# Visualization PNA FISH

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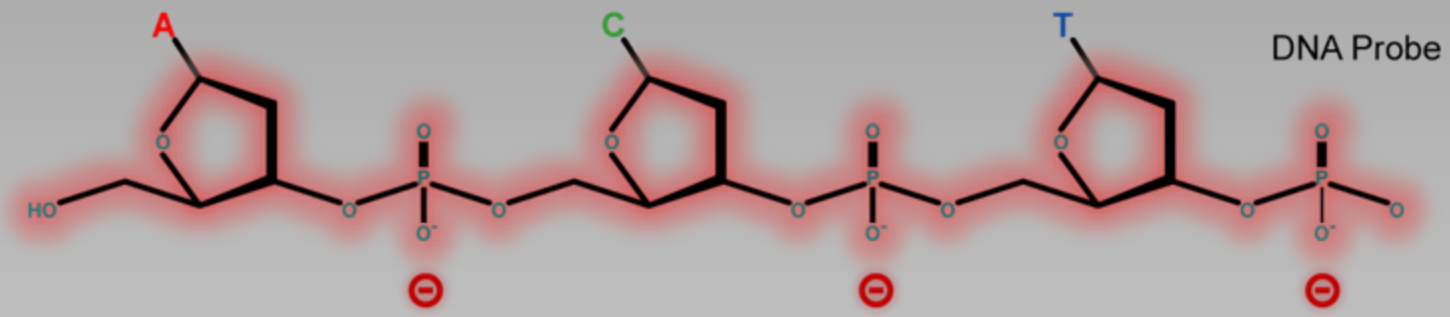
# PNA FISH

- **Peptide Nucleic Acid Fluorescence In Situ Hybridization**
- Fluorescent PNA probe hybridizing to ribosomal RNA
- Procedure takes 2 hours + microscopy
  - Expectorates
  - Lung tissue
  - Wound material
  - culture smears
  - etc.

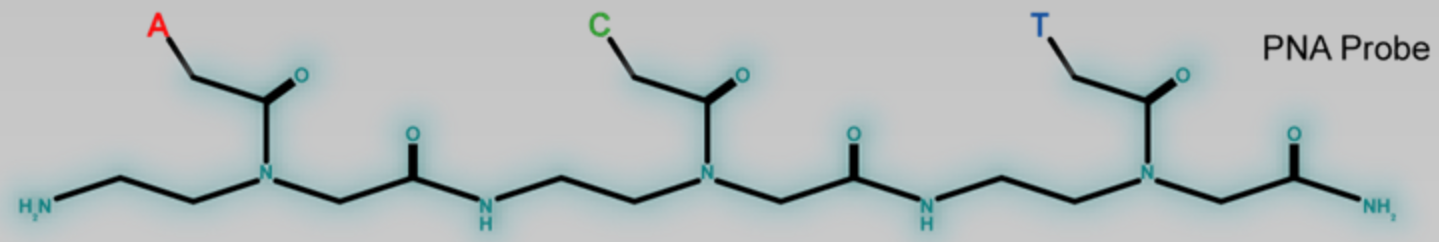
# PNA vs. DNA Technology

- ✓ The Backbone is the difference

DNA



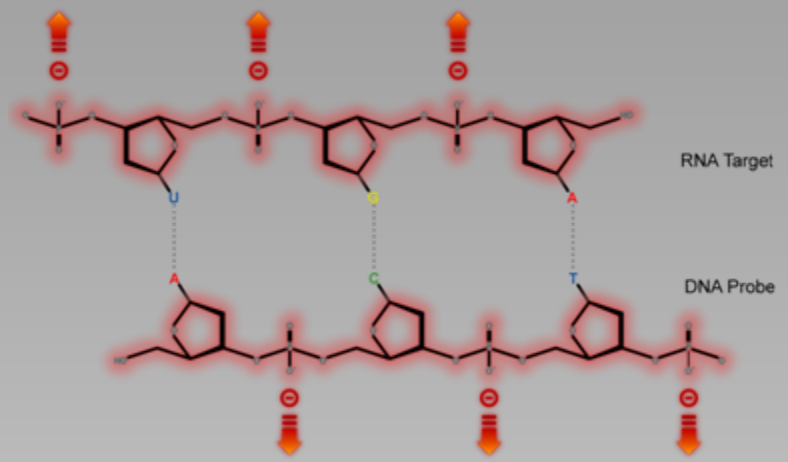

PNA

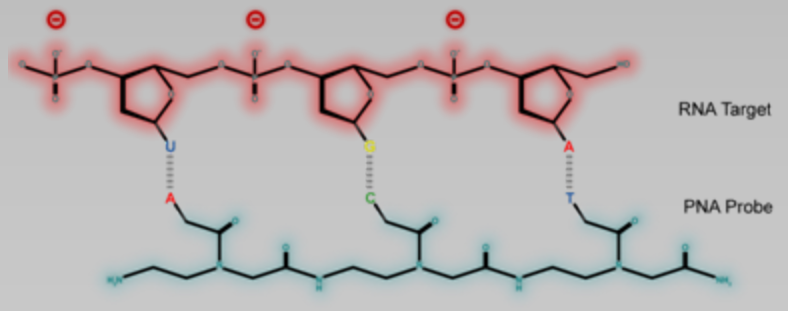



Egholm, M. et al. Nature 1993; 365:556-568.

