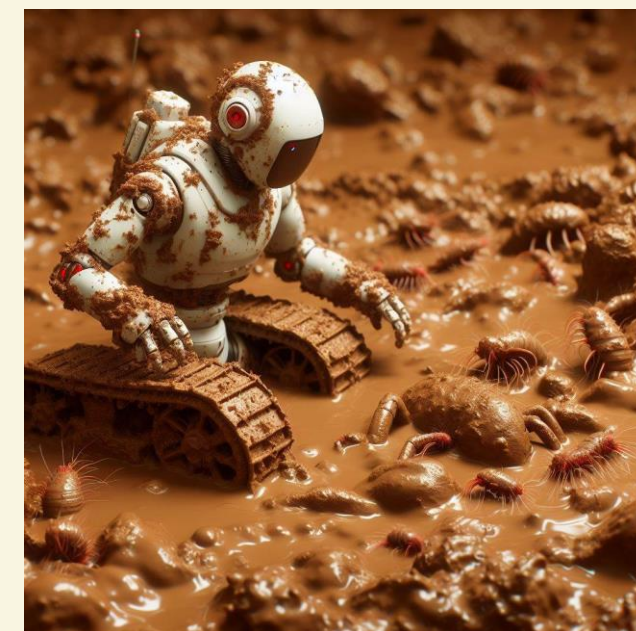




“Parasite AI Has Entered the Chat”: Parasitology Finally Leads the Field in Clinical Microbiology

Marc Roger Couturier Ph.D., D(ABMM)



Disclosures

Relevant:

- Techcyte – research reagents, collaboration

Less relevant:

- Seegene – Scientific Advisory Board
- Biorad – Scientific Advisory Board
- Genetic Signatures – speaker fees, clinical trials activities
- biomérieux – consultant, research support, speaker fees, stock ownership, spousal income
- The College of American Pathologists – Microbiology Resource Committee
- American Society for Clinical Pathology – educational committee, examination committee

Practical:

- ARTIFICIAL INTELLIGENCE is **NOT** intelligent! It needs guidance & direction to reach a set goal
-

Objectives

- Review the use of traditional parasitology testing for gastrointestinal parasites
- Recognize the value and role of AI for trichrome stain interpretation and screening
- Recognize the diagnostic yield and role of wet-mount screening with AI
- Understand other emerging applications of AI in microbiology

Microscopy for Parasites

- **Pros:**

- Detect anything you can see
- Fun!

- **Cons:**

- Insensitive
- Requires well-trained personnel
- Maintaining competence
- Time-consuming
- Scope fatigue/burnout/retiring workforce...lack of new interest



Ova & Parasite Exam

- Standard O&P (most conventional parasites)
 - **Wet mount stain:** specimen + iodine
 - **Trichrome stain:** specimen on slide + stain

***Coccidia & Cryptosporidium stain:**
(modified acid fast or safranin)
Same process as trichrome (not done on all O&Ps)



O&P Use

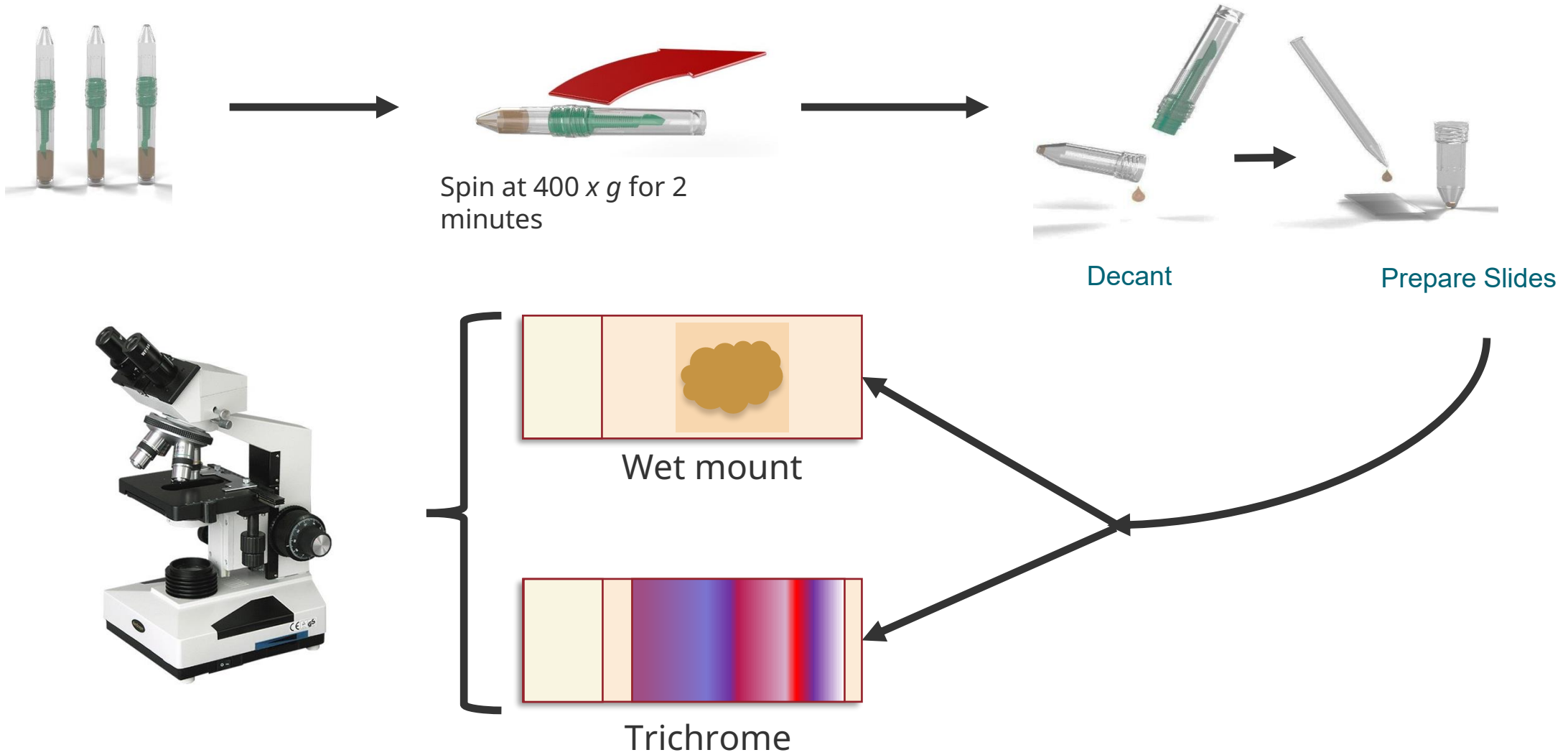
- **Recommended:** 3 specimens/patient
 - Reasonable exposure/risk
- **Reality:** EVERYONE with diarrhea (exaggeration)
- ~120k orders/year
 - ~240k preps/examinations

Content credentials

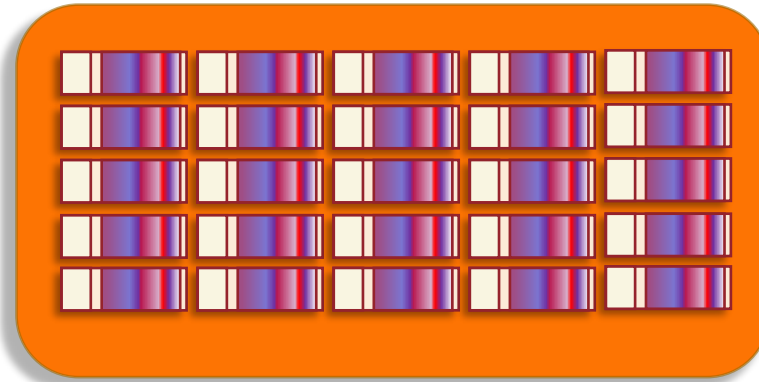
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Preparation for an O&P



Reading an O&P Run



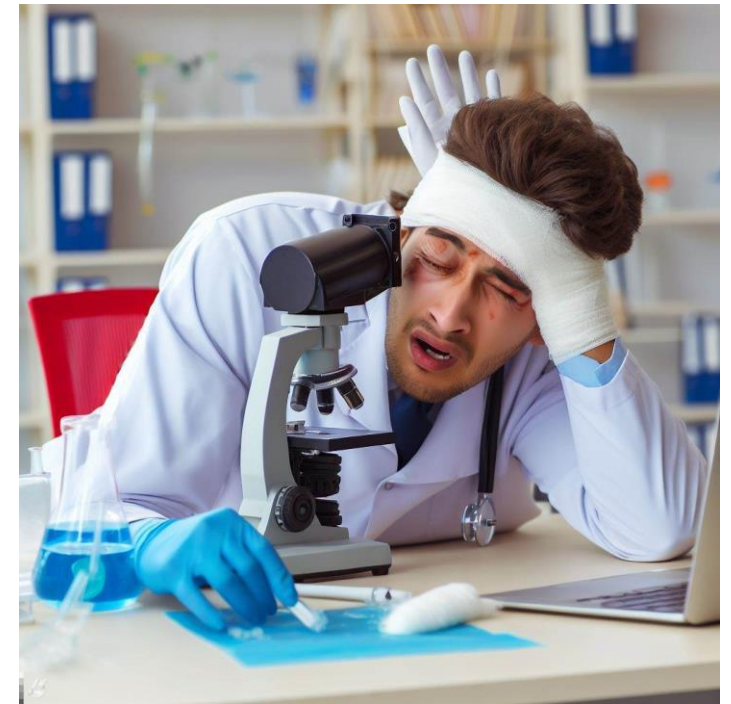
- 30 trichrome slides
- 30 wet mounts
- ✓ ~2.5 – 3 hours/run
- ✓ ~98% negative
- ✓ Positives back-read

2-5 min/slide (technologist variable)

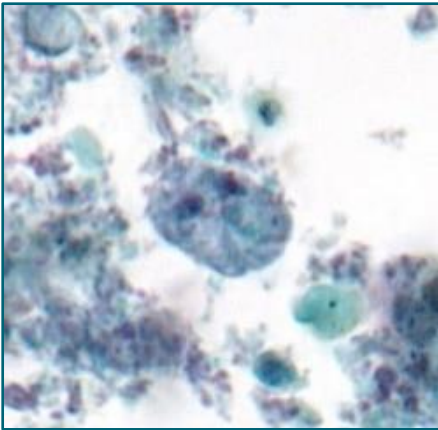
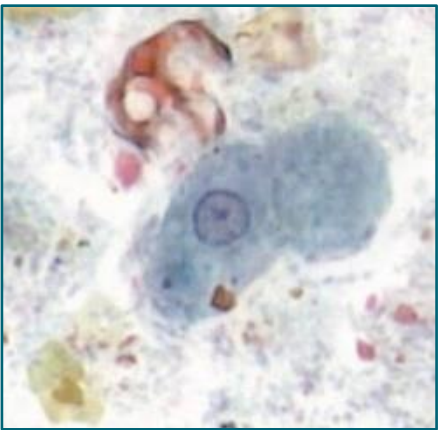
“Questionable artifacts” can take longer

Concerns for O&P Reading

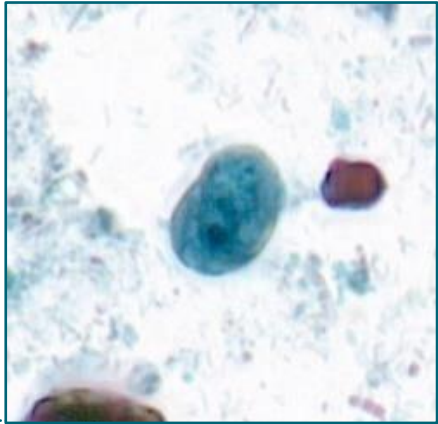
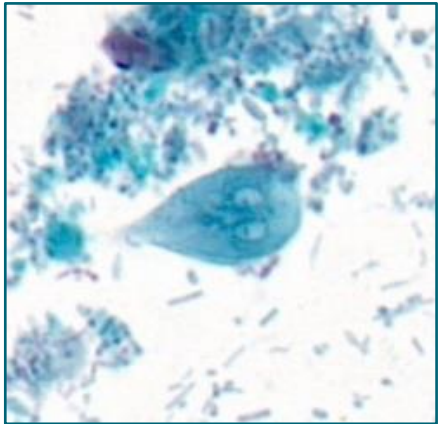
- ✓ Eye strain
- ✓ Neuromuscular strain
- ✓ Burnout/low Satisfaction
- ✓ Decreased Accuracy
 - Individual
 - Time of day
 - Workload



Content credentials
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PM



Digital imaging & machine learning can make this process more efficient & accurate?

