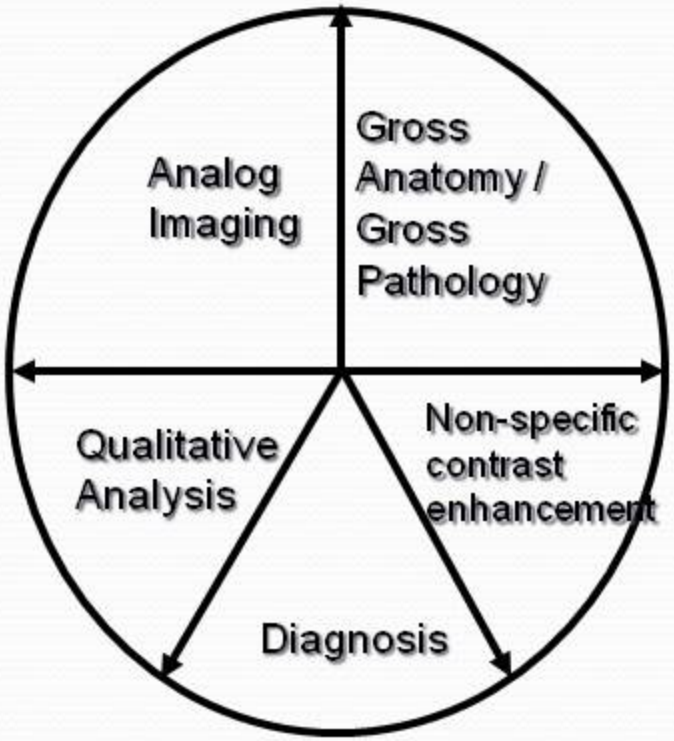
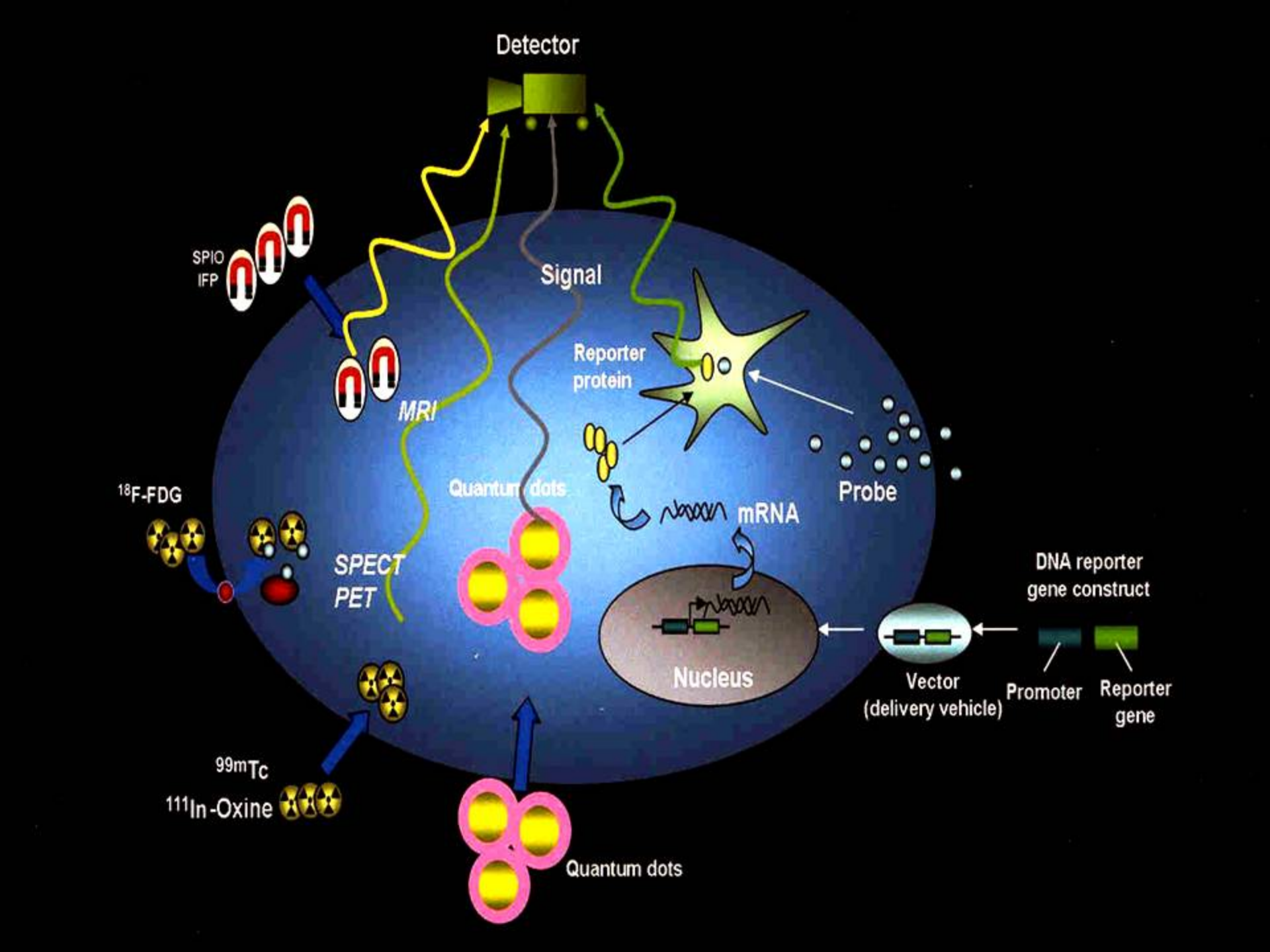
Radioimmunoassay

核子醫學部
朱力行 醫師

WHERE WE WERE IN THE FIRST CENTURY OF MEDICAL IMAGING

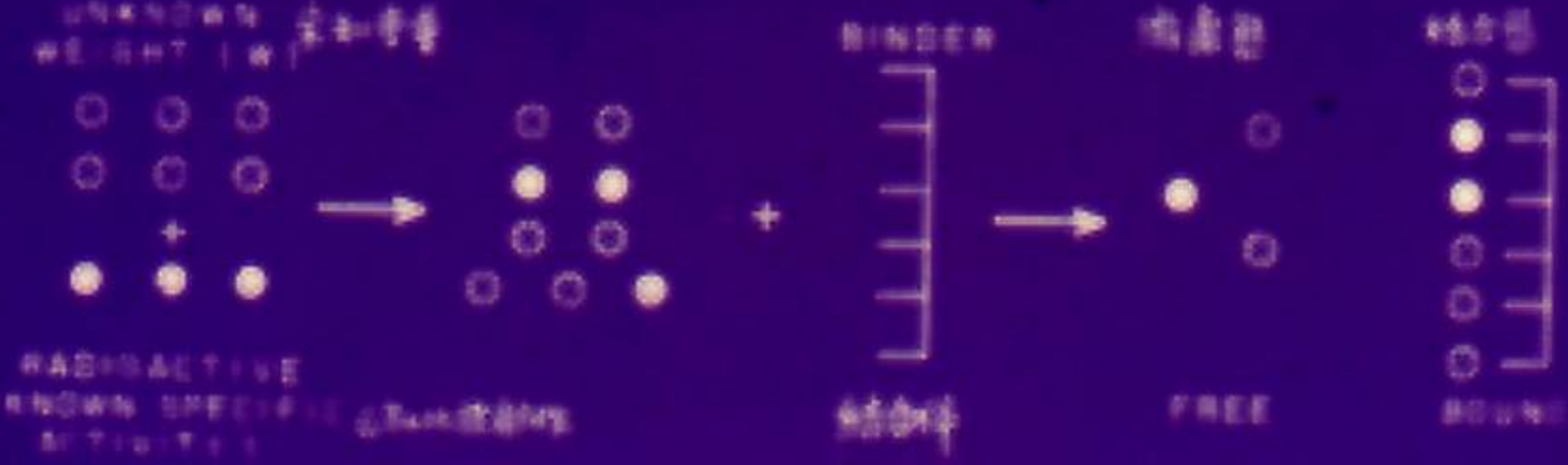



Detector
SPIO
IFP
Signal
Reporter
protein
MRI
Probe
^{18}F-FDG
Quantum dots
mRNA
SPECT
PET
DNA reporter
gene construct
Nucleus
Vector
(delivery vehicle)
Promoter
Reporter
gene
^{99m}Tc
^{111}In-Oxine
Quantum dots

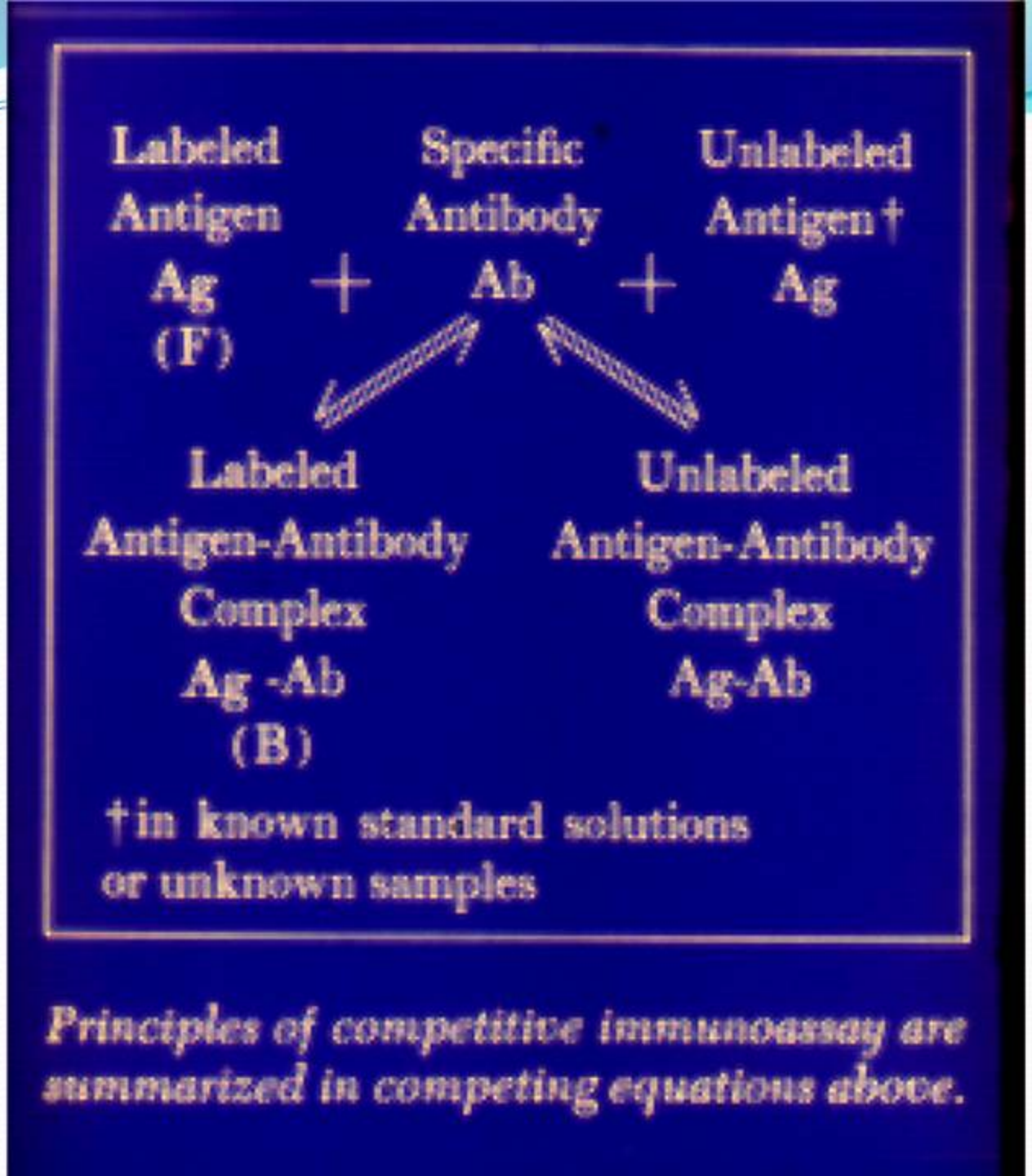
NUCLEAR MEDICINE

- In vitro study
- In vitro radio bioassay (RIA)
- * Radionuclide labeled ligand (tracer)
- * Immunologic reaction response
- * In vitro micro-biotechnique

E 飽和分析原理(Principle of saturation analysis)



结合態与游离態放射性之比值与原有物質重量(W)呈一函数関係变化。



Principles of competitive immunoassay are summarized in competing equations above.

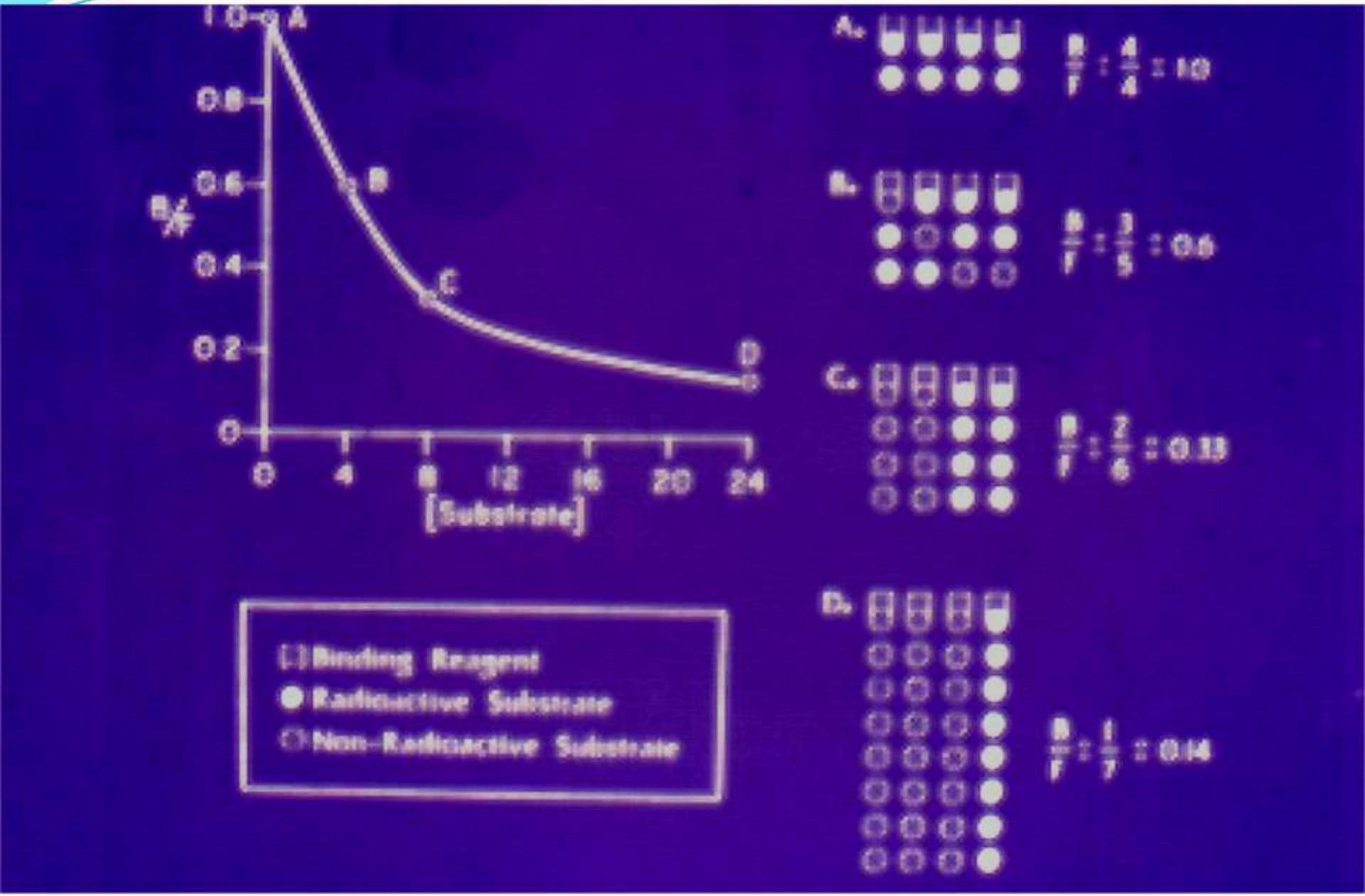
COMPETITIVE BINDING ASSAY(CBA)

