LyteStar 2019-nCoV RT-PCR Kit 2.0 Workflow

Kit Components

What is in the kit?

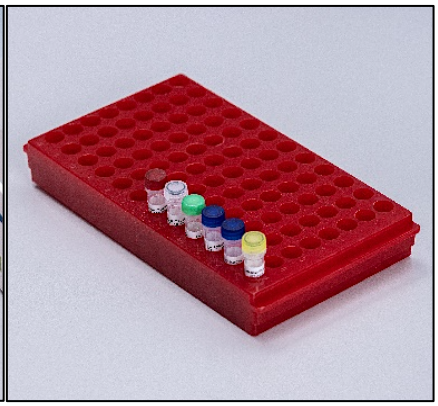
2x Master A (yellow cap)
4x Master B (blue cap)
1x Internal Control (green cap)
1x Positive Control (red cap)
1x Negative Control (clear cap)

1x Master A + 2x Master B
= 48 reactions

Master Mix Setup for 48 reactions

1. Remove the following tubes from the box and let thaw completely:

1x Master A (yellow cap)
2x Master B (blue cap)
1x Internal Control (green cap)
1x Positive Control (red cap)
1x Negative Control (clear cap)

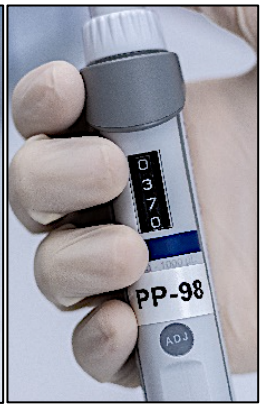


2. After all components have thawed completely, briefly vortex and centrifuge.

3. Prepare PCR Master Mix by adding both tubes Master B to Master A.

Set Pipette to **370** µl and pipette the complete content of the 1st tube Master B (blue cap) into Master A (yellow cap).

Repeat the process with the 2nd tube of Master B.

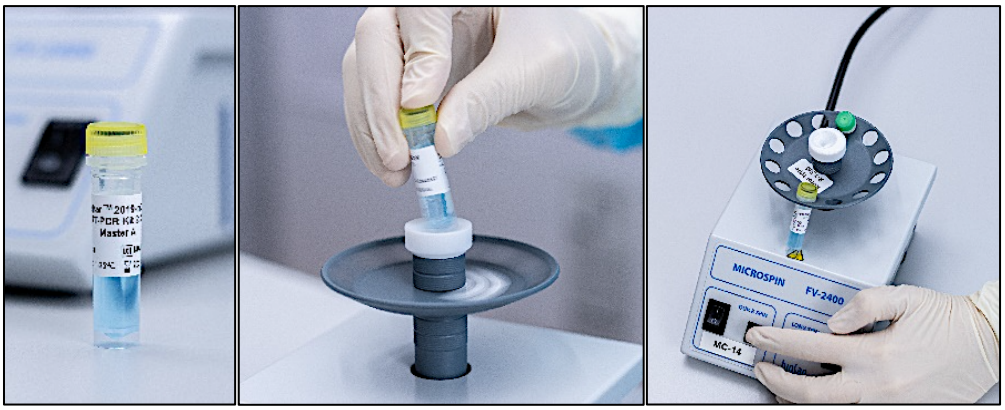


4. Add Internal Control into PCR Master Mix.

Set Pipet to **27** µl

and pipette 27 µl of the Internal Control (green cap) into the PCR Master Mix.

5. Briefly vortex and centrifuge the PCR Master Mix.



PCR reactions Setup

6. Pipette 20 µl of PCR Master Mix into each PCR tube.

Set the Pipet to **20** µl

and add 20 µl of PCR Master Mix to each required Mic PCR tube.

7. Add the Negative Control

Set the Pipet to **5** µl

and add 5 µl of PCR grade water to the appropriate PCR tubes for NTC.

Make sure to use a fresh pipet tip for each Negative Control.

8. Add the Samples

Add 5 µl of each sample to the appropriate PCR tubes.

Make sure to use a fresh pipet tip for each sample.

9. Add the Positive Control

Add 5 µl of Positive Control to the appropriate PCR tubes.

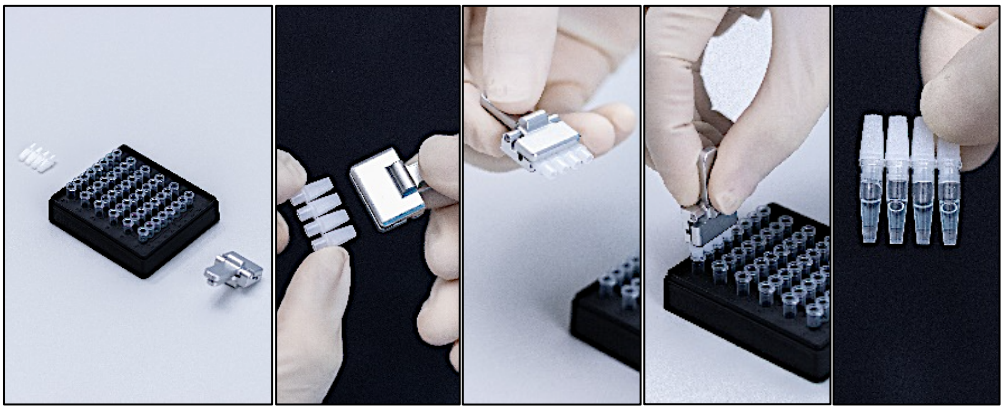
Make sure to use a fresh pipet tip.

10. Close the PCR tubes

Tightly close the PCR tubes with the appropriate caps.

For Mic PCR tubes, use the provided capping tool to fit the caps properly.

Only for ultraSMBS24, add one drop of PCR mineral oil into each PCR tube before closing the tube.

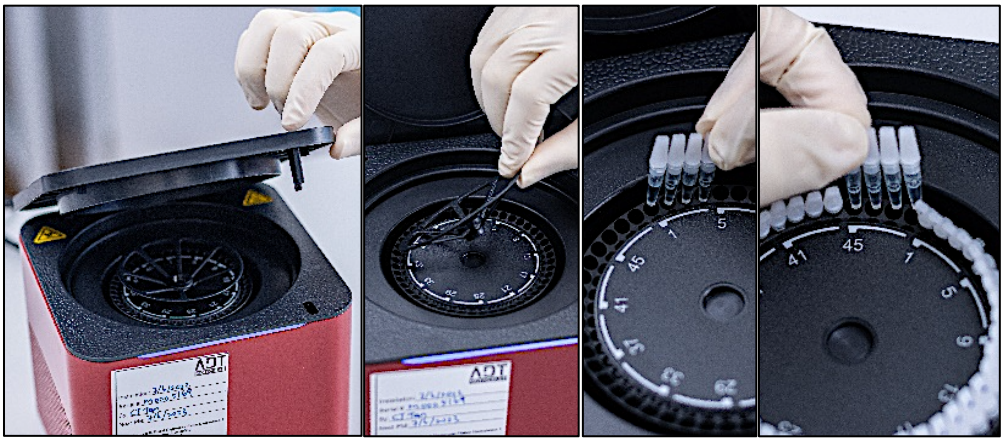


11. Load the PCR cycler

Open the qPCR cycler and remove the tube clamp.

