IMI2 Project ID 101005077

CARE – Corona Accelerated R&D in Europe

WP2 – Target-based drug discovery and design

D2.2. A HTS assay for the RTC under a 384-well plate format suitable to expedite focused screening of inhibitors and hit-to-lead efforts

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Document History

Version	Date	Description
V1	21 Mar 2021	First draft
V2	21 Apr 2021	Final draft

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Abstract

The Aim of CARE WP2 is to prepare SARS-CoV1 and 2 proteins for enzymatic assays, structural studies, targeted drug design, and mechanism-of-action studies. Beyond this virus, the scope will be expanded to include additional beta-coronaviruses and alpha-coronaviruses, aiming to obtain *pan*-coronavirus inhibitors. Available enzymes will support focused screening campaigns originating from virtual screening, partners' in-house libraries, and lead discovery in conjunction with WP1 and WP3.

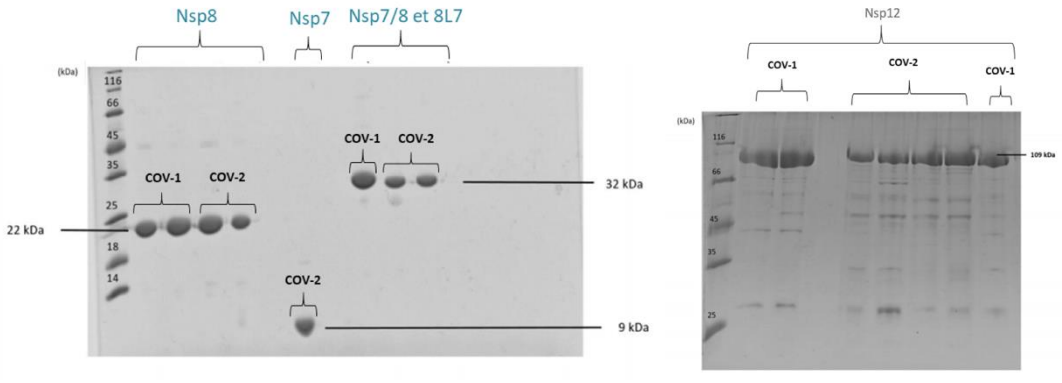
This report describes a robust Coronavirus Replication/Transcription Complex (RTC) assay. After optimisation, the assay has been set-up to perform HTS campaigns under a 384 well plate format, which is a universal standard in pharmaceutical companies and research laboratories. P2-Janssen, P12-LUMC, and P18-UHAM have contributed to the establishment of the assay through benchmarking with 384 well plate standards, cDNA clones to select the best expression systems, and synthesis of 5'-triphosphate analogues, respectively.

The HTS assay has been used to perform several medium scale screens (2000 - 3000 compounds), which will be reported during the next reporting period.

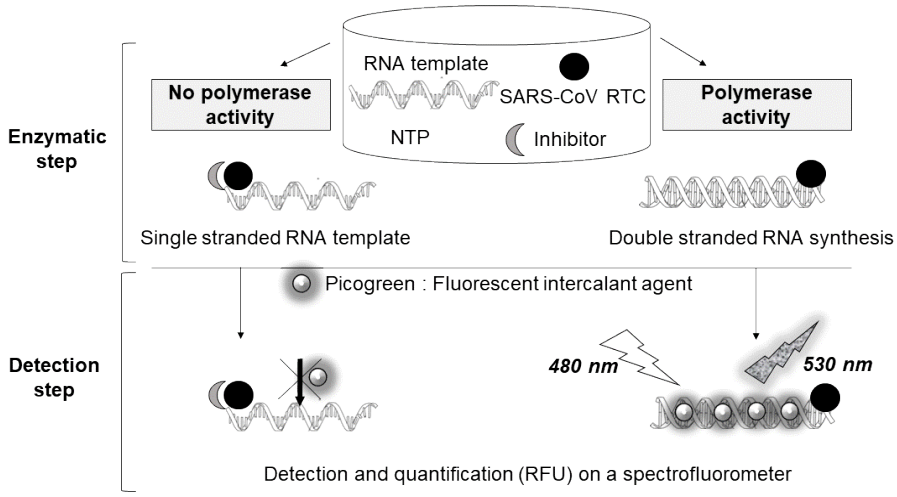
Methods & Results

To achieve the proposed work, we have expressed and purified the proteins required to perform a RdRp activity assay for SARS-CoV-1 and SARS-CoV-2. These are nsp7, nsp8, and nsp12.
For the sake of simplicity, we have also expressed a fusion of nsp7 with nsp8 (named nsp7L8 and nsp8L7, designed with P12-LUMC), which allows reconstitution of the complex with mixing of only two proteins (nsp7L8 and nsp12).
All proteins are expressed in *E.coli*, purified, and assembled with methods described in Eydoux et al., J. of Virological Methods, 2021 Feb;288:114013.

