Lab-3

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Laboratory Diagnosis

- **Microscopic ex**
- **Virus cultivation**
- **Serological tests**
- **Detection of N/A**

Virus cultivation

- Laboratory animal
- Embryonated egg
- Cell culture

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Cell culture

- Conventional C/C
  - detected by CPE
    Hemadsorption
    Interference
    EM
  - Identified by IF
- Shell vial tech.

Serological tests

- Definition
- Types

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Antibody assays

- Measure total Abs& class-specific Ab
  - IgM & IgG
- IgM → recent inf. single serum
- IgG
  - 2 sera; 1 during the acute phase of dis.
    2 during the convalescent phase
  - A 4fold or greater increase in IgG
    → recent inf

Antigen assays

- They are used
  - Detection of virus in specimen
  - Identification after culture

Applications

- Diagnose infectious diseases
- Evaluate the course of an infection
- Determine the stage of a slower or chronic inf.
  - EBV
    - Ab to VCA detected first
    - Ab to nuclear antigen later

Applications

- Determine the nature of inf.
  - A primary or reinfection or reactivation
  - A cute or chronic
- Determine the immune status of individuals
- Screen ;

  blood donors for blood borne viruses
- Determine the prevalence, spread& control of infectious dis.

Serological methods

- Precipitation
- Agglutination
- C.F.T
- Solid phase immunoassays
  - ELISA &its modification
  - RIA
  - IF

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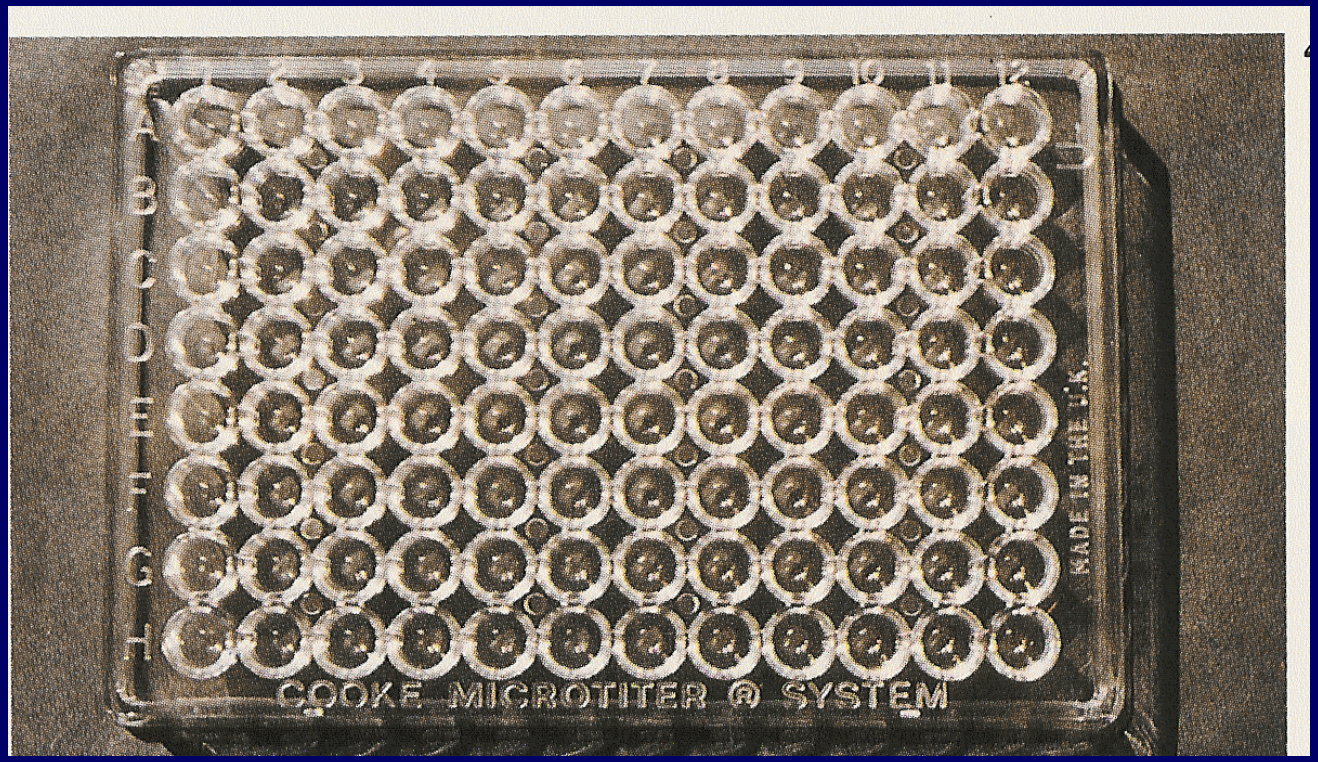
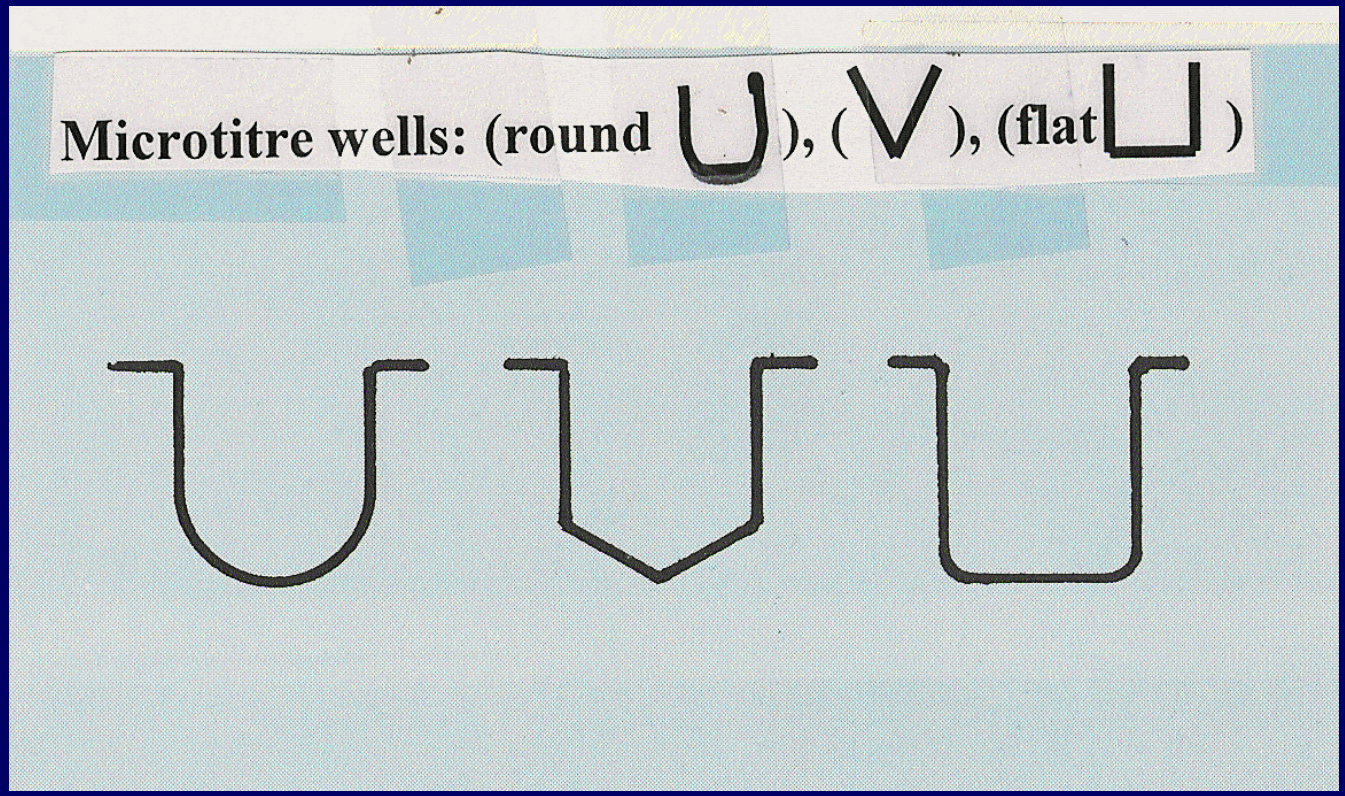
- Ab detection assays are not recommended for immun ↓ ed

