

药物分析期末

Main content

- The definition of TRFIA
- The principle of TRFIA
- The advantages of TRFIA
- The clinical application of detecting HBV infection

Definition

TRFIA is a analysis that use **trivalent rare earth ions and chelating agents** as a **tracer**, instead of fluorescent substances, isotopes or enzyme, marking up protein, hormone, antibody, nucleic acid probes or biological activity cells. When the reaction occurs, TRF instrument detect the fluorescent intensity of final product. According to the fluorescence intensity to determine concentration of the substances.

Unique fluorescent marker of rare earth metals- Lanthanide

元素周期表

族 周期	I A	元素周期表										0	电子层	电子数											
1	1 H 氢 $1s^1$ 1.008	<div style="display: flex; justify-content: space-between;"> <div style="width: 20%;"> <p>原子序数</p> <p>元素名称注* 的是人造元素</p> </div> <div style="width: 20%; text-align: center;"> <p>92 U</p> <p>铀</p> <p>$5f^3 6d^1 7s^2$</p> <p>238.0</p> </div> <div style="width: 40%;"> <p>元素符号, 红色指放射性元素</p> <p>非金属</p> <p>金属</p> <p>过渡元素</p> <p>外围电子层排布, 括号指可能的电子层排布</p> <p>相对原子质量</p> </div> </div>										2 He 氦 $1s^2$ 4.003	K	2											
2	3 Li 锂 $2s^1$ 6.941	4 Be 铍 $2s^2$ 9.012											5 B 硼 $2s^2 2p^1$ 10.81	6 C 碳 $2s^2 2p^2$ 12.01	7 N 氮 $2s^2 2p^3$ 14.01	8 O 氧 $2s^2 2p^4$ 16.00	9 F 氟 $2s^2 2p^5$ 19.00	10 Ne 氖 $2s^2 2p^6$ 20.18	L K	8 2					
3	11 Na 钠 $3s^1$ 22.99	12 Mg 镁 $3s^2$ 24.31	III B	IV B	V B	VI B	VII B	VIII	IB	II B	13 Al 铝 $3s^2 3p^1$ 26.98	14 Si 硅 $3s^2 3p^2$ 28.09	15 P 磷 $3s^2 3p^3$ 30.97	16 S 硫 $3s^2 3p^4$ 32.07	17 Cl 氯 $3s^2 3p^5$ 35.45	18 Ar 氩 $3s^2 3p^6$ 39.95	M L K	8 8 2							
4	19 K 钾 $4s^1$ 39.10	20 Ca 钙 $4s^2$ 40.08	21 Sc 钪 $3d^1 4s^2$ 44.96	22 Ti 钛 $3d^2 4s^2$ 47.87	23 V 钒 $3d^3 4s^2$ 50.94	24 Cr 铬 $3d^5 4s^1$ 52.00	25 Mn 锰 $3d^5 4s^2$ 54.94	26 Fe 铁 $3d^6 4s^2$ 55.85	27 Co 钴 $3d^7 4s^2$ 58.93	28 Ni 镍 $3d^8 4s^2$ 58.69	29 Cu 铜 $3d^{10} 4s^1$ 63.55	30 Zn 锌 $3d^{10} 4s^2$ 65.39	31 Ga 镓 $4s^2 4p^1$ 69.72	32 Ge 锗 $4s^2 4p^2$ 72.61	33 As 砷 $4s^2 4p^3$ 74.92	34 Se 硒 $4s^2 4p^4$ 78.96	35 Br 溴 $4s^2 4p^5$ 79.90	36 Kr 氪 $4s^2 4p^6$ 83.80	N M L K	8 18 8 2					
5	37 Rb 铷 $5s^1$ 85.47	38 Sr 锶 $5s^2$ 87.62	39 Y 钇 $4d^1 5s^2$ 88.91	40 Zr 锆 $4d^2 5s^2$ 91.22	41 Nb 铌 $4d^4 5s^1$ 92.91	42 Mo 钼 $4d^5 5s^1$ 95.94	43 Tc 锝 $4d^5 5s^2$ [99]	44 Ru 钌 $4d^7 5s^1$ 101.1	45 Rh 铑 $4d^8 5s^1$ 102.9	46 Pd 钯 $4d^{10}$ 106.4	47 Ag 银 $4d^{10} 5s^1$ 107.9	48 Cd 镉 $4d^{10} 5s^2$ 112.4	49 In 铟 $5s^2 5p^1$ 114.8	50 Sn 锡 $5s^2 5p^2$ 118.7	51 Sb 锑 $5s^2 5p^3$ 121.8	52 Te 碲 $5s^2 5p^4$ 127.6	53 I 碘 $5s^2 5p^5$ 126.9	54 Xe 氙 $5s^2 5p^6$ 131.3	O N M L K	8 18 18 8 2					
6	55 Cs 铯 $6s^1$ 132.9	56 Ba 钡 $6s^2$ 137.3	57-71 La-Lu 镧系	72 Hf 铪 $5d^2 6s^2$ 178.5	73 Ta 钽 $5d^3 6s^2$ 180.9	74 W 钨 $5d^4 6s^2$ 183.8	75 Re 铼 $5d^5 6s^2$ 186.2	76 Os 锇 $5d^6 6s^2$ 190.2	77 Ir 铱 $5d^7 6s^2$ 192.2	78 Pt 铂 $5d^9 6s^1$ 195.1	79 Au 金 $5d^{10} 6s^1$ 197.0	80 Hg 汞 $5d^{10} 6s^2$ 200.6	81 Tl 铊 $6s^2 6p^1$ 204.4	82 Pb 铅 $6s^2 6p^2$ 207.2	83 Bi 铋 $6s^2 6p^3$ 209.0	84 Po 钋 $6s^2 6p^4$ [209]	85 At 砹 $6s^2 6p^5$ [210]	86 Rn 氡 $6s^2 6p^6$ [222]	P O N M L K	8 18 32 18 8 2					
7	87 Fr 钫 $7s^1$ [223]	88 Ra 镭 $7s^2$ 226.0	89-103 Ac-Lr 锕系	104 Rf 钨* $(6d^2 7s^2)$ [261]	105 Ha 铪* $(6d^4 7s^2)$ [262]	106 * $(6d^4 7s^2)$ [263]	107 * $(6d^5 7s^2)$ [262]	108 * $(6d^6 7s^2)$ [265]	109 * $(6d^7 7s^2)$ [266]																
镧系	57 La 镧 $5d^1 6s^2$ 138.9	58 Ce 铈 $4f^1 5d^1 6s^2$ 140.1	59 Pr 镨 $4f^3 6s^2$ 140.9	60 Nd 钕 $4f^4 6s^2$ 144.2	61 Pm 钷 $4f^5 6s^2$ [147]	62 Sm 钐 $4f^6 6s^2$ 150.4	63 Eu 铕 $4f^7 6s^2$ 152.0	64 Gd 钆 $4f^7 5d^1 6s^2$ 157.3	65 Tb 铽 $4f^9 6s^2$ 158.9	66 Dy 镝 $4f^{10} 6s^2$ 162.5	67 Ho 铈 $4f^{11} 6s^2$ 164.9	68 Er 铒 $4f^{12} 6s^2$ 167.3	69 Tm 铥 $4f^{13} 6s^2$ 168.9	70 Yb 镱 $4f^{14} 6s^2$ 173.0	71 Lu 镥 $4f^{14} 5d^1 6s^2$ 175.0										
锕系	89 Ac 锕 $6d^1 7s^2$ 227.0	90 Th 钍 $6d^2 7s^2$ 232.0	91 Pa 镤 $5f^2 6d^1 7s^2$ 231.0	92 U 铀 $5f^3 6d^1 7s^2$ 238.0	93 Np 镎 $5f^4 6d^1 7s^2$ 237.0	94 Pu 钚 $5f^6 7s^2$ [244]	95 Am 镅* $5f^7 7s^2$ [243]	96 Cm 锔* $5f^7 6d^1 7s^2$ [247]	97 Bk 锫* $5f^9 7s^2$ [247]	98 Cf 锿* $5f^{10} 7s^2$ [261]	99 Es 镱* $5f^{11} 7s^2$ [262]	100 Fm 镆* $5f^{12} 7s^2$ [267]	101 Md 镎* $(5f^{13} 7s^2)$ [268]	102 No 铈* $(5f^{14} 7s^2)$ [269]	103 Lr 铈* $(5f^{14} 6d^1 7s^2)$ [260]										

