

NATIONAL STANDARD METHOD

INVESTIGATION OF VESICULAR RASHES

QSOP 55

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections



UK Clinical Virology Network



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INVESTIGATION OF VESICULAR RASHES

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AMENDMENT PROCEDURE

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Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
1/ 06.07.09	1	2	1	Front page	NIMAG Logo added
			14	Glossary	Glossary added to the document
			18	References	References reviewed and updated
			All	All	PDF links to other documents inserted
			All	All	Department name changed to DEST

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Types of specimen:	Serum
	Plasma
	Whole blood
	Vesicle fluid
	Swabs
	Faeces

INTRODUCTION

Vesicles are circumscribed, elevated lesions filled with clear fluid. When over 0.5 cm diameter they are usually termed bullae². They result from a disturbance of cohesion of epidermal cells or components of the basement membrane zone associated with influx of fluid into or beneath the site of the lesion³.

Vesicular lesions are principally associated with immune disorders and other non-infectious conditions including insect bites, acne, atopic eczema, drug reactions, herpes gestationis, etc. Vesicular rashes are also seen in a number of viral infections, especially varicella-zoster and herpes simplex. Outside the classic childhood exanthemata atypical exanthemata with vesicles are usually, but not exclusively, due to enterovirus infections⁴. Recently there has been increasing interest in poxvirus infections, many of which have lesions that vesiculate.

Bacterial and fungal infections also need to be considered in evaluating vesicular rashes. Vesicles can occur in conditions as diverse as impetigo, candidiasis, echthyma gangrenosum associated with pseudomonal infection, and anthrax.

Erythema nodosum, which may present as a disseminated vesicular and pustular rash, may be seen in the bacteraemic stages of gonococcal and less frequently meningococcal infection.

Notes on selected infective causes of vesicular rashes follow in Sections 2 to 17. Images of vesicular rashes are available in standard textbooks and on-line. The Centres for Disease Control and Prevention offer images of smallpox, vaccinia, and varicella-zoster lesions via their website <http://www.bt.cdc.gov/>.

Diagnosis of Vesicular Rash

Certain vesicular rashes are usually diagnosed clinically. Chickenpox for example is usually diagnosed in the community without reference to medical professionals or laboratory tests. When laboratory examination is indicated, vesicular fluid or an impression smear from a lesion may be collected for electron microscopy (EM) or virus antigen detection (DIF). Fluid or a swab from the base of a vesicle may also be collected into viral transport medium for virus culture or PCR. If a bacterial cause is suspected eg impetigo, swabs should be collected into bacterial transport medium for Gram stain and bacterial culture.

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1 HAND FOOT AND MOUTH DISEASE

Enteroviral infections occur worldwide and may cause illnesses characterized by vesicular lesions on the hands, feet and in the mouth⁵. Such cases are caused by Coxsackie A viruses notably A10 and A16 (but also A5 and A9), enterovirus 71, ECHO 1 and 4 and Coxsackie B5. Enterovirus type 71 has caused epidemics in the Far East and in Australia in recent years and has been associated with significant morbidity and mortality from neurological complications (including encephalitis and flaccid paralysis), myocarditis, and pulmonary complications⁶⁻⁹. In hand foot and mouth disease there is usually a prodromal illness with fever, sore throat and anorexia, which is followed by vesicles appearing on the cheeks, gums, and tongue after about 2 days, often going on to ulcerate⁴. A nonpruritic skin rash then develops over the next day or two, beginning as small red papulovesicular lesions especially on the palms, soles, and buttocks. The illness resolves in about 7 days. Most cases are seen in young children who are often given out patient care with symptomatic treatment¹⁰.

1.1 DIAGNOSIS OF ENTEROVIRUS INFECTION

In typical cases of hand foot and mouth disease this is usually made clinically. Laboratory confirmation can include cell culture, particularly if RD cells are used⁵. Serological assays for enterovirus IgM may detect the majority of cases even within the first week of illness⁷. Molecular detection methods are becoming the tests of choice and offer advantages over other detection methods in terms of sensitivity with the methodology constantly progressing^{8,11}. Enterovirus culture from faeces is also a very sensitive method⁵ which can support but not prove a diagnosis of enterovirus infection.

2 HERPES B VIRUS (CERCOPITHECINE HERPESVIRUS 1)

Human infections occur occasionally as a result of contamination with herpes B virus from infected macaque monkeys or from infected tissues derived from macaques. A vesicular rash, which may be accompanied by pain, tingling or numbness, appears at the inoculation site in many of the cases¹². Early diagnosis is critical as there is a high mortality rate from encephalomyelitis unless antiviral treatment is begun as soon as possible.

2.1 DIAGNOSIS OF HERPES B INFECTION

Virus culture (which is undertaken by a reference laboratory with Category 4 containment level) is insensitive when lesions which have been washed are sampled¹². PCR offers a more sensitive, more rapid and safer alternative^{13,14}.

3 HERPES SIMPLEX

Herpes simplex infections are common and are often acquired asymptotically¹⁵. Vesicular lesions are the principal signs of both herpes simplex type 1 and type 2 infections. Lesions that are caused by these two viruses are clinically indistinguishable¹⁶. Symptomatic primary HSV-1 infection usually presents with gingivostomatitis and pharyngitis, often accompanied by a systemic febrile illness. Lesions in the mouth ulcerate. Primary genital herpes is characterised by fever, headache, malaise, myalgia, with local pain, itching, dysuria, vaginal and urethral discharge, and inguinal lymphadenopathy. Lesions may be present in varying stages, including vesicles, pustules, or ulcers¹⁶. Lesions due to HSV-2 are more likely to reactivate and recur in the genital area than HSV-1, while for oral-labial herpes HSV-1 is more likely to recur¹⁷.

