# Pooled Testing: The Uganda Experience

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Pooled testing is an option to address high demand of SARS COV-2 testing and unstable supply chain of test reagents

- Pool testing also known as group testing
  - Many samples are testing in one reaction ,saving on the number of tests
  - If a pool of 8 samples tests negative, all samples must be negative
  - If the pool is positive, all 8 samples must be repeated to identify the positive

- WHO included pool testing in the SARS-Cov-2 diagnostic guidelines
  - could be considered in population groups with a low/very low expected prevalence
  - but not for in clinical care and for contact tracing purposes is not recommended
  - must be validated in the appropriate populations and settings

- key constrains pooling to be aware of
  1) loss of sensitivity
  2) complexity may cause errors
  3) Results turn around time

32 Samples in 4 tests

Additional 8 tests
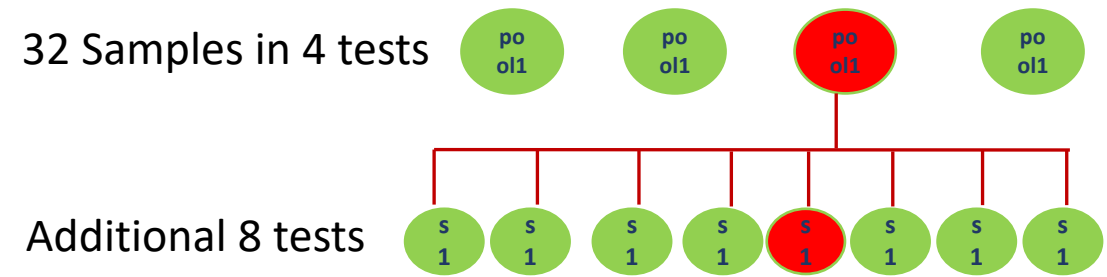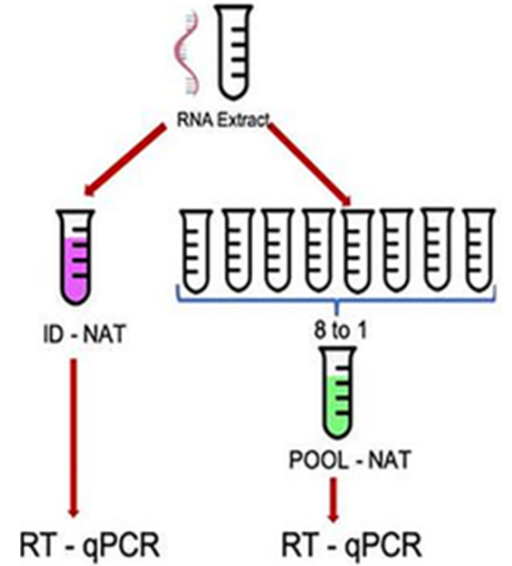
32 samples completed in 12 tests, with a saving of 75%

"Finally, when resource availability is sufficient to meet testing demand, consider whether the risks of reduced test sensitivity with pooling continue to outweigh the benefits of resource conservation" **WHO** SARS-Cov-2 diagnostic guidelines

Validation of Pooling on the Cobass8800 to determine the suitable pool size without losing the sensitivity of the test

Frequency distribution of CT values for
Positive SARS CoV-2 samples tested on Cobass8800

n= 283
median[max,min]= 29.55 [15.35,36.72]

