

Smallpox (*Variola virus*)

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for smallpox.

Authorisation: PHLN

Consensus date: 29 July 2019

1 PHLN Summary Laboratory Definition

1.1 Condition:

Acute smallpox infection.

1.1.1 Definitive Criteria

- Detection of *variola virus* nucleic acid in clinical specimens; or
- Isolation of *variola virus* from clinical specimens

1.1.2 Suggestive Criteria

- Detection of a *poxvirus* resembling *variola virus* by electron microscopy from clinical specimens

1.1.3 Comments

Smallpox has been eradicated as a natural occurring infectious illness with no known animal vector. Its cause, the *variola virus*, has been effectively contained. The last naturally acquired case of smallpox in the world occurred in 1977. Global eradication was certified by the World Health Organization (WHO) in 1979.

A single probable or confirmed case of smallpox would constitute a public health emergency of international concern under the International Health Regulations (IHRs). States party to the IHRs are required to immediately notify the WHO of any confirmed case of smallpox.

The smallpox vaccination remains the only way to prevent smallpox. Routine smallpox vaccination of the Australian public ceased in the early 1970s.

There are only two WHO approved virus research and repository laboratories, which are the Centers for Disease Control and Prevention (CDC) in Atlanta USA and the State Research Centre of Virology and Biotechnology in Novosibirsk, Russia. It is possible that other clandestine stocks may exist.

Laboratory diagnosis where there is a high pre-test probability of variola infection (refer to Section 3.1 below) requires specimen processing in a Physical Containment level 4 (PC4) laboratory. Access to laboratory testing should be through the Chief Medical Officer (CMO). Requests for testing should come via the Chief Health Officer (CHO) of the state or territory once satisfied that grounds for testing exist i.e.:

- i. An illness as judged by an expert physician that is compatible with smallpox with credible smallpox exposure within the past 3 weeks (associated with a specific threat, a known or suspected bioweapon release, or close exposure to material allowing respiratory/mucous membrane transmission such as viral cell culture in which smallpox can't be excluded) OR
- ii. An illness as judged by an expert physician that is compatible with smallpox where relevant differential diagnoses including non-variola *orthopoxviruses* (e.g. *monkeypox* or *cowpox*) and viruses causing vesicular rashes (e.g. *varicella zoster virus*, *herpes simplex virus* and *enteroviruses*) have been excluded.

Smallpox testing may be recommended in the absence of overt smallpox exposure after expert case review. As variola virus is a potential bioterrorism agent, appropriate 'chain of custody' should be observed for the handling of any specimens from suspect smallpox cases.

Isolation of *variola virus* in vitro could be done at the National High Security Quarantine Laboratory (NHSQL), but would only be for reference purposes. Nucleic acid test is the diagnostic modality of choice, although validation and experience with nucleic acid tests for detection of *variola virus* is limited.

Variola, vaccinia, cowpox, monkeypox and other *orthopoxvirus* virions cannot be definitively distinguished by electron microscopy.

2 Introduction

Variola or *smallpox virus* is a double stranded DNA virus. It is a member of the family Poxviridae, genus *orthopoxvirus*. *Variola virus* has a host range strictly limited to humans. Of the other animal orthopoxviruses, three (vaccinia, monkeypox, and cowpox) may also infect humans. All have brick shaped virions between 220 to 450 nm in length ¹⁻³.

Smallpox manifested as two diseases associated with different variola variants. Variola major circulated in Asia and had a case fatality rate of 15–45% ^{1,3}, including 3% among the vaccinated ⁴. Variola minor circulated in South Africa, the Americas and Europe and had a case fatality rate of approximately 1%. Smallpox had winter and early spring seasonality similar to varicella and measles outbreaks.