Starting from **Position 1**, place the PCR tubes in the qPCR cycler. Fill the unused wells with PCR tubes that are pre-loaded with 25 µl of water.

Place the tube clamp back on and close the lid of the instrument.



Programming the ultraSBMS24 real-time PCR instrument

1.

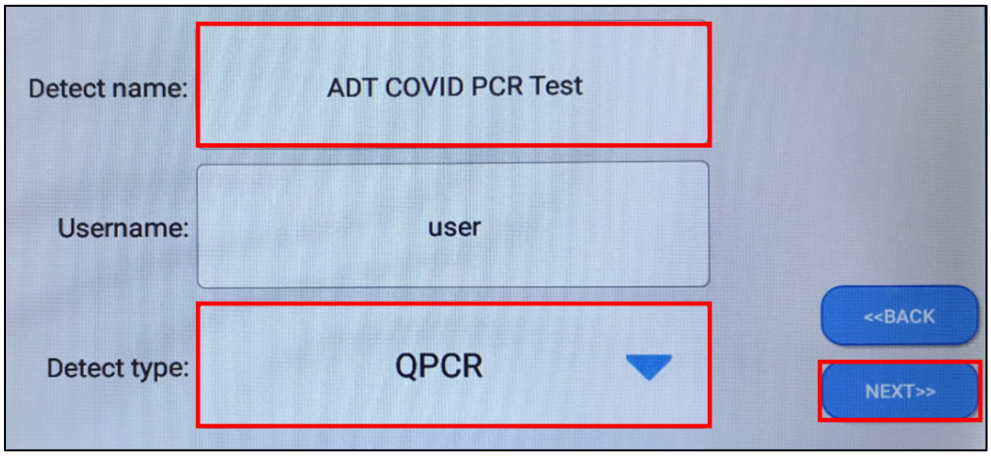
Click the main interface function *NEW DETECT*

2.

Enter *ADT COVID PCR Test* as the Detect Name

Select *QPCR* as the Detect Type

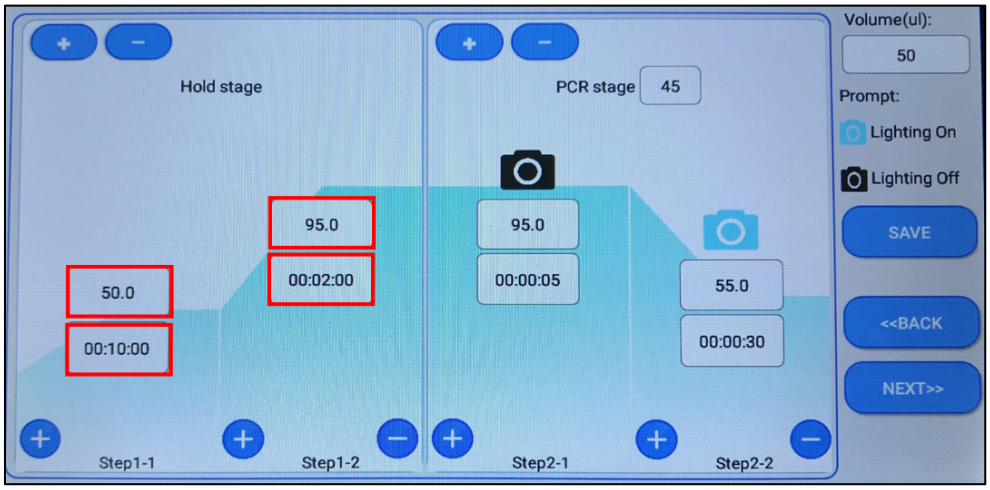
Click *NEXT*



3.

Set the Hold stage Step 1-1 temperature and time as *50* degrees for *10* minutes (Reverse Transcription step)

Set the Hold stage Step 1-2 temperature and time as *95* degrees for *2* minutes (Initial Activation step)

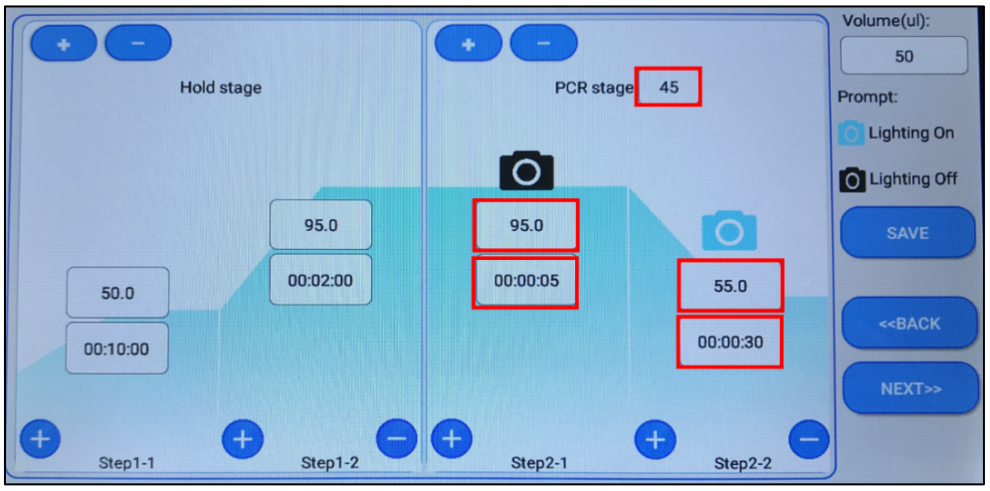


4.

