

Sheet: 18

Refer to slide no. 1 (Dr. Khatib) *Not necessary*

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## Pox Virus

This virus has been eradicated, no new cases since almost 2000. This virus is one of the oldest types of viruses known to human.

### Smallpox

- Smallpox is a serious, contagious and sometimes fatal disease.
- There is no specific treatment for smallpox, and the only prevention is vaccination.
- The name smallpox is derived from the latin word “spotted” and refers to the raised bumps that appear on the face and body of an infected person.
- Smallpox is one of the Orthopoxvirus genus of viruses.
  - Very serious disease.
  - Very infective
  - Transmission is high, very contagious
  - It can be fatal; up to 30% of cases end in death
  - No specific treatment, only prevention by vaccination which is why it is eradicated
  - Spots are a bit elevated

### First Case of Smallpox

- There is no animal reservoir, and no human carriers.
- First certain evidence comes from the mummified remains of Ramses. (1157 B.C.)
- Written descriptions did not appear until the 10th century in Southwestern Asia.
  - In ancient Egypt, the first case was reported. Ramses had contracted the disease and is noticed on his mummified body.
  - Maybe it started in Egypt or India we don't know for sure

### Variolation

- Ground scabs, pus, vesicles used to vaccinate
  - China, powdered scabs blown into nostrils
    - Pills from fleas of cows
  - India, application of scab or pus to scarified skin
  - Turks wear used red dress used by patients

- Children exposed to mild smallpox
  - Inoculated James Phipps with fluid from milkmaid's pustule
- Development of vaccine using cowpox, Protective for smallpox
- Cows used in early 19<sup>th</sup> century for vaccine production
- It was the first "vaccination" invented.
- Variola: Transmission of the disease from a person to another.
- When we talk about Variola, the disease will end up in the production of crusts on the infection areas (rashes)
- They get the crust, crush it then throw it in the air to be inhaled and act as a vaccination "prevent the person from contacting the disease"
- Different variolation procedure depending on the country. For example, the turkish used the clothes of patient and give it to normal people to transmit the crusts and to get some sort of immunity.
  
- Variolation came to Europe early 18<sup>th</sup> century
- 1715, Lady Mary Wortley Montague
- 1745, London Smallpox Inoculation Hospital founded
- 1777, George Washington had all soldiers variolated
- 1796, England, May Edward Jenner (1749-1823)
- 1796, England, May
- Inoculated James Phipps with fluid from milkmaid's pustule
- Subsequent variolation of boy produced no reaction
- Development of vaccine using cowpox
- Protective for smallpox

Edward Jenner had found that there's a similar disease in cows, so the milk maid who milked the cows didn't get the disease. So he formulated an idea that there must be something she took from the cows to stop her from having the disease. He used cows' pox to "vaccinate" children to stop the disease. And from here, the word "Vaccine" came, the virus's name was vaccinia.

In London, a fine lady Mary got the disease and she was disfigured. She was able to viriolate her 2 sons and was able to make an inoculation hospital for the infected patients. Also, in Washington, George Washington had used variolation to vaccinate his troops during the American war. This disease was very very contagious it killed almost 500 million people during the war.

### Smallpox History

- Last naturally-occurring case in 1977 (*23 year old Al Maow Maalin of the Somali*)

- High case fatality rate (30%)
- Caused at least 500 million deaths in the 20<sup>th</sup> century
- Routine vaccination ceased in 1972
- May 8, 1980, official declaration by WHO - Smallpox Eradicated!
- Except some accidents that have happened to researchers that were working with the virus, and gotten infected by mistake.

