Corona Detective Clinical Validations
Swiss samples

22.8.22, La Chaux-de-Fonds

Corona Detective reactions were run with clinical samples obtained from Reto Leinhard and Antony Croxatto, of AdMed in La Chaux-de-Fonds, Switzerland.

For these tests, using the PoP Corona Detective reactions made by EVIK in Canada,
x3 8-tube strips of reactants were taken from batches kept at room temperature (RT) lab conditions - over 35 ºC on some days - since 28.6.22, so over more than 7 weeks, and
x1 8-tube strip used was always kept in the refrigerator at 4 ºC.

Extracted RNA samples and samples from swabs in Amies buffer were provided 'blind' by RL. (all already tested). AMIES samples (AD) were treated with 1x TCEP/EDTA at 95 ºC, unless otherwise noted. RNAs (R1-R4) were used as provided.
The standard 10+10 protocol was followed, and initially run only 1.5h at 63ºC.

Replicate reactions, with the first set of tubes always at 4 ºC versus and another tube strip (1) from the RT batch were made with samples as as follows:
('F' and '1')
tube 1 – water (negative control)
tube 2 – inactivated viral control
tube 3 – AD2212 5457
tube 4 – AD2210 5123
tube 5 – AD2261 3755
tube 6 – AD2107 255
tube 7 – R1 820433
tube 8 – R2 820439

The other two sets of tubes (from RT in the lab) were used for further reactions.
Reactions were set up in the 2nd RT 8-tube strip ('2') as follows:
tube 1 – water (negative control)
tube 2 – inactivated viral control
tube 3 – AD2107 260
tube 4 – AD2107 256
tube 5 – AD2260 0984 (had swab in it still – bigger tube)
tube 6 – Aimies alone (to control for any autofluor)
tube 7 – AD2106 5212
tube 8 – AD2261 3768

and in the 3rd RT 8-tube strip ('3'):
tube 1 – water (negative control)
tube 2 – inactivated viral control
tube 3 – R1 820433
tube 4 – R2 820439
tube 5 – R3 841775
tube 6 – R4 842000
tube 7 – AD2106 5212 (not treated with tcep/edta)

tube 8 – AD2261 3768 (not treated with tcep/edta)
Incubations were performed in a PCR machine, at a constant 63 °C.

Figure 1 shows an example of the the dry lyospheres:

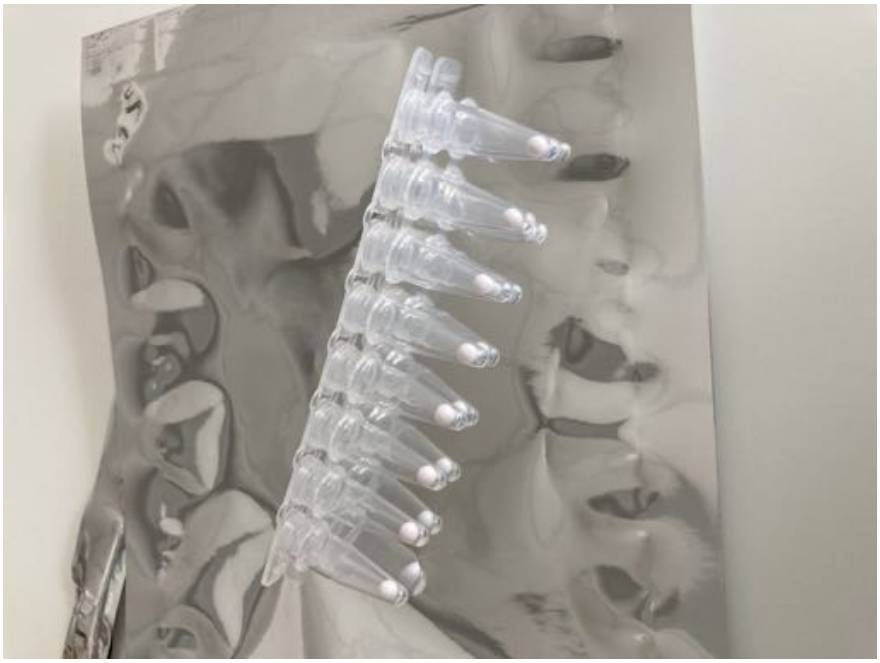

Figure 1: Lyospheres of Corona Detective reagents (EVIK)

Figure 2 shows the first images taken of the 4 sets of tubes after 45 min incubation.

