THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

Training on EQA and National TB Laboratory Network

Module 3: Sample Rechecking Procedures

Date

Uganda Supranational Reference Laboratory

Content Outline

- Keeping and collecting the slides
- Restaining the slides
- Reading the slides
- Evaluating the smear quality

Keeping the Slide Boxes (1)

STEPS:

- Label slides with lab. number and result column number or code
- After slide reading
    - Let oil soak into absorbent paper by putting slides with smear downwards on strip of toilet paper or news paper
    - Do not remove oil with xylene or other solvent
    - Do not rub
- Keep slides of all patients in a slide box until sample is taken

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Keeping the Slide Boxes (2)

STEPS

- Arrange slides in boxes according to serial number
- Leave empty slots for specimens not yet examined (2nd, 3rd diagnostic smear)
- Start filling new boxes each quarter
- Ensure sufficient boxes for slide storage for 2 quarters

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Sample Selection (1)

- Sample collection by TB supervisor, never by laboratory staff
- Feedback on previous EQA performance to motivate lab staff to keep slides and improve performance if applicable
- If sampling during visit not possible:
  - forward all slides and copy Lab. Register to rechecking coordinator for sample taking, filling forms and forwarding to first controller

Sample Selection (2)

- Collect during each visit fraction of sample covering period since last visit
- Mark period in Lab. Register, draw line under last entry of the period
- Count total number of smears examined during the period, or use numbers recorded by lab.staff
- Calculate sampling step by dividing total number of smears by required sample size
  - e.g. 675 smears examined, 24 samples to take, gives sampling step of 675/24= 28

Sample Selection (3)

- Fill two blinded rechecking forms of sputum smear examinations for AFB
  - one form with and one without smear results
- Use random number, e.g. last digit of bank note to select first slide.
  - If 5, the first slide is the 5th in Lab. Register.
  - The second slide is the 33rd slide, and so on, until 24 slides have been selected

Sample Selection (4)

- Note identification number of the slides on the rechecking form
- Request staff of the centre to retrieve the selected slides from the slide boxes
- Check slides against the list
- Mark missing slides on the form; replace missed slide with next slide in Lab. Register
- Ensure that each slide has identification number

Sample Selection (5)

- If no slides with positive/scanty results in the sample, add first two positive/scanty slides in Lab. Register from start of the period
- Ensure that all not selected slides are discarded
- Arrange slides in transport box in order of the form, and give box, together with copy of the form without the results to first controller
- Give form with results to rechecking coordinator

Restaining slides; Ziehl-Neelsen (ZN) Stained Slides

- Fuchsin stain fades with high temperature and high humidity
- Speed of fading is unpredictable
- More extreme on low positives and poorly stained smears, not only in extreme climates
- Consequences of fading of fuchsin stain
  - FP reported by first controller and/or second controller
  - Missed low positives at microscopy centre not identified as FN by first controller and/or second controller

Ziehl-Neelsen (ZN) Stained Slides (2)

- Options when fading may be a problem

  1. Re-staining of all discordant slides at the second control level

  But re-staining of all sample slides is needed if:

  - first controllers miss many scanty and 1+
  - and/or quantifications are systematically lower for first controllers

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Ziehl-Neelsen (ZN) Stained Slides (3)

2. Restaining of all slides at the first control level

- Advised if
  - Quality of staining solution not well controlled; no control on chemicals
  - Decentralized preparation of solutions
- Advantages
  - Detection of gross deficiencies of staining or staining solutions
  - Takes care of fading
- Disadvantages
  - Increased work load for controllers

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Restaining Slides: Auramine Stained Slides

- Auramine stain fades with light, high temperature and high humidity.
- Restaining of all slides at the first control level
- Restaining of discordant slides at the 2nd control level if slides not protected against fading since restaining at the 1st control level
- Justified based on experience, anecdotal evidence and theoretical considerations.

Restaining Technique

- Remove the oil from the slides.
- No need to decolorize.
- Restain with same staining solution type and same staining procedure as in routine.

Reading Slides and Evaluating Smear Quality

- First level rechecking is completely blinded

-First controller has no access to the results

-Only slides and copy of rechecking form with identification numbers are provided

- Clean all oil-immersion examined slides with xylene

Reading Slides and Evaluating Smear Quality (Cont....)

