

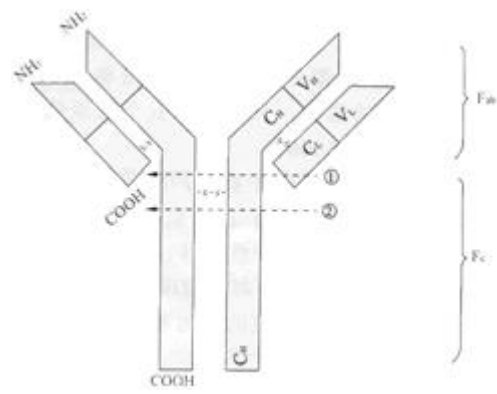
ELISA

ELISA -

1.2

1.2.1

			(immunoglobulin, Ig)	Ig		IgG	IgA	
IgM	IgD	IgE	Ig	IgG	IgM	Ig	L	
	H		Ig	κ	kappa	λ	Lambda	
Ig				IgG	IgM		γ	gamma
	μ	mu	IgG					



IgG

Fab

Fab

Fc

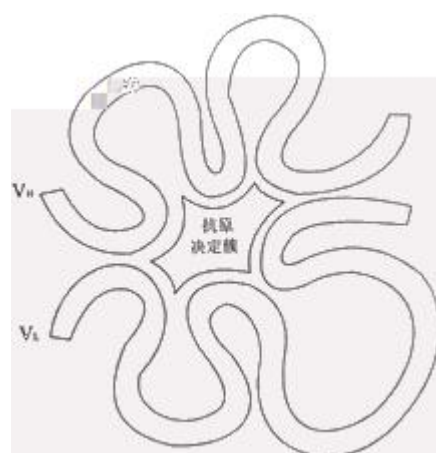
IgG

IgG

Fab

F ab' 2

Fc



IgM

10

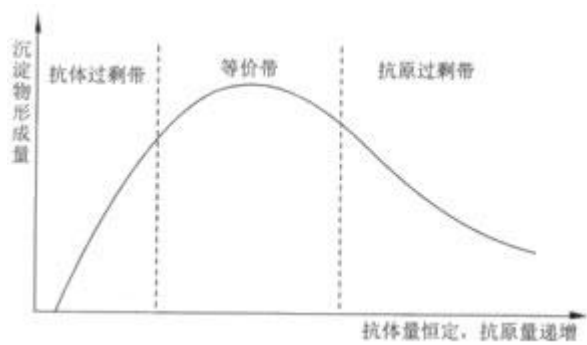
