ALDATU BIOSCIENCES

PANDAA qDx™ SARS-CoV-2

Analytical and Clinical Performance

Emergency Use Authorization (EUA)
Validation Data Summary

1. PANDAA enables uniquely durable detection of SARS-CoV-2

In order to evaluate the long-term inclusivity and cross-reactivity of the primers and probes used in the PANDAA qDx™ SARS-CoV-2 assay, they must be considered in the context of the unique advancements of the PANDAA technology.

Limitations of Conventional qPCR

PCR and real-time PCR (qPCR)-based methodologies continue to be the gold standards in diagnostic virology, the clinical acceptability of which demands high sensitivity and specificity for the selected viral nucleic acid target(s). Analytical specificity comprises the inclusivity and exclusivity of a diagnostic to characterize the extent to which all viral phylogenetic variants are captured at the exclusion of the target's genetic near neighbors. This is facilitated by targeting primer and probe design to evolutionarily conserved regions through alignments of all available sequences and identifying geographical and temporal genomic variability.

Uncharacterized genetic variation and ongoing viral evolution significantly reduce traditionally-designed PCR diagnostic sensitivity when polymorphisms arise in the oligonucleotide-binding sites, leading to reduced assay efficiency or complete failure. Therefore, frequent in silico re-evaluation of primer and probe design is necessary to identify escape variants, which then requires assay redesign, optimization, and clinical validation, which is a significant time and economic burden and represents a bottleneck during an epidemic response.

Pan-Degenerate Amplification and Adaptation (PANDAA)

Pan-Degenerate Amplification and Adaptation (PANDAA), is a platform technology that addresses high genomic variability by normalizing probe-binding regions and has inherent sensitivity tolerance to *de novo* sequence diversity. PANDAA insures against performance issues associated with this *de novo* diversity, and maintains assay inclusivity to safeguard against false negative results while maintaining assay exclusivity (i.e. without sacrificing specificity). Using degenerate primers with fixed-sequence adaptor regions that overlap with the probe-binding site, the target genome is adapted through site-directed mutagenesis during the initial qPCR cycles to replace any secondary polymorphisms flanking the