3.1 DIAGNOSIS OF HERPES SIMPLEX INFECTION

Rapid methods for laboratory diagnosis of HSV infection include cytological examination of a smear from the base of a lesion, the Tzanck smear, and electron microscopy with negative staining. Neither of these methods distinguishes between herpes simplex and varicella-zoster and they are not very sensitive. Until recently cell culture methods (particularly with MRC5 or similar fibroblast lines) have been regarded as the 'gold standard' for HSV diagnosis¹⁸. The

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sensitivity of the method is very good in early vesicular lesions but lessens considerably with time. The time required for viral cytopathic effect to be seen can range from 1-5 days¹⁹. Direct antigen detection has similar sensitivity, by using a combination of direct immunofluorescent antibody detection and cell culture, sensitivity of HSV detection is further improved²⁰. Use of shell-vial methods has been suggested to allow earlier detection of HSV in cell culture²¹. However this method may offer no advantage²² compared with conventional culture in cell lines such as mink lung. The commercial enzyme-linked viral inducible system (ELVIS) which employs genetically engineered baby hamster kidney cells gives similar sensitivity and specificity to shell vial assays with the advantages of rapidity, ease of handling, and the option of using multiwell plate formats²³. The stably transformed cell line (BHKICP6LacZ-5) expresses beta-galactosidase only after infection with HSV, histochemical staining allows visualisation of infected cells²⁴. Molecular detection offers considerable advantages over other methods¹⁹. PCR methods can increase the diagnostic yield of HSV from genital lesions by between 13% and 27% compared to virus culture²⁵. LightCycler real-time PCR methodology melting curve analysis allows HSV typing^{26,27}.

4 HUMAN HERPES VIRUS 6

Although classically causing the febrile exanthem roseola infantum in infants HHV6 may rarely cause vesicular lesions (See [QSOP 56-Investigation of Red Rash](#)).

4.1 DIAGNOSIS OF HUMAN HERPES VIRUS 6

Laboratory diagnosis can be difficult due to cross-reactivity among the herpesviruses. The development of microarray technology has also led to the development of a method for the simultaneous detection of the seven human herpes viruses²⁸. Antibody assays looking for seroconversion or low IgG avidity are employed, together with IgM detection and PCR positivity in blood²⁹.

5 VARICELLA ZOSTER

The rash of chickenpox typically has lesions in various stages of evolution. After an incubation period of 10 days to 3 weeks there may be a mild prodrome of fever and malaise. The rash then starts as small red papules, which later become raised and itchy and form vesicles on a red background. Vesicles then become pustular and resolve and scab over. The rash is pruritic. New crops of lesions tend to occur every 2 to 4 days, and continue appearing for about 7 days. There are therefore lesions in chickenpox at different stages of development. The distribution of the rash is typically on the trunk initially, then later on the face, arms and legs. The palms and soles are seldom affected^{2,30}. Herpes zoster (shingles) results from reactivation of VZ virus latent in sensory ganglia. The virus moves down the sensory neuron causing initial pain in the dermatomal distribution of the nerve. Erythema appear in a few days or even weeks followed by a vesicular rash along the distribution of the nerve. The face (particularly trigeminal nerve) and trunk are the principal sites for the lesions of shingles in adults. However the cervical and sacral dermatomes are more commonly involved in children³¹.

5.1 DIAGNOSIS OF CHICKENPOX AND ZOSTER

Diagnosis can usually be made clinically. Note that the rash of zoster is frequently mistaken for herpes simplex³². If laboratory confirmation is required, particularly if there are atypical features or where there are infection control implications, a range of investigations is available. The traditional Tzanck smear is simple and rapid. In this cytological technique a smear from the base of a lesion is stained with Giemsa and examined under the microscope for multinucleated giant cells³³. It is seldom used now as it cannot differentiate between herpes simplex and varicella-zoster virus and it is relatively insensitive³⁴. The same considerations apply to electron microscopy. Negative stain EM³⁵ although rapid cannot differentiate between HSV and VZ and has relatively poor sensitivity (48% compared with cell culture)³⁶.

Isolation of VZV in cell culture (human embryo lung fibroblasts) has been reported as having 100% sensitivity from vesicles in the first three days of illness but declines substantially by the

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sixth day³⁷. It is also lower if samples are collected after antiviral therapy has commenced. The use of the shell vial (DEAFF) technique for VZ culture improves the isolation rate by around 50% compared with conventional cell culture. This method gives a definitive result in 3 days compared to over 7 days for conventional culture^{34,38}. Direct antigen detection using fluorescence-labelled anti-VZ monoclonal antibodies generally has higher sensitivity than cell culture, over 90% sensitive in some reports^{34,39}. Concerns that direct IF detection with generally available monoclonal antibodies might have a significant false negative rate due to VZV gE mutant viruses seem to be unfounded at the present time⁴⁰.

Serology can suggest recent infection with chickenpox. VZ IgG seroconversion and presence of IgM can be diagnostic. A rise in IgG, detection of IgM and high titre IgA are consistent with recent zoster⁴¹. Reactivity in VZ IgG tests can however be due to cross reactivity in a herpes simplex infection⁴². Antibody capture VZ IgM EIA similarly shows cross-reaction with HSV and has a specificity as low as 66%⁴³.

6 VARICELLA ZOSTER VACCINE

Individuals who are vaccinated against varicella may develop a rash in which the vaccine Oka strain of varicella can be detected. A small number of vesicular lesions appear, some 3-5% have lesions at the site of the vaccination and in 3-5% lesions appear elsewhere on the body. The vaccine rash usually appears around 3-4 weeks post-vaccination but may be seen as late as 6 weeks. Zoster lesions may also be seen after varicella vaccination, with rates around 2% in vaccinated children who are leukaemic^{44,45}. In this context accurate diagnosis depends upon virus PCR with differentiation of wild type and vaccine virus (Oka) strains^{46,47}. Evaluations have been carried out on a VZV/HSV PCR reaction and shown that PCR is a valuable alternative to electron microscopy in virus isolation and detection⁴⁸.

7 COWPOX

Cowpox in humans is quite uncommon, and is usually acquired by contact with infected cats. The reservoir of infection is thought to be wild rodents⁴⁹. There is usually a single lesion, commonly on the lower limb, which begins as a papule and develops into a vesicle which becomes haemorrhagic and finally ulcerates. The vesicle is often umbilicated and surrounded by erythema and oedema. Lymphangitis and lymphadenopathy are common, as are fever and myalgia⁵⁰.