### The Organism

- Double stranded DNA
- Large virus Diameter 400 nm
- Orthopoxvirus
  - Variola, cowpox, vaccinia, monkeypox,
- Variola major or minor
- Stable out side host
  - Retains infectivity
- Last case, 1977
- Eradicated, 1980
- Largest virus that can be seen even under light microscope
- Variola in humans
- Variola major or minor causes major or minor disease respectively
- Resistant to environmental conditions so they remain in the crust for long period

### MORPHOLOGY OF THE VIRION

- Have an brick-like shape; dimensions 400x200nm can
- be seen by light microscope
- Four major elements:
  - ❖ 1. core ( 9 nm thick membrane, biconcave disk, a tightly compressed nucleoprotein)
  - ❖ 2. lateral bodies ( unknown function)
  - ❖ 3. outer membrane ( a protein shell 12nm thick, the surface consists of irregularly arranged tubules)
  - ❖ 4. envelope ( an inconstant element, proteins are glycosylated and acylated)
- Circular ds-DNA (*the doctor said circular, but wrote linear.*)
- Lateral body is seen inside it
- Envelope is composed from host body cell membrane during budding
- Replicate in cytoplasm of the cell, contains a m-RNA otherwise it will replicate in nucleus

## CHARACTERISTICS SHARED BY SPECIES OF ORTHOPOXVIRUS

- The largest and most complex viruses
- They contain a linear genome of a single
  - double-stranded DNA
- They replicate in the cytoplasm of the host cell,
  - therefore they must provide their own mRNA and
  - DNA synthetic machinery
  - (including DNA-dependent RNA polymerase)
- Inclusion bodies: type B and type A Virions have a brick-like shape and are present in 2 forms, both are infectious:
- EEV (Extracellular Enveloped Virus)
- IMV (Intracellular Mature Virus)
- Serological cross-reactivity
- Produce a hemagglutinin antigen (HA)
- Vaccinia is the most intensively studied member of the poxvirus family

It has a DNA-polymerase to produce the nucleic acid of the virus. In addition it has certain antigenic structures like EEV which is extracted envelope virus antigen, and intracellular mature virus, an antigen, too. Antigens are used to diagnose the virus; we can look for antibodies against these antigens, or use the virus membrane structure to look for virus.

## TAXONOMY

- FAMILY: POXVIRIDAE
- SUBFAMILY: CHORDOPOXVIRINAE (infect vertebrates)
  - GENERA: ORTHOPOXVIRUS (variola, vaccinia, cowpox, monkeypox)
- AVIPOXVIRUS (fowlpox)
- CAPRIPOXVIRUS (sheep-pox)
- LEPORIPPOXVIRUS (myxoma)
- SULPOXVIRUS (swinepox)
- 2. SUBFAMILY: ENTOMOPOXVIRINAE (infect arthropods)

It can infect pigs, too. This is a very widely distributed virus.

## Variola Virus

- Occurs in 2 strains
  - variola major
    - 90% of cases are clinically characteristic
    - 30% case fatality rate
  - variola minor
    - Less severe
    - 1% case fatality rate

At the time the doctor lived in a village, when the virus invades it will kill 10-15 people.

### Smallpox Transmission

- Person-to-person (Inhalation of droplets)
- Direct contact (With infected body fluids)
- Scabs
- Contaminated objects (Bedding, clothing, bandages)
- Aerosol (Rarely)

No reservoir, not even human since the infected person either dies or survives who has permanent immunity, so he will not get the disease a second time. Transmission by direct contact with infected body fluids.

### Smallpox Transmission

- Spread more easily in cool, dry winter months
  - Can be transmitted in any climate
- No transmission by insects or animals
- Transmission from a smallpox case
  - Prodrome phase, less common
    - Fever, no rash yet
  - Most contagious with rash onset
    - First 7-10 days
- Contagious until last scab falls off

Prodrom phase: Incubation period where there is no clinical manifestation yet. This phase is the most contagious. After the last crust falls, the person is no more contagious.

