

Unexpected Mycobacterial cases from the University of Wisconsin's Pathology Department

Byron Barksdale, MD

Erin Brooks, MD, FCAP

Catherine Leith, MB, BChir

University of Wisconsin Hospital and Clinics

Laura Louison, MLS

Wisconsin State Lab of Hygiene



School of Medicine
and Public Health

UNIVERSITY OF WISCONSIN-MADISON

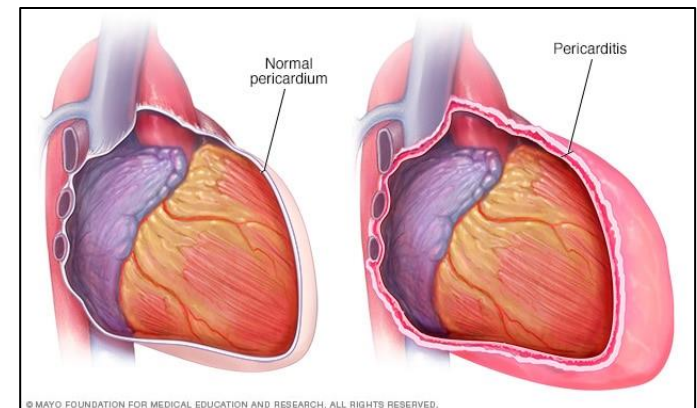
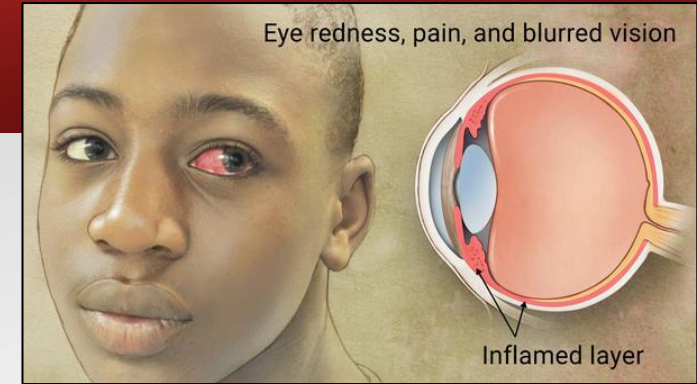
Learning objectives

- Unexpected presentations of Mycobacterial infections
 - Briefly review granulomatous diseases and sarcoidosis
- Specimen preparation in pathology
- Molecular testing on select samples
- Reporting of *Mycobacterium tuberculosis* in Wisconsin
- Infection with *Mycobacterium genevense* mimicking a post-transplant lymphoproliferative disorder



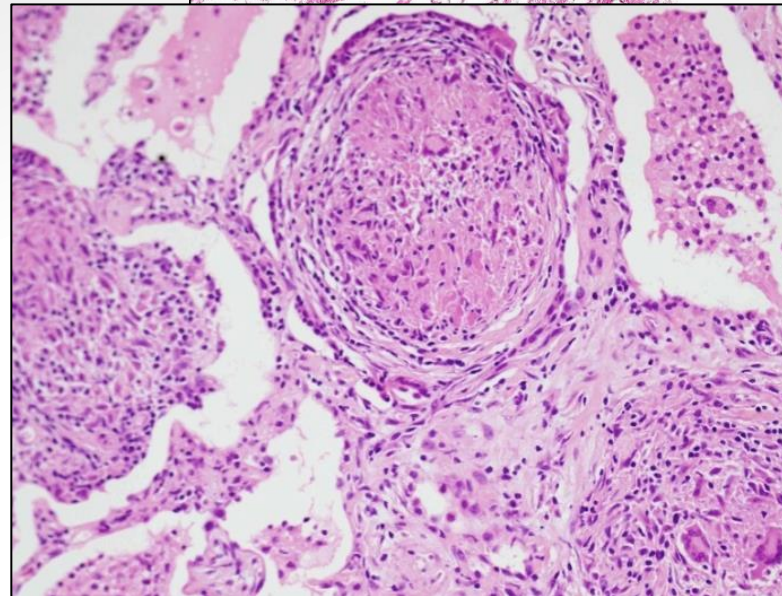
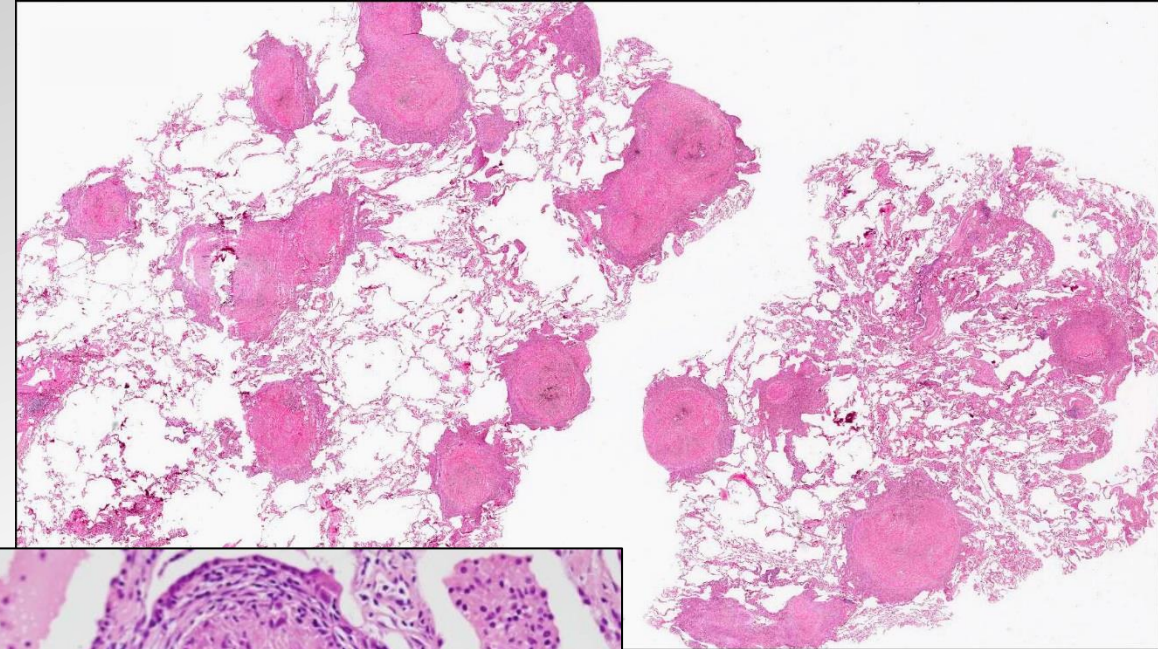
Case 1 Clinical History

- 47 y/o woman
- PMH: hypertension, type II diabetes, obesity, sarcoidosis (diagnosed in 2008 at OSH). At diagnosis, she reportedly had:
 - Radiologic lung nodules (which were biopsied and revealed non-necrotizing granulomas on histology)
 - Ocular disease: optic neuritis, bilateral anterior uveitis
 - Joint disease: inflammatory arthritis
 - Presumed pericardial disease: pericarditis



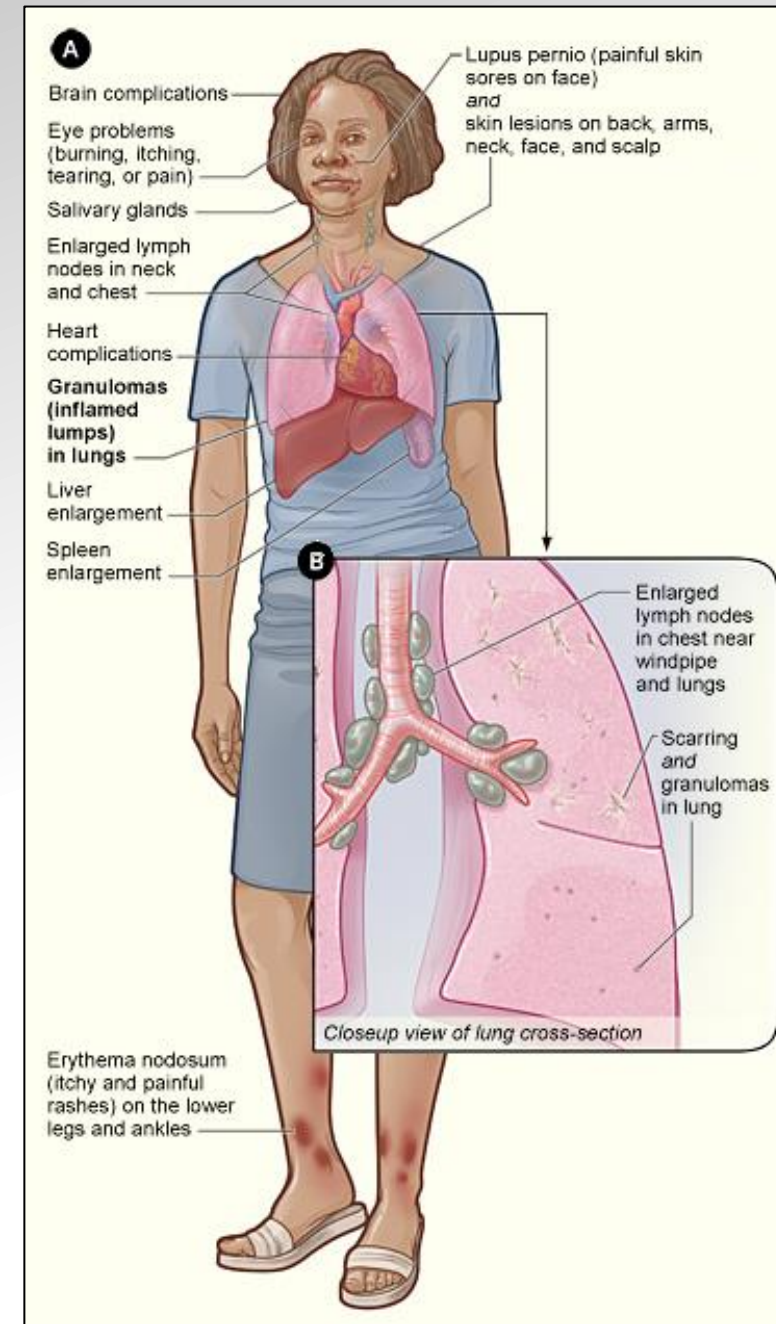
What is a Granuloma?

- Granuloma formation is a cellular attempt to contain what is not easily eradicated
- Granulomatous inflammation is characterized by:
 - Activated macrophages (a.k.a. 'epithelioid' cells)
 - Often MNGs
 - Surrounding collar of lymphs



What is Sarcoidosis?

- A systemic disease that manifests with non-caseating granulomatous inflammation.
 - Granulomas are predominantly seen in lungs and hilar lymph nodes
 - Can also involve eyes, skin, liver/spleen, heart, CNS, and other organs.
- Etiology is still unknown. A diagnosis of exclusion.
- More common in younger patients (i.e. < 40 y/o olds), African-Americans (prevalence 10x > whites), and women.



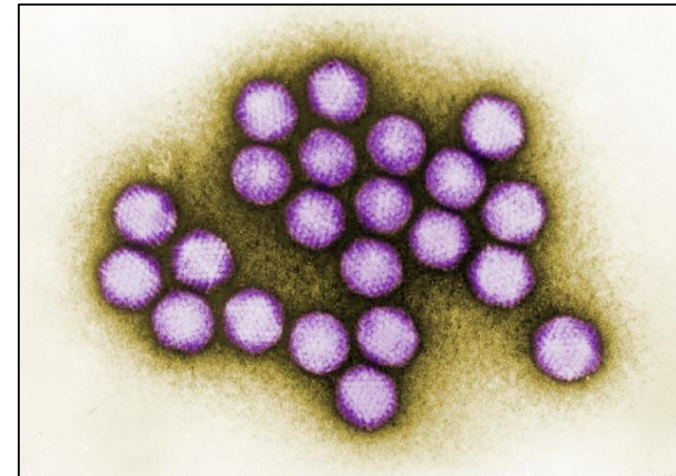
Clinical History

- Her sarcoidosis was treated with methotrexate and high dose steroids
- In September 2017 she developed vision loss in one eye and had a cortical infarct
 - She was suspected to have CNS involvement by sarcoidosis and was treated with high dose steroids, cyclophosphamide, and infliximab



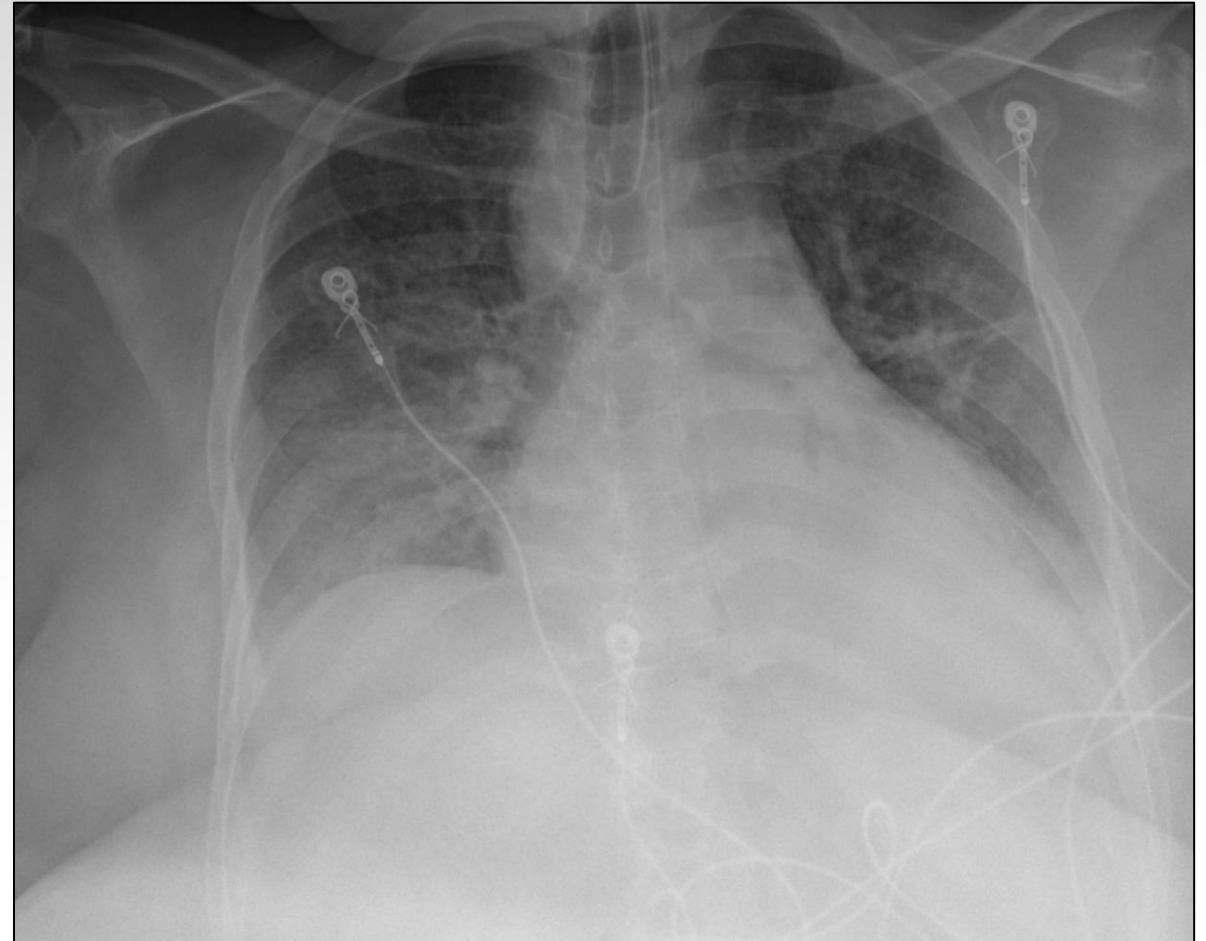
Clinical History

- HPI: In April 2018, presented to UWHC with worsening weakness, malaise, and fever (up to 39.4 C).
- Lab results:
 - Elevated Inflammatory Markers: ESR (105 mm/hr), CRP (18.2 mg/dL)
 - CBC with Increased Immature Granulocytes: 220/uL
 - Oropharyngeal Respiratory Viral Panel PCR (+) Adenovirus
 - Blood qualitative PCR (+) Adenovirus
 - Low level CMV & EBV viremia:
 - » Blood quantitative EBV DNA by PCR: 3720 IU/mL
 - » Blood quantitative CMV DNA by PCR: 407 IU/mL



Clinical History

- HPI:
 - On 5/5, she developed altered mental status with dyspnea
 - Started on respiratory ventilatory support
 - CXR showed paratracheal lymphadenopathy and tiny diffuse lung nodules (presumed infection)
 - Condition continued to decline, developing HLH: on 5/8, went into cardiac arrest and expired despite CPR
 - Autopsy requested to determine COD

