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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Hernaez B, Muñoz-Gómez A, Sanchiz A, et al. Monitoring monkeypox virus in saliva and air samples in Spain: a cross-sectional study. *Lancet Microbe* 2022; published online Nov 24. https://doi.org/10.1016/S2666-5247(22)00291-9.

Appendix

Monitoring monkeypox virus in saliva and air samples in Spain: a cross sectional study

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SUPPLEMENTARY METHODS

STUDY DESIGN

We used a cross sectional design, with consecutive patients who visited our STI Clinics (Centro Sandoval) or Hospital Clínico San Carlos, Madrid between May 18th and July 15th 2022. Our database of patients is composed of all individuals who tested positive in one of our centers, starting in May 18th 2022. From each patient with vesicular-umbilicated and pseudo-pustular skin lesions suspicious of MPXV infection, demographic information (biological sex and age) is collected as well as a complete clinical and epidemiological survey which includes gender, sexual orientation, smallpox vaccination reported by the patient, first symptom, symptoms present at the time of the visit, date of onset of the disease, previous contacts with infected population and other risk factors, such as concomitant STIs, number of sexual partners or sexually unprotected intercourses. From each patient a sample of the cutaneous lesion, saliva, mask and air was obtained. All patients signed an informed consent to be included in the study.

PARTICIPANTS AND VARIABLES

Current study included all the patients who tested positive for MPXV in any suggestive skin lesion between May 18th and July 15th 2022. In patients with two samples, the most recent sample from the first visit was selected when this information was used to associate clinical and laboratory data. For the statistical analysis, clinical information (time since the onset of symptoms, first symptom, symptoms at the time of the visit, location of typical cutaneous lesions) were considered. Table S1 shows the clinical data, with symptoms referred at any time point (sum of first symptom and symptoms at the evaluation). Just vesicular-umbilicated or pseudo-pustular skin lesions are counted for the analysis. The location of cutaneous lesions was classified in 8 sites: facial (all the skin in the face but the perioral area), perioral (if the lesion involved the lips or the buccal mucosa), upper limbs, lower limbs, palms and soles, trunk, genital (if external genitals showed lesions) or anal. If at least one lesion was found in any of these sites, the area was considered as affected. Subsequent broader areas are considered, such as anogenital area which is the sum of the genital and anal area, and any facial area which is the sum of perioral and facial area. If the patient had perioral lesions or odynophagia at the time of the visit was considered to have oral involvement. The count of affected skin regions took into account the eight pre-defined areas.

A 28 year-old male patient was hospitalized for community-acquired pneumonia, along with a pattern of skin lesions and lymphadenopathy characteristic of MPXV. In addition, parainfluenza A virus and human rhinovirus/enterovirus was detected in a multiarray of respiratory viruses (Biofire respiratory Panel 2.1 Plus, bioMérieux Spain, Madrid) in a nasopharyngeal exudate. The patient was admitted to an isolation room with symptomatic treatment; pneumonia and MPXV symptoms evolved favorably.

CONDITIONS OF AIR SAMPLING

The air samples were obtained from the two participating centers, the hospital's infectious disease consultation and the Sandoval center's consultation. The hospital's office is a large room of about 125 m^2 with a 2.5 m ceiling, with the hospital's internal ventilation (Fig. S3A, B). However, it does not have a formal air renewal system. In the clinic, the office is a 15 m^2 room with a height of 3.5 m, with a window that was kept open while the patients were in it (Fig.S3C, D). In both offices, air samples were obtained as indicated in the Methods section, through 47-mm nanofiber filters connected to air pumps, placed at 2-3 m from the patient. These filters were connected when the patient entered the office; the patient was there for 30-45 min with an FFP2 mask on, removing it for ~30 sec during exploration of the oral cavity and oropharynx. We turned off the filter at the end of each consultation. A new filter was put on when the next patient entered the same room. There were no specific ventilation procedures between patients; therefore, we cannot rule out that the viral material detected during consecutive patient visits could have remained in suspension in the air of the previous patient. The hospital room where the patient was admitted was a negative pressure isolation room of 15 m^2. The nanofiber filter was located at 2-3 m from the patient and no room cleaning or changing of bed linen took place during the air sampling. The patient was not wearing a mask during the sampling period.