10

10

IgM

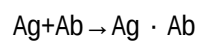
900000 IgG

150000



1.3

1.3.1



affinity

K

$$K = \frac{[Ag \cdot Ab]}{[Ag][Ab]}$$



IgG

IgG

1.3.2

1-4

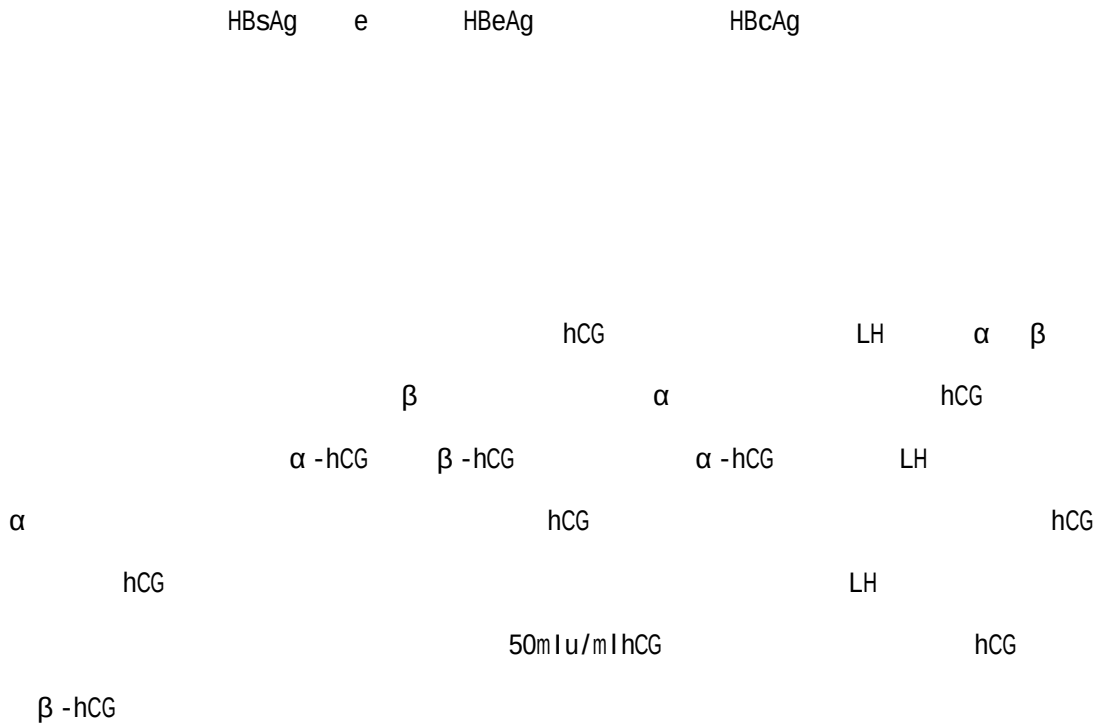
zone of equivalence

zone phenomenon

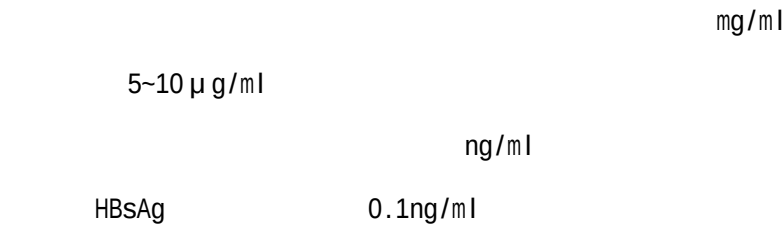
prezone

postzone

1.3.3



1.3.4



1.4

1)

2)

3)