## Disease in Humans

### Progression of Smallpox

- 1) Incubation Period
- 2) Prodrome Stage
- 3) Macules
- 4) Papules
- 5) Vesicles
- 6) Pustules
- 7) Scabs
- 8) Scars

## Smallpox Pathogenesis

- Incubation: 12-14 days (range 7-17d)
- Infection occurs after implantation of virus on the oropharyngeal or respiratory mucosa
- Day 3-4: viral multiplication in regional lymph nodes; asymptomatic viremia
- Viral spread to spleen, bone marrow, lymph nodes
- Day 8: secondary viremia followed by fever and toxemia

In 1<sup>st</sup> viremia no fever or increase temperature, the 2<sup>nd</sup> viremia there is.

## Smallpox Prodrome

- a) Incubation period 12 days (range 7-17 d)
- b) Prodrome
  - (1) abrupt onset of fever >101°F
  - (2) malaise, headache, muscle pain, nausea, vomiting, backache
  - (3) lasts 1- 4 days

After incubation period there's an abrupt onset of fever (39 C-40 C)

## Smallpox Rash

- Enanthem (mucous membrane lesions) appears ~ 24 hours before skin rash
  - Minute red spots on the tongue and oral/pharyngeal mucosa
  - Lesions enlarge and ulcerate quickly
  - Become infectious from lesions in mouth
  - Virus titers in saliva highest and most infectious during first week of exanthem (skin rash)

Now the rashes can be seen all over the body. All these rashes are full of viruses. It can be seen by direct examination or by taking a smear.

- Exantham (skin rash) – (21 days)
  - Stages: macules, papules, vesicles, pustules, scabs
  - Pustules raised, round, firm - like small beads in the skin (“shotty”)
  - Umbilication common
- Begins and most dense on face and extremities (centrifugal distribution)
- Lesions on palms and soles (≥50% of cases)
- Lesions in same stage and evolve slowly (1-2 days/stage)
- Around 30% will die



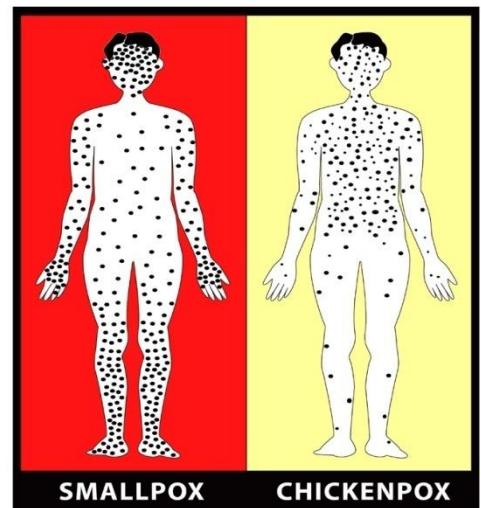
- At the beginning, there are large vesicles.
- Later on by day 5-6 you will see them all over the body.
- You can see raised rashes with *pin?* in the middle
- At 13 days, rashes will start scraping off

### Rash Distribution

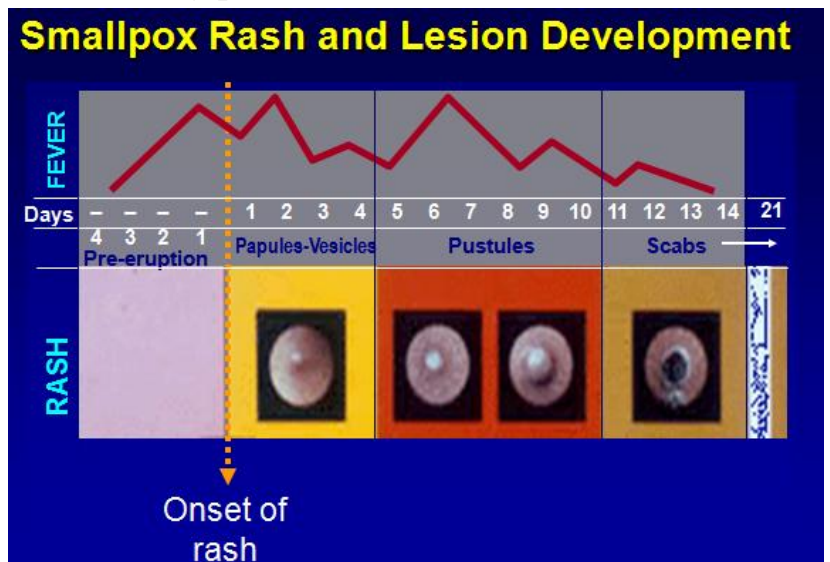
The relative density of rash on different parts of the body should be carefully observed. This diagram illustrates the differences that are usually seen.

