Microscopy studies: Reference Standards

Andrew Ramsay
WHO Tropical Disease Research (TDR)
RamsayA@who.int

Evaluating Microscopy Methods
References, standards, comparability

Spt Microscopy Methods

"Low and variable sensitivity"

20 – 60 %

HIV-associated TB

High specificity

Sputum Culture Ref Std

High sensitivity and specificity

High 90%s?

Less affected by HIV

Evaluating Microscopy Methods

Spt Microscopy Methods

No of specimens/smears

Ziehl-Neelsen (Hot? Cold?)
Fluorescence microscopy?
Specific stains?
Condition of stain?

Microscope type? (mono-, bi-, student, lenses)

Microscope condition? (lenses, condenser, iris, light source)

Microscopist? (experience, exposure, workload, colour vision).

Specimen collection?

Smear preparation?

Smear examination? (Number of microscopic fields examined? Time spent to negative? Monitoring time spent – morning vs afternoon? FM – screening magnification vs confirmation?)

Internal Quality Control (IQC)
External Quality Assessment (EQA)

Evaluating Microscopy

Sputum Culture Reference Std

Solid culture? (Egg-based? Agar-based? Glycerol/pyruvate?)

Liquid culture (MGIT manual/auto, MODS, others).

Speciation method (to Mtb complex level).

Specimen collection?

Specimen transit? (duration, conditions, additives)

Delays/batching in laboratory processing?

Volume/No of specimens?

Digestion/decontamination procedure? (agent type, concentration of agent, specimen contact time, agitation)

Centrifugation (refrigerated, RCF used, once/twice)
Neutralisation/washing step?

Volume of inoculum?

Incubation? Duration?

Reading

Internal Quality Control (IQC)
External Quality Assessment (EQA)

Relating smear results to culture – dealing with discrepancies

Number of bacilli observed	Estimated concent of bacilli per ml sputum	Probability of a positive culture result
0 in ≥ 100 HPF	Less than 1,000	Less than 10%
1 – 2 in 300 HPF	5,000 – 10,000	50%
1 – 9 in 100 HPF	Approx 30,000	80%
1 – 9 in 10 HPF	Approx 50,000	90%
1 – 9 per HPF	Approx 100,000	96.2%
≥ 10 per HPF	Approx 500,000	99.95%

H.L David. Bacteriology of Mycobacterioses. US Dept of Health, Education and Welfare, Public Health Service, CDC, Atlanta, USA, 1996.

Solid culture vs liquid culture as reference standard

Liquid culture more sensitive
Less discrepancies? More contamination?

Is solid culture acceptable?

Are non-culture methods acceptable?

Getting published?