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AN EVALUATION OF THE POOLED LOLLI-METHOD RT-qPCR TESTING FOR COVID-19 SURVEILLANCE IN SINGAPORE

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AUTHORS' CONTRIBUTIONS

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ABSTRACT

Background: Following the success of the Lolli-Method or Lolli-Test as a surveillance method in Germany, the Ministry of Health, Singapore investigated the feasibility of deploying the method as a rostered routine testing in vulnerable individuals such as children, nursing homes and frontline workers; and evaluated the sensitivity and ideal pooling ratio of the Lolli-Method.

Methods: The study was conducted in two phases – the first phase was to assess the operational feasibility of the Lolli-Method. It was held in conjunction with air sampling at a childcare centre with children ages 2 to 6 years old across 40 days. The second phase was to evaluate the sensitivity of the Lolli-Method with different pooling ratios and was conducted in collaboration with the National Centre for Infectious Diseases (NCID) where each pool was spiked with one Lolli swab from a COVID-positive patient. All patients enrolled in this study have their viral load cycle threshold (CT) levels assessed prior to admission via a mid-turbinate oropharyngeal (MTOP) polymerase chain reaction (PCR) swab.

Results: The sensitivity of the pooled Lolli-Test was similar to antigen rapid tests with 100% sensitivity (3/3) in a pooling ratio of 20:1 for patients with viral loads of cycle threshold (CT) levels below 20. For individuals with lower viral loads, the sensitivity of the Lolli-Test was 66.7% (2/3) in a pooling ratio of 20:1 and 100% (2/2) in a smaller pooling ratio of 15:1. The operational feasibility of the Lolli-Test was assessed to be high amongst study participants although students were noted to require some additional assistance from teachers.

Conclusion: The Lolli-Test is an effective surveillance method with adequate sensitivity to detect a COVID-19 infected individual in a pool of up to 20 albeit largely dependent on the viral load. Furthermore, the Lolli-Test also provides a less invasive alternative sample collection method for individuals who cannot tolerate or have

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contraindications for the regular nasal or oropharyngeal swabs such as young children. More studies should be done to assess the Lolli-Test's true limit of detection and to evaluate the use of the Lolli-Method in infants and for other respiratory diseases such as influenza.

Keywords: Lolli-test; lolli-method; COVID-19; SARS-CoV-2; infectious diseases; respiratory diseases; surveillance; PCR.

ABBREVIATIONS

S/N	*Phrase*	*Abbreviation*
1	*National Centre for Infectious Diseases*	*NCID*
2	*Antigen Rapid Test or Rapid Antigen Test*	*ART*
3	*Polymerase Chain Reaction*	*PCR*
4	*Nasopharyngeal*	*NP*
5	*Mid-turbinate Oropharyngeal*	*MTOP*
6	*Oropharyngeal*	*OP*
7	*Cycle Threshold*	*CT*

1. INTRODUCTION

Since COVID-19 was categorised by the World Health Organisation as a global pandemic in early 2020 [1], there has been an urgent need to find appropriate diagnostics in order to detect COVID-19 reliably. The Lolli-Method or Lolli-Test was first developed by the Institute of Virology at the University Hospital in Cologne, Germany. This method was rolled out between 9 November 2020 to 23 December 2020 as the B-FAST study across 14 primary and secondary schools in five communities in Germany. Subsequently, the method was employed as rostered routine testing in day-care centres, primary schools, schools with primary level and special schools across multiple cities in Germany for children aged 6 months old and above [2,3].

Based on the Institute of Virology's study [2,3], the overall sensitivity of the individual Lolli-Method when compared to nasopharyngeal or oropharyngeal swabs was 81%. The Institute of Virology has also found that utilising the Lolli-Test regularly at an interval of twice weekly for rostered routine testing managed to reduce COVID-19 transmissions by 80% [3].

There are several advantages of the Lolli-Test method of sucking the swab over existing methods of nasal ART and PCR using nasopharyngeal or mid-turbinate oropharyngeal samples which are currently being employed in Singapore for COVID-19 diagnosis. These include ease of testing particularly in young children and elderly with dementia, and ability to conduct testing in individuals who are contraindicated for nasal swabbing [2,3]. Following the success of Lolli-Test in Germany, Singapore decided to evaluate the feasibility of conducting similar testing methodology locally. The objectives of this study were to evaluate the sensitivity of the Lolli-Test, and to assess the feasibility of conducting the Lolli-Test in young children.