4) HBsAg HBeAg

5) -HBs

5

5~10 μ g/ml

Linked immunosorbent assay

ELISA

ELISA -

1. ELISA

ELISA

2. ELISA

ELISA

1

11

" immunosorbent

2

" " conjugate

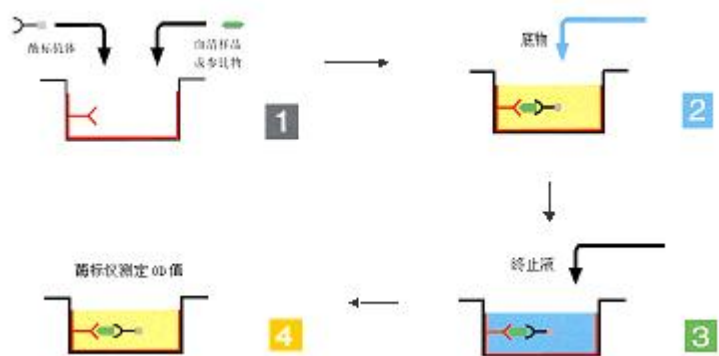
3

ך

ך

ר' י

ך



1

2

3

4

HBsAg HBeAg AFP hCG

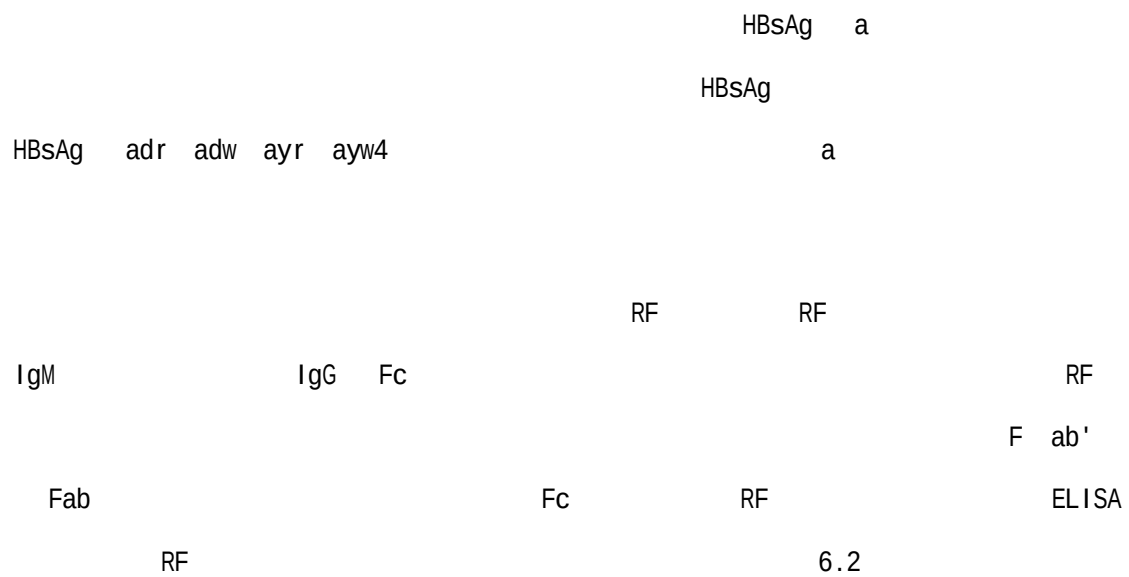
"

"

