# A MICROSCOPIA ELETRÓNICA NO DIAGNÓSTICO RÁPIDO DE AGENTES INFECIOSOS EM SITUAÇÕES DE EMERGÊNCIA.

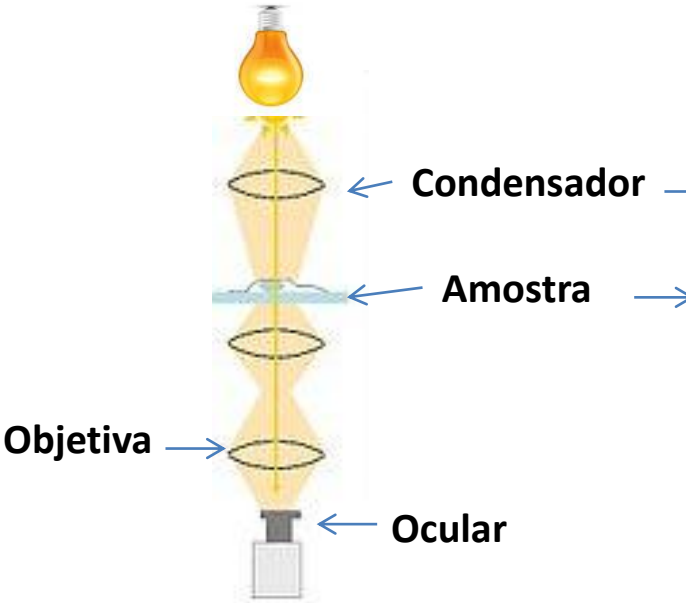
**Luísa Jordão, INSA**

## Sumário

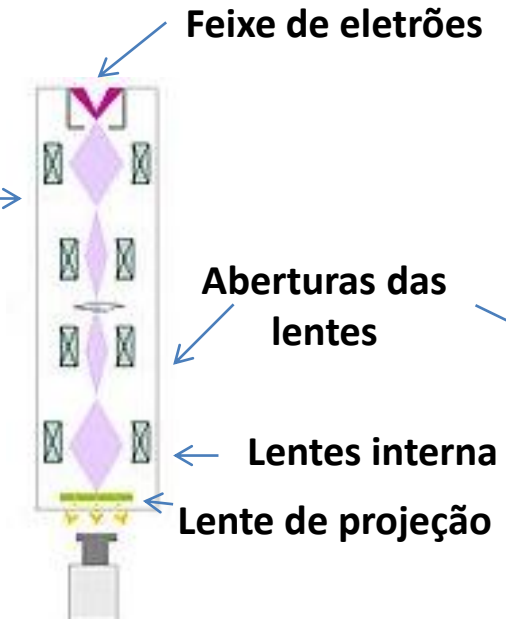
- **Introdução**
- **Metodologias**
- **Vantagens e Desvantagens associadas à microscopia eletrónica**
- **Aplicações da microscopia eletrónica no diagnóstico**

# Breve Fundamento Teórico

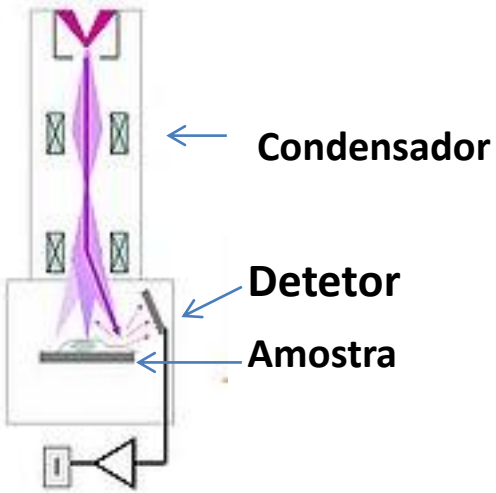
## Microscópio óptico




## Microscópio eletrónico de Transmissão (TEM)

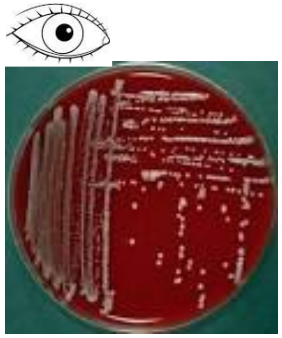



## Microscópio eletrónico de Varrimento (SEM)

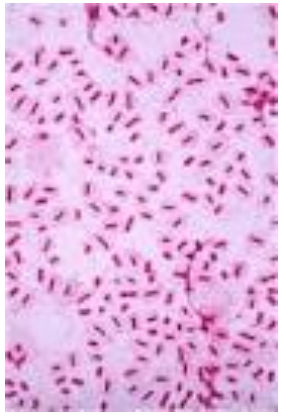


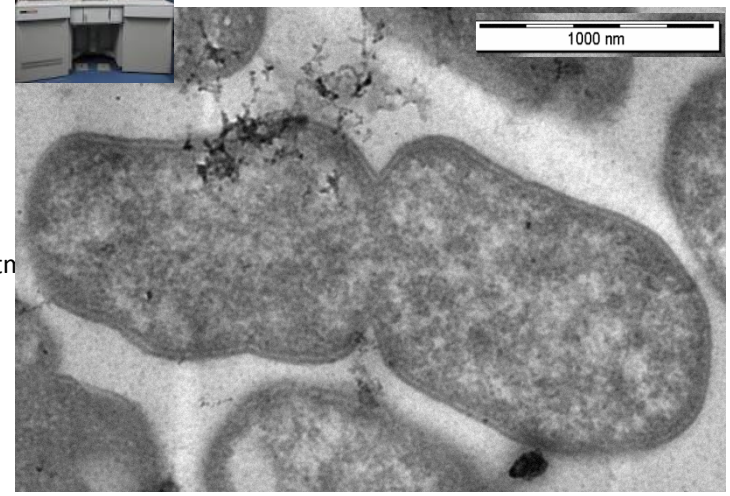

| | | | |
|---|---|---|---|
| Ampliação | ~ 2000 x | ~1 500 000 x | ~ 1 000 000 x |
| Resolução | 200 nm | 0,1 nm | 0,5 nm |