2. METHODS

This study was conducted in two phases. The first phase was conducted as a surveillance method and feasibility assessment at a childcare centre with 80 students aged 2 years and older from five different classes. Children aged 2 to 6 years old were recruited to participate in the Lolli-Test on two weekday mornings across 4 weeks over a two-month period. All parents were provided with an information sheet with detailed description of the Lolli-Test and air sampling before the school obtained consent from the parents to conduct the Lolli-Test on their children. Every morning, the children were directed to suck on the swabs for 30 seconds under close supervision from teachers and school staff. The swabs were then collected dry in a swab container according to the students' classes without any medium and pooled in a maximum ratio of 20 swabs to 1 container [2]. Pooled samples were then tested with PCR using Perkin Elmer or Taqpath assays. For pools with one or both COVID-19 gene targets identified, the pooled UTM were re-tested using a fast PCR (Roche Liat) to quickly confirm the presence of COVID-19 within the pool. Should the pooled PCR be tested positive, an individual confirmatory test would then be conducted on Lucira – a loop-amplified molecular test [4].

The second phase of this study was conducted concurrently in the hospital to assess the sensitivity of different pooling ratios for the Lolli-Test. Each pool consisted of one Lolli-Test swab from a known COVID-19 positive patient and the remaining Lolli-Test swabs samples to make up the pool were collected from healthy individuals who were healthcare workers undergoing rostered routine testing in parallel. A tiered approach to viral load was taken based on the COVID-19 positive individuals' CT values. Taking reference from the Cologne study [2,3], the base pooling ratio used in this study was 20:1, with smaller pooling ratio used for higher CT values. In total, there were 10 pools of swabs evaluated.

At the end, all participants and all those who assisted in the administration of the Lolli-Test were also given a questionnaire to assess the operational feasibility of conducting the Lolli-Test.

3. RESULTS

3.1 Pooled Lolli-Test Results

For the first phase of the study, approximately 70 to 80 students aged 2 years and older from five different classes participated in the bi-weekly Lolli-Test during the school holiday season. The swabs were pooled according to the children's classes with each pool consisting of a maximum of 20 individual swabs. During the second phase of the study, 10 COVID-19 positive individuals aged 23 to 71 with CT values ranging from 16.1 to 28.1 were recruited for the Lolli-Test along with 6 healthy individuals. Each pool consisted of one Lolli-Test swab from a COVID-19 positive individual and 19 or 14 Lolli-Test swabs from the 6 healthy individuals to make up the pooling ratio of 20:1 or 15:1 respectively.

Results showed that the pooled Lolli-Test was able to reliably detect (100%) individuals with CT values less than 20 in a larger pool size of 20:1. For individuals with lower viral loads or CT values ranging between 20 to 25, the Lolli-Test was able to detect 66.7% of the time in a larger pool of 20:1 and 100% of the time in a smaller pool of 15:1. The pooled Lolli-Test was unable to detect individuals with CT values above 25 in pooling ratio of 15:1.

3.2 Feasibility Assessment

There were no issues recruiting all students, with mostly 100% participation rates in the childcare centre. Most parents did not raise any concerns. The teachers felt that the Lolli-Test was relatively easy to administer, and students tolerated the Lolli-Test better than a regular anterior nasal antigen rapid test. Some students, mostly the younger children, required additional assistance as they accidentally contaminated the swabs by touching the cotton swab end. However, this may be improved over time when children are more used to the Lolli-Test. There were mixed reactions from the teachers with some preferring the Lolli-Test to air sampling while others felt that more manpower was required to supervise the children for the Lolli-Test.

All participants and Lolli-Test administrators from the hospital had good feedback on the Lolli-Test for its tolerability and ease of administration. There were no concerns raised by the participants and all did not foresee any issues should the Lolli-Test be performed regularly. From the Lolli-Test administrators' perspective, all participants were cooperative and there were no difficulties to recruit participants for the study. No biosafety concerns, such as cross-contamination or spillage, were raised.

4. DISCUSSION

Both phases of the study were conducted amidst the beginning and peak of Omicron wave in Singapore when Omicron was the main variant of concern [1,5]. Although there were no S-gene dropout performed to determine the strain of COVID-19 that our participants had, it was presumed that all participants had the Omicron variant as the study was conducted when there was an estimated 85% Omicron prevalence amongst NCID's hospitalised patients. Although the sensitivity of a pooled Lolli-Test was similar to antigen rapid tests, the Lolli-Test was less vulnerable to variants as PCR testing required a minimum of two genes for detection.