- If slides are routinely restained at the first control level:
  - -Inspect smears with naked eye
  - -Write appreciation on size, thickness, evenness and background color
  - -Note that restaining was done

Reading the Slides (2)

- If slides are not routinely restained at the first control level:

-Write appreciation on size, thickness, evenness,

-AFB color and artefacts after microscopic reading

- Restain all auramine stained slides

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Reading the Slides (Cont…)

- Restain all ZN stained slides, if NTP policy
- Check smears using original microscope: bright field for ZN, fluorescence for FM and standard magnification
- Check same number of fields as in routine- usually 100 fields or one length

Reading the Slides (3)

- Note results on copy rechecking form in column" first controls"
- Add overall appreciation on smearing and staining at bottom of form
- Do not recheck slides with unclear identification or severely damaged smears; report as excluded/ID or excluded/damaged.

Reading the Slides (4)

- Let oil soak in paper (toilet paper, news paper), do not rub
- Re-arrange slides in original box
- Keep box in fridge if fading is expected
- Send rechecking form to rechecking coordinator

Evaluating Smear Quality

- Smear quality concerns
  - quality of specimen: real sputum or saliva
  - quality of smear: size, thickness, evenness
  - quality of staining: color of AFB and background

- Smears should be evaluated for specimen quality (sputum vs. saliva), appropriate size and thickness, and quality of staining by the controller.
- Problems identified should be noted on the sampling form

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Smear Quality (1)

- Equal spreading of the material over an area of 2 cm by 3 cm or 2 cm by 1 cm
- Microscopic appearances
  - good sputum: mucus threads with white blood cells
  - bad sputum: thin smear with epithelial cells
- Follow-up smear may be salivary
- Remarks on poor quality smear only if several thin smears without white blood cells

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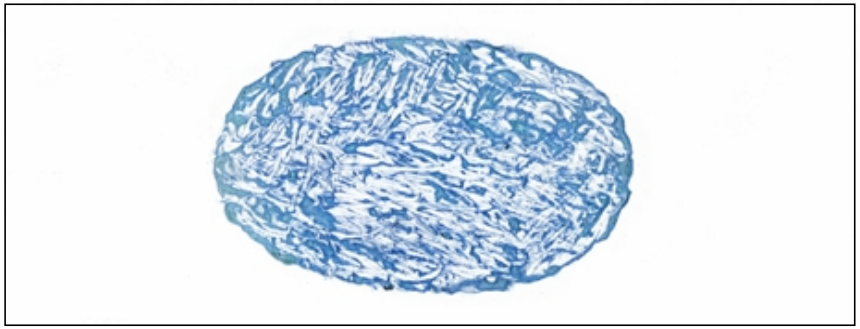
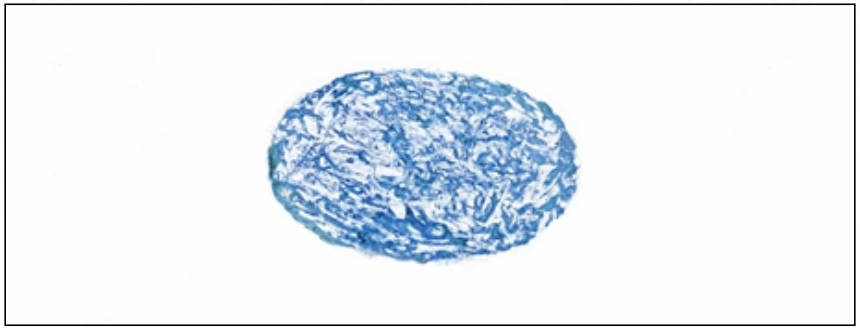
Macroscopic Appearance of a Good Smear





