

1 **Supplementary material**

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31 **1. Supplementary notes.**

32 **A. Helsinki University scent dog validation protocol**

33 1. Positive/negative disease status of volunteers providing each of the samples is confirmed in advance  
34 using an approved reference method.

35 2. Only novel samples i.e. new to the dog (no previous scenting), are used.

36 3. A power calculation is performed to obtain the lowest acceptable numbers of positive and negative  
37 samples for determining the sensitivity and specificity while striving for a lower CI value of 80 %.

38 4. The ratio between positive and negative samples is predetermined but can be different in different  
39 validation setups. All tracks are randomized using a computer-generated list, programmed to comprise  
40 different amounts of positive samples per track as well as a predetermined proportion of “blank” tracks,  
41 i.e. not including any positive samples.

42 5. The validation experiment is triple-blinded: the dog, the handler and the assistant presenting the  
43 samples to the dog and handler inside the same testing space, are all blinded to the status of each track  
44 (zero or more positive samples) and each sample (positive or negative).

45 6. Dogs are allowed to go directly to the positive sample and mark it. This is counted as a successful  
46 result if there is a maximum of one single positive sample per track, thereby not necessitating sniffing  
47 of the other samples (if agreed, after removing the positive sample, the dog may be allowed to continue  
48 sniffing the remaining ones, but this part of the experiment is to be considered single-blinded (thereafter  
49 only the dog is blinded).

50 7. To avoid regressing and to reinforce the target scent, the dogs are rewarded after each positive result.  
51 Each sample will thus become unblinded onsite immediately after the validator has confirmed the result.

52 8. During validation each dog will sniff the predetermined number of samples. The working days  
53 required for validation may vary in number, since the maximal amount of samples one dog can sniff in  
54 one day depends on the dog and the setup.

55 9. All validations are video recorded using at least 3 cameras at different angles of the validation setup.  
56 This allows any external evaluator to check the process and the results afterwards if needed.

57 10. An external pre-determined controller with societal credibility and expertise in scent dog work, has  
58 to be present at all validation experiments, confirming that the process follows the pre-determined  
59 protocol in detail. His/her report will be included in the study dossier.

60 11. The environment, such as sound and stress level, temperature and humidity should be optimal for  
61 the dog.

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## 64 B. Complete list of protocol violations

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66 1. Four video cameras recorded the validations all the time. Cameras 1, 2 and 3 were linked  
67 together and camera 4 was separate from the others, placed to show the runs and to allow the  
68 external evaluator to see the interior of the cubicle from all angles, and to ensure that no one  
69 acts in violation of the triple-blind protocol. There were occasions when the research team forgot  
70 to start one or several of the cameras: 1) twice camera 4 was started late (E.T's, validations 6  
71 and 7) yet the three other cameras were running from the start; 2) Rele's whole validation 6 was  
72 recorded only on camera 4; 3) Silja's validation 3 had both camera systems off.

73 Evaluation: The researchers and the external evaluator considered these omissions of minor  
74 significance to the implementation of the study.

75 2. The humidity in the working cubicle was suboptimal for the dogs: The temperature was quite  
76 stable and not too warm (21-22°C) but the humidity was relatively low (21-25 %). Evaluation:  
77 An undetermined, presumably moderate impact on performance, since humidity is known to  
78 affect the volatility of the odour molecules and dogs adjust their behaviour to detect these  
79 molecules when the humidity drops making it more demanding for scent work (1).

80 3. The Helsinki University scent dog validation protocol recommends a calm environment free of  
81 external noise and stress factors.

82 Evaluation: An undetermined, presumably moderate impact on performance. As the validation  
83 was conducted at the airport, we allowed normal airport stress factors (people rushing, loud  
84 noises from suitcases and high heels, dogs coming to greet their owners, children crying,  
85 loudspeakers giving corona advice every 3 minutes etc.) since we wanted to validate the dogs  
86 in their operational work environment. Unfortunately, on top of all these factors, the Helsinki-  
87 Vantaa airport had noisy construction work going on during the whole validation period, the  
88 worst being concrete drilling on the next level, just above the cubicle. The noise level was  
89 measured using a mobile phone app, showing values of up to 80 decibel during a validation.  
90 This was unfortunate but neither the construction work nor the validation could be postponed.  
91 The impact of this noise remains unclear.

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93 Summary: none of the protocol violations were considered to have a major impact on conducting the  
94 study or the dogs' performance.

