

# Contributing to surveillance of communities affected by SARS-CoV-2



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## First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community

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Please:

- Turn your video off during the presentation
- Mute your microphone
- Leave questions to the end

# HUGE THANKS TO (from acknowledgement)

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Margaret Butler and Helen Pennington (ACE, UQ)

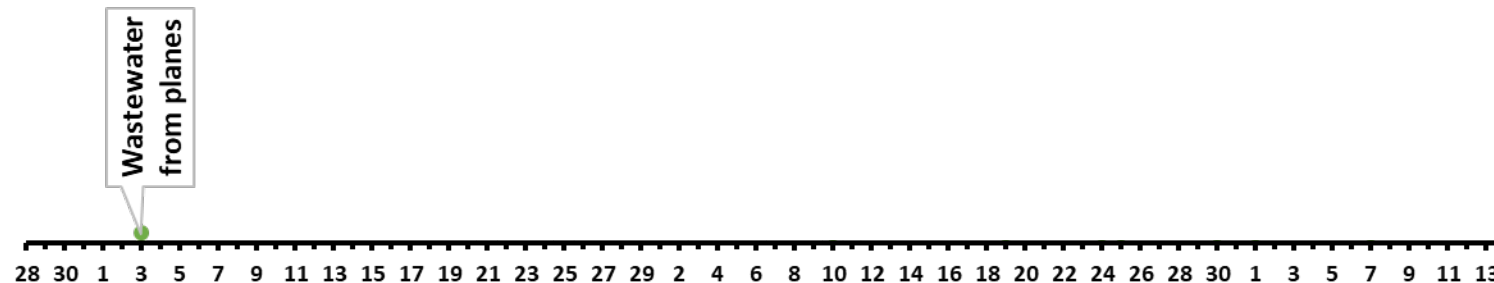
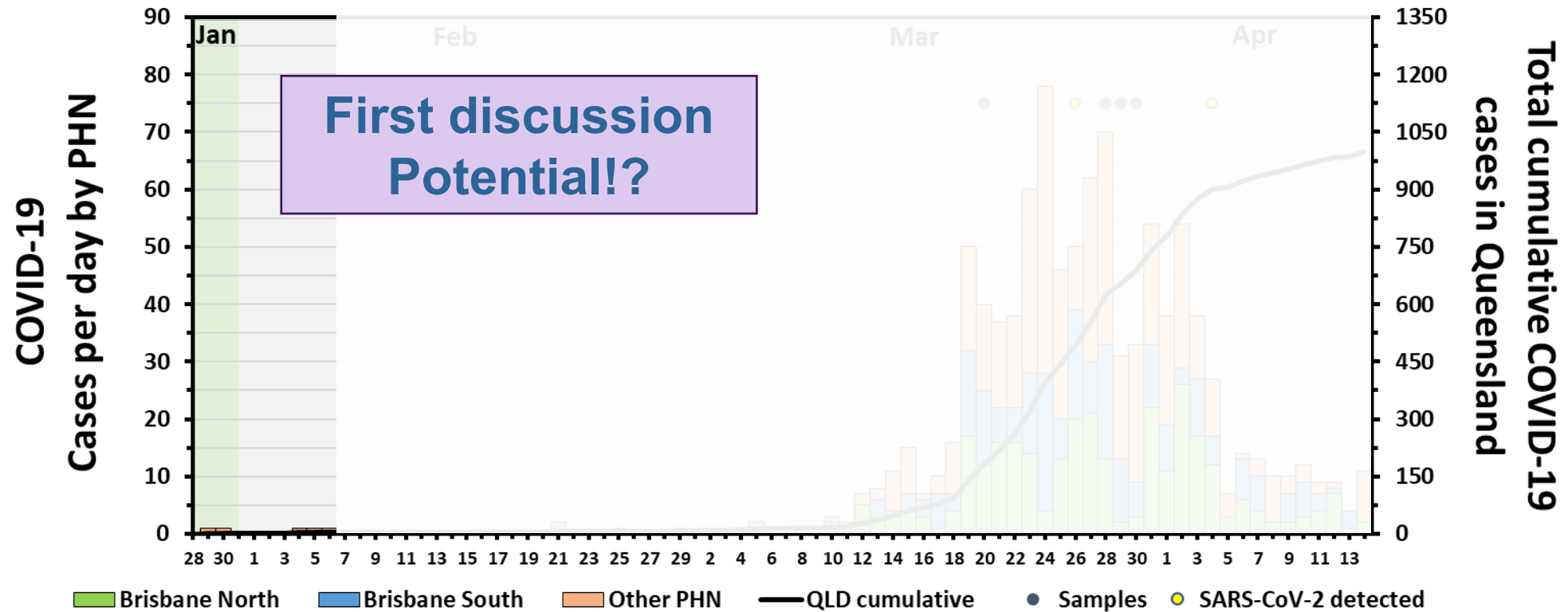
Ian Hosegood (Qantas)

Martyn Kirk (ANU)

