

التشخيص المخبري للكورونا فيروس

مديرية مخابر الصحة العامة

الدكتور جميل الخباز

Coronaviruses

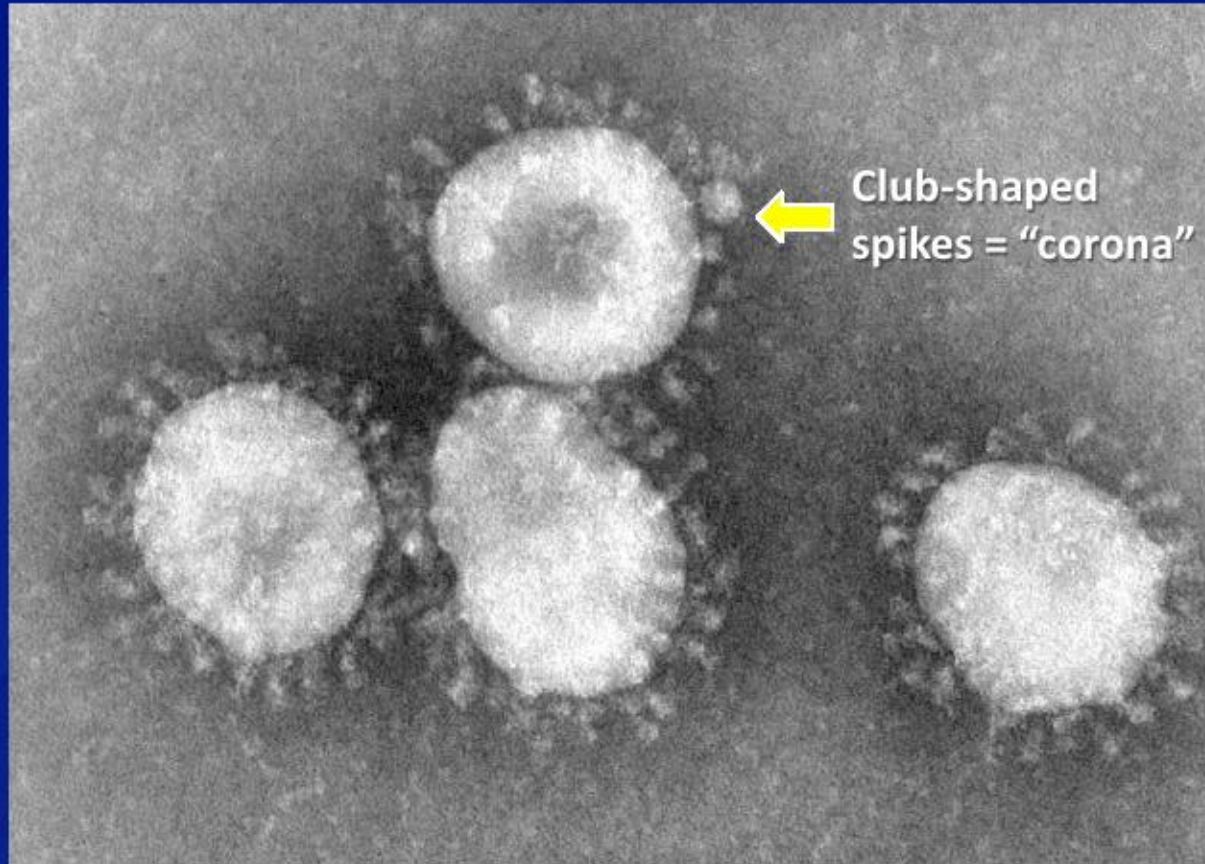
Background

- Enveloped positive strand RNA viruses
- Human CoVs (HCoVs) isolated in the 1960s
 - HCoV-229E
 - HCoV-OC43
 - HCoV-NL63
 - HCoV-HKU1
 - SARS-CoV
 - MERS-CoV



Coronaviruses

Particles



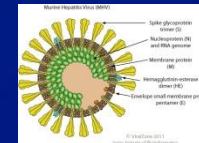
Size: 120-160 nm diameter



Coronaviruses

Classification

- Alpha
 - Human examples: HCoV-229E, HCoV-NL63
 - Pig, dog, and cat CoVs
- Beta
 - HCoV-OC43, HCoV-HKU1, SARS-CoV
 - MHV, rat, pig and cow CoVs
 - **MERS-CoV**
- Gamma
 - Chicken and turkey CoVs
- Delta
 - Bird CoVs



Coronaviruses

Genome

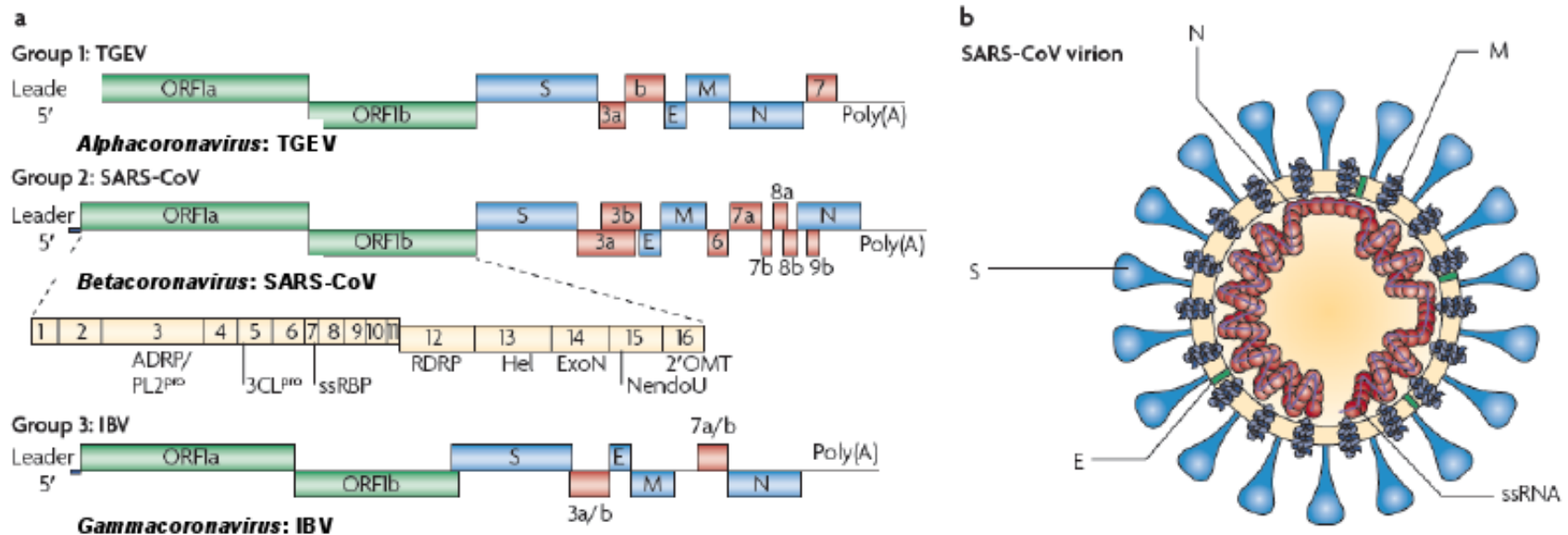
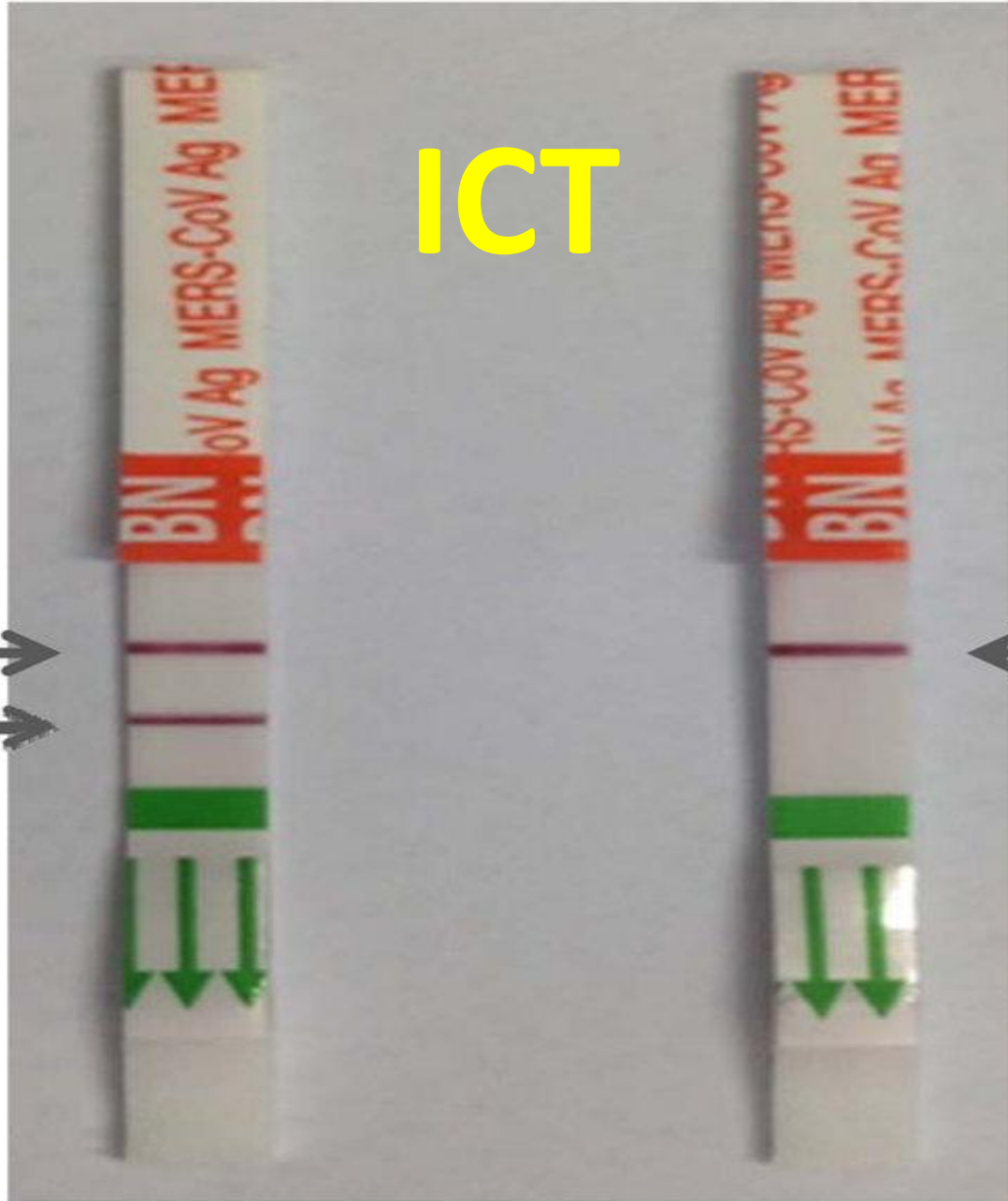