MPXV DNA ISOLATION

Saliva was inactivated under BSL3 containment by addition of 1 volume of 2X CLB (Promega) in a DNA low binding tube (Eppendorf) before DNA isolation. Maxwell® RSC Viral Total Nucleic Acid Purification kit (Promega) was used for DNA extraction in combination with a Maxwell® RSC 48 Instrument (Promega) following manufacturer's instructions. Briefly, mask filters, air filters and saliva in CLB were mixed by vortexing, and 400, 600 and 400 µl, respectively, were used for DNA isolation. To enhance the

recovery of DNA, 100 ng of human DNA (from HeLa cells) were added to each sample from mask and air filters as DNA carrier. DNA was finally eluted in 50 µl of nuclease-free water. A blank sample containing CLB was included in each DNA extraction batch to control potential contamination. DNA samples were stored at -20º C until qPCR analysis.

DETECTION AND QUANTITATION OF MPXV DNA BY qPCR

MPXV DNA detection was performed by qPCR in a CFX Opus 384 Real-Time PCR system (Bio-Rad) using specific primers and fluorescent-labelled probes previously described (2): MPXV_WA-F, 5'-CACACCGTCTCTTCCACAGA-3'; MPXV_WA-R, 5'-GATACAGGTTAATTTCCACATCG-3'; and MPXV_WA-P, 5'-FAM-AACCCGTCGTAACCAGCAATACATTT-BHQ-3' (Metabion). As human DNA control, partial sequence of the hypoxanthine phosphoribosyltransferase (HPRT) gene was amplified in a duplex reaction with MPXV_WA: HPRT1_F, 5'-TTCTTTGCTGACCTGCTGGA-3'; HPRT1_R, 5'-ACAATCAAGACATTCTTTCCAGTT-3'; HPRT1_P, 5'-HEX-ATTGGTGGA/ZEN/GATGATCTCTCAACT/3IABkFQ/-3' (IDT). The 85 bp specific MPXV amplicon was cloned into pGEM-T easy vector (Promega) and 10-fold serially diluted to prepare a standard curve from 10^6 to 1 copy/µl. qPCRs were performed in a final volume of 10 µl containing 3 µl of isolated DNA samples, 2X iTaq Universal Probe Supermix (Bio-Rad), 800 nM MPXV_WA primers, 200 nM MPXV_WA probe, 100 nM HPRT primers 200 nM HPRT probe. Amplification protocol consisted of 5 s at 95ºC followed by 40 cycles of 30 s at 95ºC and 20 s at 62ºC. All measurements were made in triplicate and non-template control together with the standard curve were included in each plate. CFX Maestro 2.2 Software (Bio-Rad) was used for data analysis. Only samples with at least 2 of 3 positive replicates, both in MPXV_WA and HPRT signals, were considered positive. After quantification by Ct interpolation in the calibration curve, data were converted to copies of MPXV DNA/ml of saliva, copies/mask-filter or copies/m^3 of captured air.

DETERMINATION OF MPXV VIABILITY FROM CLINICAL SAMPLES

Virus infectivity from clinical samples were tested in BSL3 facilities. A 20% volume of Dulbecco´s modified Eagle medium containing 2% fetal bovine serum, 0.5 µg/ml amphotericin B, 100 U/mL penicillin and 100 µg/ml streptomycin was added to saliva samples as soon as they arrived to the BSL3 facility. BSC-1 cells (ATCC CCL-26) seeded in 12-well plates at 70–80% of confluency were inoculated within the first 72 h after sample collection (except where indicated) with 200 µl of saliva samples or 300 µl of mask and air filter samples. Virus adsorption was performed at 37ºC for 90 min and then cells were extensively washed with PBS before the addition of 0.5 ml Dulbecco´s modified Eagle medium containing 2% fetal bovine serum, 0.5 µg/ml amphotericin B, antibiotics and 10 mM HEPES. Plates were sealed and incubated at 37ºC for daily examination of cytopathic effect.

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Table S1. Clinical and epidemiological features of the study patients with monkeypox infection, H. Clinico San Carlos and Centro Sanitario Sandoval, Madrid (n=44 patients).