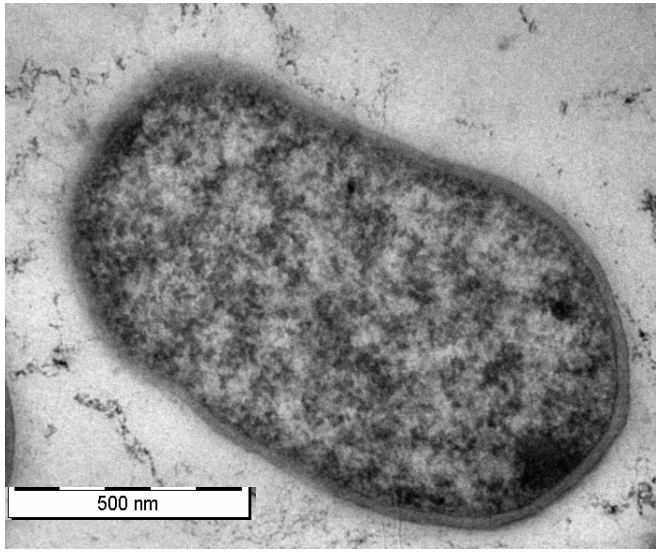
# Método / Imagem

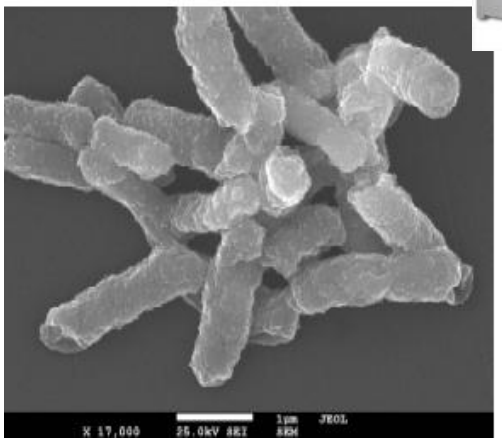


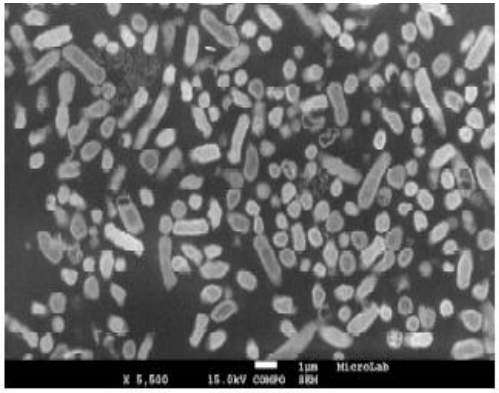
http://klebsiella-pneumoniae.org/treatment.htn



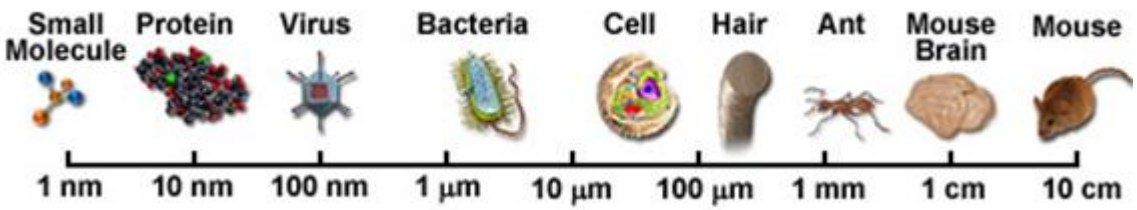


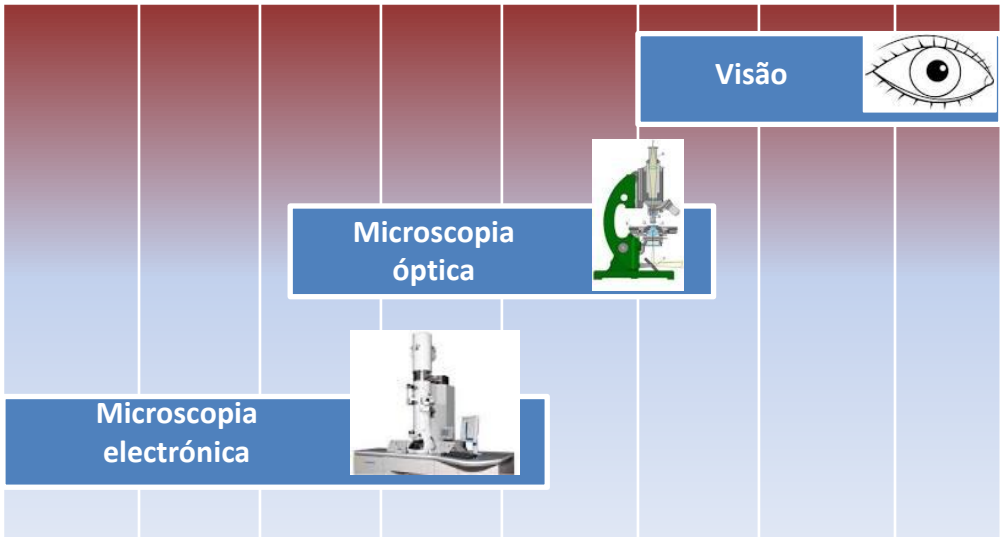



# Resolução de várias técnicas de visualização de amostras biológicas




Adaptado de: http://zeiss-campus.magnet.fsu.edu/print/superresolution/introduction-print.html