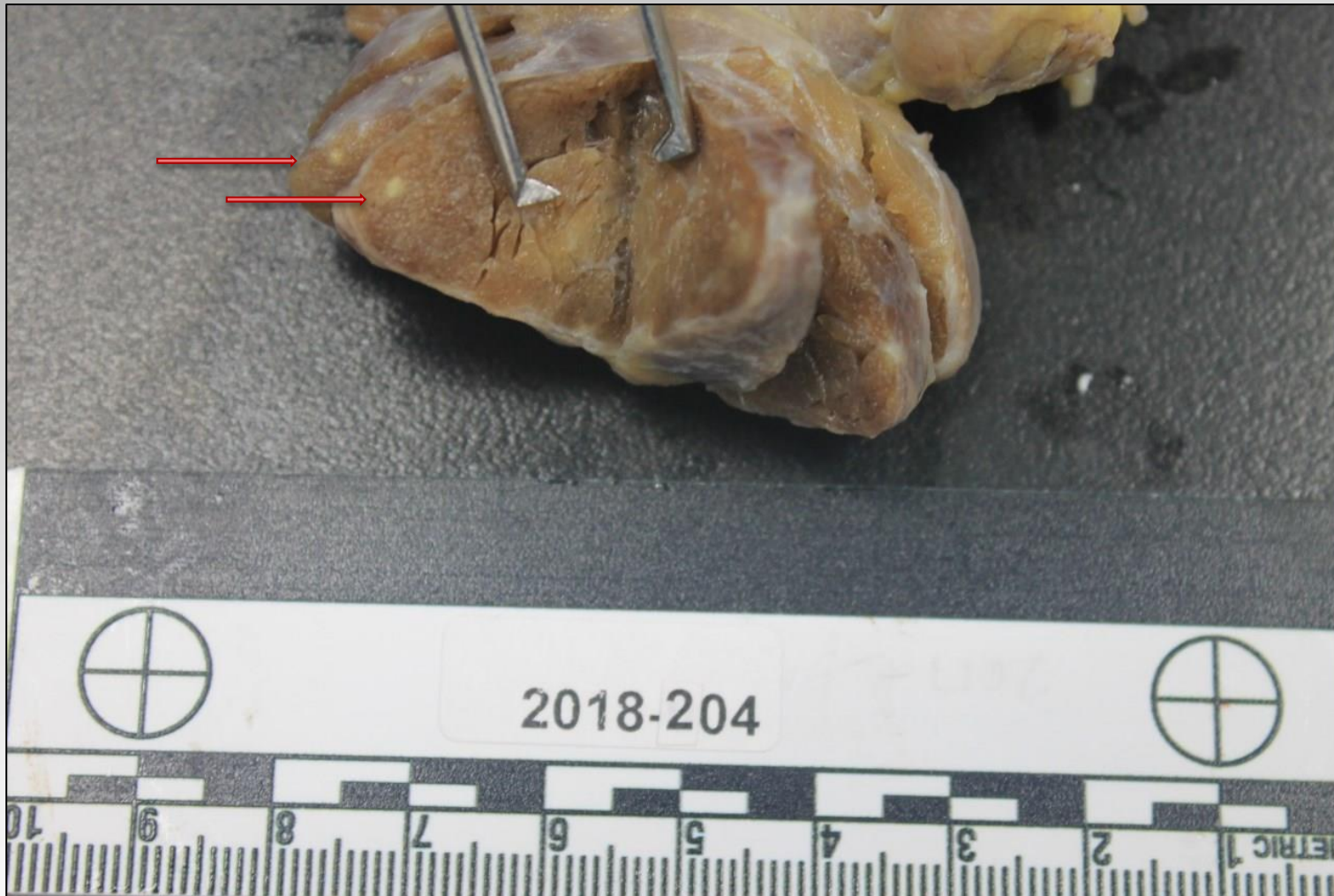


Autopsy

- External exam revealed an obese (BMI: 40.7) female with evidence of medical intervention including:
 - Endotracheal intubation
 - Triple lumen catheter (right neck)
 - Peripheral access lines (2 in left arm and 2 in right)
 - EKG adhesive patches
- Internal exam revealed minute lesions involving multiple organs



Thyroid Lesions



- Scattered yellow ovoid (0.1-0.2 cm) parenchymal lesions

Pulmonary Involvement



Bilateral Renal Involvement

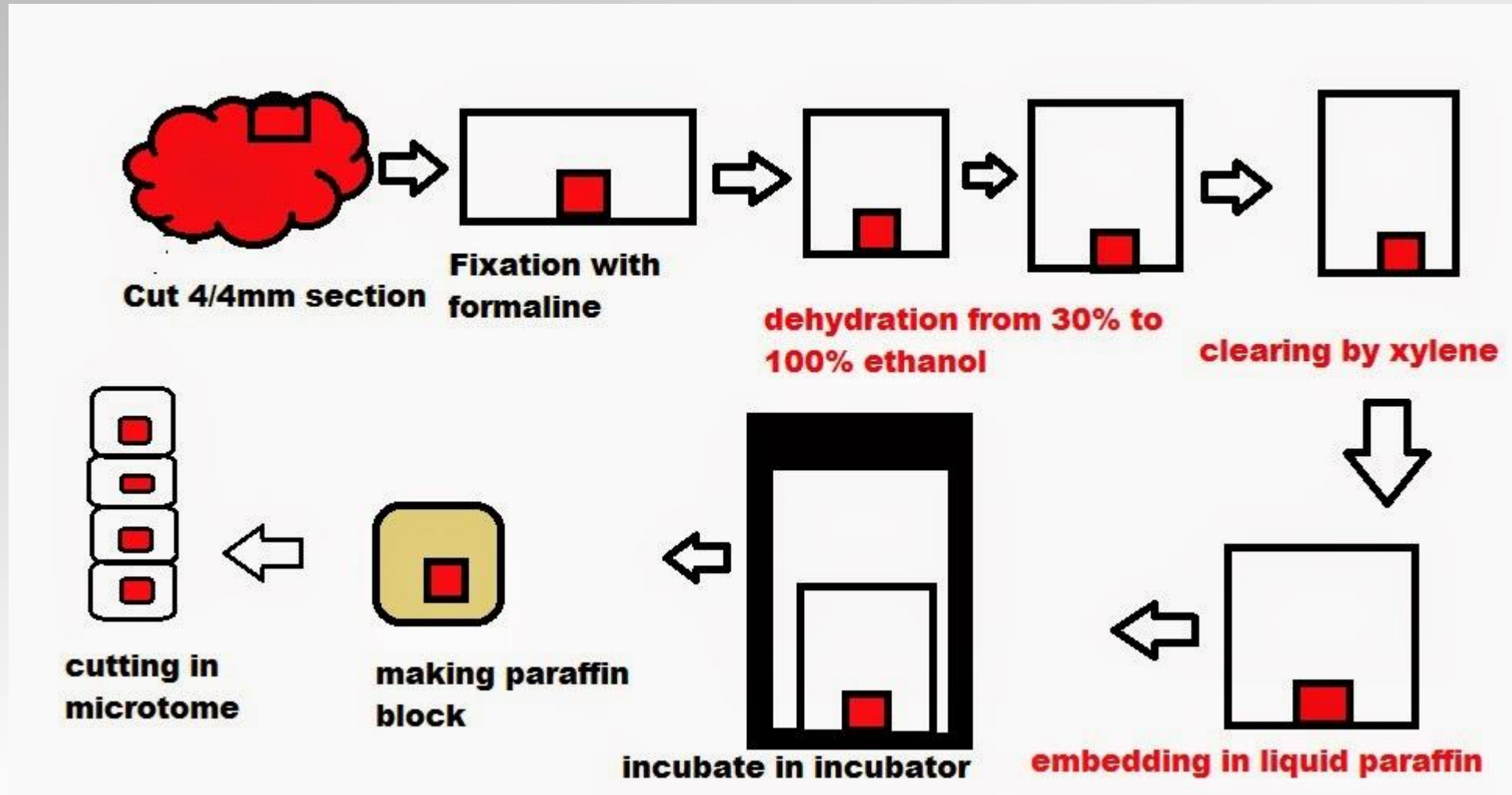


Extensive Splenic Involvement

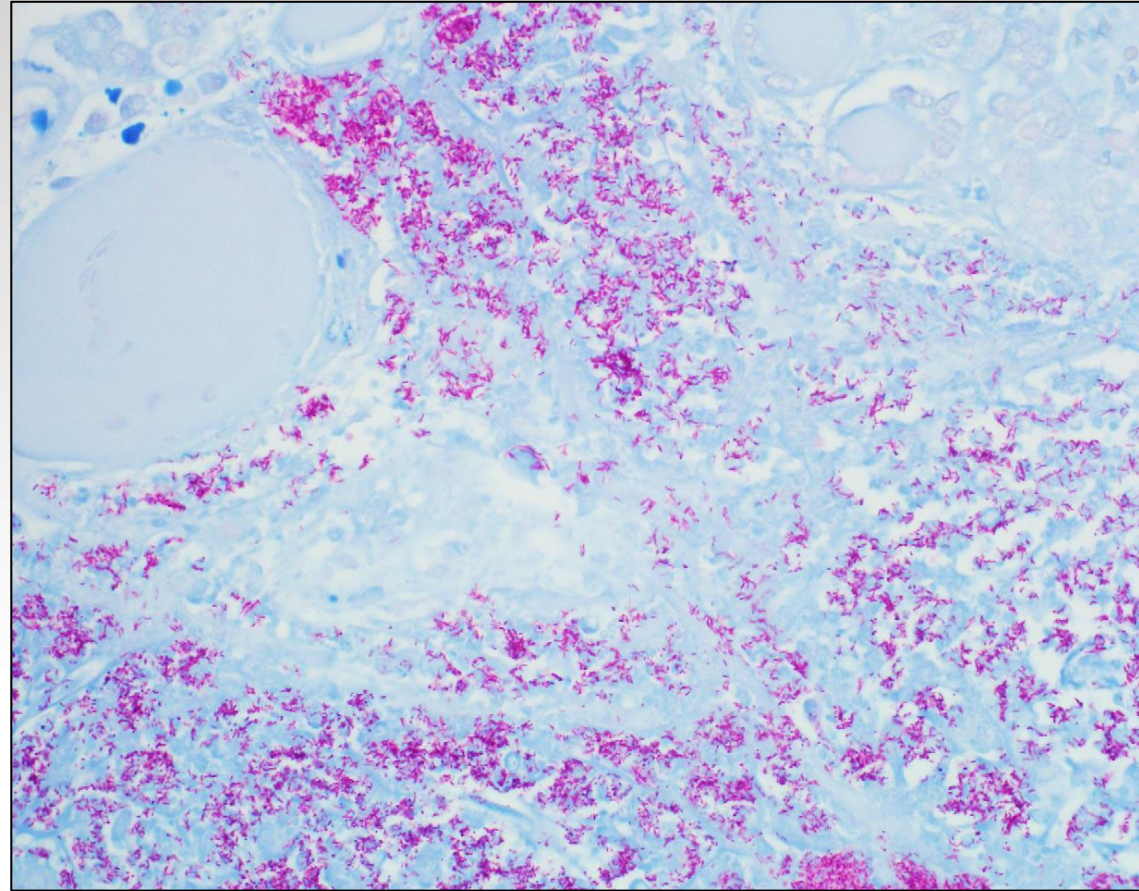
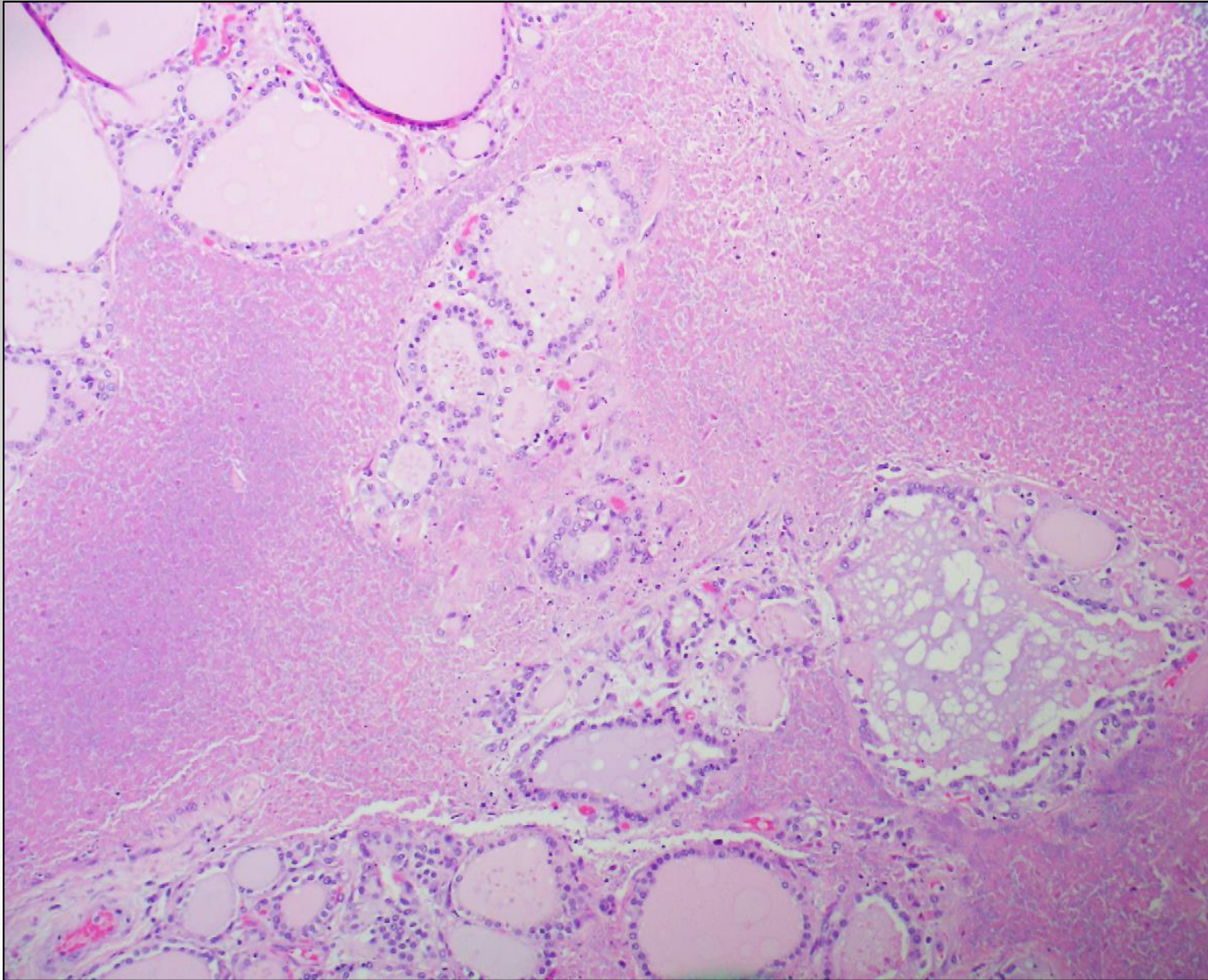


Larger confluent yellow parenchymal and capsular lesions measuring up to 0.4 -0.5 cm

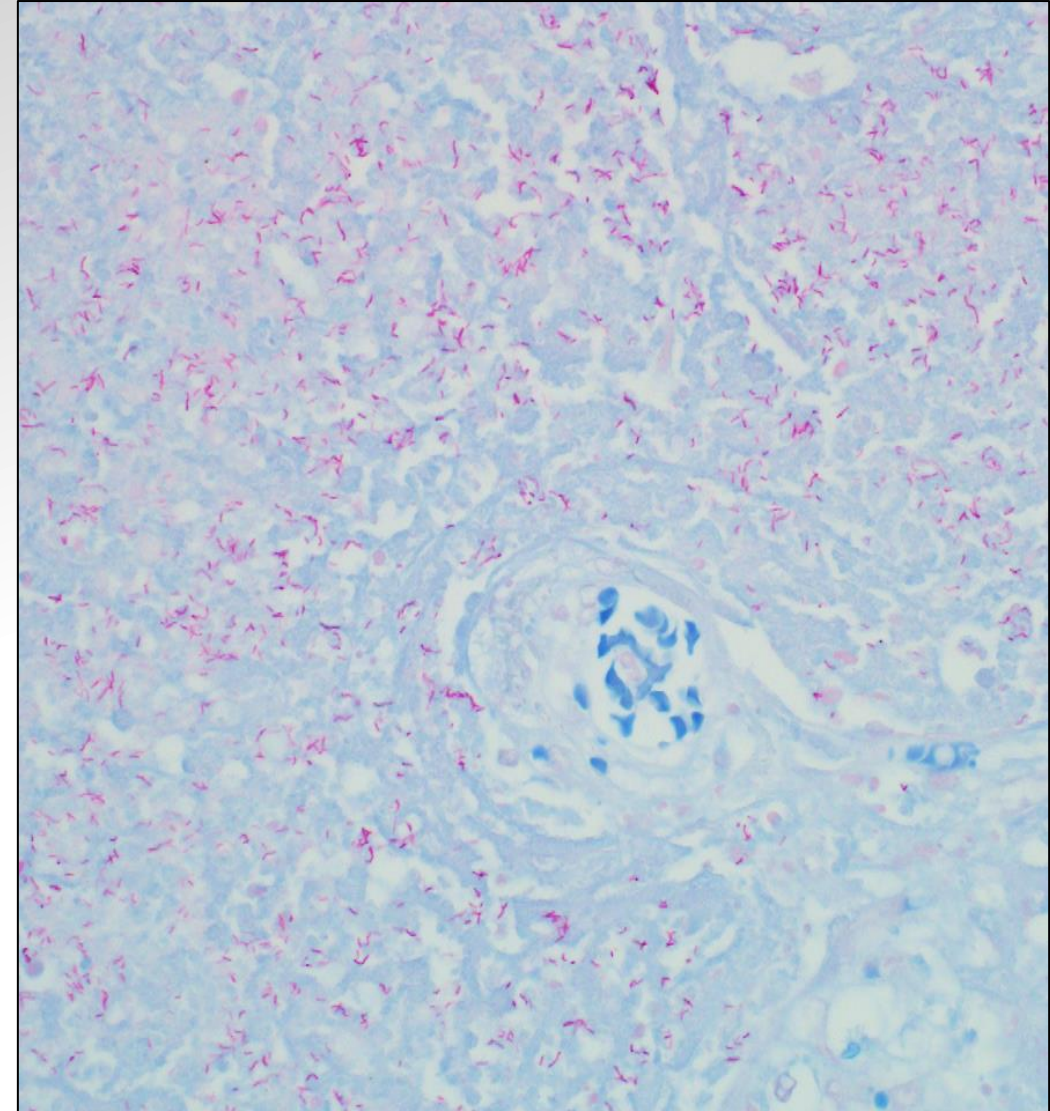
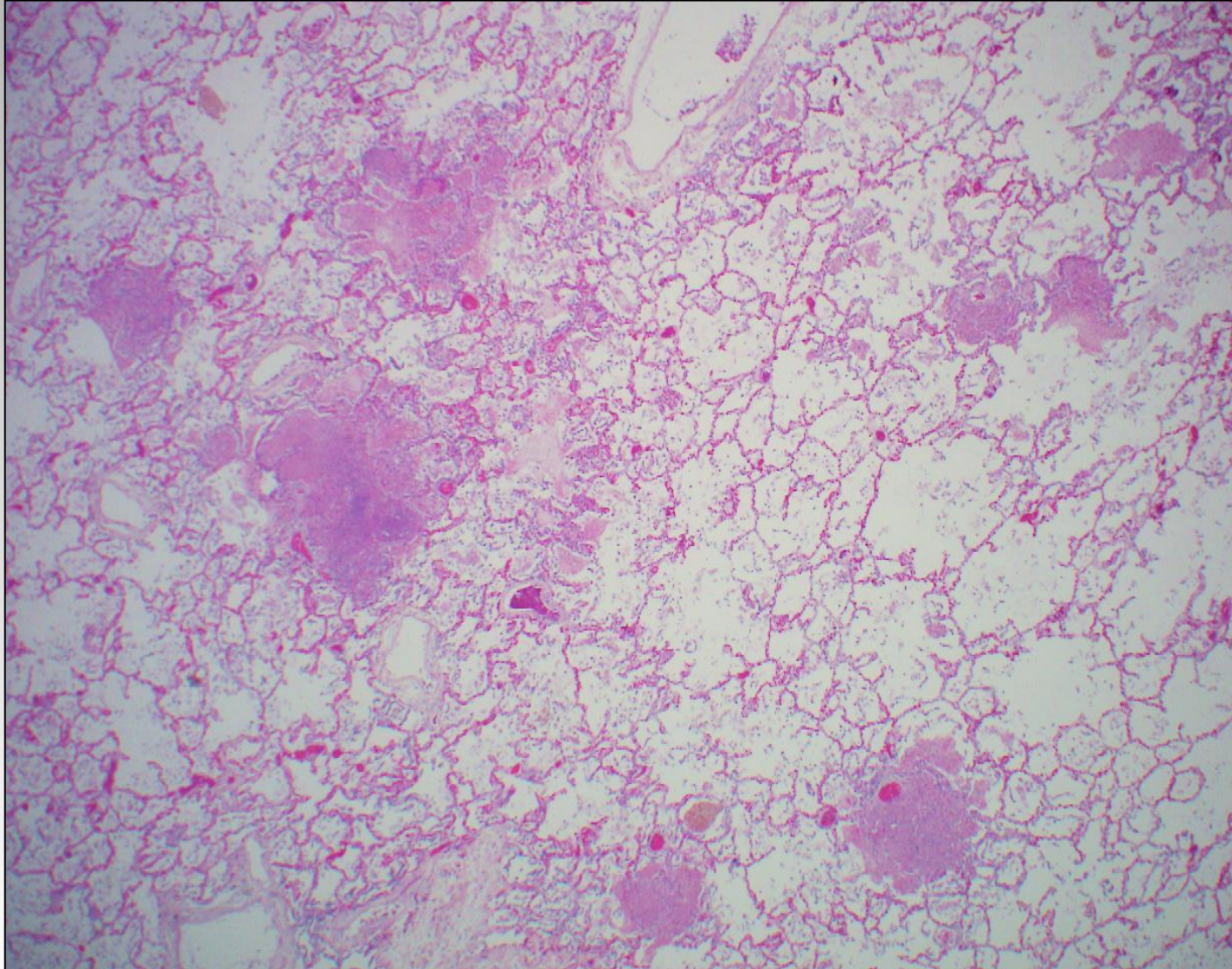
Tissue Processing



