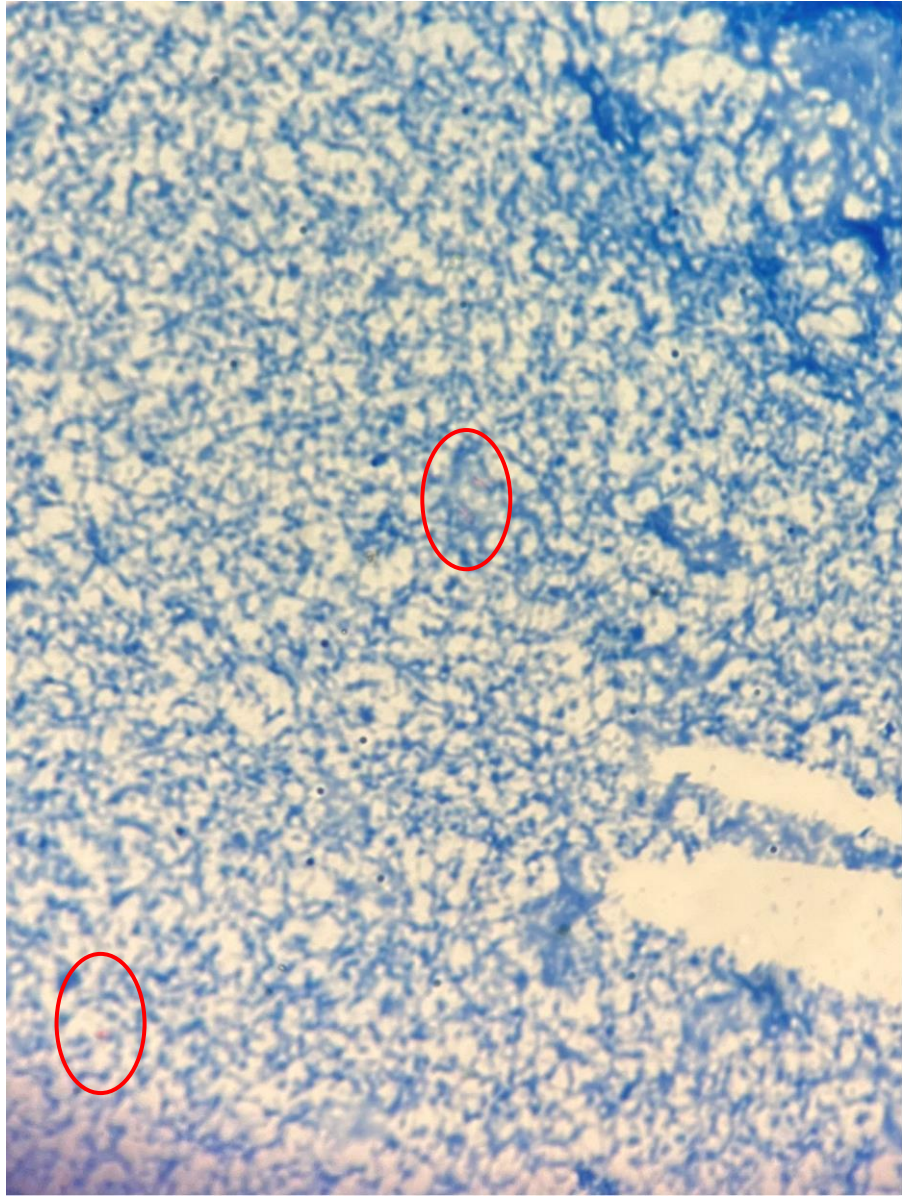




AFB Slide Contamination

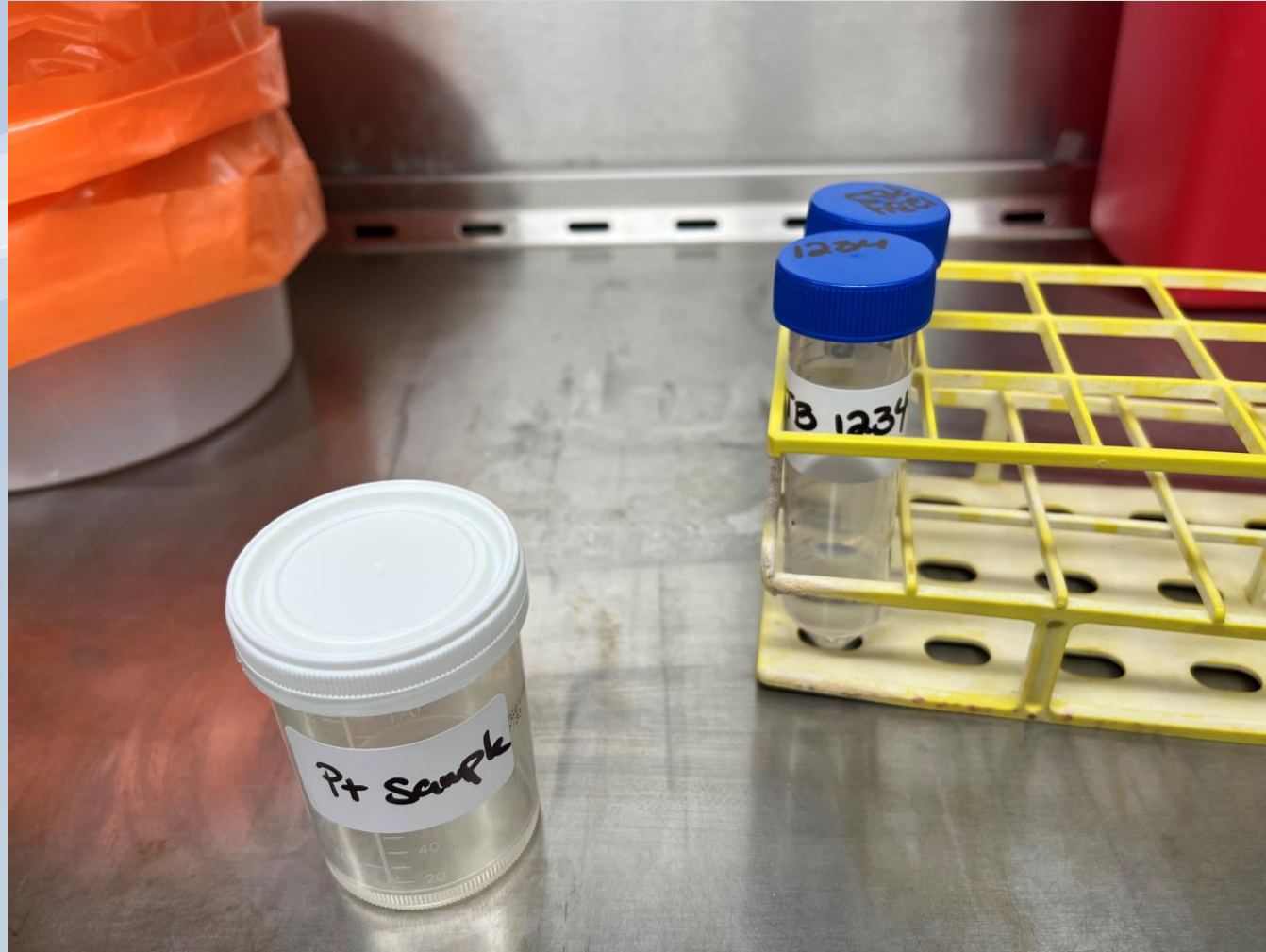
By Melissa Fisher

- Numerous Auramine O Concentrate Positive samples from various sites in our VISN and patients.
- Slides are double read by another technologist.
- Kinyoun stain was performed over these slides.
- Organisms were Kinyoun Positive, but very short rods.
- Concentrates were sent to the WSLH for MTB and MAC PCR on the patient's first positive sample.
- No Mycobacterium tuberculosis or Mycobacterium avium complex detected via PCR.
- Cultures were determined to be negative for Mycobacterium in 8 weeks.



AFB Processing

- Preprocessing of necessary samples- grinding tissue or concentrating fluids
- Label 2 sterile 50 mL conical tubes with patient accession number on side and cap.
- One tube is for NAC50/3.5% NaOH/ patient sample
- One tube is for sterile PBS
- Add a volume of NAC50 /3.5% NaOH that is approximately equal to amount of patient sample.



- Specimens are incubated at room temperature for 15 minutes.
- After the 15 minute incubation, specimens are vortexed.
- Using the corresponding labeled PBS conical tube, pour over PBS to bring the volume to 50 mL.
- Conical tubes are inverted multiple times and centrifuged for 15 minutes.
- All of the supernatant from each tube is poured off into the biohazard waste container.

- The concentrate is then used to prepare a slide.
- A prepared MGIT containing growth supplement and PANTA is inoculated using 0.5 mL of patient's sample.
- 0.3 mL patient's sample is inoculated on solid media.



AFB Staining Procedure

- Slides are heat fixed and placed on an absorbent mat for staining.
- A purchased Remel QC slide is stained with each run.
- TB Auramine O is flooded on the slides for 15 minutes.
- Slides are rinsed and then decolorized for 3 minutes.
- Slides are counterstained with Potassium Permanganate for 3 minutes.

