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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.

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# SUPPLEMENTARY APPENDIX

## Persistence of monkeypox virus at oral and rectal sites.

Towns, Lim *et al*

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**SUPPLEMENTARY METHODS**

### *Case Clearance*

In Victoria, Australia, the Department of Health stipulated that a person with mpox (previously monkeypox disease) could leave isolation when all the following criteria were met: a) they are clinically well and all systemic symptoms have resolved; b) there have been no new lesions for at least 48 hours, there are no mucous membrane lesions and all lesions in exposed areas have re-epithelialised; and c) lesions on unexposed skin, if not fully healed, must be covered at all times when in contact with other people.[1,2] *Re-epithelialised* was defined as the formation of new skin once the crusts/scabs had fallen off lesions. The decision to clear a patient from isolation was made by the patient's treating clinician, who notified the Department of Health. The Victoria guidelines were aligned with the UK NHS virtual management of confirmed mpox cases, which stated that after self-isolation has ended, any remaining lesions should be covered when leaving the house or having close contact with people, until they are re-epithelialised.[3]

### *Clinical samples and storage*

This study was conducted at Melbourne Sexual Health Centre, the main sexually transmitted infections clinic in Melbourne, Australia. Patients attending the clinic with symptoms suggestive of, or as a contact of mpox, had samples collected. This included clinician-collected lesion swabs and self-collected first-pass urine, oral and anal swabs on the day of first presentation (day 0) and then weekly until lesion resolution and clinical clearance was achieved. The first and second review visits occurred on a median of 7 days and on a median of 14 days after the first presentation. Oral and anal swabs were placed in viral transport medium and sent with the urine samples to the Victorian Infectious Diseases Reference Laboratory (VIDRL) at the Peter Doherty Institute for Infection and Immunity. The samples were stored at 4° Celsius for up to 24 hours prior to testing.

### *Monkeypox virus PCR, culture, and serology*

Monkeypox virus polymerase chain reaction (PCR) testing has been previously described.[4] Monkeypox PCR-positive specimens were cultured in a 24-well cell-culture plate containing a Vero cell monolayer in an incubator at 37˚C with 5% $CO_2$. Wells were monitored for virus-induced cytopathic effect (CPE) and CPE readings were recorded by two independent readers for each sample. Serological testing for anti-Monkeypox IgG was conducted using indirect immunofluorescent assay (IFA) developed at VIDRL. Patient sera were incubated with microscope slides fixed with both Monkeypox-infected and non-infected Vero cells, followed by incubation with anti-human fluorescein-conjugated secondary antibody and visualisation by two independent readers.

### *Statistical analysis*

All data analysis, including frequency analyses and calculation of proportions was performed using SPSS version 29.0.0.0.

