# The SARS-CoV-2 panel by Agena Bioscience is suitable for sample pooling: An example using the FACT strategy

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## INTRODUCTION

Globally COVID-19 has already infected over 23 million people and over 814,000 people have passed away. Many countries have limited the mobility of their residents and are looking for ways to increase testing options to contain the virus and open their country. To assist in this effort, Agena Bioscience has developed a genotyping panel for the detection of the SARS-CoV-2 virus on the MassARRAY® System. This panel provides a robust option to test for the presence of SARS-CoV-2 virus in human samples. The single well panel utilizes RNA extraction and a one-step RT-PCR reaction to reverse transcribe viral RNA into cDNA and amplify the nucleic acid material in the same reaction. This biochemistry is shown to work robustly with an input containing as few as 0.3-0.4 genome copy number equivalents (GCE) of the SARS-CoV-2 virus per µl (300-400 GCE/ml) of matrix tested. The high-throughput MassARRAY® System enables laboratories to process up to 6,122 samples per day with a single instrument. The panel has been tested with the 96 and 384 CPM as well as with the RS1000 spotting device. This case study describes the iPLEX®-based SARS-CoV-2 panel being a sensitive method to reliable be used for sample pooling strategy to fight the COVID-19 pandemic. The pooling concept FACT, or Frankfurt adjusted COVID-19 testing, demonstrated excellent performance to identify a single positive sample in pools of 5, 10 and 20 samples (Schmidt *et al.*, 2020, Hanel & Thurner, 2020). This pooling strategy will help to meet the high demand for testing in the mass coronavirus screening programs needed in the early identification and isolation of asymptomatic individuals.

## MATERIALS & METHODS

### SARS-CoV-2 Panel

The Agena Bioscience SARS-CoV-2 Panel consists of five SARS-CoV-2 specific assays - three in the *N* (nucleocapsid) region and two in the *ORF1ab* region - as well as a control assay for the MS2 phage, all multiplexed into a single reaction. Assay details are provided in Figure 1 and Table 1. The SARS-CoV-2 assays were designed based on the 165 available SARS-CoV-2 genomes on March 29, 2020. The MS2 assay is a control for the RNA extraction, reverse transcription and PCR amplification. The panel is tested according to the FDA Emergency Use Authorization Guideline (2020) for LoD determination, confirmation and clinical evaluation.

### Assay Design Verification (Inclusivity of SARS-CoV-2 genomes)

Alignment against a consensus sequence of the SARS-CoV-2 genome, generated using 10948 complete genomes available as of 18th August 2020, was performed. Conserved regions were identified from the consensus sequence and used to verify primer homology (two PCR primers and one extension primer) for each assay in the panel. All assay components showed 100% sequence homology to conserved SARS-CoV-2 regions except for the forward PCR primer for the SC2_N2 assay. According to the most recent sequencing data (08/18/2020), 19% of the SARS-CoV-2 sequences have a novel multi-nucleotide polymorphism that overlaps with the first three 5' end nucleotides of the SC2_N2 forward PCR primer (AAC vs. GGG at the 5' end). This results in 97.3% weighted PCR primer homology. However, since the mismatch is located at the 5' end of the PCR primer it will not affect the test performance because 5' end mismatches are tolerated by the PCR enzyme.

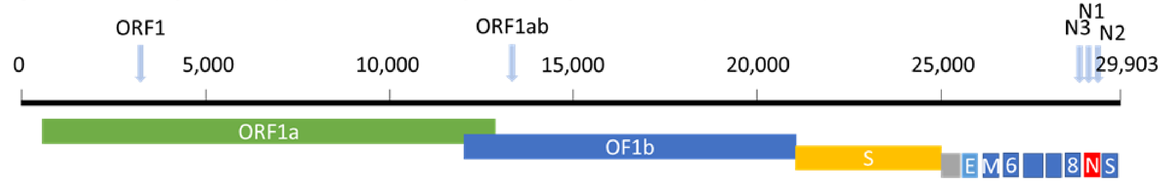
### Assay Design Verification (Cross-reactivity vs. selected other micro-organisms)

NCBI nucleotide BLAST was used to test for cross-reactivity of the primers *in silico* using a list of FDA-recommended micro-organisms (see Appendix A). The combination of primers for each assay (two PCR primers and one extension primer) exhibit 100% sequence homology to the conserved SARS-CoV-2 regions. However, individual primers (one of two PCR primers and/or the extension primer) for three of the assays exhibit higher than the 80% homology to a cross-reactive species. As the reverse PCR primer did have <80% homology vs. any of the tested micro-organisms, the reverse transcription should be specific for SARS-CoV-2 only. The individual primers will not create a template for extension, the likelihood of false positive results is extremely unlikely in each of these cross-reactive cases.

| *Assay name* | *Region* | *Genome area covered* |
|---|---|---|
| *SC2-N1* | Nucleocapsid | 28,653-28,760 |
| *SC2-N2* | Nucleocapsid | 28,880-28,978 |
| *SC2-N3* | Nucleocapsid | 28,076-28,190 |
| *SC2-ORF1* | ORF1ab/nsp3 | 3,223-3,335 |
| *SC2-ORF1ab* | ORF1ab/nsp10 | 13,342-13,432 |
| *MS2 phage* | | |

