General Cross-validation plan

NYS SARS-CoV-2 assay: EZ1 to eMAG and easyMAG

LOD

Using the Qiagen DSP Viral extraction kit, 50µl of culture supernatant from the BEI isolate, was extracted and eluted into 60µl. 540µl of elution buffer was added to bring the total volume of the extracted viral RNA to 600µl. A serial dilution was performed of the RNA from 10^{-1} up to 10^{-9} and cross titrated against a quantified transcript of known concentration, designed by the Wadsworth Center and manufactured by Biosynthesis Inc.

The following extractions will be performed using 110µl of sample eluted into 110µl, using the bioMerieux easyMAG Generic protocol.

For the range finding experiments, 11µl of quantified RNA from the above culture extract will be spiked into previously lysed pooled sputum at:

Gene copies/ul	equivalent gene copies/5ul reaction of eluate
10E4	5,000
10E3	500
10E2	50
10E1	5
10E0	0.5
10E-1	0.05

Each of the dilutions will be set up, extracted in triplicate and tested on the Wadsworth EUA N1, N2 and RP targets.

It is anticipated that the LOD will fall in the range of 1-50.
Therefore, further testing will be performed at 50, 25, 5 and 1 gene copies per reaction, in triplicate, to fine tune the range-finding experiment.

Once the LOD is estimated, 60 sputum samples will be pre-lysed and spiked with viral RNA at the quantity of virus that is at the estimated LOD of the assay.

20 samples and a HEL control will be extracted on the bioMerieux easyMAG.
20 samples and a HEL will be extracted on the bioMerieux eMAG.
20 samples and a HEL will be extracted on the Qiagen EZ1 Advance XL.

From this the LOD for the easyMAG and eMAG will be compared to that of the Qiagen EZ1 Advance XL.

		gene copies per ul of RNA	gene copies of RNA per 11µl	gene copies of RNA per µl of extract	gene copies RNA per 5µl reaction	Est Real-time Ct
Range finding	Sample 1	10,000	110,000	1,000	5,000	26
	Sample 2	1,000	11,000	100	500	29
	Sample 3	100	1,100	10	50	32
	Sample 4	10	110	1	5	35
	Sample 5	1	11	0.10	0.50	38
	Sample 6	0.1	1	0.01	0.05	41
Fine tune	Sample 7	100	1100	10.00	50.00	32
	Sample 8	50	550	5.00	25.00	32
	Sample 9	25	275	2.50	12.50	32
	Sample 10	12.5	137.5	1.25	6.25	35
20 @LOD	Samples 1-20easy	10	110	1	5	35
	Samples 1-20 eMAG	10	110	1	5	35

	gene copies per ul of RNA	gene copies of RNA per 12µl	gene copies of RNA per µl of extract	gene copies RNA per 5µl reaction	Est Real-time Ct
Sample 1-20 EZ1	10	120	1	5	35

Clinical Evaluation

Extract 30 negative/non-reactive sputum specimens.

Extract 30 positive sputum specimens, spiked to produce the following viral load:

- 20 with 1-2x the LOD
- 5 with 3 x the LOD
- 5 with 4 x the LOD

The above 60 samples should be blinded and extracted on the easyMAG and eMAG.

Inclusivity

In-silico testing already performed by CDC.
Does this need to be repeated?

Cross-Reactivity

This will include empirical testing of the test assay against a large panel of agents that are causative of similar disease and/or likely to be found in the same specimen type(s). Pathogens will be tested at concentrations that represent the higher end of that found in biological samples, generally at least 10E6 per ml.
This will be a large range of respiratory agents, including:

- influenza viruses
- parainfluenza viruses
- respiratory adenoviruses (multiple subtypes)
- RSV
- hMPV
- rhinoviruses
- enteroviruses (multiple subtypes)
- various respiratory bacterial pathogens

For those agents that we do not have cultured samples (e.g. some seasonal coronaviruses), the absence of cross-reactivity will be demonstrated/inferred by in silico analysis and the absence of, or minimal homology, in large data sets from available sequence data for that agent.

Other agents commonly found in respiratory specimens that we would include in the above specificity analysis include viruses such as measles, CMV and EBV.