1.3.2 1-4

hook effect

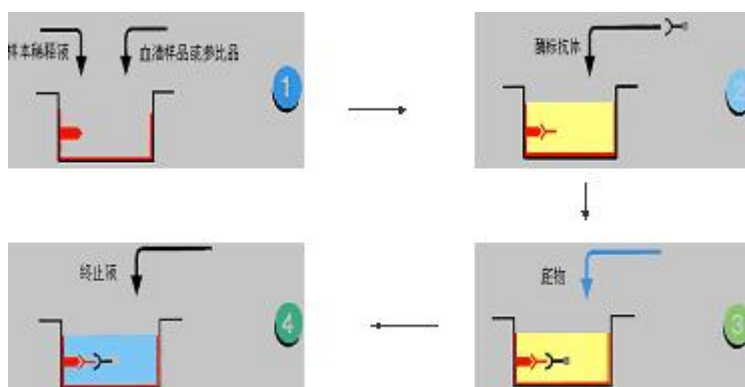
HBsAg AFP hCG



2.2.2

HBs

2.2.3



2-3

1

2

3

Ig

IgG

IgG

4

E.Coli i

E.Coli i

E.Coli i

E.Coli i

Ig

Ig

IgG

IgG

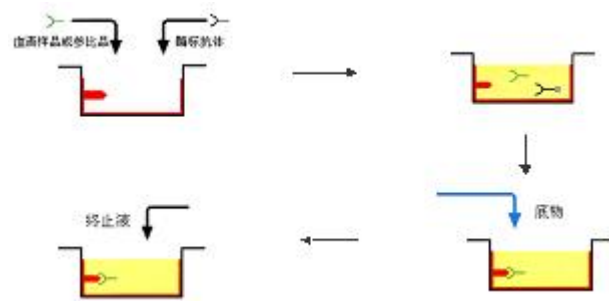
IgG

IgG

blocking

1:40~1:200

2.2.4



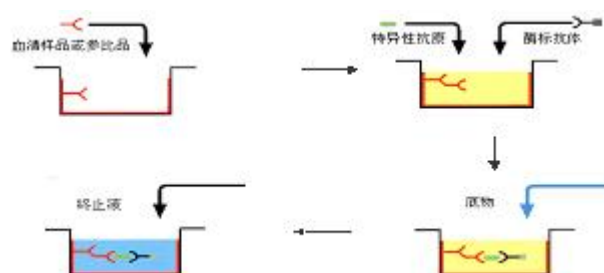
HBe ELISA

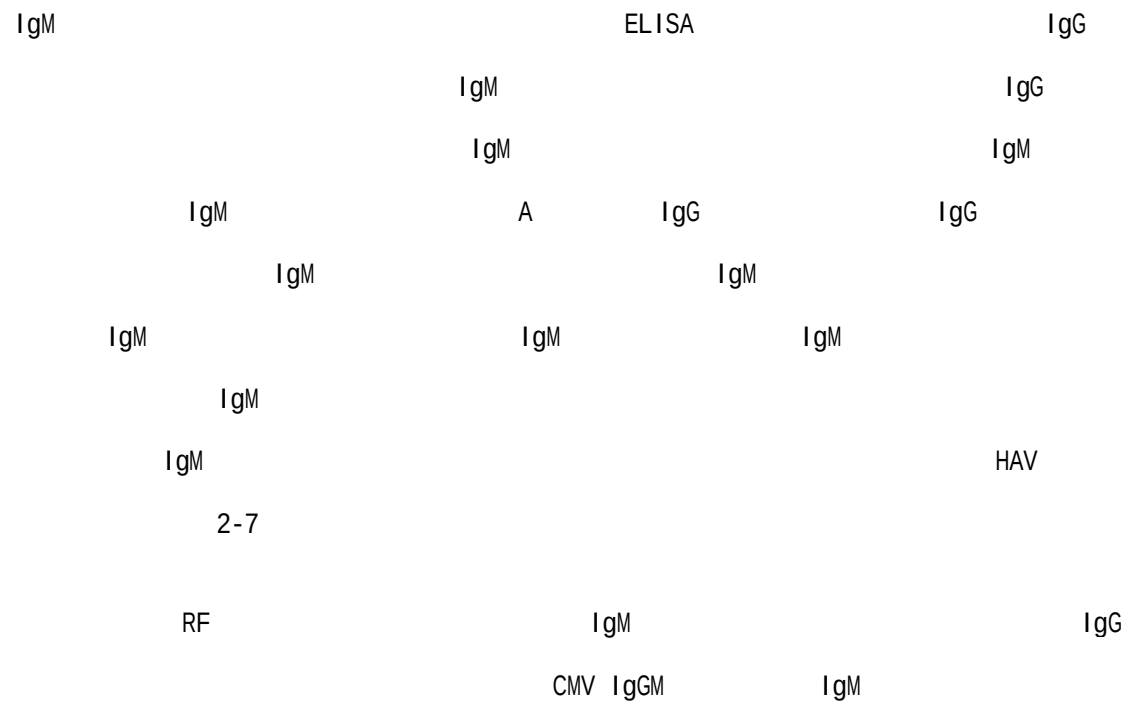
HBe

2.2.5

ELISA

2.2.6





2.2.7 ABS-ELISA

ABS	(avidin)	(biotin)	(system)
60000	4		244
		-	

2.2 ELISA

ELISA

- 1
- 2
- 3
- 4
- 5
- 6
- 7

3.1

2~8 6

3.1.1

ELISA ELISA

ELISA
EILSA ELISA 8 × 12 96
8 12 ELISA
ELISA
ELISA

ELISA

ELISA

ELISA

IgG

10ng/ml

ELISA

IgG

0.8

10%

ELISA

ELISA

ELISA

ELISA

ELISA

0.6cm

ELISA

200mm²

1000mm²

ELISA

5

ELISA

ELISA

100-200uI

ELISA

ELISA

3.1.2

(coat ing)

