



b

	Pilot 1 - initial test of three viral BCM	Pilot 2 - Expanding number of samples and include Illumina 8-plex	Pilot 3 - continue with RVI-seq method and test laboratory cost savings	Production plate 1 RVI-seq and standard SPRI	Production plate 2 RVI-seq and standard SPRI
Number of clinical and synthetic samples used	veSEQ 8-plex (n32) RVI-seq 8-plex (n32) Illumina 3-plex (n32)	veSEQ 8-plex (n96) RVI-seq 8-plex (n96) Illumina 3-plex (n96) Illumina 8-plex (n96)	1X cDNA synthesis, standard SPRI (n48) 1.6X cDNA synthesis, standard SPRI (n48) 1.6X cDNA synthesis, stringent SPRI (n48) 1.6X cDNA synthesis, stringent SPRI (n48)	Repeat of Pilot 3 samples (n48) for reproducibility testing, plus additional clinical samples (n48)	Repeat of Pilot 3 samples (n48) for reproducibility testing, plus additional clinical samples (n48)
Clinical SARS-CoV-2 positive	8	22	16	16	16
Clinical SARS-CoV-2 negative	8	16	2	2	2
Twist HCoVs (NL63+OC43+229E)	4	4	4	4	4
Twist H3N2	5	5	5	5	5
Twist H1N1 + hPIV1 + SC2	6	6	6	6	6
Water negative	1	1	1	1	1
Twist SC2 + clinical SC2 positive (Omicron)		1			
NIBSC H3N2 + hPIV1		7			
NIBSC H1N1 + Rhinovirus		7			
NIBSC Flu B		5			
NIBSC RSV B		6			
NIBSC human Metapneumovirus		1			
NIBSC human		1			
NIBSC multi plex Flu+RSV+SARS-CoV-2		1			
Nasal swap negative		5			
Nasal swap negative + viral spike in		5			
ZeptoMetrix controls		2	2	2	2
Extraction control		1			
Twist SARS-CoV-2			7	7	7
NIBSC Commercial RSV A			5	5	5
NIBSC Commercial RSV B				4	4
Clinical Flu B				4	2
Clinical Flu A				23	9
Clinical hPIV 2,3,4				7	16
Clinical RSV A + B				8 + 1	
Clinical HCoV					13
Clinical Adenovirus + Extraction Controls					3 + 1

Figure S1 - Flowchart outlining bioinformatics pipeline and samples used for validating three viral bait capture methods (BCM).

a. The bioinformatics pipeline includes programs for short read taxonomic classification, reference mapping, de novo assembly, BAM file and contigs analysis tools. **b.** Samples include clinical samples of SARS-CoV-2 (SC2), influenza A (H1N1 and H3N2), influenza B, human parainfluenza viruses (hPIV 2, 3, and 4), respiratory syncytial viruses (RSV A and B), non-SARS human coronaviruses (hCoV), and adenovirus. Additionally, synthetic RNA from Twist and reference RNA from the National Institute for Biological Standards and Control (NIBSC), as well as samples from ZeptoMetrix NATtrol™ Respiratory Panel 2.1 (RP2.1) controls, were included. Abbreviations: SPRI = Solid-Phase Reversible Immobilisation.