

# Comparison of stained versus unstained simulated *Mycobacterium tuberculosis* microscopy smears for proficiency testing

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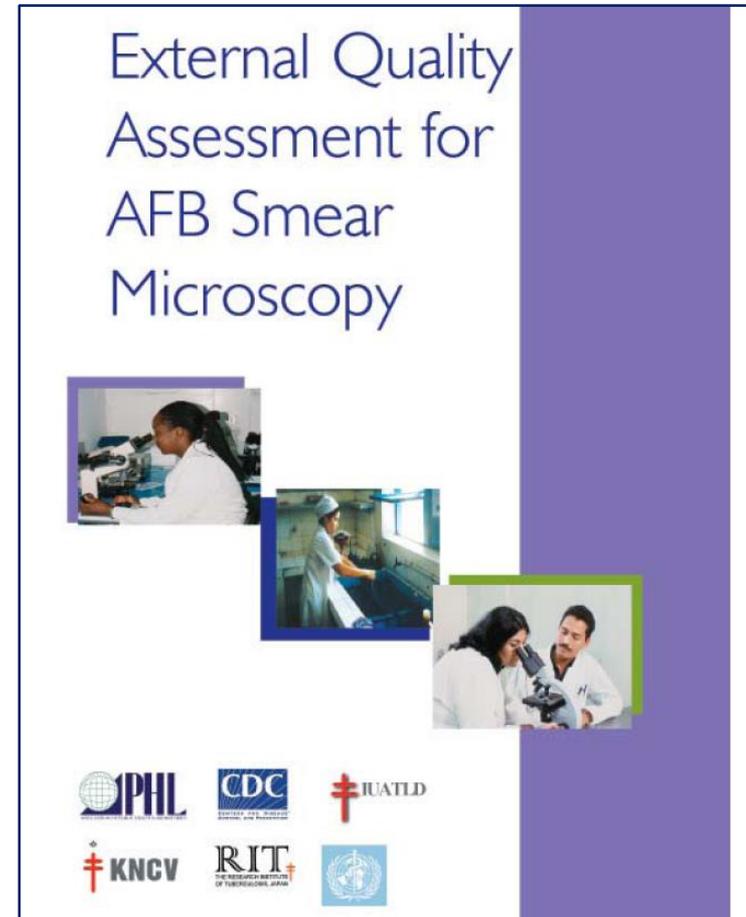
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# Background

- *Mycobacterium tuberculosis* (TB) smear microscopy is extensively used in most laboratories despite advances in molecular diagnosis.
- The TB Microscopy Proficiency Testing Scheme (PTS) is provided by the Microbiology External Quality Assessment Reference Laboratory (MEQARL), at the Centre for Opportunistic, Tropical and Hospital Infections (COTHI) at the National Institute Communicable Diseases (NICD), a division of the National Health Laboratory Service (NHLS).

# Background

- MEQARL prepare and quality assure smears for the TB microscopy PTS.
- The method used has been adapted from the WHO publication: “External Quality Assessment for AFB smear microscopy” published by the Association of Public Health Laboratories in September 2002.
- The PTS is conducted every four months.
- Each survey consists of 10 simulated smears.
- Results are monitored continuously to indicate timely interventions when problems are identified.



[http://www.aphl.org/AboutAPHL/publications/Documents/External\\_Quality\\_Assessment\\_for\\_AFB\\_Smear\\_Microscopy.pdf](http://www.aphl.org/AboutAPHL/publications/Documents/External_Quality_Assessment_for_AFB_Smear_Microscopy.pdf)

# Objectives

- To analyse trends in TB microscopy performance from 2012-3 to 2013-3
- To evaluate change in the TB microscopy programme with unstained smears 2014-1 to 2014-2

# Methods

- Participants are required to use the International Union Against Tuberculosis and Lung Disease grading system for reporting of smears.

Carbol-fuchsin stain under oil (1000x)	Report
- No AFB -	No acid-fast bacilli observed/ negative
1 to 9 AFB/100 fields	record exact number (scanty)
10 to 99 AFB/100 fields	1+
1 to 10 AFB/field	2+
>10 AFB/field	3+

<http://www.sahealthinfo.co.za/tb/microrecording.htm>

# Methods

## Classification of errors

Result obtained by the participating laboratory	Results as deemed by Proficiency Testing Scheme Provider				
	Negative	Scanty	1+	2+	3+
Negative	Correct	LFN	HFN	HFN	HFN
Scanty	LFP	Correct	Correct	QE	QE
1+	HFP	Correct	Correct	Correct	QE
2+	HFP	QE	Correct	Correct	Correct
3+	HFP	QE	QE	Correct	Correct

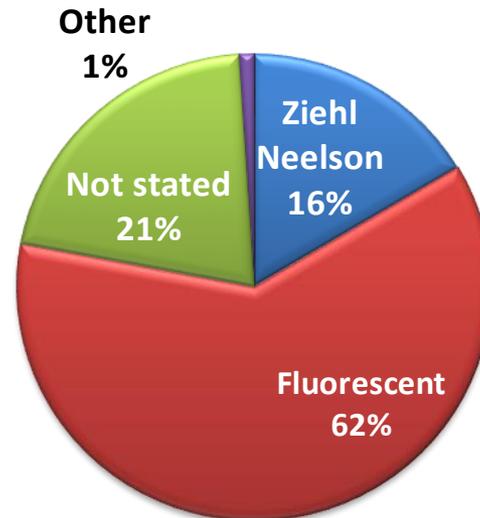
# Methods

## Interpretation and Scoring

Interpretation		Error type	Performances	Score
Correct	Correct response	No error	Acceptable	10 points
QE	Quantification error	Minor error	Acceptable	5 points
LFN	Low false negative	Minor error	Acceptable	5 points
LFP	Low false positive	Minor error	Acceptable	5 points
HFN	High false negative	Major error	Unacceptable	0 points
HFP	High false positive	Major error	Unacceptable	0 points

# Methods

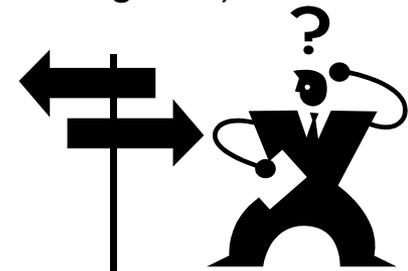
- Five batches of different quantifications (scanty, 1+, 2+, 3+, negative)
- Each batch is equally split into stained and unstained smears.
  - The stained smears were stained Ziehl Neelson by MEQARL using an automated staining machine.
  - Participants are requested to stain the unstained smears using the method they would routinely use in their laboratory (ZN/ fluorescence).



- The results for each batch should be comparable for stained smears and unstained smears.
- A comparison of stained and unstained smears was performed on 4 successive surveys from 2012-3 to 2013-3.

# Methods

- It was identified that there was better performance with the unstained smears than the stained smears.
- It was agreed at the EQA Advisory Committee meetings held at the beginning of the year (2014) that smears will be sent to the participants unstained.
  - MEQARL was unable to guarantee homogeneity and quality of the stained product produced.
- Results of the two survey where smears were sent unstained have been analysed.
  - Five batches of different quantifications (scanty, 1+, 2+, 3+, negative)
  - Each batch is equally split (unstained vs unstained)
- The last survey of 2014 has yet to be analysed.



# Methods



Why do the unstained smears reflect better performance?

- How many laboratory personnel examine at the smears and report on them?
  - Is there one person for stained and one person for unstained?
  - Does the laboratory look at them as a collective?
- Staining method used?
  - Ziehl Neelson
  - Fluorescence (conversion factor)
  - Does the type of staining method used make a difference in the results obtained?
- Are they stained in the labs as “special” samples or stained with a routine batch of TB smears?