Figure 2 | **Structure of coronavirus genome and virion.** **a** | Schematic diagram of representative genomes from each of the coronavirus groups. Approximately the first two-thirds of the 26–32 Kb, positive-sense RNA genome encodes a large polyprotein (ORF1a/b; green) that is proteolytically cleaved to generate 15 or 16 non-structural proteins (nsps; nsps for severe acute respiratory syndrome coronavirus (SARS-CoV) are illustrated). The 3'-end third of the genome encodes four structural proteins — spike (S), membrane (M), envelope (E) and nucleocapsid (N) (all shown in blue) — along with a set of accessory proteins that are unique to each virus species (shown in red). Some group 2 coronaviruses express an additional structural protein, haemagglutinin-esterase (not shown). **b** | Schematic diagram of the coronavirus virion. 2'OMT, ribose-2'-O-methyltransferase; ExoN, 3'→5' exonuclease; Hel, helicase; IBV, infection bronchitis virus; NendoU, uridylylate-specific endoribonuclease; RDRP, RNA-dependent RNA polymerase; ssRBP, single-stranded RNA binding protein; ssRNA, single-stranded RNA; TGEV, transmissible gastroenteritis virus.

Perlman & Netland. Nature Reviews. 2009;7:439-450.

C
T



ICT

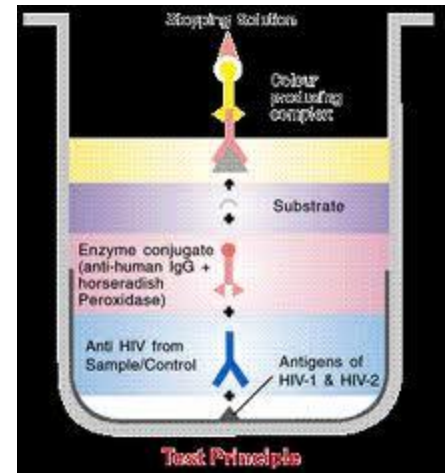
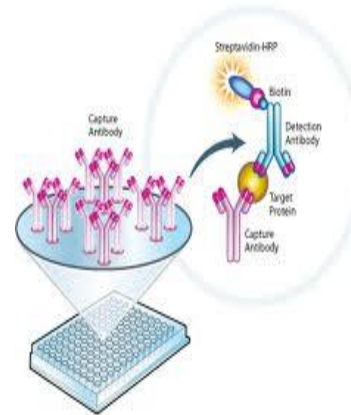
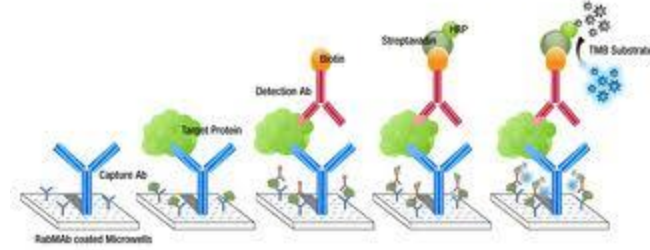