Enter the PCR Stage as *45*

Set the PCR stage Step 2-1 temperature and time as *95* degrees for *5* seconds (Denaturation step)

Set the PCR stage Step 2-2 temperature and time as *55* degrees for *30* seconds (Annealing/Extension step)

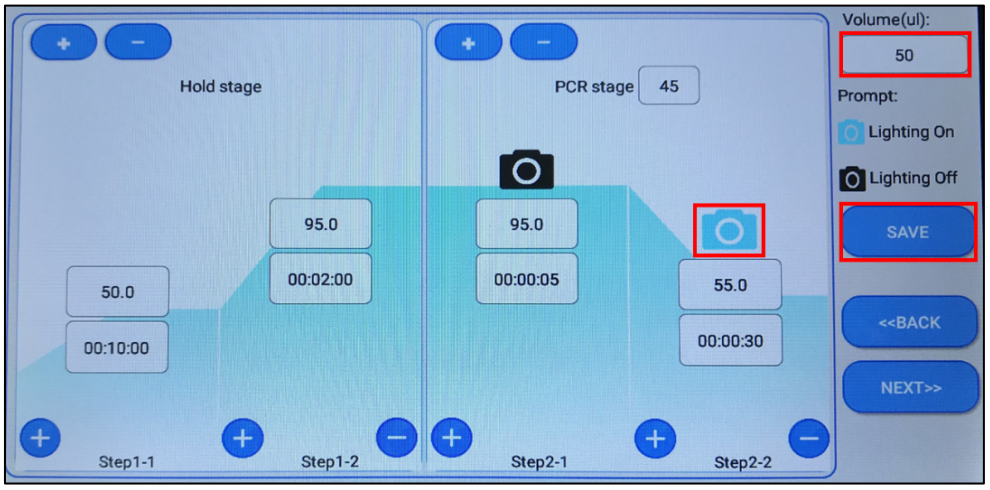


5.

Ensure that *Lighting On* is set on PCR stage Step 2-2

Set Volume (ul) as *50*

Click *SAVE*

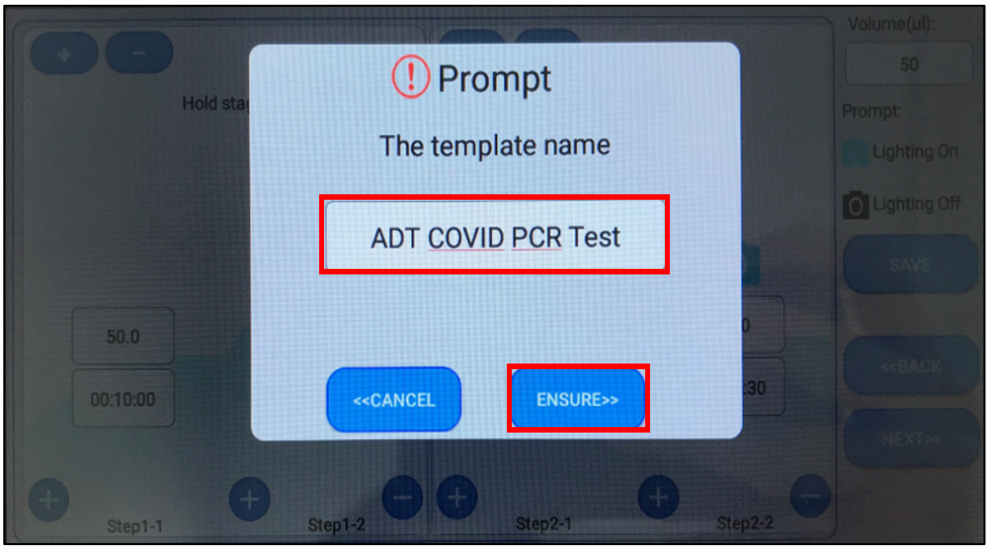


6.

Enter *ADT COVID PCR Test* as the Template Name

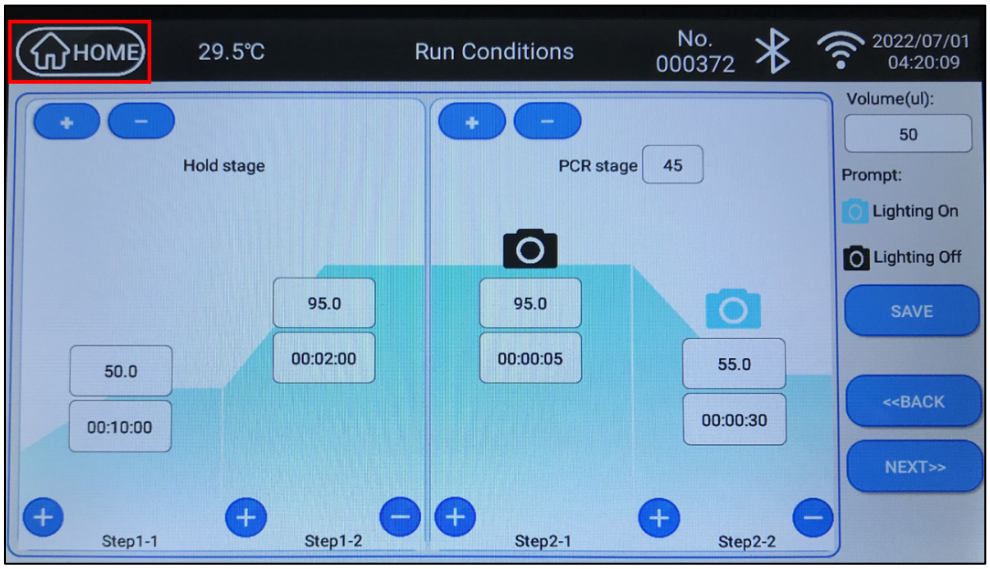
Click *ENSURE* to save the template

Once the run profile has been saved as a template you do not need to repeat the programming of the ultraSBMS24 cycler but can simply open the template from the *Load Template* on the main interface for any new PCR runs.



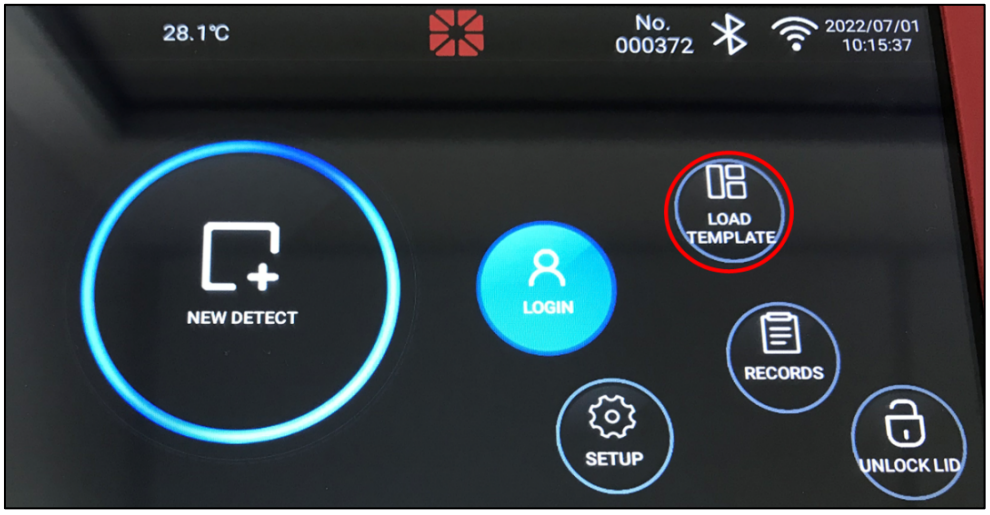
7.

