

# Replacement of Dryvax™ vaccine with ACAM2000™

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[http://www.cdc.gov/mmwr/preview/mmwrhtml/  
mm5708a6.htm](http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5708a6.htm)

## Newly Licensed Smallpox Vaccine to Replace Old Smallpox Vaccine

CDC has begun distribution of a new-generation smallpox vaccine, ACAM2000™ (Acambis, Inc., Cambridge, Massachusetts), to civilian laboratory personnel, the military, and state public health preparedness programs. ACAM2000 is a live, vaccinia virus smallpox vaccine that was licensed for use in the United States by the Food and Drug Administration in August 2007 (1).\* ACAM2000 will be replacing Dryvax® smallpox vaccine (Wyeth Pharmaceuticals, Inc., Marietta, Pennsylvania) because of withdrawal of the Dryvax license. ACAM2000 is a live vaccinia virus derived from plaque purification cloning from Dryvax. The safety data available from the ACAM2000 clinical trials indicate a similar safety profile to Dryvax.

Wyeth intends to withdraw the Dryvax license and asks that all remaining quantities of vaccine held by civilian and military users be quarantined by February 29, 2008, for the purpose of destruction. This withdrawal is not necessitated by any safety, purity, or quality concerns with

\*ACAM2000 package insert and medication guide are available at <http://www.acambis.com/acam2000>.

the product but rather is consistent with a contract agreement between CDC and Wyeth.<sup>†</sup> All lots of Dryvax vaccine will expire on February 29, 2008, and should not be used after that date.

All Dryvax vaccine should be destroyed on site. Vaccine vials can be 1) dropped into the hospital sharps container and autoclaved or 2) disposed of following the procedure for all other biohazard materials. In sites where medical waste is buried, soaking the medical waste in a 1:10 dilution of bleach for at least 10 minutes before disposal is advised. All programs that hold supplies of Dryvax vaccine must provide documentation of Dryvax vaccine destruction to the CDC Drug Service by March 31, 2008. These programs are advised to use the Dryvax vaccine destruction form.<sup>§</sup>

CDC will continue to provide ACAM2000 smallpox vaccine to protect responders as part of state public health preparedness programs (2) and civilian laboratory personnel who risk exposure to orthopoxviruses (3). Unlike Dryvax, ACAM2000 expires 18 months after release from the CDC Strategic National Stockpile. Requests for smallpox vaccine should be directed to the CDC Drug Service by e-mail ([drugservice@cdc.gov](mailto:drugservice@cdc.gov)) or telephone (404-639-3670).

## References

1. Food and Drug Administration. Product approval information. Available at <http://www.fda.gov/cber/products/acam2000.htm>.
2. CDC. Recommendations for using smallpox vaccine in a pre-event vaccination program: supplemental recommendations of the Advisory Committee on Immunizations Practices (ACIP) and the Healthcare Infection Control Practices Advisory Committee (HICPAC). MMWR 2003;52 (Dispatch).
3. CDC. Smallpox vaccine available for protection of at-risk laboratory workers. MMWR 1983;32:543-4.

<sup>†</sup> Additional information regarding the withdrawal is communicated in a letter, dated February 1, 2008, from Wyeth to the CDC Drug Service; the letter is available at [http://emergency.cdc.gov/agent/smallpox/vaccination/pdf/ltr\\_cdc\\_010208\\_dryvax.pdf](http://emergency.cdc.gov/agent/smallpox/vaccination/pdf/ltr_cdc_010208_dryvax.pdf).

<sup>§</sup> Available at [http://emergency.cdc.gov/agent/smallpox/vaccination/pdf/dryvax\\_destruction\\_note\\_gen.pdf](http://emergency.cdc.gov/agent/smallpox/vaccination/pdf/dryvax_destruction_note_gen.pdf).



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*Recommendations  
and  
Reports*

***Inside: Continuing Education Examination***

## **Vaccinia (Smallpox) Vaccine**

**Recommendations of the Advisory Committee  
on Immunization Practices (ACIP), 2001**

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES**  
Centers for Disease Control and Prevention (CDC)  
Atlanta, GA 30333



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# Vaccinia (Smallpox) Vaccine

## Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001

### Summary

*These revised recommendations regarding vaccinia (smallpox) vaccine update the previous Advisory Committee on Immunization Practices (ACIP) recommendations (MMWR 1991;40; No. RR-14:1–10) and include current information regarding the nonemergency use of vaccinia vaccine among laboratory and health-care workers occupationally exposed to vaccinia virus, recombinant vaccinia viruses, and other Orthopoxviruses that can infect humans. In addition, this report contains ACIP's recommendations for the use of vaccinia vaccine if smallpox (variola) virus were used as an agent of biological terrorism or if a smallpox outbreak were to occur for another unforeseen reason.*

## INTRODUCTION

Variola virus is the etiological agent of smallpox. During the smallpox era, the only known reservoir for the virus was humans; no known animal or insect reservoirs or vectors existed. The most frequent mode of transmission was person-to-person, spread through direct deposit of infective droplets onto the nasal, oral, or pharyngeal mucosal membranes, or the alveoli of the lungs from close, face-to-face contact with an infectious person. Indirect spread (i.e., not requiring face-to-face contact with an infectious person) through fine-particle aerosols or a fomite containing the virus was less common (1,2).

Symptoms of smallpox begin 12–14 days (range: 7–17) after exposure, starting with a 2–3 day prodrome of high fever, malaise, and prostration with severe headache and backache. This preeruptive stage is followed by the appearance of a maculopapular rash (i.e., eruptive stage) that progresses to papules 1–2 days after the rash appears; vesicles appear on the fourth or fifth day; pustules appear by the seventh day; and scab lesions appear on the fourteenth day (Figures 1,2) (3). The rash appears first on the oral mucosa, face, and forearms, then spreads to the trunk and legs (3,4). Lesions might erupt on the palms and soles as well. Smallpox skin lesions are deeply embedded in the dermis and feel like firm round objects embedded in the skin. As the skin lesions heal, the scabs separate and pitted scarring gradually develops (Figure 2) (4). Smallpox patients are most infectious during the first week of the rash when the oral mucosa lesions ulcerate and release substantial amounts of virus into the saliva. A patient is no longer infectious after all scabs have separated (i.e., 3–4 weeks after the onset of the rash).

During the smallpox era, overall mortality rates were approximately 30%. Other less common but more severe forms of smallpox included a) flat-type smallpox with a mortality rate >96% and characterized by severe toxemia and flat, velvety, confluent lesions that did not progress to the pustular stage; and b) hemorrhagic-type smallpox, characterized by severe prodromal symptoms, toxemia, and a hemorrhagic rash that was almost always fatal, with death occurring 5–6 days after rash onset (4).

**FIGURE 1. Man with smallpox**

**Source:** CDC/Public Health Images Library, identification no. 131. Photographer: Barbara Rice. Available at <<http://phil.cdc.gov/Phil/>>; accessed on May 16, 2001.

\* Additional smallpox images are available at <<http://www.bt.cdc.gov/Agent/Smallpox/Smallpox.asp>> (accessed April 20, 2001).

**FIGURE 2. Progression of smallpox lesions from, left to right, pustules to scabs to scars**

**Source:** Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. Smallpox and its eradication. Geneva, Switzerland: World Health Organization (WHO), 1988. Reprinted with permission from WHO.

\* Additional smallpox images are available at <<http://www.bt.cdc.gov/Agent/Smallpox/Smallpox.asp>> (accessed April 20, 2001).

Vaccinia vaccine is a highly effective immunizing agent that enabled the global eradication of smallpox. The last naturally occurring case of smallpox occurred in Somalia in 1977. In May 1980, the World Health Assembly certified that the world was free of naturally occurring smallpox (5). By the 1960s, because of vaccination programs and quarantine regulations, the risk for importation of smallpox into the United States had been reduced. As a result, recommendations for routine smallpox vaccination were rescinded in 1971 (6). In 1976, the recommendation for routine smallpox vaccination of health-care workers was also discontinued (7). In 1982, the only active licensed producer of vaccinia vaccine in the United States discontinued production for general use, and in 1983, distribution to the civilian population was discontinued (8). All military personnel continued to be vaccinated, but that practice ceased in 1990. Since January 1982, smallpox vaccination has not been required for international travelers, and International Certificates of Vaccination forms no longer include a space to record smallpox vaccination (9).

In 1980, the Advisory Committee on Immunization Practices (ACIP) recommended the use of vaccinia vaccine to protect laboratory workers from possible infection while working with nonvariola Orthopoxviruses (e.g., vaccinia and monkeypox) (10). In 1984, those recommendations were included in guidelines for biosafety in microbiological and biomedical laboratories (11). The guidelines expanded the recommendations to include persons working in animal-care areas where studies with Orthopoxviruses were being conducted. They further recommended that such workers have documented evidence of satisfactory smallpox vaccination within the preceding 3 years. CDC has provided vaccinia vaccine for these laboratory workers since 1983 (12). In 1991, ACIP further expanded smallpox vaccination recommendations to include health-care workers involved in clinical trials using recombinant vaccinia virus vaccines and lengthened the recommendations for revaccination for persons working with vaccinia virus, recombinant vaccinia viruses, or other nonvariola Orthopoxviruses to every 10 years (13).

Currently, international concern is heightened regarding the potential use of smallpox (variola) virus as a bioterrorism agent (14,15). Because of these concerns, ACIP has developed recommendations for vaccinia (smallpox) vaccine regarding the potential use of smallpox virus as a biological weapon. Additionally, recommendations regarding vaccination of persons working with highly attenuated strains or recombinant vaccines derived from highly attenuated strains of vaccinia virus have been revised.

## VACCINIA VACCINE

Dryvax,<sup>®</sup> the vaccinia (smallpox) vaccine currently licensed in the United States, is a lyophilized, live-virus preparation of infectious vaccinia virus (Wyeth Laboratories, Inc., Marietta, Pennsylvania). Vaccinia vaccine does not contain smallpox (variola) virus. Previously, the vaccine had been prepared from calf lymph with a seed virus derived from the New York City Board of Health (NYCBOH) strain of vaccinia virus and has a minimum concentration of 10<sup>8</sup> pock-forming units (PFU)/ml. Vaccine was administered by using the multiple-puncture technique with a bifurcated needle. A reformulated vaccine, produced by using cell-culture techniques, is now being developed.

## Vaccine Efficacy

Neutralizing antibodies induced by vaccinia vaccine are genus-specific and cross-protective for other Orthopoxviruses (e.g., monkeypox, cowpox, and variola viruses) (16–18). Although the efficacy of vaccinia vaccine has never been measured precisely during controlled trials, epidemiologic studies demonstrate that an increased level of protection against smallpox persists for  $\leq 5$  years after primary vaccination and substantial but waning immunity can persist for  $\geq 10$  years (19,20). Antibody levels after revaccination can remain high longer, conferring a greater period of immunity than occurs after primary vaccination alone (3,19). Administration of vaccinia vaccine within the first days after initial exposure to smallpox virus can reduce symptoms or prevent smallpox disease (2–4).

Although the level of antibody that protects against smallpox infection is unknown, after percutaneous administration of a standard dose of vaccinia vaccine, >95% of primary vaccinees (i.e., persons receiving their first dose of vaccine) will experience neutralizing or hemagglutination inhibition antibody at a titer of  $\geq 1:10$  (21). Neutralizing antibody titers of  $\geq 1:10$  persist among 75% of persons for 10 years after receiving second doses and  $\leq 30$  years after receiving three doses of vaccine (22,23). The level of antibody required for protection against vaccinia virus infection is unknown also. However, when lack of local skin response to revaccination with an appropriately administered and potent vaccine dose is used as an indication of immunity, <10% of persons with neutralizing titers of  $\geq 1:10$  exhibit a primary-type response at revaccination, compared with >30% of persons with titers <1:10 (24). Lack of major or primary-type reaction can indicate the presence of neutralizing antibody levels sufficient to prevent viral replication, although it can also indicate unsuccessful vaccination because of improper administration or less potent vaccine.

## Recombinant Vaccinia Viruses

Vaccinia virus is the prototype of the genus Orthopoxvirus. It is a double-stranded DNA (deoxyribonucleic acid) virus that has a broad host range under experimental conditions but is rarely isolated from animals outside the laboratory (25,26). Multiple strains of vaccinia virus exist that have different levels of virulence for humans and animals. For example, the Temple of Heaven and Copenhagen vaccinia strains are highly pathogenic among animals, whereas the NYCBOH strain, from which the Wyeth vaccine strain was derived, had relatively low pathogenicity (3).

Vaccinia virus can be genetically engineered to contain and express foreign DNA with or without impairing the ability of the virus to replicate. Such foreign DNA can encode protein antigens that induce protection against one or more infectious agents. Recombinant vaccinia viruses have been engineered to express immunizing antigens of herpesvirus, hepatitis B, rabies, influenza, human immunodeficiency virus (HIV), and other viruses (27–32).

