

# Efficiency of a third serial sputum smear examination in the diagnosis of tuberculosis in Moldova and Uganda

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## SUMMARY

**SETTING:** Twenty-four and 30 tuberculosis (TB) microscopy laboratories in Moldova and Uganda, respectively.

**OBJECTIVE:** To estimate the workload required to identify one additional case of TB with a third serial sputum smear examination.

**METHODS:** Retrospective laboratory register study to determine the prevalence and the incremental yield of TB cases from a third serial sputum smear examination among suspects in Moldova and Uganda, with the reciprocal of the product of these two fractions providing the number of examinations required to identify one additional TB case.

**RESULTS:** In Moldova, 9% (1141/12 525) and in Uganda 20% (7280/36 054) of suspects met the TB case defini-

tion with at least one positive sputum smear. The incremental yield from the third examination was 4% in Moldova and 3% in Uganda. To detect one additional TB case on a third smear, 273 examinations (95%CI 200–389) in Moldova and 175 (95%CI 153–222) in Uganda were thus required. This corresponded to an average of 11 days (8–16) and 7 days (6–9), respectively, to diagnose one additional case of TB.

**CONCLUSION:** In both countries, the third serial sputum smear examination was inefficient in diagnosing sputum smear-positive TB.

**KEY WORDS:** case finding; incremental yield; microscopy; tuberculosis; workload

SPUTUM MICROSCOPY remains a cornerstone of pulmonary tuberculosis (TB) diagnosis. The World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (The Union) recommend the examination of three smears before excluding sputum smear-positive TB.<sup>1,2</sup> Studies done in different countries under programme conditions indicate a minimal gain in the proportion of TB cases diagnosed by the third smear.<sup>3–8</sup> The rationale for limiting the recommended number of serial examinations to three smears is the diminishing return from serial examinations, i.e., an increasing amount of work is required to identify one additional TB case with each successive examination. The recommendations do not take into account an upper limit of examinations or working days that can reasonably be required to identify one additional TB case, although it is recognized that a full-time microscopist cannot be expected to prepare and examine more than 25 slides per working day.<sup>2</sup>

Technical personnel and policy makers were consulted in Moldova and Uganda to decide on a critical maximum number of examinations that could be reasonably required to identify one additional TB case following two negative examinations, above which the

requirement of three negative serial smears before declaring a suspect as a 'non-smear-positive suspect' would be excessive in their setting. In Moldova, this upper critical number was 100 and in Uganda it was 50. The study was thus designed to determine retrospectively the actual number of smear examinations recorded in laboratory registers that yielded one additional TB case of sputum smear-positive TB following two negative examinations.

## METHODS

### *Study design and setting*

The study was designed to retrospectively test the hypothesis that not more than 100 and 50 slides in Moldova and Uganda, respectively, were needed to identify an additional TB case with a third examination following two negative results. With a 2003 population estimate of 4.3 and 25.8 million for Moldova and Uganda, respectively, the WHO estimated the respective prevalence of all TB cases to be 108 and 652 per 100 000 population, and the respective incidence of sputum smear-positive TB to be 62 and 179/100 000.<sup>9</sup> TB control in the two settings follows the WHO DOTS

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strategy, using sputum smear microscopy in the diagnosis of pulmonary TB. At the time of the study, 56 laboratories performed sputum smear microscopy in Moldova and used a standard TB register to record the results of examinations, thus providing these services to populations of approximately 80 000 each. In Uganda, 240 laboratories offered sputum smear microscopy, corresponding to approximately one laboratory per 100 000 population. Although the number of laboratories seems to be adequate,<sup>2</sup> geographical coverage is poor in many rural areas.<sup>10</sup>

#### *Eligibility criteria*

As a first step, all laboratories offering TB sputum microscopy and using a standard TB laboratory register<sup>2</sup> were enumerated. From these country lists, 30 were to be randomly selected for inclusion in the study.

In Moldova, 56 laboratories met the inclusion criteria. Of these, only 24 had complete records for at least one year (i.e., January–December 2003). These were therefore included in the study.

In Uganda, 240 laboratories met the inclusion criteria. A random sample of 30 laboratories for the period January 1999–December 2001 was selected.

#### *Data collection*

A uniform data entry form was created using EpiData Entry (EpiData Association, Odense, Denmark).<sup>\*</sup> To test the study hypothesis, data on the reason for examination (diagnosis or follow-up) and the results obtained for up to three examinations were required. Data on age and sex were also obtained from the register. Data collection was at the level of the laboratory. A unique code was assigned to each laboratory.

To minimize data entry errors, data were entered twice, each time by two different persons, one reading and the other recording, and the data they could enter were restricted to pre-determined legal values. The two resulting files were compared for discordances using a key identifier created from the laboratory serial number and the registration year. A final dataset had the discordances resolved and corrected where needed by consulting the physical laboratory register.

#### *Analysis*

EpiData analysis was used for the analysis and supplemented by manipulating output with a spreadsheet (OpenOffice, Sun Microsystems Inc, Santa Clara, CA, USA<sup>†</sup>).