IgG

Fc

2.2.4

1/10 /100

DNA

DNA

30W

75cm

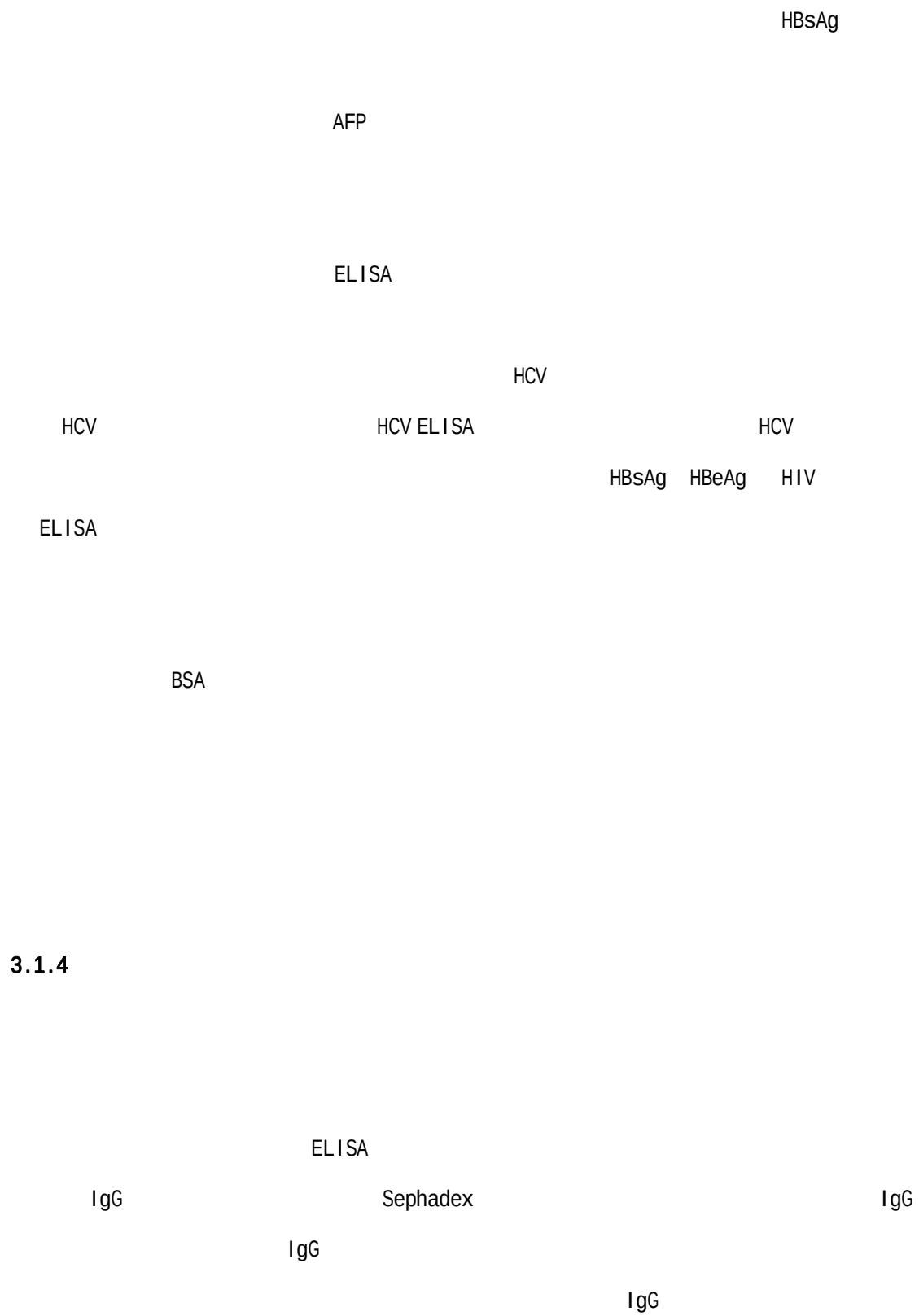
12

DNA

ELISA

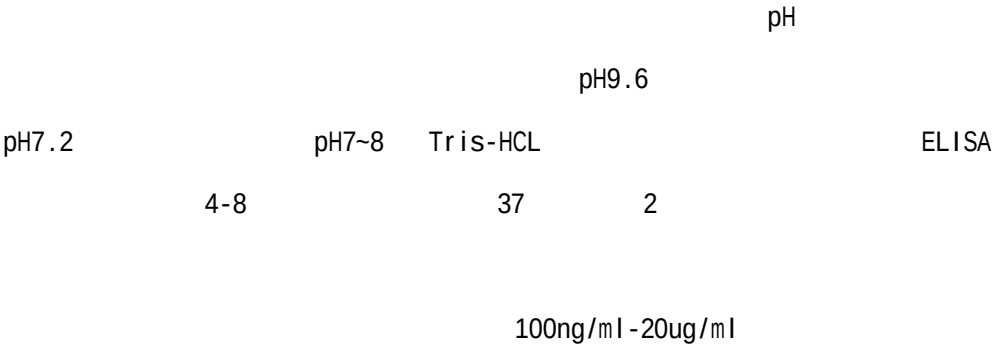
ELISA

3.1.3



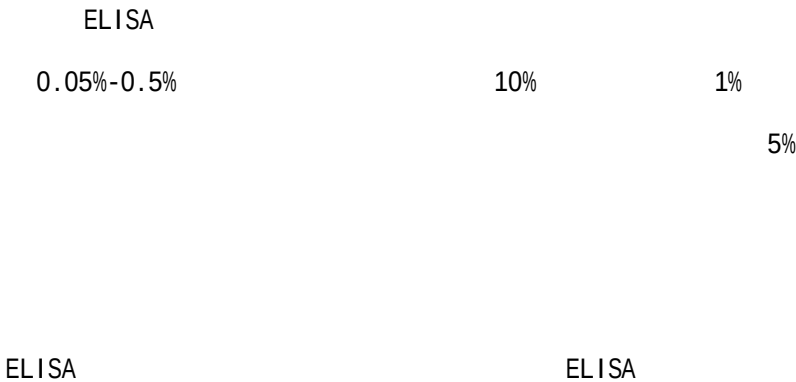
IgG

3.1.5



3.1.6

(blocking)



ELISA ELISA

2.2.2 ELISA

ELISA -

3.2

ELISA

3.2.1

ELISA

ELISA (alkaline phosphatase, AP) (horseradish peroxidase, HRP) ELISA β -D-

ELISA HRP HRP 18% 44000 275nm 403nm HRP RZ (Reinheit Zahl) 403nm 280nm HRP RZ ≥ 1.0 HRP ELISA RZ

ELISA AP AP AP 80000 pH 8.0 100000 pH 9.6 ELISA AP HRP AP HRP

3.2.2

IgG
F(ab')₂ RF ELISA

3.2.3

1

ELISA

60%-70%

1:1

2

HRP

Schiff

/ =1-2/1

HRP

ELISA

ELISA

HRP	IgG	1:5000	1:5000
IgG	ELISA		ELISA

" "

3.2.4

ELISA

3.2.5

ELISA

4-8

6

HRP

AP

3.2.5

1%

0.05%

3.3

3.3.1 HRP

HRP

H2O2

DH2+ H₂O₂ D+ H₂O

DH2

H₂O₂

ELISA

DH2

(0-phenylenediamine, OPD)