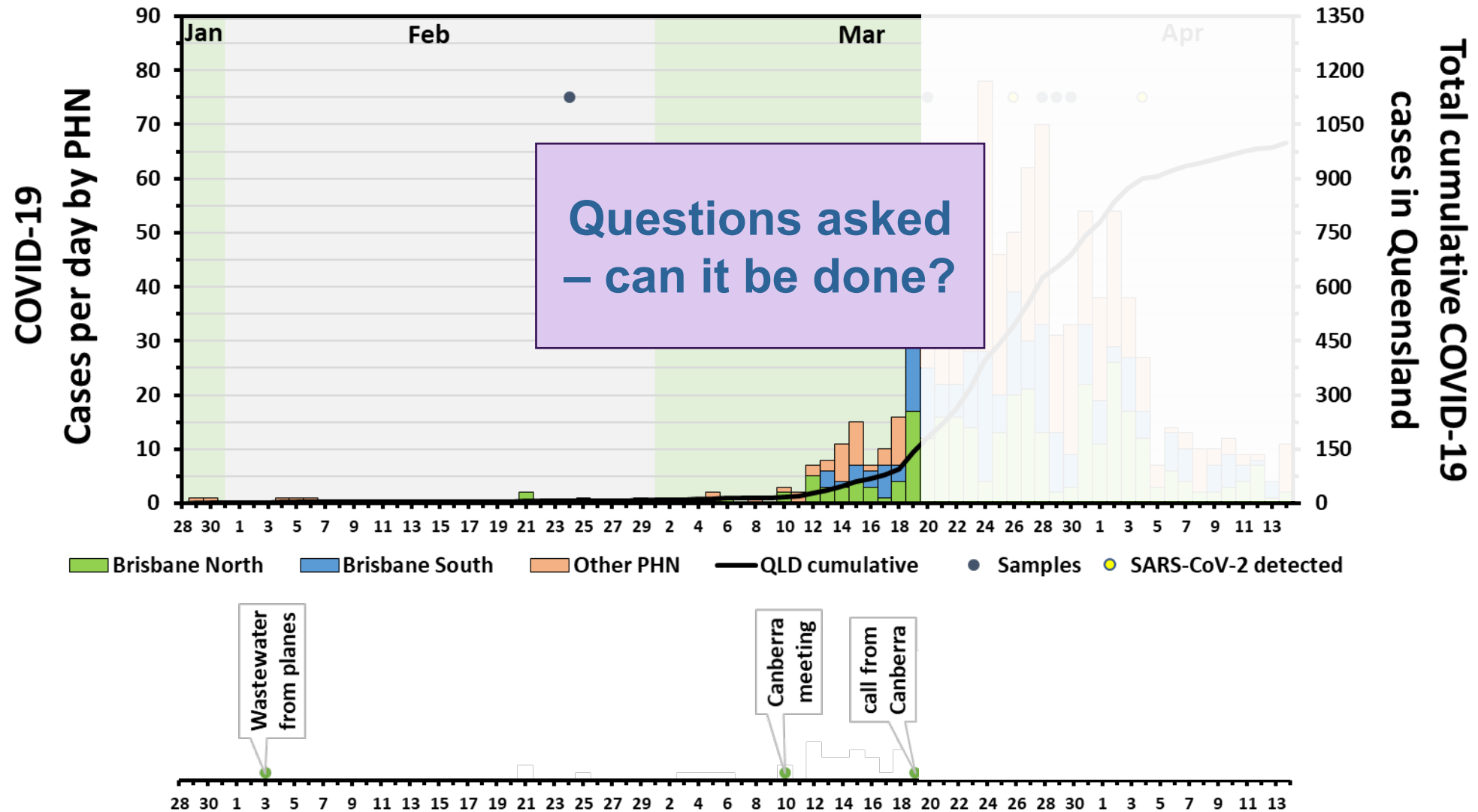
Nick Crosby (Melbourne Water)

Water Research Australia

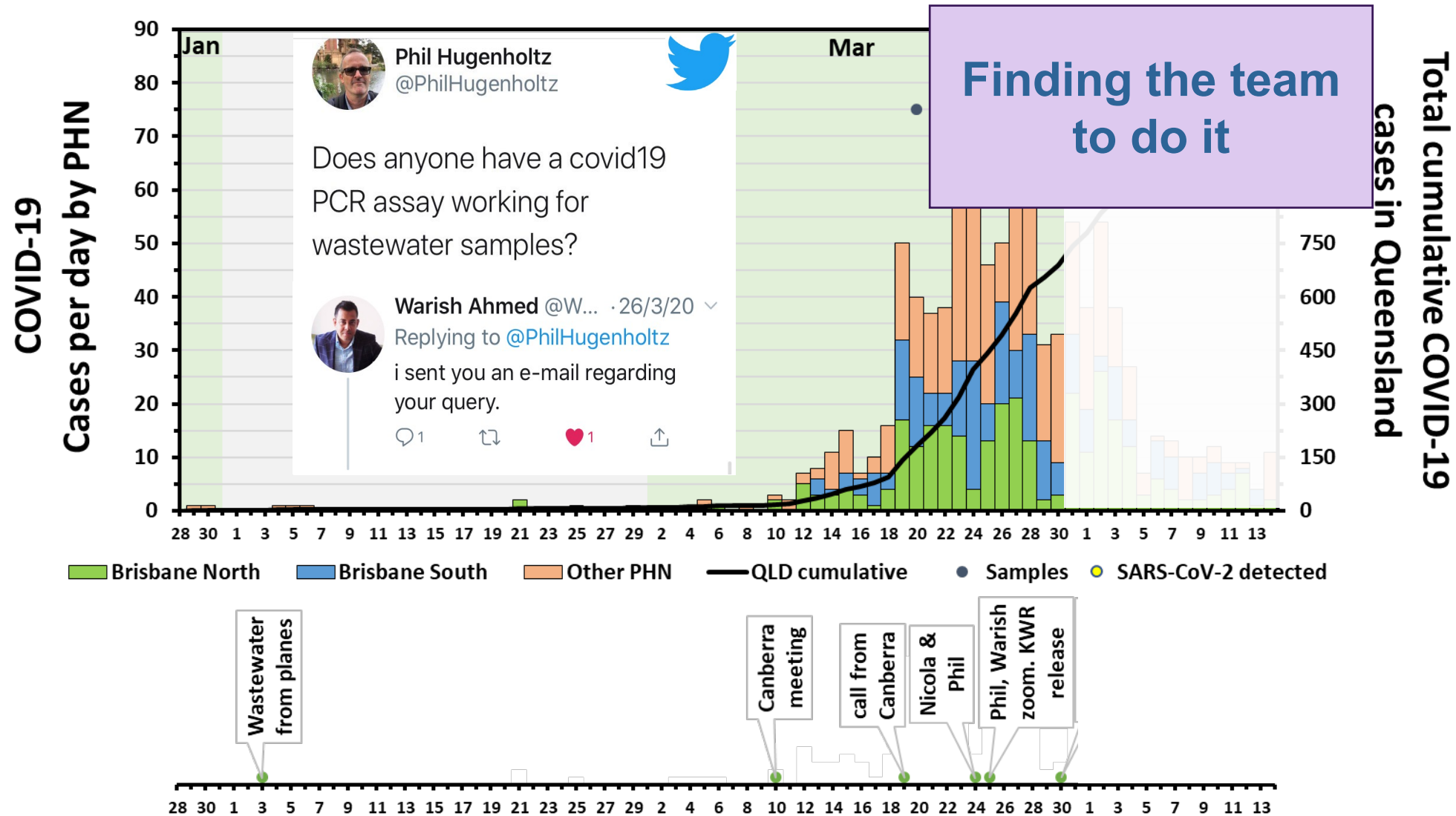
# How the project evolved – relative to Covid19 inf. in Qld