Recombinant vaccinia viruses have been created from different strains of vaccinia virus. In the United States, recombinants have been made from a nonattenuated NYCBOH strain, or a mouse neuroadapted derivative, the WR strain. Recombinants have also been made by using the Copenhagen and Lister vaccinia strains, which are more pathogenic among animals than the NYCBOH strain. Additionally, certain highly attenuated, host-restricted, non- or poorly replicating poxvirus strains have been developed for use as substrates in recombinant vaccine development. These strains include the

Orthopoxviruses, modified vaccinia Ankara (MVA) and NYVAC (derived from the Copenhagen vaccinia strain), and the Avipoxviruses, ALVAC and TROVAC (derived from canarypox and fowlpox viruses, respectively) (33–36) (Table 1).

**TABLE 1. Highly attenuated poxvirus strains used for recombinant vaccine development**

Strain	Parent virus strain	Biosafety level
MVA	Vaccinia virus (Ankara)	2
NYVAC	Vaccinia virus (Copenhagen)	1
TROVAC	Fowlpox virus	1
ALVAC	Canarypox virus	1

Animal studies indicate that recombinants are less pathogenic than the parent strain of vaccinia virus (37). Laboratory-acquired infections with nonhighly attenuated vaccinia and recombinant viruses derived from nonhighly attenuated vaccinia strains have been reported (38–41). However, highly attenuated poxvirus strains (MVA, NYVAC, ALVAC, and TROVAC) are unable to replicate (MVA, ALVAC, and TROVAC) or replicate poorly (NYVAC) in mammalian host cells; therefore, highly attenuated poxvirus strains do not create productive infections (36).

These highly attenuated strains have also been reported to be avirulent among normal and immunosuppressed animals (MVA, NYVAC, ALVAC, or TROVAC) and safe among humans (MVA) (33,35,42,43). Although no formal surveillance system has been established to monitor laboratory workers, no laboratory-acquired infections resulting from exposure to these highly attenuated strains or recombinant vaccines derived from these strains have been reported in the scientific literature or to CDC. Because of the biological properties and accumulated attenuation data for NYVAC, ALVAC, and TROVAC, the Recombinant DNA Advisory Committee of the National Institutes of Health (NIH) reduced the biosafety level for these viruses to biosafety level 1 (44). The Occupational Safety and Health Board of NIH no longer requires vaccinia (smallpox) vaccination for personnel manipulating MVA or NYVAC in a laboratory where no other vaccinia viruses are being manipulated (45).

During human trials of recombinant vaccines, physicians, nurses, and other health-care personnel who provide clinical care to recipients of these vaccines could be exposed to both vaccinia and recombinant viruses. This exposure could occur from contact with dressings contaminated with the virus or through exposure to the vaccine. Although the risk for transmission of recombinant vaccinia viruses to exposed health-care workers is unknown, no reports of transmission to health-care personnel from vaccine recipients have been published. If appropriate infection-control precautions are observed (46,47), health-care workers are at less risk for infection than laboratory workers because of the smaller volume and lower titer of virus in clinical specimens compared with laboratory material. However, the potential does exist of nonhighly attenuated vaccinia viruses or recombinant viruses derived from these strains being transmitted to health-care personnel. Therefore, those workers who have direct contact with contaminated dressings or other infectious material from volunteers in clinical studies where such strains are used can be offered vaccination. Vaccination is not indicated for health-care personnel who are exposed to clinical materials contaminated with highly attenuated poxvirus strains used to develop vaccine recombinants.

Laboratory and other health-care personnel who work with highly attenuated strains of vaccinia virus (e.g., MVA and NYVAC) do not require routine vaccinia vaccination. Laboratory and other health-care personnel who work with the Avipoxvirus strains ALVAC and TROVAC also do not require routine vaccinia vaccination because these viruses do not grow in mammalian cells and, therefore, do not produce clinical infections among humans. In addition, antibodies induced by vaccinia vaccine are genus-specific (16) and would probably not inhibit the expression of genes incorporated into recombinant vaccines derived from ALVAC and TROVAC. Therefore, vaccination would provide no theoretical benefit in preventing seroconversion to the foreign antigen expressed by a recombinant virus if an inadvertent exposure occurred. Laboratory and other health-care personnel who work with viral cultures or other infective materials should always observe appropriate biosafety guidelines and adhere to published infection-control procedures (46–48).

### **Routine Nonemergency Vaccine Use**

Vaccinia vaccine is recommended for laboratory workers who directly handle a) cultures or b) animals contaminated or infected with, nonhighly attenuated vaccinia virus, recombinant vaccinia viruses derived from nonhighly attenuated vaccinia strains, or other Orthopoxviruses that infect humans (e.g., monkeypox, cowpox, vaccinia, and variola). Other health-care workers (e.g., physicians and nurses) whose contact with nonhighly attenuated vaccinia viruses is limited to contaminated materials (e.g., dressings) but who adhere to appropriate infection control measures are at lower risk for inadvertent infection than laboratory workers. However, because a theoretical risk for infection exists, vaccination can be offered to this group. Vaccination is not recommended for persons who do not directly handle nonhighly attenuated virus cultures or materials or who do not work with animals contaminated or infected with these viruses.

Vaccination with vaccinia vaccine results in high seroconversion rates and only infrequent adverse events (see Side Effects and Adverse Reactions). Recipients of standard potency vaccinia vaccine (Dryvax) receive controlled percutaneous doses (approximately  $2.5 \times 10^5$  PFU [3]) of relatively low pathogenicity vaccinia virus. The resulting immunity should provide protection to recipients against infections resulting from uncontrolled, inadvertent inoculation by unusual routes (e.g., the eye) with a substantial dose of virus of higher or unknown pathogenicity. In addition, persons with preexisting immunity to vaccinia might be protected against seroconversion to the foreign antigen expressed by a recombinant virus if inadvertently exposed (41). However, persons with preexisting immunity to vaccinia might not receive the full benefit of recombinant vaccinia vaccines developed for immunization against other infections (31,49).

### **Routine Nonemergency Revaccination**

According to data regarding the persistence of neutralizing antibody after vaccination, persons working with nonhighly attenuated vaccinia viruses, recombinant viruses developed from nonhighly attenuated vaccinia viruses, or other nonvariola Orthopoxviruses should be revaccinated at least every 10 years (13). To ensure an increased level of protection against more virulent nonvariola Orthopoxviruses (e.g., monkeypox), empiric revaccination every 3 years can be considered (17).

## Side Effects and Adverse Reactions

### *Vaccine Recipients*

**Side Effects and Less Severe Adverse Reactions.** In a nonimmune person who is not immunosuppressed, the expected response to primary vaccination is the development of a papule at the site of vaccination 2–5 days after percutaneous administration of vaccinia vaccine. The papule becomes vesicular, then pustular, and reaches its maximum size in 8–10 days. The pustule dries and forms a scab, which separates within 14–21 days after vaccination, leaving a scar (Figure 3). Primary vaccination can produce swelling and tenderness of regional lymph nodes, beginning 3–10 days after vaccination and persisting for 2–4 weeks after the skin lesion has healed. Maximum viral shedding from the vaccination site occurs 4–14 days after vaccination, but vaccinia can be recovered from the site until the scab separates from the skin (50).

A fever is also common after the vaccine is administered. Approximately 70% of children experience  $\geq 1$  days of temperatures  $\geq 100$  F for 4–14 days after primary vaccination (21), and 15%–20% of children experience temperatures  $\geq 102$  F. After revaccination, 35% of children experience temperatures  $\geq 100$  F, and 5% experience temperatures of  $\geq 102$  F (24). Fever is less common among adults after vaccination or revaccination (CDC, unpublished data, undated).

Inadvertent inoculation at other sites is the most frequent complication of vaccinia vaccination and accounts for approximately half of all complications of primary vaccination and revaccination (Tables 2,3). Inadvertent inoculation usually results from autoinoculation of vaccinia virus transferred from the site of vaccination. The most common sites involved are the face, eyelid, nose, mouth, genitalia, and rectum (Figure 4). Most lesions heal without specific therapy, but vaccinia immunoglobulin (VIG) can be useful for cases of ocular implantation (see Treatment for Vaccinia Vaccine Complications). However, if vaccinal keratitis is present, VIG is contraindicated because it might increase corneal scarring (51).

**FIGURE 3. Vaccine site major reaction and progression after primary smallpox vaccination or revaccination after a prolonged period between vaccinations, using multiple-puncture technique**



Source: CDC

\* Additional smallpox images are available at <<http://www.bt.cdc.gov/Agent/Smallpox/Smallpox.asp>> (accessed April 20, 2001).

**TABLE 2. Vaccine adverse reactions and vaccinia immunoglobulin (VIG) indications**

Adverse reactions	VIG treatment
<b>Mild to moderate</b>	
Inadvertent inoculation	Usually not required; might be indicated for ocular implantation*
Erythematous or urticarial rashes	Not indicated†
Bullous erythema multiforme (Stevens-Johnson syndrome)	Not indicated†
<b>Moderate to severe</b>	
Eczema vaccinatum	Indicated in severe cases
Generalized vaccinia	Usually not required but might be indicated if patient is severely ill or has serious underlying illness
Progressive vaccinia (vaccinia necrosum)	Might be effective, depending on immune defect
Postvaccinial encephalitis	Not indicated†
Vaccinial keratitis	Contraindicated*

\* VIG contraindicated if vaccinial keratitis present because increased scarring can occur.

† VIG is not effective in treatment of these adverse reactions.

**TABLE 3. Rates of reported complications\* associated with vaccinia vaccinations† (cases/million vaccinations)**

Age (yrs) and status	Inadvertent inoculation <sup>§</sup>	Generalized vaccinia	Eczema vaccinatum	Progressive vaccinia <sup>¶</sup>	Postvaccinial encephalitis	Total**
<b>Primary vaccination</b>						
<1	507.0	394.4	14.1	— <sup>††</sup>	42.3	<b>1549.3</b>
1–4	577.3	233.4	44.2	3.2	9.5	<b>1261.8</b>
5–19	371.2	139.7	34.9	— <sup>††</sup>	8.7	<b>855.9</b>
≥20	606.1	212.1	30.3	— <sup>††</sup>	— <sup>††</sup>	<b>1515.2</b>
<b>Overall rates<sup>††</sup></b>	<b>529.2</b>	<b>241.5</b>	<b>38.5</b>	<b>1.5</b>	<b>12.3</b>	<b>1253.8</b>
<b>Revaccination</b>						
<1	— <sup>††</sup>	— <sup>††</sup>	— <sup>††</sup>	— <sup>††</sup>	— <sup>††</sup>	— <sup>††</sup>
1–4	109.1	— <sup>††</sup>	— <sup>††</sup>	— <sup>††</sup>	— <sup>††</sup>	<b>200.0</b>
5–19	47.7	9.9	2.0	— <sup>††</sup>	— <sup>††</sup>	<b>85.5</b>
>20	25.0	9.1	4.5	6.8	4.5	<b>113.6</b>
<b>Overall rates<sup>§§</sup></b>	<b>42.1</b>	<b>9.0</b>	<b>3.0</b>	<b>3.0</b>	<b>2.0</b>	<b>108.2</b>

\* See text for descriptions of complications.

† Adapted from Lane JM, Ruben FL, Neff JM, Millar JD. Complications of smallpox vaccination, 1968: results of ten statewide surveys. *J Infect Dis* 1970;122:303–9.

§ Referenced as accidental implantation.

¶ Referenced as vaccinia necrosum.

\*\* Rates of overall complications by age group include complications not provided in this table, including severe local reactions, bacterial superinfection of the vaccination site, and erythema multiforme.

†† No instances of this complication were identified during the 1968 10-state survey.

§§ Overall rates for each complication include persons of unknown age.

**FIGURE 4. Inadvertent autoinoculation of lower eyelid with vaccinia virus**

**Source:** Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. Smallpox and its eradication. Geneva, Switzerland: World Health Organization (WHO), 1988. Reprinted with permission from WHO.

\* Additional smallpox images are available at <<http://www.bt.cdc.gov/Agent/Smallpox/Smallpox.asp>> (accessed April 20, 2001).

Erythematous or urticarial rashes can occur approximately 10 days after primary vaccination and can be confused with generalized vaccinia. However, the vaccinee is usually afebrile with this reaction, and the rash resolves spontaneously within 2–4 days. Rarely, bullous erythema multiforme (i.e., Stevens-Johnson syndrome) occurs (52).

