

# Lab Director's Conference

April 19, 2007

# Behold the Mold!

Mycology Workshops  
as a part of  
Laboratory Training at DSHS

# A tradition of wet workshops

- For over 35 years this laboratory has offered a variety of wet workshops
- Changes in clinical laboratory science have reduced the importance of some topics and eliminated the need to teach some classical hands-on techniques.
- Notably, **parasitology** and **mycology** remain largely tied to microscopic observation to identify etiologic agents and contaminating organisms



# The National Laboratory Training Network, our strong partner in hands-on training since 1989

“a training system sponsored by the Association of Public Health Laboratories (APHL) and Centers for Disease Control and Prevention (CDC)”

# Hands-on, “wet” mycology workshops

- Few sources- Mayo Clinic, CDC, DSHS
- Other mycology training exists, but usually without\* the hands-on component.

\*At least one mycologist, Davise Larone, teaches by microscopic examination of previously prepared slides.

In the last 23 years, this laboratory has presented hands-on mycology workshops -

- In at least eight cities in Texas
- In at least 19 other states (mostly cosponsored by our laboratory and the NLTN) – with luck, soon 21 states
- Multiple times in some states

Our two day course:  
Identification of Medically Important  
Filamentous Fungi

A natural question might be: “Why the emphasis on filamentous fungi when yeasts are the most commonly isolated pathogenic fungi from human sources?”

Technology has rescued the clinical mycologist when it comes to dealing with yeast identification. (Big business and big \$\$\$ for makers of miniaturized and/or automated systems)

No automation “life preserver” for the clinical lab to help i.d. filamentous fungi. Developing such instrumentation is not cost effective. In spite of some probe technology, filamentous fungi are still studied by microscopy.



# “Identification of Medically Important Filamentous Fungi”

what it's all about...

Thanks to Tom Mitchell  
prioritizing workshop objectives...

# “Identification of Medically Important Filamentous Fungi”

- **Review of mycological terminology**
- Review of categories of fungal infection
- Presentation of several diseases in each category of infection
- Participants prepare and examine tease mounts of about 60 different filamentous fungi
- Participants compare tease mounts with prepared slide culture mounts of the same fungi
- Presentation of several methods and techniques developed in our laboratory which may simplify some of the work in the mycology laboratory

# Reproductive structures of fungi

- Spores – sexual reproductive forms.  
Several types occur among fungi
- Conidia – asexual reproductive forms also of several types

# “new, improved” terminology

- Don't say asexual form, say “anamorph”
- Don't say sexual form, say “teleomorph”  
(Now, don't we sound educated!!)
- Bear in mind that numerous fungi may reproduce by both sexual and asexual means

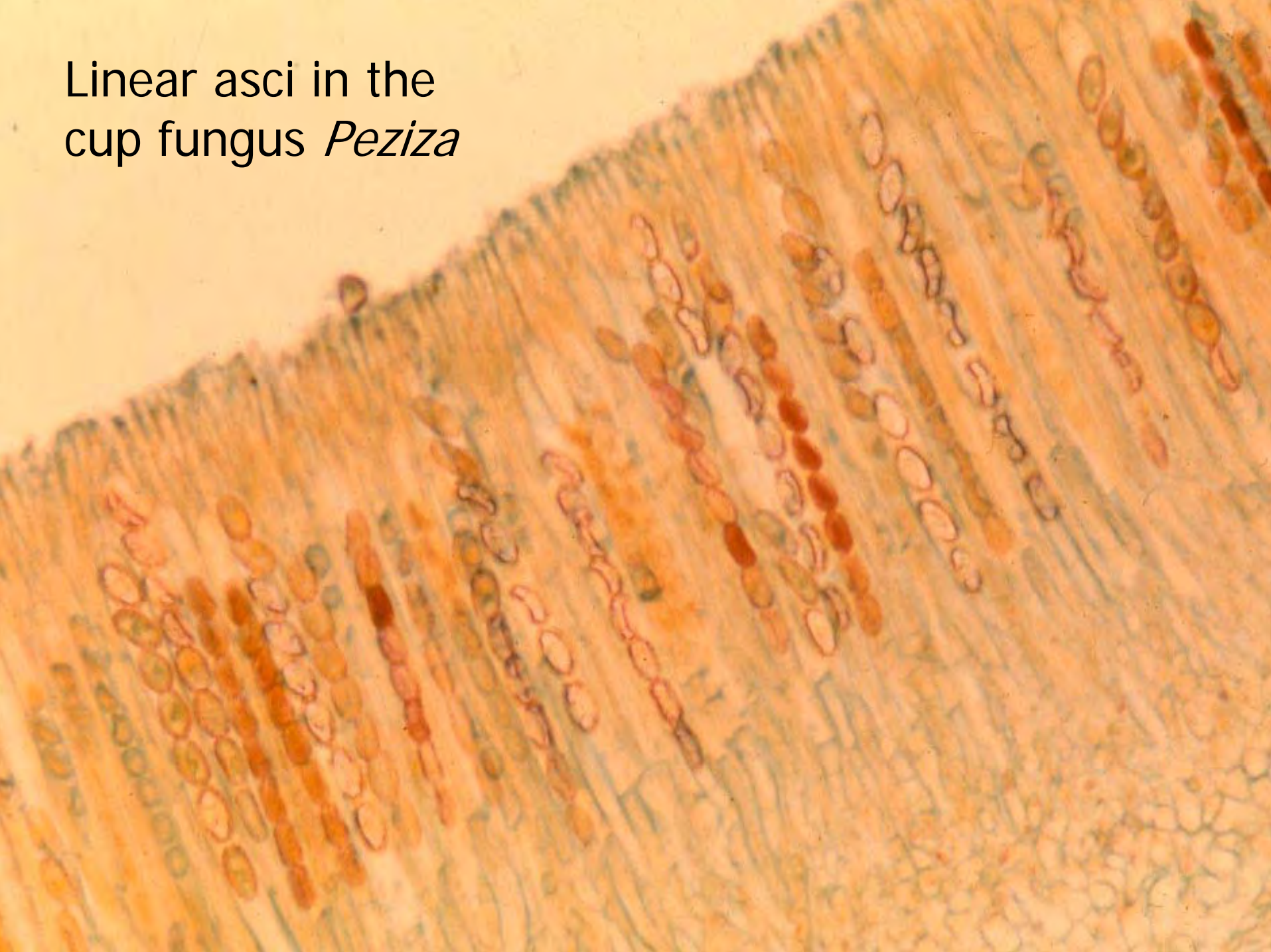
# Spore types

- Ascospore
- Basidiospore
- Zygospor

*Peziza*, a cup fungus



Linear asci in the  
cup fungus *Peziza*

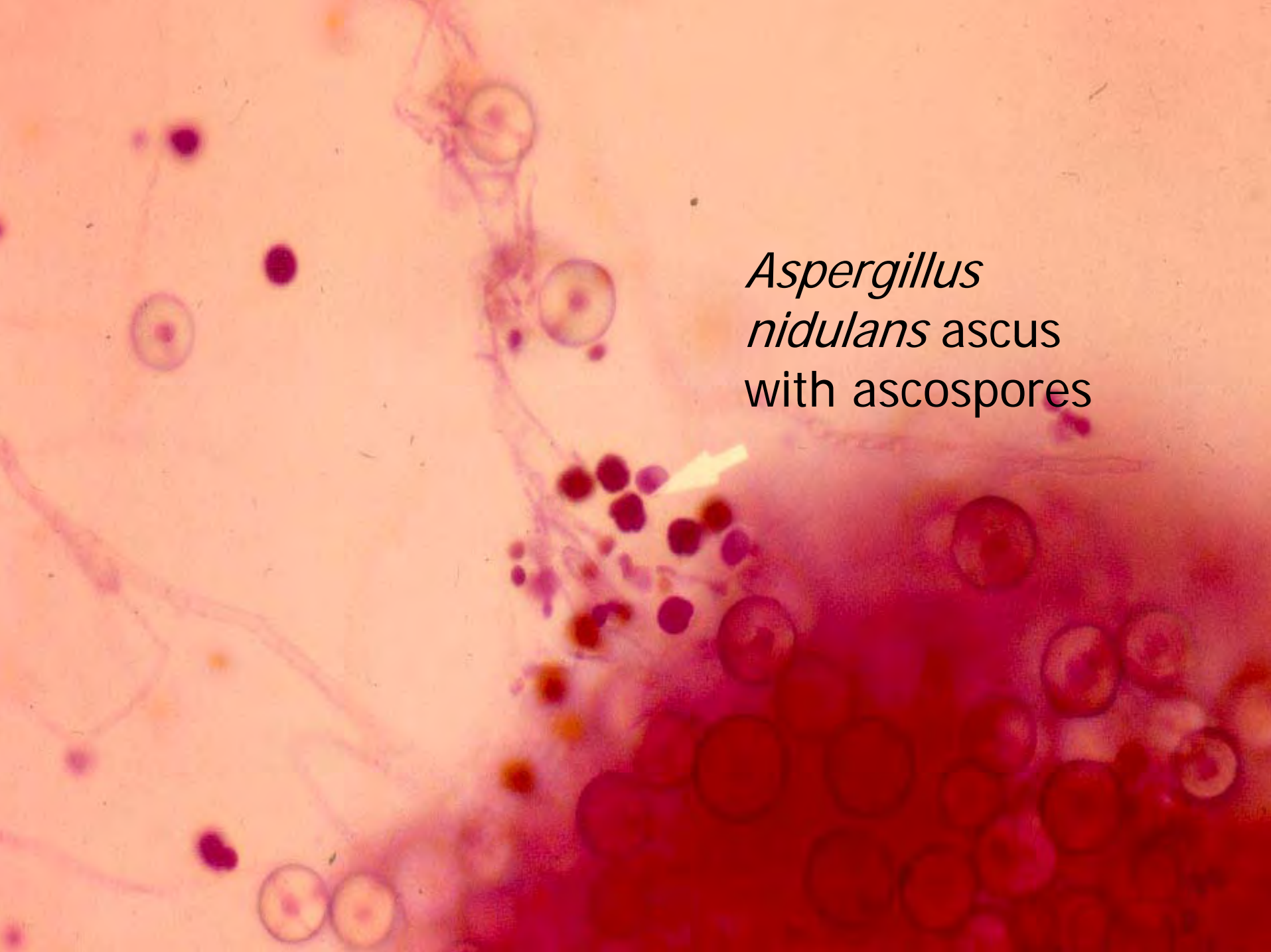




More linear asci.  
Note 8 ascospores in  
each ascus



*Aspergillus  
nidulans* ascus  
with ascospores





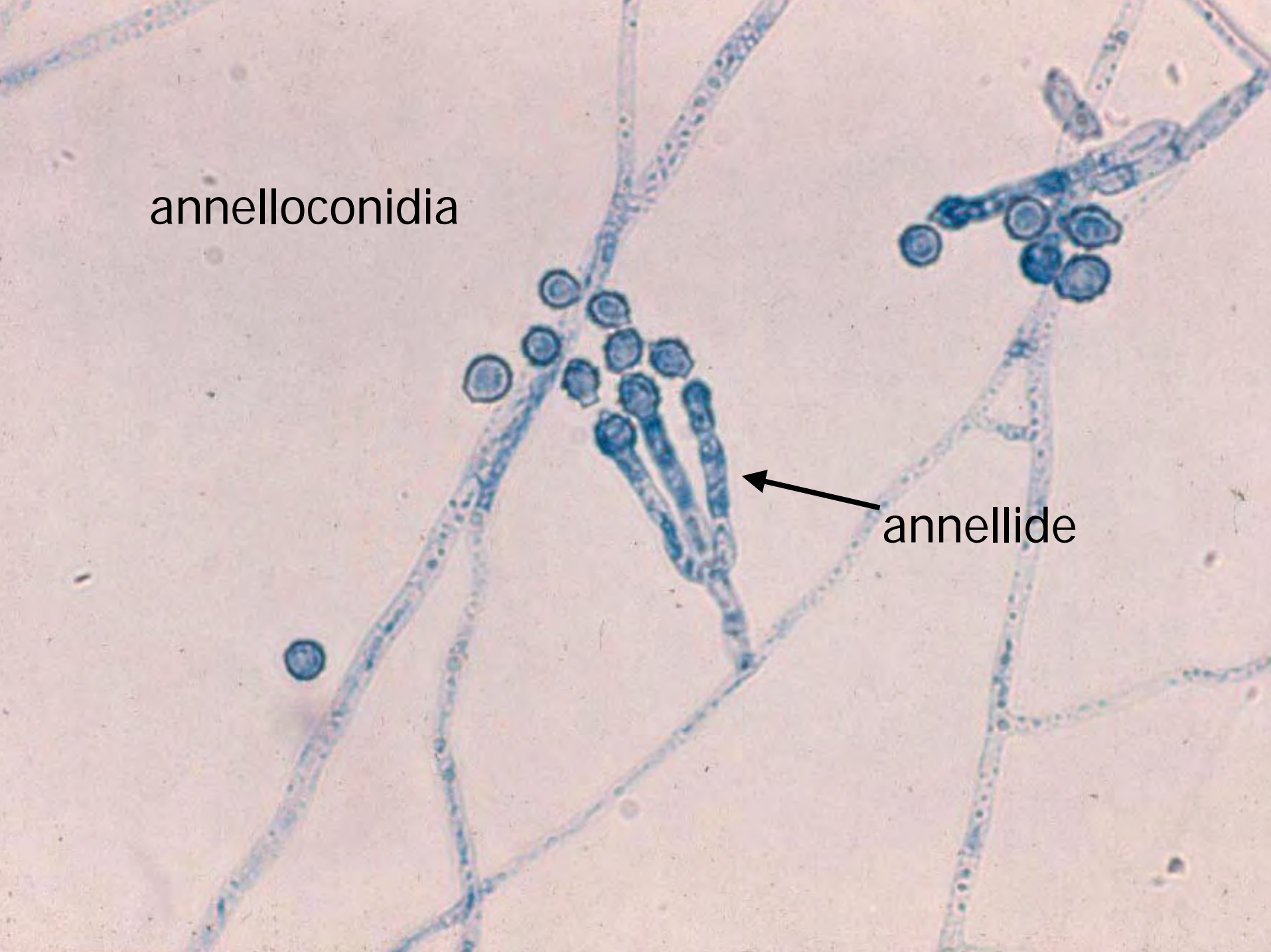
Ascospores of  
*Saccharomyces cerevisiae*