(3,3',5,5'-tetramethylbenzidine, TMB) ABTS[2,2'-azino-di-(3-ethylbenziazobine sulfonate-6)]

OPD

492nm

HRP

OPD

OPD · 2HCL

OPD

OPD

OPD

H₂O₂

OPD

ELISA

0.02% H₂O₂

,

OPD

TMB HRP

TMB

H₂O₂

TMB

ELISA

HCL

H₂SO₄

TMB

405nm

ABTS

OPD

TMB

HRP 3-(4-) [3-(4-hydroxy)phenyl propionic acid, HPPA]
 HRP ELISA

HRP H₂O₂ (urea
 peroxide) H₂O₂ H₂O₂
 2 8 1

3.3.2 AP

AP (p-nitrophenyl phosphate, p-NPP)
 405nm NaOH

AP 4- ELISA

3.4

ELISA 0.05% 20

3.5

HRP ELISA
 2mol/L

3.6

(positive control) (negative control)

ELISA

(recalcified human plasma)

		HBsAg		HBsAg	HBs
				HBsAg	ELISA
	0.5ng/ml		10ng/ml		
3.7					
	ELISA				
		4-5			
ELISA	-				
4.1					
	ELISA				
				ELISA	
		ELISA			
				HRP	ELISA
				HRP	
				ELISA	
	5		4		

3.2.4

4.2

ELISA

pH

4.3

ELISA	3
LEISA	

ELISA

1

4.4

ELISA

(incubat ion)

ELISA

ELISA

ELISA
ELISA

ELISA
ELISA
ELISA

20-25

ELISA

4.5

ELISA

ELISA

ELISA

ELISA

1

a.

b.

c.