# PNA vs. DNA Probe Hybridization

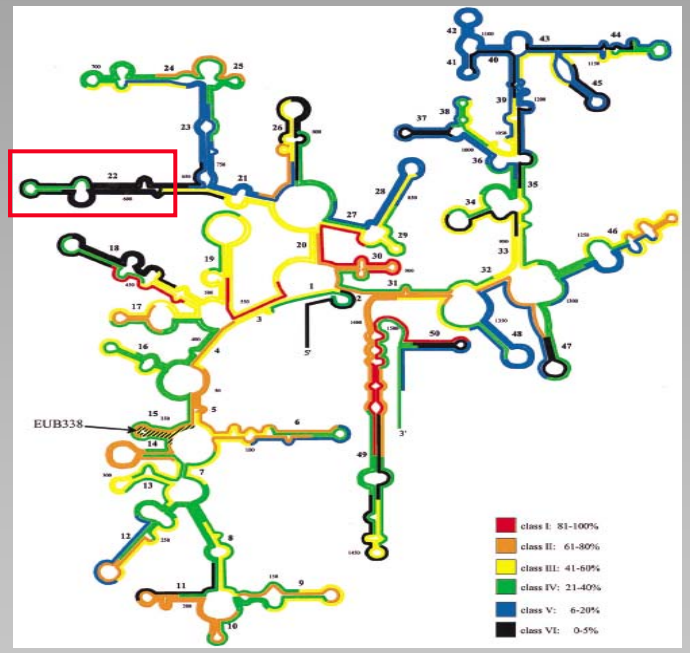
- Charge - neutral
- No natural repulsion with charged backbones
- More specific binding to target DNA or RNA
- Greater sensitivity
- No nuclease and protease degradation



# PNA FISH Targets rRNA

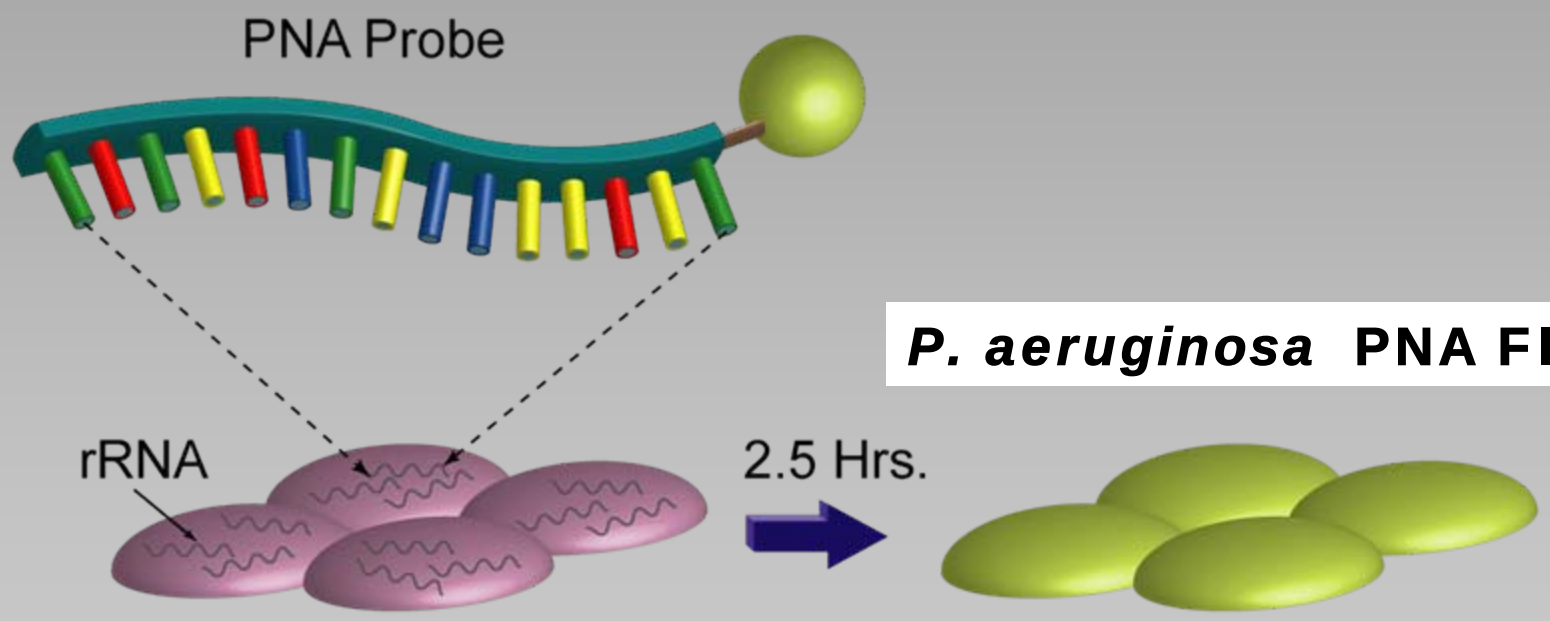
*E. coli* 16S rRNA




- Sequences are known and unique between species
- Highly abundant target
- PNA binds in highly conserved regions not accessible to DNA
- Enables highly sensitive assay with PNA FISH
- Used at low salt concentrations (destabilizing DNA)