7.1 DIAGNOSIS OF COWPOX

Diagnosis is made by direct electron microscopy (EM) of material from lesions, vesicle fluid or reconstituted dry scabs. EM can also demonstrate orthopoxviruses in biopsy material. Cowpox virus can be grown in tissue culture, where it can be detected by EM. Cowpox may not be differentiated from molluscum contagiosum by EM, but PCR methods may offer a specific diagnosis^{51,52}.

8 MONKEYPOX

Formerly confined to the rainforests of central and western Africa monkeypox recently emerged in the USA as a result of importation of exotic pets. Of the 35 proven cases all had had contact with ill prairie dogs, premises where prairie dogs had been housed, or human monkeypox cases⁵³. The prairie dogs had been in contact with animals from a consignment of African rodents imported into the USA from Ghana, among which a giant Gambian rat and two rope squirrels were confirmed as positive for monkeypox by PCR and virus isolation. In the human after an incubation period of about 12 days there is a prodrome with fever, headache, myalgia and sweats and sometimes a non-productive cough. The rash in US cases tended to begin on the face, then appear on the head, trunk, and extremities. Many had satellite lesions on the hands and feet⁵⁴. The rash evolves through papules, vesicles, pustules, umbilication and crusting. Unlike smallpox, monkeypox lesions crop and may be seen in different stages of development. In Africa most cases are seen in young children who have contacted animals especially squirrels, with a secondary attack rate of 8 - 9% among family members (without prophylactic smallpox vaccination). Increasing numbers of cases

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have been seen in humans in recent years. The major differential diagnosis is chickenpox. In some outbreaks in the Democratic Republic of the Congo both varicella and monkeypox have co-circulated^{55,56}.

8.1 DIAGNOSIS OF MONKEYPOX

Electron microscopy on scabs, vesicle material or biopsy specimens show large numbers of brick-shaped orthopoxviruses⁵⁷. Histology of monkeypox lesions is essentially identical to that of tanapox or the papulonecrotic stage of smallpox, with necrosis of the stratum basale, adjacent dermal papillae and stratum spinosum. Structures resembling Guarnieri bodies in the cytoplasm of infected epidermal cells⁵⁸ are also seen. PCR methods⁵², typing methods including RFLP and microarrays using *crmB* gene regions can differentiate the various orthopoxviruses^{57,59}.

9 ORF

Orf (also known as contagious ecthyma, contagious pustular dermatitis, or scabby mouth) is a disease of sheep seen in all parts of the world in which they are raised. It can also affect goats and humans. It is caused by a parapoxvirus. In young sheep vesicles which evolve to pustules and then scabs occur mainly around the mouth. Humans may be infected by contact with animals, and usually have solitary lesions on the hands or forearms. Widespread papulovesicular lesions on the hands and face are occasionally associated with orf infection⁶⁰. A history of recent contact with sheep and the characteristic lesion make orf an easy condition to diagnose⁶⁰. The typical lesions are large, 2-3 cm, and go through the following weekly stages: the initial maculopapular stage, vesiculation, bullous lesion, weeping nodule, and firm crusted nodule. Large nonresolving lesions may occur in the patients who are immunocompromised⁶¹.

9.1 DIAGNOSIS OF ORF

The diagnosis is usually made clinically⁶⁰ but can be confirmed by EM or by PCR⁶².

10 VACCINIA

Large numbers of civilians and military personnel have recently been vaccinated against smallpox. This process is likely to continue in many parts of the world⁶³. In primary vaccination inoculation of vaccine into the upper layers of the skin with a bifurcated needle leads to development of a papule at the inoculation site 3 to 4 days later. The papule progresses to a vesicle with surrounding erythema by about day 5 or 6. The centre of the vesicle umbilicates and forms a pustule, which crusts. A scab forms by about the twelfth day after vaccination, and detaches by 3 weeks, leaving a scar. Pain is usual and local lymphadenopathy is common (25-50%). Fever and systemic symptoms are also not infrequent. Satellite lesions may be seen within 2.5 cm of the inoculation site, together with oedema and sometimes lymphangitis (2-6%). Vesicular lesions are not common after revaccination. Generally there is no difficulty in recognising the lesion of vaccinia, and there will be a history of recent vaccination^{64,65}.

More widespread vesicular lesions or solitary lesions in unusual sites may be seen in individuals who have been infected with vaccinia through contact with a recently inoculated individual, or, in the inoculated person where virus has been spread to other skin sites (or the eye) by scratching the vaccination site. Lesions are easily diagnosed through the history and from the fact that their natural history is the same as for the primary vaccine lesion. Atypical lesions are often widespread and extensive, they may be seen in areas of damaged skin (eg nappy rash). Inoculation onto current or apparently healed eczematous areas produces extensive lesions (eczema vaccinatum) which can resemble herpes. This is a life-threatening situation with potential viraemia and systemic spread⁶⁴.

Generalized vaccinia results from viraemic dissemination of vaccinia. Lesions can appear on any part of the body, especially on the trunk and abdomen, less often the face and limbs. While resembling the typical vaccinia inoculation lesion they are generally smaller and evolve rapidly (only 5 or 6 days to scarring). It is particularly difficult to differentiate the lesions of

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generalized vaccinia from those of modified smallpox where vaccination has been given after a smallpox exposure. PCR testing may be vital in these cases. Progressive vaccinia (vaccinia gangrenosum) in the patients who are immunocompromised is usually fatal. Most cases have been seen in children with cellular immune defects. The primary lesion fails to heal, and viraemia occurs with new progressive lesions, occurring anywhere on the body. Lesions may coalesce and become necrotic. Patients are toxic and often succumb to septicaemia with a secondary infection⁶⁴. Patients with AIDS are likely to be at risk^{64,66}.

10.1 DIAGNOSIS OF VACCINIA

In the laboratories protocols similar to those that are used for variola (smallpox) are employed⁶⁷. The efficacy of an algorithm using real-time PCR and EM has recently been demonstrated in a case of generalised vaccinia⁶⁸. The PCR methodology that was used was that of the CDC Laboratory Response Network (CDC LRN) which employs three assays, a vaccinia virus-specific assay, a varicella-zoster virus (VZV)-specific assay, and an endogenous control *Escherichia coli* 16S ribosomal DNA assay to identify PCR inhibition⁶⁸.

