042) : Baxter Travenol, USA.

125 I-TBG (Lot no. 0214) : Baxter Travenol, USA.

125 I-T3 (Lot no. 2167) : DPC, USA.

125 I-T4 (Lot no. 407) : DPC, USA.

125 I-PRL (lot no. 291) : DPC, USA.

2,4,6,7-3H-E₂ (250 uCi, batch no. 103) : Amersham, England

1,2,6,7,³H-P (100 uCi, batch no. 68) : Amersham,

England.

3. Anteserum and Standards

Rabbit antiserum to hCG (R13, PoolD) : NIH, USA.

Rhesus pituitary LH reference preparation

(NICHD-rhLH or WP-XV-20) : NIH, USA.

Rabbit anti-hTSH serum (Lot NO.4870) : Baxter, Travenol,

USA.

hTSH standards (Lot NO. 4869 , calibrated with Medical

Research council, WHO human pituitary TSH 68/38) : Baxter, Travenol USA. Rabbit anti-T4 serum coated tubes : Baxter Travenol, USA. T4 serum standard (Lot No.4461) : Baxter Travenol, USA. Sheep anti-TBG serum (Lot No.5148) : Baxter Travenol, USA. hTBG standard (Lot No. 5659) : Baxter Travenol, USA. Rabbit anti-hPRL serum (Lot No. 1109) : DPC, USA. hPRL standard (Lot No. 6033, WHO second IRP 83/562) : DPC, USA. Rabbit anti-hT4 serum (Lot No. 710) : DPC, USA. hT4 standard (Lot No. 7033) : DPC, USA. Rabbit anti-hT3 serum (Lot No. 1029) : DPC, USA. hT3 standard (Lot No. 5042) : DPC, USA. E_2 anteserum (Lot K. 158330) : WHO Program E₂ standard (Lot K. 079610) : WHO Program P anteserum (Lot K. 873610) : WHO Program P standards (Lot K. 079410) : WHO Program

4. Miscellaneous

Goat anti-Rabbit-r-globulin

N R S (K 478410) : WHO Program

Donkey anti-Rabbit-r-globulin (K 288210) : WHO Program

: DPC, USA.

Goat anti-Rabbit-r-globulin (for TSH assay)

: Baxter, Travenol, USA.

Appendix II

Instruments

Balances : Sartorius 2004 MP, E2000D USA.

Centrifugator (Dynac II) : Clay Adams, USA.

Refrigerated Centrifugator : Mistral 3000, MSE, England.

Foam decanting rack : DPC, USA.

Magnetic stirrer and spin bar

: Nuova II Sybron / Thermolyne

: Framo-Geratetechnik M 22/1 Germany

Micropipettes (Separated volumns of 10,20,50,100,200,250,500 and 1000ul

:Eppendrof, Germany.

Repetitive pipettings ($Multipette^{R}$ model 4780)

: Eppendrof, Germany.

Syringse repetitive pipetting dispenser model 8100

: Nichiryo, Japan.

Adjustable micropipette (10ul-100ul and 500ul-1000ul)

: Eppendrof, Germany.

Dispenser 511 : Socorex Switzerland.

Dispenser (Zippette): Jencons (Scientific) LTD.,

England.

Microsyringse (5,15ul): SGE, Australia.

pH-meter : Orion Research, USA.

Beta-counter model 1214 : LKB Wallac, Finland

Gamma-counter model 1270,1270 rack gammaII,1280

:LKB Wallac, Finland.

Heating Cabinets (series FE)

: WTB Binder, Germany.

Water batch : GFL, USA.

Refrigerator (- 4°C) : Electrolux, USA.

Refrigerator (-20°C): MAK, Thailand.

Refrigerator (-40°C): Vestfrost, USA.

Stop-watch clock : Heuer, Germany.

Ultrasonic cleanser : Metter Electronics, USA.

: Branson Cleaning Equipment

Company, USA.

Disposable plain 12x75 mm polystylene tubes

:Elkay, USA.