The virus is shed from the oropharynx and skin, and transmitted mainly via droplets to the respiratory mucosa of contacts. Approximately 30% to 80% of close contacts of both variola major and minor are

likely to be infected ⁵. The period of infectivity is generally from the onset of rash until scab formation, typically within 7 to 10 days. Indirect spread by fomites or fine particle aerosol is less common. Variola aerosols might survive in the environment between 6 and 24 hours ¹. Variola is much more durable in scabs, surviving weeks to even years, but scabs are thought to be minimally infectious for humans ¹.

Following an incubation period of 12 – 14 days (range of 7 – 17) ^{1,6}, the variola major patient typically experiences a 2 – 3 day prodrome of high fever, malaise and prostration with headaches and backaches. A maculopapular rash then appears on the oral and pharyngeal mucosa, face and forearms before spreading to the trunk and legs (centrifugal spread). Unlike *monkeypox virus* infection, lymphadenopathy is usually absent. The rash then progresses to become vesicular and finally pustular. Lesions are often found in one stage of development in the body, with each stage lasting 1 – 2 days. The smallpox lesion is typically hard and deep, well-circumscribed and umbilicated. With recovery, scabs separate and scarring develops. Haemorrhagic and malignant forms of smallpox also exist. Variola minor manifests as a less severe illness with few constitutional symptoms and a relatively sparse rash.

A rapid presumptive laboratory diagnosis of an *orthopoxvirus* infection could be made by expert electron microscopic examination of vesicle fluid, or cells from a lesion base ¹⁻³. However, monkeypox, cowpox and vaccinia virions appear similar, and cannot be reliably distinguished on electron microscopy appearances alone. Definitive identification requires detection of *variola virus* nucleic acid or isolation of *variola virus*. Testing for *orthopoxviruses* is performed in a PC4 laboratory, and specimens should be collected, packaged and transported appropriately by trained personnel using the correct personal protective equipment.

3 Tests

3.1 Access to diagnostic testing

While smallpox remains eradicated, the pretest probability of smallpox is considered vanishingly low, and in the unlikely event that testing is requested, is decentralised to jurisdictional PHLN laboratories who will ensure testing for other differential diagnoses is undertaken, and if necessary facilitate testing for variola, either via local test capability if they have it, or via referral. This is intended to facilitate timely & responsive testing, at an acceptable low risk.

Should the pretest probability of smallpox rise, through a specific threat, a deliberate release or cases occurring overseas, then testing for both *variola virus* and its differential diagnoses is centralised at the NNSQL, VIDRL at the Peter Doherty Institute for Infection and Immunity, Melbourne. This is intended both to maximise containment of laboratory processes, minimise risk to laboratory staff, and to tightly control the consistency of laboratory testing even further than can be achieved by collaborating PHLN laboratories. The same centralised approach is taken should there be an initial case, or case cluster in Australia.

NNSQL should be notified through the relevant State or Territory CHO. However, direct contact with the medical microbiologist on call at VIDRL is essential to arrange receipt of specimens and obtain advice on specimen collection, safe packaging and transport.

The VIDRL on-call microbiologist can be contacted on mobile (0438 599 437). If attempts to reach the VIDRL on-call microbiologist fail the designated back up is the VIDRL on-call laboratory manager (0438 599 439), or failing this the Royal Melbourne Hospital switchboard (+613 9342 7000) who will attempt to reach VIDRL staff on the caller's behalf.

Nucleic acid testing (NAT) is the primary diagnostic modality employed for detection of smallpox by the NHSUK, and collection of appropriate vesicle swabs for this purpose is a priority. Electron microscopy and viral culture can be done, but are inferior in speed and sensitivity to NAT. In practice their use would be confined to reference purposes after a positive NAT result.

In the event that community transmission of smallpox becomes established, then the associated changes in pre-test probability, requirement for containment, relative risk to laboratory staff from the workplace compared to the community, and demands on test capacity will drive decentralisation of diagnostic variola testing to other PHLN laboratories.

