The SARS-CoV-2 panel by Agena Bioscience®: Improved FAST cycling protocols allows for doubling the throughput

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INTRODUCTION

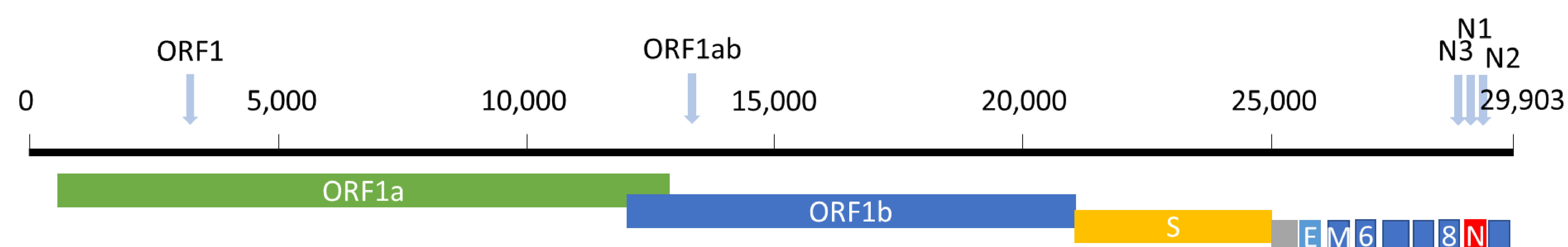
Globally COVID-19 has infected over 28 million people and over 900,000 people have passed away. Many countries have limited the mobility of their residents and are looking for ways to increase testing options to contain the virus and open their country. To assist in this effort, Agena Bioscience® has 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[1]. 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 plate format CPM instruments as well as with the RS1000 spotting device. This case study describes improvements in the biochemistry workflow of the iPLEX® Pro-based SARS-CoV-2 panel to reduce the time to result and increase the sample throughput and thus increase testing capacity to fight the COVID-19 pandemic. The FAST optimizations of the biochemistry workflow allows a reduction of 3 hours in workflow and thus increases throughput per day with the same amplification equipment.

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 quality control assay for the MS2 bacteriophage, 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 quality 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[2].

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



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

Alignment against a consensus sequence of the SARS-CoV-2 genome, generated using 10,948 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 minimally affects 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 bacteriophage		

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

WorkFlow Improvements

Nasopharyngeal samples were taken with a swab and stored dry for transport. Subsequently 600 µl TE buffer was added to each of the individual sample, then incubated at room temperature while shaken for 30 min after which 300 uL were extracted using the 'Chemagic MSM I instrument' and the 'Chemagic Viral DNA/RNA 300 kit special H96' (Perkin Elmer). Ten µl MS2 RNA were added to each of these samples before the RNA extraction. After extraction, the SARS-CoV-2 panel was further processed according to the standard protocol (Agena Bioscience) or processed using the cycling programs and steps as specified in Figure 2. Duplicate reactions using 3 µl of the extracted RNA were tested. The analysis was performed using the SARS-CoV-2 Reporter software (Agena Bioscience) .

RESULTS

Archived (dry) swabs of 8 samples previously tested positive for SARS-CoV-2 with different Ct values (4 strong, 2 moderate and 2 weak positive samples) and 8 previously tested Negative samples were tested using the standard protocol as well as a FAST protocol. In parallel, the RNA samples were also analyzed using RT-PCR. The positive SARS-CoV-2 sample could be reliably detected with both protocols. Furthermore, the MS2 assay was also positive in all samples. As is shown in table 2, all positive samples showed the same high consistency of 4 or more assays positive in each sample. All negative samples showed up negative in all cases. Therefore our PPA is 100% and our NPA is also 100%.