ID Sample	Age (years)[a]	Concomitant STIs[b]	Smallpox vac	Days from the onset[c]	Location of skin lesions[d]	Other symptoms[e]
1	32	HIV	NO	7	UL, T, F, LL, A, PS	F, R, ADi, O, A, M, S
2	25	HIV, CT	NA	7	A	A, M, P, S
3	45	HIV	NA	4	UL, T, PO, LL, G,	A, M, S
4	32	HIV, NG	NO	7	UL, T, F, LL	F, R, S
5	32	HIV	NO	4	T, F	**F**, **R**, ADi, H, O, A, S
6	27	NO	NO	4	G	**F**, ADi, H, O, A, U, P, S
7	25	HIV	NO	2	UL, T, PO, F, LL, G, PS	**F**, ADi, AD, O, A, M, U, C, S
8	21	NO	NO	4	G	**F**, ADi, H, O, A, M, U, P, S
9	49	HIV	YES	8	PO, A	**F**, **R**, AD, H, O, A, S
10	35	HIV	NO	1	G	**F**, ADi, S
11	23	NO	NO	6	UL, T, F, A, PS	F, ADi, AD, O, **A**, M, P, S
13	30	HIV	NO	3	UL, T, PO, F, LL, PS	**F**, R, AD, O, A, M, S
14	43	NO	YES	8	UL, T, PO, F, LL, PS	ADi, **S**
15	32	HIV, NG	NO	7	UL	ADi, O, P, S
16	49	HIV	NO	5	T, PO, LL, PS	**F**, O, A, P, S
17	37	HIV	NO	4	UL, T, F, LL, G	F, ADi, AD, H, O, **A**, M, NC, C, S
18	52	HIV, CT	NA	5	T, F, G, A, UL, LL	R, ADi, H, **A**, M, U, P, S
19	40	NA	NA	4	UL, T, F, LL, G, PS	F, **R**, ADi, A, M, S
20	28	TP	NO	3	PS	**F**, ADi, A, S
21	46	HIV, MG	YES	4	UL, T, PO, F, LL, G	F, R, ADi, AD, H, **O**, A, M, P, S
22	38	HIV	YES	6	UL, T, PO, F, LL, G	F, ADi, AD, H, A, M, **S**
23	34	NO	YES	3	LL	F, ADi, AD, H, **O**, A, M, S
24	36	HIV, MG	NO	6	G	R, AD, O, **S**
25	33	NO	YES	4	T, F, G	F, ADi, A, M, **S**
26	47	HIV	NO	5	UL, T, PO, LL, G	H, A, C, **S**
27	35	HIV	NO	8	UL, T, PO, F, LL	F, ADi, AD, O, **A**, M, P, S
28	35	HIV	NO	8	UL, T, PO, F, LL	F, **AD**, H, O, A, M, S
29	52	HIV	YES	7	UL, T, PO, F, LL, A	F, H, A, M, S
30	42	NO	YES	4	UL, T, F, LL, A	**F**, AD, H, A, M, NC, C, S
31	32	CT	NO	7	UL, T, PO, F, LL, A	ADi, **A**, M, P, NC, C, D, S
32	33	HIV	NO	7	UL, T, PO, F, LL, G, A	**F**, O, P, S
33	36	NO	NO	3	PO, G, A	H, O, A, M, U, P, **S**
34	32	NO	NO	6	G	**F**, A, M, P, S

35	58	NO	YES	9	A	**F**, H, **A**, P, S
36	27	NG	NA	9	UL, T, PO, LL, G	**F**, R, AD, H, A, M, S
37	48	NG	NO	5	F, A	**F**, ADi, H, O, A, P, S
38	54	NO	NO	2	G	ADi, **H**, **M**, C, S
39	35	NO	YES	5	UL, T, F, LL, G	F, ADi, AD, H, A, M, **S**
41	34	HIV	NO	4	UL, PO, G	**F**, **R**, ADi, H, O, A, M, P, S
42	30	HIV	NA	4	A	**F**, **R**, **H**, **A**, **M**, **P**, ADi, S
43	29	TP	NO	3	PO, G	ADi, AD, H, O, A, S
44	44	NO	YES	6	PO	**F**, **AD**, H, M, S
45	33	NG	NO	4	UL, T, F, A	**H**, F, A, M, P, S
46	41	NO	NO	5	G	F, ADi, O, A, M, **S**

[a]All patients were men.
[b] HIV – human immunodeficiency virus; CT – *Chlamydia trachomatis*; NG – *Neisseria gonorrhoeae*; TP – *Treponema pallidum* (Syphilis); MG – *Mycoplasma genitalium*. STI- sexually transmitted infection.
[c] Days from the onset of symptoms to acquisition of saliva, filter-mask and air-filter samples
[d] T- Trunk, PO- periorally; F- facial; G- genital; A – anal; PS – palm/sole; UL- upper limbs; LL- lower limbs
[e] F- fever; R- Rash; H- Headache; O- Odynophagia; A- Asthenia; M – Myalgia; U – Urethritis; P- Proctitis; NC – nasal congestion; C – cough; D – Dyspnea; ADi – Inguinal adenopathy; AD- Other adenopathy; S – Skin lesions. Bold and underline indicate first symptoms reported.
NA – not available.

