

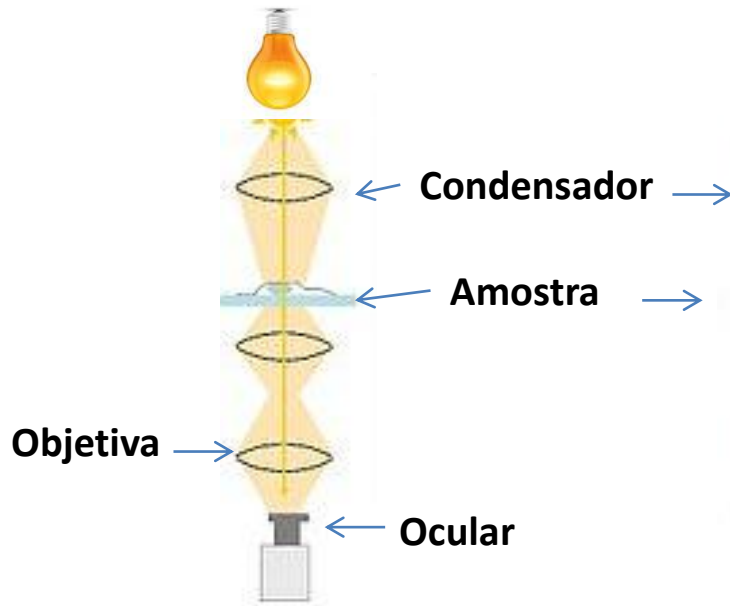
A MICROSCOPIA ELETRÓNICA NO DIAGNÓSTICO RÁPIDO DE AGENTES INFECIOSOS EM SITUAÇÕES DE EMERGÊNCIA.

Luísa Jordão, INSA

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

Breve Fundamento Teórico

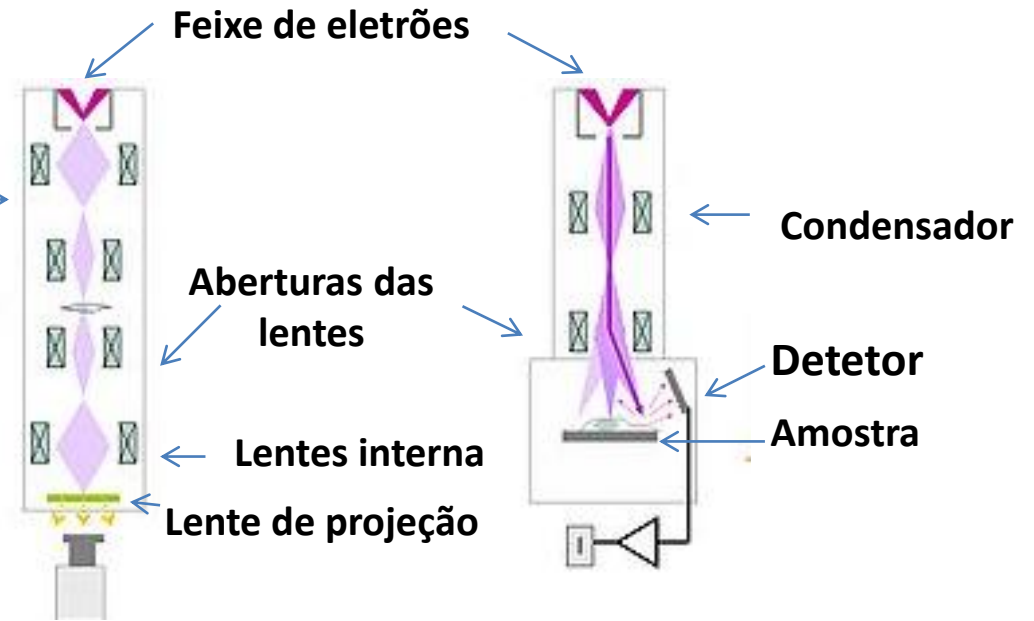
Microscópio óptico



Ampliação ~ 2000 x

Resolução 200 nm

Microscópio eletrónico de Transmissão (TEM) Varrimento (SEM)



