Case Study in Implementation Research: LED Microscopy

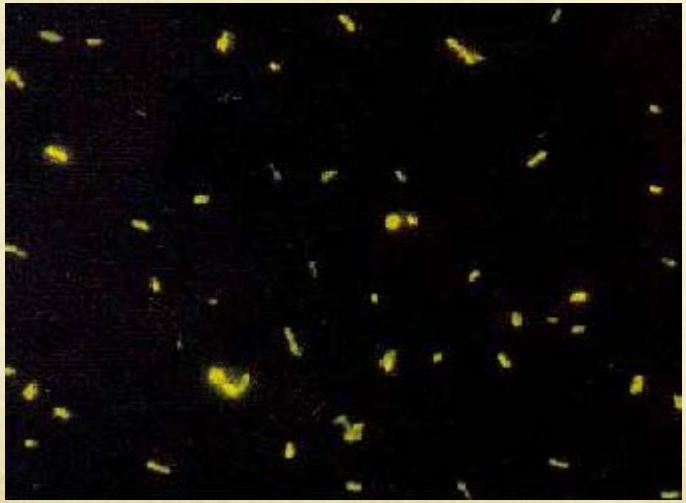
Advanced TB Diagnostic Research Course
Montreal, QC, Canada
Jessica Minion, MD MSc FRCPC
July 11, 2012
jessicaminion@gmail.com

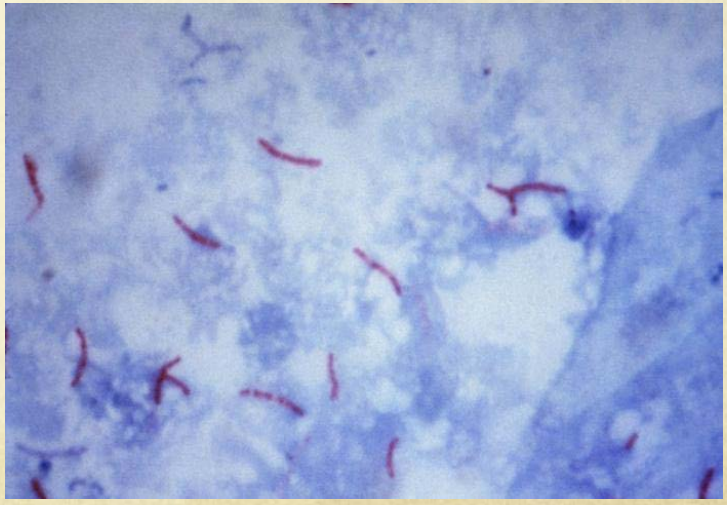
Outline

- Introduction: Why we were doing this?
- Methods: How did we do it?
- Results: What did we find?
- Discussion: What did we learn?

Smear Microscopy

- Cheap, quick, relatively simple
- Poor Sensitivity
  - Less in HIV, extrapulmonary, pediatrics





LED Fluorescent Microscopy

- Fluorescence → 10% higher sensitivity & 46% less time to read smears
- LED Fluorescence
  - Equipment less expensive, more durable, less maintenance
  - No hazardous bulbs, half life 10+ years
  - Does not require a dark room

Table 1. Comparison of commercial light-emitting diode products currently available for TB diagnostics.

Device	Manufacturer	Standalone microscope	Attachment	Light transmission	Battery powered	Weight (kg)	Cost (US $)
Primo Star iLED 	Carl Zeiss, Oberkochen, Germany	Yes	NA	Epifluorescent	Yes	9.5	4825*
Lumin™ 	LW Scientific, Lawrenceville, GA, USA	No	Objective lens replacement (20, 40, 60 and 100× oil)	Epifluorescent	Yes	0.448	700–2000*
ParaLens 	QBC™ Diagnostics, Philipsburg, PA, USA	No	Objective lens replacement (40, 60 and 100× oil)	Epifluorescent	Yes	1.27	995[§]
FluoLED 	Fraen Corporation Srl, Settimo Milanese, Italy	No	Adaptor attached to base and filter installed on head of microscope	Transfluorescent	Yes	5	1977–3530[¶]
CyScope® 	Partec, Gorlitz, Germany,	Yes	NA	Epifluorescent	Yes	2.7	2372–3699*