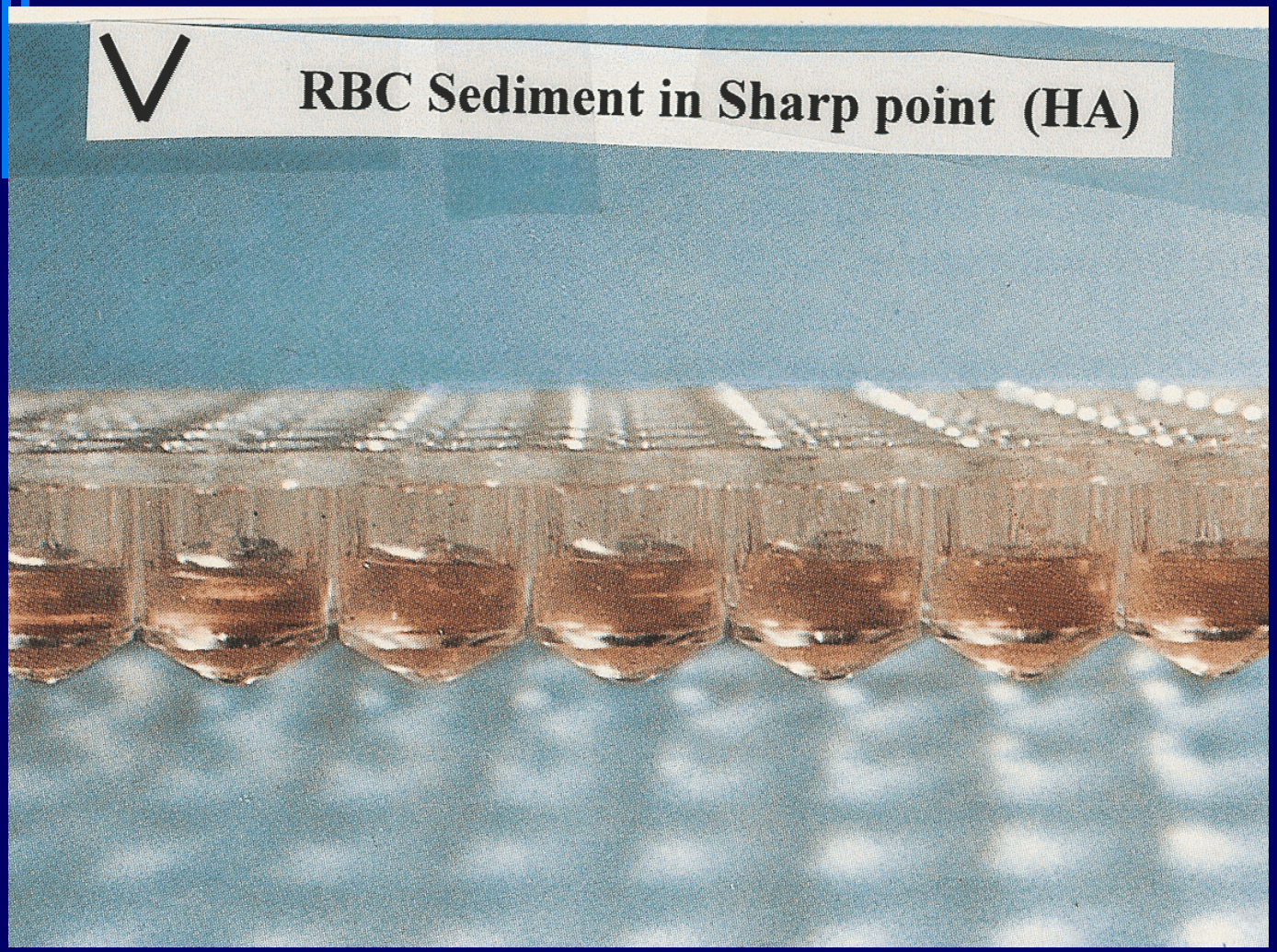
Serological methods

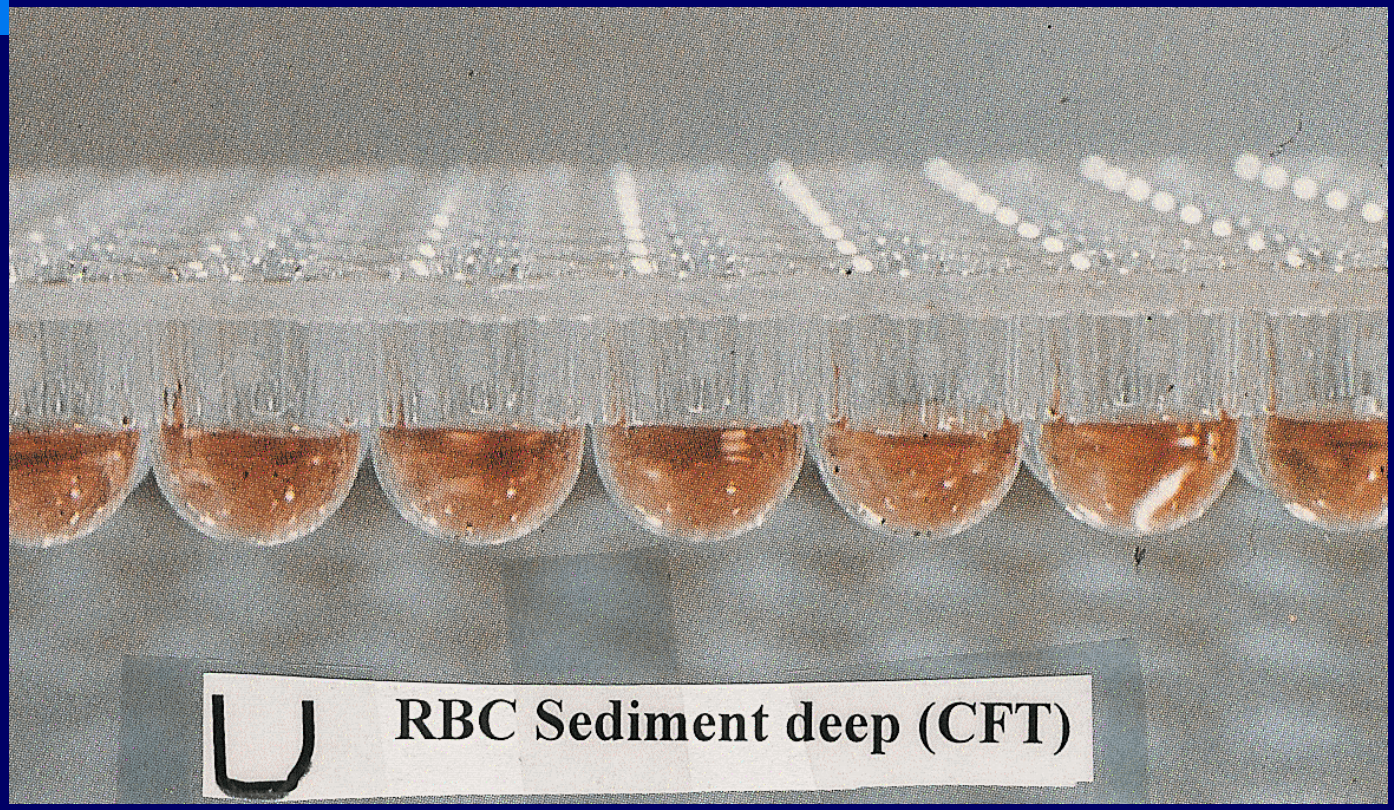
- Complement fixation test ; CFT
- Haemagglutination inhibition test ; HI
- Reverse passive Haemagglutination test ; RPHA


A
B
C
D
E
F
G
H
COOKE MICROTITER ® SYSTEM