C

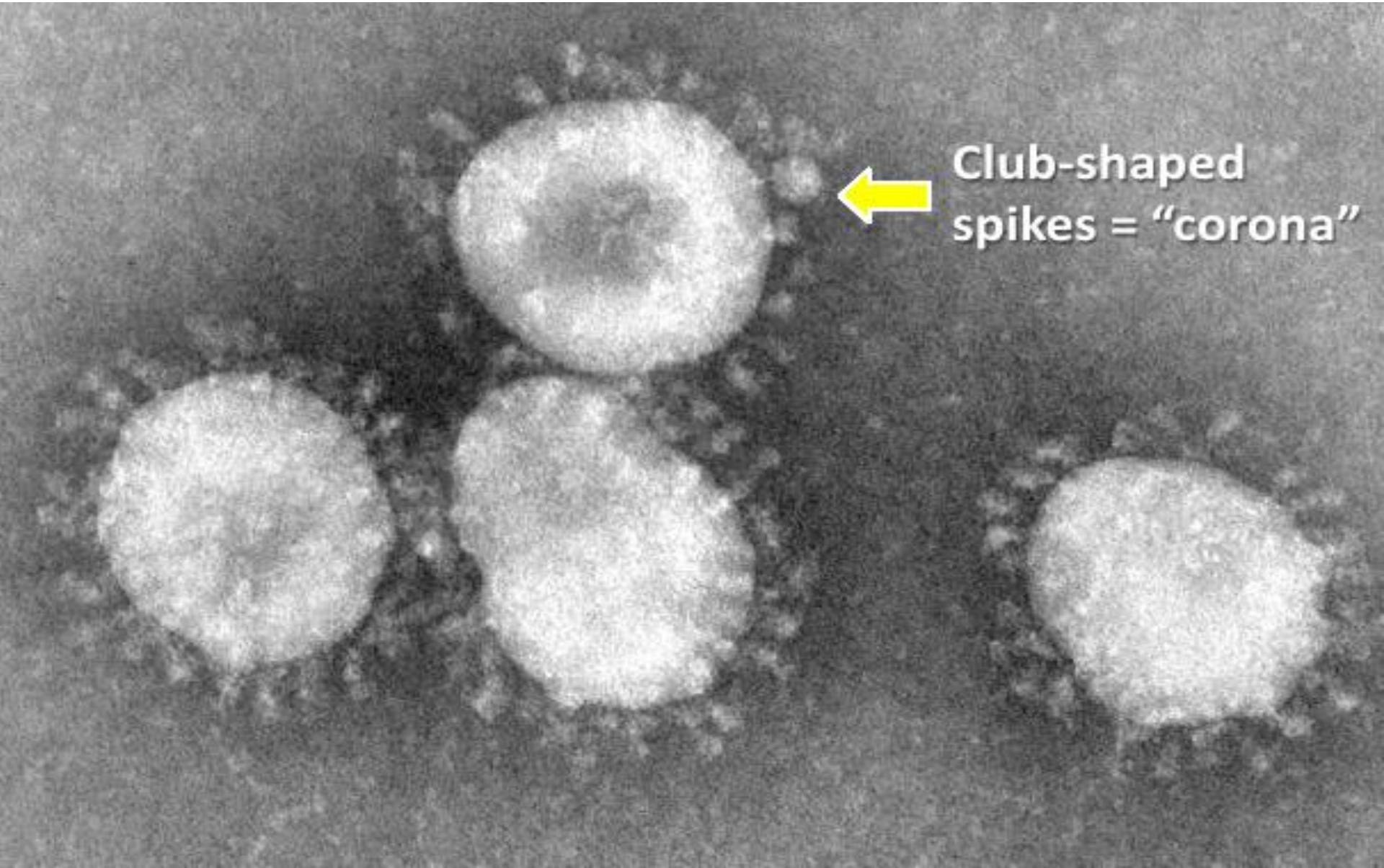
(A) Positive

(B) Negative

تقنية الإليزا



المجهر الإلكتروني

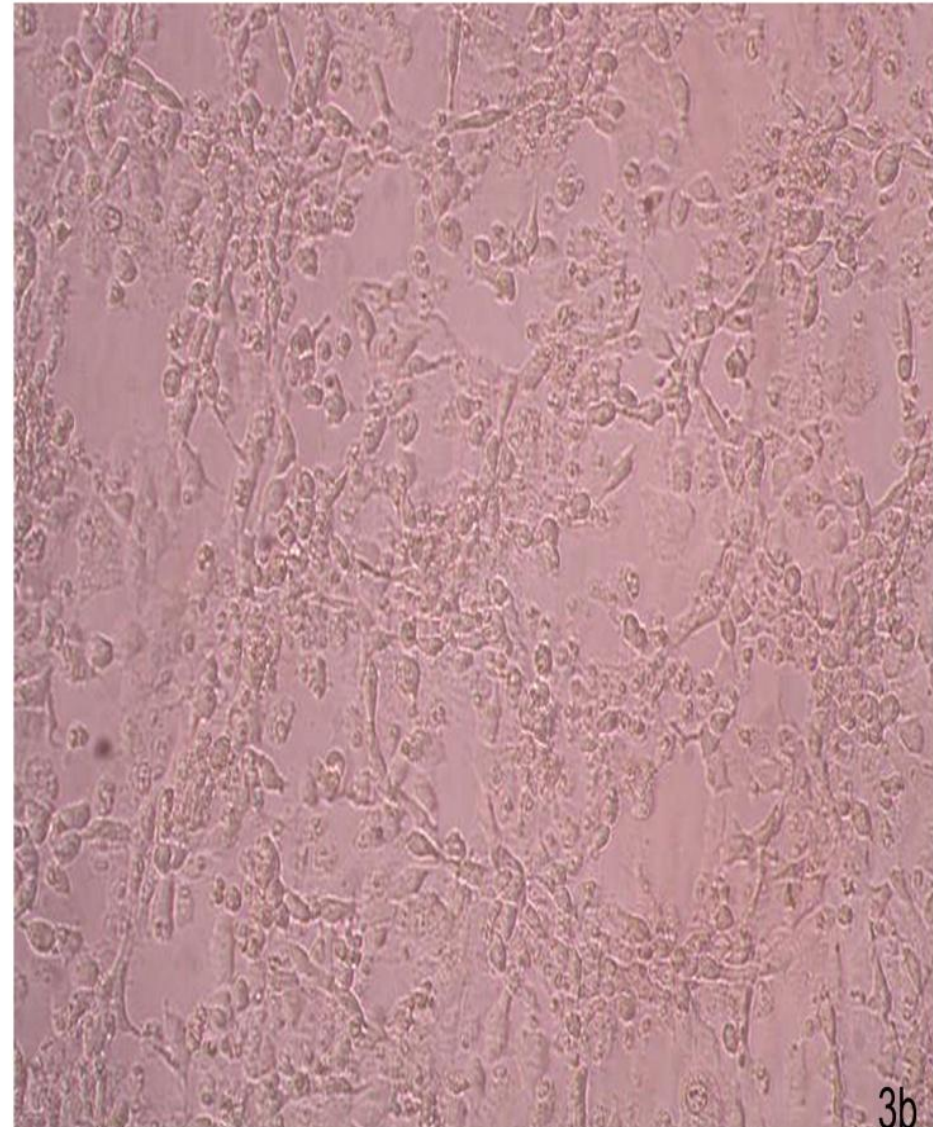
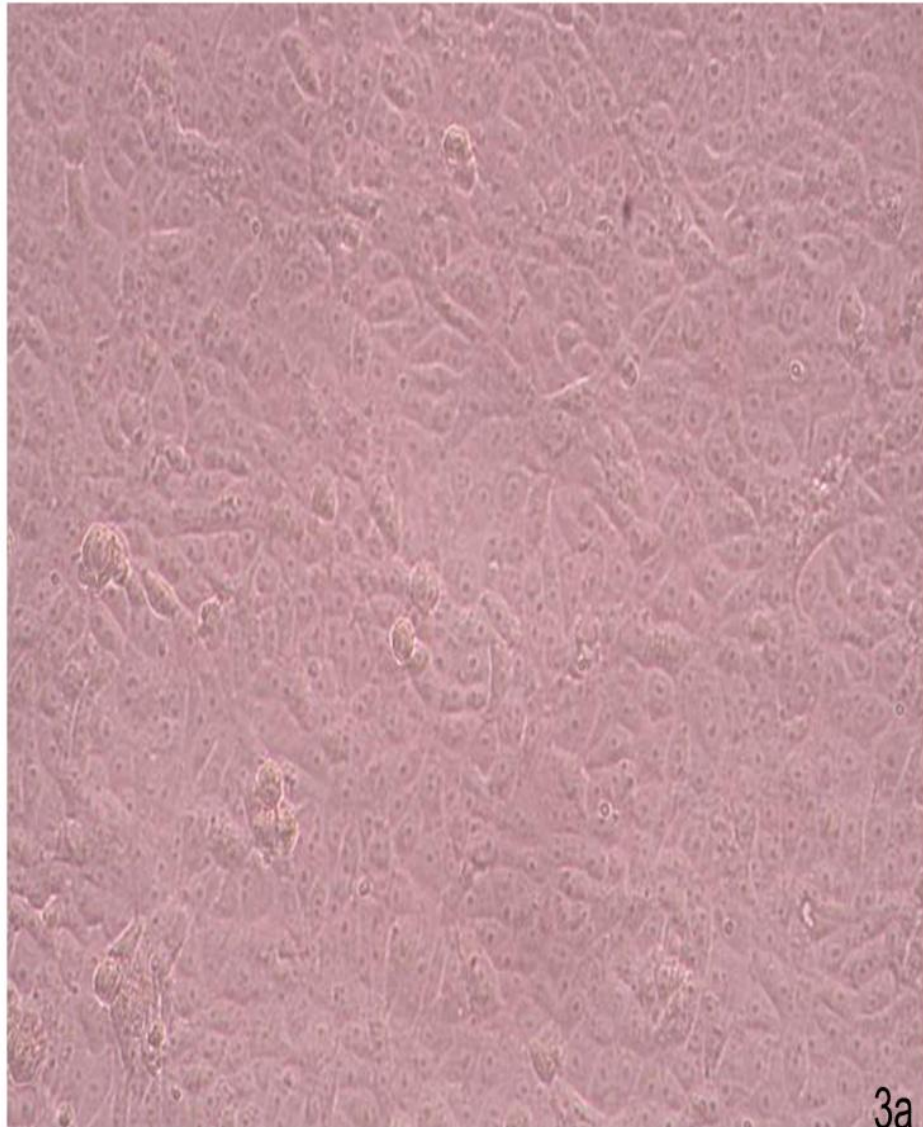


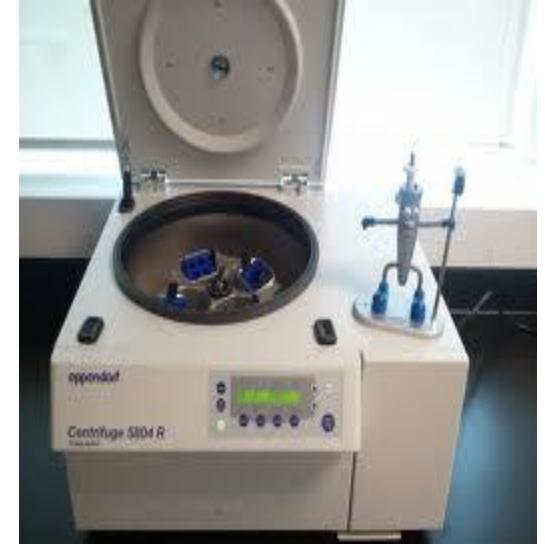
Club-shaped
spikes = "corona"