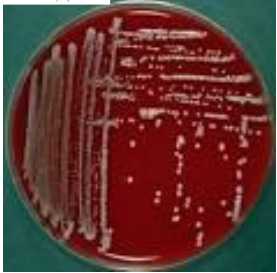
~1 500 000 x

0,1 nm

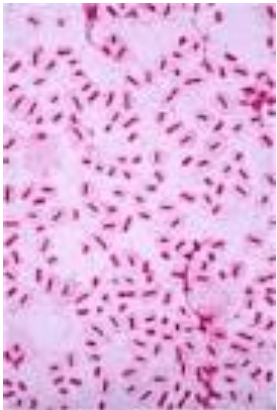
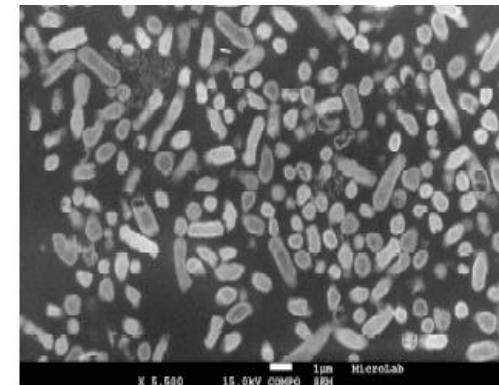
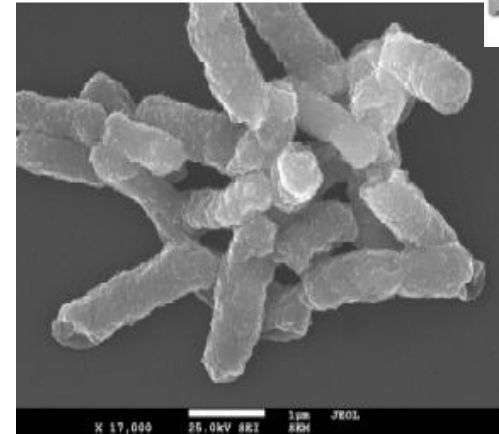
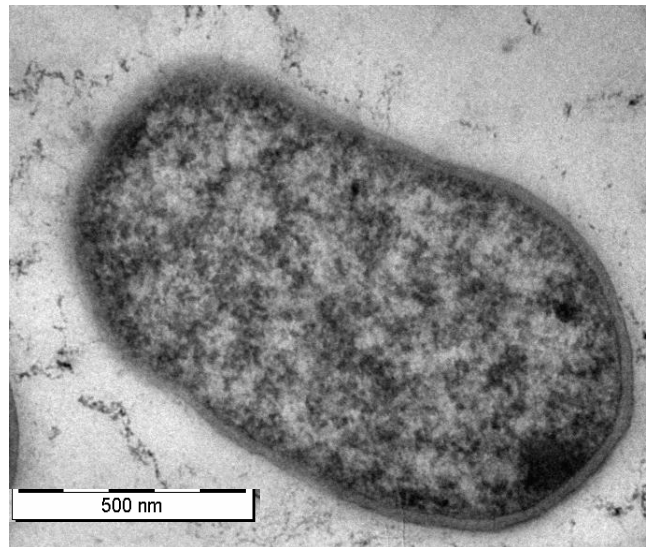
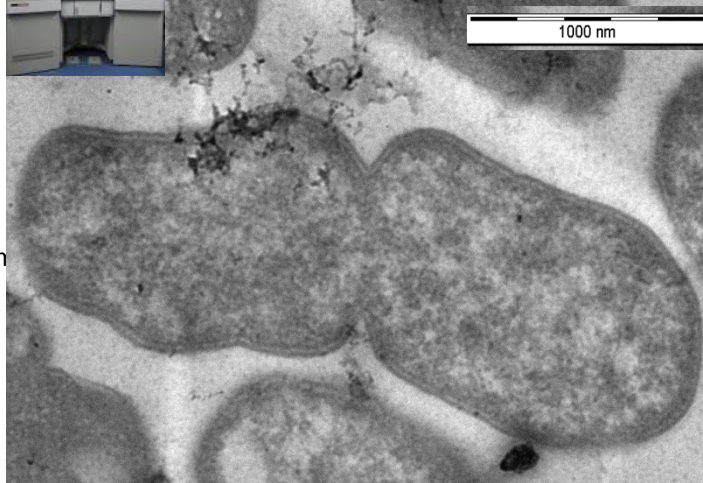
~ 1 000 000 x

0,5 nm

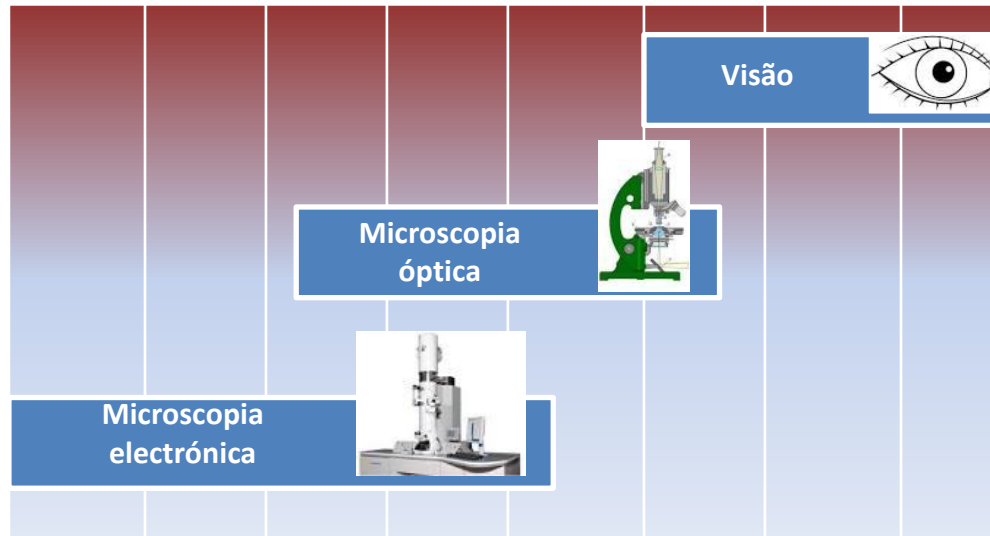
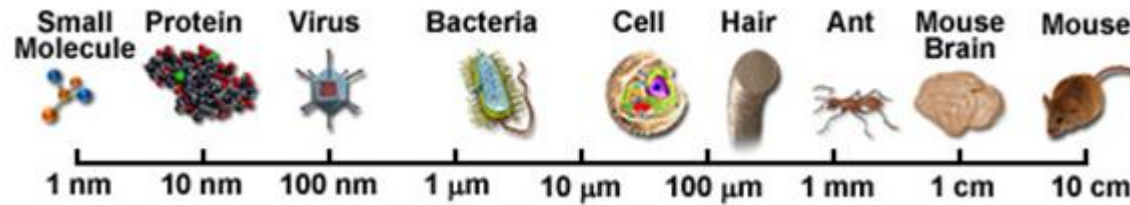
Método / Imagem



