Use of Artificial Intelligence and a Convolutional Neural Network for the Detection of Intestinal Parasites

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Disclosures

- Disclosures Related to this Talk:
  - » None
- Other Professional Disclosures:
  - » American Society for Microbiology (speaker/honoraria; Editorial Board, *Journal of Clinical Microbiology*)
  - » Infectious Diseases Society of America (speaker/honoraria)
  - » Seegene (speaker/honoraria)
  - » Techcyte Inc. (collaborator)
  - » Apacor (collaborator)
  - » SWACM (speaker/sponsored travel)

Learning Objectives

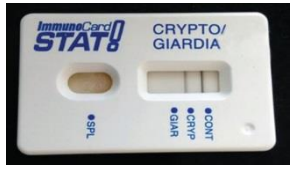
- Recall detection of intestinal parasites, with an emphasis on microscopy
- Describe the development of machine learning software to detect intestinal parasites
- Discuss the implementation of image analysis software in a clinical diagnostic lab, the benefits derived therefrom, and possible challenges to overcome

Reviewing Microscopic Diagnostic Methods for Stool Parasites

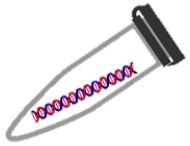
Conventional Diagnosis of Intestinal Parasites

- Morphologic Analysis

- Antigen Detection



- Molecular Detection



- Artificial Intelligence and Machine Learning

Conventional Diagnosis of Intestinal Parasites

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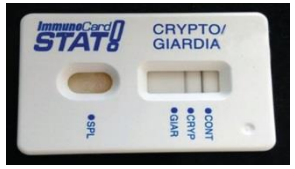

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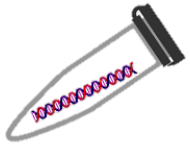

- Artificial Intelligence and Machine Learning

Morphologic and Microscopic Methods for the Detection of Intestinal Parasites

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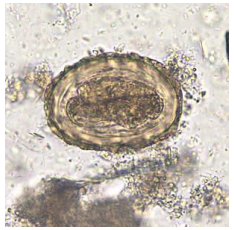
- Wet Mount (direct or concentrate)



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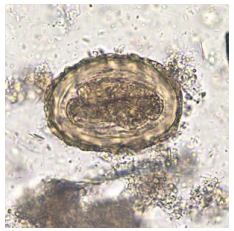
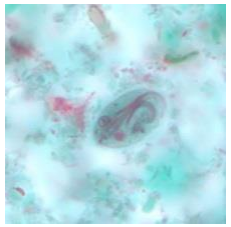


- Trichrome Smear



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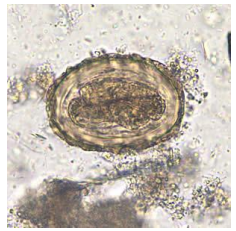

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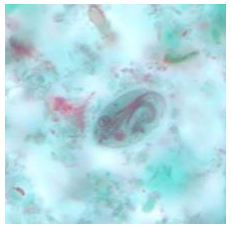
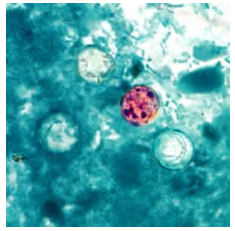


- Modified Acid-Fast/Safranin Stains



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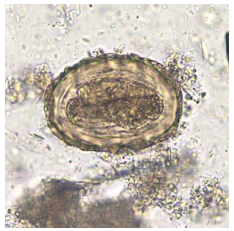

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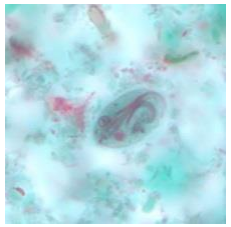

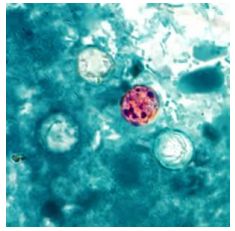

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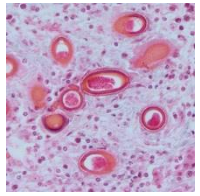

- Histopathology

Morphologic and Microscopic Methods for the Detection of Intestinal Parasites

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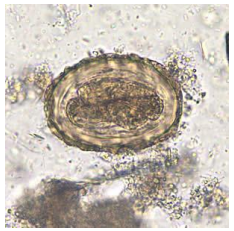

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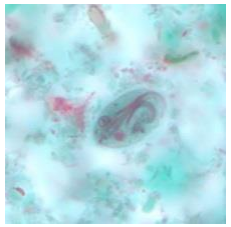

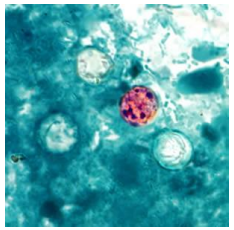

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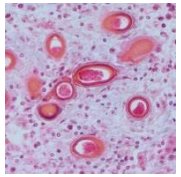
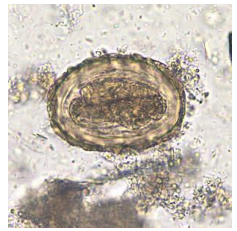


- Histopathology

- Gross Examination of Helminths

Morphologic and Microscopic Methods for the Detection of Intestinal Parasites

- Wet Mount (direct or concentrate)



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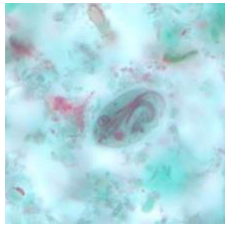

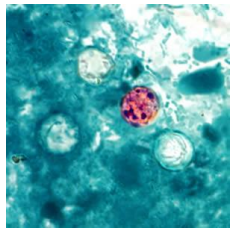

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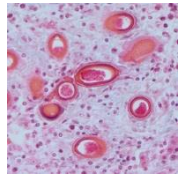

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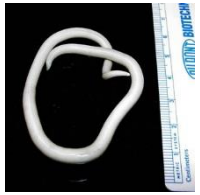


- Gross Examination of Helminths

Understanding the insanity of the method

Microscopy and The Ova and Parasite Exam

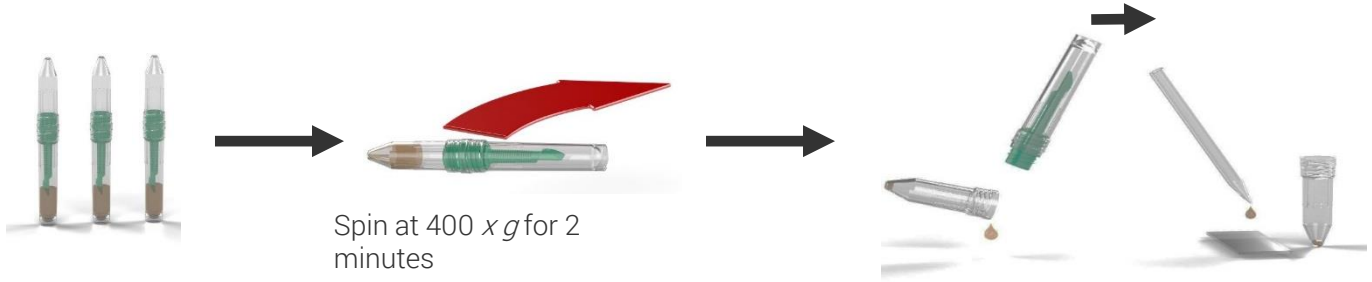
Ova and parasite exam

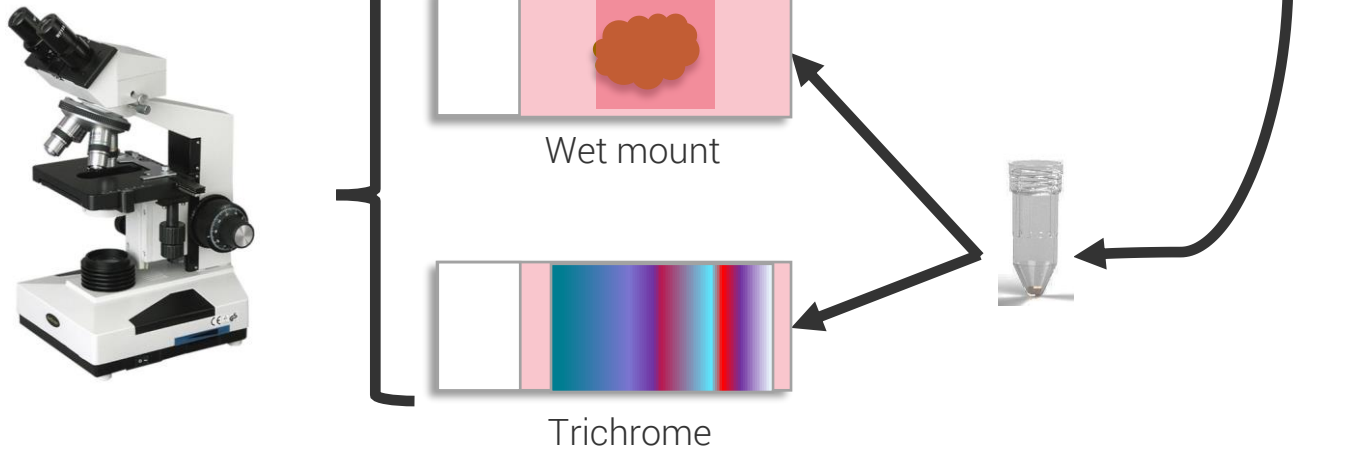
- Fixed stool
  (formalin & polyvinyl alcohol or formalin free fixative)
  - » Specimen is concentrated (↑ sensitivity)
  - » 2 Components of an O&P
    - **Wet Mount:** specimen added to slide, mixed with iodine (optional) and visualized unfixed
    - Trichrome stain: specimen smeared on slide & stained

O&P Recommended Use

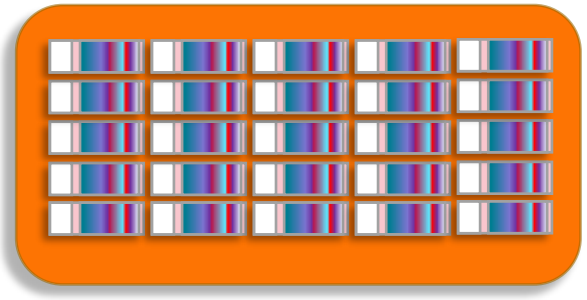
- 3+ unique specimens/patient
- Not recommended for patients with hospital onset diarrhea
- Only for patients with high pre-test probability
  - » Immunocompromised patients
  - » Pertinent exposure history (immigrants, hikers, splash parks)
  - » Pertinent travel history
  - » Persistent (>15d)/chronic(>30d) diarrhea with no alternative Dx

What goes into an O&P Exam





Reading an O&P Run



Run tray

- 30 trichrome slides
- 30 wet mounts

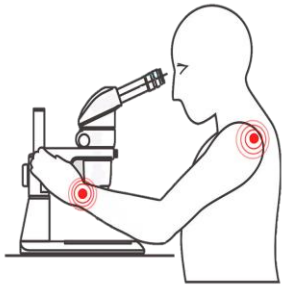
✓ ~2.5 – 3 hours/run
✓ ~98% negative
✓ Positives back-read

Technologist scans specimens looking for parasites
- Anywhere from 2-5 min/slide (technologist variable)

  - "Questionable Negatives" can take longer

Concerns for O&P Reading

- Eye strain
- Neuromuscular strain
- Burnout/Satisfaction
- Accuracy
  - » Technologist (experience, rest, distractions, *etc*)
  - » AM vs PM
  - » Run 1 vs Run 2 vs Run 3
  - » Low parasite burden challenges interpretation
    - ▪ Bias, perceptions over time



How can we make this process more efficient, more accurate….and possibly more fun?

Digital imaging and machine learning!

Terminology Used in this Talk

- CNN – convolutional neural network
- Class – a classification based on shared morphologic features
  - » Usually, one organism is a class
- Class confusion – software detects a parasite but misidentifies it
  - » e.g., software detects *Chilomastix* but calls it *Giardia*
- Failed scan – unsuccessful scans (usually do to insufficient fecal material on slide)
- Incomplete scan – scanner doesn't cover the entire scan area

Digital Imaging

Capture images as seen in a microscope and “thread” into a virtual slide for machine or human evaluation

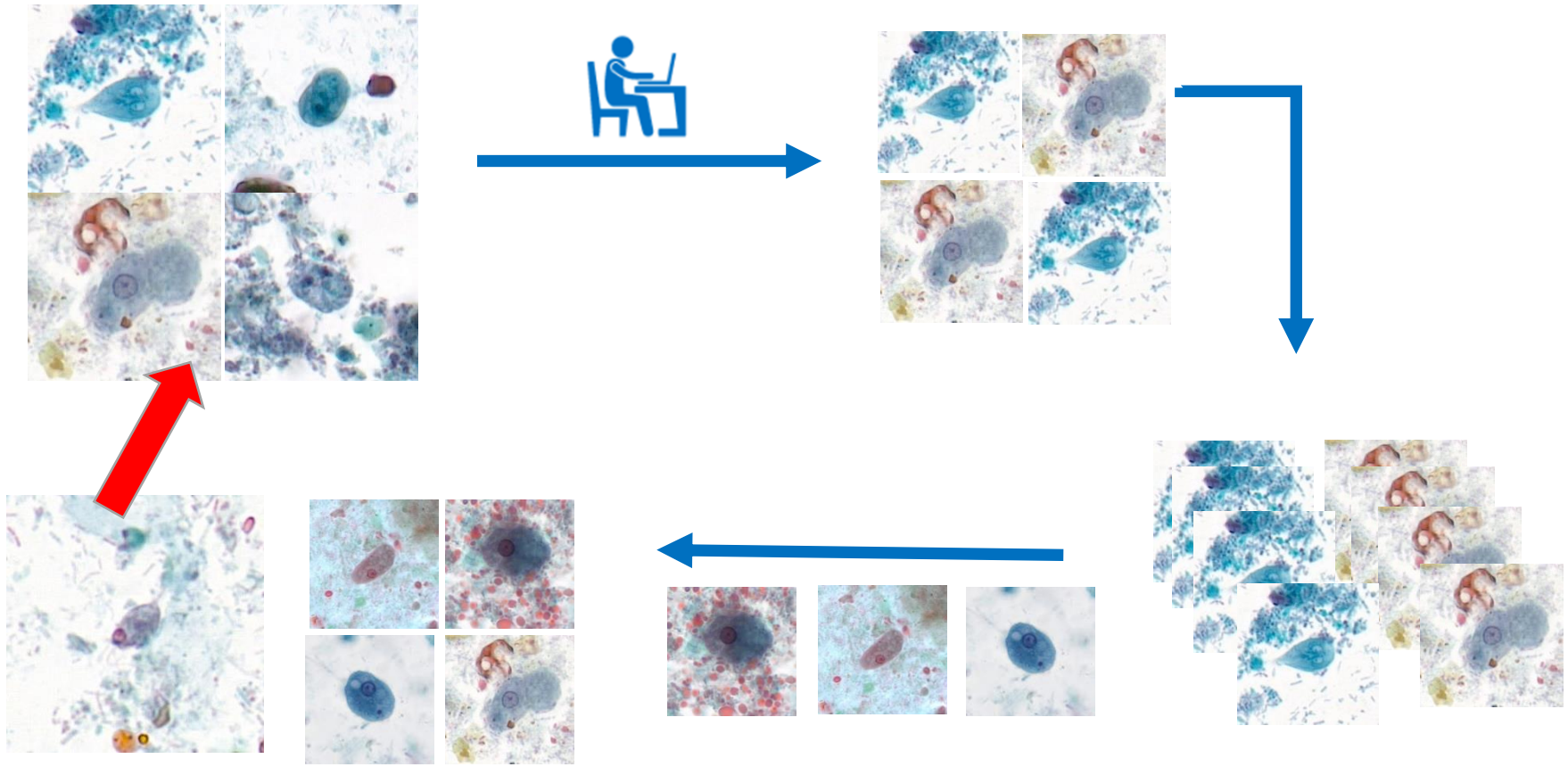
- ✓Must be high resolution for fine detail determination
- ✓Must improve ease of review
- ✓Must be time-effective for scan time considering test volumes
- ✓ Must be user friendly
- ✓ Must be equal or better than what is seen through an eyepiece