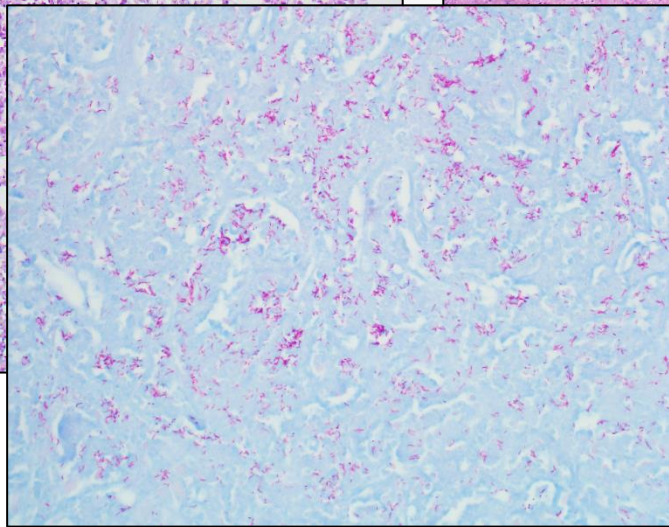
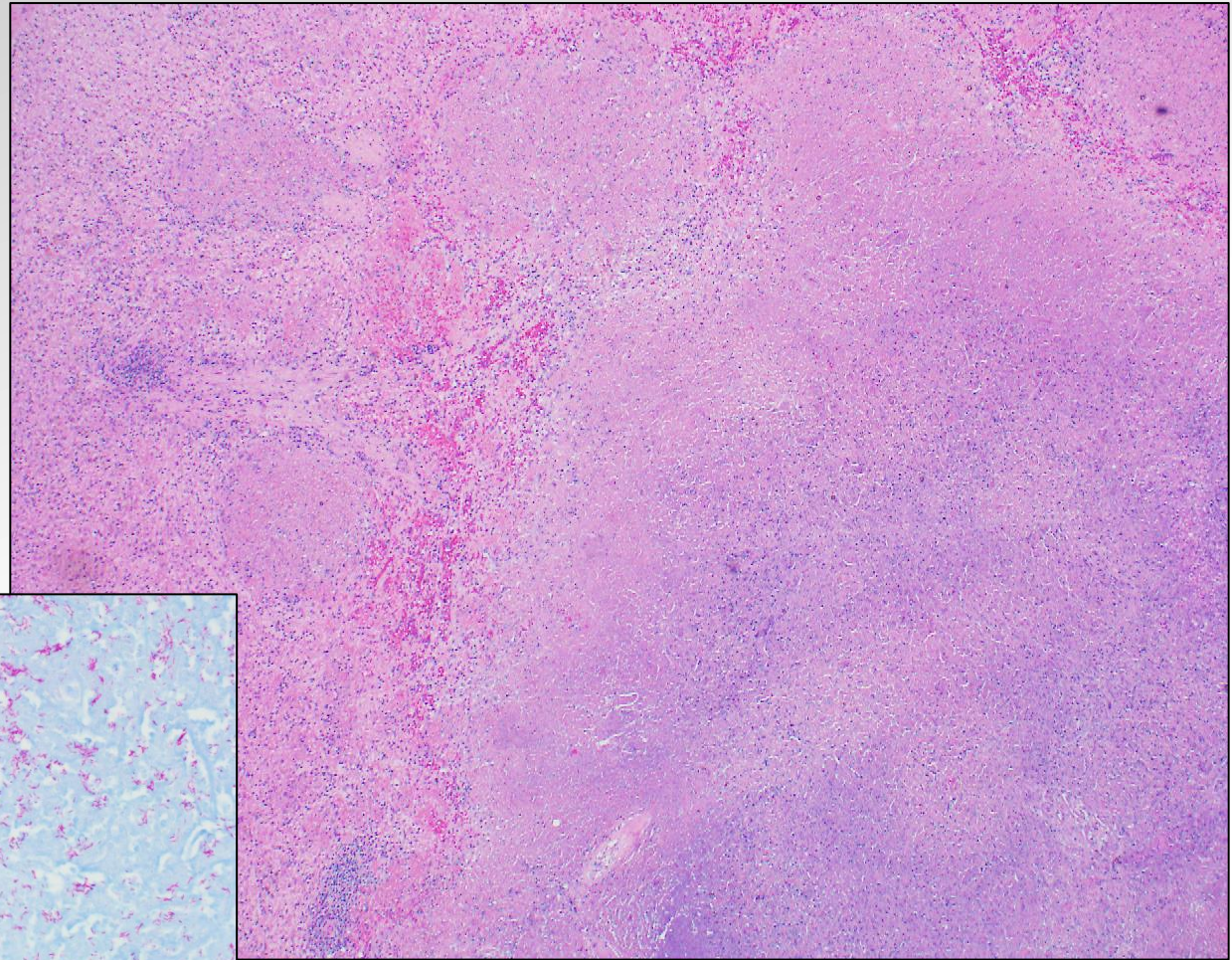
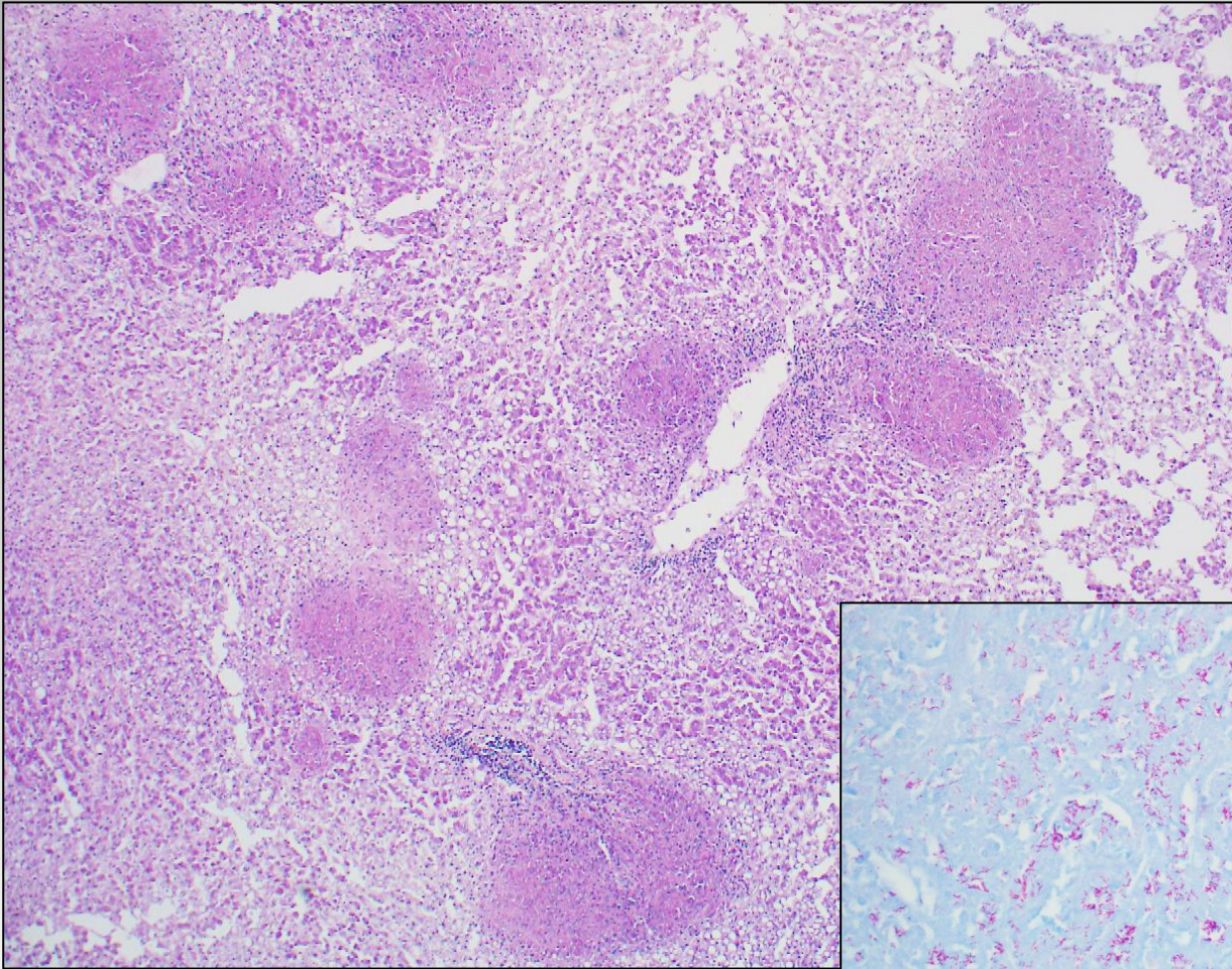
Thyroid Histology



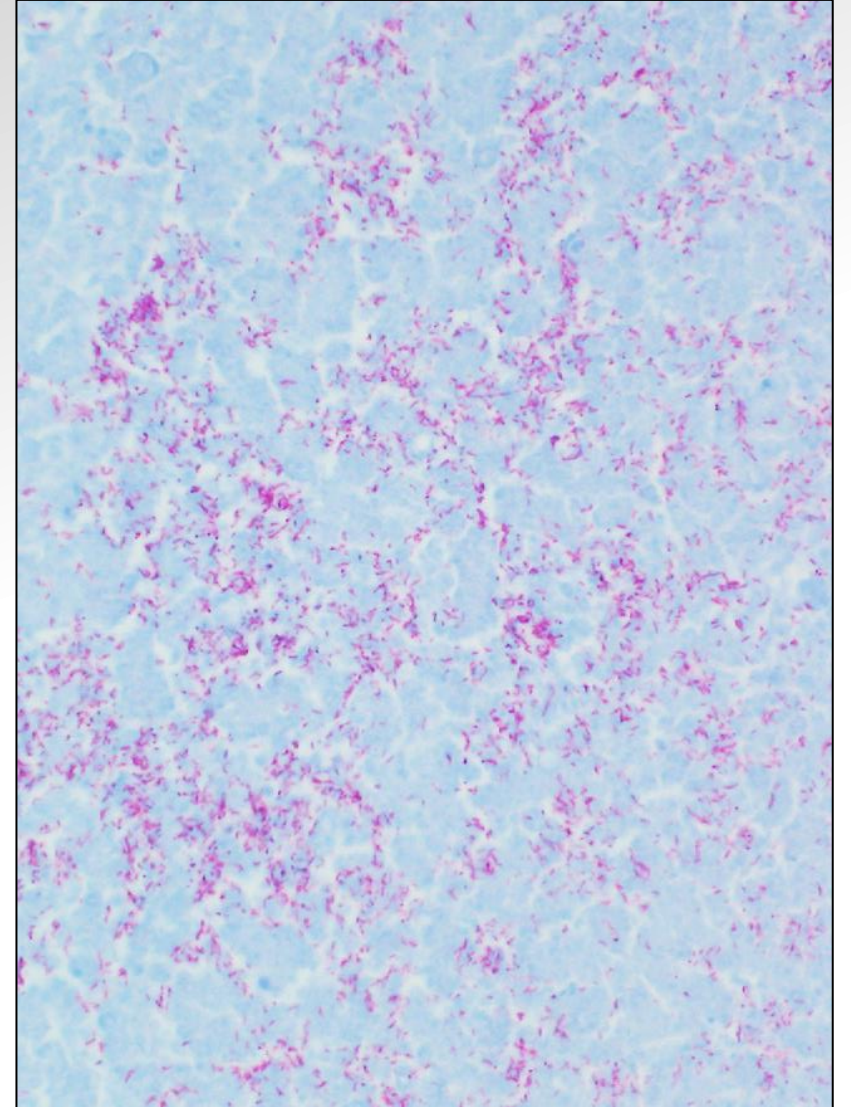
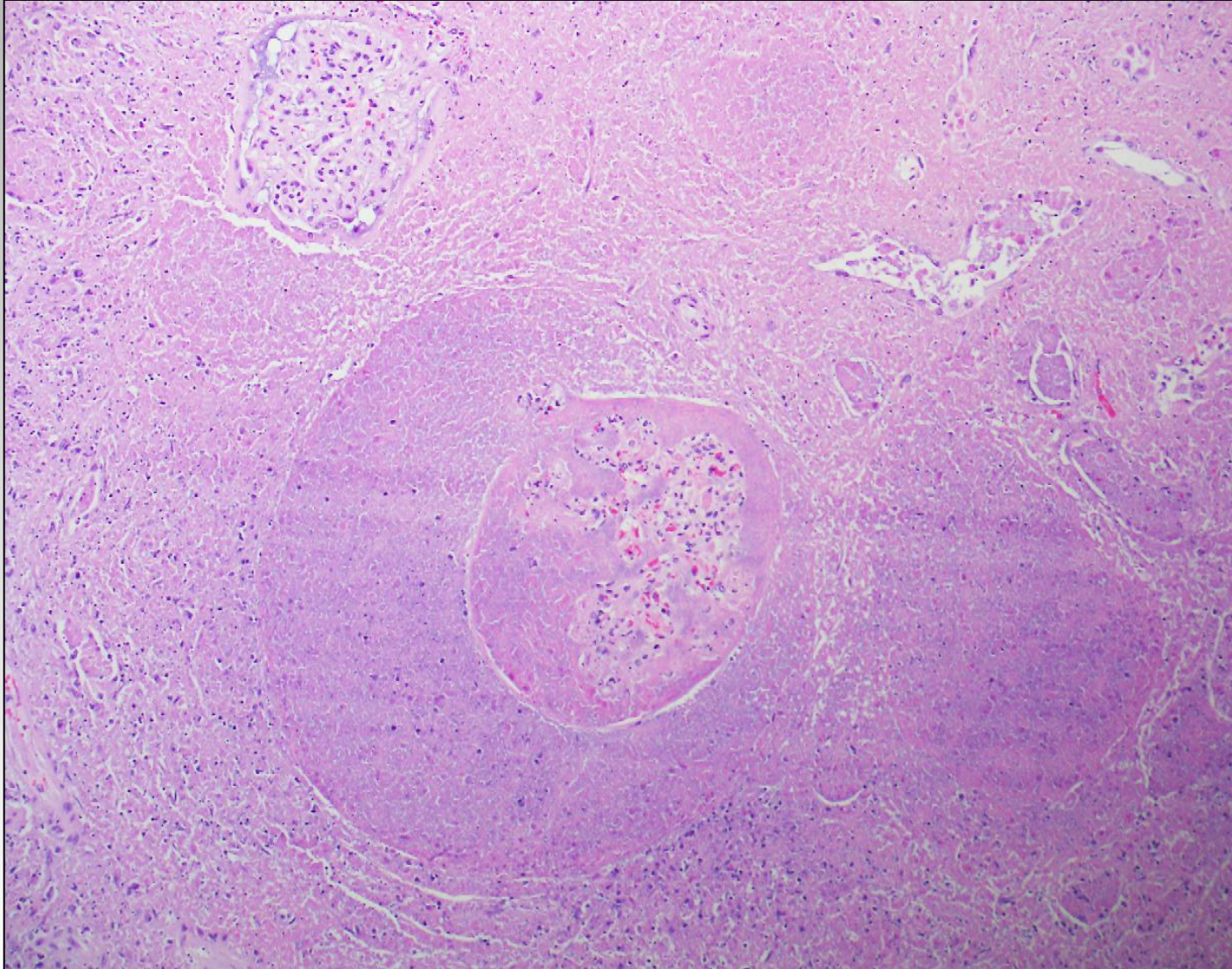
Lung Histology



Hepatosplenic Histology



Kidney Histology



Further Diagnostic Testing

- Reported case to UW Infection Control and Hospital Epidemiology
- Concerns raised about hospital staff exposure and whether TB prophylaxis was needed
- Questioned whether it was possible to speciate the acid fast organism?

