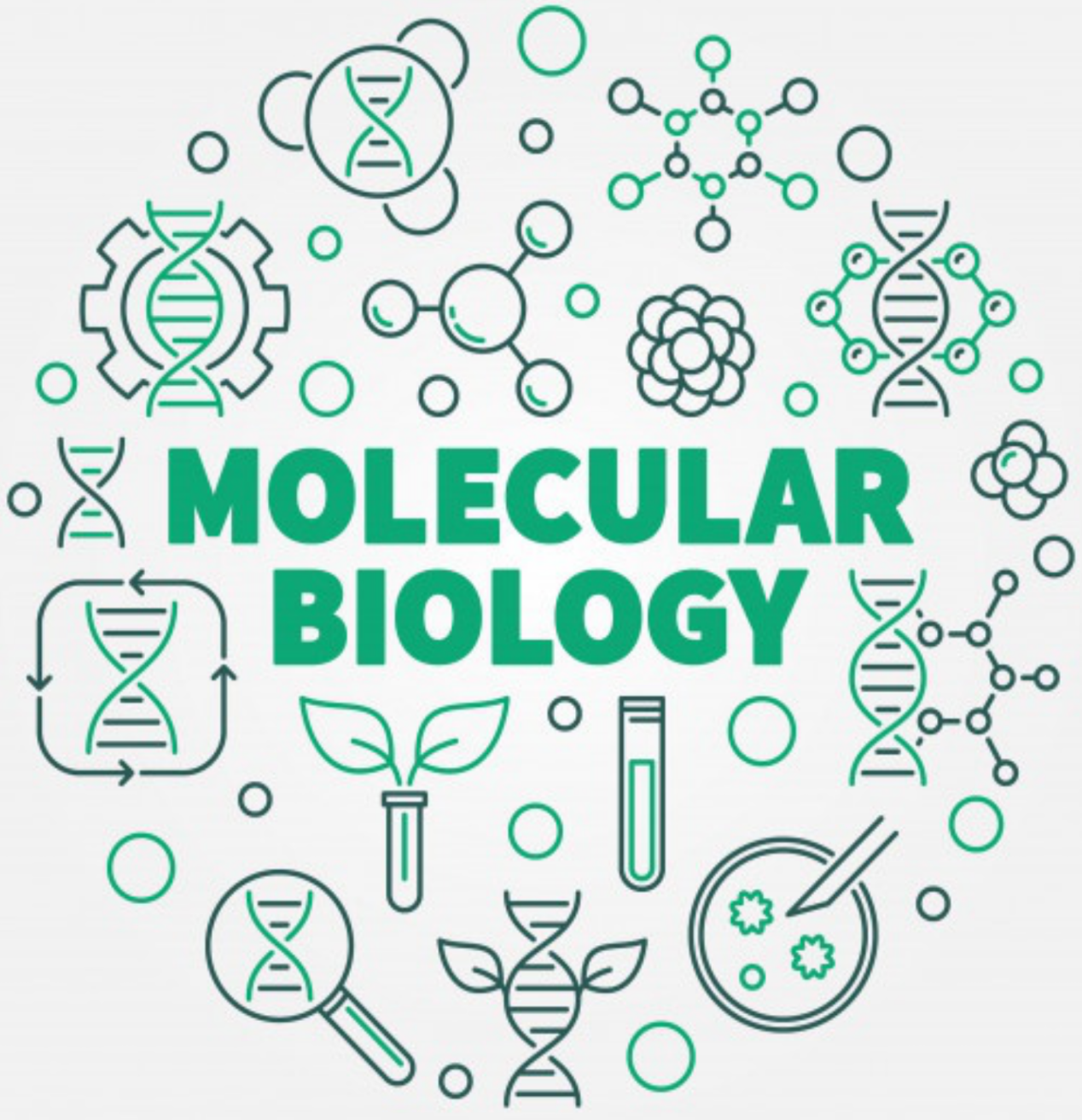


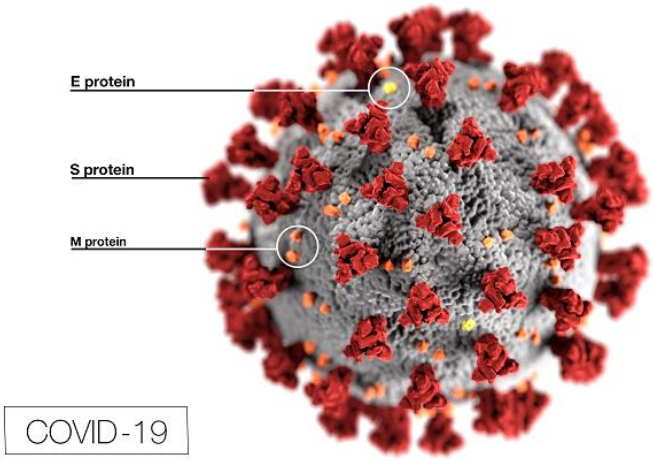
Lab 5 notes Dr.Nesrin

Done by Sojoud Nasser

Wateen batch

COVID-19 Testing using RT-qPCR

* حتى اعمل pCR
لازم تكون عينة DNA
اذا كان RNA بحوله إلى DNA



Dr. Nesrin Mwafi

Biochemistry & Molecular Biology Department

Faculty of Medicine, Mutah University

COVID-19

- Coronavirus disease (also known as COVID-19) is an infectious disease
- Caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
- Initially started in Wuhan province in China and has now affected > 200 countries worldwide
- Declared as pandemic by WHO (world health organization)
- The virus primarily affects the respiratory system causing flu-like illness with symptoms such as a cough, fever, and in more severe cases, difficulty breathing

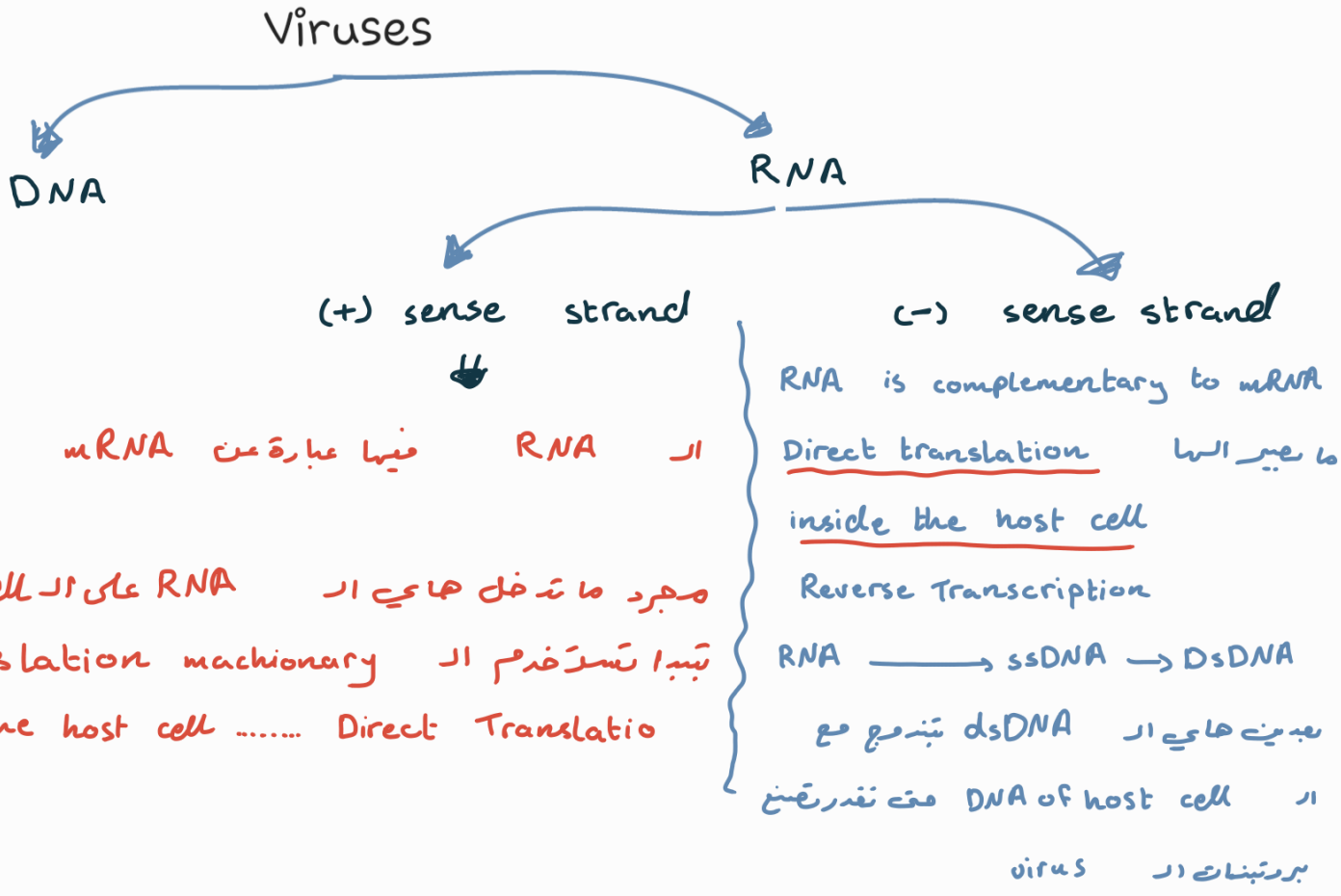
COVID-19

حالات الوفاة

- Mortality is high in older age group individuals (> 60 years of age) and people with other morbid conditions
- Coronaviruses (CoVs) are a group of enveloped viruses. They are pathogenic (virulent) and have a positive sense single-stranded RNA genome
- SARS-CoV-2 is more pathogenic compared to the previously identified SARS-CoV I (2002) and Middle East respiratory syndrome coronavirus (MERS-CoV, 2013)
- All of the three coronaviruses (SARS-CoV-I, MERS-CoV and SARS-CoV-2) are transmitted and spread among humans through close contact.