Table S2. Obtained data regarding monkeypox vDNA detection and quantification by qPCR and determination of MPXV viability from clinical samples (saliva, filter from the mask and air filter). Ct interpolation was performed in a calibration curve with LOD of 1 copies/μl of vDNA. Virus infectivity from clinical samples was tested in cell culture.

ID Sample	Saliva qPCR result	Mean Ct	Viral load (copies/ml)	Viability assay	Mask qPCR result	Mean Ct	Viral load (copies/mask)	Viability assay	Air qPCR result	Mean Ct	Viral load (copies/m^3)
1	POSITIVE	20.70	1.83E+06	POSITIVE	POSITIVE	33.50	7.98E+01	NEGATIVE	POSITIVE	34.74	3.57E+01
2	POSITIVE	26.30	2.03E+04	POSITIVE	NEGATIVE	36.30	9.53E+00	NEGATIVE	POSITIVE	31.50	6.93E+02
3	POSITIVE	28.50	8.76E+03	NEGATIVE	POSITIVE	34.00	5.93E+01	NEGATIVE	NEGATIVE	36.70	1.12E+01
4	NEGATIVE	36.20	2.33E+01	UNTESTED	NEGATIVE	35.20	7.33E+01	NEGATIVE	UNDETERMINED	34.60	4.92E+01
5	POSITIVE	26.60	3.95E+04	POSITIVE	NEGATIVE	35.30	1.83E+01	NEGATIVE	POSITIVE	34.00	2.63E+02
6	UNDETERMINED	34.40	8.10E+01	UNTESTED	POSITIVE	34.00	5.46E+01	NEGATIVE	POSITIVE	34.30	6.67E+01
7	POSITIVE	22.60	1.13E+06	UNTESTED	POSITIVE	28.90	3.11E+03	POSITIVE	POSITIVE	31.20	7.49E+02
8	NA	NA	NA	NA	NEGATIVE	35.70	1.41E+01	NEGATIVE	NA	NA	NA
9	POSITIVE	21.82	4.22E+06	UNTESTED	POSITIVE	31.99	3.82E+02	NEGATIVE	POSITIVE	33.31	1.77E+02
10	POSITIVE	28.15	2.20E+04	NEGATIVE	POSITIVE	32.41	2.55E+02	NEGATIVE	NEGATIVE	35.26	1.10E+02
11	POSITIVE	18.21	7.66E+07	POSITIVE	POSITIVE	28.53	6.56E+03	NEGATIVE	UNDETERMINED	34.10	9.14E+01
13	POSITIVE	26.04	1.92E+05	POSITIVE	POSITIVE	26.28	3.93E+04	NEGATIVE	POSITIVE	33.18	2.42E+02
14	POSITIVE	28.31	6.33E+04	NEGATIVE	POSITIVE	32.21	3.20E+02	NEGATIVE	POSITIVE	28.58	9.43E+03
15	NEGATIVE	35.95	2.93E+01	NEGATIVE	POSITIVE	25.81	6.48E+04	NEGATIVE	POSITIVE	31.66	7.14E+02
16	POSITIVE	22.46	1.09E+06	NEGATIVE	POSITIVE	33.83	7.74E+01	NEGATIVE	POSITIVE	30.64	1.66E+03
17	POSITIVE	20.17	1.28E+07	POSITIVE	POSITIVE	29.89	2.18E+03	NEGATIVE	POSITIVE	33.47	1.54E+02
18	POSITIVE	24.22	1.14E+06	POSITIVE	POSITIVE	34.24	2.62E+02	NEGATIVE	NA	NA	NA
19	POSITIVE	22.23	2.75E+06	POSITIVE	POSITIVE	30.81	9.87E+02	NEGATIVE	NEGATIVE	34.83	6.63E+01
20	POSITIVE	29.70	1.73E+04	NEGATIVE	POSITIVE	33.14	3.12E+02	NEGATIVE	NEGATIVE	37.14	2.77E+01
21	POSITIVE	24.52	9.17E+05	POSITIVE	POSITIVE	29.81	7.83E+03	POSITIVE	POSITIVE	33.59	3.44E+02
22	POSITIVE	27.08	2.01E+05	POSITIVE	NEGATIVE	36.49	6.63E+01	NEGATIVE	NEGATIVE	35.66	1.37E+02
23	POSITIVE	25.92	5.79E+05	POSITIVE	UNDETERMINED	34.82	1.00E+02	NEGATIVE	NEGATIVE	35.84	6.91E+01
24	NEGATIVE	35.20	1.88E+02	NEGATIVE	POSITIVE	31.00	1.46E+03	NEGATIVE	NEGATIVE	37.10	4.48E+01
25	POSITIVE	28.49	1.65E+04	NEGATIVE	UNDETERMINED	34.85	9.46E+01	NEGATIVE	NEGATIVE	36.10	6.05E+01
26	POSITIVE	24.31	3.81E+05	NEGATIVE	NEGATIVE	35.51	4.54E+01	NEGATIVE	NEGATIVE	37.2	1.25E+01
27	POSITIVE	27.70	3.32E+04	POSITIVE	POSITIVE	35.36	3.27E+01	NEGATIVE	POSITIVE	28.74	8.02E+03
28	POSITIVE	19.75	1.48E+07	POSITIVE	POSITIVE	35.69	2.72E+01	NEGATIVE	POSITIVE	33.47	1.80E+02
29	NA	NA	NA	POSITIVE	POSITIVE	30.58	1.31E+03	NEGATIVE	POSITIVE	31.01	1.31E+03
30	POSITIVE	24.07	3.70E+05	POSITIVE	POSITIVE	32.42	2.87E+02	NEGATIVE	UNDETERMINED	34.29	1.09E+02
31	POSITIVE	21.86	2.73E+06	POSITIVE	POSITIVE	32.46	2.74E+02	NEGATIVE	POSITIVE	33.65	1.65E+02
32	POSITIVE	21.86	2.71E+06	POSITIVE	POSITIVE	35.54	2.47E+01	NEGATIVE	POSITIVE	34.23	1.16E+02