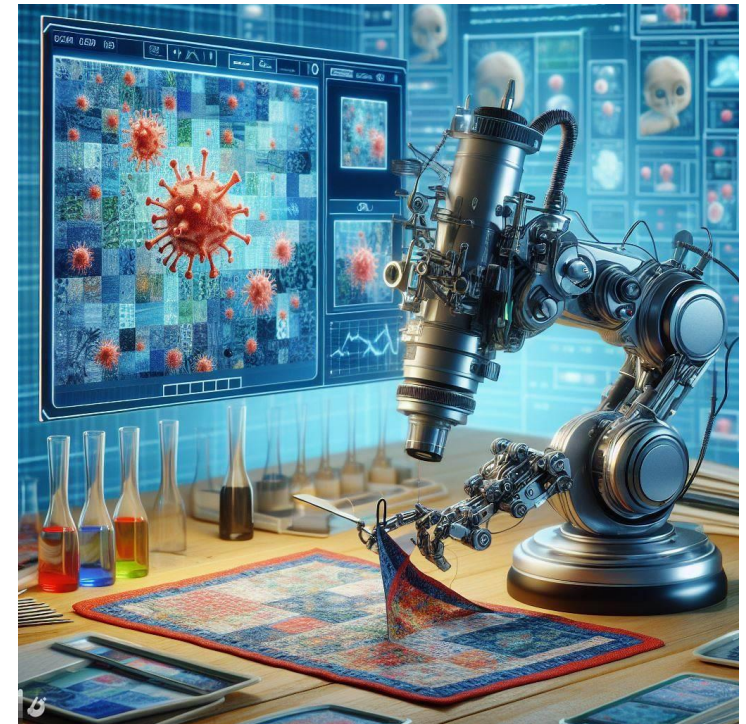


Digital Imaging

Capture images & “thread” into virtual slide

Must be:

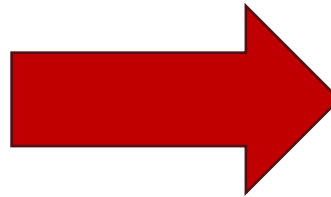
- ✓ High resolution
- ✓ User friendly
- ✓ Equal or better than microscopy
- ✓ Time-efficient for scans



Content credentials
Generated with AI - November 20, 2023 at 1:20 PM

Think Google Image Search

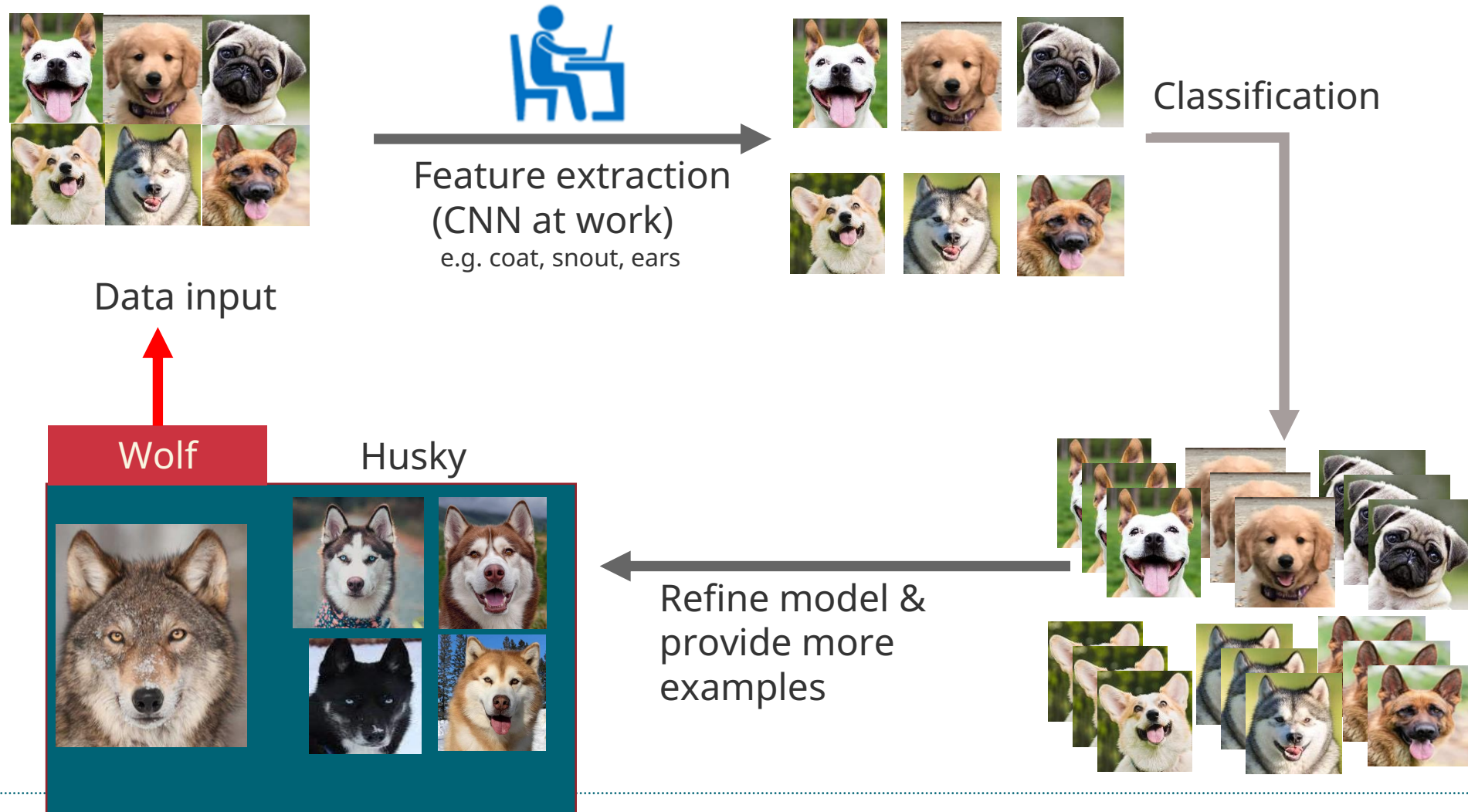
- “Ok Google” show me a husky



Now show me more huskies



Machine Learning & Convolutional Neural Network (CNN) – Simplified



Supervised Learning

- Expert boxes organisms = “truth”
- Software eventually predicts organisms
 - Expert confirms or denies
- Software runs iterations
 - Expert remediates software...software learns
- ~ >1000 remediated examples: predictions = accurate