Table 1. Sensitivity of pooled Lolli-Test across various CT values and pooling ratios

CT Range	Pooled 20:1	Pooled 15:1
CT < 20	100% (3/3)	-
CT 20 to 25	66.7% (2/3)	100% (2/2)
CT > 25 to 30	-	0% (0/2)

Table 2. Feasibility assessment and feedback from participants

Aspects of Feasibility Assessment	Childcare	Hospital		
	Teachers	Patients	Healthcare Workers	Administrator
Recruitment	+++	NA	NA	+++++
Administration	+++	+++++	+++++	+++++
Operations	++	+++++	+++++	+++++
Sample Retrieval	+++	+++++	+++++	+++++
Performing Lolli-Test Regularly	++	+++++	+++++	+++++

This study was also conducted during the holiday season and the number of students participated varied from week to week and were lower than anticipated. This resulted in a relatively low positive sample pick-up and may have affected the comprehensiveness of our study. A larger sample size with varying pool sizes may be done with COVID-19 positive individuals and healthy individuals to further evaluate the sensitivity of more pooling ratios for the Lolli-Test. This may be particularly useful in the younger age groups given that there was no COVID-19 vaccine approved at the time of the study, and the current lack of ARTs available for children below the age of 2 years [6,7,8].

There have been several COVID-19 surveillance techniques implemented in various countries for pre-event and rostered routine testing [9,10,11]. These techniques cover an array of sample types including wastewater testing [12,13,14,15] and air sampling [16]. Amongst these techniques, Lolli-Test remains one of the most promising surveillance methods as it is less invasive and easily implementable amongst participants of different ages. It also has the potential to provide more granularity in identifying the infected individual compared to other surveillance techniques like air sampling and wastewater testing. Furthermore, it is relatively cheaper compared to other surveillance methods. The estimated cost of implementing bi-weekly Lolli-Test in a group of 20 individuals, inclusive of courier, is S$230 which equates to approximately S$5.75 for each Lolli-Test per pax. This price is comparable to some of the most affordable regular ARTs in Singapore which usually retails around S$4.90 for single packs [17,18,19]. The price of conducting the Lolli-Test may be further reduced with either an in-house lab facility which eliminates the need for courier or having economies of scale from mass testing with a risk-tiered approach.

With the recent shift to endemicity, Singapore has been moving away from using PCR as the primary COVID-19 diagnostic tool to the more convenient, portable, and accessible antigen rapid tests [20,21]. The Lolli-Test remains a useful surveillance technique to keep onboard for future variants as it can utilise existing infrastructure and lab facilities, which makes it readily implemented and easily scaled up [21,22]. Thus, the Lolli-Test may be useful as the first-line surveillance method to be rolled out for future COVID-19 waves amongst high-risk individuals (such as frontline healthcare and border control workers) and facilities with high transmissibility (such as nursing homes, infant-care, childcare and eldercare centres, schools, and dormitories) [4].

5. CONCLUSION

The Lolli-Method or Lolli-Test is an effective COVID-19 surveillance technique with adequate sensitivity in detecting a COVID-19 individual with viral loads within the infectious range up to a pooling ratio of 20:1. Furthermore, the Lolli-Test offers a less invasive alternative sample type which is generally more preferred for use in young children when compared to the traditional gold standard nasopharyngeal or mid-turbinate oropharyngeal swab. Given that the Lolli-Method uses PCR as its analysis, it is largely dependent on the sensitivity of the assay selected and will be resistant to variants due to its multiple gene targets. Further studies may be conducted to validate its use in infants, or for the diagnosis and surveillance of other respiratory diseases.

FUNDING

Ministry of Health, Singapore.

CONSENT

All parents were provided with an information sheet with detailed description of the Lolli-Test and air sampling before the school obtained consent from the parents to conduct the Lolli-Test on their children.