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98 **2. Supplementary Tables**

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Supplementary Table 1. Review of the previously published COVID-19 scent dog validation studies

Reference	Dogs (n)	Training period (weeks)	Sample type	RT-PCR <sup>1</sup> positive samples (n)	RT-PCR negative samples (n)	Validation test	Success rate sensitivity / specificity
14	10	1	Saliva, urine, sweat	Hospitalized and non-hospitalized (46)	Healthy non-hospitalized and other respiratory infections (47)	Blinding: dog, handler, Randomization:1+/6- Automated device (total 7 samples)	Overall success rate 92% Saliva: 93% / 82% / 96% Urine: 95% / 95% / 98% Sweat:93% / 91% / 94%
13	8	1	Saliva, trachea-bronchial secretion	Hospitalized (7)	Non-hospitalized (no recent signs of infection) (7)	Blinding: dog, handler, observer Randomization:1+/6- Automated device (total 7 samples)	94% / 83% / 96%
15	6	1-3	Sweat	Hospitalized (95)	Hospitalized non-covid or healthy HCW <sup>2</sup> (82)	Blinding: dog, handler Randomization:1+/2-3- /mocks Line of cones (total 3 or 4 samples)	76-100% / NA <sup>3</sup> / NA
17	6	7	Pharyngeal secretion, face masks/clothes	ICU <sup>4</sup> (1. test 26, 2. test 50)	Hospitalized non-covid and healthy (1. test 54, 2. test 70)	Blinding: single-blind Randomization:1-3+/7-10- Training wheel (total of 10 samples)	1. test: NA / 65% / 89% 2. test: NA / 86% / 93%
16	9	5	Urine, saliva	Hospitalized children (11) and adults (5)	Emergency department children (14) and asymptomatic, healthy, non-hospitalized adults (4)	Blinding: dog Randomization: 1+ / 3-4- / 7-8 distractors Training wheel (total of 12 samples)	Test 1: 94%/71%/ 99% Test 2: 94%/71%/98% Test 3:100%/75%/98% Test 4:62%/62%/98% Test 5:96%/68%/99% Test 6:11%/18%/41% Test 7:67%/22%/100% Test 8:100%/11%/94%

101 1. RT-PCR, reverse transcription polymerase chain reaction, 2. HCW, healthcare worker, 3. NA, not available, 4. ICU, intensive care unit. +  
 102 = PCR positive, - = PCR negative

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Supplementary Table 2. Dogs performance by validation test

VAL 1–7 and dates of sample collection	Order of validation days	Dog indication	PCR pos <sup>1</sup>	PCR neg <sup>2</sup>	Se <sub>sniff</sub> <sup>3</sup>	Sp <sub>sniff</sub> <sup>4</sup>	PPV <sub>sniff</sub> <sup>5</sup>	NPV <sub>sniff</sub> <sup>6</sup>
<b>SILJA</b>								
VAL-1 Dec 2020	1	Pos Neg	16 0	0 35	100 %	100 %	100 %	100 %
VAL-2 Dec 2020–Jan 2021	2	Pos Neg	18 0	0 26	100 %	100 %	100 %	100 %
VAL-3 Jan 2021	3	Pos Neg	15 1	2 25	94 %	93 %	88 %	96 %
VAL-4 Jan–Feb 2021	4	Pos Neg	14 1	1 33	93 %	97 %	93 %	97 %
VAL-5 Feb 2021	5	Pos Neg	15 0	0 26	100 %	100 %	100 %	100 %
<b>VAL1–5<sup>†</sup></b>		<b>Pos Neg</b>	<b>78 2</b>	<b>3 145</b>	<b>98 % (90–100)</b>	<b>98 % (94–99)</b>	<b>96 % (89–99)</b>	<b>99 % (95–100)</b>
VAL-6 Feb–March 2021	6	Pos Neg	13 1	2 31	93 %	94 %	87 %	97 %
VAL-7 March 2021	7	Pos Neg	8 5	6 30	62 %	83 %	57 %	86 %
<b>VAL6–7<sup>†</sup></b>		<b>Pos Neg</b>	<b>21 6</b>	<b>8 61</b>	<b>78 % (57–90)</b>	<b>88 % (79–95)</b>	<b>72 % (52–87)</b>	<b>91 % (81–96)</b>
<b>RELE</b>								
VAL-1 Dec 2020	5	Pos Neg	16 0	2 28	100 %	93 %	89 %	100 %
VAL-2 Dec 2020–Jan 2021	1	Pos Neg	18 0	0 29	100 %	100 %	100 %	100 %
VAL-3 Jan 2021	4	Pos Neg	17 0	0 29	100 %	100 %	100 %	100 %
VAL-4 Jan–Feb 2021	2	Pos Neg	16 0	3 33	100 %	92 %	84 %	100 %
VAL-5 Feb 2021	3	Pos Neg	15 0	1 31	100 %	97 %	94 %	100 %
<b>VAL1–5<sup>†</sup></b>		<b>Pos Neg</b>	<b>82 0</b>	<b>6 150</b>	<b>100 % (94–100)</b>	<b>96 % (91–98)</b>	<b>93 % (85–97)</b>	<b>100 % (97–100)</b>
VAL-6 Feb–March 2021	6	Pos Neg	11 3	6 24	79 %	80 %	65 %	89 %
VAL-7 March 2021	7	Pos Neg	11 4	6 23	73 %	79 %	65 %	85 %
<b>VAL6–7<sup>†</sup></b>		<b>Pos Neg</b>	<b>22 7</b>	<b>12 47</b>	<b>76 % (56–89)</b>	<b>80 % (67–89)</b>	<b>65 % (46–80)</b>	<b>87 % (74–94)</b>
<b>KOSTI</b>								
VAL-1 Dec 2020	4	Pos Neg	15 1	2 27	94 %	93 %	88 %	96 %
VAL-2 Dec 2020–Jan 2021	7	Pos Neg	16 0	2 22	100 %	92 %	89 %	100 %