Rash distribution: All over the body, more densely in the face and extremities.

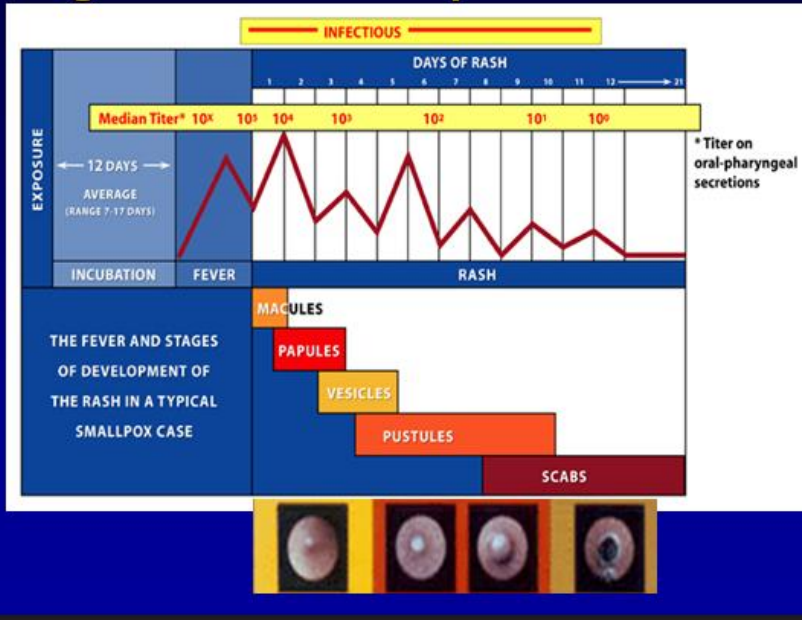
Smallpox rashes can be mixed with other viruses' rashes like chickenpox. Chickenpox rashes will be mainly on the abdomen and few on the face and extremities. But the main difference between them is that the palms and soles in smallpox are invaded by rashes while rarely invaded in chickenpox. Chickenpox is extremely painful since it will affect the root of nerves.



Predorm phase: no rash



# Progression of Smallpox



When the scabs are scrapped off, the number of viruses decreases, till it becomes zero.

## Smallpox Differential Diagnosis

- Varicella (chickenpox)
- Vaccinia
- Monkeypox
- Cowpox
- Herpes zoster
- Drug-induced rashes
- Sulfonamide reaction
- Morbilliform rash
- Coxsackie virus
- Secondary syphilis
- Molluscum lesions

All these can be confused with smallpox.

## Differentiating Smallpox from Chickenpox

	SMALLPOX	CHICKENPOX
FEVER ONSET	2 to 4 days before rash	At rash onset
RASH		
Evolution	Lesions at same stage	Lesions appear in crops
	Lesions evolve at same rate	Lesions in different stages
Distribution	Rash centrifugal	Rash centripetal
	Rash on palms and soles	Never on palms or soles
Development	Slow	Rapid
	Pox don't burst when probed	Lesions burst when probed
MORTALITY	30%	Rare

Chickenpox is most commonly confused with smallpox.

At smallpox the lesions are all the same size and the crust will come in certain time and then the scar but in chickenpox you can find all stages of the lesions at the same time



## Chicken Pox



Rash is rare on back and extremities.