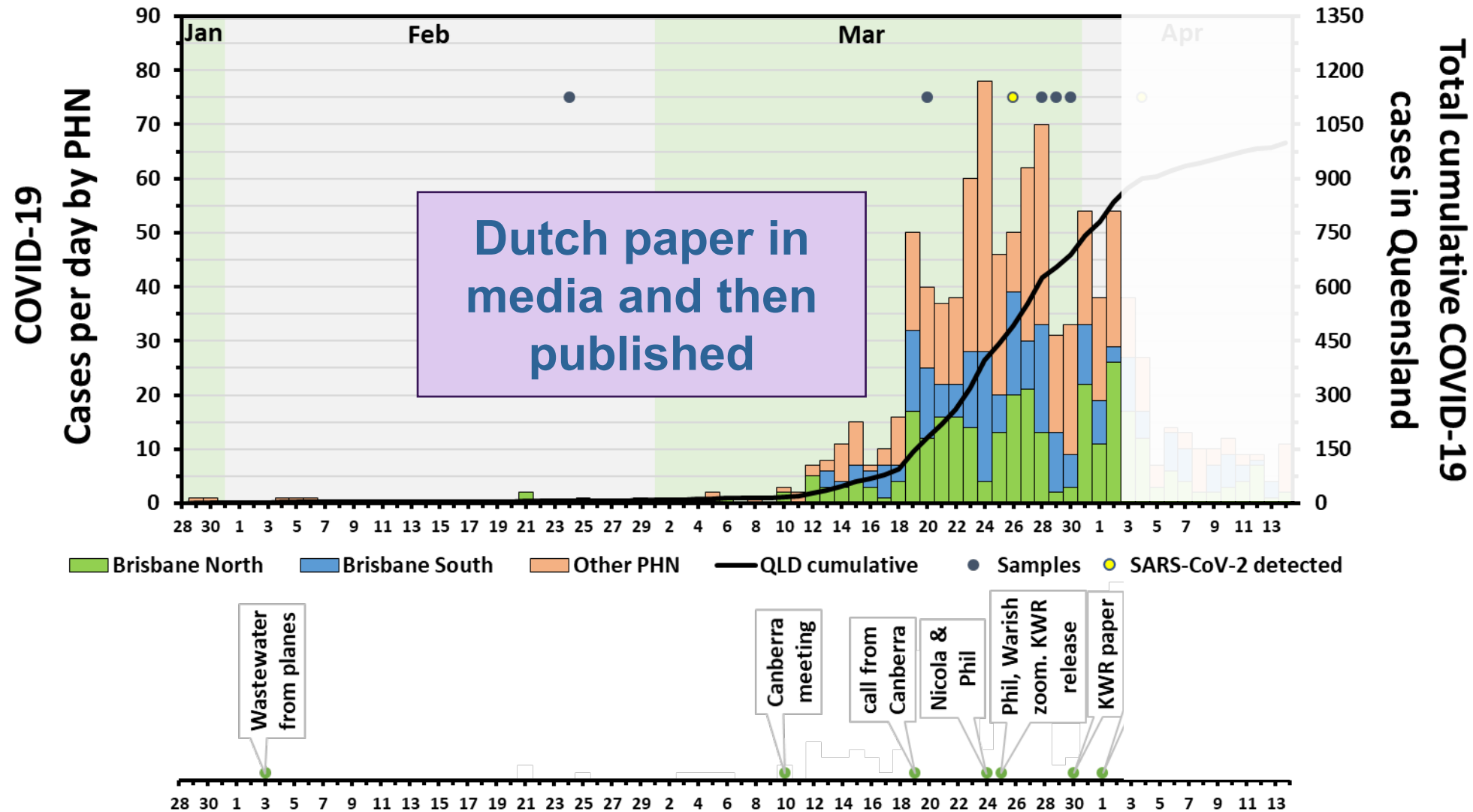
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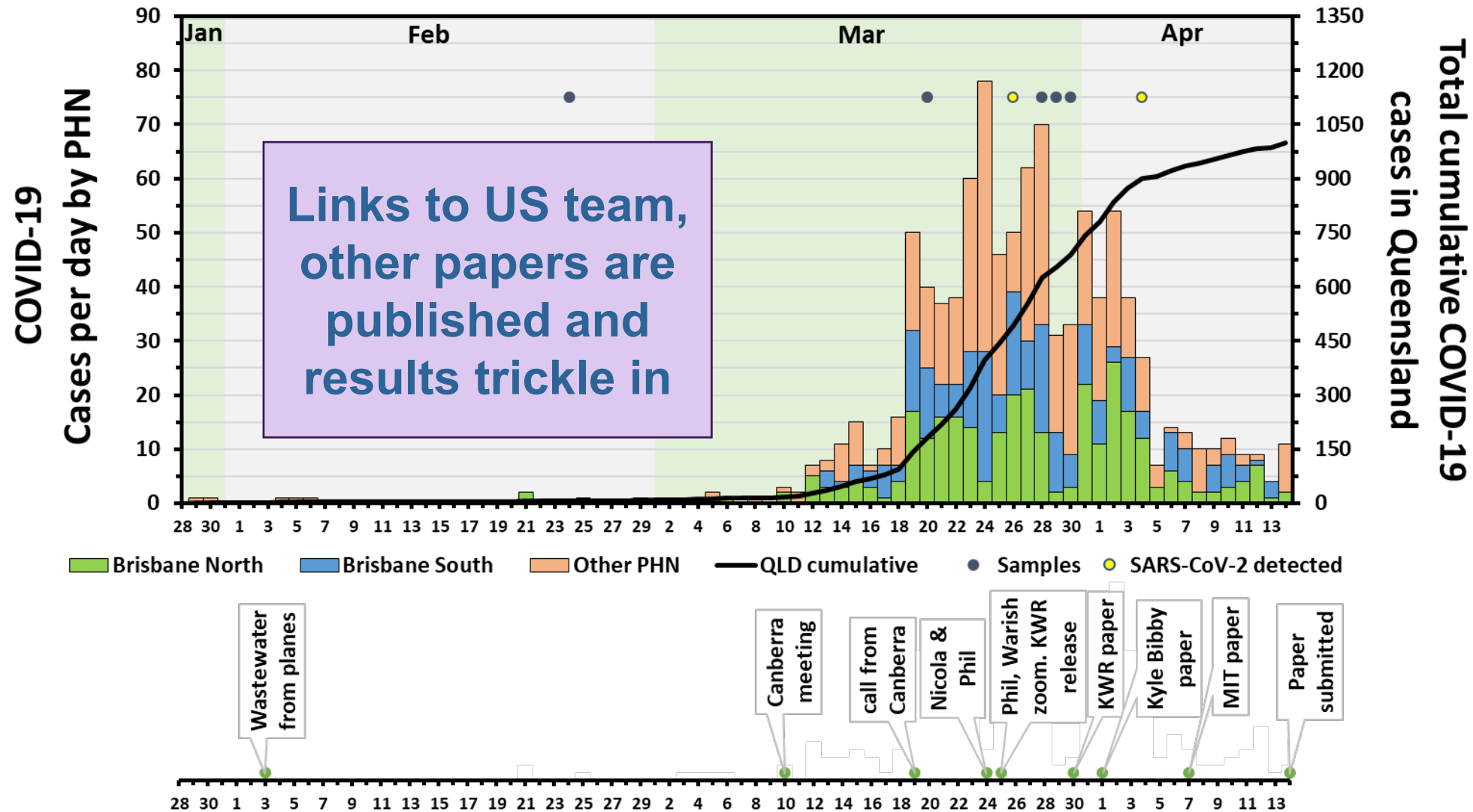