الزراع الفيروسي



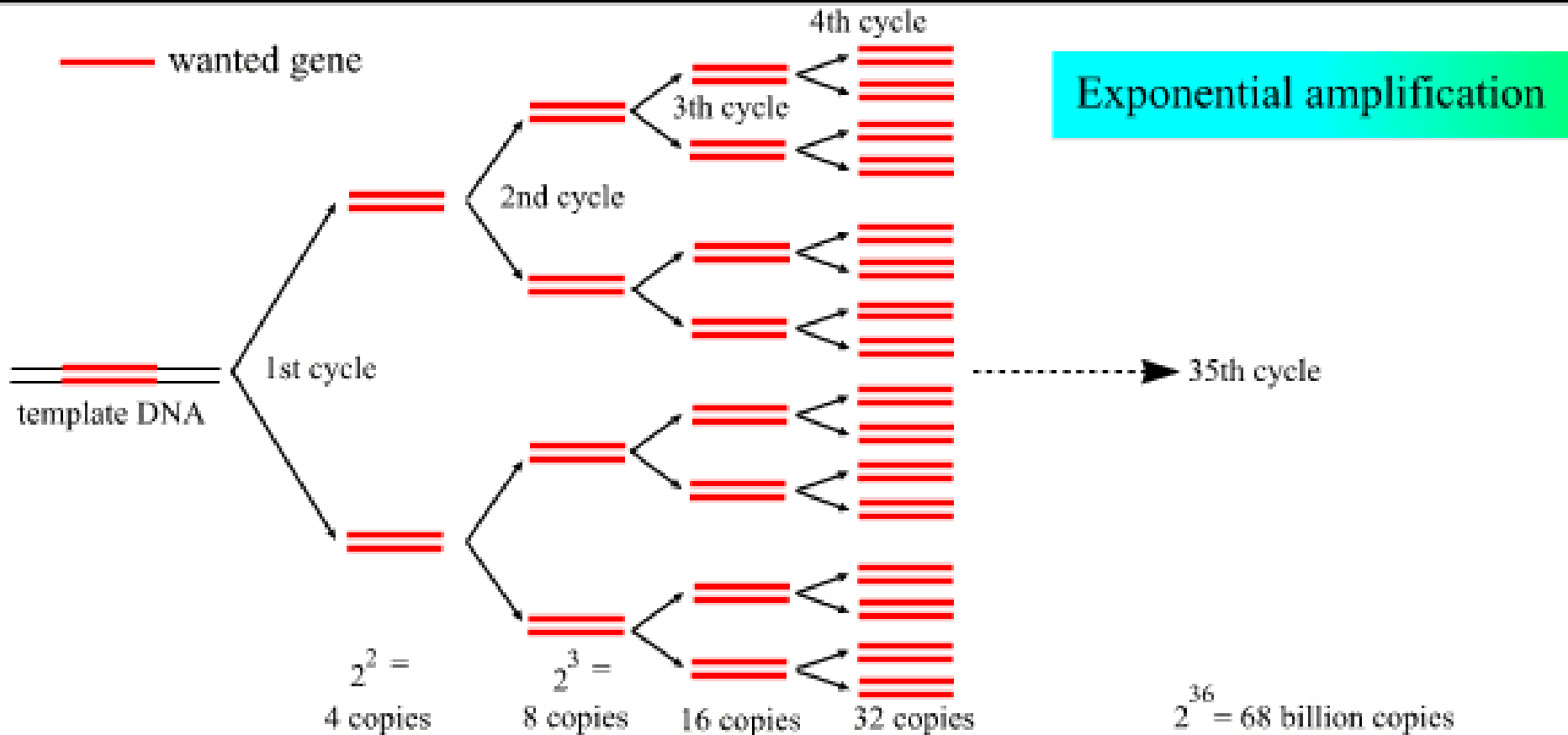
CPE

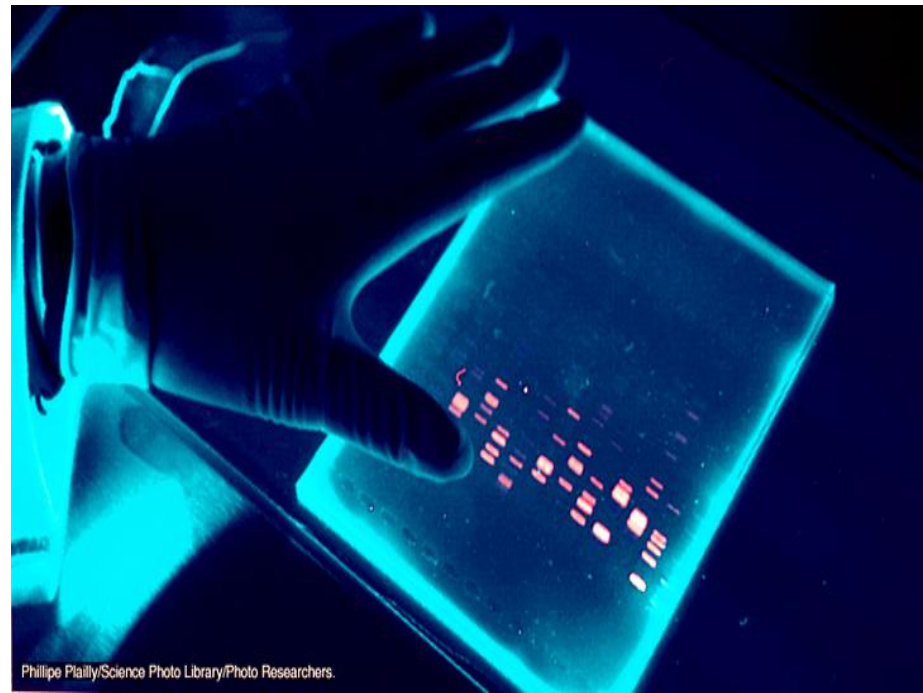
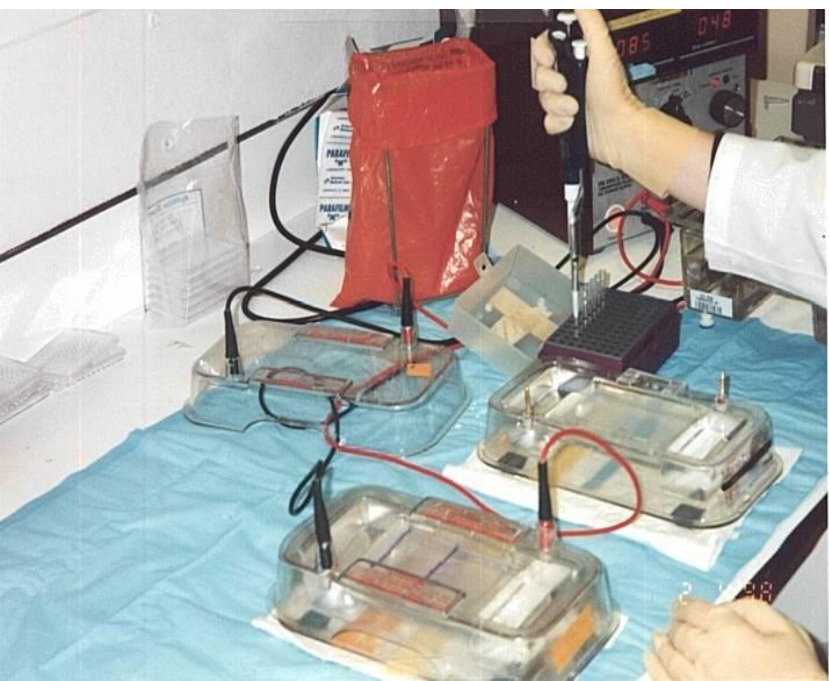




PCR

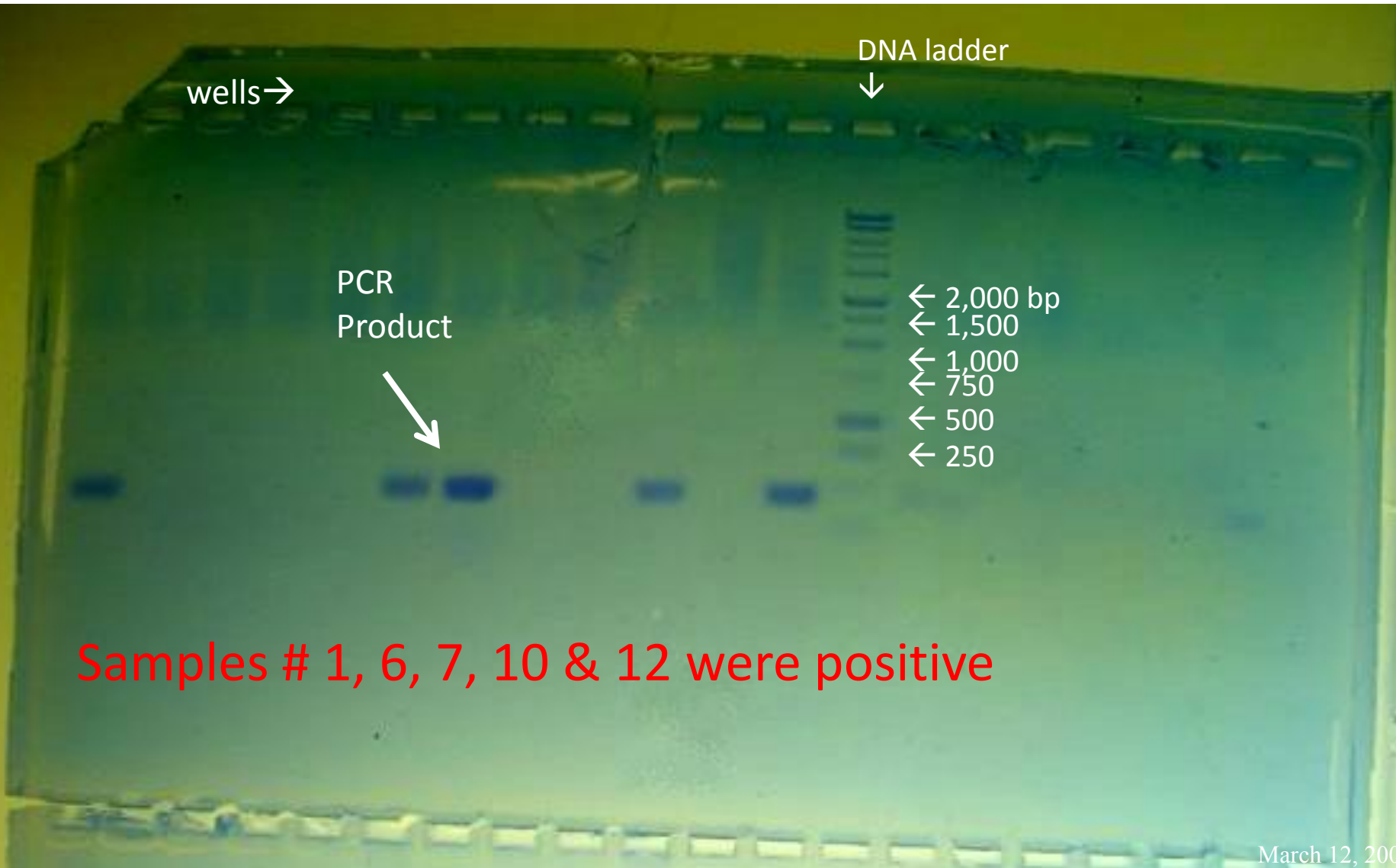
Exponential Amplification of template DNA





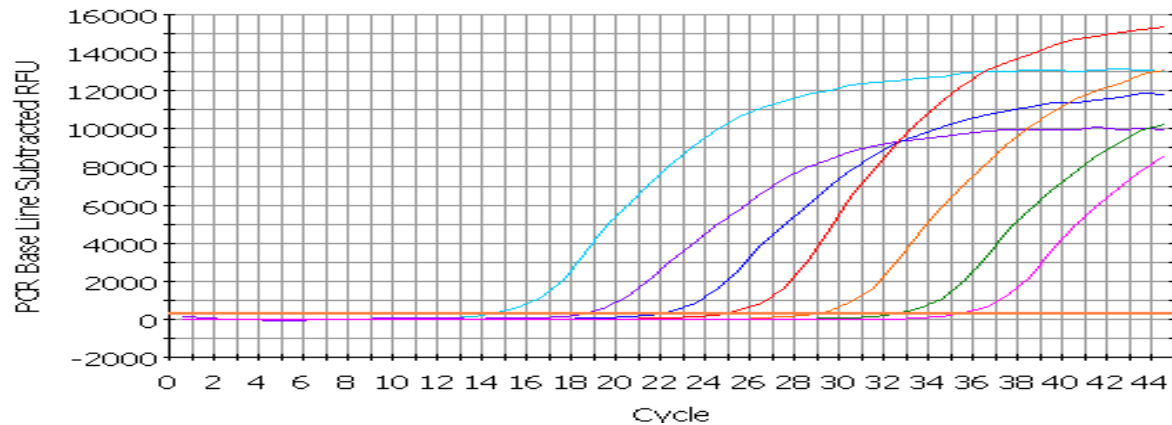
Phillipe Plailly/Science Photo Library/Photo Researchers.