# Methods



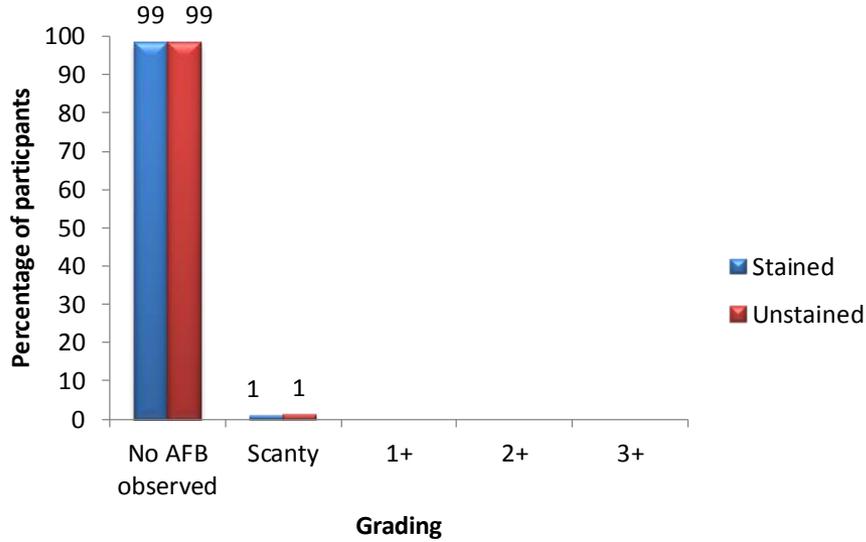
- Internal Quality Control (IQC)
  - Staff competence
  - Expired reagents
  - When is the QC performed on the smears?
  - Where is the QC performed?
  - Why perform internal QC?
- How are the smears stained during the QC process?
- Ziehl Neelson: **Carbol fuchsin stain**
  - Difficult to purchase
  - Short shelf-life
  - Adjust intensity depending on the smear
  - Fading – direct sunlight, high humidity and high temperatures
    - Factors: consistency of the smear, AFB clumping, staining quality



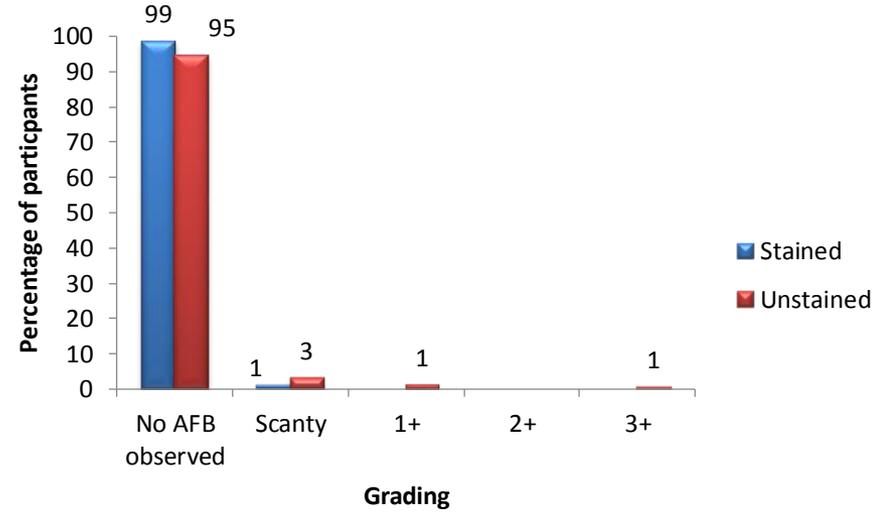
# Expected result: No AFB observed (2012-3 to 2013-3)