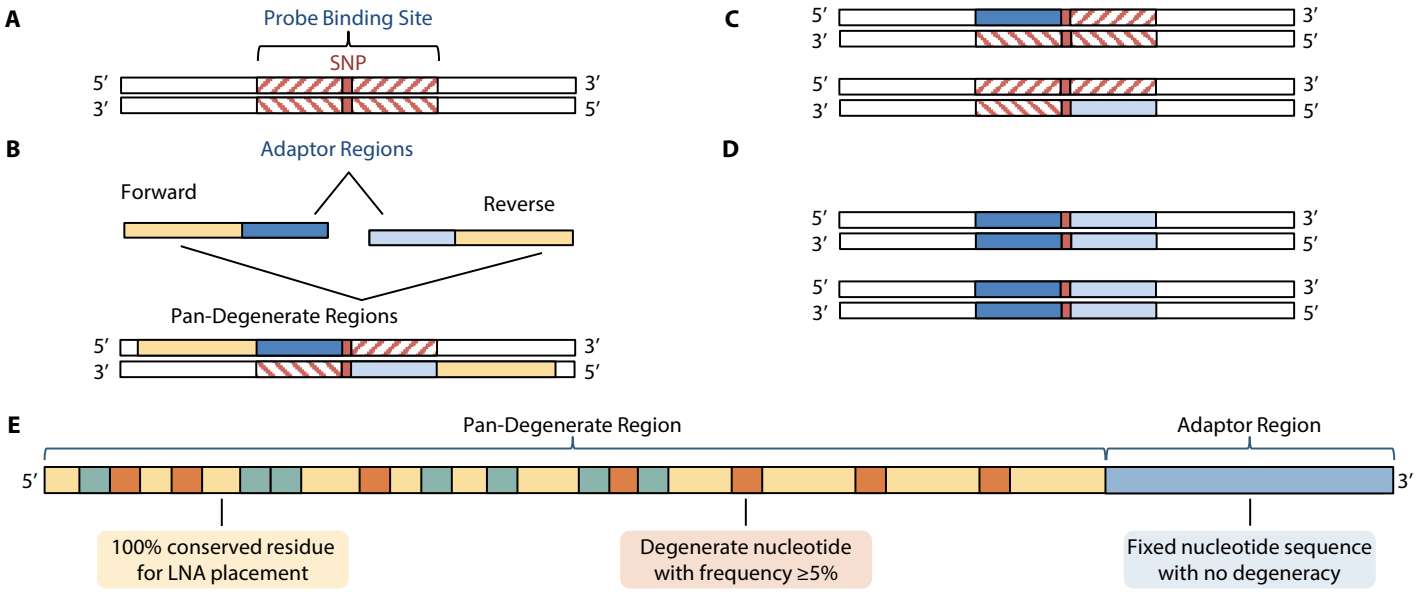

Figure 1: Overview of PANDAA method and primer design.(**A**) Heterogeneous genomes contain secondary polymorphisms within the probe-binding site, reducing diagnostic sensitivity. (**B**) PANDAA primers overlap with the probe-binding site, adapting secondary polymorphisms that would otherwise abrogate probe hybridization. (**C**) and (**D**) As qPCR proceeds, newly-generated amplicon will contain probe-binding sites that are perfectly complementary to the probe. (**E**) PANDAA primers contain two key features: a 3' adaptor region that is matched to the probe-binding site and a pan-degenerate (PDR) region that incorporates the nucleotide degeneracy observed in the primer-binding site of the target. The PDR is designed to account for the high degree of variability in primer-binding sites. LNA bases are incorporated into the primer 5' region at 100% conserved positions to offset the thermodynamic instability of mismatches between the primer ADRs and the template and further increase diagnostic specificity.

primary target sequence or SNP (**Figure 1**, A–D). This approach generates an amplicon population with a homogenous probe-binding site allowing for target detection with consensus probes.

PANDAA Diagnostics – A Proven Method

The PANDAA technology has been employed in a number of formats for HIV drug resistance genotyping. As the prototypic rapidly-evolving RNA virus, HIV-1 presents significant challenges to the design of qPCR-based diagnostics, which had been precluded from drug resistance genotyping in HIV clinical care due to the high rate of false negative results. Additionally, PANDAA has been used to develop the first pan-lineage diagnostic for Lassa virus, the causative agent of Lassa fever, which has substantial inter-lineage diversity.

The design of PANDAA qDx™ SARS-CoV-2 leverages the core features of the PANDAA technology to enable uniquely durable detection of SARS-CoV-2. With PANDAA's ability to mitigate the effects of *de novo* polymorphisms in the assay target site(s), it can insure against genetic changes that may arise as the SARS-CoV-2 virus continues to evolve, and uniquely preserve test performance in spite of those changes. As such, the PANDAA qDx™ SARS-CoV-2 test can offer distinct longevity and utility for COVID-19 testing.

2. Non-Clinical Performance Evaluations

Analytical Sensitivity (Limit of Detection)

Limit of Detection (LoD) studies were performed to determine the lowest detectable concentration of SARS-CoV-2 at which ≥95% of all true positive replicates test positive. Gamma-irradiated SARS-CoV-2 was obtained from BEI Resources at 1.7 x 10^9 genome equivalent (GE) copies / mL. Unless otherwise noted, all analytical sensitivity evaluations were performed by spiking SARS-CoV-2 into simulated clinical matrix (SCM) following the recipe as recommended by the US CDC, consisting of 2.5% (w/v) porcine mucin, 2.5% (v/v) human whole blood in 0.85% sodium chloride (NaCl) formulated in 1x PBS solution with 15% glycerol and 0.00002% w/v human genomic DNA, which was then diluted 1:5 in universal transport media (UTM).