Table 2 Acid-Fast Staining Organisms*

Bacterial:

Nocardia spp., *Rhodococcus equi*, *Legionella micdadei*,
Tsukamurella spp., *Gordonia* spp.

Mycobacterial:

Mycobacterium tuberculosis and nontuberculous mycobacteria (ie,
M. kansasii, *M. marinum*)

Parasitic:

Cryptosporidium spp., *Cyclospora cayetanensis*, *Isospora belli*,
Sarcocystis hominis

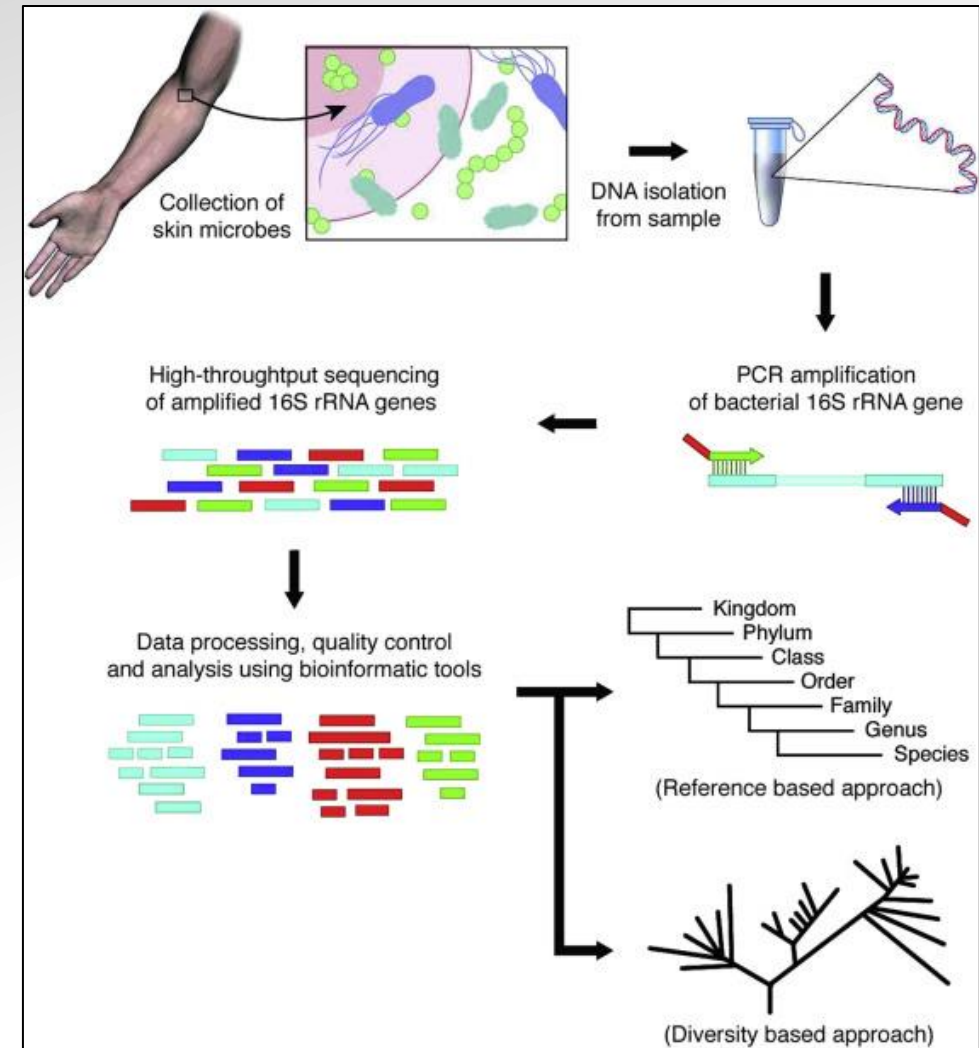
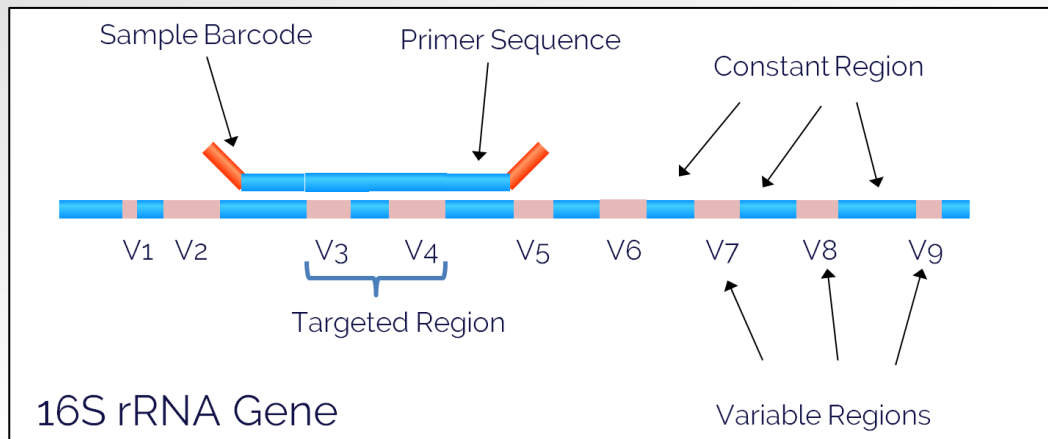
Fungal:

Blastomyces dermatitidis

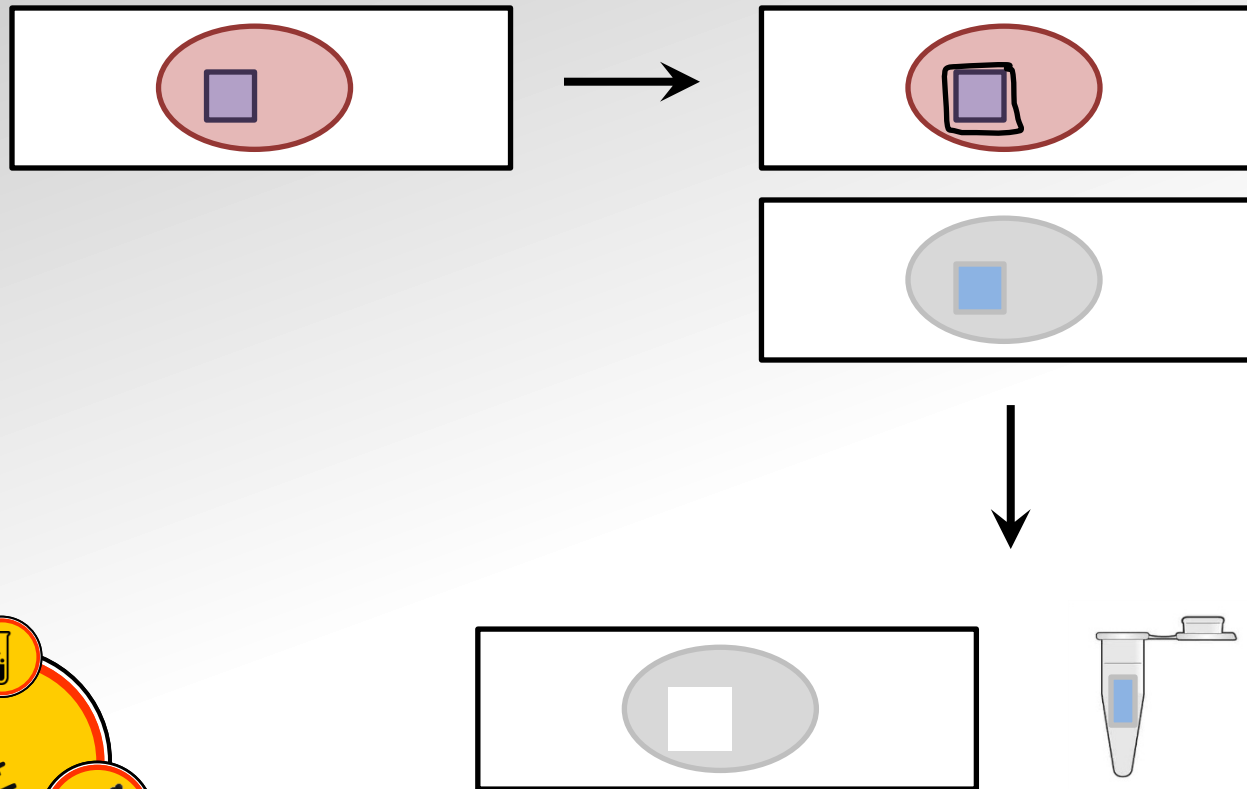
*Acid-fast or modified acid-fast staining pattern.

Further Diagnostic Testing

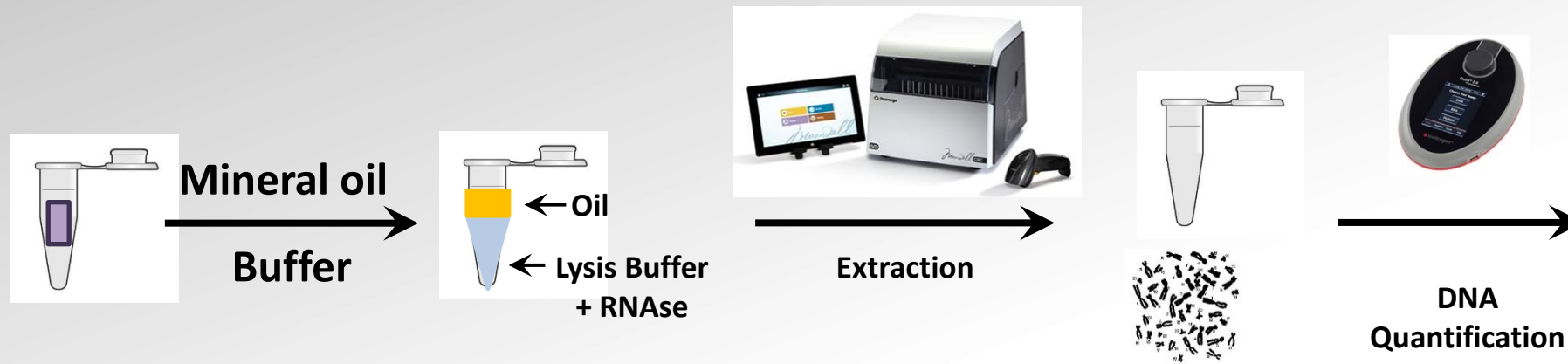
- *16S rRNA* gene: ubiquitous in bacteria
 - Has conserved regions (good for universal PCR amplification primers)
 - Has variable regions (good for discriminating between bacterial species)
 - PCR then gene sequencing (works on FFPE)



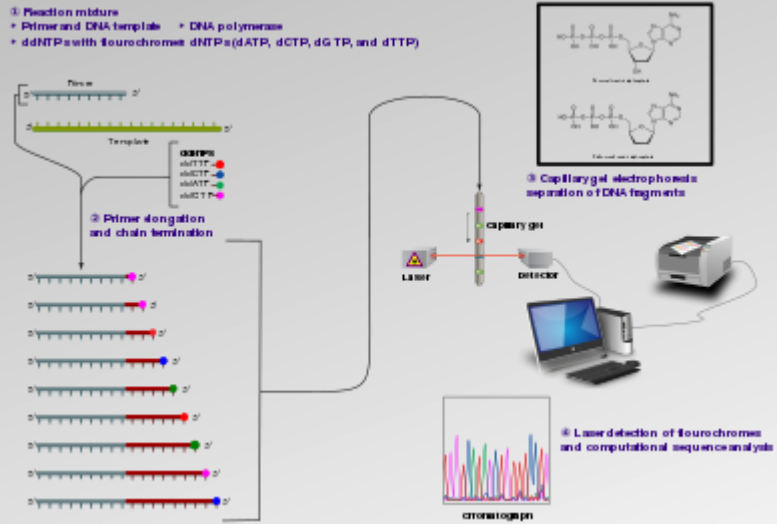
DNA extraction method



DNA extraction



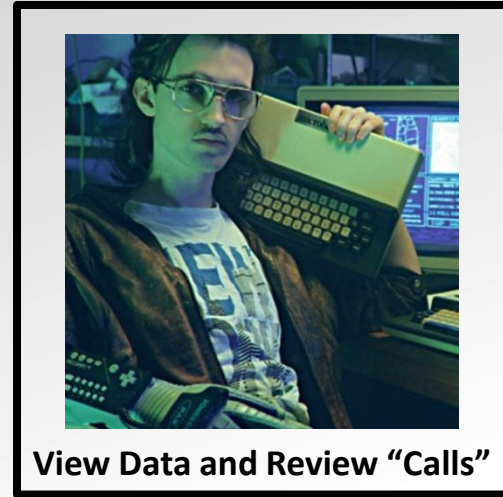
Sequencing, BLAST



Sanger Sequencer



Processing of Raw Data



View Data and Review "Calls"



Create Report



Results

- FFPE tissue section positive for *Mycobacterium tuberculosis*
- Reported to UW Infection Control and Hospital Epidemiology

Result

Final identification: **Mycobacterium tuberculosis CDC1551**

Closest reference found: **Mycobacterium tuberculosis CDC1551 AE000516 1308 (99.70%)**

Remark:

Similarity search summary

Scanned databases (Db):
D1 - IDNS 16S Centroids

Rank	Db	AC - Accession	OS - Organism	Species group size	Gen. confidence value	Seq. Length	Identities	Mismatches	Match Length	Score
1	D1	AM408590	Mycobacterium bovis BCG str. Pasteur 1173P2	29	100.0	1536	1308 (99.70%)	4	1312	1364.0
2	D1	CP016401	Mycobacterium caprae	4	100.0	1536	1308 (99.70%)	4	1312	1364.0
3	D1	AE000516	Mycobacterium tuberculosis CDC1551	405	100.0	1536	1308 (99.70%)	4	1312	1364.0
4	D1	CP010334	Mycobacterium africanum	12	100.0	1536	1308 (99.70%)	4	1312	1364.0
5	D1	CP010333	Mycobacterium microti	10	100.0	1536	1307 (99.62%)	5	1312	1360.0
6	D1	AF502574	Mycobacterium pinnipedii	1	100.0	1381	1307 (99.62%)	5	1312	1360.0
7	D1	CP000854	Mycobacterium marinum M	69	100.0	1535	1298 (98.93%)	14	1312	1330.0
8	D1	AY005147	Mycobacterium shottsii	4	100.0	1491	1298 (98.93%)	14	1312	1330.0
9	D1	AP017624	Mycobacterium ulcerans subsp. shinshuense	26	100.0	1535	1295 (98.70%)	17	1312	1319.0
10	D1	AF406783	Mycobacterium lacus	8	100.0	1470	1294 (98.55%)	19	1313	1312.0

Histopathology in the immunocompromised



ELSEVIER

Available online at www.sciencedirect.com



Pathology – Research and Practice 204 (2008) 155–161

PATHOLOGY
RESEARCH AND PRACTICE

www.elsevier.de/prp

ORIGINAL ARTICLE

Lung granulomas from *Mycobacterium tuberculosis*/HIV-1 co-infected patients display decreased *in situ* TNF production

Almério L.L. de Noronha^{a,b}, André Báfica^c, Lucas Nogueira^{a,b}, Aldina Barral^{a,b},
Manoel Barral-Netto^{a,b,*}