*Fuchs et al. 1998. AEM 64:4973-4982*

# PNA FISH Targets rRNA – *P. aeruginosa*




***P. aeruginosa* PNA FISH**

**Bacteria in biofilms**

***P. aeruginosa***

# Multiple PNA probes

- *P. aeruginosa* txRED
- BacUni FITC
- DAPI as counter stain
- etc

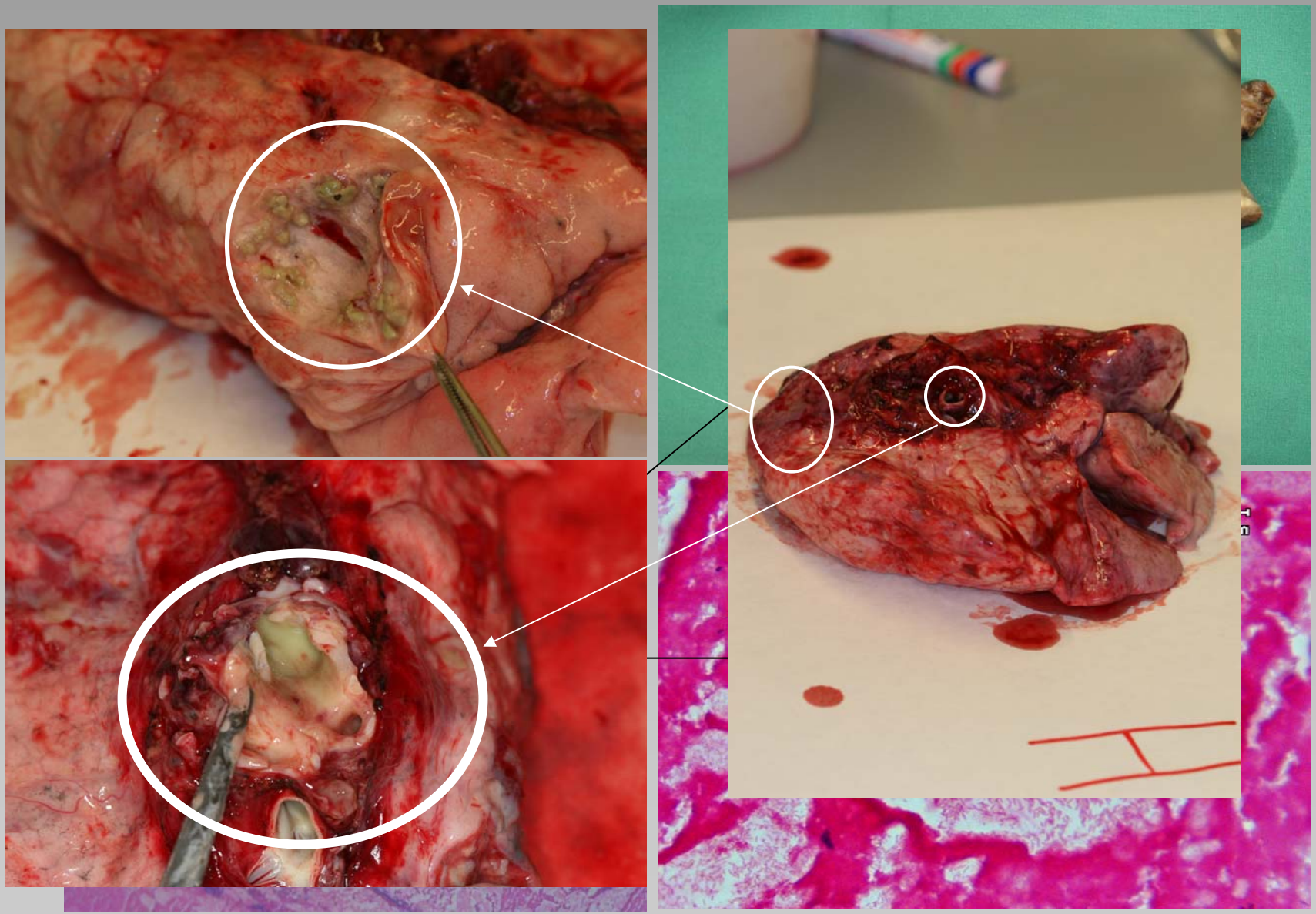
# PNA FISH Procedure

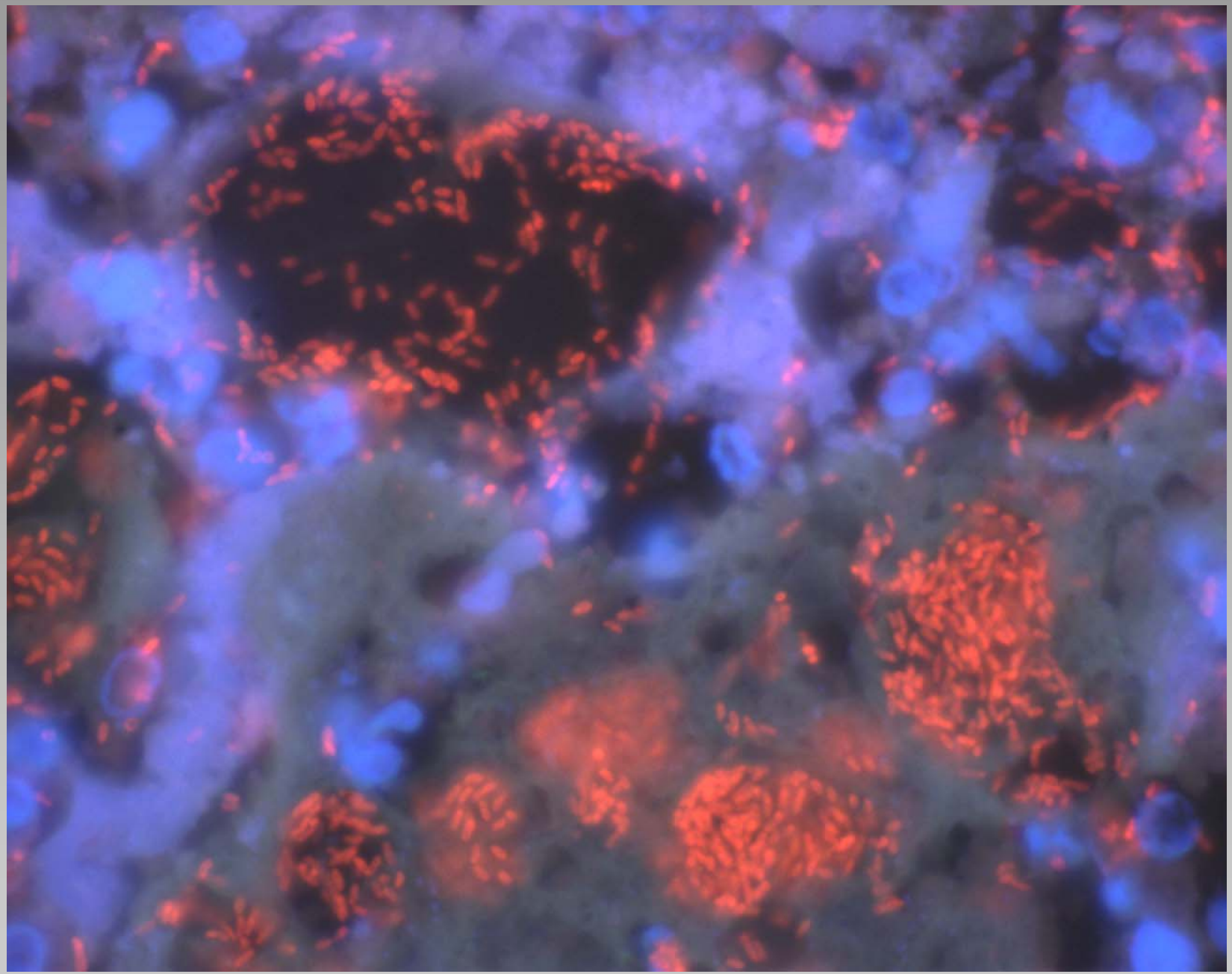
1. Fix tissue section or smear- onto microscope slide
2. Hybridize - with PNA Probe
3. Wash & Mount - remove excess probe
4. Examine - results