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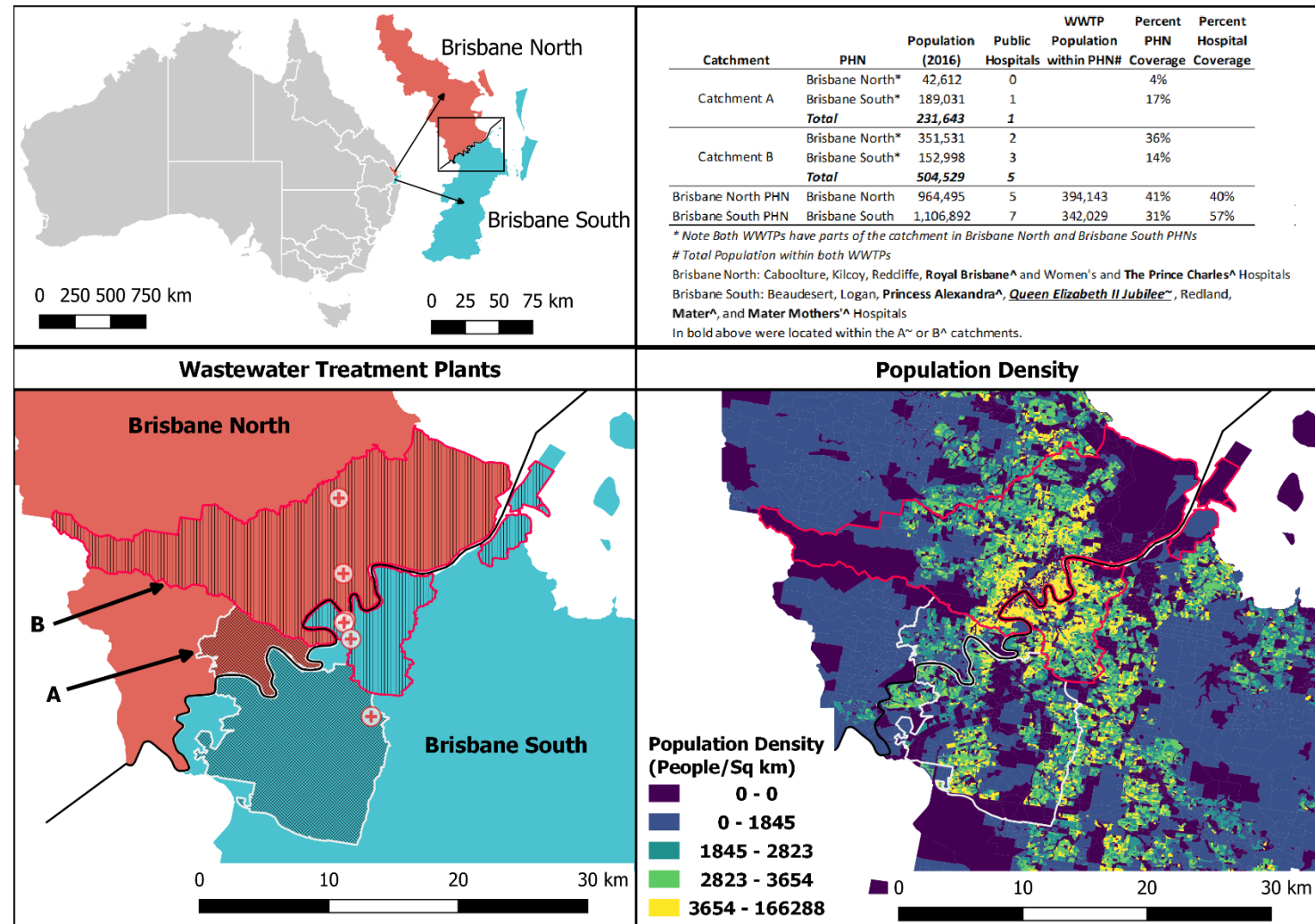