^a*Centro de Pesquisas Goncalo Moniz, Fiocruz, Bahia, Brazil*

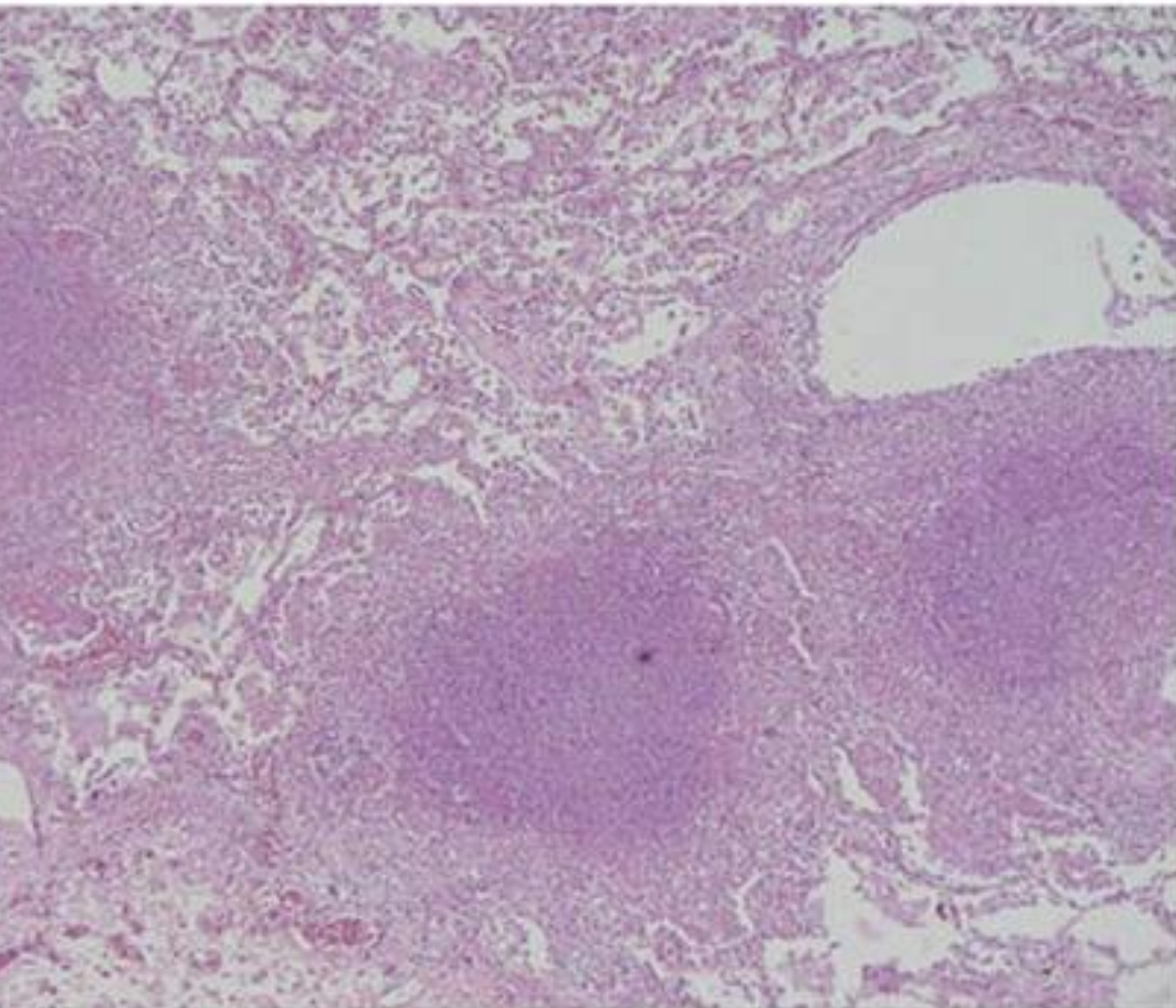
^b*Faculdade de Medicina da Bahia, UFBA, Salvador, Bahia, Brazil*

^c*Division of Immunology, Microbiology and Parasitology Department, Federal University of Santa Catarina, Florianopolis-Santa Catarina, Brazil*

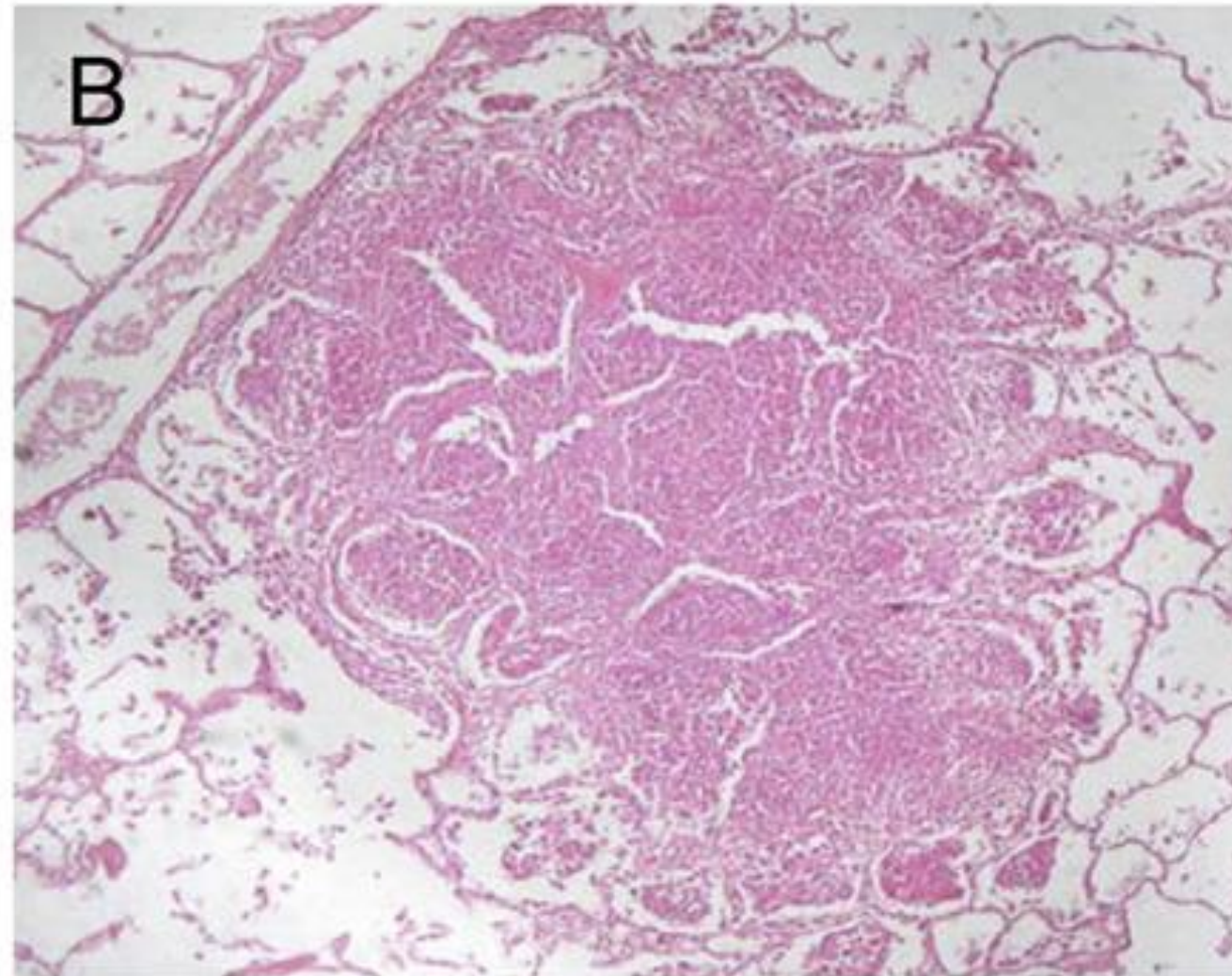
Received 2 January 2007; accepted 22 October 2007

Histopathology in the immunocompromised

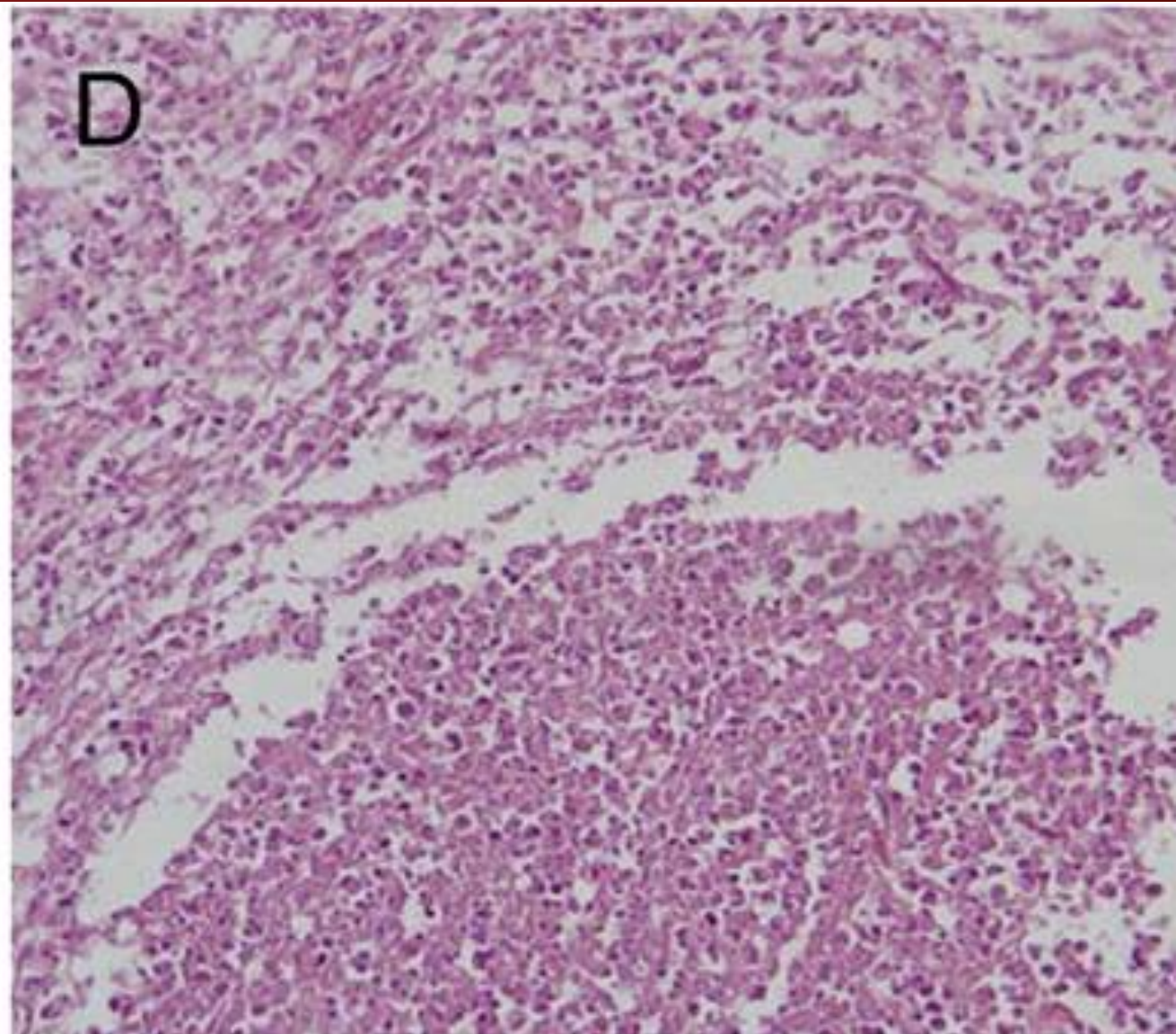
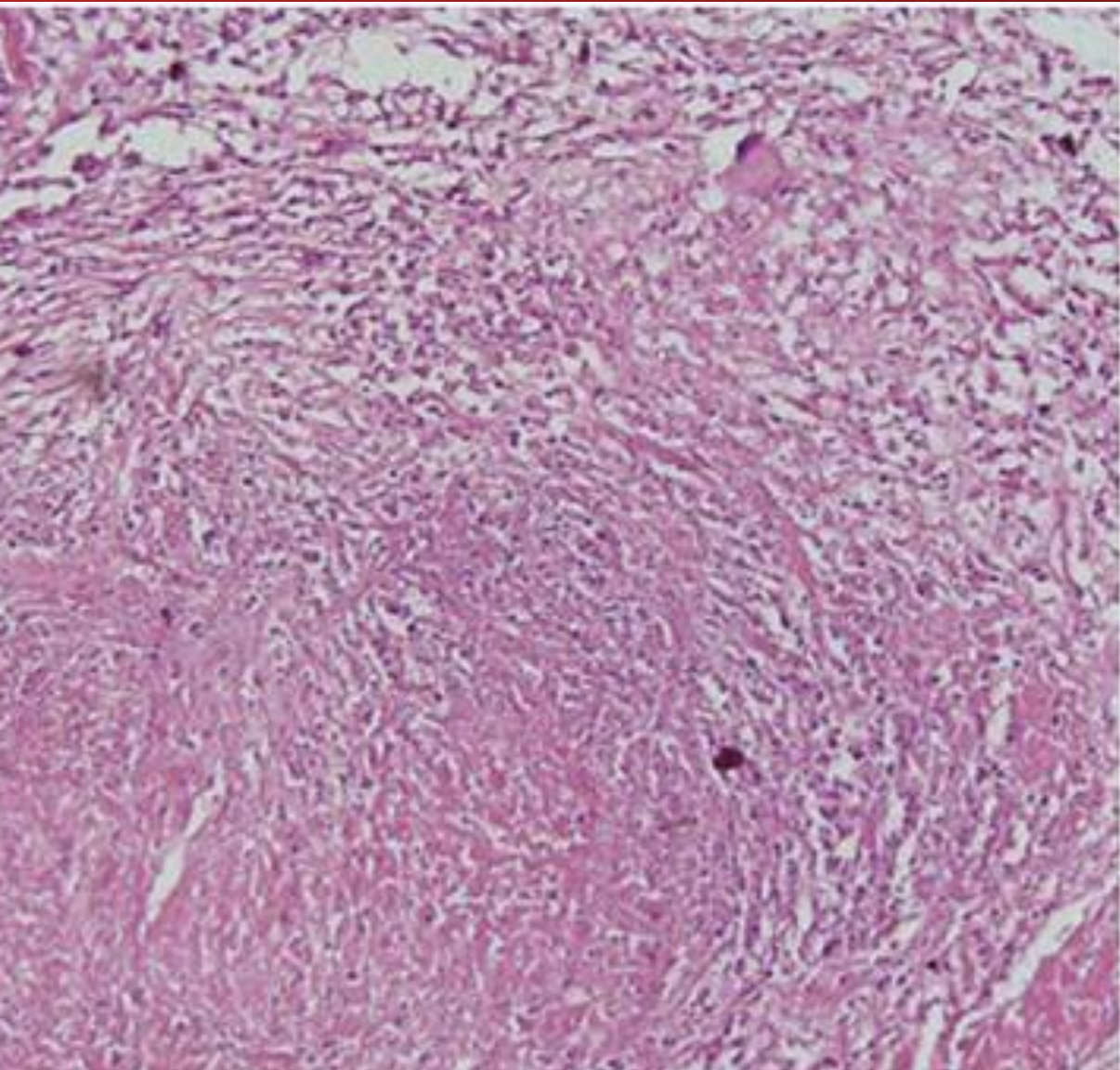
TB