Minion et al. *Exp Rev Med Dev* 2009

WHO Recommendation

2009

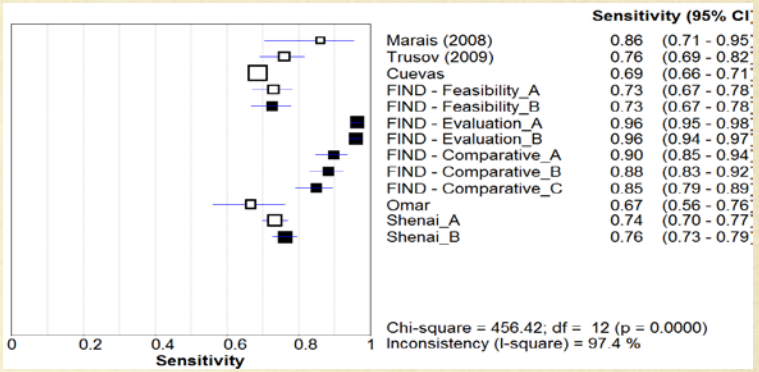
FINAL RECOMMENDATION

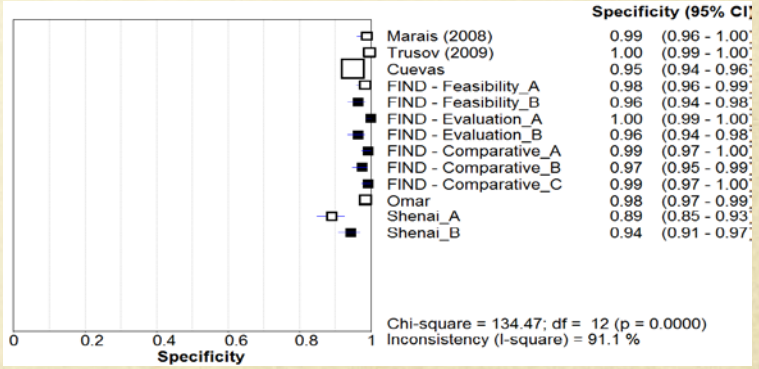
The Expert Group felt that there was sufficient generalisable evidence that LED microscopy

- should replace conventional fluorescence microscopy;
- is a better alternative to ZN light microscopy in both high and low volume laboratories;

OVERALL QUALITY OF EVIDENCE	**MODERATE**
STRENGTH OF RECOMMENDATION	**STRONG**

www.who.int/tb/dots/laboratory/egmreport_microscopymethods_nov09.pdf





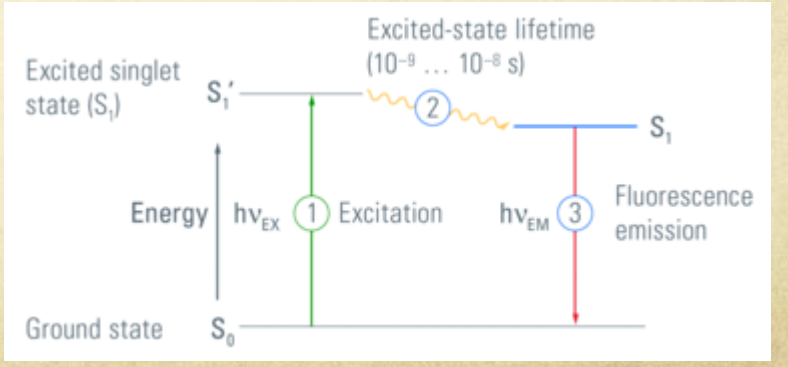
Minion, et al. in WHO Expert Group Meeting, Geneva. 2009

LED head-to-head evaluation

- How comparable is the first reading vs. the second reading?

Fluorochrome-based Stains

- Fade over time
  - "photobleaching"
  - Molecule has limited number of photons available for emission during its chemical lifespan
- Rate of fading is unique to particular fluorochrome used
- Known to be affected by
  - Exposure to light
  - Oxygen
  - Temperature
  - pH



Effect on Implementation?

- External Quality Assurance program
  - Reliant on "re-reading" select number of ZN stained smears
  - Slides stored and sent to reference laboratory ~quarterly

- CAN THIS STILL BE DONE WITH FLUORESCENT STAINS??

Previous Literature

- Nada
- Seriously??
- Ok, well, maybe…
- Van Deun et al (1999) looked at fading of Ziehl-Neelsen stained slides
  - Fading was significant (over period of months)
  - Fading enhanced by light, heat, humidity
  - Concentrated smears more effected (thinner consistency?)