<http://klebsiella-pneumoniae.org/treatment.htm>



Resolução de várias técnicas de visualização de amostras biológicas



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

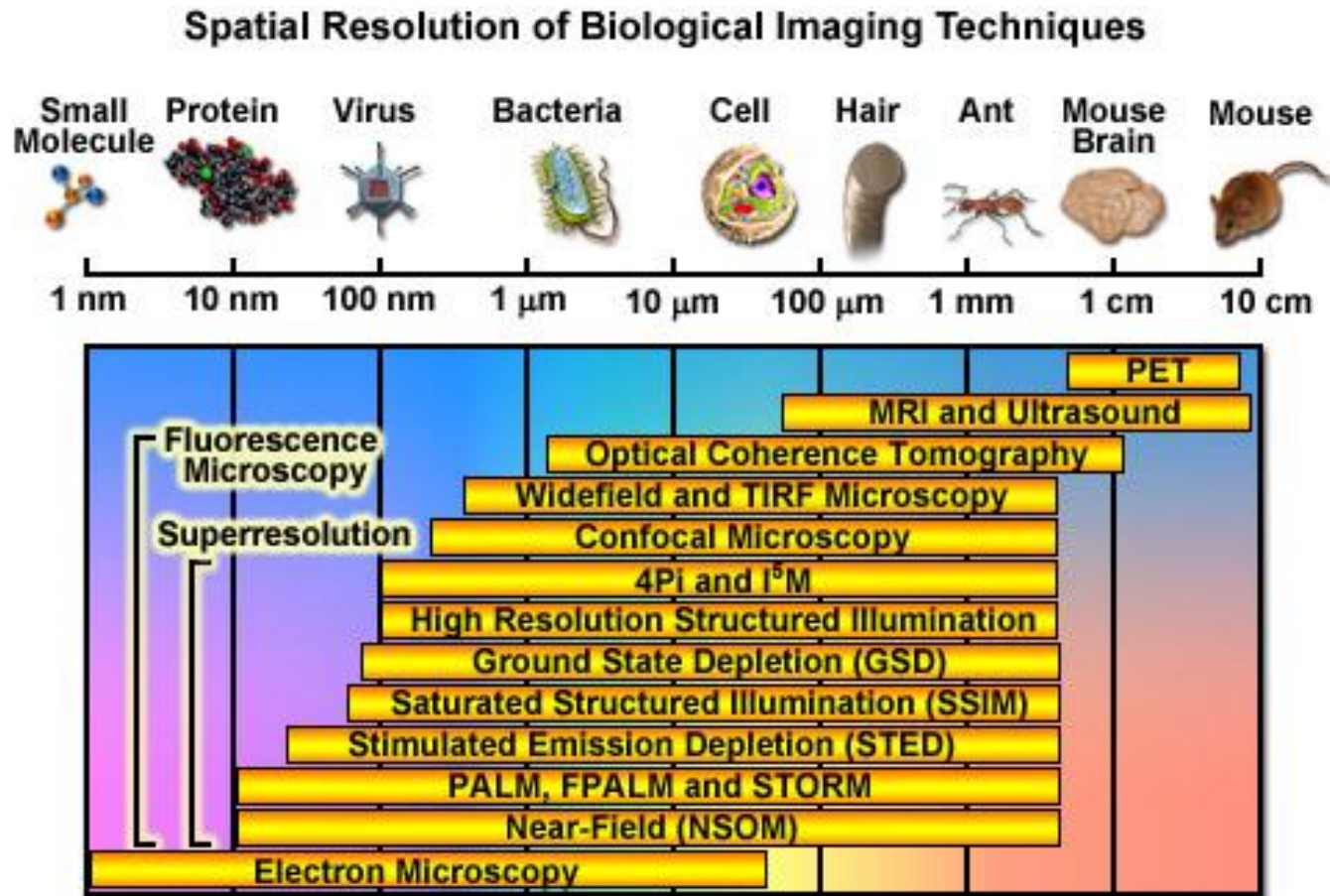
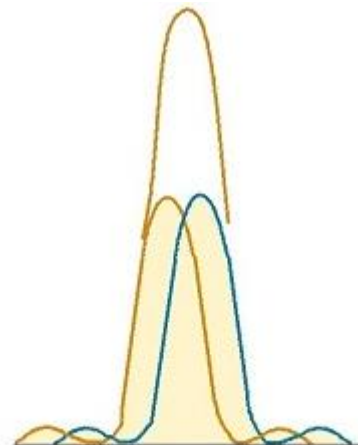
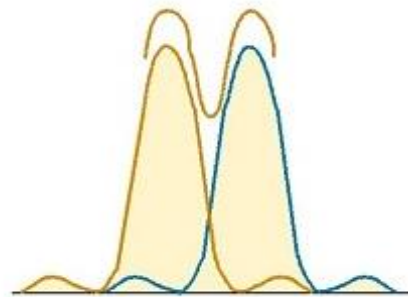
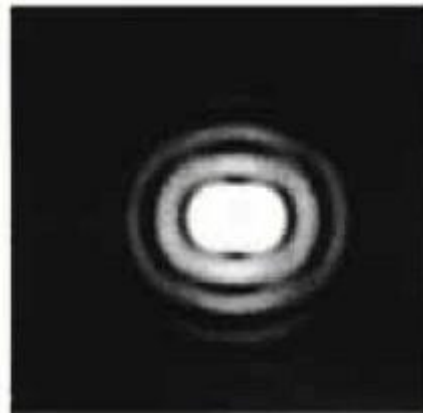
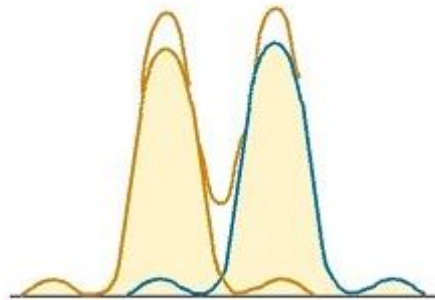
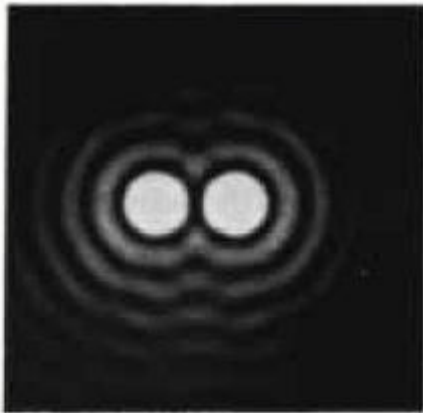