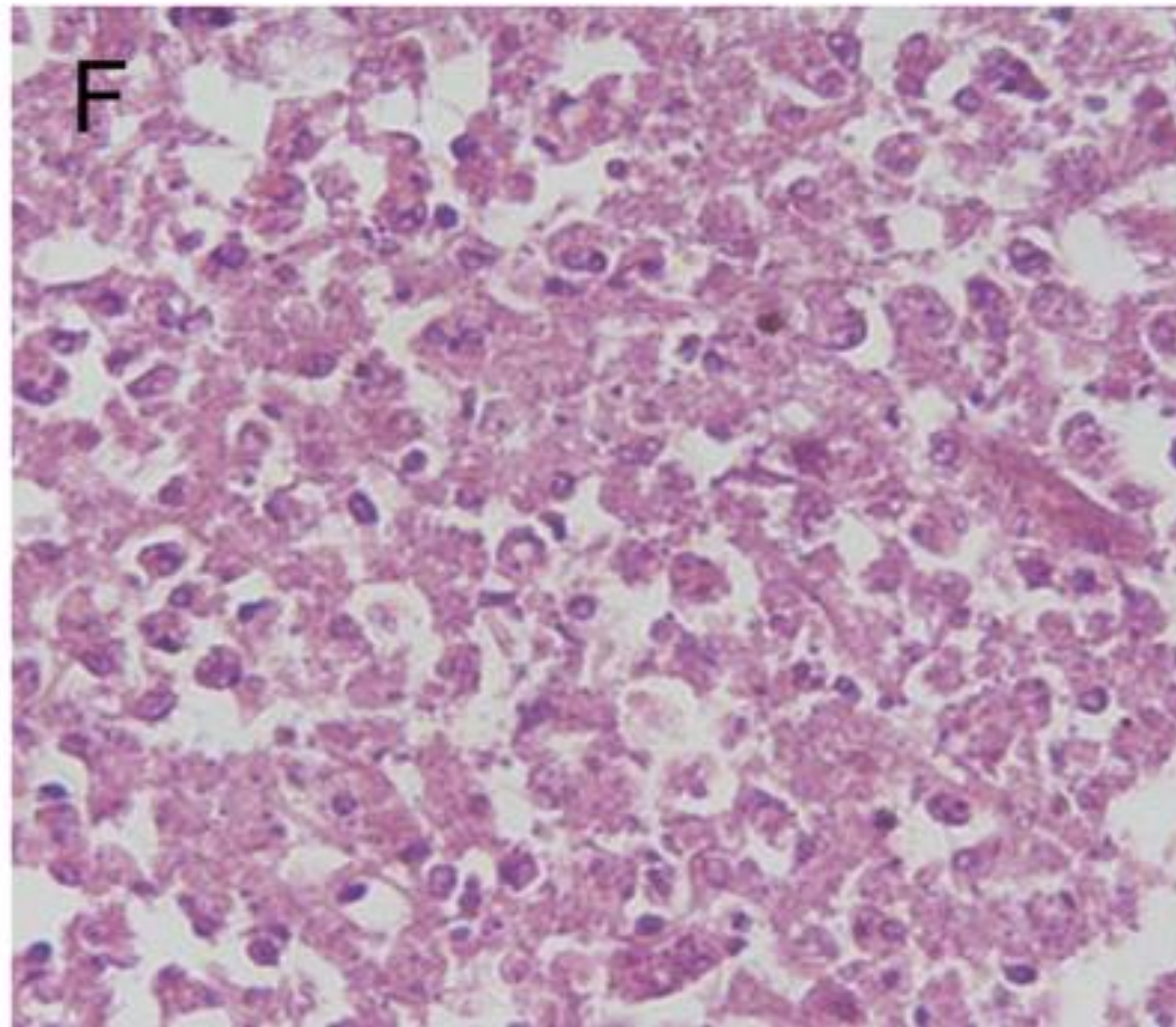
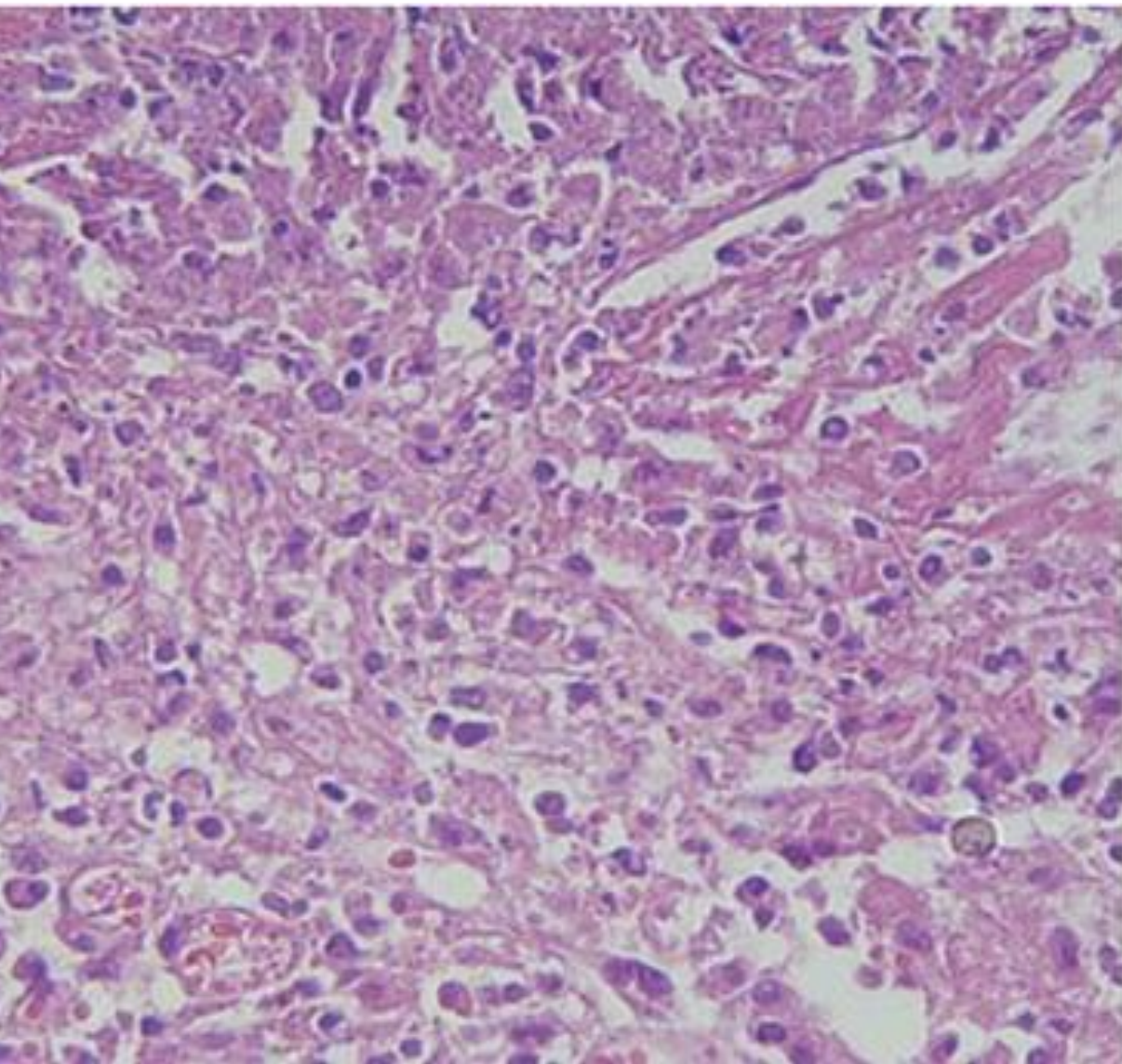
TB/HIV-1



Histopathology in the immunocompromised

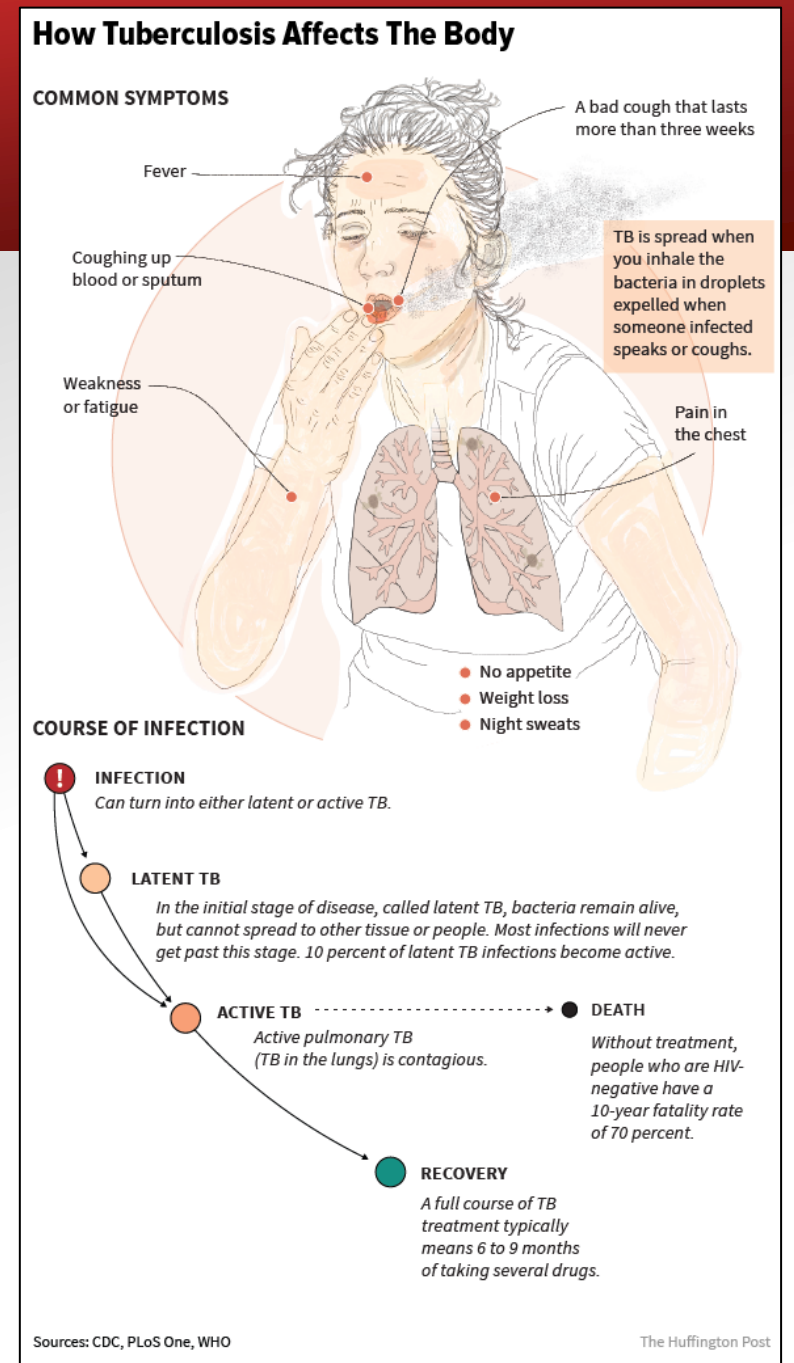


Histopathology in the immunocompromised



Natural History of TB Infection

- Transmitted via cough aerosol
- In most patients (>90%), primary infection is asymptomatic
 - Either the immune system eliminates TB entirely or else latent TB develops
- In a minority, active tuberculosis develops
 - Primary TB: following initial infection
 - Secondary TB: following reactivation of latent TB



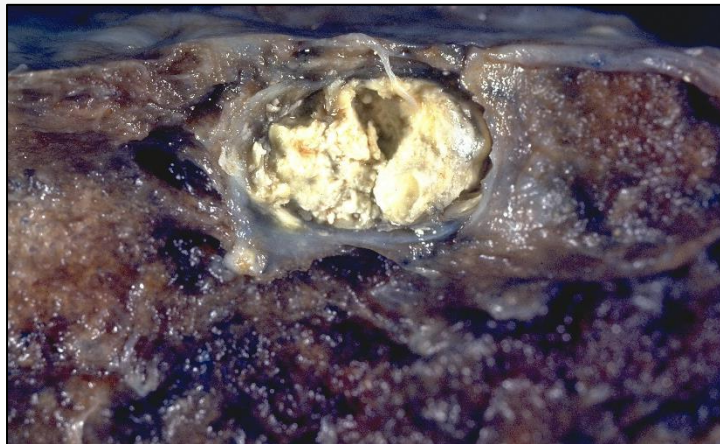
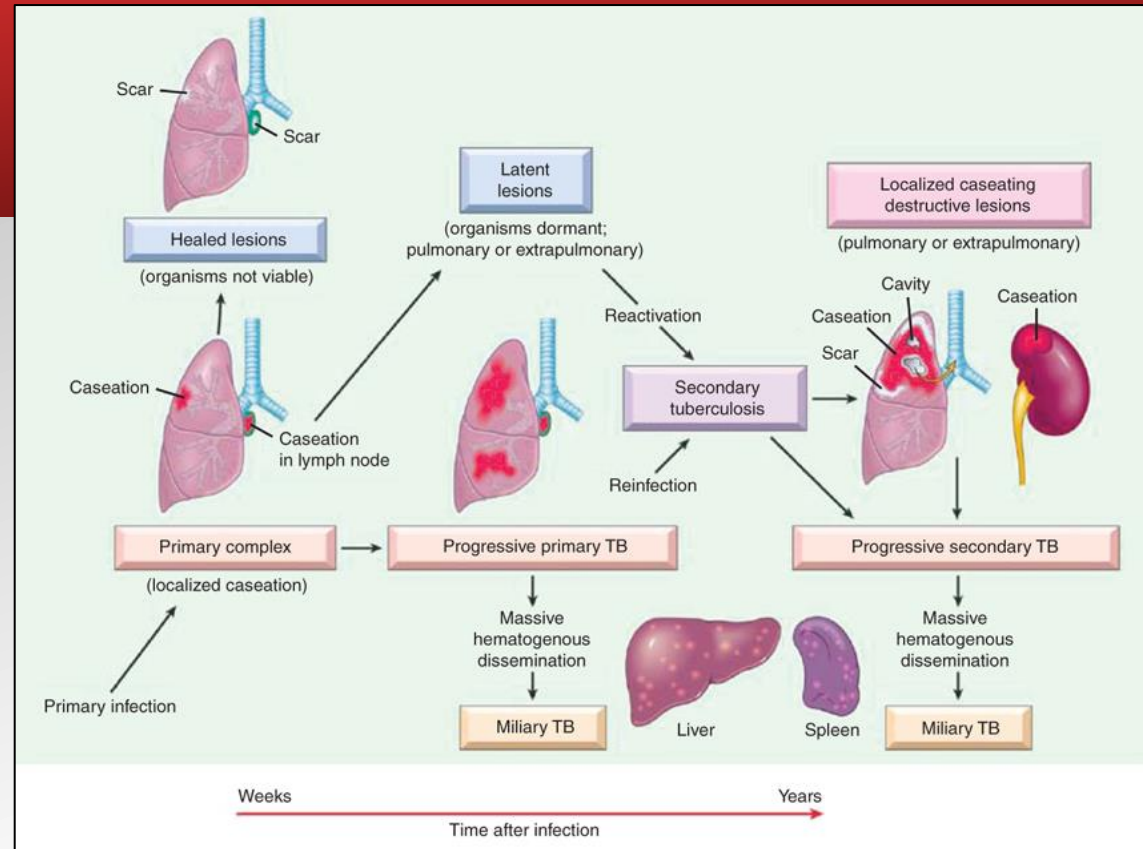
TB: Pathogenesis

- **Latent infection**

- Ghon lesion
(granulomatous lesion, usually subpleural, avg. 2-3 cm)

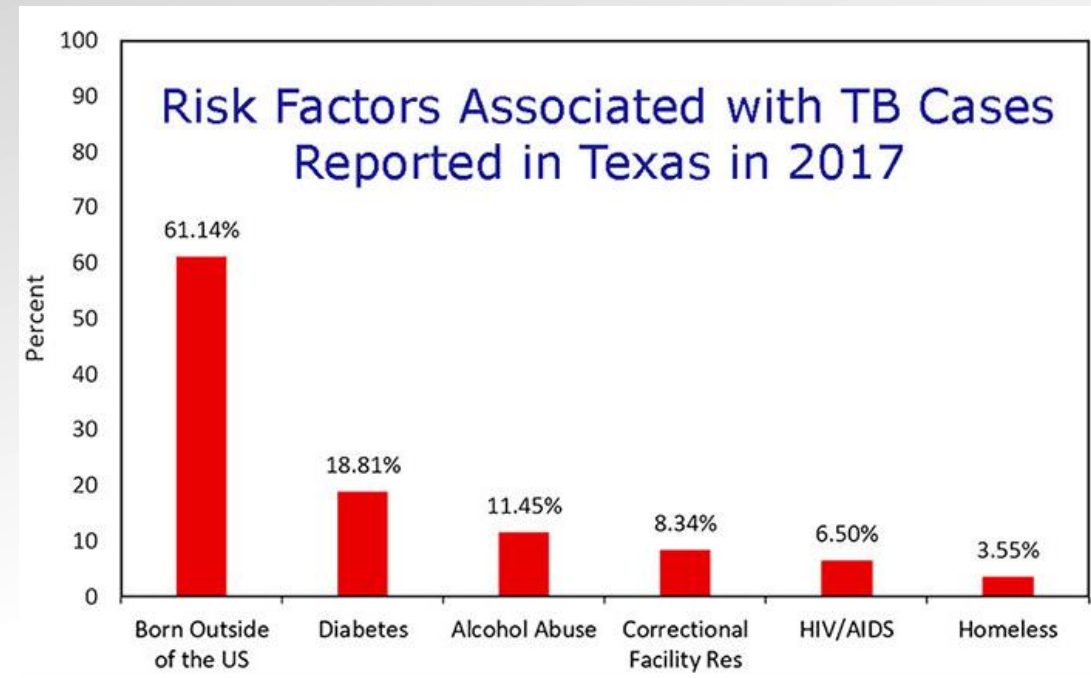
- **Active infection**

- Locally destructive cavitary lesions
- Widespread hematogenous spread, i.e. 'miliary' TB



Further Record Review: Social History

- The patient had grown up in the crowded housing projects of Chicago
- She had been incarcerated in Jacksonville FL (1995)
- She had been homeless in Madison WI (1999)
- She had a history of alcohol abuse (quit 2007)
- She had a sister dx'd with latent TB at age 14 (~ 20 years ago)
- The patient herself had been previously tx'd for TB: 12/1999-7/2000



TB: Statistics

- 1/4th of the world's population is infected w/ TB
 - In 2017 there were 1.3 million TB-related deaths worldwide
- In 2017 in the US, > 9000 TB cases were reported (2.8 cases per 100,000 persons)
 - In 2017 US spent \$463 million on TB health care costs
- Timely reporting of cases is crucial to minimize exposures, provide prophylaxis and prompt treatment

TAKE ON TB

Too many people in our country still suffer from tuberculosis (TB).

9,105 TB CASES REPORTED IN THE U.S. IN 2017

528

TB Deaths in 2016

A Typical TB Case Requires:

180

days of medications

PLUS

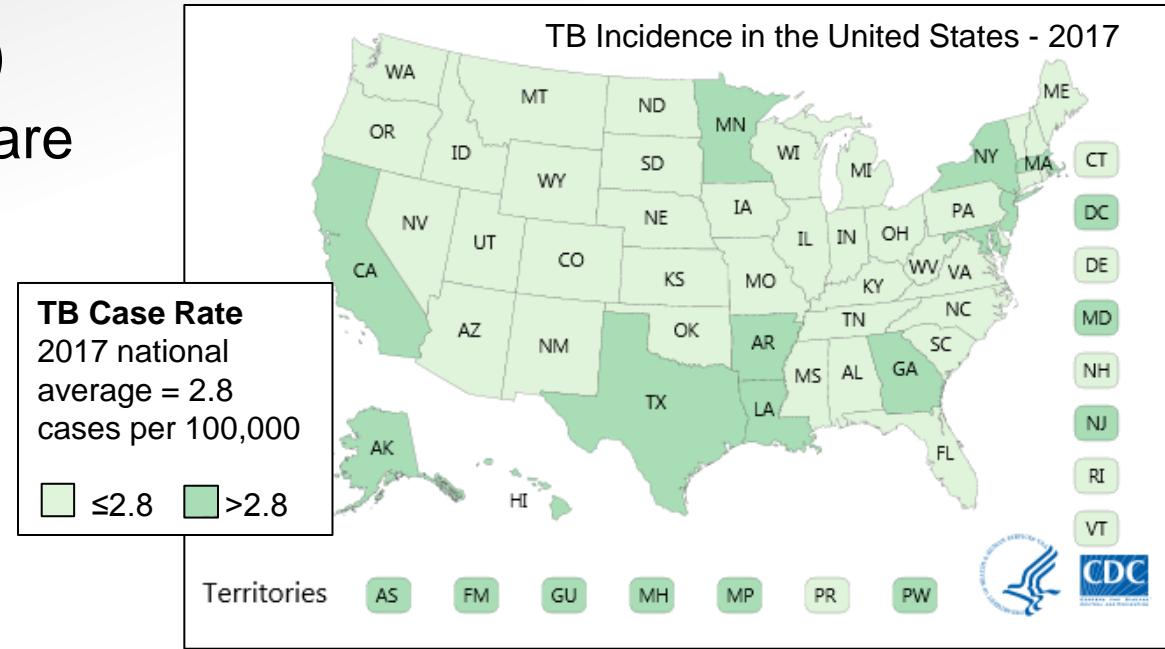
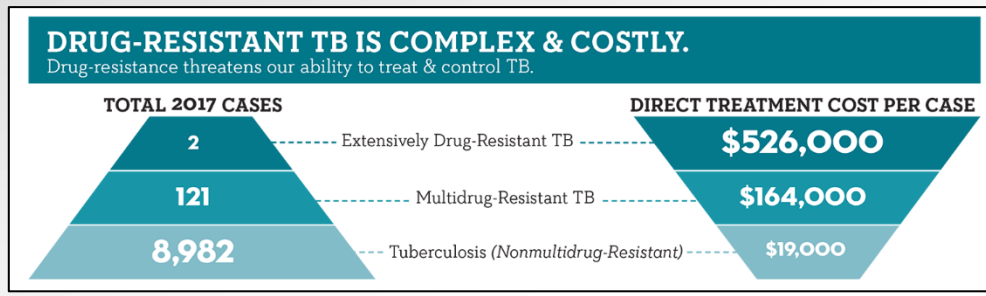
- X-rays
- Lab tests
- Follow-up & testing of contacts

\$463

MILLION

Total cost to U.S. for TB cases in 2017.