3.2 Specimen collection

Skin lesions are considered the best source of specimens for laboratory diagnosis of *poxvirus* infections ¹⁻³. Specimens should be collected by smallpox immune staff (vaccination that day will suffice) wearing gown, gloves and a mask. There are vaccinated staff in many Australian laboratories due to the use of vaccinia constructs in molecular biological research.

In the smallpox era, laboratory diagnosis was applied only to material derived from clinical cases, so the negative predictive value of these laboratory tests when applied to environmental material has not been established. Notably the infectious dose of *variola virus* may be as low as 10–100 virions, while the analytical sensitivity of electron microscopy is approximately 10⁵ virions. Culture is generally considered less sensitive than electron microscopy ².

3.2.1 Equipment for collection of specimens for nucleic acid testing

Appropriate equipment includes:

- Personal protective equipment
- A small scalpel blade or 25G needle for removing the roofs and upper tissue from lesions
- 'O' ring sealed tubes containing viral transport medium
- Dry swabs plus 'O' ring sealed tubes containing viral transport medium (or commercial swab/viral transport medium combination, provided there is certainty that this is leak-proof)
- A fine tip permanent marker pen
- A waterproof sharps container for needles, syringes, scalpels
- Waterproof plasters
- A sealable plastic specimen bag. Absorbent packaging material and a strong metal outer container plus bio hazard tape to seal it and 0.1% hypochlorite solution to clean the outside before transport to the laboratory
- 'High Risk' labels
- A clinical waste bag for disposal of discarded dressings and personal protective equipment

3.2.2 Procedure for collection of specimens for nucleic acid testing

The procedure for collecting specimens of vesicle fluid is as follows:

- Put on personal protective equipment
- Gently de-roof a vesicle using a scalpel blade or 25G needle
- Firmly rub a dry swab on the base of the lesion using a rotary motion. The objective is to absorb fluid from the vesicle onto the swab, and to dislodge cellular material from the lesion base which will also adhere to the swab.
- Place the swab into an 'O' ring tube of viral transport medium, carefully break or snip the swab shaft to allow closure of the tube, and replace the lid.
- At least 3 vesicles containing clear (i.e. non-postular) fluid should be sampled in the manner and the swabs pooled in a single 'O' ring tube.
- Label the tube with patient identifying data, place it in zip-lock plastic specimen bag and seal.
- Repeat to generate a duplicate set of 3 swabs in a second tube, labelled and bagged as above.

3.2.3 Transport of specimens to the laboratory

The outside of each specimen container should be wiped with disinfectant (0.1% hypochlorite solution) and a label should be attached bearing the patient's name, hospital identification, the date of collection and the nature of the suspected infection. The specimens should be double bagged in secure, airtight and watertight bags, which have been similarly labelled. Bags containing specimens should be sponged with disinfectant before removed from the patient's room.

Samples to be tested by local PHLN laboratories should be packaged as below, and then transported to the relevant PHLN jurisdictional laboratory following local protocols.

Samples to be transported to the NHSUK should be packaged, labelled and transported as follows:

Samples should be identified as: "Infectious Substances Affecting Humans (smallpox sample)" and packaged and handled as required by the International Air Transport Association (IATA) packing instruction 620.

The specimens should be packaged as follows:

- Place the specimens in a tightly sealed, watertight primary container, such as a 'O' ring screw-cap plastic tube or vial, and seal the cap with tape. Ensure plastic containers are resistant to temperatures as low as -80 °C.
- Wrap the primary container in sufficient absorbent material (e.g. tissue) to absorb the entire contents in case the container leaks or breaks.
- Place the wrapped, sealed primary container into a durable, watertight, screw-cap mailing tube or metal can. This secondary container should be sealed with tape.
- Several primary containers may be placed in one secondary container to a maximum of 50 mL of specimen material.