Click the *HOME* button to return to the main interface.



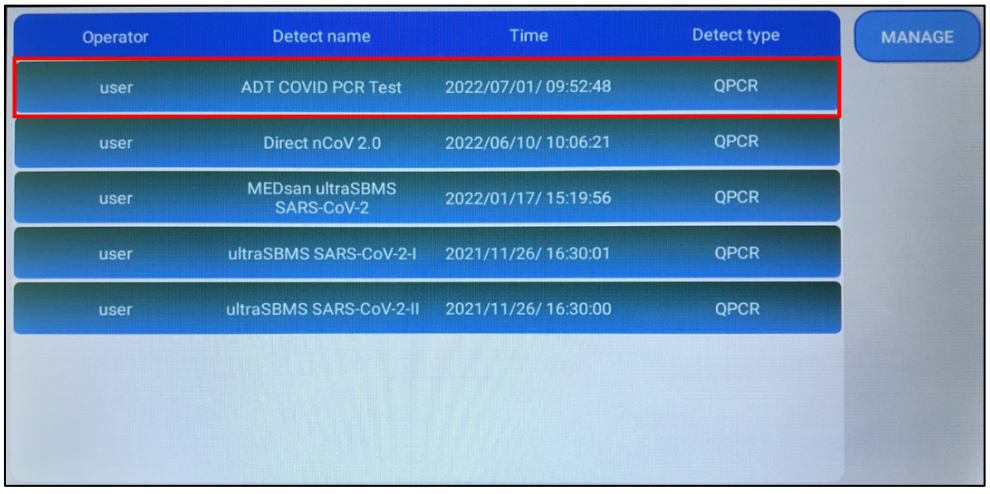
8.

Click *LOAD TEMPLATE* on the main interface.



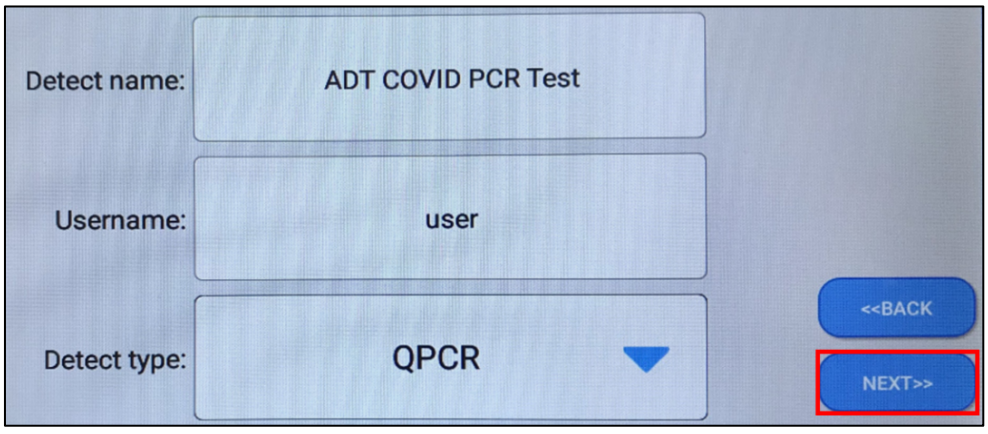
9.

To choose the template, click on *ADT COVID PCR Test*.



10.

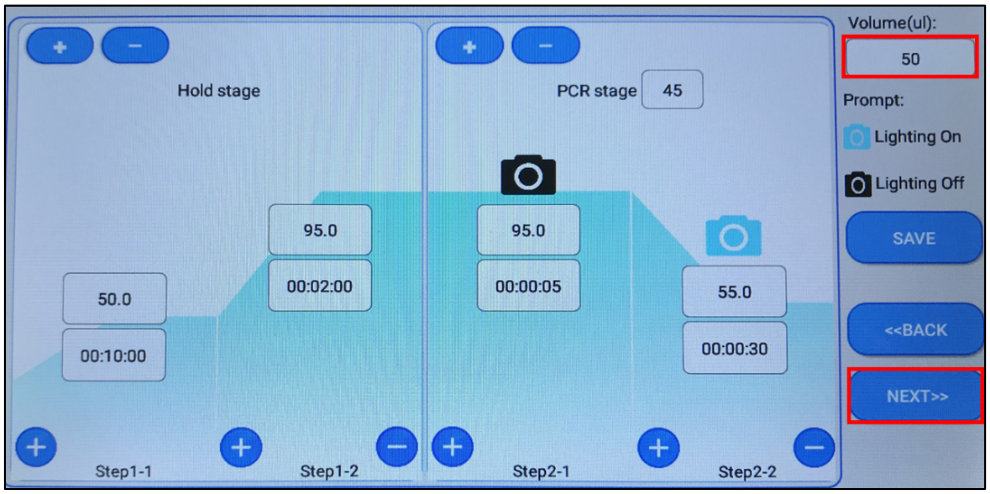
Click *NEXT* to enter the Run Conditions Interface



11.

Enter Volume (ul) as *50*

Click *NEXT* to enter the Sample Setup interface



12.

Select the sample well position to be tested and click *EDITOR* to edit the sample information of this well.

13.