KingFisher™ Flex / Apex Extraction with QuantStudio™ 3 and 5 Real-Time PCR Systems

Initial Evaluation of Limit of Detection (LoD)

SARS-CoV-2 was serially diluted in 400 µL simulated clinical matrix to target concentrations of 500, 400, 300, 200, 100, 75, 50 and 25 copies / mL. Extractions were performed on the KingFisher™ Apex (functionally equivalent to the KingFisher™ Flex) using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Thermo Fisher, Cat No. A42352) according to the manufacturer's instructions, using 400 µL sample volume and 75 µL elution volume. Five replicates of each extraction were performed at each concentration including the negative control (i.e. un-spiked simulated clinical matrix). Extracted nucleic acid was tested with the PANDAA qDx™ SARS-CoV-2 on the QuantStudio™ 3 and QuantStudio™ 5 Real-Time PCR Systems.

With the QuantStudio™ 3 Real-Time PCR System, 5/5 replicates were detected at 50 copies / mL; with the QuantStudio™ 5 Real-Time PCR System, 5/5 replicates were detected at 75 copies / mL and 4/5 replicates at 50 copies / mL (**Table 1**). Internal Control was detected in all reactions. The preliminary LoD was considered to be 50 – 75 copies / mL.

Table 1. Preliminary LoD of PANDAA qDx™ SARS-CoV-2 on the QuantStudio™ 3 and QuantStudio™ 5.

	QuantStudio 3			QuantStudio 5		
Concentration	**n**	**SARS-CoV-2 Median Ct (IQR)**	**Internal Control Median Ct (IQR)**	**n**	**SARS-CoV-2 Median Ct (IQR)**	**Internal Control Median Ct (IQR)**
500 copies / mL	5/5	21.25 (± 0.1)	17.84 (± 0.3)	5/5	21.64 (± 1.0)	17.77 (± 0.5)
400 copies / mL	5/5	21.20 (± 0.1)	17.55 (± 0.3)	5/5	21.70 (± 0.4)	17.75 (± 0.1)
300 copies / mL	5/5	22.08 (± 0.2)	17.86 (± 0.2)	5/5	23.12 (± 1.3)	17.73 (± 0.3)
200 copies / mL	5/5	22.92 (± 0.9)	17.83 (± 0.1)	5/5	23.36 (± 1.2)	17.78 (± 0.2)
100 copies / mL	5/5	23.91 (± 0.4)	17.75 (± 0.2)	5/5	23.67 (± 0.2)	17.68 (± 0.5)
75 copies / mL	5/5	24.15 (± 0.1)	17.56 (± 0.2)	5/5	24.58 (± 1.3)	17.61 (± 0.3)
50 copies / mL	5/5	24.94 (± 0.1)	17.78 (± 0.3)	4/5	24.86 (± 1.4)	18.19 (± 0.3)
25 copies / mL	3/5	25.21 (± 1.5)	18.06 (± 0.5)	2/5	28.17 (± 3.7)	18.20 (± 0.5)

Confirmation of Limit of Detection (LoD)

SARS-CoV-2 was serially diluted in 400 µL simulated clinical matrix to target concentrations of 75 and 50 copies / mL. 20 replicates of each extraction were performed at both concentrations including the negative control (i.e. un-spiked simulated clinical matrix). Extracted nucleic acid was tested with the PANDAA qDx™ SARS-CoV-2 on the QuantStudio™ 3 and QuantStudio™ 5 Real-Time PCR Systems.

With both the QuantStudio™ 3 and QuantStudio™ 5 Real-Time PCR Systems the lowest concentration at which ≥19 replicates were detected was 75 copies / mL (Table 2). Thus, the LoD for the Thermo FIsher MagMAX™ Viral/Pathogen Nucleic Acid Isolation kit on the KingFisher™ Flex / Apex automated extraction instruments was confirmed as 75 copies / mL for both the QuantStudio™ 3 and QuantStudio™ 5 Real-Time PCR Systems.

Table 2. LoD Confirmation of PANDAA qDx™ SARS-CoV-2 on the QuantStudio™ 3 and QuantStudio™ 5.