Jess Kohan



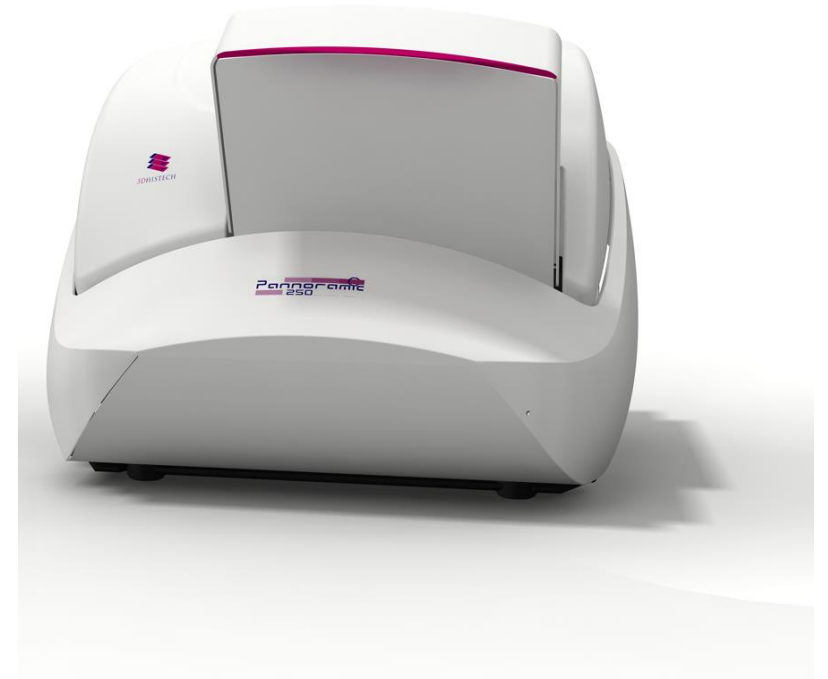
Katie Knight

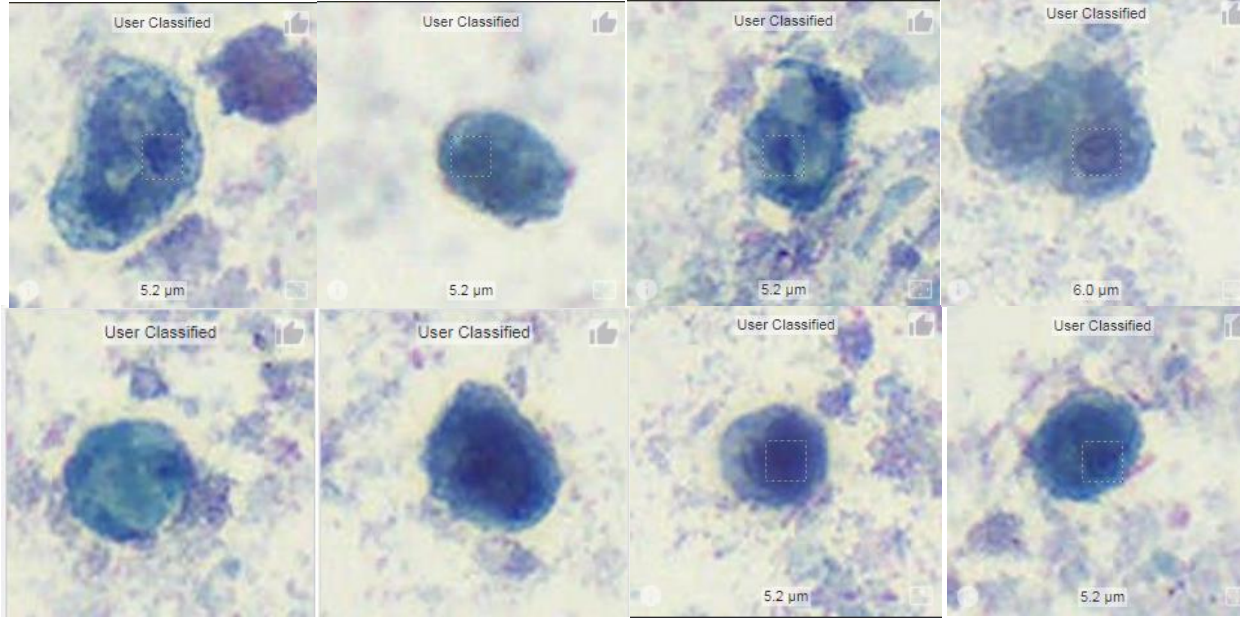


'The' Blaine Mathison

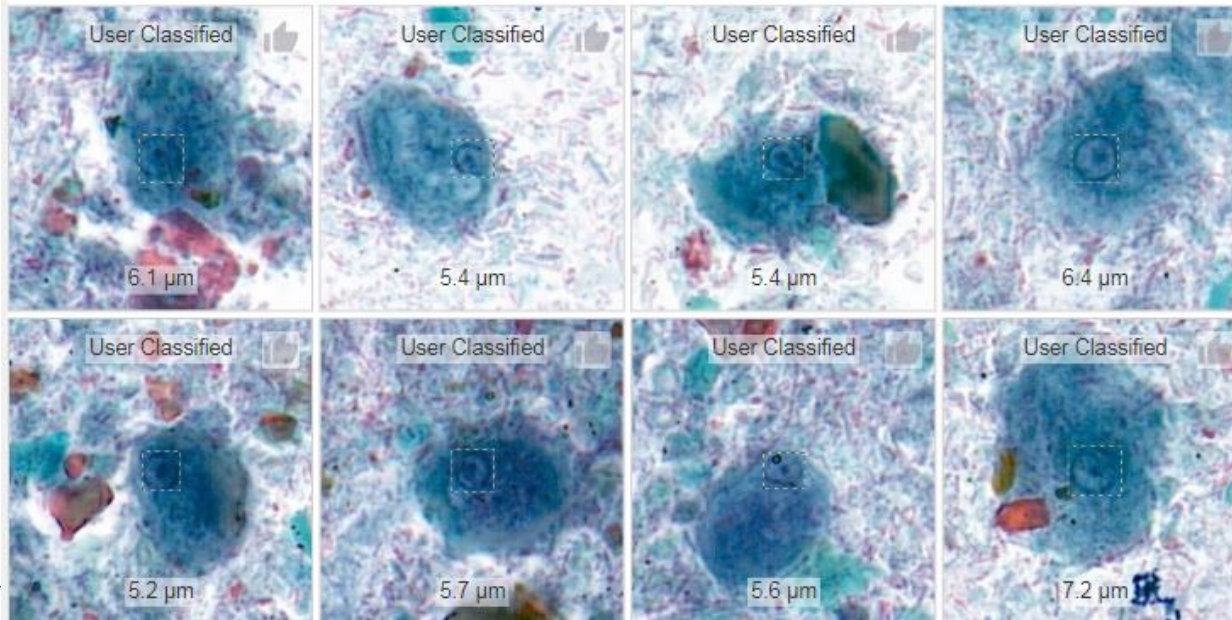
3DHISTECH Pannoramic 250

- 80X virtual magnification
- 250 slide capacity
- Extended focus
 - Automated “good depth” image
- BUT - Slides load on angle (problem for wet-mounts)





Entamoeba Troph Nucleus



Motic scanner

VS

3D HISTECH Scanner

Wish List Metrics for Success (circa 2018)

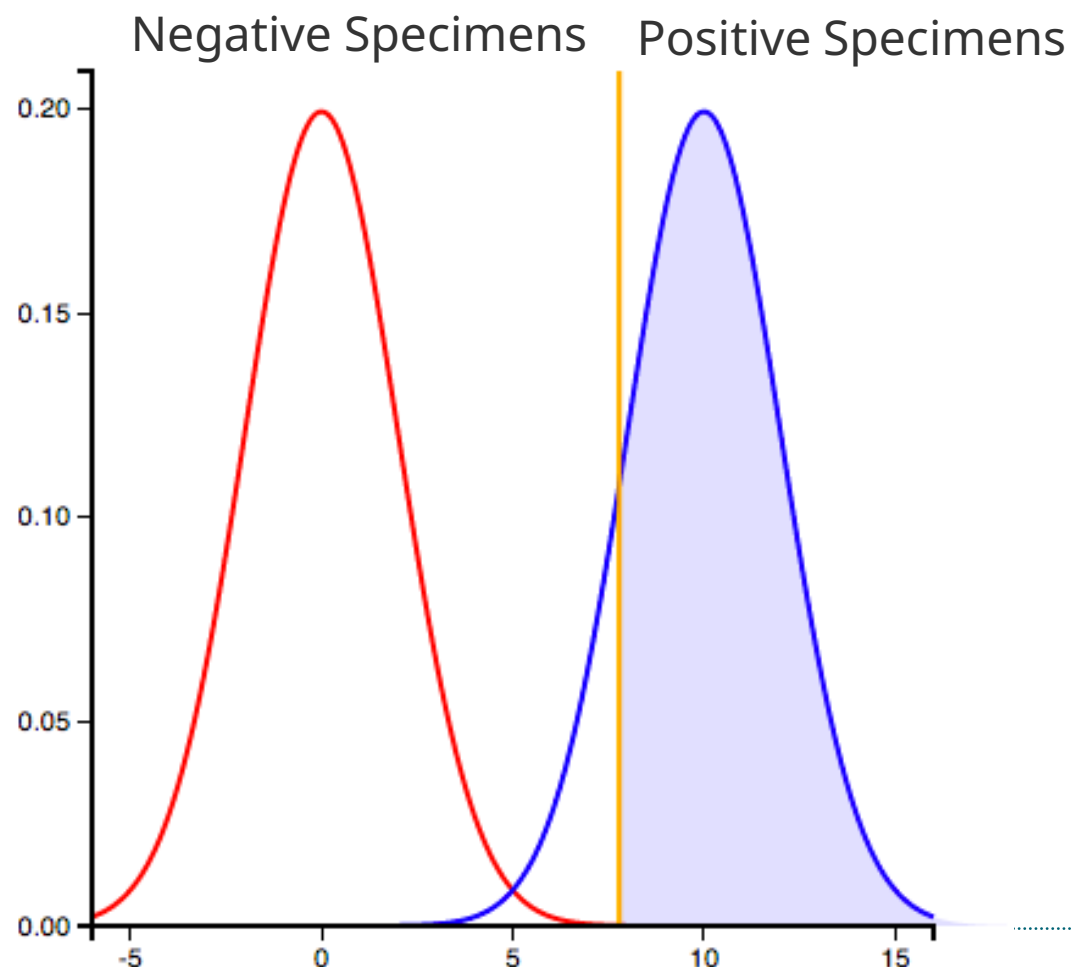
- ✓ Faster
- ✓ Easier
- ✓ Less hands on

Goal: Screen out 70% of negative specimens w/no microscope.



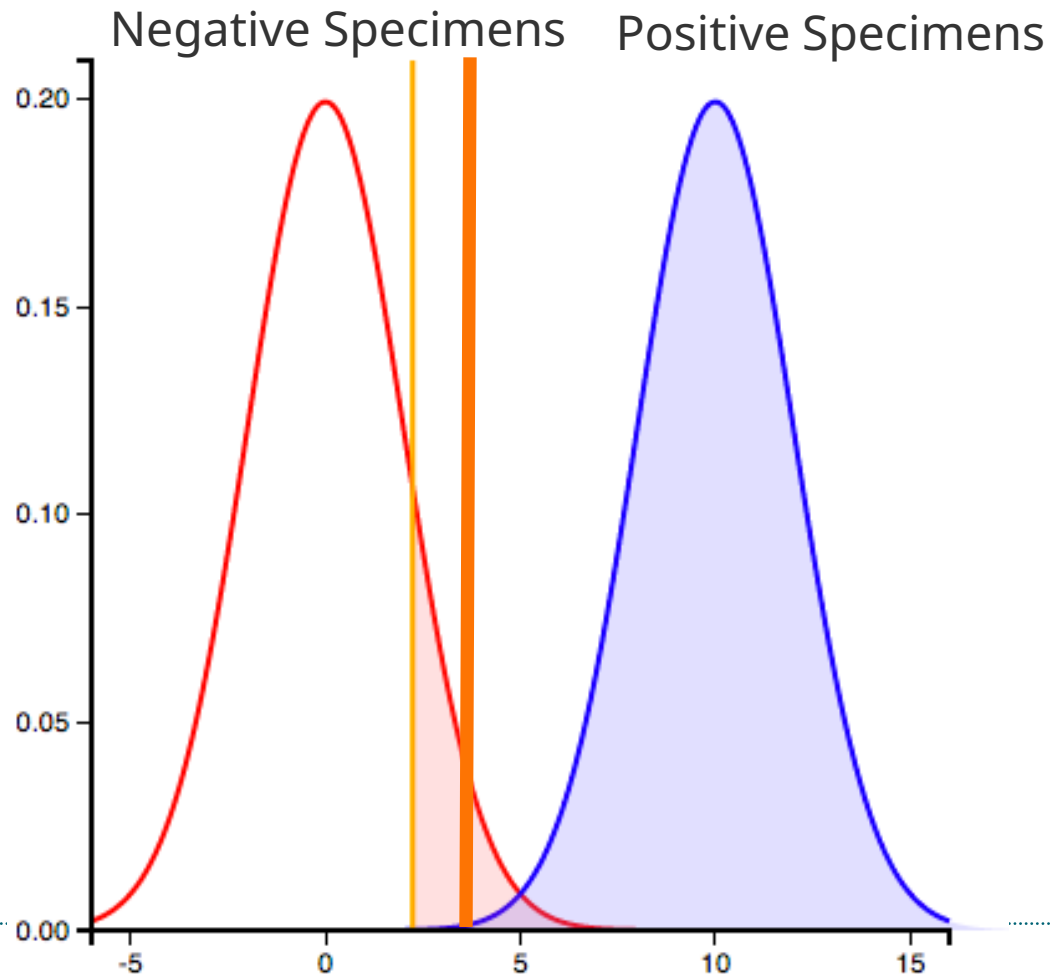
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Perfect Specificity, Lower Sensitivity...



- Everything identified is true
- Will miss some positives

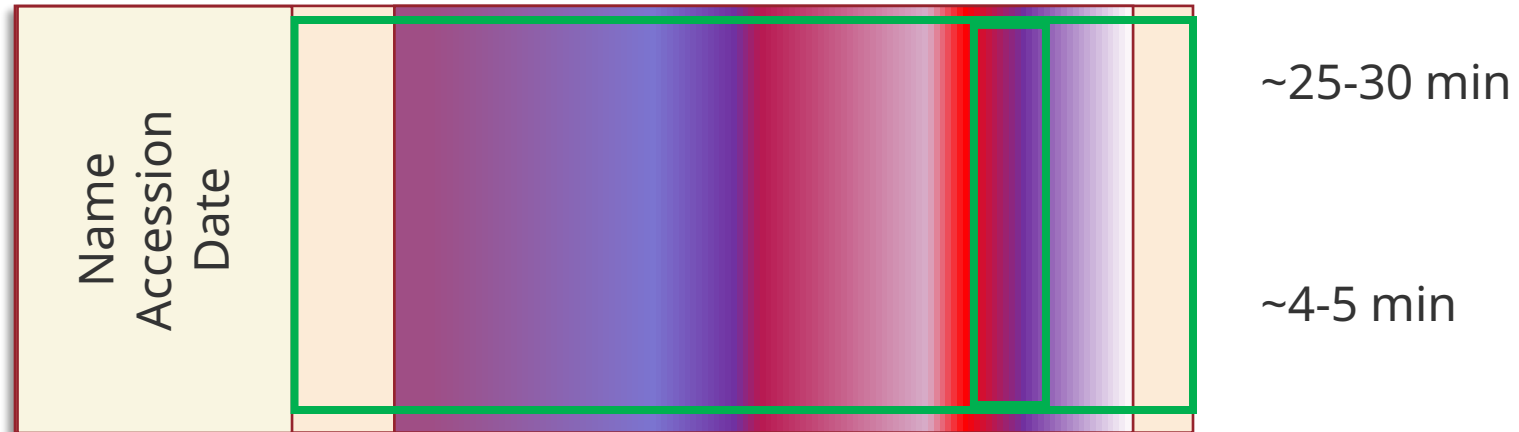
Perfect Sensitivity, Lower Specificity...