**Moderate to Severe Adverse Reactions.** Moderate and severe complications of vaccinia vaccination include eczema vaccinatum, generalized vaccinia, progressive vaccinia, and postvaccinial encephalitis (Table 2). These complications are rare but occur  $\geq 10$  times more often among primary vaccinees than among revaccinees and are more frequent among infants than among older children and adults (53–55) (Table 3). A study of Israeli military recruits aged  $\geq 18$  years, who were vaccinated during 1991–1996, reported rates of the severe complications progressive vaccinia (i.e., vaccinia necrosum rate: 0/10,000 vaccinees) and postvaccinial encephalitis (rate: 0/10,000 vaccinees) similar to those reported in previous studies (56).

Eczema vaccinatum is a localized or systemic dissemination of vaccinia virus among persons who have eczema or a history of eczema or other chronic or exfoliative skin conditions (e.g., atopic dermatitis) (Figure 5). Usually, illness is mild and self-limited but can be severe or fatal. The most serious cases among vaccine recipients occur among

**FIGURE 5. Eczema vaccinatum**

**Source:** John M. Leedom, M.D.

\* Additional smallpox images are available at <<http://www.bt.cdc.gov/Agent/Smallpox/Smallpox.asp>> (accessed April 20, 2001).

primary vaccinees and are independent of the activity of the underlying eczema (57). Severe cases have been observed also after contact of recently vaccinated persons with persons who have active eczema or a history of eczema (see Contacts of Vaccinees) (Figure 6).

**FIGURE 6. Eczema vaccinatum resulting from contact with recently vaccinated child; patient recovered without sequelae or permanent ocular damage**



**Photographer:** John M. Leedom, M.D.

\* Additional smallpox images are available at <<http://www.bt.cdc.gov/Agent/Smallpox/Smallpox.asp>> (accessed April 20, 2001).

Generalized vaccinia is characterized by a vesicular rash of varying extent that can occur among persons without underlying illnesses (Figure 7). The rash is generally self-limited and requires minor or no therapy except among patients whose conditions might be toxic or who have serious underlying immunosuppressive illnesses (e.g., acquired immunodeficiency syndrome [AIDS]) (58).

**FIGURE 7. Generalized vaccinia in an otherwise healthy child; the child recovered without sequelae**



**Photographer:** John M. Leedom, M.D.

\* Additional smallpox images are available at <<http://www.bt.cdc.gov/Agent/Smallpox/Smallpox.asp>> (accessed April 20, 2001).

**FIGURE 8. Progressive vaccinia (vaccinia necrosum), which was fatal, in a child with an immunodeficiency**



**Source:** Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. Smallpox and its eradication. Geneva, Switzerland: World Health Organization (WHO), 1988. Reprinted with permission from WHO.

\* Additional smallpox images are available at <<http://www.bt.cdc.gov/Agent/Smallpox/Smallpox.asp>> (accessed April 20, 2001).

Progressive vaccinia (vaccinia necrosum) is a severe, potentially fatal illness characterized by progressive necrosis in the area of vaccination, often with metastatic lesions (Figure 8). It has occurred almost exclusively among persons with cellular immunodeficiency. The most serious complication is postvaccinial encephalitis. In the majority of cases, it affects primary vaccinees aged <1 year or adolescents and adults receiving a primary vaccination (3). Occurrence of this complication was influenced by the strain of vaccine virus and was higher in Europe than in the United States. The principle strain of vaccinia virus used in the United States, NYCBOH, was associated with the lowest incidence of postvaccinial encephalitis (3). Approximately 15%–25% of affected vaccinees with this complication die, and 25% have permanent neurological sequelae (52–54). Fatal complications caused by vaccinia vaccination are rare, with approximately 1 death/million primary vaccinations and 0.25 deaths/million revaccinations (54). Death is most often the result of postvaccinial encephalitis or progressive vaccinia.

### **Contacts of Vaccinees**

Transmission of vaccinia virus can occur when a recently vaccinated person has contact with a susceptible person. In a 1968 10-state survey of complications of vaccinia vaccination, the risk for transmission to contacts was 27 infections/million total vaccinations; 44% of those contact cases occurred among children aged  $\leq 5$  years (53). Before the U.S. military discontinued routine smallpox vaccination in 1990, occurrences of contact transmission of vaccinia virus from recently vaccinated military recruits had been reported, including six cases resulting from transmission from one vaccine recipient (59–61).

Approximately 60% of contact transmissions reported in the 1968 10-state survey resulted in inadvertent inoculation of otherwise healthy persons. Approximately 30% of the eczema vaccinatum cases reported in that study were a result of contact transmission (53). Eczema vaccinatum might be more severe among contacts than among vaccinated persons, possibly because of simultaneous multiple inoculations at several sites (54,62). Contact transmission rarely results in postvaccinial encephalitis or vaccinia necrosum.

## Precautions and Contraindications

### ***Routine Nonemergency Laboratory and Health-Care Worker Contraindications***

The following contraindications to vaccination apply to routine nonemergency use of vaccinia vaccine (see Smallpox Vaccine for Bioterrorism Preparedness for information regarding precautions and contraindications to vaccination during a smallpox outbreak emergency) (Table 4). Before administering vaccinia vaccine, the physician should complete a thorough patient history to document the absence of vaccination contraindications among both vaccinees and their household contacts. Efforts should be made to identify vaccinees and their household contacts who have eczema, a history of eczema, or immunodeficiencies. Vaccinia vaccine should not be administered for routine nonemergency indications if these conditions are present among either recipients or their household contacts.

**TABLE 4. Vaccination contraindications and precautions for nonemergency and emergency use contraindications\***

Contraindications for nonemergency vaccine use	Contraindications during smallpox emergency
History or presence of eczema <sup>†</sup>	Exposure to smallpox virus — no contraindications
Other acute, chronic, or exfoliative skin conditions <sup>‡§</sup>	No virus exposure — same contraindications as nonemergency use
Immunosuppression <sup>¶</sup>	—
Pregnancy <sup>†</sup>	—
Aged <18 yrs	—
Vaccine component allergy	—

\* See text for explanation.

† Vaccination also not recommended for persons who live in household with others who have these conditions.

§ Vaccination may be administered after condition resolves.

¶ Conditions include human immunodeficiency virus, acquired immunodeficiency syndrome, leukemia, lymphoma, generalized malignancy, solid organ transplantation, cellular or humoral immunodeficiencies, or therapy with alkylating agents, antimetabolites, radiation, or high-dose corticosteroids.

### ***History or Presence of Eczema or Other Skin Conditions***

Because of the increased risk for eczema vaccinatum, vaccinia vaccine should not be administered to persons with eczema of any degree, those with a past history of eczema, those whose household contacts have active eczema, or whose household contacts have a history of eczema. Persons with other acute, chronic, or exfoliative skin conditions (e.g., atopic dermatitis, burns, impetigo, or varicella zoster) might also be at higher risk for eczema vaccinatum and should not be vaccinated until the condition resolves.

### ***Pregnancy***

Live-viral vaccines are contraindicated during pregnancy; therefore, vaccinia vaccine should not be administered to pregnant women for routine nonemergency indications. However, vaccinia vaccine is not known to cause congenital malformations (63). Although <50 cases of fetal vaccinia infection have been reported, vaccinia virus has been reported to cause fetal infection on rare occasions, almost always after primary vaccination of the mother (64). Cases have been reported as recently as 1978 (55,65). When fetal vaccinia does occur, it usually results in stillbirth or death of the infant soon after delivery.

### ***Altered Immunocompetence***

Replication of vaccinia virus can be enhanced among persons with immunodeficiency diseases and among those with immunosuppression (e.g., as occurs with leukemia, lymphoma, generalized malignancy, solid organ transplantation, cellular or humoral immunity disorders, or therapy with alkylating agents, antimetabolites, radiation, or high-dose corticosteroid therapy [i.e.,  $\geq 2$  mg/kg body weight or 20 mg/day of prednisone for  $\geq 2$  weeks] [66]). Persons with immunosuppression also include hematopoietic stem cell transplant recipients who are  $< 24$  months posttransplant, and hematopoietic stem cell transplant recipients who are  $\geq 24$  months posttransplant but who have graft-versus-host disease or disease relapse. Persons with such conditions or whose household contacts have such conditions should not be administered vaccinia vaccine.

### ***Persons Infected with HIV***

Risk for severe complications after vaccinia vaccination for persons infected with HIV is unknown. One case of severe generalized vaccinia has been reported involving an asymptomatic HIV-infected military recruit after the administration of multiple vaccines that included vaccinia vaccine (58). Additionally, a 1991 report indicated that two HIV-infected persons might have died of a progressive vaccinia-like illness after treatment with inactivated autologous lymphocytes infected with a recombinant HIV-vaccinia virus (67). No evidence exists that smallpox vaccination accelerates the progression of HIV-related disease. However, the degree of immunosuppression that would place an HIV-infected person at greater risk for adverse events is unknown. Because of this uncertainty, until additional information becomes available, not vaccinating persons (under routine nonemergency conditions) who have HIV infection is advisable.

### ***Infants and Children***

Before the eradication of smallpox, vaccinia vaccination was administered routinely during childhood. However, smallpox vaccination is no longer indicated for infants or children for routine nonemergency indications.

### ***Persons with Allergies to Vaccine Components***

The currently available vaccinia vaccine (i.e., Dryvax) contains trace amounts of polymyxin B sulfate, streptomycin sulfate, chlortetracycline hydrochloride, and neomycin sulfate. Persons who experience anaphylactic reactions (i.e., hives, swelling of the mouth and throat, difficulty breathing, hypotension, and shock) to any of these antibiotics should not be vaccinated. Vaccinia vaccine does not contain penicillin. Future supplies of vaccinia vaccine will be reformulated and might contain other preservatives or stabilizers. Refer to the manufacturer's package insert for additional information.

## **Treatment for Vaccinia Vaccine Complications**

### ***Using VIG***

The only product currently available for treatment of complications of vaccinia vaccination is VIG, which is an isotonic sterile solution of the immunoglobulin fraction of plasma from persons vaccinated with vaccinia vaccine. It is effective for treatment of eczema vaccinatum and certain cases of progressive vaccinia; it might be useful also in the

treatment of ocular vaccinia resulting from inadvertent implantation (68,69). However, VIG is contraindicated for the treatment of vaccinia keratitis (51,54). VIG is recommended for severe generalized vaccinia if the patient is extremely ill or has a serious underlying disease. VIG provides no benefit in the treatment of postvaccinia encephalitis and has no role in the treatment of smallpox. Current supplies of VIG are limited, and its use should be reserved for treatment of vaccine complications with serious clinical manifestations (e.g., eczema vaccinatum, progressive vaccinia, severe generalized vaccinia, and severe ocular viral implantation) (Table 2).

The recommended dosage of the currently available VIG for treatment of complications is 0.6 ml/kg of body weight. VIG must be administered intramuscularly and should be administered as early as possible after the onset of symptoms. Because therapeutic doses of VIG might be substantial (e.g., 42 ml for a person weighing 70 kg), the product should be administered in divided doses over a 24- to 36-hour period. Doses can be repeated, usually at intervals of 2–3 days, until recovery begins (e.g., no new lesions appear). Future reformulations of VIG might require intravenous administration, and health-care providers should refer to the manufacturer's package insert for correct dosages and route of administration. CDC is currently the only source of VIG for civilians (see Vaccinia Vaccine Availability for contact information).

### **Other Treatment Options for Vaccinia Vaccine Complications**

The Food and Drug Administration has not approved the use of any antiviral compound for the treatment of vaccinia virus infections or other Orthopoxvirus infections, including smallpox. Certain antiviral compounds have been reported to be active against vaccinia virus or other Orthopoxviruses in vitro and among test animals (70–75). However, the safety and effectiveness of these compounds for treating vaccinia vaccination complications or other Orthopoxvirus infections among humans is unknown. Questions also remain regarding the effective dose and the timing and length of administration of these antiviral compounds. Insufficient information exists on which to base recommendations for any antiviral compound to treat postvaccination complications or Orthopoxvirus infections, including smallpox. However, additional information could become available, and health-care providers should consult CDC to obtain up-dated information regarding treatment options for smallpox vaccination complications (see Consultation Regarding Complications of Vaccinia Vaccine).

### **Consultation Regarding Complications of Vaccinia Vaccine**

CDC can assist physicians in the diagnosis and management of patients with suspected complications of vaccinia vaccination. VIG is available when indicated. Physicians should telephone CDC at (404) 639-3670 during Mondays–Fridays, except holidays, or (404) 639-3311 during evenings, weekends, and holidays. Health-care workers are requested to report complications of vaccinia vaccination to the Vaccine Adverse Event Reporting System at (800) 822-7967, or to their state or local health department.