	QuantStudio 3			QuantStudio 5		
Concentration	**n**	**SARS-CoV-2 Median Ct (IQR)**	**Internal Control Median Ct (IQR)**	**n**	**SARS-CoV-2 Median Ct (IQR)**	**Internal Control Median Ct (IQR)**
75 copies / mL	20/20	24.46 (± 1.5)	17.94 (± 0.5)	20/20	24.41 (± 1.2)	18.20 (± 0.3)
50 copies / mL	12/20	25.02 (± 0.7)	17.95 (± 0.4)	17/20	25.11 (± 0.9)	17.92 (± 0.3)
Negative	0/20	-	18.42 (± 0.3)	0/20	-	18.46 (± 0.4)

PANDAA qDx™ SARS-CoV-2 as an LDA on the Abbott *m*2000 RealTi*me* System

The PANDAA qDx™ SARS-CoV-2 test was also validated on the Abbott *m*2000 RealTi*me* System in laboratory developed assay (LDA) mode. In all cases, LDA programs with the following parameters were used on the *m*2000*sp* sample preparation platform and *m*2000*rt* real-time PCR system:

- *m*2000*sp* Sample Extraction: 500 µL sample input, 200 µL dead volume; 90 µL elution
- *m*2000*sp* Master Mix Addition: 10 µL elution, 20 µL master mix
- *m*2000*rt* Protocol: PANDAA qDx SARS-COV LDA 2.3

Evaluation of Limit of Detection (LoD)

SARS-CoV-2 was serially diluted in 700 µL UTM to target concentrations of 500, 400, 300, 200, 100, 80, 60, 40 and 20 copies / mL. Eight replicates of each extraction were performed on the Abbott *m*2000*sp* sample preparation platform at each concentration including the negative control (i.e. un-spiked UTM). At the presumptive LoD of 100 copies / mL, 21 replicate extractions were performed. Extracted nucleic acid was tested with the PANDAA qDx™ SARS-CoV-2 on the Abbott m2000*rt* real-time PCR system.

With PANDAA qDx™ SARS-CoV-2 on the *m*2000*rt* real-time PCR system, 7/8 replicates were detected at 80 copies / mL; 21/21 replicates were detected at 100 copies / mL (**Table 3**). Internal Control was detected in all reactions. The LoD was therefore considered to be 100 copies / mL.

Table 3. LoD of PANDAA qDx™ SARS-CoV-2 as an LDA on the Abbott *m*2000 RealTi*me* System.

	PANDAA qDx™ SARS-CoV-2		
Concentration	**n**	**SARS-CoV-2 Median Ct (IQR)**	**Internal Control Median Ct (IQR)**
500 copies / mL	8 / 8	20.65 (± 0.6)	16.19 (± 0.3)
400 copies / mL	8 / 8	21.04 (± 0.7)	16.06 (± 0.2)
300 copies / mL	8 / 8	21.53 (± 0.3)	16.02 (± 0.4)
200 copies / mL	8 / 8	22.49 (± 1.4)	16.00 (± 0.2)
100 copies / mL	21 / 21	23.33 (± 0.9)	16.13 (± 0.1)
80 copies / mL	7 / 8	23.91 (± 0.7)	15.97 (± 0.2)
60 copies / mL	8 / 8	24.12 (± 1.4)	15.96 (± 0.2)
40 copies / mL	4 / 8	25.72 (± 2.8)	15.87 (± 0.3)
20 copies / mL	4 / 8	25.48 (± 0.7)	16.02 (± 0.1)

3. Inclusivity

Agreement with Published Sequences

PANDAA qDx™ SARS-CoV-2 primers and probes were aligned to 10,450 SARS-CoV-2 sequences obtained from GISAID and NCBI databases. **100% of published SARS-CoV-2 sequences are detectable with the two-target assay.**

N Gene Target

The PANDAA qDx™ SARS-CoV-2 forward primer had 100% identity with the *N* gene target site in 10,397 sequences. 53 sequences, primarily from the United Kingdom, had a single mismatch in the forward primer-binding site. Due to the two-target, one-channel design of the assay, together with the general features of the PANDAA primer design, this polymorphism in a very small fraction of forward primer-binding sites is not expected to reduce overall assay performance.

No mismatches were present in the reverse primer-binding site nor in the binding sites for the SARS-CoV-2-specific probe.