- Identifies everything
- Shows some junk, but human arbitrates it
- Find a sweet spot: catches all, but limits junk!

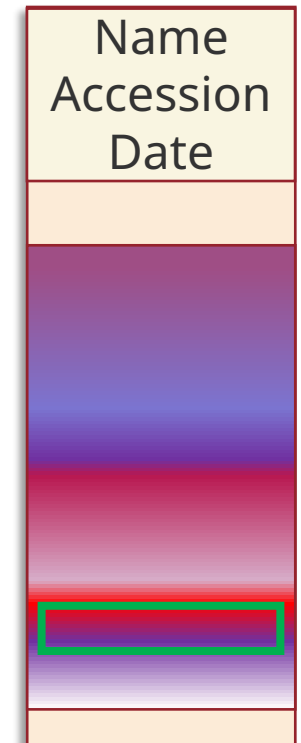
Whole Slide Scanning

- Not time-effective, not standard of care
- Theoretical slide scan area (= to manual read fields)
 - Minimize scan time & maintain equal or better accuracy than human



Are We Still Sensitive? (@ slide level)

- Serially diluted *Giardia* & *Blastocystis* in negative stool
- Duplicate slides of each dilution
 - Manual read in lab (blindly integrated into run)
 - Scanned & read by AI
- Compare analytical sensitivity with new scan area



Analytical Sensitivity – Scan Area Test

Dilution	Technologist read	AI read
Neat	Giardia + Blastocystis	Giardia (276) + Blastocystis (129)
1:1	Giardia + Blastocystis	Giardia (95) + Blastocystis (19)
1:2	Giardia + Blastocystis	Giardia (68) + Blastocystis (17)
1:4	Giardia + Blastocystis	Giardia (79) + Blastocystis (46)
1:8	Negative	Giardia (70) + Blastocystis (13)
1:16	Giardia + 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)

AI (with constrained scan region) was 4-6 fold more sensitive than a human

AI Enters Validation

- Software is locked down: no further learning
- “Holdout” slides used to validate software – Techcyte (commercial partner)
- ARUP validates w/ validation slide set
 - Development >12 months, validation < 2 weeks
- Train lab
- Go Live (invisible to anyone external)

[illegible]

Limit of Detection

- Human: No dilutions series (n=4) detected below 1:16 dilution
- AI: Detected all to 1:256



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Slide-Level Accuracy

- **False Positive:** AI images cannot be excluded as “False” without review of slide.

O&P Examination

CNN Model Analysis



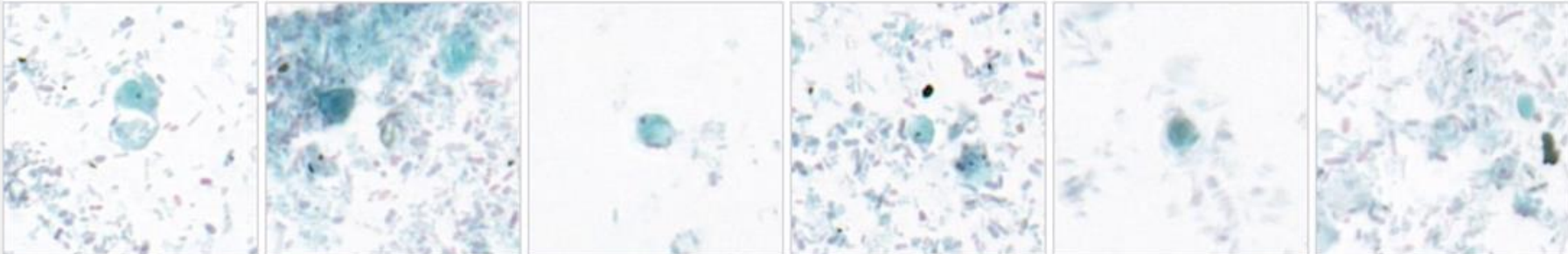
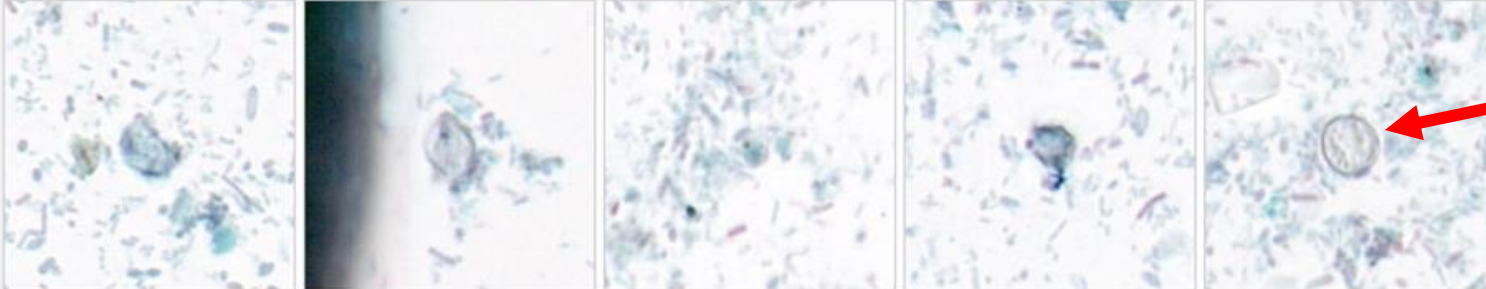
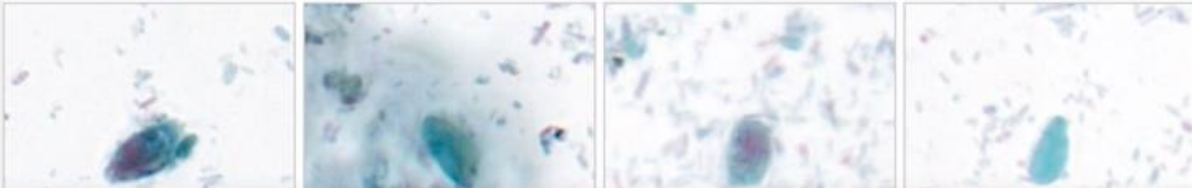
	Positive	Negative
Positive	86	2
Negative	1	104

Positive percent agreement: 98.88% [95% CI 93.76% to 99.98%]

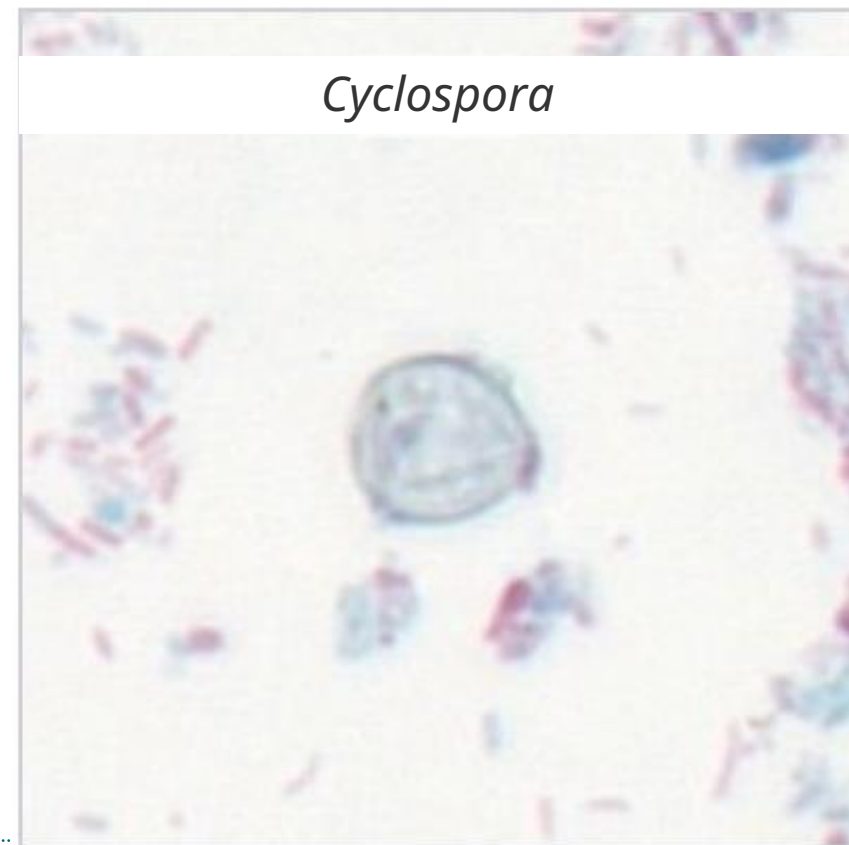
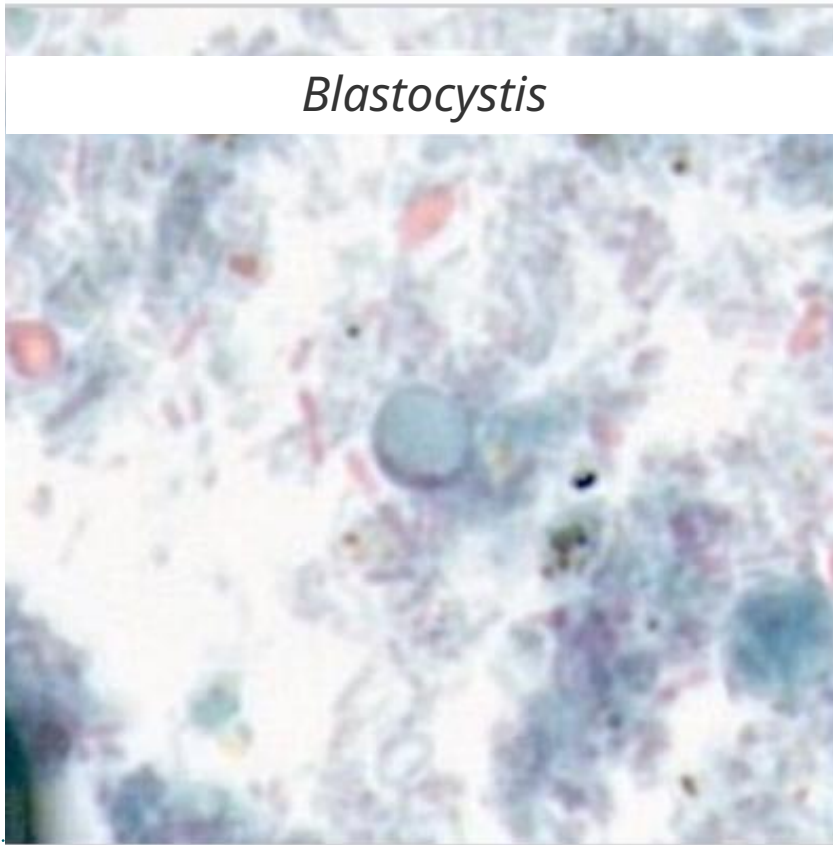
Negative percent agreement: 98.11% [95% CI 93.35% to 99.77%]