MADE IN THE U.K.


Microtitre wells: (round), ( ), (flat)

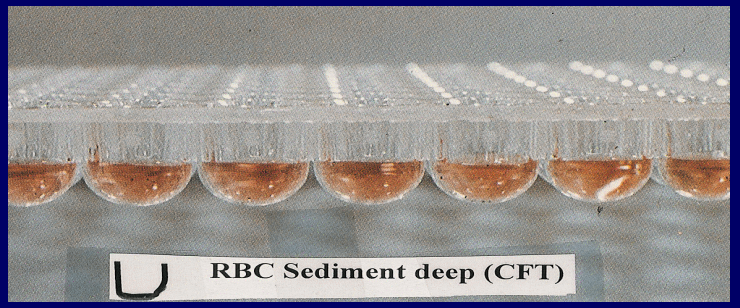

For T.culture, EIA

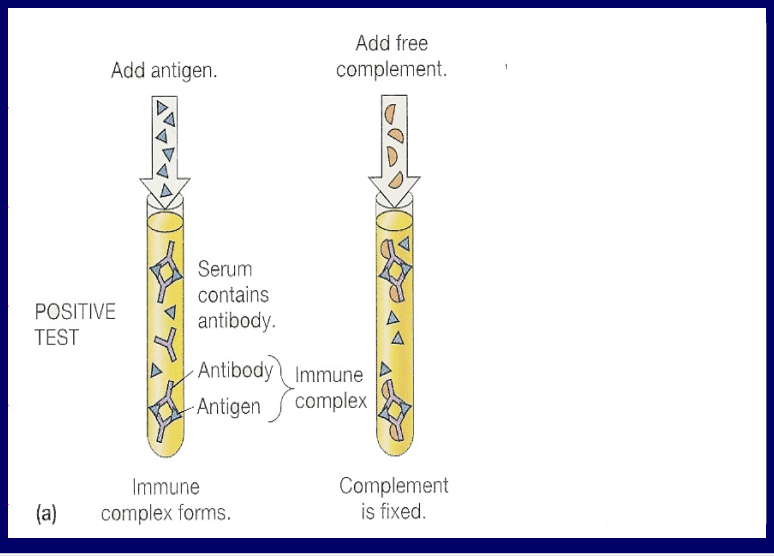

V
RBC Sediment in Sharp point (HA)

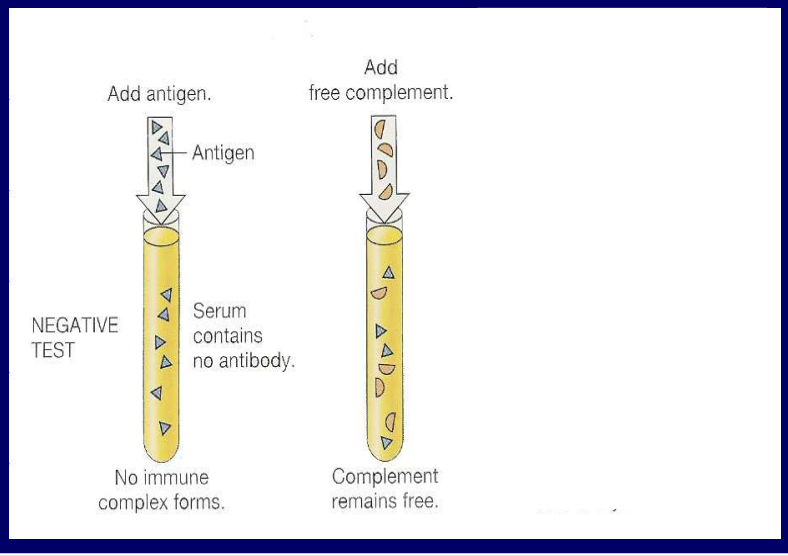

U
RBC Sediment deep (CFT)