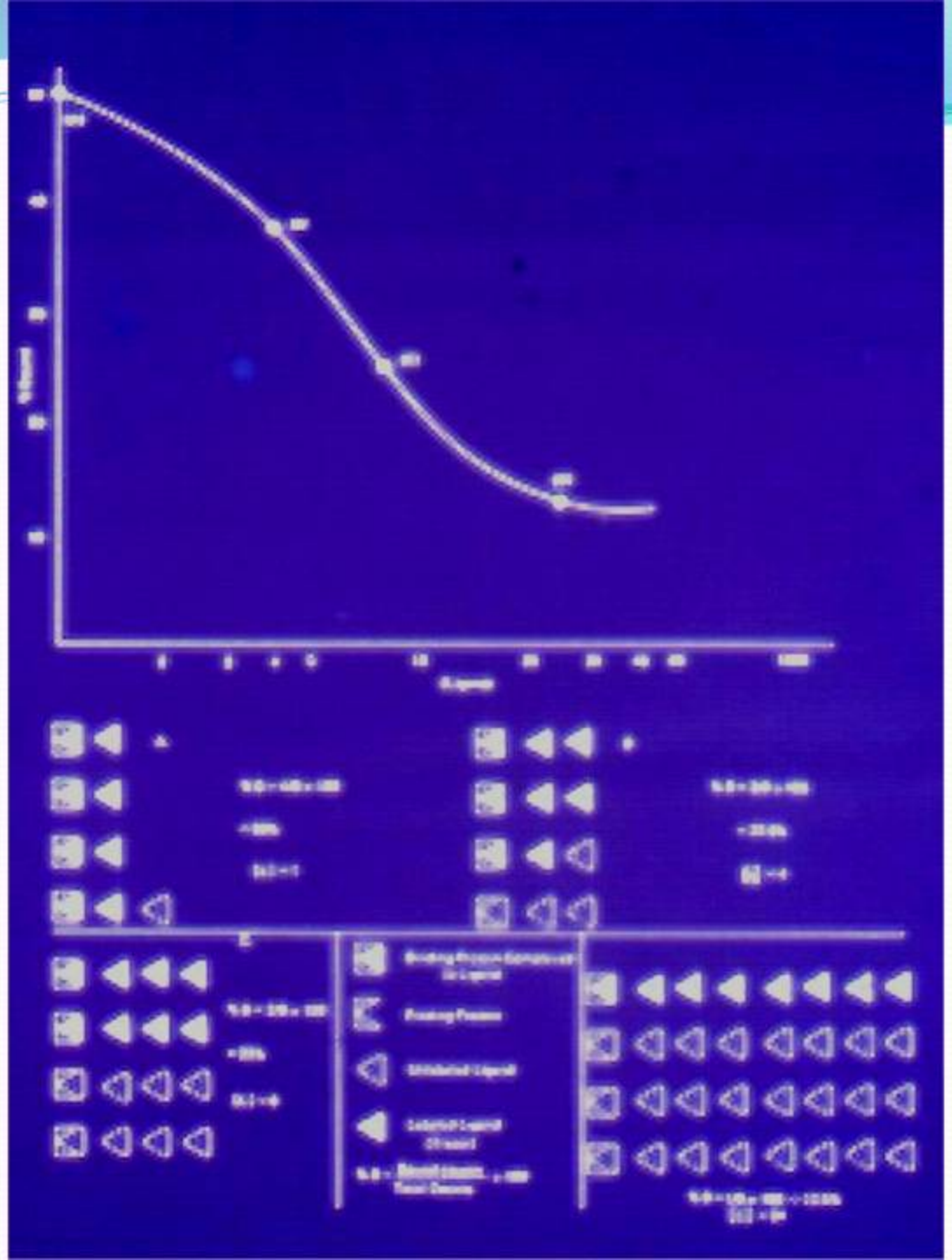
- Competitive binding principle
- Ag*
- Ag
- Ab (inadequate Qnt.)
- Ag* and Ag had similar immunochemical character, the competition produced by Ag
- Ag*-Ab and Ag-Ab complex

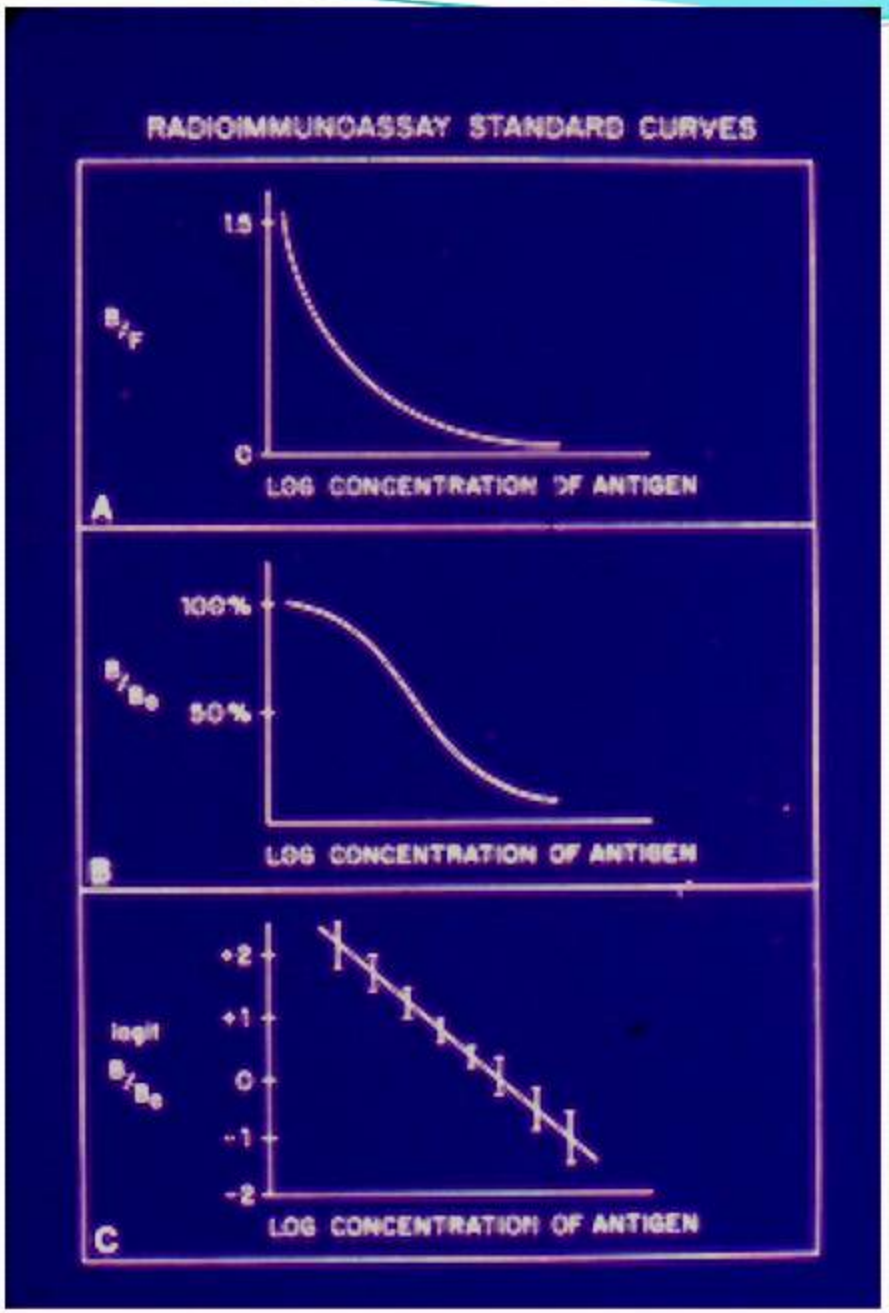
Four Basic Requirements for RIA

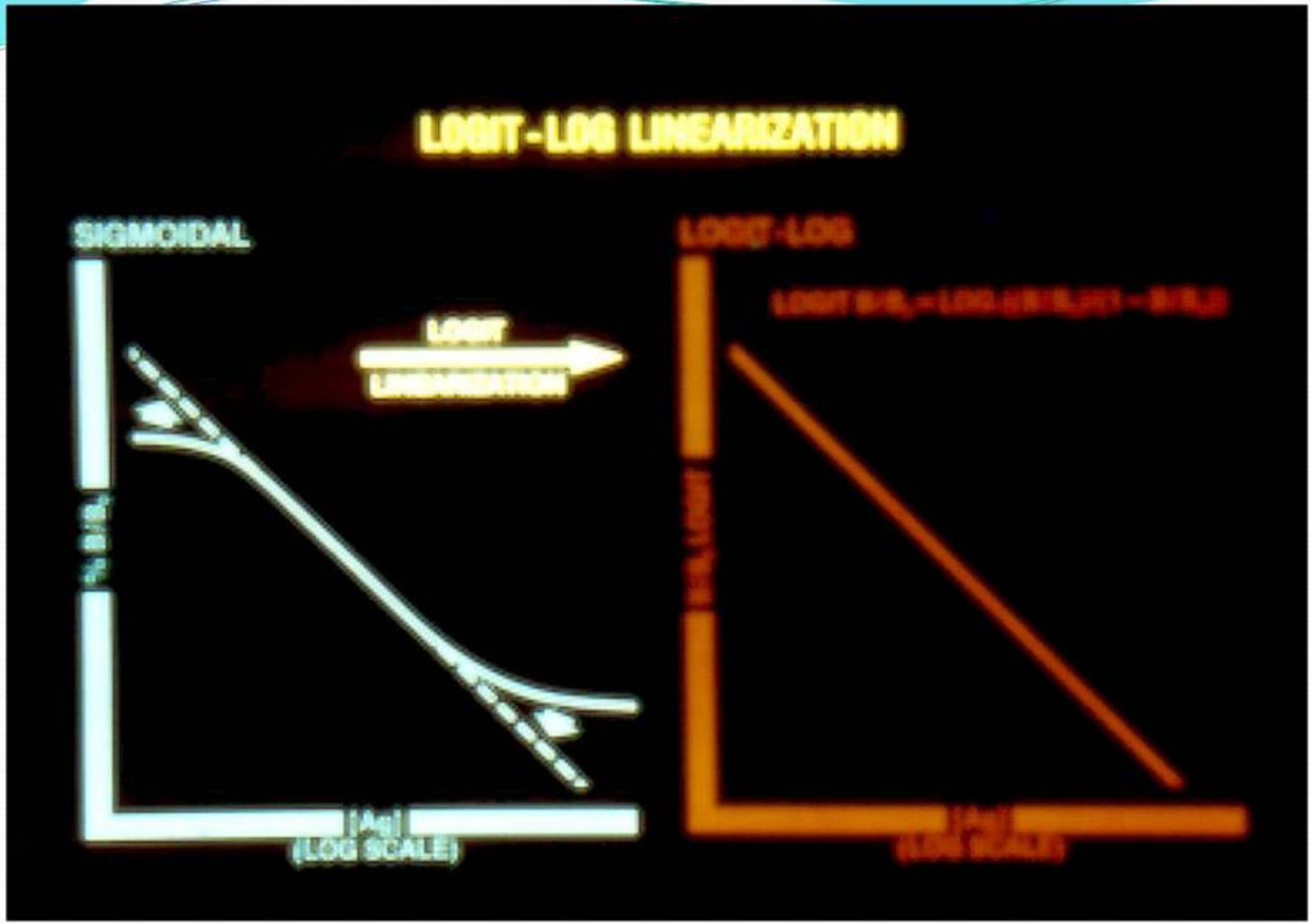
- Antibody- specificity, sensitivity, and titer.
- A purified antigen (Ag)
- An lsotope labeling method (Ag*)
- A separation technique (--Standard Curve)

- STANDARD CURVE
- B/F Vs. Ligand (ng/ml)
- B/T Vs. Ligand (ng/ml)
- B/Bo Vs. Ligand log (ng/ml)
- B/BoLogit Vs. Ligand log (ng/ml)


1.0
0.8
0.6
0.4
0.2
0
B/F
A
B
C
D
0
4
8
12
16
20
24
[Substrate]
A.
B.
C.
D.
Binding Reagent
Radioactive Substrate
Non-Radioactive Substrate




RADIOIMMUNOASSAY STANDARD CURVES
1.5
B/F
0
LOG CONCENTRATION OF ANTIGEN
A
100%
B/Bo
50%
LOG CONCENTRATION OF ANTIGEN
B
+2
+1
log it
B/Bo
0
-1
-2
LOG CONCENTRATION OF ANTIGEN
C


LOGIT-LOG LINEARIZATION
SIGMOIDAL
LOGIT-LOG
LOGIT
LINEARIZATION
% Bound
[Ag]
(LOG SCALE)

Separation techniques

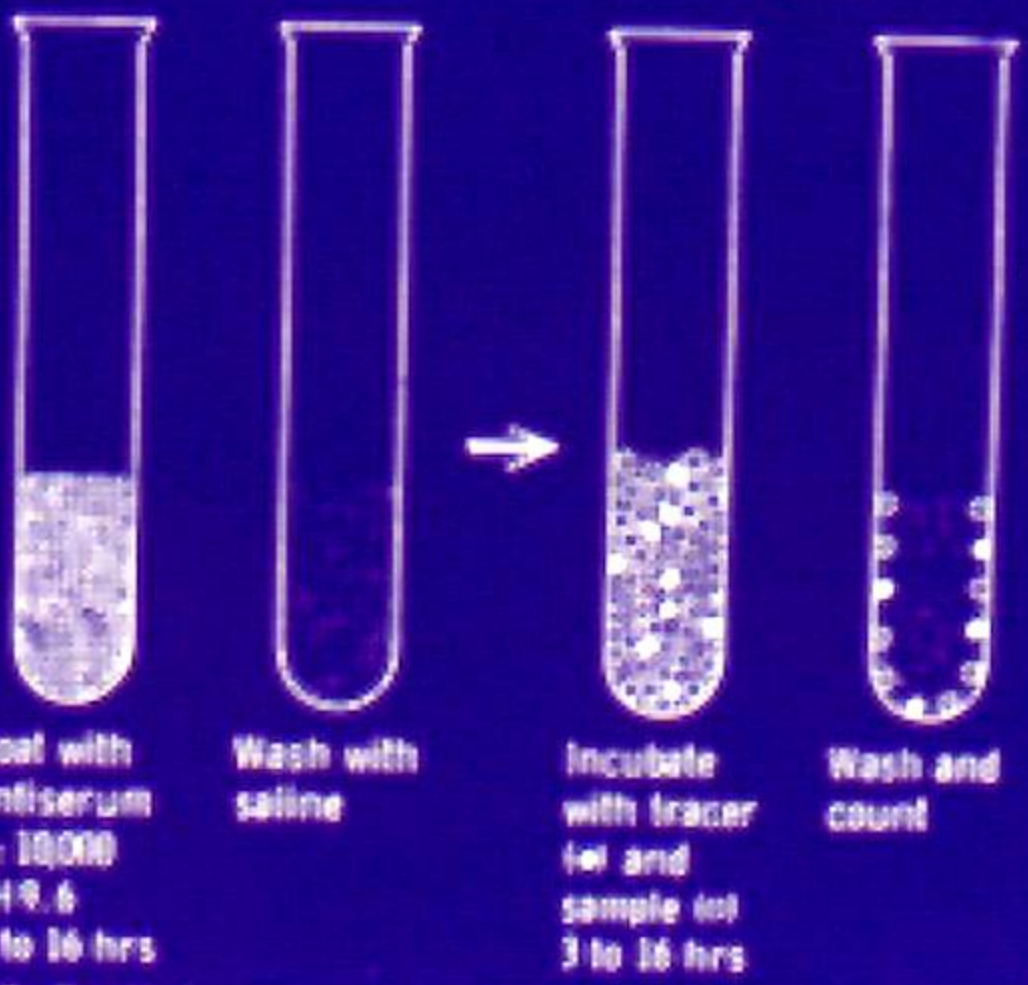
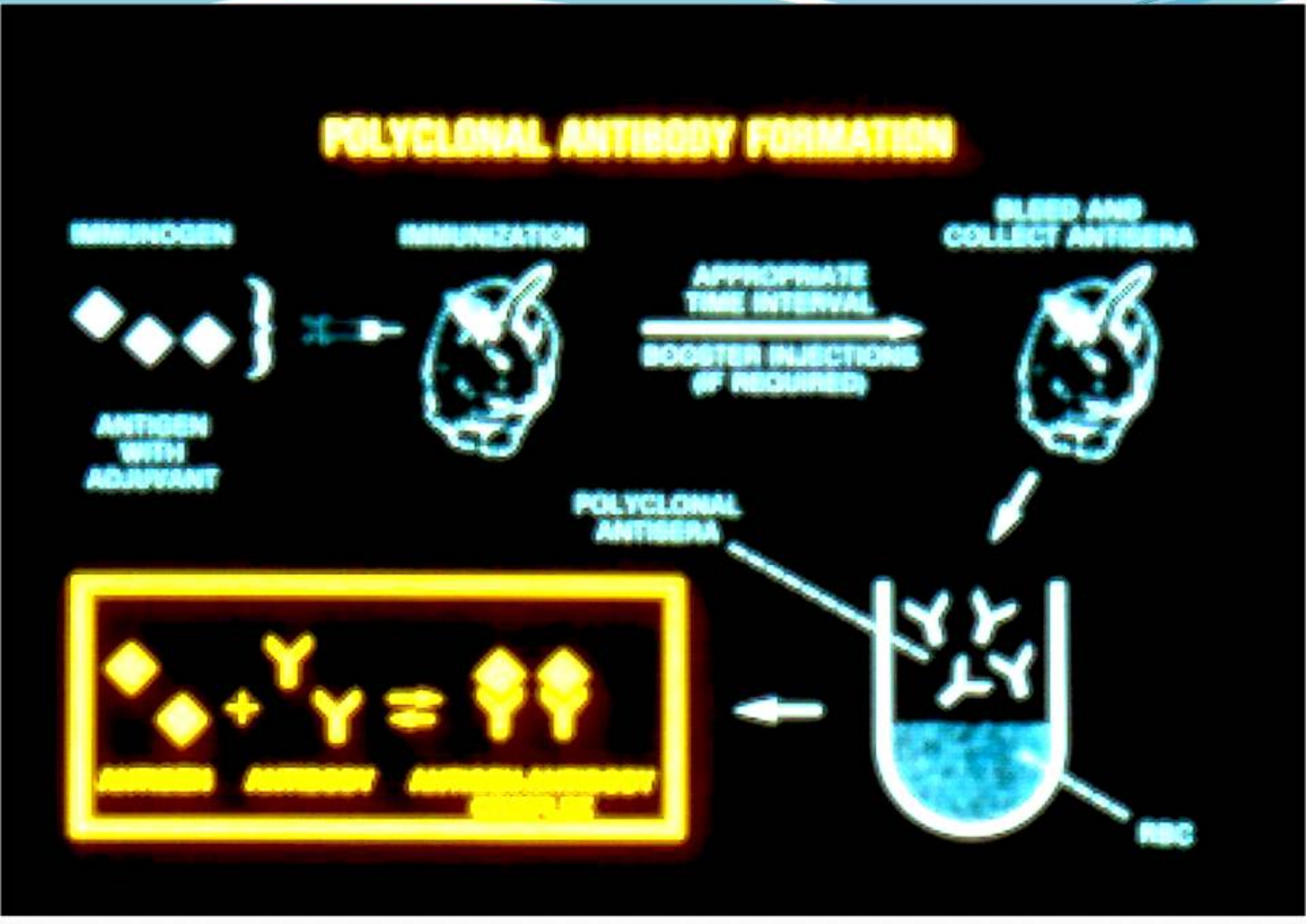