注:

1. 相对原子质量录自1995年国际原子量表, 并全部取4位有效数字。
2. 相对原子质量加括号的为放射性元素的半衰期最长的同位素的质量数。

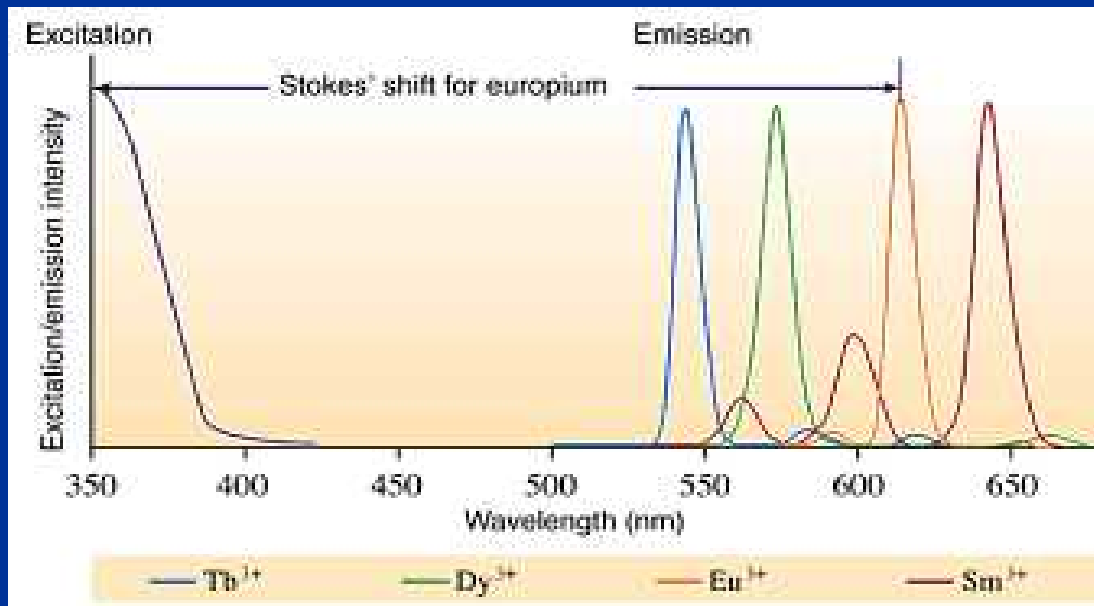
1
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Characteristics of lanthanide fluorescence :