- On the outside of the secondary container, attach the specimen labels and other relevant information.
- Place the second container in a secure box or mailing tube addressed to:

National High Security Quarantine Laboratory
 Victorian Infectious Diseases Reference Laboratory
 The Peter Doherty Institute for Infection and Immunity
 792 Elizabeth Street, VIC 3000
 Ph 0438 599 437

Use a competent door-to-door courier. Because individual commercial and non-commercial carriers or shipping services may apply different regulations for transporting biological specimens, contact a representative of the chosen carrier beforehand to ensure that all necessary formalities are fulfilled.

Notify the on-call VIDRL medical microbiologist of the dispatch of the specimen and flight time and number, courier or airway bill number as appropriate. If transport is by air, a dangerous goods declaration must be made (refer to the IATA Dangerous Goods regulations).

3.3 Laboratory procedures

3.3.1 Specimen handling

Specimen collection and laboratory testing should only be undertaken by appropriately trained personnel who are wearing appropriate PPE and should have had a successful vaccination. Should it be necessary to conduct work other than in a PC4 laboratory, a full risk assessment must be conducted.

In the early stages of a suspected outbreak of smallpox, specimens from potential cases will be sent to one of the designated PHLN jurisdictional laboratories for testing of differential diagnoses including (but not limited to) *varicella-zoster virus* (VZV), *herpes simplex virus* (HSV), *enterovirus* and other *orthopoxviruses*. Electron microscopy may be performed if available, but is relatively insensitive, and not definitive. Investigation will require:

- Handling of samples in a Class I or II cabinet based within PC3 laboratory (if consistent with a risk assessment as above), preferably by vaccinated staff.
- Inactivation of the sample by 5% formalin for electron microscopy or inactivation of the sample by guanadinium for NAT, to be undertaken within a class II or III cabinet in a PC3 laboratory (refer to next section).

The *poxvirus* infection, molluscum contagiosum, is endemic in Australia. If viruses are visualised by electron microscopy, the result should be discussed urgently with the referring clinician. Differential diagnoses such as *varicella zoster virus*, *herpes simplex virus* and *enteroviruses* should be excluded to enable a rapid risk assessment.

3.3.2 Occupational health and safety issues

Staff at the designated PHLN jurisdictional laboratories will be vaccinated in the Stand-by Phase of a response (refer to appendix A for more information). Wider vaccination of laboratory staff is not justified at, since the risks of adverse effects from vaccination outweigh those from the risk of smallpox in this situation. However, staff liable to be involved in diagnostic work should be identified and screened in advance for suitability for vaccination.

3.3.3 Nucleic acid tests

Specimens should be referred to the NHSUK for analysis by PCR. Preparation of variola nucleic acid for NAT from cases with a high pre-test probability of variola infection should be undertaken only in a PC4 laboratory. Once non-infectious nucleic acid has been prepared the specimen can be removed from the PC4 laboratory for further processing.

Protocols for extraction of *orthopoxvirus* nucleic acid from lesion material, and specific PCR amplification for variola, vaccinia, cowpox and monkeypox among others, have recently been published ^{8,9}. These have the potential to provide rapid and sensitive laboratory diagnosis of these viruses. Validation and experience with variola diagnosis is limited however. The availability of positive control material for variola is problematic for most laboratories.

PCR protocols have been developed targeting the acidophilic inclusion protein (ATI) or haemagglutinin (HA) of *orthopoxviruses*, with subsequent specific identification by restriction digest of PCR amplicons ^{8,9}.

The ATI-PCR targets a region prone to extreme hypervariability which may limit the effectiveness of the assay ⁹, and only identification of 5 *orthopoxviruses* (*cowpox*, *vaccinia*, *ectromelia*, *camelpox* and *monkeypox*) has been described ⁸.

The HA-PCR employing primers EACP1&2 and restriction digest with Taq 1 distinguishes 7 “old world” *orthopoxviruses* including vaccinia, variola and monkeypox ^{7,8}. Variola major and minor viruses are not distinguished by this method ⁹. Other “new world” *orthopoxviruses* not affecting humans may be detected with a second primer set and restricted digest protocol.

