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Comparison of extraction kits performance for SARS-CoV-2 detection in wastewater

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Abstract: A fundamental element of a successful SARS-Cov-2 sewage surveillance program is to accurately detect the presence of the virus within the sewage. As there is no standard method employed in sewage surveillance, understanding the performance of different extraction kits in the recovery of SARS-CoV-2 and the impact PCR inhibitors have on quantification is essential to minimise data variation originating from sample extraction. With many commercial RNA extraction kits available, the performance of three commonly used kits were evaluated for recovery of *in situ* SARS-CoV-2 from two South Australian wastewater matrices—a major metropolitan and regional centre.

Keywords: extraction; wastewater; SARS-CoV-2

Sewage surveillance programs for SARS-CoV-2 in wastewater have been utilised across different states of Australia as part of an ongoing support measure to help health authorities monitor the virus within the community. Firstly, to restrict, then manage and finally monitor the progression of the pandemic. With public presentations for standard diagnostic testing decreasing, active monitoring of SARS-CoV-2 in wastewater is becoming increasingly important to understand the incidence of SARS-CoV-2 within a community in order that health authorities can be duly prepared.

To better understand the performance of extraction kits to accurately detect the presence of the virus within the sewage, two wastewater matrices were investigated, a major metropolitan (Bolivar WWTP) and a regional centre (Port Augusta West WWTP). Samples were collected using two approaches reflective of that used in the SARS-CoV-2 sewage surveillance programme undertaken at SA Water—24-hour raw sewage liquid composites and membrane loaded passive samplers deployed over time (Schang *et al*. 2021). Liquid samples were pre-centrifuged, and the supernatant was pH adjusted with 2N hydrochloric acid within the range of 3-4 before being filtered through a membrane (Ahmed *et al*. 2020). Both liquid and passive samples were spiked with an internal recovery control, MS2. Concentrated samples were then processed using the following kits: Qiagen RNeasy PowerSoil Total RNA kit (PS), Qiagen RNeasy PowerMicrobiome Kit (PMB) and ThermoFisher MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit (MM), with modifications incorporated within the lysis step (e.g. addition of Zymo DNA/RNA Shield, phenol/chloroform/isoamyl alcohol etc). Extracts were quantified using the PerkinElmer SARS-CoV-2 Nucleic Acid detection kit and standards prepared utilizing the Twist Bioscience synthetic SARS-CoV-2 RNA control. Extractions were performed side by side to better compare the performance of the kits.

The addition of DNA/RNA shield to the lysis step was found to improve the recovery of SARS-CoV-2 across all kits, and this was more pronounced when using the PS kit, previously shown to result in poor recovery of the virus from wastewater samples (WaterRA, 2021). Inclusion or exclusion of a solid—pellet from pre-centrifugation—within the lysis step affected recoveries and was kit dependent, with the PS kit performing better with inclusion of the solid, whereas PMB and MM recoveries were improved without. Both PS and PMB were less impacted from PCR inhibitors, with little or no inhibition evident when 10-fold sample dilutions were undertaken on the

neat extracts. (Figure 1.1 and 1.2). However, for samples extracted using MM, significant inhibition was detected, with a 2-3 fold increase in SARS-CoV-2 detected in the dilutions after taking the dilution factor into consideration. This difference could likely be ascribed to the incorporation of patented Inhibitors Removal Technology® within the Qiagen kits, absent in the MM kit.

Overall, the MM kit had better recovery of SARS-CoV-2 from the samples tested, followed by PMB and PS (Table 1.1). The performance of PMB when compared to the MM kit was strongly influenced by sample matrix, with poorer recoveries for liquid samples collected from the major metropolitan WWTP Bolivar, but improved recovery from passive samples obtained from the same site. However, recoveries using PMB for both sample types (liquid and passive) collected from the regional centre were consistently less. Recoveries and inhibitor removal using the PS kit were consistently poorer across both matrices when compared to MM and PMB kits. Depending on equipment and reagent availability, the MM kit would be recommended for future sewage surveillance work of SARS-CoV-2, followed by the PMB kit. Under adverse conditions—such as the SARS-CoV-2 pandemic—the preferred choice of kit may not always be available; it is therefore essential that appropriate kits are sourced that give the sample matrix due consideration so sensitivity is not compromised.