Before

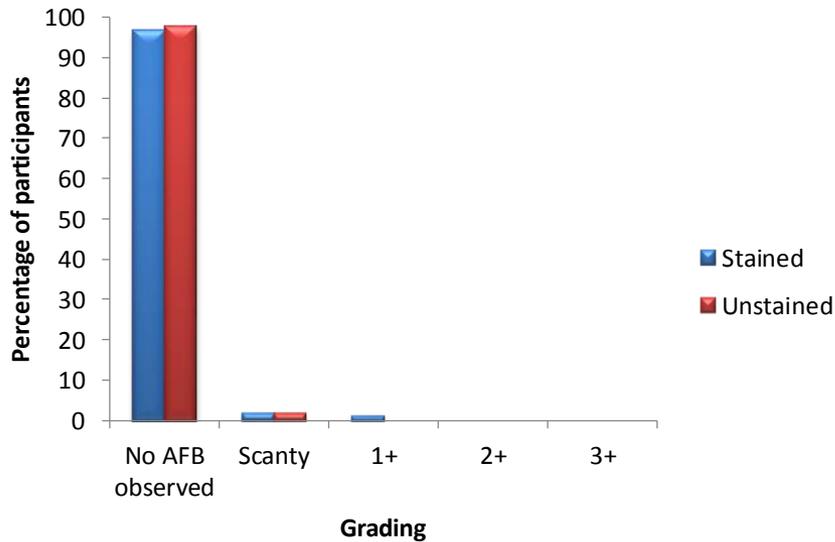
## Survey 2012-3



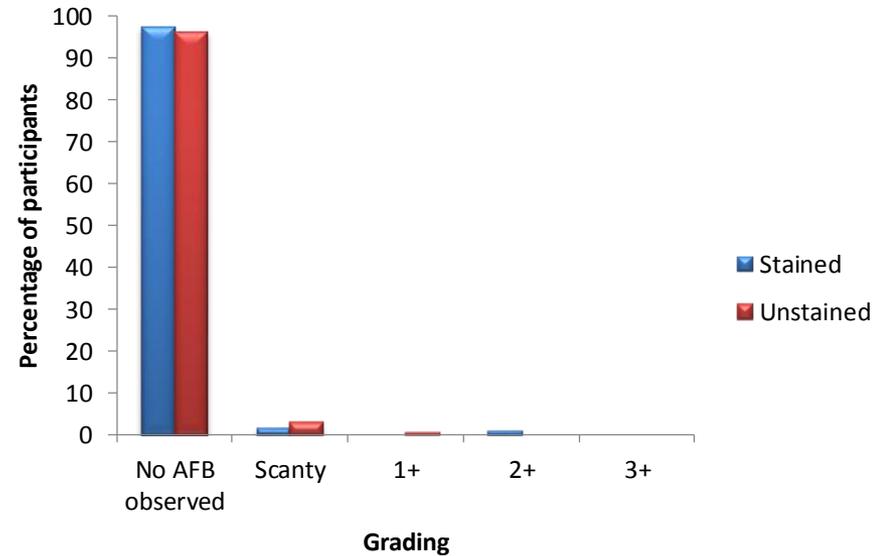
## Survey 2013-1



## Survey 2013-2



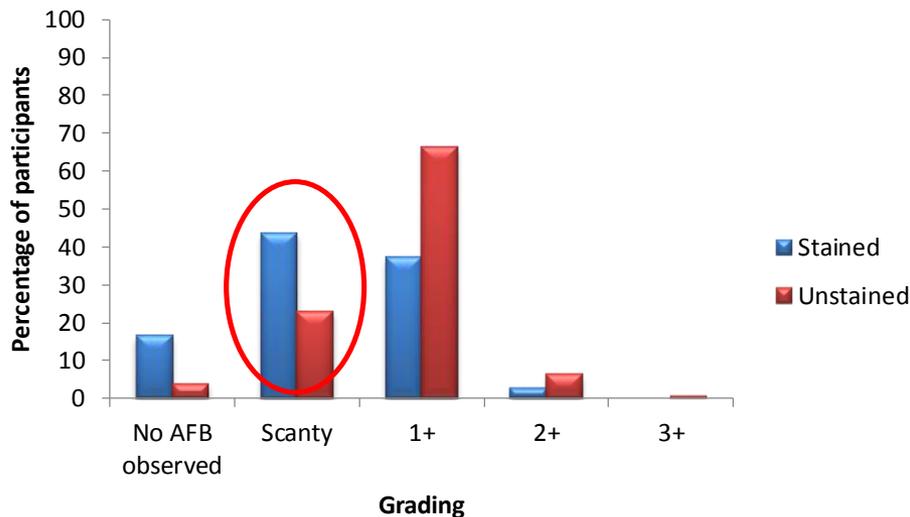
## Survey 2013-3



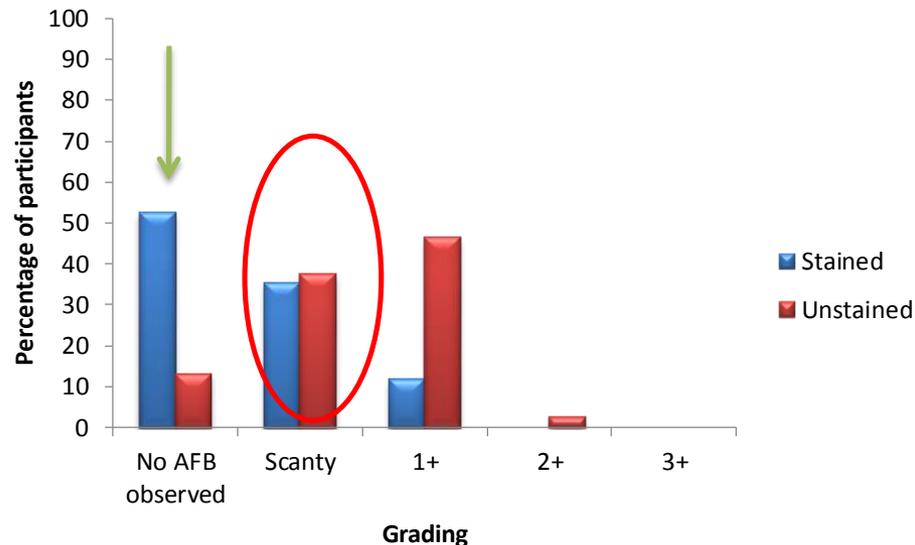
**Expected result: Scanty (2012-3 to 2013-3)**

**Before**

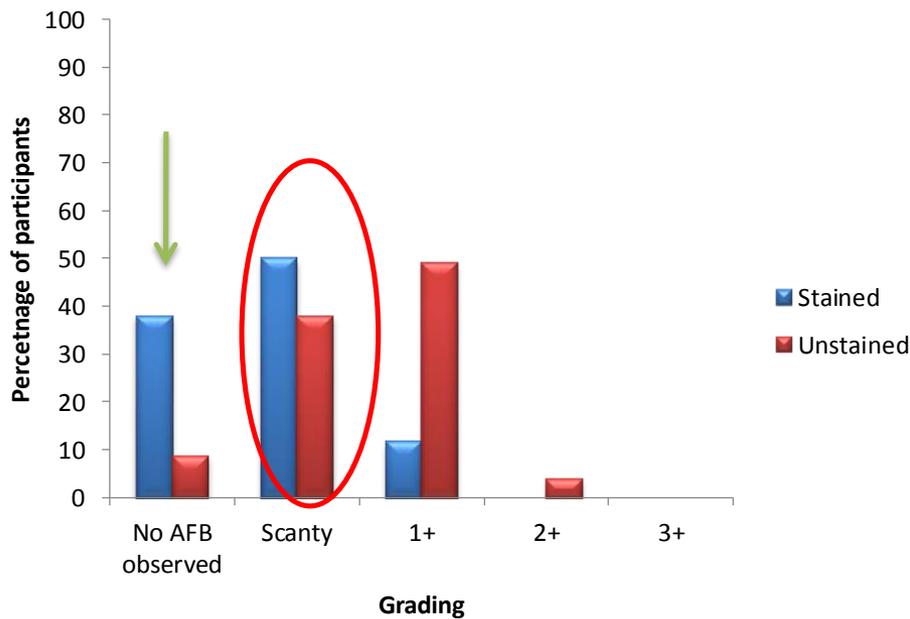
**Survey 2012-3**



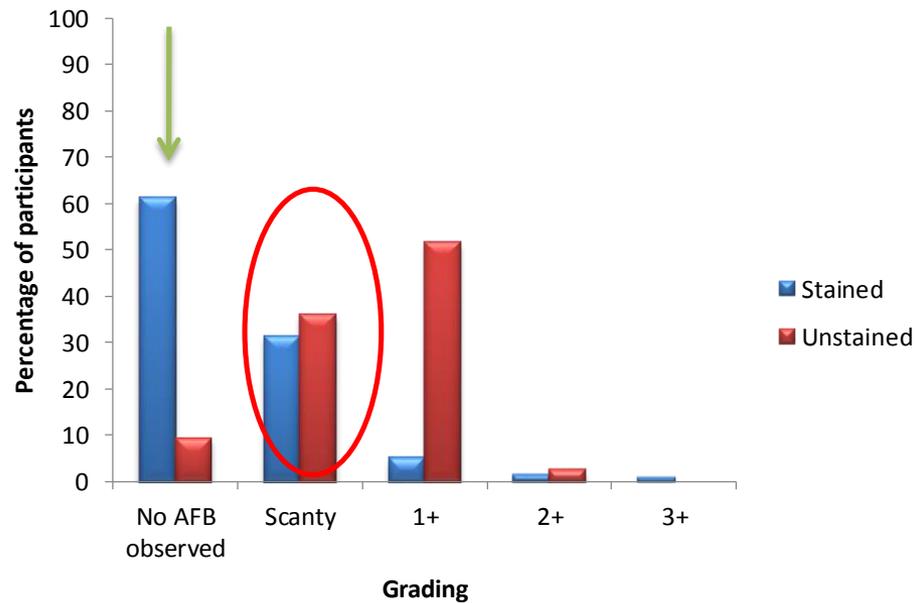
**Survey 2013-1**



**Survey 2013-2**



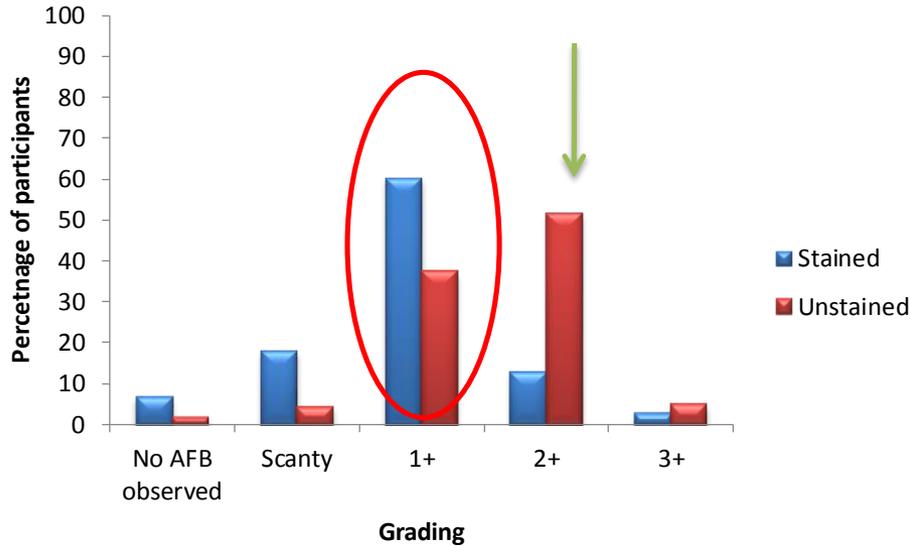
**Survey 2013-3**



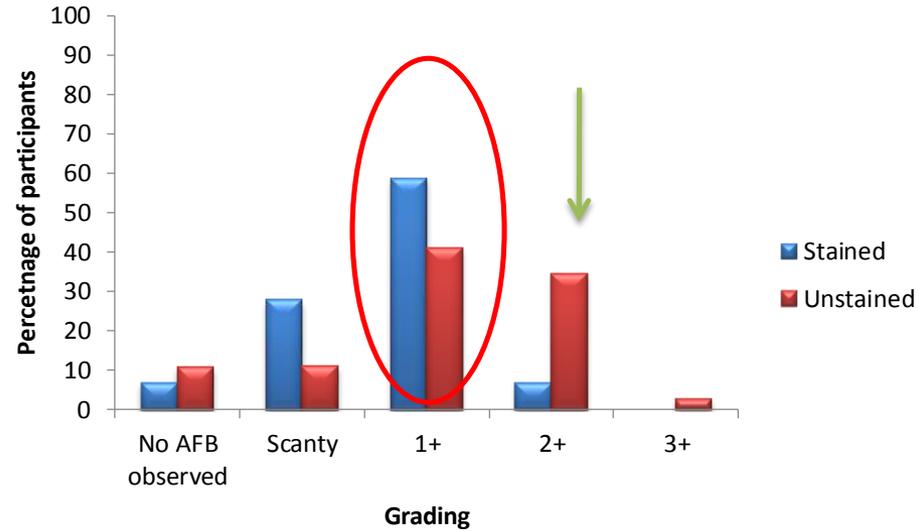
Expected result: 1+ (2012-3 to 2013-3)