Complement Fixation test

- Inactivated serum is serially diluted &
- known Ag & complement → incubation







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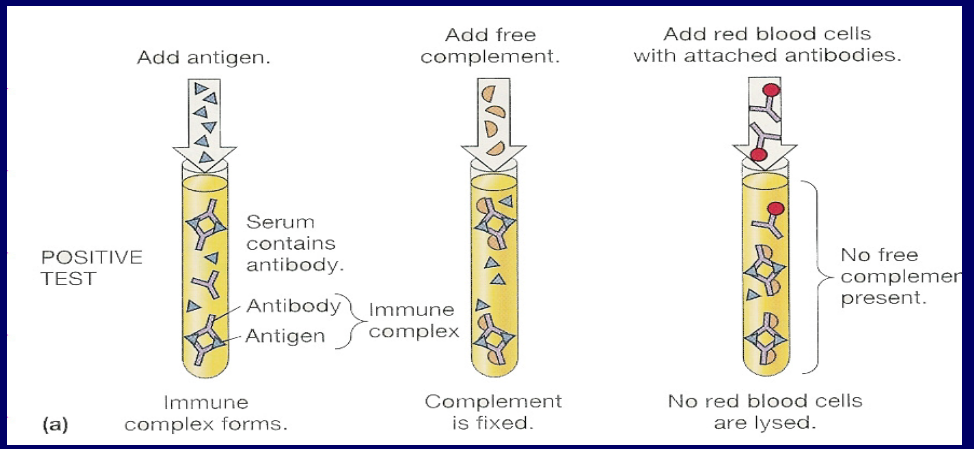
An indicator system [sensitized SRBC] is added

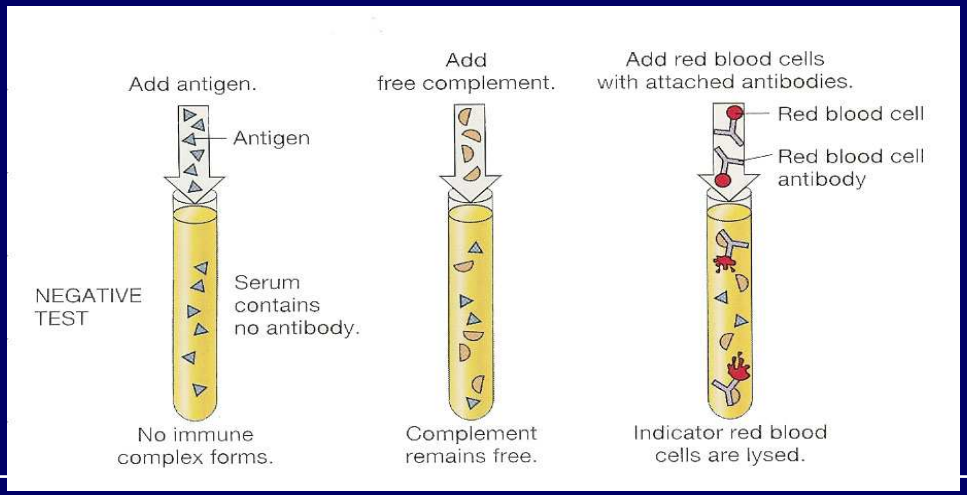
- If the C is fixed in the 1 step,

  It will not lyse the RBC (+)

- If the C is free ,

  it will lyse the RBC (-)





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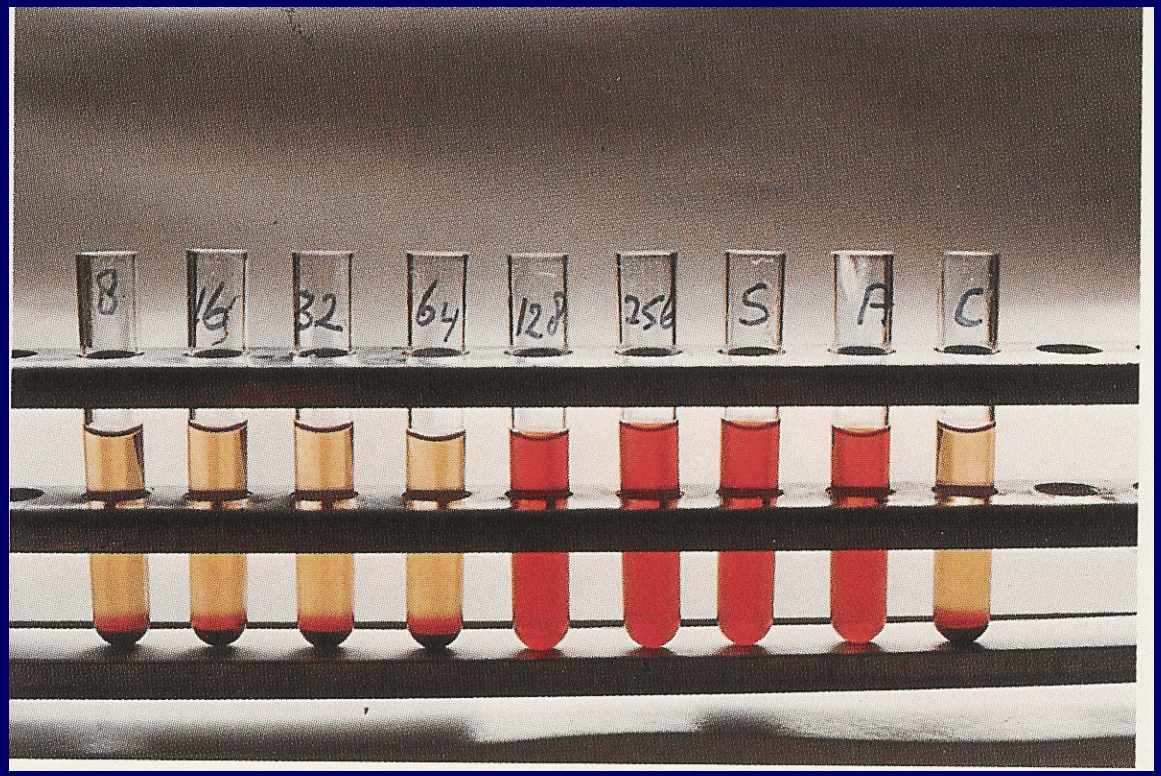
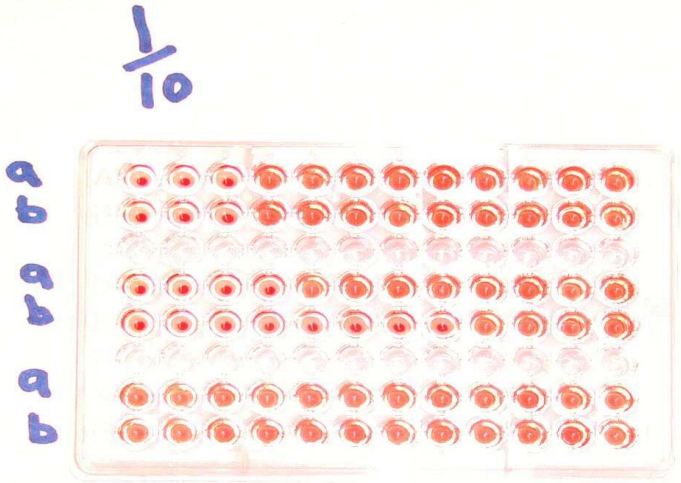