Mathison et al. 2020 *J Clin Microbiol*

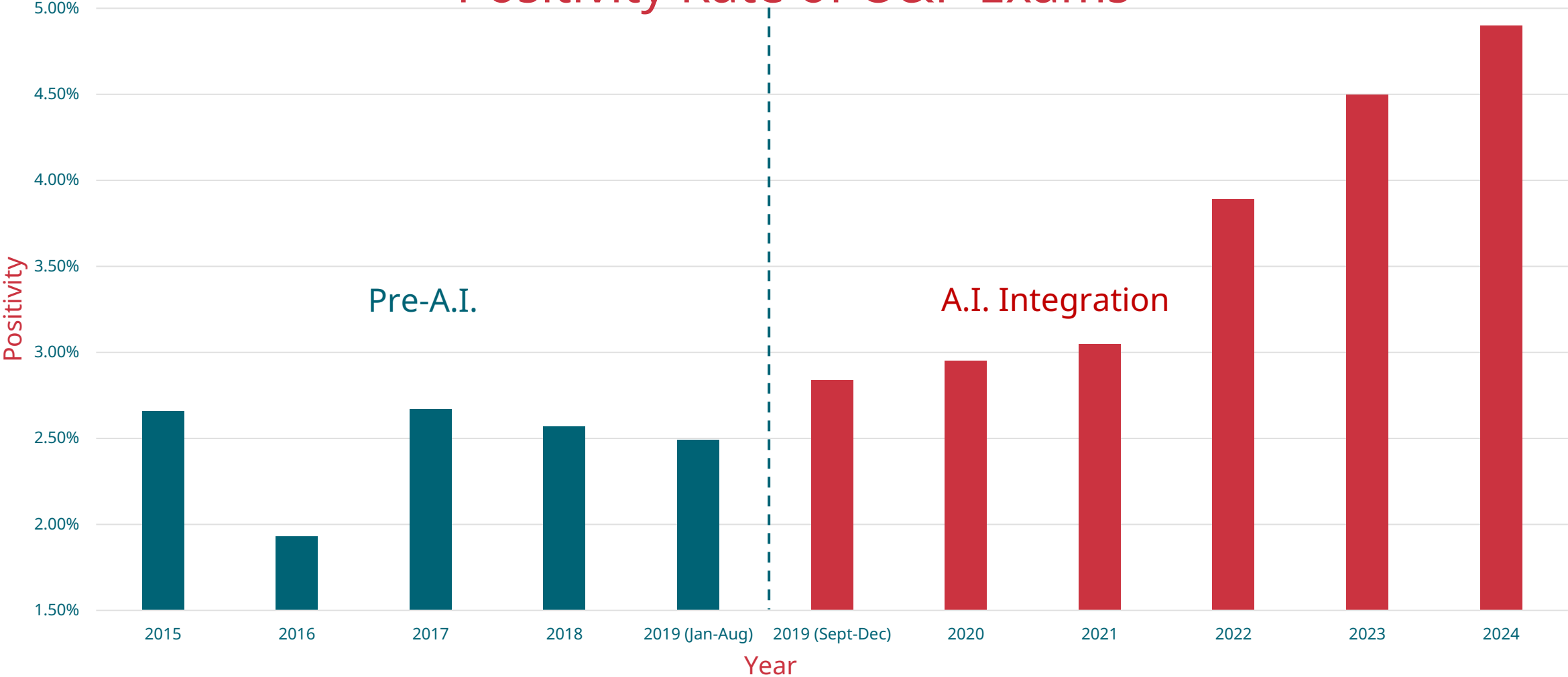
Cyclospora in Trichrome NOT Blastocystis (circa Aug. 2019)

	count	total	present	
Fecal Smear	0	32		
Blastocystis sp.	12		☐	Blastocystis sp. 
Giardia duodenalis	0	6		
Giardia Cyst	4		☐	Giardia Cyst 
Giardia Troph	2		☐	
Endolimax/Iodamoeba Troph	2		☐	Giardia Cyst 
Entamoeba hartmanni				

Cyclospora in Trichrome NOT *Blastocystis*

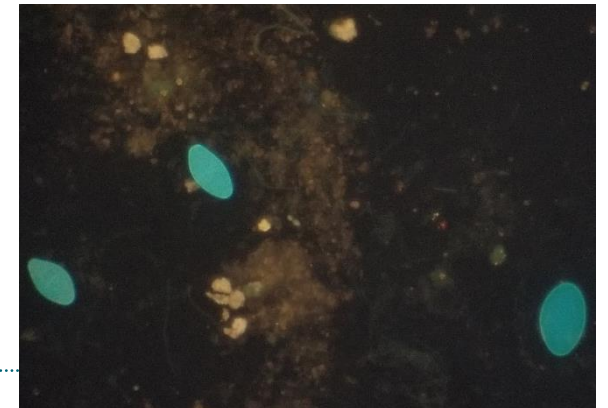
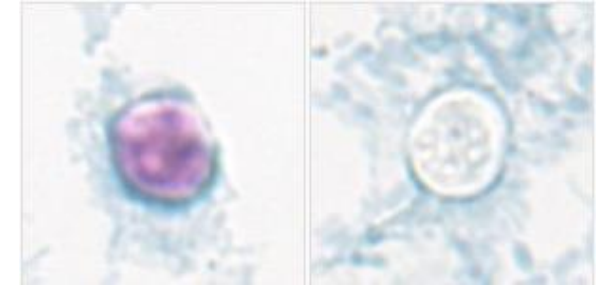


Positivity Rate of O&P Exams



Modified Acid Fast v1 – step 2 toward complete stool parasite AI

- Stain for *Cryptosporidium*, *Cyclospora*, & *Cystoisospora*
 - *Cyclospora* does not retain trichrome stain well
 - “Ghost” forms can be detected by human...but difficult
 - *Cystoisospora* better detected by wet-mount (bright field & UV)
 - Rare, not trained
(in progress v2 & wet-mount)



MAF - Validation Summary

- 20 *Cryptosporidium* / 20 *Cyclospora* / 100 Negative

Original Results

		Positive	Negative
Software Analysis	Positive	40*	3
	Negative	0	97

*one slide positive for *C. cayetanensis* was backread as the software detected suspect *Cryptosporidium* (false positive)

Positive percent agreement: 100% [95% CI 91.19% to 100.00%]

Negative percent agreement: 97.0% [95% CI 91.48% to 99.38%]

Mathison *et al.* In Preparation

MAF Limit of Detection

Same trend as trichrome

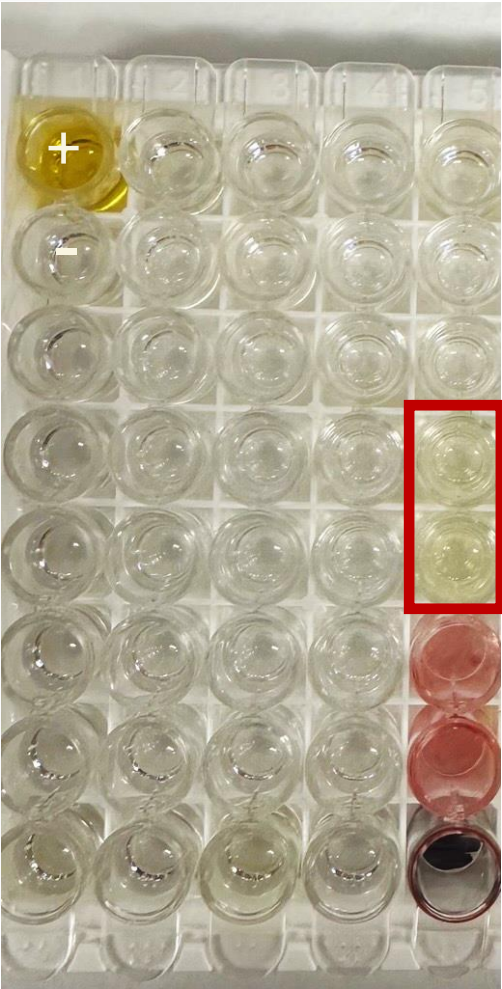
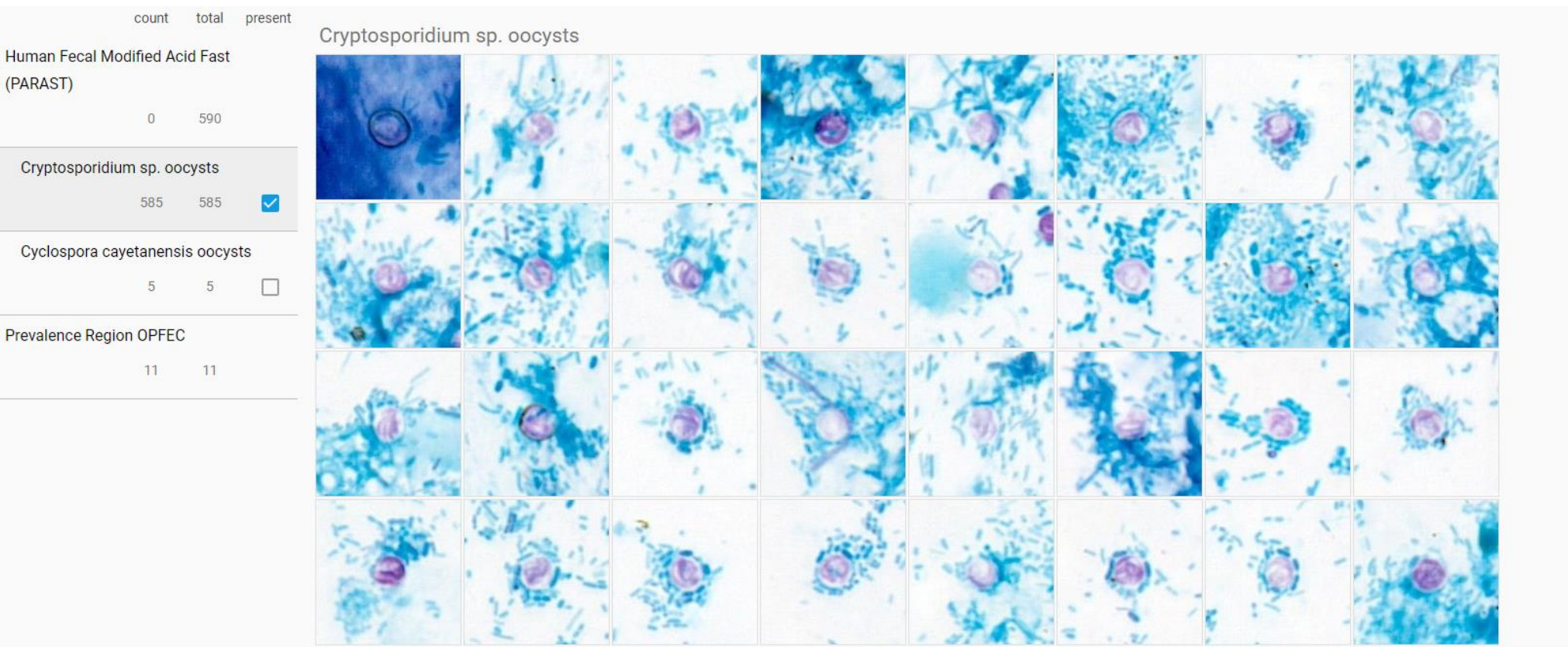
- **Human:** 1:1, 1:2, & 1:8
- **AI:** 1:256 (n=2) & 1:512 (n=1)