Our current strategies are not enough to achieve TB elimination in this century.



Reporting TB Cases

WI Communicable Diseases and Other Notifiable Conditions

- 3 Categories: I, II, III
- **Category I:**
 - Diseases that are of urgent public health importance and shall be reported IMMEDIATELY by telephone to the patient's local health officer, or to the local health officer's designee, upon identification of a case or suspected case.
 - Reported case to WSLH TB Laboratory Program Coordinator (who reported to WI-DHS and CDC)

Category I Disease	Notes
<u>Tuberculosis</u>	1, 2, 3, 4, 5

Notes Key:

1. Infectious disease or other condition designated as notifiable at the national level.
2. Required Wisconsin or CDC follow-up form completed by public health agency.
3. High-risk assessment by local health department is needed to determine if patient or member of patient's household is employed in food handling, daycare or health care.
4. Source investigation by local or state health department is needed.
5. Immediate treatment is recommended, i.e., antibiotic or biologic for the patient or contact or both.

Case 2

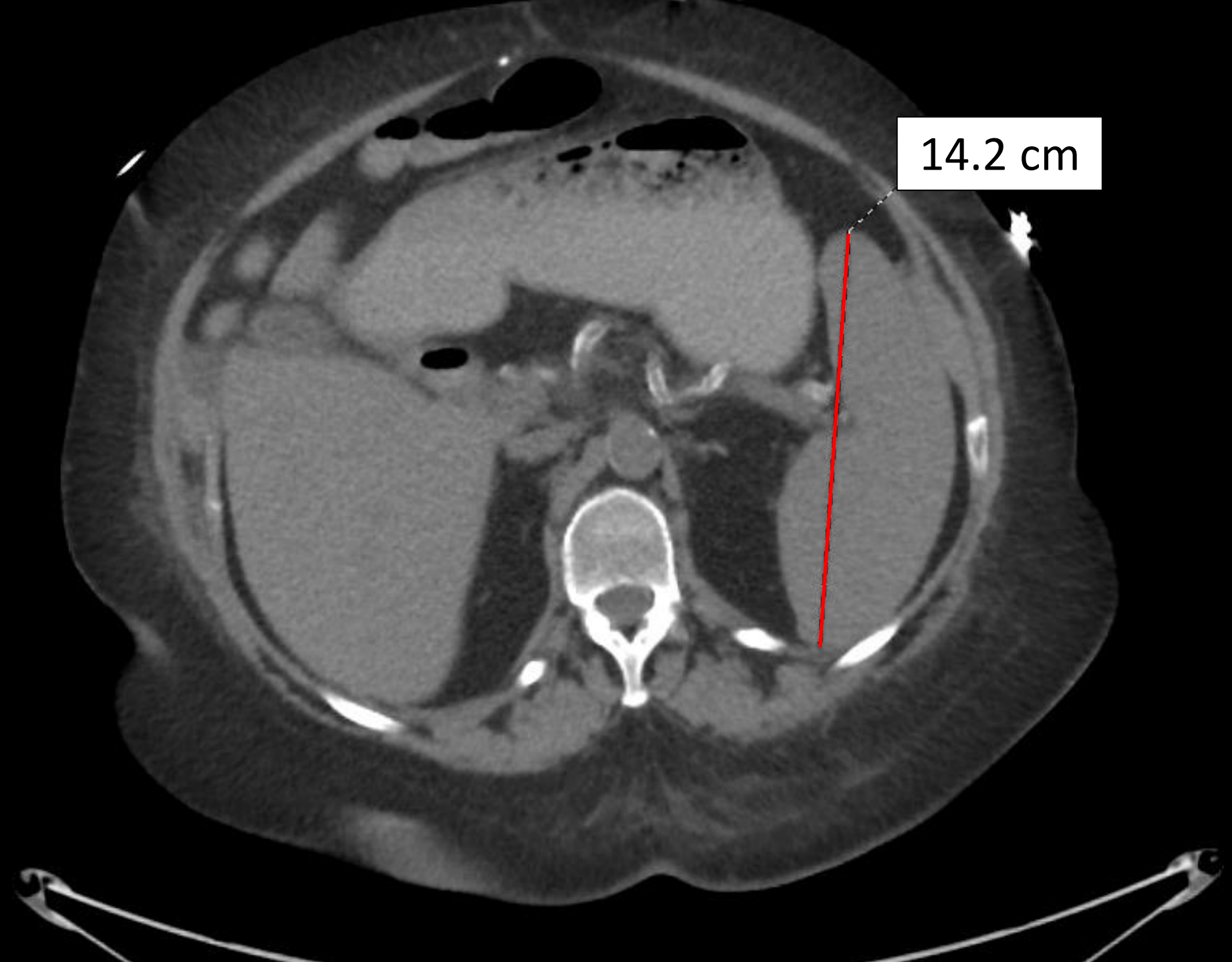
- 60 year old woman with multiple transplants (Pancreas in 1996, Pancreas and Kidney in 2004)
- On chronic immunosuppression
- Presents with fever, tachycardia and urinary tract infection
- Complaint of abdominal pain, diarrhea, and fatigue
- Immunosuppression: cyclosporin, mycophenolate, prednisone

Clinical Course

- Admitted, given fluids, started on antibiotics
- Urine culture positive for *Klebsiella pneumoniae*
- Rash found that was positive for HSV-2 via PCR
- Condition improved over the next few days, but then developed fever of unknown etiology
 - Concern for occult infection or post-transplant lymphoproliferative disorder

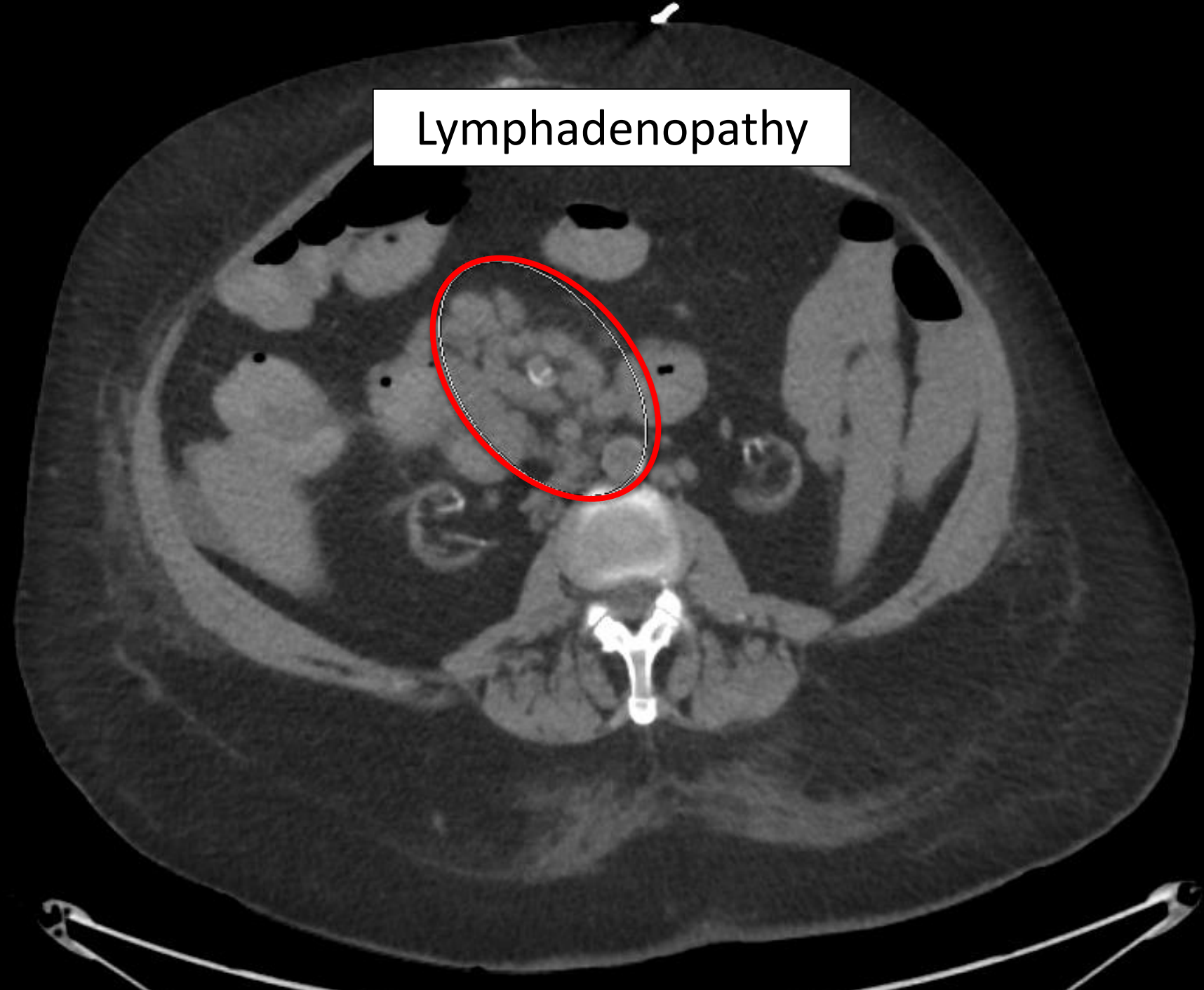
Diagnostic workup

- Workup for CMV and EBV as well as other infections were negative
- CT abdomen performed revealing...