### Smallpox Laboratory Procedures

- Specimens should be collected by recently vaccinated personnel
- Vesicular or pustular fluid, scabs, scraping of skin lesions, blood samples, tonsillar swabs
- Diagnosis confirmed by PCR and electron microscopy

Clinically it is very obvious. Specimen should be collected by recently vaccinated person; otherwise technician will contract the disease. This is a must.

### Sample requirements for Poxvirus DNA identification

- ✓ Lesion 'roofs' and crusts
- ✓ Vesicular fluids (touch prep)
- ✓ Biopsy, autopsy
- ✓ Others (e.g. CSF?) (*CSF in case of neurological diseases*)

### Confirmation of Orthopoxviruses, Laboratory Methods

- PCR-related methods for DNA Identification:
  - ✓ Real-time PCR
  - ✓ Single-gene PCR/RFLP
  - ✓ Pan-genomic methods, if indicated
- Electron Microscopy (we use negative stain )
- Histopathology
- Culture
- Serology?

Variety of older and more modern laboratory methods that can be used to confirm an orthopoxvirus diagnosis. List of methods currently used at CDC to confirm and to identify an orthopoxvirus infection, whether it is vaccinia, monkeypox, or variola (smallpox).

Serologic methods may help to evaluate the extent of an immune response, but will not be helpful to determine if replicative vaccinia is the cause of a vaccine associated adverse event.

Negative staining can also be used (we stain the background while the virus will not be stained)

TB stain: Z-Neelsen stain or Acid fast stain

Negative staining is used in C. neoformans, india-ink stain , specimen: we use CSF

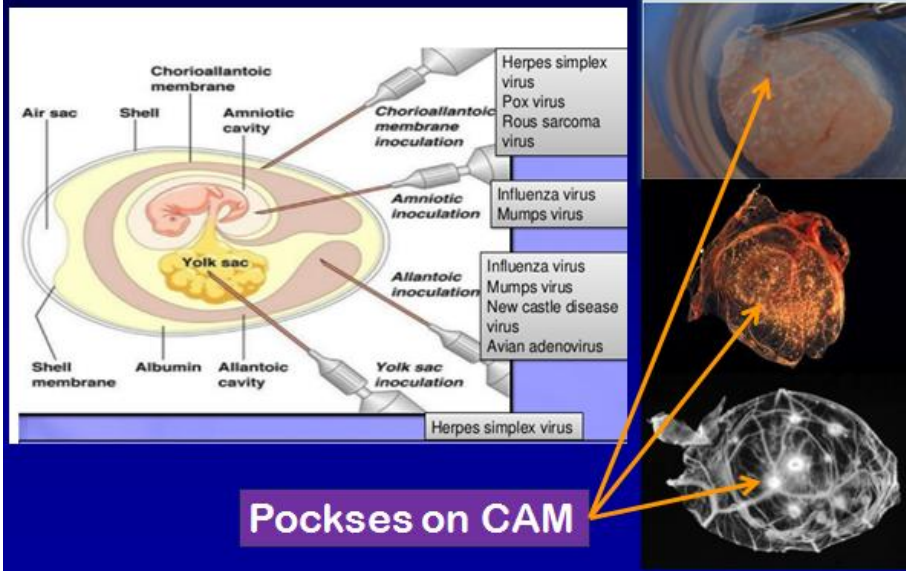
Diagnosis of organisms generally:

- ✓ Direct examination
  1. unstained
  2. stained, depending on organism
  3. specific staining, direct immunofluorescence
- ✓ Culture (not direct)
- ✓ Serological reactions (not direct)
- ✓ Animal inoculation

Rule Out Other Rash Illnesses Laboratory Testing	
Disease	Test
Varicella Zoster	•DFA •PCR •EM •Immunohistochemistry
Herpes Simplex	•PCR •EM •Immunohistochemistry •Culture
Streptococcus, Staphylococcus	•Gram stain •Rapid Tests •Culture

Disease	Test
Enterovirus	•PCR •Immunohistochemistry •Culture
Scabies	•Evidence of Organisms
Drug Eruptions, Allergies	•Skin Biopsy •Pathology
Others	•Biopsy

## Growth of Viruses in Embryonated Eggs



We have to rule out the diseases that can be mixed with the diseases.