# More descriptive terms for conidia

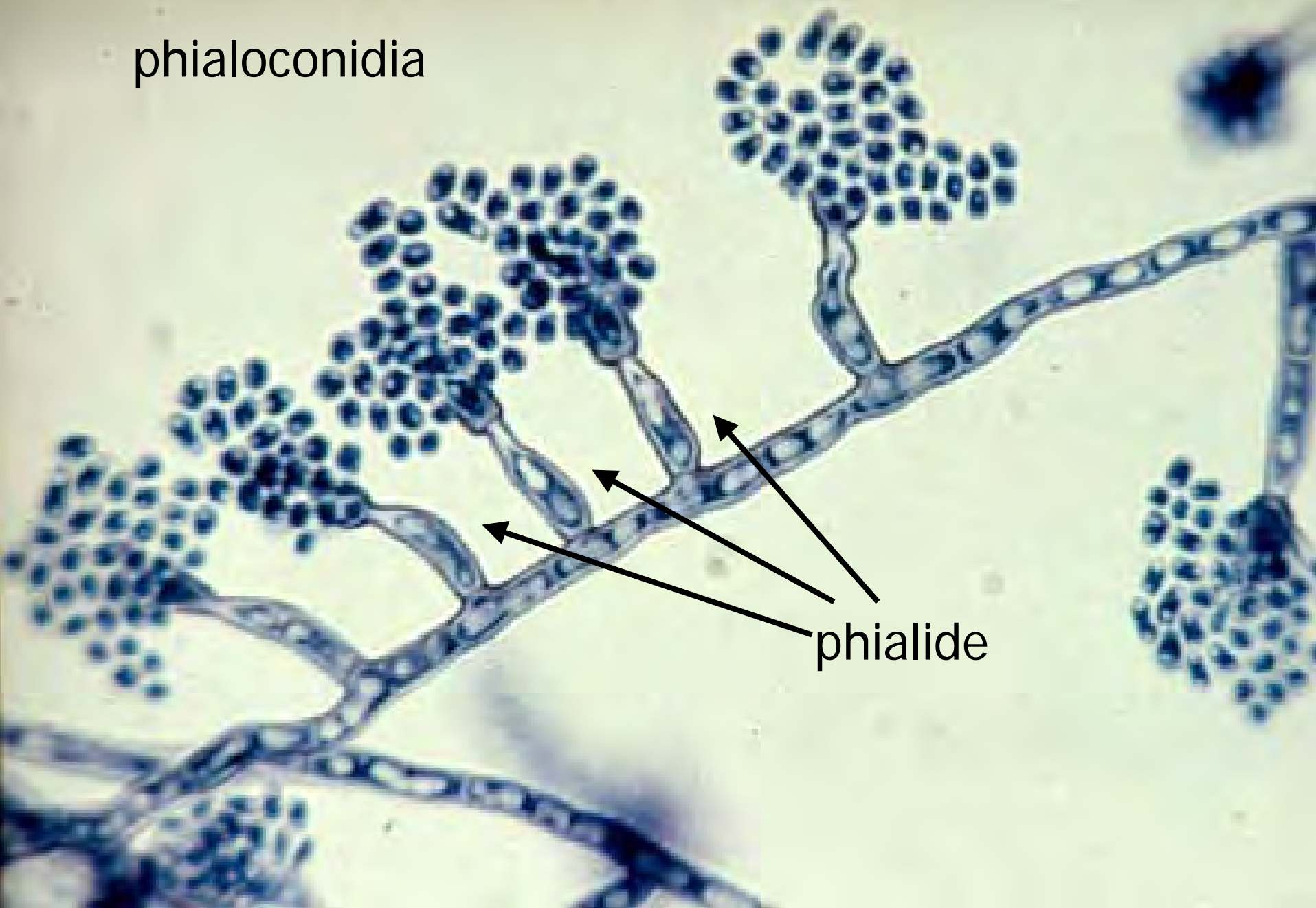
- Anneloconidia
- Phialoconidia
- Arthroconidia
- Chlamydoconidia
- Sporangiospore

annelloconidia

annellide



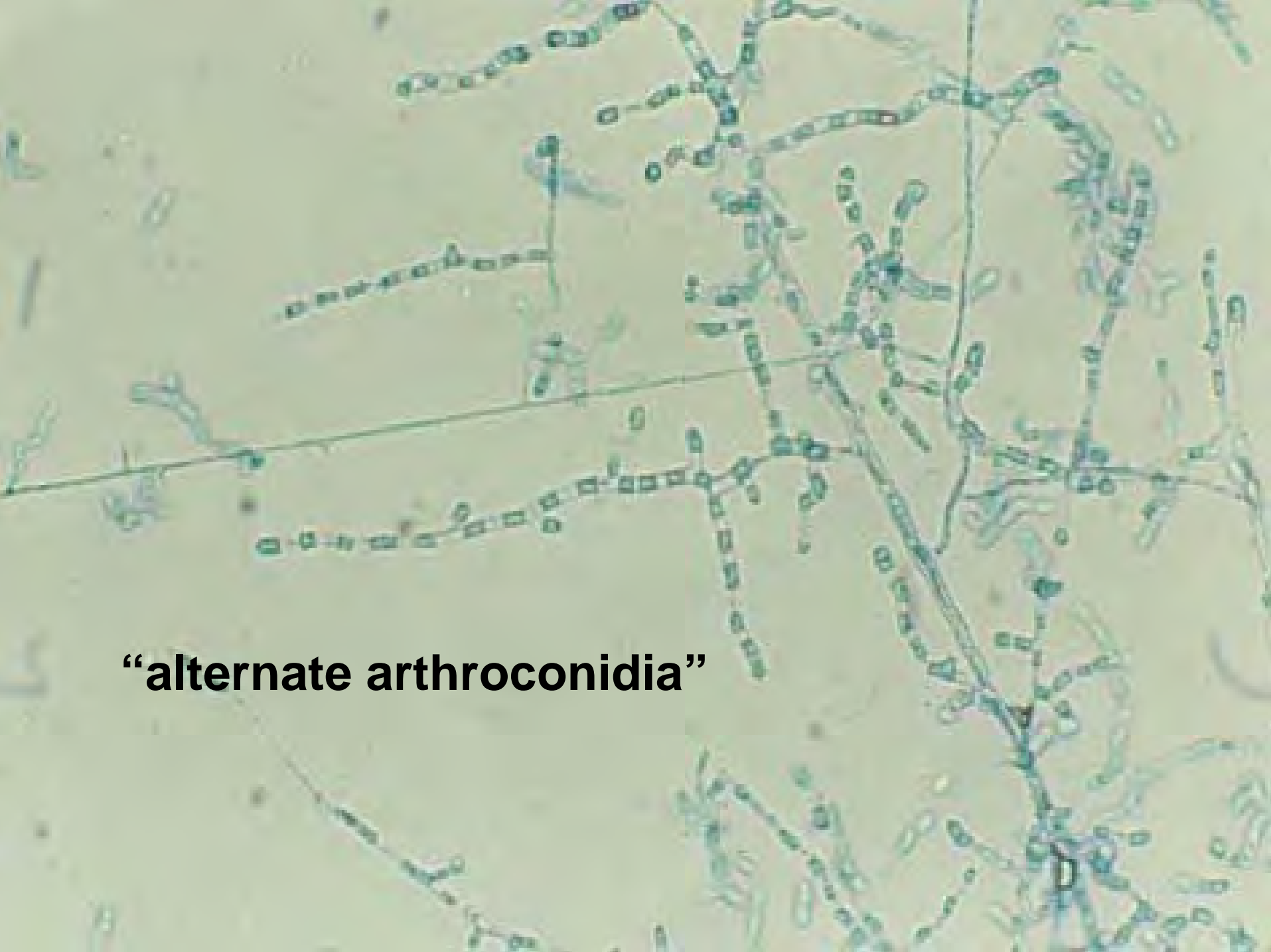
phialoconidia



phialide

A light micrograph showing numerous chains of arthroconidia. The chains are composed of dark blue, oval-shaped spores. The spores are arranged in long, thin chains, with some chains appearing to be broken or fragmented. The background is a light, pale blue color. The text "arthroconidia" is overlaid in the center of the image.

**arthroconidia**

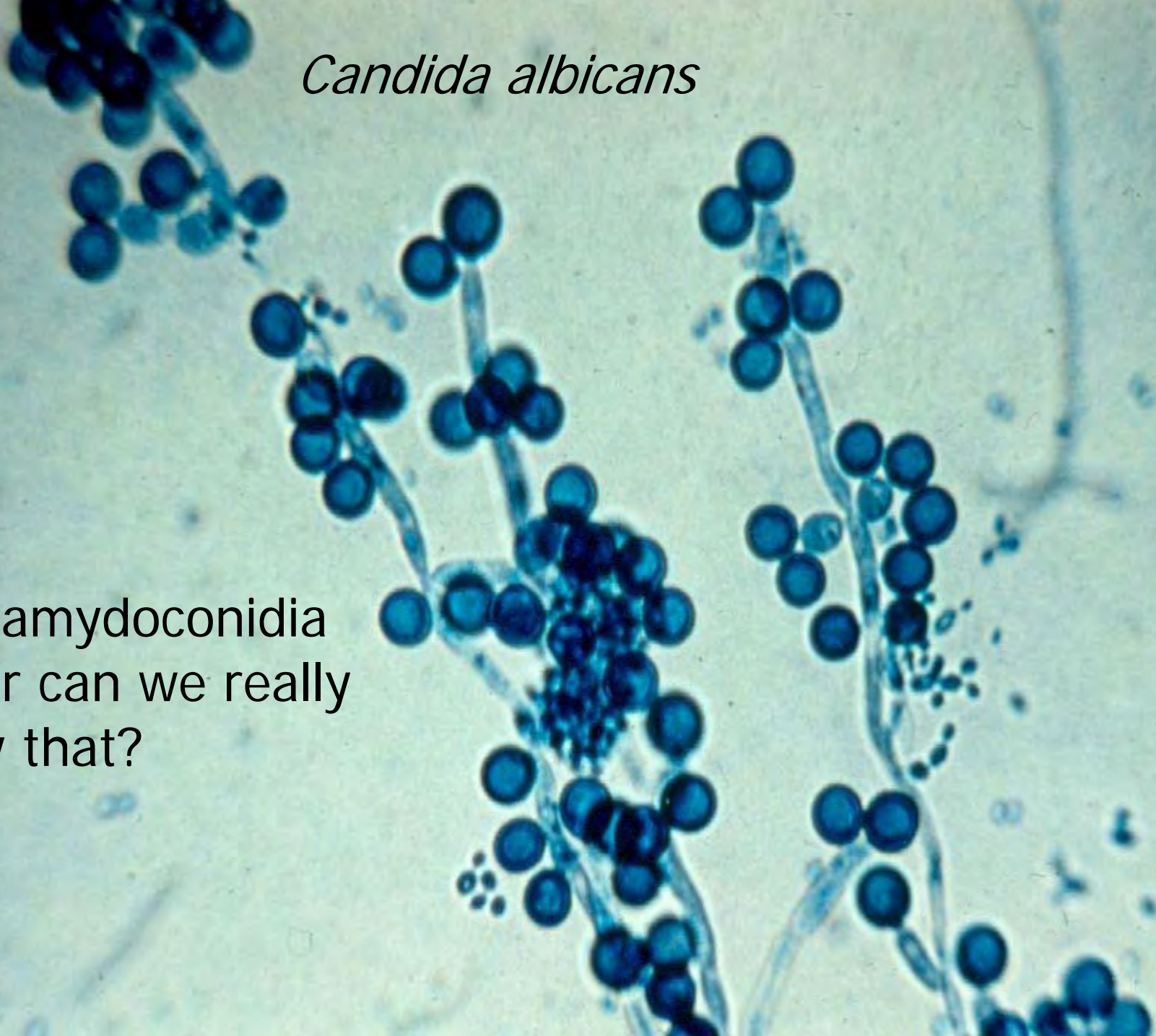


**“alternate arthroconidia”**



*Candida albicans*

Chlamydoconidia  
– or can we really  
say that?



*Rhizopus* species

sporangiospores



# More useful terms

- Hyaline – colorless
- Dematiaceous – dark pigmented
- Macroconidium/Microconidium
- Pleomorphic
- Dimorphic (diphasic)
- Echinulate
- Keratinophilic

Ringworm or Tinea - informal  
terms for Dermatophytosis

Tinea is derived from Latin for “worm” - we couple it with other Latin words to give specificity to the site of infection, e.g. *Tinea barbae*





tinea barbae( beard)

tinea capitis (head)

tinea corporis (body)

tinea manum (hand)

tinea cruris (groin)

tinea pedis (foot)



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# Classification of mycoses

- Superficial
- Cutaneous
- Subcutaneous
- Systemic
- Rare/opportunistic

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**tinea nigra palmaris**

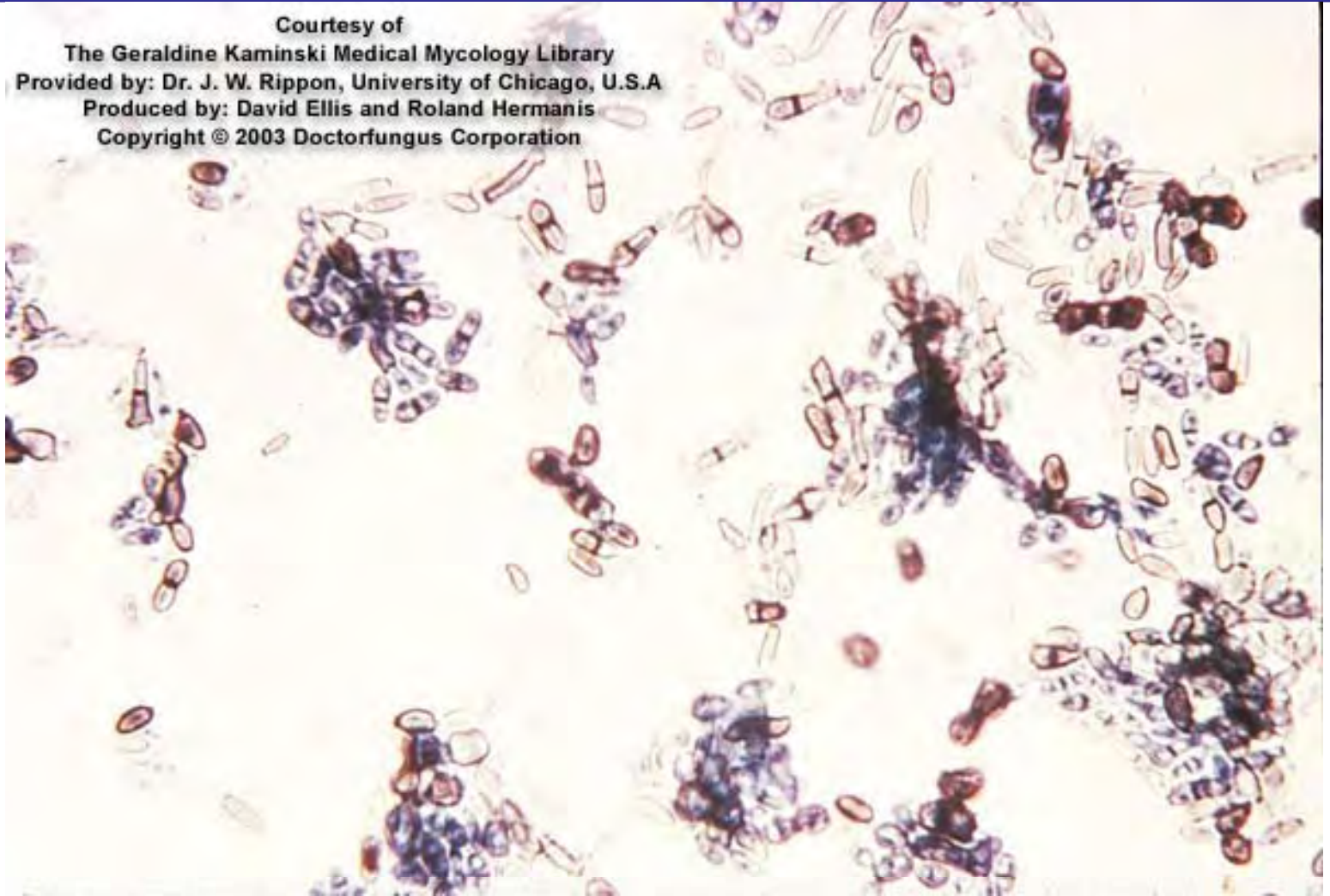


# Tinea nigra palmaris

- *Hortaea werneckii* (a.k.a. many, many wonderful names over the years e.g. *Phaeoannellomyces*, *Cladosporium* *Exophiala*)
- black, yeastlike fungus
- pigmented lesions on skin, frequently on hands or feet

# *Exophiala werneckii*

Courtesy of  
The Geraldine Kaminski Medical Mycology Library  
Provided by: Dr. J. W. Rippon, University of Chicago, U.S.A  
Produced by: David Ellis and Roland Hermanis  
Copyright © 2003 Doctorfungus Corporation



• **Genus/Species:** *Exophiala werneckii*

• **Image Type:** Microscopic Morphology

• **Slide Reference #:** GK 544

• **Disease(s):** Tinea nigra

**tinea versicolor**





Early lesion of chromoblastomycosis –  
but how do you **know**?