# Resolução de várias técnicas de visualização de amostras biológicas

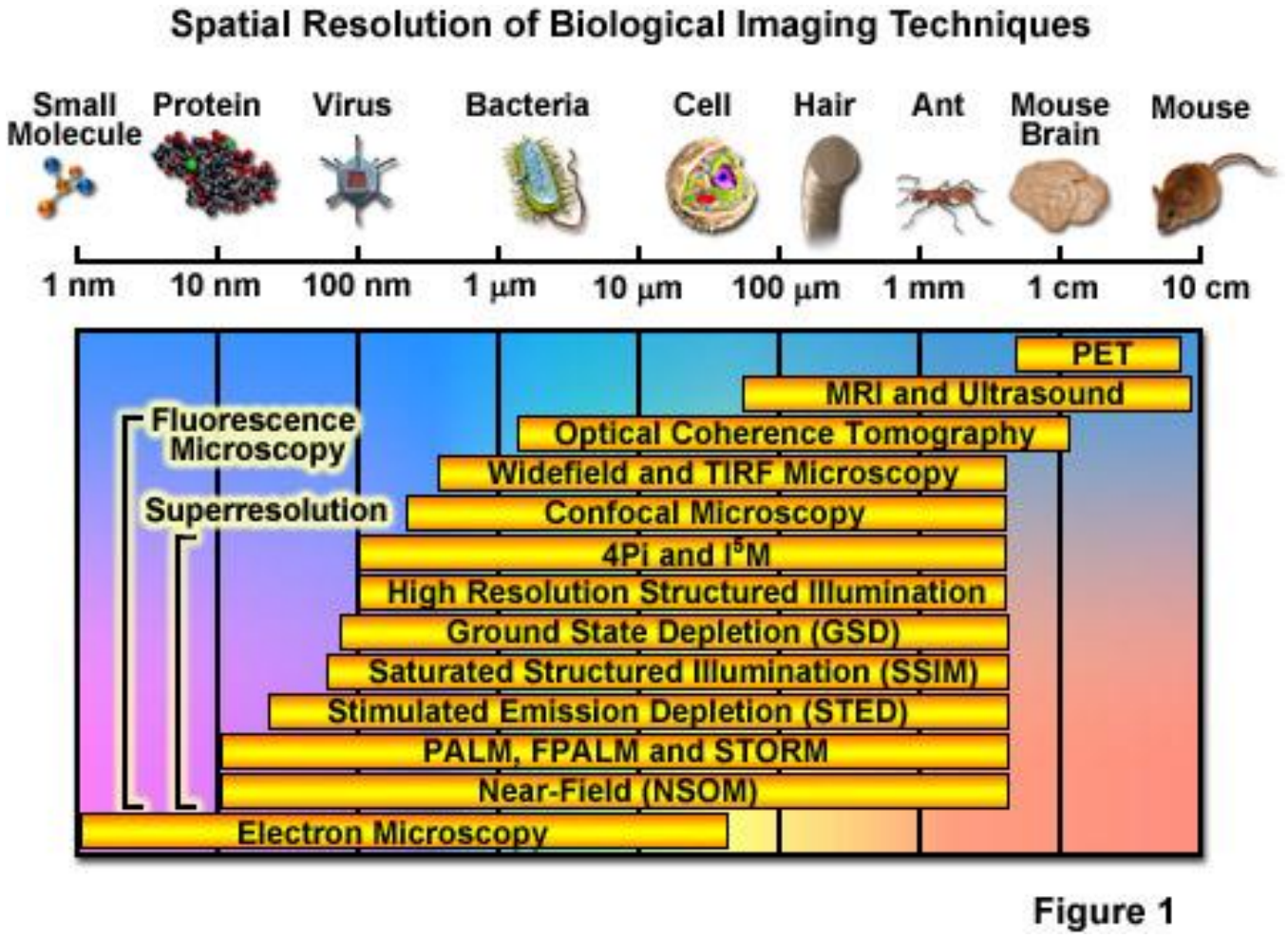
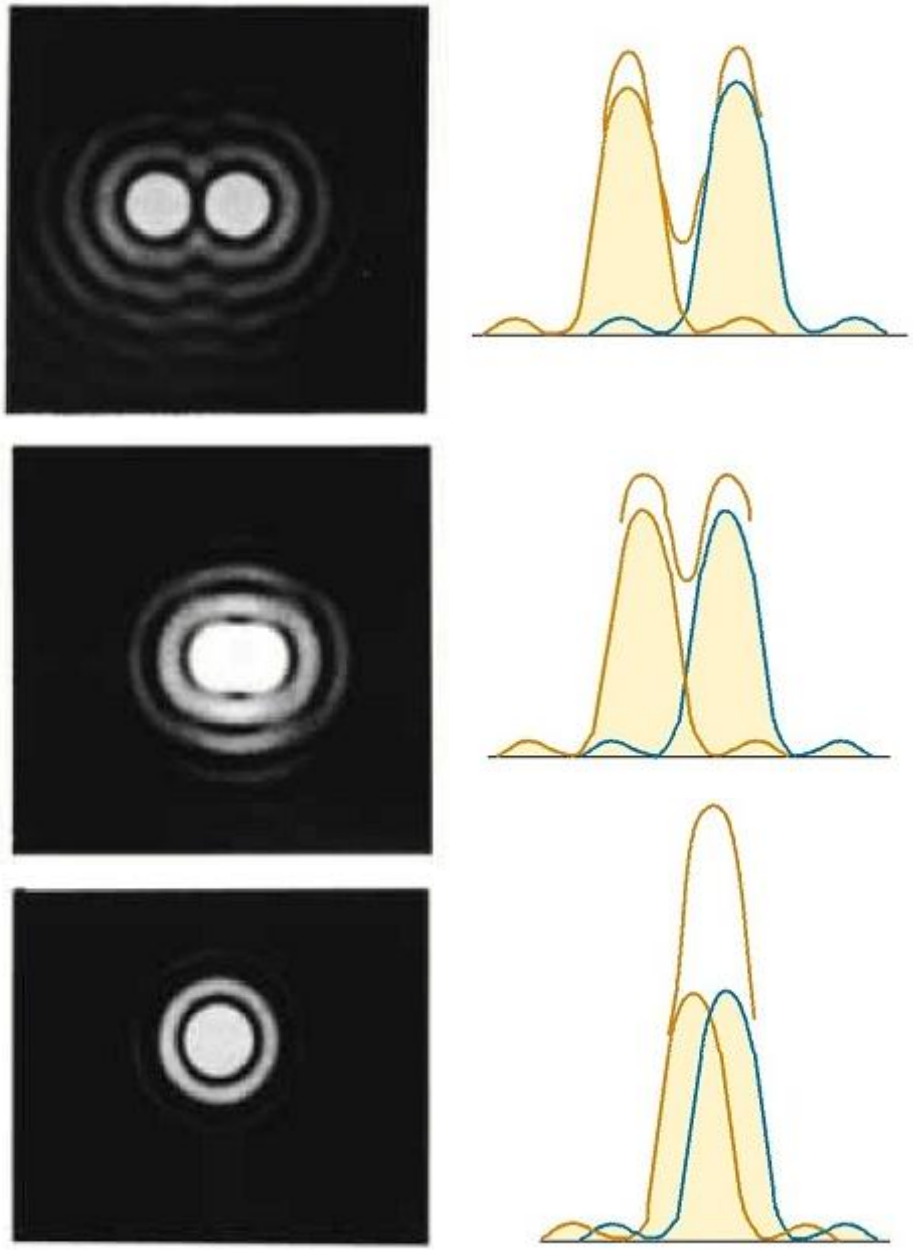



Figure 1

http://zeiss-campus.magnet.fsu.edu/print/superresolution/introduction-print.html



$\rho = 0.6\lambda/(\eta \sin\alpha)$

| Light Microscope | Electron Microscope |
|---|---|
| $\lambda = 0.5\ \mu m$ | $\lambda \sim \sqrt{\frac{150}{V_o}} = 0.055\ Å$ (@50 kV) |
| $\eta = 1.5$ (glass) | $\eta = 1.0$ (Vacuum) |
| $\alpha = 70^o$ | $\alpha = 1^o$ |
| $\rho = 0.2\ \mu m = 2000\ Å$ | $\rho = 0.00016\ \mu m = 1.6\ Å$ |