**Table 1:** Assays and their location on the SARS-CoV-2 genome

**Figure 1:** SARS-CoV-2 genome and approximate locations of targets in in the Agena SARS-CoV-2 panel




### Sample pooling strategy

Nasopharyngeal samples were taken with a swab and stored dry for transport. After arriving to the laboratory, first the swabs were placed in individual containers with 600 µl TE buffer to create an "individual reserve pool" after which 5 or 10 swabs were placed together in another container with 600 µl TE buffer making the pooled samples (Figure 2). Subsequently 300 µl each of the individual and the pooled samples were extracted using the 'Chemagic MSM I instrument' and the 'Chemagic Viral DNA/RNA 300 kit special H96' (Perkin Elmer) and tested using the SARS-CoV-2 panel according to the IFU (Agena Bioscience).

## RESULTS

The original (dry) swabs of 6 samples previously tested positive for SARS-CoV-2 with different Ct values (2 strong, 2 moderate and 2 rather weak positive samples) were "eluted" a second time in 600 µl TE buffer each, together with 4 or 9 SARS-CoV-2 negative samples. 10 µl MS2 RNA were added to each of these 6 pools before the RNA extraction. Then 3 µl of the extracted (pooled) RNA were used in the analysis according to the Agena protocol.

In parallel, the pooled RNA samples were also analyzed using RT-PCR. The positive SARS-CoV-2 sample could be reliably detected in all pools. Furthermore, the MS2 assay was also positive in all pools. As a comparison, the SARS-CoV-2 positive samples were also prepared and analyzed individually (without pooling) giving the same results. See Table 2 for results.

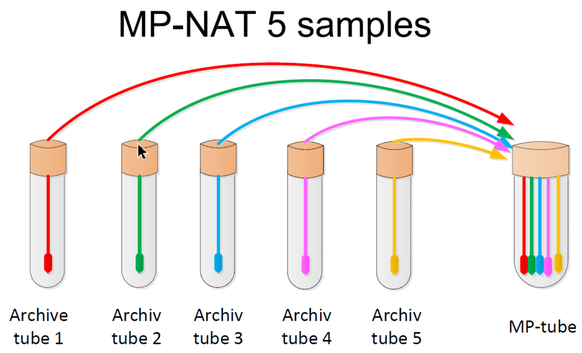
With this pooling concept, which is designed for dry smears, pools of 5 or 10 samples are created without affecting the sensitivity, since there is no dilution effect.

Based on calculations, in a population of 10,000 individuals, an infection rate of 1%, and a pool size of 10 this can reduce the testing from 10,000 individual tests down to 1,956 tests (1,000 pools and 956 individuals based on positive pools) for almost 5x higher efficiency.

### Key Takeaways

- The Agena SARS-CoV-2 Panel has a sensitivity down to 400 copies/ml
- Because of this high sensitivity it is ideally suited to be used for pooling strategies
- The current data shows that in pools of 5 or 10 samples the SARS-CoV-2 panel always gave the correct outcome showing the possibility to reduce the number of tests significantly.

**Figure 2:** The mini-pool method allows for increasing test capacities to detect the virus with no decrease in sensitivity while the individual samples are archived.




| Sample | Est. copy number/µl | Individual | Pooled | | IND-CPM | Pooled-CPM |
|---|---|---|---|---|---|---|
| 33 | 3000 | 21.5 | 21.9 | 5 | 5/5 assays detected | 5/5 assays detected |
| 96 | 1500 | 22.8 | 23.8 | 5 | 5/5 assays detected | 5/5 assays detected |
| 126 | 500 | 24.6 | 24.5 | 5 | 5/5 assays detected | 5/5 assays detected |
| 91 | 4000 | 21.1 | 23.6 | 10 | 5/5 assays detected | 5/5 assays detected |
| 129 | 900 | 23.6 | 24.8 | 10 | 5/5 assays detected | 5/5 assays detected |
| 188 | 260 | 25.6 | 24.3 | 10 | 5/5 assays detected | 5/5 assays detected |

**Table 2:** The sample results generated by RT-PCR (Individual and pooled columns) and CPM96 (IND-CPM and Pooled-CPM columns). Pooled column shows Pooled Ct and size of the pools.

## CONCLUSIONS

With this pooling concept, which is designed for dry smears, pools of 5 or 10 samples were successfully created without affecting the sensitivity. This may allow a more comprehensive screening and thus help in containing the spread of COVID-19

## REFERENCES

Hanel, R. & Thurner, S. Boosting test-efficiency by pooled testing strategies for SARS-CoV-2. (2020).

Schmidt, M. *et al. FACT- Frankfurt adjusted COVID-19 testing- a novel method enables high-throughput SARS-CoV-2 screening without loss of sensitivity. medRxiv* (2020). doi:10.1101/2020.04.28.20074187

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