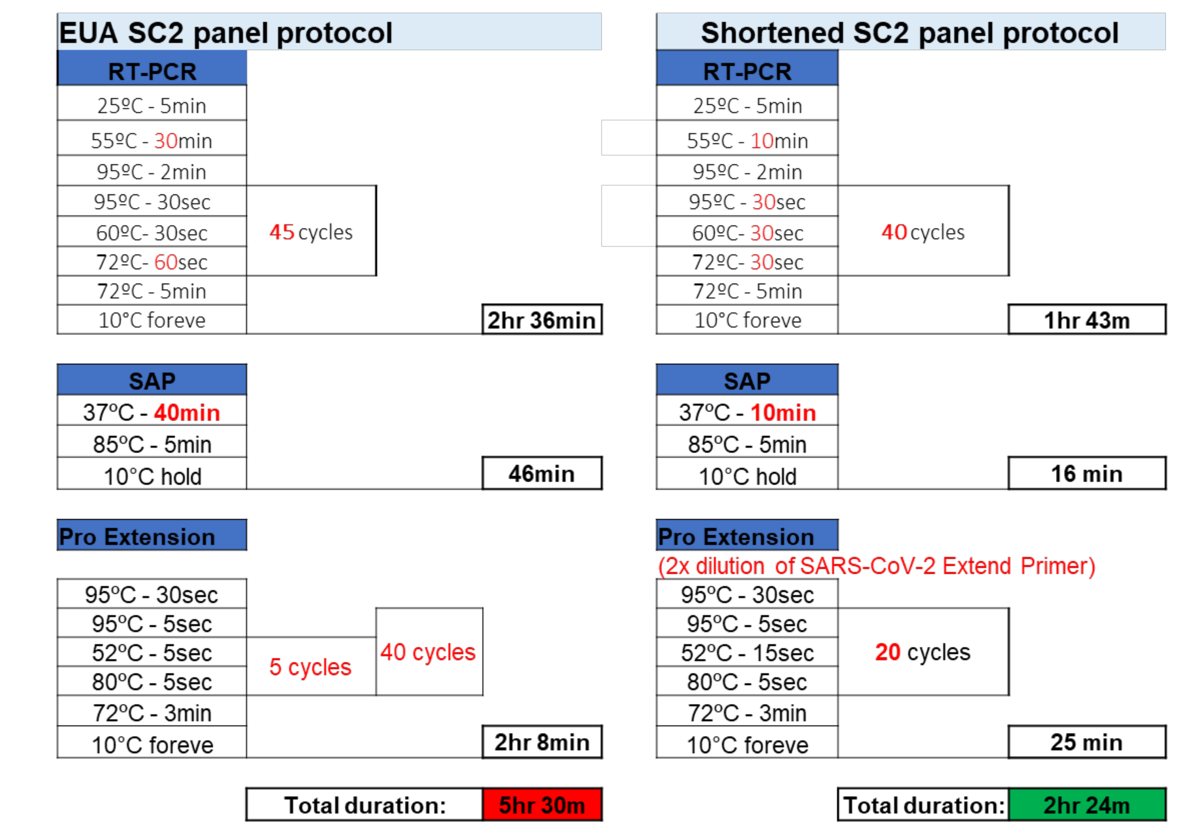
For the new workflow, the optimized cycle step duration and number of cycles for each step in the program are shown in Figure 2. In short, the RT-step was reduced by 20 minutes, and the PCR was optimized saving in total 50 min. SAP incubation was shortened by 30 min and the Extend program was shortened by 1:30 hr.

Based on the changes in the protocol as described, the biochemistry workflow is reduced from almost 6 hours to less than 3 hours. Practically this enables a doubling of sample throughput without adding thermal cycler instruments.

Key Takeaways

- The Agena SARS-CoV-2 Panel has a sensitivity down to 400 copies/ml
- The current data shows that with a FAST protocol the SARS-CoV-2 panel still provided the correct outcome, showing the possibility to speed up the sample to answer time by 3 hours
- Reducing the biochemistry from 6 hours to 3 hours allows the same amplification equipment to significantly improve throughput

Figure 2: Description of the standard IFU-based method and the FAST method as tested in this poster.



Sample	RT-PCR	STD-CPM1	STD-CPM2	FAST-CPM1	FAST-CPM2
10	20.45	5/5 assays detected	5/5 assays detected	5/5 assays detected	5/5 assays detected
11	21.49	5/5 assays detected	5/5 assays detected	5/5 assays detected	5/5 assays detected
52	25.20	5/5 assays detected	5/5 assays detected	5/5 assays detected	5/5 assays detected
60	28.96	5/5 assays detected	5/5 assays detected	5/5 assays detected	5/5 assays detected
72	20.80	5/5 assays detected	5/5 assays detected	5/5 assays detected	5/5 assays detected
86	28.72	5/5 assays detected	5/5 assays detected	4/5 assays detected	4/5 assays detected
109	17.34	5/5 assays detected	5/5 assays detected	5/5 assays detected	5/5 assays detected
111	21.90	5/5 assays detected	5/5 assays detected	5/5 assays detected	5/5 assays detected

Table 2: Comparison of Cq values and MassARRAY–based outcomes for the duplicates of all POS samples.

CONCLUSIONS

- With this improved FAST protocol, 8 positive and 8 negative clinical samples were successfully analyzed without affecting the sensitivity.
- This allows for a higher throughput, potentially up to double the current 6,122 samples per day, and thus help in containing the spread of COVID-19 by increasing testing capacity significantly.

REFERENCES

[1]Wandernoth, P. *et al.* Detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by Mass Spectrometry. Viruses (2020). doi:10.3390/v12080849

[2]FDA. Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised). (2020).