### **PREVENTING CONTACT TRANSMISSION OF VACCINIA VIRUS**

Vaccinia virus can be cultured from the site of primary vaccination beginning at the time of development of a papule (i.e., 2–5 days after vaccination) until the scab separates from the skin lesion (i.e., 14–21 days after vaccination). During that time, care must be

taken to prevent spread of the virus to another area of the body or to another person by inadvertent contact. Thorough hand-hygiene with soap and water or disinfecting agents should be performed after direct contact with the site or materials that have come into contact with the site to remove virus from the hands and prevent accidental inoculation to other areas of the body (76). In addition, care should be taken to prevent contact of the site or contaminated materials from the site by unvaccinated persons. The vaccination site can be left uncovered, or it can be loosely covered with a porous bandage (e.g., gauze) until the scab has separated on its own to provide additional barrier protection against inadvertent inoculation. An occlusive bandage should not be routinely used because maceration of the site might occur. Bandages used to cover the vaccination site should be changed frequently (i.e., every 1–2 days) to prevent maceration of the vaccination site secondary to fluid buildup. Hypoallergenic tape should be used for persons who experience tape hypersensitivity. The vaccination site should be kept dry, although normal bathing can continue. No salves or ointments should be placed on the vaccination site. Contaminated bandages and, if possible, the vaccination site scab, after it has fallen off, should be placed in sealed plastic bags before disposal in the trash to further decrease the potential for inadvertent transmission of the live virus contained in the materials. Clothing or other cloth materials that have had contact with the site can be decontaminated with routine laundering in hot water with bleach (2,4).

Recently vaccinated health-care workers should avoid contact with unvaccinated patients, particularly those with immunodeficiencies, until the scab has separated from the skin at the vaccination site. However, if continued contact with unvaccinated patients is unavoidable, health-care workers can continue to have contact with patients, including those with immunodeficiencies, as long as the vaccination site is well-covered and thorough hand-hygiene is maintained. In this setting, a more occlusive dressing might be required. Semipermeable polyurethane dressings (e.g., Opsite®) are effective barriers to vaccinia and recombinant vaccinia viruses (31). However, exudates can accumulate beneath the dressing, and care must be taken to prevent viral contamination when the dressing is removed. In addition, accumulation of fluid beneath the dressing can increase the maceration of the vaccination site. Accumulation of exudates can be decreased by first covering the vaccination site with dry gauze, then applying the dressing over the gauze. The dressing should also be changed at least once a day. To date, experience with this type of containment dressing has been limited to research protocols. The most critical measure in preventing inadvertent implantation and contact transmission from vaccinia vaccination is thorough hand-hygiene after changing the bandage or after any other contact with the vaccination site.

## VACCINATION METHOD

The skin over the insertion of the deltoid muscle or the posterior aspect of the arm over the triceps muscle are the preferred sites for smallpox vaccination. Alcohol or other chemical agents are not required for skin preparation for vaccination unless the area is grossly contaminated. If alcohol is used, the skin must be allowed to dry thoroughly to prevent inactivation of the vaccine by the alcohol. The multiple-puncture technique uses a presterilized bifurcated needle that is inserted vertically into the vaccine vial, causing a droplet of vaccine to adhere between the prongs of the needle. The droplet contains the recommended dosage of vaccine, and its presence within the prongs of the bifurcated needle should be confirmed visually. Holding the bifurcated needle perpendicular to the

skin, 15 punctures are rapidly made with strokes vigorous enough to allow a trace of blood to appear after 15–20 seconds (3). Any remaining vaccine should be wiped off with dry sterile gauze and the gauze disposed of in a biohazard waste container.

## EVIDENCE OF IMMUNITY AND VACCINATION-RESPONSE INTERPRETATION

Appearance of neutralizing antibodies after vaccination with live vaccinia virus indicates an active immune response that includes the development of antibodies to all viral antigens and increased vaccinia-specific cell-mediated immunity. In a person with normal immune function, neutralizing antibodies appear approximately 10 days after primary vaccination and 7 days after revaccination (3). Clinically, persons are considered fully protected after a successful response is demonstrated at the site of vaccination.

The vaccination site should be inspected 6–8 days after vaccination and the response interpreted at that time. Two types of responses have been defined by the World Health Organization (WHO) Expert Committee on Smallpox. The responses include a) major reaction, which indicates that virus replication has taken place and vaccination was successful; or b) equivocal reaction, which indicates a possible consequence of immunity adequate to suppress viral multiplication or allergic reactions to an inactive vaccine without production of immunity.

### Major Reaction

Major (i.e., primary) reaction is defined as a vesicular or pustular lesion or an area of definite palpable induration or congestion surrounding a central lesion that might be a crust or an ulcer. The usual progression of the vaccination site after primary vaccination is as follows:

- The inoculation site becomes reddened and pruritic 3–4 days after vaccination.
- A vesicle surrounded by a red areola then forms, which becomes umbilicated and then pustular by days 7–11 after vaccination.
- The pustule begins to dry; the redness subsides; and the lesion becomes crusted between the second and third week. By the end of approximately the third week, the scab falls off, leaving a permanent scar that at first is pink in color but eventually becomes flesh-colored (77).

Skin reactions after revaccination might be less pronounced with more rapid progression and healing than those after primary vaccinations. Revaccination is considered successful if a pustular lesion is present or an area of definite induration or congestion surrounding a central lesion (i.e., scab or ulcer) is visible upon examination 6–8 days after revaccination (3).

### Equivocal Reaction

Equivocal reaction, including accelerated, modified, vaccinoid, immediate, early, or immune reactions, are defined as all responses other than major reactions. If an equivocal reaction is observed, vaccination procedures should be checked and the vaccination repeated by using vaccine from another vial or vaccine lot, if available. Difficulty in determining if the reaction was blunted could be caused by immunity, insufficiently po-

tent vaccine, or vaccination technique failure. If the repeat vaccination by using vaccine from another vial or vaccine lot fails to elicit a major reaction, health-care providers should consult CDC or their state or local health department before attempting another vaccination.

## MISUSE OF VACCINIA VACCINE

Vaccinia vaccine should not be used therapeutically for any reason. No evidence exists that vaccinia vaccine has any value in treating or preventing recurrent herpes simplex infection, warts, or any disease other than those caused by human Orthopoxviruses (78). Misuse of vaccinia vaccine to treat herpes infections has been associated with severe complications, including death (54,79,80).

## VACCINIA VACCINE AVAILABILITY

CDC is the only source of vaccinia vaccine and VIG for civilians. CDC will provide vaccinia vaccine to protect laboratory and other health-care personnel whose occupations place them at risk for exposure to vaccinia and other closely related Orthopoxviruses, including vaccinia recombinants. Vaccine should be administered under the supervision of a physician selected by the institution. Vaccine will be shipped to the responsible physician. Requests for vaccine and VIG, including the reason for the request, should be referred to

Centers for Disease Control and Prevention  
Drug Services, National Center for Infectious Diseases  
Mailstop D-09  
Atlanta, GA 30333  
Telephone: (404) 639-3670  
Facsimile: (404) 639-3717

## SMALLPOX VACCINE FOR BIOTERRORISM PREPAREDNESS

Although use of biological agents is an increasing threat, use of conventional weapons (e.g., explosives) is still considered more likely in terrorism scenarios (81). Moreover, use of smallpox virus as a biological weapon might be less likely than other biological agents because of its restricted availability; however, its use would have substantial public health consequences. Therefore, in support of current public health bioterrorism preparedness efforts, ACIP has developed the following recommendations if this unlikely event occurs.

### Surveillance

A suspected case of smallpox is a public health emergency. Smallpox surveillance in the United States includes detecting a suspected case or cases, making a definitive diagnosis with rapid laboratory confirmation at CDC, and preventing further smallpox transmission. A suspected smallpox case should be reported immediately by telephone to state or local health officials and advice obtained regarding isolation and laboratory specimen collection. State or local health officials should notify CDC immediately at (404) 639-2184, (404) 639-0385, or (770) 488-7100 if a suspected case of smallpox is reported.

Because of the problems encountered previously in Europe with health-care–associated smallpox transmission from imported cases present in a hospital setting (82,83), health officials should be diligent regarding use of adequate isolation facilities and precautions (see Infection Control Measures). Currently, specific therapies with proven treatment effectiveness for clinical smallpox are unavailable. Medical care of more seriously ill smallpox patients would include supportive measures only. If the patient's condition allows, medical and public health authorities should consider isolation and observation outside a hospital setting to prevent health-care–associated smallpox transmission and overtaxing of medical resources. Clinical consultation and a preliminary laboratory diagnosis can be completed within 8–24 hours. Surveillance activities, including notification procedures and laboratory confirmation of cases, might change if smallpox is confirmed.

### **Prerelease Vaccination**

The risk for smallpox occurring as a result of a deliberate release by terrorists is considered low, and the population at risk for such an exposure cannot be determined. Therefore, preexposure vaccination is not recommended for any group other than laboratory or medical personnel working with nonhighly attenuated Orthopoxviruses (see Routine Nonemergency Vaccine Use).

Recommendations regarding preexposure vaccination should be on the basis of a calculable risk assessment that considers the risk for disease and the benefits and risks regarding vaccination. Because the current risk for exposure is considered low, benefits of vaccination do not outweigh the risk regarding vaccine complications. If the potential for an intentional release of smallpox virus increases later, preexposure vaccination might become indicated for selected groups (e.g., medical and public health personnel or laboratorians) who would have an identified higher risk for exposure because of work-related contact with smallpox patients or infectious materials.

### **Postrelease Vaccination**

If an intentional release of smallpox (variola) virus does occur, vaccinia vaccine will be recommended for certain groups. Groups for whom vaccination would be indicated include

- persons who were exposed to the initial release of the virus;
- persons who had face-to-face, household, or close-proximity contact (<6.5 feet or 2 meters) (84) with a confirmed or suspected smallpox patient at any time from the onset of the patient's fever until all scabs have separated;
- personnel involved in the direct medical or public health evaluation, care, or transportation of confirmed or suspected smallpox patients;
- laboratory personnel involved in the collection or processing of clinical specimens from confirmed or suspected smallpox patients; and
- other persons who have an increased likelihood of contact with infectious materials from a smallpox patient (e.g., personnel responsible for medical waste disposal, linen disposal or disinfection, and room disinfection in a facility where smallpox patients are present).

Using recently vaccinated personnel (i.e., <3 years) for patient care activities would be the best practice. However, because recommendations for routine smallpox vaccination in the United States were rescinded in 1971 and smallpox vaccination is currently recommended only for specific groups (see Routine Nonemergency Vaccine Use), having recently vaccinated personnel available in the early stages of a smallpox emergency would be unlikely. Smallpox vaccine can prevent or decrease the severity of clinical disease, even when administered 3–4 days after exposure to the smallpox virus (2,4,85). Preferably, healthy persons with no contraindications to vaccination, who can be vaccinated immediately before patient contact or very soon after patient contact (i.e., ≤3 days), should be selected for patient care activities or activities involving potentially infectious materials. Persons who have received a previous vaccination (i.e., childhood vaccination or vaccination >3 years before) against smallpox might demonstrate a more accelerated immune response after revaccination than those receiving a primary vaccination (3). If possible, these persons should be revaccinated and assigned to patient care activities in the early stages of a smallpox outbreak until additional personnel can be successfully vaccinated.

Personnel involved with direct smallpox patient care activities should observe strict contact and airborne precautions (47) (i.e., gowns, gloves, eye shields, and correctly fitted N-95 masks) for additional protection until postvaccination immunity has been demonstrated (i.e., 6–8 days after vaccination). Shoe covers should be used in addition to standard contact isolation protective clothing to prevent transportation of the virus outside the isolation area. After postvaccination immunity has occurred, contact precautions with shoe covers should still be observed to prevent the spread of infectious agents (see Infection Control Measures). If possible, the number of personnel selected for direct contact with confirmed or suspected smallpox patients or infectious materials should be limited to reduce the number of vaccinations and to prevent unnecessary vaccination complications.

Children who have had a definite risk regarding exposure to smallpox (i.e., face-to-face, household, or close-proximity contact with a smallpox patient) should be vaccinated regardless of age (20,52). Pregnant women who have had a definite exposure to smallpox virus (i.e., face-to-face, household, or close-proximity contact with a smallpox patient) and are, therefore, at high risk for contracting the disease, should also be vaccinated (52). Smallpox infection among pregnant women has been reported to result in a more severe infection than among nonpregnant women (3). Therefore, the risks to the mother and fetus from experiencing clinical smallpox substantially outweigh any potential risks regarding vaccination. In addition, vaccinia virus has not been documented to be teratogenic, and the incidence of fetal vaccinia is low (52,63,86,87). When the level of exposure risk is undetermined, the decision to vaccinate should be made after assessment by the clinician and patient of the potential risks versus the benefits of smallpox vaccination.