Oligonucleotide	Sequence Identity
Forward Primer	99.5% (n=10,397)
Reverse Primer	100% (n=10,450)
SARS-CoV-2 Probe	100% (n=10,450)

RdRp Gene Target

No mismatches were present in the forward or reverse primer-binding sites nor in the binding sites for the SARS-CoV-2-specific probe.

Oligonucleotide	Sequence Identity
Forward Primer	100% (n=10,450)
Reverse Primer	100% (n=10,450)
SARS-CoV-2 Probe	100% (n=10,450)

4. Cross-Reactivity

In Silico Analysis

The *in silico* analysis for possible cross-reactions with all the organisms listed below was conducted by mapping primers and probes used in the PANDAA qDx™ SARS-CoV-2 assay individually to the reference sequences available from NCBI (Table 4). If both the forward and reverse primers mapped to a sequence, then laboratory testing was performed. **No potential unintended cross reactivity is expected based on this in silico analysis.**

As >80% identity was found with one or more oligonucleotides to SARSr-CoV and MERS-CoV, then *in vitro* laboratory testing was performed to confirm that no cross-reactivity occurred (see **Table 5**, below).

Table 4: Percent Identity to SARS-CoV-2

Strain	Forward Primer	Probe	Reverse Primer
SARS coronavirus	91.9%	84.6%	90.3%
Middle East Respiratory Syndrome (MERS) coronavirus	48.6%	61.5%	80.6%
Human coronavirus 229E	43.2%	30.8%	51.6%
Human coronavirus HKU1	40.5%	61.5%	67.7%
Human coronavirus NL63	35.1%	46.2%	54.8%
Human coronavirus OC43	35.1%	61.5%	67.7%

Microorganisms with No Identity

No alignment was found for the following microorganisms:

- Adenovirus (Ad. 71)
- Bacillus anthracosis
- Bordetella pertussis
- Candida albicans
- Chlamydia pneumoniae
- Chlamydia psittaci
- Corynebacterium diphtheriae
- Coxiella burneti
- Enterovirus Type 71
- Haemophilus influenzae
- Human Metapneumovirus (hMPV)
- Influenza A (H1N1)
- Influenza A /(H3N2)
- Influenza B
- Legionella pneumophila
- Legionella non-pneumophila
- Leptospirosis
- *Moraxella cararrhalis*
- *Mycobacterium tuberculosis*
- *Mycoplasma pneumoniae*
- Neisseria elongate and meningitides
- Parainfluenza virus 1
- Parainfluenza virus 2
- Parainfluenza virus 3
- Parainfluenza virus 4
- Parechovirus
- Pneumocystis jirovecii
- Pseudomonas aeruginosa
- Respiratory syncytial virus
- *Staphylococcus epidermis*
- *Staphylococcus salivarius*
- *Streptococcus aureus*
- *Streptococcus pneumoniae*
- *Streptococcus pyrogenes*

In Vitro Analyses

Severe Acute Respiratory Syndrome-Related Coronavirus (SARSr-CoV)

Using *in vitro* transcribed RNA encoding the nucleocapsid gene, we confirmed that the SARS-CoV-2 specific probe of the PANDAA qDx™ SARS-CoV-2 assay did not hybridize to SARSr-CoV (**Table 5**, below).

Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

Using *in vitro* transcribed RNA encoding the nucleocapsid gene, we confirmed that the SARS-CoV-2 specific probe of the PANDAA qDx™ SARS-CoV-2 assay did not hybridize to MERS-CoV (**Table 5**, below).

Specificity Controls

We confirmed the high specificity of PANDAA qDx™ SARS-CoV-2 using two controls:

- a negative control of highly complex, non-target nucleic acid (human genomic DNA) to confirm that off-target amplification does not occur;
- a no template control (nuclease-free water) to confirm that non-specific product formation through primer dimerization in the absence of nucleic acid does not occur.

Both controls demonstrated that non-specific amplification does *not* occur.