Morbid conditions or chronic diseases 👇 أكثر عرضة للوفاة

Chronic renal failure,,,DM,,autoimmune diseases.



and > 90% sequence identity for essential enzymes and structural proteins

معظم الجينات ب covid 19

Codes for proteins required for the function of this virus

وهاي لبروتينات متشابهة مع السلالات اللي قبل بنسبة 82%

🧁Spike protein

مسؤول عن انتشار المرض وال infection وبدخل جوا lung cells

🧁Nucleocapsid👉 محيط بال RNA

لما اعمل primers للcovid 19 عشان ال pcr لازم اختار مناطق على الفيروس تكون مختلفة عن sars and mers اختار مناطق unique على الفيروس

لما عملوا ال primer حسب ال S لقوا انه هاي المنطقة كل فترة بصير فيها mutations....بالنسبة لل E ,N ,M لقوا انه فيه تشابه بينهم وبين sars and mers لكن N كانت بتختلف شوي عنهم فاختاروا ال protein N عشان يعملوا primer منها

COVID-19

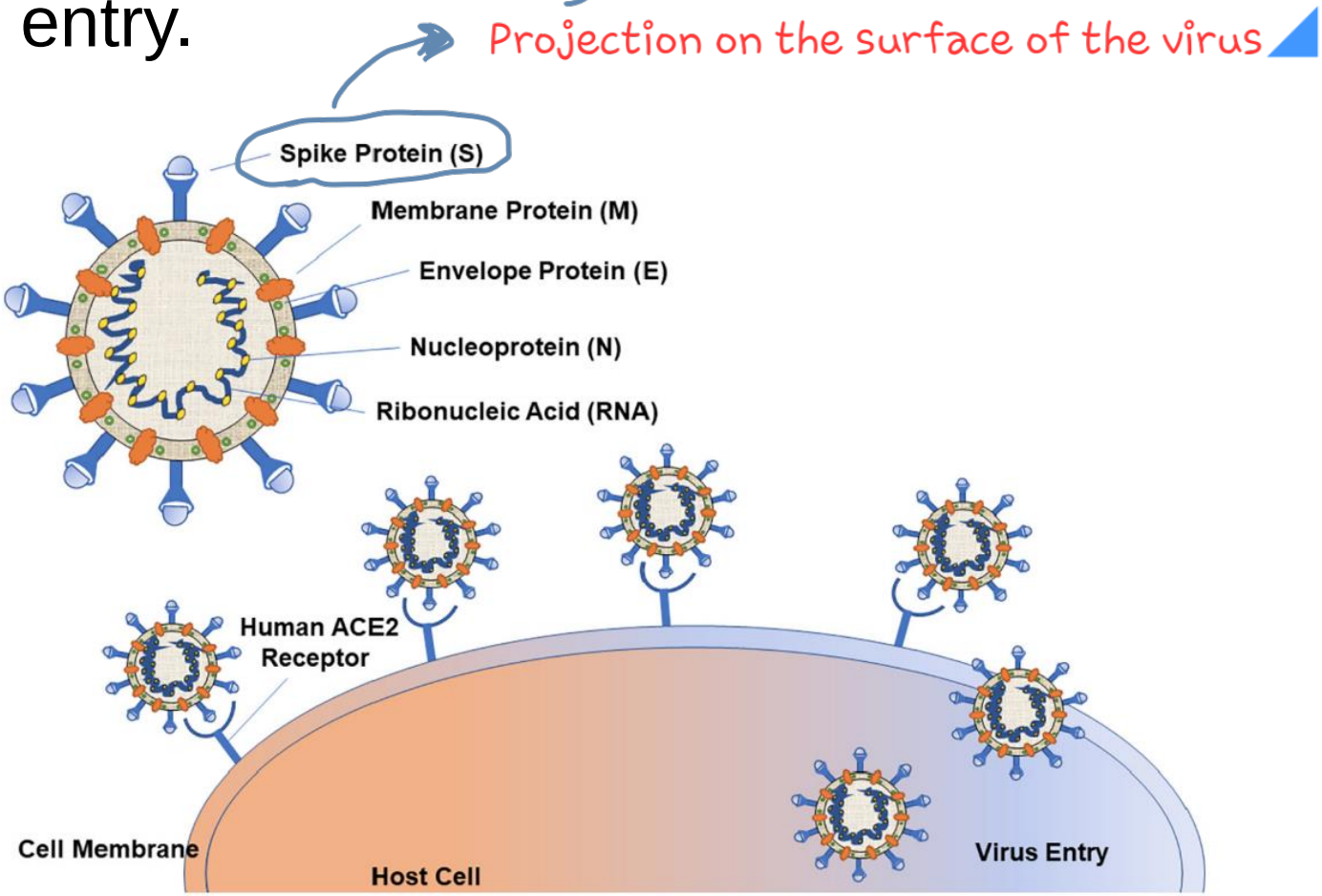
- SARS-CoV-2 has genome with length of 29.9 kb (29900 nucleotides)
- The SARS-CoV-2 genome share about 82% sequence identity with SARS-CoV and MERS-CoV and > 90% sequence identity for essential enzymes and structural proteins

Structure of the virus بتدخل بال

- Structurally, SARS-CoV-2 contains four structural proteins: spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins. These proteins share high sequence similarity to the sequence of the corresponding protein of SARS-CoV, and MERS-CoV

COVID-19

- Spike (S) proteins are responsible for the binding to the host cell surface receptor (ACE2) during host cell entry.



دور ال Spike protein بانتقال العدوى:

ال Spike protein اله Receptor خاص على سطح الخلية angiotensin converting enzyme type 2 receptors

These receptors have high affinity to bind with the virus

Found in

🍯kidney, arteries, heart ,small intestine

Lung cells (Alveolar cells type 2)الجزء الأخطر منها موجود بال

Binding between virus and the receptor will induce endocytosis

فبتدخل المادة الوراثية للفيروس عالخلية،،، ال m RNA جاهز بروح مباشرة على ال Ribosomes,,وتصنع بروتينات الفيروس

منطقة التداخل بينهم unique

مش موجودة بالأنواع الثانية

ممكن نستخدمها حتى نعملها primer

Only 4 proteins are structural,,the rest are non structural proteins

The non structural are responsible for replication and transcription

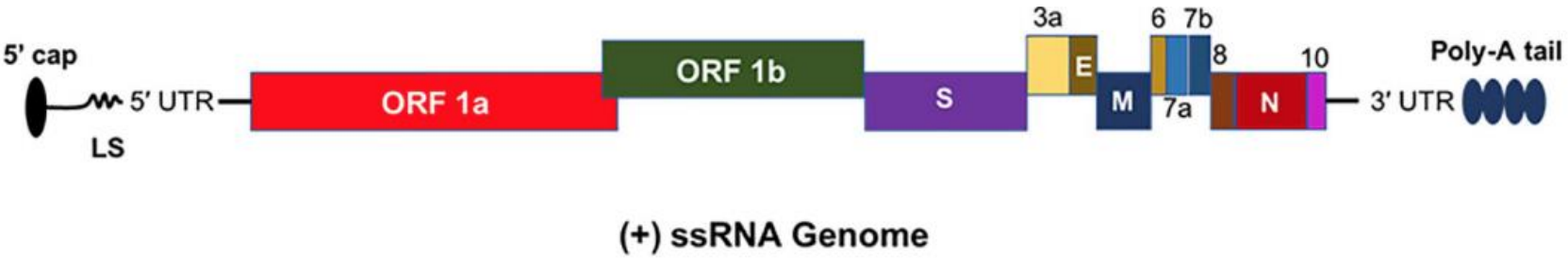
ORF 1a and ORF 1b ,,their genes encode proteins required for the replication of the virus ,,transcription and translation of the virus

منطقة conserved

يعني ثابتة صعب يصير عليها mutation وتتغير

COVID-19

- Viral genome is composed of ORF1a, ORF1b, Spike (S), ORF3a, Envelope (E), Membrane (M), ORF6, ORF7a, ORF7b, ORF8, Nucleocapsid (N), and ORF10

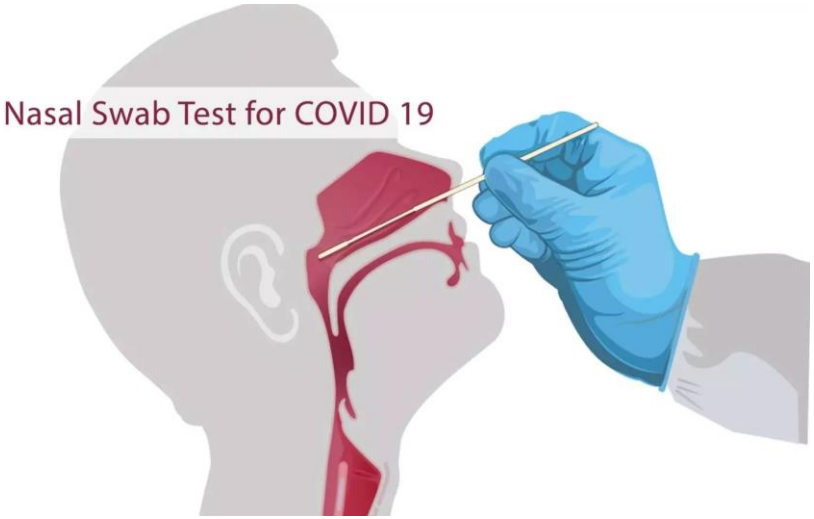


(+) ssRNA Genome

- Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N) are the four structural proteins
- We diagnose patients using nasal swab sample


المملكة الأردنية الهاشمية
وزارة الصحة
COVID - 19 LAB

Stage I: Sample Collection

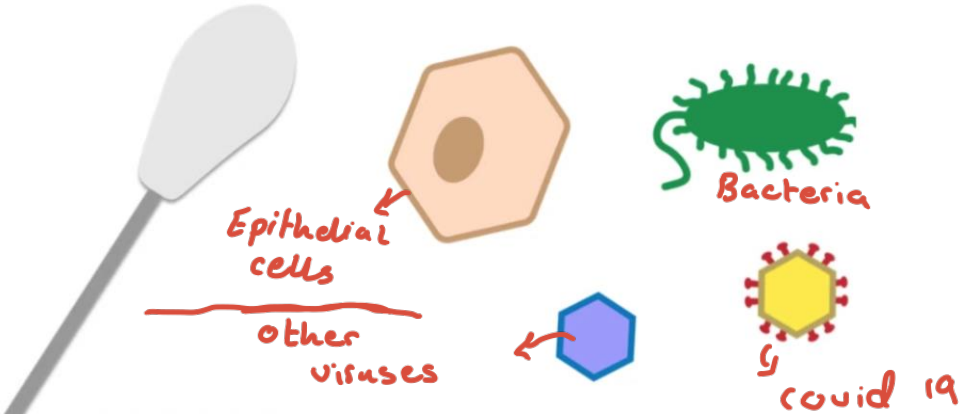


collecting tube

بحتوي على مادة حافظة للعينة

What's in that sample?