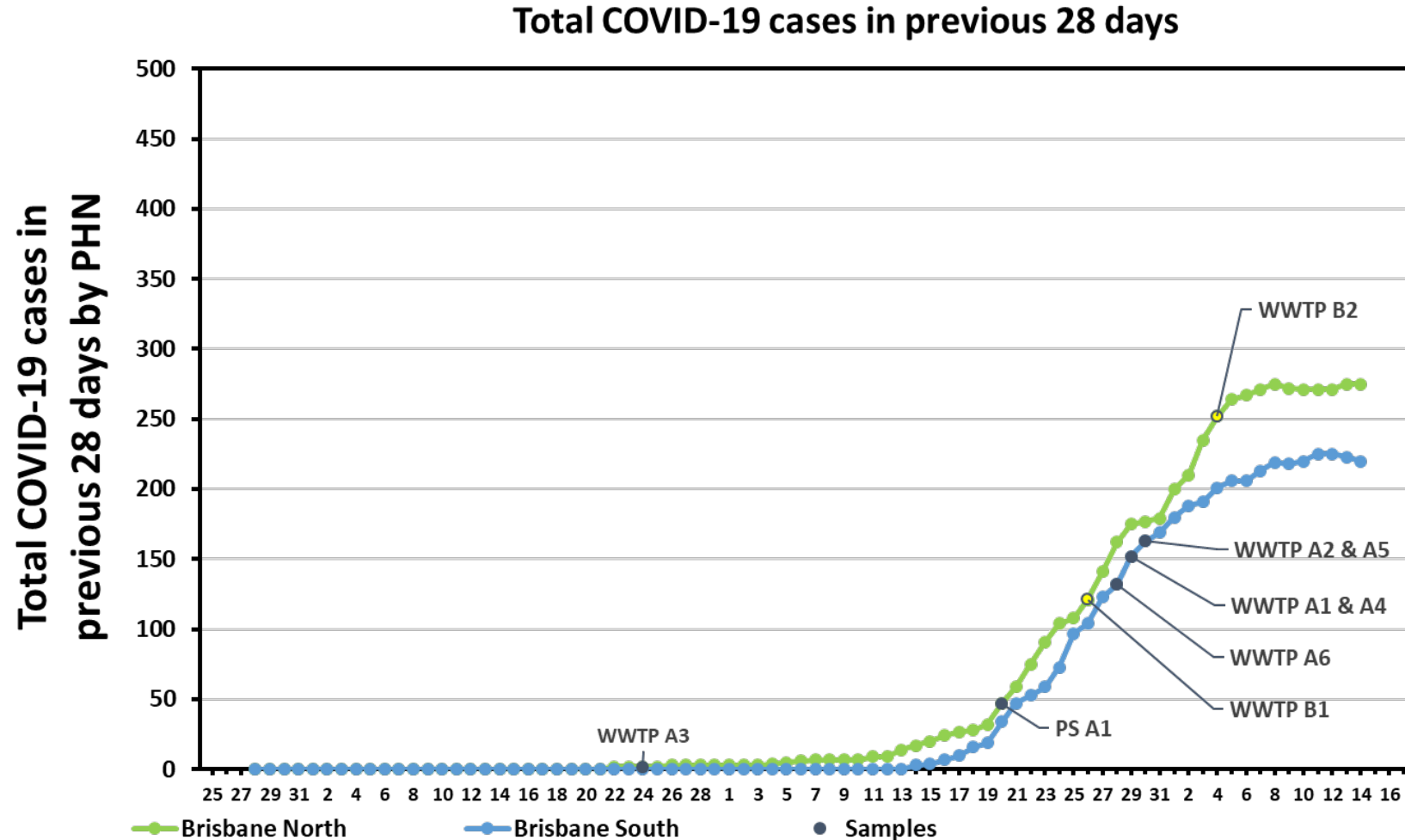
# Samples collected from 2 WWTPs and a Pumping Station