1-2

d.

e.

c

d

3-4

20

0.05%-0.2%

0.2%

2

4.6

4.6.1

ELISA

OPD

37

20-30

OPD

OPD

TMB

TMB HRP

40

2

TMB

SDS

12-24

450nm

4.6.2

96

optical density OD

absorbance

A

A

492nm

"A492nm"

"OD492nm"

OPD

4.6.3

ELISA

		0.001	± 1%	0.5%
A	1.083		A	1.083 ± 0.01(1.073~1.093
	A	1.083 ± 0.05	1.078~1.088	
		0.000~2.000		2.900
	A	"*" "over"		
				0.000~2.900
0.000~2.000		ELISA		
			15~30	

ELSIA

3.6

sample S

P

N

a

cut-off value

A

2ng/ml

NC_x

0.400

HBsAg

2

A

3

PCX

3

A

HBsAg

A

P-N

$\geq 0.5 \times NC_x$

NC_x

$\leq 1.5 \times NC_x$

P=9 ±

A

A

=NC_x+0.05

A

0.05

" "

b. /

ELISA

patient

N A

S/N

P/N

P

S/N

S

(positive)

S/N

N

N<0.05

0.05

2

ELISA

1.0-1.5

ELISA

S P N

a.

HBc

HBc

125 ± 100u/ml

A

NC_x

0.300

2

A

HBc

3

PCX

3

A

2

N-P

2.000

$\geq 0.5 \times NC_x$

$\leq 1.5 \times NC_x$

$\times NC_x$

2

A

A

$=0.4 \times NC_x + 0.6 \times PCX$

A

$\leq$

A

b.

%

=

A

-

A

$\times 100\%$

/

A

$\geq 50\%$

50%

ELISA -

1949 College of American Pathologists CAP
Leverry Jennings 1950

70 -- Good Laboratory
Parctice GLP 80 GLP " "

ELISA

ELISA

1988

5.1

5.1.1 Quaility Control,Q.C

1

2

3

3

4

5

"

"

"

"

"

"

"

"

5.3.

5.1.2

5.1.3

ELISA

20

5-1

X

SD

X ± 1SD

0.68

X ± 2SD

0.95

X ± 3SD

0.99

ELISA

			X	± 1SD	
68%	X ± 2SD	95%	X ± 3SD		99%
	X ± 2SD	95%			

5.1.4

5.1.5 accuracy

$$= \frac{\text{Number of correct results}}{\text{Total number of results}} \times 100\%$$

5.1.6 Precision

5.1.7

1 WHO

2 WHO

3

5.1.8 clinical decision level

5.2

5.2.1

3-6 4 5 -20

5.2.2

5.2.3

1
2 56 10
3
4 10% PBS
5 20
6 20-30 > ± 2SD

5.3

1
2
3
4

5.3.1 optimal conditions variance, OCV

20-30

SD

HBsAg ELISA OCV HBsAg 5ng/ml

2 A X

20 20 X X1.....X20 20 OCV X SD

5.3.2 routine conditions variance-known value, RCVK

20

OCV RCV SD OCV SD OCV RCVK

SD RCVK SD OCV OCV RCVK

OCV RCVK OCV RCVK

5.3.3 , (routine conditions variance-unknown value RCVU)

, RCVU

RCVK

5.3.4

RCVK SD

S/C0 -2SD , " "

ELISA

2SD 2SD " "

2SD " "

	OCV		OCV		
3SD		2SD	3-5	2SD	5 7

5.3.5 --" "

			ELISA		
			ELISA	ELSIA	
20		"	"	3	3
"	"				

1)

2) X SD

3) SDI SDI

4) SDI SDI SDI

SDI	SDI	n2SD			
SDI	SDI	n2SD	n2SD	2SD~3SD	" "
SDI	SDI	n2SD	3SD	" "	" " "
"					

ELISA

	20		"	"
20	SD		21	

5.4 external quality assessment, EQA

ELISA -

ELISA evaluation

ELISA

ELISA

ELISA

6.1

100%

" " panel

a b c d

$$(\%) = a / (a + c) \times 100\%$$

$$(\%) = b / (b + d) \times 100\%$$

$$(\%) = (a + d) / (a + b + c + d) \times 100\%$$

>90%

6.2

1

2

3

4

5

6

7

RF

6.3

-HBc- IgM

abbott

-HBc- IgM

70

29

41

-HBc- IgM

70

7

-HBc- IgM

22

16

5	1	-HBe-IgM	40
---	---	----------	----

"HD-HOOK"