8
16
32
64
128
256
S
A
C

CFT

Test

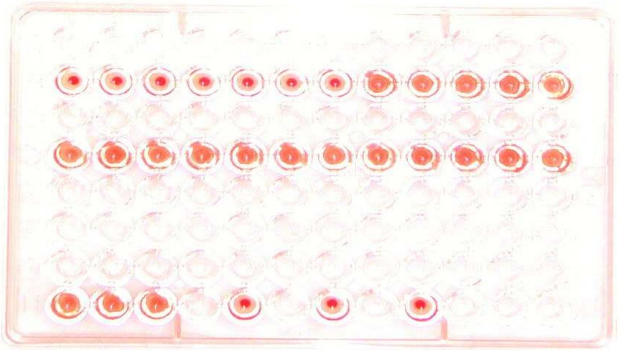


Pt-1

Pt-2

Pt-3

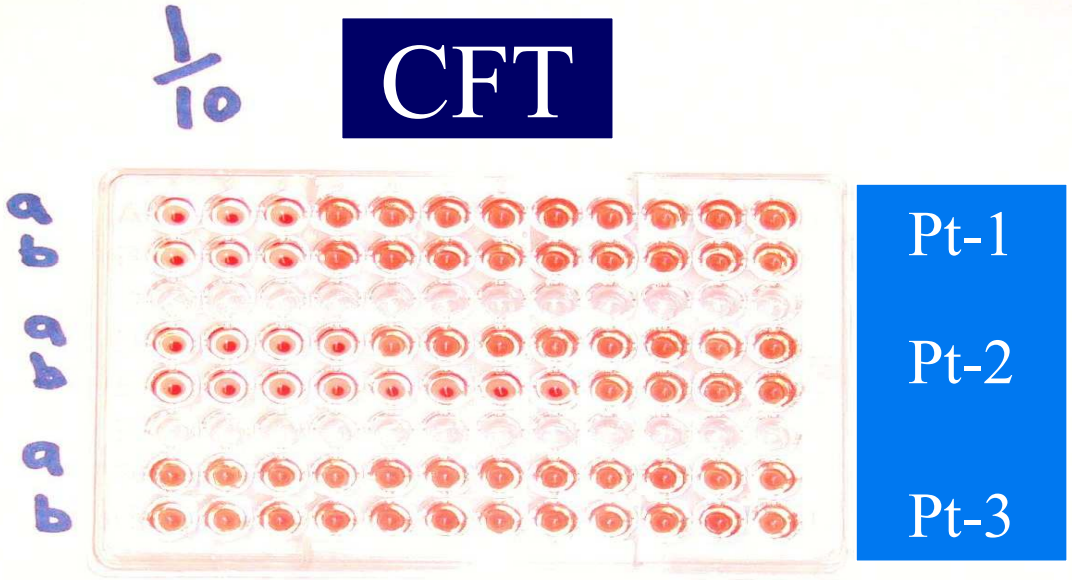
Control



+ ve control

- Ve conttrol

CFT



**Results are recorded in a table as follows:*

	Acute titer	conval. Titer	ratio(conval./acute)
Pt-1	*40*	*40*	*40/40 = 1*
Pt-2	*80*	*1280*	*1280/80= 16*
Pt-3	*-ve*	*-ve*	*--*

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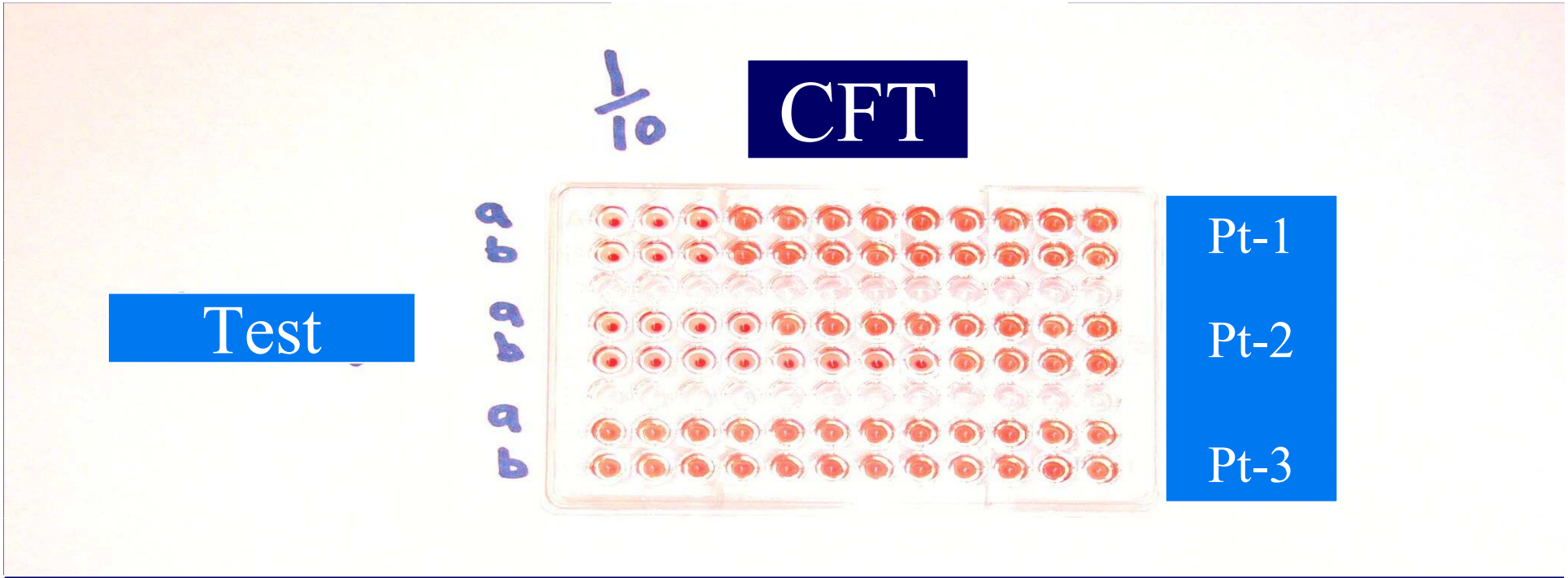