VAL-3 Jan 2021	3	Pos Neg	16 1	2 30	94 %	94 %	89 %	97 %
VAL-4 Jan–Feb 2021	1	Pos Neg	13 2	3 37	87 %	93 %	81 %	95 %
VAL-5 Feb 2021	2	Pos Neg	15 0	2 30	100 %	94 %	88 %	100 %
<b>VAL1–5†</b>		<b>Pos Neg</b>	<b>75 4</b>	<b>11 146</b>	<b>95 % (87–98)</b>	<b>93 % (88–96)</b>	<b>87 % (78–93)</b>	<b>97 % (93–99)</b>
VAL-6 Feb–March 2021	5	Pos Neg	15 0	2 33	100 %	94 %	88 %	100 %
VAL-7 March 2021	6	Pos Neg	9 4	10 24	69 %	71 %	47 %	86 %
<b>VAL6–7†</b>		<b>Pos Neg</b>	<b>24 4</b>	<b>12 57</b>	<b>86 % (66–95)</b>	<b>83 % (71–90)</b>	<b>67 % (49–81)</b>	<b>93 % (83–98)</b>
<b>E.T.</b>								
VAL-1 Dec 2020	3	Pos Neg	16 0	1 26	100 %	96 %	94 %	100 %
VAL-2 Dec 2020–Jan 2021	5	Pos Neg	16 0	2 27	100 %	93 %	89 %	100 %
VAL-3 Jan 2021	2	Pos Neg	13 3	3 27	81 %	90 %	81 %	90 %
VAL-4 Jan–Feb 2021	6	Pos Neg	14 2	1 37	88 %	97 %	93 %	95 %
VAL-5 Feb 2021	1	Pos Neg	11 2	4 31	85 %	89 %	73 %	94 %
<b>VAL1–5†</b>		<b>Pos Neg</b>	<b>70 7</b>	<b>11 148</b>	<b>91 % (81–96)</b>	<b>93 % (88–96)</b>	<b>86 % (77–93)</b>	<b>95 % (91–98)</b>
VAL-6 Feb–March 2021	4	Pos Neg	10 4	6 27	71 %	82 %	63 %	87 %
VAL-7 March 2021	7	Pos Neg	10 1	6 26	91 %	81 %	63 %	96 %
<b>VAL6–7†</b>		<b>Pos Neg</b>	<b>20 5</b>	<b>12 53</b>	<b>80 % (59–92)</b>	<b>82 % (70–90)</b>	<b>63 % (44–78)</b>	<b>91 % (80–97)</b>