For the study conducted in NCID, all participants were also provided with an information sheet consisting of a detailed description of the Lolli-Test before consent was obtained by NCID.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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ANNEX

Table 1. Infographics used for the studies

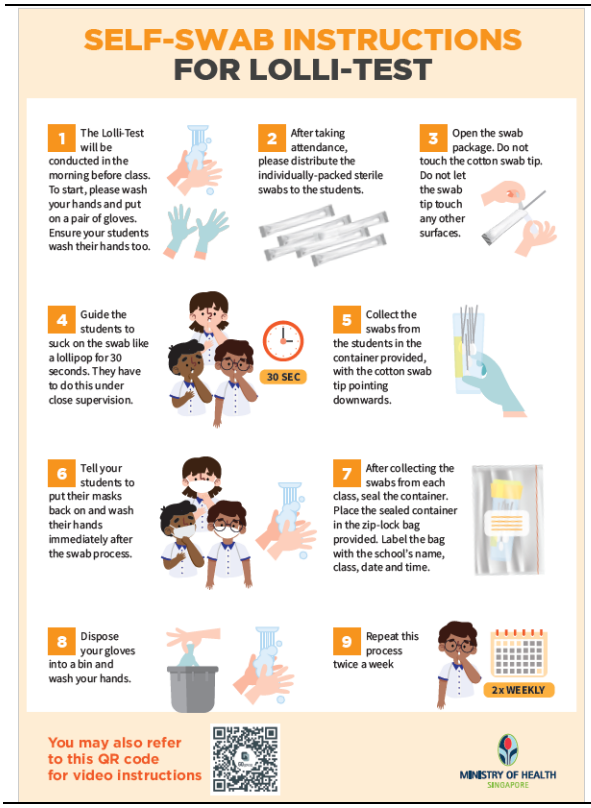
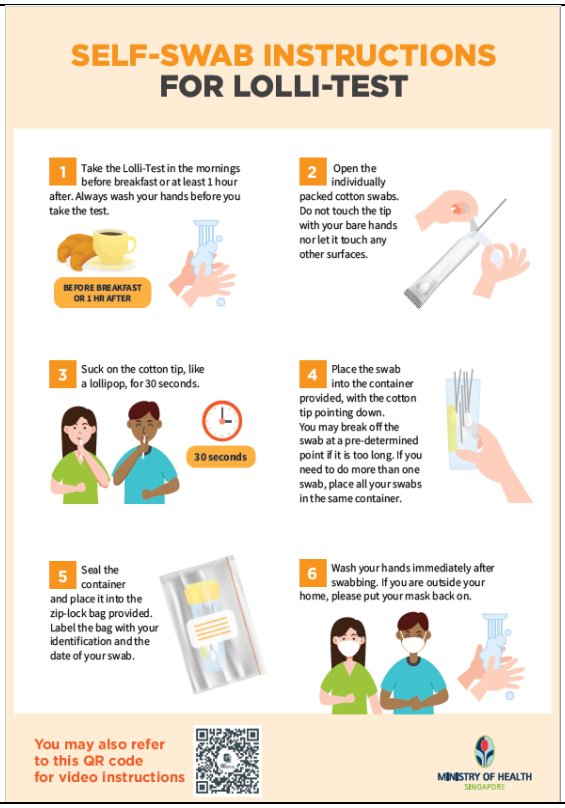
First Phase	Second Phase
	

Table 2. Lab Protocols, with inputs from Cologne

Innoquest's	Cologne's[8,9]
1. Add 3 mL of viral transport medium (VTM) into 50 mL tube with the collected swabs. 2. Vortex the tube for 30 seconds. 3. Aliquot 700 uL of VTM into secondary tubes. 4. Load the samples into Biomek i7 Automated workstation for RNA extraction and aliquoting of PCR reagents (TaqPath COVID-19 Combo Kit). 5. Sample with the PCR master mix will then be sent to Quantstudio 5 (PCR thermocycler) for PCR	1. 3 ml PBS were pipetted in one centrifugation tube. 2. The tube was vortexed for 30 seconds. 3. Of all samples, 1 ml each was used for SARS-CoV-2 detection. 4. For SARS-CoV-2 detection, either COBAS 6800 (Roche Diagnostics) and Alinity m (Abbott) instruments equipped with their respective SARS-CoV-2 detection kits, or the Quantstudio 5 (Thermofisher) instrument, using the Quick-RNA Viral Kits (Zymo Research) for RNA isolation and GeneFinder™ COVID-19 Plus RealAmp was used.