Catchments:

“WWTP” ≠ “Health”



# Samples collected during main 'growth phase'



Site A – autosampler

Site B and from pumping station we had to rely on grab samples

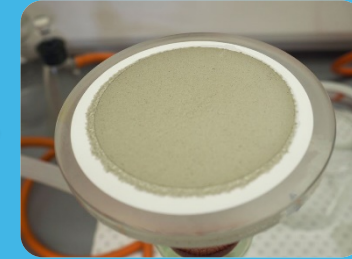
# RNA isolation from wastewater

Electronegative  
membrane

Filtration



Untreated  
wastewater



Captured viruses



Bead tube



Bead beating



RNeasy Power  
Microbiome



Extraction robot



RNA

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# RNA isolation from wastewater

Ultrafiltration  
filter



Untreated  
wastewater



Centricon 70



Centrifuge



Concentrate



Bead tube



RNeasy Power  
Microbiome



Extraction robot

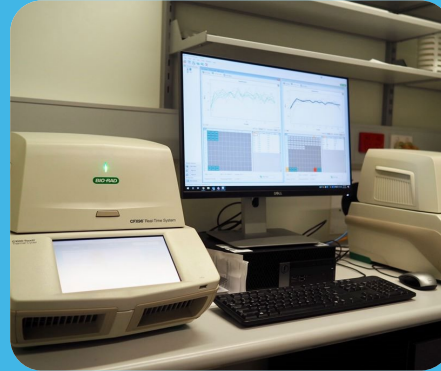


RNA

# RT-qPCR and sequencing



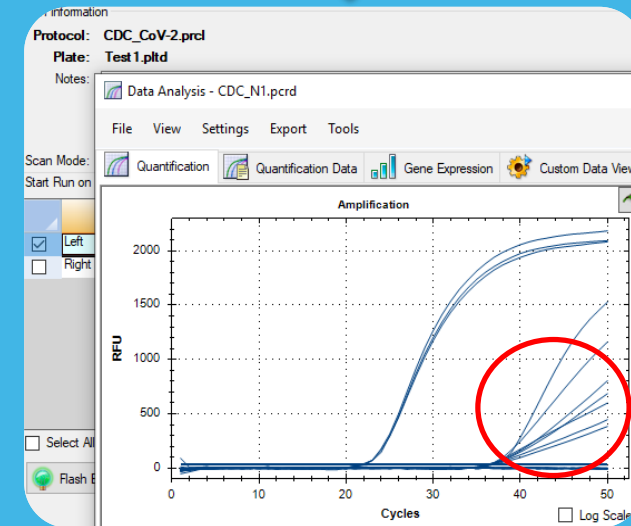
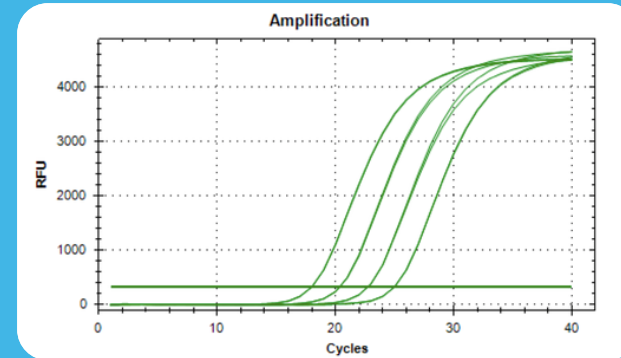
**Liquid Handler**



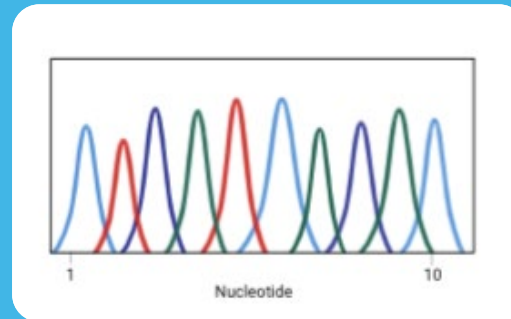
**Bio-Rad CFX-96**



**Standard curve**



**RT-PCR amplification**



**Sequencing**





# Prevalence of SARS-CoV-2 in wastewater

Sources of wastewater and sample ID	Sampling date	Concentration methods	
		Electronegative membrane	Ultrafiltration filter
PS	20/3/2020	ND	ND
WWTP A-1	24/02/2020	ND	ND
WWTP A-2	28/03/2020	ND	ND
WWTP A-3	29/03/2020	ND	ND
WWTP A-4	29/03/2020	ND	ND
WWTP A-5	30/03/2020	ND	ND
WWTP A-6	30/03/2020	ND	ND
WWTP B-1	26/03/2020	+ ~12 copies/100 mL	ND
WWTP B-2	01/04/2020	ND	+ ~2 copies/100 mL