Figure 1



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

Light Microscope	Electron Microscope
$\lambda = 0.5 \mu\text{m}$	$\lambda \sim \sqrt{\frac{150}{V_0}} = 0.055 \text{ \AA} \text{ (@50 kV)}$
$\eta = 1.5 \text{ (glass)}$	$\eta = 1.0 \text{ (Vacuum)}$
$\alpha = 70^\circ$	$\alpha = 1^\circ$
$\rho = 0.2 \mu\text{m} = 2000 \text{ \AA}$	$\rho = 0.00016 \mu\text{m} = 1.6 \text{ \AA}$

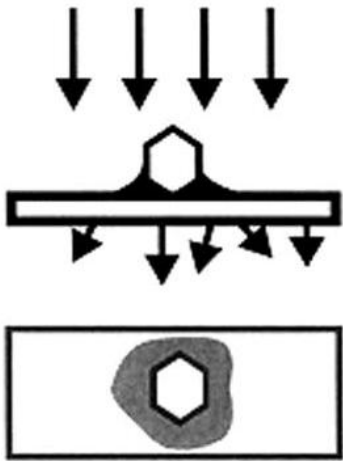
Critério de Rayleigh

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

Estrutura

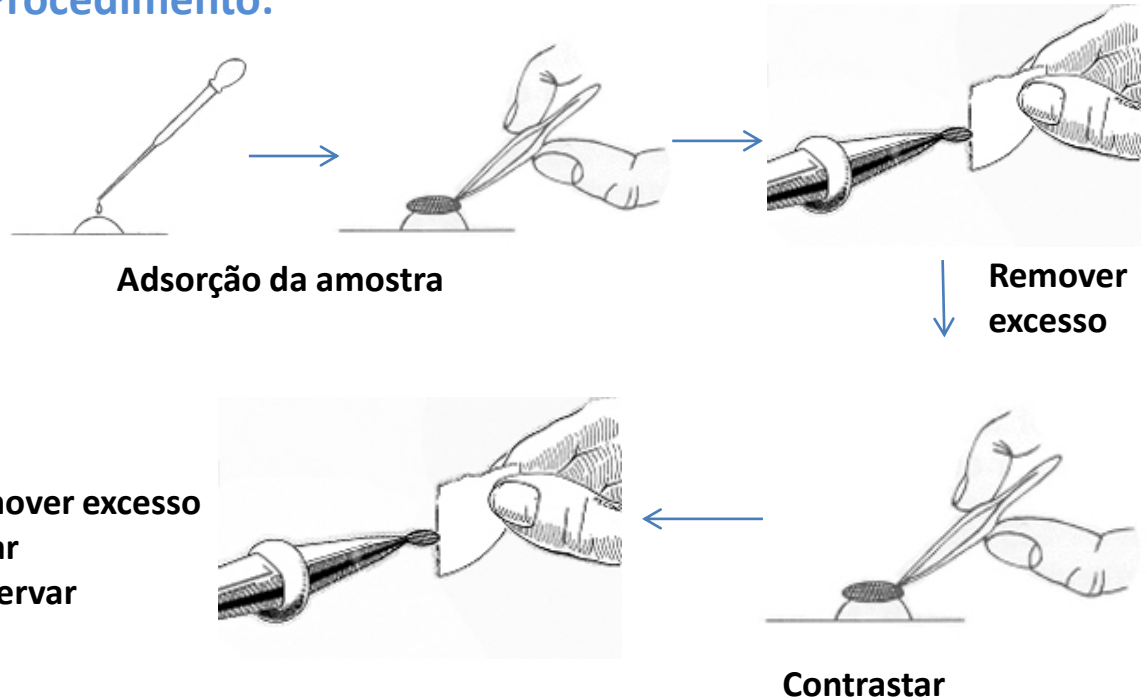
Immunolabelling

Negative Contrast



Herpes vírus

Procedimento:



Adsorção da amostra

Remover excesso

Remover excesso
Secar
Observar

Contrastar

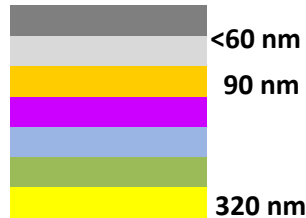
Rápido

Muito utilizado na análise de vírus

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

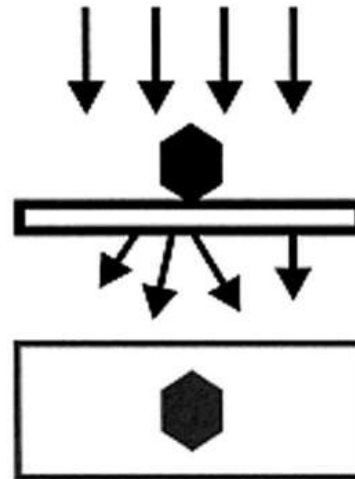
Procedimento:

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



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

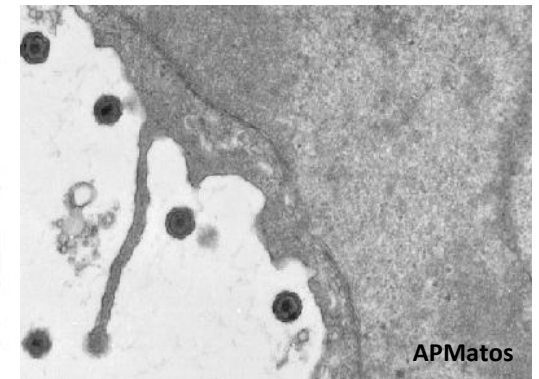
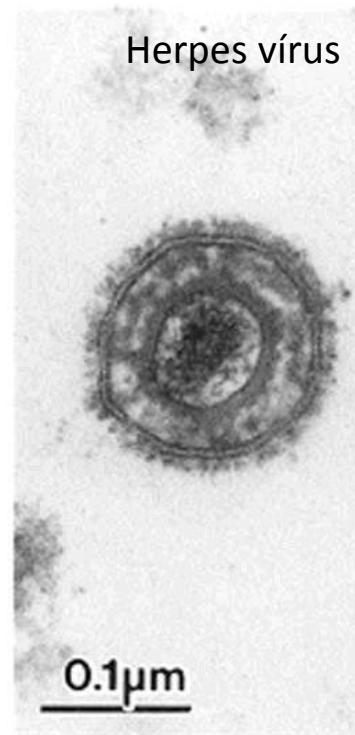
Positive Contrast