11 SMALLPOX (VARIOLA)

Although the last case of endemic smallpox in the world occurred in 1977 the possibility of the use of smallpox as a biological weapon has become a serious consideration. After an incubation period of 7-17 days (mean 10–12 days) a prodromal illness appears abruptly with severe headache, backache, and fever. The temperature often reaches 40°C and then subsides over 2-3 days. The rash of smallpox begins as small, reddish macules, which become 2-3 mm papules over 1–2 days. These papules become vesicles of 2-5 mm diameter after a further one or two days. The lesions occur first and are most prominent on the face and extremities but gradually cover the body. Pustules of 4-6 mm diameter develop about 4-7 days after the onset of the rash and remain for 5-8 days, followed by umbilication and crusting. Sometimes there is a second temperature spike 5-8 days after the onset of the rash, especially if there is secondary bacterial infection. The crusts begin separating by the second week of the eruption. The principal differential diagnosis is from chickenpox⁶⁹. High fever is more a feature of smallpox and the lesions of smallpox have a peripheral or centrifugal distribution (often lasting longest on the palms and soles of the feet) and are all at the same stage of development. In smallpox an enanthem over the tongue, mouth, and oropharynx may be seen a day before the skin rash⁷⁰.

11.1 DIAGNOSIS OF SMALLPOX

At the present time in Britain, in the absence of any smallpox activity in the world (Alert Level 0) laboratory confirmation of a suspected case of smallpox would be carried out under Category 4 containment conditions at the Centre for Infections, Health Protection Agency, Colindale, London or at Health Protection Agency, Porton Down, Salisbury⁷¹. In Scotland samples should be sent to West of Scotland Specialist Virology Centre Gartnavel General Hospital Glasgow. Diagnosis would be based on EM on skin lesion scraping supported by virus culture and PCR^{67,71}. While there are several PCR assays available for smallpox diagnosis⁷² one of the important tasks in smallpox diagnosis will be to exclude the main differential diagnosis, varicella-zoster virus infection, by PCR⁶⁷.

12 RICKETTSIAL DISEASE

Vesicles are seen in rickettsialpox (due to *Rickettsia akari*). Small central vesicles develop within erythematous macules. The geographic area where the infection was acquired (eastern USA, Ukraine, Croatia) and the presence of an eschar at the site of a house mouse mite bite suggest possible rickettsialpox^{73,74}. Similarly vesicles may occur in African tick bite fever, *Rickettsia africae* infection (particularly in the elderly, where discrete vesicular lesions are common⁷⁵) and in boutonneuse fever, *R.conori* infection⁷⁵⁻⁷⁸. Diagnosis of rickettsial infection

The Weil-Felix test which was formerly used for rickettsial diagnosis is insensitive and non-specific. Serodiagnosis should be carried out in reference laboratories using antibody detection by immunofluorescence or Western blot. Antigen detection by immunofluorescence,

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shell-vial culture, and nucleic detection by PCR offer rapid alternatives for early diagnosis from blood or skin^{77,79}.

13 ANTHRAX

The primary lesion of cutaneous anthrax is usually a painless, pruritic papule that appears 3-5 days after the inoculation of endospores. In 24-36 hours, the lesion forms a vesicle that undergoes central necrosis and dries, leaving a characteristic black eschar surrounded by oedema and a number of purplish vesicles. There is usually a history of contact with animals or animal products, or of material suspected to be linked to bioterrorism activities. The head, neck and extremities are the most commonly involved sites⁸⁰.

13.1 DIAGNOSIS OF CUTANEOUS ANTHRAX

Swabs should be taken in bacterial transport medium for bacterial culture. Serological assays for antibody to *Bacillus anthracis* toxin protective antigen offer high sensitivity and specificity⁸¹. A number of PCR assays for diagnosis of anthrax bacilli have been described. PCR has been demonstrated to be more sensitive than conventional culture in diagnosing anthrax infection in humans, particularly after antibiotic therapy has begun⁸¹. Real-time assays targeting the virulence plasmids pX01 and pX02 can identify and confirm the pathogenicity of strains of *Bacillus anthracis*⁸².

14 CANDIDIASIS

The rash of *Candida albicans* infection in babies, including cases of congenital cutaneous candidiasis⁸³, may have a vesicular or bullous component. Differential diagnosis includes varicella and herpes simplex infection⁸⁴. Lesions are most common in the nappy area but may be widespread. Systemic candidiasis should be considered in low birth weight babies presenting with *Candida* dermatitis⁸⁵.

14.1 DIAGNOSIS OF CANDIDIASIS

Swabs should be collected into bacterial transport medium for Gram staining and for fungal culture. If systemic infection is a possibility then blood cultures (with terminal subculture)⁸⁶ should be taken for candida, and EDTA-anti-coagulated blood should be considered for candida PCR if available.

15 CUTANEOUS LARVA MIGRANS

Self-limiting skin eruptions can result from nematode infections acquired in the tropics, particularly from beaches contaminated with dog or cat faeces containing eggs of the hookworms *Ancylostoma caninum* and *Ancylostoma brasiliense*. Clinical presentation of itchy erythematous linear or serpiginous lesions, commonly on the feet, buttocks and legs, are usually diagnostic. However atypical cases do occur, including vesicular lesions⁸⁷.

15.1 DIAGNOSIS OF CUTANEOUS LARVA MIGRANS

Diagnosis is usually clinical. Histopathology may be necessary in atypical cases. Peripheral blood eosinophilia is uncommon, and should lead to investigation for other parasitic infections⁸⁷.

16 IMPETIGO

A common skin infection in children, impetigo is caused by infection with *Streptococcus pyogenes* and/or *Staphylococcus aureus*, often at sites where the skin has been damaged². Impetigo commonly begins with a single erythematous macule of 2-3 mm which quickly progresses to a vesicle or pustule. The vesicle ruptures, leaving a honey-coloured exudate. Impetigo is readily spread to adjacent and distant sites by auto-inoculation. The less common bullous impetigo presents with superficial fragile bullae, usually due to a staphylococcus infection. Bullous impetigo is usually seen in children under 2 years of age.

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16.1 DIAGNOSIS OF IMPETIGO

Swabs should be collected into bacterial transport medium for Gram stain and bacterial culture. Serum antibody titres to anti-streptolysin O and anti-DNAse B are frequently raised⁸⁸.