➤ *Stokes shift is large*

Eu: emission 613nm, excitation 340nm

Fluorescence near 280nm



➤ *Fluorescence lifetime is long*

Lanthanide chelates (60 ~ 900 us) <Eu: 714us>

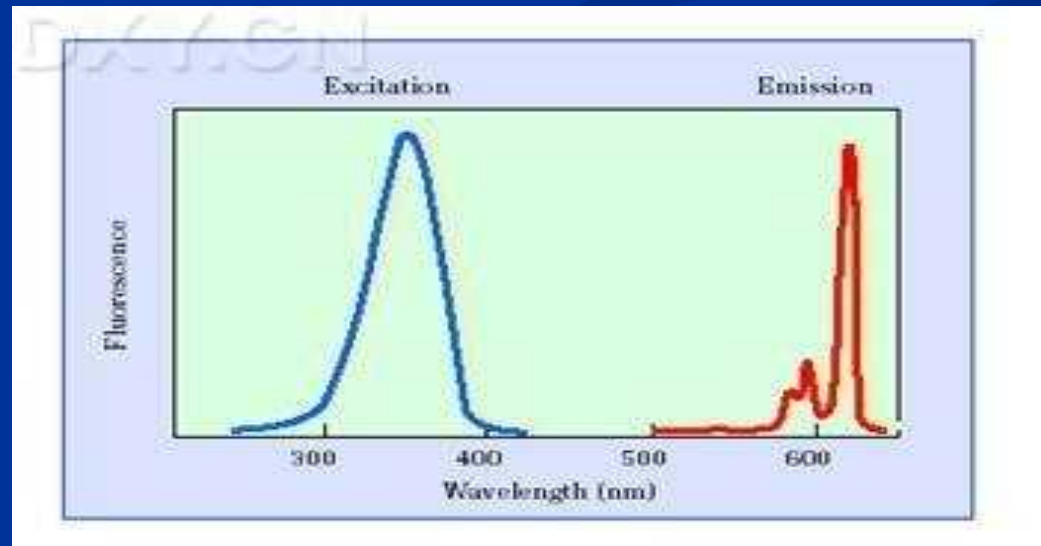
Fluorophores of common immunofluorescence : 1 ~ 100ns

Protein fluorescence: 1 ~ 10ns, easy to quenching

➤ *Fluorescence specificity*

Emission band is narrow, even less than 10nm

Dissociation - Enhancement technology enhance intensity **100 million times**



Rare earth ion excitation and emission light and Stokes shift

Rare earth ion chelate	Excitation wavelength (nm)	Emission wavelength (nm)	Stokes shift (nm)
Eu chelate	340	613	273
Sm chelate	340	600	260
Tb chelate	295	490/543	195/248
Dy chelate	295	573	278

What is Time-resolved?

