THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory
Timely Accurate Diagnostics for a TB-Free Africa

Training on Proficiency Testing Scheme (Microscopy PT)

Module 6: Validation of microscopy PT items

24th- 29th April 2018

Uganda Supranational Reference Laboratory

Content outline

- Methods of Validation
- Coding of Microscopy slides
- Microscopy PT composition
- implementation of PT Scheme.

Group exercise-5 minutes

1) Prepare a written checklist for all materials and tools required during Validation of microscopy PT items.

Introduction

- Mandatory requirement!

Pre-validation:

Validation of consistency of panel batches prior to sending test panels out to periphery

Post-validation:

Validation of panel slides / batches after receiving aggregate results from all laboratories

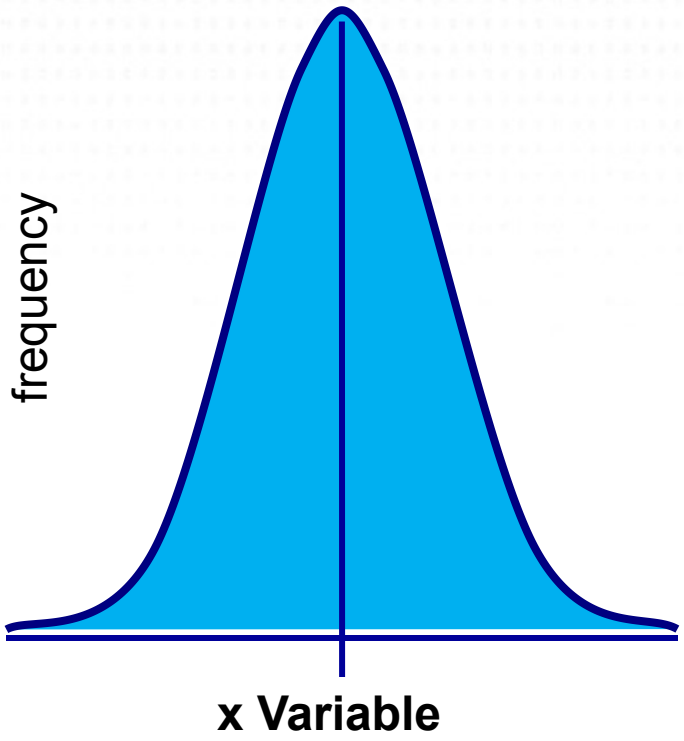
Keep accurate records of batches prepared and detailed results of the validation process

Pre-validation.

- Stain at least 6 slides from each batch to be examined independently by 3 or more technicians
- Calculate the average results and standard deviation (SD)
- The average minus 2 SD should be > 0 to accept the batch
- Use the Validation Log to record results

Materials required.

- All values are symmetrically distributed around the mean
- Characteristic “bell-shaped” curve
- Assumed for all quality control statistics
- tubes



What is Standard Deviation?

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The principle calculation used in the laboratory to measure dispersion of a group of values around a mean

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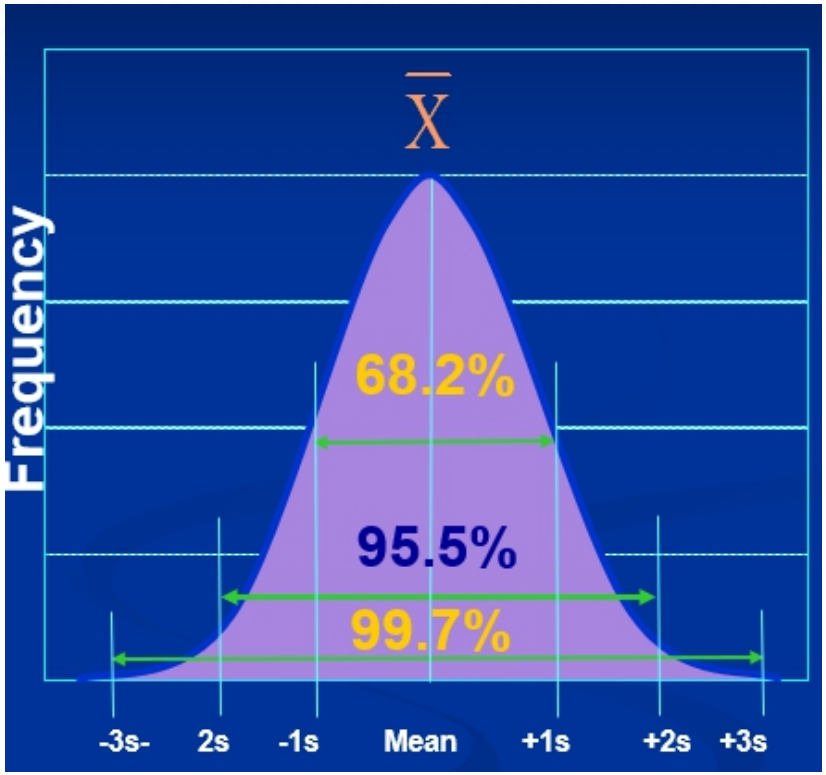
$$SD = \sqrt{\frac{\sum (Xi - \bar{X})^2}{n - 1}}$$