Before

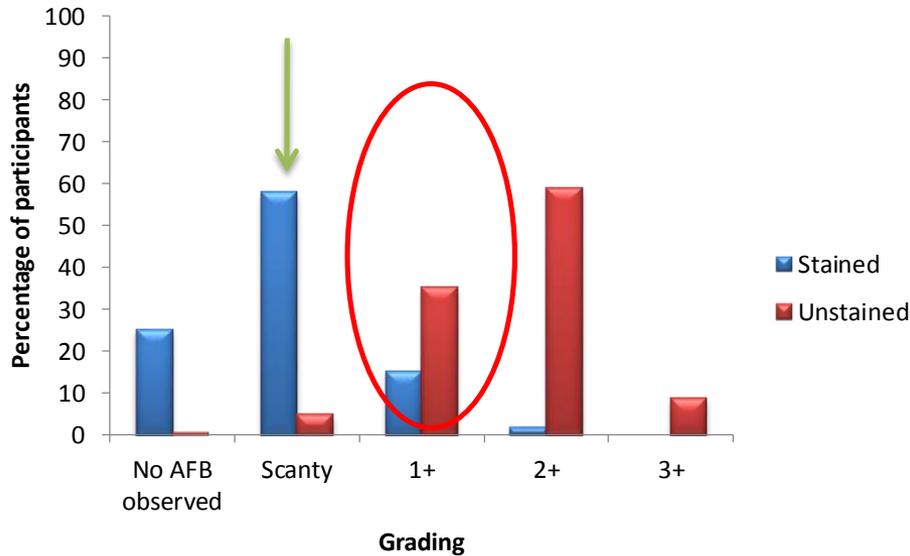
Survey 2012-3



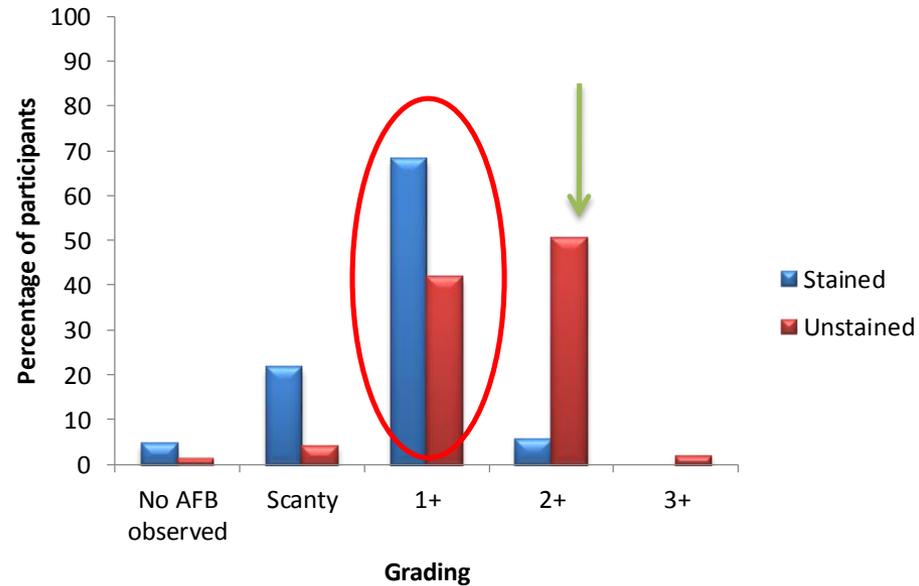
Survey 2013-1



Survey 2013-2



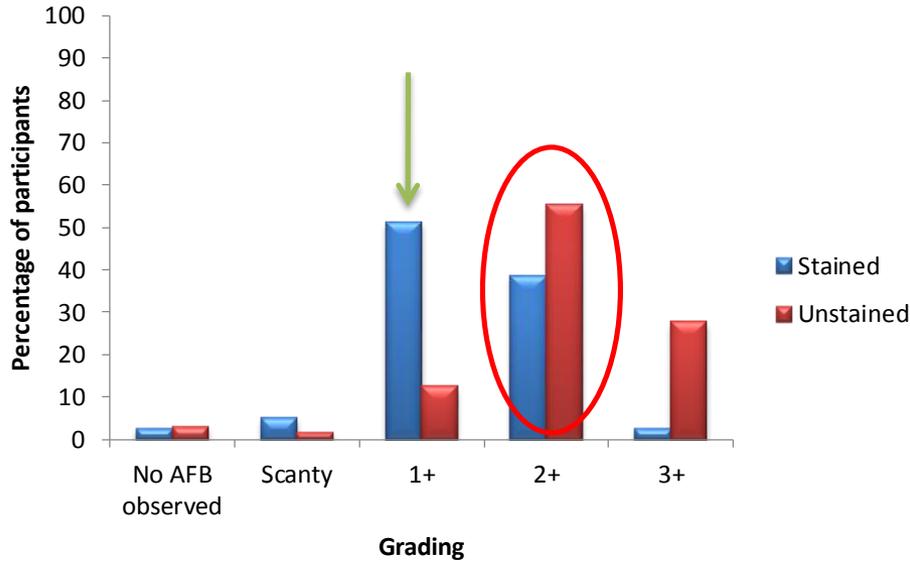
Survey 2013-3



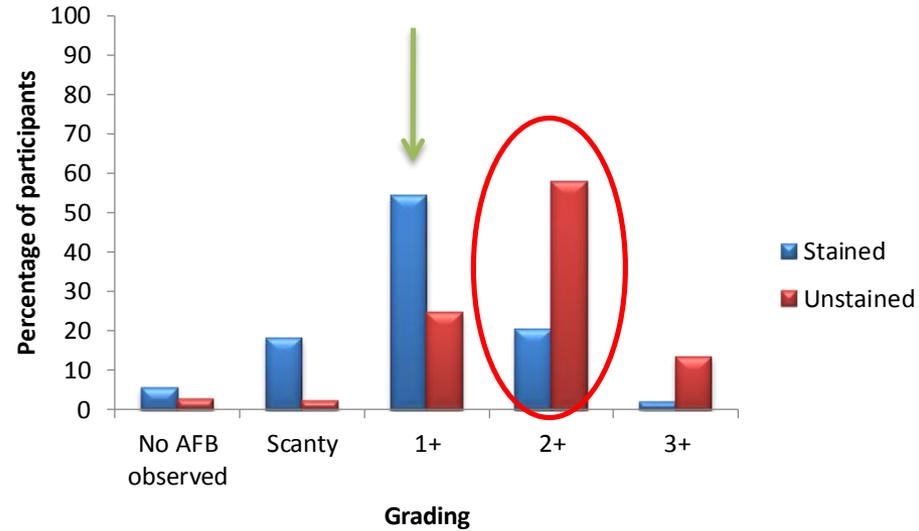
Before

Expected result: 2+ (2012-3 to 2013-3)