Important Concepts going into Development

- This is a **SCREENING TOOL,** not a 'parasite definitive identification tool'
  - » Definitive identification would be made based on manual backreading any slides flagged as suspect positive by the software
  - » The software does not declare a specimen negative; all specimens will have to have at minimum their images analyzed.
- The goal would be to have 70-80% of negative slides screened out after image analysis.
- The wet mount would still be read normally

The software is to **COMPLEMENT** the Technologist, not replace the Technologist

Simplified Machine Learning: Supervised Machine Learning



What classes did we train the software on? Trichrome

- What was trained
  - *Giardia duodenalis* trophs
  - *Giardia duodenalis* cysts
  - *Blastocystis* spp.
  - *Entamoeba* spp. (non-*hartmanni*) trophs
  - *Entamoeba hartmanni* trophs
  - *Endolimax nana*/ *Iodamoeba buetschlii* trophs
  - *Dientamoeba fragilis*
  - *Chilomastix mesnili*
  - *Cyclospora* spp.
  - Red Blood Cells
  - White Blood Cells

- What the end-user sees
  - *Giardia duodenalis*
  - *Blastocystis* sp.
  - *Entamoeba* spp. (non-*hartmanni*) trophs
  - *Entamoeba hartmanni* trophs
  - *Endolimax nana*/ *Iodamoeba buetschlii*/*Dientamoeba fragilis* trophs
  - *Chilomastix mesnili*
  - *Cyclospora* spp.
  - Red Blood Cells
  - White Blood Cells

*yeast were trained as an 'anti-class'

What classes did we train the software on? MAF

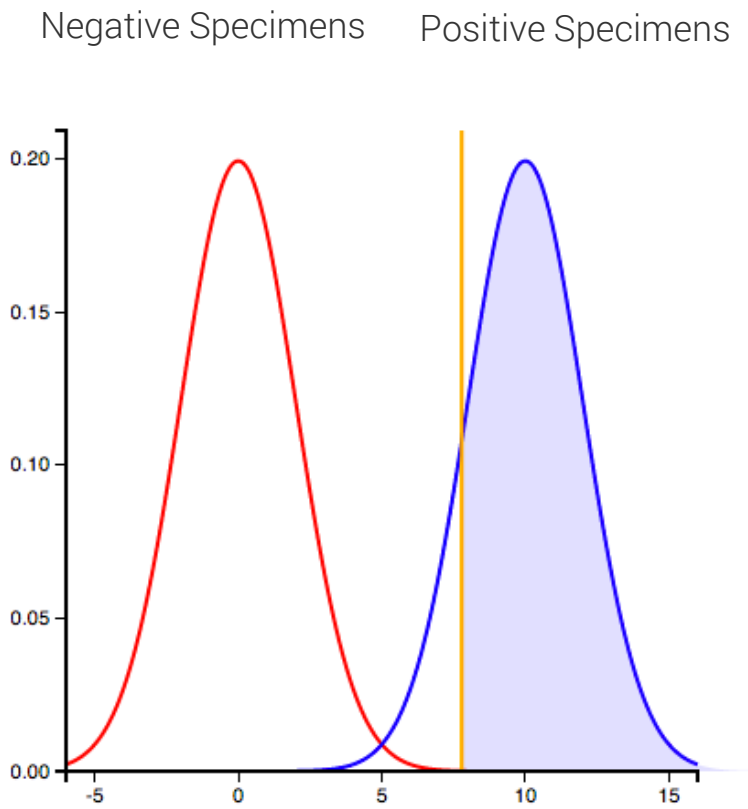
- What was trained
  - *Cyclospora* spp. –stained
  - *Cyclospora* spp. – 'ghost'
  - *Cryptosporidium* spp. – stained
  - *Cryptosporidium* spp. – 'ghost'
- What the end-user sees
  - *Cyclospora* spp.
  - *Cryptosporidium* spp.

*yeast were trained as an 'anti-class'

**Cystoisospora belli* was originally not trained due to lack of material and because our lab screens MAF specimens by UV

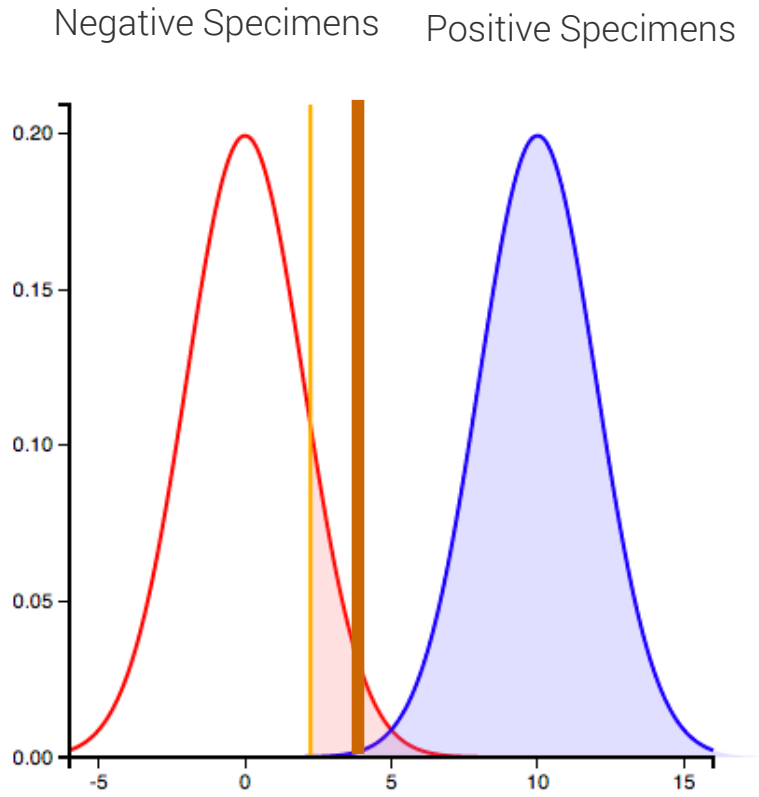
Perfect Specificity, Lower Sensitivity…

- Everything identified is true
- Will miss some positives



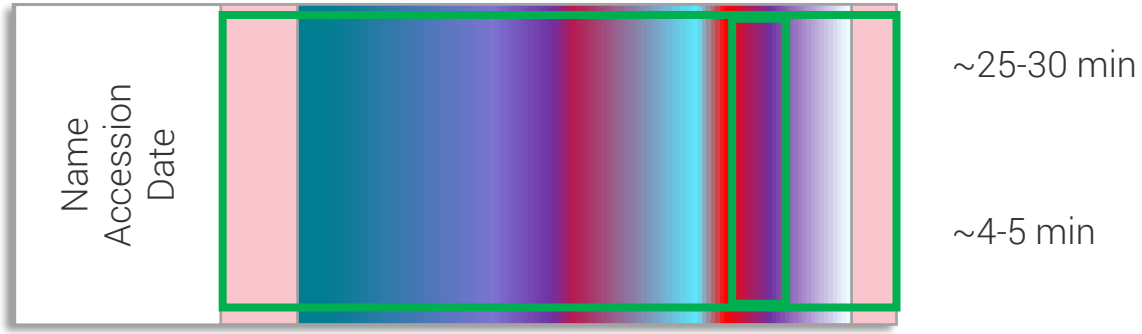
Perfect Sensitivity, Lower Specificity…

- Identifies everything
- Also shows you some junk, but the human arbitrates it
- Find a sweet spot that catches all, but limits junk!



Designing a Scan Area

- Not time effective
- Determine slide scan area necessary to minimize scan time and maintain equal or better accuracy than human



Limit of Detection - Trichrome

Dilution	Technologist read	Software Analysis
Neat	*Giardia* + 1+ *Blastocystis*	*Giardia* (276) + *Blastocystis* (129)
1:1	*Giardia* + 1+ *Blastocystis*	*Giardia* (95) + *Blastocystis* (19)
1:2	*Giardia* + 1+ *Blastocystis*	*Giardia* (68) + *Blastocystis* (17)
1:4	*Giardia* + 1+ *Blastocystis*	*Giardia* (79) + *Blastocystis* (46)
1:8	Negative	*Giardia* (70) + *Blastocystis* (13)
1:16	*Giardia* + 1+ *Blastocystis* (rare)	*Giardia* (12) + *Blastocystis* (10)
1:32	Negative	*Giardia* (16) + *Blastocystis* (5)
1:64	Negative	*Giardia* (15) + *Blastocystis* (2)
1:128	Negative	*Giardia* (9) + *Blastocystis* (1)
1:256	Negative	*Giardia* (15) + *Blastocystis* (1)

Limit of Detection – MAF

Number	Dilution Series	Software Results	Technologist Results
26	C Neat	*Cyclospora cayetanensis* (79 called; 50 of first 50 valid)	Negative
25	C 1:1	*Cyclospora cayetanensis* (131 called; 50 of first 50 valid)	*Cyclospora cayetanensis*
4	C 1:2	*Cyclospora cayetanensis* (60 called; 47 of first 50 valid)	Negative
32	C 1:4	*Cyclospora cayetanensis* (25 called; 21-22 valid)	Negative
13	C 1:8	*Cyclospora cayetanensis* (5 called; 4 valid)	*Cyclospora cayetanensis*
30	C 1:16	*Cyclospora cayetanensis* (16 called; all valid)	Negative
12	C 1:32	*Cyclospora cayetanensis* (2 called; 0-1 valid)	Negative
20	C 1:64	Negative	Negative
15	C 1:128	Negative (1 *C. cayetanensis* called, doesn't appear valid)	Negative
28	C 1:256	*Cyclospora cayetanensis* (6 called; 1 valid)	Negative
3	C 1:512	Negative	Negative

Real life applications in a diagnostic parasitology laboratory

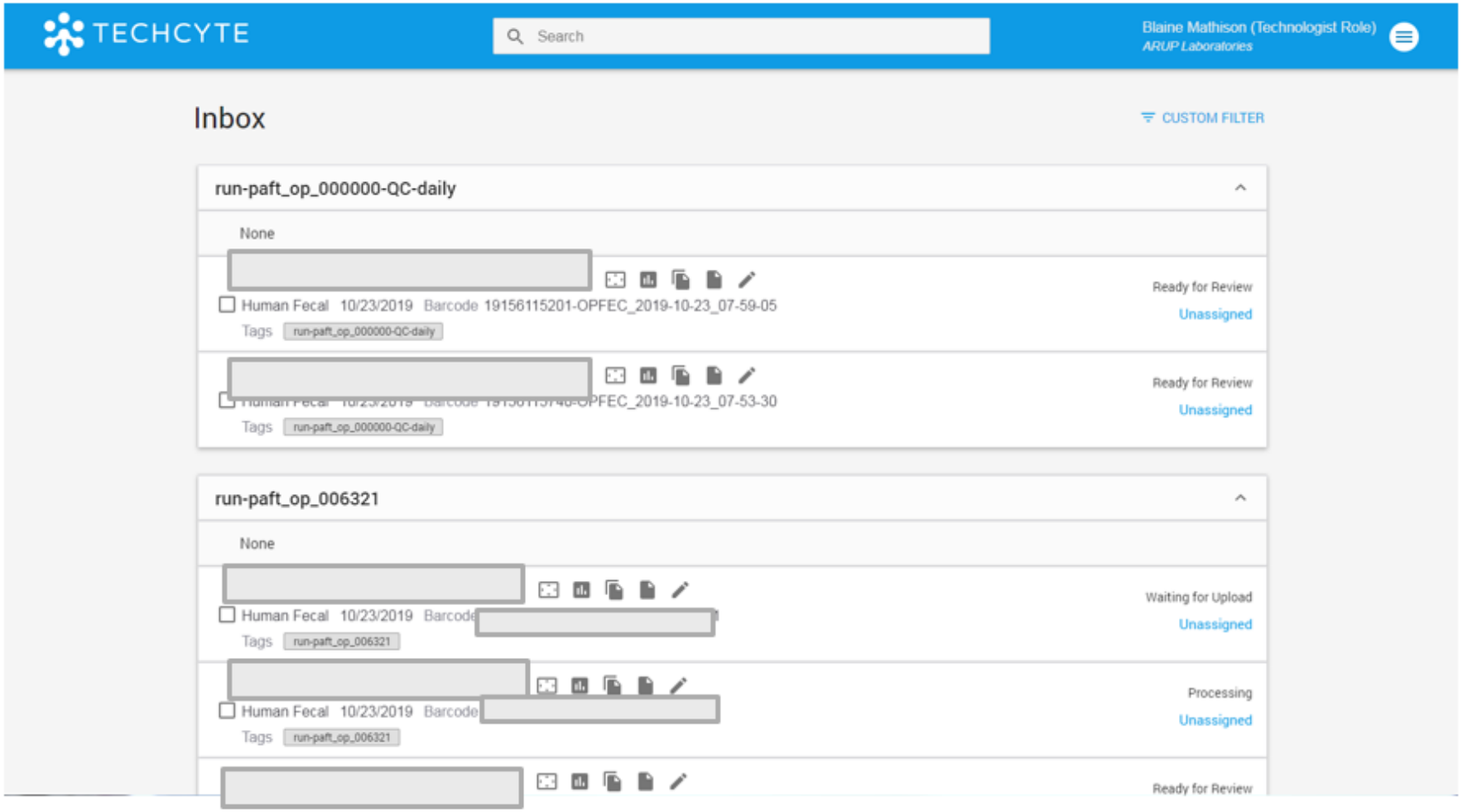
Challenges to consider and overcoming them!

