Feces as alternative convenient specimen for COVID-19 diagnostics

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Data of the first few hundred study were used. Briefly, SARS-CoV-2 PCR positive patients in primary diagnostics were used to contact families and follow up at first contact by research nurse (d1) and approximately 14 days later. Jars for feces collection were left and asked to submit to RIVM the same day or the day after the contact with the research nurse.

One inoculation loop of feces was transferred to 1 ml MEM + Hanks' salts and penicillin/streptomycin in a 1.5 ml screw-top tube, thoroughly vortexed for 15 seconds and 1 minute centrifuged at 16,000 rcf in tabletop minicentrifuge. Two hundred µl supernatant is transferred to 275 µl MagNApure blue extraction buffer with EAV internal control and yeast tRNA; 450 µl is used for extraction and eluted in 50 µl; 5 µl is used in the PCR. For molecular detection we use in-house implementation of E-gene Corman et al. real-time RT-PCR on LC480 II using fast virus master mix chemistry.[1]

Data of 240 included persons were analysed; 54 cases and 186 contacts.

Taking any specimen type positive in PCR as a COVID-19 case, using respiratory specimens only or feces only a number of cases would be missed in children as well as adults (Table 1). Especially at day 14/15 most positive children were positive in feces only and most positive adults were positive in respiratory specimens only.

Table 1. SARS-CoV-2 detection in respiratory specimens and feces of persons for which both specimens were collected at day 1 and day 15 by age group and day of collection

Age group	D1				D15			
0 - <18 (n=117)			106 pairs				101 pairs	
			Feces				Feces	
			+	-			+	-
	Respiratory specimens	+	20	5	Respiratory specimens	+	5	4
		-	3	78		-	15	77
>=18 (n=123)			72 (120) pairs[1]				113 pairs	
			Feces				Feces	
			+	-			+	-
	Respiratory specimens	+	19 (42)	8 (35)	Respiratory specimens	+	8	17
		-	5 (5)	40 (38)		-	7	81

[1] number between brackets: although for most cases no respiratory results were available at day 1, these are considered respiratory positive as this is inclusion criterion. However day 1 in the study can be multiple days later than the case was diagnosed with COVID-19 and therefore the respiratory result and feces result are not from approximately the same day.

Proportionally for persons with feces +ve at day 1 children have more frequently positive feces at day 15 compared to adults (Table 2).

Table 2. SARS-CoV-2 detection in feces of persons for which feces was collected at day 1 and day 15 by age group

Age group				
0 - <18 (n=117)			105 pairs	
			Day 1	
			+	-
	Day 15	+	16	4
		-	6	79
>=18 (n=123)			113 pairs	
			Day 1	
			+	-
	Day 15	+	15	0
		-	30	68

More than half of the children with +ve feces at day 1 showed no symptoms whereas at day 15 this is reversed, similar to in adults of whom most with +ve feces showed symptoms at day 1 and 15 (Table 3). For respiratory specimens at day 1 results are similar, of 25 positive children at day of specimen collection 14 did not show symptoms and for adults this was only 5 out of 27.

Table 3. SARS-CoV-2 detection in feces for persons showing symptoms or not; by age group

Age group	D1				D15			
0 - <18 (n=117)			109 pairs				106 pairs	
			Feces				Feces	
			+	-			+	-
	Symptoms	+	11	27	Symptoms	+	16	36
		-	13	58		-	5	49
>=18 (n=123)			118 pairs				111 pairs	
			Feces				Feces	
			+	-			+	-
	Symptoms	+	40	43	Symptoms	+	14	76
		-	7	28		-	1	20

As oral fluid has been suggested as a good alternative for respiratory specimens, especially for children the previously analysed oral fluid data [2], were combined with the current dataset and analysed (Table 4). In children who are positive in respiratory specimens feces outperforms oral fluid compared to adults. However, either way some of the respiratory positive persons would be missed using oral fluid and/or feces. However, as is clearly shown in table 1 also persons who test negative in respiratory specimens can test positive in feces. In the pilot study for oral fluid, none of the selected persons negative in respiratory specimens tested positive in oral fluid.[2].

Table 4. SARS-CoV-2 detection in feces and/or oral fluid of a selection of respiratory positive or negative persons at day 1 for which oral fluid was analysed; by age group

Age group	Respiratory positive				Respiratory negative			
0 - <18 (n=28)			21 pairs				6 pairs	
			Feces				Feces	
			+	-			+	-
	Oral fluid	+	12	1	Oral fluid	+	0	0
		-	5	3		-	0	6[1]
>=18 (n=17)			15 pairs				2 pairs	
			Feces				Feces	
			+	-			+	-
	Oral fluid	+	12	2	Oral fluid	+	0	0
		-	0	1		-	1[2]	1

[1] 2 are feces only positive at d14; 1 is respiratory and feces positive at d14

[2] is respiratory and feces positive at d14

In conclusion, feces as a convenient specimen for children, and especially young children, is a good alternative to taking nasopharyngeal and oropharyngeal swabs (or nasal aspirate). Although numbers are low, in comparison with collection of oral fluid feces seem to outperform oral fluid in positivity rate among children with positive respiratory specimens. We can also conclude that feces is a good addition to the diagnostic repertoire, especially if respiratory specimens test negative and the suspicion for COVID-19 is strong.

Proposal for specimens collection in young children at 'basisschool' age and below, depended on what a laboratory can process:

1. In case of outbreak/source-contact investigation we propose for very young children to collect feces, preferably in combination with oral fluid collected with Oracol sponge (S10 or S14). As usual, if a contact person in the outbreak gets symptoms additional testing is recommended with feces and preferably also oral fluid collection.

2. MHS (GGD) testing street. For symptomatic children any age preferably nose (oropharyngeal or midturbinate) and oropharyngeal swab. If not possible, similar to point 1. above collect feces preferably in combination with oral fluid collected with Oracol sponge (S10 or S14) for very young children or for older children collected by drooling/spitting.

If testing capacity does not allow two specimens per child at very young age, feces is the preferred specimen.

Implementation should be organized by LCDK in discussion with GGD and associated laboratory as the laboratory should have the capability to process feces and oral fluid specimens. Both oral fluid collected with Oracol sponge and feces require additional handlings, centrifugation, before the specimen can be extracted.

References

1. [redacted], [redacted] Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020 Jan;25(3).
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