Clinical lab integration early 2021



Mathison *et al.* In Preparation

PCR -/Ag- vs Microscopy +



User Look at Trichrome

https://app.techcyte.com/worklist?sort=-created_at&status=processing_complete&assigned_to=-%2Cme

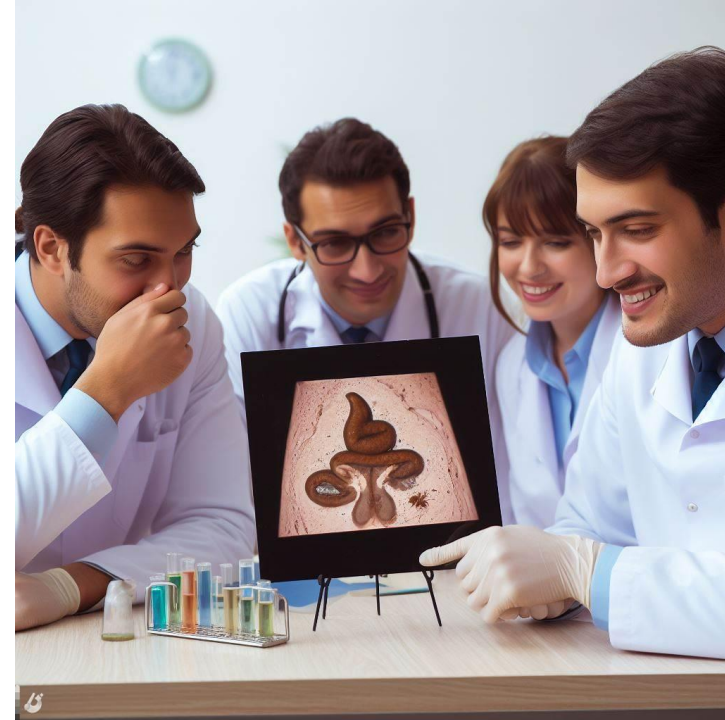


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Wet-mount exam development

Part 3 of total O&P AI

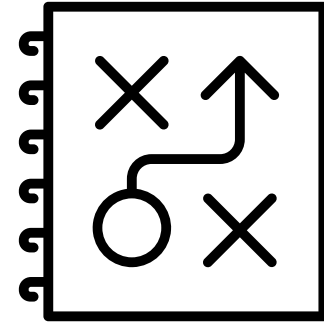


Prompt: “A sneak preview of stool wet mount slide being shown to a group of pathologists”

Content credentials **Generated with AI** · November 20, 2023 at 2:04 PM

Practical challenges

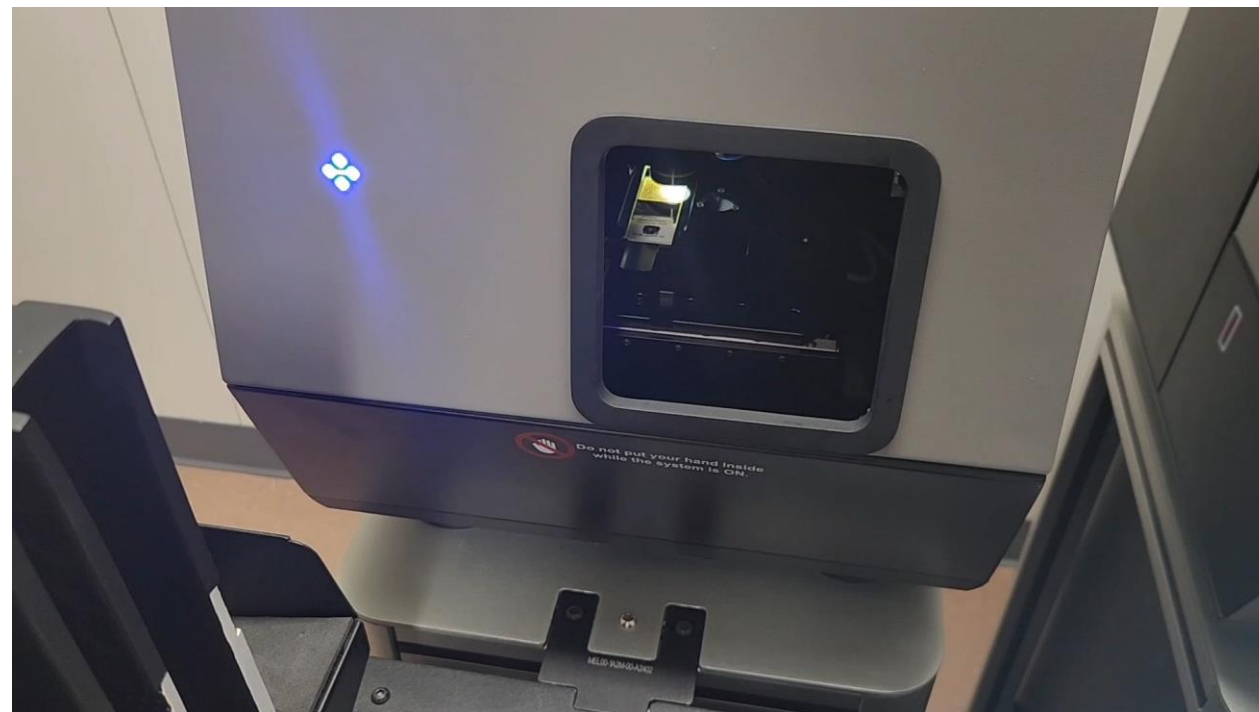
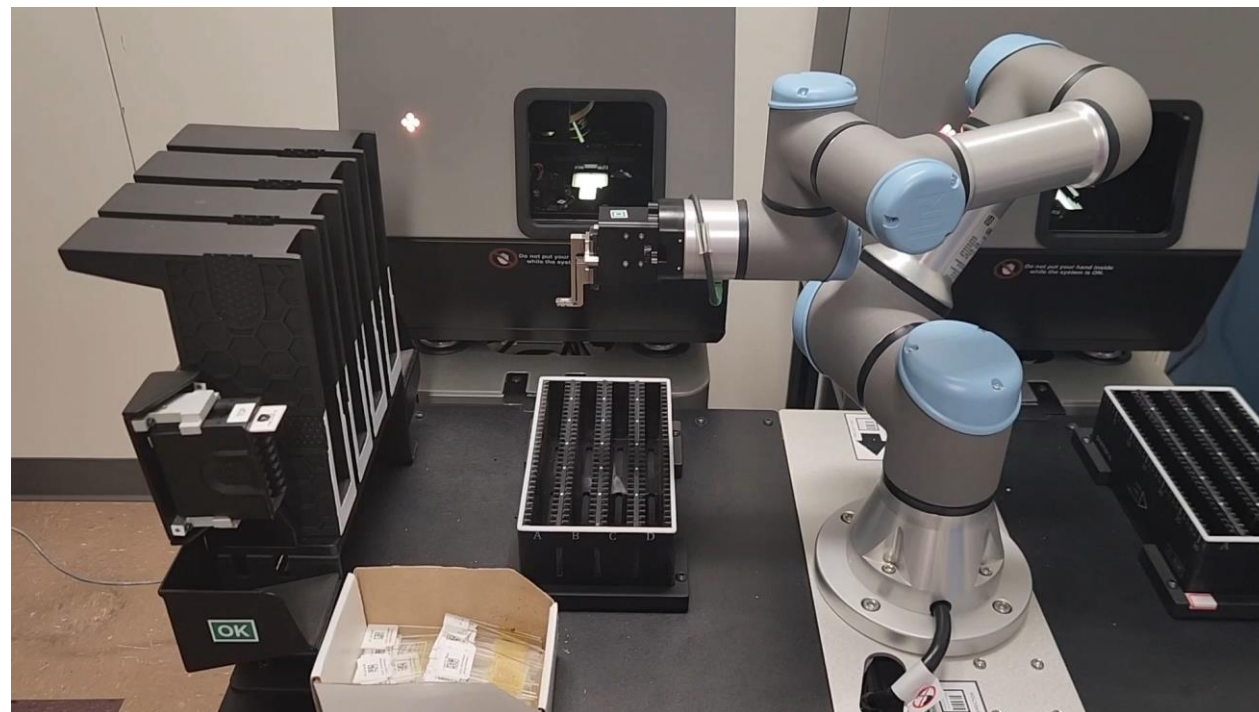
1. Scanner: 3DH loads at an angle (spills)
2. “Depth” of specimen
3. Dry time: iodine mounts can dry fast (~20-30 mins)
4. Brownian motion & resolution: things float too fast & grainy textures



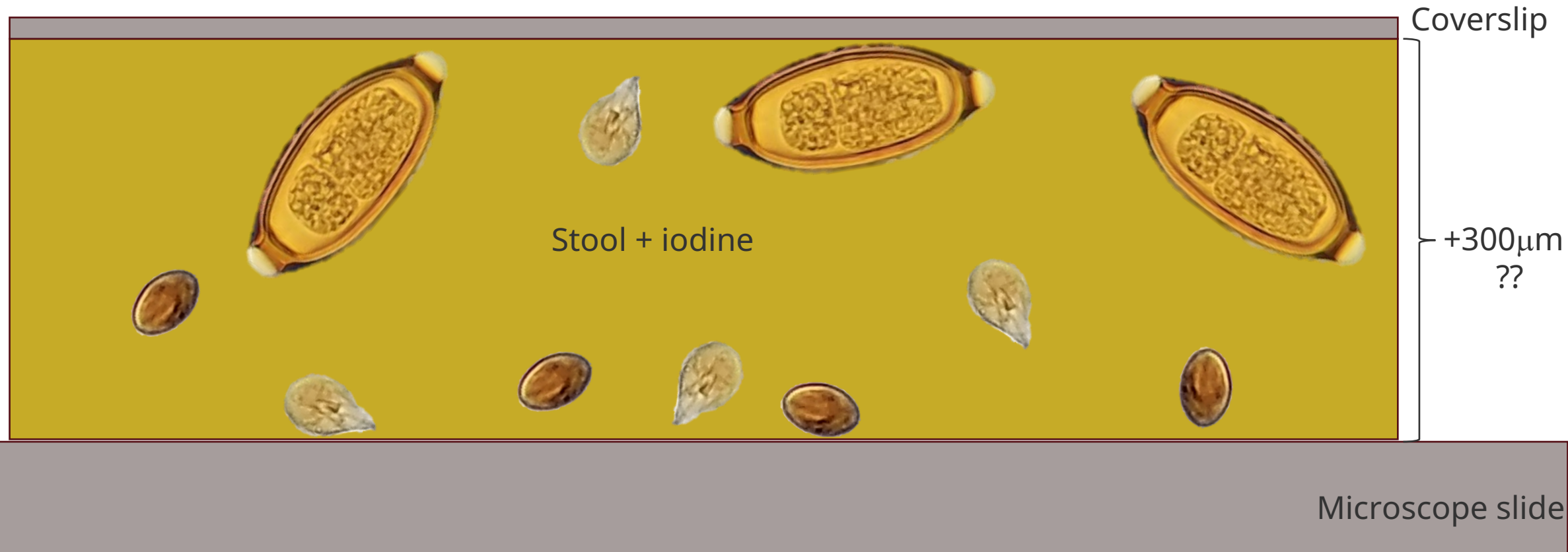
Pramana spectralHT



Pramana spectralHT



Depth and focus issues



Modified Mounting Medium

- **Iodine:** dries fast & grainy backgrounds
- **Fluidity:** scanning challenges
- **Solution:** (Trial & error) homogenized iodine & glycerol medium