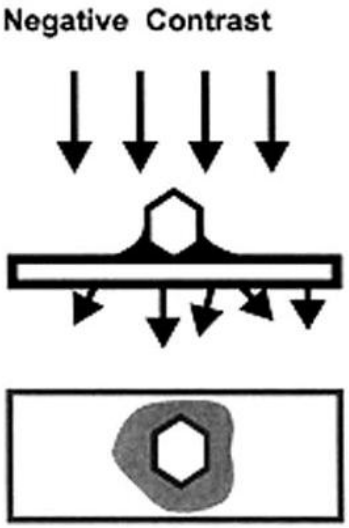
Critério de Rayleigh

Adaptado de http://www.kshitij-iitjee.com/resolution-of-single-slit-and-circular-apertures; PAC, I curso teórico/ Prático de Microscopia Electrónica, INSA, 2014

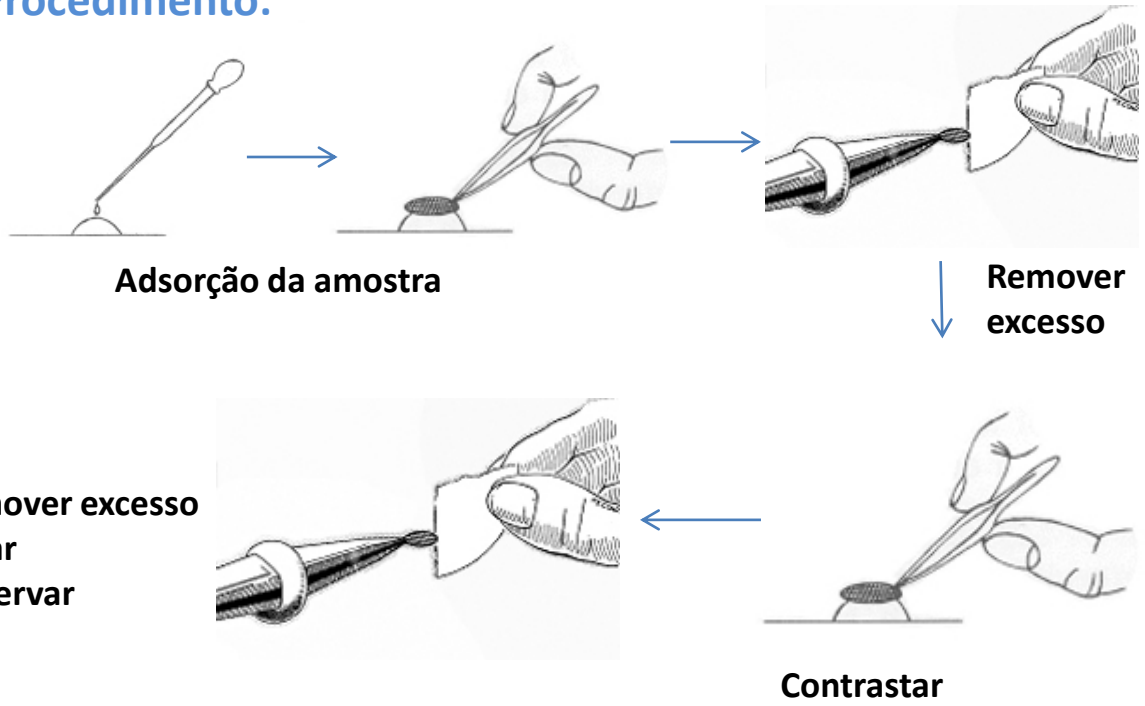
- **Contraste negativo**
- **Observação de cortes ultrafinos:**

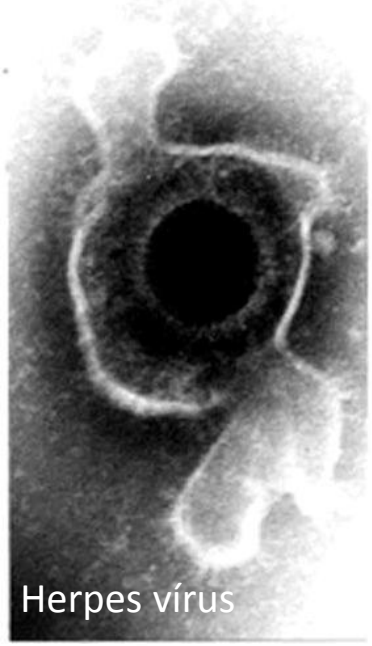
**Estrutura**

***Immunolabelling***




## Procedimento:



Herpes vírus

**Rápido**

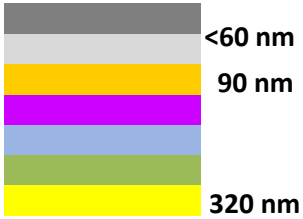
**Muito utilizado na análise de vírus**

**A adição ac específicos pode aumenta a probabilidade de detetar um determinado agente (*SPIEM: Solid-phase immunoelectron microscopy*)**

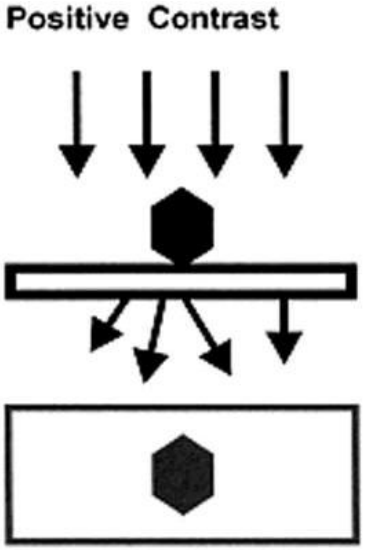
Adaptado de:http://www.iymenet.de/iiteratur/imelectronmicroscopy/electronmic.htm

## Procedimento:

Fixação da amostra
Tratamento com metais pesados
Desidratação
Inclusão em resina
Polimerização



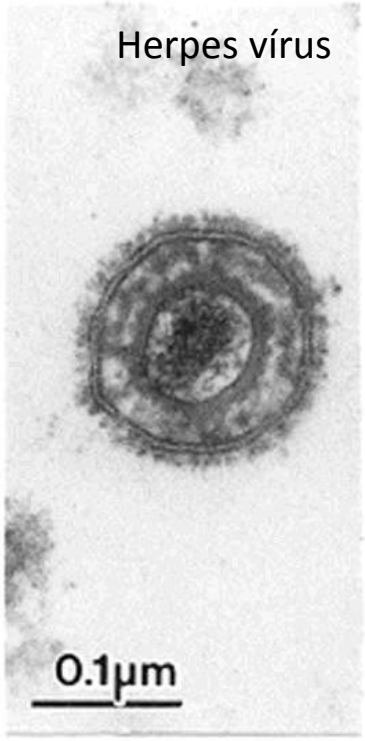

Contrastação com metais pesados
Observação….. ……
Vários dias após o início do procedimento

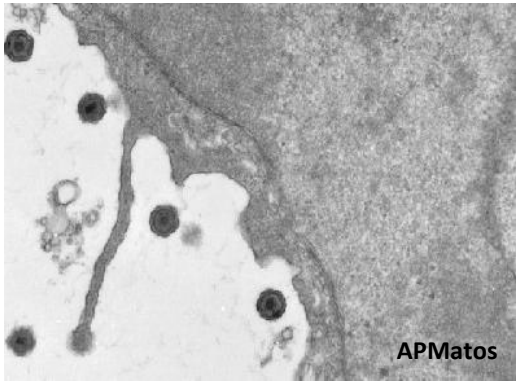



O procedimento é muito demorado
e
tecnicamente exigente.