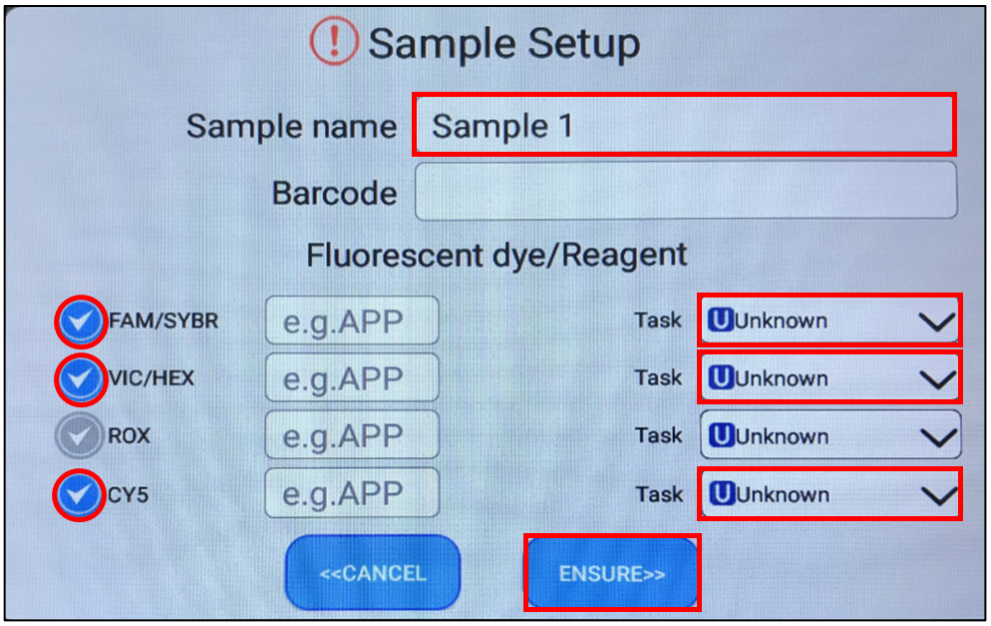
In the Sample Setup box, enter the Sample name and select the following Fluorescent dye/Reagent:

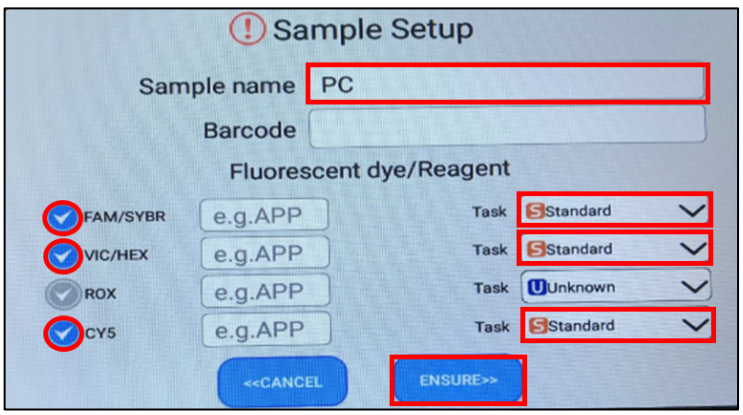
- *FAM/SYBR*
- *VIC/HEX*
- *CY5*

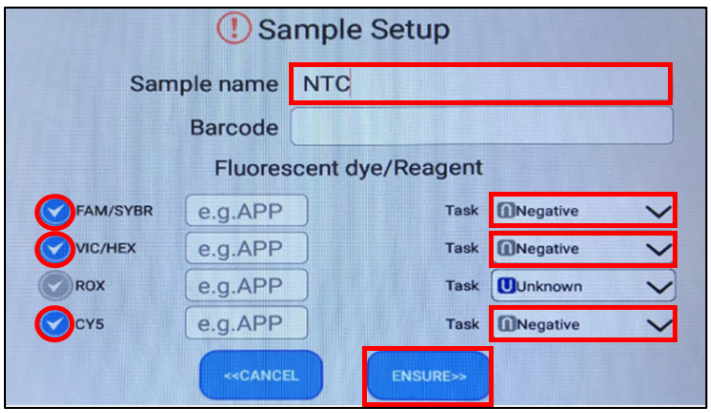
For each Fluorescent dye/Reagent selected, choose the appropriate Task:

- Sample – *Unknown*
- Positive Control (PC) – *Standard*
- No Template Ctrl (NTC) - *Negative*

Click *ENSURE* after each sample setup is complete



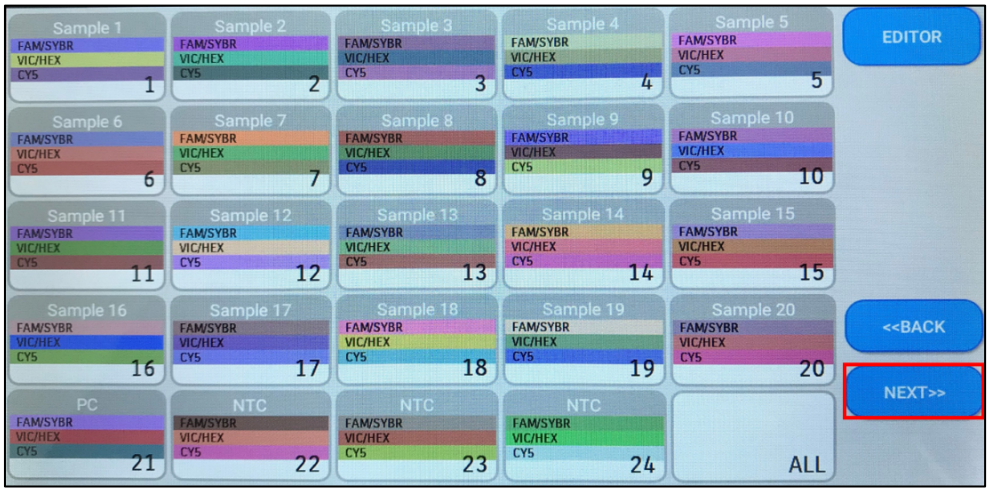




14.

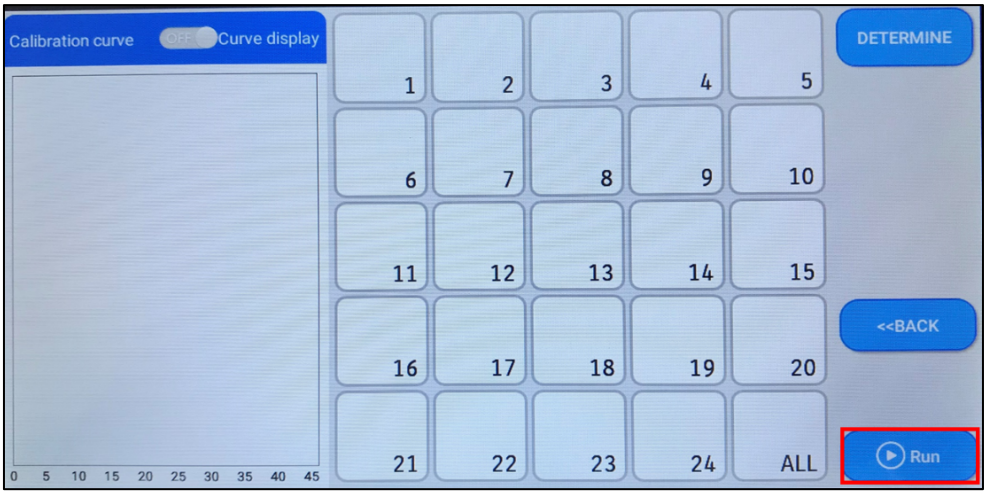
Check to ensure all sample wells are labelled correctly and the correct fluorescent dyes are selected.