Fig. 2. Radioimmunoassay in antibody-coated tubes.


POLYCLONAL ANTIBODY FORMATION
IMMUNOGEN
IMMUNIZATION
APPROPRIATE TIME INTERVAL
BOOSTER INJECTIONS (IF REQUIRED)
BLEED AND COLLECT ANTISERA
ANTIGEN WITH ADJUVANT
POLYCLONAL ANTISERA
RBC

抗體

- RIA的特異性、靈敏度與抗體的品質有很大關係。
- 以被測物質爲抗原注入動物體內，在動物血清中即可出現特異結合性的抗體，分子量大於5000的較能產生抗體。
- 低分子量的可用Conjugate方法產生抗體。

單株抗體

- 細胞融合瘤技術也可以製備高特異性的單株抗體。
- 親和力大、滴度高、特異性強者，所製作的試劑品質佳 。

POLYCLONAL ANTIBODY FORMATION

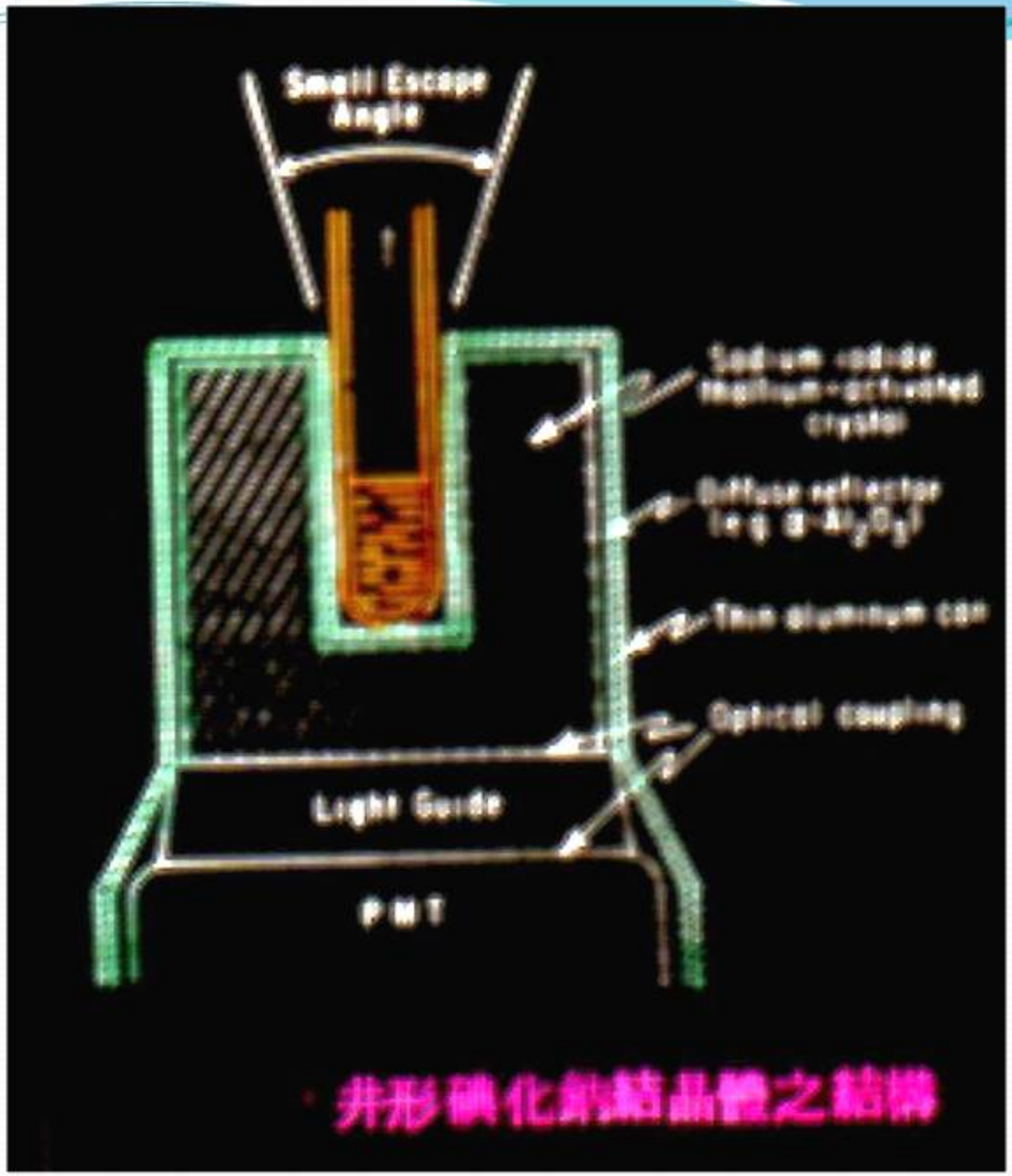
- Purified Ag- Immunogen
- Immunization
- Specific antibody (antiserum- Mw>5000 polypeptide complex with protein)
- Antibody affinity
- Antibody titer

Radionuclide Labeled Antigen

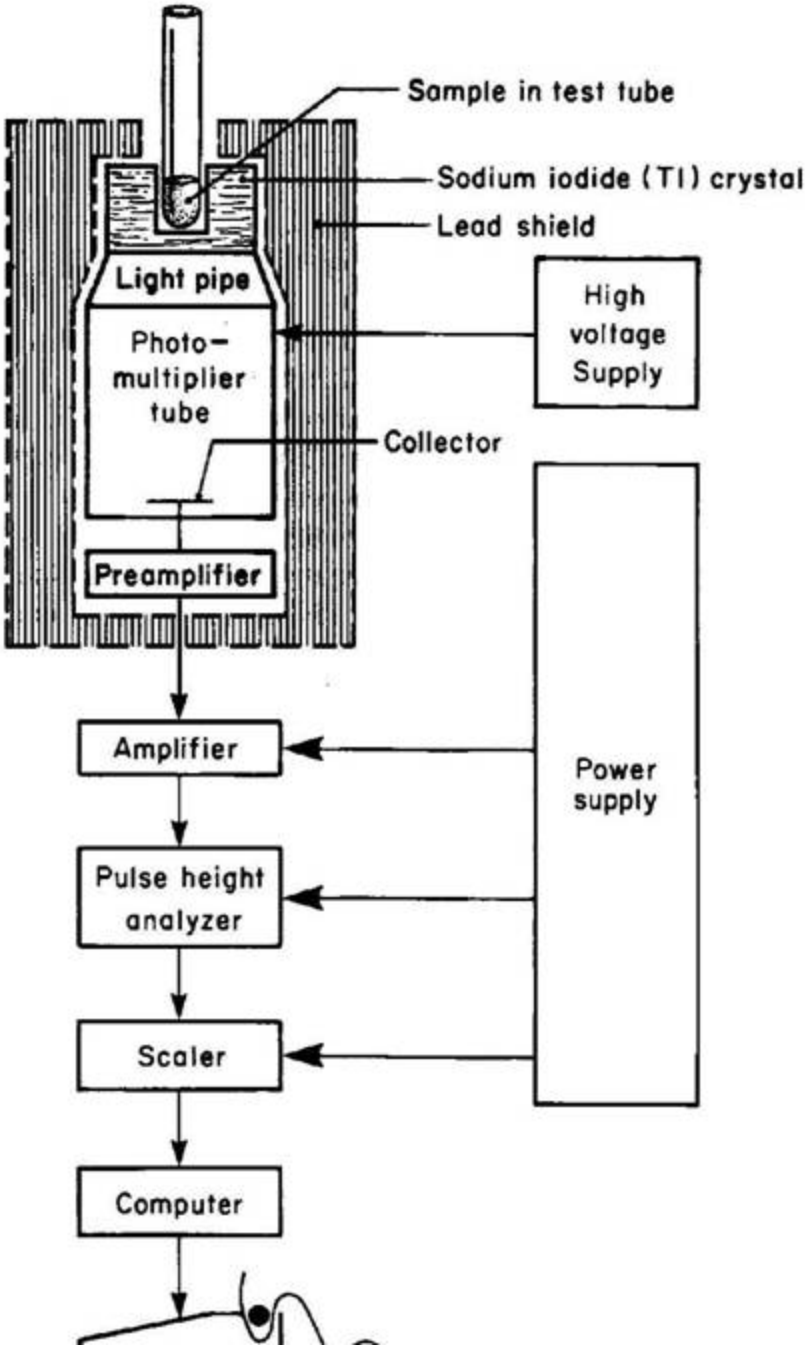
- Tracer preparation
- C-14,H-3 (Liquid Scintillation counter)
- I-131
- I-125 –(for labeling tyrosine 酥氨酸-)
- Iodination
- (Iodine 原子-replace the 烃基鄰位氫原子)
- (for non-tyrosine– prelinking酥氨酸甲 酯)

Requirement for Labeled Antigen

- Ag* Specific.activity=
- Ag* radioactivity/Ag*mas
- Ag* Immunoactivity(If more iodine-
- activity change?)
- Ag* Available counting
- RIA radiobio-purity >90%
- (Radiobio-purity= ImmunoAg* Spc. act. /
- Ag* total activity)



井形碘化鈉結晶體之結構


Sample in test tube
Sodium iodide (TI) crystal
Lead shield
Light pipe
Photo–
multiplier
tube
High
voltage
Supply
Collector
Preamplifier
Amplifier
Power
supply
Pulse height
analyzer
Scaler
Computer

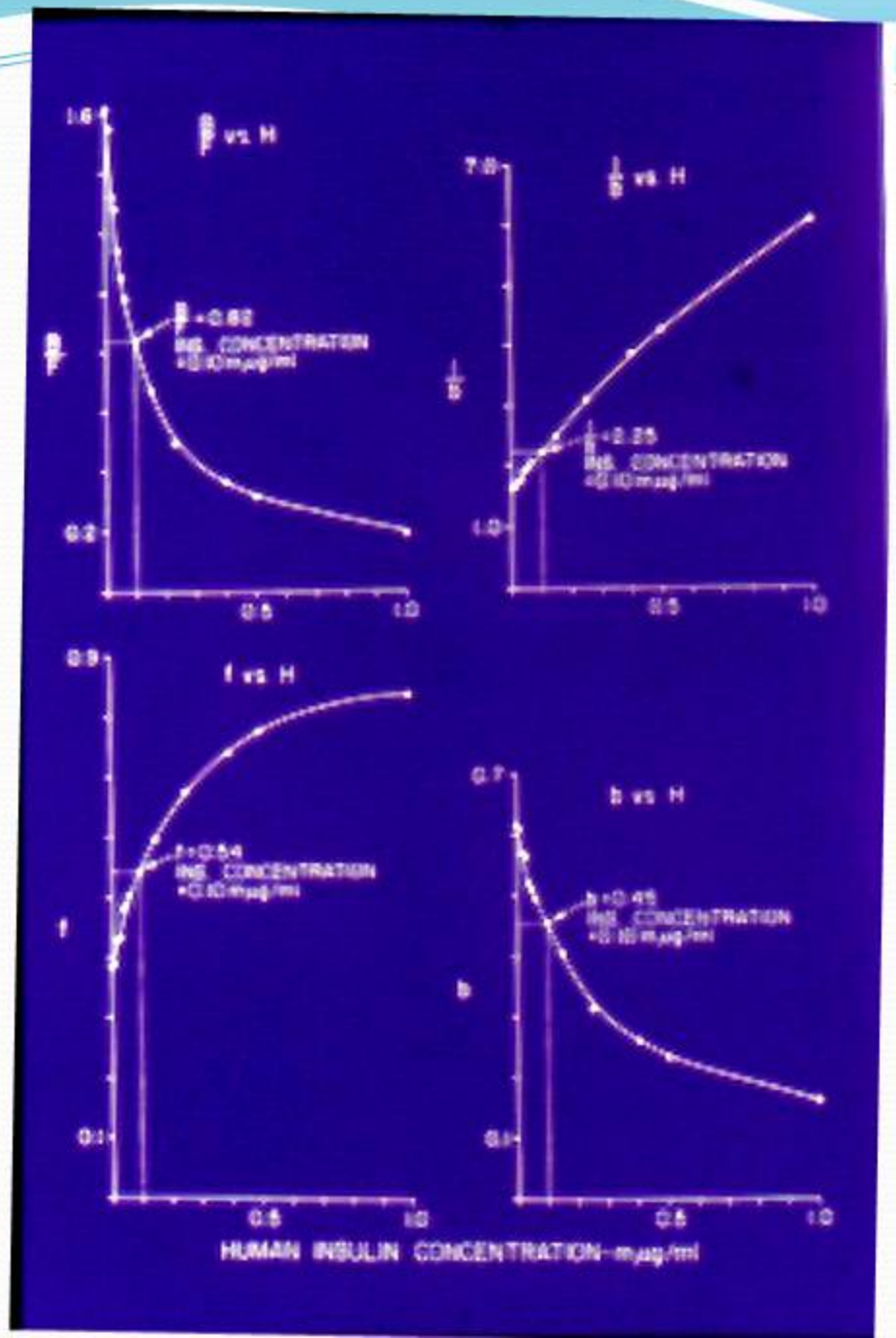
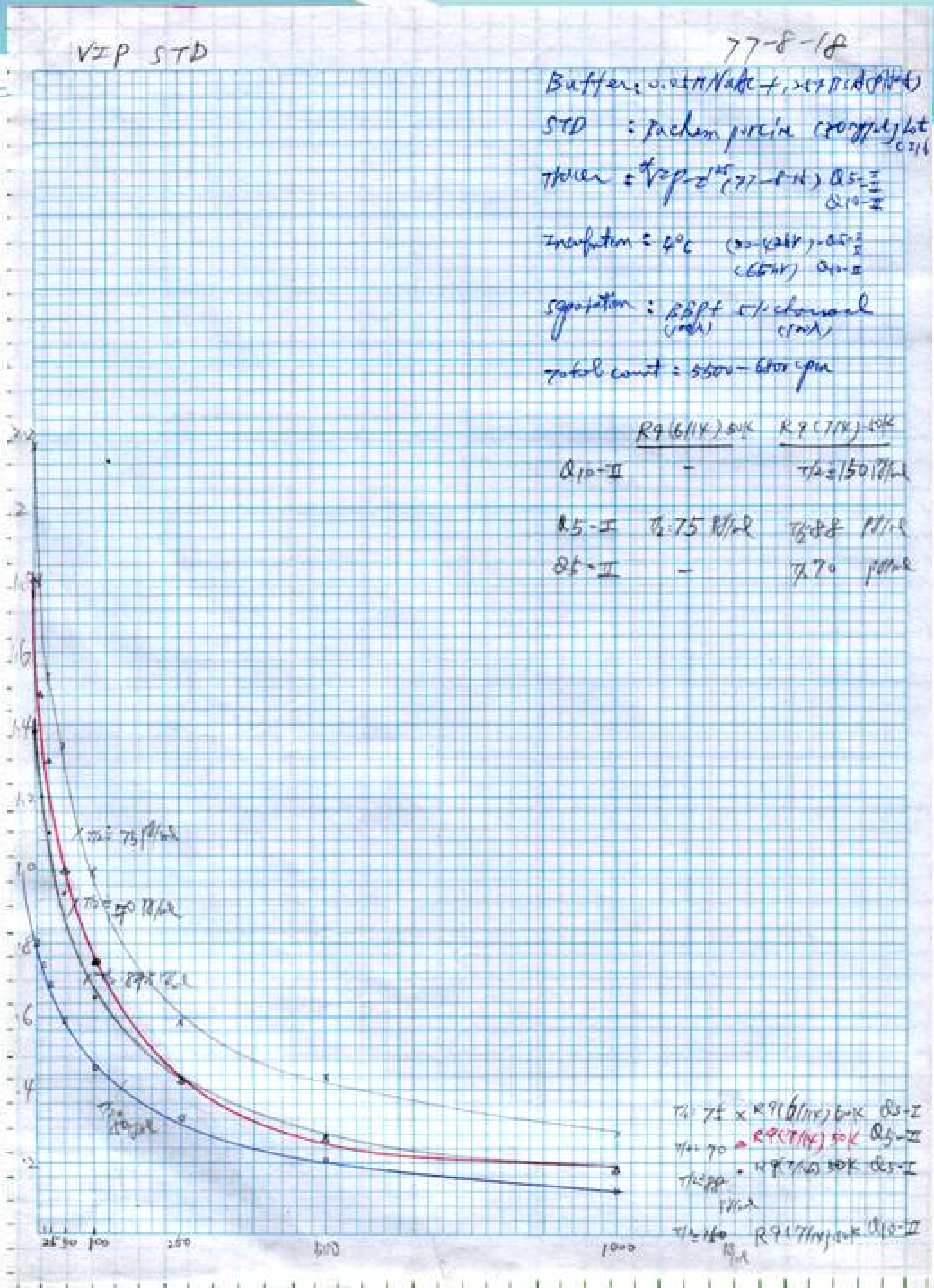