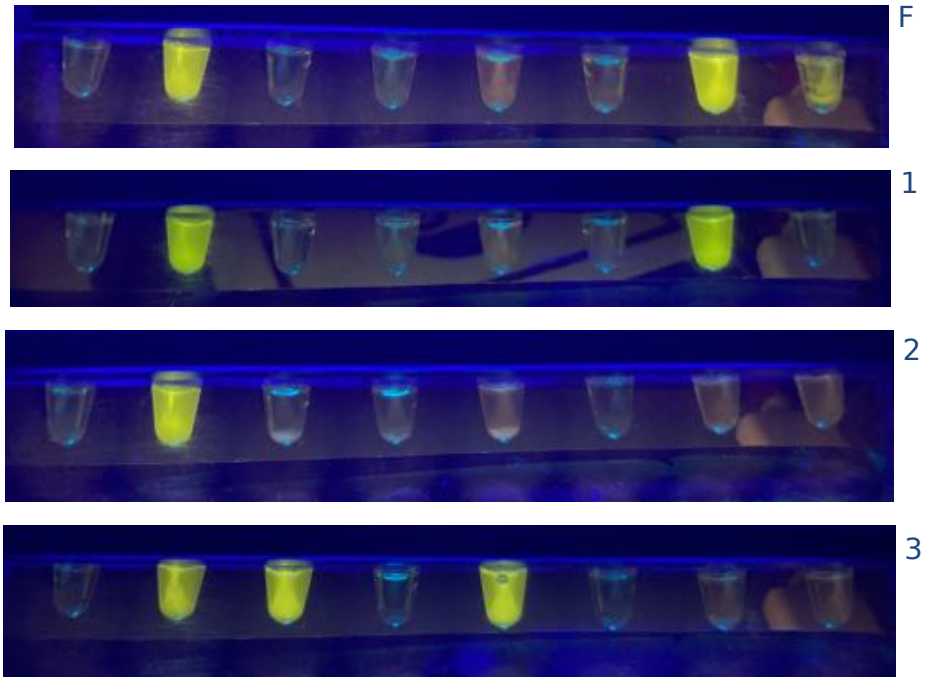

Figure 2: Initial reaction results after a half hour incubation.

The positive controls and some of the RNA samples were the first clearly fluorescent signals, another 15 min incubation (for 1h) only added a bit more green to the 8th tube of 'F'.

Figure 3 shows more images after another 30 min incubation (1h30min total)

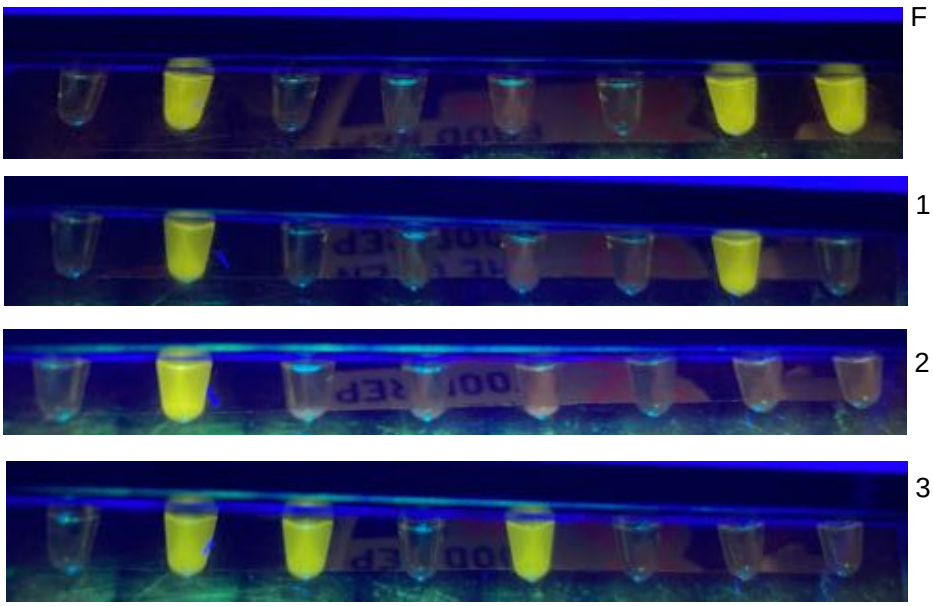

Figure 3: Reaction results after a one and a half hours at 63 °C.