Click *NEXT*



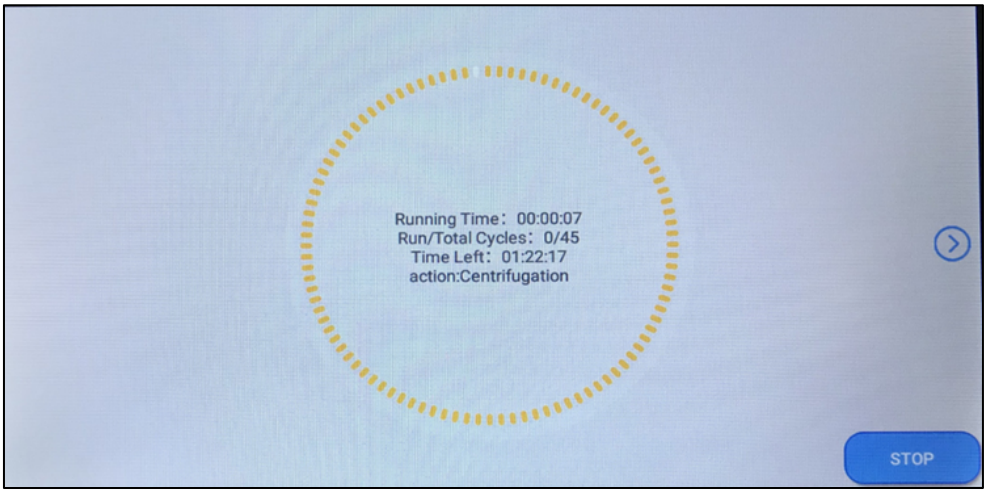
15.

Start the run by clicking the *RUN* button.



16.

In this interface, the progress and completion of the test are displayed.

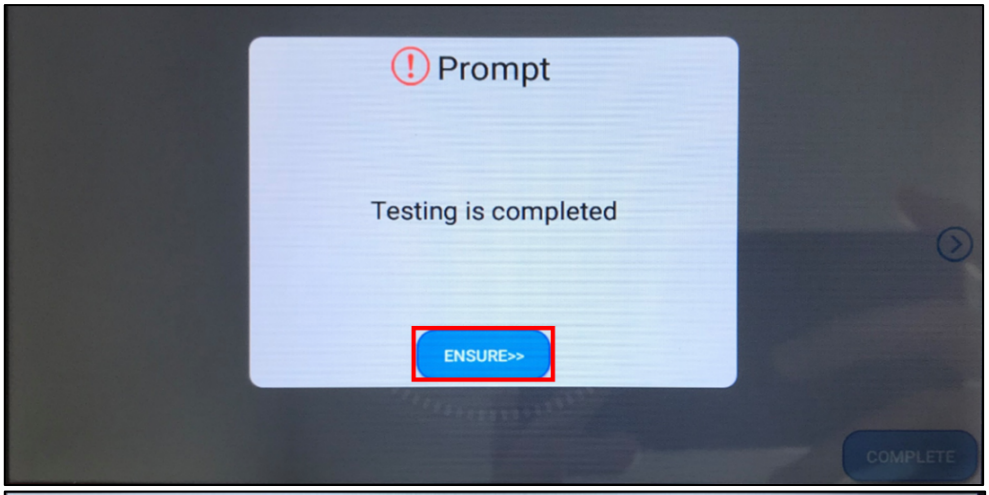


Data Analysis the ultraSBMS24

Data Analysis

1.

Once the run test has completed, click *ENSURE* on the Prompt



2.

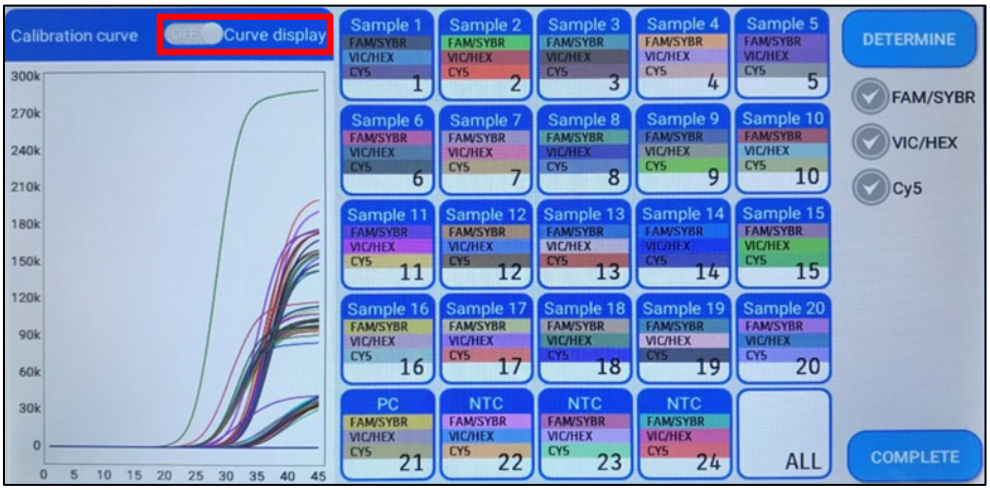
Click the *Arrow* to move to the Run Conditions interface

3.

Click the *Arrow* to move to the Run Curve interface

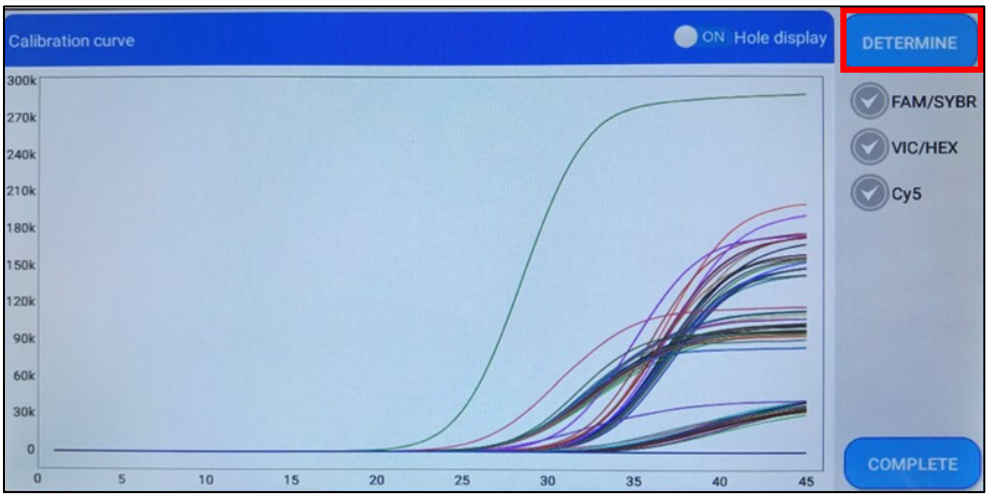
4.