You could just wait...



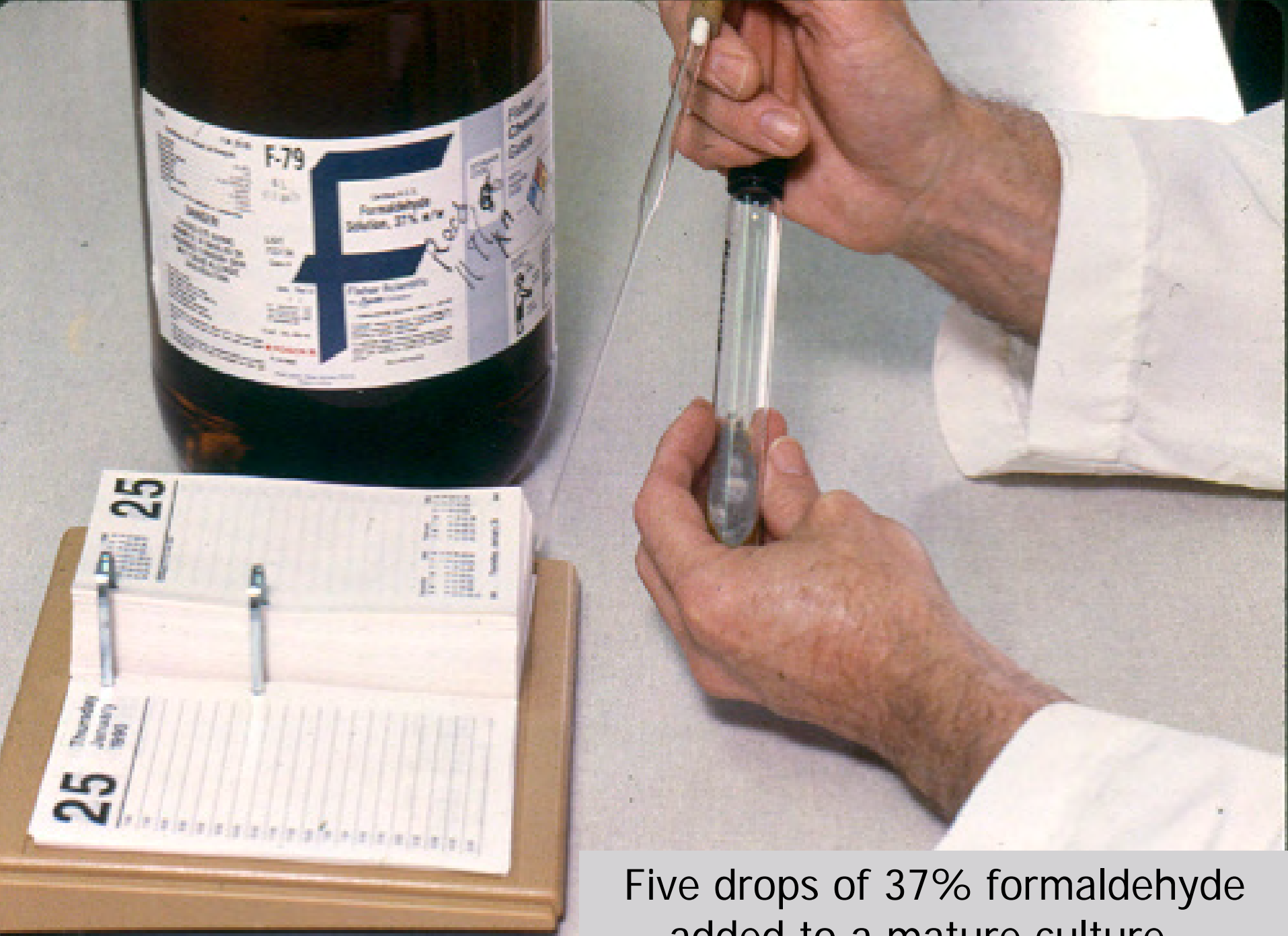
and wait...



Slide credit: Paul Jacobs  
Mycology Collection

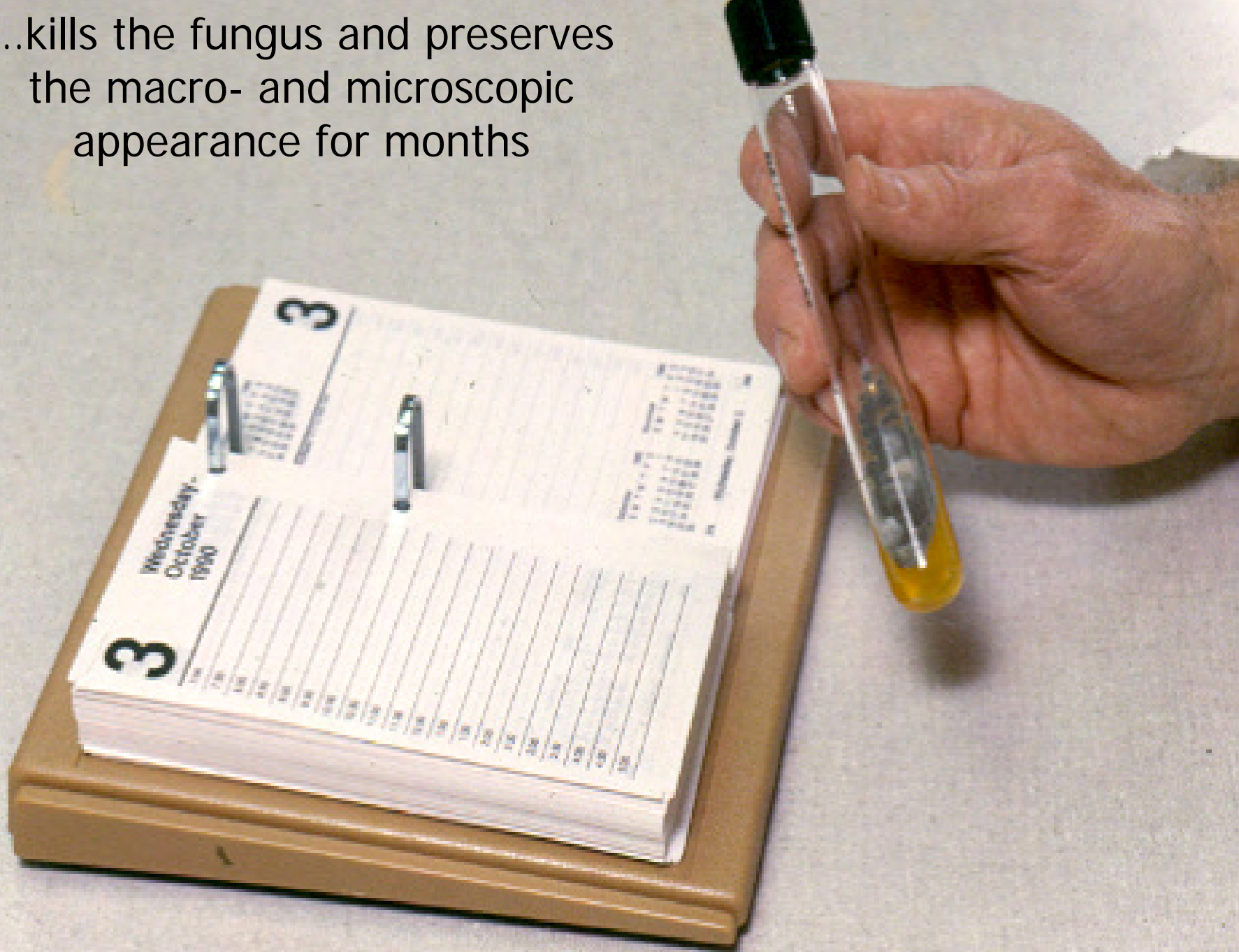
# “Identification of Medically Important Filamentous Fungi”

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Five drops of 37% formaldehyde added to a mature culture...

...kills the fungus and preserves  
the macro- and microscopic  
appearance for months



Slide culture mounts in  
lactophenol aniline blue with  
polyvinyl alcohol remain good  
for years as teaching aids

# Hands-on

a major distinction and value  
aspect of our workshop:  
manipulating fungi and looking at  
them through a microscope

(Our hands “teach” our brains!)

**Never underestimate  
the value of hands-on training**





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# Locally developed methodologies presented in our two day, filamentous fungus workshop

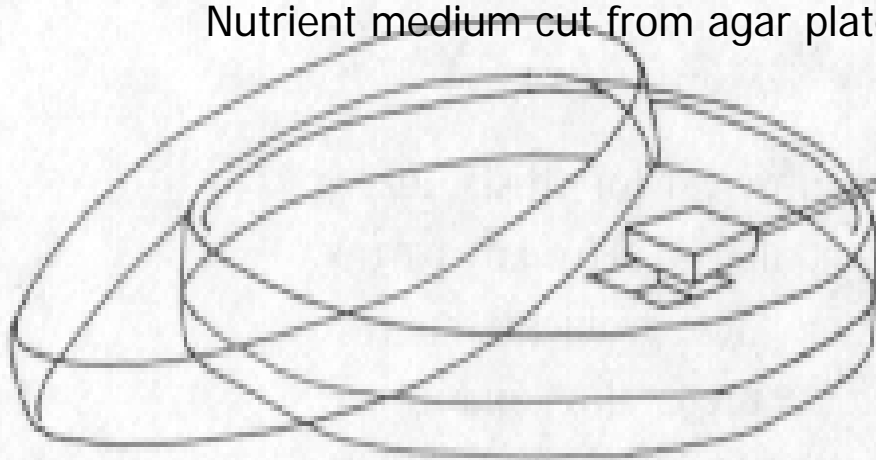
- Modified slide culture method
- Modified tape touch technique

# Traditional slide culture method

## (Riddell Slide Culture)

- Culture dish
- Filter paper and bent glass rod
- Sterile slide with block of nutrient medium
- Sterile water to be added as needed