Tamanho amostra

Valioso para identificar algumas patologias

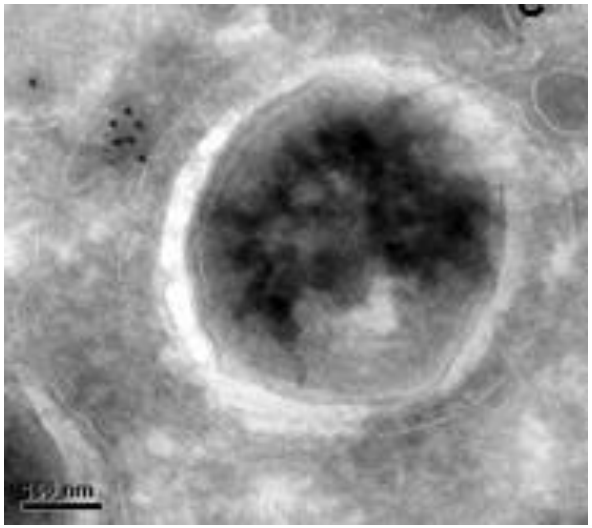


## Procedimento:

(….)
Recolher os cortes ultrafinos em grelhas
Deixar secar
Incubar com solução de bloqueio
Incubar com Ac primario
Lavar
Incubar com Protein A gold

Contrastação com metais pesados
Observação….. ……



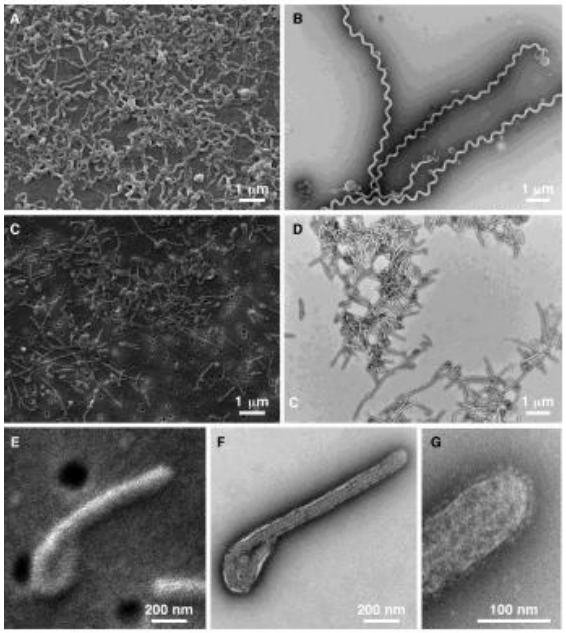
**Cathepsin S immunogold labeling of dendritic cells infected with BCG after 3 hours of infection (bar 100nm).**

O procedimento é ainda mais demorado e exigente

mas

Mais seletivo

# TEM: Vantagens e Desvantagens




Elevada resolução

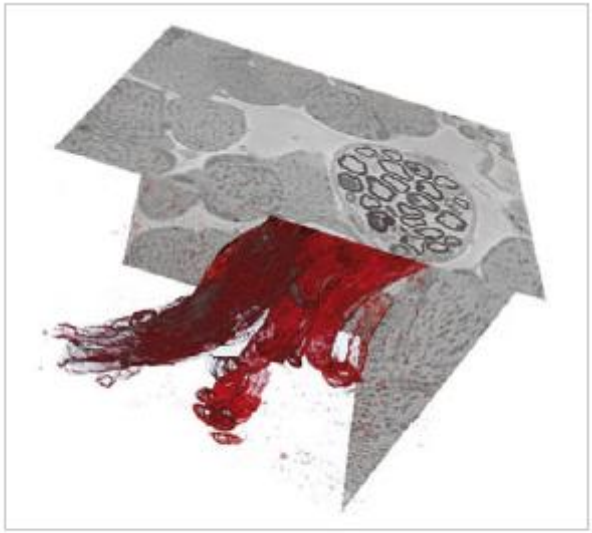
Técnica generalista (“ catch all”)

Protocolos extensos

Requer técnicos especializados (preparação, observação e interpretação)

Correlação com microscopia ótica.

Tamanho da amostra analisada pode levar a resultados erróneos.



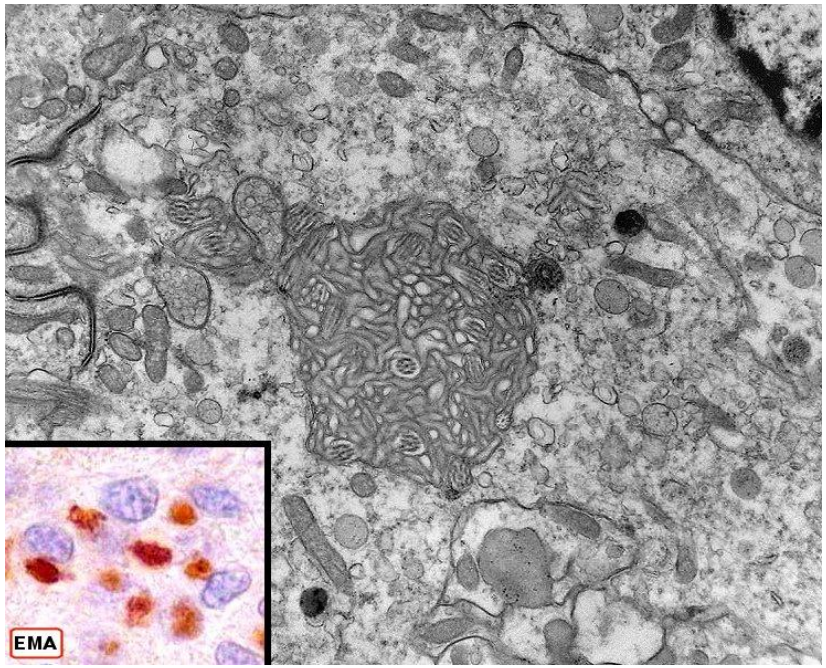
*Mouse extraocular muscle with reconstructed peripheral nerves, 100 x 100 x 100 micrometer 3D dataset with 1,000 slices*