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

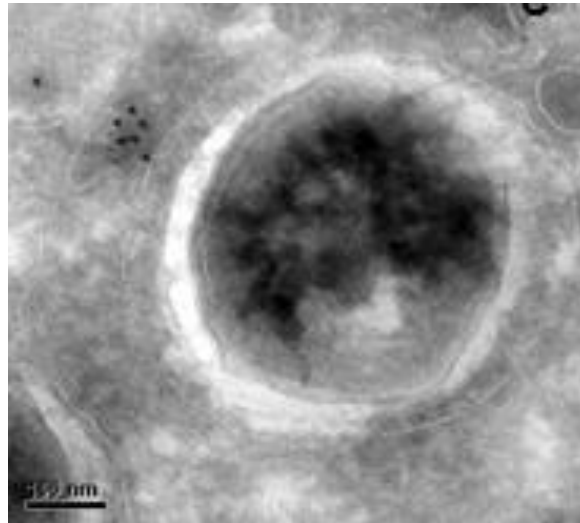
Incubar com Ac primario

Lavar

Incubar com Protein A gold

Contrastação com metais pesados

Observação.....

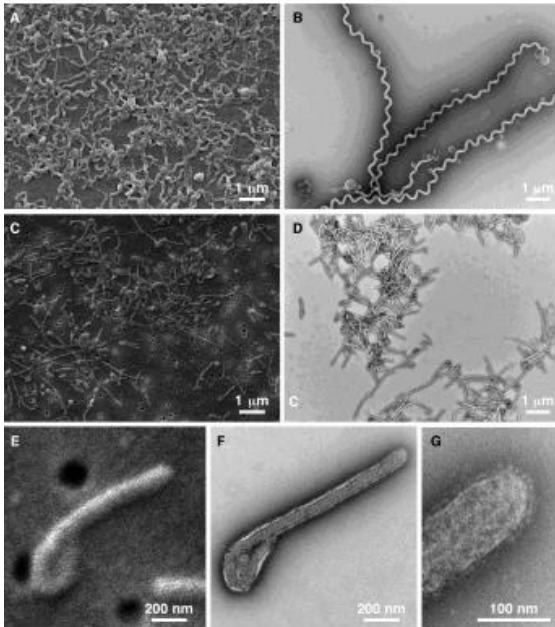


Cathepsin S immunogold labeling of dendritic cells infected with BCG after 3 hours of infection (bar 100nm).

O procedimento é ainda mais demorado e exigente

mas

Mais seletivo



Elevada resolução

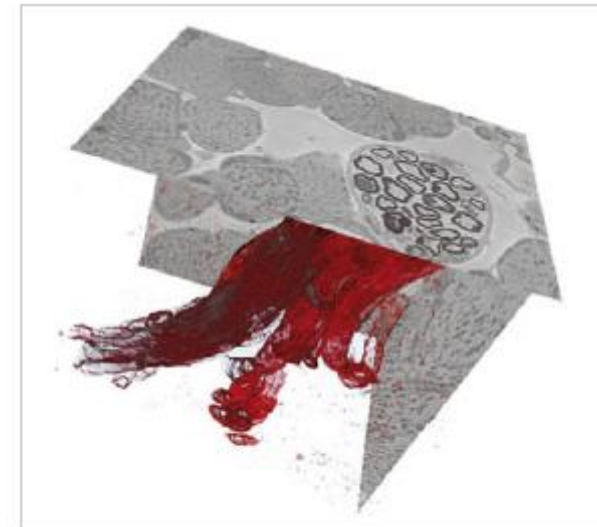
Técnica generalista (“catch all”)

Protocolos extensos

Requer técnicos especializados (preparação, observação e interpretação)

Correlação com microscopia ótica.

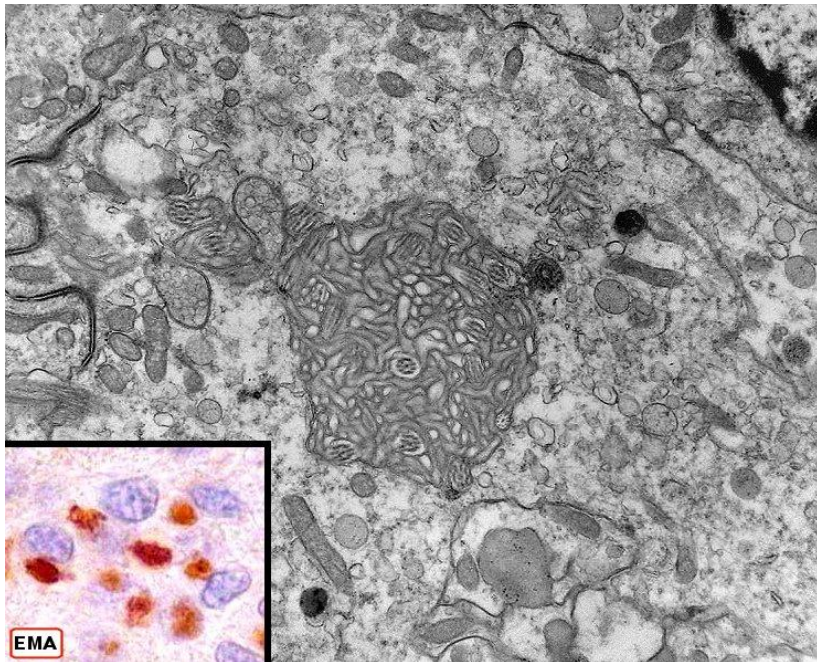
Tamanho da amostra analisada pode levar a resultados erróneos.