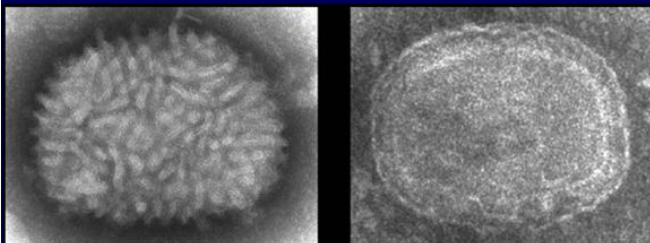
We can culture, we use an embryonated egg. After fertilization, egg is incubated for 1 week and during this period an embryo will form.

Depending on the specimen to be culture we place it in the right area.

For vaccinia virus, we place it on the membrane right below the shell. After 2-3 days we break the shell, and isolate the membrane and examine

Under microscope, we will notice that smallpox virus are beginning to be seen on the membrane

## Negative Stain Electron Microscopy



vaccinia

~1/2 hour per sample (for experienced microscopist)

### Smallpox Infection Control

- ✓ Strict adherence to airborne and contact precautions
- ✓ Isolate suspected case in negative air pressure room
- ✓ Healthcare providers should be immunized and use standard, airborne and contact precautions
- ✓ Virus destroyed with standard disinfectants and heat

Wear mask and cover all your body to prevent any contact with the infected person. When we disinfect surfaces using detergent, we will destroy the envelope of the virus and it will no longer attach to infect. The main thing here is the vaccination.

## History of Smallpox Vaccination

1805	Growth of virus on the flank of a calf in Italy.
1864	Publicity about vaccine production at a medical congress.
After WWI	Most of Europe smallpox free.
After WWII	Transmission interrupted in Europe and North America.
1940's	Stable freeze-dried vaccine perfected by Collier.

\*Henderson DA, Moss M, Smallpox and Vaccinia in Vaccines, 3<sup>rd</sup> edition, 1999

### Vaccination

- The smallpox vaccine is actual live *vaccinia* virus, unlike other vaccines which use dead virus; for this reason the vaccination site must be cared for to prevent spread
- Smallpox vaccine is administered using a bifurcated needle, not an injection, unlike any other vaccine
- The bifurcated needle is dipped into the vaccine and then used to prick the skin 15 times in about 3 seconds in a 5mm radius area
- It is administered into the **superficial** layer of the skin

Vaccine reaction looks like the disease itself. After vaccine, we will see redness at site of injection, then rash (7<sup>th</sup> day), after 14 days crust will form, and at 21 days crusts will fall. It is exactly like the disease. But the vaccine itself may form complications like: lymph adenopathy, fever, encephalitis, progressive vaccination disease, eczema, and sometime death.