JOURNAL OF CLINICAL MICROBIOLOGY, May 2011, p. 2024–2026 Vol. 49, No. 5
0095-1137/11/$12.00 doi:10.1128/JCM.00507-11

Fading of Auramine-Stained Mycobacterial Smears and Implications for External Quality Assurance[▽]

Jessica Minion,[1,2,3] Shubhada Shenai,[4] Viral Vadwai,[4] Tejashree Tipnis,[4] Christina Greenaway,[5] Dick Menzies,[1,2] Andrew Ramsay,[6] Camilla Rodrigues,[4] and Madhukar Pai[1,2]*

Department of Epidemiology, Biostatistics, and Occupational Health, McGill University, Montreal, Canada[1]; Respiratory Epidemiology and Clinical Research Unit, Montreal, Canada[2]; Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Canada[3]; P. D. Hinduja Hospital and Medical Research Centre, Mumbai, India[4]; Department of Laboratory Medicine, Division of Infectious Diseases, SMBD—Jewish General Hospital, McGill University, Montreal, Canada[5]; and UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, World Health Organization, Geneva, Switzerland[6]

Study Objectives

- How quickly do auramine-stained smears fade?
- What is the effect of storage conditions on the rate of fading?
- What is the effect of repeated readings on the rate of fading?

Study Plan (in brief)

- Hinduja National Hospital and Medical Research Centre, Mumbai, India
  - High volume, CAP accredited
- All specimens submitted for TB had an extra slide prepared and stained with auramine-rhodamine
  - As a part of a concurrent LED microscopy evaluation
- Unblinded researcher selected "batches" of 30 slides per week for storage and re-reading
  - All smear reading performed in duplicate, by same microscopists blinded to previous results, during routine daily work
  - Re-reading results compared to "reference standard" of the initial reading at Day 0. Re-labeling/re-assortment to maintain blinding
  - Only slides with 2 identical initial readings included

Storage Conditions

- Room Temperature, 22° C
  - Dark (closed smear boxes)
  - Light (open smear boxes)
- Incubator, 30° C + humidity
- Refrigerator, 4° C

Reading Schedules

- Weekly
- Monthly
- Quarterly (q3 months)

Sample Size

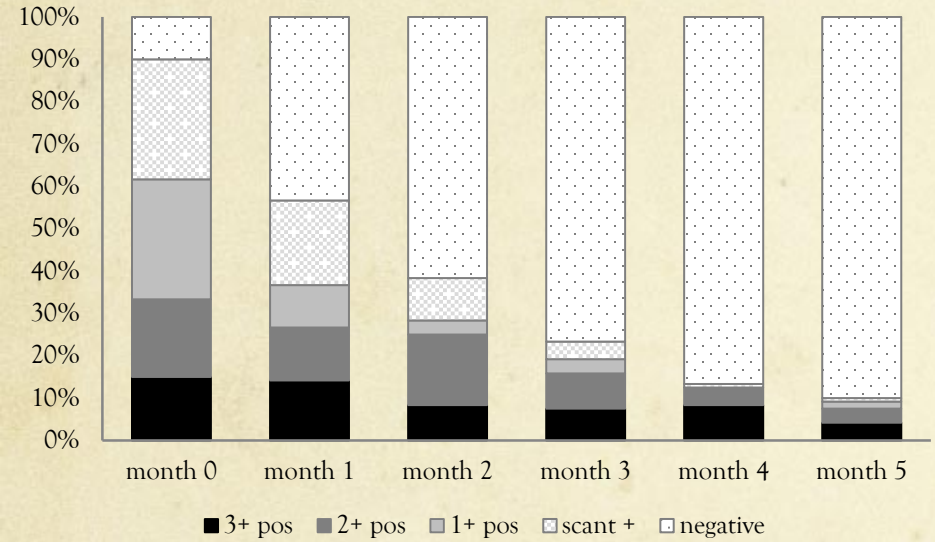
- One-sided hypothesis of equivalence
- 100% slides known positive, 80% power to detect 10% drop in “positivity” with 0.05 significance
- Batches needed to be 25 slides
- Selected batches of 27 positive slides, with 3 negative slides per batch for blinding