الخلايا اللي بتحتويها العينة :

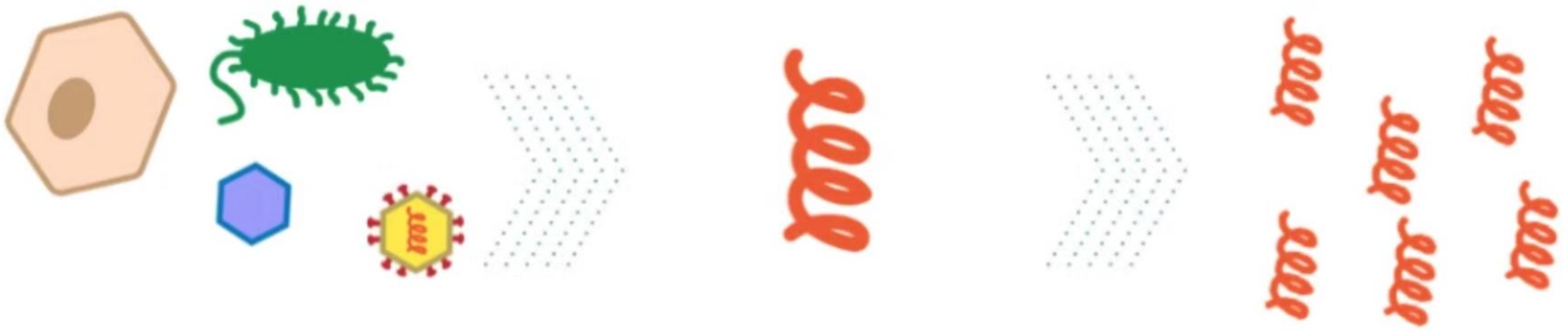


Diagnosis of COVID-19

Stage 1

Stage 2

Stage 3



Nasal swab

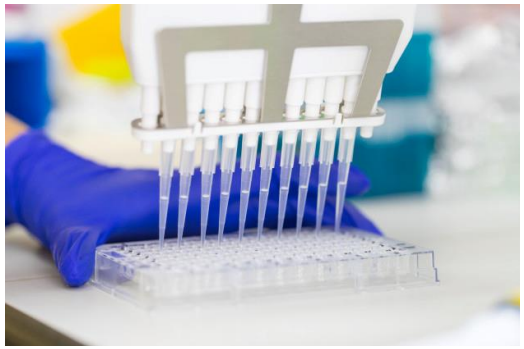
Extraction of viral RNA

Amplification of viral RNA by qPCR

Stage II: Viral RNA Extraction

- Nucleic acid extraction Kit is used to extract the viral RNA using the magnetic beads method
- Advantages:

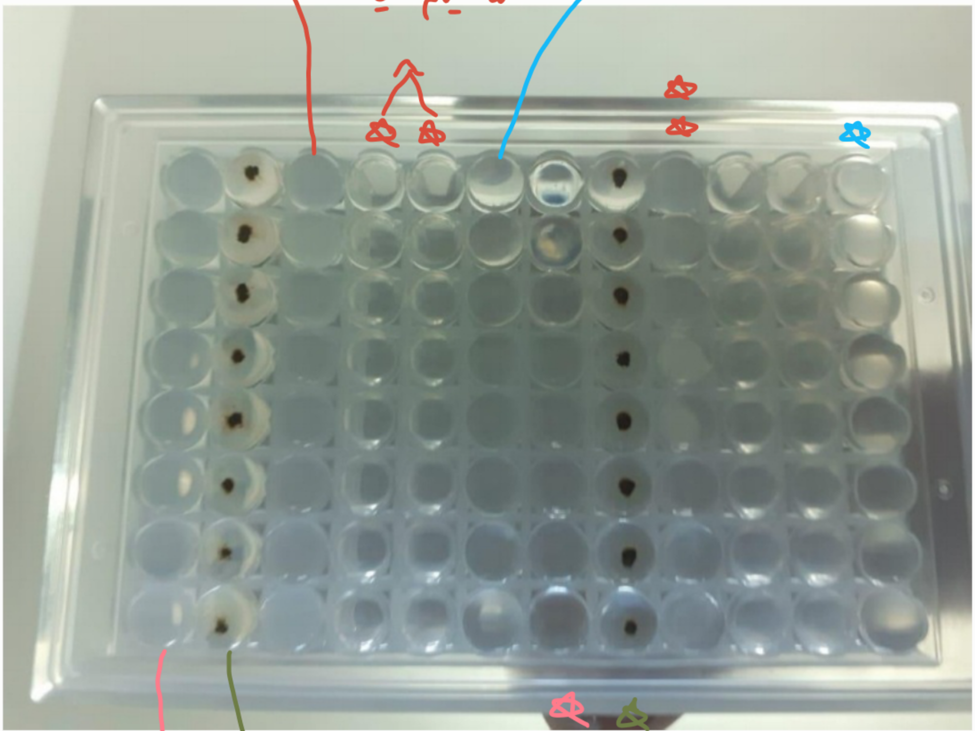
1. High quality of the extracted nucleic acid
2. The kit contains two 96-well plates (each can be used to extract material from 16 samples)
3. Reliable extraction process for 32 samples in 10 minutes only
4. Nucleic acid extraction reagents are pre-packaged in the 96-well plates
5. Easy, simple and convenient
   (Only add 200µl sample and
   15 µl proteinase K, 1µl internal control)



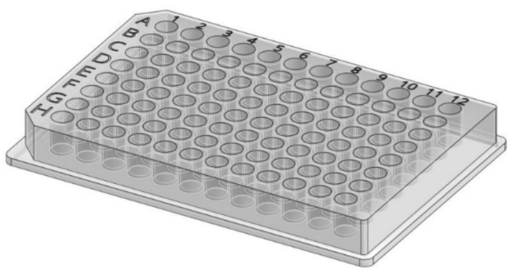
ال kit العادي ما بنفع لجائحة كورونا لانه عدد العينات بكون اقل لهيك الشركات