Chilomastix



Giardia



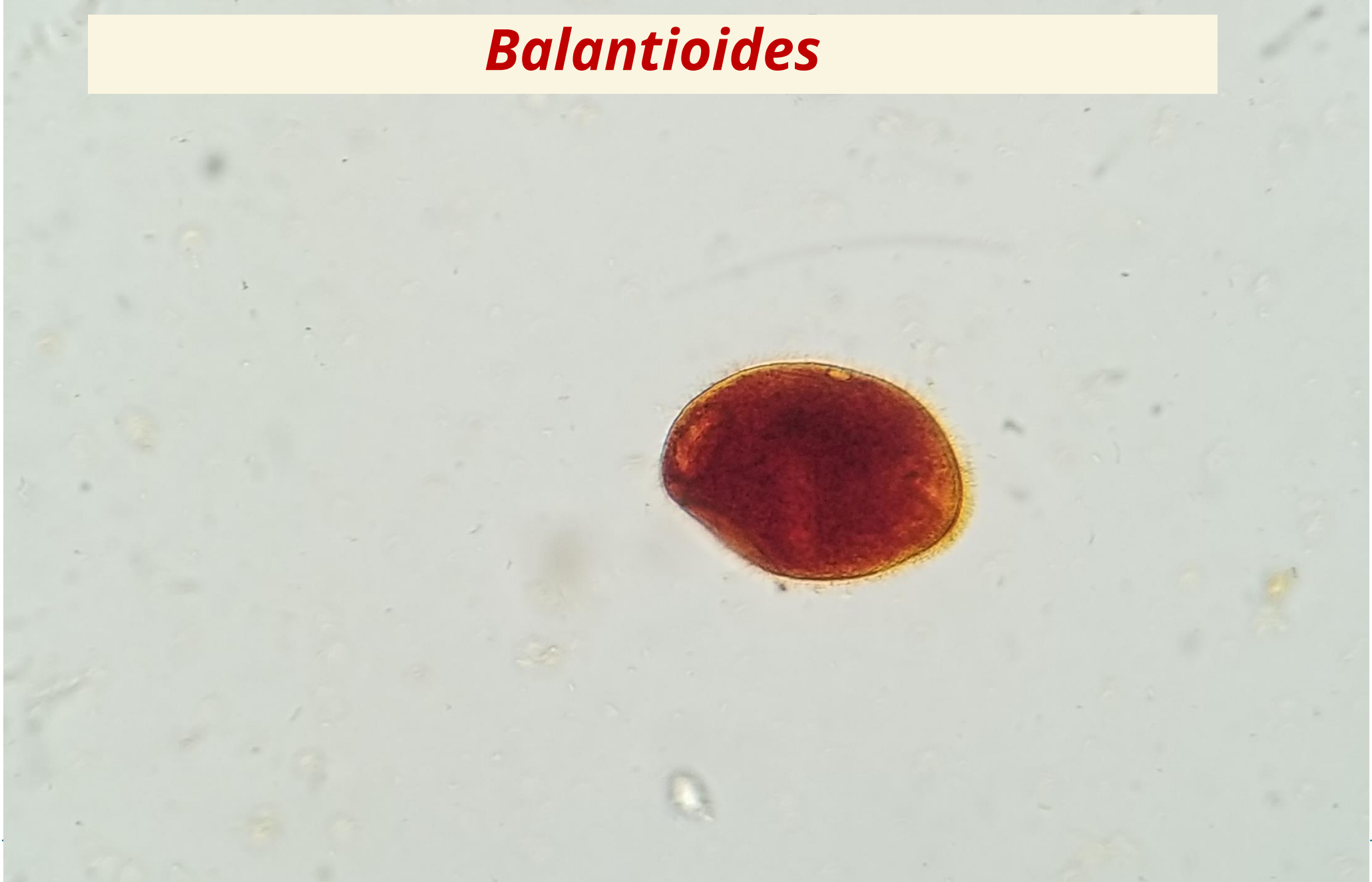
Strongyloides



Trichuris



Balantioides



Training & Validation Set

- **Fixatives:** Formalin, Alcorfix, TOTAL-FIX, EcoFix, SAF, Unifix
- **Origin:** ARUP, Mayo, CDC, Africa, Asia, Europe
- **Concentration method:** Vertical filtration, ethyl acetate sedimentation, gravity filtration

Training Set Data

Category (class)	No. of unique scans per class	No. of unique examples per class
Blastocystis species	131	1968
Entamoeba species non-hartmanni cysts	168	1401
Entamoeba species non-hartmanni trophozoites	116	1650
Endolimax nana cysts	92	1964
Iodamoeba buetschlii cysts	61	1474
Miscellaneous Small Protozoans ¹	182	3116
Giardia duodenalis cysts	83	3611
Giardia duodenalis trophozoites	51	1782
Chilomastix mesnili cysts	63	1438
Chilomastix mesnili trophozoites	28	1546
Balantioides coli cysts	133	466
Balantioides coli trophozoites	65	1398
Cyclospora species oocysts	23	1813
Cystoisospora belli oocysts	17	1296
Ascaris lumbricoides fertile eggs	134	983
Ascaris lumbricoides infertile eggs	183	894

Trichuris trichiura eggs	207	1037
Hookworm/Trichostrongylus eggs	421	1268
Strongyloides species L1 larvae	33	1215
Paracapillaria philippinensis eggs	201	1044
Enterobius vermicularis eggs	246	796
Fish Tapeworm eggs (Diphylobothrium-complex)	333	987
Taenia species eggs	97	1283
Rodentolepis nana eggs	58	1018
Hymenolepis diminuta eggs	224	1023
Schistosoma mansoni eggs	262	1088
Schistosoma japonicum eggs	719	989
Paragonimus species eggs	116	1044
Fasciola species/Fasciolopsis buski eggs	219	504
Clonorchis sinensis/Opisthorchis species eggs	28	1095
Background/Artifacts	1778	38339

Mathison et. al.
Accepted, JCM.

Accuracy: Initial

Target 10+ & 10 -

Mathison et. al.
Accepted, JCM.

Organism	Positive Accuracy	Negative Accuracy
<i>Dientamoeba fragilis</i>	9/10 (90%)	99/100 (99%)
<i>Giardia duodenalis</i>	10/10 (100%)	100/100 (100%)
<i>Endolimax nana</i>	10/10 (100%)	99/100 (99%)
<i>Cyclospora</i> species	10/10 (100%)	100/100 (100%)
<i>Entamoeba hartmanni</i>	10/10 (100%)	99/100 (99%)
<i>Blastocystis</i> species	15/15 (100%)	100/100 (100%)
<i>Chilomastix mesnili</i>	6/10 (60%)	99/100 (99%)
<i>Entamoeba</i> species	10/10 (100%)	99/100 (99%)
<i>Iodamoeba buetschlii</i>	10/10 (100%)	99/100 (100%)
<i>Cystoisospora belli</i>	10/10 (100%)	100/100 (100%)
<i>Balantiodides coli</i>	10/10 (100%)	100/100 (100%)
<i>Ascaris lumbricoides</i>	9/10 (90%)	100/100 (100%)
<i>Trichuris trichiura</i>	8/10 (80%)	100/100 (100%)
Hookworm/ <i>Trichostrongylus</i> sp.	10/10 (100%)	100/100 (100%)
Fish Tapeworm	9/10 (90%)	100/100 (100%)
<i>Taenia</i> species	10/10 (100%)	100/100 (100%)
<i>Enterobius vermicularis</i>	10/10 (100%)	100/100 (100%)
<i>Strongyloides</i> species	9/10 (100%)	100/100 (100%)
<i>Rodentolepis nana</i>	10/10 (100%)	100/100 (100%)
<i>Hymenolepis diminuta</i>	10/10 (100%)	100/100 (100%)
<i>Schistosoma mansoni</i>	10/10 (100%)	100/100 (100%)
<i>Paracapillaria philippinensis</i>	10/10 (100%)	100/100 (100%)
<i>Paragonimus</i> species	10/10 (100%)	100/100 (100%)
<i>Fasciola</i> sp./ <i>Fasciolopsis buski</i>	10/10 (100%)	100/100 (100%)
<i>Clonorchis</i> / <i>Opisthorchis</i> spp.	10/10 (100%)	100/100 (100%)
<i>Schistosoma japonicum</i>	5/10 (50%)	100/100 (100%)
Total Agreement	250/265 (94.3%)	94/100 (94%)

Accuracy: Resolved

66% increased diagnostic yield

Mathison et. al.
Accepted, JCM.

Organism	Positive	Negative
<i>Dientamoeba fragilis</i>	11/11 (100%)	102/103 (99%)
<i>Giardia duodenalis</i>	26/26 (100%)	103/104 (99%)
<i>Endolimax nana</i>	21/21 (100%)	102/103 (99%)
<i>Cyclospora</i> species	11/11 (100%)	103/104 (99%)
<i>Entamoeba hartmanni</i>	12/12 (100%)	102/103 (99%)
<i>Blastocystis</i> species	61/61 (100%)	103/110 (94%)
<i>Chilomastix mesnili</i>	13/16 (81%)	102/104 (98%)
<i>Entamoeba</i> species	49/49 (100%)	102/112 (91%)
<i>Iodamoeba buetschlii</i>	20/20 (100%)	102/104 (98%)
<i>Cystoisospora belli</i>	10/10 (100%)	103/103 (100%)
<i>Balantiodides coli</i>	13/13 (100%)	103/103 (100%)
<i>Ascaris lumbricoides</i>	17/18 (95%)	103/104 (99%)
<i>Trichuris trichiura</i>	30/30 (97%)	103/103 (100%)
Hookworm/ <i>Trichostrongylus</i> sp.	26/26 (100%)	103/103 (100%)
Fish Tapeworm	10/10 (100%)	103/103 (100%)
<i>Taenia</i> species	10/10 (100%)	103/103 (100%)
<i>Enterobius vermicularis</i>	11/11 (100%)	103/103 (100%)
<i>Strongyloides</i> species	17/18 (94%)	103/104 (99%)
<i>Rodentolepis nana</i>	13/13 (100%)	103/103 (100%)
<i>Hymenolepis diminuta</i>	10/10 (100%)	103/103 (100%)
<i>Schistosoma mansoni</i>	10/10 (100%)	103/103 (100%)
<i>Paracapillaria philippinensis</i>	10/10 (100%)	103/103 (100%)
<i>Paragonimus</i> species	10/10 (100%)	103/103 (100%)
<i>Fasciola</i> sp./ <i>Fasciolopsis buski</i>	10/10 (100%)	103/103 (100%)
<i>Clonorchis</i> / <i>Opisthorchis</i> spp.	10/10 (100%)	103/103 (100%)
<i>Schistosoma japonicum</i>	9/9 ² (100%)	103/103 (100%)
Misc. Small Protozoans ¹	22/22 (100%)	102/111 (92%)
Total Agreement	472/477 (98.9%)	Variable by target*

Relative Limit of Detection

Mathison et. al.
Accepted, JCM.