1.6
0.2
0.5
1.0
7.0
1.0
0.9
0.1
0.7
0.1
HUMAN INSULIN CONCENTRATION-mμg/ml

TABLE 17-2. Common Separation Techniques

Liquid Phase Separation	Solid Phase Separation	
	Liquid Reagent	Solid Reagent
Dextran-coated charcoal	Second antibody	Macro beads
Resin	Polyethylene glycol (PEG)	Coated tubes
	Second antibody/PEG Micro beads	

RIA DESIGN

- Information flow in clinical analytical work
- Hormone analyses (list of analyses with sampling instructions)
- Age variation
- Menstrual cycle variation



VIP STD
77-8-18
Buffer
STD
4°C
total count
Q10-II
Q5-I
Q5-II
250
500
1000

Historical Reviews

- 1959 Yalow & Berson First reported principles of RIA
- 1960 Yalow & Berson Applied RIA to mesaure human insulin
- 1960 Ekins First reported CPBA by using TBG as a specific binder
- 1960 Hunter , Green-Wood et al.Using Chloramine T method as a radioiodination procedure

- 1961 Hager et al To assay serum glucagon
- 1961 Rothenburg An assay for B12 using charcoal separated
- 1965 Rothenburg Firstly developed radioassaymatic assay
- 1968 Rodbard et al. QC. of RIA
- 1971 Lofkowita Assay for ACTH using cell-receptor with a specific binders
- 1977 Yalow Recived the 1977 Nobel Prize for Medicine

- Analysis information: Radioimmuno-analysis
- Analysis frequency
- Specificity: No cross reaction against other
- Sensitivity : 0.5 ug/l
- Calibration : high purified human FSH
- Precision: Intra-assay Variation=5%
- Between –assay V. = 8%

Competitive Binding Assay

- A group of in vitro analytical methods
- Based on non-covalent , reversible binding of a small molecule or ligand to a specific binding protein

Competitive Binding Assay

- As addition of increasing amounts of unlabeled ligand to reaction mixtures
- Containing known , constant amounts of
- labeled ligand and specific binding protein

COMPETITIVE (limited reagent)

Simultaneous

$$Ab + Ag + Ag\text{–}L \rightleftharpoons Ab{:}Ag + Ab{:}Ag\text{–}L$$

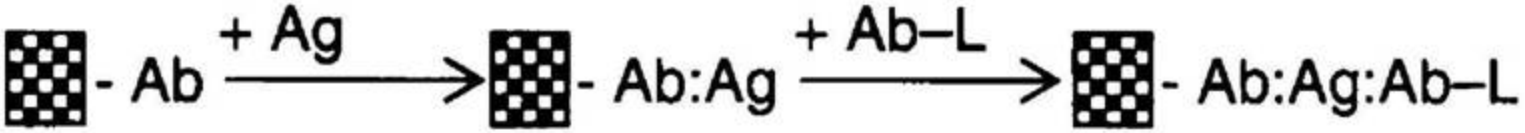
(free) (bound)

Sequential

$$\text{Step 1} \quad Ab + Ag \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Ab{:}Ag + Ab$$

$$\text{Step 2} \quad Ab{:}Ag + Ab + Ag\text{–}L \rightleftharpoons Ab{:}Ag + Ab{:}Ag\text{–}L + Ag\text{–}L$$

NONCOMPETITIVE (excess reagent, two-site, sandwich)



Types of Labels

- Radioisotopes
- Enzymes
- Fluorophores

Radioisotopes

- Isotope emulsion Mx.Sp.ac. t1/2 counter
- H-3 Beta 9.6x103 12.3y LS
- C14 Beta 4.5Ci/g 5730y LS
- P32 Beta 2.85x103 14.2d LS
- I125 Gamma 1.74x104 60 d crystal
- Co-57 Gamma 8.48x103 270d crystal
- *1Ci= 3.7xE+10 disintegration per second.

RIA isotope

- 130-Te (n, γ) 131 Te ->β--→131 I
- 8.04d

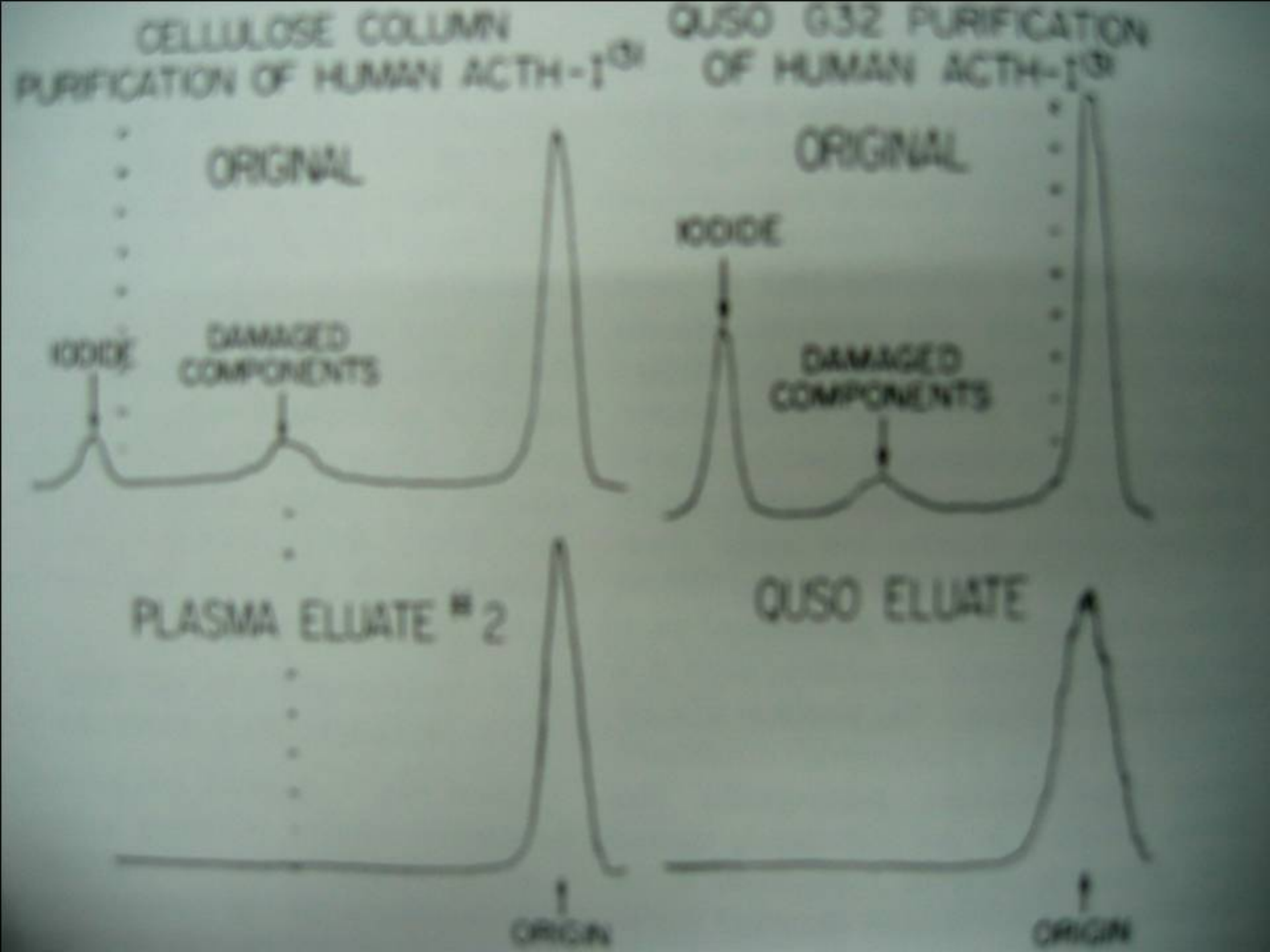
- 124Xe (n, γ) 125 Xe -> EC →125 I
- 60.14 d

Assay Design and Radioligand Quality

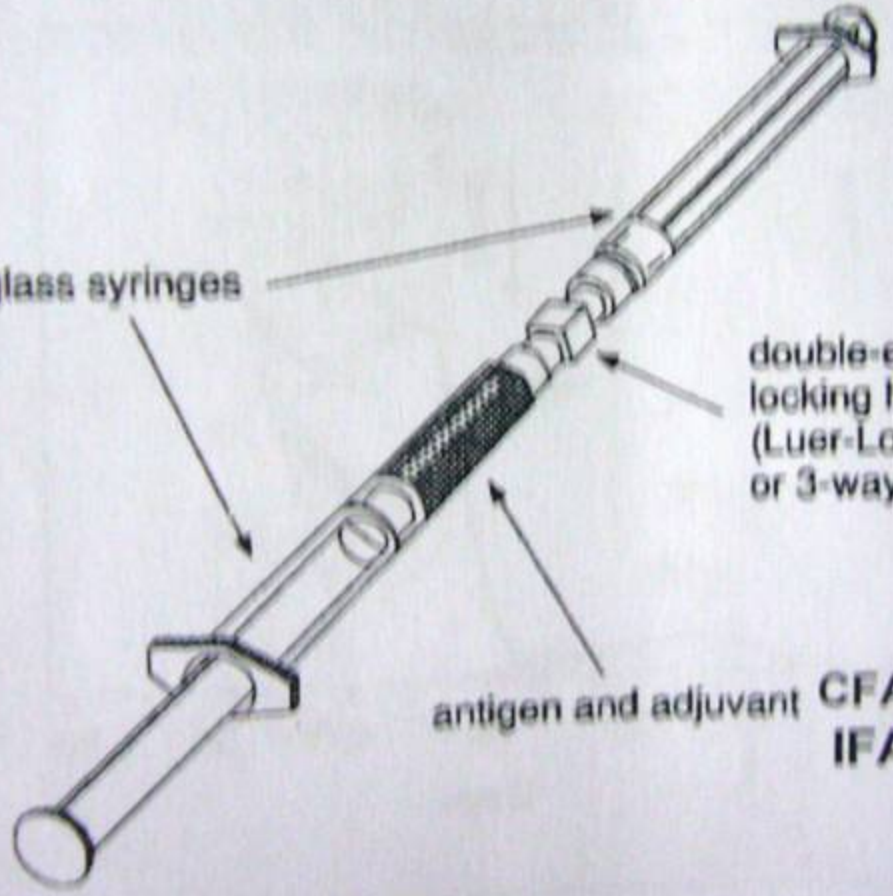
- Defining purposes- the assay
- Selection or production of reagents:
- antigen for labelling
- Iodination
- Assessment and purification of Ag

放射性測量

- 用γ井型計數器測定^{125}I，用液態閃爍計數器測定^{3}H。
- 校正；power, energy, background
- Multi-well counter；cross talk；χ^2
- High Count
- Low Count


CELLULOSE COLUMN
PURIFICATION OF HUMAN ACTH-1(3)
ORIGINAL
IODIDE
DAMAGED
COMPONENTS
PLASMA ELUATE #2
ORIGIN
QUSO G32 PURIFICATION
OF HUMAN ACTH-1(3)
ORIGINAL
IODIDE
DAMAGED
COMPONENTS
QUSO ELUATE
ORIGIN

Double-syringe device
(antigen-adjuvant emulsion)



Antibody-antigen interactions

- The interactions can be disrupted by
  - **high salt concentrations**
  - **extremes of pH**
  - **detergents**

Use of antibodies

- Abs can be tagged to **fluorophores, radioisotopes and enzymes**
- Antibodies can be used to:
  - **detect antigen**
  - **purify proteins**
  - **locate antigen**

ELISA = EIA

(enzyme linked immunosorbent assay)

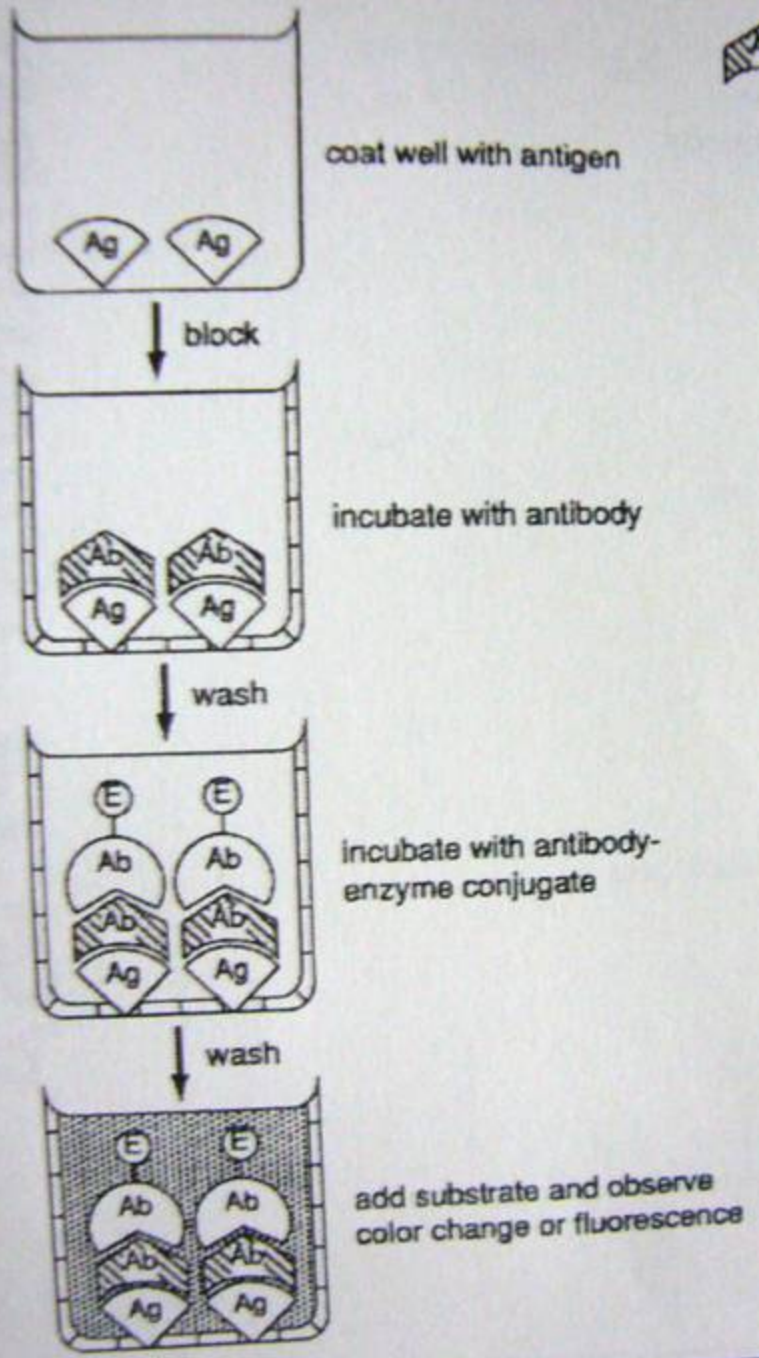
direct ELISA (tests for virus),

indirect ELISA (tests for Ab).