طورت kit جديد يفحص عدد كبير من العينات لحد ٣٢ عينة كل مرة

خطوة ال extraction صارت automated



4,5 → ما فيهم اشي مش ما يصير contamination



1,7 → proteinase K بحط فيهم 200 ML من كل عينة ومعاهم

2,8 → magnetic Beads فيهم

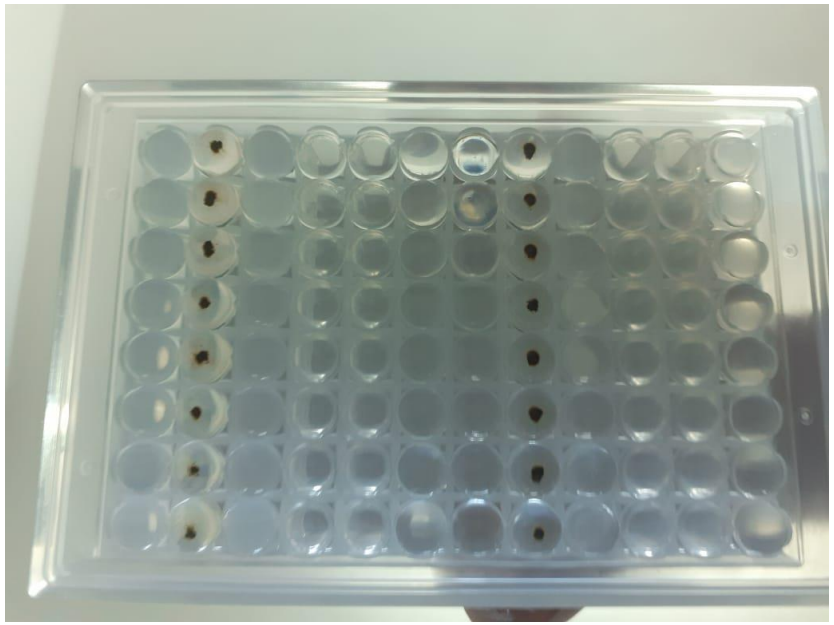
3,9 → whashing Buffer

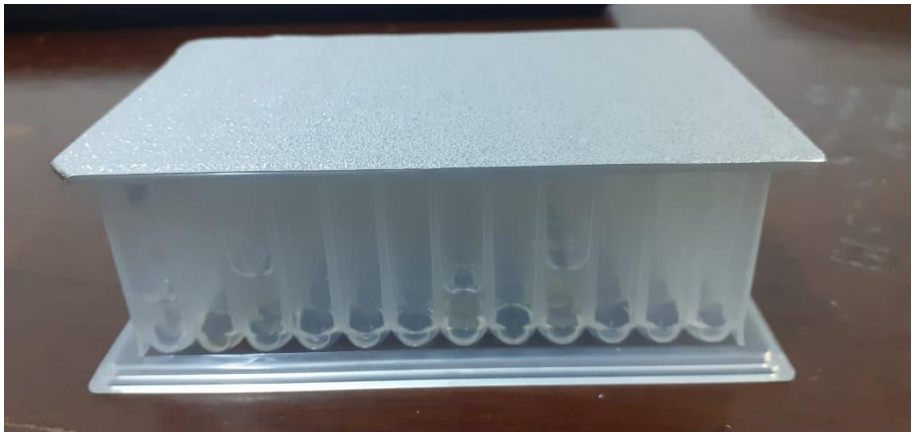
4,5 ,10, 11 → Empty

6,12 → elution buffer

الجهاز يحتوي على multichannel pipette بسحب ال magnetic beads وبضيفهم على العينات

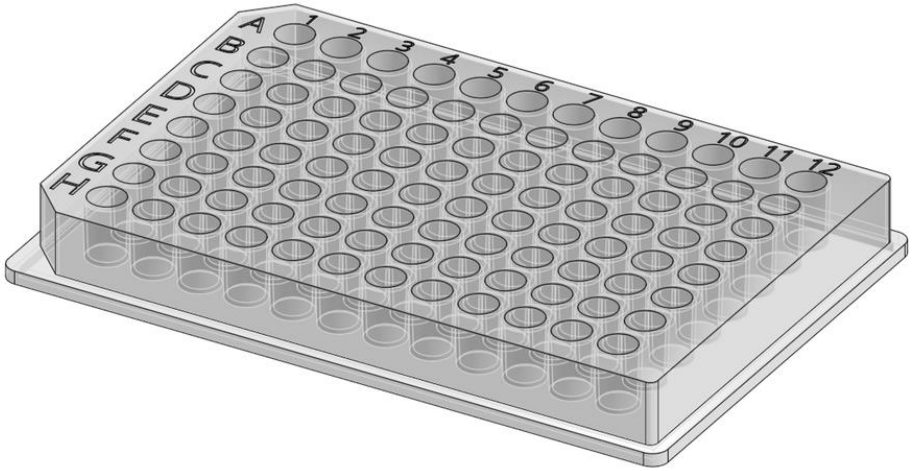
Pre-packaged 96-Well Plate





Pre-packaged 96-Well Plate

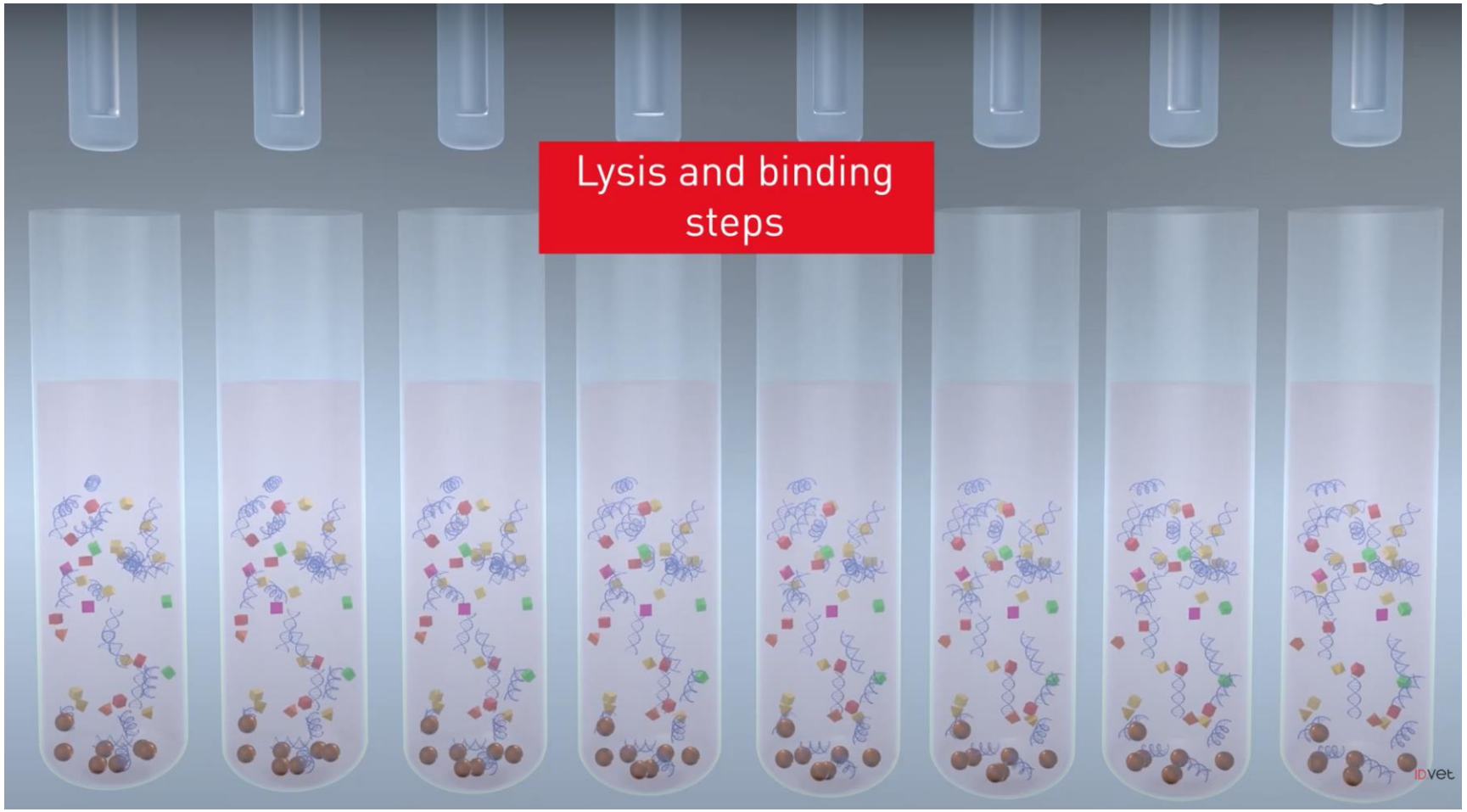
- Extraction reagent I: pre-loaded at positions (A1-H1 & A7-H7)
- Magnetic beads solution: pre-loaded at positions (A2-H2 & A8-H8)
- Extraction reagent II: pre-loaded at positions (A3-H3 & A9-H9)
- Elution buffer (deionized water): pre-loaded at positions (A6-H6 & A12-H12)
- These wells are empty (A4-H4, A5-H5, A10-H10 & A11-H11)



Automated Nucleic Acid Extraction System

- Zybio EXM3000 is designed as a high-tech instrument to isolate and purify the nucleic acid through magnetic beads method
- More efficient extraction and safer operation
- Place the 96-well plates (Don’t forget to position the combs at their appropriate location

Magnetic Beads



The Extraction Program

- Click “start” to run the extraction program
- Each extraction cycle takes 10 min

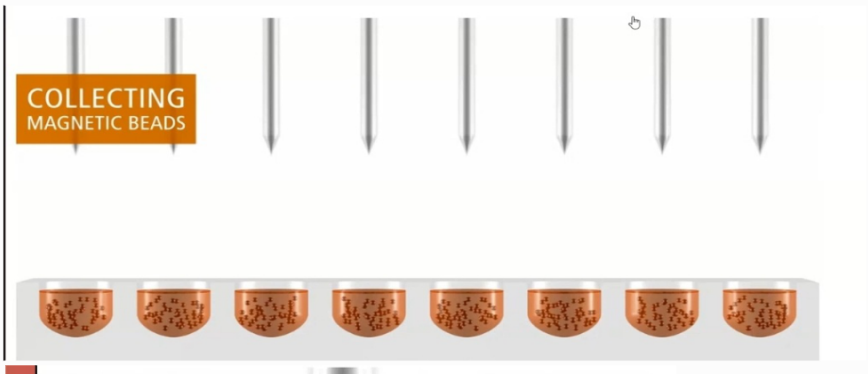
No.	Position	Name	Waiting Time (min)	Mixing Time (min)	Absorption Magnetic Bead Time (sec)	Mixture Velocity	Volume	Temperature State	Temperature(℃)
1	2	Move	0	0	30	Slow	150	Closed	0
2	1	Lysis and binding	0	4	60	Slow	500	Heating for Lysis	55
3	3	Wash	0	1	60	Slow	600	Closed	0
4	6	Elution	0	2	30	Slow	50	Heating for Elution	80
5	1	Move	0	0	0	Slow	150	Closed	0

Stage II: Viral RNA Extraction

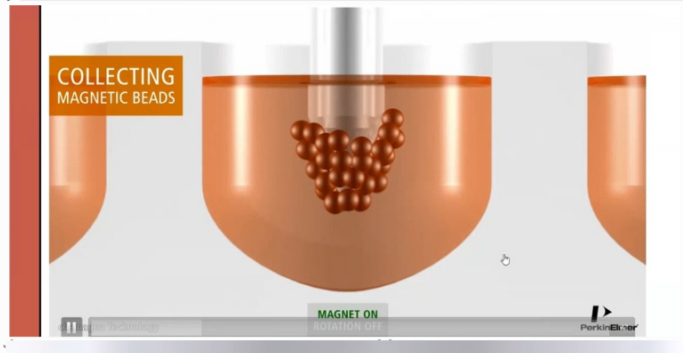
PerkinElmer®

For the Better

بركب على الـ multichannel pipette بركب عليها الـ comb
The comb is disposable to avoid contamination
لما اخلص الفحص بكب الـ comb وبتظل الـ multichannel pipette
نظيفة

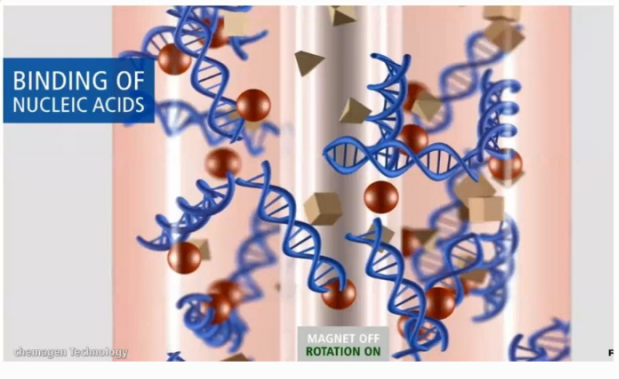


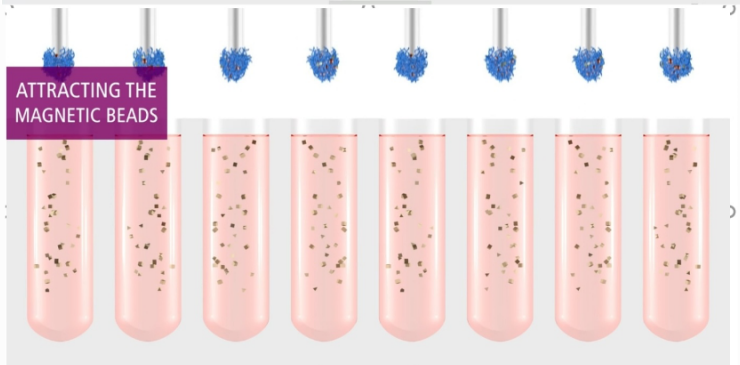
الجهاز بحرك الـ multichannel pipettes لعند
الـ2nd well اللي فيها magnetic beads
الجهاز بده 30s حتى يسحبهم
بينقلهم على 1st well

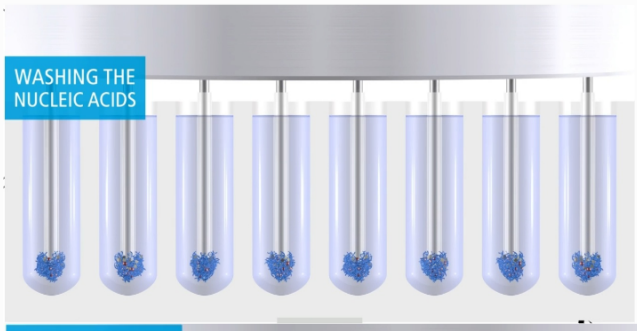


Inside the 1st well
Binding of nucleic acids and lysis
mixing for بعمل magnetic beads الجهاز لما يضيف الـ
4m وبرفع الحرارة إلى 55 داج يصيد