Back to the O&P Workflow – Challenges to Consider

- Potential changes to the O&P processing:
  - » Slide preparation (flat, 'homogenous' slides vs. 'hills and valleys')
  - » Coverslipping of slides required for optimal clarity of scans
  - » Possible organizing of your workforce (technologists vs. technicians)
- Developing and maintaining QC for scans (this is not the same as QC for the stain)
- How to handle failed or incomplete scans
- Have a backup plan when there are problems with any step in the process (coverslipping, scanning, software analysis)
- How well will employees embrace such a change (older vs. younger workforce)

Lab Workflow

- Analysis of the Wet Mount
- Analysis of the Images
- Manual Backreading of the Trichrome/MAF slide if:
  - » Suspect organisms are seen in the images
    - ▪ Medical Director review if convincing organisms seen in images cannot be found on the slide (e.g. low parasitemia)
  - » Discrepant results between wet mount and images
  - » Failed, Invalid, or Incomplete scans
- End goals:
  - » 1. Successfully Detect common intestinal protozoa
  - » 2. Screen-out a minimum of 70-80% of negative slides


TECHCYTE
Search
Blaine Mathison (Technologist Role)
ARUP Laboratories
Inbox
CUSTOM FILTER
run-paft_op_000000-QC-daily
None
Human Fecal 10/23/2019 Barcode 19156115201-OPFEC_2019-10-23_07-59-05
Tags run-paft_op_000000-QC-daily
Ready for Review
Unassigned
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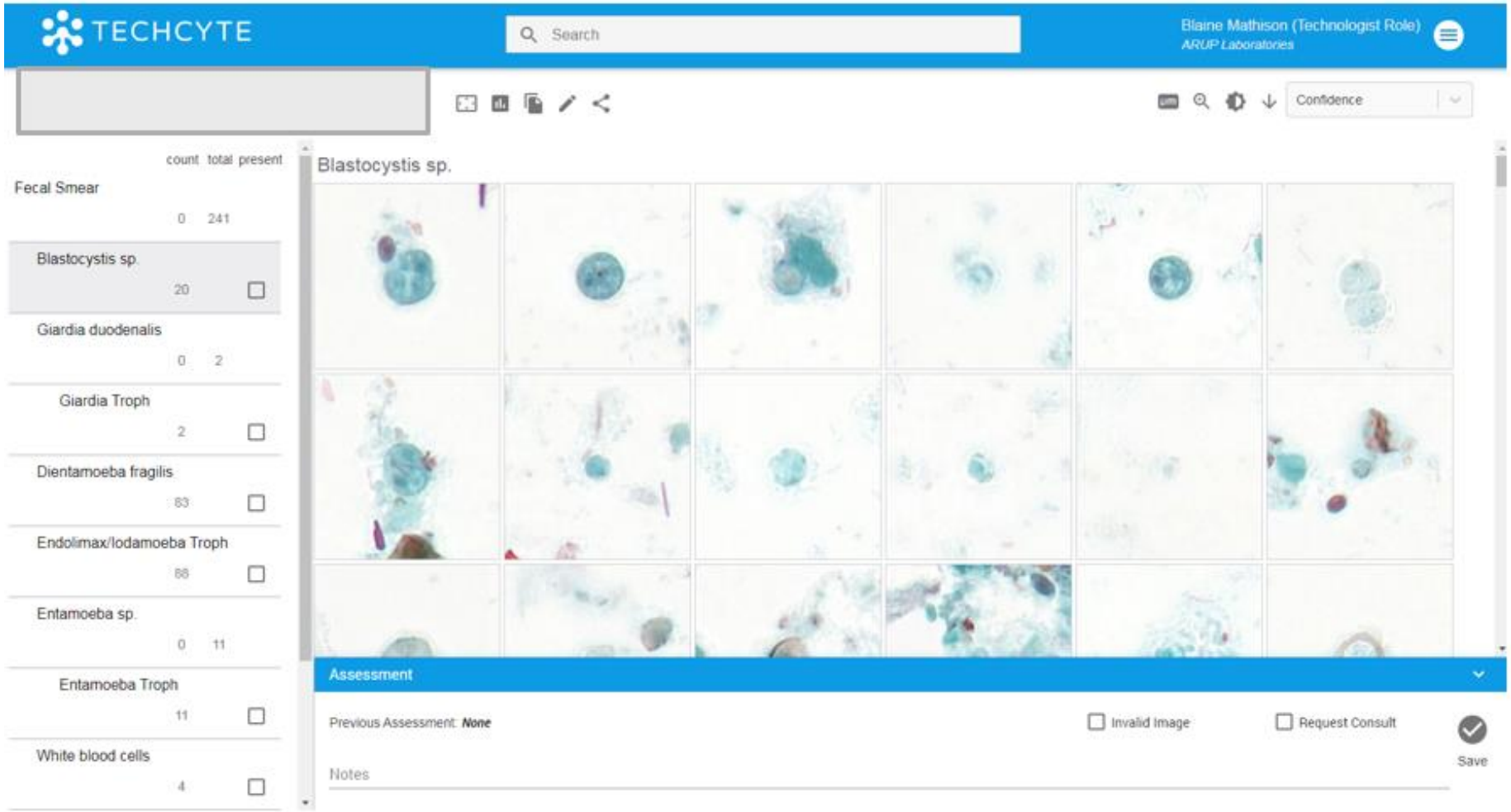
Processing
Unassigned
Ready for Review



Giardia duodenalis

0 2

Giardia Troph

2

Dientamoeba fragilis

83

Endolimax/Iodamoeba Troph

88

Entamoeba sp.