ELISA is similar to the immunofluorescent assays, but differs in the type of molecule that is tagged to the antibodies that are used.

- The molecule that is attached to an antibody in an ELISA assay is an enzyme.
- The presence of the enzyme is detected by adding a substrate to the enzyme which when acted upon by the enzyme produces **a colored product**.
- An indirect ELISA test is used to screen individuals for HIV infection (Ab).

Direct ELISA



Antibody Production

- Antibody Immunization
- Immunogen
- Booster

Antiserum Properties of RIA

- Characteristics of Biological active substance of significance of RIA
- Molecular heterogeneity
- Molecular homology
- The widespread synthesis
- Different processing : met- enkephalin –> B-endorphin ---- precursor= proopiomelanocorticotropin(POMC)

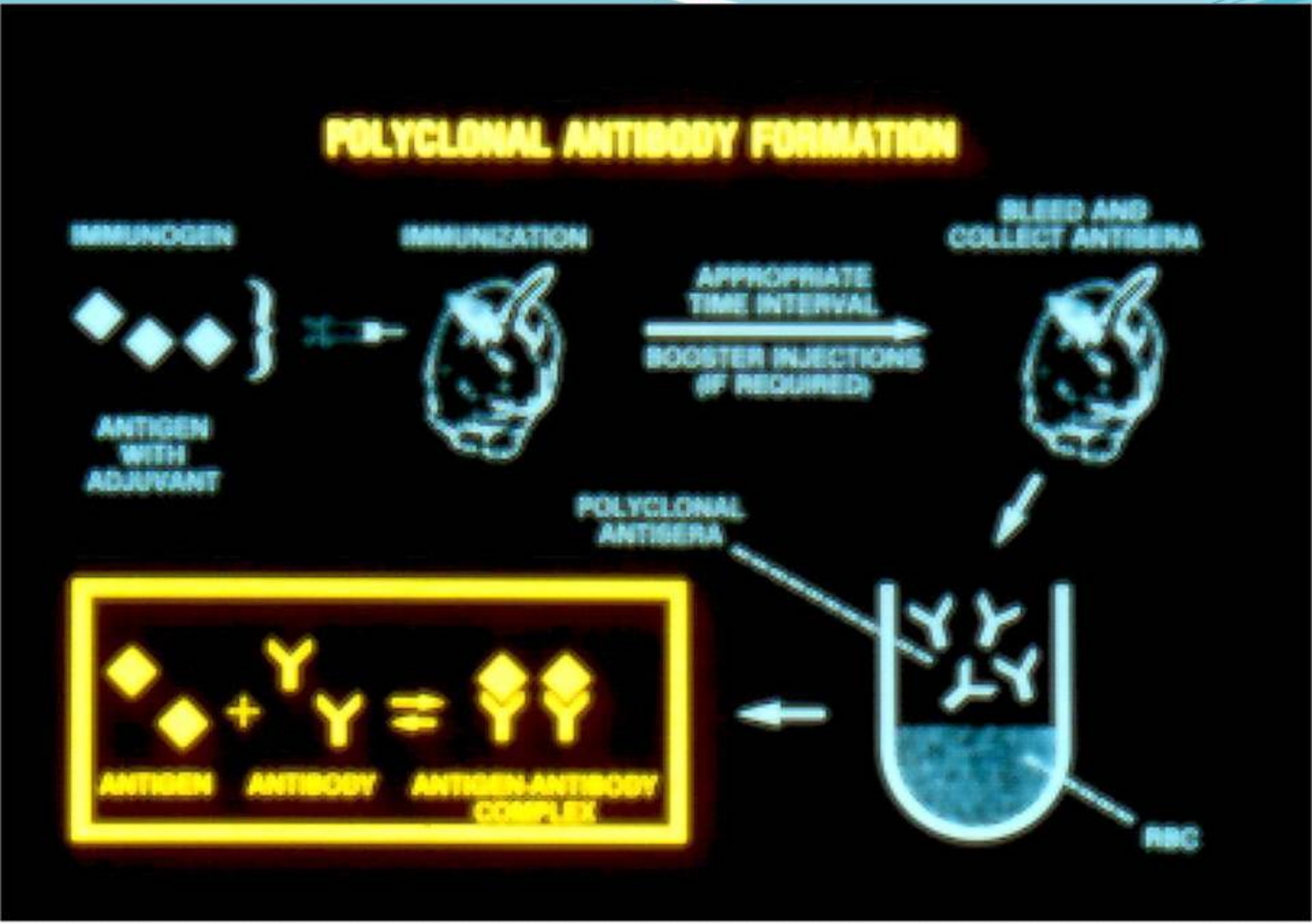
Parameters of Significance for RIA

- Titer (or working dilution) number of samples If: below :1000 are of limited value –unspeific interference----- 10 3to 108 (common 10 6)
- Avidity– ultimate detection limit is

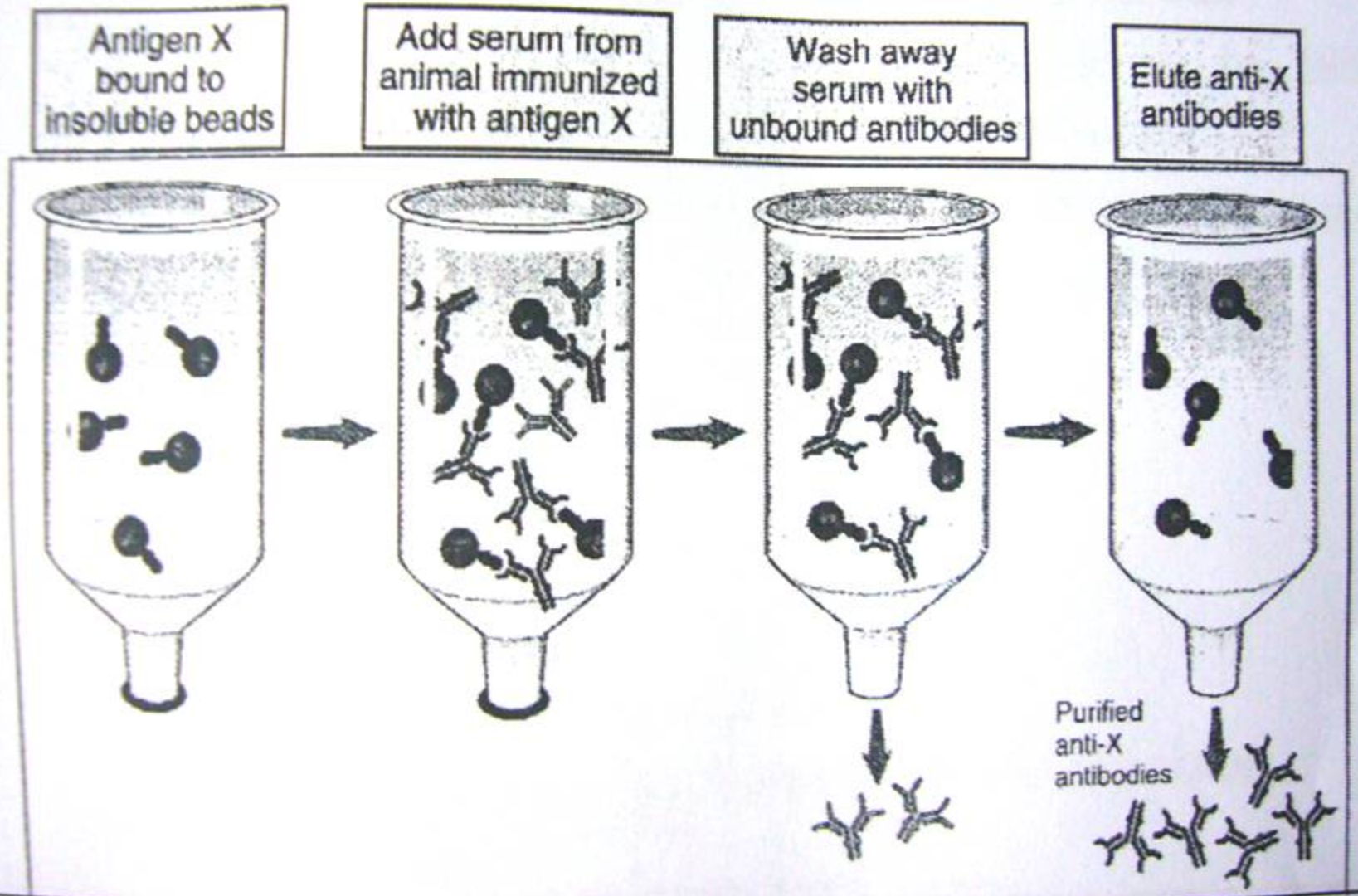
  2E/ ko eff

Specificity –directed 3-6 residues of AA. Hexoses or nucleotides

Homogeneity

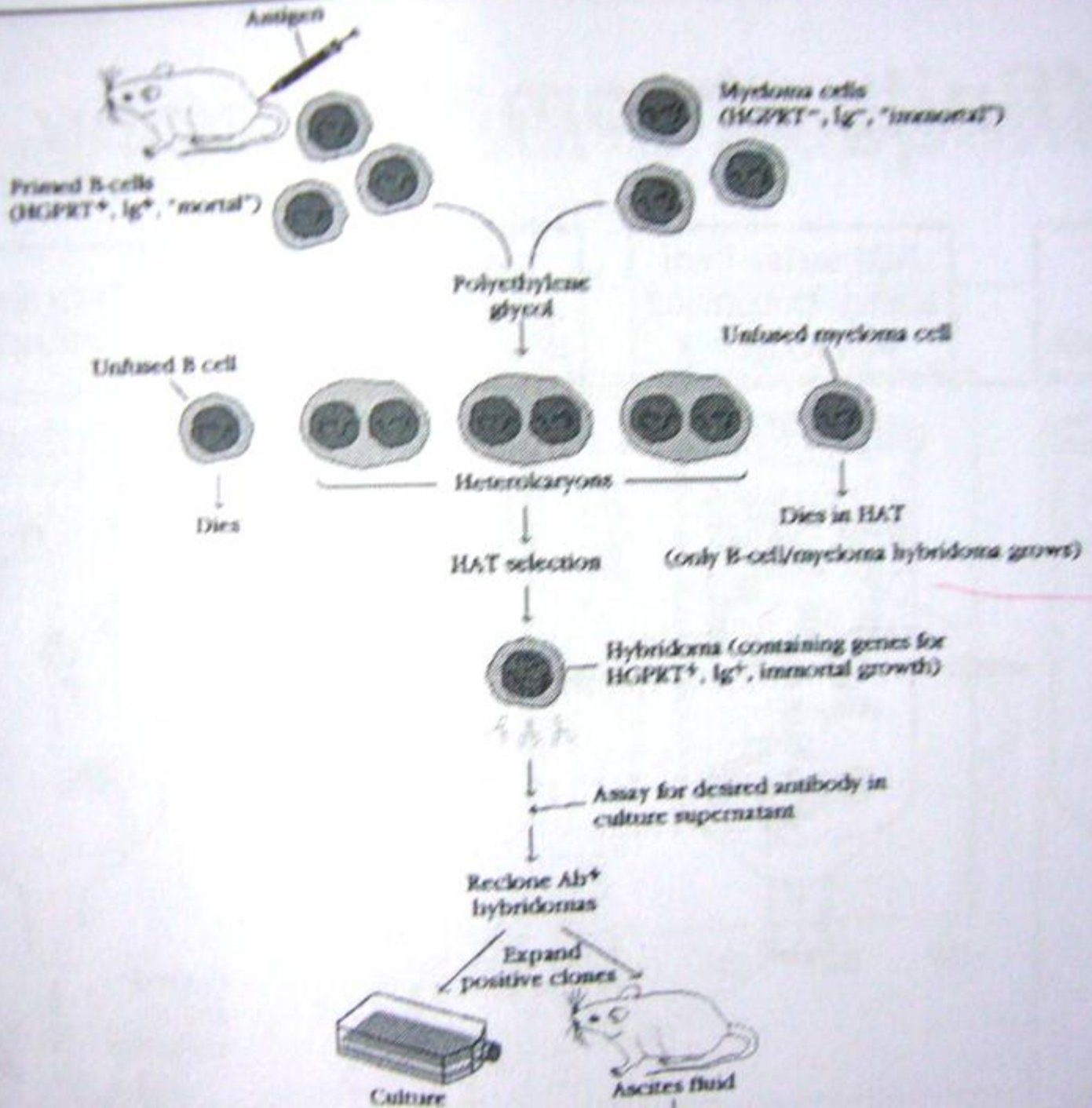

POLYCLONAL ANTIBODY FORMATION
IMMUNOGEN
ANTIGEN WITH ADJUVANT
IMMUNIZATION
APPROPRIATE TIME INTERVAL
BOOSTER INJECTIONS (IF REQUIRED)
BLEED AND COLLECT ANTISERA
POLYCLONAL ANTISERA
ANTIGEN
ANTIBODY
ANTIGEN-ANTIBODY COMPLEX
RBC

Affinity chromatography



POLYCLONAL ANTIBODY FORMATION

- Purified Ag- Immunogen
- Immunization
- Specific antibody (antiserum- Mw.>5000 polypeptide complex with protein)
- Antibody affinity
- Antibody titer


Antigen
Primed B-cells
(HGPRT+, Ig+, "mortal")
Myeloma cells
(HGPRT-, Ig-, "immortal")
Polyethylene glycol
Unfused B cell
Unfused myeloma cell
Dies
Heterokaryons
Dies in HAT
(only B-cell/myeloma hybridoma grows)
HAT selection
Hybridoma (containing genes for HGPRT+, Ig+, immortal growth)
Assay for desired antibody in culture supernatant
Reclone Ab+ hybridomas
Expand positive clones
Culture
Ascites fluid

IMMUNORADIOMETRIC ASSAY

- IRMA (Sandwich method)
- Ab- Monoclonal Ab
- Ag
- Ab*
- Separation technique by washer

FIGURE 17-7. An example of an IRMA technique utilizing a macro bead. The patient is first reacted with excess antibody on the bead. A radiolabeled antibody is then added which binds only to any patient already bound to the primary antibody. Excess labeled antibody is discarded and the bead is then counted. The amount of radioactive counts on the bead is directly proportional to the patient concentration.