Disposable plain 10x75 mm polystylene tubes

: Nunc, Denmark.

Disposable microfuge tubes : Elkay, USA.

Disposable Volumetric pipette (10ml)

: Costar, USA.

Survey meter (model SML-2): Technical Association,

CA, USA.

Appendix III

1. Preparations of steriod hormone assay buffers

Steriod assay buffer (S)

This buffer(s) is used as the diluent for all reagents in the steriod assays. 1.0 gm of gelatin was

dissolves in warm distilled water 300 ml completely, then added the chemicals as below:

2.70g Sodium dihydrogen phosphate monohydrate ($\mathrm{NaH}_2\mathrm{PO}_4$.

 H_2O , MW 137.99)

- 14.50g Disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$, MW178)
- 8.80g Sodium chloride (NaCl)
- 0.10g Thiomersal (merthiolate)

The solution was added up to 1 litre with distilled water, mixed until the chemicals dissolved absolutely and then checked the pH, and adjusted to be ranging 7.2-7.4. This steriod buffer should be stored and stable for up to 1 month at 4°C .

Charcoal suspension

0.0625 gm of dextran was dissolved in 100 ml of assay buffer (BS) completely, then added activated charcoal 0.625gm and shaked vigorously by magnetic stirrer for 30 seconds. This suspension should be kept at 4° c and stable for up to 1 mounth . When using, it must be mixed throughly at 4° c in ice batch.

Counting solution

Toluene 3 litres, liquidfluor 128 ml and dioxane 600 ml were mixed homogeneously and stored in brown bottles.

- 2. Preparations of LH radioimmunoassay
- 2.1 Phosphate buffer (0.5 M)
- -2.1.1. Monobasic sodium phosphate (Stock solution A)
- $69.0~\rm gm$ of $\rm NaH_2PO_4$. $\rm H_2O$ was dissolved in 500 ml distilled water and then brought total volume to 1 litre with distilled water. This stock solution should be kept at 4 $^{\rm O}{\rm C}$ and stable for 6 month.
- -2.1.2. Dibasic sodium phosphate (Stock solution B)
- $89.0~\rm gm$ of $\rm Na_2HPO_4$. 2 $\rm H_2O$ was dissolved in 500 ml distilled water and brought total volume to 1 litre with distilled water. This stock solution should be stored at $\rm 4^{\circ}C$.
- -2.1.3. Phosphate buffer (0.5 M, pH 7.5)

Both stock solution A (100 ml) and stock solution B (200 ml) were mixed and then adjusted the pH. This buffer shoul be stored at $4^{\circ}\mathrm{C}$.

- 143.0 gm of NaCl was dissolved in 500 ml distilled water and then added 150 ml of 0.5 M monobasic sodium phosphate. Both 200 ml of 0.5 M dibasic sodium phosphate and 200 gm of merthiclate were then added and mixed. 17.5 litres of distilled water was finally added, and then

adjusted pH to 7.0. This solution should be stored at 4 $^{\rm o}{\rm C}\,.$

2.3 0.1% gel-PBS (pH 7.0)

1.0 gm of gelatin (Merck) was mixed with 300ml PBS and then dissolved on hot plate with constant stirring. Then, total volumn was brought up to 1 litre with PBS and adjusted the pH. This buffer should be kept at 4° C.

2.4 EDTA-PBS (0.05M,pH7.0)

 $18.6~{\rm gm}$ of EDTA was mixed with 800 ml PBS and dissolved on hot plate with constant stirring. Total volumn aws brought up to 1 litre with PBS and adjusted the pH to 7.0. This solution should be stored at $4^{\circ}{\rm C}$.

2.5 1:400 NRS in 0.05 M EDTA-PBS

2.5~ml of NRS was aliquoted and added to 1 litre of 0.05 M EDTA-PBS. This solution should be kept at $4^{\,\mathrm{O}}\mathrm{C}$.

2.6 Chloramine T (1mg/1ml)

20 mg of chloramine T was dissolved in 20 ml of 0.05 M phosphate buffer, pH 7.5. This solution must be fresh prepared before using.