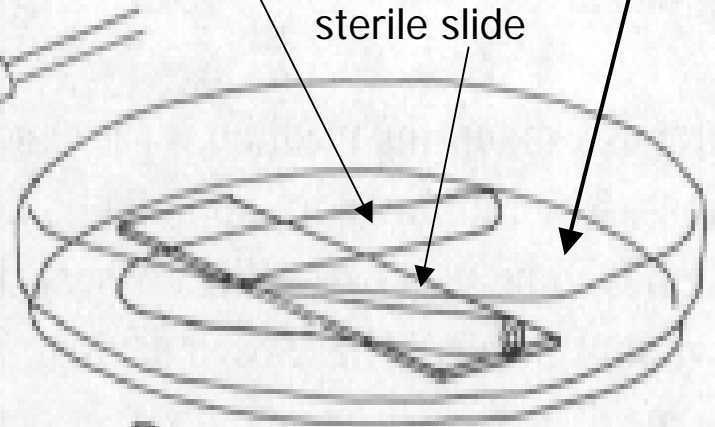
Nutrient medium cut from agar plate



A

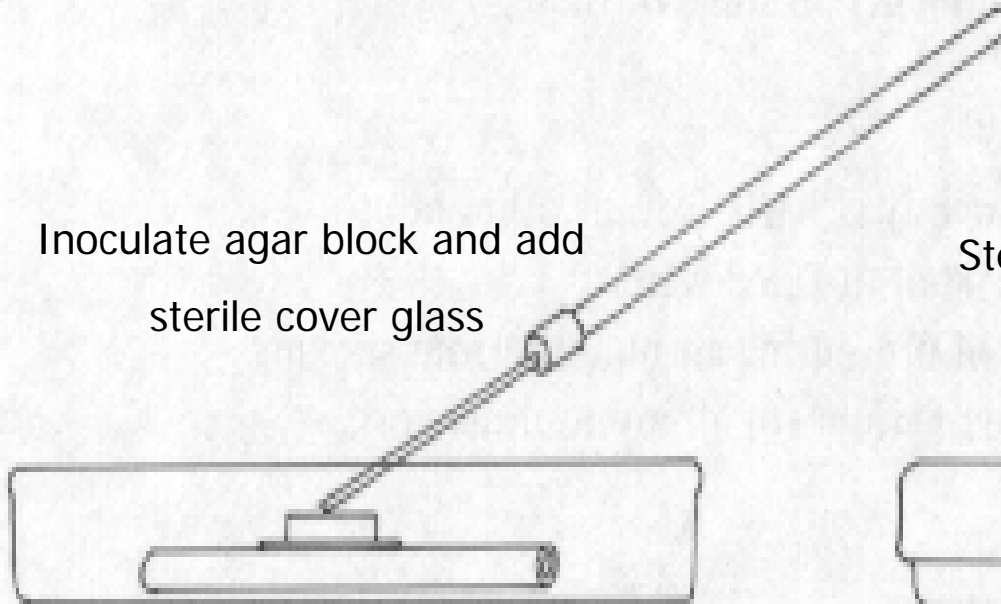
Bent glass rod filter paper

sterile slide



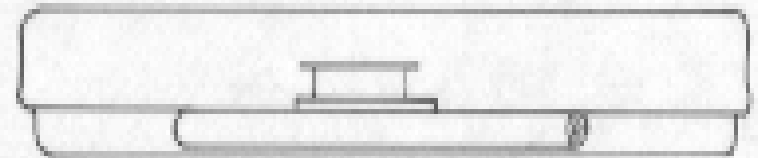
B

Inoculate agar block and add  
sterile cover glass



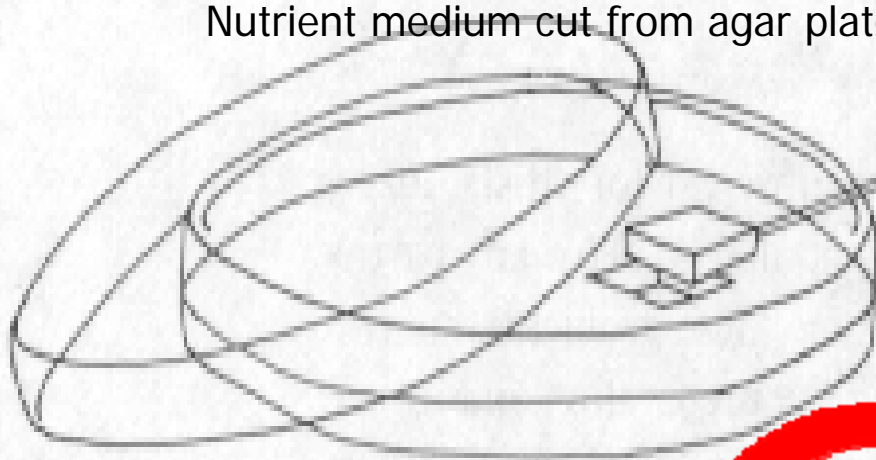
C

Sterile water added to  
filter paper



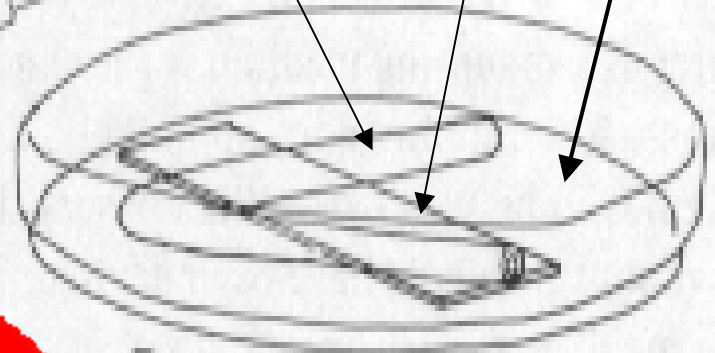
D

Nutrient medium cut from agar plate

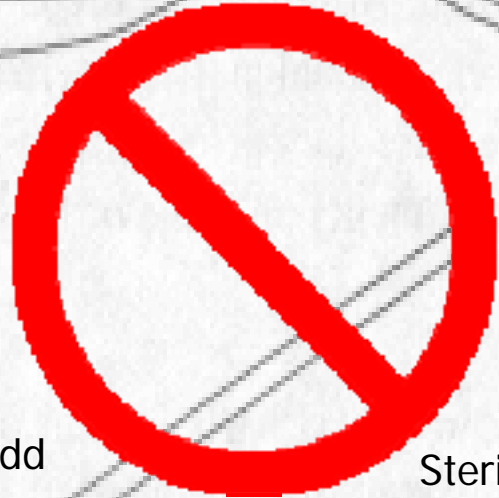


A

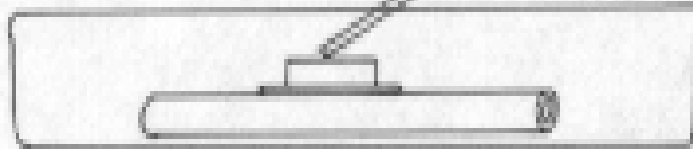
Bent glass rod  
sterile slide  
filter paper



B

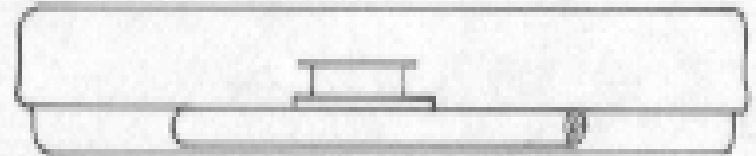


Inoculate agar block and add  
sterile cover glass



C

Sterile water added to  
filter paper



D

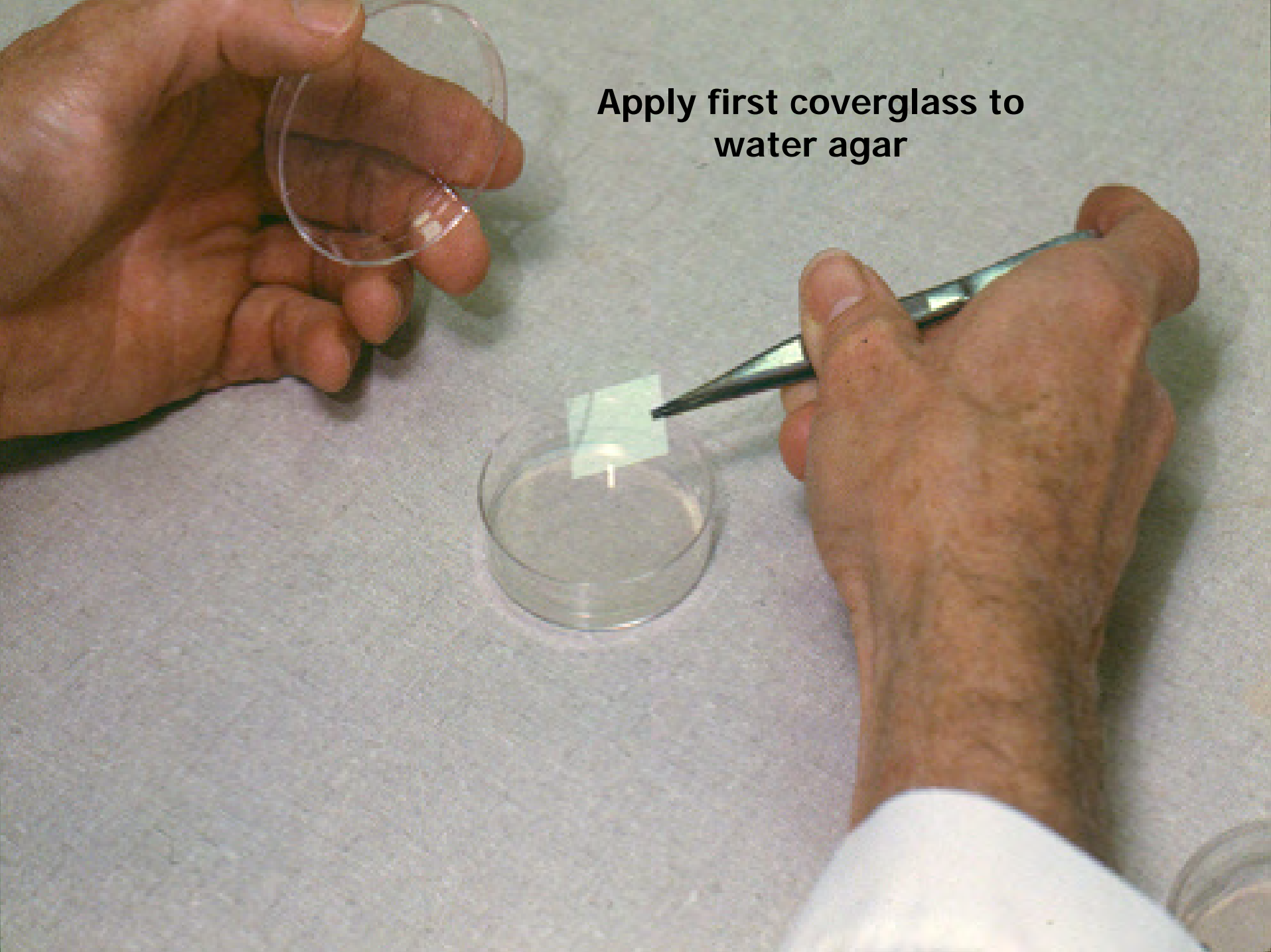
# Modified slide culture method

Journal of Clinical Microbiology

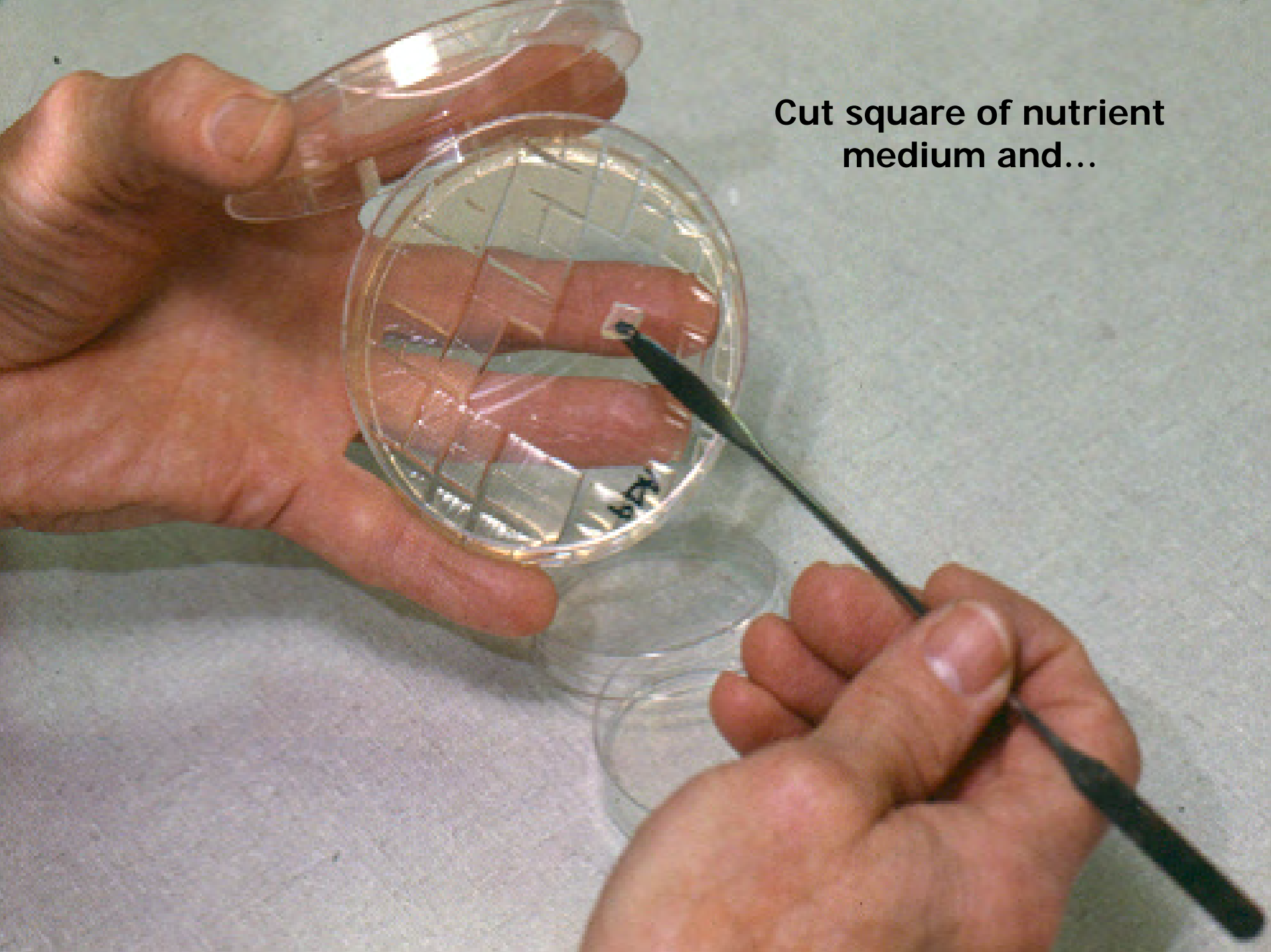
Vol 24, No. 3, p. 460-461

- Water agar plate
- Sterile cover glasses
- Sterile applicator sticks
- Nutrient medium
- Mounting medium

**Apply first coverglass to  
water agar**

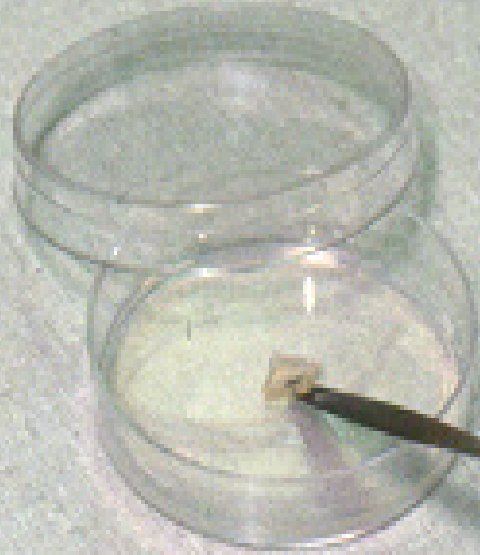


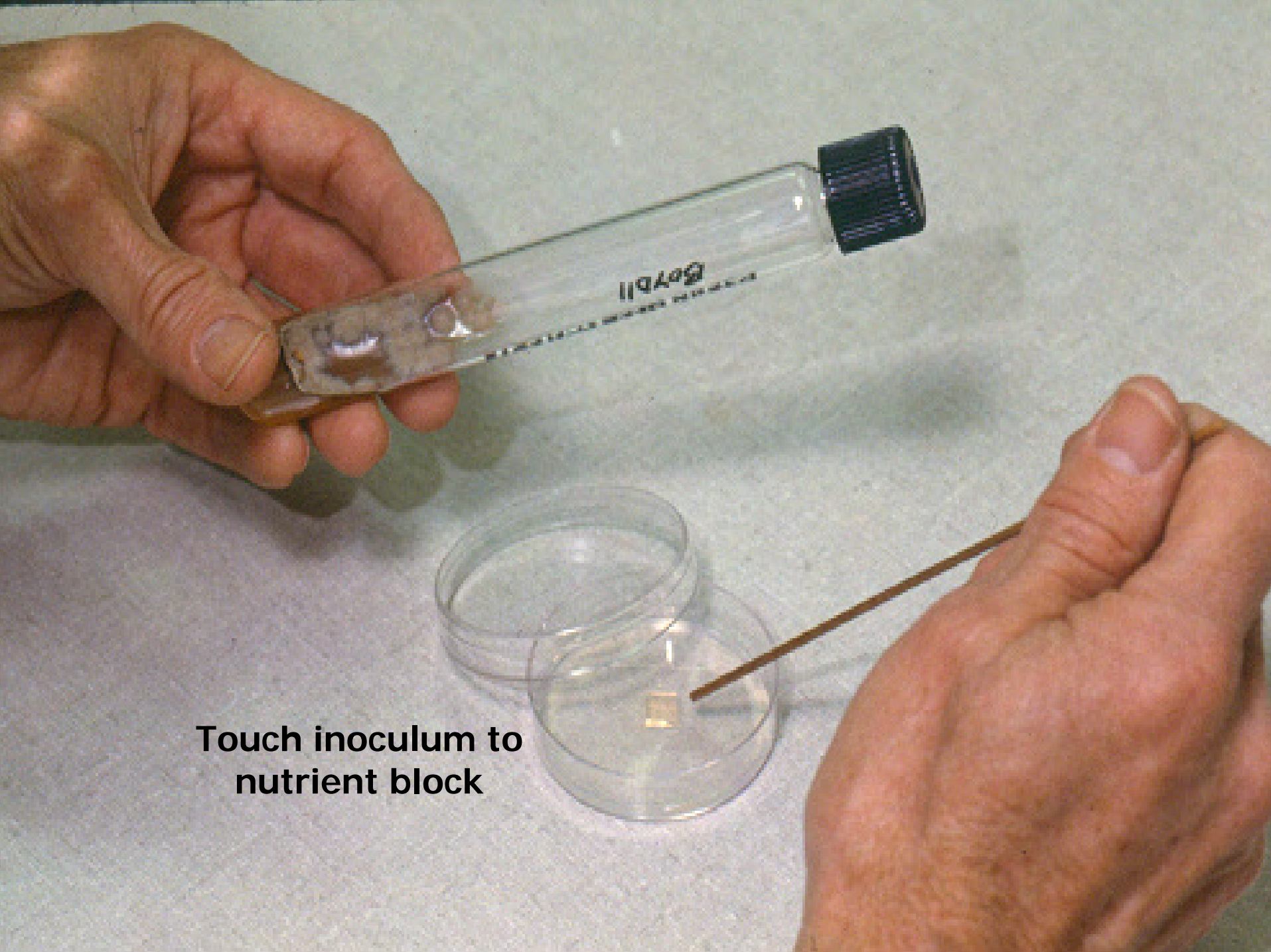
**Cut square of nutrient  
medium and...**