■ *Fluorescent of biological products*

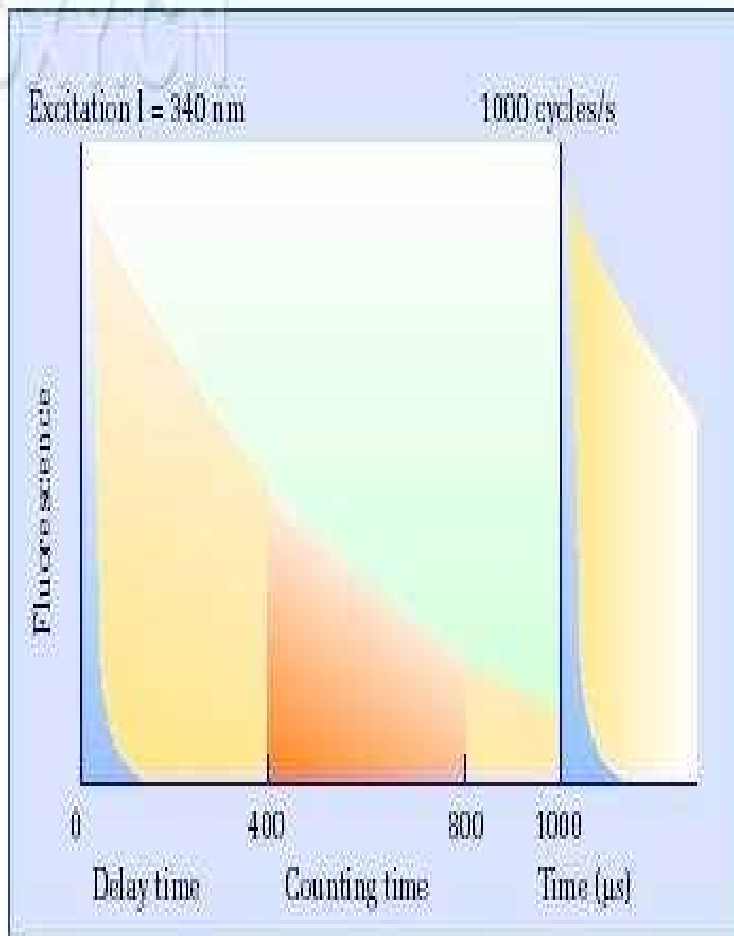
- a) Proteins fluorescence wavelength 400-600nm
- b) Protein fluorescence decay time is about 1-10ns

Detecting by Ordinary fluorescent, interference is very large

■ *Fluorescent of lanthanide chelates*

- a) Fluorescence intensity is strong
- b) Fluorescence lifetime is long(10-1000us)

Higher 5-6 orders of magnitude than the ordinary fluorescent markers



- Delaying measurement time
 - Fluorescence of short-lived sample decay completely
 - Detection of rare earth ion chelate fluorescence signal
- Eliminated non-specific fluorescence from the sample, reagents

This is the “Time-resolved”

Dissociation enhanced lanthanide fluoroimmunoassay

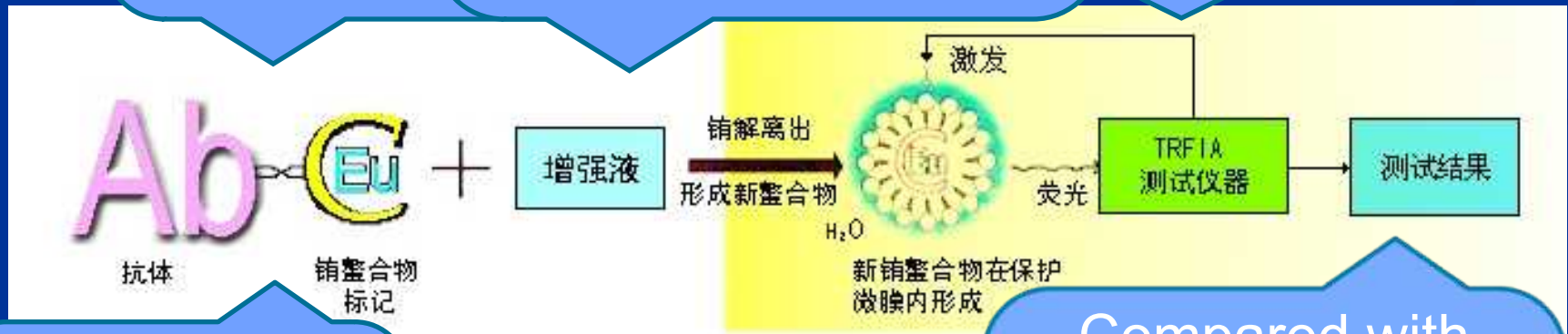
- DELFIA is **unique**
- After immune response completed, part of the marker binding to the solid phase carrier. Then washing off the unbound marker
- Addition enhancement solution with **low PH value (PH2 ~ 3)**, dissociating Eu or Sm from immune complexes and combination with Chelate in enhanced fluid (β -ketone) to form a new chelate
- It makes fluorescence intensity of markers enhance nearly **a million times**