0 11

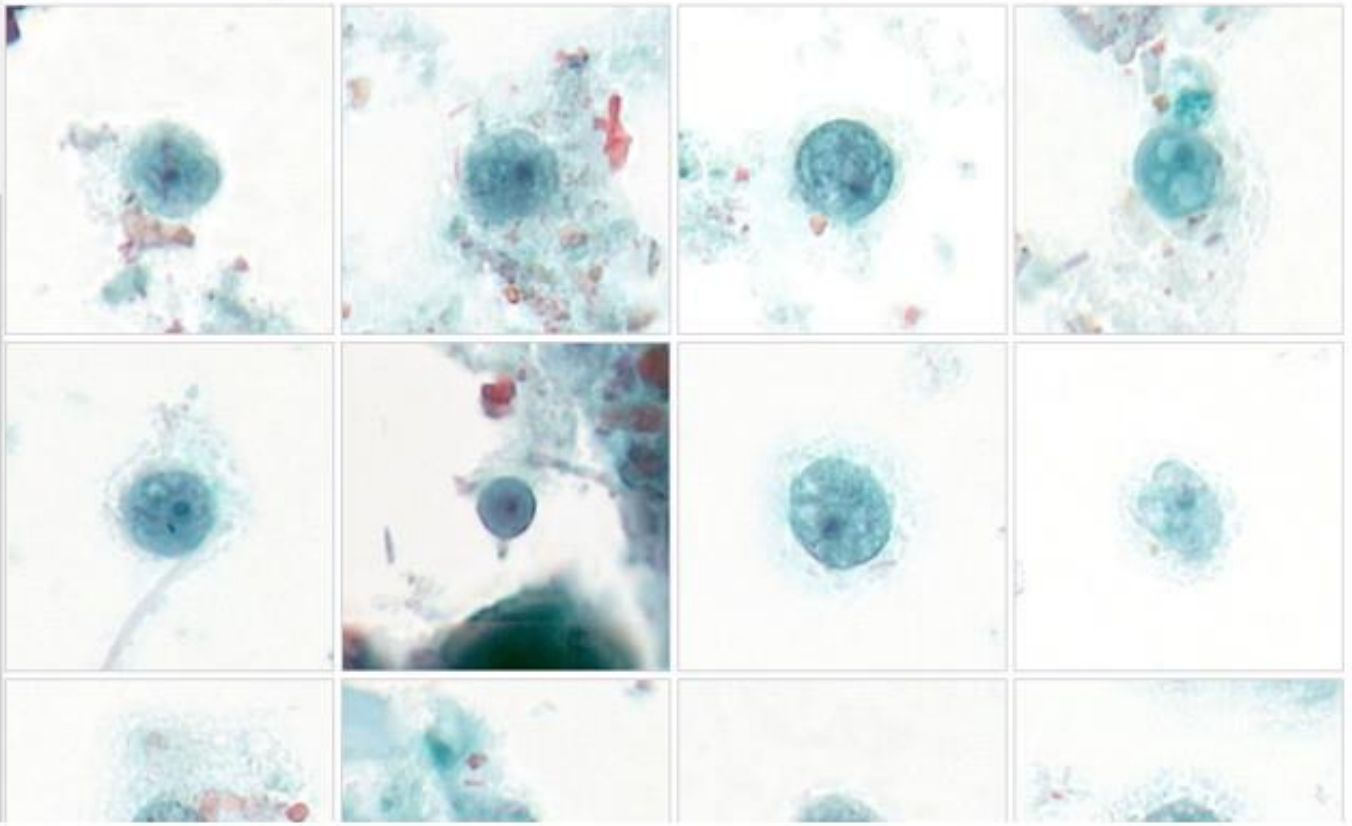
Entamoeba Troph

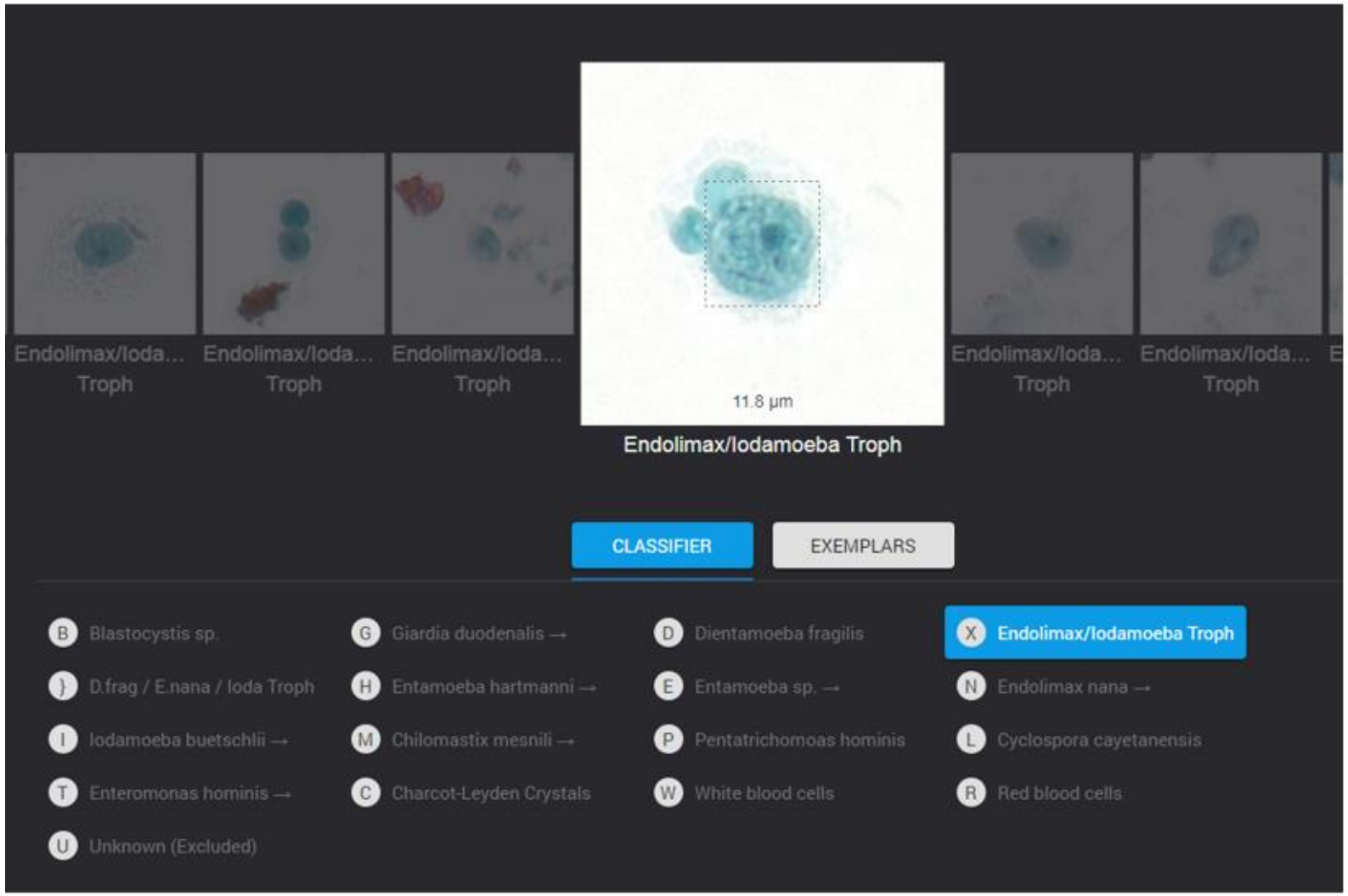
11

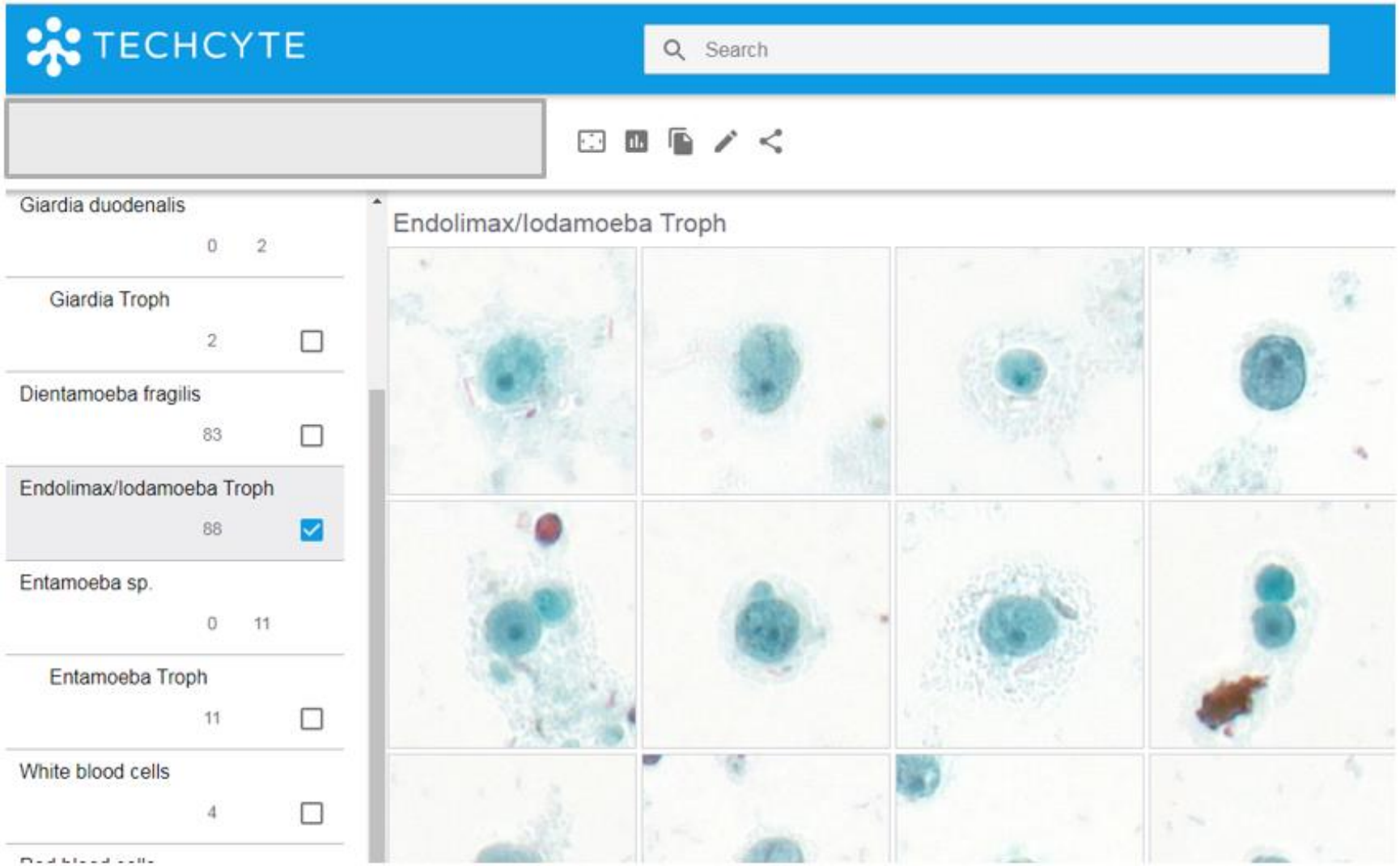
White blood cells

4

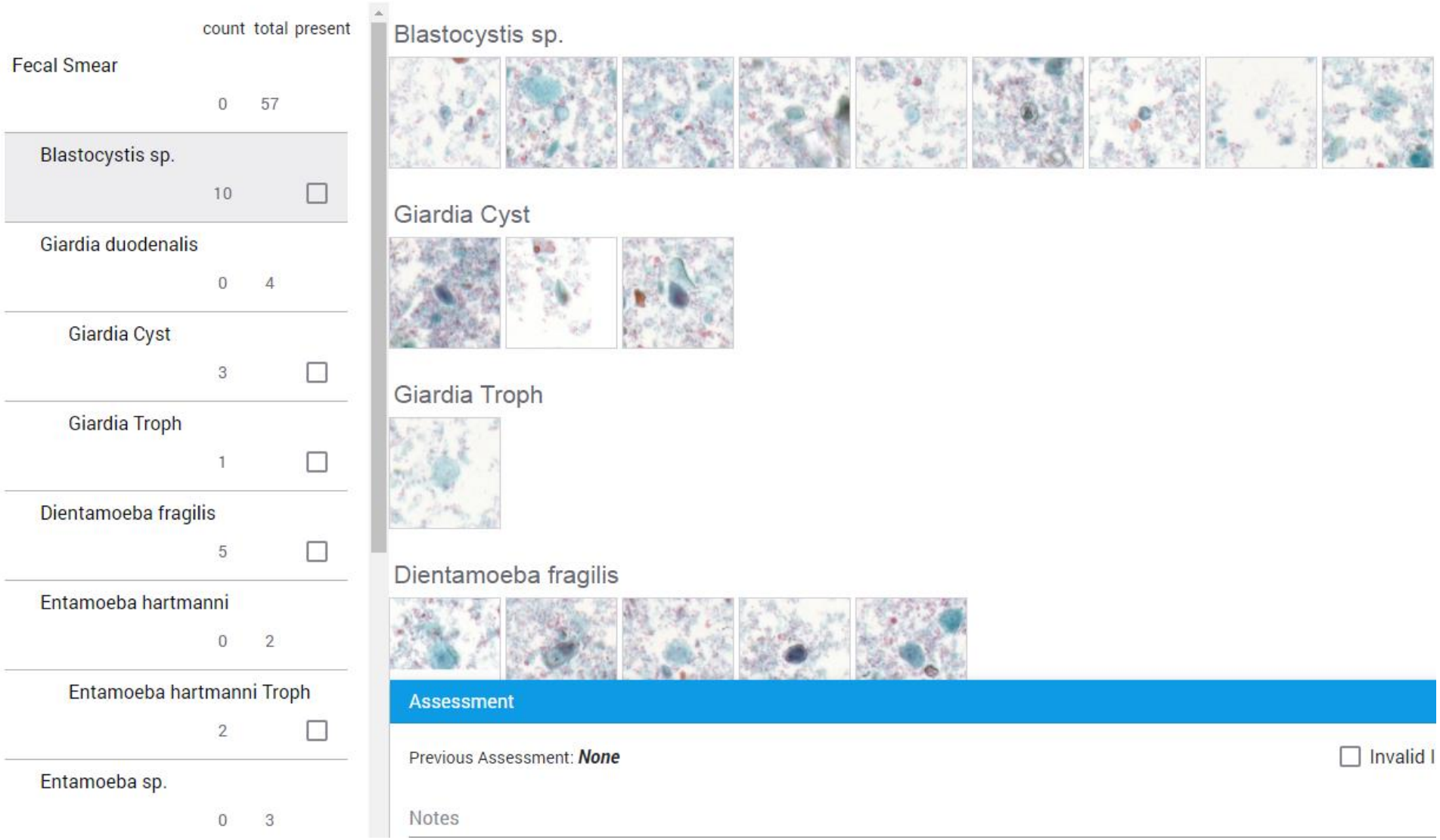
Dientamoeba fragilis



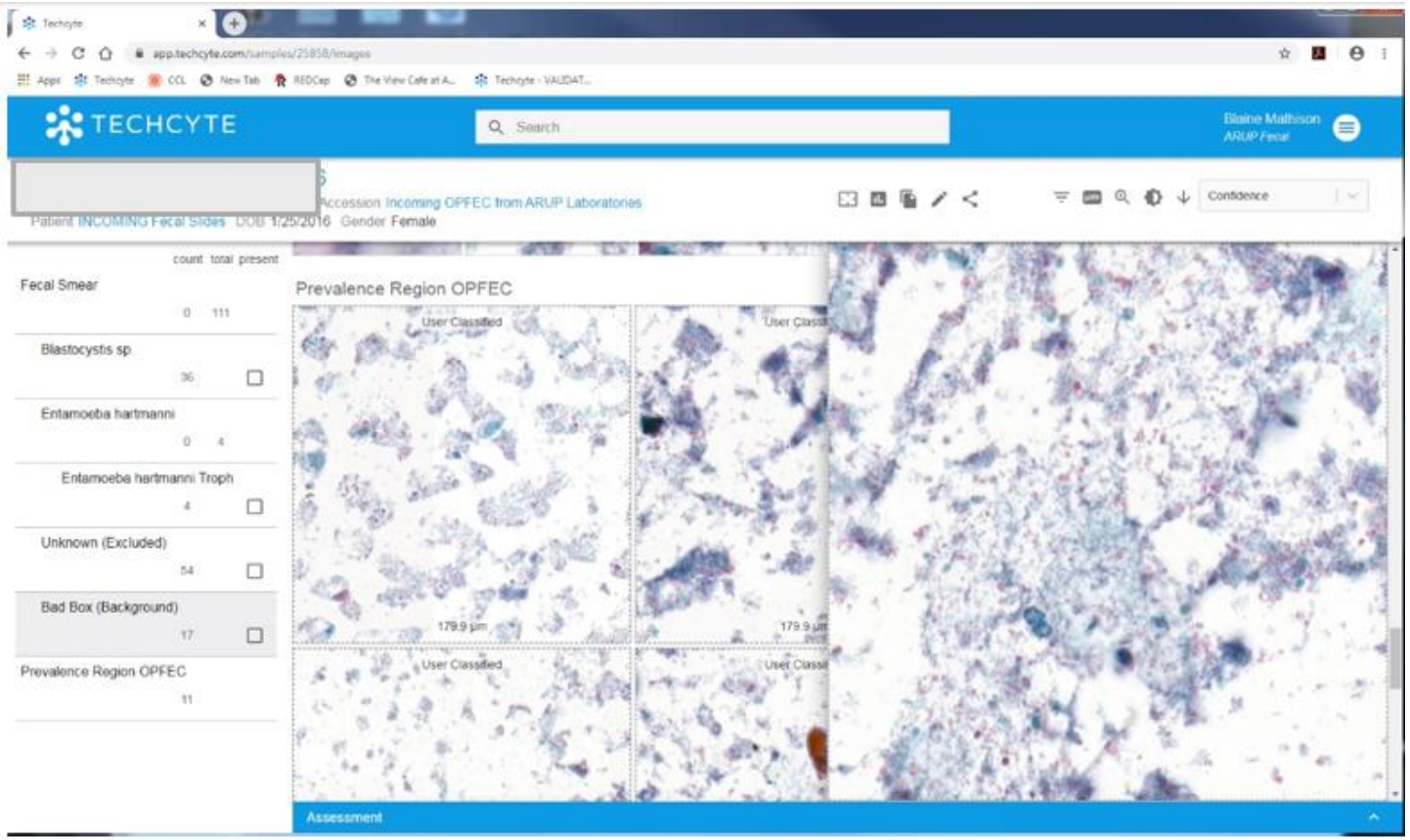




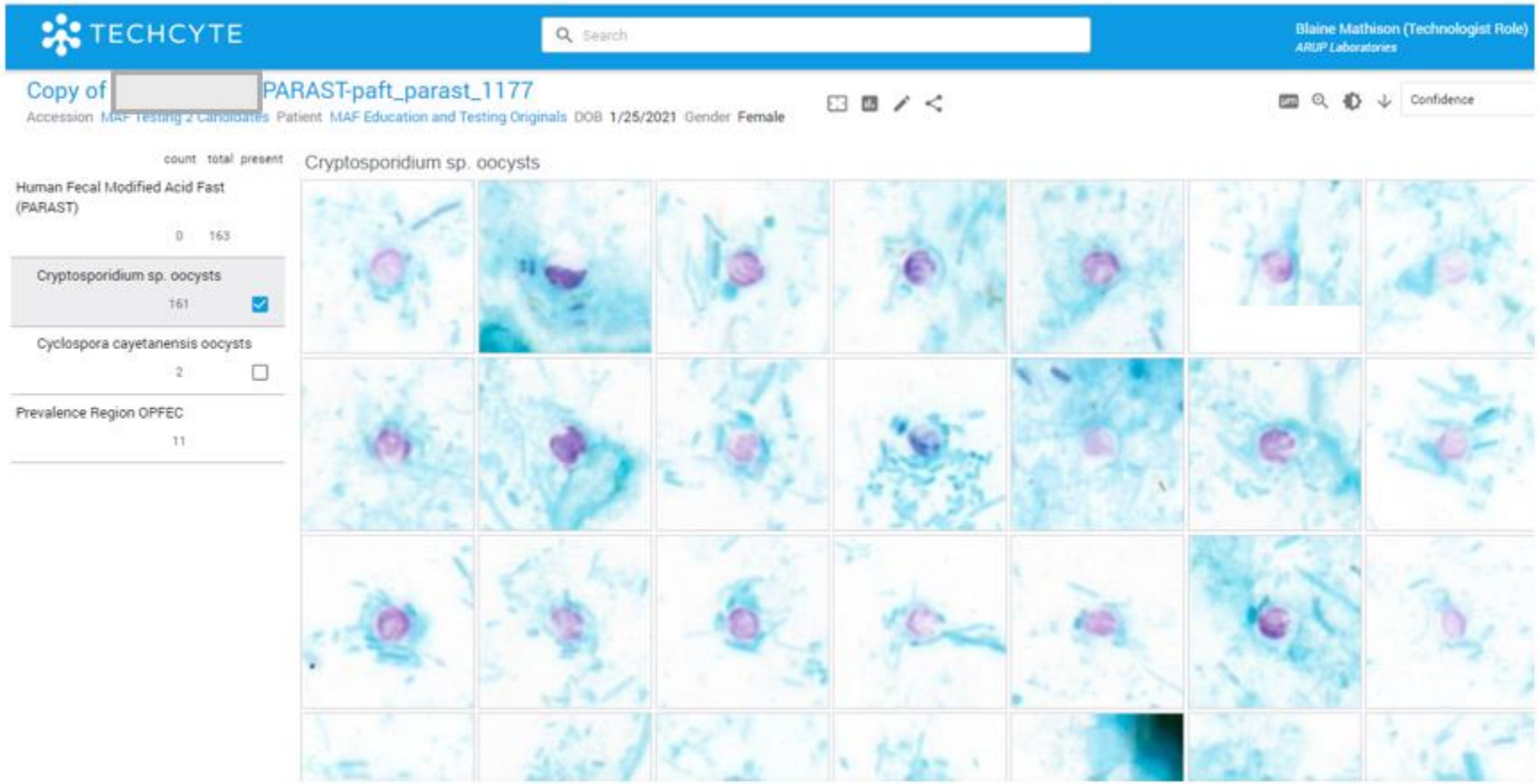