ELISA

-
-
- ,
-
-

ELISA

- PVDF
-

ELISA

-

HBsAg	HBeAg	HBcAb- IgG	HBcAg- IgM	HBeAb	HBsAb		
+	+	-	-	-	-	HBV	HBV
+	+	+	+	-	-	HBV	HBV
+	-	+	+	-	-	HBV	HBV
+	-	+	+	+	-	HBV	HBV
+	-	+	-	+	-	HBV	
-	-	+	+	-	-	HBV	HbsAg
-	-	+	-	-	-	HBV	-HBs
-	-	+	+	+	-	HBs	HBV
-	-	+	-	+	+	HBV	

-	-	+	-	-	+	HBV
+	+	+	+	-	+	
+	-	-	-	-	-	
-	-	-	-	-		

1

2

ELISA

CV

15%

37

37

4-10

Panel

SOP

ELISA

5-100 I

$\pm 10\%$

± 1

2uI

1

UV-2201

-

$\pm 0.3\text{nm}$

200uI

0.500A

8

1

490nm

,

2

8

96

200uI

0.100A

, 490nm

$\pm 1.96s$

n

8

8

200uI

490nm

30

4

1

3

200uI

3

490nm

20

,

CV

S	0.038	0.008	0.			
008	0.008	0.018	0.018	0.018	0.018	0.018
P	> 0.05	> 0.05	< 0.01	< 0.01	< 0.01	<
0.01	<0.01	<0.01				

ELISA

ELISA

, (SOP)

) 1/3

;

30

37 ° C

ELISA

1/3

2ng/ml HBsAg

ELISA

1

2

3

2-3

4

5

HRP

37 ° C 10-15

0.2

.

30

OPD

TMB

TMB

OPD

490nm

TMB

450nm

630nm

S/C.O

S

A

C.O

Cut Off

)

 $S/C.O \geq 1$ $S/C.O \geq 1$

Cut Off

A C.O=2.1 × N N 0.05 0.05

 $P/N > 2.1$

B C.O=0.5 × N

C C.O=N+C C

D) C.O=C × P+N C

ELISA

ELISA

ELISA

CO

+

1 C.O × 1 ± CV

CV CV (15-20%

2 C.O ± 2s s ROC s

HD-HOOK

ELISA

HIGH DOSE HD

HOOK MILES 1974

"HD-HOOK"

" " " "

Quality Control QC

Whitehead

4

OCV

RCV

SPR

EQA

IQC

GLP

QC

IQC

QC

Quality Control QC

ELISA

QC

S/C.0 ≥ 1

S/C.0 ≥ 10

A 0.05-0.07

"HOOK"

QC

(Quality Control)

ELISA

AFP CEA CA

(Quality Control)

" "

3

20

RCV X s

1

X1

Xn

2 X s

3 SI

SI = X -X /S SI = X -X /S

SI

SI ≤

SI >

(Quality Control)

SI

N

3

4

5

6

7

8

9

10

11

N(3s) 1.15 1.49 1.75 1.94 2.10 2.22 2.32 2.41 2.48

N (2s) 1.15 1.46 1.67 1.82 1.94 2.03 2.11 2.18 2.23

12	13	14	15	16	17	18	19	20
2.55	2.61	2.66	2.71	2.75	2.79	2.82	2.85	2.88
2.29	2.33	2.37	2.41	2.44	2.47	2.50	2.53	2.56

(, 3-6

56 10

10% PBS

Cut Off

-20

≤ 0.05 - >0.8 2s

> 0.80 ;

Quality Aprovement QA

100

X

s

SI

SI=

-

X

/

s

SI

0.0-0.9

,1.0-1.9

,SI

SI=

-

X

/

s

SI

0-1.0

,1.1-2.0

,>2.0

SI

Quality Improvement QI

- 1
- 2
- 3