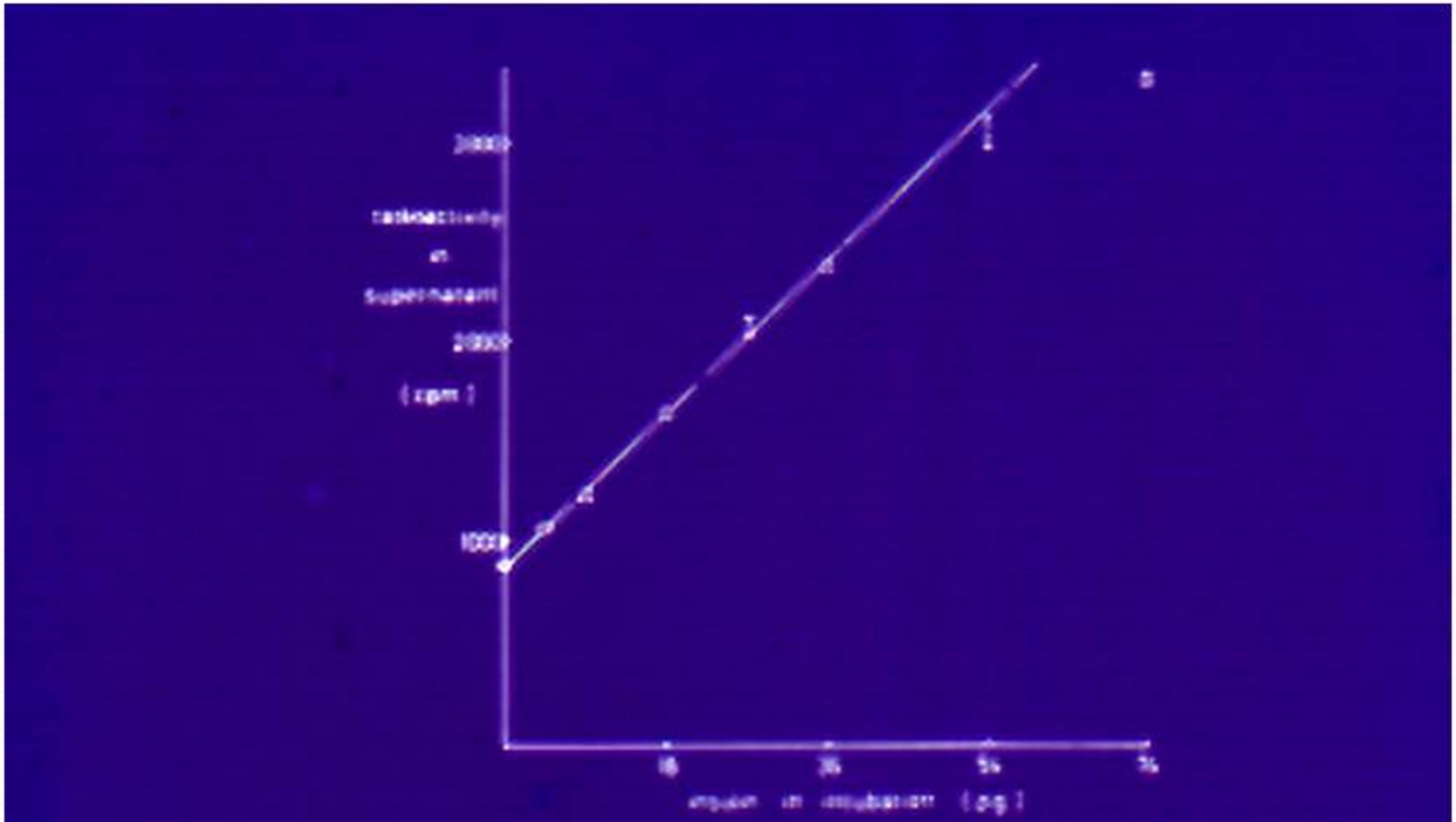
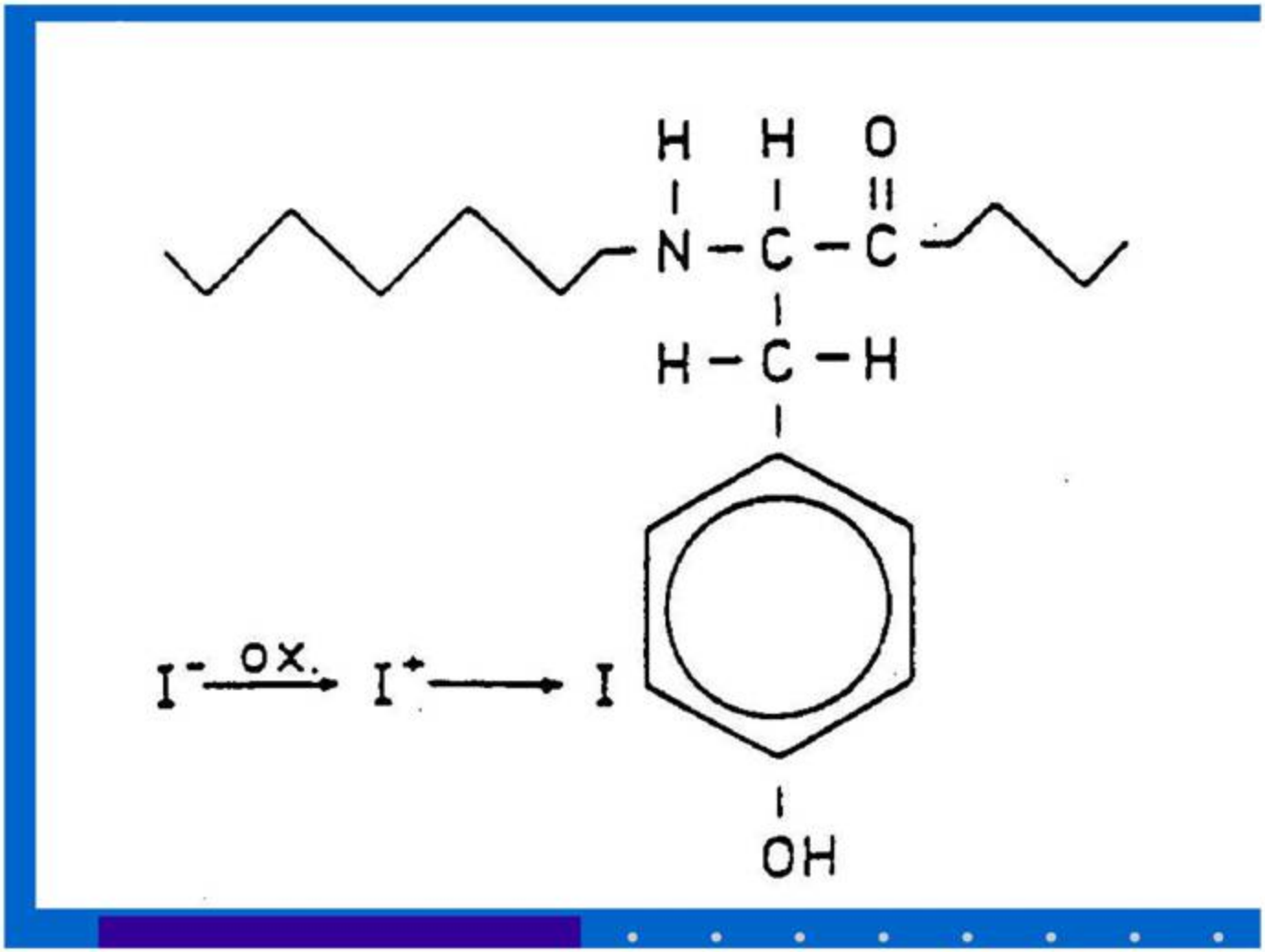


Fig. 6.11. A standard curve for an immunoradiometric assay, performed according to the principle shown in Fig. 6.10.

Radionuclide Labeled Antigen

- Tracer preparation
- C-14,H-3 (Liquid Scintillation counter)
- I-131
- I-125 –(for labeling tyrosine 酥氨酸-)
- Iodination
- (Iodine 原子-replace the 烃基鄰位氫原子)
- (for non-tyrosine– prelinking酥氨酸甲 酯)


H H O
N C C
H C H
OH
I⁻ ox. I⁺ I

Preparation of Tracer

- A minor modification of technique mentioned by Hunter, using the chloramine -T method
- 0.25N phosphate buffer, pH 7.0 20ul
- Natural XXX hormone 25ul
- Carrier-free Na 125-I 3-5ul
- Chloramine –T 10ul
- Sodium metabisulfite 20ul
- Blood –bank plasma 2-3ul

Requirement for Labeled Antigen

- Ag* Specific.activity=
- Ag* radioactivity/Ag*mas
- Ag* Immunoactivity(If more iodine-
- activity change?)
- Ag* Available counting
- RIA radiobio-purity >90%
- (Radiobio-purity= ImmunoAg* Spc. act. /
- Ag* total activity)

Single Incubation RIA

- Haptens
- Small peptides when sensitivity is not required
- The equilibrium reach in 2-4 h and to avoid within-assay drift
- Diluent requirement :pH, ionic strength, ionic/protein carrier content, bacteriostatic and anti-coagulant ,detergents – low NSB

Non-Sensitive , Single Incubation RIA

- Designing for sensitivity

Separation

- Assay design:
- Optimization–/ incubation/ applicable separation system
- double antibody (DA) ppt./incubate 16h at 4 C
- assisted double antibody (ADA) /1-4h at RT
- solid phase II Ab (DASP)/ 0.5-1h at RT

Influences of Separation Methods

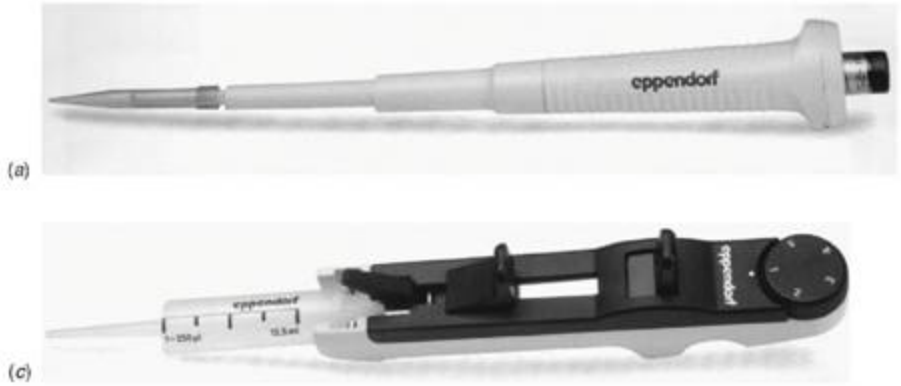
- Choice of separation method
- Double antibody precipitation
- First antibody solid phase
- Adsorption methods
- Fractional separation-

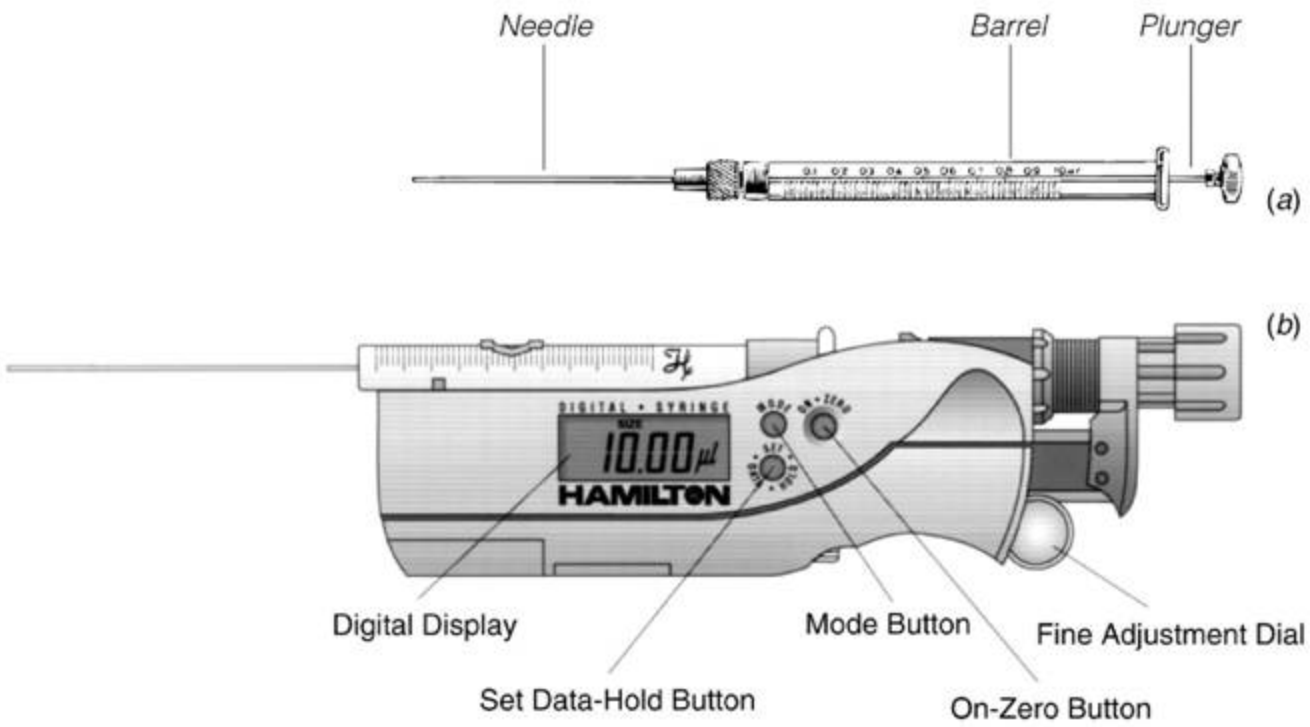
  Polyethylene glycol (PEG) – ppt.

  white color.

Materials and Methods

- Pipette
- Pipette quality control
- Pipette trouble shooting


(a)
eppendorf
(c)
eppendorf
eppendorf
(b)


Needle
Barrel
Plunger
(a)
(b)
DIGITAL SYRINGE
10.00 µl
HAMILTON
Digital Display
Set Data-Hold Button
Mode Button
On-Zero Button
Fine Adjustment Dial

放射性測量

- 用 γ 井型計數器測定 ^{125}I，用液態閃爍計數器測定 ^{3}H。
- 校正；power, energy, background
- Multi-well counter；cross talk；χ^2
- High Count
- Low Count

NaI(Tl) Crystal

Advantages

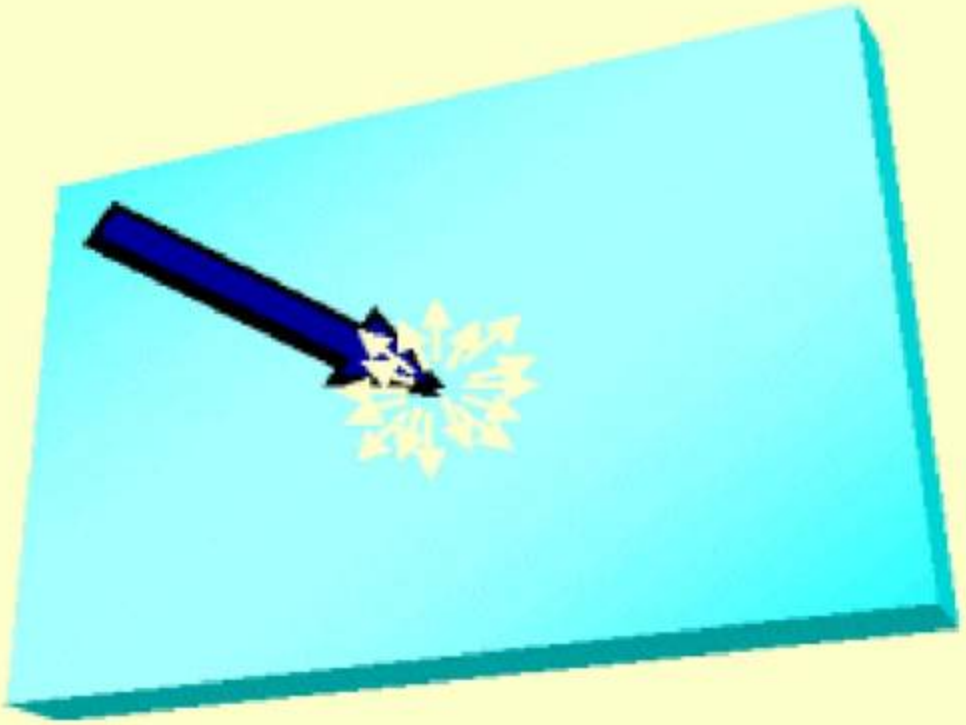
- > 85% sensitivity @ 140 keV
- Moderate energy resolution (9-10% @ 140 keV)
- Moderate cost

Disadvantages

- Hygroscopic (requires hermetic seal)
- Limiting component in count rate performance (200 nSec scintillation decay time)

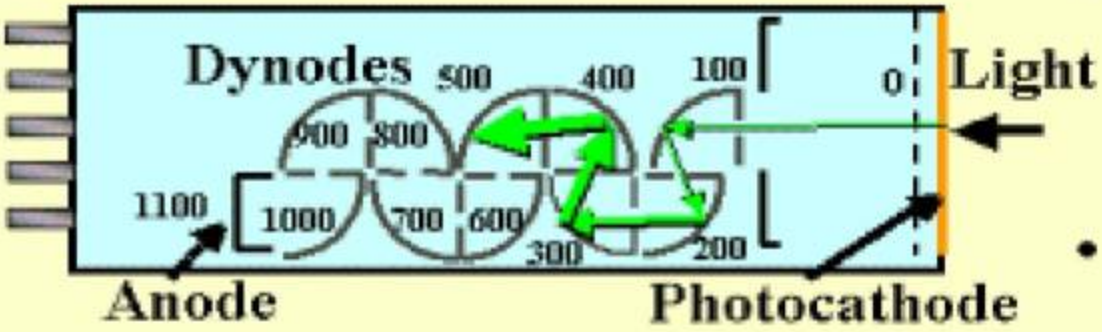
NaI(Tl) Scintillator

- Sensitive material for γ ray detection.
- *Single large (40 x 50 cm), thin (9.5 mm) crystal.
- Converts γ ray energy into visible light (Total absorption of a 140 keV γ-ray yields 5000 photons).
- Fragile: Sensitive to trauma and temperature changes.