Standard Deviation and Probability

- For a set of data with a normal distribution, a value will fall within a range of:
  - +/- 1 SD 68.2% of the time
  - +/- 2 SD 95.5% of the time
  - +/- 3 SD 99.7% of the time
- Laboratories use the +/- 2 SD criteria for the limits of the acceptable range for a control value
- When the QC measurement falls within that range, there is 95.5% confidence that the measurement is correct



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Sample Form: Validation Log for AFB Panel Testing Slide Batches (pre-validation)

VALIDATION LOG FOR AFB PANEL TESTING SLIDE BATCHES

Batch №	Slide Preparation		Slide evaluation										
	Date slides made	Number of slides made	Slide test results (AFB per 100 fields)							Standard deviation (SD)	Consistency (average minus 2 standard deviations)	ACCEPT or REJECT?	Report result
			1	2	3	4	5	6	Average				
1	7/6/2006	17	10	10	50	15	10	11	17.7	15.96	-14.2	**reject**	1+
2	9/6/2006	34	0	0	0	0	0	0	0.0	N/A	N/A	**accept**	negative
3	9/6/2006	40	7	2	4	3	9	2	4.5	2.88	-1.3	**reject**	scanty
4	10/6/2006	23	5	6	2	9	12	9	7.2	3.54	0.1	**accept**	scanty
5	15/6/2006	17	30	27	28	36	43	50	35.7	9.22	17.2	**accept**	1+
6	16/6/2006	30	3500	3700	1500	1700	2600	2900	2650.0	907.19	835.6	**accept**	3+
7													
8													
9													
10													

- Intended positives should never be negative
- Intended negative smear should never be positive
- Quantification differences should not reach 2 steps on scale

Post-validation

- The same smear error reported by a majority of technicians may represent a problem with the panel slide / batch:
  - Technical difficulties in preparing panel slides
  - Error in the pre-validation
  - Incorrect recording of the expected result
  - Fading of smears during transportation to peripheral sites

Infectious material: all steps have to be performed in a BSC at BSL3 Laboratory

Logbook of Panel Slides Sets / Post-validation

Form PT2: PANEL SETS' LOGBOOK / AGGREGATE RESULTS

(Record of a set of 10 slides selected from Form PT1)

Central Laboratory administering panel test: _____

District where panel testing is conducted: _____

Date slide set(s) sent to peripheral laboratories: _____

Slide set(s)' number(s): _____

Slide No	Batch No	Stained or unstained	Expected result (from Form PT1)	Slide Sets Numbers										Comments
				Peripheral Laboratories' Results										
				1	2	3	4	5	6	7	8	9	10	
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														

Supervisor: _____

Coding of Panel Smears

- Ensure that result can not be guessed by an examinee – to avoid reading bias
- Make identification of a panel smear clear to a supervisor in charge of a panel testing exercise

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Make identification of a panel smear clear to a supervisor in charge of a panel testing exercise

Example of a smear's code:

62-45-1

- a panel set number
- a batch number
- a smear serial number in a panel

Panels' Composition

- The composition of a panel set is determined by NRL
- Number and types of slides to reassure that correct or incorrect results are not accidental
  - At least 10 slides provides a valid and fair test
  - Batch of stained and unstained smears
- Unstained smears:
  - Evaluate staining technique; provide information about stain preparation and quality

Preparation of PT item for Microscopy

- A panel test should represent a challenge in terms of difficulty:
- - some scanty and low-positive smears

1 slide graded 3+	1 slide graded 3+	1 slide graded 2-3 +
1 Slide graded 2+	1 slide graded 2+	2 slides graded 1+
1 slide graded 1+	2 slides graded 1+	3 slides graded
2 slides graded	3 slides graded	1-9 / 100 fields
1-9 / 100 fields	1-9 / 100 fields	4 negative slides
5 negative slides	3 negative slides	

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Implementation of Panel Testing

- Responsibility of the NRL- from preparation of slides to analysis of results and feedback
- Determine the number of AFB technicians who will participate in PT (ensure preparation of the needed number of panels)
- Communicate with Public Health Directors regarding EQA activities
- Prepare the schedule for panel testing in each location
- Collaborate with intermediate laboratories

Assessment

1. Mention methods to valid microscopy PT items preparation.
2. What method is better for your laboratory and why?

References

- GLI TB training package http://www.stoptb.org/wg/gli/trainingpackages.asp
- www.hain-lifesciences.com

Acknowledgments

KOFIH
Korea Foundation for International Healthcare
한국국제보건의료재단