In a postrelease setting, vaccination might be initiated also for other groups whose unhindered function is deemed essential to the support of response activities (e.g., selected law enforcement, emergency response, or military personnel) and who are not otherwise engaged in patient care activities but who have a reasonable probability of contact with smallpox patients or infectious materials. If vaccination of these groups is initiated by public health authorities, only personnel with no contraindications to vaccination should be vaccinated before initiating activities that could lead to contact with suspected smallpox patients or infectious materials. Steps should be taken (e.g.,

reassignment of duties) to prevent contact of any unvaccinated personnel with infectious smallpox patients or materials.

Because of increased transmission rates that have been described in previous outbreaks of smallpox involving aerosol transmission in hospital settings (1,82,83), potential vaccination of nondirect hospital contacts should be evaluated by public health officials. Because hospitalized patients might have other contraindications to vaccination (e.g., immunosuppression), vaccination of these nondirect hospital contacts should occur after prudent evaluation of the hospital setting with determination of the exposure potential through the less-common aerosol transmission route.

## **Contraindications to Vaccination During a Smallpox Emergency**

No absolute contraindications exist regarding vaccination of a person with a high-risk exposure to smallpox. Persons at greatest risk for experiencing serious vaccination complications are also at greatest risk for death from smallpox (20,52). If a relative contraindication to vaccination exists, the risk for experiencing serious vaccination complications must be weighed against the risk for experiencing a potentially fatal smallpox infection. When the level of exposure risk is undetermined, the decision to vaccinate should be made after prudent assessment by the clinician and the patient of the potential risks versus the benefits of smallpox vaccination.

## **Infection Control Measures**

Isolation of confirmed or suspected smallpox patients will be necessary to limit the potential exposure of nonvaccinated and, therefore, nonimmune persons. Although drop-let spread is the major mode of person-to-person smallpox transmission, airborne transmission through fine-particle aerosol can occur. Therefore, airborne precautions using correct ventilation (e.g., negative air-pressure rooms with high-efficiency particulate air filtration) should be initiated for hospitalized confirmed or suspected smallpox patients, unless the entire facility has been restricted to smallpox patients and recently vaccinated persons (88,89). Although personnel who have been vaccinated recently and who have a demonstrated immune response should be fully protected against infection with variola virus (see Evidence of Immunity and Vaccination-Response Interpretation), they should continue to observe standard and contact precautions (i.e., using protective clothing and shoe covers) when in contact with smallpox patients or contaminated materials to prevent inadvertent spread of variola virus to susceptible persons and potential self-contact with other infectious agents. Personnel should remove and correctly dispose of all protective clothing before contact with nonvaccinated persons. Reuseable bedding and clothing can be autoclaved or laundered in hot water with bleach to inactivate the virus (2,4). Laundry handlers should be vaccinated before handling contaminated materials.

Nonhospital isolation of confirmed or suspected smallpox patients should be of a sufficient degree to prevent the spread of disease to nonimmune persons during the time the patient is considered potentially infectious (i.e., from the onset of symptoms until all scabs have separated). Private residences or other nonhospital facilities that are used to isolate confirmed or suspected smallpox patients should have nonshared ventilation, heating, and air-conditioning systems. Access to those facilities should be limited to recently vaccinated persons with a demonstrated immune response. If suspected small-

pox patients are placed in the same isolation facility, they should be vaccinated to guard against accidental exposure caused by misclassification as someone with smallpox.

In addition to isolation of infectious smallpox patients, careful surveillance of contacts during their potential incubation period is required. Transmission of smallpox virus rarely occurs before the appearance of the rash that develops 2–4 days after the prodromal fever (3). If a vaccinated or unvaccinated contact experiences a fever >101 F (38 C) during the 17-day period after his or her last exposure to a smallpox patient, the contact should be isolated immediately to prevent contact with nonvaccinated or nonimmune persons until smallpox can be ruled out by clinical or laboratory examination.

### **VIG for Prophylaxis and Treatment of Adverse Reactions During a Smallpox Emergency**

If vaccination of persons with contraindications is required because of exposure to smallpox virus after an intentional release as a bioterrorism agent, current stores of VIG are insufficient to allow its prophylactic use with vaccination. Because of the limited stores of VIG, its use in such a scenario should be reserved for severe, life-threatening complications (e.g., progressive vaccinia, eczema vaccinatum, or severe, toxic generalized vaccinia). If additional VIG becomes available in sufficient quantities to allow its prophylactic use, VIG should be administered intramuscularly as a dose of 0.3 mg/kg along with vaccinia vaccine to persons with contraindications who require vaccination.

## **RESEARCH PRIORITIES**

### **Development and Evaluation of New Vaccinia Vaccine**

Current supplies of vaccinia vaccine are limited to remaining stores of vaccine that were produced before the discontinuation of production by Wyeth Laboratories, Inc., in 1981. Although viral titer evaluations have indicated that the vaccine has remained potent, additional quantities of vaccine are needed to augment the current stores and replace expired vaccine. Previous methods of vaccine production that used calf lymph are no longer available; therefore, virus produced for use in a new vaccine must be grown by using a Food and Drug Administration-approved cell-culture substrate. Any new cell-culture vaccine should be evaluated for safety and efficacy by direct comparison with Dryvax by using appropriate animal models, serologic and cell-mediated immunity methods, and cutaneous indicators of successful vaccination (major reaction).

### **Treatment and Prevention Alternatives for Vaccine Adverse Reactions**

Regarding alternatives to VIG for potential treatment and prevention of vaccine adverse reactions, research priorities include a) evaluating antivirals for activity against vaccinia virus by using in vitro assays and test animals that demonstrate vaccinia virus pathogenicity, and b) developing and evaluating monoclonal antibodies against vaccinia virus. Antivirals or monoclonal antibodies that demonstrate activity against vaccinia virus in vitro and efficacy in protecting against dissemination of vaccinia virus among test animals without compromising vaccine effectiveness could provide medical personnel with alternatives to VIG.

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## Laboratory-Acquired Vaccinia Exposures and Infections — United States, 2005–2007

The last case of naturally acquired smallpox disease, caused by the orthopoxvirus variola virus (VARV), occurred in 1977, and the last laboratory-acquired case occurred in 1978. Smallpox was eradicated largely as the result of a worldwide vaccination campaign that used the related orthopoxvirus, vaccinia virus (VACV), as a live virus vaccine. Routine childhood vaccination for smallpox in the United States was terminated by 1972, but vaccination continues or has been reintroduced for specific groups, including laboratory workers who may be exposed to orthopoxviruses, members of the military, selected health-care workers, and first responders. Severe complications of VACV infection can occur, particularly in persons with underlying risk factors, and secondary transmission of VACV also can occur (1). VACV is used in numerous institutions

for various research purposes, including fundamental studies of orthopoxviruses and use as a vector for the expression of foreign proteins (often antigens or immunomodulators) in eukaryotic cells and animal models. The widespread use of VACV for research has resulted in laboratory-acquired VACV infections, some requiring hospitalization. The current Advisory Committee on Immunization Practices (ACIP) guidelines recommend VACV vaccination for laboratory workers who handle cultures or animals contaminated or infected with nonhighly attenuated VACV strains or other orthopoxviruses that infect humans (2). This report describes five recent occurrences of laboratory-acquired VACV infections and exposure and underscores the need for proper vaccination, laboratory safety, infection-control practices, and rapid medical evaluation of exposures in the context of orthopoxvirus research.

### Case Reports

During 2005–2007, five cases of laboratory-acquired VACV infection were reported to CDC from state health departments and health-care providers in the United States. No national surveillance system exists to track laboratory-related VACV exposures, and the five cases were reported to CDC informally in the course of seeking consultation on treatment and prevention. All five cases involved the Western Reserve (WR) vaccinia strain. Cases 1–4 involved recombinant VACVs with an insertion at the thymidine kinase (TK) locus. Case 5 also involved a recombinant VACV, but details of the virus are not known (Table).

**Case 1.** In March 2005, a laboratory worker at an academic institution in Connecticut experienced a needlestick

**TABLE. Characteristics of laboratory-acquired vaccinia virus (VACV) cases — United States, 2005–2007**

Case	State	VACV vaccination history	Met ACIP* recommendations?	Vaccinia strain	Type of vaccinia virus	Time from incident to initial medical care	Location of initial medical care
1	Connecticut	Twice, most recently 10 years prior	Questionable; due for revaccination	Western Reserve	Recombinant, insert in TK <sup>†</sup> locus	3 days	Occupational health clinic
2	Pennsylvania	No previous vaccination	No; declined vaccination	Western Reserve	Recombinant, insert in TK locus	6 days	Health-care provider
3	Iowa	No previous vaccination	No; declined vaccination	Western Reserve	Recombinant, insert in TK locus	11 days	Emergency department
4	Maryland	6 years prior; no take	No; failed take; no follow-up vaccination	Western Reserve	Recombinant, insert in TK locus	Same day	Occupational health clinic
5	New Hampshire	No previous vaccination	No; declined vaccination	Western Reserve	Recombinant, details not known	8 days	Emergency department

\* Advisory Committee on Immunization Practices. Vaccinia vaccine is recommended for laboratory workers who directly handle cultures or animals infected with nonhighly attenuated vaccinia viruses. Revaccination is recommended at least every 10 years. CDC. Vaccinia (smallpox) vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001. MMWR 2001;50(No. RR-10).

<sup>†</sup>Thymidine kinase.

to a finger while injecting mice with recombinant VACV. The laboratory worker was admitted to a hospital 3 days later with fever, lymphadenopathy, lymphangitis, and a hemorrhagic bulla at the site of the injury. The laboratory worker had been vaccinated with VACV as a child, and a second time approximately 10 years before the incident. Symptoms improved rapidly, and the laboratory worker was released after one night in the hospital. Infection with an orthopoxvirus was confirmed by testing in the state's Laboratory Response Network (LRN) laboratory.

**Case 2.** In October 2006, a laboratory worker at an academic institution in Pennsylvania experienced a needlestick injury on the thumb while injecting a mouse with a recombinant WR VACV strain. The laboratory worker had previously declined VACV vaccination. Six days after the incident, the laboratory worker sought medical care, with a primary lesion at the site of the inoculation and a secondary lesion near the thumbnail. Nine days after inoculation, the laboratory worker reported malaise, and on the following day, had a fever of 102.0°F (38.9°C) and lymphadenopathy. By day 13, the laboratory worker was feeling better; on day 14, a surgeon debrided the lesion near the thumbnail. VACV infection was confirmed by polymerase chain reaction and viral culture at CDC.

**Case 3.** In May 2007, a laboratory worker at an academic institution in Iowa who had no previous history of VACV vaccination was unsheathing a sterile needle and received a needlestick in a finger. The laboratory worker continued with the experiments, which involved two recombinant VACVs, and did not change gloves or wash hands until finished. The typical challenge dose for this set of experiments was  $3 \times 10^6$  plaque-forming units (pfu). Approximately 11 days after the needlestick, the laboratory worker developed symptoms of VACV infection, including fever and chills, and noted a lesion and swelling at the site of the needlestick. The laboratory worker sought medical attention at an urgent-care facility and informed the clinical staff of the incident. A diagnosis of VACV infection was confirmed by the state LRN laboratory. The laboratory worker recovered fully.

**Case 4.** In August 2007, a laboratory worker at a government facility in Maryland unintentionally inoculated a finger with approximately 5  $\mu$ L of a solution containing VACV, after injection of a research animal. The inoculum contained up to  $10^4$  pfu of the virus, which was a recombinant strain of WR VACV. The laboratory worker did not wash the exposed area immediately, but instead immersed the wound in a disinfectant containing hypochlorite for a few minutes.

The laboratory worker had received a primary VACV vaccination in 2001, but immunization was unsuccessful (i.e., no lesion developed at the site of the vaccination). On the day of the incident, the laboratory worker went to the occupational health clinic and was revaccinated with VACV. Vaccinia immunoglobulin was not administered. When the worker was reevaluated on days 3, 4, and 5 postvaccination, no evidence of VACV infection was observed at the site of inoculation, and a characteristic lesion developed at the site of vaccination, evidence of a take.

**Case 5.** In September 2007, a laboratory worker at an academic institution in New Hampshire who had no history of vaccination incurred a minor scratch to a finger with a small-gauge needle containing  $5 \times 10^4$  pfu/mL of recombinant WR VACV, which was being used for injecting mice. The laboratory worker felt pain, but did not bleed, and so continued working. Seven days later, the laboratory worker noted a pustule at the site of the scratch, sought medical attention the following day, and was hospitalized when red streaking appeared from the site of the scratch and extended into the axilla. Samples from the pustule were submitted to the state LRN laboratory, where VACV infection was confirmed. The laboratory worker was afebrile and recovered without specific therapy.