Reading AFB concentrates:

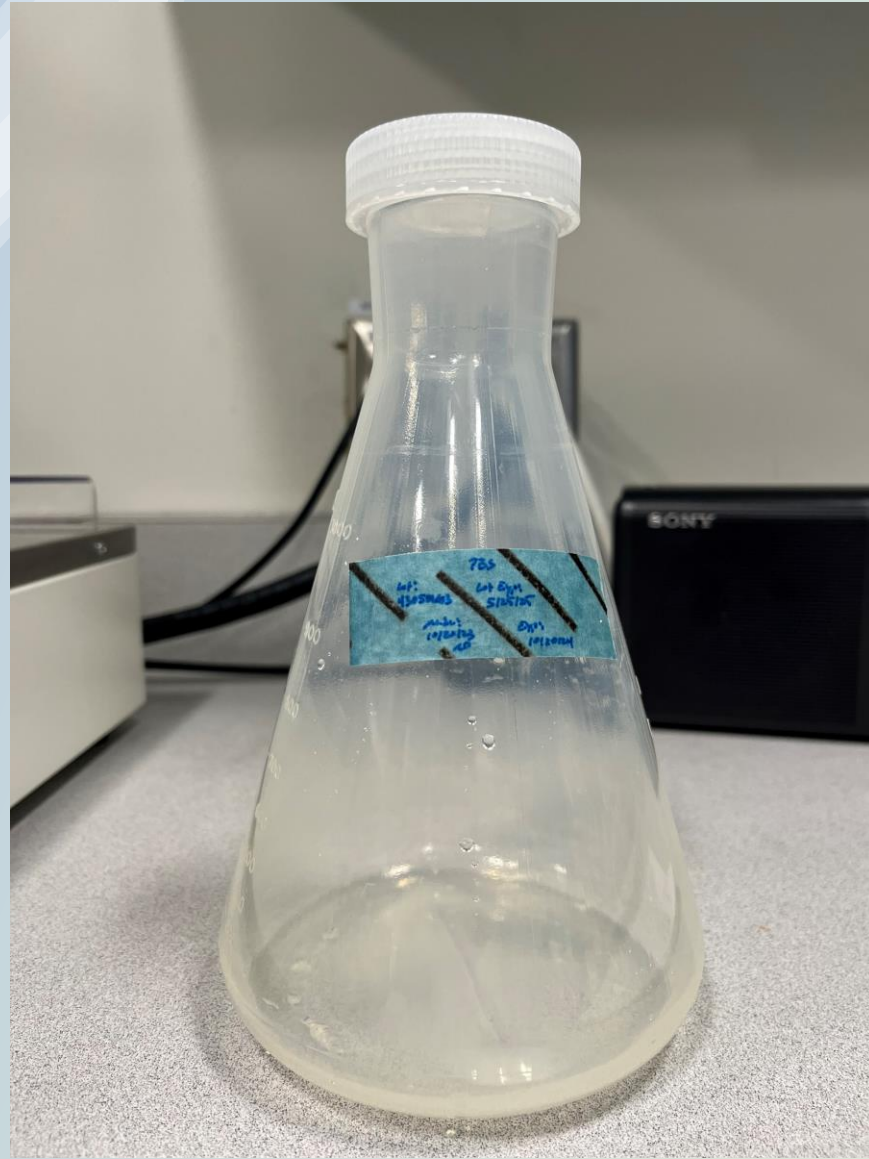
- 3-9 AFB rods per slide Concentrate Positive 1+
- 10-15 AFB rods per slide Concentrate Positive 2+
- 16-20 AFB rods per slide Concentrate Positive 3+
- >20 AFB rods per slide Concentrate Positive 4+

Troubleshooting

- Ruled out a staining issue. QC slide was acceptable.
- Ruled out a competency issue. All positive smears are double checked by an additional technologist
- Potential slide contamination- placed cleaned slides in 95% alcohol before making smears.
- Potential reagent contamination

Reagents Prepared for AFB Processing

- 3.5% NaOH
- Sterile Phosphate Buffered Saline (PBS)
- NAC 50 (N-acetyl-L-cysteine)/ 3.5% NaOH



- Stock supplies of PBS and NaOH were sequestered and tested
- 200 mL of PBS and NaOH were concentrated, and slides made.
- Both Auramine O and Kinyoun slides were separately made on the reagents
- Slides were Kinyoun Negative, no organisms seen
- For the Auramine O slides, each had areas that had fluorescence. A Kinyoun stain was performed over these slides and was negative. There was rod like debris present on the slide.

- AFB culture were held for 8 weeks.
- Terminal stains on MGITS were performed and were Kinyoun positive.
- 2 MGITs were sent to WSLH for DNA sequencing
- WSLH detected an environmental contaminate-
Cutibacterium acnes from both MGITs

- We attempted to change one reagent variable at a time.
- We use reagent logs to track when reagents are made and put into use.
- We purchased new flasks for PBS and NaOH.

Conclusion

- We ultimately determined that the flasks were contaminated. The organisms were potentially present, but not viable after autoclaving.
- We continue to monitor the issue and will potentially change our processing procedure.