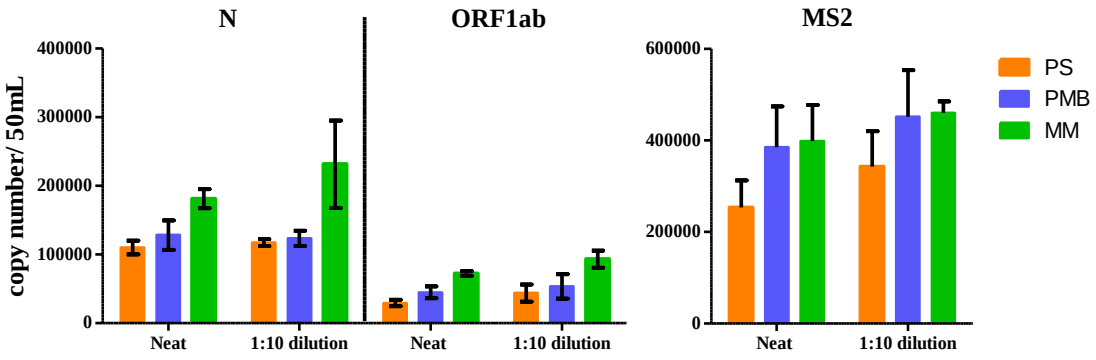

Figure 1.1 The impact of PCR inhibition on Bolivar WWTP liquid samples (experiment 1).

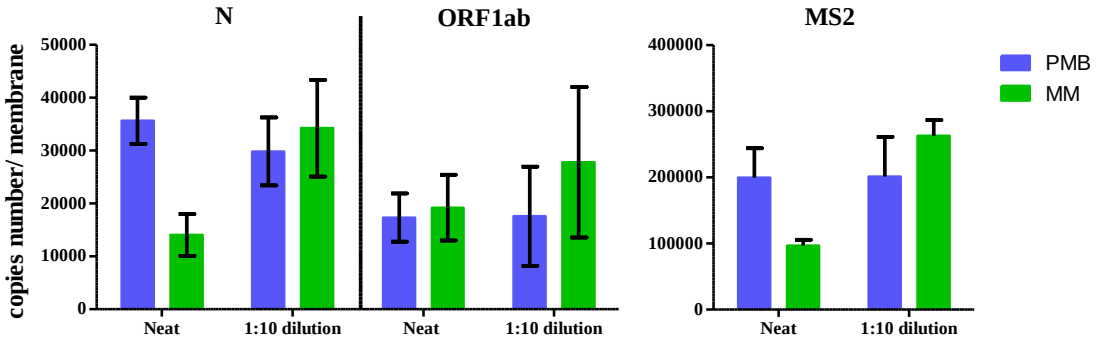

Figure 1.2 The impact of PCR inhibition on passive samples deployed at Bolivar WWTP.

Table 1.1 SARS-CoV-2 (N and ORF1ab) and recovery control (MS2) detection in gene copies per 50 mL or per membrane for the PS, PMB and MM extraction kit.

			PS		PMB		MM	
WWTP	Exp.	Gene	Mean	% CV	Mean	% CV	Mean	% CV
24 hrs composite (copies/ 50 mL)								
Bolivar	1	N	110093	9.17	128051	16.83	181208	7.78
		ORF1ab	29258	15.78	44592	18.97	72332	4.83
		MS2	253327	23.36	384534	23.33	398412	19.82
	2	N	62473	41.02	155166	7.57	173260	7.10
		ORF1ab	31044	47.24	56681	10.58	99512	8.49
		MS2	141742	32.52	468427	12.74	358628	4.13
PAW	1	N	179058	34.15	138567	6.01	354996	8.07
		ORF1ab	70371	26.03	28443	4.79	108960	14.16
		MS2	71769	10.80	207363	4.13	163520	8.14
	2	N	43644	15.64	12997	41.02	88364	12.07
		ORF1ab	16116	19.68	6528	42.31	47488	16.04
		MS2	53745	35.47	64313	55.00	101320	28.70
Passive - Laboratory (copies/ membrane)								
Bolivar	1	N	ND	ND	14040	49.39	7207	12.57
		ORF1ab			9245	23.71	4363	23.25
		MS2			440383	2.08	249108	9.85
	2	N	NT	NT	30800	48.73	22963	36.57
		ORF1ab			15186	18.89	11070	37.18
		MS2			197573	5.86	196188	32.83
	3	N	NT	NT	13844	9.65	8145	22.17
		ORF1ab			10148	13.39	5530	32.30
		MS2			308220	23.05	167131	7.58
PAW	1	N	NT	NT	10442	6.52	34513	12.49
		ORF1ab			6681	21.30	20542	2.54
		MS2			222173	5.12	243972	3.63
Passive - plant deployment (copies/ membrane)								
Bolivar	1	N	NT	NT	29827	21.58	34212	26.70
		ORF1ab			19266	40.15	36196	41.87
		MS2			199359	22.24	262896	9.12

*ND=Not Detected/Determined; NT=Not Tested

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