A second PCR approach also targets the HA gene of *orthopoxviruses* using specific primer pairs to selectively amplify variola, vaccinia, monkeypox, cowpox and others ^{7,8}. Some retrospective validation of primer pair VAR1/VAR2 has been carried out, successfully amplifying 15 stored smallpox scab specimens from the CDC Smallpox Virus Specimen Repository ⁸. These PCR primers rely on high stringency of PCR amplification conditions for their specificity, with the potential for loss of sensitivity or specificity if the reaction conditions are varied. Nucleic acid sequencing of PCR amplification products is recommended to provide definitive virus identification ⁸.

3.3.4 Electron microscopy

Electron microscopic examination should only be undertaken by an operator expert in application of this technique in virology. Grids should be examined for 20 minutes before being considered negative. At least two convincing virus particles must be seen and photographed before reporting as positive. All possible viruses and virus-like particles should be photographed.

Viewed by electron microscope, the virions of variola, vaccinia, cowpox and monkeypox viruses are brick-shaped and approximately 220 to 450nm in length. In the smallpox era, clinical presentation and history allowed cowpox and vaccinia to be distinguished from smallpox. Monkeypox only occurs naturally in tropical rainforest areas of Africa and could also be excluded on the basis of travel history.

As large numbers of *poxvirus* particles are present in vesicular lesions, electron microscopic examination is considered highly reliable in smallpox diagnosis provided an adequate volume of specimen is referred ^{1,2}.

For additional technical detail regarding electron microscopy refer to appendix C.

3.3.5 Virus growth assays

Poxvirus virus growth assays are laborious and slow, and rely on expertise that is not generally available in Australia. They also tend to be relatively insensitive ². Culture work can only be undertaken in a PC4 laboratory. Generally culture of *poxviruses* is being supplanted by NAT for diagnostic purposes in the few laboratories undertaking this type of work ^{2,5}.

Culture may be attempted from vesicle fluid which contains large numbers of viral particles, or from lesion scrapings.

- i. Chorioallantoic membrane culture
Definitive detection of *orthopoxviruses* traditionally relied on growth on the chorioallantoic membrane of 12 day old chicken embryos. Morphologically characteristic pocks typical of each virus were produced after incubation for 72 hours at 35 OC.
- ii. Cell culture
Variola will grow in a variety of cell lines, including human embryonic lung, primary rhesus monkey kidney, Vero and LLC-MK2. After several days a cytopathic effect appears consisting of ballooning, cell fusion and destruction. Subculture to chorioallantoic membrane allowed definitive identification of other *poxviruses* by the characteristic pock morphology each produced.

3.3.6 Serology

A variety of serological assays have been described for detecting exposure to *orthopoxviruses*. In general, serological tests do not distinguish between antibodies to the various *orthopoxviruses* ², and requires testing of acute and convalescent sera, limiting their use in the acute diagnosis of variola infection. At present only neutralisation tests, Western blot and enzyme immunoassay are regularly used, and then generally for research purposes only by laboratories such as CDC, Atlanta.

4. Quality assurance

The Royal College of Pathologists of Australasia Quality Assurance Program (RCPAQAP) Biosecurity program offers a Smallpox proficiency testing panel for PHLN member laboratories.