Example of a ‘clean’ negative scan

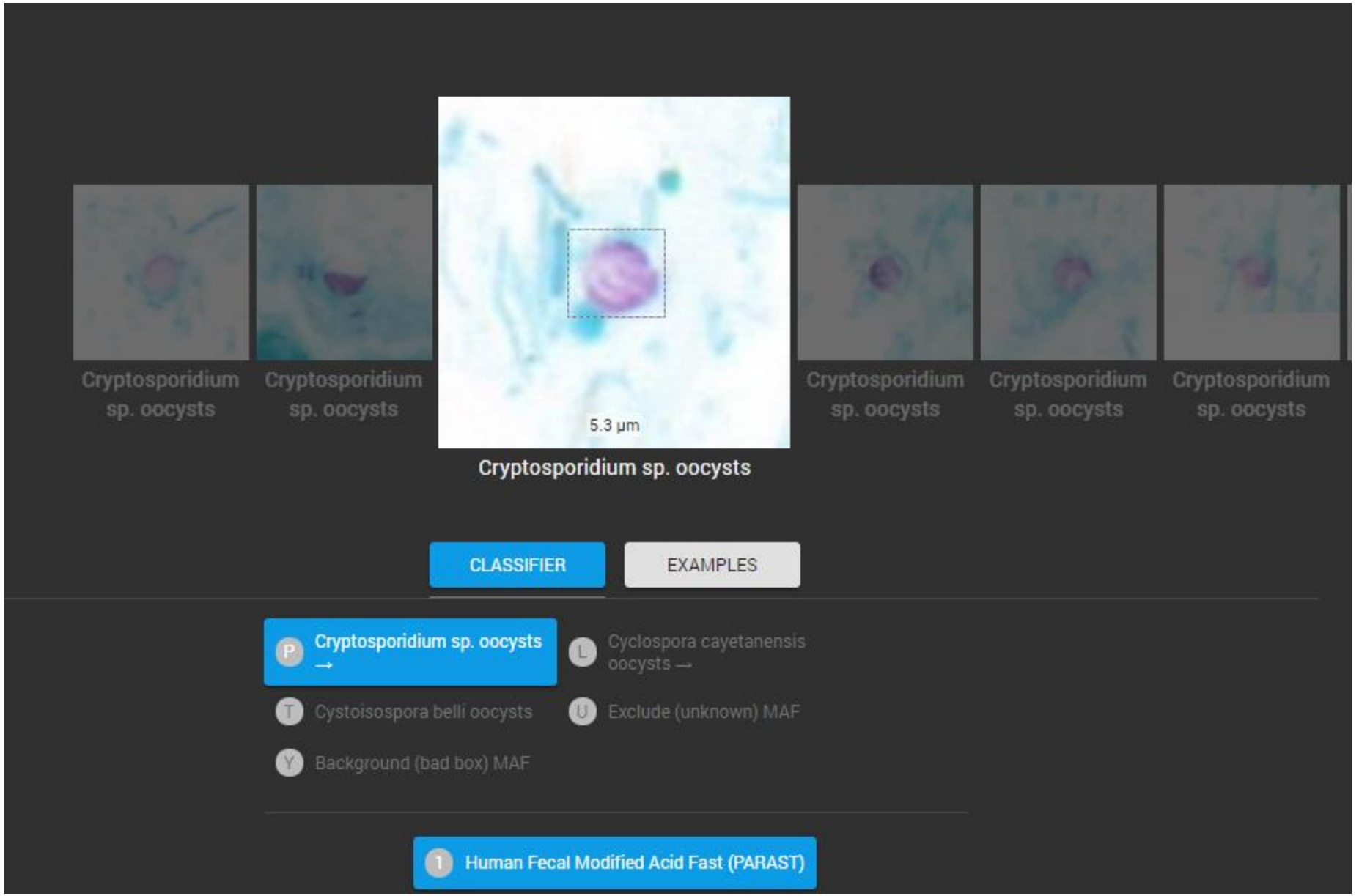


Prevalence Regions

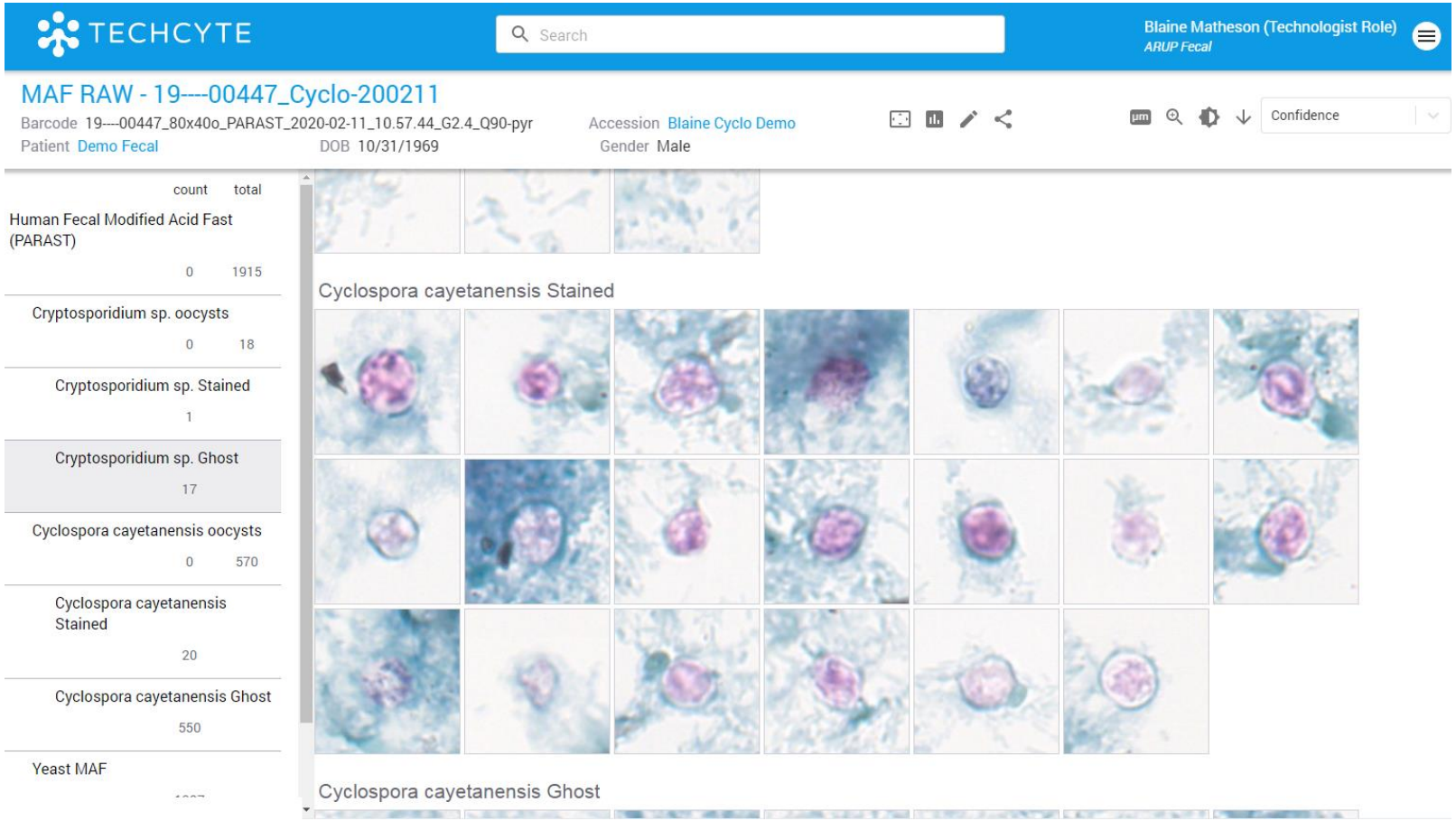


MAF Model – *Cryptosporidium* sp.

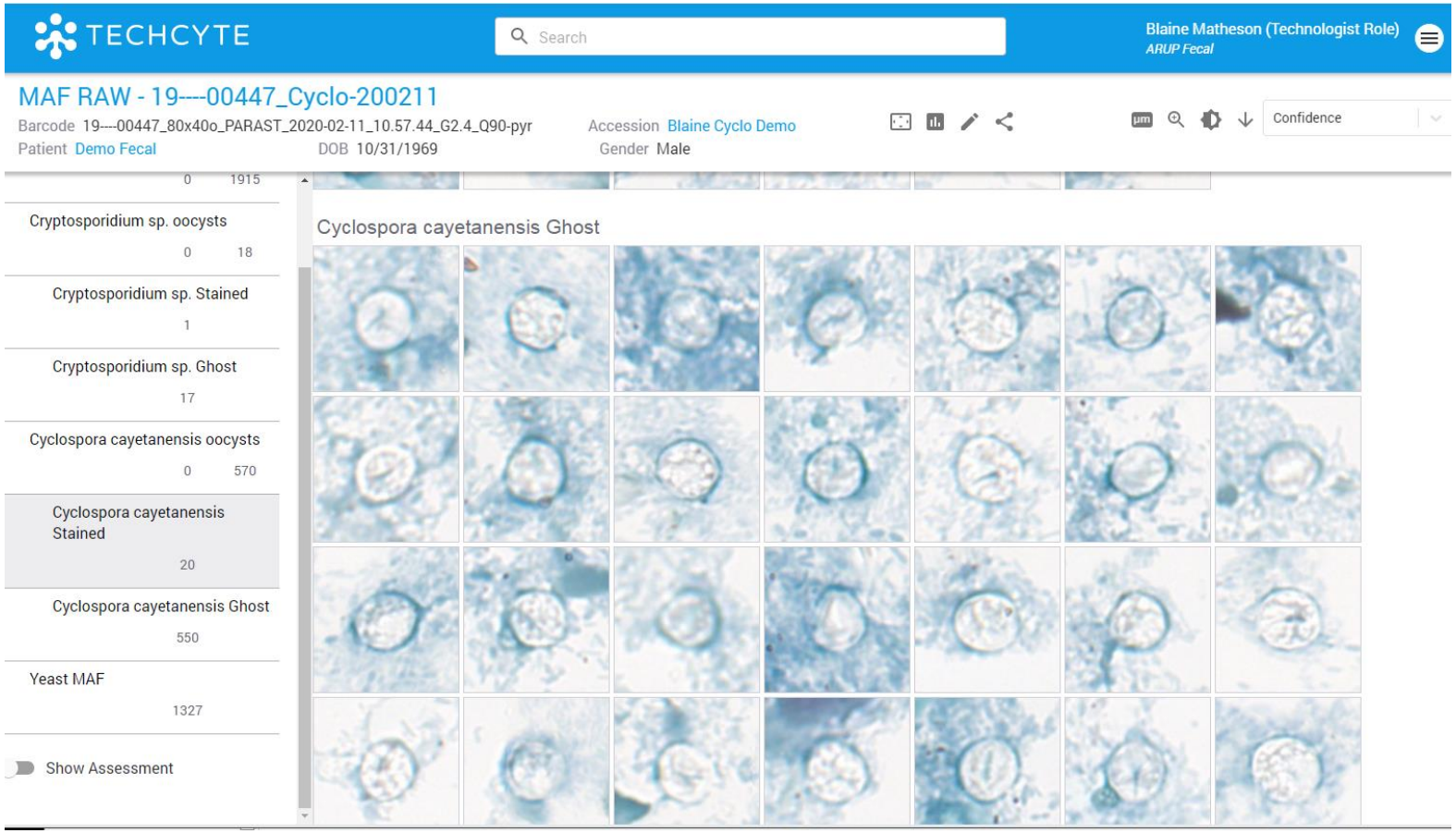




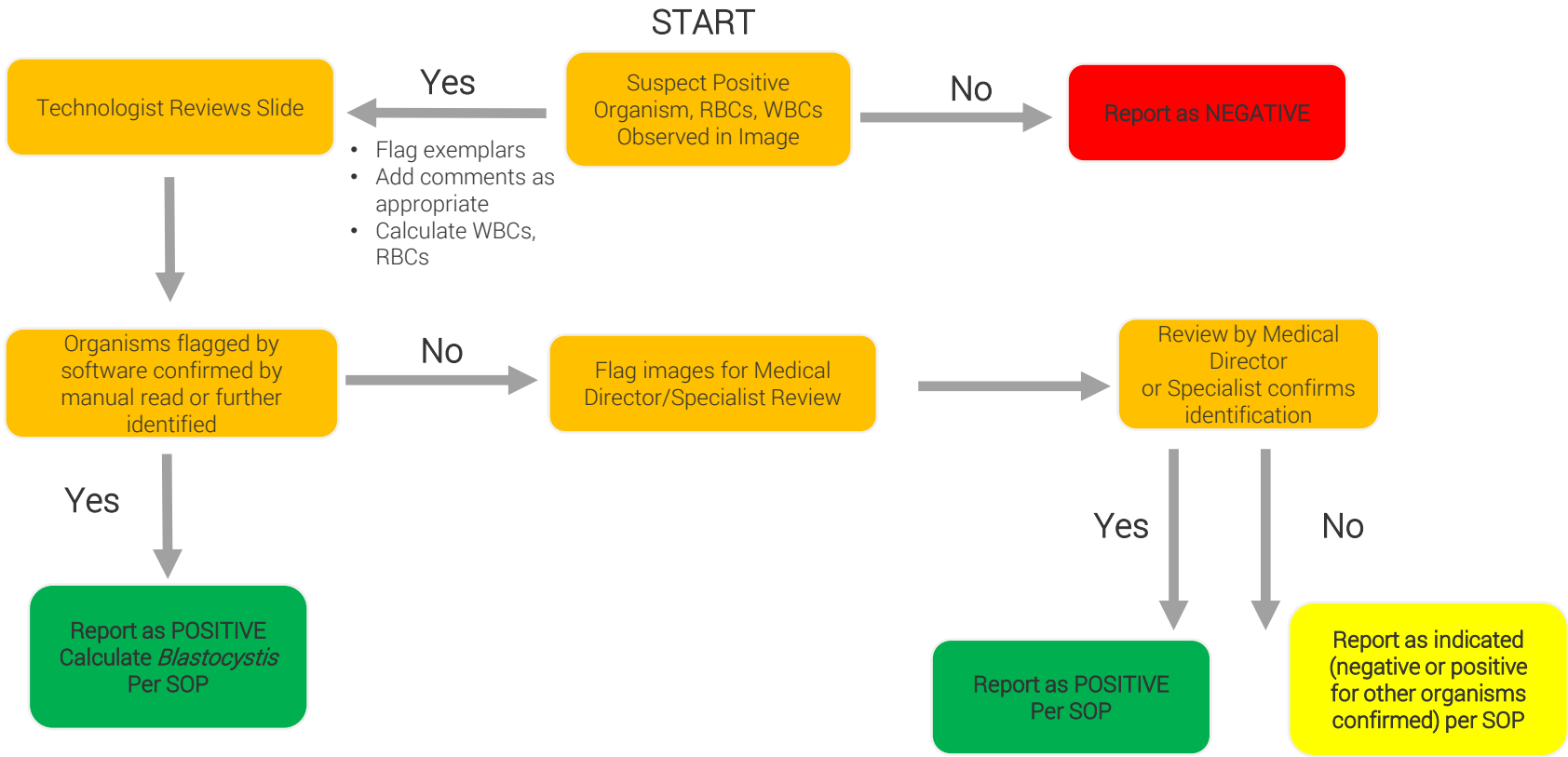
MAF Model – *Cyclospora cayetanensis* - stained