Visualizing the DNA

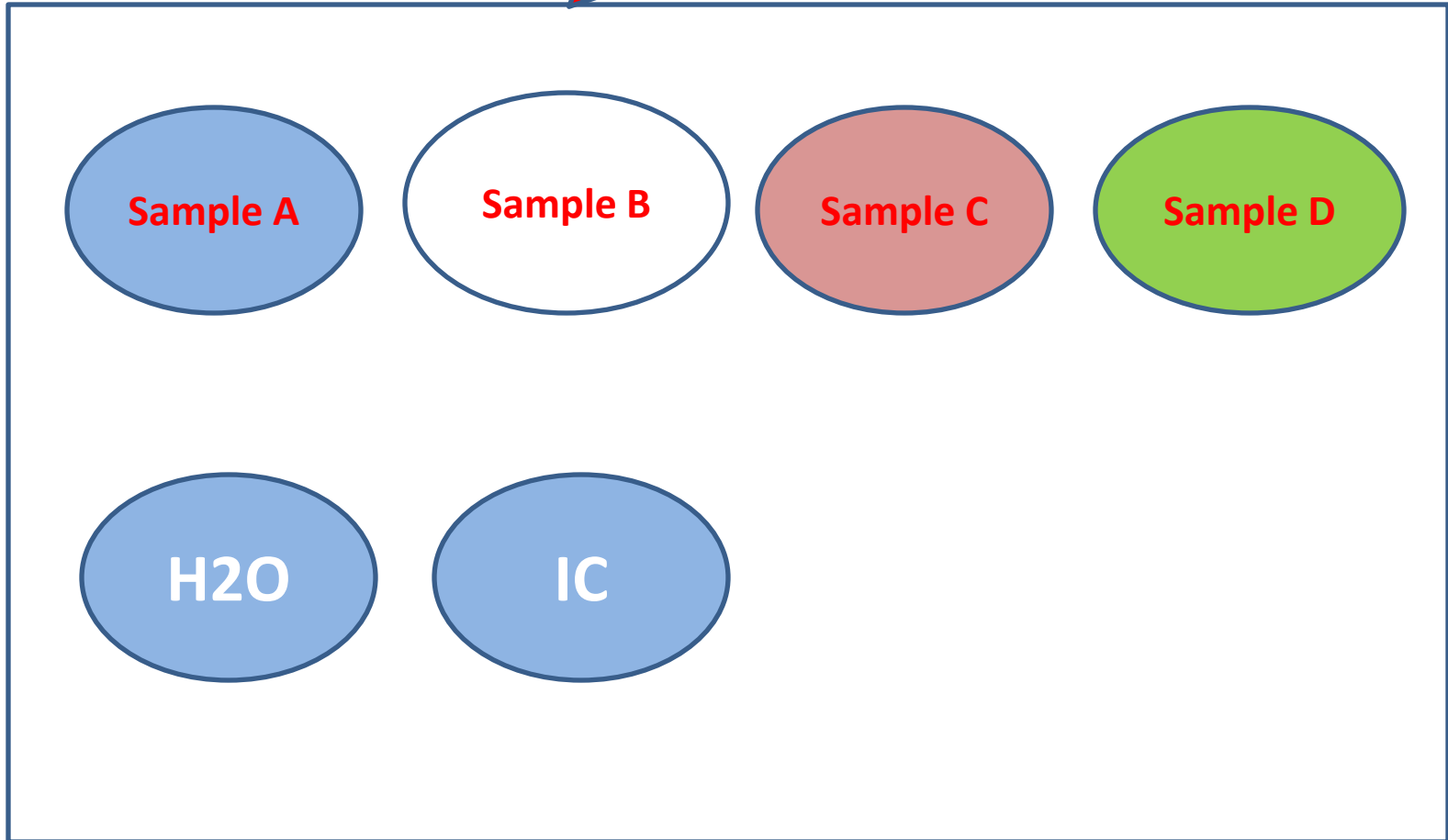


Samples # 1, 6, 7, 10 & 12 were positive

REAL TIME PCR



RNA Extraction Layout

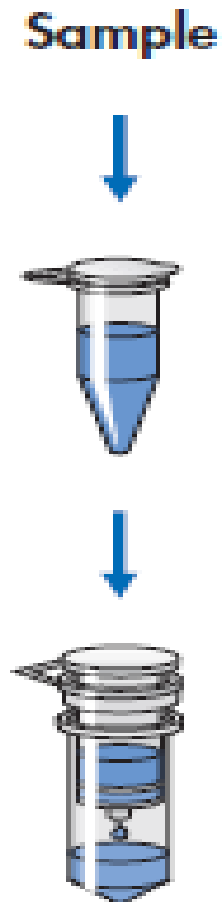


QIAamp® Viral RNA Mini Handbook

For purification of viral RNA from

- plasma
- serum
- cell-free body fluids
- cell-culture supernatants

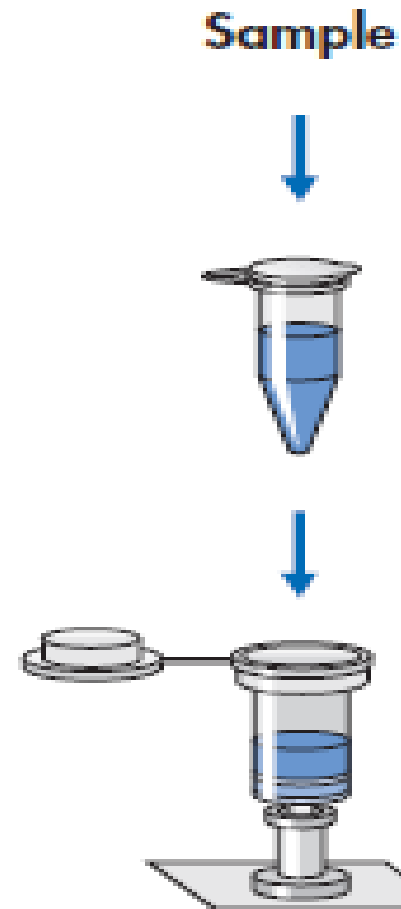
QIAamp Viral RNA Mini Spin Procedure

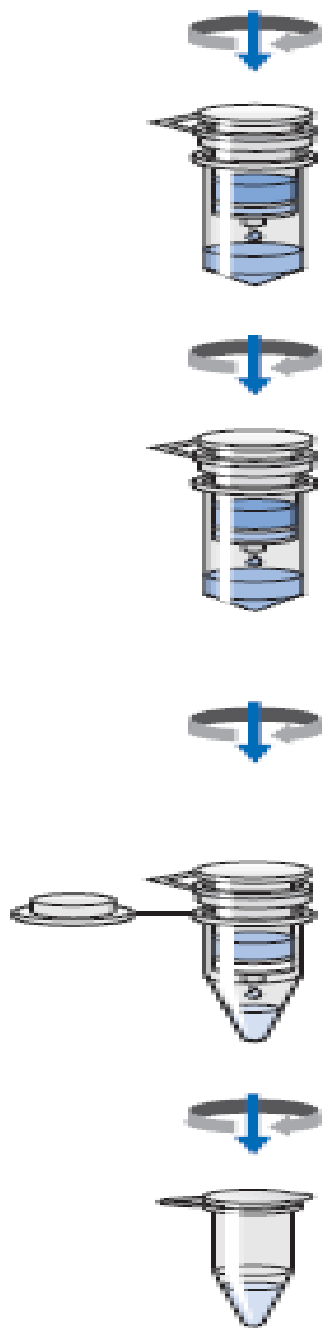


Lyse

Bind

QIAamp Viral RNA Mini Vacuum Procedure

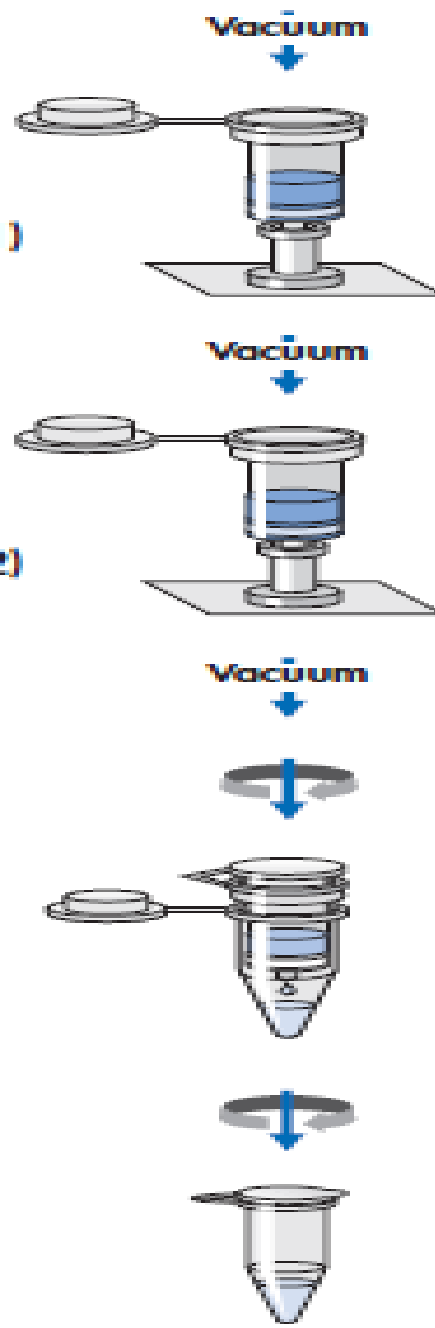




Wash
(Buffer AW1)