References

1. Henderson DA, Inglesby TV, Bartlett JG. (1999). Smallpox as a biological weapon, medical and public health management. JAMA. 281:p 2127-37.
2. Buller M. (2000). Poxviruses. Chapter 34. In: Specker S, Hodinka R, Young S, (Eds). Clinical Virology Manual, 3rd Edition, ASM, Washington.
3. Behbehani A. (1999). Human Poxviruses. Chapter 35. In: Lennette E, Smith T, (Eds). Laboratory diagnosis of viral infections, 3rd Edition, Marcel Detter, New York.
4. Eitzen E, Paulin J, Cieslak T, Christopher G, Culpepper R. (1998). Medical Management of Biological Casualties Handbook. Fort Detrick, Frederick, Maryland.
5. Ropp S, Esposito J, Loparev V. (1999). Poxviruses infecting humans. Chapter 91. In: Murray P, Baron E, Pfaller M, Tenoer F, Tenover R, (Eds). Manual of Clinical Microbiology, 7th Edition, ASM, Washington.
6. Center for Disease Control and Prevention. (2001). Vaccinia (smallpox) vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR. 50 (No RR-10):
7. Keckler MS, Reynolds MG, Damon IK, Karem KL. (2013). The Effects of Post-Exposure Smallpox Vaccination on Clinical Disease Presentation: Addressing the Data Gaps Between Historical Epidemiology and Modern Surrogate Model Data. Vaccine. 31(45): p5192-5201.
8. Meyer M, Ropp S, Esposito J. (1998). Poxviruses. Chapter 15. In: Stephenson J, Warnes A, (Eds). Methods in Molecular Medicine, Vol 12: Diagnostic Virology Protocols, Humana Press, New Jersey.
9. Ropp S, Jin Q, Knight J. (1995). PCR strategy for identification and differentiation of smallpox and other orthopoxviruses. J. Clin. Microbiol. 33:p 2069-76.

Appendix A - Laboratory test procedures for smallpox by stage

Stages	Testing characterized by	Laboratory action
Preparedness Smallpox remains eradicated	Decentralised exclusion of smallpox: <ul style="list-style-type: none"> • Low pretest probability of smallpox • Moderate containment requirement • Modest throughout demand 	<ul style="list-style-type: none"> • Designated PHLN laboratories are lead agencies • PC3 processing + immune staff • Differential diagnoses tested (VZV, HSV) • Negative samples tested for smallpox by EM plus referral to NHSQI for PCR ^(a)
Response (Stand-by) <ul style="list-style-type: none"> • Specific threat <i>or</i> • Release/case(s) overseas 	Centralised screening for the first smallpox case: <ul style="list-style-type: none"> • Elevated pretest probability of smallpox • High containment requirement • High throughput demand 	<ul style="list-style-type: none"> • NHSQI lead agency • PC4 processing + immune staff • Smallpox tested (PCR) • NHSQI tests differential diagnoses
Response (Action) (1) Release case or cluster of cases in Australia	Centralised screening for the first smallpox case: <ul style="list-style-type: none"> • Increasing pretest probability of smallpox • High containment requirement • High throughput demand 	<ul style="list-style-type: none"> • NHSQI lead agency • PC4 processing + immune staff • Smallpox tested (PCR) • NHSQI tests differential diagnoses

Stages	Testing characterized by	Laboratory action
Response (Action) (2) Community transmission of smallpox in Australia	Decentralised confirmation of wide-spread smallpox: <ul style="list-style-type: none"> • High pretest probability of smallpox • Diminished containment requirement • High throughput demand 	Case epidemiologically linked to laboratory proven smallpox <ul style="list-style-type: none"> • Laboratory confirmation not necessary No epidemiologic link <ul style="list-style-type: none"> • PHLN laboratories are lead agencies • PC3 processing + immune staff • Smallpox tested (IM, or referral to NHSQL) • PHLN lab tests differential diagnoses • Undiagnosed cases referred to NHSQL

^a: during the prevention stage, confirmatory PCR assays could be batched.

Note: Planning, management of the preparation for, and the response to a deliberate release of smallpox will be a staged process. Australian Governments follow the comprehensive approach to emergency management and look at emergencies as part of an ongoing cycle of activities in the four areas of: prevention; preparedness; response and recovery. Please refer to the Domestic Health Emergency Response Plan for a Deliberate Release of Smallpox for more information.