MAF Model – *Cyclospora cayetanensis* – 'ghost' forms



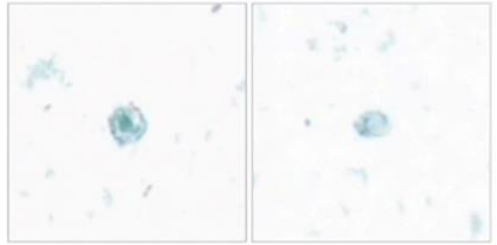
Lab Workflow



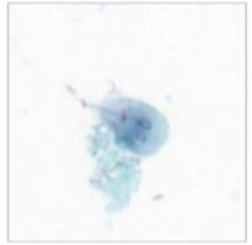
Would the lab have missed this?

	count	total	present
Fecal Smear	0	42	
Blastocystis sp.	2		☐
Giardia duodenalis	0	1	
Giardia Troph	1		☐
Entamoeba sp.	0	2	
Entamoeba Troph	2		☐
White blood cells	4		☐
Red blood cells	33		☐
Prevalence Region OPFEC	11		

Blastocystis sp.

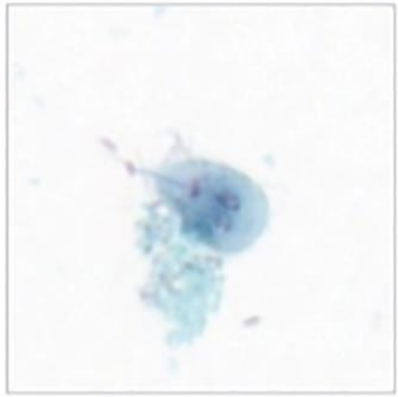


Giardia Troph



Entamoeba Troph

Giardia Troph



Future developments

Wet mounts!

Development of a Model for Parasite Detection in concentrated Wet Mounts – Challenges to Consider!

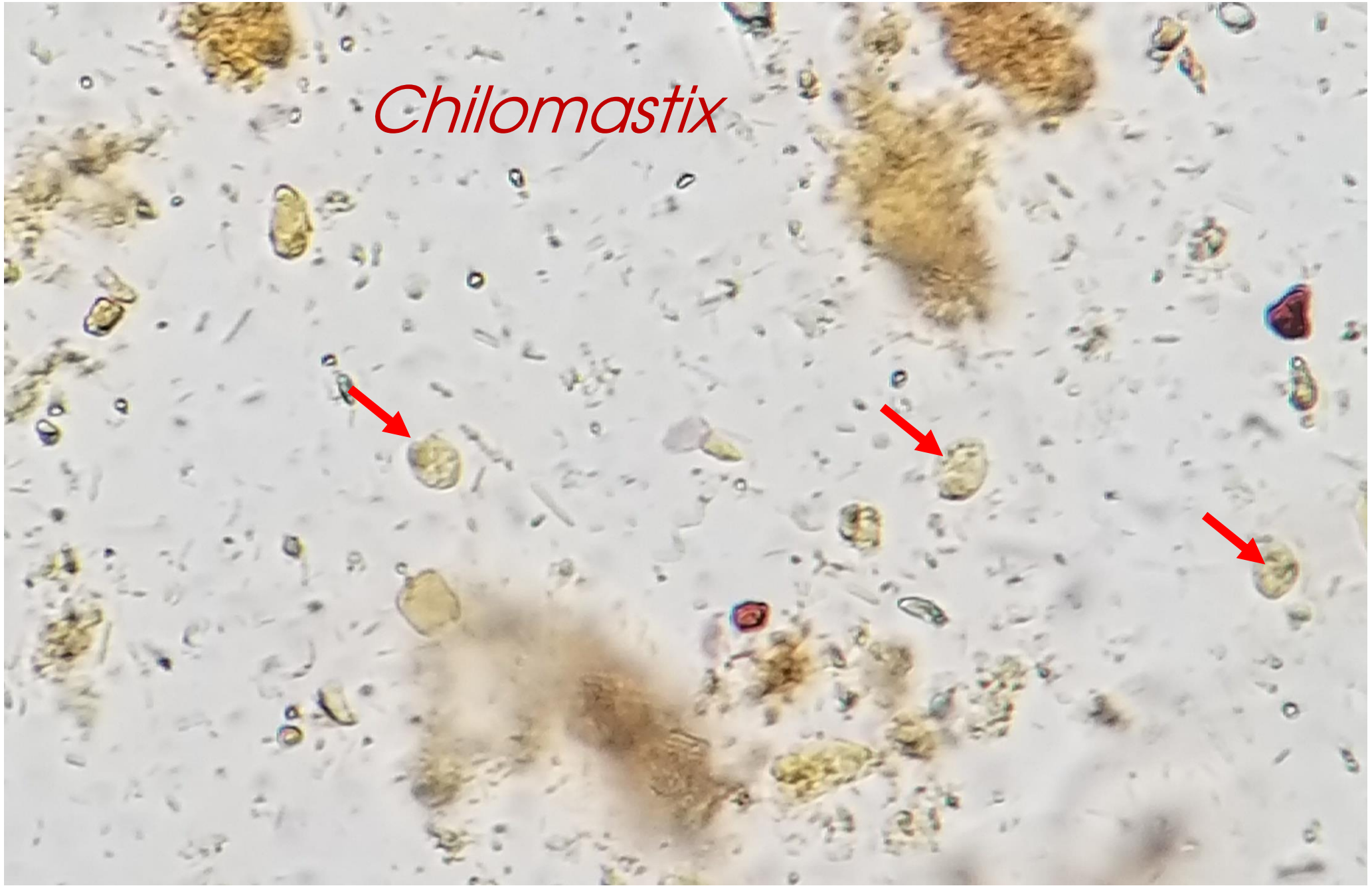
- Acquisition of helminths and other rare classes
  - » must train for common and familiar helminths, even those not endemic to North America.
- Development of a mounting medium to slow drying
  - » Drying studies to see how long 20 slides last
- Use of a scanner where slides are loaded flat
  - » Make sure loading and unloading of the slides doesn't cause shifting of the coverslip!
- Development of the scan area
  - » Must scan as much of the 22x22 mm coverslip area as possible
- Finding the optimal levels to scan
  - » Protozoan cysts and helminth eggs/larvae do not settle in the same planes!
- After clinical validation, designing workflow for complete AI screening of the O&P workflow!

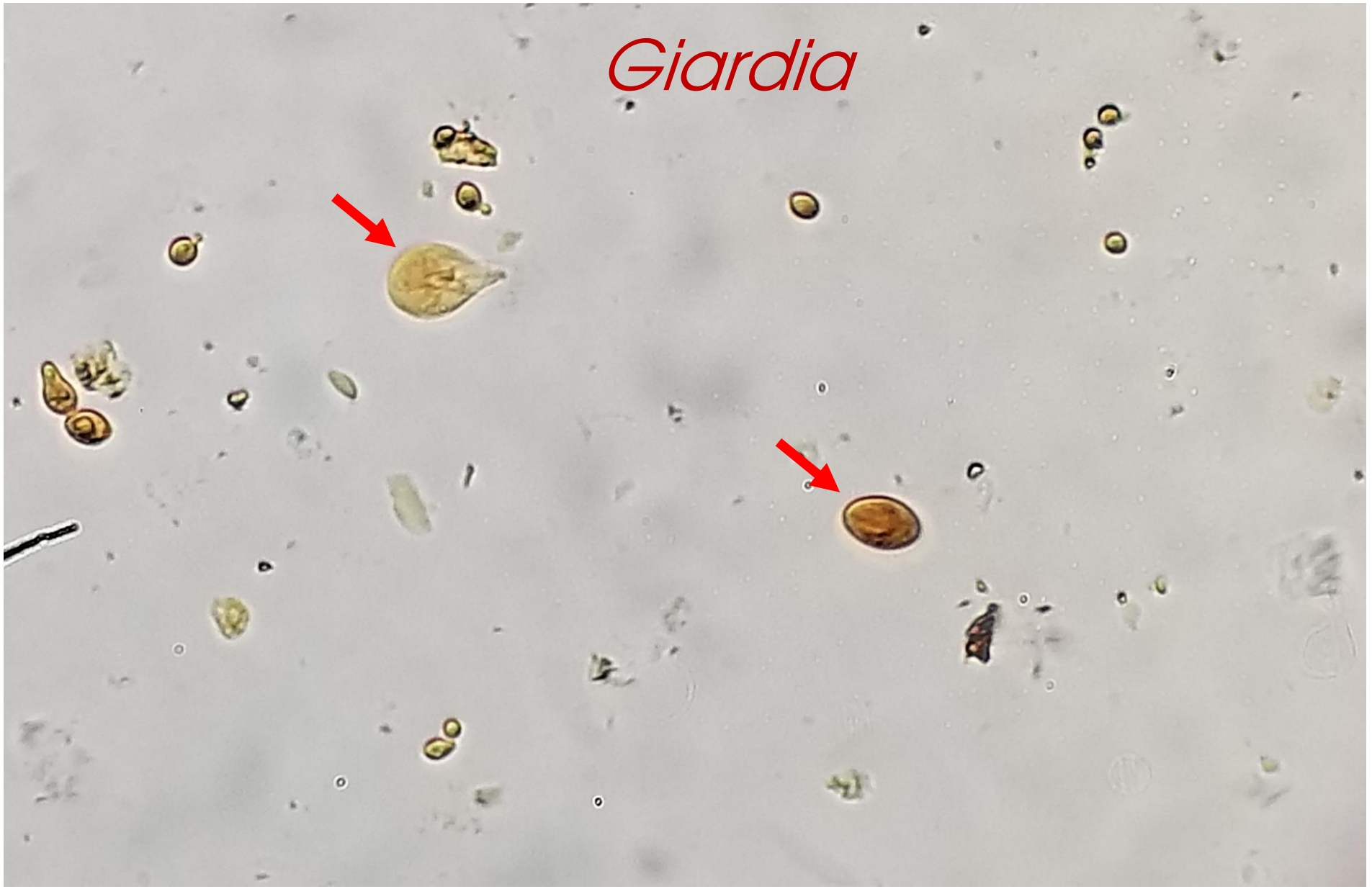
Wet Mount – Protozoan Classes Trained

- *Blastocystis* spp.
- *Giardia duodenalis* (cysts)
- *Giardia duodenalis* (trophs)
- *Entamoeba* spp. (cysts)
- *Entamoeba* spp. (trophs)
- *Chilomastix mesnili* (cysts)
- *Endolimax nana* (cysts)
- *Iodamoeba buetschlii* (cysts)
- Misc. small trophs (*Dientamoeba fragilis*, *E. nana*, *I. buetschlii*, *E. hartmanni*, *C. mesnili*)
- *Balantioides coli*
- *Cyclospora* spp.
- *Cystoisospora belli*