No. of samples

CT value (ORF1a/b)
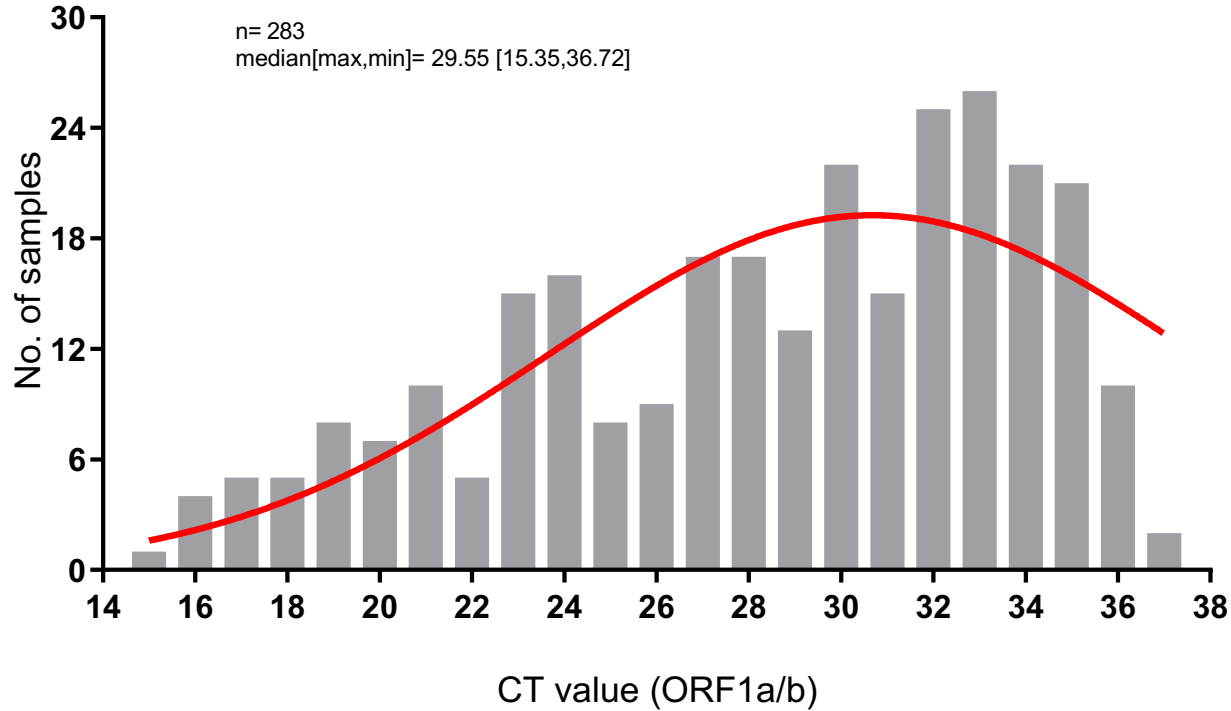
- Perfumed a analysis of the of the positive by the ct values

- Selected 40 positive samples with the bias for sample with high ct values
  - ct<30   10
  - ct 31-33   10
  - ct>34   20

- The samples included were tested within 48 hours prior to the pooling and were stored at fridge

- Positive samples were pooled with negative samples at 1/10, 1/5, 1/3

- The pool that registered a CT value for any of the two targets was considered positive