	<i>Entamoeba</i>					
	Series A [8 years exp.]		Series B [<1 year exp.]		Series C [3.5-4 years exp.]	
Dilution	Software	Technologist	Software	Technologist	Software	Technologist
Neat	Detected (n=185)	Detected	Detected (n=261)	Detected	Detected (n=141)	Detected
1:1	Detected (n=99)	Detected (n=2)	Detected (n=101)	Detected	Detected (n=120)	Detected
1:2	Detected (n=75)	Detected (rare)	Detected (n=70)	Detected (rare)	Detected (n=44)	Detected
1:4	Detected (n=25)	Detected (rare)	Detected (n=40)	Detected	Detected (n=26/27)	Detected
1:8	Detected (n=10)	Detected (n=2)	Detected (n=6)	Not Detected	Detected (n=16)	Detected
1:16	Detected (n=2/3)	Not Detected	Detected (n=3)	Not Detected	Detected (n=2)	Not Detected
1:32	Detected (n=2)	Not Detected	Detected (n=2)	Not Detected	Detected (n=1)	Detected (suspicious)
1:64	Detected (n=1)	Not Detected	Detected (n=1/2)	Not Detected	Detected (n=3)	Not Detected
1:128	Not Detected	Not Detected	Not Detected	Detected (possible, rare)	Not Detected	Not Detected
1:256	Not Detected	Not Detected	Detected (n=1?)	Not Detected	Not Detected	Not Detected
1:512	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected

Relative Limit of Detection

Mathison et. al.
Accepted, JCM.

	<i>Trichuris trichiura</i>					
	Series A [8 years exp.]		Series B [<1 year exp.]		Series C [3.5-4 years exp.]	
Dilution	Software	Technologist	Software	Technologist	Software	Technologist
Neat	Detected (n=49)	Detected	Detected (n=49)	Detected	Detected (n=45)	Detected
1:1	Detected (n=12)	Detected	Detected (n=15)	Detected	Detected (n=22)	Detected
1:2	Detected (n=10)	Detected	Detected (n=16)	Detected	Detected (n=11)	Detected
1:4	Detected (n=1)	Not Detected	Detected (n=5)	Detected (rare)	Detected (n=9)	Detected
1:8	Detected (n=1)*	Not Detected	Detected (n=1)*	Not Detected	Detected (n=1)	Not Detected
1:16	Not Detected	Not Detected	Detected (n=1)	Not Detected	Not Detected	Not Detected
1:32	Not Detected	Not Detected	Detected (n=1)	Not Detected	Not Detected	Not Detected
1:64	Detected (n=1)	Not Detected	Not Detected	Detected (rare)**	Not Detected	Not Detected
1:128	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
1:256	Detected (n=1)	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
1:512	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected

Relative Limit of Detection

Mathison et. al.
Accepted, JCM.

	<i>Ascaris lumbricoides</i>					
	Series A [8 years exp.]		Series B [<1 year exp.]		Series C [3.5-4 years exp.]	
Dilution	Software	Technologist	Software	Technologist	Software	Technologist
Neat	Detected (n=23)	Detected	Detected (n=23)	Detected	Detected (n=23)	Not Detected
1:1	Detected (n=3)	Detected	Detected (n=7)	Detected	Detected (n=12)	Detected
1:2	Detected (n=5)	Detected	Detected (n=1/2)	Detected	Detected (n=5)	Detected
1:4	Detected (n=1)	Not Detected	Detected (n=2)	Detected	Detected (n=1)	Not Detected
1:8	Detected (n=2)	Not Detected	Detected (n=1)	Detected (rare)	Not Detected	Not Detected
1:16	Detected (n=1)	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
1:32	Not Detected	Detected (n=1)*	Not Detected	Not Detected	Not Detected	Not Detected
1:64	Not Detected	Not Detected	Not Detected	Detected (possible, rare)*	Not Detected	Not Detected
1:128	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
1:256	Not Detected	Not Detected	Detected (n=1)	Not Detected	Not Detected	Not Detected
1:512	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected

Relative Limit of Detection

Mathison et. al.
Accepted, JCM.

	Hookworm					
	Series A [8 years exp.]		Series B [<1 year exp.]		Series C [3.5-4 years exp.]	
Dilution	Software	Technologist	Software	Technologist	Software	Technologist
Neat	Detected (n=2)	Not Detected	Detected (n=2)	Not Detected	Detected (n=4)	Not Detected
1:1	Detected (n=2)	Detected (n=1)	Detected (n=2)	Detected (rare)	Detected (n=1)	Not Detected
1:2	Not Detected	Not Detected	Not Detected	Not Detected	Detected (n=1)	Not Detected
1:4	Not Detected	Not Detected	Not Detected	Not Detected	Detected (n=1)	Not Detected
1:8	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
1:16	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
1:32	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
1:64	Not Detected	Not Detected	Not Detected	Not Detected	Detected (n=1)	Detected
1:128	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
1:256	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
1:512	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected

User Look at Wet-mount Development/Validation

https://app.techcyte.com/worklist?sort=-created_at&status=processing_complete&assigned_to=-%2Cme



Content credentials Generated with AI · November 21, 2023 at 8:13 AM

Wet-Mount Conclusions

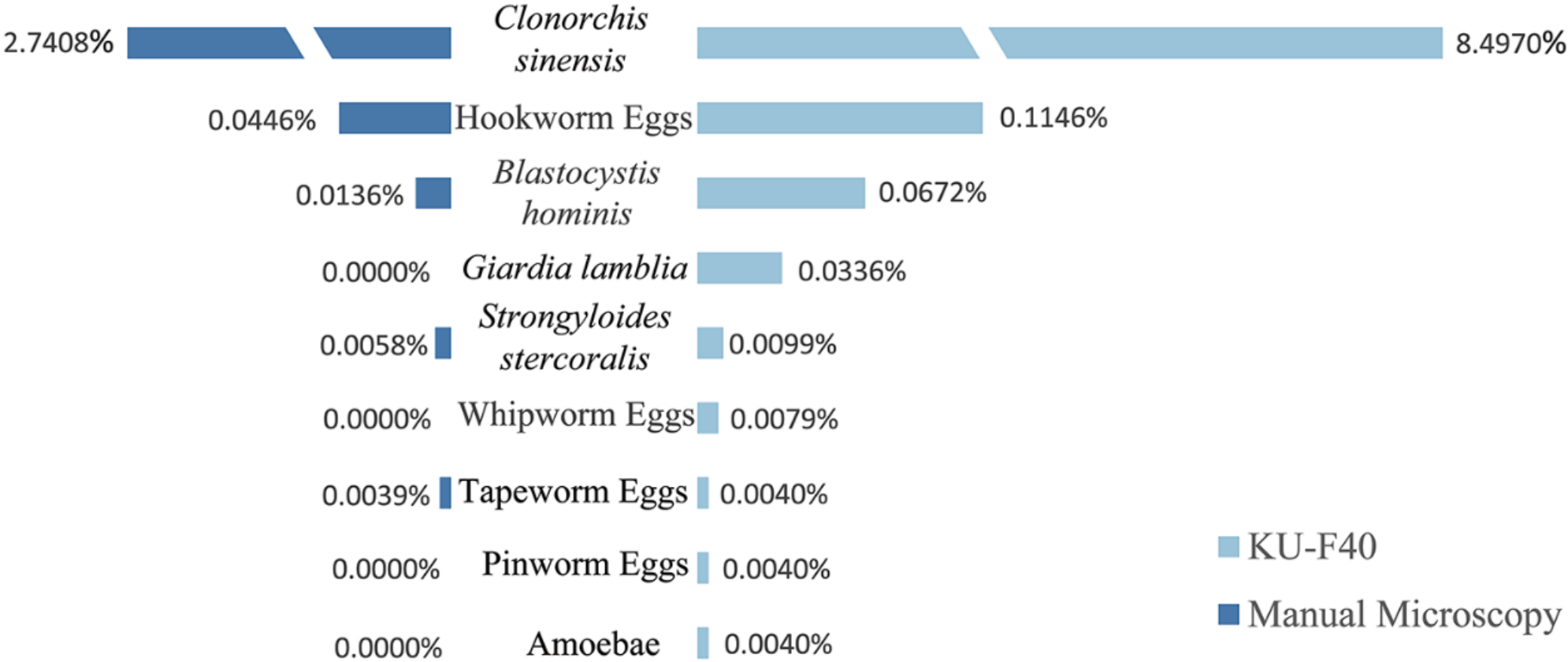
- Excellent screening tool for positive and negative slides
- Large diversified training and validation set
- Excellent performance for all pathogens in model
- Increased diagnostic yield vs manual O&P (66% ↑)
- Increased analytic sensitivity vs humans
- Less scope time

KU-F40 Fully Automated Feces Analyzer

- Wet-mount only
- High throughput
- Multiple focal depth imaging
- Flotation and sedimentation
 - +/- iodine
- Currently O-US



KU-F40 System retrospective study

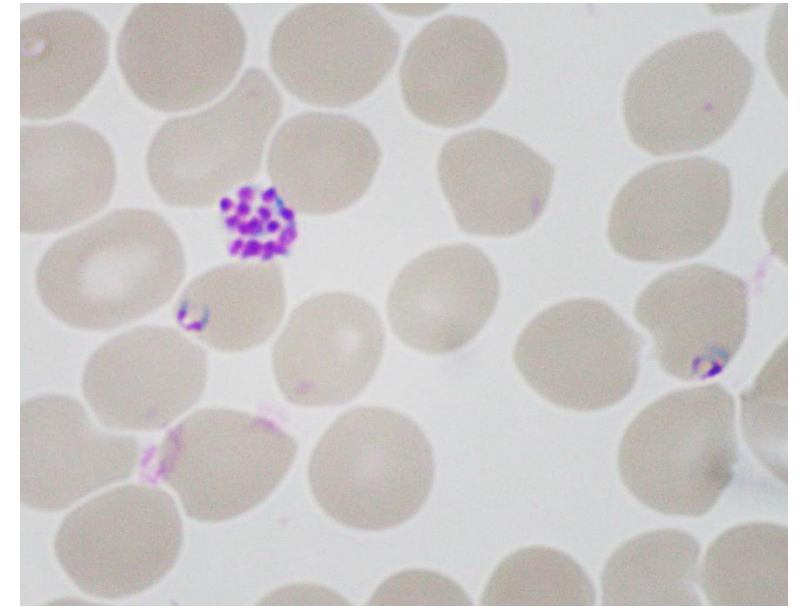


Tornado diagram for parasite (egg) species detection levels by manual microscopy and KU-F40. Note: Due to the significant difference in detection levels between *Clonorchis sinensis* eggs and other parasites (eggs), an open section is used to represent the omitted portion of the actual detection level

Huang et al. Nature Scientific Reports 15, 2025

Blood smears

- Giemsa stains for malaria
- **Early findings:** Imperfect sensitivity, high specificity (for *Plasmodium* sp.)
- **Challenges**
 - Species-level ID for 4 species is poor
 - Some systems just do *P. falciparum* and *P. vivax*
 - Does not detect other blood parasites
- **Commercial example(s)** – EasyScan GO, miLab MAL



A.I. Here and Now in Microbiology

- Culture plate interpretation – colony predictions
- Gram stain interpretation
- AFB stains
- AST interpretation
- Pap smear interpretation
- Nugent score vaginal Gram stains

Acknowledgments



R&D

- **Blaine Mathison**
- **Katie Knight**
- **Jessica Kohan**
- Jonia Cook
- Kim Frizzell
- Dr. Adam Barker

TechCyte

- **John Walker**
- Ken Dixon
- Jill Potts
- Fallon Markow
- Kellie Hicken
- Ben Cahoon
- Ben Martin
- Rick Smith
- Rob Fotheringham

ARUP Parasitology Lab

- Madison Sant
- Alicia Backman
- Cole Neider
- Garrin Abbott
- Ryan Jensen
- Everyone else who embraced it

Thank You



Questions?

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