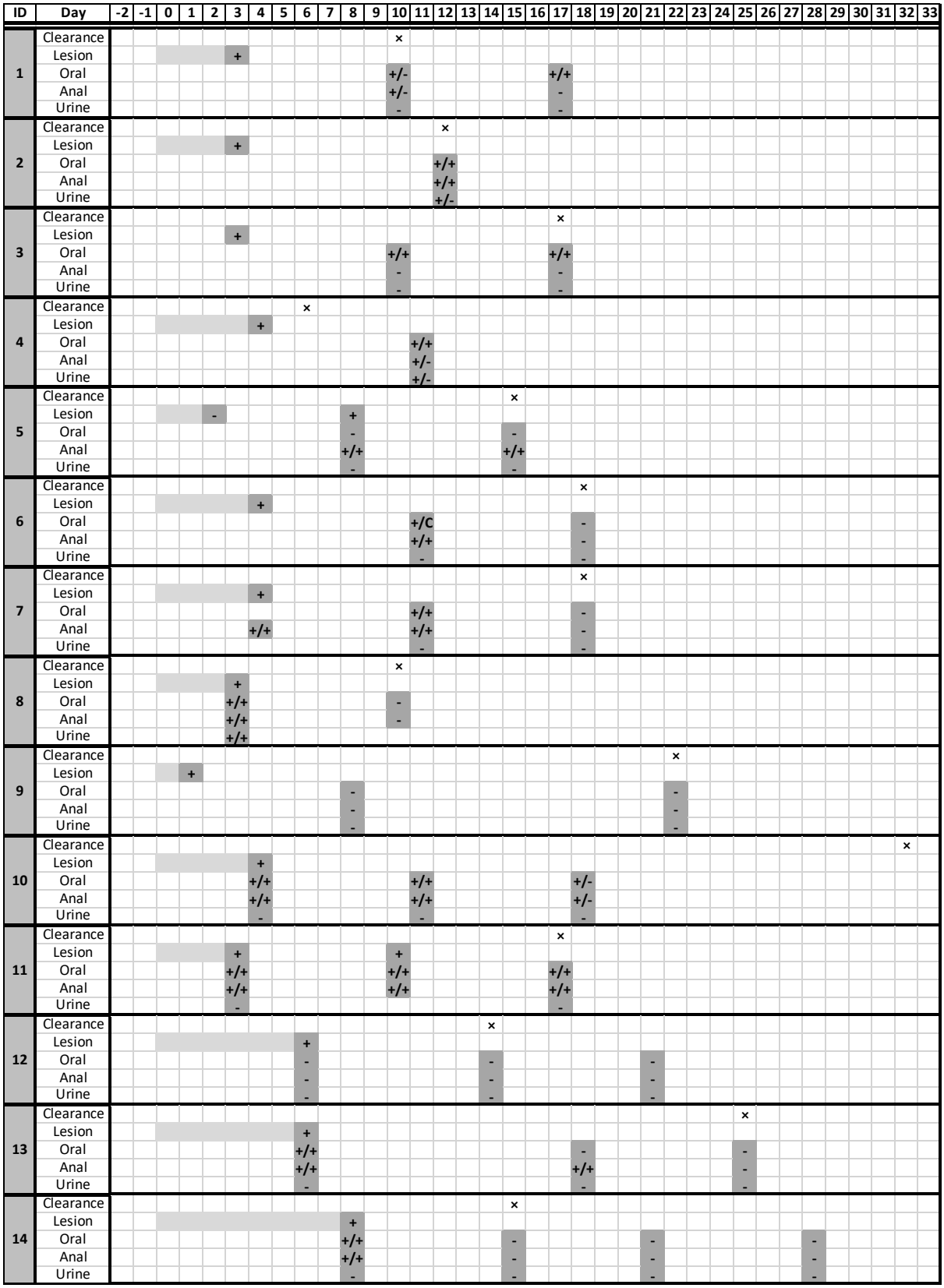
**SUPPLEMENTARY TABLE 1**

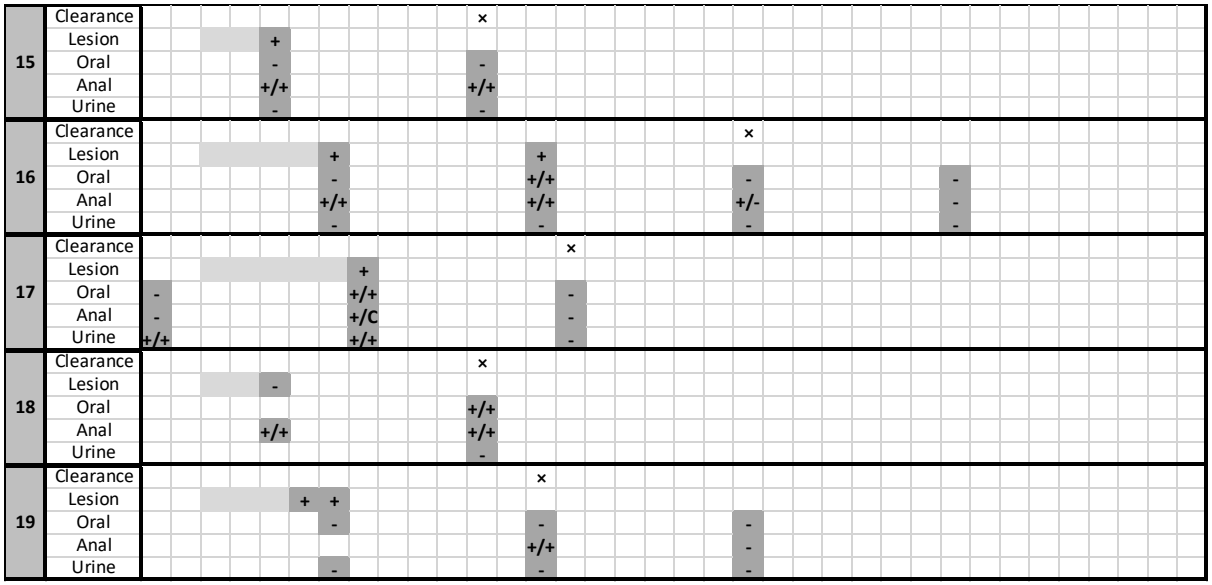
**S Table 1: Clinical and laboratory characteristics of 19 men with mpox.**