A pool of 5 samples results in loss of sensitivity of positive samples with CT value above 33 from 100% to 69%
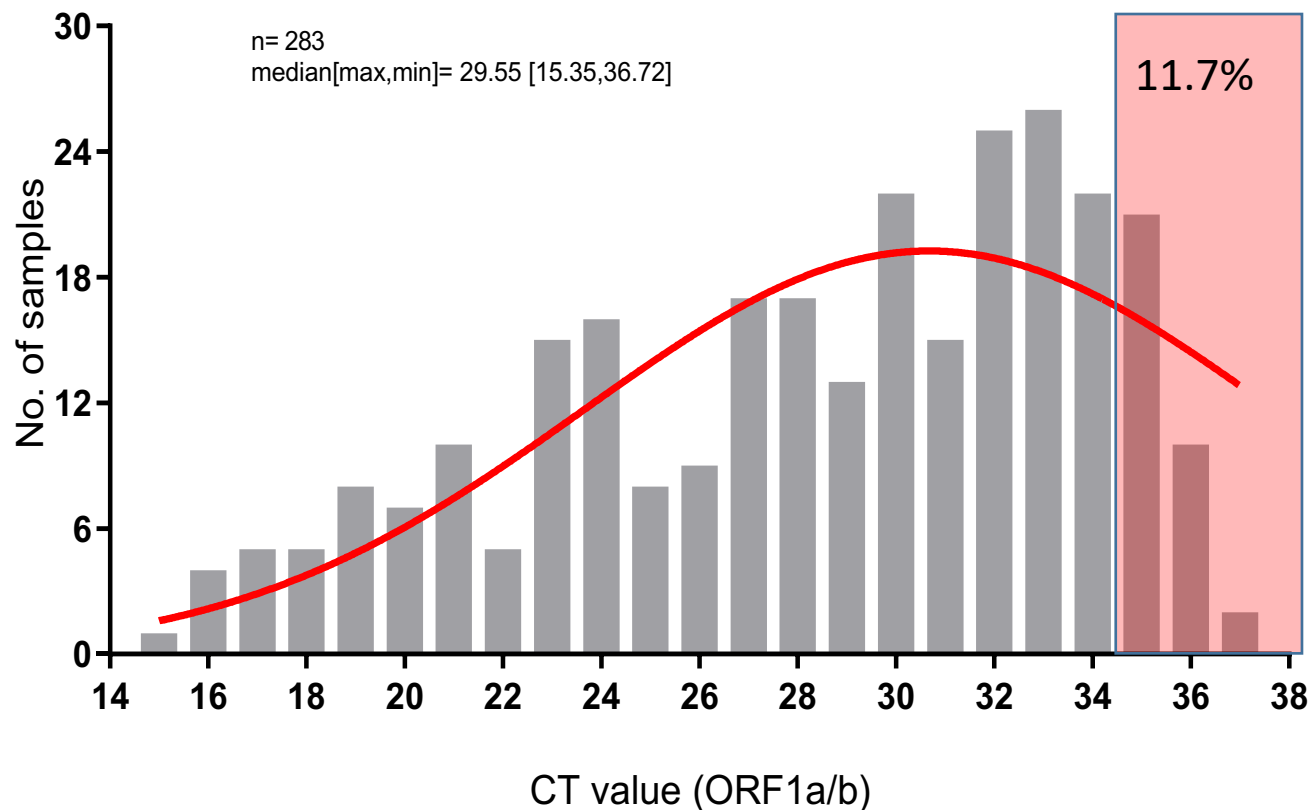
	unpooled		X5 POOL		
SAMPLE	CT1	CT2	CT1	CT2	Score
IQ 2	15.35	15.52	16.75	16.96	Positive
IQ 3	17	17.17	18.6	18.83	Positive
IQ 4	21.39	21.61	21.9	22.1	Positive
IQ 1	23.26	23.48	25.05	25.42	Positive
IQ 5	24.04	24.73	25.01	25.78	Positive
2Q 4	28.26	29.08	28.93	29.75	Positive
2Q 3	29.47	29.96	30.63	31.55	Positive
2Q 5	30.72	31.7	31.84	33.21	Positive
2Q 1	31.34	32.45	31.25	32.4	Positive
2Q 2	31.76	32.86	31.69	33.21	Positive
3Q 7	31.92	33.59	33.18	35.14	Positive
3Q 3	32.08	33.2	31.98	33.66	Positive
3Q 4	32.13	33.65	32.87	33.94	Positive
3Q 9	32.45	33.68	32.74	34.93	Positive
3Q 1	32.53	34.22	32.74	34.37	Positive
3Q 6	32.77	34.91	34.34	35.53	Positive
3Q 5	33.07	36.16	34.36	36.75	Positive
3Q 8	33.28	34.99	34.27	0	Positive
3Q 10	33.6	35.49	33.83	35.79	Positive
3Q 2	33.66	35.32	34.77	36.48	Positive
4Q 15	33.71	35.39	0	37.75	Positive
4Q 4	33.84	36.47	34.33	36.68	Positive
4Q 14	33.91	35.99	32.87	35.38	Positive
4Q 5	33.99	37.35	34.18	36.94	Positive
4Q 19	34.17	36.12	34.82	37.46	Positive
4Q 2	34.46	37.9	0	0	Negative
4Q 12	34.55	36.68	34.65	36.98	Positive
4Q 7	34.56		0	38.02	Positive
4Q 3	34.67	36.76	0	0	Negative
4Q 16	34.83	36.75	34.27	35.96	Positive
4Q 11	34.85	37.15	35.15	37.57	Positive
4Q 13	34.89	41.68	33.82	37.79	Positive
4Q 6	34.9	35.82	35.14	37.65	Positive
4Q 20	34.93	36.96	0	0	Positive
4Q 17	35.13	37.75	35.49	38.28	Positive
4Q 8	35.4		0	0	Negative
4Q 18	35.44	37.5	0	0	Positive
4Q 9	35.77	37.4	0	0	Negative
4Q 10	35.79		0	38.69	Positive
4Q 1	36.54		0	0	Negative