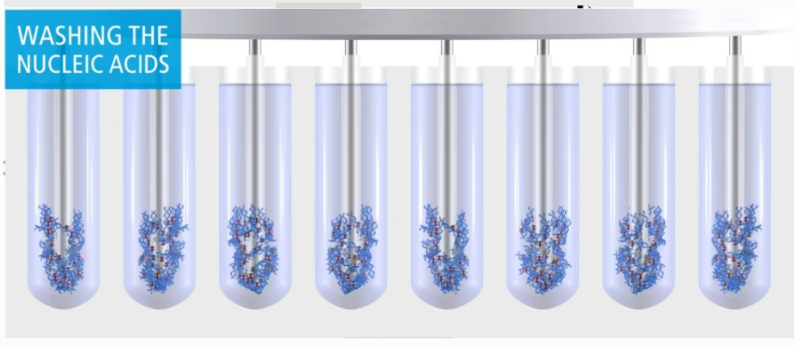
Lysis of viral cells
RNA binds specifically with beads
Application of a magnetic field to absorb beads and RNA
Absorption time is 1m

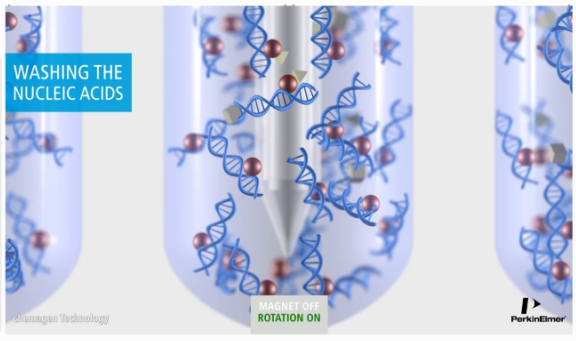


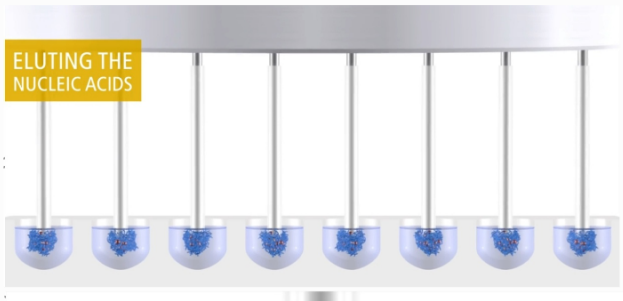




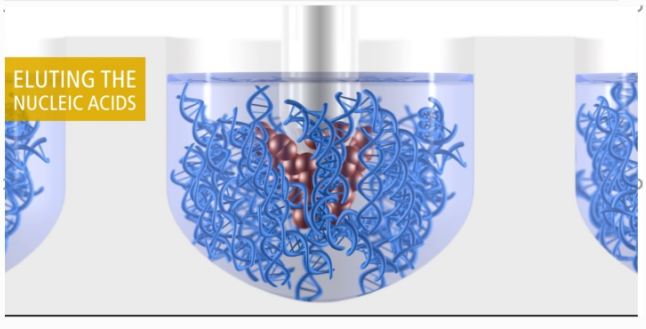
Washing the sample
First step mixing for 1 m
2nd absorption for 1m

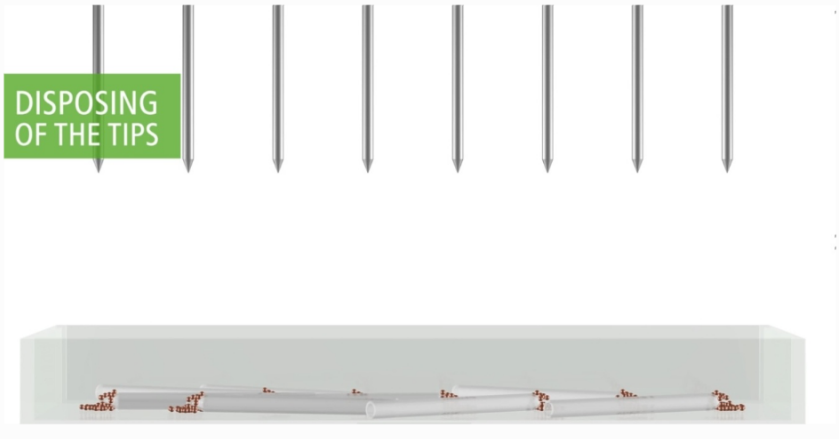






Elution step
RNA and magnetic beads بفك الرابطة بين ال
The elution buffer is deionized water 💧
Mixing for 2m
Absorption for 30s

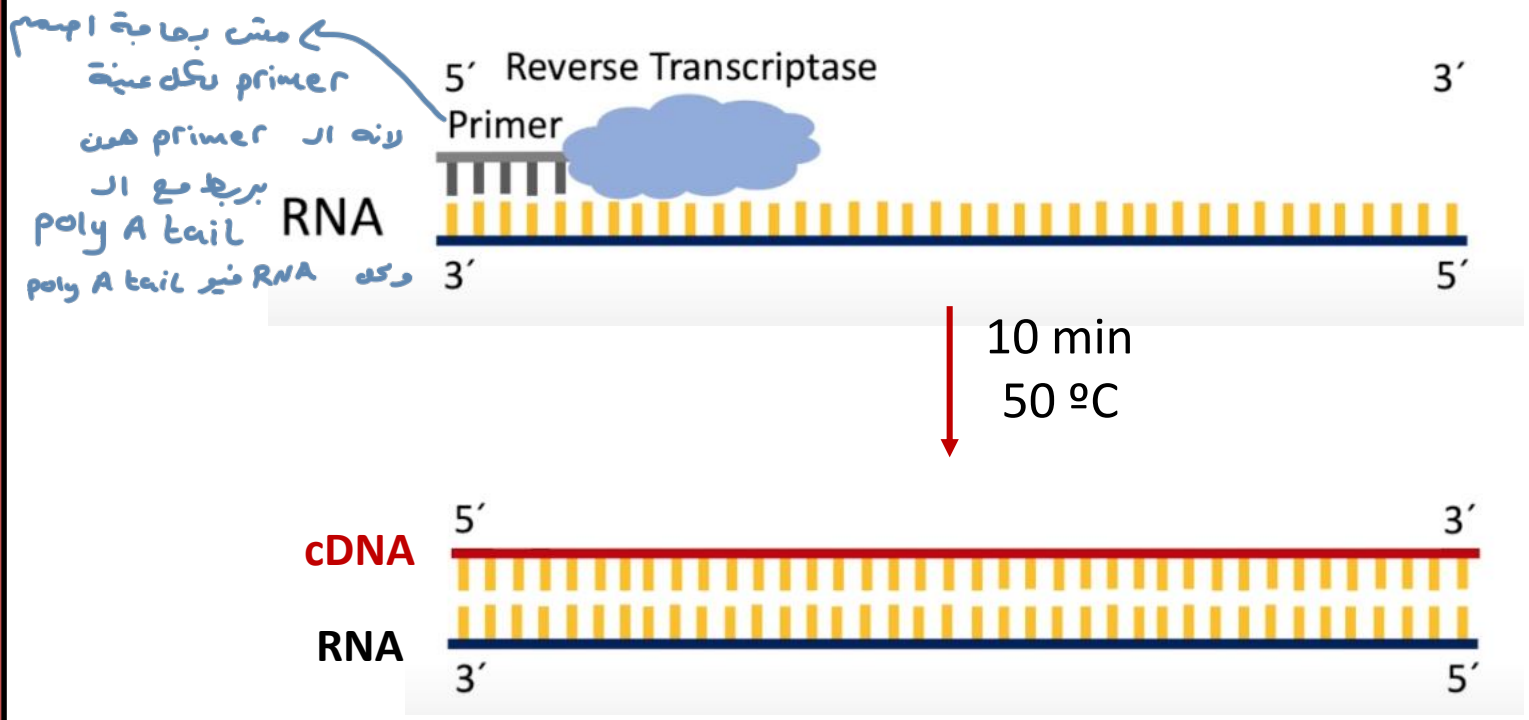





PCR Unit

RT-qPCR

- We can't do PCR directly on viral RNA !!!! and it should be converted first to complementary DNA known as cDNA by reverse transcription (RT)
- RT is catalyzed by reverse transcriptase enzyme



فيه طريقتين للشغل 👇👇👇

▢ One shot or one approach

اعمل reverse transcription and qPcr مع بعض بنفس ال kit
بضيف كل اشي بحتاجه لل reverse transcription and qpcr بنفس ال well اللي فيو العينة اللي
عملتلها extraction وبضبط البرنامج على الجهاز انه اول 10m بكونوا reverse transcription
بعدي بجهز برنامج ال pcr
بستخدموها لفحص كورونا

▢ Two approaches

بتكون على مرحلتين ،،،بحالة اذا كنت بدي اخزن ال cDNA
لما اخلص transcription باخذ جزء من ال cDNA اللي بدي اعمل اله pcr والباقي بخزنه على حرارة20 –

الطريقة الأولى هي اللي بستخدمها لفحص كورونا لكن بضيف على التجربة
اشياء مثل positive , negative and internal controls

Control ==reference point

🍜Negative control: الشخص غير مصاب

🍜Positive control 👇

بيين اذا كان مصاب كيف راح تكون ال curve للمصاب

🍜Internal control 👇

الهدف منها 👈 عشان اذا ما ربطت التجربة اعرف وين الخلل بالتحديد ،،اذا الخلل بال primer او
خلل بالجهاز او kit او أثناء ال extraction
بحدد وين الخلل

°°°°°°°°°°°°°°

بكون parallel to patient's sample شو ما بصير على عينة المريض بصير عليها
مثلا °°اذا كان الخلل بعينة المريض أثناء ال extraction ال IC ما راح يصير اله
extraction مثل عينة المريض بالتالي ما راح يطلع الها curve
او اذا عينة المريض ما الها curve لكن ال IC الها يعني العينة سليمة
الهدف من ال IC حتى اتجنب false negative or false positive result