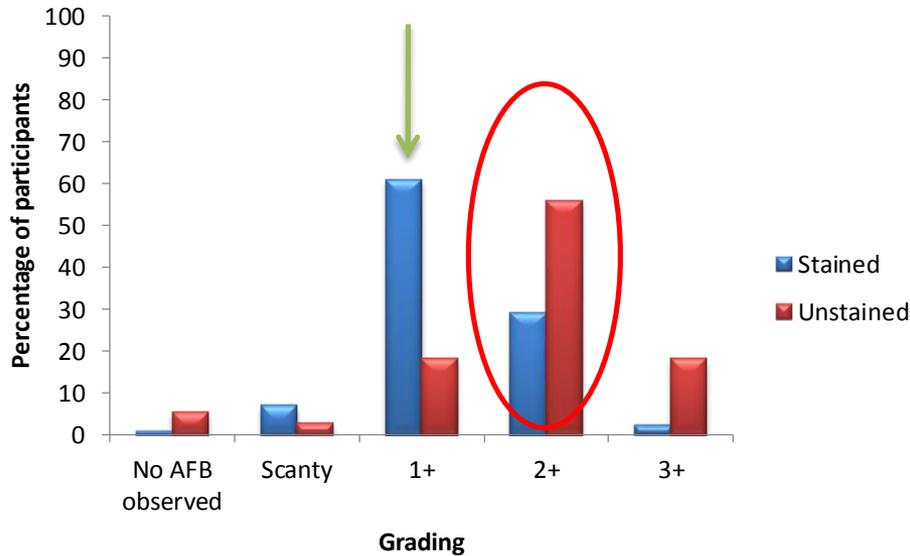
Survey 2012-3



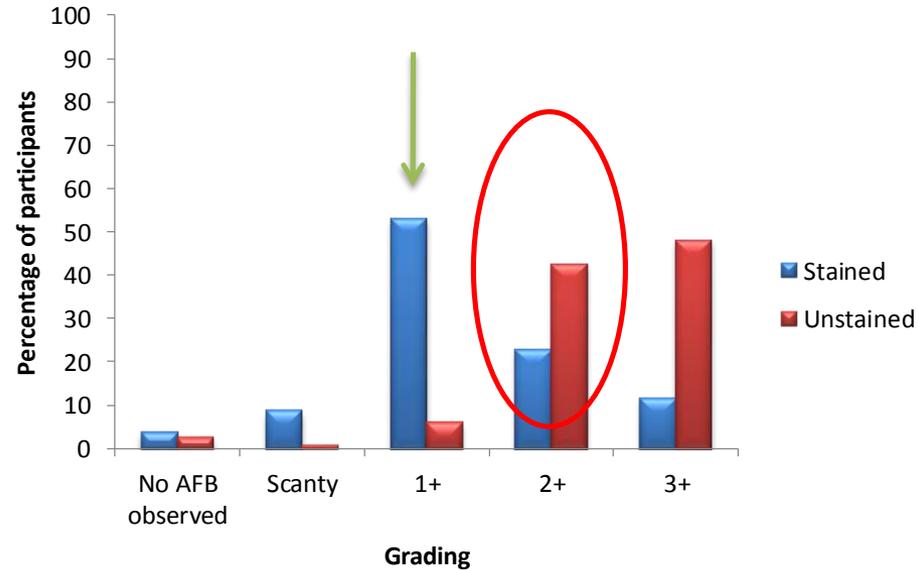
Survey 2013-1



Survey 2013-2



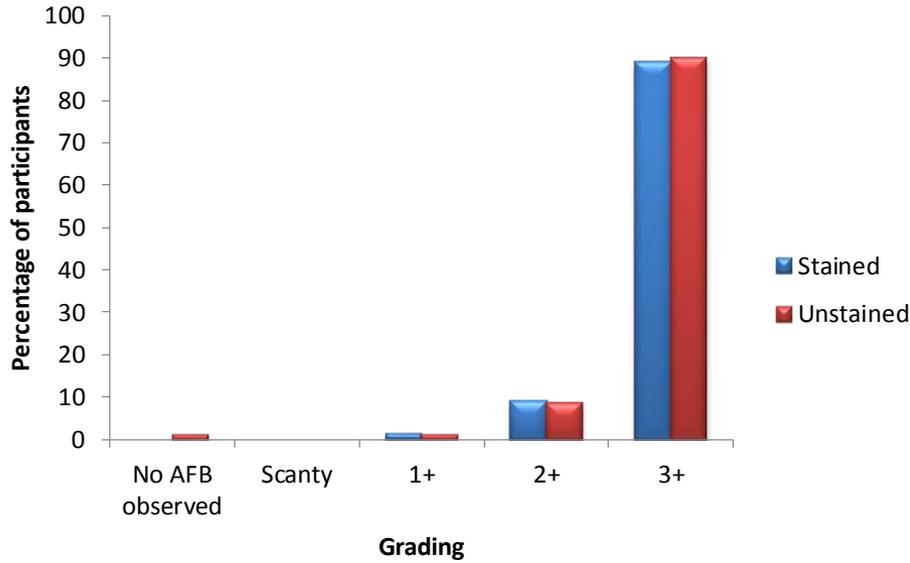
Survey 2013-3



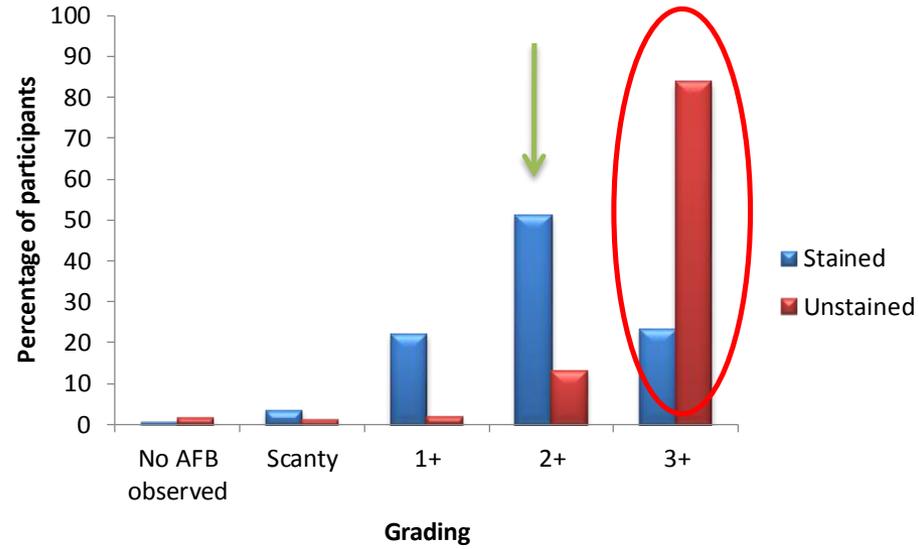
Expected result: 3+ (2012-3 to 2013-3)