WWW. Zeiss.com

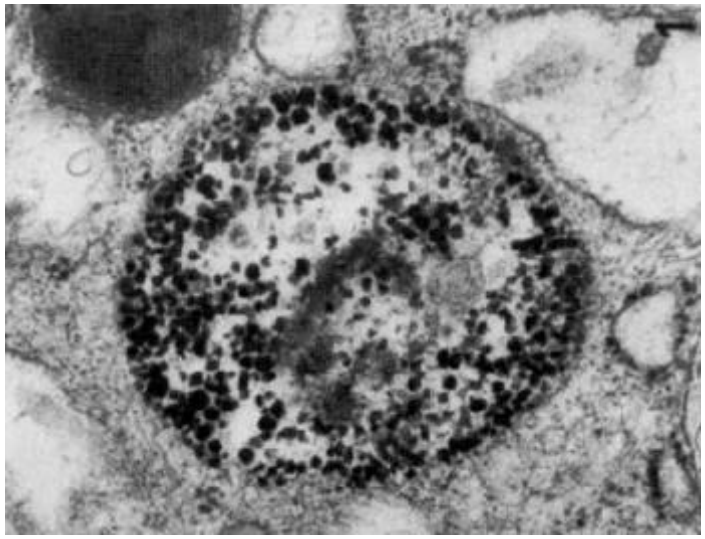
- **Patologias renais**
- **Neoplasias**
- **Doenças infeciosas**
- **Doenças metabólicas**
- **Patologias de causa desconhecida**

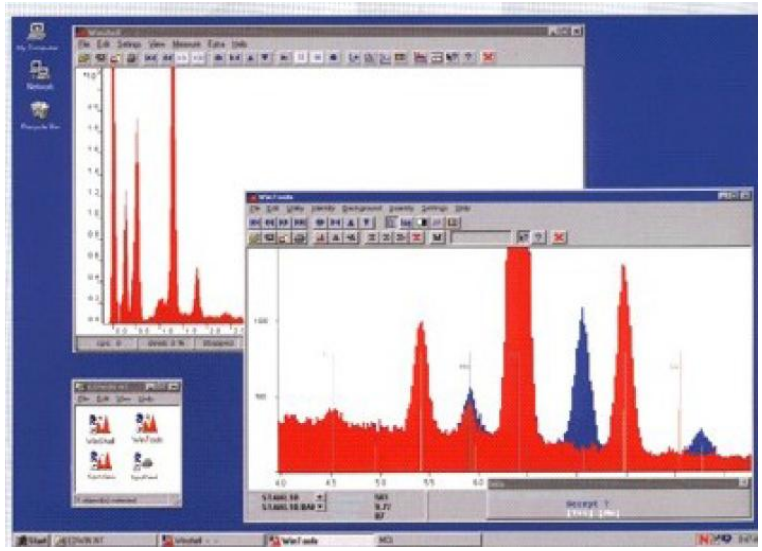
# Aplicações da microscopia eletrónica no diagnóstico

http://anatpat.unicamp.br/nptependimoma17c.html



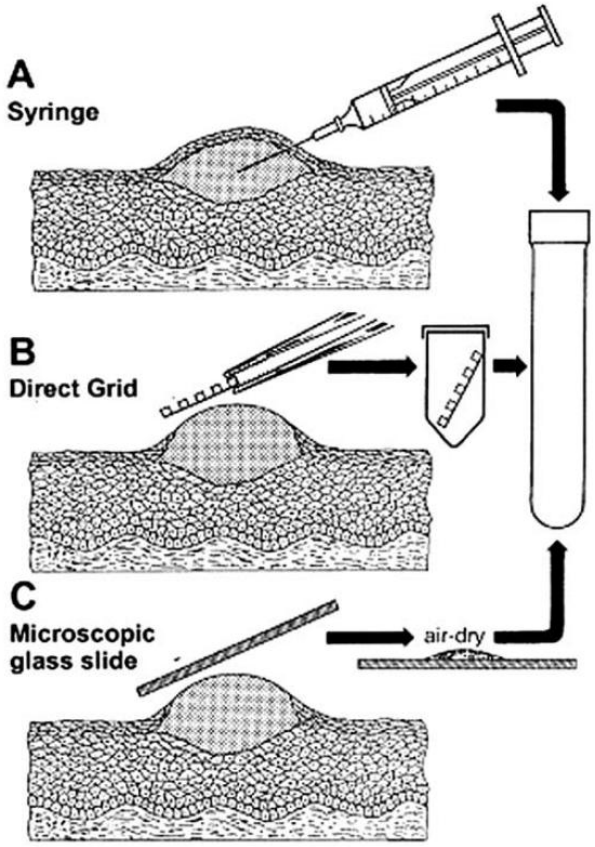

**Neoplasia – Microscopia óptica / TEM**
**Adenocarcinoma**
**EMA: *Epitheleal membrane antigen***





**Patologia de causa desconhecida**
**Deposição de metais - TEM-EDS**

**APMatos**




Emerging Infectious Diseases • Vol. 9, No. 3, March 2003

**Urina, LCR, saliva, lágrimas, etc**
Podem ser usadas diretamente.

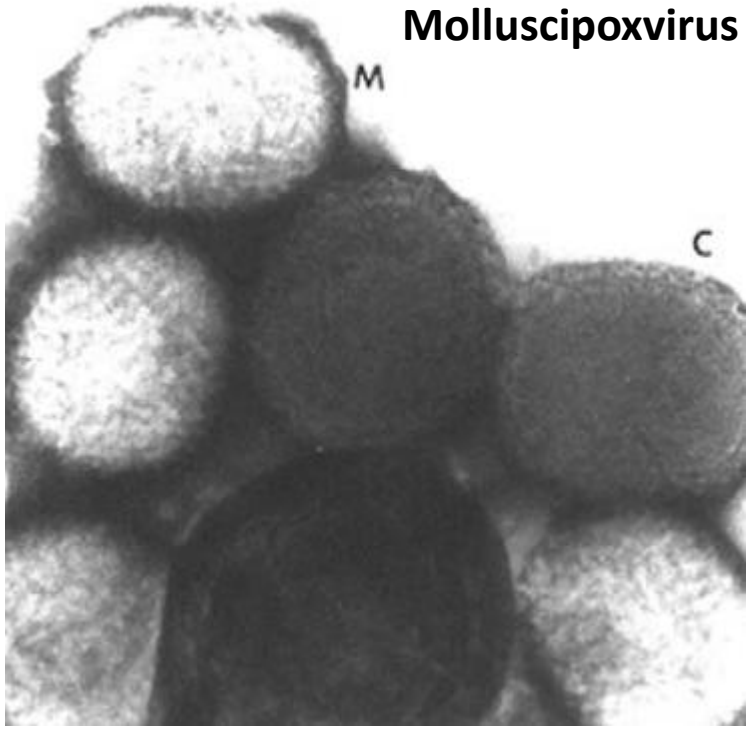
**Fezes**
Diluir, homogeneizar, descartar interferentes por centrifugação a baixa velocidade