Other Respiratory Pathogens

Cross reactivity of PANDAA qDx™ SARS-CoV-2 assay was evaluated *in vitro* by testing genomic material listed below in **Table 5**. Synthetic RNA for SARSr-CoV and MERS-CoV were generated in-house by Aldatu Biosciences. All other nucleic acid samples were obtained from BEI Resources and used at approximately 5×10^5 copies / reaction. No cross-reactivity was observed.

Table 5: PANDAA qDx™ SARS-CoV-2 Cross-Reactivity Summary

Microorganism	Result
SARS coronavirus (synthetic RNA)	0 / 24
MERS coronavirus (synthetic RNA)	0 / 24
Influenza A Virus, A/Victoria/361/2011 (H3N2)	0 / 6
Influenza A Virus, A/New Jersey/8/1976 (H1N1)	0 / 6
Influenza B Virus, B/Texas/06/2011 (Yamagata Lineage)	0 / 6
Influenza B Virus, B/Nevada/03/2011 (Victoria Lineage)	0 / 6
Human Respiratory Syncytial Virus, A2001/2-20	0 / 6
Human Coronavirus (HCoV), NL63	0 / 6
Alphacoronavirus 1, Purdue P115	0 / 6
Avian Coronavirus, Massachusetts	0 / 6
Porcine Respiratory Coronavirus, ISU-1	0 / 6
Human Metapneumovirus, TN/83-1211	0 / 6

5. Clinical Validation of PANDAA qDx™ SARS-CoV-2 as an LDA on the Abbott *m*2000 RealTi*m*e System

The performance of PANDAA qDx™ SARS-CoV-2 assay was validated with 178 nasopharyngeal swab clinical samples (88 prospective, 90 retrospective) collected from patients with signs and symptoms of an upper respiratory infection and suspected of COVID-19 disease. As the workflow with the highest LoD (vs. the Thermo Fisher workflow), the PANDAA qDx™ SARS-CoV-2 assay was clinically evaluated using the Abbott *m*2000 LDA sample extraction and real-time PCR workflow.

Samples were tested for SARS-CoV-2 at Tufts Medical Center (Boston, USA) with the Abbott RealTi*m*e SARS-CoV-2 assay and designated positive or negative based on the manufacturer's instructions.

Prospective Samples (n=88): We were blinded to results as prospective samples of unknown SARS-CoV-2 status were used. Sample extraction was initiated using the Abbott RealTime SARS-CoV-2 workflow, and then residual samples were immediately processed with the PANDAA qDx™ SARS-CoV-2 workflow.

Retrospective Samples (n=90): Samples were selected that had been tested within the previous 72 hours by the Abbott RealTi*m*e SARS-CoV-2 assay. These samples were then re-tested with the PANDAA qDx™ SARS-CoV-2 assay. 59 of these samples were called as positive by Abbott, and 31 of these samples were called as negative by Abbott.

Results from the prospective and retrospective sample panels were combined for clinical concordance analysis. The positive percent agreement (PPA) was 100% [95% CI: 83.9% - 100.0%] and negative percent agreement (NPA) was 98% [95% CI: 92.8% - 99.8%] (**Table 6**). Two prospective samples were excluded from the combined analysis as they were determined to be 0.3x and 0.04x of the PANDAA qDx™ SARS-CoV-2 LoD, based on the samples' target Ct values as determined by the Abbott RealTime SARS-CoV-2 assay; in a discordant sample analysis, PANDAA qDx™ SARS-CoV-2 results for both excluded samples were determined to be concordant with the CDC 2019-Novel Coronavirus Real Time RT-PCR Diagnostic Panel (negative by both the CDC assay and the PANDAA qDx™ SARS-CoV-2 assay).

Table 6. **PPA and NPA of the PANDAA qDx™ SARS-CoV-2 assay (Abbott *m*2000 LDA workflow); combined prospective and retrospective samples**

	Comparator [Positive]	Comparator [Negative]	Total
PANDAA qDx™ [Positive]	80	2	**82**
PANDAA qDx™ [Negative]	0	96	**96**
Total	**80**	**98**	

PPA	(80 / 80)	100.0% [95% CI: 95.5% - 100.0%]
NPA	(96 / 98)	98.0% [95% CI: 92.8% - 99.8%]