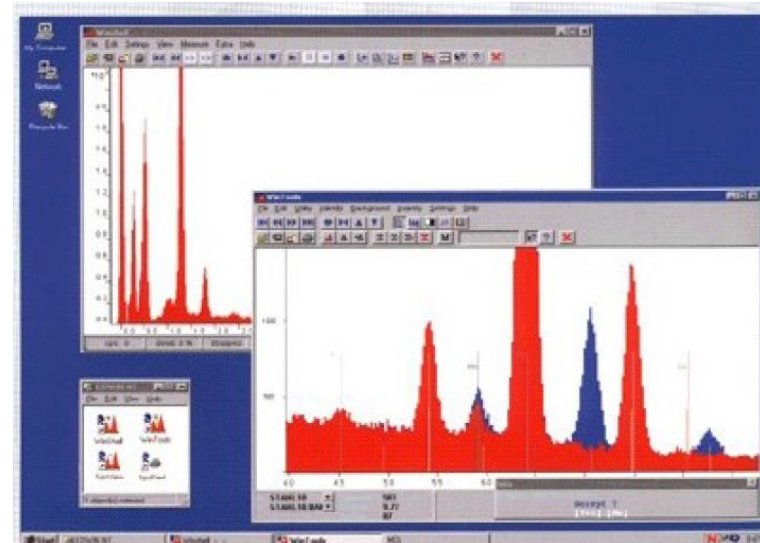
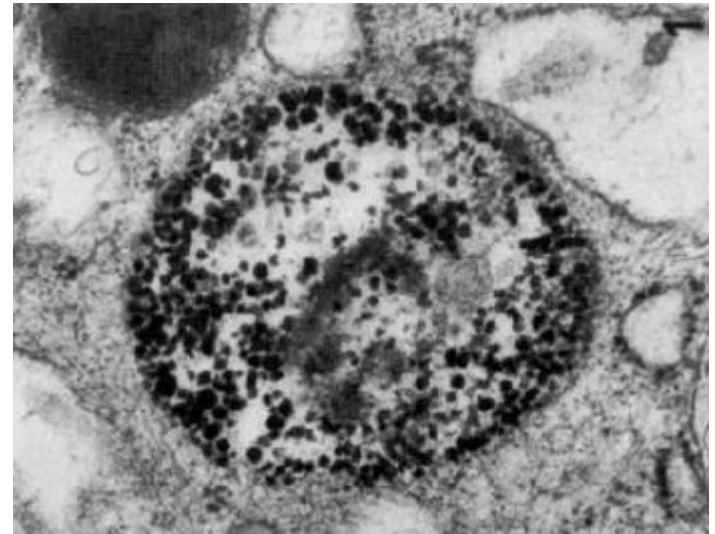
Mouse extraocular muscle with reconstructed peripheral nerves,
100 x 100 x 100 micrometer 3D dataset with 1,000 slices
WWW. Zeiss.com

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

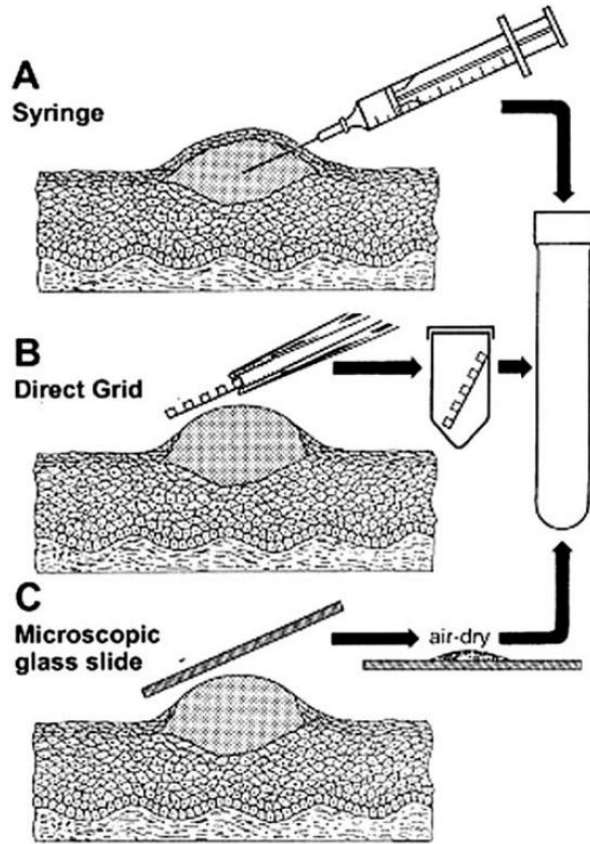
<http://anatpat.unicamp.br/nptependimoma17c.html>