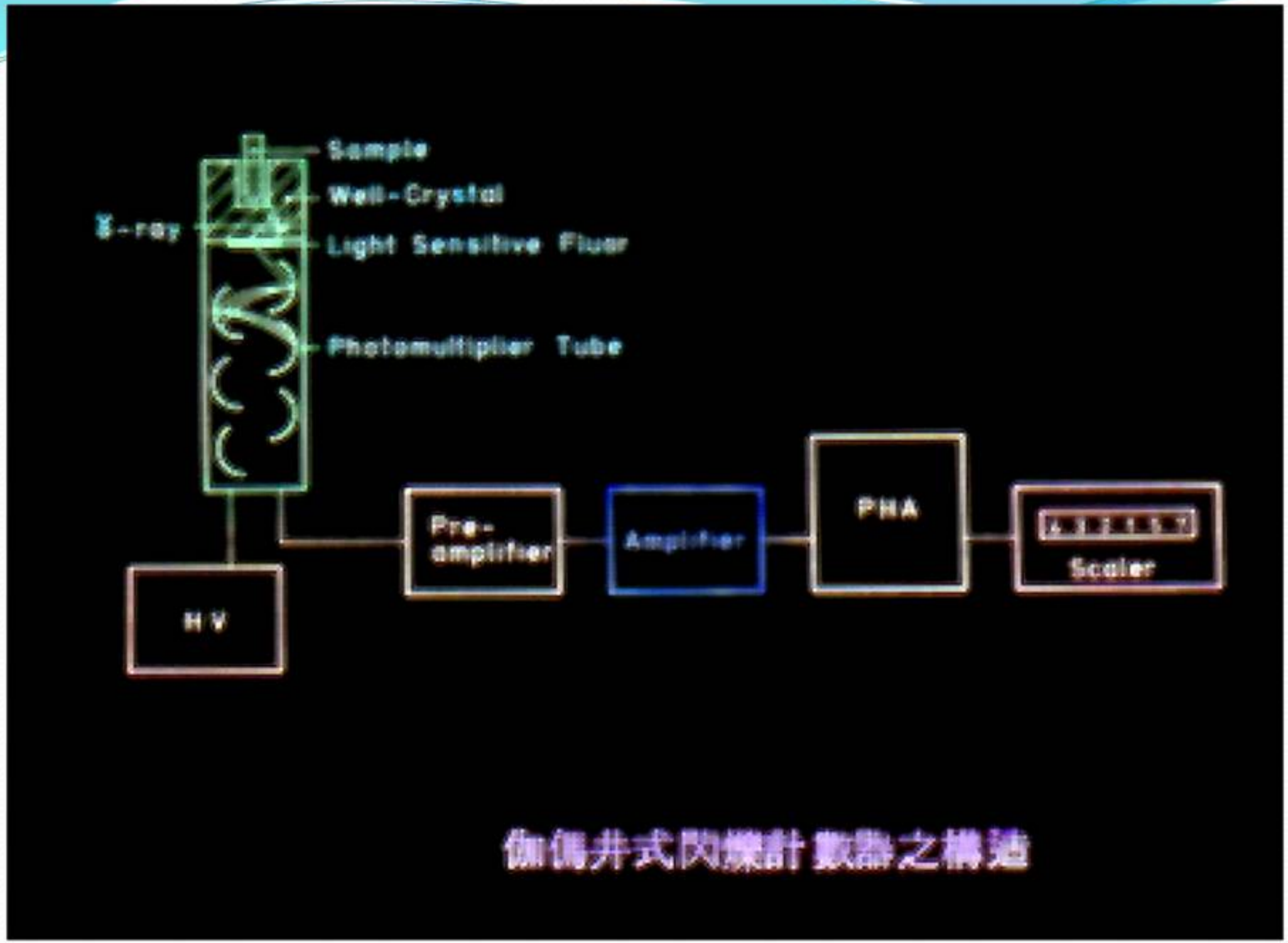
* This is a typical crystal size, but other dimensions and thicknesses are available.

Photomultiplier Tube (PMT)



- Converts visible light (scintillation) into an electronic pulse
- Electron amplification through a series of dynodes
- Overall gain ~ 10^6

Light hitting the photocathode liberates electrons which are accelerated through a series of dynodes. The electron gain at each dynode is ~ 4.



伽傌井式閃爍計數器之構造

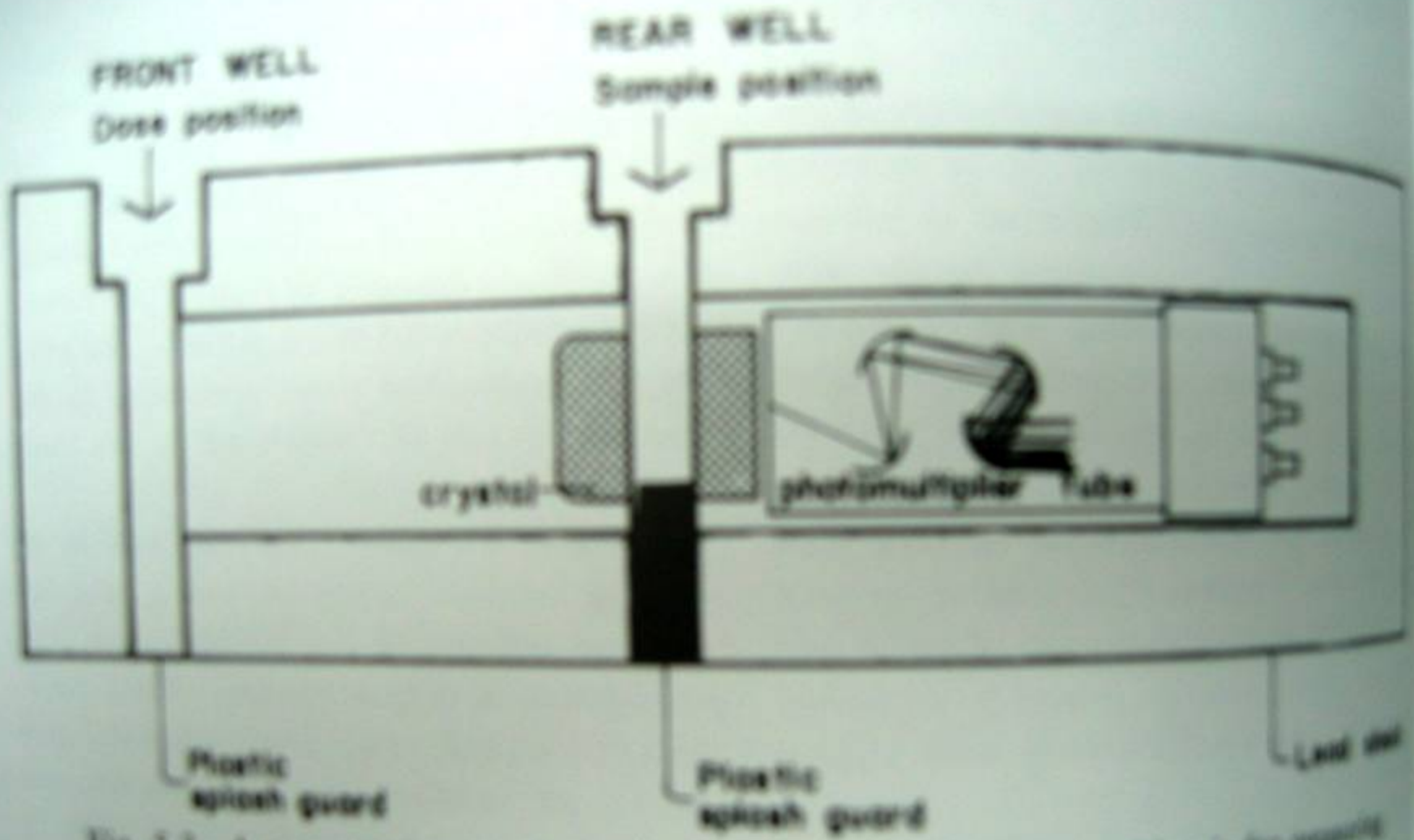
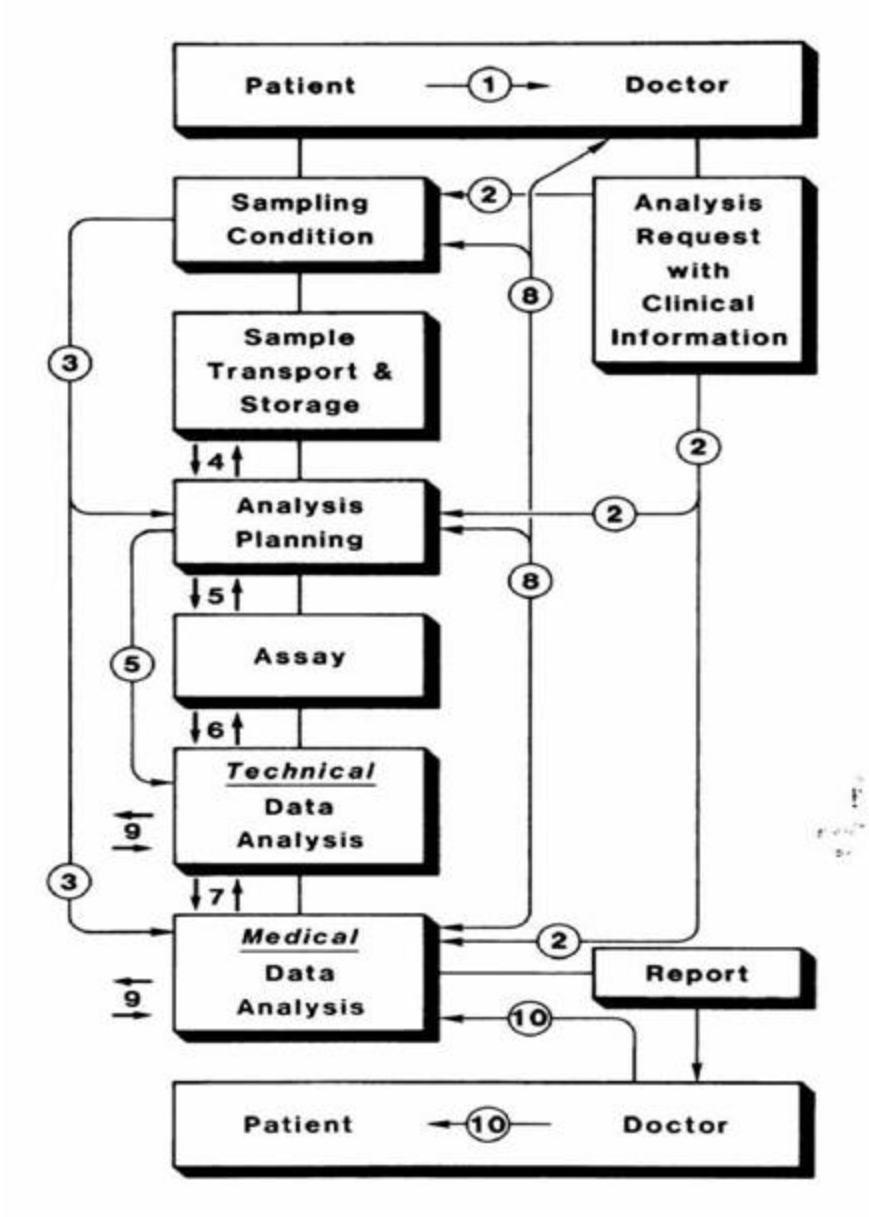
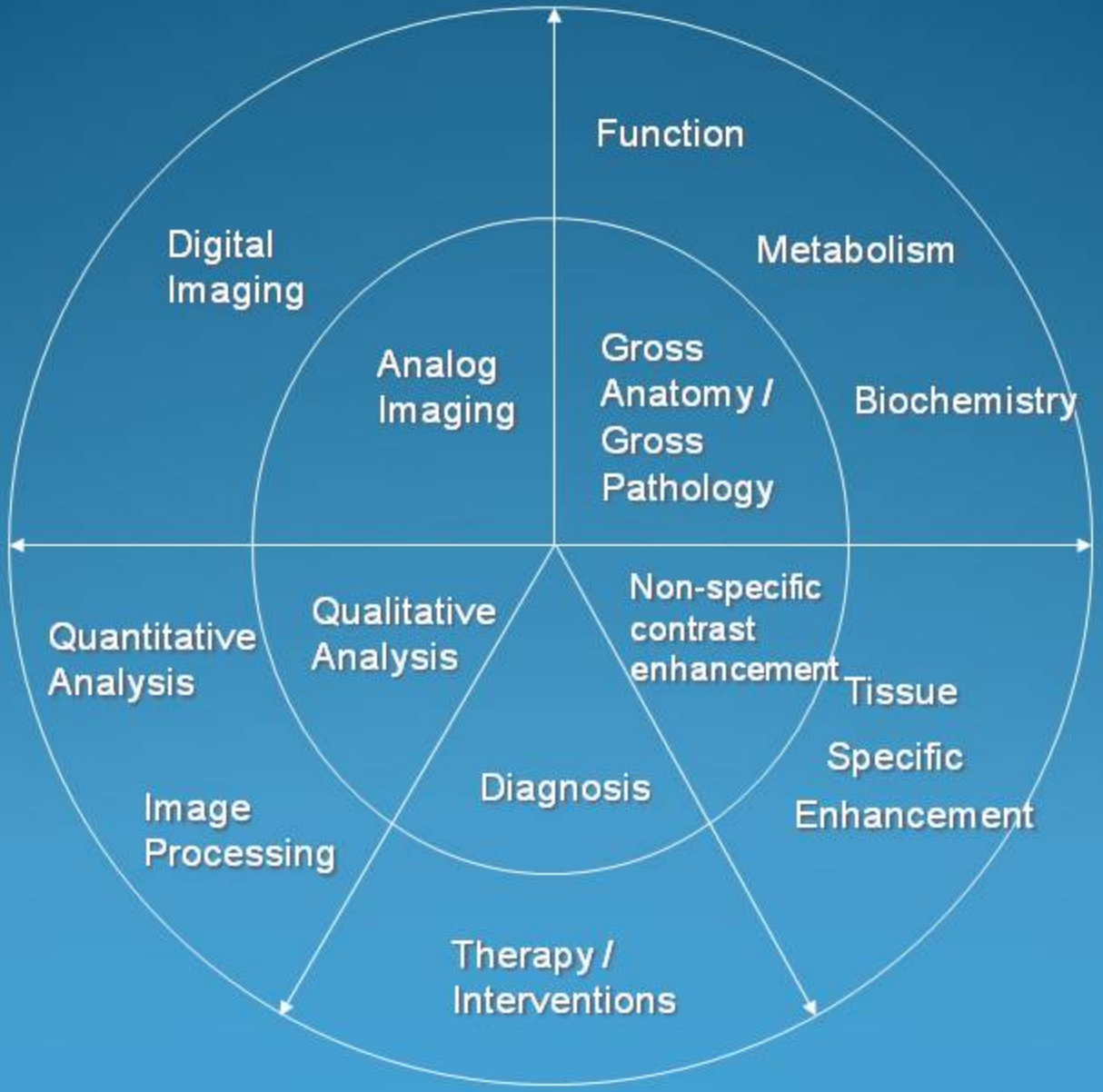


Fig. 5-3. A cross-section of the 2-position detector. The dose position is for measuring standard in plastic syringes—total volume exposed to sensing crystal is 7 to 8 ml. The well position (sample position) through the crystal opening accommodates disposable plastic test tubes. The patient background and a sample analysis are performed in the position.