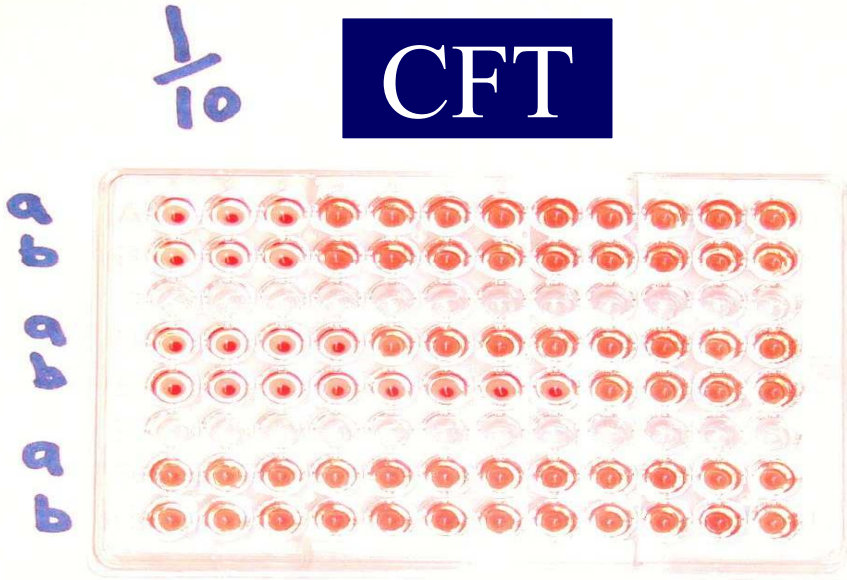
Interpretation of results

1- conval-titer / acute-titer > 4 recent infect.

2- conval-titer / acute titer < 4 immunity

3- negative negative not exposed

CFT



**Results are recorded in a table as follows:*

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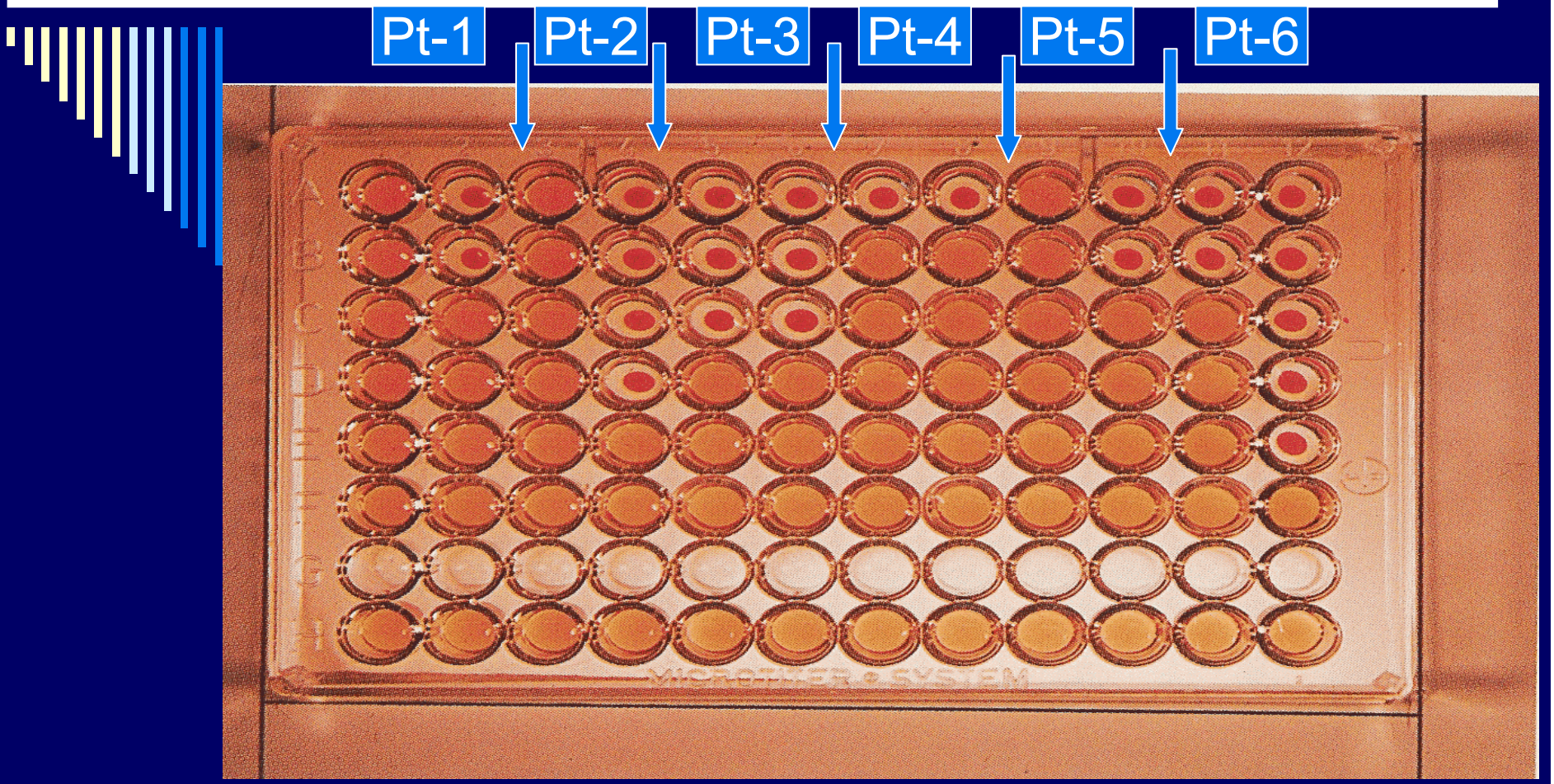
Pt-1	40	40	40/40 = 1	immunity.
Pt-2	80	1280	1280/80= 16	recent inf
Pt-3	-ve	-ve	--	not exposed