بضيف ال IC مع العينة لما اعمل extraction

RT-qPCR

- The next step is the amplification reaction using the cDNA as template, specific primers for the target fragments of the virus (ORF 1ab and N genes), dNTPs, Taq DNA polymerase, probes
- Negative control (NaCL solution) and positive control (containing the target fragment sequence of the virus)
  ↳ ORF1a and N genes
- Also the kit contains internal control IC (should be added to the wells before the extraction process)
- Purpose of IC: allow accurate detection of **PCR** inhibition and failure of extraction so avoid false negative results

SARS-CoV-2 Nucleic Acid Detection Kit

- Is enough for 96 tests or samples
- The one approach RT-qPCR (reverse transcriptase and Taq DNA polymerase)
- Contains 5 tubes: positive control, negative control, internal control, PCR reaction solution (master mix) and enzyme solution

بعينات المرضى فقط IC

بعني لعد 94

RT-qPCR

Negative control

Positive control

بضيفهم على كل العينات
بعد رقم 96

- Add 8µl master mix, 2µl enzyme solution, 10µl sample or negative or positive control (total 20µl)

بقدر أضيف 94 عينة

رقم 95 بحطفيها negative control

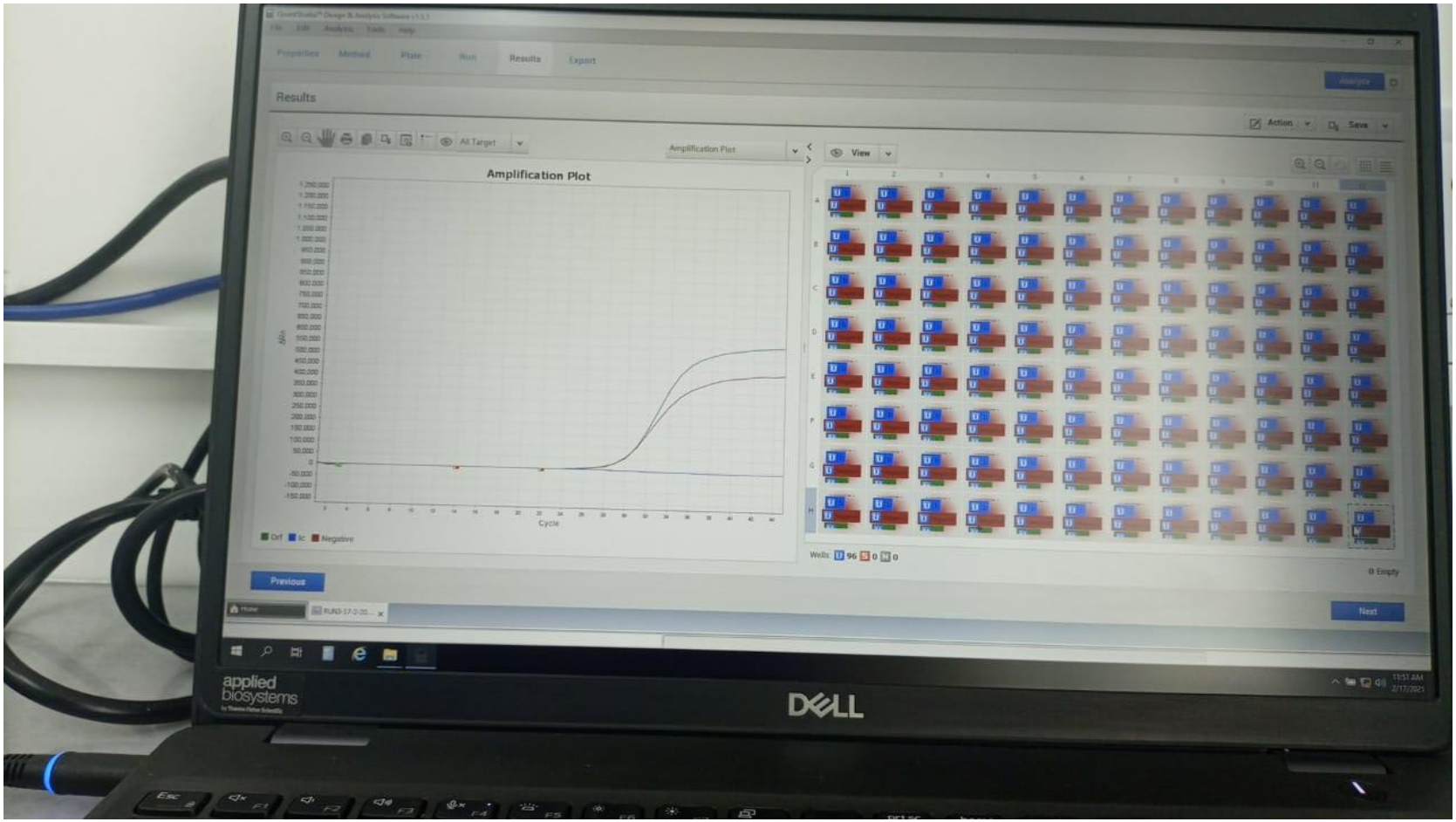
94 لعد فقط

رقم 96 بحط ال positive control

عند 95 فقط

عند 96 فقط

Analysis of the Results



positive control

2curves فيه يكون
الأخضر والأحمر

Green colored curve

detection curve for ORF 1ab

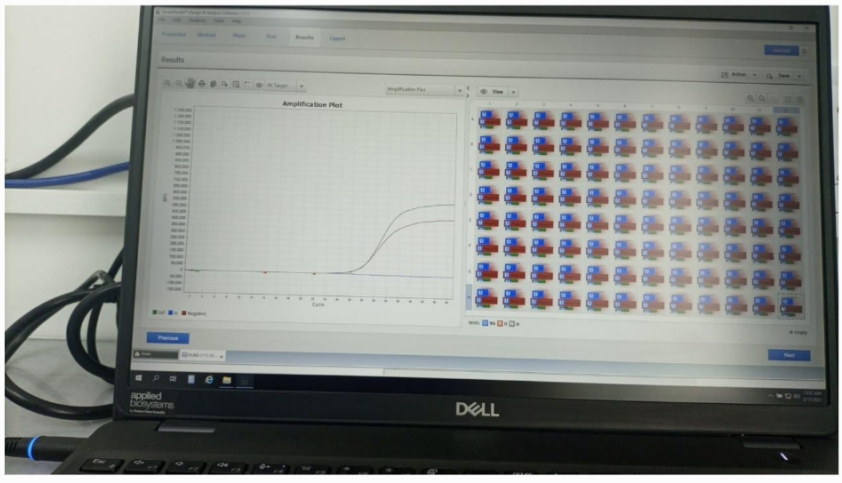
Red colored curve

Detection curve for N gene

Blue curve

مو موجود هون

Detection curve for Internal control



ال curve الناتج بكون S shaped sigmoid curve لانه عملية ال amplification is exponential