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**Editorial Note:** Although laboratory-related VACV exposures are rare, the cases described in this report demonstrate the need for laboratory workers to comply with ACIP vaccination recommendations (3,4). The total number of laboratories or researchers using nonhighly attenuated-VACV strains is unknown; therefore, estimating the incidence of VACV infection among at-risk laboratory workers is not possible. However, CDC does continue to receive reports of laboratory-related VACV exposures (fewer than five per year).

Laboratory-acquired exposure to VACV can lead to severe or atypical infections; exposures can be associated with a high inoculum or can occur through a route that has a high risk of complications, such as ocular VACV infection (5). Recombinant strains of VACV are commonly

generated by insertion of genetic material in the TK locus of the virus. Because inactivation of the TK locus has been associated with decreased VACV virulence in mice (6), some laboratory workers might perceive TK insertion mutants as attenuated; however, at least four of the infections and attendant illnesses described in this report involved VACV strains that had insertions at the TK locus. Additionally, recombinant strains of VACV commonly encode foreign gene products, and the possibility exists that resultant recombinant strains might have increased pathogenicity in humans.

ACIP currently recommends VACV vaccination at least every 10 years for laboratory workers who handle cultures or animals infected with nonhighly attenuated orthopoxviruses (2), including the WR strain of VACV. Reasons the five persons described in this report failed to meet ACIP recommendations included refusal of vaccination, absence of follow-through on a failed vaccination take, and overdue revaccination. Because some laboratory workers are hesitant to receive VACV vaccination for fear of side effects, laboratory directors and occupational health programs are encouraged to provide education regarding the risks and potential benefits of vaccination, including, for the latter, the prevention or reduction of severe complications from laboratory-acquired VACV infection. This benefit accrues from receiving a carefully measured (rather than undetermined) dose of a well-characterized vaccine formulation, which results in local infection at a predetermined site on the body, and resultant memory-immune response on subsequent exposure. Laboratory workers should adhere to the vaccination schedule recommended by ACIP (2). Persons who have a contraindication to VACV vaccination should consider carefully the possible consequences of a laboratory-acquired VACV infection in their decisions to work with nonhighly attenuated VACV.

Laboratory directors, research staff, and institutional biosafety officials can further minimize the likelihood of inadvertent VACV exposure by reinforcing proper laboratory safety procedures, such as proper use of personal protective equipment and safe needle-handling practices when handling VACV-infected cultures or animals.

When a potential exposure occurs, the laboratory worker should immediately and thoroughly wash the affected body part with water and the available cleaning product sanctioned by their biosafety office; eyewash protocols should be followed for ocular exposures. The laboratory worker should then report the incident and strain to which they might have been exposed to the laboratory director and

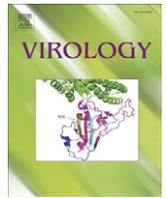
the occupational health clinic of the institution. VACV vaccination shortly after an exposure might help minimize the effects of inadvertent VACV infection. If severe illness or ocular infection occur, arrangements can be made with CDC for the administration of vaccinia immunoglobulin (2,3). The laboratory worker in case 4 immediately disinfected the wound and received prompt postexposure vaccination the day of the laboratory incident; this might have contributed to preventing infection at the site of the needlestick.

Secondary spread of VACV represents an additional public health concern. Patients with suspected VACV infection should be instructed by their caregivers in appropriate lesion care (2) as a precaution against spread of infection to another body site or to another person. Special care must be taken to avoid transmission to social contacts and persons in the health-care setting, particularly those with increased risk for severe illness from exposure to VACV, such as persons with atopic dermatitis, pregnant females, and immunocompromised persons.

Finally, occupational health clinics and health-care workers who might provide primary care for a laboratory worker exposed to VACV should become familiar with protocols for recognition and diagnosis of suspected poxvirus infections (3). Laboratory workers also should be instructed to seek care from appropriately trained health-care providers at their supporting institution. Appropriate infection-control measures should be instituted at the time of presentation of a patient with a suspected case, and whenever possible, clinical care should be provided by persons who have been vaccinated with VACV. Clinics also should review procedures for communication with and confirmation of orthopoxvirus infection through the LRN or the Poxvirus Program (404-639-4129) at CDC.

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## Minireview

Risks associated with vaccinia virus in the laboratory<sup>☆</sup>Adam MacNeil<sup>\*</sup>, Mary G. Reynolds, Inger K. Damon

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## ABSTRACT

Vaccinia virus (VACV) is used commonly in research laboratories. Non-highly attenuated strains of VACV are potentially pathogenic in humans, and VACV vaccination and biosafety level 2 facilities and protocols are currently recommended for vaccinated laboratory workers in the United States who handle non-highly attenuated strains of the virus. Despite this, laboratory-related VACV exposures continue to occur and a number of recent instances of VACV infection in non-vaccinated laboratory workers have been documented. We provide a discussion of the usage and risks associated with VACV in laboratory research.

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## Background

The practice of immunizing with orthopoxviruses dates back over 200 years, in which the concept of vaccination was introduced by Edward Jenner, to protect against variola virus infection, the causative agent of smallpox. While it is not entirely clear what orthopoxvirus was first used for vaccination practices, vaccinia virus (VACV) was eventually (if not initially) used as the viral agent for smallpox vaccination, and its successful usage was largely responsible for the global eradication of smallpox. Similarly, a natural reservoir for VACV has yet to be identified and the origins of the virus are not known (Baxby, 1977); explanations for the origin of VACV include (i) the possibility that this was a zoonotic virus that has since gone extinct in nature, (ii) that VACV was artificially derived from another orthopoxvirus through serial passage during vaccine production, and (iii) that VACV may be a recombinant between variola and the original species of orthopoxvirus that was used for vaccination (Binns and Smith, 1992; Shchelkunov et al., 2005). Genetically, VACV is closely related to horsepoxvirus, however, the presence of gene disruptions in the horsepox genome, that are intact in the VACV genome, does not support the origin of VACV from horsepox (Tulman et al., 2006).

Vaccination with VACV provides long-lasting protection against a variety of orthopoxviruses, including variola and monkeypox viruses (Fine et al., 1988; Jezek et al., 1987, 1988). Among human vaccines,

VACV is unique in that it is delivered as a live, non-attenuated virus, by puncture of the skin overlying the deltoid with a bifurcated needle. Because of this, adverse events following vaccination are known to occur, particularly among those with high risk conditions, such as individuals with eczema, atopic dermatitis, or other skin conditions, individuals who are immunocompromised or pregnant, and those less than 1 year of age (Casey et al., 2006; Cono et al., 2003; Wharton et al., 2003). Secondary transmissions to close contacts of vaccinees can also occur. Out of 37,901 volunteers vaccinated under the US Department of Health and Human Services preparedness program, 722 nonserious adverse events and 100 serious events, including 85 hospitalizations, were reported (Casey et al., 2005). Similarly, in a study of laboratory workers receiving VACV vaccine, a wide variety of post-vaccination symptoms were identified, although most common symptoms tended to be relatively mild (Baggs et al., 2005). In August 2007, the Food and Drug Administration (FDA) licensed the new-generation ACAM2000 vaccine, which is now used in place of the Dryvax vaccine, in the United States (CDC, 2008) and clinical trials suggest similar safety, as well as efficacy (Greenberg and Kennedy, 2008).

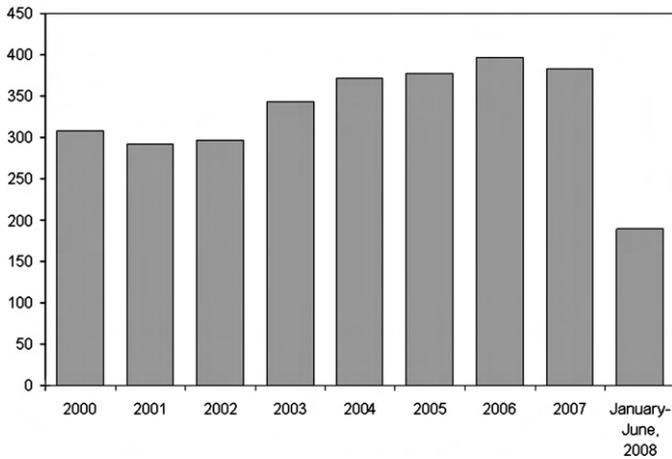
## VACV in laboratory research

VACV is commonly used in modern molecular biology research. Fig. 1 shows cumulative yearly numbers of publications (from National Library of Medicine's *Pubmed* database) with 'Vaccinia' in the title or abstract. While numbers of publications have increased slightly over the past decade, it is apparent that VACV usage has long been well-established in laboratory research. Consistent with this observation, VACV is used in the laboratory for a wide variety of purposes. As an example, we classified all abstracts, with 'Vaccinia' in the title or

<sup>☆</sup> The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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**Fig. 1.** Number of abstracts with 'Vaccinia' listed in the title or abstract, by year of publication (National Library of Medicine's *Pubmed* database).

abstract, with publication dates between January–June 2008, on the basis of the role of VACV in the research (Fig. 2). These include both studies involving VACV that are directly related to the understanding of orthopoxviruses, as well as usage of VACV as a functional tool for other areas of research.

In terms of research directly related to orthopoxviruses, the largest proportion of VACV-related studies fall in the area of basic virology (25% of abstracts). Other areas of research directly related to orthopoxviruses, that commonly involve VACV, include studies involving immune response to VACV infections, studies involving experimental orthopoxvirus vaccines, studies on anti-orthopoxvirus drugs or compounds, and studies related to public health issues surrounding orthopoxviruses.

As shown in Fig. 2, nearly half of all studies involving VACV are not related to orthopoxvirus research, but instead use VACV as a research tool. These types of studies can be broadly grouped into two areas: studies that use VACV as a protein expression or antigen delivery tool for basic immunology research (22% of abstracts) and studies that use VACV as a backbone for expression of foreign antigens for development of vaccines against unrelated agents (25% of abstracts).

VACV serves as an effective vector in the expression of foreign proteins for a variety of reasons. First, VACV infects a variety of cell types and results in high levels of protein expression. Additionally, viral replication occurs in the cytoplasm, and thus does not necessitate nuclear processing and transport of RNA. Finally, straight-forward, well-developed systems currently exist for the generation of recombinant VACV (Moss, 1996). Currently, most recombinant VACV strains are generated through insertional recombination in the viral thymidine kinase (TK) locus, resulting in a recombinant TK-minus viral strain (Chakrabarti et al., 1985; Mackett et al., 1984; Moss, 1996).

While the widespread use of TK-minus strains of VACV clearly demonstrate that this gene is nonessential for viral replication, studies do suggest that abrogation of TK function results in a modest attenuation in virulence. For instance, Buller et al. (1985) observed lower levels of infectivity and virulence of TK-minus strains of VACV in mice when delivered by intracerebral and intraperitoneal inoculations and similarly, Lee et al. (1992) observed a decrease in intracranial virulence in TK-minus strains of VACV. It should be noted, though, that both studies reported similar viral titers for wildtype and TK-minus strains on the skin, following intradermal inoculation (Buller et al., 1985; Lee et al., 1992). Additionally, human infections with TK minus strains of VACV following laboratory-related exposure have been well-documented (2008; Jones et al., 1986; Lewis et al., 2006; Mempel et al., 2003; Openshaw et al., 1991), indicating that recombinant TK-insertion strains of VACV are still pathogenic in humans.

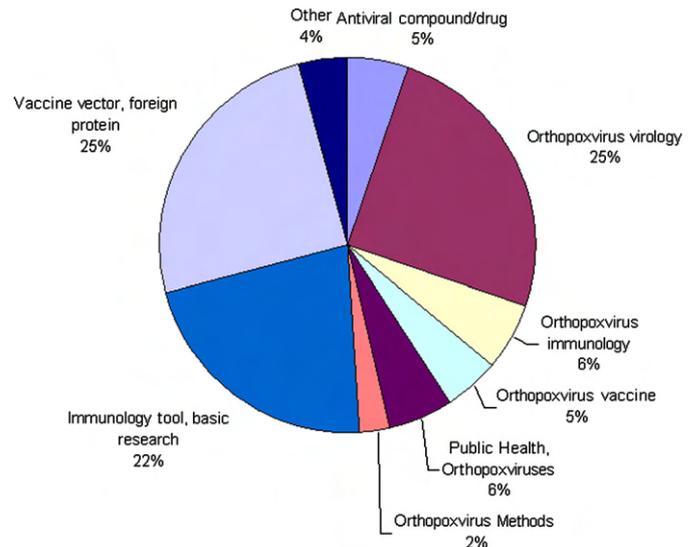
A VACV strain is considered non-highly attenuated if the virus maintains the capacity to replicate productively in mammalian cells. Numerous non-highly attenuated strains of VACV currently exist. Of central importance to current laboratory research is the Western Reserve (WR) strain of VACV which was selected by serial intracerebral passage in mice, for neurotropic potential. Other strains used in the laboratory commonly are related to the New York City Board of Health (NYCBH) strain, from which the Dryvax and ACAM2000 vaccine strains, as well as VACV WR, were derived (ATCC, 2008; Wokatsch, 1972). In contrast, attenuated strains of VACV do not have the capacity to replicate in mammalian cells. Of note is the modified vaccinia Ankara (MVA) strain, which is able to infect and result in protein expression, but not replicate, in mammalian cells (Sutter and Moss, 1992), and may serve as a possible alternative in the future to the currently licensed VACV vaccine (Phelps et al., 2007).