Appendix B. Guidance notes on EM of suspected smallpox specimens

Appropriate Equipment for Obtaining Specimens

- Personal protective equipment
- A 'tuberculin' syringe and needle for aspirating fluid from vesicles
- A small scalpel blade for removing the roofs and upper tissue from lesions, and for lifting scabs
- Clean plastic microscope slides
- 'O' ring tube or bijou container for transporting crusts
- A permanent marker pen

- A slide container for the safe transfer of slides
- A waterproof sharps container for needles, syringes, scalpels and unused slides
- Waterproof plasters
- A sealable plastic specimen bag, absorbent packaging material and a strong metal outer container, plus bio hazard tape to seal the bag
- Fresh 0.1% hypochlorite solution to clean the outside of the container before transport to the laboratory
- 'High-Risk' labels
- A clinical waste bag for the disposal of discarded dressings and personal protective equipment.

Procedure for Collecting Specimens of Vesicle Fluid

- Put on personal protective equipment
- Puncture a vesicle with the tuberculin syringe, draw up fluid and express it onto a clean plastic microscope slide
- Cover the punctured vesicle with a waterproof plaster
- Allow the slide to air dry—do not wave it in the air
- Mark the slide clearly to indicate the surface used and the position of the sample
- Use a plastic slide carrier to transfer the slide to the laboratory
- Do not submit vesicle fluids to the laboratory in hypodermic syringes or in capillary tubes, as this could be hazardous to laboratory staff extracting the specimen.
- Swabs or specimens in viral transport medium are not suitable for electron microscopy.
- Vesicle crusts may be removed and sent for examination in a small sealed container ('O' ring tube or bijou).

Suspected smallpox specimens must be inactivated before processing. All work must be carried out in a cabinet (class I or II)

Formalin

Solutions of formalin at 5% and 10% (v/v) should be freshly prepared from 40% (v/v) stock solution just before use.

Vesicle fluid specimens

- Rehydrate the specimen in 10¹ to 50⁻ solution of 5% formalin in distilled water.
- Continue with staining and grid preparation according to local protocol.
- Reconstitution of the specimen in formalin does not disrupt the virus particles but does destroy infectivity.
- Some detail of surface structure may be lost, but herpes group and pox group viruses should be clearly recognisable.

- It may not be possible to distinguish between orthopoxvirus and molluscum contagiosum (although infection with these viruses should be clinically distinct).

Vesicle crust specimens

- Place crust in a plastic Griffith's tube with a few drops of 5% formalin in distilled water and grind to disrupt.
- Alternatively, disrupt crusts on a clean microscope slide with a few drops of 5% formalin solution using forceps.
- The resulting homogenate may be used for grid preparation.

Other liquid material (e.g. TCF)

Mix equal volumes of sample and 10% formalin solution to inactivate virus. Process as usual.

Microscope

As a guide viruses measure roughly 200 by 250nm, so an electron microscope with good resolution is required. An electron microscopist will normally scan a grid (on the large fluorescent screen) at a screen magnification of x 40 000 and check any possible virus using the binoculars and small screen. Viruses would normally be photographed at between x 40 000 and x 60 000. The microscope must be able to resolve well beyond x 40 000. The tested resolution of the electron microscope should be better than 1 nm.

Calibration

The microscope should be properly calibrated, using a cross grating and if possible catalase crystals or similar. Do not trust the magnification given by the instrument: old microscopes, in particular, can give incorrect readings unless calibrated. Size is important in virus identification. Smallpox measures about 250 nm x 200 nm.

Stains and grids

Usually a negative stain such as phosphotungstic acid is used. Good results are achieved with pH 7. Homemade Formvar carbon-coated copper 400-mesh grids are best.

Literature

There are a number of good atlases of virus morphology. Recommended texts are Virus Morphology (Madeley and Field 1988) and Electron Microscopy in Diagnostic Virology (Doane and Anderson 1987).

Notes:

1. PC4 facilities to guarantee containment, and central diagnostic responsibility, are important during screening for initial cases at elevated risk.
2. PC3 facilities and immune staff provide acceptable staff safety, maximal laboratory capacity and simplified logistics when wide-spread community circulation of smallpox makes high-security laboratory containment irrelevant.