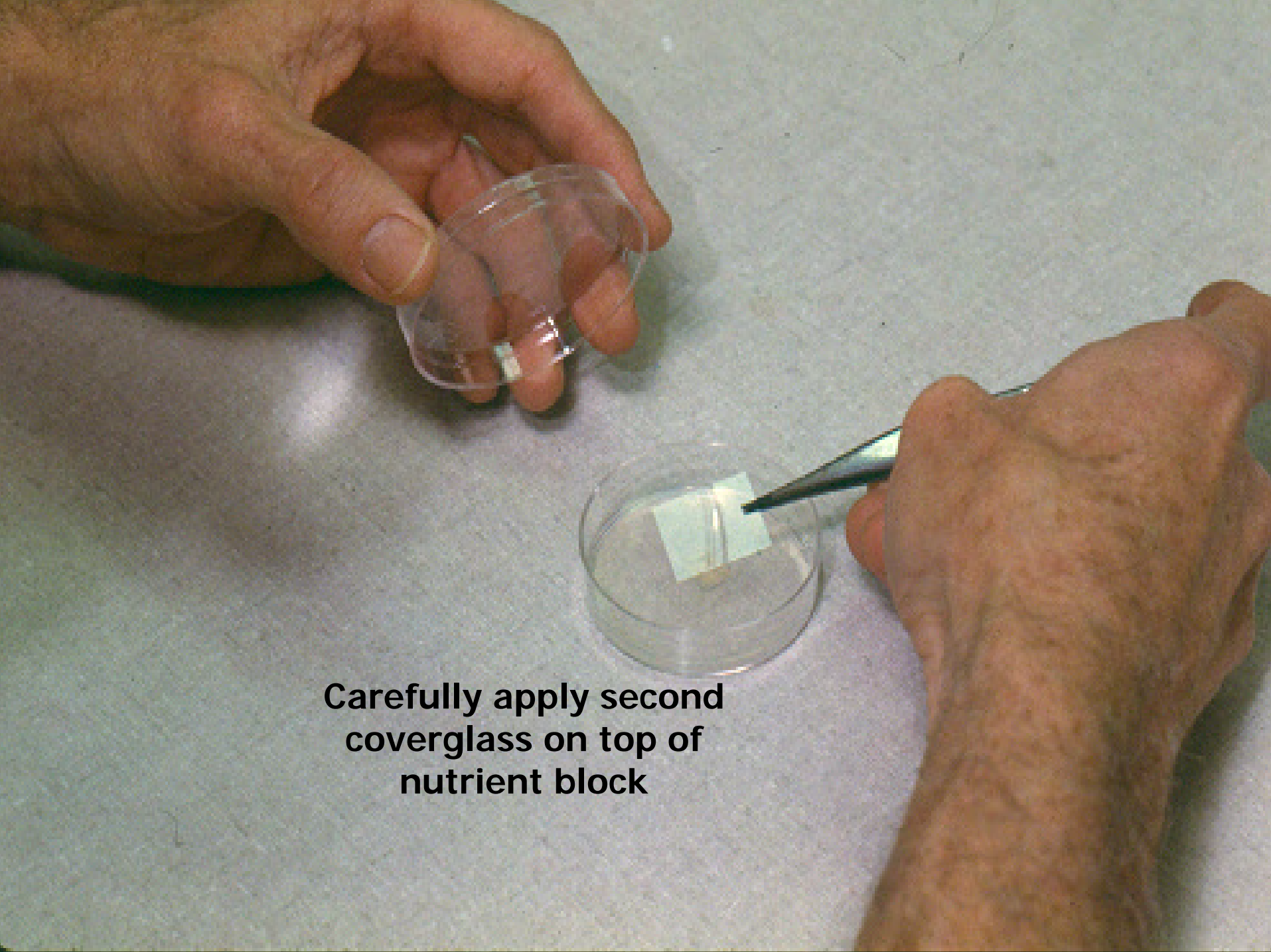


....apply to center of  
coverglass



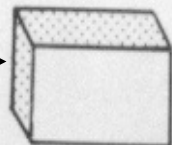
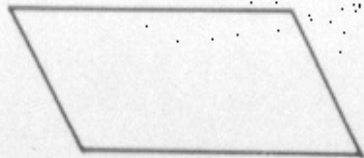
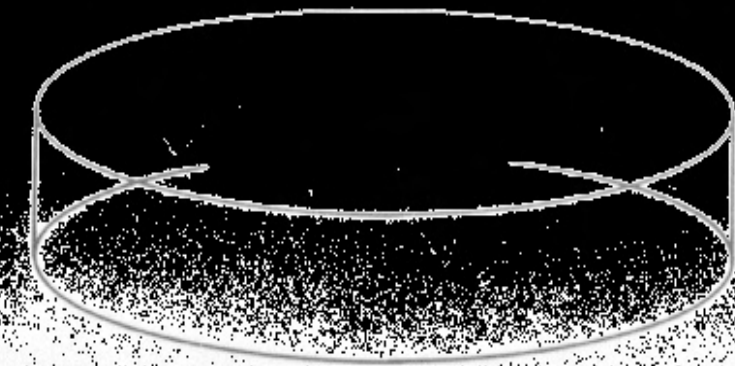


**Touch inoculum to  
nutrient block**

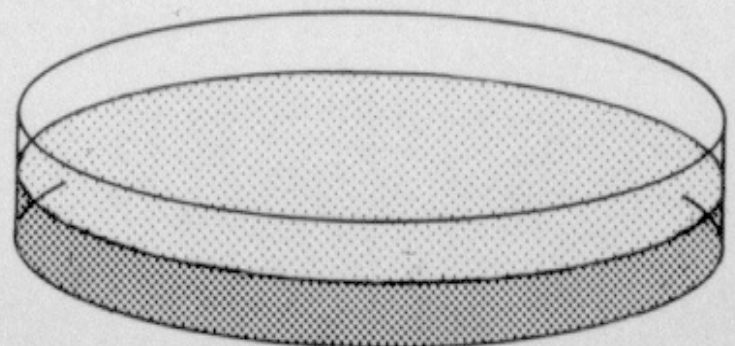
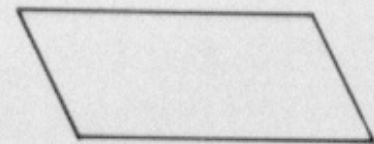


**Carefully apply second  
coverglass on top of  
nutrient block**

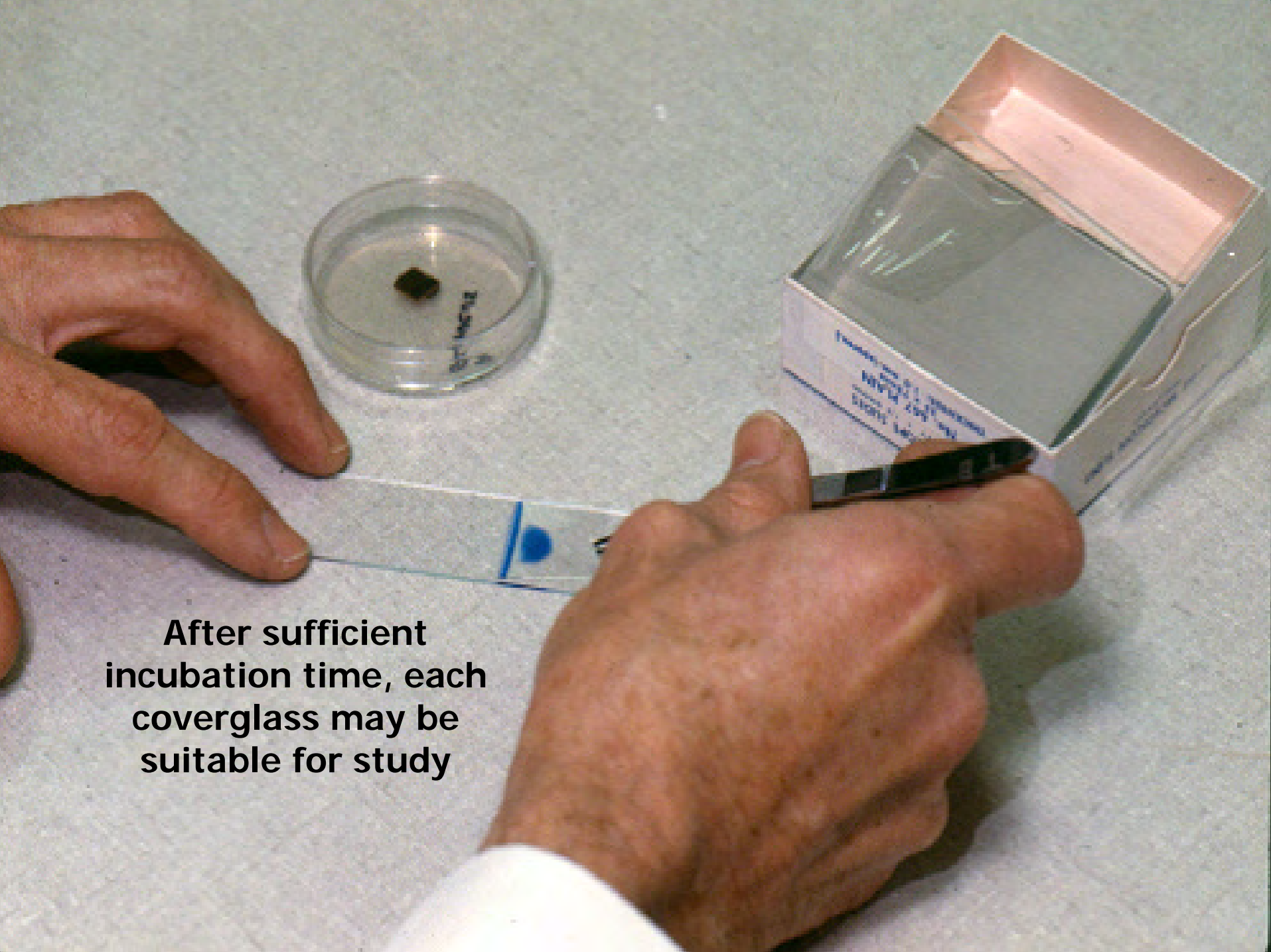
**Inoculum  
applied here** →



**NUTRIENT AGAR**




**WATER AGAR**



**After sufficient incubation time, each coverglass may be suitable for study**

One more plus -small dishes can incubate in small space





Slide culture is easily viewed  
at low magnification before  
mounting

*Aspergillus niger*

# The Tape Touch Technique

In the last 15 or so years, it has become a common practice to lift fungal growth from an agar surface with clear tape, stick it on a slide and examine it microscopically.



# Troubles with the Traditional Tape Touch Technique using clear cellophane tape:

- Tape - sticks to gloves
- ripples and curls
- is difficult to tear

# Safe, Low Distortion Tape Touch Method for Fungal Slide Mounts

Journal of Clinical Microbiology

Vol 38, No. 12, p. 4683-4684

# Modified tape touch mounts

- No more tape stuck to gloves
- Mounts lie flat – easier viewing
- Uses readily available frosted tape
- Fingers are kept out of the fungi

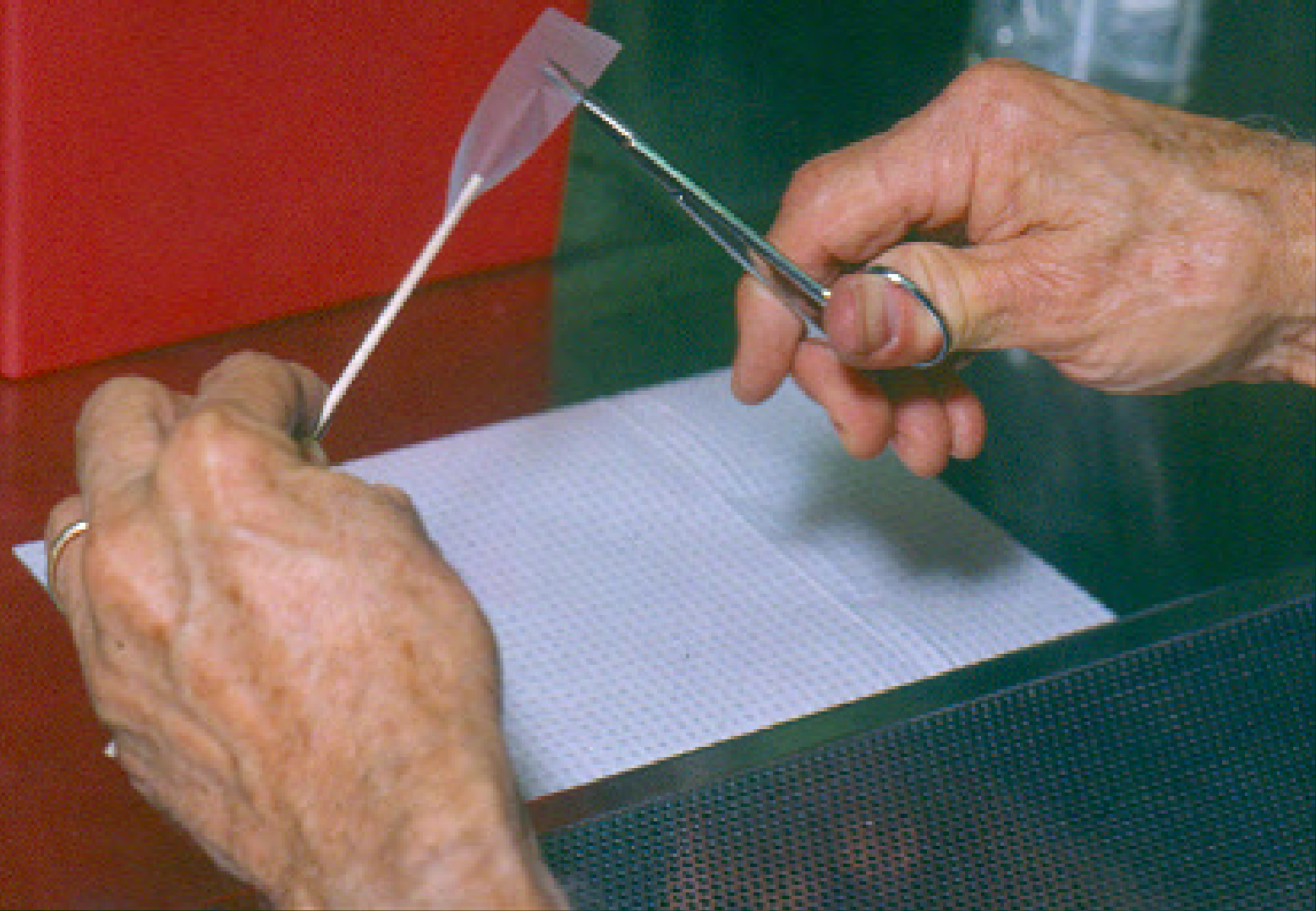
**Apply a two inch piece  
of frosted tape to end  
of applicator stick**