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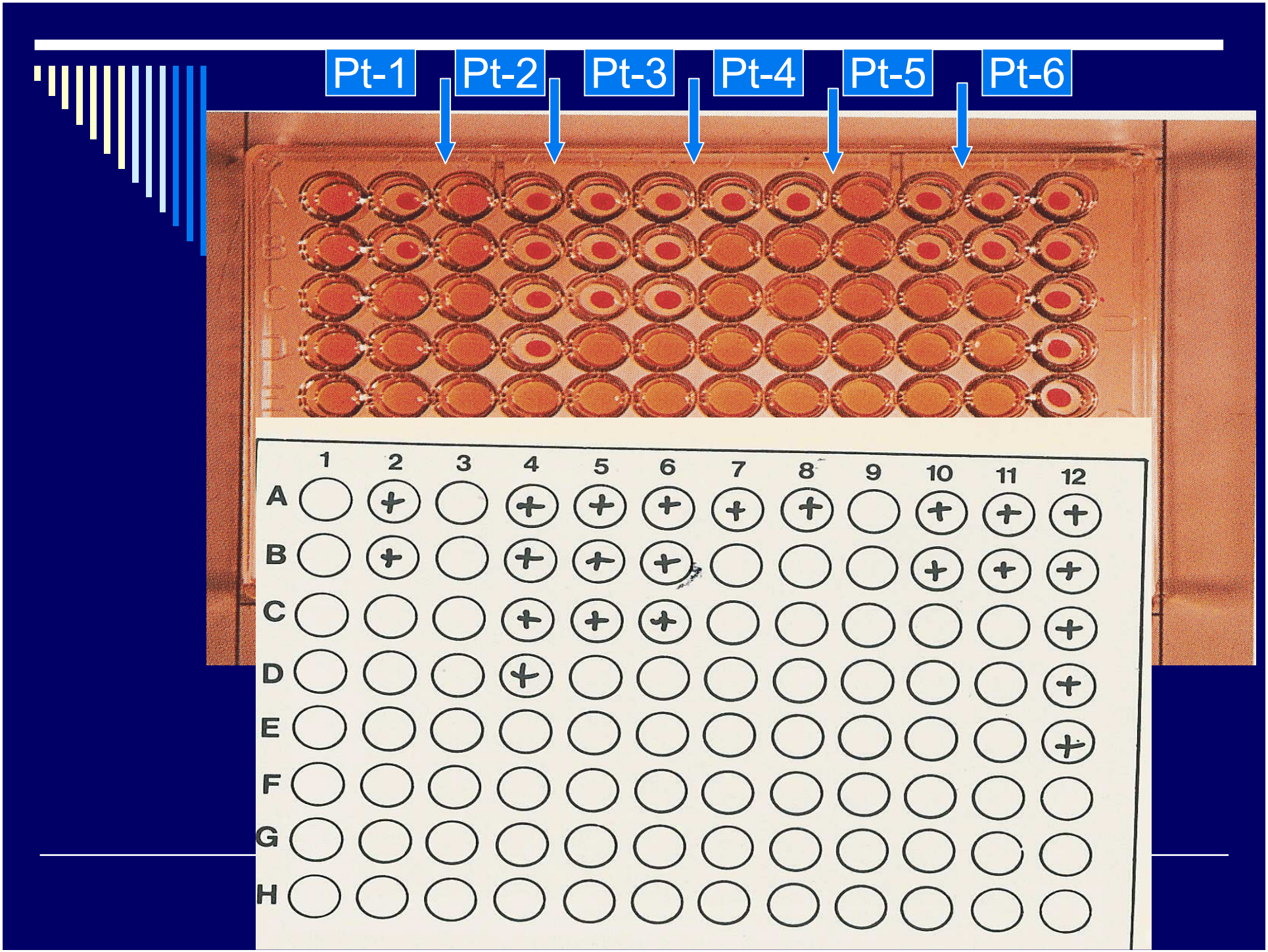

Pt-1
Pt-2
Pt-3
Pt-4
Pt-5
Pt-6
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H

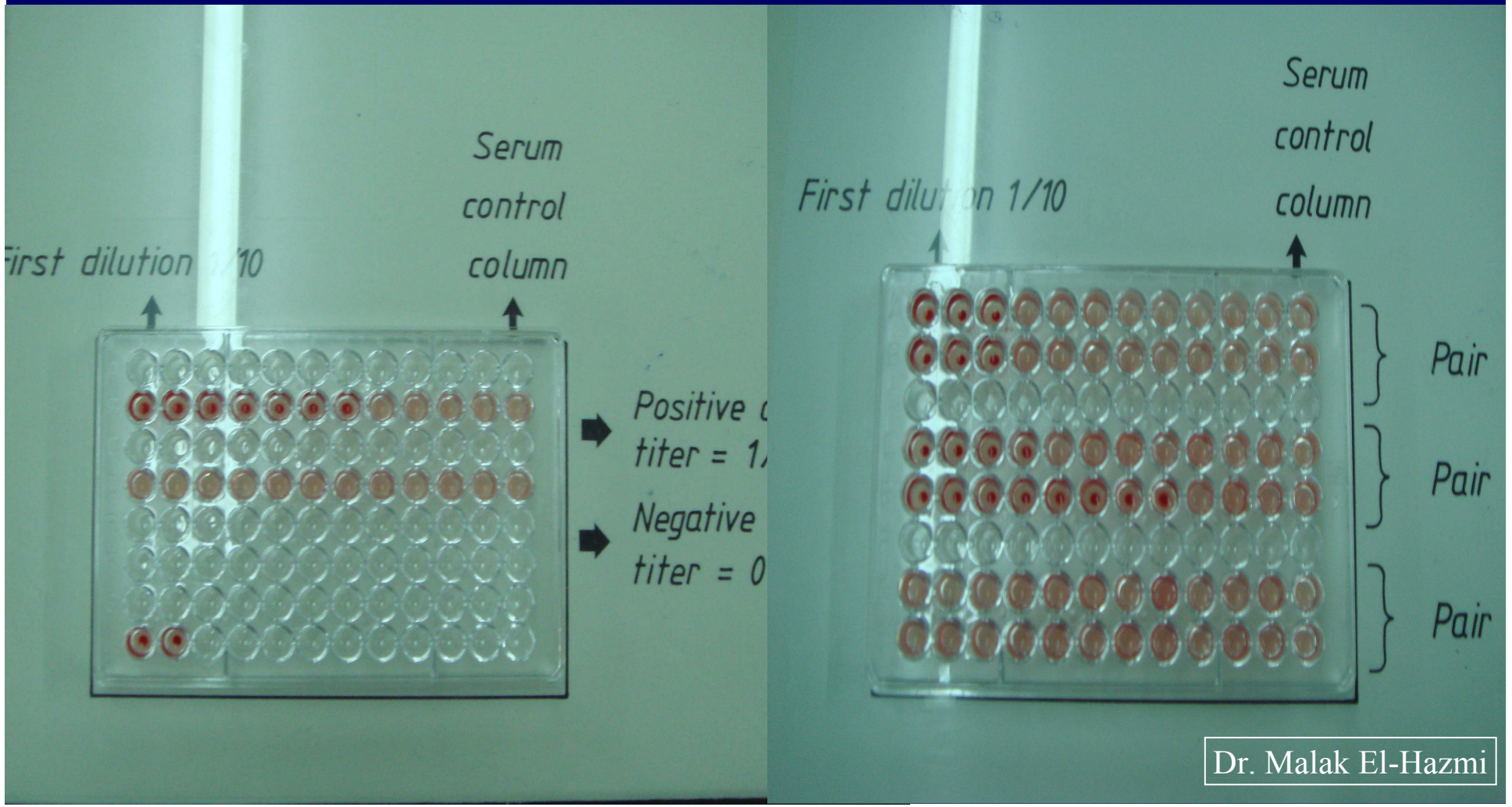
Haemagglutination inhibition test (HI)