33	NEGATIVE	35.76	1.98E+01	NEGATIVE	**POSITIVE**	32.98	1.80E+02	NEGATIVE	**POSITIVE**	33.30	2.07E+02
34	**POSITIVE**	27.04	6.76E+04	NEGATIVE	**POSITIVE**	31.85	4.46E+02	NEGATIVE	**POSITIVE**	33.25	2.19E+02
35	**POSITIVE**	31.48	1.33E+03	NEGATIVE	**POSITIVE**	32.15	3.54E+02	NEGATIVE	**POSITIVE**	32.26	4.84E+02
36	**POSITIVE**	23.27	4.50E+05	**POSITIVE**	**POSITIVE**	29.84	2.24E+03	NEGATIVE	**POSITIVE**	32.36	4.79E+02
37	**POSITIVE**	20.97	2.78E+06	**POSITIVE**	**POSITIVE**	32.61	2.45E+02	NEGATIVE	**POSITIVE**	33.41	1.98E+02
38	**POSITIVE**	32.25	1.13E+03	NEGATIVE	**POSITIVE**	33.06	1.24E+02	NEGATIVE	NA	NA	NA
39	**POSITIVE**	20.20	6.85E+06	**POSITIVE**	**POSITIVE**	32.55	1.86E+02	NEGATIVE	**POSITIVE**	33.79	2.96E+02
40	NEGATIVE	37.72	8.73E+00	NEGATIVE	NEGATIVE	36.16	7.14E+01	NEGATIVE	UNDETERMINED	34.21	2.93E+00
41	**POSITIVE**	21.85	7.23E+06	**POSITIVE**	NEGATIVE	37.08	4.75E+01	NEGATIVE	**POSITIVE**	31.19	4.25E+03
42	NA	NA	NA	NA	UNDETERMINED	34.75	1.89E+02	NEGATIVE	**POSITIVE**	32.53	1.44E+03
43	**POSITIVE**	21.71	8.58E+06	**POSITIVE**	**POSITIVE**	30.29	6.13E+03	NEGATIVE	**POSITIVE**	31.51	7.04E+03
44	**POSITIVE**	25.79	3.53E+05	**POSITIVE**	NEGATIVE	36.12	1.08E+02	NEGATIVE	NEGATIVE	34.90	2.52E+02
45	**POSITIVE**	17.67	3.90E+07	NEGATIVE	**POSITIVE**	31.15	7.88E+02	NEGATIVE	**POSITIVE**	32.44	4.14E+02
46	NA	NA	NA	NA	NEGATIVE	35.96	2.11E+01	NEGATIVE	UNDETERMINED	34.10	1.49E+01

* Samples no. 38 and 40 were collected from the same patient (No. 38 in Table S1) before and after confirmation of MPXV infection.
UNDETERMINED- Below qPCR limit of detection (LOD) or only 2 out of 3 wells were positive with mean Ct between 34 and 35 (baseline of positivity).
Amplification reaction up to 40 cycles. NA – not available.

