# Automated and high throughput digital fluorescence microscopy for diagnosis of

1. **pulmonary tuberculosis.**

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1. Smear-microscopy is still the most frequently used method for diagnosis of tuberculosis (TB) and the primary
2. tool used to monitor treatment response in low income settings. However, smear-microscopy is laborious
3. and time consuming and prone to human error. Automated smear-microscopy systems could enable high
4. laboratory throughput with limited staff time and standardise results in diagnostic centres with large
5. numbers of samples.1 32
6. We evaluated an automated fluorescence microscopy system (Fluorobot, ConsultaSK Ltd., Budapest,
7. Hungary) capable of the automated reading of smears using an ultrabright light emitting diode (LED) and
8. image interpretation for the detection of bacilli on slides.2 The system reads slides stained with auramine-O,
9. which are mounted in a conveyer belt, conduct automated image focus, image capture and interpretation.3
10. These steps include auto-focusing, image capture, filtering by normalization of noise levels and setting the
11. number of view-fields for examination. The default number of fields examined is 100, corresponding to an
12. area of 2 mm.4 The examination however is halted at 40 view fields if the slide reaches a 2+ grade , and at 20
13. fields if 3+. The platforms automatically finds the fields of interest (FOI), determines independent and
14. complex morphological parameters and classifies the FOIs using proprietary deep machine learning
15. algorithms trained on a repository of 100,000 positive and 100,000 negative smears.2 43
16. This was a prospective evaluation of the Fluorobot diagnostic accuracy, and was conducted at the National
17. TB Reference Laboratory at Chiril Dragniuc Pthisiopneumology Institute (PPI), Chisinau, Republic of Moldova.
18. Consecutive sputum samples of adults (≥ 18 years old) with presumptive TB were (Sept-Dec 2016)
19. decontaminated and centrifuged to conduct liquid and solid TB culture 5. Aliquots of the processed sputum
20. sediment were used to prepare auramine-O-stained smears. Smears were graded manually by experienced
21. microscopist unaware to the Fluorobot results and with the automated Fluorobot system. Slides were
22. examined manually using the 20X objective and with 26X magnification for Fluorobot, without oil immersion.
23. Smears were graded negative, scanty, 1+, 2+ and 3+.5 Smears graded manually as scanty by a first
24. microscopist were confirmed by a second microscopist and discordant readings were discussed to reach
25. consensus. Smears graded scanty by Fluorobot were selected by the software and the digital images were

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1. presented to a microscopist for manual confirmation. Fluorobot smears were classified according to the
2. number of images resembling bacilli per FOI and were considered negative if there was no or 1 bacilli per FOI
3. 5; scanty if 2-9 bacilli, 1+ if 10-99 bacilli, 2+ if 100-1000 bacilli and 3+ if >1000 bacilli. Culture was considered
4. the reference standard for comparison and one positive solid or liquid culture was considered as positive.
5. Patients with contaminated or missing cultures were excluded. Statistical analyses was performed in MedCalc
6. Statistical Software (MedCalc bvba, Ostend, Belgium; [https://www.medcalc.org](https://www.medcalc.org/); 2019, version 19.0.5).
7. Analysis included a straight comparison of the manual and automated readings. This was followed by a
8. second analysis comparing the manual and the automated readings supplemented by the manual
9. confirmation of automated scanty smears. Ethical approval was obtained from the Research Ethics
10. Committees of the PPI, Moldova and the Liverpool School of Tropical Medicine, UK.
11. Four-hundred and twenty-two sputum samples were available for analysis, of which 80 (18.9%) were culture-
12. positive. In the first analysis, 56 of 80 culture-positive samples were graded as smear-positive (sensitivity of
13. 70%, 95%CI 58.7% - 79.7%) and 335 of 342 culture-negative samples were graded smear-negative (specificity
14. 97.9%, 95%CI 95.8% - 99.2%) by manual examination. Fluorobot graded smear-positive 56 of the 80 culture-
15. positive smears (sensitivity 70.0%, 95%CI 58.7% - 79.7%) and smear-negative 280 of 342 culture-negative
16. smears (specificity 81.8%, 95%CI 77.4% - 85.8%). Sixty-six smears had been graded scanty by Fluorobot and
17. these were reviewed by a microscopist for confirmation, which resulted in a revised scale to regrade smears
18. with 2-4 bacilli per FOI as negative. This second analysis with regraded scanty smears resulted in 51 of 80
19. culture-positive smears graded smear-positive (sensitivity 63.8%, 95%CI, 52.2 - 74.2) and 329 of 342 culture-
20. negative specimens graded smear-negative (specificity 96.2%, 95%CI, 93.6 - 98.0). The difference of Area
21. Under the Curve (AUC) of the manual and automated readings for the second analysis was not statistically
22. significant (AUC manual grading - AUC fluorobot alone = 0.044, p=0.07; AUC manual grading - AUC fluorobot partially revised = 0.036;
23. p = 0.06), as shown in the figure.
24. Our findings indicate that the fully automated Fluorobot has similar sensitivity but lower specificity than
25. manual smear-microscopy. However, the selection of a small proportion of smears (15%) graded scanty for
26. confirmation resulted in similar sensitivity and specificity to manual readings. The selection of digital images

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1. with scanty smears can be automated and images can be transmitted electronically for confirmation. The
2. confirmation step only requires reviewing four view fields per smear.
3. The integration of automated microscopy tools such as Fluorobot in locations where smear-microscopy is
4. still conducted for the diagnosis of large numbers of patients and to monitor treatment response by
5. documenting conversion to smear-negative after two and five months has the potential to reduce the
6. workload while generating reproducible results that are less prone to human error and dependent on human
7. competency.
8. Our study has several limitations, as it was conducted in a single laboratory and in a setting with relatively
9. low HIV prevalence. HIV status was not available for analysis, which precluded stratifying by this important
10. factor 6. Moreover, all participants were adults and further studies in children are needed.
11. Automated smear microscopy systems could facilitate processing high sample numbers in busy laboratories
12. and has the potential to standardize reading procedures, enabling consistency in smear gradings, shorter
13. turnaround times, while reducing systematic human errors due to fatigue and low proficiency .1,7,8 As the
14. digital images can be transmitted electronically, quality assurance can be conducted remotely, without the
15. need of physical visits or sample transport. Despite these advantages, the system also requires stable
16. electricity, a computer, internet access and establishing procedures for the procurement of proprietary
17. smears and stains and the maintenance and calibration of the platform. Although commercial prices are not
18. available, it is intended the system would have comparable prices to a good quality, general purpose,
19. microscope and further cost-benefit analyses are warranted.
20. The WHO recommends conducting sputum smear-microscopy for the diagnosis of TB in locations where
21. GeneXpert is not available. 9 Smear microscopy also continues to be one of the mainstay methods to monitor
22. TB treatment response. The Fluorobot system therefore could be suitable for settings with high laboratory
23. workloads, where staff can be overworked and fatigued and could facilitate the establishment of a uniform
24. acid-fast microscopy service with minimal human involvement. Further evaluations of the platform are
25. warranted in high burden settings.

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Figure. Area under the curve (AUC) of manual, Fluorobot and Fluorobot plus scanty confirmation smear microscopy using culture as reference standard.

For

Review



*Solid line* Manual smear-microscopy (AUC 0.84; 95%CI 0.81 - 0.87)

*Dashed line* Fluorobot smear-microscopy with confirmation of scanty smears (AUC - 0.81. 95%CI 0.77 - 0.85)

*Pointed line* Fluorobot alone (AUC - 0.80; 95%CI 0.76 0.84).

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