As the usual protocol for these isothermal amplification reactions is 45min, then another 45min, no further signal in tubes (in particular tube 8 of '1') was not taken as a good sign, and a train was caught soon thereafter, back to Lausanne, with all the tubes in double zip-lock bags in a backpack.

Nonetheless, the next day, another observation seemed worthwhile.
In fact, two more tubes had fluorescent signal, tube 4 of both the 'F' and '1' replicates.
Thus, the 4 sets of strip-tubes were incubated for at least another hour and a half at 63 °C. Tube 5 of the set '1' also got bright. The final results are shown in Figure 4.

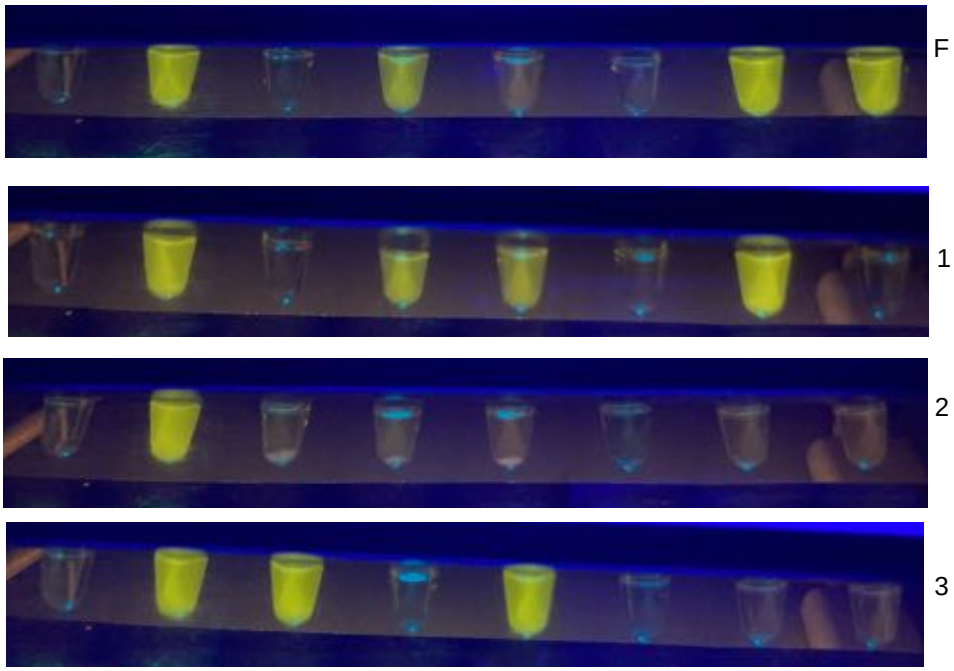

Figure 4: Reaction results after at least 3 hours of incubation.

This blinded experiment was successful, in a sense, as the positive controls came up quickly and the negative controls reliably stayed negative. Additionally, not only RNA samples that had been extracted already, but treated Amies samples could be detected, although the latter came up much more slowly than the ordinary saliva samples for the standard Corona Detective protocol.

However, these results leave more than a few questions.
Why didn't the 5th tube of 'F' or the 8th tube of '1' give positive signals?
Why didn't any of the treated Amies samples in tube-strip '2' give positive signals?
(Did the long time at room temperature have different effects on reaction efficacy, varying from strip to strip and tube to tube?)
Was RNA 2 (820439) at a relatively low concentration, that it came up positive only once for 3 different tube strips? (Was RNA 1 (820433) at a higher concentration, that it gave positive signals in all 3 cases?)

Finding out the ct values for each tested sample will help resolve these issues.
The ultimate utility of this method clearly remains to be determined with more clinical validations.

To summarize, here are samples run and the results obtained:

negative control – negative (4/4)
positive control – positive (4/4)

Samples in Amies media:
- AD2212 5457 – negative (2/2)
- AD2210 5123 – positive (2/2)
- AD2261 3755 – positive (1/2)
- AD2107 255 – negative (2/2)
- AD2107 260 – negative (1/1)
- AD2107 256 – negative (1/1)
- AD2260 0984 – negative (1/1)

Amies alone – negative (1/1)
- AD2106 5212– negative (2/2) treated or untreated
- AD2261 3768– negative (2/2) treated or untreated

extracted RNAs
- 820433 – positive (3/3)
- 820439 – positive (1/3)
- 841775 – positive (1/1)
- 842000 – negative (1/1)

(ct's to be added, please...)