| *Characteristics of men* | n/N (%) | % |
|---|---|---|
| Median age: 36 years (IQR 30 – 40 years) | | |
| People living with HIV | 3/19 | 15.8 |
| Taking HIV PrEP | 10/16 | 62.5 |
| Previous smallpox vaccination | 2/19 | 10.5 |
| Proctitis | 11/19 | 57.9 |
| Pharyngitis | 2/19 | 10.5 |
| Prodrome | 11/19 | 57.9 |
| Hospitalised | 1/19 | 5.3 |
| Lesion sites: | | |
| Oral | 1/19 | 5.3 |
| Anal | 9/19 | 47.4 |
| Genital | 9/19 | 47.4 |
| Other | 6/19 | 31.6 |
| *Monkeypox virus PCR* | | |
| Mucocutaneous lesion swab | 18/19 | 94.7 |
| Oral swab PCR positive | 14/19 | 73.7 |
| Oral/facial lesion present at PCR positive site | 2/14 | 14.3 |
| Anal swab PCR positive | 16/19 | 84.2 |
| Anal lesion present at PCR positive site | 9/16 | 56.3 |
| Urine PCR positive | 4/19 | 21.1 |
| Genital lesion present if urine PCR positive | 3/4 | 75.0 |
| No. of patients with any positive PCR at clinical clearance | 10/19 | 52.6 |
| Oral PCR positive | 6/19 | 31.6 |
| Anal PCR positive | 9/19 | 47.4 |
| Urine PCR positive | 2/19 | 10.5 |
| *Monkeypox virus culture* | | |
| No. of patients with any positive culture at clinical clearance | 8/19 | 42.1 |
| Oral culture positive | 6/6 | 100 |
| Anal culture positive | 6/9 | 66.6 |
| Urine culture positive | 0/2 | 0 |
| *Monkeypox virus serology* | | |
| Anti-monkeypox IgG seropositivity* | 8/16 | 50.0 |
| *Serology specimens collected at day 0 (n = 10), day 4 (n = 1) and days 13-15 (n = 5) | | |

**SUPPLEMENTARY FIGURE 1**

**S Figure 1.** Time of detection of monkeypox virus by PCR and culture from urine, oral and anal samples compared with clearance of mpox lesions at other sites.






S Figure 1 legend:

| | |
|---|---|
| | Symptom duration prior to clinic presentation |
| 0 | Day of first clinic attendance |
| × | Day of clinical clearance |
| + | Positive PCR |
| - | Negative PCR |
| +/- | Positive PCR, negative viral culture |
| +/+ | Positive PCR, positive viral culture |
| C | Contaminated viral culture |
| "Clearance" | Day of clinical lesion clearance, and release from isolation |
| "Lesion" | Day positive lesion swab collected |

Subject #17 was screened as a contact of mpox and had a positive urine sample prior to developing mpox symptoms.

The latest detection of monkeypox virus from an anal swab was 18 days from symptom onset, n = 3 men with anal positive PCR result, including 1 with a positive viral culture.

The latest detection of monkeypox virus from the oral cavity was 18 days by oral PCR (n = 1) from symptom onset, and 17 days by oral PCR and viral culture (n = 3).

Four patients with persistent lesions were cleared from isolation with remaining lesions to be covered by clothing: two with persistent positive oral viral culture and two with persistent anal viral culture.

**SUPPLEMENTARY REFERENCES**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