The principle of TRFIA

Rare earth
chelating agent
tracer, macro
prote

Addition enhancement
solution, Eu
dissociation and
generation new
chelate

TRFIA instrument
detect the intensity
of fluorescence



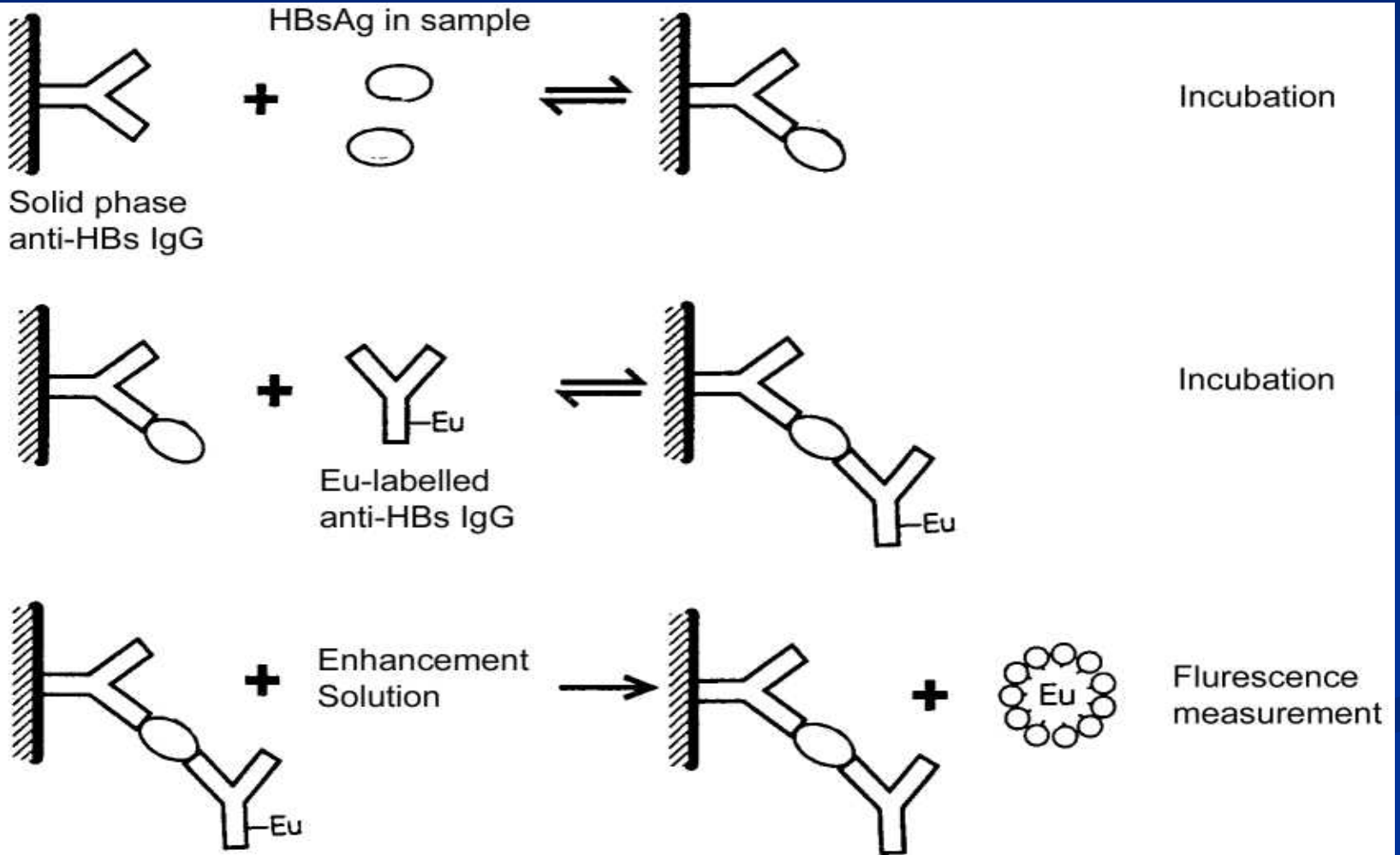
Special
immune
complex
occurs

Compared with
standard curve,
then calculate the
concentration of
detected sample