On the Calibration curve bar turn the Curve display *ON* to display the all the collected data curves.



5.

Click on *DETERMINE* to enter the results analysis interface.



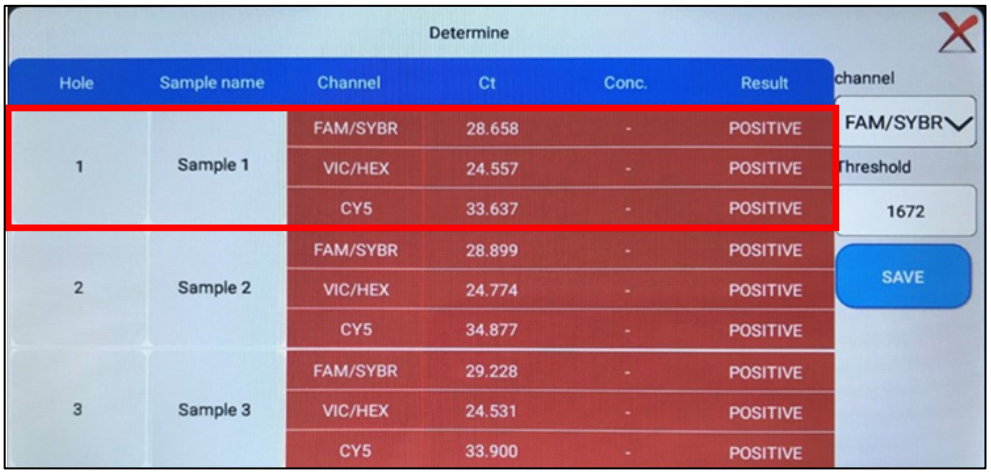
6.

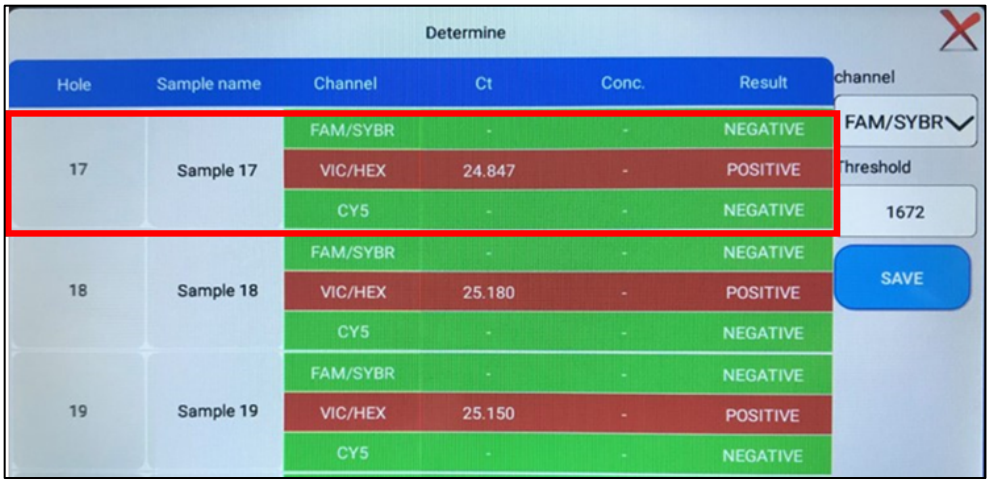
This page displays the detailed information of the detection result, including the quantification cycle (Ct) value (4th column) and the interpretation of positive/negative results of the sample (6th column).

A positive result is indicated by a Ct value and a *POSITIVE* label in the table (e.g. Sample 1). A negative result is indicated by an absence of a Ct value and a *NEGATIVE* label in the table (e.g. Sample 17).

The “threshold” displays the positive threshold of intelligent interpretation that is set by the machine. This does not need to be altered.

To view each sample individually, click on the Sample of interest.