Wet Mount – Helminth Classes Trained

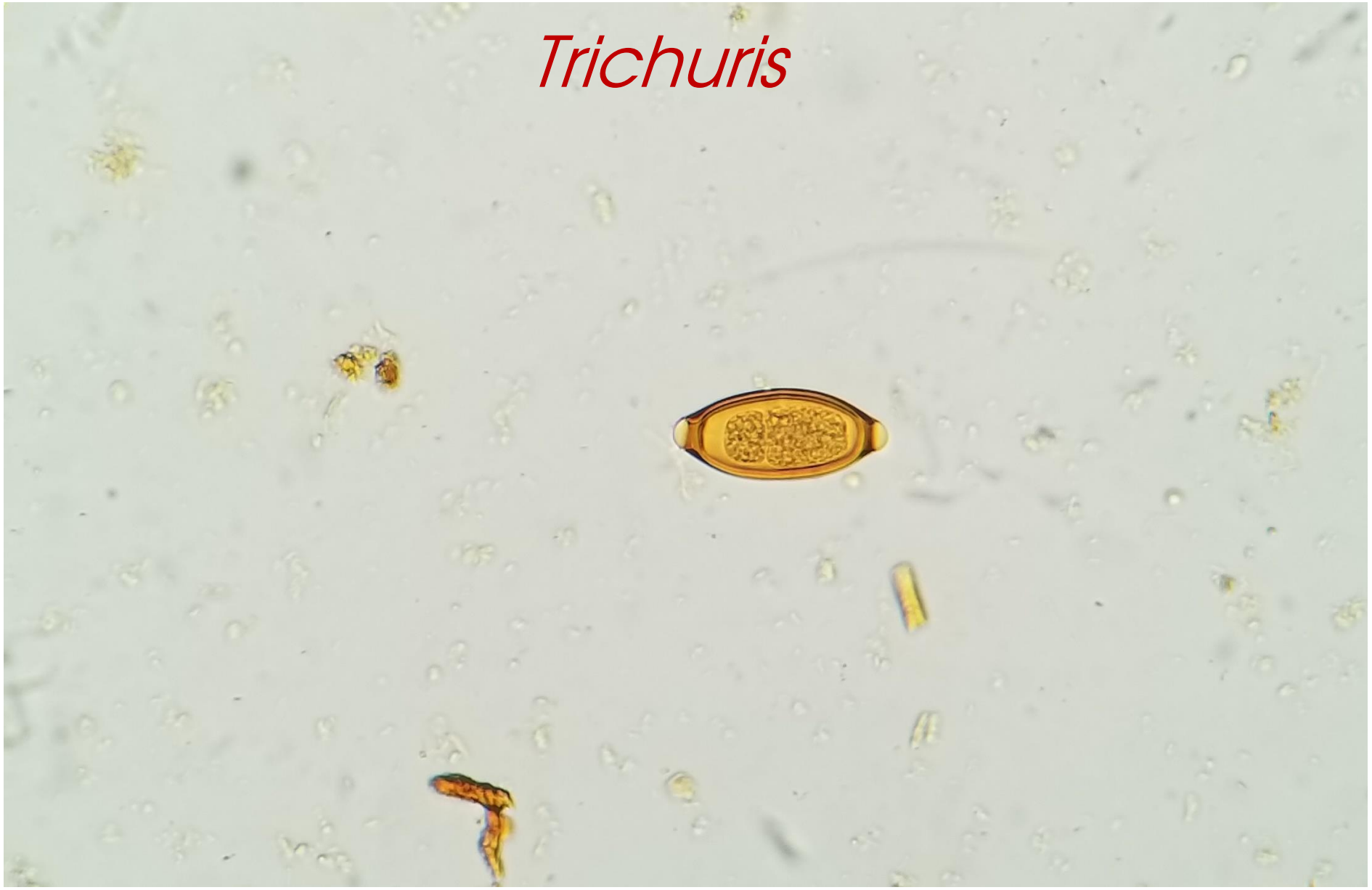
- *Ascaris lumbricoides* (fertile eggs)
- *Ascaris lumbricoides* (infertile eggs)
- *Trichuris trichiura* (eggs)
- Hookworm/ *Trichostrongylus* (eggs)
- *Strongyloides stercoralis* (L1 larvae)
- *Enterobius vermicularis* (eggs)
- *Paracapillaria* (formerly *Capillaria*) *philippinensis* (eggs)
- *Taenia* spp. (eggs)
- *Rodentolepis* (formerly *Hymenolepis*) *nana* (eggs)
- *Hymenolepis diminuta* (eggs)
- Fish tapeworm (eggs)
- *Schistosoma mansoni* (eggs)
- *Schistosoma japonicum* (eggs)
- *Clonorchis*/ *Opisthorchis* (eggs)
- *Paragonimus* spp. (eggs)
- *Fasciola*/ *Fasciolopsis* (eggs)*