17 RELEVANT NATIONAL STANDARD METHODS

For additional details on specific areas of diagnosis refer to the following NSMs available through the Department for Evaluations, Standards and Training web page (www.hpa-standardmethods.org.uk).

[BSOP 11 – Investigation of skin and superficial wound swabs](#)

[QSOP 56 – Investigation of red rash.](#)

[VSOP 13 – Investigation of clinical specimens by electron microscopy using the floatation \(direct\) method](#)

[VSOP 17 – Isolation of herpes simplex virus associated with herpes genitalis](#)

[VSOP 23 – Investigation of human herpes viruses \(excluding herpes genitalis\)](#)

[VSOP 24 – Isolation of enteroviruses and parechoviruses](#)

[VSOP 34 – Investigation of pregnant patient with rash illness](#)

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19 GLOSSARY

TERM	DEFINITIONS
Coalesce	The red spots from the rash merging together to form a single abnormal area
Dermatomal	Pertaining to a dermatome – where the dermatome is an area of skin that is associated with a pair of dorsal nerve roots from the spine or a specific cranial nerve. They are used in neurology for finding the site of damage to the spine
Dysuria	Difficult or painful discharge of urine
Enanthem	Skin eruption on a mucous membrane, for example in the mouth. Often associated with exanthem, the term used to describe the eruption or rash on the skin
Encephalitis	Inflammation of the brain. Encephalitis can result in confusion, abnormal behaviour, weakness in some parts of the body, or seizures (fits)
Encephalomyelitis	A description for inflammation of the brain and spinal cord
Endemic	Present in a community at all times but in relatively low frequency. Something that is endemic is typically restricted or peculiar to a locality or region
Erythema	Is the redness of the skin caused by capillary congestion
Eschar	The scab formed when a wound or skin is sealed by the heat of cautery or burning. Also the dark crusted ulcer (tache noire) at the site of the chigger (mite larva) bite in scrub typhus
Exanthem	An eruption of the skin (plural exanthemata)
Febrile	The presence of a fever

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Fibroblast	<ol style="list-style-type: none"> 1. A cell ubiquitous in connective tissue that makes and secretes collagen 2. A connective-tissue cell of mesenchymal origin that secretes proteins and especially molecular collagen from which the extracellular fibrillar matrix of connective tissue forms
Gingivostomatitis	Inflammation of the gums and of the mouth
Guarnieri Bodies	A minute inclusion body characteristic of smallpox and cowpox. Named after Giuseppe Guarnieri, (1856-1918), an Italian pathologist. In 1893 Guarnieri reported his discovery of certain inclusion bodies found in the specific lesions of smallpox and cowpox. Guarnieri believed that these bodies were the causative organism of these diseases
Haemorrhagic	Relating to bleeding
Impetigo	An acute contagious staphylococcal or streptococcal skin disease characterized by vesicles, pustules, and yellowish crusts
Lymphadenopathy	Abnormal enlargement of the lymph nodes
Lymphangitis	Inflammation of the lymphatic vessel
Mollusum	Any of various skin diseases marked by the occurrence of soft spherical tumors on the face or the body
Mollusum contagiosum	A contagious disease of the skin marked by the occurrence of rounded soft tumors of the skin caused by the growth of a virus (one that belongs to the virus family called the Poxviridae)
Morbidity	A diseased condition/state
Mortality	Relating to death
Myalgia	Refers to muscular pain, a symptom of many diseases and disorders

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Necrosis	The death of living cells or tissues. Necrosis can be due to ischaemia (lack of blood flow)
Oedema	The presence of excessive amounts of fluid in the intercellular tissue spaces of the body, due to increased transudation of fluid from the capillaries
Papillae	A small nipple-shaped projection, such as the conoid papillae of the tongue and the papillae of the dermis that extend from collagen fibres, the capillary blood vessels, and sometimes the nerves of the dermis
Papulonecrotic	Marked by the formation of papules (the solid elevation of the skin with no visible fluid) that tend to break down and form open sores (papulonecrotic tuberculids)
Papulovesicular	Marked by the presence of both papules and vesicles (a papulovesicular rash)
Pyoderma Gangrenosum	A condition where the skin has painful ulceration. The ulcers often have a purulent surface and a blue-black edge
Roseola Infantum	A mild virus disease of infants and children that is characterized by fever lasting three days followed by an eruption of rose-colored spots and is caused by a herpesvirus (species Human herpesvirus 6 of the genus Roseolovirus)
Septicaemia	An Invasion of the bloodstream by virulent microorganisms (as bacteria, viruses, or fungi) from a focus of infection that is accompanied by acute systemic illness -- called also blood poisoning
Seroconversion	The production of antibodies in response to an antigen
Serpiginous	Slowly spreading; especially : healing over in one portion while continuing to advance in another (serpiginous ulcer)
Shingles	Shingles is a skin rash caused by the reactivation of the same virus that causes chickenpox. The rash usually has dermatomal distribution. The virus responsible for these conditions is called the Varicella zoster virus

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Stratum Spinosum	The layers of prickle cells over the layer of the skin basale capable of undergoing mitosis – also called prickle cell layer --
Stratum	A layer of tissue
Ulcerate	To become affected with or as if with an ulcer

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This NSM should be used in conjunction with the series of NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