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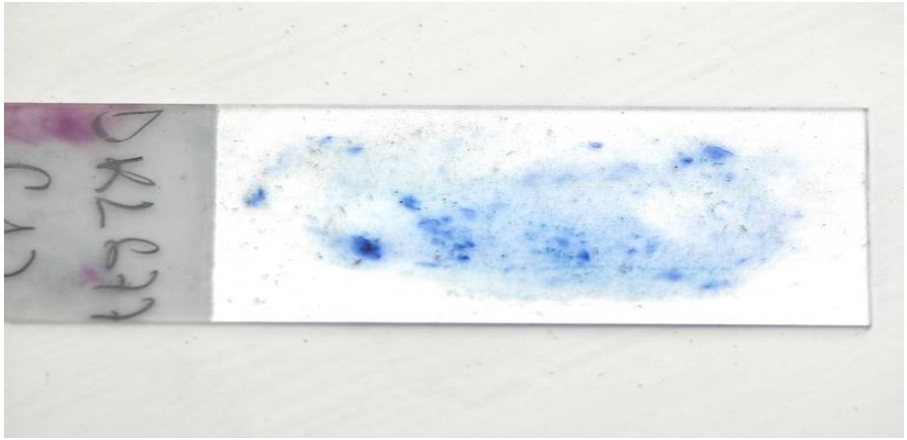
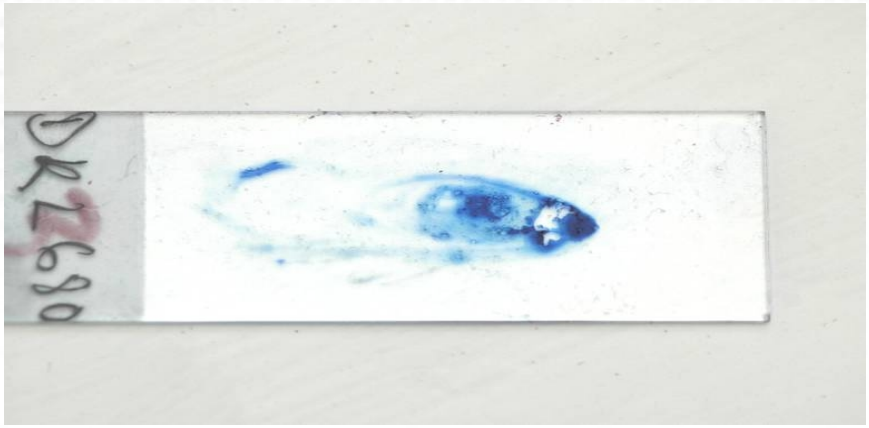
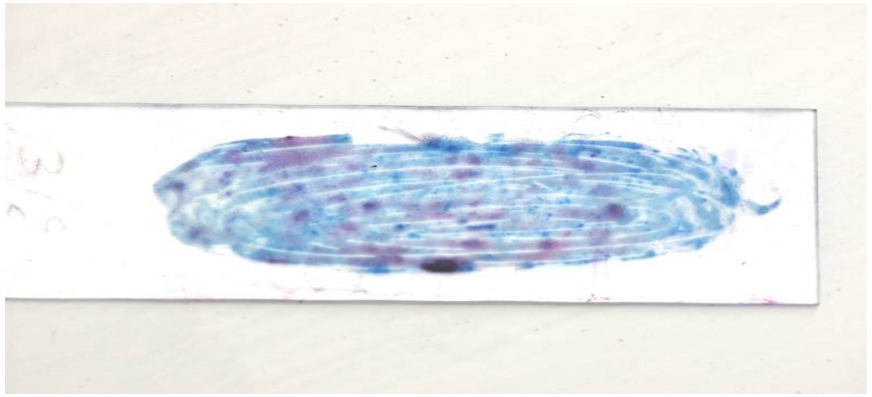
Macroscopic Appearance of a Poor Slide







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Staining Quality

- A well stained ZN smear shows strong red AFB against a weak blue background
- A well stained auramine smear (FM) shows yellowish fluorescent rods against a dark background
- Background should not show much remaining red (ZN) or fluorescence and no red or fluorescent artifacts

Smear Quality Remarks

- Note qualitative aspects of smearing and staining on the sampling form and feedback report
- There will always be some less good smears, but no serious problem if no errors are found

Assessment

- Who is responsible for sample collection?
- How long should slides be kept in the laboratory?
- What causes fading of ZN smears and Auramine smears?

SUMMARY

- Slides from all patients, irrespective of their results, should be kept in the laboratories until sampling is done
- Sampling should be random and first level rechecking must be completely blinded
- Fading of fuchsin staining can falsify the results of rechecking. Restaining prior to controlling is needed for ZN, and auramine stained smears.
- Evaluation of smear quality should be done by controlers.

Acknowledgments