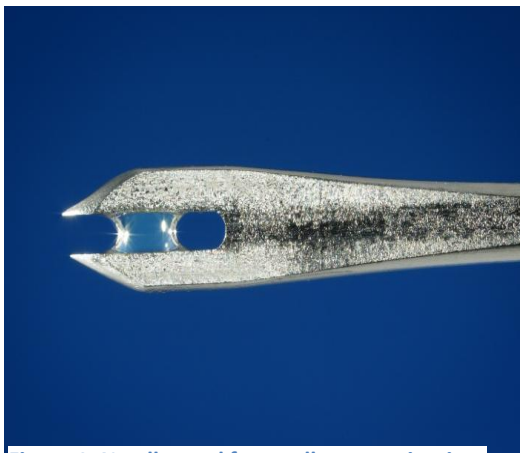


Figure 1: Needle used for small pox vaccination

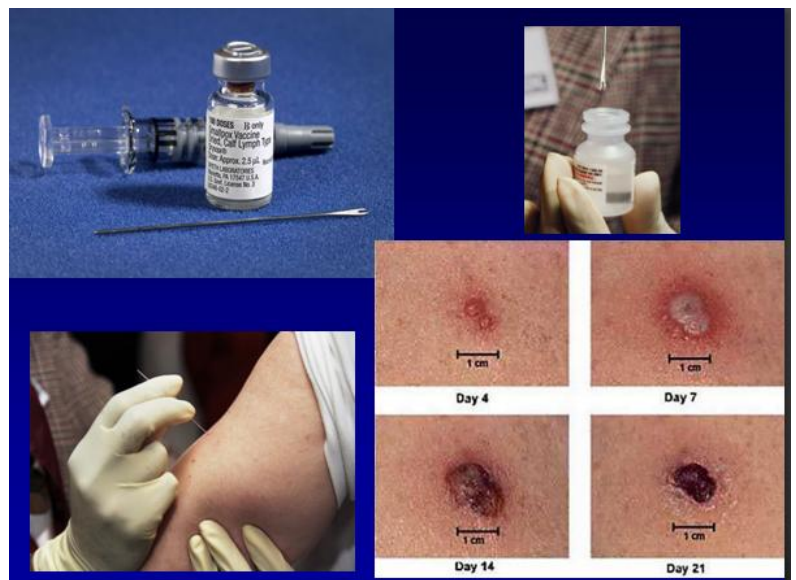


Figure 2: Smallpox vaccination

## Smallpox Vaccination Adverse Events

- 1/10,000 persons have serious side effects including:
  - ◆ lymphadenopathy
  - ◆ fever
  - ◆ encephalitis (1/300,000)
  - ◆ progressive vaccinia (1/2,000)
  - ◆ eczema vaccinatum (1/26,000)
  - ◆ death (1-2/1,000,000)

## Vaccine Contraindications (Pre-exposure), For Vaccinees and Potential Contacts

1. Immunodeficiency
  - a. e.g., HIV infection, AIDS, many cancers, lupus
2. Immunosuppressive therapy
  - a. Cancer, transplants, steroid therapy\*, topical steroids for skin dz, inhaled steroids\*\*
3. Eczema/Atopic Dermatitis
  - a. Hx or presence of eczema, including “healed” eczema, atopic dermatitis
4. Skin Disorders\*\*\*
  - a. Disruptive or eruptive, e.g., acne, burns, impetigo, zoster, wounds, contact dermatitis, current surgical incision wounds
5. Pregnancy
  - a. Current or planning within 4 weeks of vaccination
  - b. Current nursing
6. Child age 1 yr or less in household
7. Eye disease of the conjunctiva or cornea (Vaccinee only)
  - a. Pruritic lesions, florid inflammation
8. Allergies to Dryvax vaccine components (Vaccinee only)
  - a. Polymyxin B sulfate
  - b. Streptomycin sulfate
  - c. Chlortetracycline hydrochloride
  - d. Neomycin sulfate
  - e. Tetracycline

## Contraindications for Vaccination

- Immunodeficiency
- Immunosuppressing therapies
- Atopic Dermatitis/Eczema; including past history
- Acute or chronic skin conditions (until resolved)
- Allergies to polymyxin B, streptomycin, tetracycline, or neomycin
- Pregnancy

## Major Complications of Smallpox Vaccination

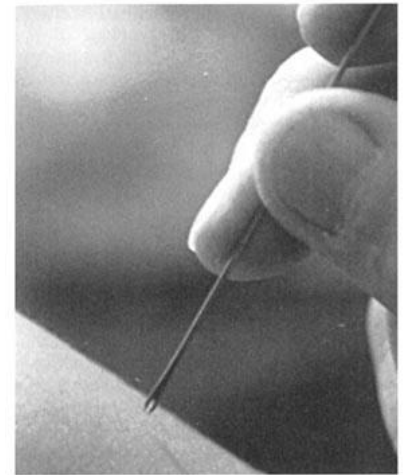
- Inadvertent autoinoculation (IA)
- Eczema vaccinatum (EV)
- Generalized vaccinia (GV)
- Progressive vaccinia (PV)
  - (vaccinia necrosum)
- Postvaccinial encephalitis (PE)