- Serum is serially diluted then mixed with viral haemagglutinin → incubation
- If Ab is specific to H
  - → Ag-Ab complex
- If Ab is not specific to H
  - → No complex

An indication system [RBC] is added

- If the Ag-Ab complex is formed in 1 step
  - → RBC will not be agglutinated & settle at the bottom of the well as a red dot (+)
- If the complex is not formed
  - → the Ag will be free to agglutinate RBC (-)

Haemagglutination inhibition test



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HAI

*Results are recorded in a table as follows:

	Acute titer	conval. Titer	ratio(conval./acute)	interpretation
Pt-1	40	40	40/40 = 1	immunity.
Pt-2	80	1280	1280/80= 16	recent inf
Pt-3	-ve	-ve	--	not exposed

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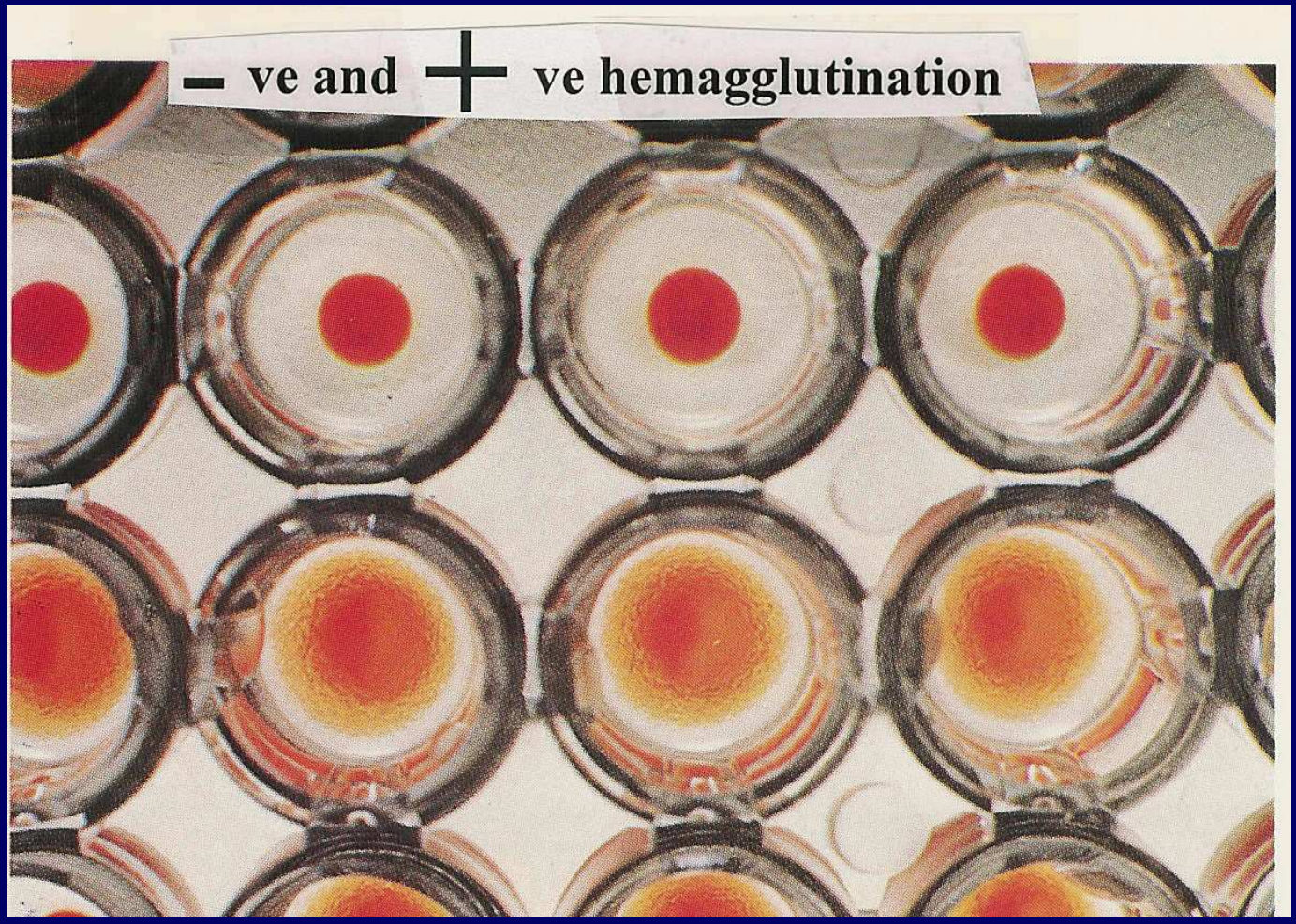
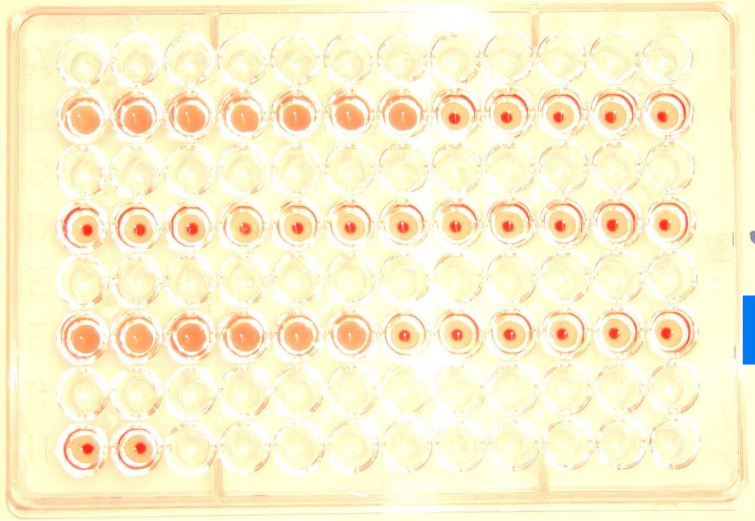
Reverse passive haemagglutination test

- *For Ag detection.*
- *One blood sample is needed*
- *Serum [Ag] is reacted with RBC coated with specific Ab*
- *If the Ag is specific ,it will bind to the Ab on the surface of the RBC and agglutinate them*


– ve and + ve hemagglutination

Reverse passive Haemagglutination test



+ ve control

- ve control

Pt serum

Cell control

- ***Positive specimen appears as red carpet.***
- ***Results are expressed as positive or negative.***

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عن أبي هريرة رضي الله عنه ، قال :

أوصاني خليلي صلى الله عليه وسلم **بثلاث** :

بصيام ثلاثة أيام من كل شهر

وركعتي الضحى ،

وأن أوتر قبل أن أرقد.