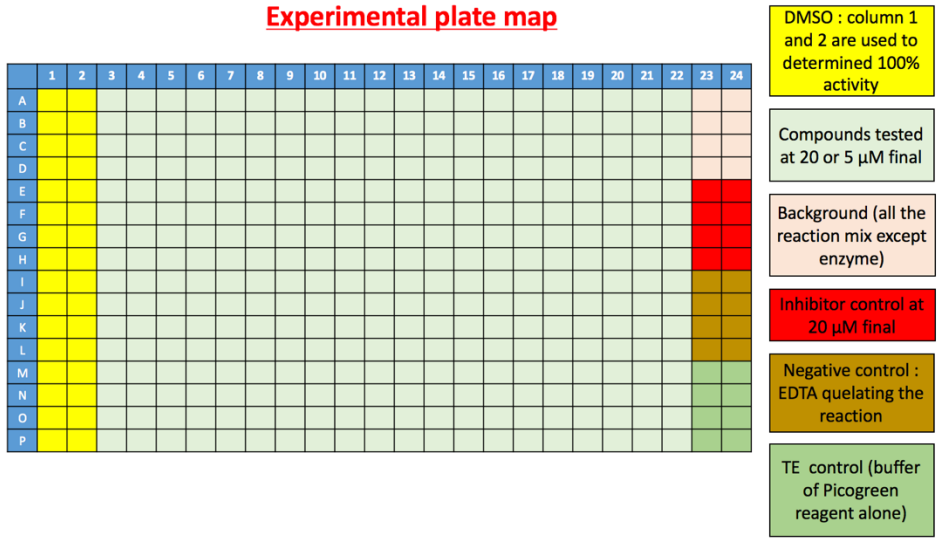
A summary of the purification experiments is shown below.



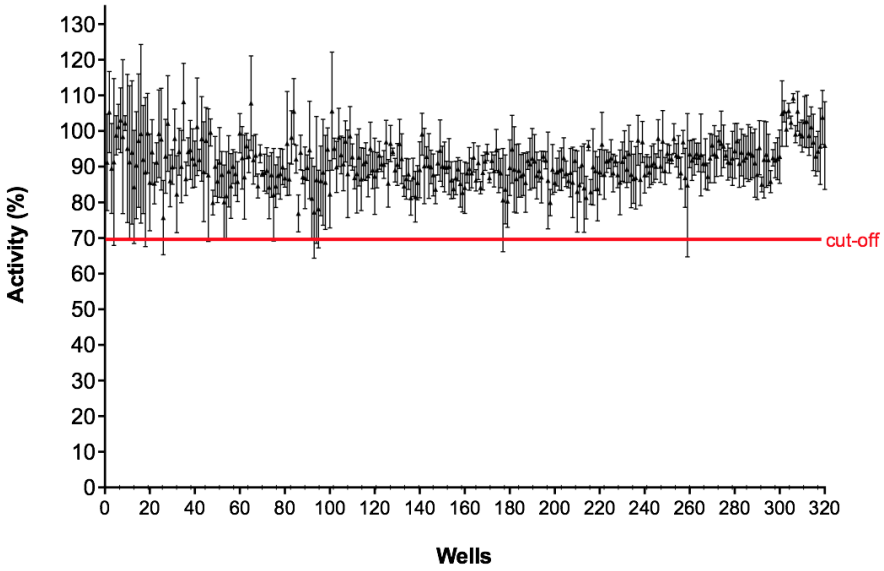
The screening assay is described below. Briefly, upon RdRp activity of the RTC complex, double stranded RNA is created, and the Picogreen intercalating agent acts as a fluorescent reporter since is glows only after insertion inside the dsRNA helix.



To provide a standardized protocol to our partners, we use the following plate design:



A typical result with DMSO controls is shown below, and used to evaluate a statistical parameters Z' > 0.75, which validates the assay:



Not shown on this result is the inhibition power of one control, the Hinikoflavone, which provides inhibition of 99.6% ± 0.6 at 20 µM. Likewise, nucleotide triphosphates provided by P18-UHAM were used to calibrate assays.

Discussion & Conclusion

We have designed a 384 well plate assay suitable for medium HTS screening. Presently, the typical output can be sustained at 30,000 compounds per month. Larger HTS will require adaptation to 1536 well plates.
The typical throughput under 384 well plates described here is clearly meeting with objectives described in the CARE project (i.e., 3,000- to-5,000 compounds twice a year, for a total 10,000/year + side campaigns upon need by WP3 hit-to-lead development).
We have started screening campaign for CARE partners, they will be reported later.
The slow steps encountered during the project are of two types, unexpectedly:
1-There are administrative bottlenecks: It took longer-than-anticipated to set-up agreements and shipping of compounds.
2- There are unexpected delays in obtaining lab reagents and consumables (e.g., resin, plates, tips), due to the overall crisis situation. Orders usually completed within 2-3 days took sometimes more than 2 months.
In any case, we had no deviation from the original plan.