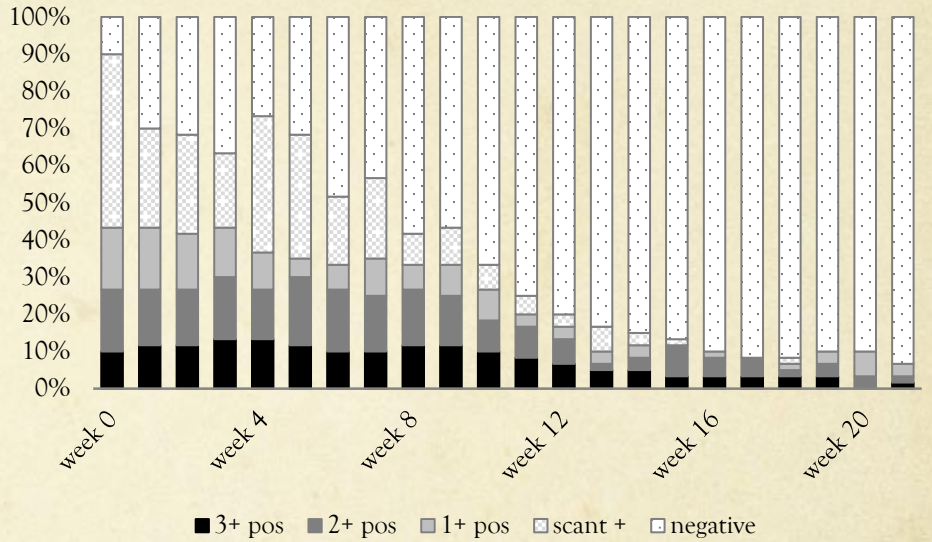
Distribution of Included Slides

Slides Read Weekly	# Slides
Room Temperature (dark)	30
Incubator (humidified, dark)	30
Refrigerator (dark)	30
Open weekly (light)	30
	Total = 120
Slides Read Monthly	
Room Temperature (dark)	60
Incubator (humidified, dark)	60
Refrigerator (dark)	60
	Total = 180
Slides Read Once at 3 Months	
Room Temperature (dark)	30
	Total = 30
TOTAL OVERALL	**330**

- Enriched for low-positive smears:
  - 25% scanty, 25% 1+, 25% 2+, 15% 3+, 10% negative (blinding)
- 50% direct, 50% concentrated

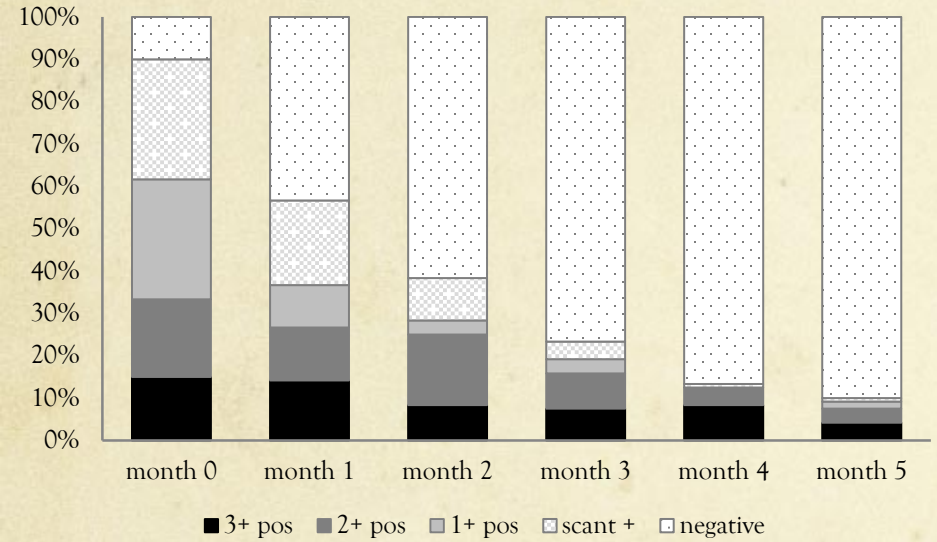
How quickly do slides fade?