Wash
(Buffer AW2)

Elute



Extraction



ESCO

1/6/2011 10:36am

MM



7



1/6/2011 12:25pm



Running

Standby

Rotor-Gene Q **GALT**

QIAGEN



Running



Standby



2 PLEX

Rotor-Gene Q





InfA

S1

S2

H2O

S3

S4

S5

IC

NC

PC

SwH1

S1

S2

H2O

S3

S4

S5

IC

NC

PC

InfB

S1

S2

H2O

S3

S4

S5

IC

NC

PC

RnaseP

S1

S2

H2O

S3

S4

S5

IC

NC

PC

1

2

3

4

5

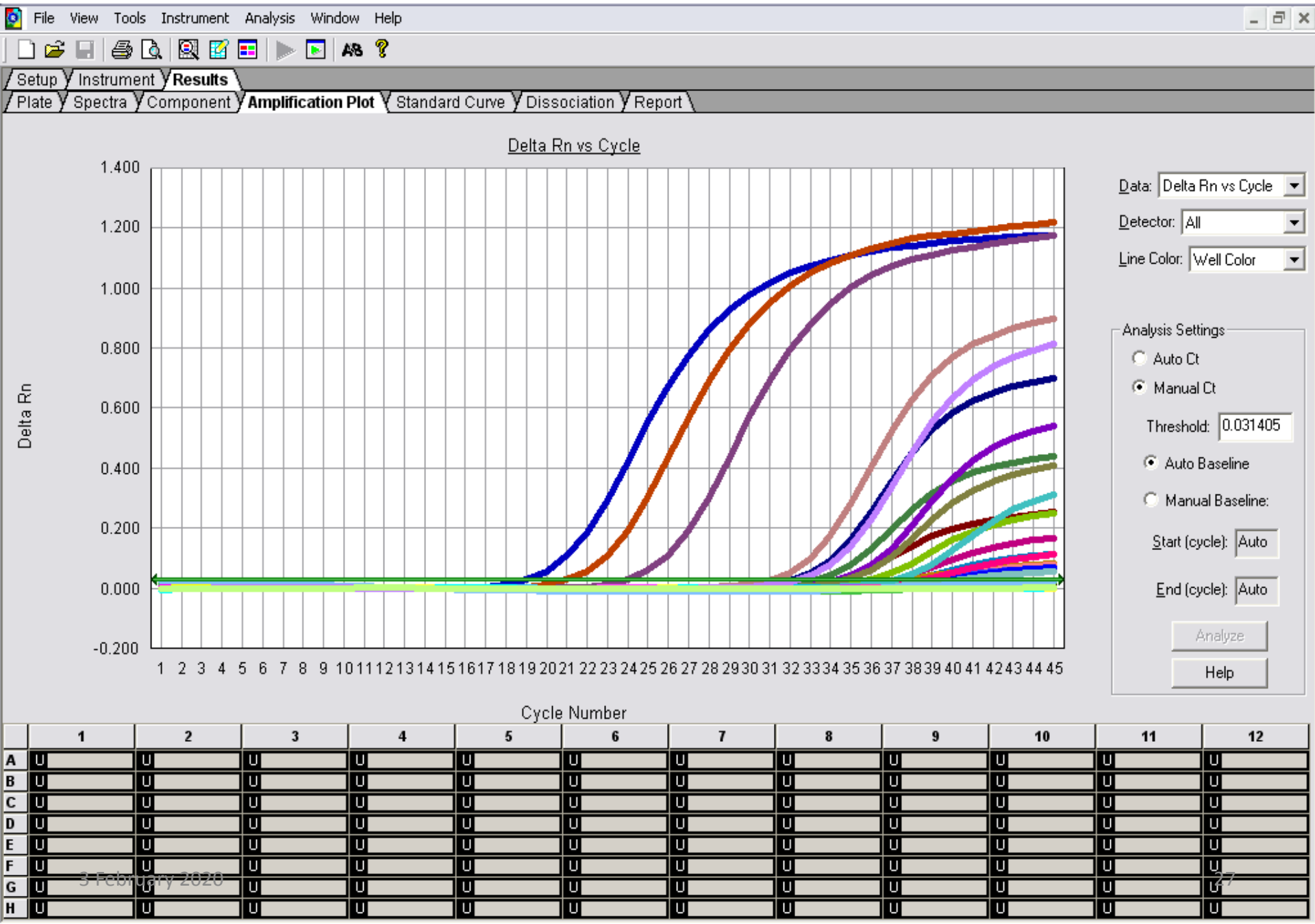
6

7

8

9

96 wells plate





WHO FLU EQAP

Sample ID:

2010-01; 2010-02; 2010-03; 2010-04; 2010-05;
2010-06; 2010-07; 2010-08; 2010-09; 2010-10;
and
Reconstitution Buffer 2010A

For use in accordance with IATA Packaging
Instruction PI 650 only.
Not for use with known infectious substances.

2/6/2011 11:39am



Centre for Health Protection
Department of Health
The Government of the Hong Kong Special Administrative Region

CERTIFICATE OF COMPLETION

This certificate is awarded to

**Emerging Diseases Laboratory, Public Health Laboratories,
Ministry of Health, Syrian Arab Republic**

In recognition of the completion of
**EXTERNAL QUALITY ASSESSMENT PROGRAMME FOR THE DETECTION OF
INFLUENZA VIRUSES BY RT-PCR**
of the World Health Organization Global Influenza Surveillance and Response System
Panel 18 with attainment of full score
2019

*Supported by:
World Health Organization*

*Organized by:
Centre for Health Protection (CHP), Department of Health (DH),
The Government of the Hong Kong Special Administrative Region (HKSAR Government)*

Dr. Dominic Tsang, CHP, DH, HKSAR Government

6 December 2019
Date

Summary table of available protocols: In house PCR or Real Time PCR

Country	Institute	Gene targets
China	China CDC	ORF1ab and N
Germany	Charité	RdRP, E, N
Hong Kong	HKU	ORF1b-nsp14, N
Japan	National Institute of Infectious Diseases, Department of Virology III	Pancorona and multiple targets, Spike protein
Thailand	National Institute of Health	N
US	US CDC	Three N primers, RdRP

Real Time PCR

1-Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR – Charité, Berlin Germany (17 January 2020)

2-Detection of 2019 novel coronavirus (2019-nCoV) in suspected human cases by RT-PCR – Hong Kong University (23 January 2020)

3-PCR and sequencing protocol for 2019-nCoV - Department of Medical Sciences, Ministry of Public Health, Thailand (Updated 28 January 2020)

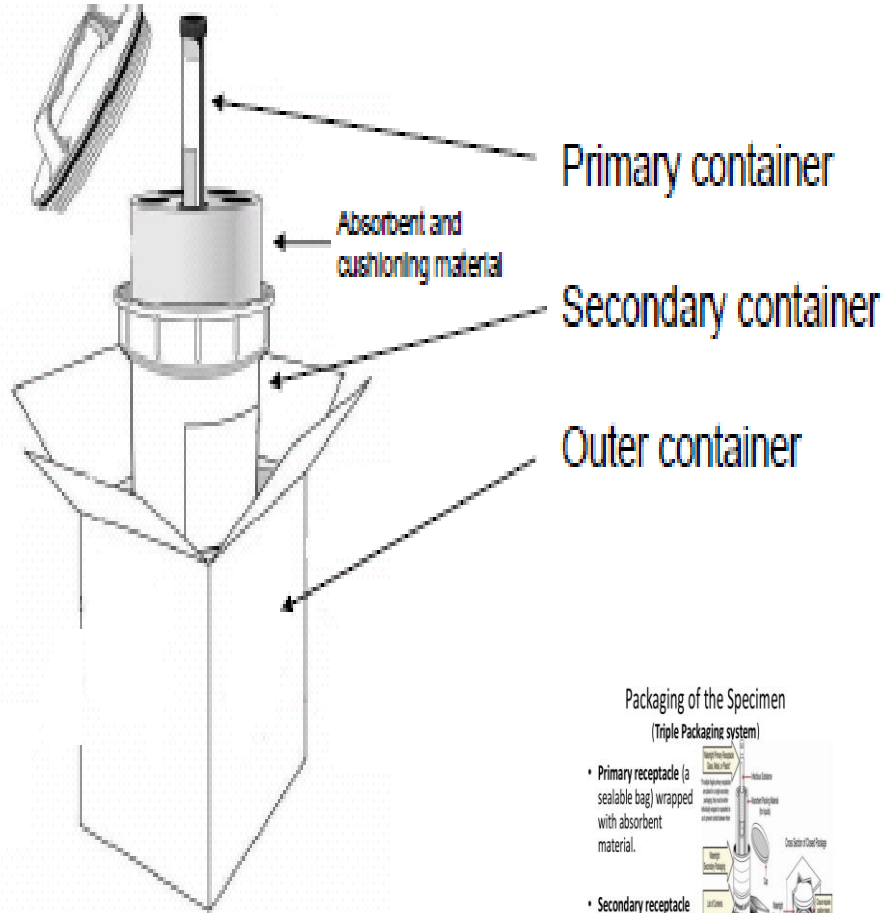
4-PCR and sequencing protocols for 2019-nCoV- National Institute of Infectious Diseases Japan (24 January 2020)