### Laboratory safety and VACV infections

Because non-highly attenuated strains of VACV are pathogenic in humans and handling virus in the laboratory presents a possible risk of infection, safety guidelines have been developed. As a vaccine, VACV has been shown to be highly efficacious in generating protective immune responses against both smallpox and monkeypox, and furthermore, studies indicate robust VACV-specific immune responses in humans following vaccination (Amanna et al., 2006). Therefore, pre-exposure vaccination with VACV is a primary intervention that can prevent or minimize the effects of accidental exposure in the laboratory. Consistent with this observation, the Advisory Committee on Immunization Practices (ACIP) recommends VACV vaccination for laboratory workers in the United States who handle cultures or animals contaminated or infected with non-highly attenuated VACV strains, at least every 10 years (Rotz et al., 2001).

In addition to vaccination, the usage of at least Biosafety Level 2 practices and facilities are currently recommended for the manipulation of viruses or animals infected with non-highly attenuated VACV strains (2007). This includes the usage of proper personal protective equipment, including gown, gloves, and eyewear protection when procedures have the risk of splash, proper laboratory facilities and safety equipment, proper decontamination of infectious material, and proper animal facilities (2007).

Despite the availability of vaccination and worker adherence to safety procedures, laboratory-acquired VACV infections do occur and



**Fig. 2.** Publications with 'Vaccinia' in the title or abstract, January–June 2008 (National Library of Medicine's *Pubmed* database). Review articles and articles not in English were excluded.

have been well documented in the literature. Inadvertent exposures have occurred through needlestick accidents or eye splash (2008; Jones et al., 1986; Lewis et al., 2006; Loeb et al., 2003; Mempel et al., 2003; Moussatche et al., 2003; Openshaw et al., 1991; Wlodaver et al., 2004). Laboratory-acquired VACV infections have commonly involved recombinant viruses, which express foreign proteins (2008; Jones et al., 1986; Lewis et al., 2006; Mempel et al., 2003; Openshaw et al., 1991) produced from non-highly attenuated strains, such as WR (2008; Jones et al., 1986; Lewis et al., 2006; Mempel et al., 2003; Moussatche et al., 2003; Openshaw et al., 1991).

There exists no formal surveillance system in place, within the United States, for instances of laboratory-related orthopoxvirus exposures or infections. However, the Poxvirus Team at the Centers for Disease Control and Prevention (CDC) has been contacted on a number of occasions in recent years regarding instances of laboratory-related orthopoxvirus exposures (Table 1). The majority of these instances have involved VACV (typically WR) harboring a foreign gene in the TK locus. Exposure to VACV most commonly occurred through accidental needlesticks or through eye splash accidents. In 6 recent instances, exposure resulted in VACV infection, and 4 of 6 infections resulted in subsequent hospitalization. Infections commonly involved fever and large focal areas of painful induration, erythema and severe swelling around the inoculation site. Hospitalizations occurred to assess the need for surgical or medical intervention. Five of the 6 cases involved infection of the hand or digits, and the remaining individual had ocular and facial involvement. None of the six infected laboratory workers had met the ACIP vaccination recommendation for working with non-highly attenuated VACV in the laboratory.

## Discussion

VACV is commonly used in laboratory research and the occurrence of laboratory-related VACV infections has been documented previously (2008; Jones et al., 1986; Lewis et al., 2006; Loeb et al., 2003; Mempel et al., 2003; Moussatche et al., 2003; Openshaw et al., 1991; Wlodaver et al., 2004). In recent years, we have received a number of reports of laboratory-related VACV exposures and infections. Additionally, laboratory-acquired VACV infections commonly have been the result of TK-minus strains of virus. Because studies in mice have suggested that inactivation of the VACV TK locus results in decreased virulence (Buller et al., 1985; Lee et al., 1992), it may be perceived there is not a risk of infection associated with handling a TK-minus VACV strain; however, it is apparent that TK-minus strains of VACV do maintain pathogenic potential in humans.

Because we currently do not have an estimate on the number of laboratories, or researchers who are potentially exposed to non-highly

attenuated VACV strains, or the actual number laboratory-associated VACV infections that occur, it is not possible to accurately assess the overall risk of VACV infection, associated with laboratory work. However, the observation that a large number of exposures, and all recent infections, involve individuals who did not follow the ACIP recommendations for vaccination warrants consideration for a number of reasons. It is apparent that many researchers are hesitant to be vaccinated, because it is perceived that the risk of adverse events associated with vaccination is higher than the risk of working with VACV. While the risks associated with vaccination are thoroughly described to those considering vaccination, the benefits of receiving vaccination are often overlooked. There are a number of reasons that researchers should consider vaccination:

1. Vaccination involves controlled delivery of the virus to the skin overlying the deltoid. This is a region of the body that can easily tolerate swelling without compromising function or causing significant pain. Accidental infection on other parts of the body (e.g., hand or digit) can result in severe pain and swelling, and possible long-term sequelae. Furthermore, many would consider the cosmetic effects of a vaccination scar on the deltoid to be less objectionable than scarring on another part of the body, such as the hand, eye, or face, which are locations commonly associated with accidental infection.
2. Vaccination involves inoculation with a controlled dosage of a well-characterized virus strain. By contrast, laboratory-related exposures can result in the delivery of a high titer of virus, as well as delivery through an atypical route, such as deep injection or ocular inoculation. Furthermore, laboratory studies commonly involve recombinant VACV strains, which have the potential to result in altered viral virulence or artificially modulated immune response to the virus.
3. Adverse events associated with vaccination are generally mild and severe adverse events are rare. For instance, 0.22% of vaccinations administered under the US Department of Health and Human Services preparedness program resulted in hospitalization (Casey et al., 2005). By contrast, 4 of the 6 (66%) recent laboratory-acquired VACV infections reported to CDC resulted in hospitalization.
4. Although the risk of exposure can be minimized by handling the virus under proper laboratory conditions and using proper techniques (for instance, the usage of eye protection can prevent ocular exposure), it is often not possible to completely eliminate the risk of accidental exposure to VACV. Personal protective equipment cannot provide complete protection from needlestick accidents, which account for a large proportion of VACV exposures.

**Table 1**  
Laboratory-related orthopoxvirus exposures reported to CDC, 2005–2008

Year	State	Virus (strain, if known)	Met ACIP vaccination?	Nature of accident	Result in infection?
2005	CA	Vaccinia	No	Eye splash	No
2005	FL	Vaccinia (rabbitpox)	Yes	Eye splash	No
2005 <sup>a</sup>	CT	Vaccinia (recombinant WR)	No	Needlestick	Yes (hospitalization)
2006 <sup>a</sup>	PA	Vaccinia (recombinant WR)	No	Needlestick	Yes
2006	CT	Vaccinia	Yes	Eye splash	No
2007 <sup>a</sup>	IA	Vaccinia (recombinant WR)	No	Needlestick	Yes
2007	NM	Vaccinia	Yes	Animal care facility	No
2007 <sup>a</sup>	MD	Vaccinia (recombinant WR)	No	Needlestick	No
2007 <sup>a</sup>	NH	Vaccinia (recombinant WR)	No	Needlestick	Yes (hospitalization)
2007	MA	Vaccinia (recombinant NYCBH)	No	Needlestick	Yes (hospitalization)
2007	MO	Monkeypox	Yes	Needlestick	No
2008	GA	Vaccinia	Yes	Animal care facility	No
2008	CA	Vaccinia (recombinant WR)	No	Eye splash	No
2008	NH	Vaccinia (recombinant WR)	No	Eye splash	No
2008	VA	Vaccinia (recombinant WR)	No	Unknown	Yes (hospitalization)
2008	FL	Vaccinia	Yes	Tube leakage	No

<sup>a</sup> Detailed account of case provided elsewhere (2008).

5. The possibility exists for inadvertent secondary transmission of VACV from an infected individual. Because of potential delays between laboratory-related exposure and recognition of symptoms, laboratory workers may put contacts, such as family members and healthcare workers, at risk for infection.

## Conclusion

The eradication of smallpox through the administration of VACV vaccine is one of the greatest public health achievements in history. VACV remains an important virus today for laboratory-based research, in the study of virology, immunology, and in the development of novel vaccines. However, VACV is potentially pathogenic in humans, and laboratory-acquired infections continue to occur. Although adverse events have been associated with VACV vaccination, post-vaccination symptoms tend to be relatively mild, and the ACIP currently recommends VACV vaccination for laboratory workers who handle non-highly attenuated VACV strains. While the highly attenuated MVA vaccine is not currently licensed in the United States, laboratory workers will hopefully benefit from this vaccine in the future. The usage of proper safety measures, including administration of VACV vaccine, can minimize the risk of conducting research which involves this virus.

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# Accidental Vaccinia Virus Exposure: Information Sheet for Laboratory Workers

If you have been exposed to vaccinia virus, or you have had a laboratory accident while working with vaccinia virus:

- **Irrigate the site of exposure\*.**

- » If exposure was by needle stick or other route which breaks the skin, wash with soap and water for 5-15 minutes and cover with a bandage.
- » If exposure was by splash to eyes or mucous membranes, irrigate thoroughly for 15 minutes at an appropriate eye wash station.

- **Report to your laboratory supervisor and occupational health IMMEDIATELY.**

- » The occurrence of vaccinia infection and its course will vary depending on route of exposure, the strain of vaccinia, the dose of exposure, your medical history and your "smallpox" (vaccinia virus) vaccine immunization status. Immediate medical "first-aid" interventions may help prevent or lessen the severity of infection.

- **In the weeks following infection take note of the following symptoms which may indicate a need for further medical attention:**

- » Lesions or swelling at the site of exposure
- » Rash
- » Fever

- **If you have already developed lesions that you suspect may be the result of a recent vaccinia Exposure:**

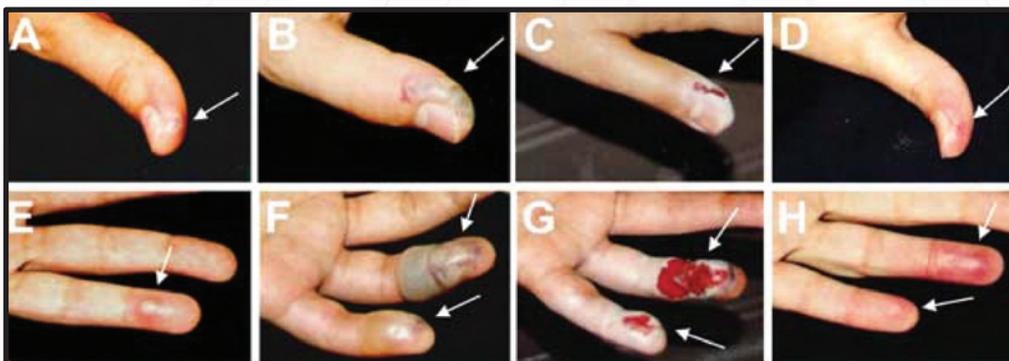
- » Cover the lesions with a bandage
- » Report to your laboratory supervisor and occupational health IMMEDIATELY (if it is after normal business hours, contact occupational health on-call)

- **Provide healthcare personnel with the following information:**

- » Whether or not you ever received a smallpox vaccination
- » Date of your last vaccination
- » The strain of vaccinia you were working with (ex. Western Reserve, Dryvax, MVA)
- » If the strain was recombinant, the identity of the inserted foreign gene
- » The dose/titer of vaccinia exposure (estimate based on concentration of virus suspension, and possible volume of inoculum)

- **The following medical conditions may increase the risk of serious complications. Notify your healthcare provider if you or any of your close contacts:**

- » Have a history of eczema/atopic dermatitis or other active skin conditions, such as acne or psoriasis
- » Have an immunodeficiency or immunosuppressive condition (taking steroids, etc.)
- » Have heart disease
- » Are currently pregnant (or could be pregnant) or nursing



**Progression of infection**  
"G" surgical debridement

# Accidental Vaccinia Virus Exposure: Information Sheet for Healthcare Personnel

If you are providing care to someone who has been exposed to vaccinia virus or who is suspected to have an active infection (ex. a laboratory worker or recent recipient of “smallpox” (vaccinia) vaccine), you should know the following:

- Standard barrier precautions should suffice to minimize infection within the health care setting.
- If lesions are active, and mucosal contact may be possible, wear goggles and/or other mucosal protection (mask/faceshield).
- Vaccinia is transmissible by direct CONTACT with lesions or contaminated materials. Risk of transmission immediately after an accidental lab exposure, before virus replication has occurred or lesions have developed, is very small. However, once lesions are present, risk of transmission is increased.
- In addition to barrier precautions, it is preferable that a patient with suspected vaccinia virus infection be cared for by someone who has a history of “smallpox” (vaccinia virus) vaccination.
- **Certain medical conditions may increase the risk of serious complications.** If vaccinated personnel are not available, it is preferable that the patient be cared for by a healthcare provider without any of the following conditions:
  - » History of eczema/atopic dermatitis or other active skin conditions, such as acne or psoriasis
  - » Immunodeficiency or immunosuppressive condition (taking steroids, etc.)
  - » Heart disease
  - » Currently pregnant (or could be pregnant) or nursing
  - » In the instance that a lesion is present, specimens can be submitted to your state or local
- Laboratory Response Network for VACCINIA testing. Contact your local health department for more information. (<http://www.cdc.gov/mmwr/international/relres.html>)
- Treatments that may be considered include vaccinia immune globulin (VIG) or post-exposure vaccination with smallpox vaccine. Contact you state health department or CDC with questions regarding treatment.