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



Patologia de causa desconhecida
Deposição de metais - TEM-EDS



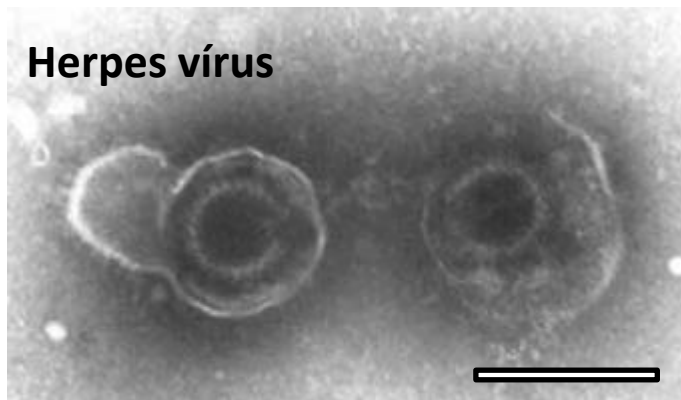
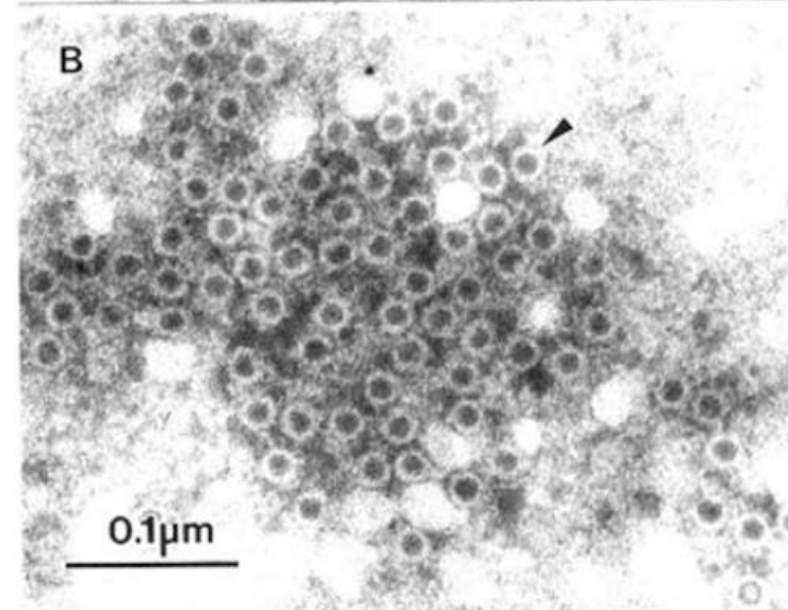
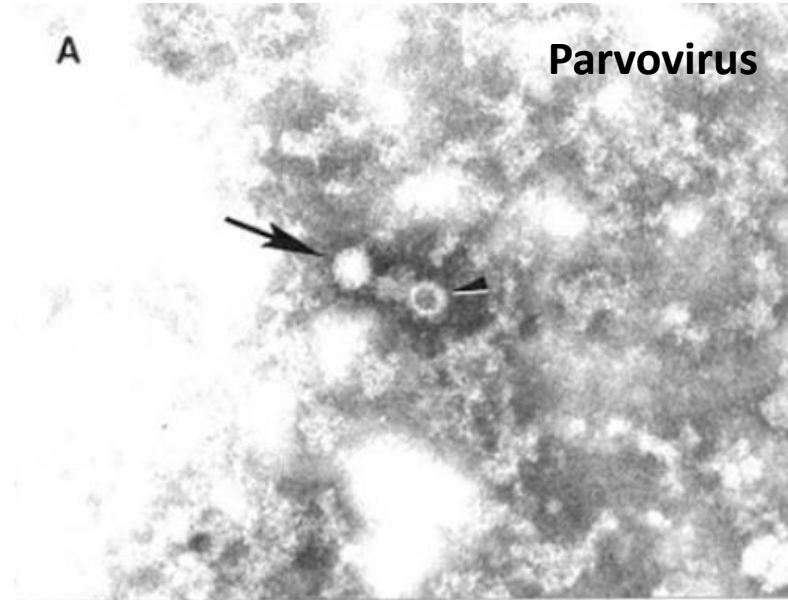
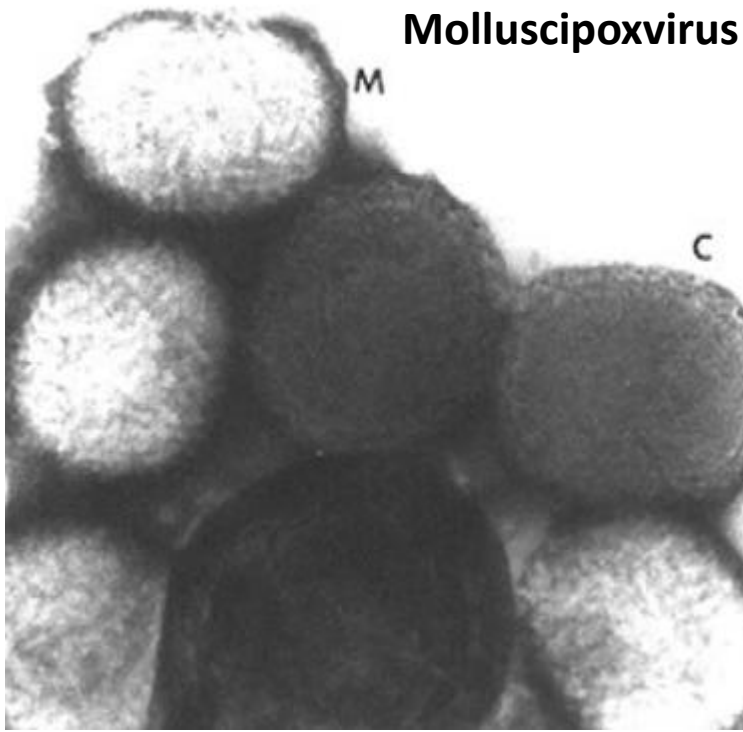
Urina, LCR, saliva,
lágrimas, etc
Podem ser usadas
diretamente.

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

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

Contraste negativo

Observação



Agente	Diagnóstico
<i>Bacillus anthracis</i>	PAC, EM, Cultura, ELISA, espectroscopia
<i>Brucella melitensis, abortus, spp.</i>	PAC, EM, Cultura
<i>Burkholderia mallei, pseudomallei</i>	PAC, EM, Cultura, ELISA, espectroscopia
Toxinas de <i>Clostridium botulinum</i>	ELISA, Ensaio funcionais, Cultura, PAC, Tipagem
<i>Coxiella burnetii</i>	PAC, EM, espectroscopia
<i>Francisella tularensis</i>	PAC, EM, Cultura, ELISA, espectroscopia
Ricin	ELISA, Ensaio funcionais, espectroscopia, PAC
Enterotoxinas estafilococicas, <i>S. aureus</i>	Cultura, ELISA, espectroscopia
<i>Variola major</i>	PAC, EM, ELISA
Vírus de febre hemorrágica	PAC, EM, Cultura, ELISA, espectroscopia
<i>Yersinia pestis</i>	PAC, EM, Cultura, ELISA, espectroscopia

PAC: pesquisa de ácidos nucleicos

Guidance for Collection, Transport, and Submission of Specimens for Ebola Virus Testing in the United States

NOTIFICATION & CONSULTATION

Hospitals should follow their state and/or local health department procedures for notification and consultation for Ebola testing requests.