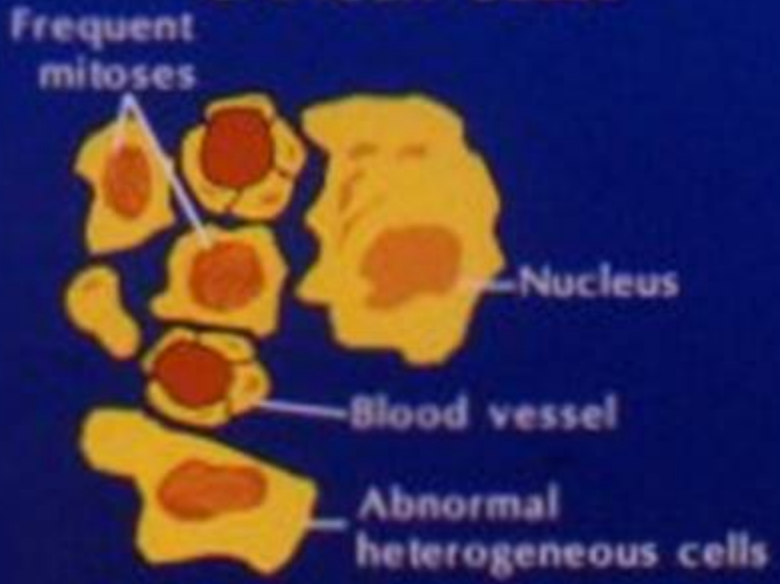

Patient
1
Doctor
Sampling Condition
2
Analysis Request with Clinical Information
8
Sample Transport & Storage
3
4
Analysis Planning
2
8
5
Assay
5
6
Technical Data Analysis
9
3
7
Medical Data Analysis
2
Report
9
10
Patient
10
Doctor

WHERE WE ARE GOING IN THE SECOND CENTURY



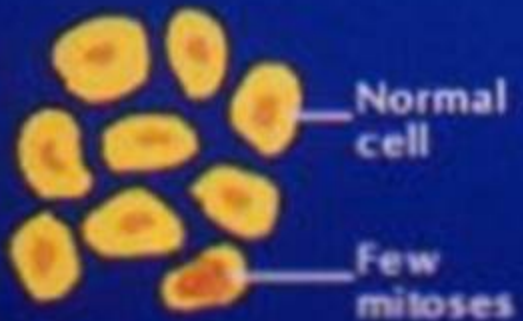
CANCER CELLS AND NORMAL CELLS

CANCER CELLS

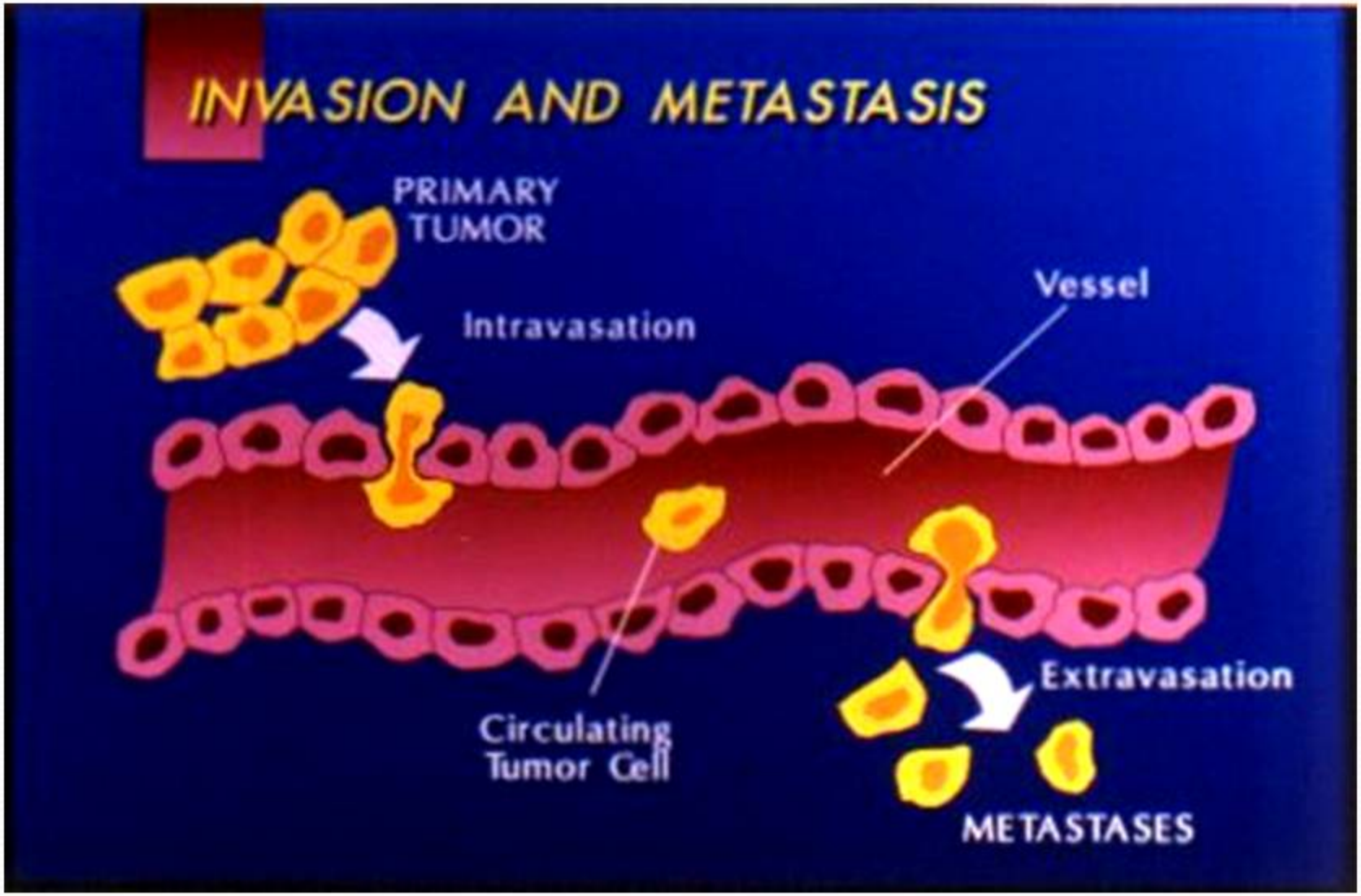


- Loss of contact inhibition
- Increase in growth factor secretion
- Increase in oncogene expression
- Loss of tumor suppressor genes

NORMAL CELLS

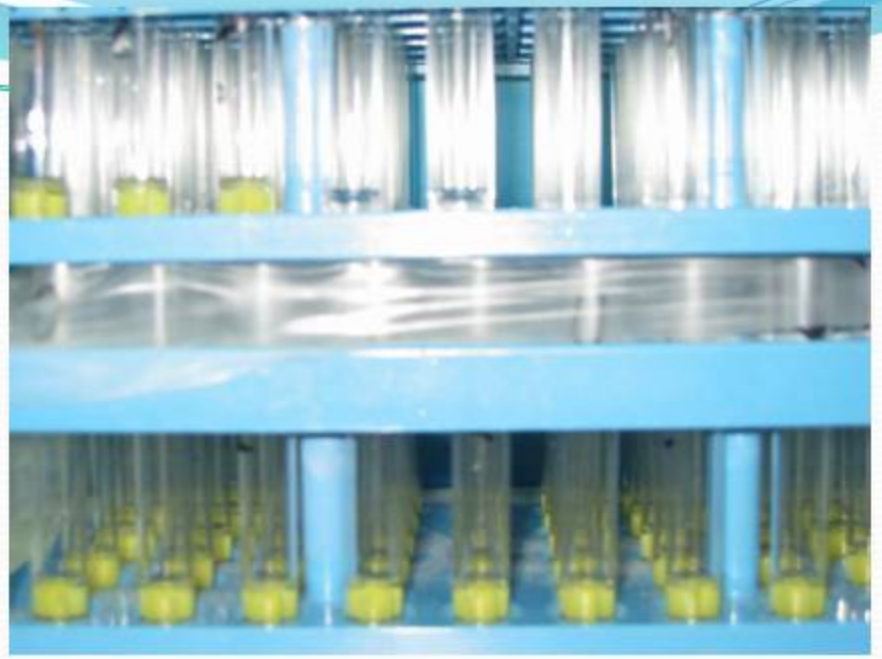


- Oncogene expression is rare
- Intermittent or co-ordinated growth factor secretion
- Presence of tumor suppressor genes

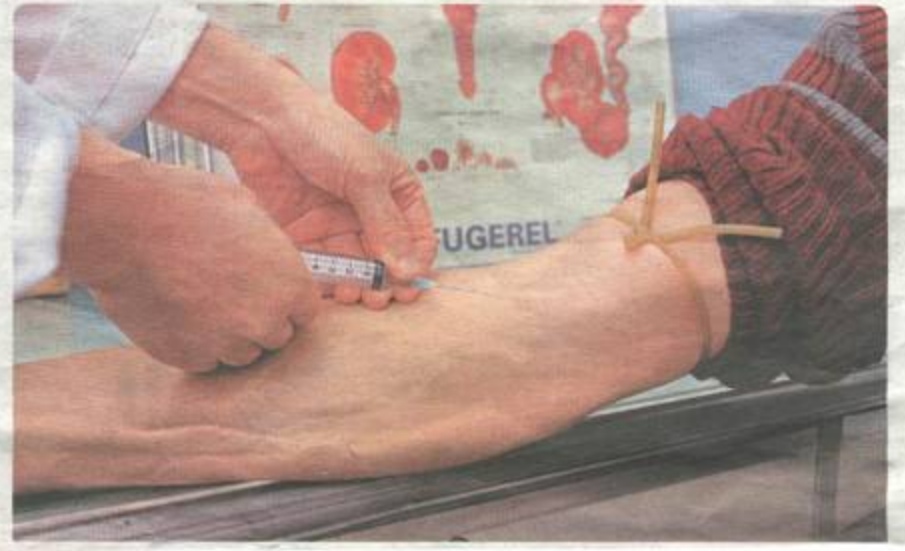

INVASION AND METASTASIS
PRIMARY TUMOR
Intravasation
Vessel
Extravasation
Circulating Tumor Cell
METASTASES

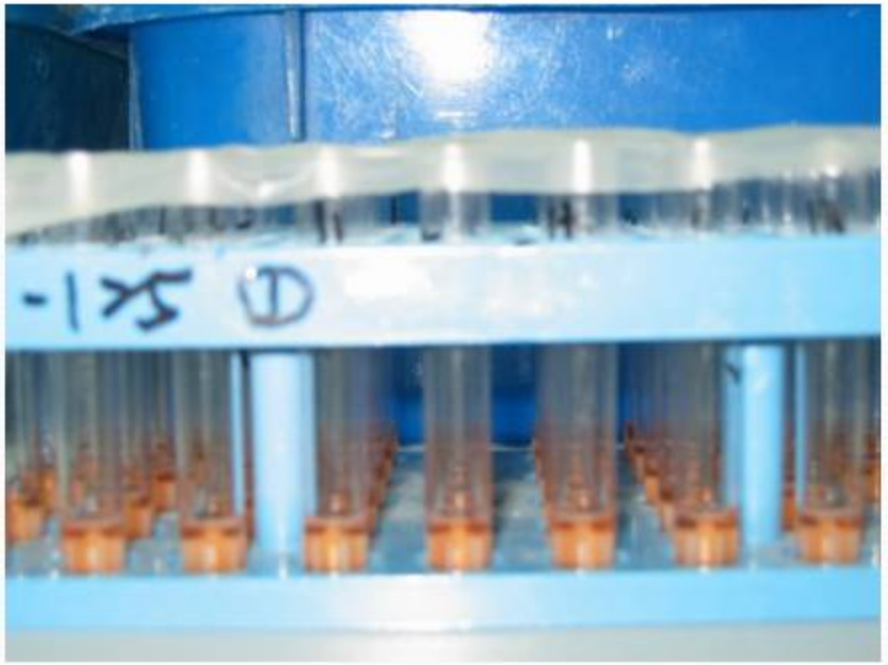
癌症篩檢

近來，國內許多知名人士如郭台成、楊德昌相繼因癌症逝世，使國人引發罹患癌症的恐懼。事實上，正確的癌症觀念以及最好的治療方式還是及早發現、及早治療。



有效幫你遠離癌症





排行	衛生署統計民國九十四年 臺灣地區十大死亡原因	死亡人數
1	惡性腫瘤	37,222
2	腦血管疾病	13,139
3	心臟疾病	12,970
4	糖尿病	10,501
5	事故傷害	8,365
6	肺炎	5,687
7	慢性肝病及肝硬化	5,621
8	腎炎、腎徵候群及腎進病變	4,822

THE LEVELS OF RADIATION

10^{6}	MEGA ROENTGEN "MR" MEGA CURIE "MCi"	DISASTER LEVEL
10^{3}	KILO ROENTGEN "KR" KILO CURIE "KCi"	RADIOTHERAPY LEVEL

THE ROENTGEN PRODUCES ONE ELECTROSTATIC UNIT IN 1cc OF AIR

THE CURIE PRODUCES 3.7×10^{10} DISINTEGRATIONS PER SECOND

10^{-3}	milli ROENTGEN "mR" milli CURIE "mCi"	THERAPY NUCLEAR MEDICINE LEVELS DIAGNOSIS
10^{-6}	micro ROENTGEN "μR" micro CURIE "μCi"	
10^{-9}	nano ROENTGEN "nR" nano CURIE "nCi"	TRACER LEVELS
10^{-12}	pico ROENTGEN "pR" pico CURIE "pCi"	LOW LEVEL INSTRUMENTS
10^{-15}	FEMTO-	BACKGROUND LEVELS
10^{-18}	ATTO-	

ARE THESE TESTS EXPENSIVE?

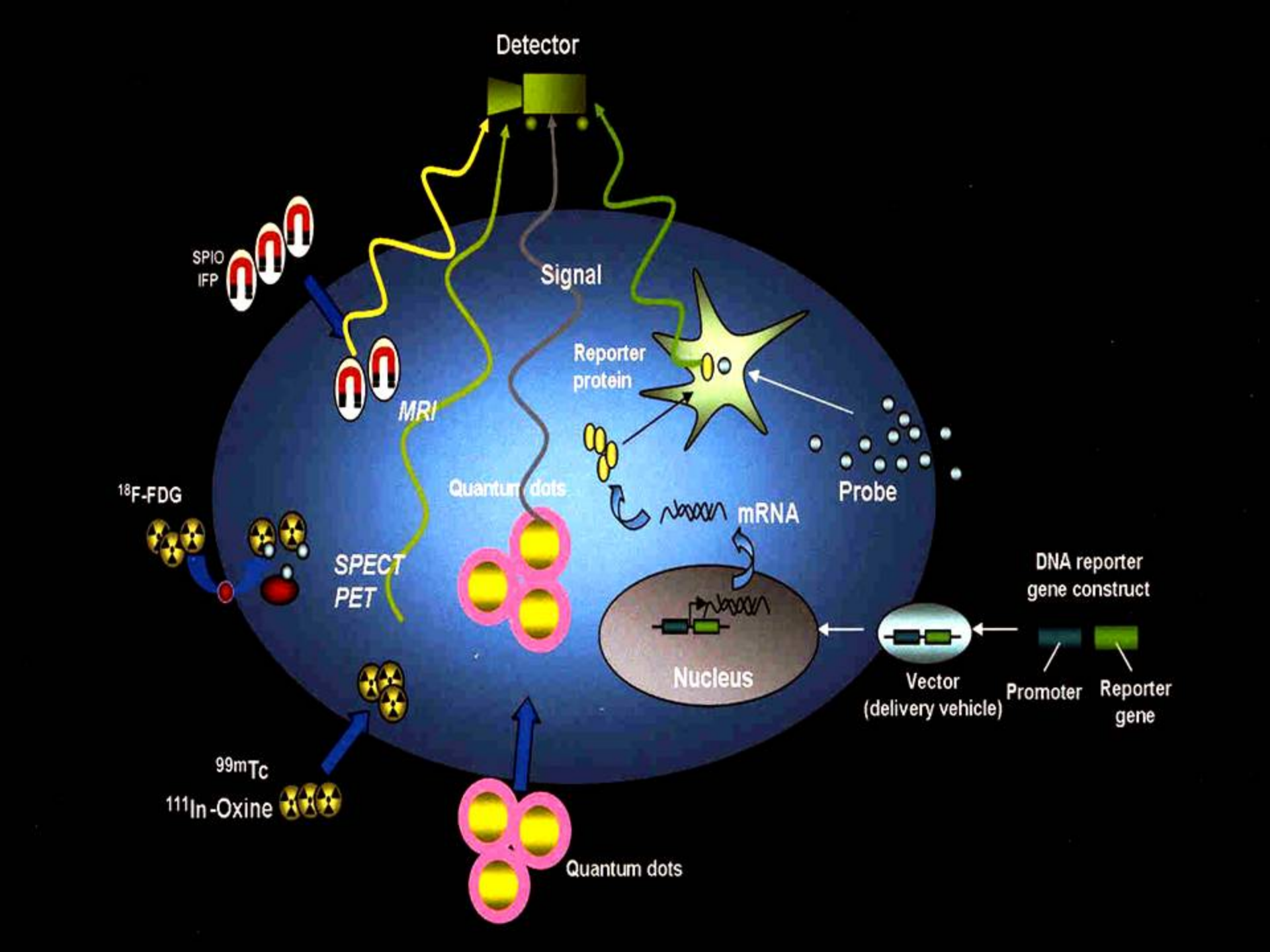
1 THE THYROID BLOOD TESTS ARE INEXPENSIVE

THE RADIOPHARMACEUTICAL IS INEXPENSIVE.

THE INSTRUMENT IS INEXPENSIVE TO BUY AND MAINTAIN.

THE TECHNICIAN CAN DO MANY TESTS EACH DAY.

THE PHYSICIAN CAN INTERPRET THE TEST RAPIDLY WITH LITTLE PREPARATION.


Detector
SPIO
IFP
Signal
Reporter
protein
MRI
Probe
^{18}F-FDG
Quantum dots
mRNA
SPECT
PET
DNA reporter
gene construct
Nucleus
Vector
(delivery vehicle)
Promoter
Reporter
gene
^{99m}Tc
^{111}In-Oxine
Quantum dots


changing
the future