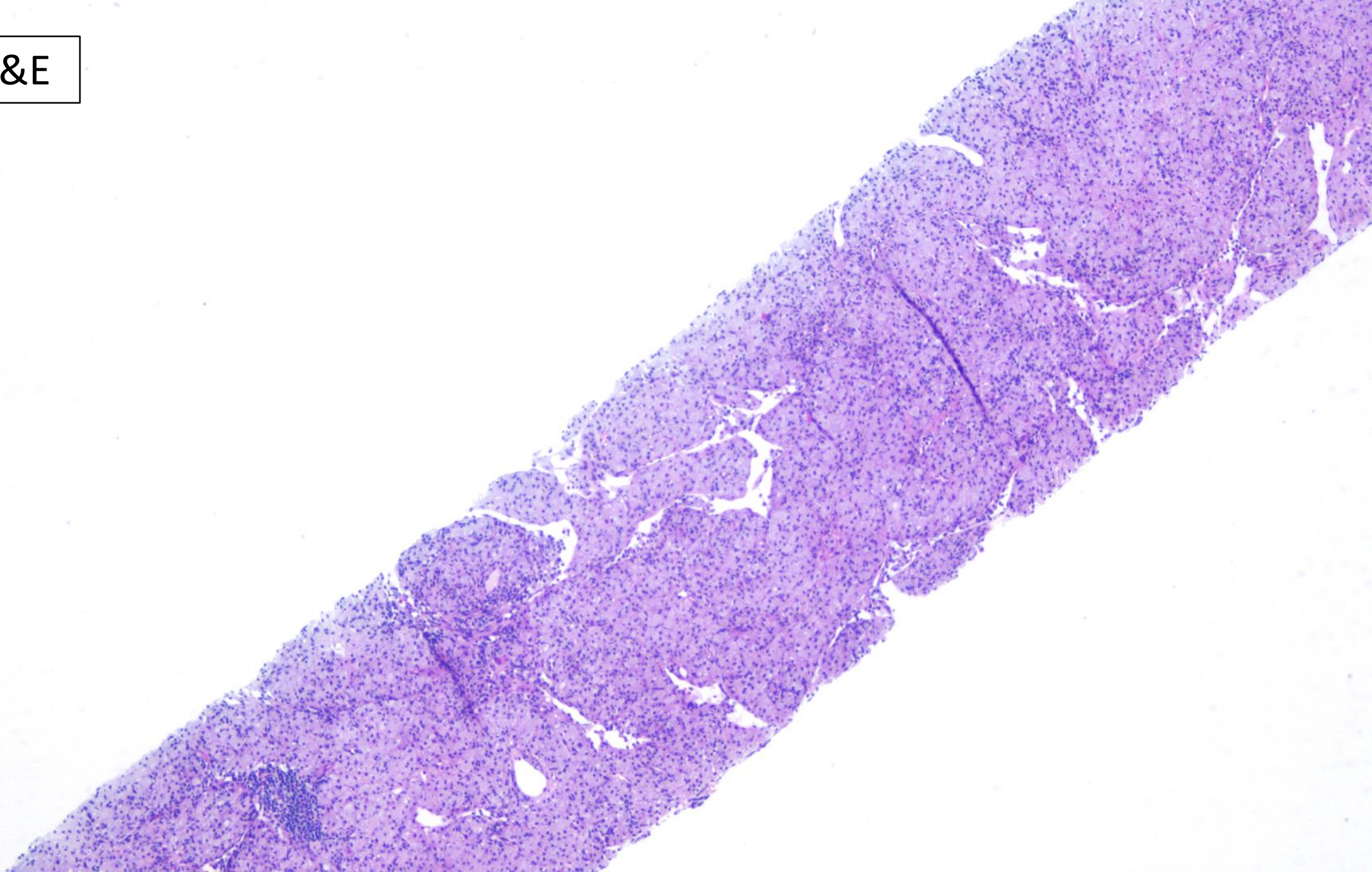


14.2 cm

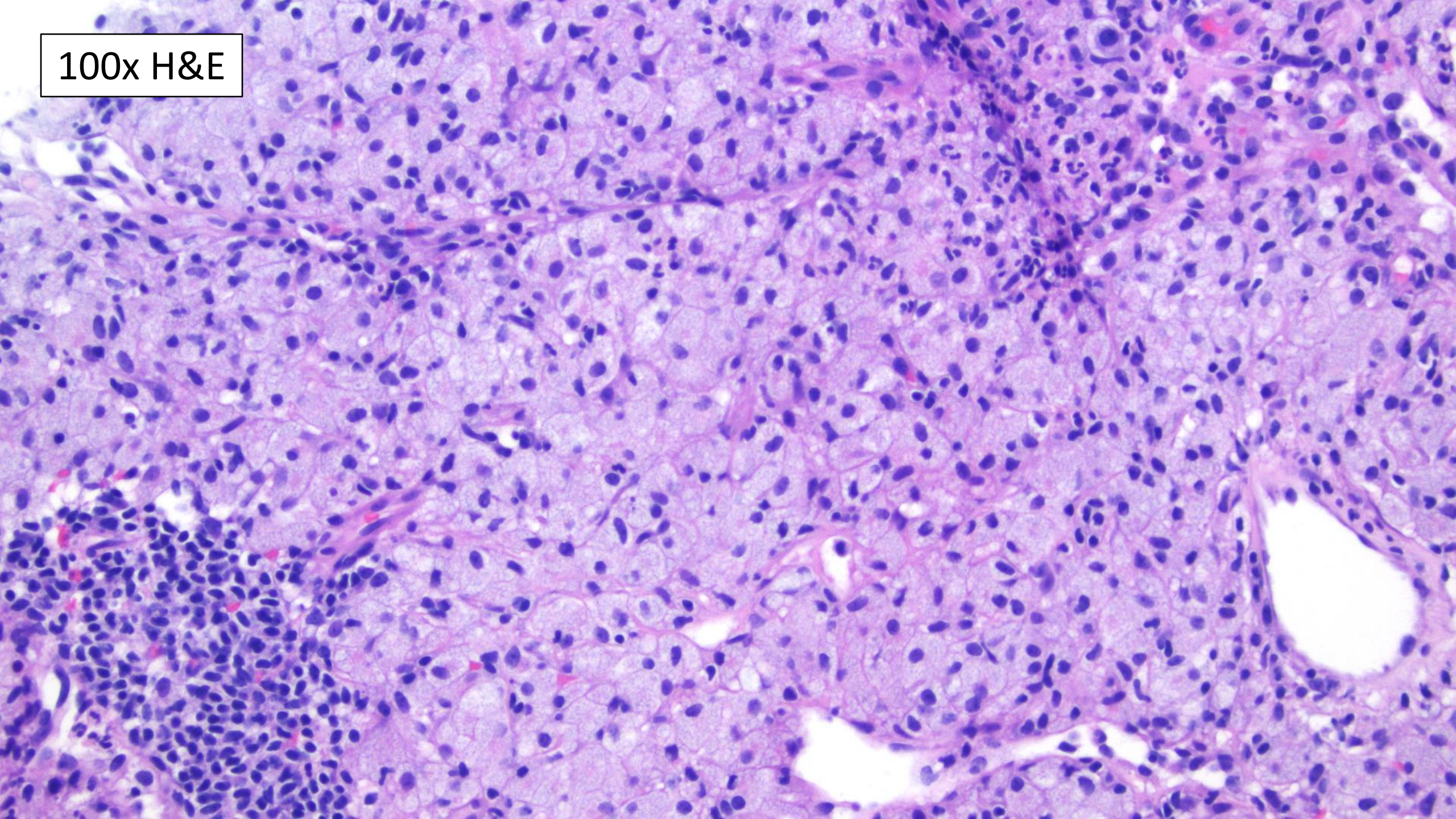
Lymphadenopathy



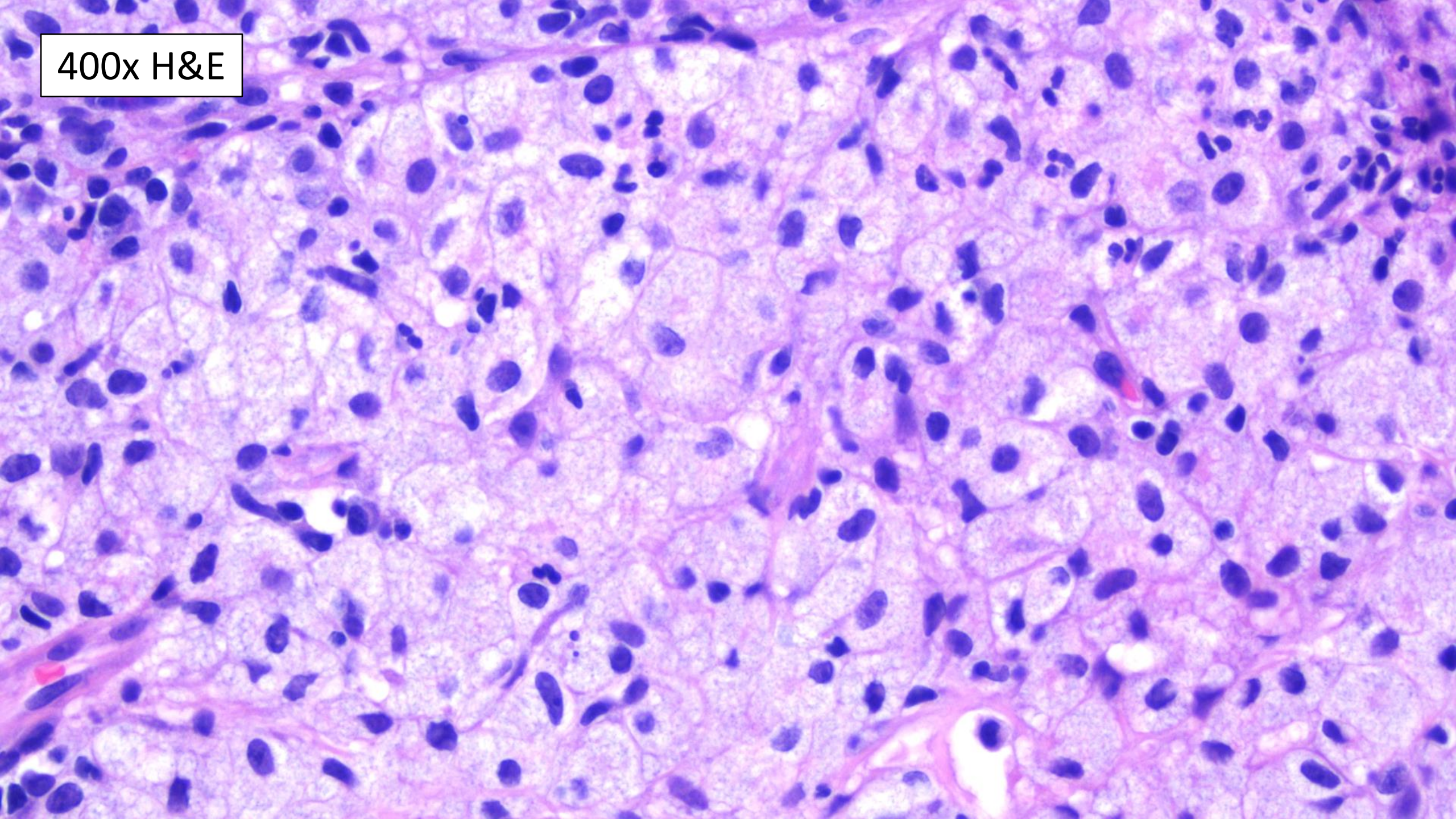
20x H&E



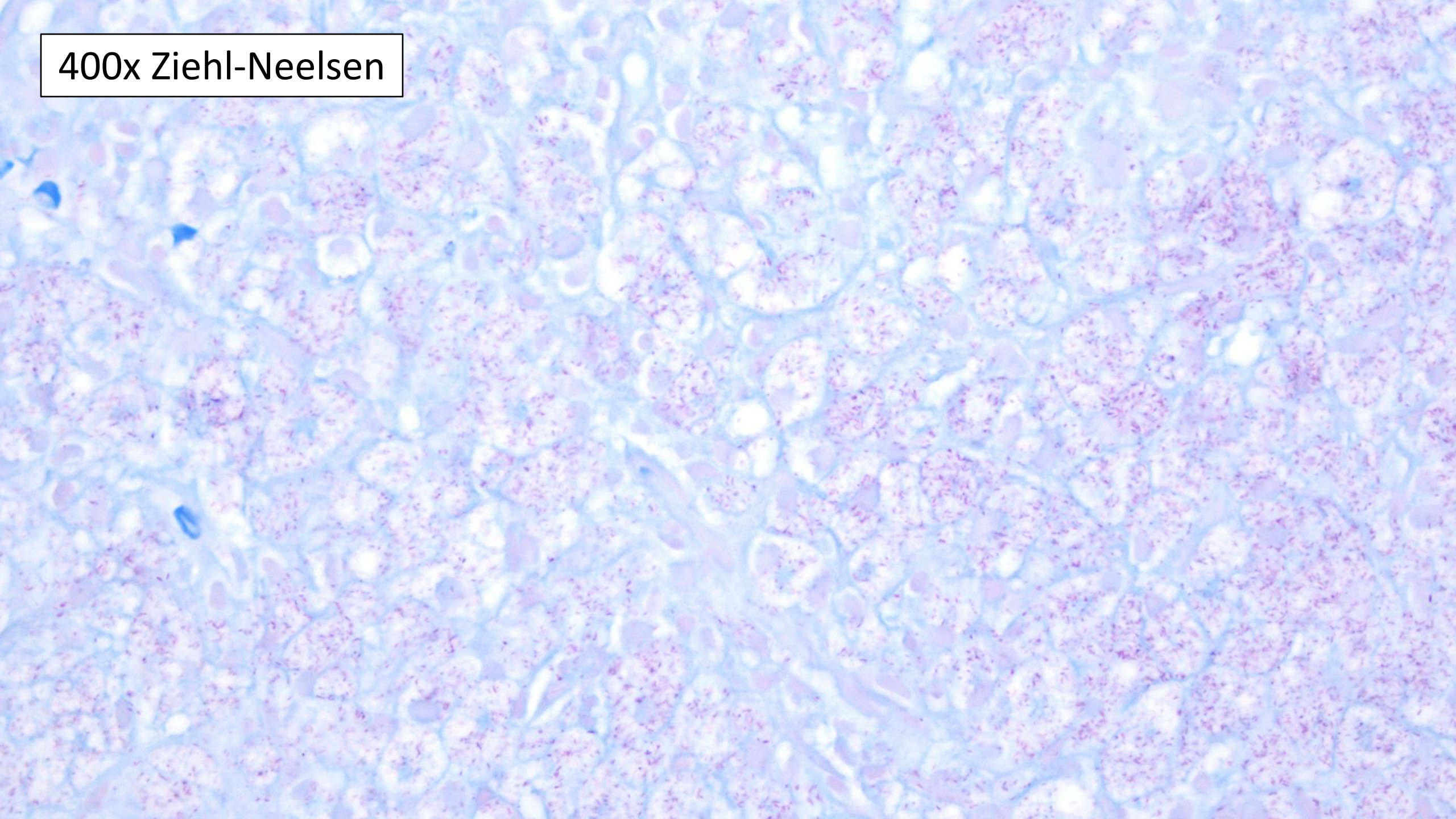
100x H&E



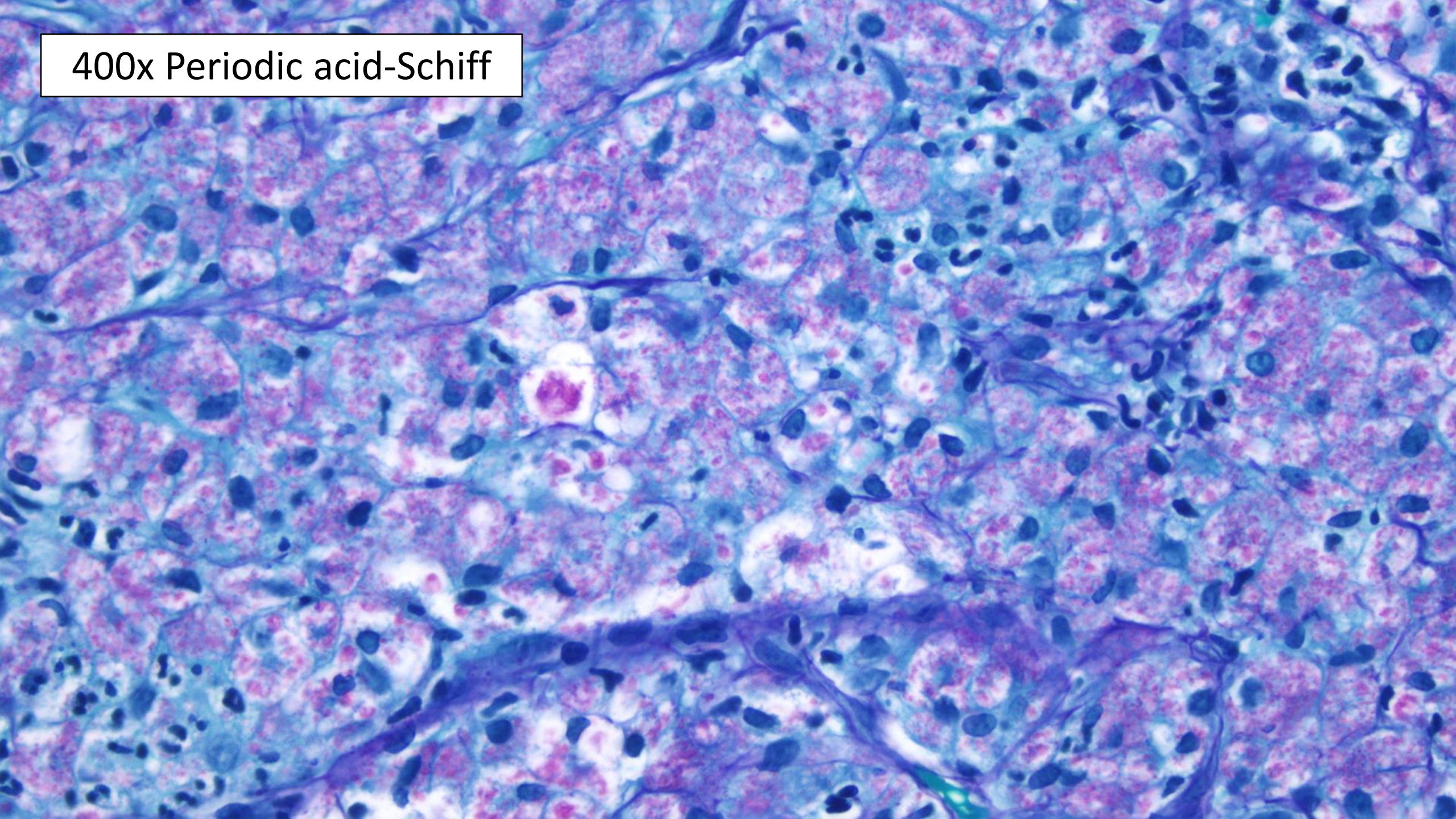
400x H&E



400x Ziehl-Neelsen



400x Periodic acid-Schiff



Diagnosis

- Lymph node effaced by histiocytes with abundant grainy cytoplasm. Few residual lymphoid cells, no evidence of lymphoma. Ziehl-Neelsen stain demonstrates **numerous acid fast organisms** that also demonstrate periodic acid-Schiff positivity
- Sent out for 16S PCR which demonstrates *Mycobacterium genavense*



Mycobacterium genavense

- First identified in 1990 in Swiss patients with AIDS
- Found in lymph nodes, bone marrow and intestinal biopsies
- Almost exclusively in patients with AIDS and CD4 counts <100
- Symptoms: fever, weight loss, diarrhea
- Associated with massive adenopathy and organomegaly



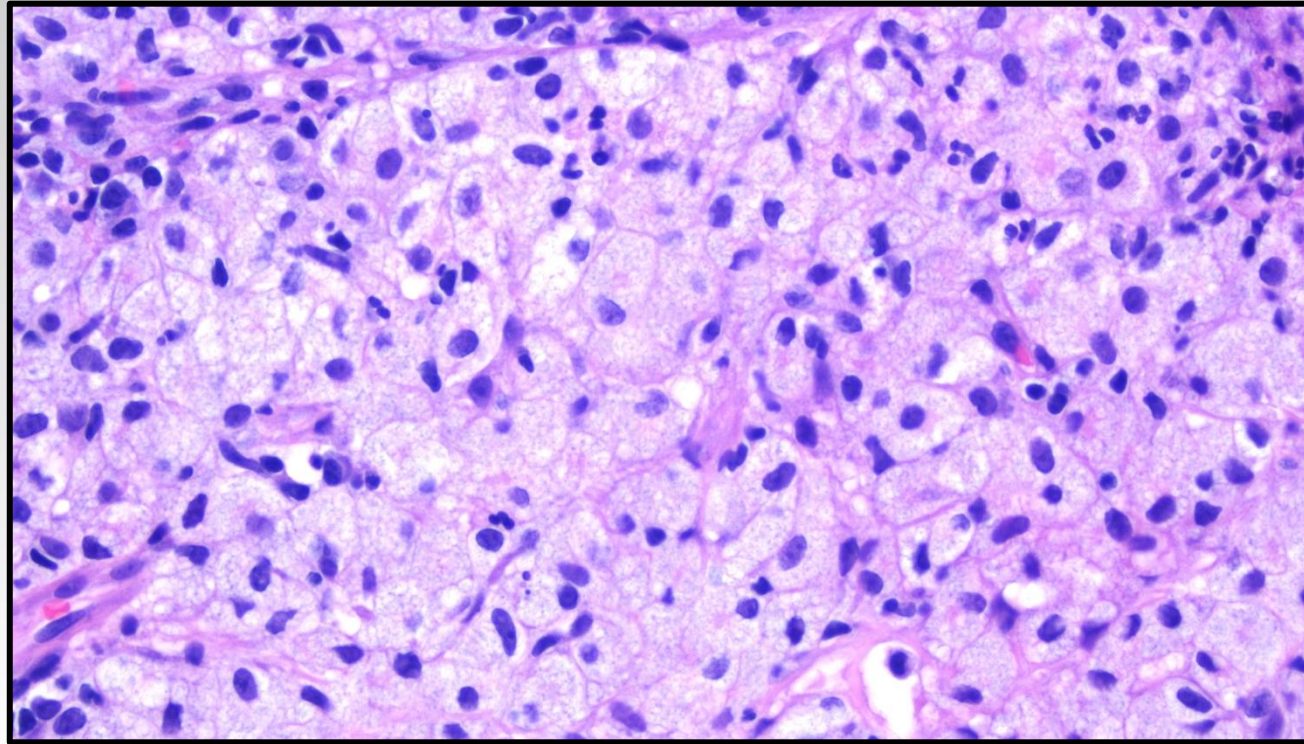
M. genavense characteristics

- Optimal isolation 37C (14-28 days)
- Niacin and Nitrate reduction test Negative
- Tween 80 hydrolysis at 10 days Positive
- Catalase, Urease, Pyrazinamidase Positive
- Non-pigmented
- Requires mycobactin J (not available in most clinical laboratories)
- Susceptible to rifampin, amikacin, clarithromycin, azithromycin
- Resistant to ethambutol and isoniazid



Histopathology

- Foamy histiocytes and ill-defined granulomas



Clinical Course

- Started on Azithromycin, moxifloxacin and rifabutin
- Resolution of abdominal pain, diarrhea and intermittent fevers as well as improvement in fatigue
- Four months post-treatment no change in imaging
 - Referral for follow-up imaging



References

1. J. Bernardo. Epidemiology and pathology of military and extrapulmonary tuberculosis. C. Fordham von Reyn, ed. UpToDate. Waltham, MA: UpToDate Inc. <http://www.uptodate.com> (Accessed May 22, 2018)
2. J. Bernardo. Clinical manifestations, diagnosis, and treatment of military tuberculosis. C. Fordham von Reyn, ed. UpToDate. Waltham, MA: UpToDate Inc. <http://www.uptodate.com> (Accessed May 22, 2018)
3. J Keane et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med. 2001, October 11; 345 (15):1098-1104.
4. L.L. Almerio et al. Lung granulomas from Mycobacterium tuberculosis/HIV-1 co-infected patients display decreased in situ TNF production. Pathol Res Pract. 2008; 204 (3): 155-161.
5. Y.M. Hui et al. Haemophagocytic lymphohistocytosis associated with Mycobacterium tuberculosis infection. BMJ Case Rep. 2015 Apr 13; 2015.
6. K.L. McClain et al. Clinical features and diagnosis of hemophagocytic lymphohistiocytosis. P. Newburger, ed. UpToDate. Waltham, MA: UpToDate Inc. <http://www.uptodate.com> (Accessed May 22, 2018)
7. Morbidity and Mortality Weekly Report: Updated Guidelines for Using Interferon Gamma Release Assays to Detect Mycobacterium tuberculosis Infection — United States, 2010. June 25, 2010, Vol 59
8. C.J. McDaniel et al. Humans and Cattle: A review of Bovine Zoonoses. Vector-Borne and Zoonotic Diseases. Vol 14, Number 1, 2014



Acknowledgements

- Erin Brooks, MD, FCAP
- Catherine Leith, MB BChir
- Laura Louison, MLS (ASCP)

Hanging out

Animal doc

Human doc