# PNA FISH Procedure - Examine

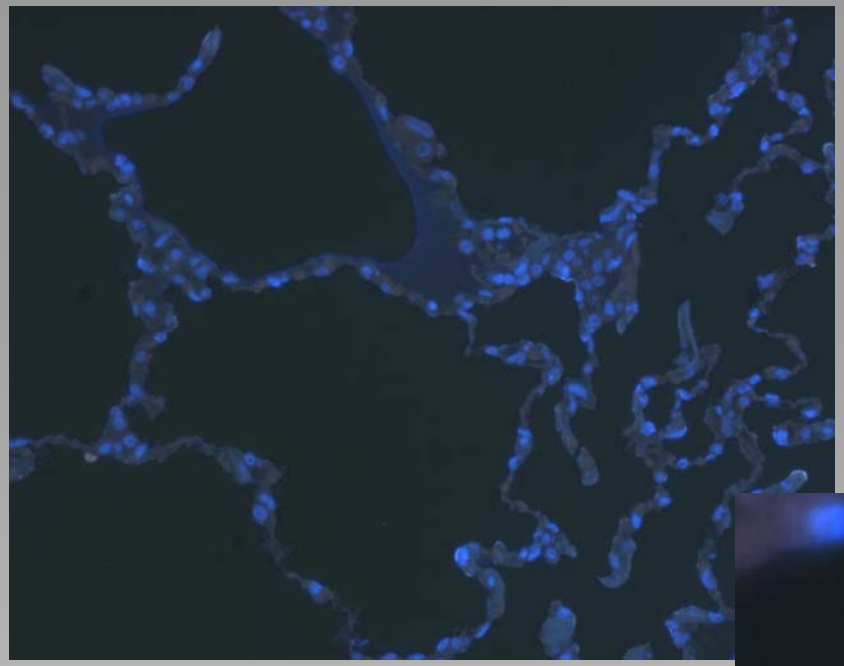
- Fluorescence Microscope using:
  - FITC
  - Texas Red
  - DAPI
  - Combination of above dual or triple Band filters