2.7 Sodium disulfite (2mg/ml)

 $10~{\rm mg}$ of sodium disulfite was dissolved in 5 ml of 0.05 M phosphate buffer, pH 7.5. Similar to chloramine T

solution, it must be prepared before using.

Appendix IV

Preparation of hormone free serum (HFS)

Hormone free serum (HFS) was used as diluent adjusting the high quality control pool serum and prepared following the North East Thames Region Immunoassay Unit (NETRIA).

A column (50 ml) was made using the dispossable syringse (50 ml) and filled with glass wool at the bottom of column with covered filter paper (Whatman no.4) and celite (fig 55). Charcoal amd celite (4:1) were mixed homogenously in 25 ml distilled water and then packed, eluted with 5 ml distilled water. After a column was settled, 50 ml of cynomolgus pooled serum adding $^{125}\mathrm{I}$ -T4 (~2000 cpm/ml) was then filtrated through this column. Void volume was collected. Subsequently, monkey column eluate was tested by (NH₄)₂SO₄ salt determining the protein in serum and then collected as hormon free serum. From this method, 98-99% of hormone free serum obtained was monitored by $^{125}\mathrm{I}$ -T4.

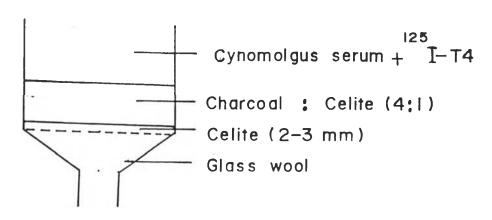


Fig 55. Illustrating preparation of hormone free serum column.

Appendix V

The precision profiles of each hormone assay were represented.

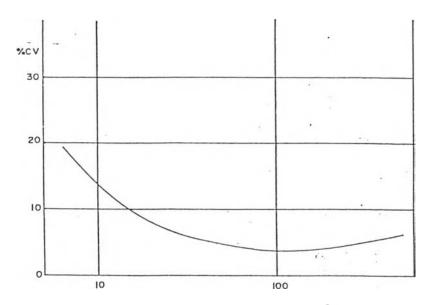


Fig 56. The precision profile of T3 assay . Plot shows % CV against dose for singletons (the precision of each tube).

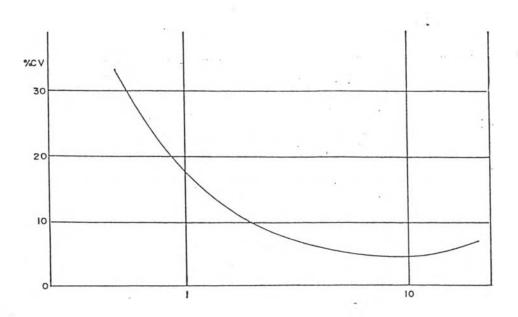


Fig 57. The precision profile of T4 assay. Plot shows % CV against dose for singletons (the precision of each tube).

Appendix V

The precision profiles of each hormone assay were represented.

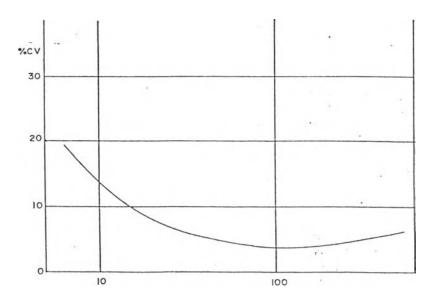


Fig 56. The precision profile of T3 assay . Plot shows % CV against dose for singletons (the precision of each tube).

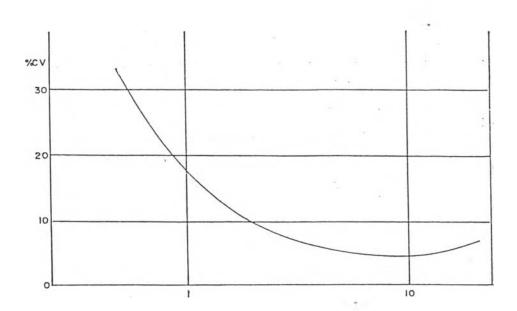


Fig 57. The precision profile of T4 assay. Plot shows % CV against dose for singletons (the precision of each tube).