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# Confirmation Sequencing of Positive Amplicon

## 1) Sanger Sequencing

- Requires clean amplicon
- Gel cut product
- F and R N\_Sarbeco Primers



## 2) Miseq

- Ligate illumine adapters to existing qPCR amplicon
- NEB UltraII Total RNA kit – from end repair step
- Sequence v3 300 cycle (150bp PE)
- Able to get sequence specific for possible SNPs



# Sanger Results

Query: None Query ID: lc1|Query\_59767 Length: 61

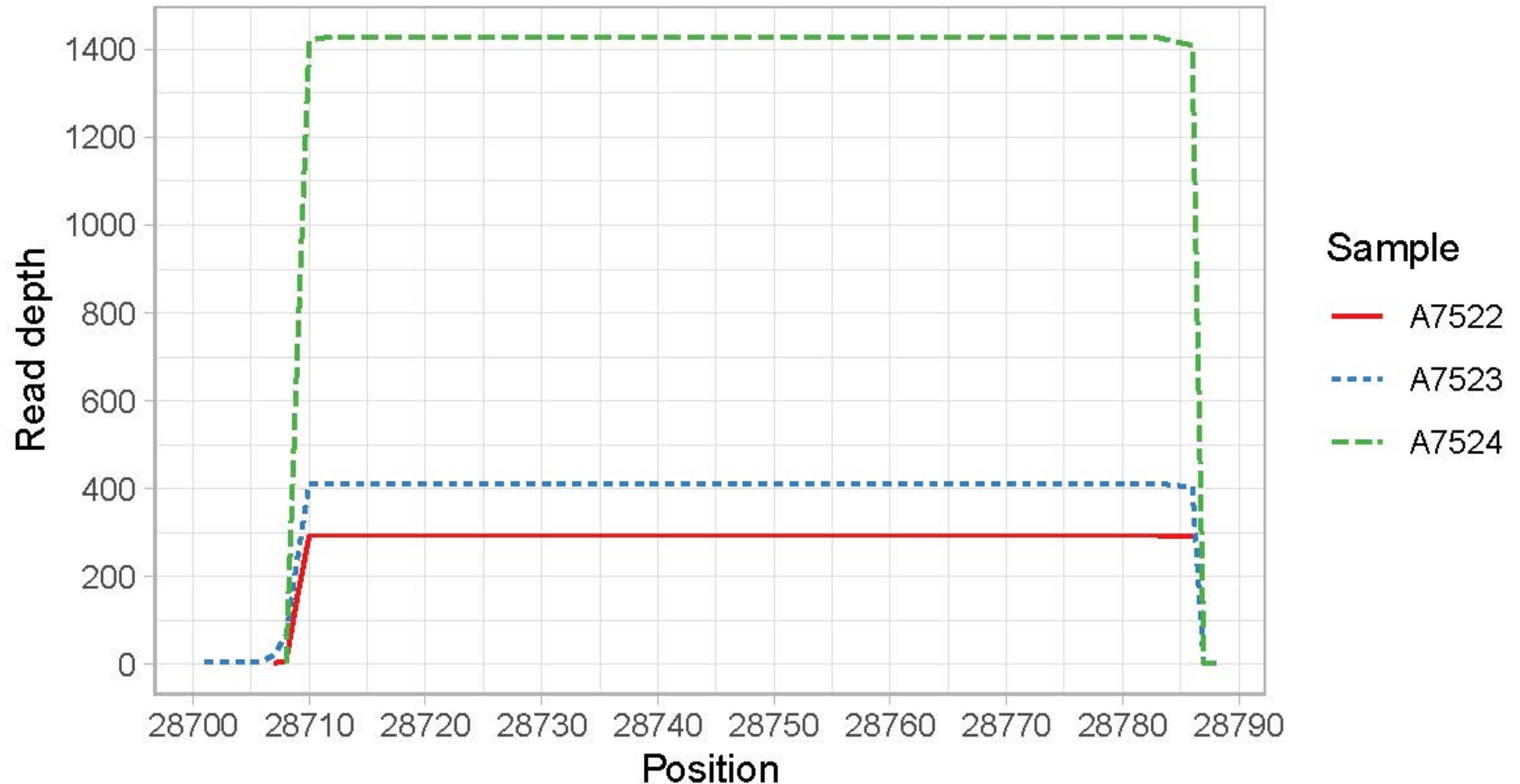
>Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/ISR\_IT0320/human/2020/ISR, complete genome  
Sequence ID: MT276598.1 Length: 29870  
Range 1: 28741 to 28786

Score:86.1 bits(46), Expect:1e-13,  
Identities:46/46(100%), Gaps:0/46(0%), Strand: Plus/Plus

Query	1	CTGCAATCGTGCTACAACCTTCCTCAAGGAACAACATTGCCAAAAGG	46
Sbjct	28741	CTGCAATCGTGCTACAACCTTCCTCAAGGAACAACATTGCCAAAAGG	28786



# MiSeq Results



MT276598.1 reference genome (SARS Cov\_2 whole genome, USA Isolate)