REFERENCES

1. Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient-identifiable information. London. December 1997.
2. Sanfilippo AM, Barrio V, Kulp-Shorten C, Callen JP. Common pediatric and adolescent skin conditions. *J Pediatr Adolesc Gynecol* 2003;16:269-83.
3. Darmstadt GL. Vesicles and Bullae. In: Long SS, Pickering LK, Prober CG, editors. *Principles and Practice of Pediatric Infectious Diseases*. 2nd ed. New York: Churchill Livingstone; 2002.
4. Drago F, Rampini E, Rebora A. Atypical exanthems: morphology and laboratory investigations may lead to an aetiological diagnosis in about 70% of cases. *Br J Dermatol* 2002;147:255-60.
5. Melnick JL, Wenner HA, Phillips CA. Enteroviruses. In: Lennette EH, Schmidt NJ, editors. *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections*. 5th ed. Washington DC: American Public Health Association; 1979. p. 471-534.
6. Lin TY, Twu SJ, Ho MS, Chang LY, Lee CY. Enterovirus 71 outbreaks, Taiwan: occurrence and recognition. *Emerg Infect Dis* 2003;9:291-3.
7. Chan KP, Goh KT, Chong CY, Teo ES, Lau G, Ling AE. Epidemic hand, foot and mouth disease caused by human enterovirus 71, Singapore. *Emerg Infect Dis* 2003;9:78-85.
8. McMinn P, Stratov I, Nagarajan L, Davis S. Neurological manifestations of enterovirus 71 infection in children during an outbreak of hand, foot, and mouth disease in Western Australia. *Clin Infect Dis* 2001;32:236-42.
9. Abubakar S, Chee HY, Shafee N, Chua KB, Lam SK. Molecular detection of enteroviruses from an outbreak of hand, foot and mouth disease in Malaysia in 1997. *Scand J Infect Dis* 1999;31:331-5.
10. Ooi MH, Wong SC, Mohan A, Podin Y, Perera D, Clear D, et al. Identification and validation of clinical predictors for the risk of neurological involvement in children with hand, foot, and mouth disease in Sarawak. *BMC Infect Dis* 2009;9:3.
11. Frydenberg A, Starr M. Hand, foot and mouth disease. *Aust Fam Physician* 2003;32:594-5.
12. Cohen JI, Davenport DS, Stewart JA, Deitchman S, Hilliard JK, Chapman LE. Recommendations for prevention of and therapy for exposure to B virus (cercopithecine herpesvirus 1). *Clin Infect Dis* 2002;35:1191-203.
13. Slomka MJ, Brown DW, Clewley JP, Bennett AM, Harrington L, Kelly DC. Polymerase chain reaction for detection of herpesvirus simiae (B virus) in clinical specimens. *Arch Virol* 1993;131:89.
14. Oya C, Ochiai Y, Taniuchi Y, Takano T, Ueda F, Yoshikawa Y, et al. Specific detection and identification of herpes B virus by a PCR-microplate hybridization assay. *J Clin Microbiol* 2004;42:1869-74.
15. Langenberg AG, Corey L, Ashley RL, Leong WP, Straus SE. A prospective study of new infections with herpes simplex virus type 1 and type 2. Chiron HSV Vaccine Study Group. *N Engl J Med* 1999;341:1432-8.
16. Corey L. Herpes Simplex Virus. In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 5th ed. Edinburgh: Churchill Livingstone; 2000. p. 1564-80.

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This NSM should be used in conjunction with the series of NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

17. Lafferty WE, Coombs RW, Benedetti J, Critchlow C, Corey L. Recurrences after oral and genital herpes simplex virus infection. Influence of site of infection and viral type. *N Engl J Med* 1987;316:1444-9.
18. Ashley RL. Laboratory techniques in the diagnosis of herpes simplex infection. *Genitourin Med* 1993;69:174-83.
19. Scoular A. Using the evidence base on genital herpes: optimising the use of diagnostic tests and information provision. *Sex Transm Infect* 2002;78:160-5.
20. Lafferty WE, Krofft S, Remington M, Giddings R, Winter C, Cent A, et al. Diagnosis of herpes simplex virus by direct immunofluorescence and viral isolation from samples of external genital lesions in a high-prevalence population. *J Clin Microbiol* 1987;25:323-6.
21. Gleaves CA, Wilson DJ, Wold AD, Smith TF. Detection and serotyping of herpes simplex virus in MRC-5 cells by use of centrifugation and monoclonal antibodies 16 h postinoculation. *J Clin Microbiol* 1985;21:29-32.
22. Johnston SL, Siegel CS. Comparison of enzyme immunoassay, shell vial culture, and conventional cell culture for the rapid detection of herpes simplex virus. *Diagn Microbiol Infect Dis* 1990;13:241-4.
23. Crist GA, Langer JM, Woods GL, Procter M, Hillyard DR. Evaluation of the ELVIS plate method for the detection and typing of herpes simplex virus in clinical specimens. *Diagn Microbiol Infect Dis* 2004;49:173-7.
24. Stabell EC, O'Rourke SR, Storch GA, Olivo PD. Evaluation of a genetically engineered cell line and a histochemical beta-galactosidase assay to detect herpes simplex virus in clinical specimens. *J Clin Microbiol* 1993;31:2796-8.
25. Filen F, Strand A, Allard A, Blomberg J, Herrmann B. Duplex real-time polymerase chain reaction assay for detection and quantification of herpes simplex virus type 1 and herpes simplex virus type 2 in genital and cutaneous lesions. *Sex Transm Dis* 2004;31:331-6.
26. Whiley DM, Mackay IM, Syrmis MW, Witt MJ, Sloots TP. Detection and differentiation of herpes simplex virus types 1 and 2 by a duplex LightCycler PCR that incorporates an internal control PCR reaction. *J Clin Virol* 2004;30:32-8.
27. Ramaswamy M, McDonald C, Smith M, Thomas D, Maxwell S, Tenant-Flowers M, et al. Diagnosis of genital herpes by real time PCR in routine clinical practice. *Sex Transm Infect* 2004;80:406-10.
28. Zheng ZB, Wu YD, Yu XL, Shang SQ. DNA microarray technology for simultaneous detection and species identification of seven human herpes viruses. *J Med Virol* 2008;80:1042-50.
29. Pellet PE, Tipples G. Human Herpesviruses 6, 7 and 8. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Tenover FC, Tenover FC, editors. *Manual of Clinical Microbiology*. 8th ed. Washington D.C: American Society for Microbiology; 2003.
30. Gershon AA, Silverstein SJ. Varicella-Zoster Virus. In: Richman DD, Whitley RJ, Hayden FG, editors. *Clinical Virology*. 2nd ed. Washington DC: ASM Press; 2002.
31. Van der Straten M, Carasco D, Tying SK. Viral infections involving the skin. In: Richman DD, Whitley RJ, Hayden FG, editors. *Clinical Virology*. 2nd ed. Washington DC: ASM Press; 2002. p. 117-34.
32. Rubben A, Baron JM, Grussendorf-Conen EI. Routine detection of herpes simplex virus and varicella zoster virus by polymerase chain reaction reveals that initial herpes zoster is frequently misdiagnosed as herpes simplex. *Br J Dermatol* 1997;137:259-61.