Table S3. Confirmation of MPXV presence from cell culture where cytopathic effect was observed after infection with samples. Detection by qPCR was performed in triplicate with DNA diluted from 1/10 to 1/100.

Sample number and kind of sample*	Mean Ct
1S	NA#
2S	NA
5S	16,28
7M	16,31
11S	16,67
13S	11,45
17S	17,13
18S	20,02
19S	21,13
21S	12,00
21M	14,09
23S	14,13
26S	17,49
27S	17,90
28S	15,20
30S	13,60
31S	15,11
32S	15,10
36S	16,77
37S	16,32
39S	13,78
41S	15,12
43S	12,60
44S	16,40
45S	22,69

*S, saliva, M, mask.
#NA, not available.

Table S4. Viral load and viability of the virus in different samples

The log_{10} transformed values of viral loads are shown, with the median [min-max] of each distribution. A Wilcoxon rank sum test was performed to test pairwise differences between total saliva, mask and air sample distributions, as well as of viable and non-viable specimens in each sample. It is shown how viable samples contain higher viral loads in all available samples. Another Wilcoxon test was performed to assess if saliva, mask and air samples were drawn from the same distribution. It is shown that saliva samples were different from mask or air samples. No difference was found between air and mask samples in viral load.

	Total	Non-viable	Viable	p value
Saliva sample	5.57 [1.3-7.88] *, †	4.23 [1.3-7.59]	6.01 [4.31-7.88]	<0.001
Mask sample	2.33 [0.98-4.81] ‡	2.27 [0.98-4.81]	3.69 [3.49-3.89]	0.03
Air sample	2.3 [0.47-7.41]	2.3 [0.47-7.41]	-	1

Footnote: * p for the comparison between saliva and mask samples < 0.001, † p for the comparison between saliva and air samples <0.001, ‡ p for the comparison between air and mask samples = 0.97

Table S5. Association of symptoms and viral load in saliva.

All viral loads (Table S2) were log10 transformed, with the median [min-max] of each distribution shown in each cell. Presence was understood as the cohort of patients which exhibited the symptom or sign, while absence was the cohort of patients which did not show that symptom (Table S1). A Wilcoxon rank sum test was performed to test the differences in viral loads between patients who reported each symptom and those who did not. It is shown how non-inguinal adenopathies, asthenia, myalgias and umbilicated papules in upper limbs, trunk and in facial locations are associated with higher viral load.

	Absence	Presence	p value
Fever	5.28 [1.3-6.93]	5.96 [1.37-7.88]	0.09
Rash	5.57 [1.3-7.88]	5.65 [1.37-6.26]	0.53
Inguinal adenopathies	5.55 [1.3-7.59]	5.96 [1.47-7.88]	0.34
Non-inguinal adenopathies	4.7 [1.3-7.59]	5.86 [2.27-7.88]	0.03
Headache	4.8 [1.37-7.88]	5.76 [1.3-7.17]	0.13

Odinophagia	5.07 [1.37-7.59]	6.05 [1.3-7.88]	0.16
Asthenia	3.7 [1.37-6.43]	5.86 [1.3-7.88]	0.01
Myalgias	4.47 [1.37-6.93]	5.86 [1.3-7.88]	0.04
Urethral pain	5.57 [1.37-7.88]	3.98 [1.3-6.06]	0.30
Rectal symptoms	5.56 [1.37-7.17]	6 [1.3-7.88]	0.84
Nasal congestion	5.55 [1.3-7.88]	6.44 [5.57-7.11]	0.21
Cough	5.42 [1.3-7.88]	5.82 [3.05-7.11]	0.54
Dyspnea	5.57 [1.3-7.88]	6.44 [6.44-6.44]	0.55
Skin lesions locations			
Upper limbs	4.34 [1.3-6.93]	6.05 [1.37-7.88]	0.02
Trunk	4.32 [1.3-6.93]	6 [1.37-7.88]	0.04
Perioral	4.71 [1.37-7.88]	5.81 [1.3-7.17]	0.27
Facial	4.34 [1.3-6.93]	6.06 [1.37-7.88]	0.01
Any facial	3.68 [1.47-5.76]	6.05 [1.3-7.88]	<0.01
Lower limbs	4.34 [1.3-7.88]	5.96 [1.37-7.17]	0.12
Genitals	5.56 [1.37-7.88]	5.62 [1.3-7.11]	0.82
Anal	5.29 [1.37-7.17]	6.35 [1.3-7.88]	0.13
Anogenital	4.8 [1.37-7.17]	5.96 [1.3-7.88]	0.16

Table S6. Progressivity in positivity and viability of the samples with increasing number of lesions.