Table 3. List of PCR Systems used, with inputs from Cologne [8,9]

Regular PCR
1. ROCHE COBAS 6800
2. Hologic Panther TMA
3. Abbott Alinity m
4. TIB MolBiol

Regular PCR
5. Qiagen NeuMoDX and Artus-Kit
6. r-Biopharm
7. BAG
8. Altona
9. Seegene
10. Taqpath
Fast PCR
1. GeneXpert Cepheid
2. Roche Liat
PCR systems that are not recommended:
1. PerkinElmer Kit: Resulted in some invalid results (3/10)

Table 4. Feedback from ECDA's Teachers

Questions, maximum score of 5	**T1**	**T2**	**T3**	**T4**	**T5**	**Average**
The Lolli-test was easy to administer by the teachers.	3	3	3	4	4	3.4
The children tolerated the Lolli-test well.	3	3	3	3	3	3
The children did not require additional assistance to complete the test.	2	1	1	2	2	1.6
Parents did not raise additional concerns regarding the testing procedure.	3	4	3	3	3	3.2
I do not foresee any issues if the Lolli-test were to be performed regularly twice a week.	3	2	3	2	1	2.2
Overall, the Lolli-test is preferable to air sampling.	3	2	1	3	4	2.6
The Lolli-test was performed without spillage of saliva.	2	2	3	4	4	3
The children were co-operative and there was no cross-contamination of saliva between children.	3	2	3	4	4	3.2
There were no additional biosafety concerns from conducting the Lolli-test.	3	3	2	3	3	2.8
The results were provided in a timely manner.	3	4	3	3	4	3.4
There were no difficulties encountered notifying parents of the results.	3	4	3	3	4	3.4
Downstream actions (e.g., Lucira test / antigen test) were performed without issues.	3	3	3	NA	NA	3

Table 5. Detailed Results of NCID Study

Date of PCR	**Patient's CT**	**Age**	**Date of Lolli**	**Days between PCR and Lolli**	**Pooled Ratio**	**Pooled Lolli Results**
22-Jan-22	16.1	69	25-Jan-22	3	20:1	Positive
19-Jan-22	16.5	68	20-Jan-22	1	20:1	Positive
18-Jan-22	18.1	40	20-Jan-22	2	20:1	Positive
19-Jan-22	20.8	26	19-Jan-22	0	20:1	Negative
20-Jan-22	20.9	23	21-Jan-22	1	20:1	Positive
24-Jan-22	21.5	71	25-Jan-22	1	20:1	Positive
19-Jan-22	22.2	69	20-Jan-22	1	15:1	Positive
23-Jan-22	24.2	30	25-Jan-22	2	15:1	Positive
18-Jan-22	25.2	36	20-Jan-22	2	15:1	Negative
20-Jan-22	28.2	46	21-Jan-22	1	15:1	Negative

Table 6. Feedback from NCID's Participants

Questions, maximum score of 10	P1	P2	P3	P4	P5	P6	P7	Average
The Lolli-test was easy to do, and the instructions were easy to follow.	10	10	10	10	10	10	10	10
I tolerated the Lolli-test well and it was easy to perform the test myself.	10	10	10	10	8	10	10	9.71
I did not face any uncertainties nor require additional assistance to complete the test.	10	10	10	10	9	10	10	9.86
I did not have additional concerns to raise regarding the testing procedure.	10	9	10	10	10	10	10	9.86
I do not foresee any issues if the Lolli-test were to be performed regularly.	10	9	10	10	10	10	10	9.86
If a repeat swab was needed, how likely would you want to do the test again?	10	9	10	10	9	10	10	9.71

Table 7. Feedback from NCID's Staff

Questions, maximum score of 10	S1	S2	Average
The Lolli-test was performed without spillage of saliva.	9	10	9.5
The participants were co-operative and there was no cross-contamination of saliva.	10	10	10
There were no additional biosafety concerns from conducting the Lolli-test.	10	10	10
The results were obtained in a timely manner.	9	10	9.5
There were no difficulties encountered recruiting participants.	10	8	9
I do not foresee any issues if the Lolli-test were to be performed regularly.	9	10	9.5

Table 8. Estimated cost breakdown for bi-weekly Lolli-Test implementation in a group of 20

S/N	Item	Unit Price (S$)	Quantity	Subtotal (S$)
1	Lolli-Test COVID 19 PCR Test (with no minimum pool size)	48.00	2	96.00
2	Swab Stick (per piece)	2.00	40	80.00
3	Swab Container	2.00	2	4.00
4	Courier	25.00	2	50.00
Total Weekly Cost for a Group of 20		S$230.00		
Total Weekly Cost Per Pax		S$11.50		
Total Cost Per Pax Per Lolli-Test		S$5.75		

**Cost may vary according to lab services employed*