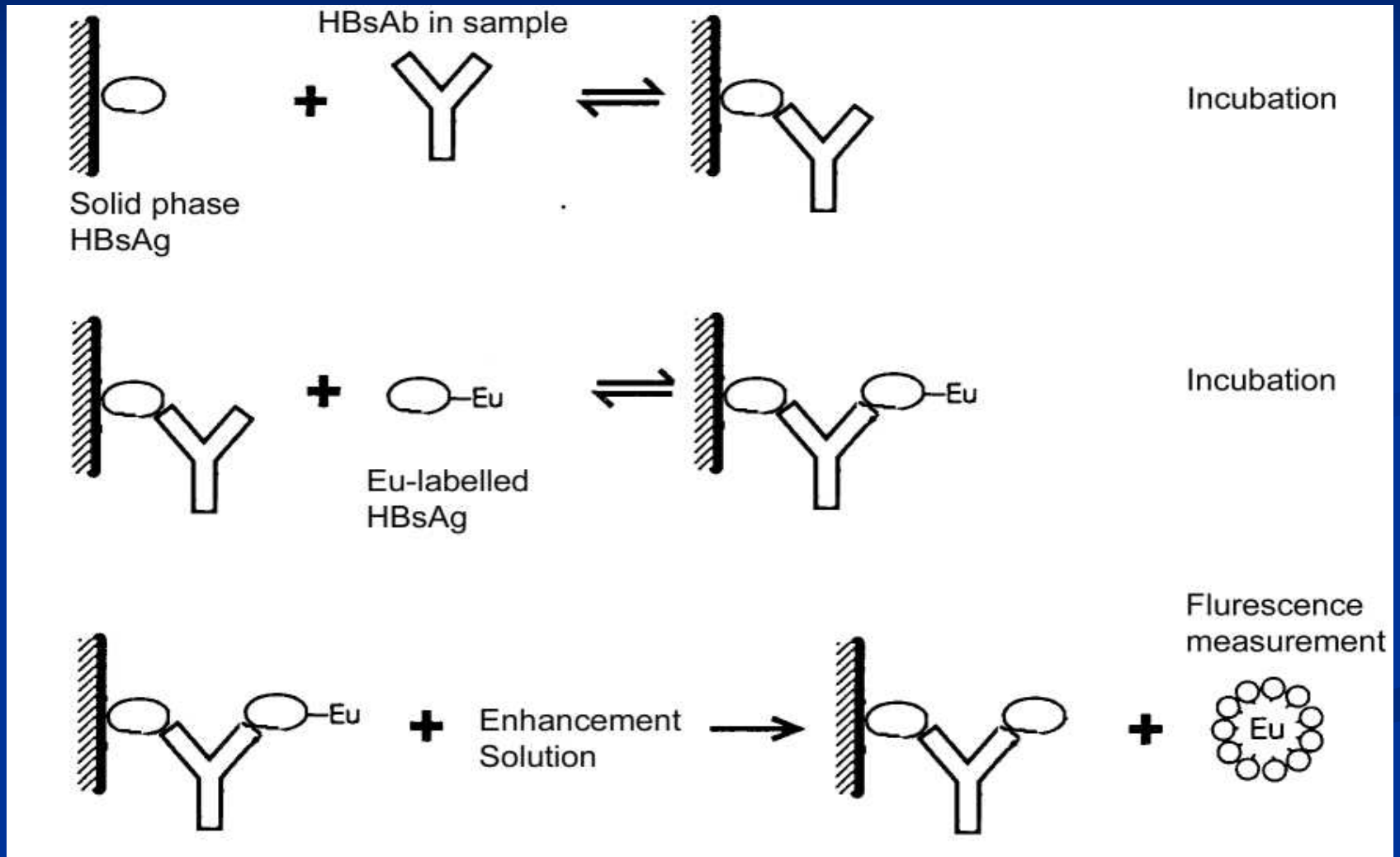
Clinical application-detection HBV

- HBsAg and Anti-HBs antibody
- HBeAg and Anti-Hbe antibody
- Anti-HBc antibody

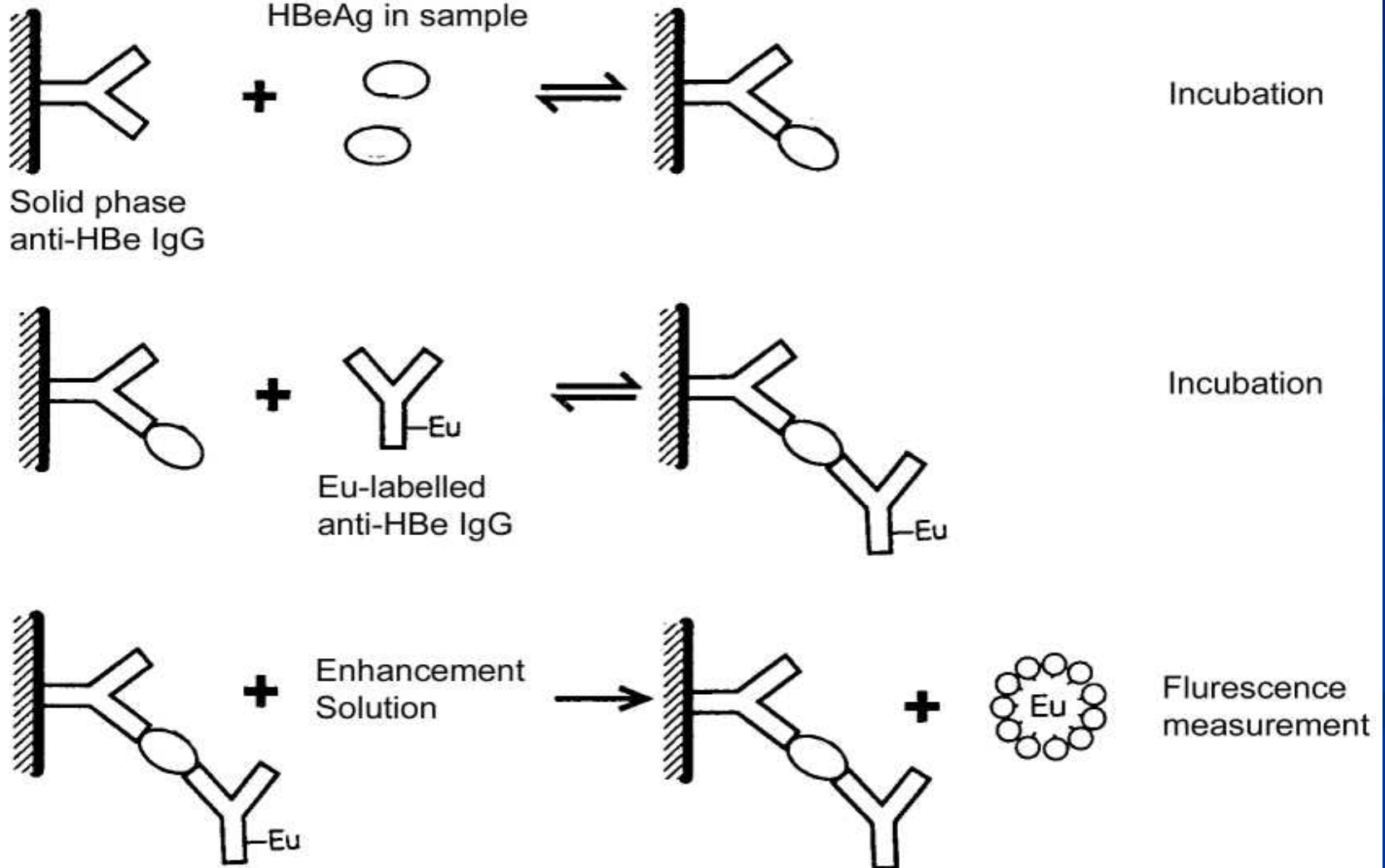
TRFIA detect HBsAg



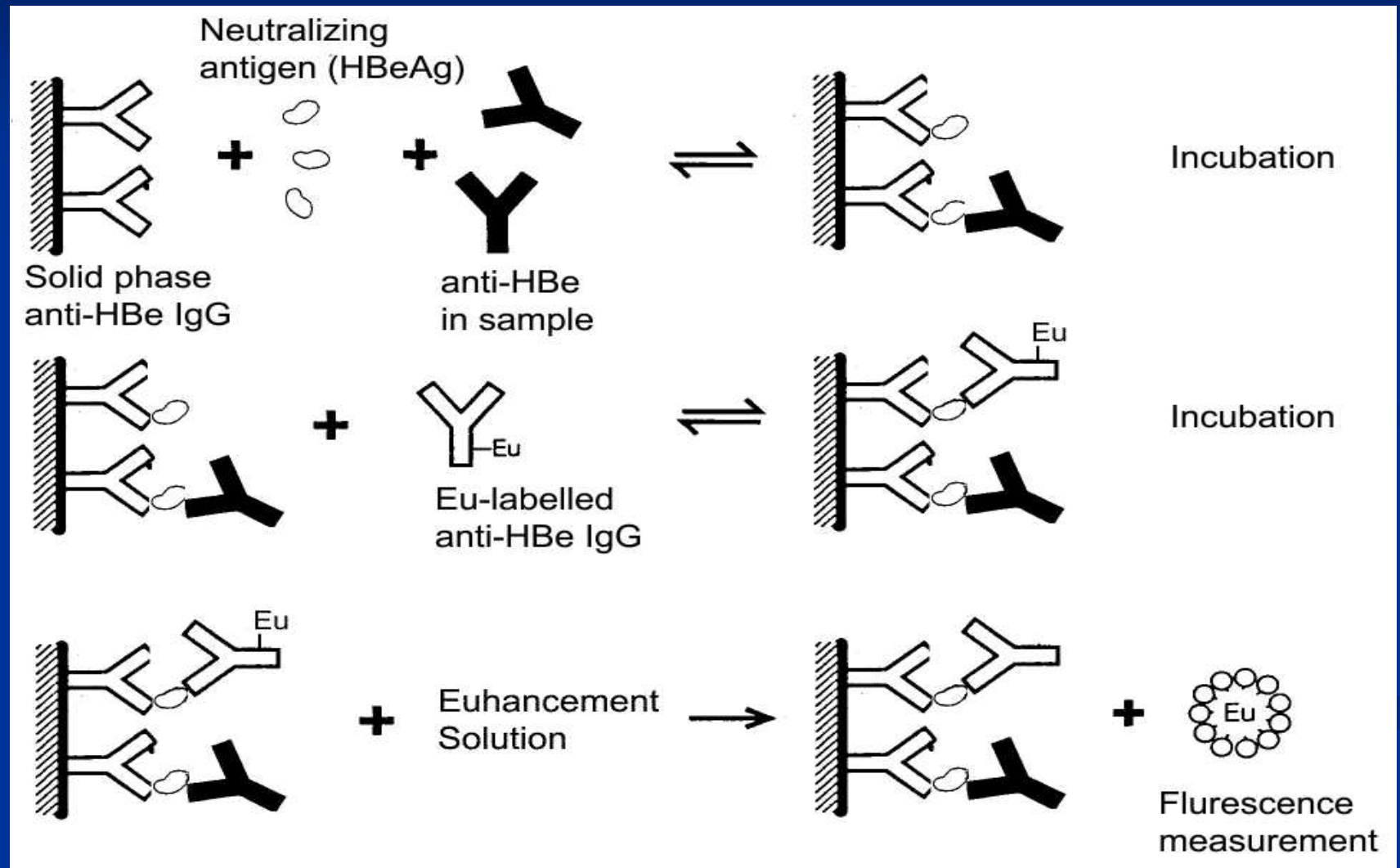