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Reference no: QSOP 55i2

This NSM should be used in conjunction with the series of NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

33. Ruocco V, Ruocco E. Tzanck smear, an old test for the new millennium: when and how. *Int J Dermatol* 1999;38:830-4.
34. Schirm J, Meulenberg JJ, Pastoor GW, Voorst Vader PC, Schroder FP. Rapid detection of varicella-zoster virus in clinical specimens using monoclonal antibodies on shell vials and smears. *J Med Virol* 1989;28:1-6.
35. Oshiro LS. Electron Microscopy. In: Schmidt NJ, Emmons RW, editors. *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections*. Baltimore: Port City Press; 1989. p. 179-203.
36. Jain S, Wyatt D, McCaughey C, O'Neill HJ, Coyle PV. Nested multiplex polymerase chain reaction for the diagnosis of cutaneous herpes simplex and herpes zoster infections and a comparison with electronmicroscopy. *J Med Virol* 2001;63:52-6.
37. Ozaki T, Kajita Y, Namazue J, Yamanishi K. Isolation of varicella-zoster virus from vesicles in children with varicella. *J Med Virol* 1996;48:326-8.
38. Perez JL, Garcia A, Niubo J, Salva J, Podzamczar D, Martin R. Comparison of techniques and evaluation of three commercial monoclonal antibodies for laboratory diagnosis of varicella-zoster virus in mucocutaneous specimens. *J Clin Microbiol* 1994;32:1610-3.
39. Dahl H, Marcoccia J, Linde A. Antigen detection: the method of choice in comparison with virus isolation and serology for laboratory diagnosis of herpes zoster in human immunodeficiency virus-infected patients. *J Clin Microbiol* 1997;35:347-9.
40. Taha YA, Quinlivan M, Scott FT, Leedham-Green M, Hawrami K, Thomas JM, et al. Are false negative direct immunofluorescence assays caused by varicella zoster virus gE mutant strains? *J Med Virol* 2004;73:631-5.
41. Gross G, Schofer H, Wassilew S, Friese K, Timm A, Guthoff R, et al. Herpes zoster guideline of the German Dermatology Society (DDG). *J Clin Virol* 2003;26:277-89.
42. Breuer J. Commentary on Herpes zoster guidelines of the German Dermatological Society. *Journal of Clinical Virology* 2003;26:291-3.
43. Oladepo DK, Klapper PE, Percival D, Vallely PJ. Serological diagnosis of varicella-zoster virus in sera with antibody-capture enzyme-linked immunosorbent assay of IgM. *J Virol Methods* 2000;84:169-73.
44. Sharrar RG, LaRussa P, Galea SA, Steinberg SP, Sweet AR, Keatley RM, et al. The postmarketing safety profile of varicella vaccine. *Vaccine* 2000;19:916-23.
45. Hardy I, Gershon AA, Steinberg SP, LaRussa P. The incidence of zoster after immunization with live attenuated varicella vaccine. A study in children with leukemia. *Varicella Vaccine Collaborative Study Group*. *N Engl J Med* 1991;325:1545-50.
46. Hawrami K, Breuer J. Analysis of United Kingdom wild-type strains of varicella-zoster virus: differentiation from the Oka vaccine strain. *J Med Virol* 1997;53:60-2.
47. Tipples GA, Safronetz D, Gray M. A real-time PCR assay for the detection of varicella-zoster virus DNA and differentiation of vaccine, wild-type and control strains. *J Virol Methods* 2003;113:113-6.
48. Beards G, Graham C, Pillay D. Investigation of vesicular rashes for HSV and VZV by PCR. *J Med Virol* 1998;54:155-7.
49. Chantrey J, Meyer H, Baxby D, Begon M, Bown KJ, Hazel SM, et al. Cowpox: reservoir hosts and geographic range. *Epidemiol Infect* 1999;122:455-60.

INVESTIGATION OF VESICULAR RASHES

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Reference no: QSOP 55i2

This NSM should be used in conjunction with the series of NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

50. Baxby D, Bennett M, Getty B. Human cowpox 1969-93: a review based on 54 cases. *Br J Dermatol* 1994;131:598-607.
51. Schupp P, Pfeffer M, Meyer H, Burck G, Kolmel K, Neumann C. Cowpox virus in a 12-year-old boy: rapid identification by an orthopoxvirus-specific polymerase chain reaction. *Br J Dermatol* 2001;145:146-50.
52. Olson VA, Laue T, Laker MT, Babkin IV, Drosten C, Shchelkunov SN, et al. Real-time PCR system for detection of orthopoxviruses and simultaneous identification of smallpox virus. *J Clin Microbiol* 2004;42:1940-6.
53. Update: multistate outbreak of monkeypox--Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin, 2003. *MMWR Morb Mortal Wkly Rep* 2003;52:642-6.
54. Maskalyk J. Monkeypox outbreak among pet owners. *CMAJ* 2003;169:44-5.
55. Mukinda VB, Mwema G, Kilundu M, Heymann DL, Khan AS, Esposito JJ. Re-emergence of human monkeypox in Zaire in 1996. Monkeypox Epidemiologic Working Group. *Lancet* 1997;349:1449-50.
56. Meyer H, Perrichot M, Stemmler M, Emmerich P, Schmitz H, Varaine F, et al. Outbreaks of disease suspected of being due to human monkeypox virus infection in the Democratic Republic of Congo in 2001. *J Clin Microbiol* 2002;40:2919-21.
57. Di Giulio DB, Eckburg PB. Human monkeypox: an emerging zoonosis. *Lancet Infect Dis* 2004;4:15-25.
58. Stagles MJ, Watson AA, Boyd JF, More IA, McSeveney D. The histopathology and electron microscopy of a human monkeypox lesion. *Trans R Soc Trop Med Hyg* 1985;79:192-202.
59. Ryabinin VA, Shundrin LA, Kostina EB, Laassri M, Chizhikov V, Shchelkunov SN, et al. Microarray assay for detection and discrimination of Orthopoxvirus species. *J Med Virol* 2006;78:1325-40.
60. Buchan J. Characteristics of orf in a farming community in mid-Wales. *BMJ* 1996;313:203-4.
61. Geerinck K, Lukito G, Snoeck R, De Vos R, De Clercq E, Vanrenterghem Y, et al. A case of human orf in an immunocompromised patient treated successfully with cidofovir cream. *J Med Virol* 2001;64:543-9.
62. Torfason EG, Gunadottir S. Polymerase chain reaction for laboratory diagnosis of orf virus infections. *J Clin Virol* 2002;24:79-84.
63. . Update: adverse events following civilian smallpox vaccination--United States, 2003. *MMWR Morb Mortal Wkly Rep* 2003;52:819-20.
64. Fulginiti VA, Papier A, Lane JM, Neff JM, Henderson DA. Smallpox vaccination: a review, part II. Adverse events. *Clin Infect Dis* 2003;37:251-71.
65. Fulginiti VA, Papier A, Lane JM, Neff JM, Henderson DA. Smallpox vaccination: a review, part I. Background, vaccination technique, normal vaccination and revaccination, and expected normal reactions. *Clin Infect Dis* 2003;37:241-50.
66. Amorosa VK, Isaacs SN. Separate worlds set to collide: smallpox, vaccinia virus vaccination, and human immunodeficiency virus and acquired immunodeficiency syndrome. *Clin Infect Dis* 2003;37:426-32.