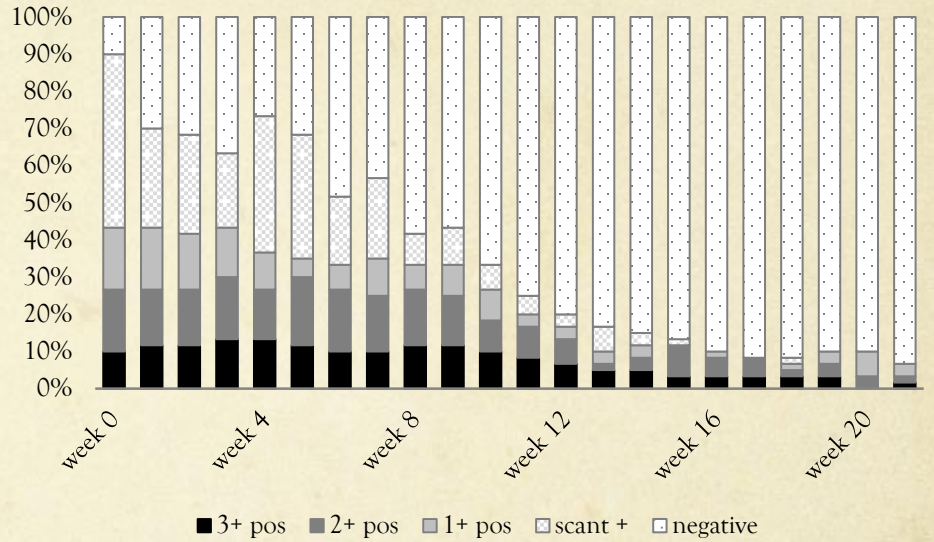




- When considering the slides originally read as positive (combined scant, 1+, 2+, 3+), the proportion of slides continuing to be read as positive was significantly lower for ALL storage environments by the FIRST re-reading regardless of the reading schedule

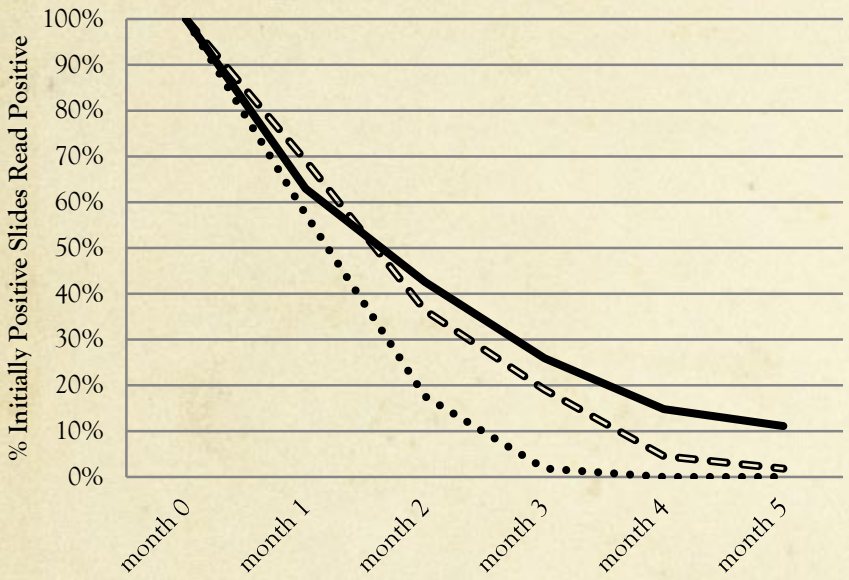
How quickly do slides fade?

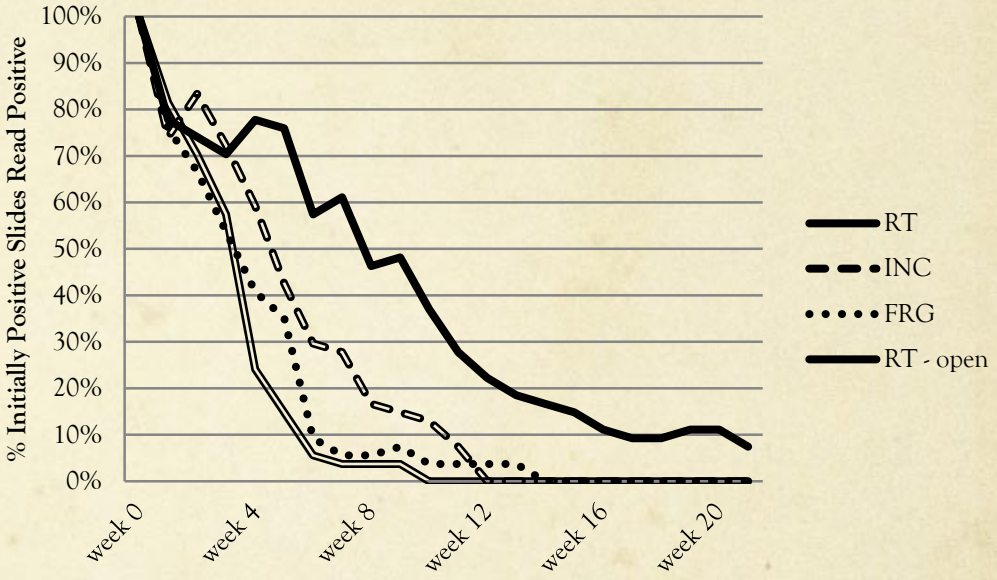




- e.g. – slides kept at room temperature (in the dark) read after 1 week → only 21 out of 27 originally positive slides remained positive (p=0.03)