Pinch sticky surface of tape  
around stick



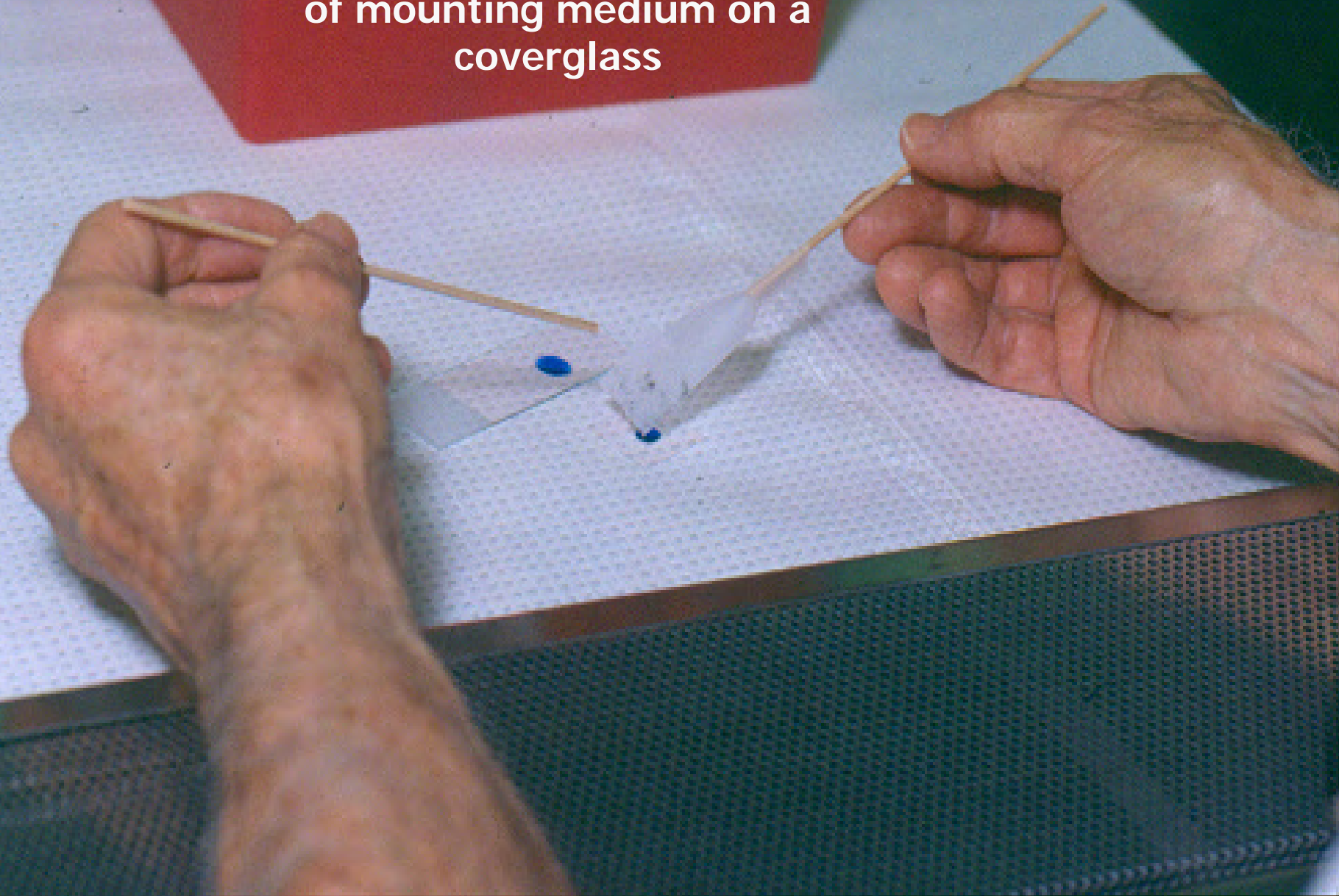
Cut about half way across  
width of tape



Press sticky side of tape onto colony using second applicator stick

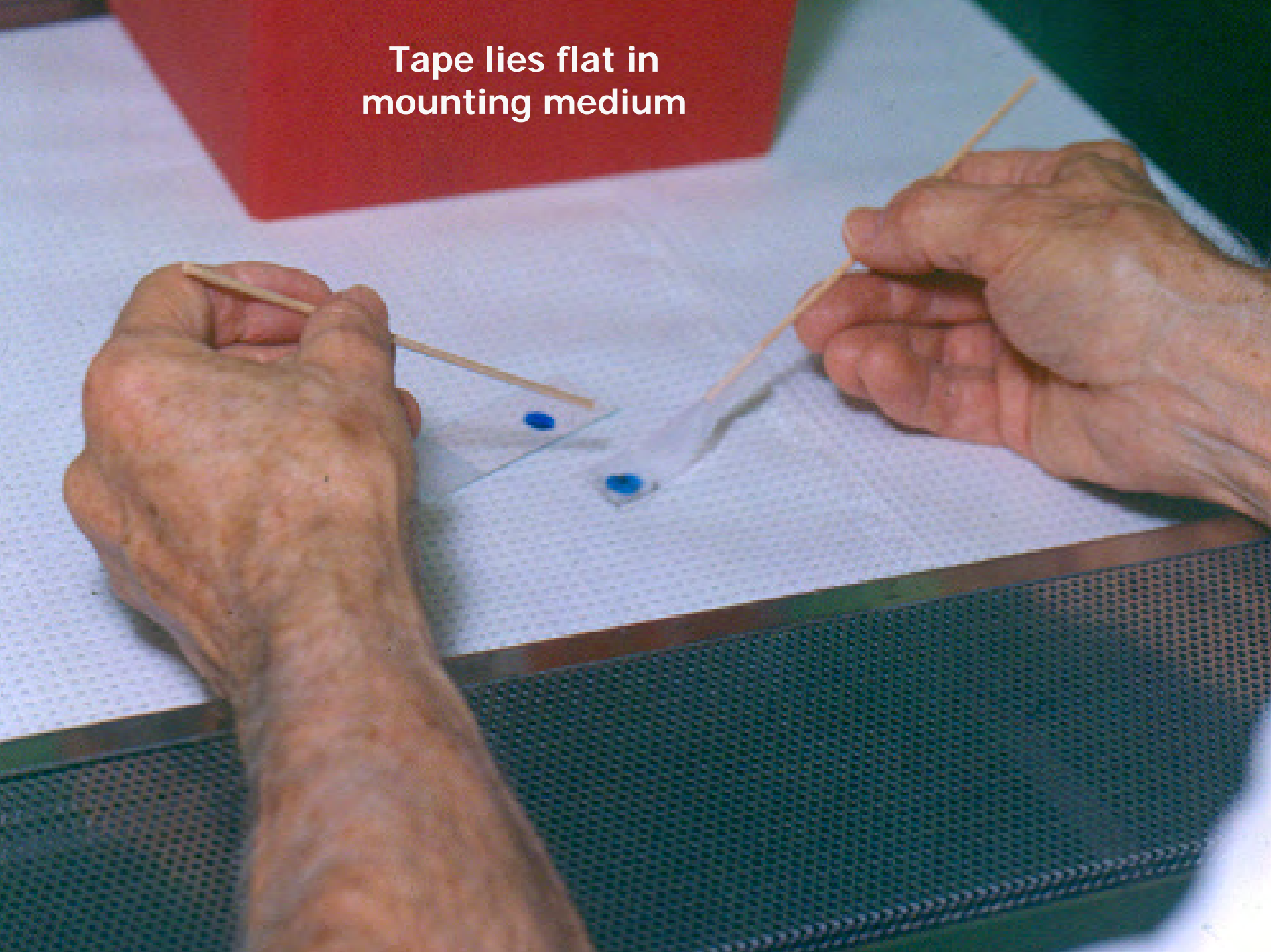


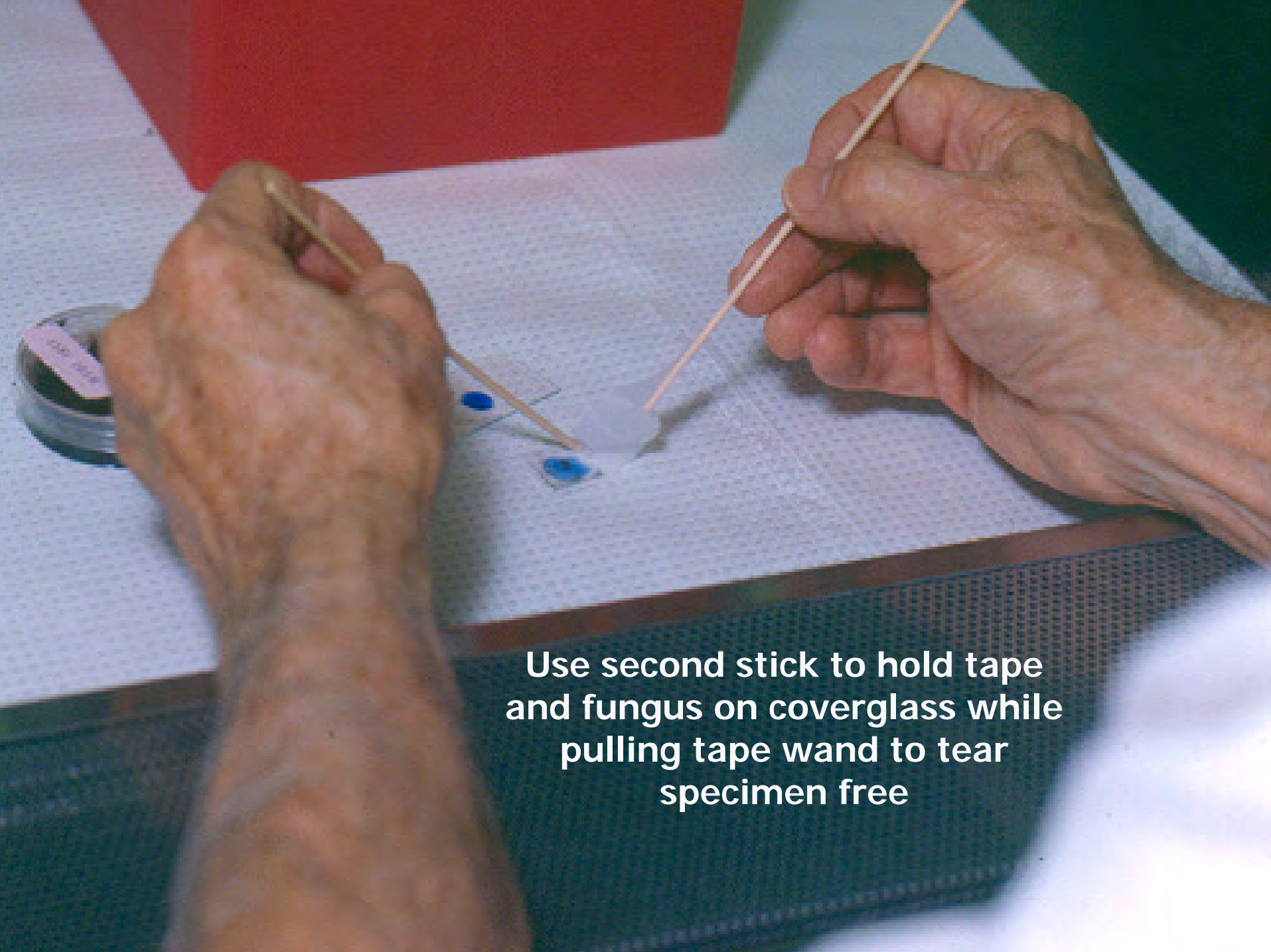
Gently lay sticky side of tape with fungus in a drop of mounting medium on a coverglass