125 1.Sniffed PCR positive, 2. Sniffed PCR negative, 3.  $Se_{sniff}$ , sensitivity on sniffed samples, 4.  $Sp_{sniff}$ , specificity on sniffed samples, 5. $PPV_{sniff}$ ,  
126 positive predictive value on sniffed samples, 6.  $NPV_{sniff}$ , negative predictive value on sniffed samples. †Shows summed sample numbers and  
127 mean values with 95% Confidence Interval (bolded). The number of SARS-CoV-2 variants per validations: VAL-1 0 alpha/16 wild-type; VAL-2 0  
128 alpha/15 wild-type; VAL-3 3 alpha/9 wild-type; VAL-4 1 alpha/13 wild-type; VAL-5 5 alpha/8 wild-type; VAL-6 7 alpha/1 wild-type; VAL-7 9 alpha/0  
129 wild-type.

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Supplementary Table 3. Data of volunteers providing spike skin swab samples with concomitant RT-PCR<sup>1</sup> verification used in real-life setting to maintain discrimination skills

Characteristics	Spike samples (n=155)
Age, median (IQR) <sup>2</sup>	33 (27)
Child, 0–12 years, n (%)	18 (11.6)
Sex, female, n (%)	83 (53.5)
male, n (%)	72 (46.5)
Sample obtained, n (%)	
Healthy screened	0 (0)
Hospitalised	0 (0)
Outpatient	155 (100)
Sample delay from PCR-test, days, median (IQR)	4 (4)
Symptoms, n (%)	
Asymptomatic	12 (7.7)
Respiratory infection	143 (92.3)
Chronic disease, n (%)	
Asthma, allergy	22 (14.2)
Cancer	6 (3.9)
Hypertension	11 (7.1)
Diabetes	5 (3.2)
Migraine	4 (2.6)

139 1. RT-PCR, reverse transcriptase polymerase chain reaction, 2. IQR, interquartile range

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151 **3. Supplementary appendix. Report of the external controller**

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Orivesi 25.5.2021

I, Lauri Kanerva, retired K-9 police sergeant from the Finnish police K-9 unit and police dog training school, have served as an external controller during the validation of the COVID-19 scent detection dogs at the Helsinki-Vantaa airport during March-April 2021. The validation was performed as a co-operation between the DogRisk research group at the University of Helsinki, led by DVM, PhD Anna Hielm-Björkman, and the Helsinki University Hospital and Professor Anu Kantele.

My duties were to assure that the validation went according to the protocol, to see that the blinded dog handlers and assistant did not have a chance to see the track maps, and to see that there was no extra communication between the blinded dog handler and the blinded sample assistant and any of those, that had the "track facit" in their possession.

The setup was well organized and 6 of the 7 persons involved were the same during all 13 sessions. The seventh person was either of three veterinary students that were involved in the project and that had to share the evaluation work, as they had compulsory teaching. The seven persons that were present were:

Inside the validation cubicle: Dog, dog handler and sample assistant, all blinded (triple-blinded)  
Outside the cubicle: One person giving the sample tracks to the sample assistant and one person taking the samples from the sample assistant. Further we were 3 persons watching the validation from a screen just outside the cubicle: One that informed the dog handler if the result was right or wrong so that the dog-handler could reward the dog, one that evaluated which of the samples the dog sniffed and which they marked, and the third was myself, as an external and independent controller.

The validation started with a "dress rehearsal" when all persons were present, and the setup was introduced to all dogs, handlers and validation crew. A whole validation with new samples was rehearsed. After that the 7 validation days per dog could be done in 12 days. It took longer that first planned since one of the dogs, E. T., got a lacrimal gland infection and had to be on antibiotics for 2 weeks. This infection also disturbed his working and the handler wanted to quit the validation 6 as she could see that the dog had no sense of smell and was merely guessing. So E. T continued his trials 15 days later. As one of the handlers also got ill, all validations stood still for 2 weeks.

From the point of dogs there were also some other disturbance: The building noise at the airport was at times so loud that even we could not hear each other. Also, the air was very dry (below 25% humidity) for dogs to be able to work at their best.

All validations were taken on video from 4 different angles (except two runs, when the researchers forgot to put on the video (7.4.2021 for dog Rele and 26.3.2021 for dog Silja). All videos can be obtained from the researchers on request.

But all in all, everything went as planned, following the protocol, and I can conclude that the validation can be considered valid.

If you have questions, please contact me on [paukku.seri@gmail.com](mailto:paukku.seri@gmail.com)

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Lauri Kanerva, Retired K-9 police sergeant

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157 **4. Supplementary references**

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