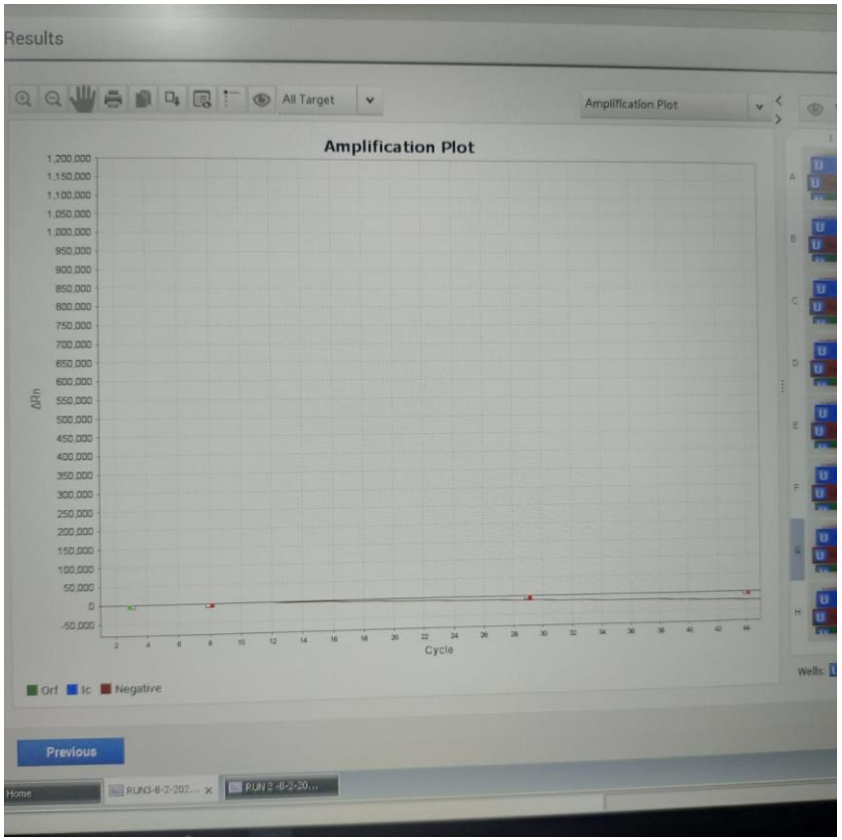
يعني عملية أسية نظام أسس

حتى تكون النتيجة positive: سهم

ال curves كلهم عشكل S

الأخضر اول بعدين الأحمر واخر اشي internal control

Analysis of the Results



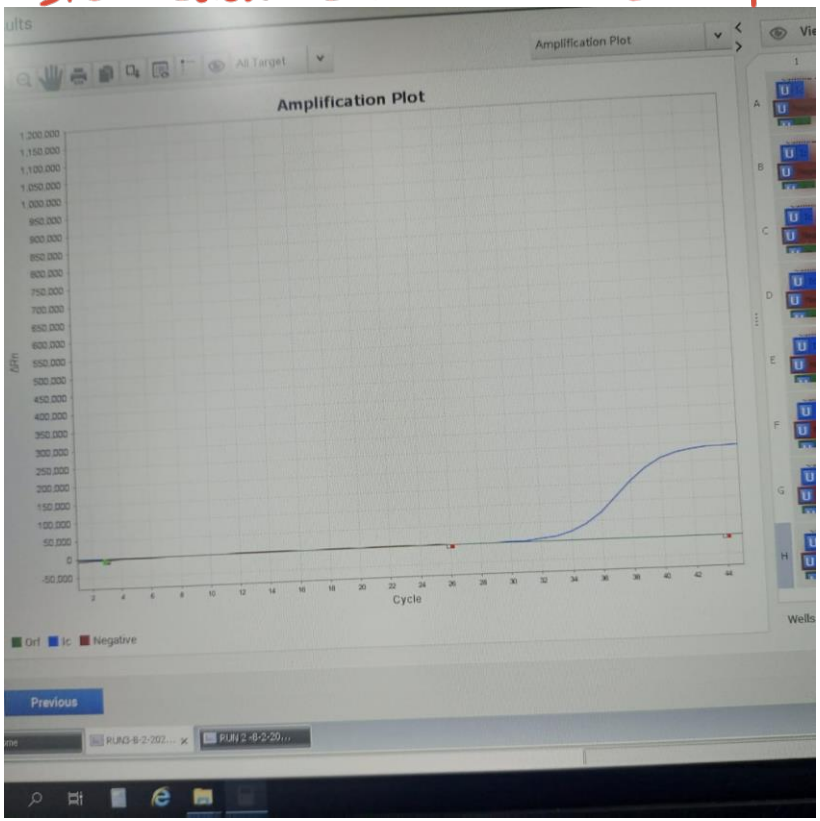
Negative control

ما بطلع السما curve

اذا ما ظهر اشي بعض العينة

False negative

بعض حمار فلك اثناء الـ pcr او extraction

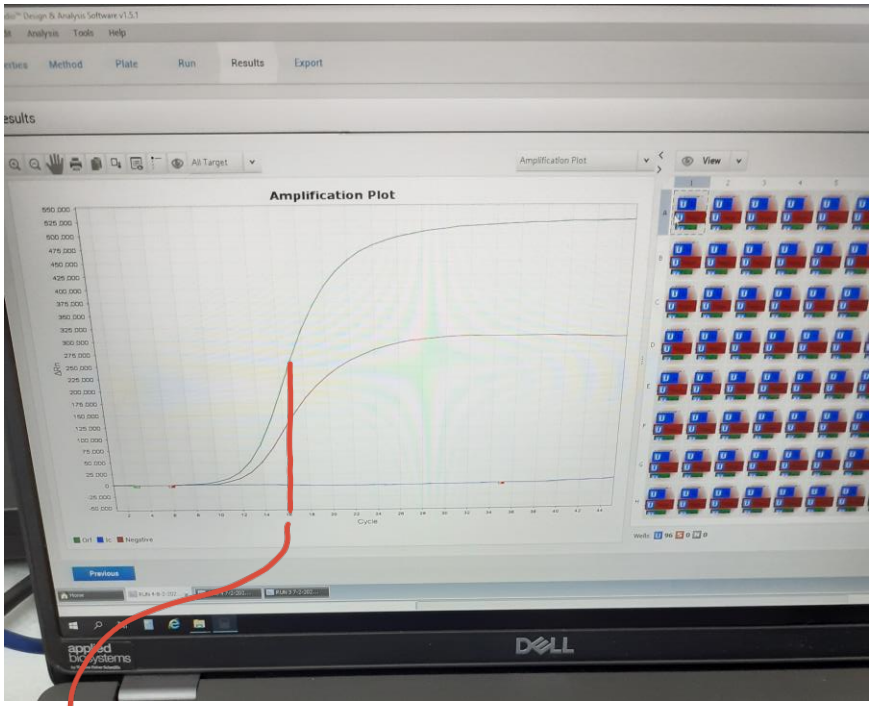


Negative result

المريض غير مصاب

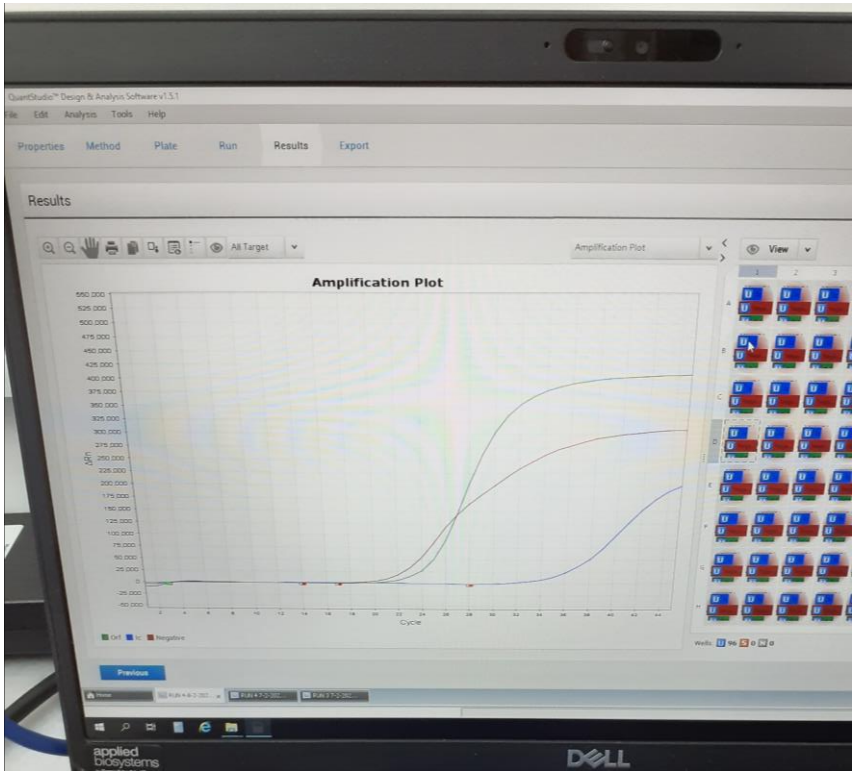
Internal control curve

Analysis of the Results



Positive (acute, strong)

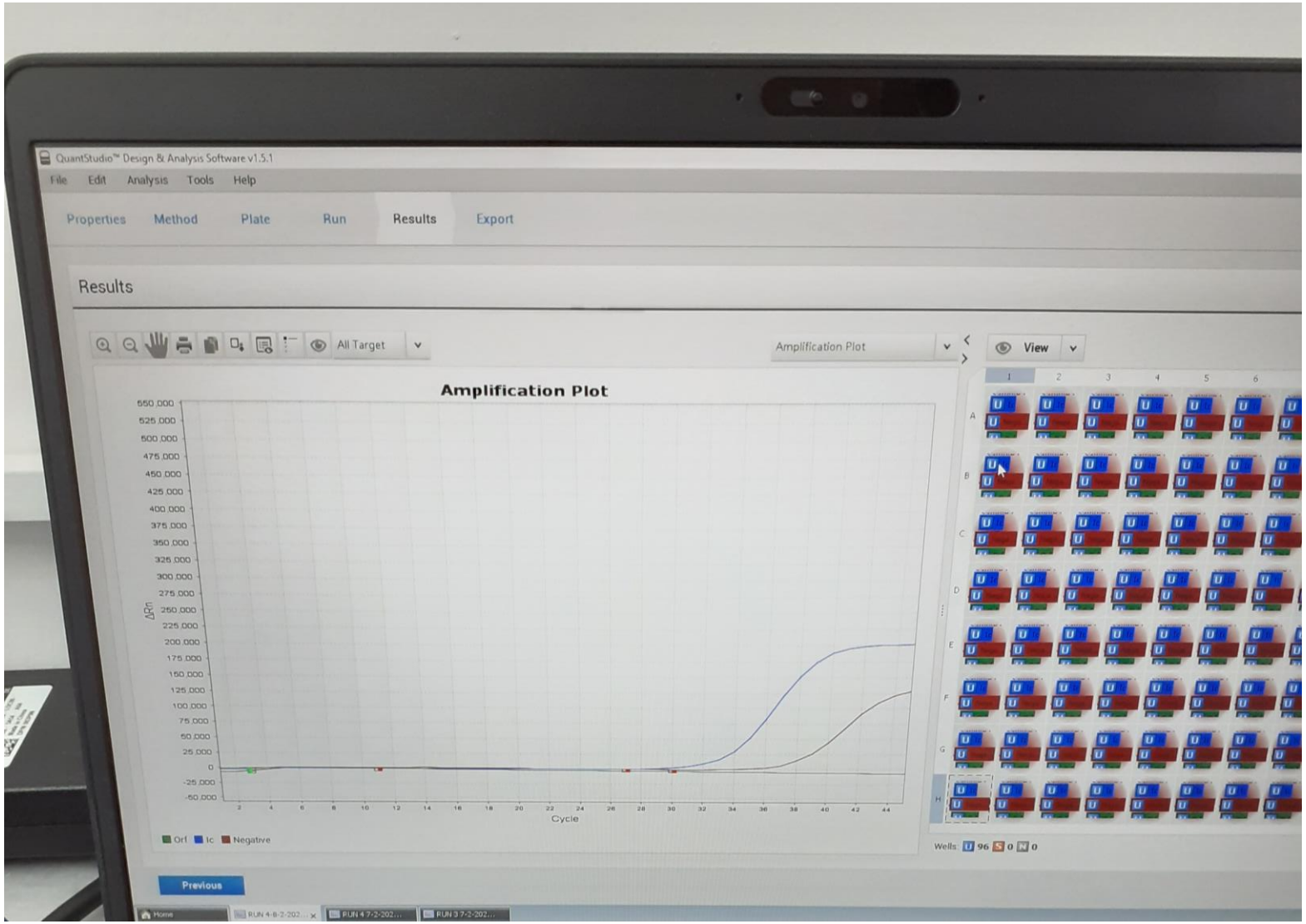
او curve طلع بسرعة او مباشرة
فالمصاب بداية المرض



Positive result

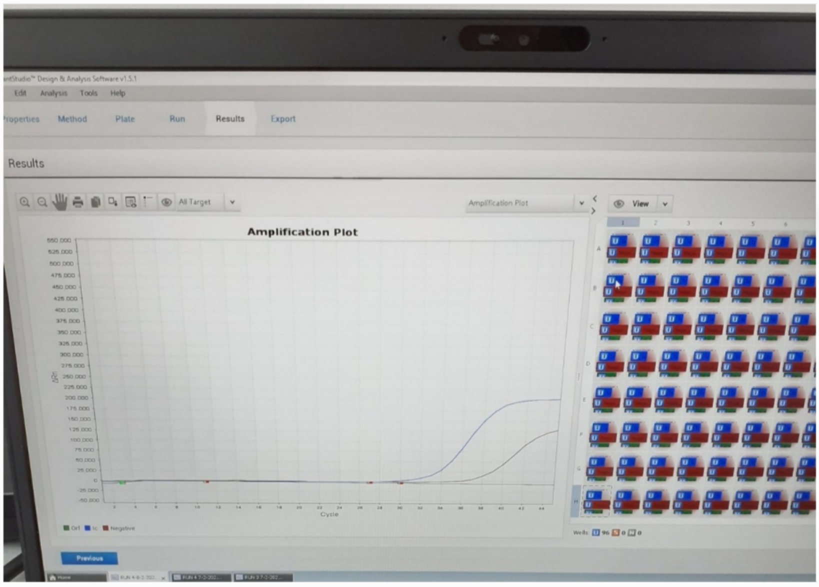
او curve انأخر حتى طلع المريض بين مرحلة المرض أو قرب يتشافا