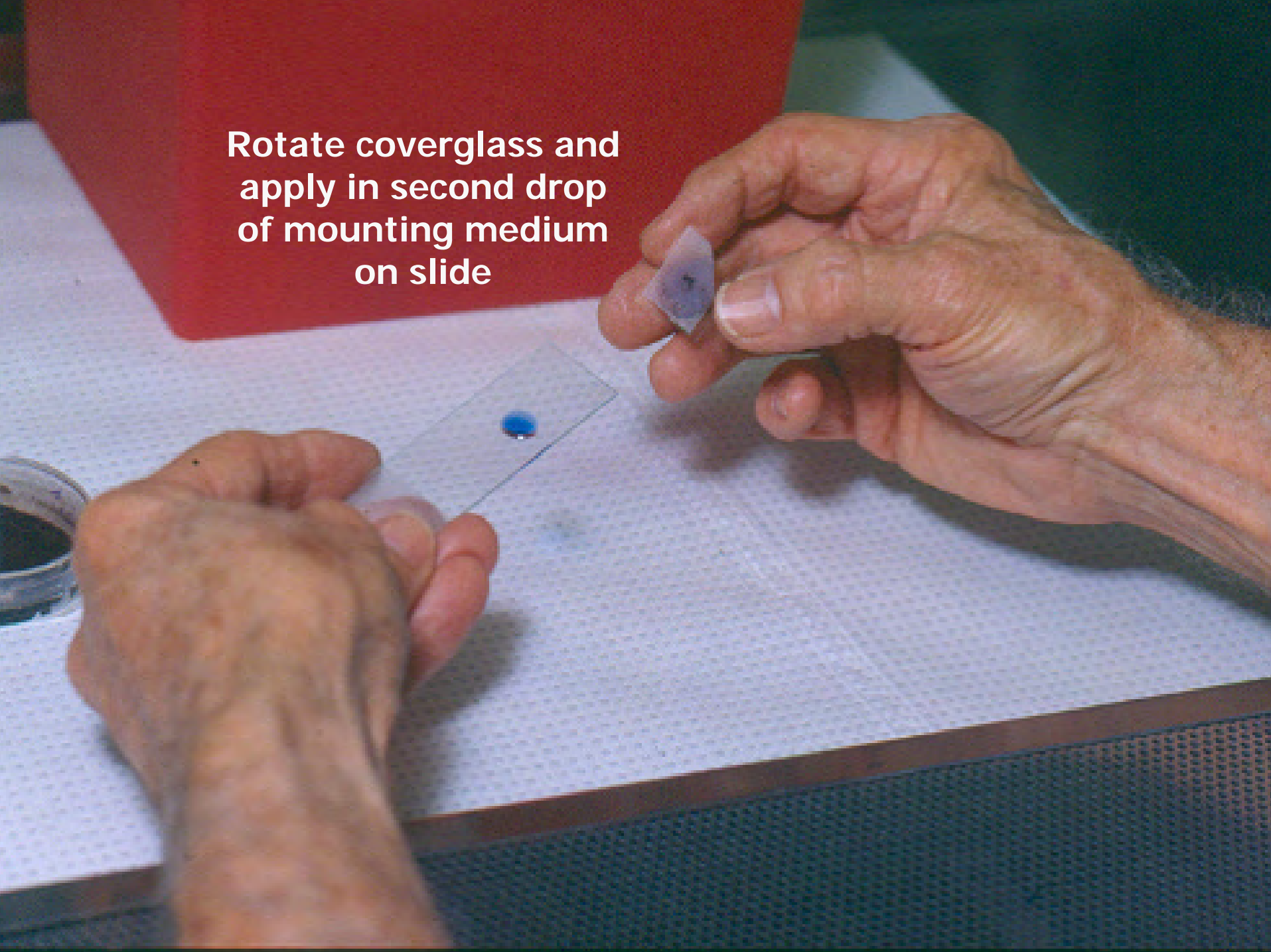
Tape lies flat in mounting medium

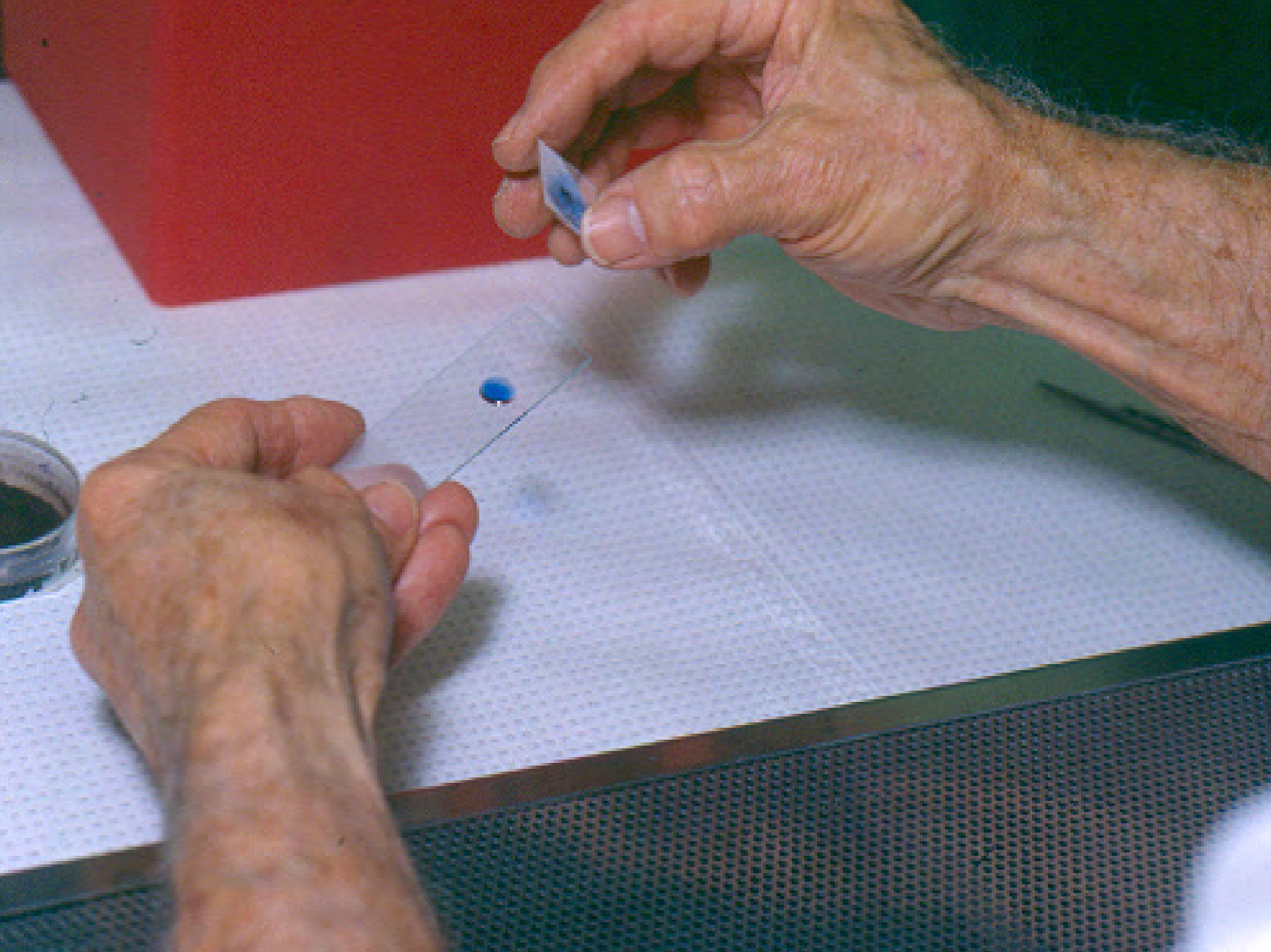


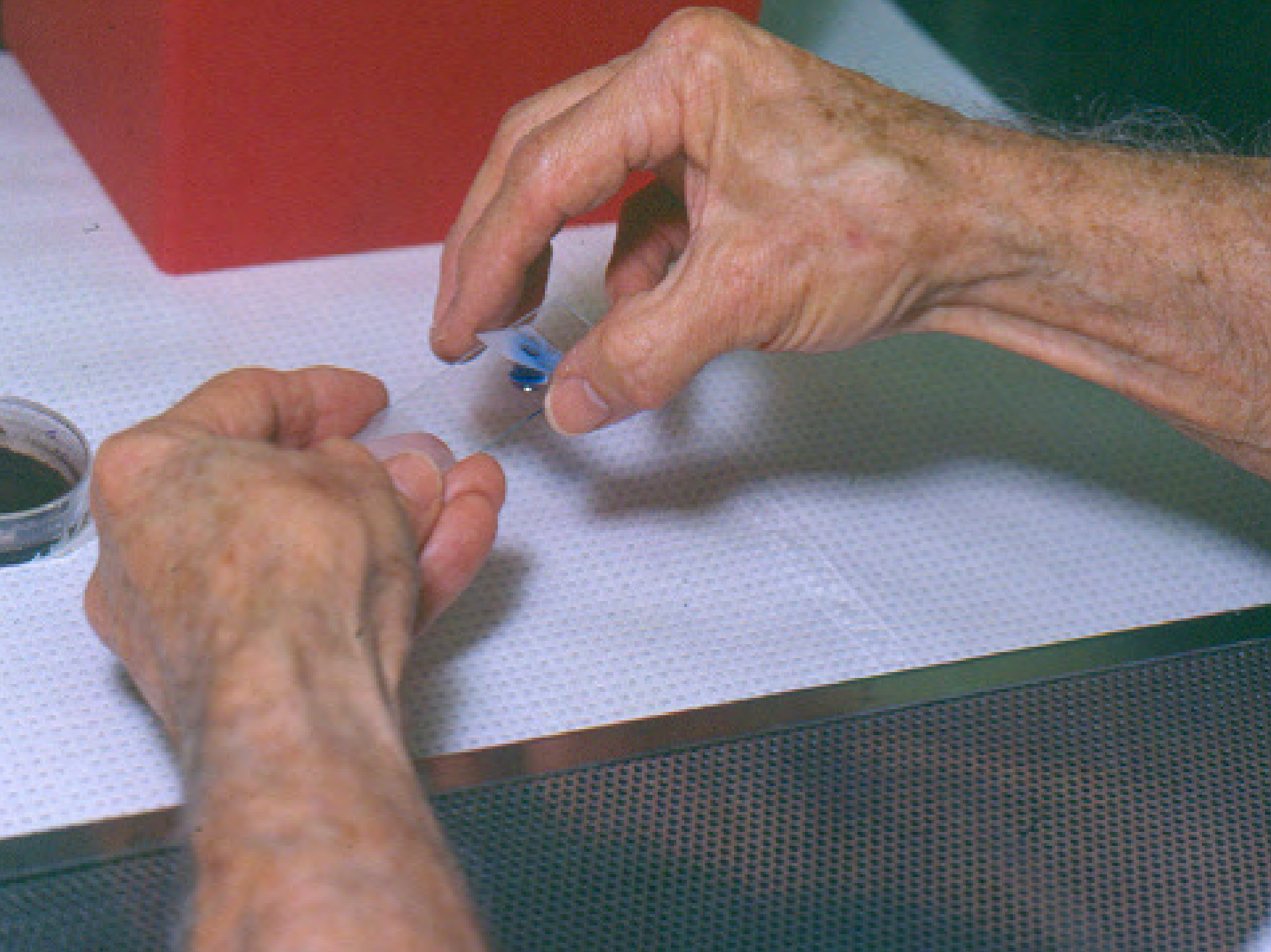


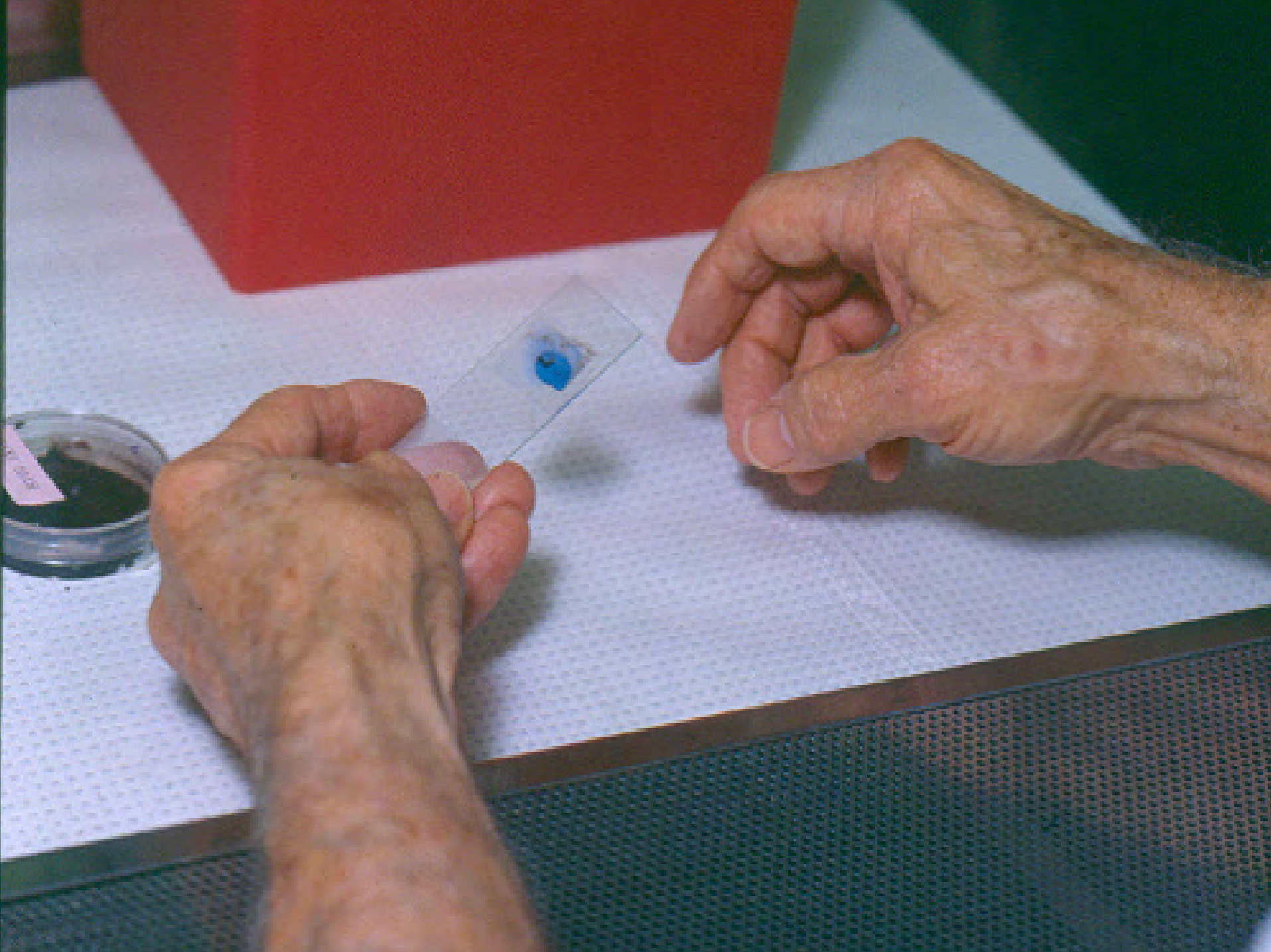
**Use second stick to hold tape and fungus on coverglass while pulling tape wand to tear specimen free**