INVESTIGATION OF VESICULAR RASHES

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Reference no: QSOP 55i2

This NSM should be used in conjunction with the series of NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

67. Besser JM, Crouch NA, Sullivan M. Laboratory diagnosis to differentiate smallpox, vaccinia, and other vesicular/pustular illnesses. *J Lab Clin Med* 2003;142:246-51.
68. Kelly CD, Egan C, Davis SW, Samsonoff WA, Musser KA, Drabkin P, et al. Laboratory confirmation of generalized vaccinia following smallpox vaccination. *J Clin Microbiol* 2004;42:1373-5.
69. Regan TD, Norton SA. Diagnosis of smallpox. *N Engl J Med* 2002;347:690-1.
70. Breman JG, Henderson DA. Diagnosis and management of smallpox. *N Engl J Med* 2002;346:1300-8.
71. UK Department of Health. Guidelines for smallpox response and management in the post-eradication era (smallpox plan). p. 1-59.
72. Nitsche A, Ellerbrok H, Pauli G. Detection of orthopoxvirus DNA by real-time PCR and identification of variola virus DNA by melting analysis. *J Clin Microbiol* 2004;42:1207-13.
73. Krusell A, Comer JA, Sexton DJ. Rickettsialpox in North Carolina: a case report. *Emerg Infect Dis* 2002;8:727-8.
74. Paddock CD, Zaki SR, Koss T, Singleton J, Jr., Sumner JW, Comer JA, et al. Rickettsialpox in New York City: a persistent urban zoonosis. *Ann N Y Acad Sci* 2003;990:36-44.
75. Cascio A, Dones P, Romano A, Titone L. Clinical and laboratory findings of boutonneuse fever in Sicilian children. *Eur J Pediatr* 1998;157:482-6.
76. Raoult D, Fournier PE, Fenollar F, Jensenius M, Prioe T, de Pina JJ, et al. *Rickettsia africae*, a tick-borne pathogen in travelers to sub-Saharan Africa. *N Engl J Med* 2001;344:1504-10.
77. Cazorla C, Socolovschi C, Jensenius M, Parola P. Tick-borne diseases: tick-borne spotted fever rickettsioses in Africa. *Infect Dis Clin North Am* 2008;22:531-x.
78. Roch N, Epaulard O, Pelloux I, Pavese P, Brion JP, Raoult D, et al. African tick bite fever in elderly patients: 8 cases in French tourists returning from South Africa. *Clin Infect Dis* 2008;47:e28-e35.
79. La Scola B, Raoult D. Laboratory diagnosis of rickettsioses: current approaches to diagnosis of old and new rickettsial diseases. *J Clin Microbiol* 1997;35:2715-27.
80. Dixon TC, Meselson M, Guillemin J, Hanna PC. Anthrax. *N Engl J Med* 1999;341:815-26.
81. Quinn CP, Semenova VA, Elie CM, Romero-Steiner S, Greene C, Li H, et al. Specific, sensitive, and quantitative enzyme-linked immunosorbent assay for human immunoglobulin G antibodies to anthrax toxin protective antigen. *Emerg Infect Dis* 2002;8:1103-10.
82. Bell CA, Uhl JR, Hadfield TL, David JC, Meyer RF, Smith TF, et al. Detection of *Bacillus anthracis* DNA by LightCycler PCR. *J Clin Microbiol* 2002;40:2897-902.
83. Darmstadt GL, Dinulos JG, Miller Z. Congenital cutaneous candidiasis: clinical presentation, pathogenesis, and management guidelines. *Pediatrics* 2000;105:438-44.
84. Miller MJ. Fungal Infections. In: Remington JS, Klein JO, editors. *Infectious Diseases of the Fetus and Newborn Infant*. 5th ed. Philadelphia: WB Saunders Company; 2001.
85. Baley JE, Silverman RA. Systemic candidiasis: cutaneous manifestations in low birth weight infants. *Pediatrics* 1988;82:211-5.

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Reference no: QSOP 55i2

This NSM should be used in conjunction with the series of NSMs from the Health Protection Agency

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Email: standards@hpa.org.uk

86. Horvath LL, Hospenthal DR, Murray CK, Dooley DP. Detection of simulated candidemia by the BACTEC 9240 system with plus aerobic/F and anaerobic/F blood culture bottles. *J Clin Microbiol* 2003;41:4714-7.
87. Blackwell V, Vega-Lopez F. Cutaneous larva migrans: clinical features and management of 44 cases presenting in the returning traveller. *Br J Dermatol* 2001;145:434-7.
88. Van Buynder PG, Gaggin JA, Martin D, Pugsley D, Mathews JD. Streptococcal infection and renal disease markers in Australian aboriginal children. *Med J Aust* 1992;156:537-40.

INVESTIGATION OF VESICULAR RASHES

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