100% sensitivity

69 % sensitivity

Frequency distribution of CT values for Positive SARS CoV-2 samples tested on Cobass8800

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11.7%

CT value (ORF1a/b)

Take home Massage;
- A pool 10 samples resulted in false negatives at the rate of 3 in 10 in patients with CT value >34, which represent 11.7% of the population.
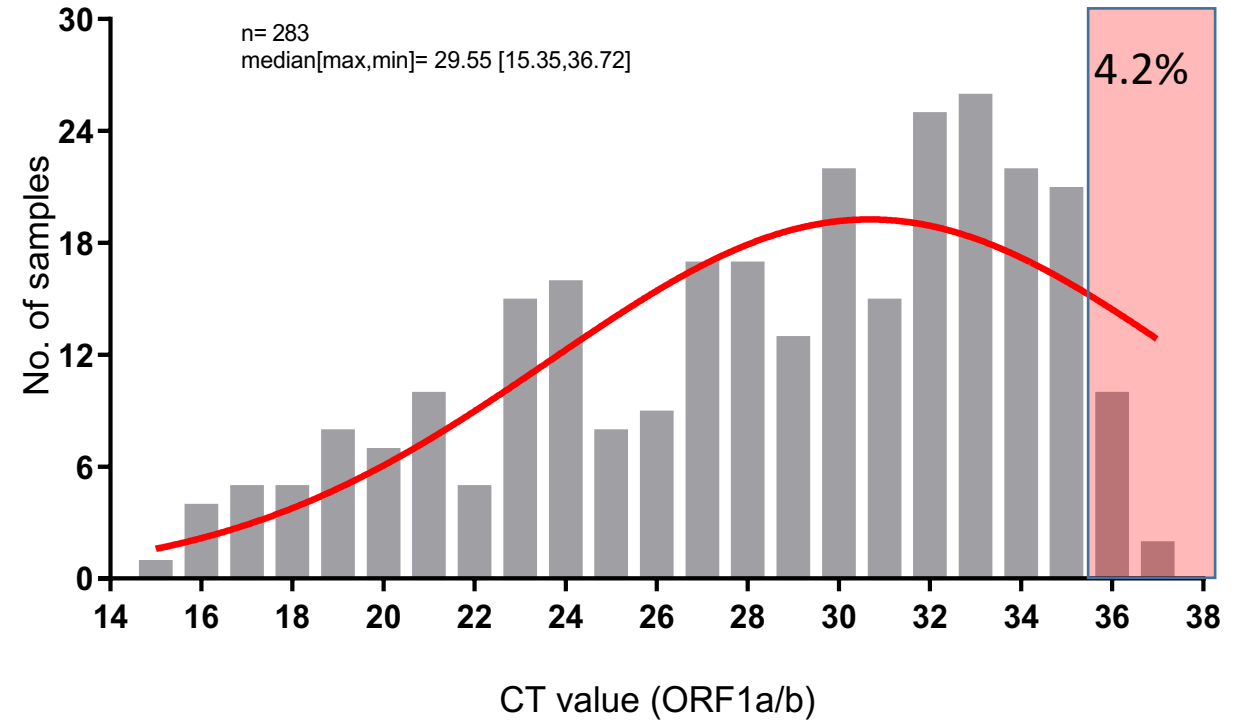
A pool of 10 samples results in loss of sensitivity of positive samples with CT value above 35 from 100% to 57%

SAMPLE ID	unpooled CT1	unpooled CT2	X5 POOL CT1	X5 POOL CT2	Score
IQ 2	15.35	15.52	16.75	16.96	Positive
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4Q 18	35.44	37.5	0	0	Positive
4Q 9	35.77	37.4	0	0	Negative
4Q 10	35.79		0	0	Negative
4Q 1	36.54		0	38.28	Positive

100% sensitivity

57 % sensitivity

Frequency distribution of CT values for Positive SARS CoV-2 samples tested on Cobass8800