Before

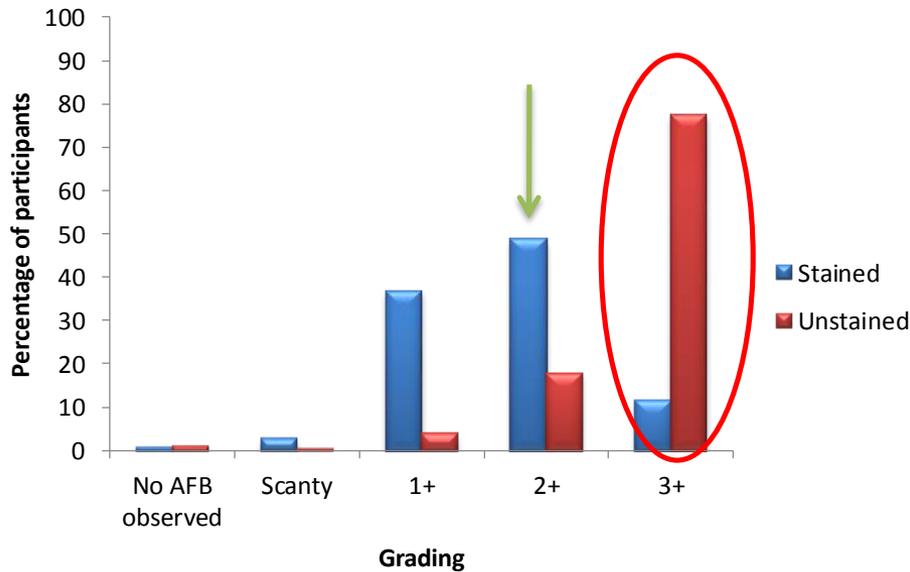
Survey 2012-3



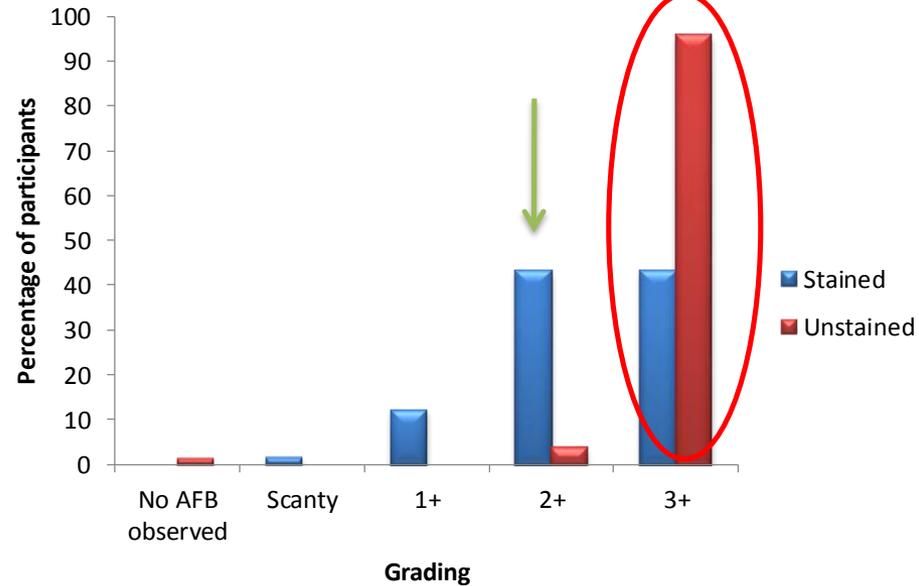
Survey 2013-1



Survey 2013-2



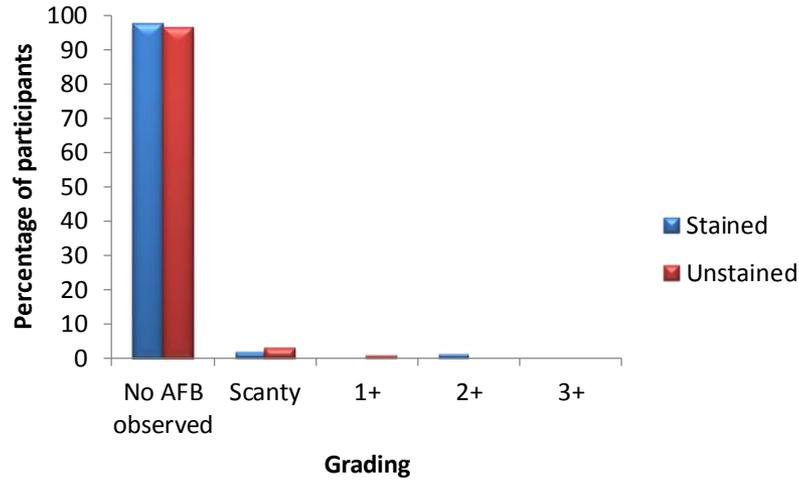
Survey 2013-3



# Expected results: No AFB observed (2014)

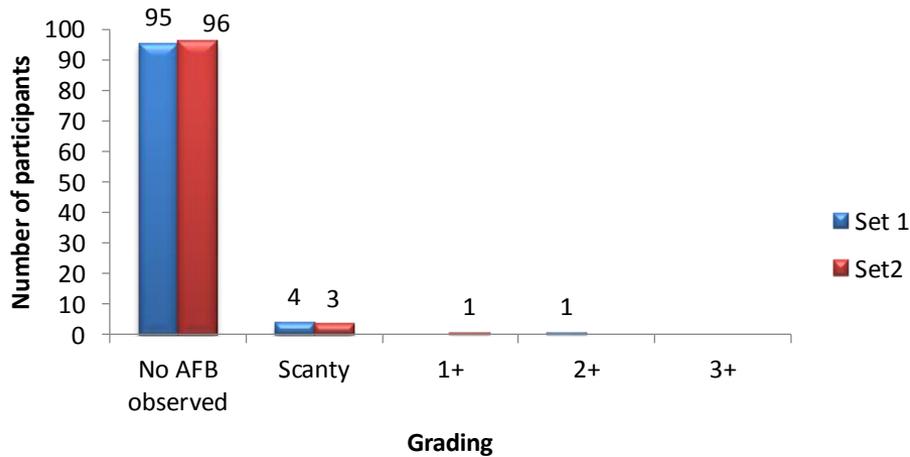
Before

### Survey 2013-3

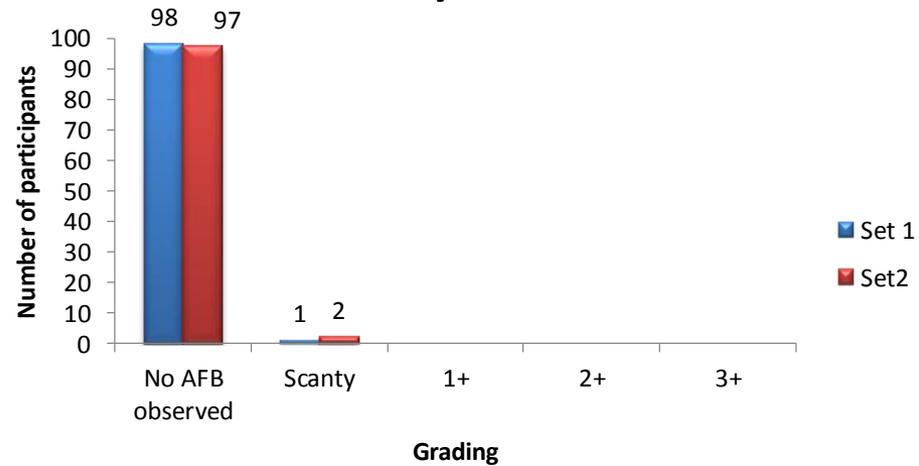


After

### Survey 2014-1



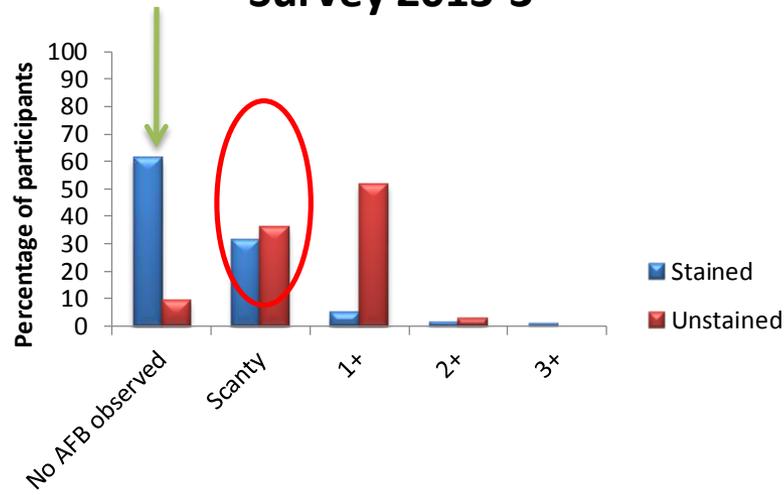
### Survey 2014-2



# Expected results: Scanty (2014)

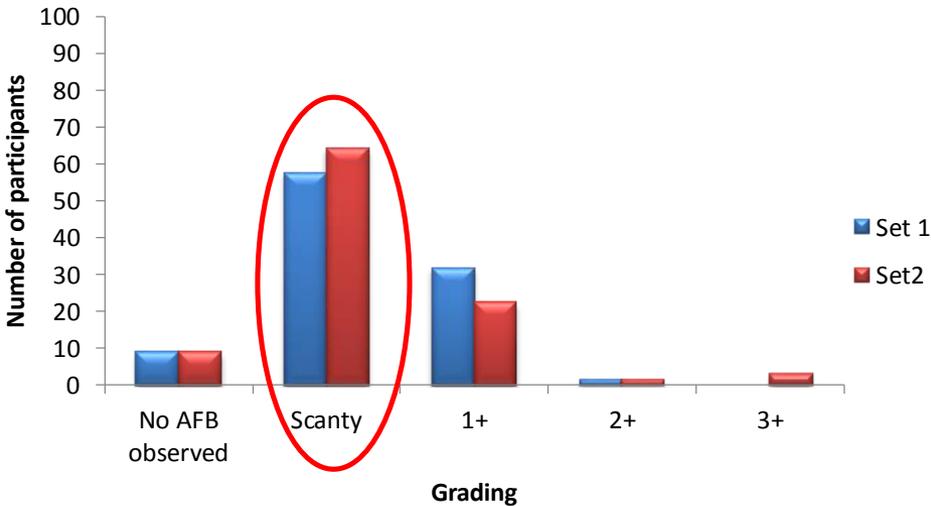
Before

## Survey 2013-3

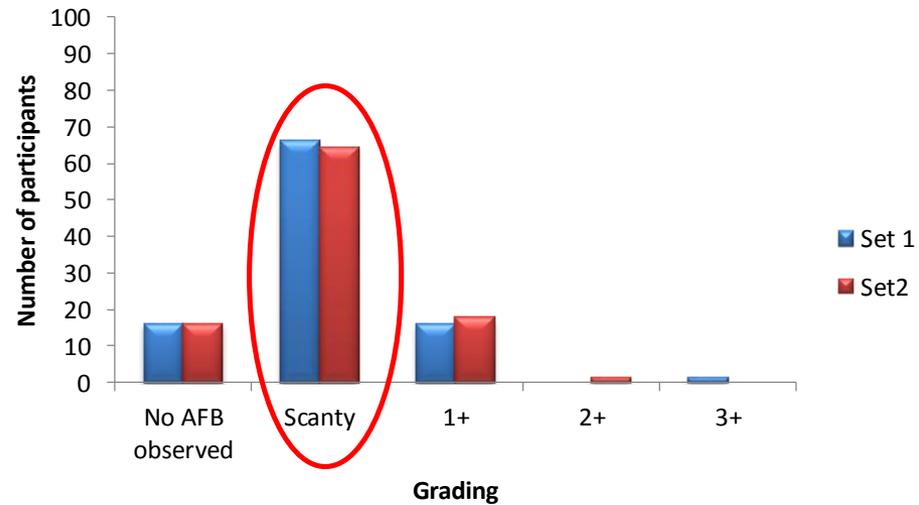


After

## Survey 2014-1



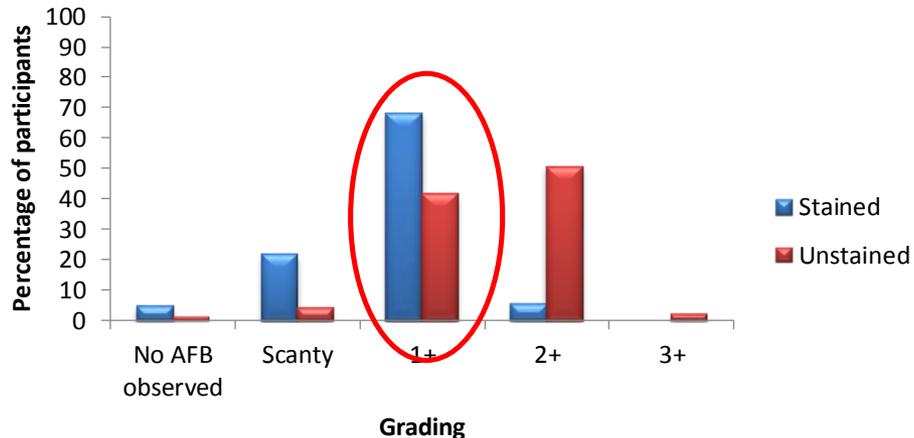