TRFIA detect Anti-HBs antibody



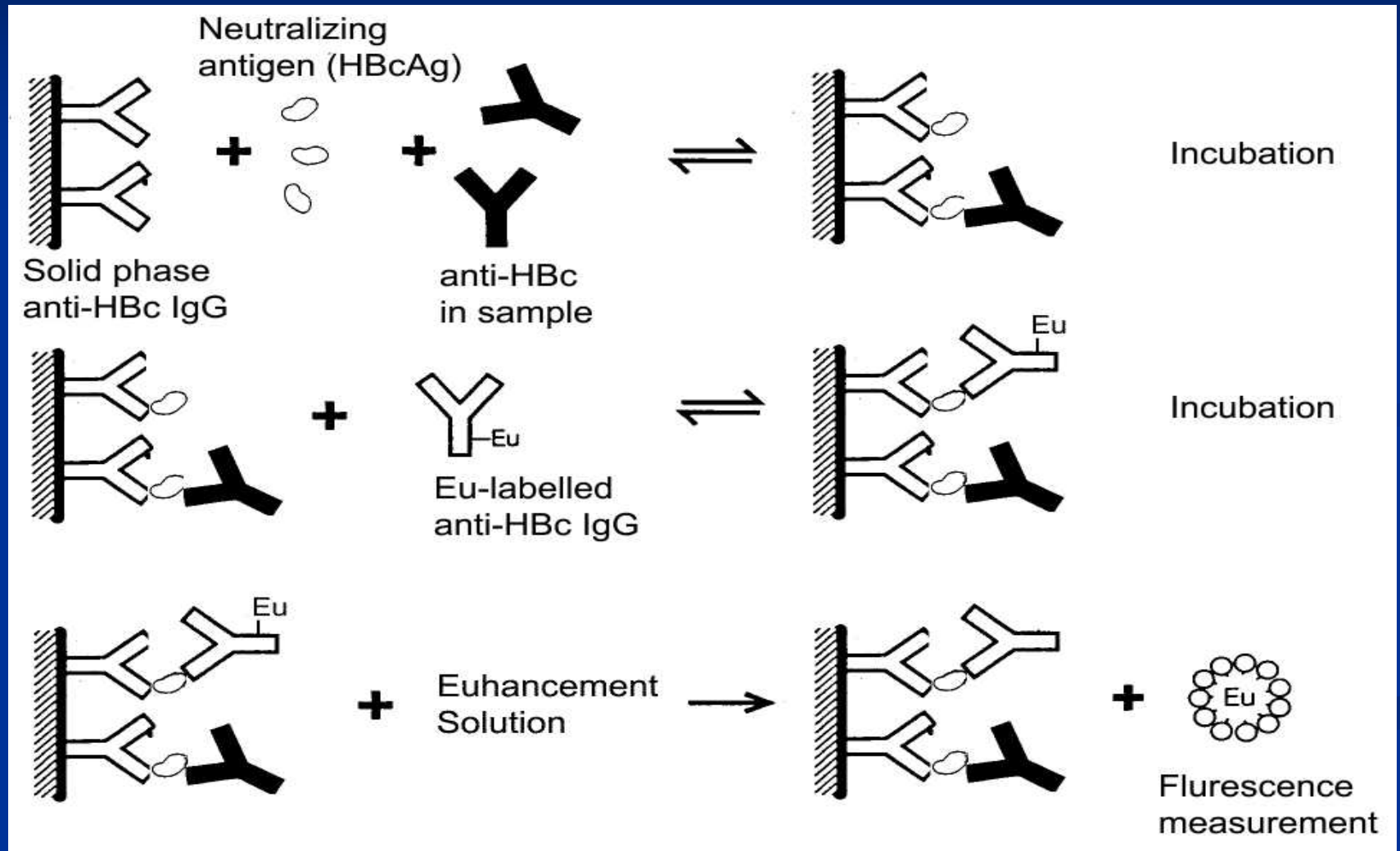
TRFIA detect HBeAg



TRFIA detect Anti-Hbe antibody



TRFIA detect Anti-HBc antibody



TRFIA brings advanced in clinical testing

技术	特点	检验先进性
时间分辨 光谱分辨	特异性荧光与非特异性荧光分离 发射荧光与激发荧光分离	0 本底、高特异性
解离-增强	稳定的荧光螯合物 荧光强度大大提高	线性范围更宽 重复性更好
原子标记	标记位点多，可达20个 对标记物结构及活性影响小 无衰变 受环境影响小	高稳定性，高精确度 试剂保质期至少一年 标准曲线保留时间长 同一批次只需两点定标
多标记	单，双，三，四标记	同一体系可同时测多个项目

时间分辨荧光免疫分析与其它 免疫学方法的比较

以上内容仅为本文档的试下载部分，为可阅读页数的一半内容。如要下载或阅读全文，请访问：<https://d.book118.com/707032055005006025>