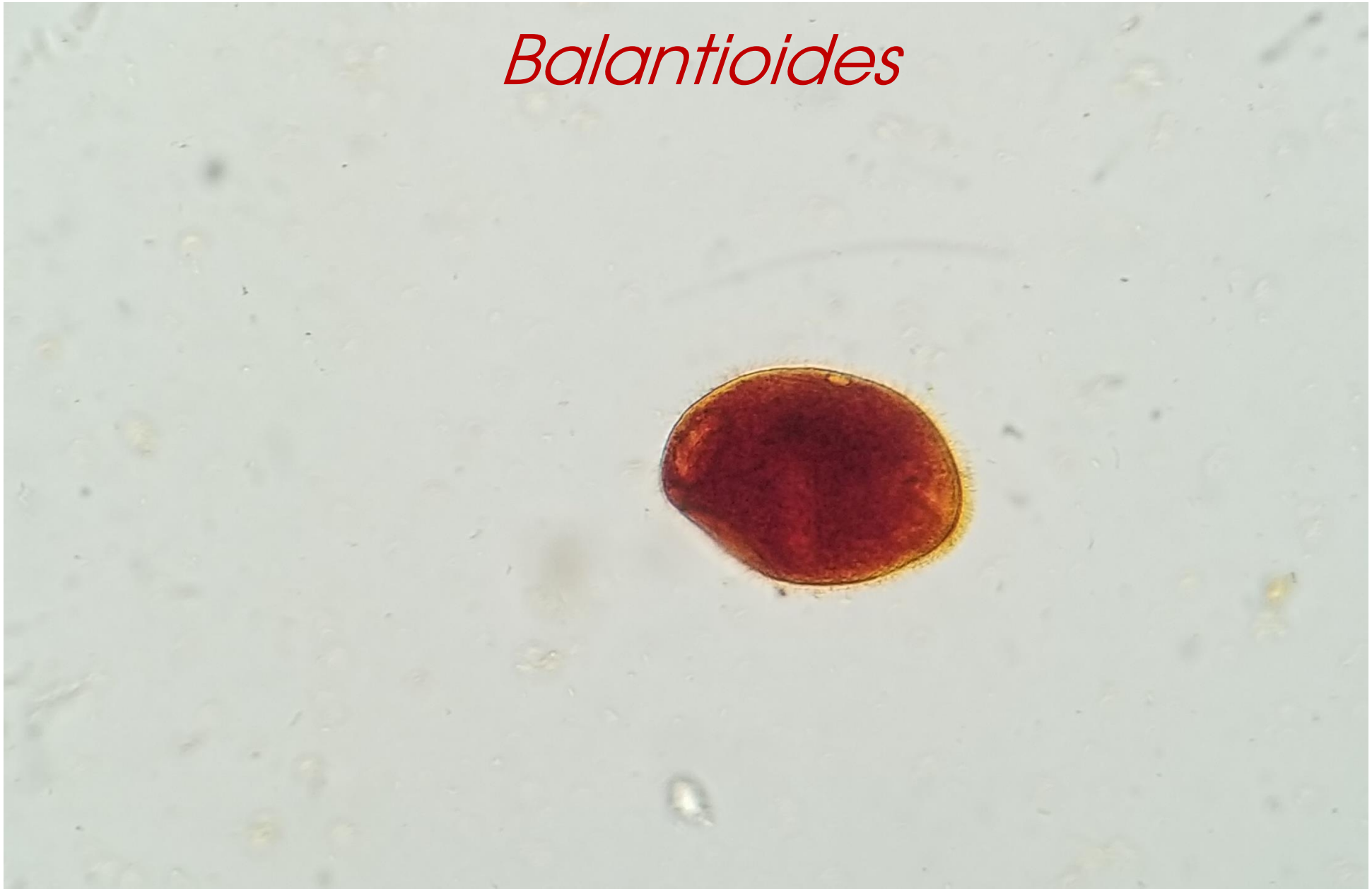


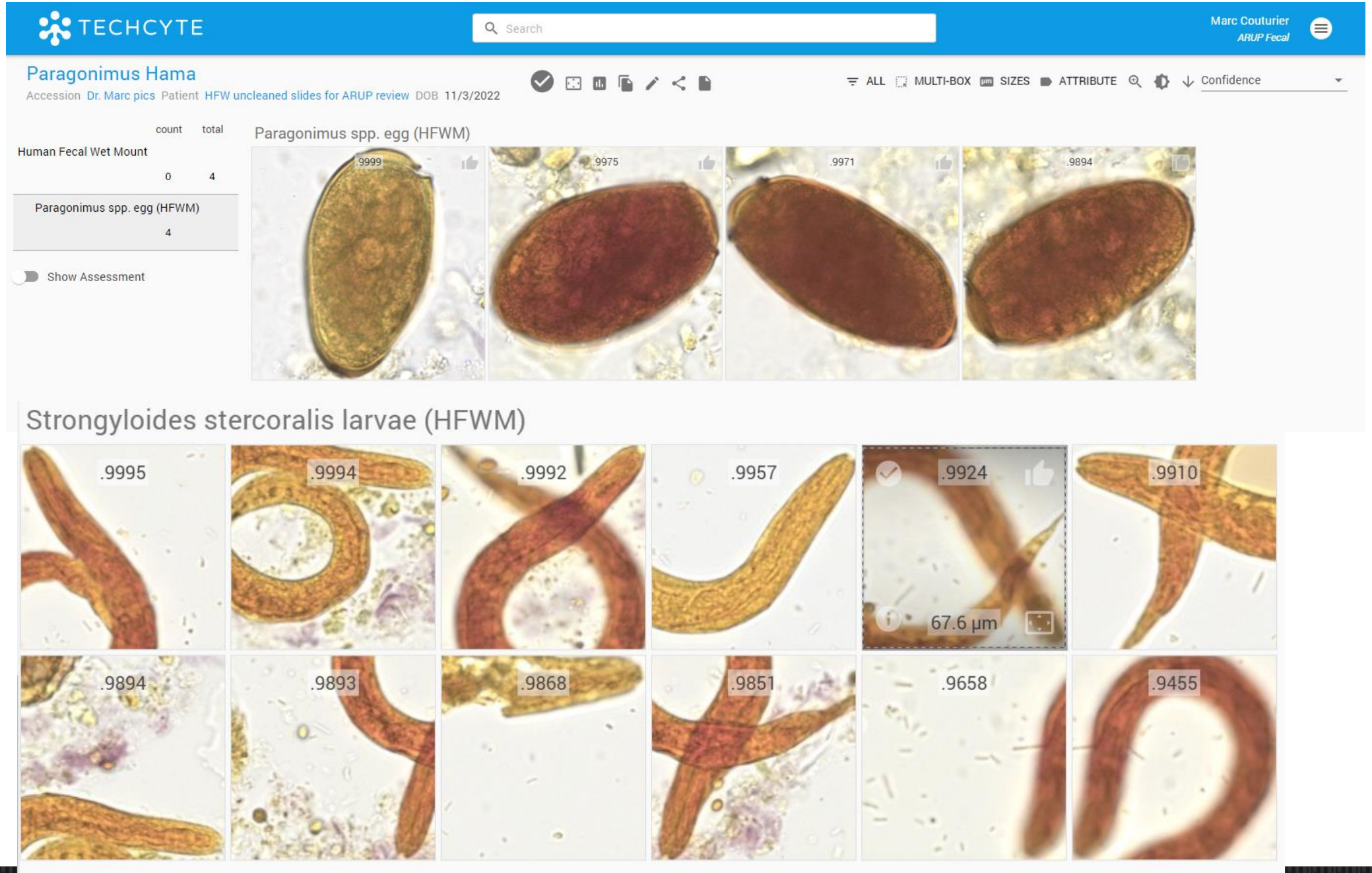
Strongyloides

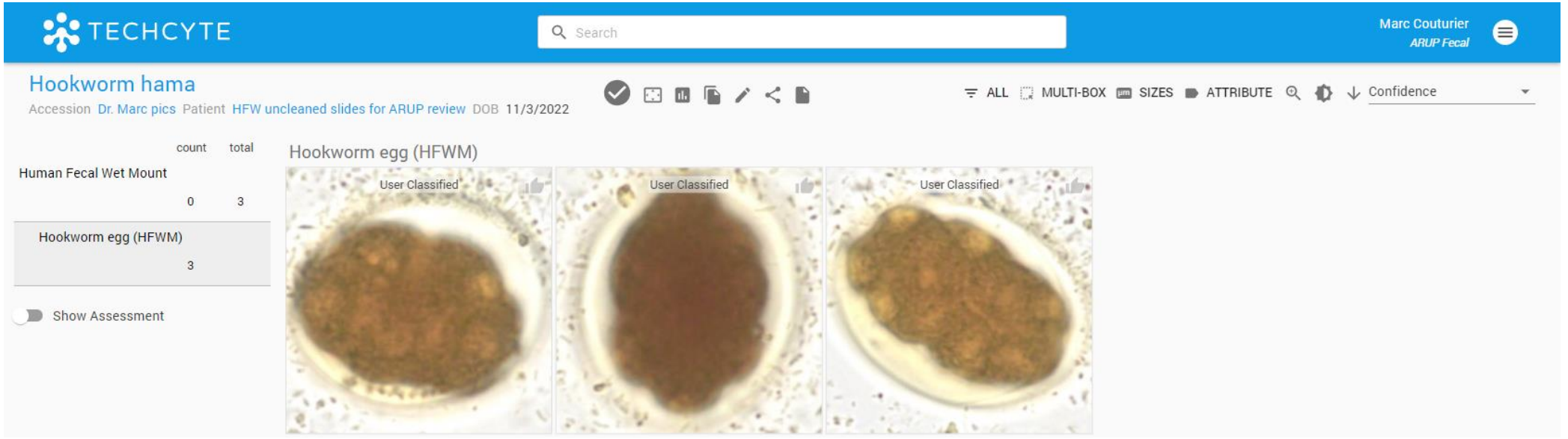
Trichuris

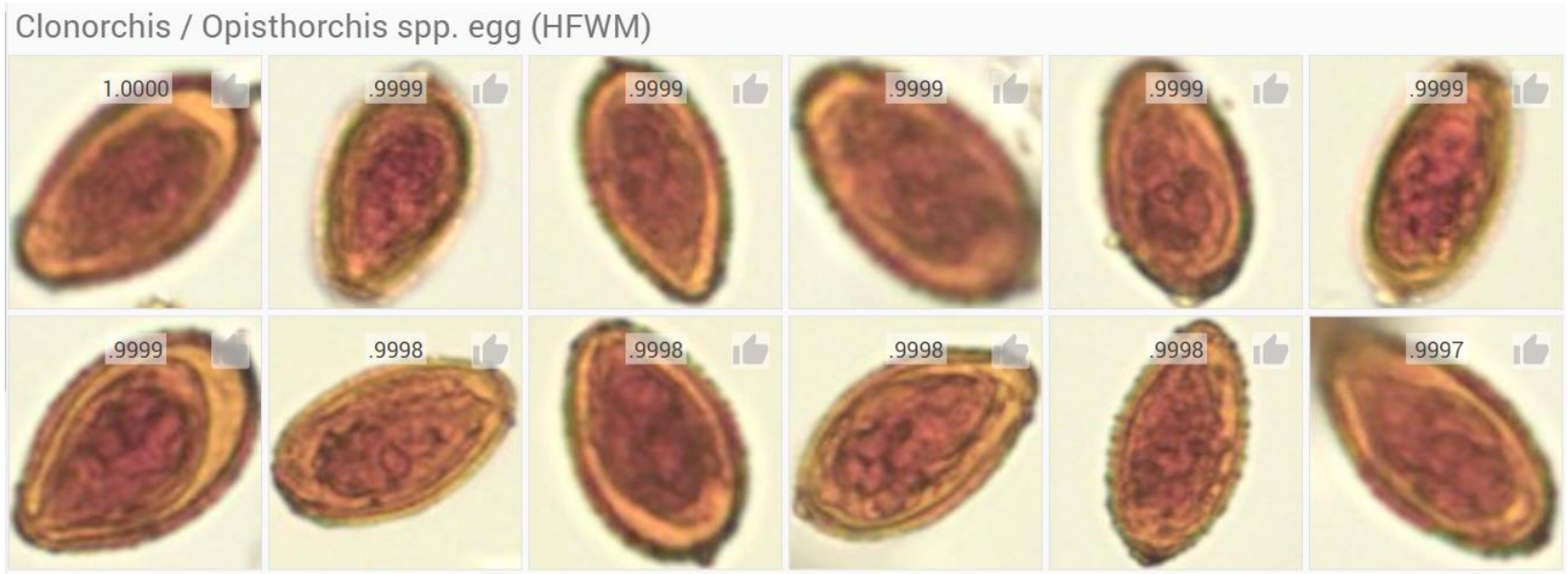


Balantioides

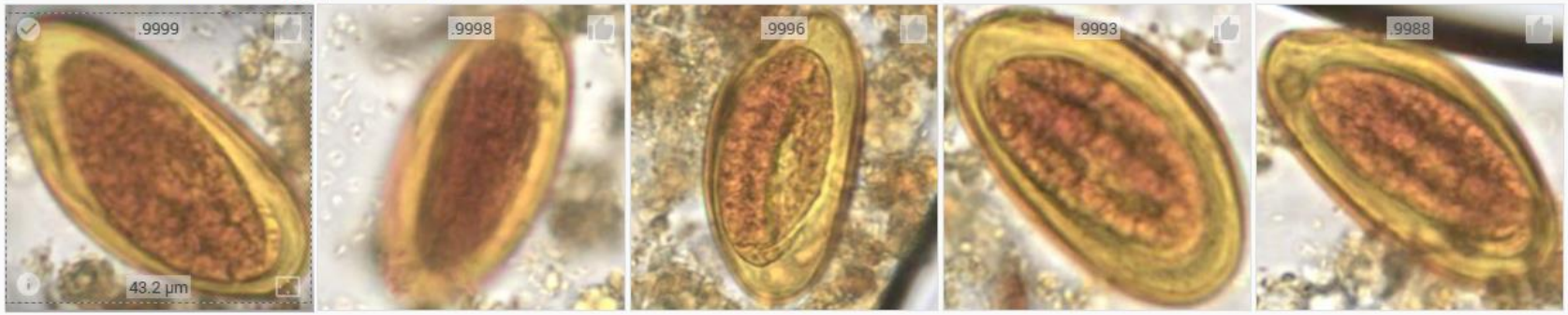




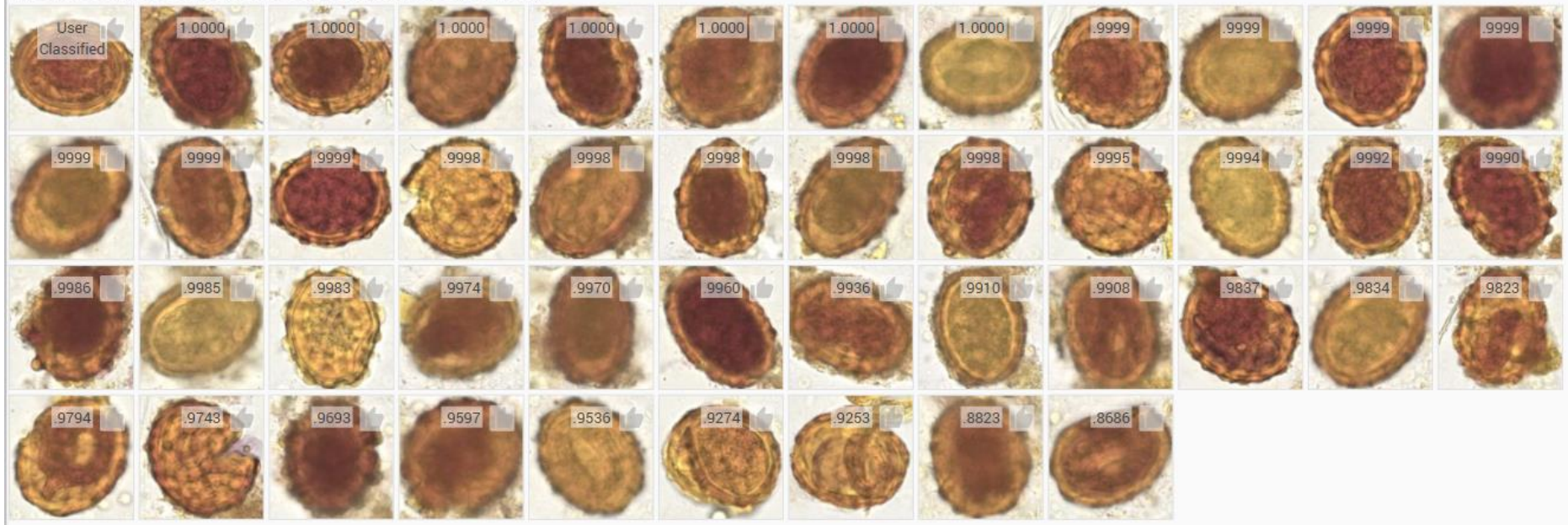




Enterobius vermicularis egg (Pinworm) (HFWM)

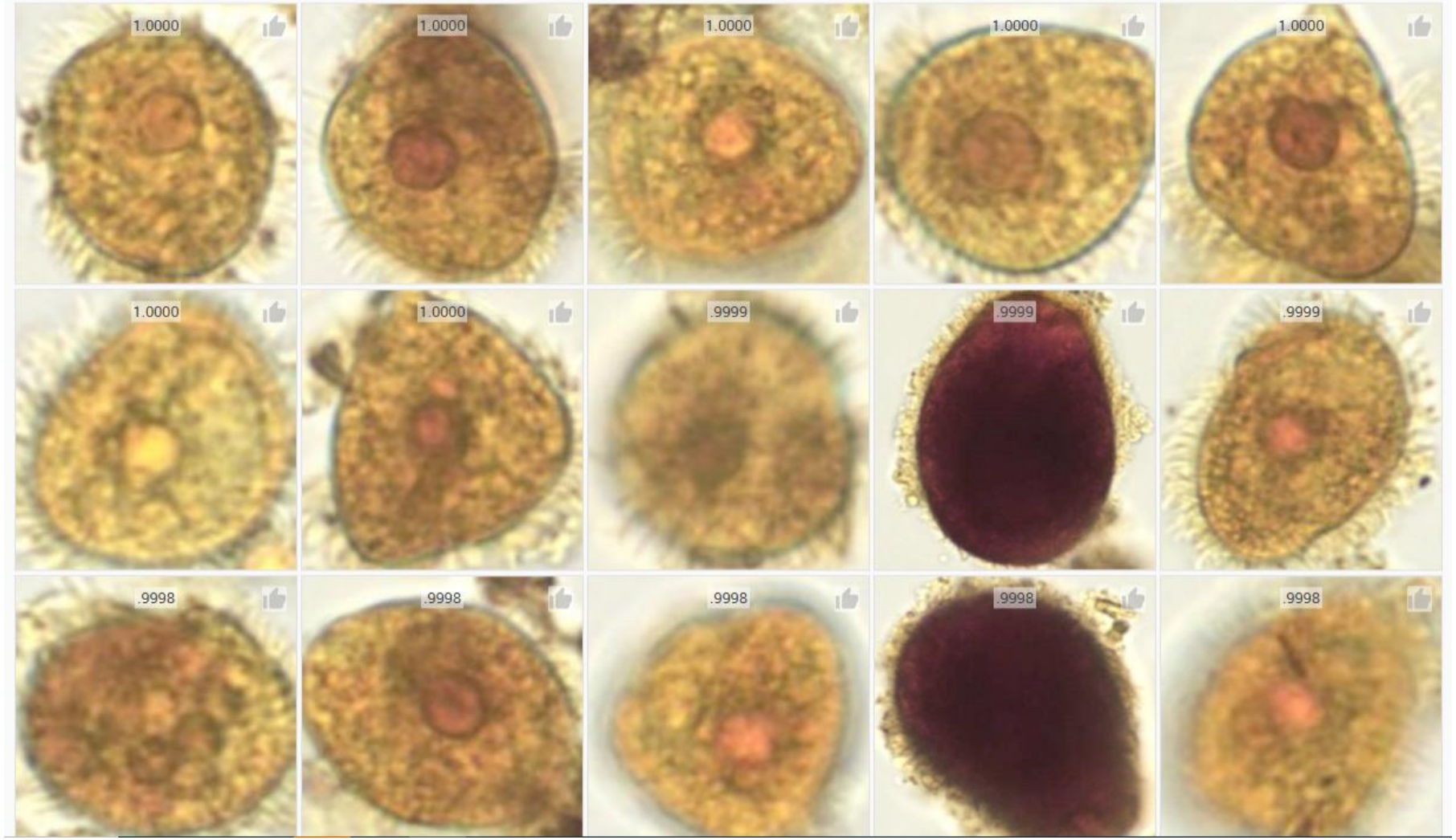


Ascaris lumbricoides, fertile egg mamillated (HFWM)

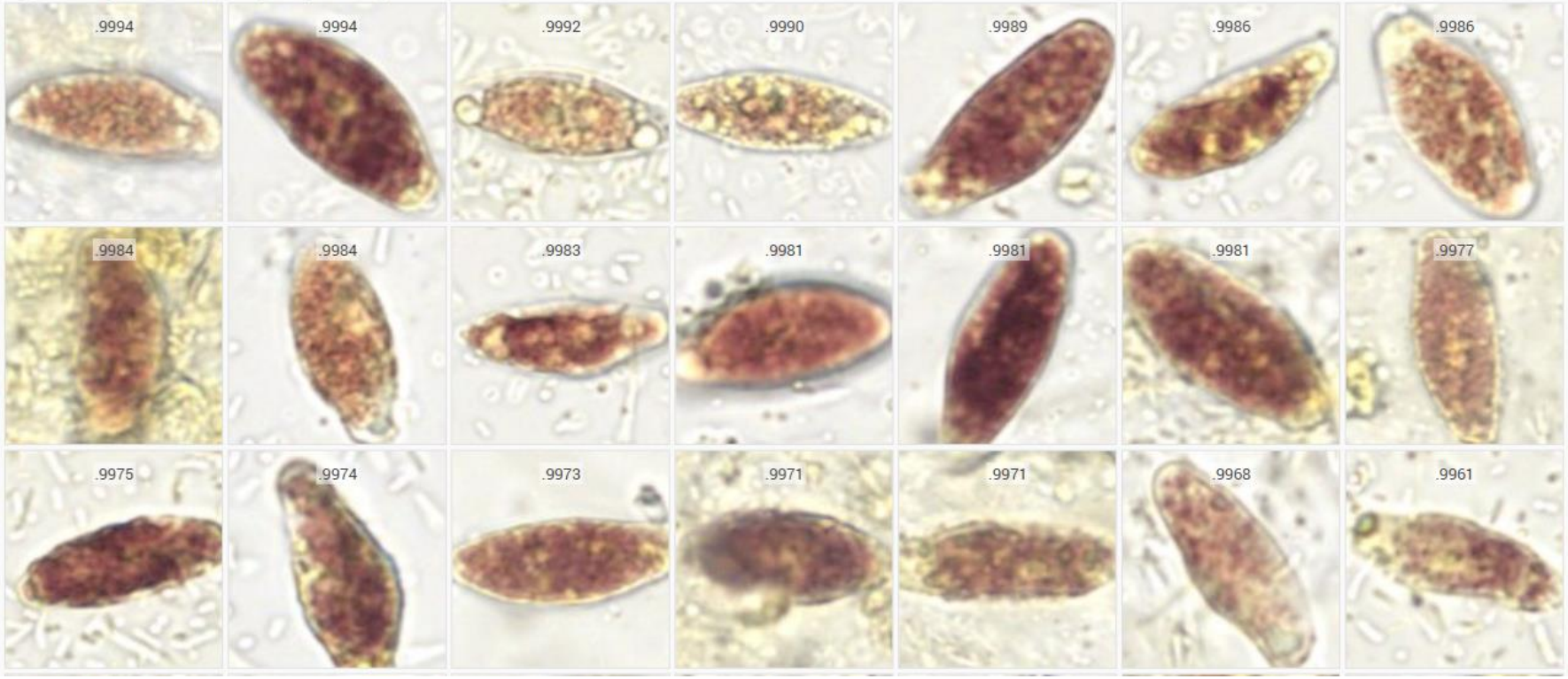


Ascaris lumbricoides, infertile egg mamillated (HFWM)

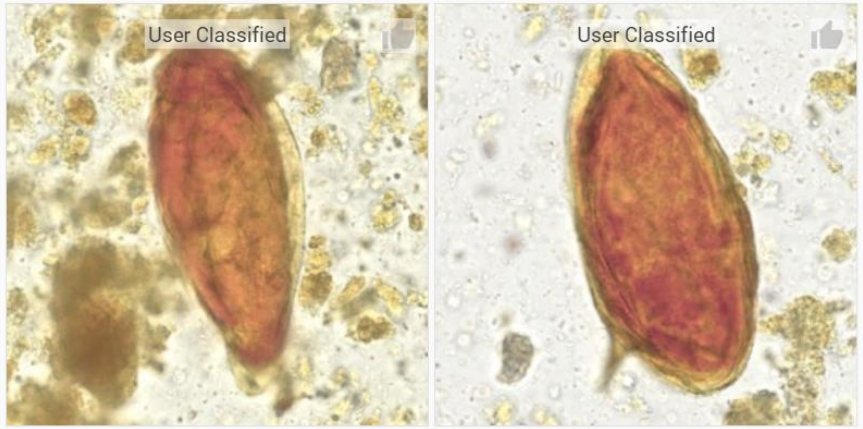
Balantioides coli troph (HFWM)



Cystoisospora belli oocysts (HFWM)



Schistosoma mansoni egg (HFWM)



In Closing…

- Artificial Intelligence such as Machine Learning can help improve the workflow in a diagnostic Parasitology lab by:
  - » Improving turnaround time (reducing the time needed to manually read trichrome and MAF slides)
  - » Screen out 70-80% negative specimens
  - » Reduce ergonomic injuries associated with prolonged microscopy
  - » Improve employee satisfaction (such as spending more time devoted to reading and confirming positive slides)
  - » More engaging for an increasingly younger workforce
  - » Allows for image analysis remotely [possible CLIA considerations]

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