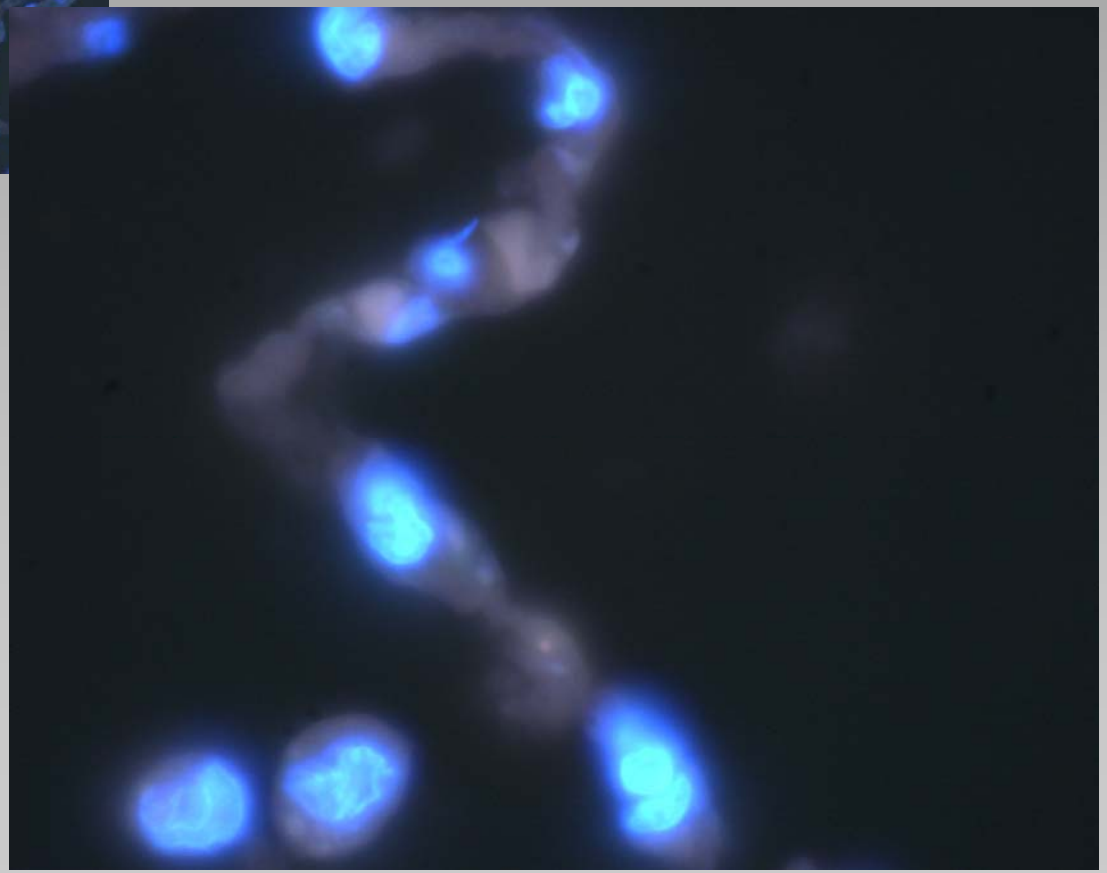
# Cystic fibrosis



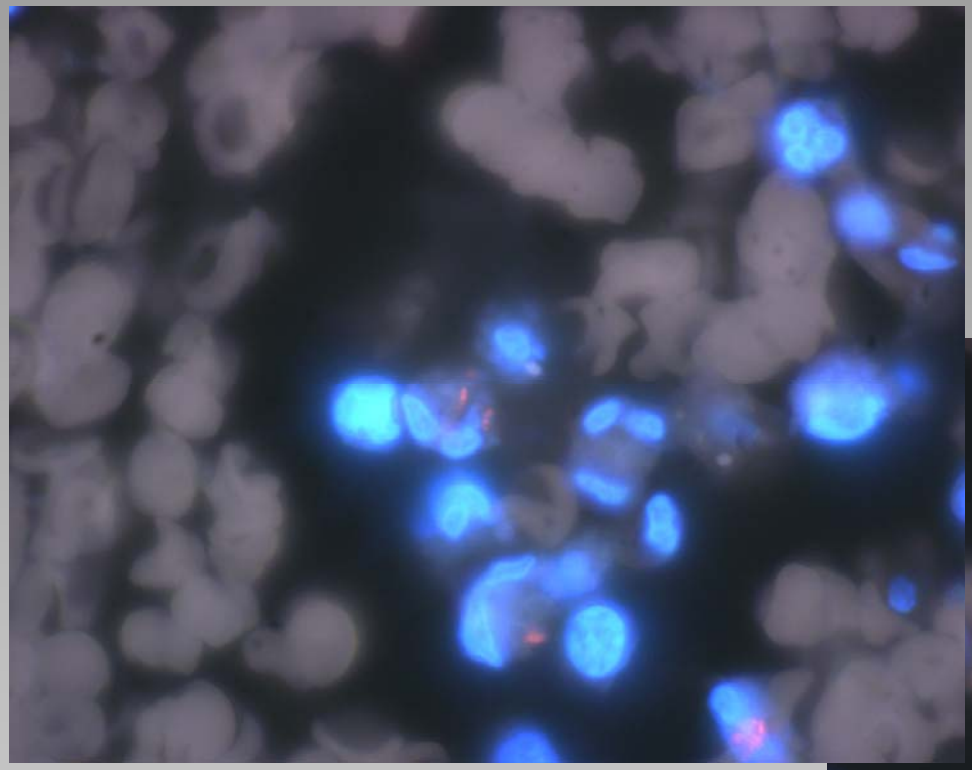


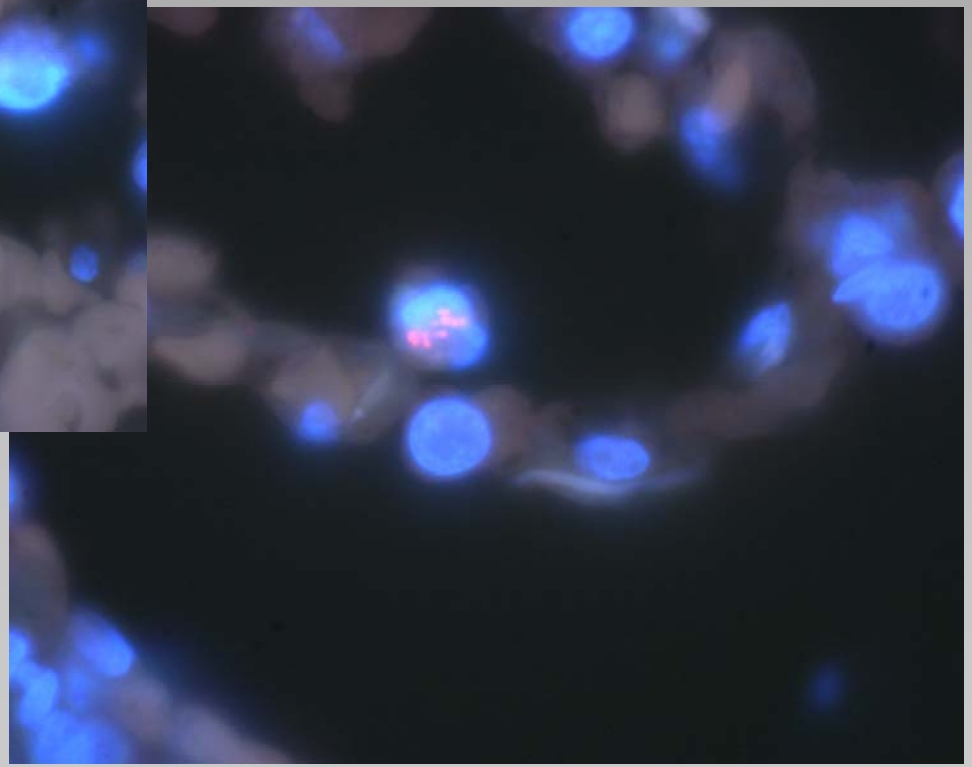
# Alveoles





# Phagocytosed bacteria





# Chronic Wounds

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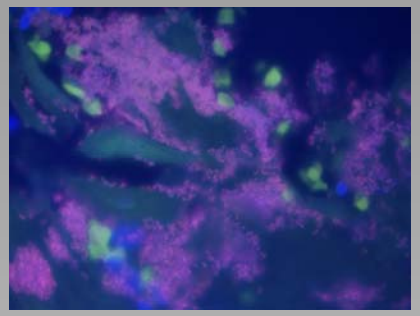
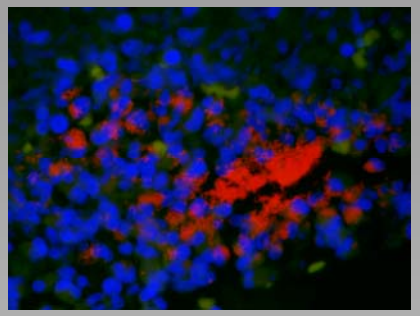
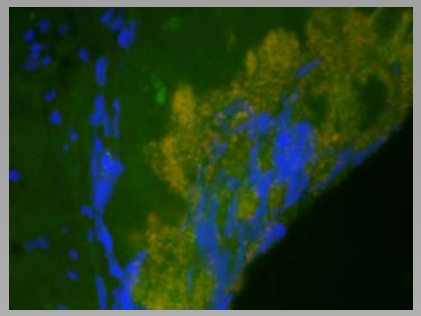