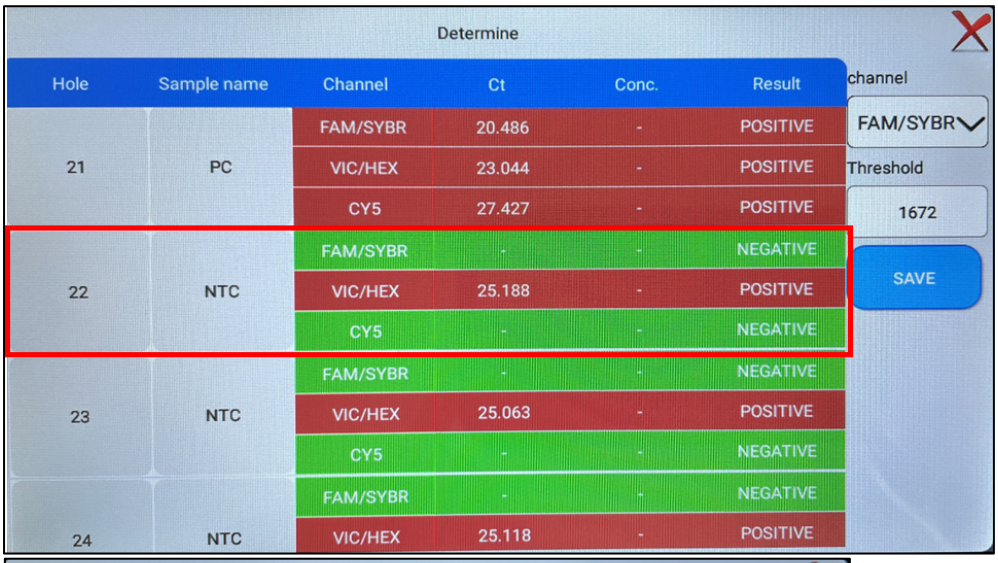


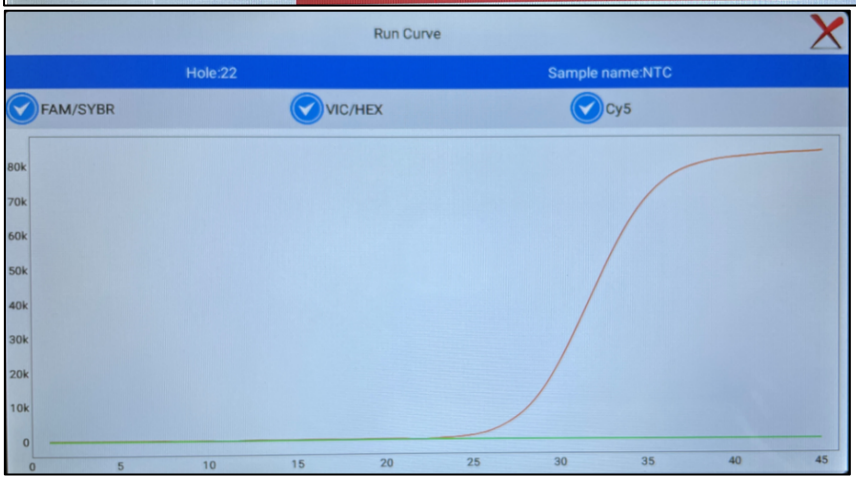


7.

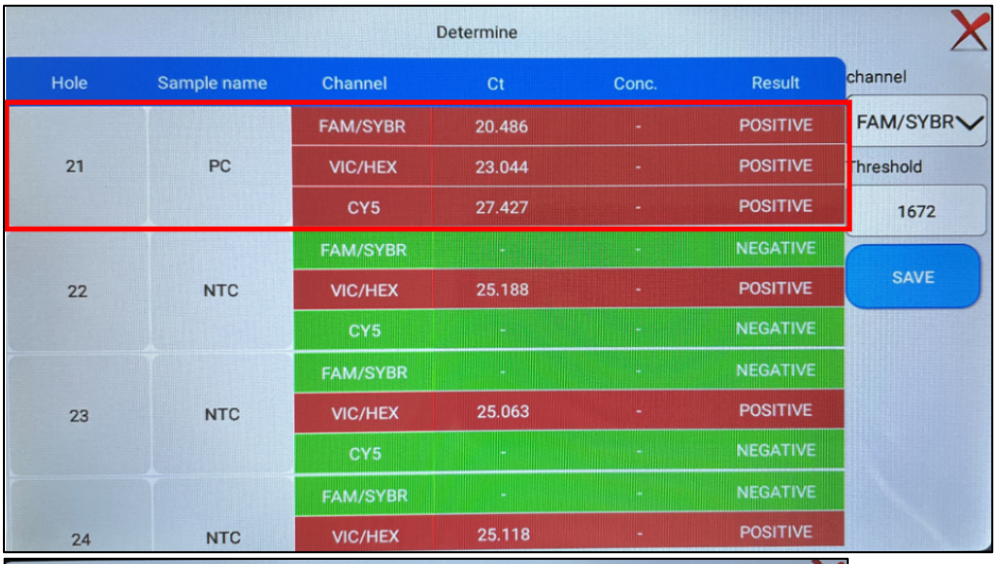
Analyse the PCR results for each individual sample and the validity of the overall PCR experiment as per the validation criteria set out in detail in the Instructions for Use; in brief,

A. The No Template Control must always be negative in the FAM and CY5 channels, and positive in the HEX channel (Sample 22)

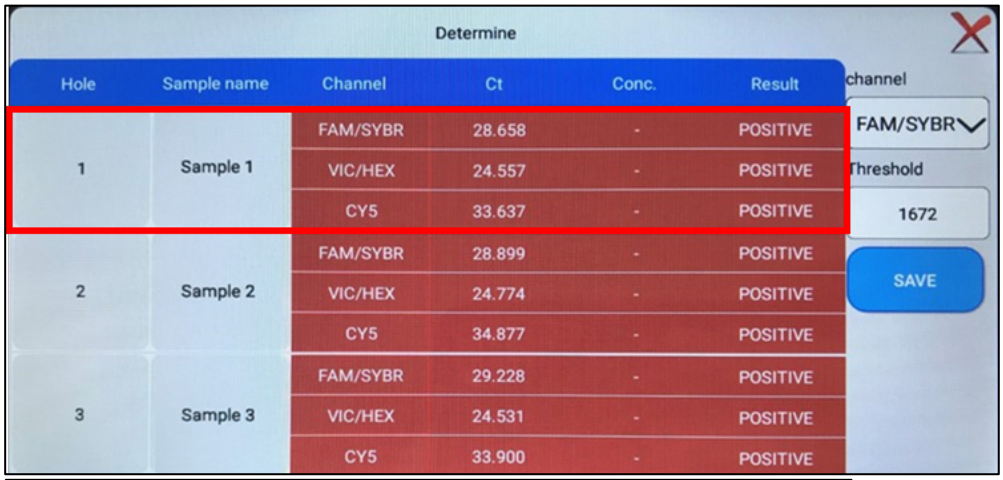


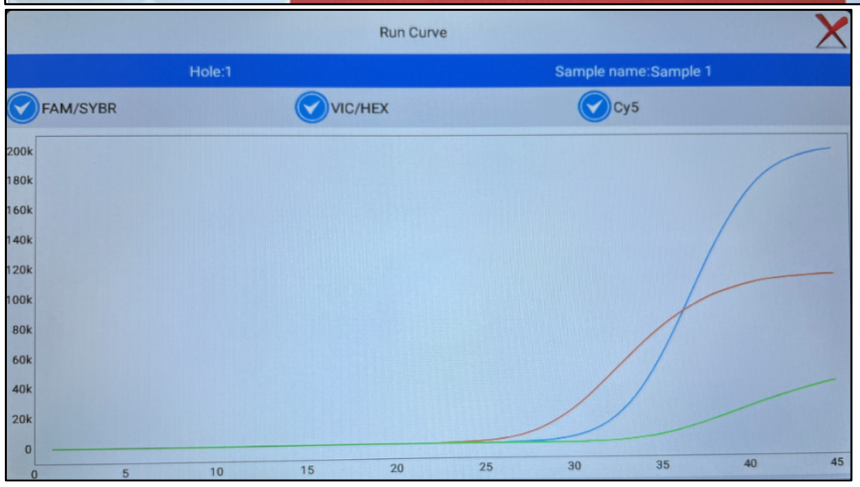


B. The Positive Control must be positive in all three channels (Sample 21)



C. A positive sample is indicated by amplification in the FAM and Cy5 channels (e.g. Sample 1)





D. A negative sample is indicated by an absence of signals in the FAM and CY5 channels, but must have amplification in the HEX (Internal Control) channel to rule out PCR inhibition. (e.g. Sample 17)