5-US CDC panel primer and probes– U.S. CDC, USAV – U.S. CDC, USA (28 January 2020)

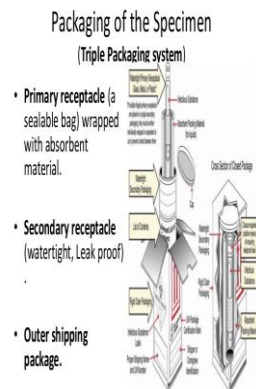
6-US CDC panel primer and probes– U.S. CDC, USA (28 January 2020)

7-China CDC Primers and probes for detection 2019-nCoV (24 January 2020)

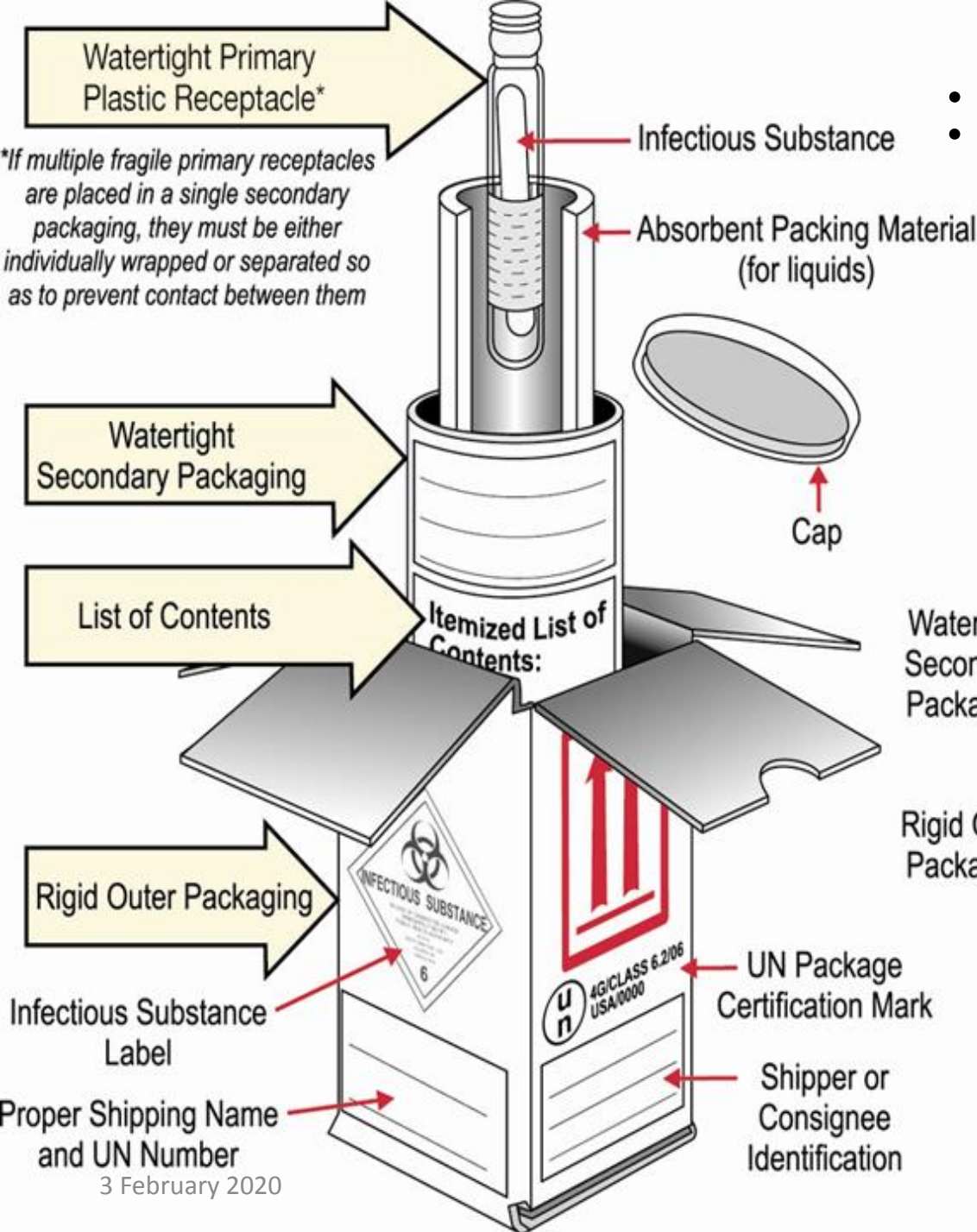
شحن العينات



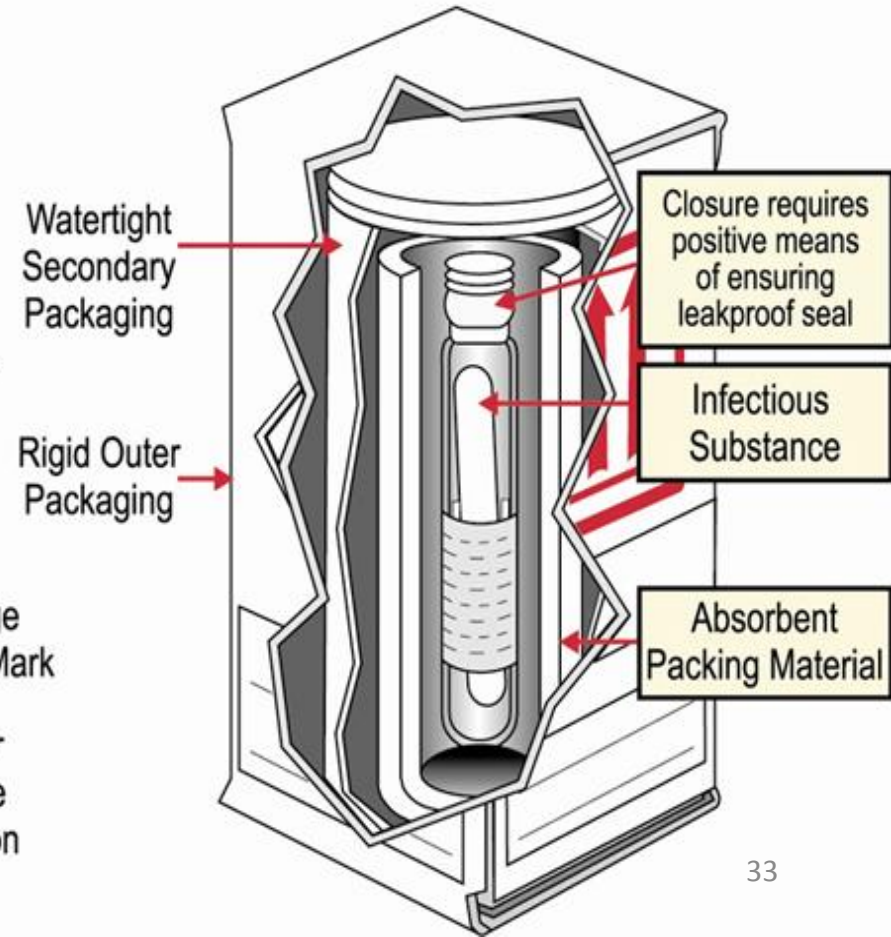
Primary container	
Secondary container	
Outer package	



شحن العينات :