Analysis of the Results



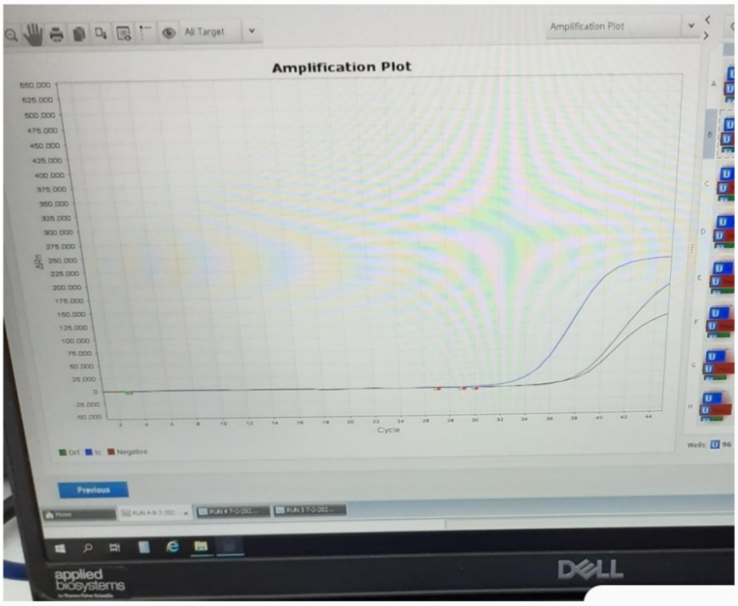
negative result

♣ العينة الـ negative الـ curve الأزرق
أعلى من الأحمر
كما انه الـ curve الأخضر ماطلع
ما بين
ما تحقق شروط الـ positive

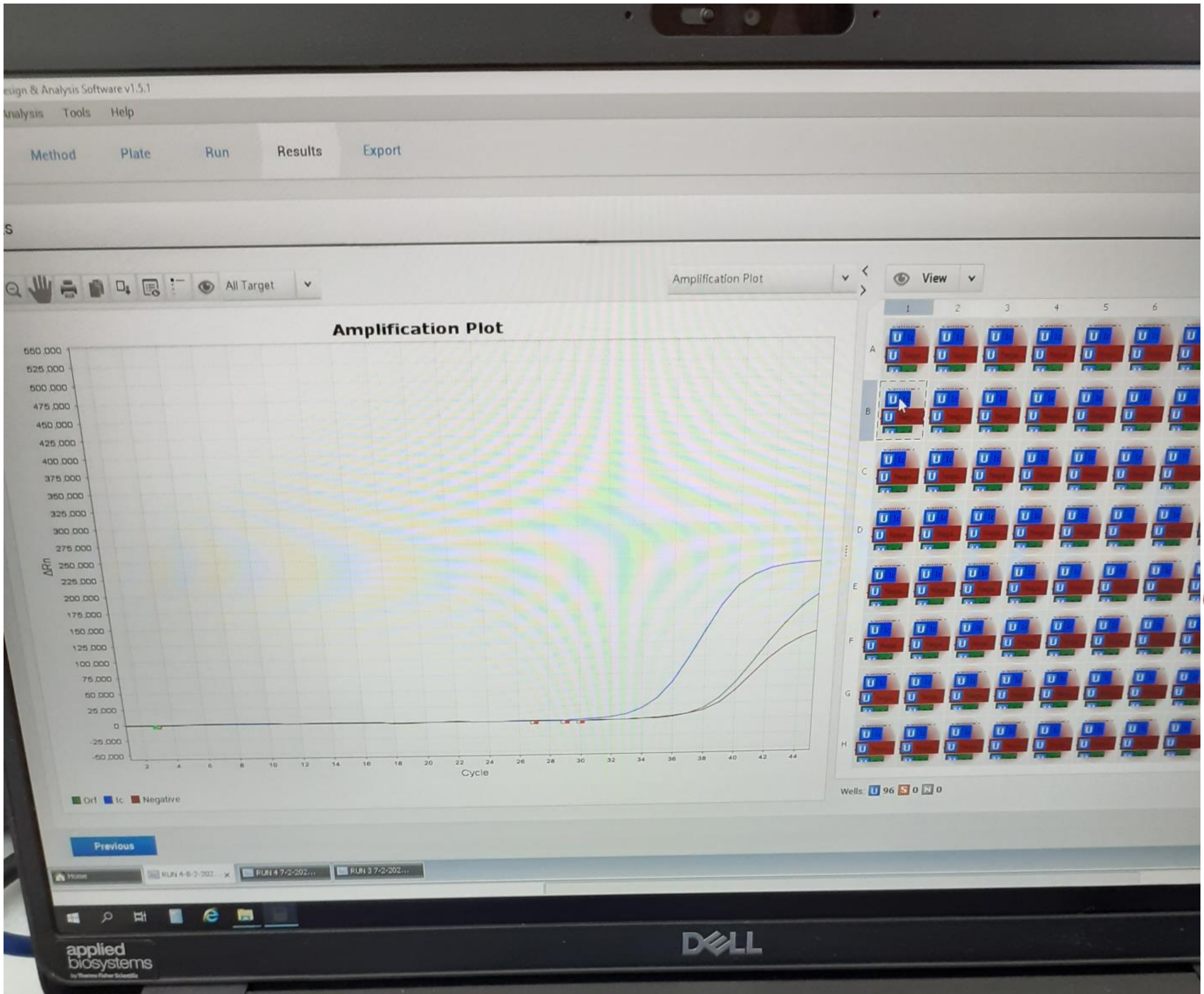


اذا طلع 3 curves والأزرق اعلى منهم
بيطلع على عدد الدورات على CT مقاحكم اذا
المريض بالمرحلة الأخيرة او لأ
اذا كانت CT بعد 38 لـ (الأخضر والأحمر) يعني
النتيجة negative

اذا طلعوا قبل 38 يعتبرها repeat
لازم تنعاد الفحص



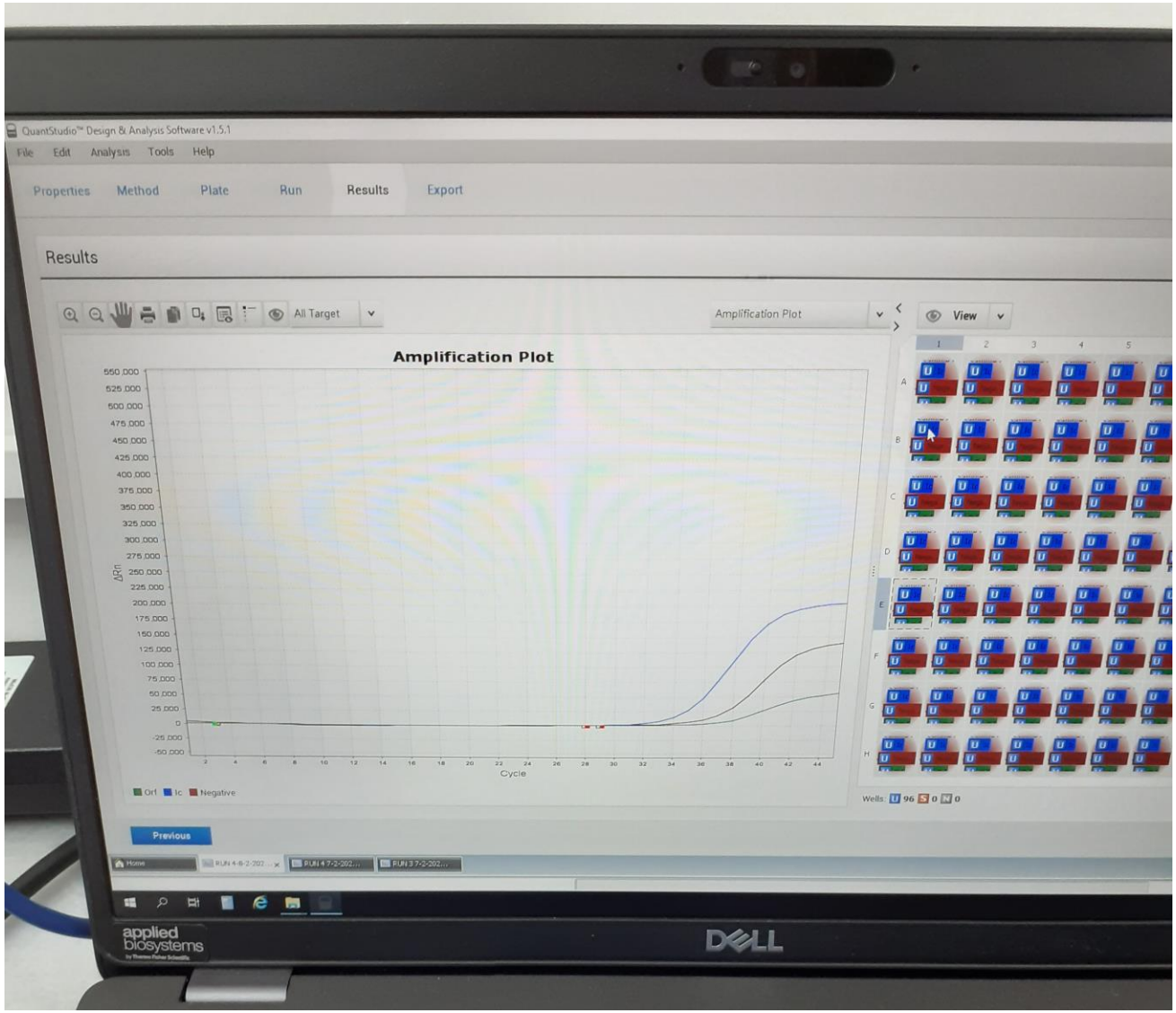
Analysis of the Results



negative result

الأزرق اعلى منهم

Analysis of the Results



Repeat (<38)