Aligned filtered reads

## Further research

- Both Sanger and MiSeq confirm product generated in qPCR
- Further evaluation of amplicon sequencing data: variants, compare to sensitivity of qPCR
- Use of sequencing to confirm qPCR – validation, different primer sets
- Evaluation of RNA quality in waste water
- Sequencing from RNA extracts
- Metagenomic approach
- Enrichment of SARS-Cov-2 fragments
- Other technologies e.g. direct RNA

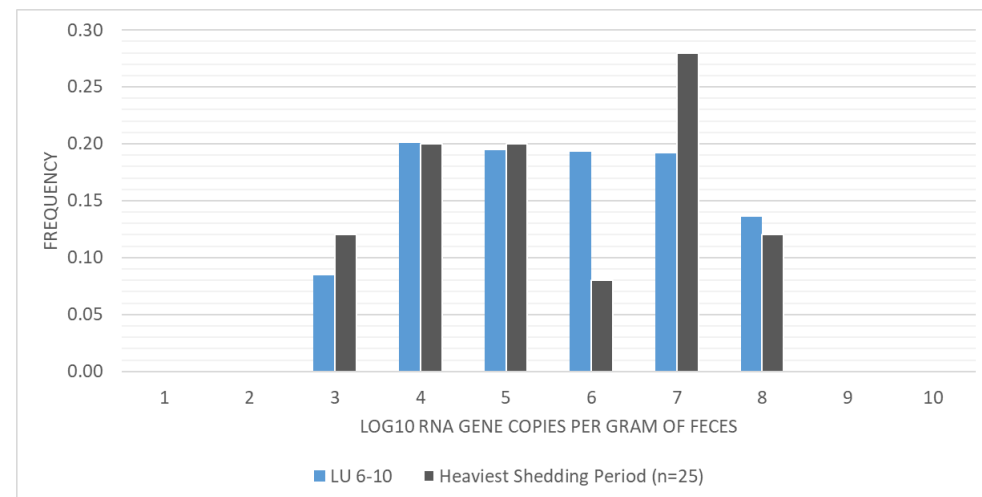
# Model Rationale: Mass Balance

$$\begin{array}{|c|} \hline \text{RNA gene} \\ \text{copies} \\ \text{per day in} \\ \text{WW} \\ \hline \end{array} = \begin{array}{|c|} \hline \text{RNA gene copies} \\ \text{per infected person} \\ \text{per day} \\ \hline \end{array} \times \begin{array}{|c|} \hline \text{Number of infected persons} \\ \text{shedding RNA} \\ \hline \end{array}$$

$$\text{Number of Persons Infected} = \frac{\left( \frac{\text{RNA copies}}{\text{liter WW}} \right) * \left( \frac{\text{liters WW}}{\text{day}} \right)}{\left( \frac{\text{g feces}}{\text{person} - \text{day}} \right) * \left( \frac{\text{RNA gene copies}}{\text{g feces}} \right)}$$

# Monte Carlo Parameters

RNA copies per liter of WW	RT-qPCR Results
Liters of WW per day	600,000 persons * 250 L/person-day
Log <sub>10</sub> g feces per person per day	Normal ( $\mu = 2.11$ , $\sigma = 0.25$ ) Median = 129 g/day
Log <sub>10</sub> RNA gene copies per g of feces	Uniform (min = 2.56, max = 7.67) Median = $1.3 \times 10^5$ gc/g



- Median number of infected persons and prevalence estimated by bootstrapping the Monte Carlo model (200 runs of 1,000 draws each)
- Sensitivity assessed by Spearman rank correlation from 10,000 draw model run

# Results, Uncertainty & Limitations

RNA copies/100 mL	Median number of shedding infections (95% CI)	Median prevalence of infection (%) (95% CI)
12	1100 (750 – 1500)	0.18 (0.12 – 0.25)
1.9	170 (120 – 230)	0.03 (0.02 – 0.04)
1.9 - 12	563 (390 – 760)	0.10 (0.06 – 0.14)

- Fecal mass is fit to data from many high-income countries and is not Australia specific (Rose *et al.* 2015)
- RNA gene copies per gram of stool is fit to clinical data for mildly ill 9 patients in Germany during the days of heaviest stool shedding (day 6 to 10 following onset) (Wolfel *et al.* 2020)
- Fecal shedding spans 5 orders of magnitude (equivalent prevalence scale 0.001% to 100%)
- Recovery not included (10% recovery would increase output 10-fold)
- Prevalence of fecal shedding among infected (27%-88%) not included

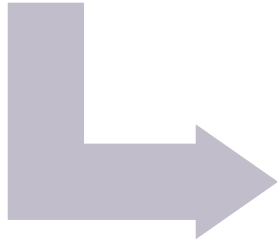
## Limitations (Note – this was a feasibility study)

- Few samples
- Few extraction methods
- Few primers
- New model
- Few data on virus shedding

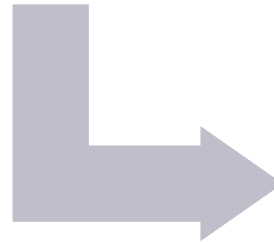
➔ Research fast and focused ➔ Rapid improvement

# Limitation and uncertainties may define application of where method is useful

Yes - No Application



Semi-Quant Application  
Spatio-temporal trend



Quantitative – Predicts  
infection in community

Work needed to get there

Thank you for your attention!

## Questions Please

(please raise your hand and we will invite you to ask your  
question  
– don't forget to unmute yourself!)