**CDC Poxvirus Inquiry line: (404) 639-4129**

**CDC Emergency Operations Center: (770) 488-7100**



\* Cohen J, Davenport D, Stewart J, Deitchman S, Hillard J, Chapman L, and the B virus working group. CID. 2002. 35: 1191-1203.

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# **Biosafety in Microbiological and Biomedical Laboratories**

**U.S. Department of Health and Human Services**

**Centers for Disease Control and Prevention**

**National Institutes of Health**

**4<sup>th</sup> Edition**

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**<http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm>**

***Agent: Poxviruses***

Sporadic cases of laboratory-associated infections with pox viruses (smallpox, vaccinia, yaba, tanapox) have been reported.<sup>61</sup> Epidemiological evidence suggests that transmission of monkeypox virus to humans from nonhuman primates or rodents

### Agent Summary Statements – Viral Agents

to humans may have occurred in nature, but not in the laboratory setting. Naturally or experimentally infected laboratory animals are a potential source of infection to exposed unvaccinated laboratory personnel. Genetically engineered recombinant vaccinia viruses pose an additional potential risk to laboratory personnel, through direct contact or contact with clinical materials from infected volunteers or animals.

*Laboratory Hazards:* The agents may be present in lesion fluids or crusts, respiratory secretions, or tissues of infected hosts. Ingestion, parenteral inoculation, and droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues, are the primary hazards to laboratory and animal care personnel. Some poxviruses are stable at ambient temperature when dried and may be transmitted by fomites.

*Recommended Precautions:* The possession and use of variola viruses is restricted to the World Health Organization Collaborating Center for Smallpox and Other Poxvirus Infections, located at the Centers for Disease Control and Prevention, Atlanta, Georgia. Biosafety Level 2 practices and facilities are recommended for all activities involving the use or manipulation of poxviruses, other than variola, that pose an infection hazard to humans. All persons working in or entering laboratory or animal care areas where activities with vaccinia, monkey pox, or cow pox viruses are being conducted should have documented evidence of satisfactory vaccination within the preceding ten years.<sup>62,63</sup> Activities with vaccinia, cow pox, or monkey pox viruses, in quantities or concentrations greater than those present in diagnostic cultures, may also be conducted at Biosafety Level 2 by immunized personnel, provided that all manipulations of viable materials are conducted in Class I or II biological safety cabinets. Immunosuppressed individuals are at greater risk of severe disease if infected with a poxvirus.<sup>64</sup>

*Transfer of Agent:* For a permit to import these agents, contact CDC. Contact the Department of Commerce for a permit to export these agents. Laboratory registration with CDC is required before sending or receiving these select agents.

## Laboratory Biosafety Level Criteria – Biosafety Level 2

**Biosafety Level 2** is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL-1 in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 2:

### A. *Standard Microbiological Practices*

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash

## Laboratory Biosafety Level Criteria – Biosafety Level 2

- of viable material with disinfectants that are effective against the agents of concern.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
  9. An insect and rodent control program is in effect (see Appendix G).

### B. *Special Practices*

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.
2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.
3. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the investigator's name and telephone number, any personal

### Laboratory Biosafety Level Criteria – Biosafety Level 2

protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.

4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).
5. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.
6. Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
7. The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
8. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
  - a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

## Laboratory Biosafety Level Criteria – Biosafety Level 2

- b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - c. Syringes which re-sheathe the needle, needleless systems, and other safety devices are used when appropriate.
  - d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.
9. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
10. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

### Laboratory Biosafety Level Criteria – Biosafety Level 2

11. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
12. Animals not involved in the work being performed are not permitted in the lab.

#### C. *Safety Equipment* (Primary Barriers)

1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
  - a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.
  - b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.
2. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.
3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory.

#### Laboratory Biosafety Level Criteria – Biosafety Level 2

This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.

4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching “clean” surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

#### D. *Laboratory Facilities* (Secondary Barriers)

1. Provide lockable doors for facilities that house restricted agents (as defined in 42 CFR 72.6).
2. Consider locating new laboratories away from public areas.
3. Each laboratory contains a sink for handwashing.
4. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.
5. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
6. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and

### **Laboratory Biosafety Level Criteria – Biosafety Level 3**

other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

7. Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.
8. An eyewash station is readily available.
9. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
10. There are no specific ventilation requirements. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

### **Biosafety Level 3 (BSL-3)**

**Biosafety Level 3** is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents.

All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropri-

### Laboratory Biosafety Level Criteria – Biosafety Level 3

ate personal protective clothing and equipment. The laboratory has special engineering and design features.

It is recognized, however, that some existing facilities may not have all the facility features recommended for Biosafety Level 3 (i.e., double-door access zone and sealed penetrations). In this circumstance, an acceptable level of safety for the conduct of routine procedures, (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, susceptibility testing, etc.), may be achieved in a Biosafety Level 2 facility, **providing** 1) the exhaust air from the laboratory room is discharged to the outdoors, 2) the ventilation to the laboratory is balanced to provide directional airflow into the room, 3) access to the laboratory is restricted when work is in progress, and 4) the recommended Standard Microbiological Practices, Special Practices, and Safety Equipment for Biosafety Level 3 are rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations should be made only by the laboratory director.

The following standard and special safety practices, equipment and facilities apply to agents assigned to Biosafety Level 3:

#### A. *Standard Microbiological Practices*

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after handling infectious materials, after removing gloves, and when they leave the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

### Laboratory Biosafety Level Criteria – Biosafety Level 3

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of aerosols.
7. Work surfaces are decontaminated at least once a day and after any spill of viable material.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Infectious waste from BSL-3 laboratories should be decontaminated before removal for off-site disposal.
9. An insect and rodent control program is in effect (see Appendix G).

#### B. *Special Practices*

1. Laboratory doors are kept closed when experiments are in progress.
2. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. Persons who are at increased risk of acquiring infection or for whom infection may have serious consequences are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. No minors should be allowed in the laboratory.

### Laboratory Biosafety Level Criteria – Biosafety Level 3

3. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures, enter the laboratory or animal rooms.
4. When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures.
5. Laboratory personnel receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing), and periodic testing as recommended for the agent being handled.
6. Baseline serum samples are collected as appropriate and stored for all laboratory and other at-risk personnel. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory.
7. A biosafety manual specific to the laboratory is prepared or adopted by the laboratory director and biosafety precautions are incorporated into standard operating procedures. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
8. Laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent expo-

### Laboratory Biosafety Level Criteria – Biosafety Level 3

asures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural changes.

9. The laboratory director is responsible for ensuring that, before working with organisms at Biosafety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques, and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in safe microbiological practices and techniques.
10. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
  - a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.
  - b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

### Laboratory Biosafety Level Criteria – Biosafety Level 3

- c. Syringes which re-sheathe the needle, needleless systems, and other safe devices are used when appropriate.
  - d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated before disposal, and disposed of according to any local, state, or federal regulations.
- 11. All open manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench. Clean-up is facilitated by using plastic-backed paper toweling on non-perforated work surfaces within biological safety cabinets.
- 12. Laboratory equipment and work surfaces should be decontaminated routinely with an effective disinfectant, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials.
  - a. Spills of infectious materials are decontaminated, contained and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material. Spill procedures are developed and posted.
  - b. Contaminated equipment must be decontaminated before removal from the facility for repair or maintenance or packaging for transport, in accordance with applicable local, state, or federal regulations.
- 13. Cultures, tissues, specimens of body fluids, or wastes are placed in a container that prevents leakage during

### Laboratory Biosafety Level Criteria – Biosafety Level 3

collection, handling, processing, storage, transport, or shipping.

14. All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) from laboratories are decontaminated before disposal or reuse.
15. Spills and accidents that result in overt or potential exposures to infectious materials are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.
16. Animals and plants not related to the work being conducted are not permitted in the laboratory.

#### C. *Safety Equipment* (Primary Barriers)

1. Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when overtly contaminated.
2. Gloves must be worn when handling infectious materials, infected animals, and when handling contaminated equipment.
3. Frequent changing of gloves accompanied by hand washing is recommended. Disposable gloves are not reused.
4. All manipulations of infectious materials, necropsy of infected animals, harvesting of tissues or fluids from infected animals or embryonate eggs, etc., are conducted in a Class II or Class III biological safety cabinet (see Appendix A).
5. When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate

### Laboratory Biosafety Level Criteria – Biosafety Level 3

combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors) are used.

6. Respiratory and face protection are used when in rooms containing infected animals.

#### D. *Laboratory Facilities (Secondary Barriers)*

1. The laboratory is separated from areas that are open to unrestricted traffic flow within the building, and access to the laboratory is restricted. Passage through a series of two self-closing doors is the basic requirement for entry into the laboratory from access corridors. Doors are lockable (see Appendix F). A clothes change room may be included in the passageway.
2. Each laboratory room contains a sink for handwashing. The sink is hands-free or automatically operated and is located near the room exit door.
3. The interior surfaces of walls, floors, and ceilings of areas where BSL-3 agents are handled are constructed for easy cleaning and decontamination. Seams, if present, must be sealed. Walls, ceilings, and floors should be smooth, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be monolithic and slip-resistant. Consideration should be given to the use of coved floor coverings. Penetrations in floors, walls, and ceiling surfaces are sealed. Openings such as around ducts and the spaces between doors and frames are capable of being sealed to facilitate decontamination.
4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and those chemicals used to decontaminate the work surfaces and equipment.

### Laboratory Biosafety Level Criteria – Biosafety Level 3

5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
6. All windows in the laboratory are closed and sealed.
7. A method for decontaminating all laboratory wastes is available in the facility and utilized, preferably within the laboratory (i.e., autoclave, chemical disinfection, incineration, or other approved decontamination method). Consideration should be given to means of decontaminating equipment. If waste is transported out of the laboratory, it should be properly sealed and not transported in public corridors.
8. Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily-traveled laboratory areas.
9. A ducted exhaust air ventilation system is provided. This system creates directional airflow which draws air into the laboratory from "clean" areas and toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building. Filtration and other treatments of the exhaust air are not required, but may be considered based on site requirements, and specific agent manipulations and use conditions. The outside exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper. It is recommended that a visual monitoring device that indicates and confirms directional inward airflow be provided at the laboratory entry. Consideration should be given to installing an HVAC control system to prevent sustained positive pressurization of the laboratory. Audible alarms

### Laboratory Biosafety Level Criteria – Biosafety Level 3

should be considered to notify personnel of HVAC system failure.

10. HEPA-filtered exhaust air from a Class II biological safety cabinet can be recirculated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system (e.g., an air gap between the cabinet exhaust and the exhaust duct). When Class III biological safety cabinets are used they should be directly connected to the exhaust system. If the Class III cabinets are connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinets (see Appendix A).
11. Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory. These HEPA systems are tested at least annually. Alternatively, the exhaust from such equipment may be vented to the outside if it is dispersed away from occupied areas and air intakes.
12. Vacuum lines are protected with liquid disinfectant traps and HEPA filters, or their equivalent. Filters must be replaced as needed. An alternative is to use portable vacuum pumps (also properly protected with traps and filters).
13. An eyewash station is readily available inside the laboratory.
14. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
15. The Biosafety Level 3 facility design and operational procedures must be documented. The facility must be

**Laboratory Biosafety Level Criteria – Biosafety Level 4**

tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified, at least annually, against these procedures as modified by operational experience.

16. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment, the site conditions, or other applicable federal, state, or local regulations.

**CDC Poxvirus Inquiry line: (404) 639-4129**

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