Adsorver à superfície da grelha
c/ ou s/ inativação e enriquencimento

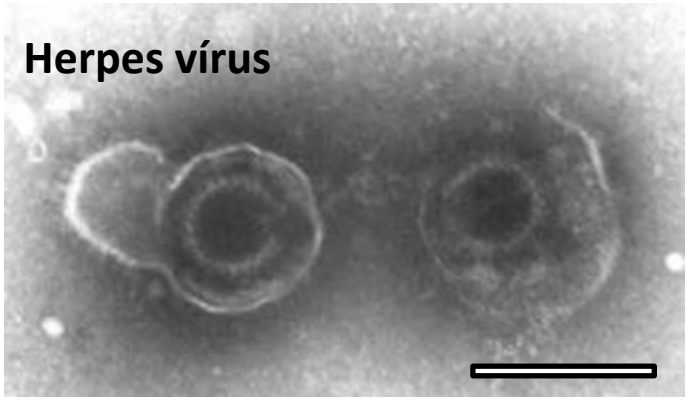
Contraste negativo

Observação

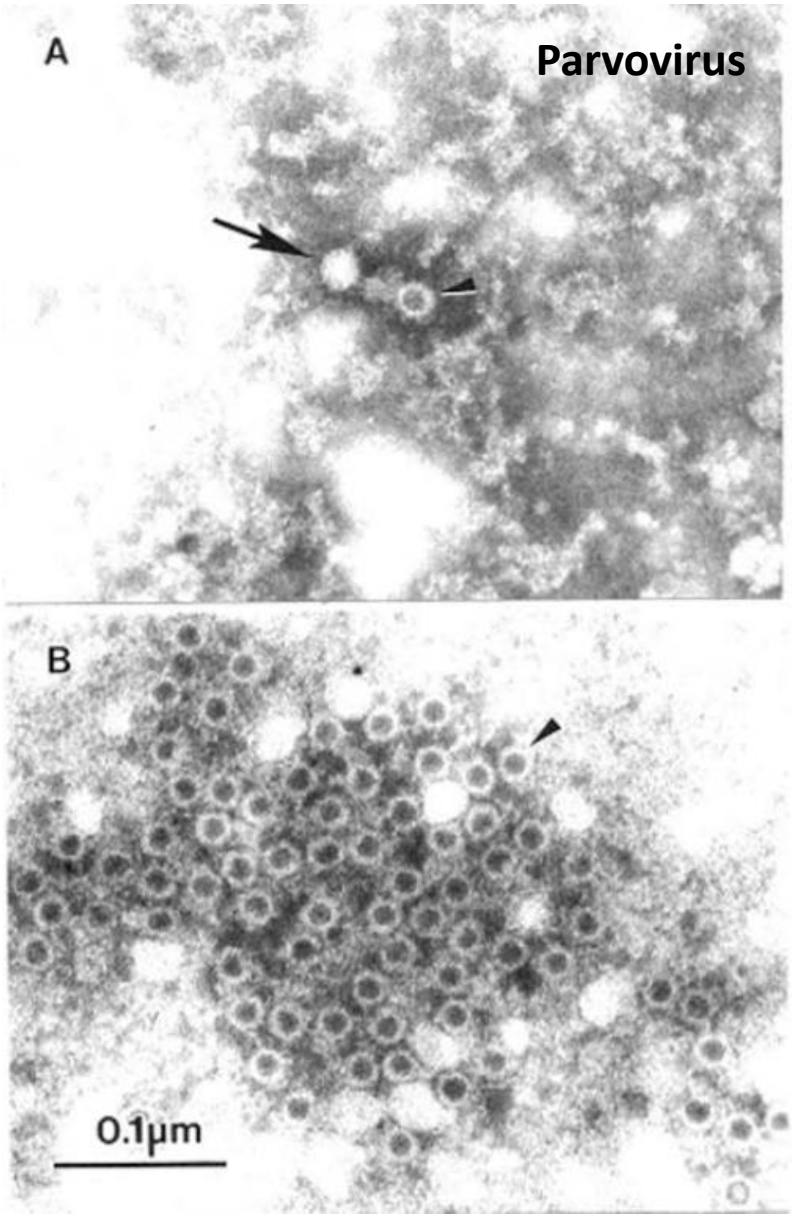
**Molluscipoxvirus**




**Herpes vírus**



**Parvovirus**

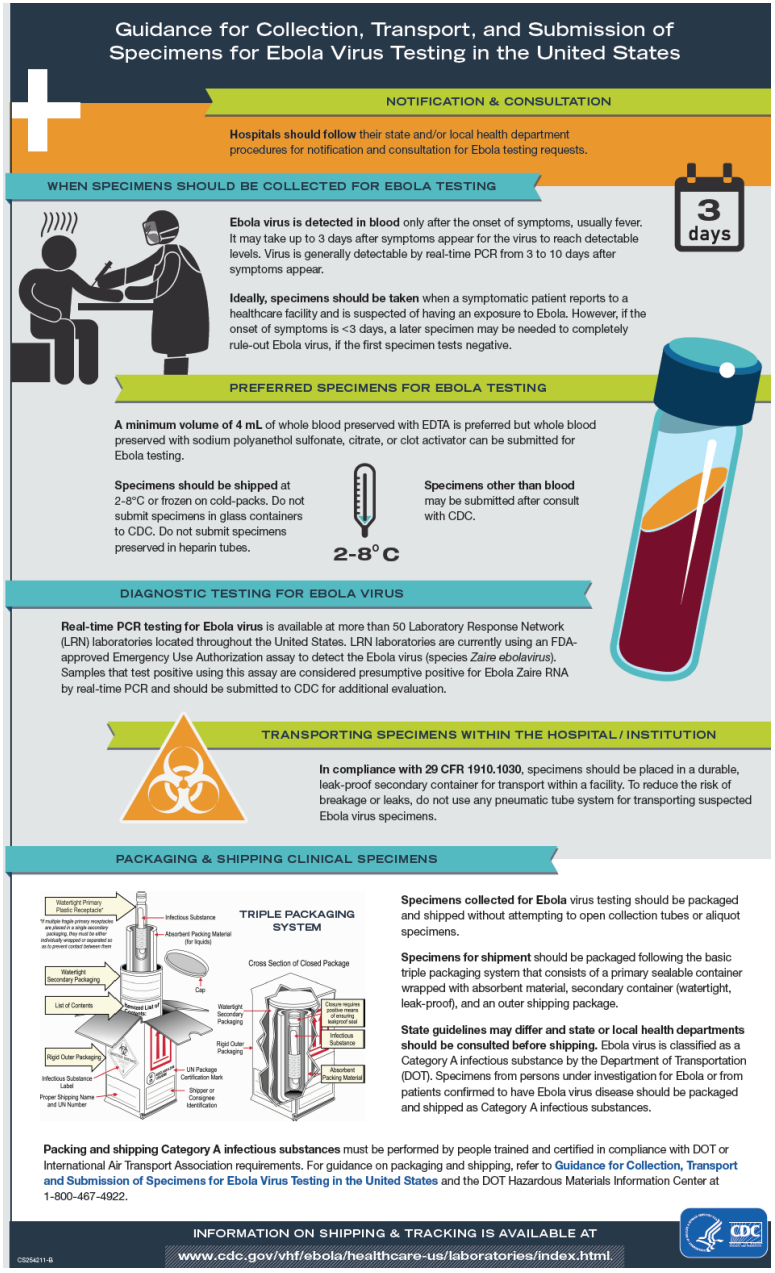



Emerging Infectious Diseases • Vol. 9, No. 3, March 2003

| Agente | Diagnóstico |
|---|---|
| *Bacillus anthracis* | PAC, EM, Cultura, ELISA, espectroscopia |
| *Brucella melitensis, abortus, spp.* | PAC, EM, Cultura |
| *Burkholderia mallei, pseudomallei* | PAC, EM, Cultura, ELISA, espectroscopia |
| Toxinas de *Clostridium botulinum* | ELISA, Ensaios funcionais, Cultura, PAC, Tipagem |
| *Coxiella burnetii* | PAC, EM, espectroscopia |
| *Francisella tularensis* | PAC, EM, Cultura, ELISA, espectroscopia |
| Ricin | ELISA, Ensaios funcionais, espectroscopia, PAC |
| Enteroxinas estafilococicas, *S. aureus* | Cultura, ELISA, espectroscopia |
| *Variola major* | PAC, EM, ELISA |
| Vírus de febres hemorrágicas | PAC, EM, Cultura, ELISA, espectroscopia |
| *Yersinia pestis* | PAC, EM, Cultura, ELISA, espectroscopia |

PAC: pesquisa de ácidos nucleicos

Adaptado de http://www.rki.de/EN/Content/Prevention/Bioterrism/Diagnostik/diagnostics-detection_node_en.html

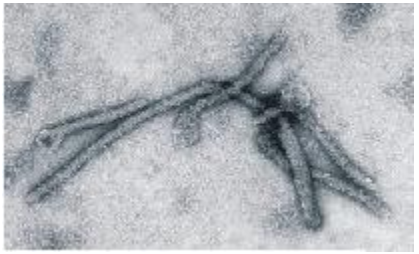