WHEN SPECIMENS SHOULD BE COLLECTED FOR EBOLA TESTING



Ebola virus is detected in blood only after the onset of symptoms, usually fever. It may take up to 3 days after symptoms appear for the virus to reach detectable levels. Virus is generally detectable by real-time PCR from 3 to 10 days after symptoms appear.



Ideally, specimens should be taken when a symptomatic patient reports to a healthcare facility and is suspected of having an exposure to Ebola. However, if the onset of symptoms is <3 days, a later specimen may be needed to completely rule-out Ebola virus, if the first specimen tests negative.

PREFERRED SPECIMENS FOR EBOLA TESTING

A minimum volume of 4 mL of whole blood preserved with EDTA is preferred but whole blood preserved with sodium polyanethol sulfonate, citrate, or clot activator can be submitted for Ebola testing.

Specimens should be shipped at 2-8°C or frozen on cold-packs. Do not submit specimens in glass containers to CDC. Do not submit specimens preserved in heparin tubes.



Specimens other than blood may be submitted after consult with CDC.



DIAGNOSTIC TESTING FOR EBOLA VIRUS

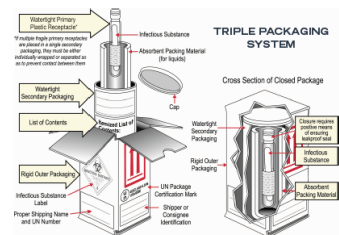
Real-time PCR testing for Ebola virus is available at more than 50 Laboratory Response Network (LRN) laboratories located throughout the United States. LRN laboratories are currently using an FDA-approved Emergency Use Authorization assay to detect the Ebola virus (species *Zaire ebolavirus*). Samples that test positive using this assay are considered presumptive positive for Ebola Zaire RNA by real-time PCR and should be submitted to CDC for additional evaluation.

TRANSPORTING SPECIMENS WITHIN THE HOSPITAL / INSTITUTION



In compliance with 29 CFR 1910.1030, specimens should be placed in a durable, leak-proof secondary container for transport within a facility. To reduce the risk of breakage or leaks, do not use any pneumatic tube system for transporting suspected Ebola virus specimens.

PACKAGING & SHIPPING CLINICAL SPECIMENS



Specimens collected for Ebola virus testing should be packaged and shipped without attempting to open collection tubes or aliquot specimens.

Specimens for shipment should be packaged following the basic triple packaging system that consists of a primary sealable container wrapped with absorbent material, secondary container (watertight, leak-proof), and an outer shipping package.

State guidelines may differ and state or local health departments should be consulted before shipping. Ebola virus is classified as a Category A infectious substance by the Department of Transportation (DOT). Specimens from persons under investigation for Ebola or from patients confirmed to have Ebola virus disease should be packaged and shipped as Category A infectious substances.

Packing and shipping Category A infectious substances must be performed by people trained and certified in compliance with DOT or International Air Transport Association requirements. For guidance on packaging and shipping, refer to [Guidance for Collection, Transport and Submission of Specimens for Ebola Virus Testing in the United States](#) and the DOT Hazardous Materials Information Center at 1-800-467-4922.

INFORMATION ON SHIPPING & TRACKING IS AVAILABLE AT

www.cdc.gov/vhf/ebola/healthcare-us/laboratories/index.html



Confirmed case:

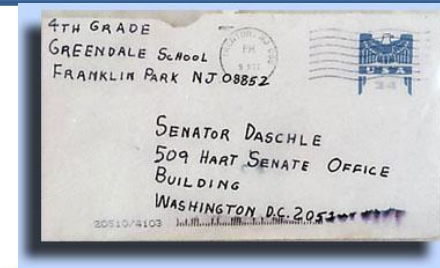
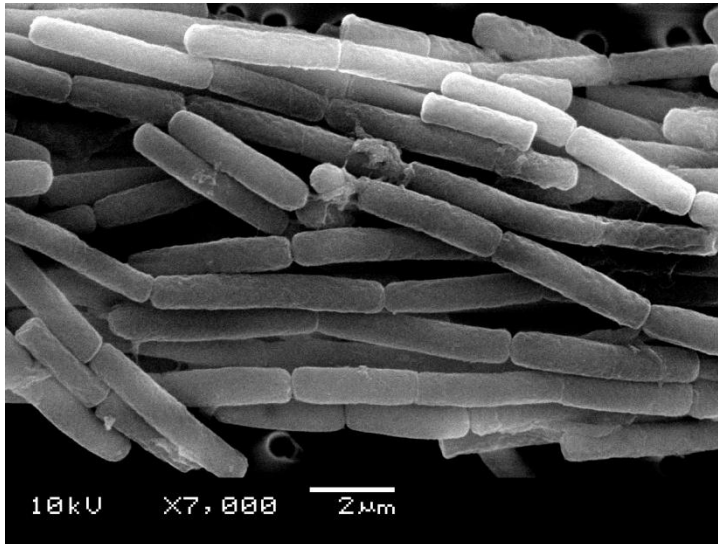
An Ebola virus Hemorrhagic Fever case is confirmed with one of the following laboratory diagnosis:

- 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_enddiseases.htm



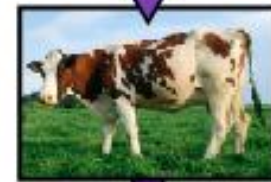
(source: H.Gelderblom)



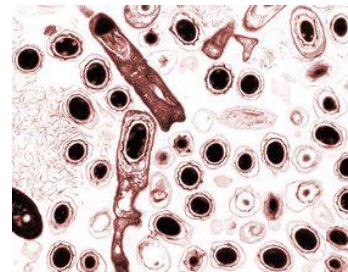
1 The bacteria known as *Bacillus anthracis* produce **spores that are dormant** (not active) and can live in the environment, like soil, for a long time, even decades.



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>