Each row includes the cumulative sum of patients that had at least the specified number of regions affected, e.g. row < 2 includes patients with less than 2 areas involved (all patients with 1 skin area involved), and row < 3 includes patients with less than 3 areas with lesions (all patients with 1 or 2 skin areas). For each group the percentage of positivity (number of positive samples divided by number of available samples) and the percentage of viability (number of viable samples divided by number of available samples) was calculated. A Cochran-Armitage test was calculated to assess the progression of the percentage in each sample. Cumulative sum was employed to overcome the small number of cases in each category.

	Positivity			Infectivity	
Number of skin areas	Saliva*	Mask †	Air ‡	Saliva ¶	Mask§
< 2 (1 area)	0/10 (0%)	0/12 (0%)	0/10 (0%)	0/10 (0%)	0/14 (0%)
< 3 (1 or 2 areas)	4/14 (28.6%)	3/16 (18.7%)	4/14 (28.6%)	0/13 (0%)	0/18 (0%)
< 4 (1,2 or 3 areas)	6/17 (35.3%)	3/18 (16.7%)	6/17 (35.3%)	1/16 (6.25)	0/21 (0%)
< 5 (1, 2, 3 or 4 areas)	8/20 (40%)	5/21 (23.8%)	6/19 (31.6%)	1/18 (5.5%)	0/24 (0%)
< 6 (1, 2, 3, 4 or 5 areas)	16/29 (55.2%)	13/30 (43.3%)	6/27 (22.2%)	9/27 (33.3%)	0/33 (0%)
<7 (1, 2, 3, 4, 5 or 6 areas)	16/37 (43.2%)	21/39 (53.8%)	13/36 (36.1%)	16/36 (44.4%)	1/42 (2.4%)
All patients	18/39 5 NA (46.1%)	23/41 3 NA (56.1%)	15/38 6 NA (39.5%)	16/37 7 NA (43.2%)	2/44 0 NA (4.5%)

p values for the Cochran-Armitage test; * = 0.01, † < 0.01, ‡ = 0.06, ¶ < 0.01, § = 0.08

Figure S1. **Flowchart diagram of the patients**. S = saliva samples, M = mask samples, A = air samples, L = data for laboratory analysis, C = data for association with clinical analysis. Samples #38 and #40 are from the same patient, so sample #40 was excluded from the clinical association analysis and just the first sample (#38) was used. Undetermined values in the qPCR were treated as NA (not available) in the clinical association analysis.