**Confirmed case:**

An Ebola virus Hemorrhagic Fever case is confirmed with one of the following laboratory diagnosis:

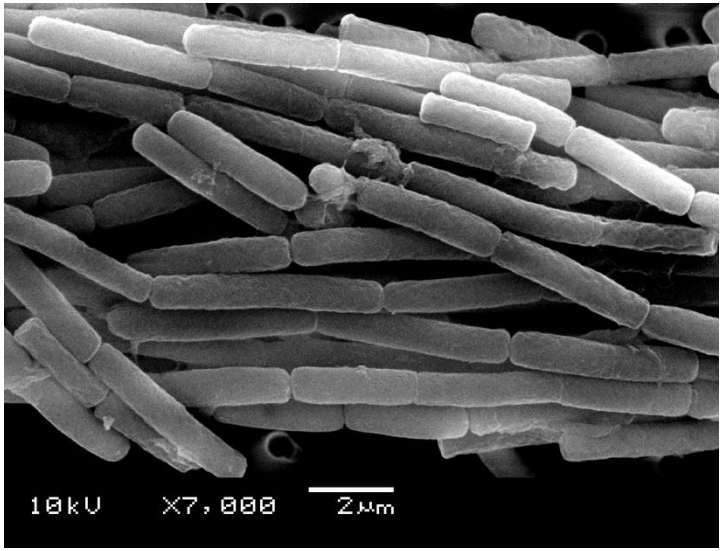
- Isolation of Ebola virus from serum or tissues
- Electronmicroscopically detection of Ebola virus in serum or tissue.
- Detection of Ebola virus nucleic acid by a sequenced RT-PCR from blood or tissues
- Detection of Ebola virus antigen in tissues by immunohistochemistry or in serum by ELISA

http://www.enivd.de/FS/fs_encdiseases.htm

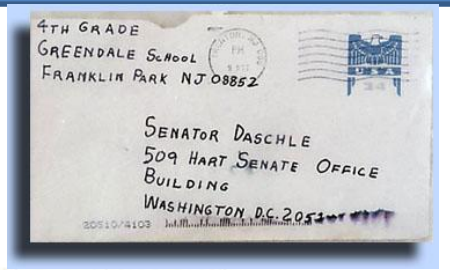


(source: H.Gelderblom)

# Aplicações práticas: *Bacillus anthracis*

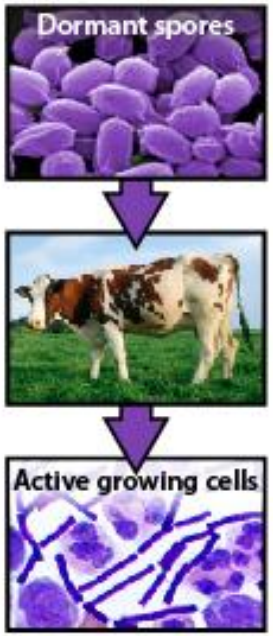

http://ncemi.org/dataman.pl?c=lib&dir=docs/photos

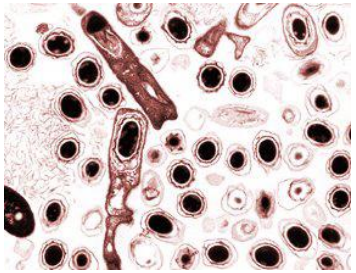



1 The bacteria known as *Bacillus anthracis* produce spores that are dormant (not active) and can live in the environment, like soil, for a long time, even decades.

2 When spores get into the body of an animal or person (a place rich with water, sugars, and other nutrients), they can be "activated" and turn into active growing cells.

3 When they become active, the bacteria can multiply, spread out in the body, produce toxins (poisons) and cause severe illness and death.



http://www.cdc.gov/anthrax/basics/index.html