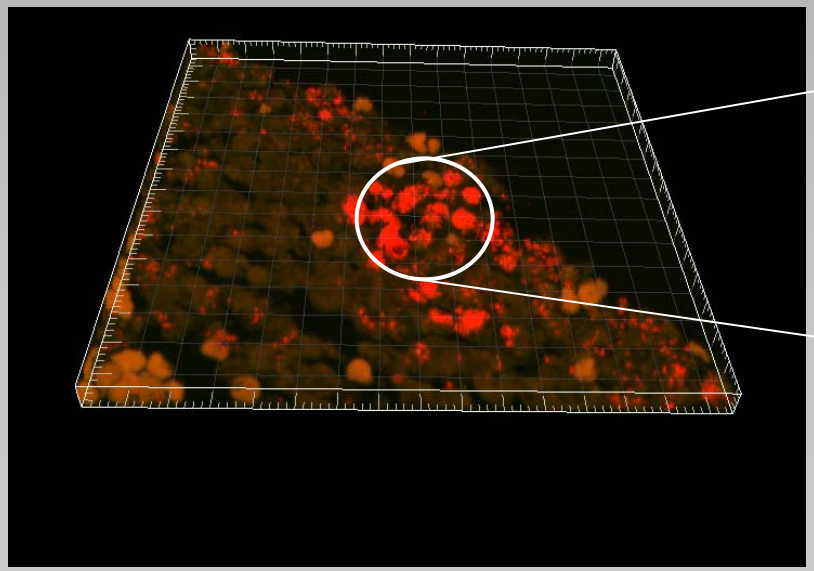
Unspecific

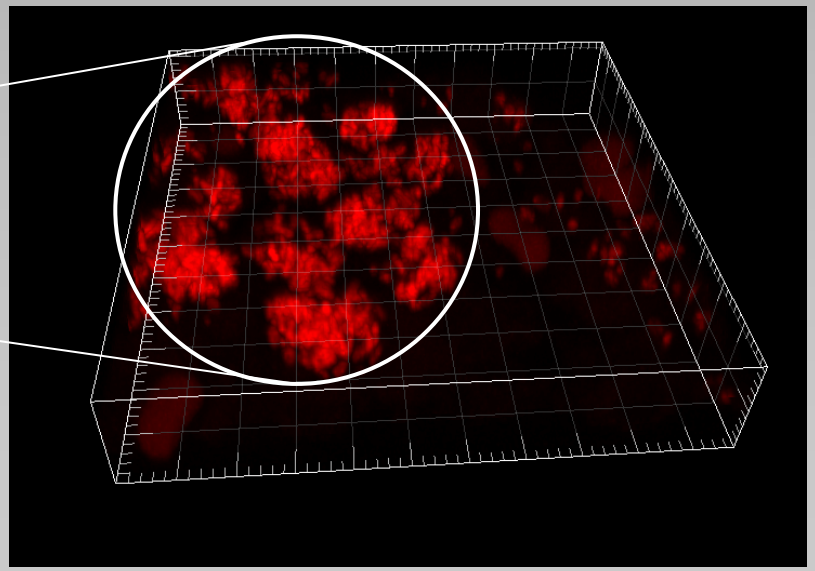


*P. aeruginosa* specific red

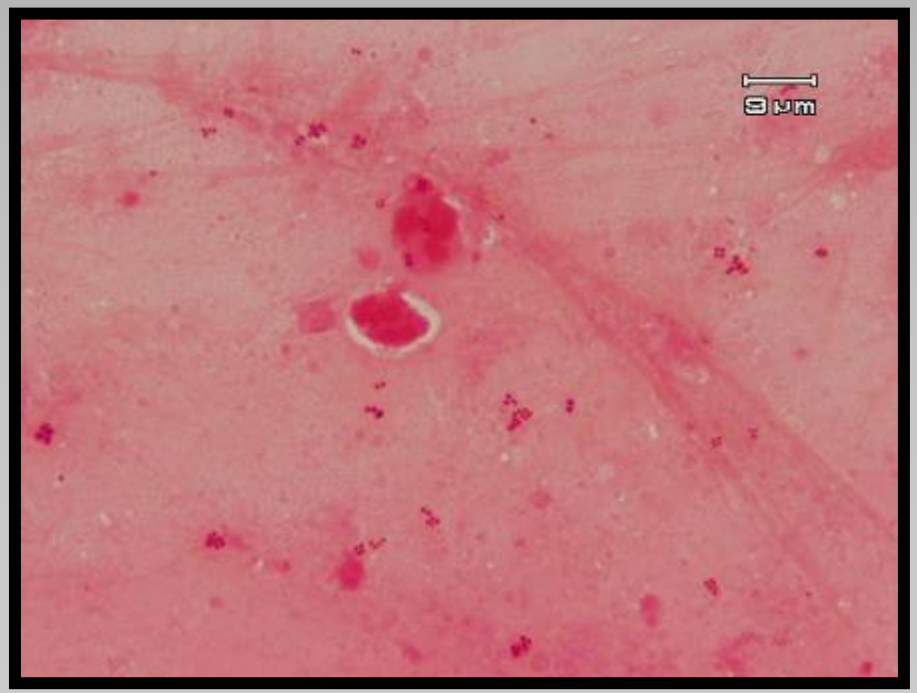


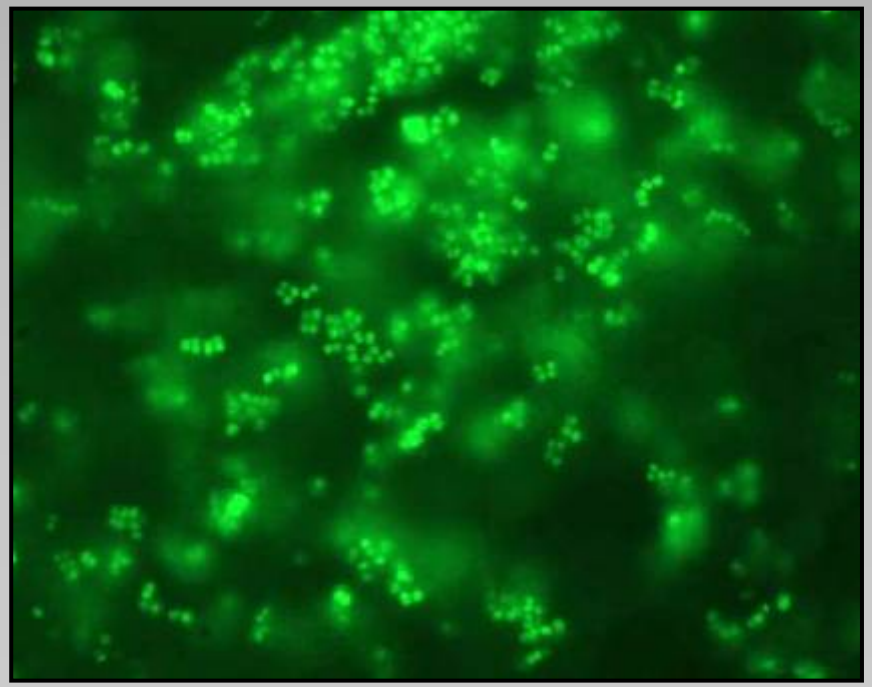
*Staph. aureus* specific green





# Chronic otitis media



# Soft tissue fillers

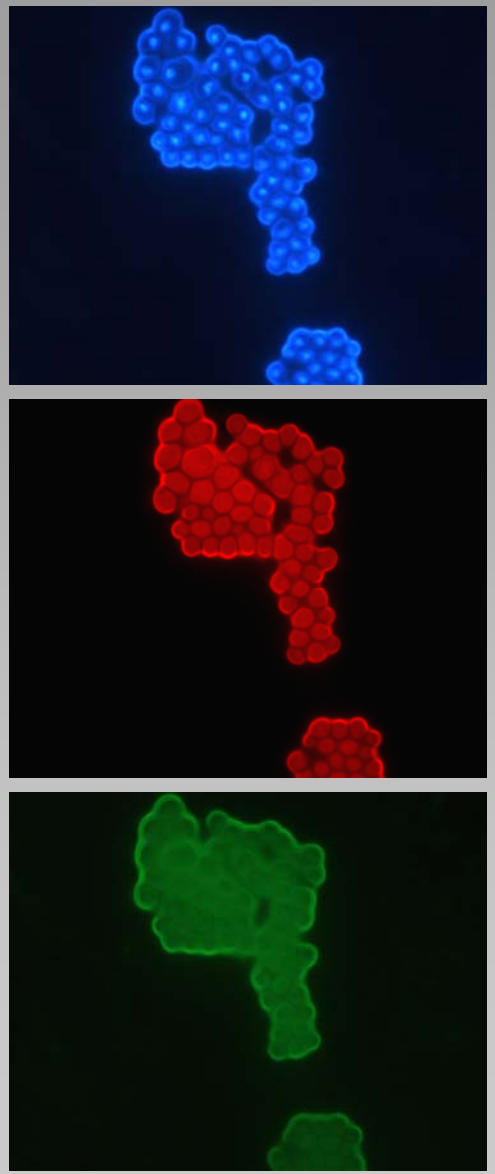
| site | Type of PAAG | Time since inj | Initial treatment | Time with AE |
|---|---|---|---|---|
| | | | | |
| Cheek | Aquamid | 2 years | Steroid inj<br>Later AB inj | 7 months |
| Lip | Aquamid | ½ year | Steroid inj<br>Later ABs | ½ year |
| Lip | Aquamid | 1 month | Steroid + ABs<br>Later ABs | 2 years |