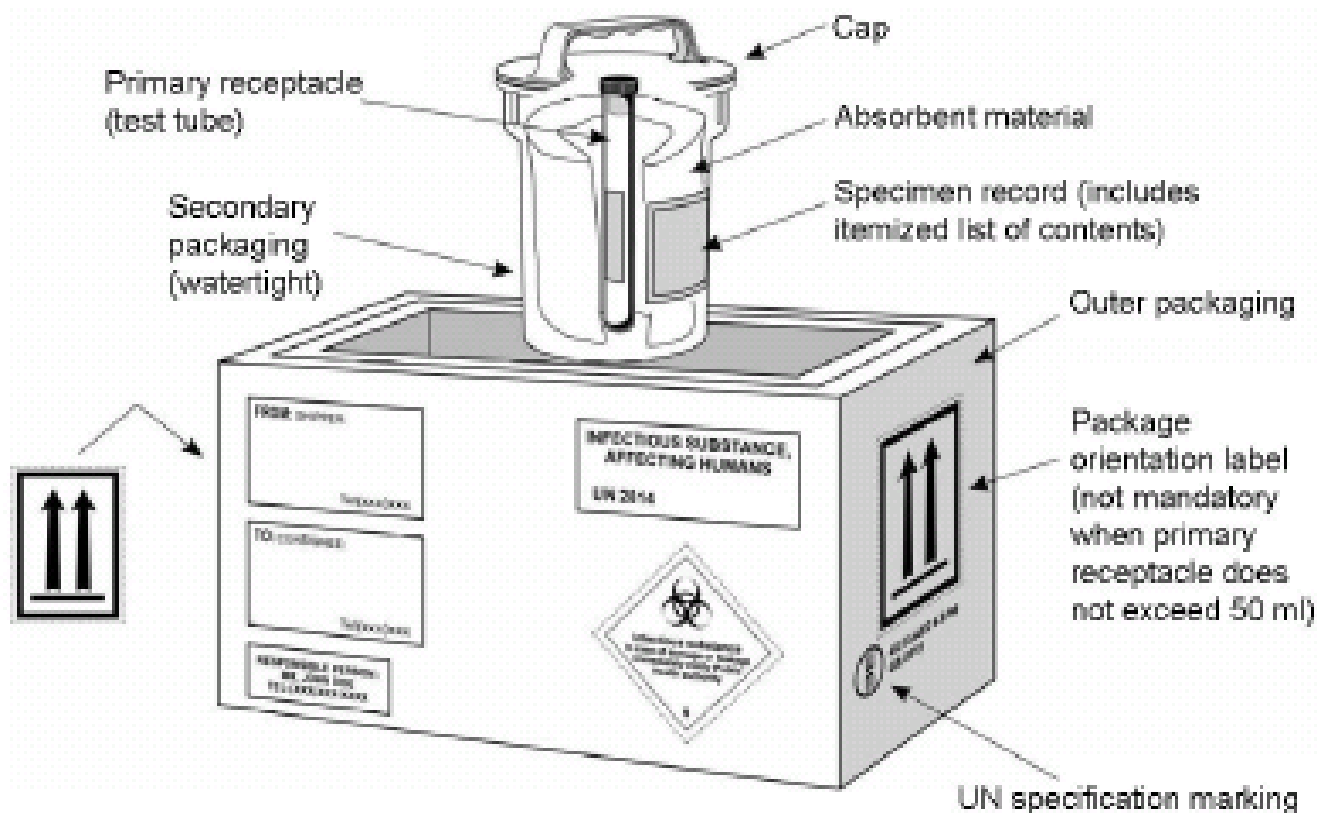


Cross Section of Closed Package

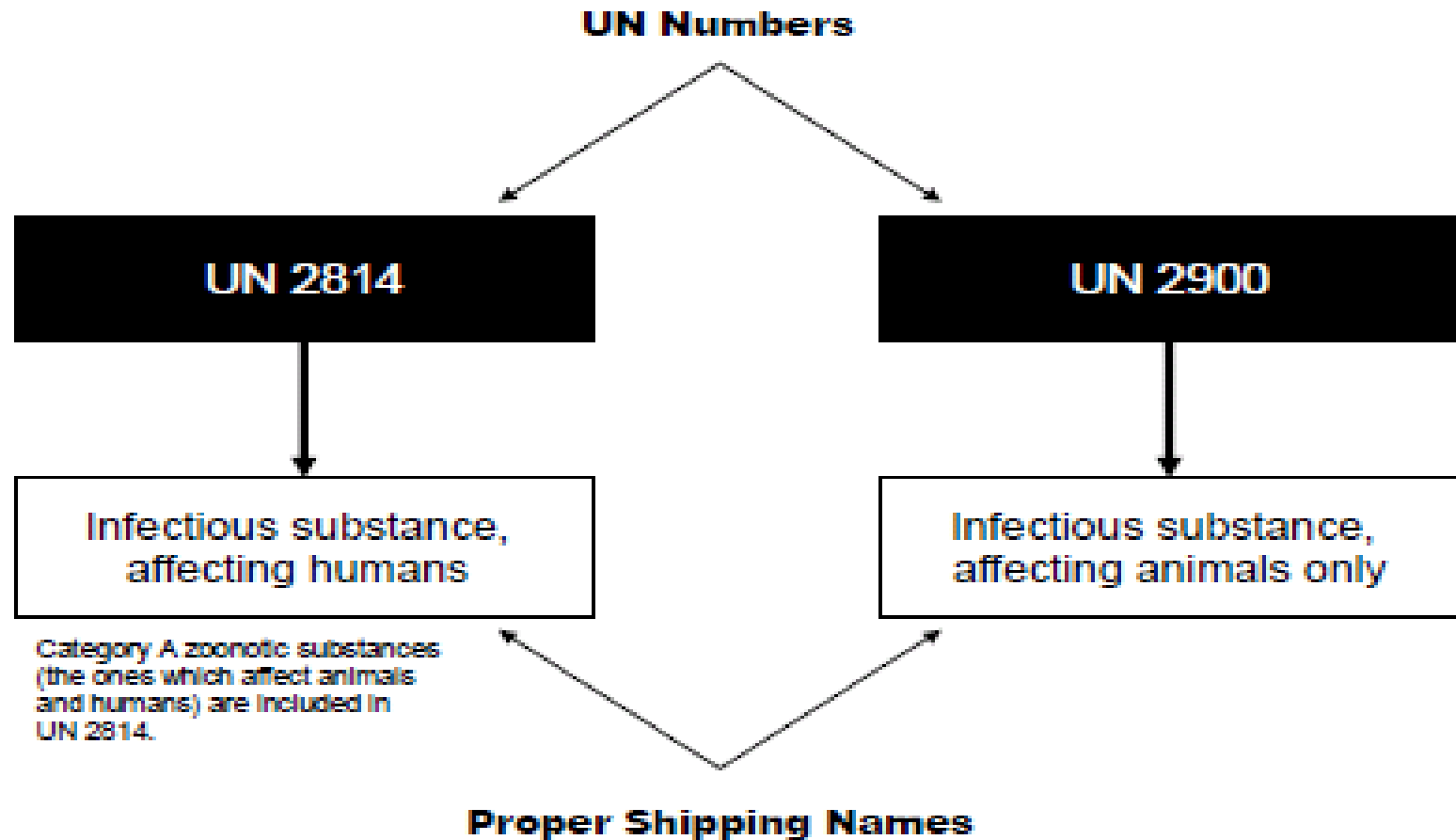


Category A packaging requirements

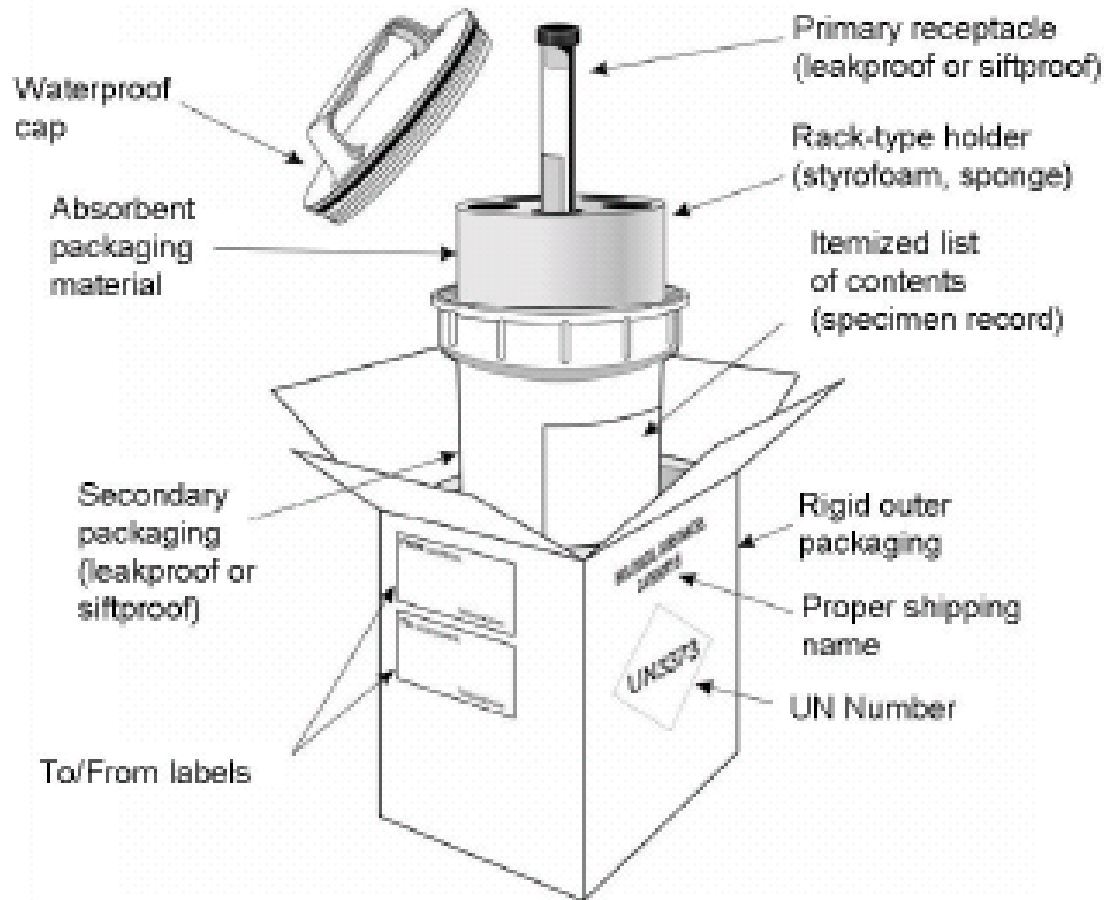
148



Category A – infectious substances



Category B packaging requirements



Category B – biological substances

UN Number

UN 3373



Biological substance,
Category B

Proper Shipping Name