## Postvaccinial Encephalitis

- Signs and symptoms of classical encephalitis, encephalopathy, demyelination, or neuropathy
- Onset 9-14 days after primary vaccination
- Highest risk among children <12 months of age
- VIG not proven useful

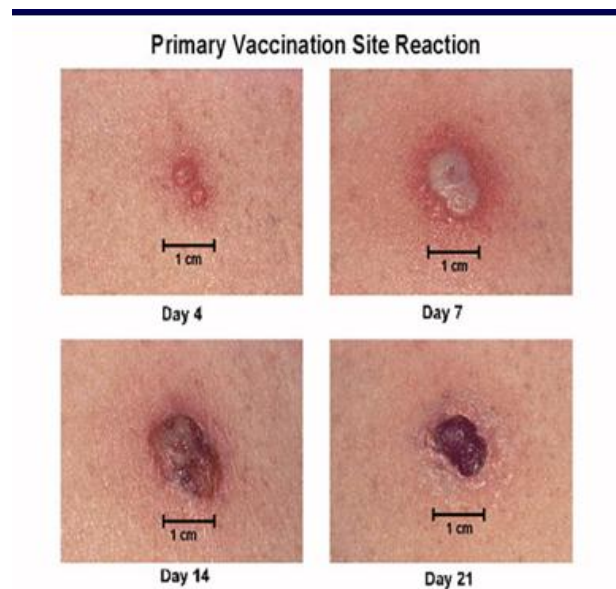
## Smallpox Vaccine Administration

- Administered via scarification with bifurcated needle
- Needle held at right angles to skin
- 15 rapid strokes in upper arm\*
- Trace amount of blood at site indicates successful vaccine delivery



## Preventing Contact Transmission

- Until a scab has formed:
  - Vaccination site must be covered
  - No touching, scratching, or rubbing vaccination site
  - Avoid person-to-person contact with susceptible persons
  - Avoid touching, rubbing or otherwise performing any maneuvers that might transfer vaccinia virus to the eye or surrounding skin
  - Carefully discard vaccination site covering
  - After handling the vaccination site covering, thoroughly wash hands with soap and running water





## Vaccinia Immune Globulin

- Vaccinia immune globulin (VIG) is used to treat persons with adverse reactions to smallpox vaccine
- Sufficient stock of VIG must be on hand before smallpox vaccinations can be administered
  - 5,000 doses available at end of 2002
- Additional VIG is being supplied from the plasma of recently inoculated persons

If person had a strong reaction to the vaccine we can give them immune Globulin

## Current Smallpox Vaccine Supply

- U.S. government has 15.4 million doses of Dryvax vaccine
- Additional 85 million doses (Aventis Pasteur) held for emergency use
- Clinical studies underway to determine safety and efficacy of other potential vaccines; Acambis vaccine in production

Smallpox vaccine is restricted to a few places. It is stored only in the United States and Russia.

## Eradication Success

- Vaccine available
- No animal reservoir
- Vaccinees easily identifiable
- Vaccinees could “vaccinate” close contacts
- Diseased easily identifiable

## Smallpox Stores

- CDC in Atlanta, Georgia, U.S.
- Vector Laboratories in Koltsovo, Russia
- Unknown others?

It is stored in other unknown places because the vaccine can be used in microbial war, and they have used it before. The U.S.A. used to get the blankets of the smallpox infected patients and gave it to the Red Indians and eradicated them.

## Smallpox as Biological Warfare

- Lord Jeffrey Amherst, Commanding General of British Forces in North America during the French and Indian War. (1754-1763)
- Used blankets (smallpox blankets) coated with smallpox dust as germ warfare to wipe out the Native American population.