| Breast | Amazing gel | 2 years | ABs<br>liposuction | 5 months |
| Tear-trough | Aquamid | 2 years | Steroid inj<br>Later ABs | ½ year |
| Naso-labial fold | Aquamid | 14 days | Steroid<br>ABs+surgery | 1½ year |
| Lip | Interfall gel | ½ year | Steroid<br>ABs+surgery | 2½ years |
| Penis | Aquamid | 2 years | Steroid<br>AB inj+surgery | 1½ years |

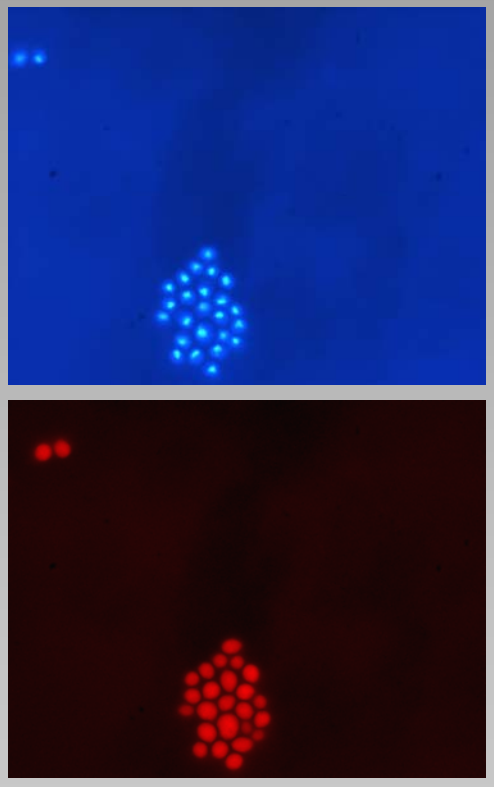
**Bjarnsholt et al**; **Detection of bacteria by FISH in culture-negative soft tissue filler lesions**; **In press Dermatol.surg.**

# PNA FISH summary

- PNA FISH enables
  - characterization of bacteria in ”difficult” tissue samples
  - detection of single bacteria in tissue, even phagocytosed bacteria