## Survey 2014-2



# Expected results: 1+ (2014)

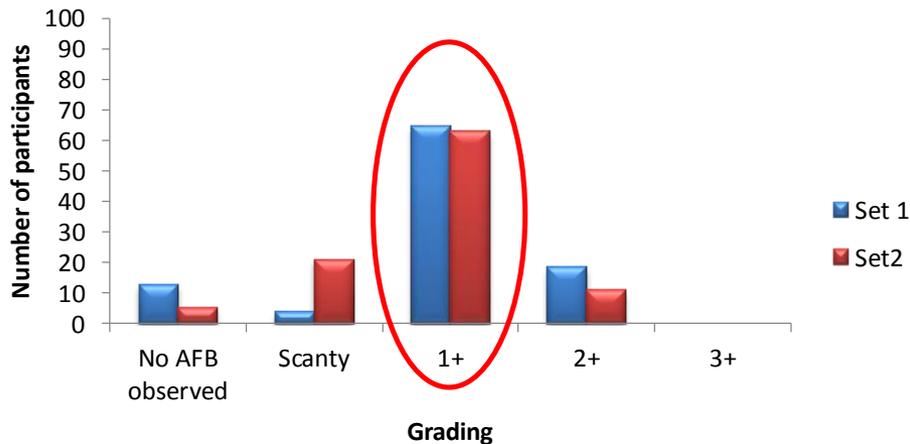
Before

## Survey 2013-3

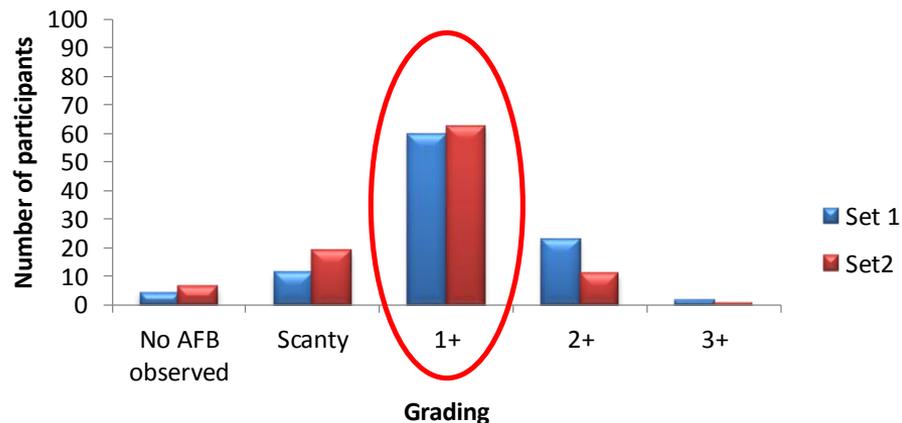


After

## Survey 2014-1



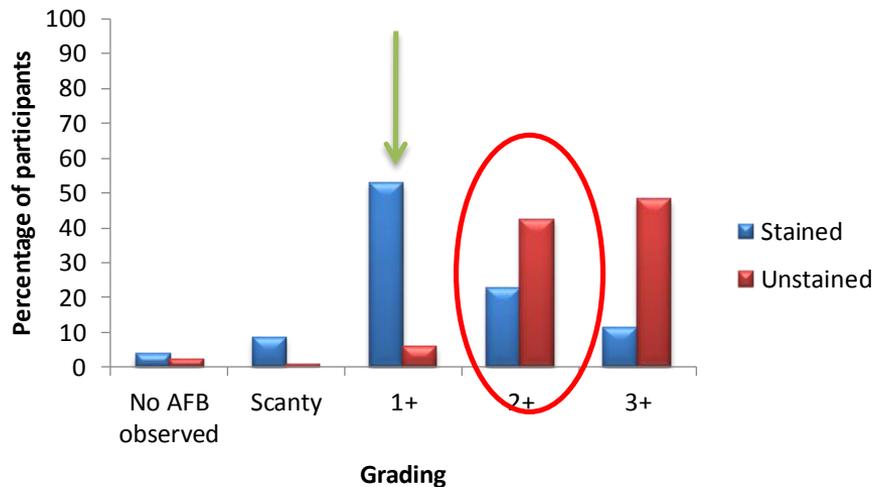
## Survey 2014-2



# Expected results: 2+ (2014)

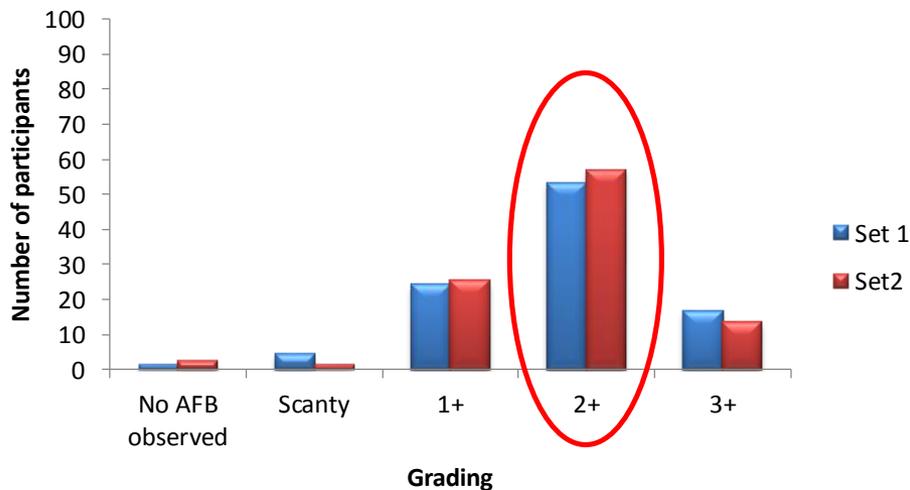
Before

## Survey 2013-3

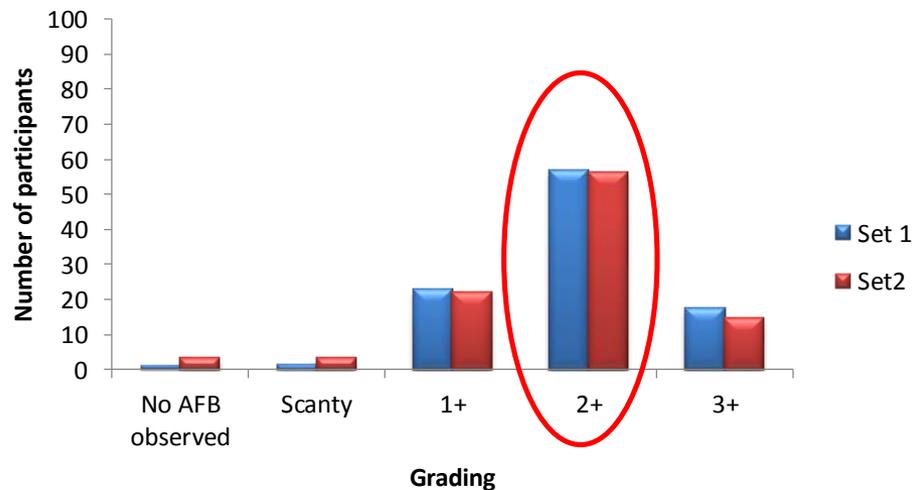


After

## Survey 2014-1



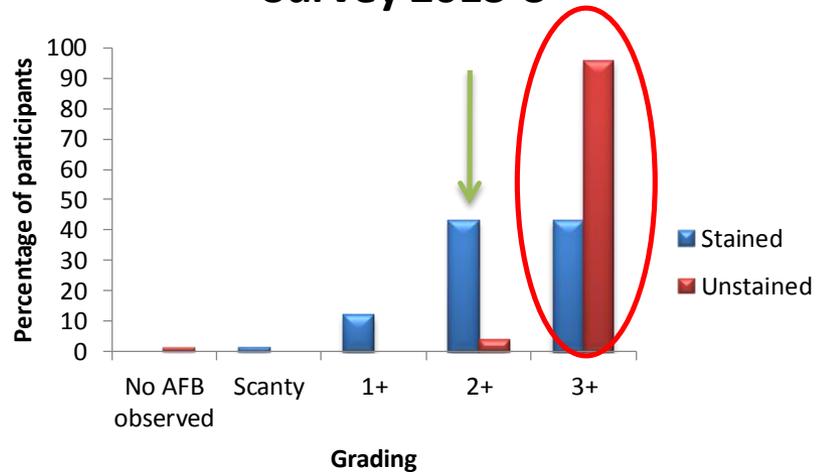
## Survey 2014-2



# Expected results: 3+ (2014)

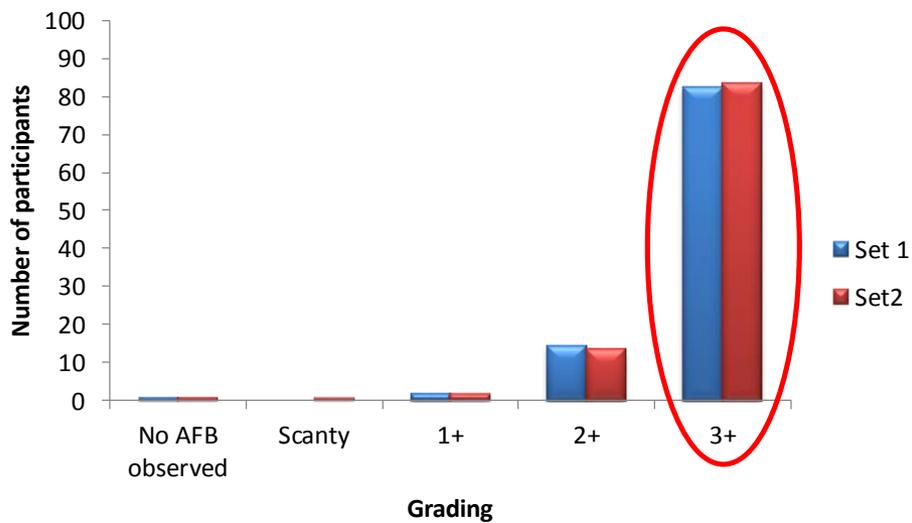
Before

### Survey 2013-3

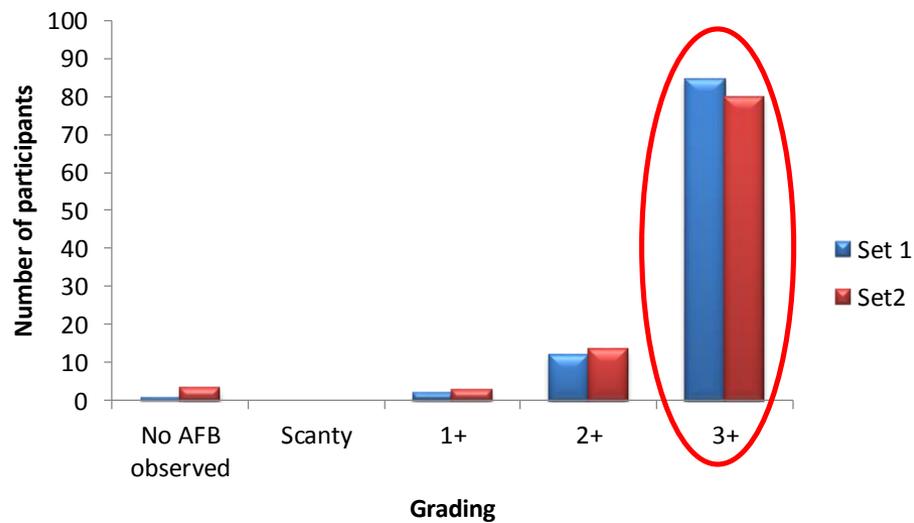


After

### Survey 2014-1



### Survey 2014-2



# Conclusion

- Although these possible areas of measurement of uncertainty still exist, namely
  - number of personnel that examine smears,
  - personal bias in reading smears,
  - the effect of environmental conditions,
  - technical competence of the individual, and
  - instrument/equipment performance,we are confident to report that the most likely cause of discrepant results has thus far been due to the automated staining technique and reagents.
- Results from surveys analysed this year to date have shown remarkable improvement in the agreement of the smears with the same quantification and expected results.

## Acknowledgments:

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