**Rotate coverglass and  
apply in second drop  
of mounting medium  
on slide**





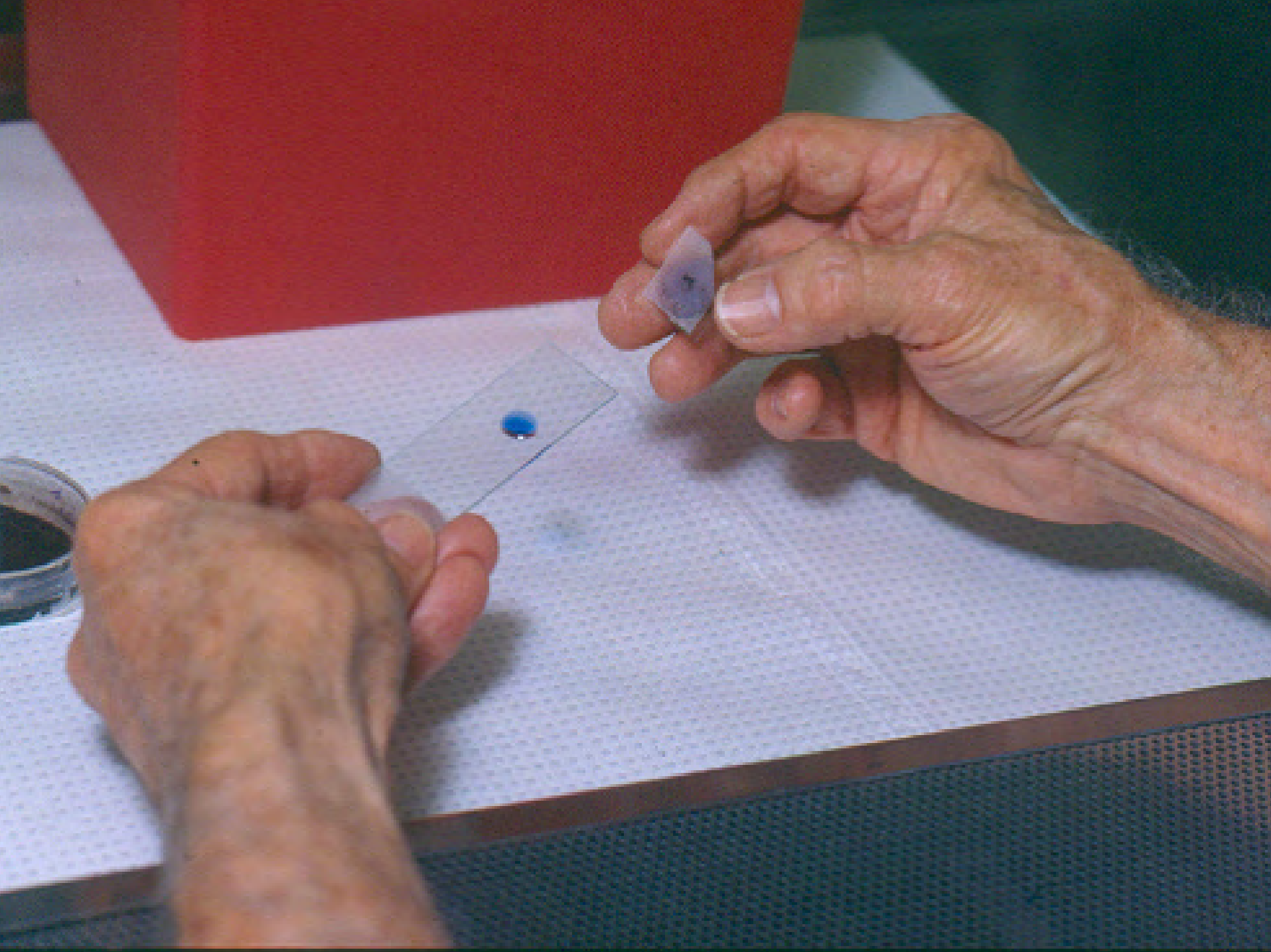




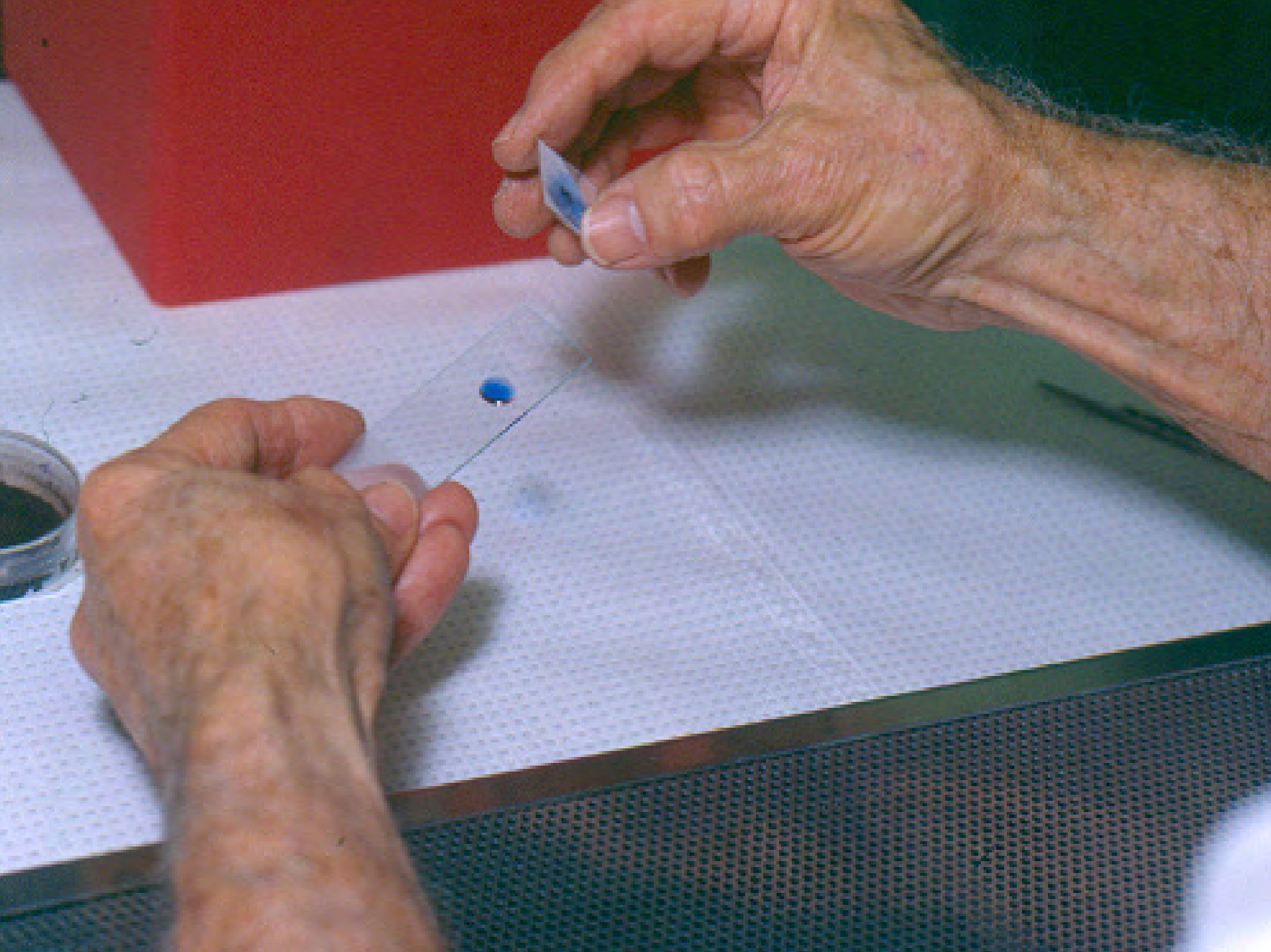
**WOW!!**

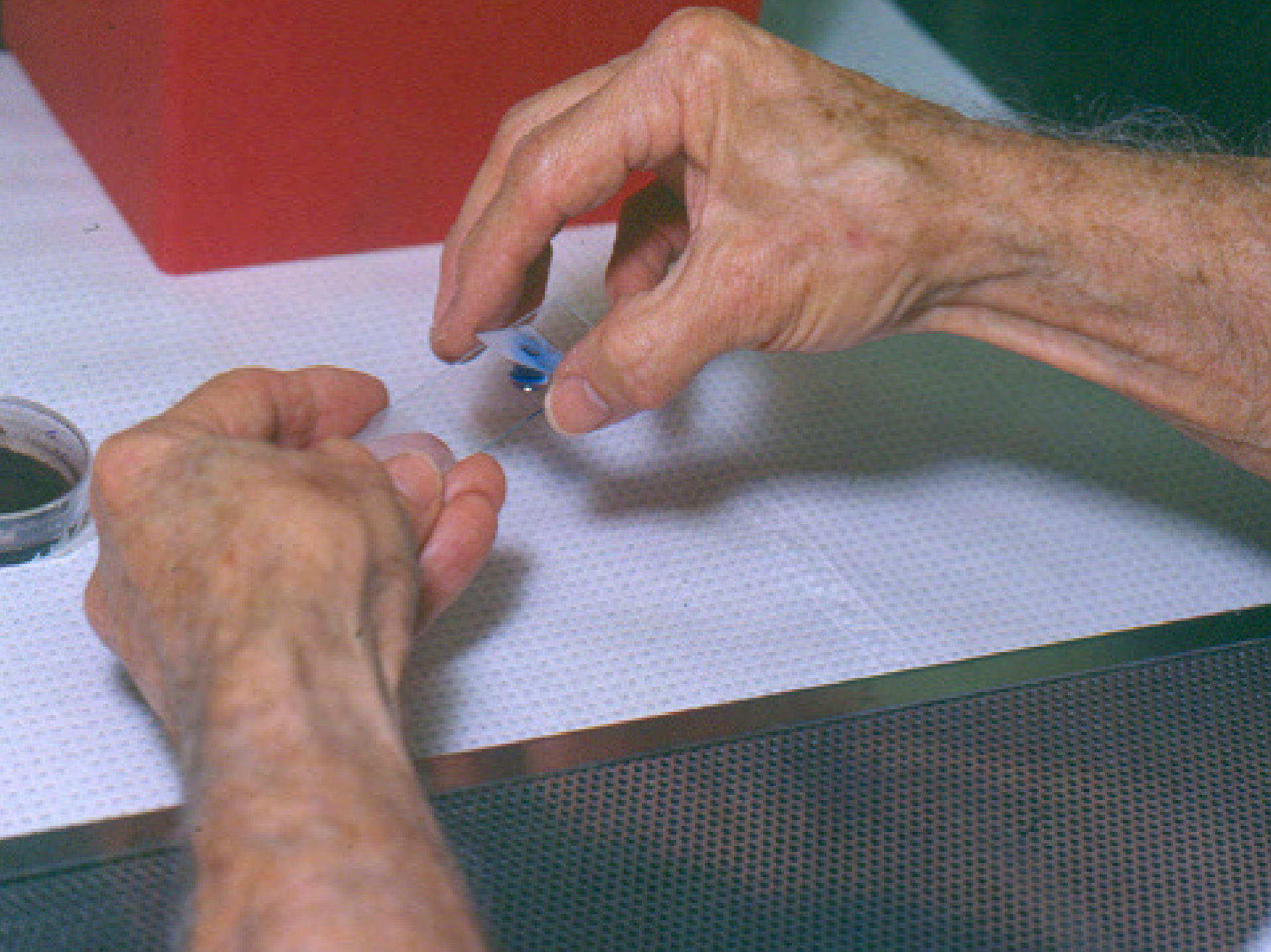
**Can we have an  
instant replay??**

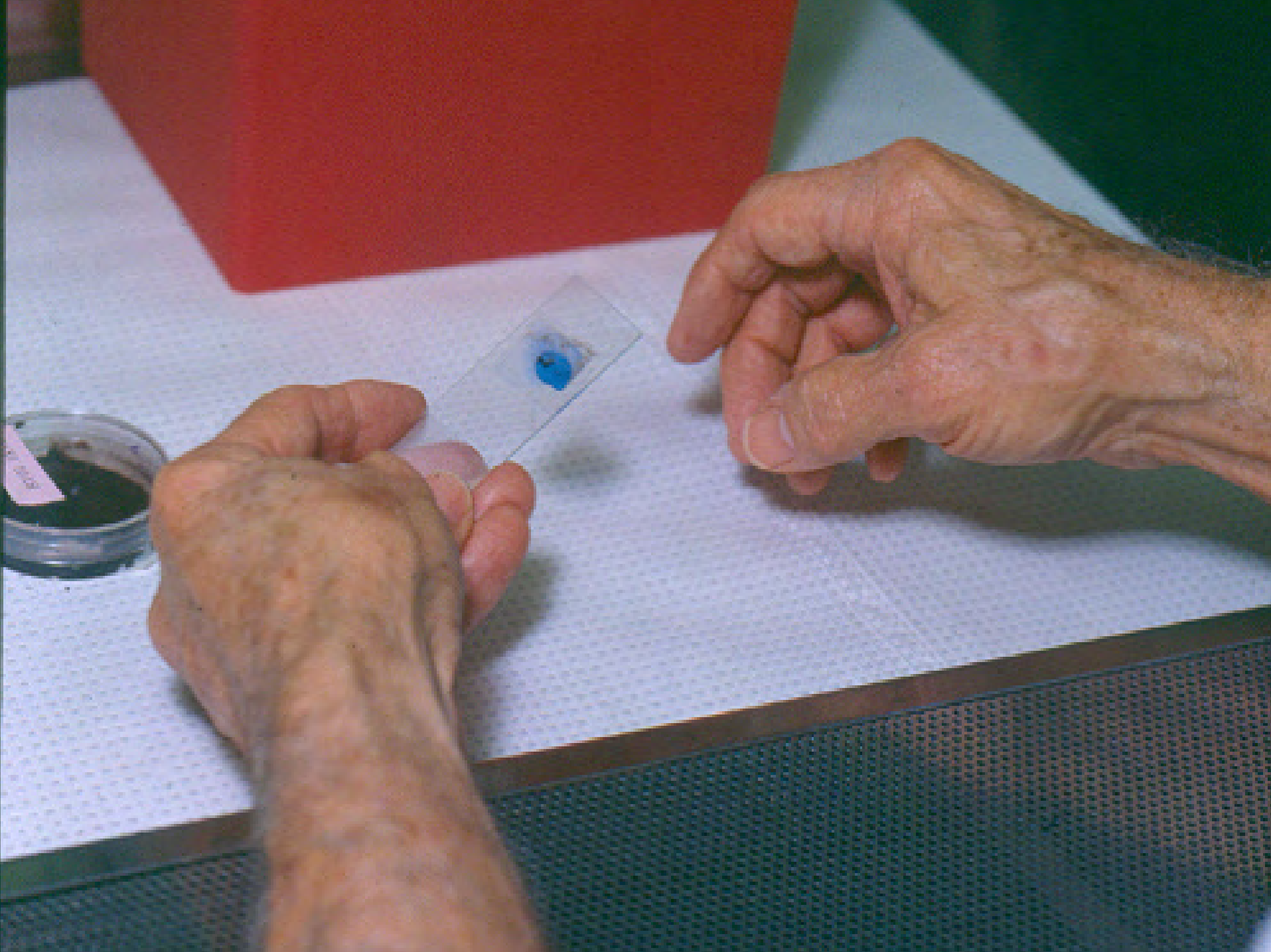












## The end result of the Modified Tape Touch Method:

- fungus is viewed in the mounting medium directly below the coverglass

- tape, lying flat behind the fungus, causes no distortion of the image

# Cautionary Note:

Workshop always includes use of traditional tease mount preparation for microscopy as an acceptable and standard method for study of filamentous fungi. On rare occasion, some critical information may be missed by slide culture or tape touch methods.

# Beyond our two day workshop, another important mycology training activity

- An intermediate to advanced mycology training event held immediately before the American Society for Microbiology annual meeting.
- Sometimes hands-on, usually one day program
- Often featuring well-known mycologists from U. S. or Canada
- Gives DSHS some national presence in training

# Still another important mycology training activity

- Specialty mycology workshops on a single genus of fungus.
- We have cosponsored with NLTN several workshops featuring internationally known experts focused on either *Aspergillus* species (Maren Klich - USDA) or *Penicillium* species (John Pitt - CSIRO in Australia)
- Participants from many places.

# And another introductory mycology training activity

- Invited lecture for medical technology students at Texas State University – even this has a hands-on component, using both tease mounts and slide cultures



None of this would currently  
be possible without approval and  
support by

Susan Neill

Eldridge Hutcheson

Liz Delamater







First documented evidence of  
Meals on Wheels

**That's all,  
folks!!**