# Artefacts





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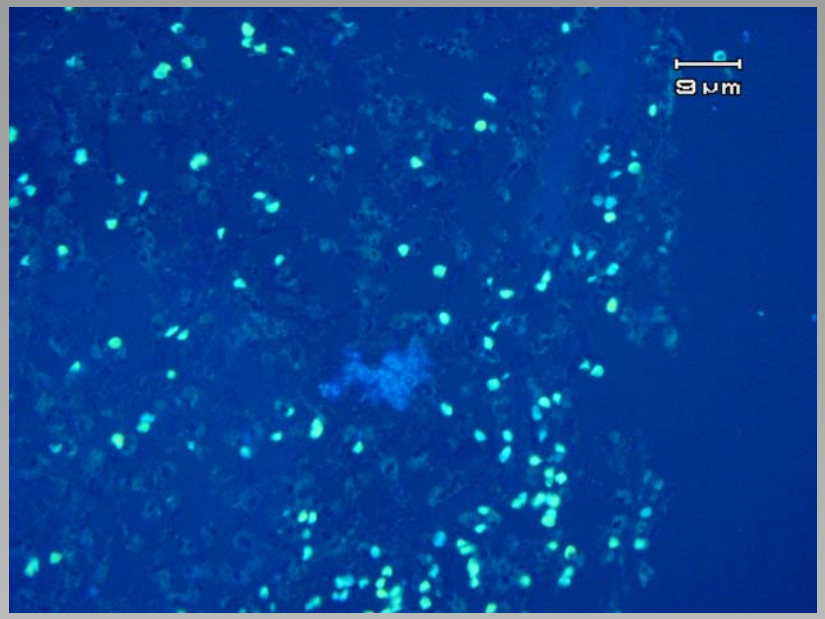


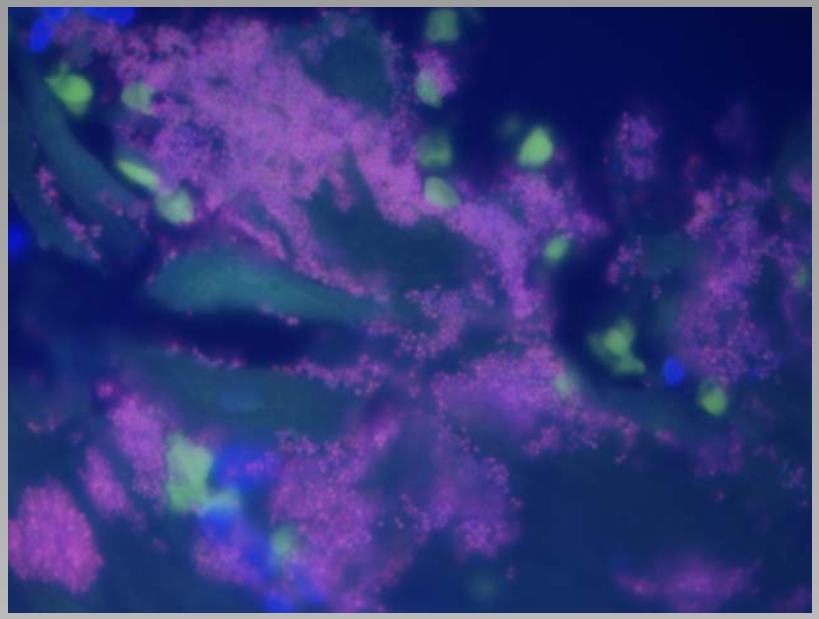

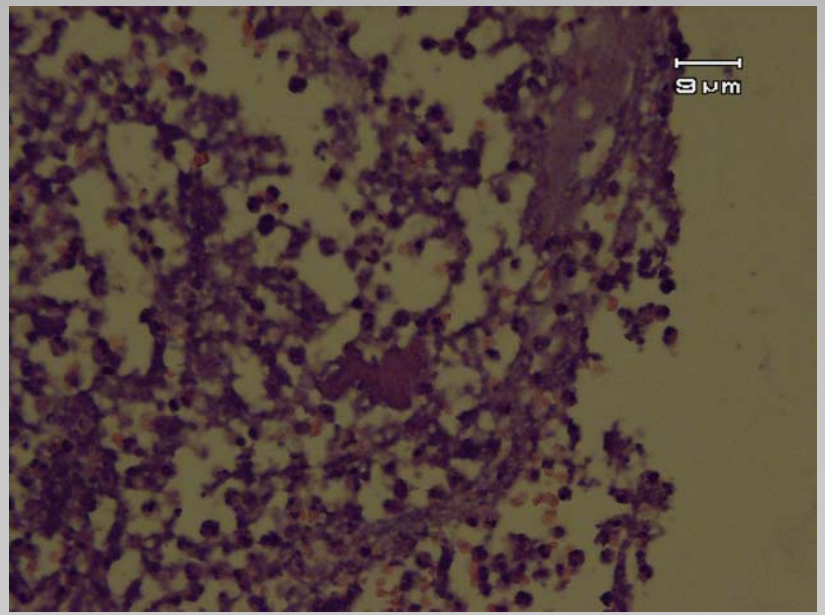


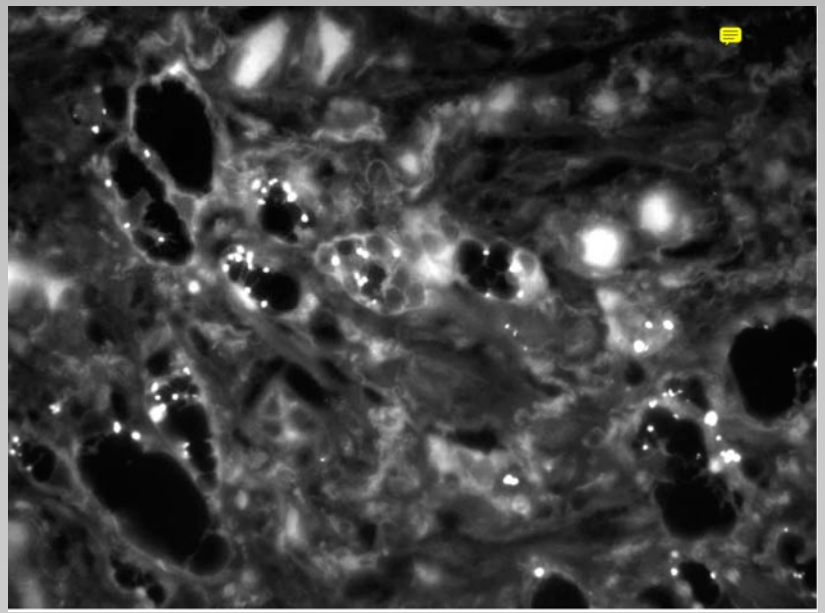

# Collaborators

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AdvanDx