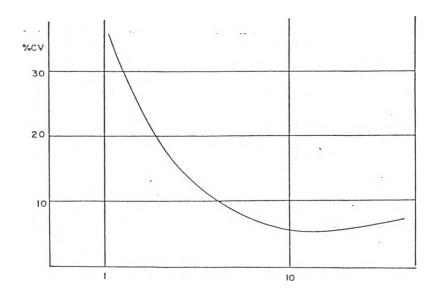


Fig 58. The precision profile of TSH assay. Plot shows % CV against dose for singletons (the precision of each tube).

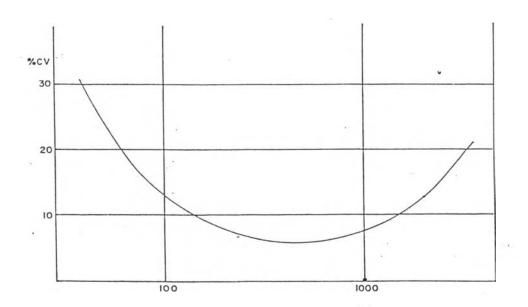


Fig 59. The precision profile of PRL assay. Plot shows % CV against dose for singletons (the precision of each tube).

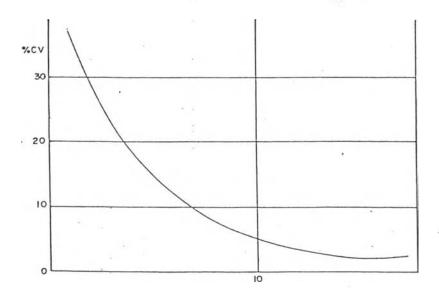


Fig 60. The precision profile of TBG assay. Plot shows % CV against dose for singletons (the precision of each tube).

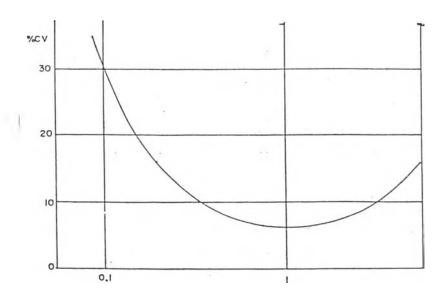


Fig 61. The precision profile of fT4 assay. Plot shows % CV ${\tt against\ dose\ for\ singletons\ (the\ precision\ of\ each\ tube).}$

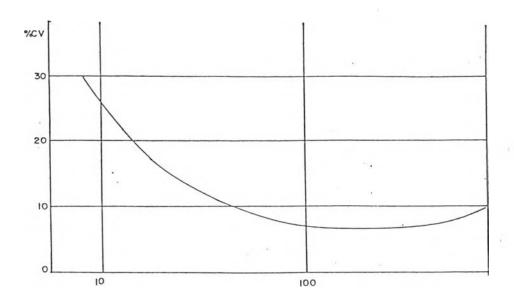


Fig 62. The precision profile of $\rm E_2$ assay. Plot shows % CV against dose for singletons (the precision of each tube).

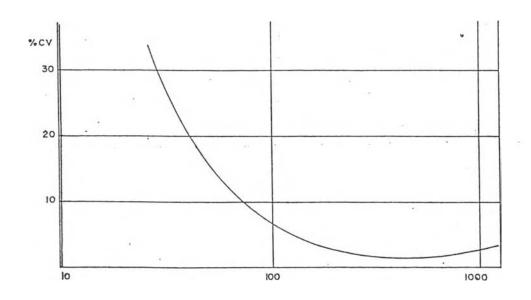


Fig 63. The precision profile of P assay. Plot shows % CV against dose for singletons (the precision of each tube).



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