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4.2%

No. of samples

CT value (ORF1a/b)

Take home Massage;
- A pool 10 samples resulted in false negatives at the rate of 4 in 10 in patients with CT value >35, which represent 4.2% of the population
- Pooling at this level was safe because loss of sensitivity was with in the detection limit zone of the assay

Other consideration in the lab to mitigate increased turn around time for results and reduce errors from complexity of pooling

- Elaborate sample Organization and accessioning
  - Samples must be accessioned in and organized on rack in a systematic fashion to allow easy pooling
  - Location of samples for repeat test is dependent on how they were organized and accessioned
- Temporary storage of samples
  - Preferably store samples at fridge for not more than 48 hours before repeat testing
  - How samples are organized in storage will allow fast retrieval at repeat test
- Use of information system
  - Automation of final results score will be best done using information systems
  - They system can be set to randomly select negative samples for repeat
  - Can flag pools that test negative on repeat
- Random repeat of negative pools to ensure that sensitivity is not lost
- Continuous monitoring of the positivity rate, turn around time and savings
  - To inform adjustment of pool size and when to stop
  - After 10% positive rate, there is no cost benefit to pooling
- Triaging samples to eliminate obvious positive or categories with high positive rates
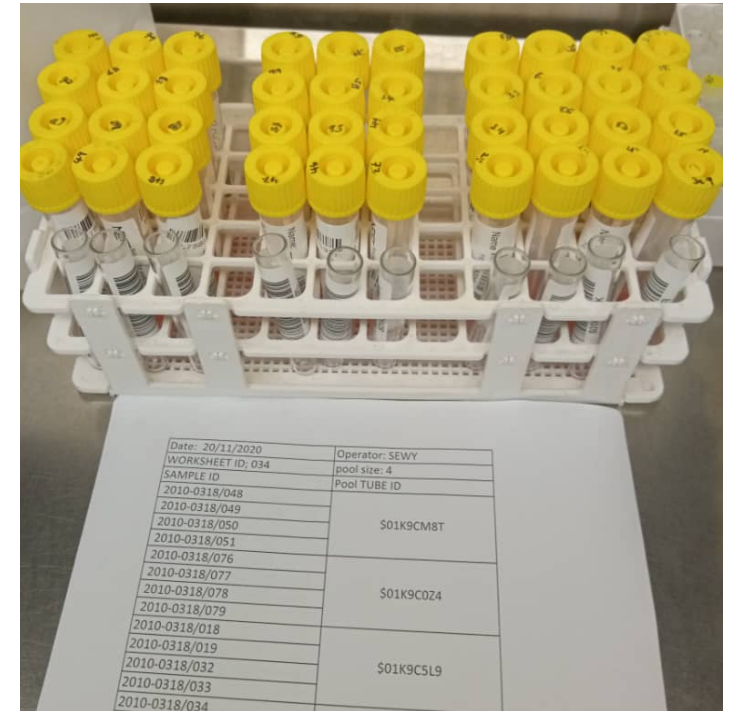
The Experience with Pool Testing: Cost saving and uninterrupted testing

- 9th Aug 2020 we stocked out of reagents for the whole country

- 12th Aug 2020received Cobass 8800 reagents worth 16,000 tests and we had accumulated a backlog of 7,000 samples

- Daily volumes peaked at 3,500 samples

- The lab would stock out even before pulling out of backlog

- High priority samples were not pooled

✓ The pool testing enabled the lab to stretch the reagent for 5 days to over 18 days

✓ the lab as saved $ 824,136 in less that 3 months

	11 Aug - 29 Aug 2020	10th sept - 3rd Nov 2020	Cumulative
Number of patients	33,600	48,000	81,600
Number of pooled tests	9,160	28,200	37,360
No of repeated tests	3,200	5,208	8,408
Total tests performed	12,360	33,408	45,768
Tests saved	21,240	14,592	35,832
Percetage of saving	63.2%	30.4%	43.9%
Total cost of savings at 23$/test (reagent 19.8$ and overhead costs 3.2%)	$488,520	$ 335,616	$ 824,136

Turnaround time from Reception to Results Release