What is the effect of storage environment?





- Proportion of originally positive slides read as positive dropped to <50% by 2 months (or 8 weeks) for all storage environments and all reading schedules
- Refrigeration did not protect against fading

What effect did the frequency of reading have?

Time	Proportion +*			
	Weekly (n=27)	Monthly (n=54)	Quarterly (n=27)	P-value†
Time=0	100%	100%	100%	
1 month	78%	63%		0.21
2 months	46%	43%		0.64
3 months	22%	26%	35%	0.79[a], 0.54[b], 0.6[c]
4 months	11%	15%		0.74
5 months	11%	11%		1.0

- Data displayed for slides stored at room temperature (dark environment)
- Also no difference between direct and concentrated smears

Did the AFB quantitation matter?

	Original Reading (n=# slides)			
	3+ n=53	2+ n=71	1+ n=78	scant n=69
T=0	100%	100%	100%	100%
1 month	83.0%	63.6%	45.2%	42.1%
2 month	61.2%	27.0%	13.8%	14.1%
3 month	35.7%	10.7%	6.0%	1.7%
4 month	16.9%	2.8%	1.2%	2.6%
5 month	14.1%	2.3%	1.2%	0.0%
Linear Regression Slope†:	-0.191 (-0.212, -0.169)	-0.241 (-0.303, -0.180)	-0.254 (-0.342, -0.166)	-0.257 (-0.349, -0.165)

- Slides with higher bacillary burden (2+ or 3+) remained positive longer
- Nevertheless, <35% of 3+ slides remained positive at 3 months

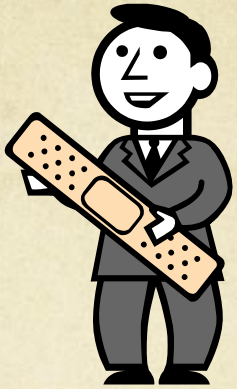
Conclusions

- Significant fading occurs quickly
- None of the storage environments evaluated would allow reliable re-checking of smears for EQA
- No significant effect of reading frequency found
  - Although non-statistically significant trend
- No significant difference between direct and concentrated smears
- Low positivity slides faded more quickly, but fading evident even for high positive slides

Implications

- Scale-up of LED fluorescent microscopy recommended by WHO (2010)
- Current EQA program appears infeasible for use with fluorescent auramine-stained smears
- Alternative EQA strategies will need to be developed and evaluated in order to maintain the laboratory QA needs for provision of smear microscopy diagnostics using fluorescent microscopy

Other solutions



- Are there staining or storage strategies that could extend fluorescence?
  - Significant potentiation needed…
- Restaining smears prior to rechecking
  - False positives introduced? (contaminating AFB)
  - False negatives introduced? (rinsing off scant AFB)
  - Increased work and costs for reference labs
  - Complaints that restained slides are more "junky" and harder to read
- Panel-based EQA
  - More suited towards ensuring competence in low incidence settings (i.e. can they recognize AFB?)
  - Less useful to assess pragmatic practice in busy high incidence settings (i.e. are they spending enough time per slide to find AFB?)

Residual Questions

- We enriched our slide population with scant/1+ smears (anticipating the need to detect less significant fading)
  - Effect magnified compared to usual
  - Reading less reliable, regression to mean

- Refrigerated slides faded quicker than those kept at room temperature or in a humidified incubator
  - Condensation? Efficiency of fluorochrome?

Residual Questions

- Effect of storage duration likely overwhelmed our ability to detect effects from multiple readings

- Slide reading was performed as part of "routine" work to maximize blinding
  - Readers taking less time to examine that would be done in an EQA program?

- Starting point → underestimated rate of fading
  - Still don't really know what is the upper limit of reliable re-reading. Days? Hours?


That's all Folks!