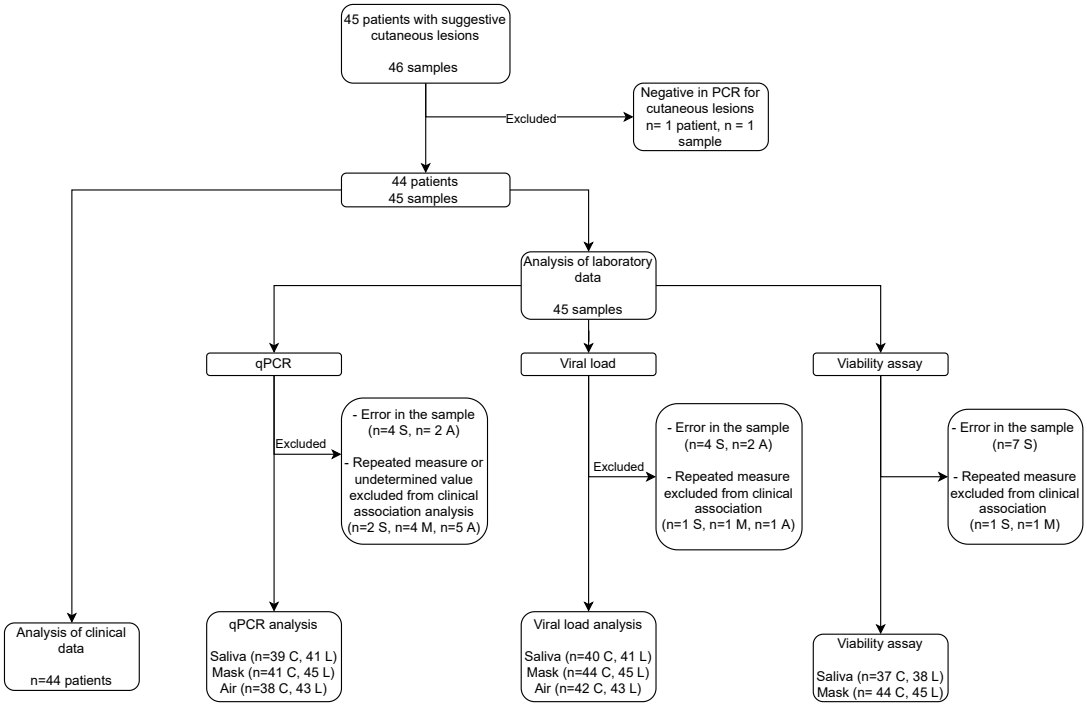

Figure S2. Scatterplot of the number of skin regions with visible lesions and viral load. Note that viral load is log transformed. Each dot represents one patient and the dashed blue line is the best fit model for the data, with the coefficients shown in the upper left corner of the figure, as well as the R2 of the model.

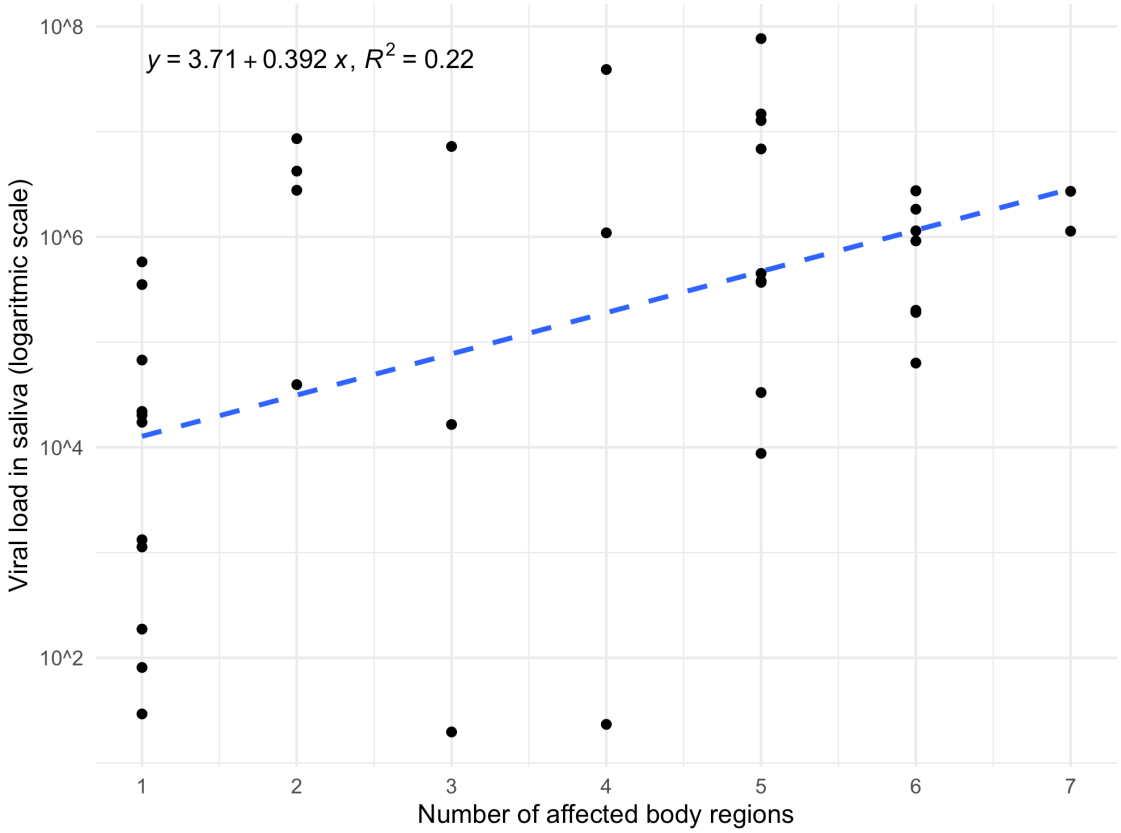

Figure S3. Photographs of the hospital's infectious disease consultation (A, B) and the Sandoval center's consultation rooms (C, D).