For the purpose of the study, a TB case was defined as a suspect with at least one acid-fast bacillus in any of the serial examinations. The fraction of suspects who were identified as TB cases on the third exami-

nation only is the product of the fraction of TB cases among suspects,  $p$ , multiplied by the fraction positive on the third examination only among all TB cases,  $f_3$ .<sup>11</sup> The reciprocal of this product,  $1/(p \cdot f_3)$ , gives the number of slides to be examined to identify one additional TB case of sputum smear-positive TB on the third diagnostic examination. Credibility intervals around the point estimate of the number of slides were computed with a Bayesian approach using the Markov Chain Monte Carlo approach (WinBUGS, Imperial College & Medical Research Council, London, UK, Version 1.4, 2003).

## RESULTS

#### *Baseline characteristics*

A total of 72 980 records of examinees were captured electronically, including 17 866 in Moldova and 55 114 in Uganda. In Moldova, the study period was the entire year of 2003 and in Uganda the 3 years from 1999 through 2001. Of these, 66 317 (90.9%) were retained for analysis, while 6663 (9.1%) examinees had to be excluded because they had no reason for the examination recorded or had a nonsensical sequence of results (e.g., the first result not recorded, followed by a valid result). The characteristics by age group and sex in each country of the retained examinees are summarized in Table 1. The proportion of examinees who were TB suspects was similar in Moldova and Uganda (between 70% and 75%). Among those for whom the sex was known, there was a male preponderance in both countries, the female-to-male ratio being 0.53 in Moldova and 0.87 in Uganda. Examinees were more than 10 years older in Moldova (mean 45.1 and median 46 years) than in Uganda (mean 34.5 and median 32 years).

#### *Proportion of TB cases among suspects*

To test the hypothesis, the analysis was limited to TB suspects. The characteristics are shown in Table 2. In Moldova, 9.1% met the definition of a study TB case compared to 20.2% in Uganda. In both countries, the yield of TB cases was lower among female than among male suspects. The age distribution in the two countries was similar among both suspects and all examinees. In both countries, TB cases were young: the median age among females was respectively 33.0 and 28.0 years in Moldova and Uganda. Among male TB cases the respective median age was 40.5 and 32.0 years.

#### *Usefulness of third smear*

Table 3 summarizes the patterns found among serial sputum smear examinations among TB suspects. In both countries, a similar proportion was identified already with the first sputum smear examination (87.9%

<sup>\*</sup> Freely available from [www.epidata.dk](http://www.epidata.dk)

<sup>†</sup> Freely available from [www.openoffice.com](http://www.openoffice.com)

**Table 1** Proportion of suspects among examinees with known reason for examination, Moldova (2003) and Uganda (1999–2001)

|                  | Moldova   |          |                       |                   | Uganda    |          |                       |                   |
|------------------|-----------|----------|-----------------------|-------------------|-----------|----------|-----------------------|-------------------|
|                  | Diagnosis |          | Follow-up<br><i>n</i> | Total<br><i>N</i> | Diagnosis |          | Follow-up<br><i>n</i> | Total<br><i>N</i> |
|                  | <i>n</i>  | Fraction |                       |                   | <i>n</i>  | Fraction |                       |                   |
| Total            | 12 525    | 0.707    | 5 183                 | 17 708            | 36 054    | 0.742    | 12 555                | 48 609            |
| Sex              |           |          |                       |                   |           |          |                       |                   |
| Female           | 4 811     | 0.781    | 1 346                 | 6 157             | 16 145    | 0.760    | 5 097                 | 21 242            |
| Male             | 7 713     | 0.668    | 3 835                 | 11 548            | 17 575    | 0.717    | 6 949                 | 24 524            |
| Unknown          | 1         | 0.333    | 2                     | 3                 | 2 334     | 0.821    | 509                   | 2 843             |
| Age group, years |           |          |                       |                   |           |          |                       |                   |
| 0–14             | 541       | 0.759    | 172                   | 713               | 1 644     | 0.828    | 342                   | 1 986             |
| 15–24            | 1 092     | 0.630    | 642                   | 1 734             | 4 900     | 0.700    | 2 096                 | 6 996             |
| 25–34            | 1 481     | 0.589    | 1 032                 | 2 513             | 9 513     | 0.713    | 3 829                 | 13 342            |
| 35–44            | 2 113     | 0.619    | 1 299                 | 3 412             | 6 034     | 0.701    | 2 568                 | 8 602             |
| 45–54            | 2 931     | 0.712    | 1 188                 | 4 119             | 3 225     | 0.730    | 1 194                 | 4 419             |
| 55–64            | 1 936     | 0.792    | 508                   | 2 444             | 1 899     | 0.777    | 545                   | 2 444             |
| 65+              | 2 371     | 0.883    | 314                   | 2 685             | 1 344     | 0.811    | 313                   | 1 657             |
| Unknown          | 60        | 0.682    | 28                    | 88                | 7 495     | 0.818    | 1 668                 | 9 163             |

in Moldova and 87.3% in Uganda). In Moldova, 3.7% and in Uganda 2.7% of all TB cases were identified with the third examination only.

Taking into account both the prevalence of TB cases among suspects and the incremental gain, the overall yield of TB cases found among suspects with the third smear examination was less than 0.4% in Moldova, corresponding to a point estimate of 273.2 slides required for each additional TB case. In Uganda, the proportion was less than 0.6%, corresponding to 174.9 slides. Given the small numbers required to arrive at the point estimate, the uncertainty was evaluated using a Bayesian approach. These results are summarized in the Figure. Clearly, the number of smears required to identify one additional case of sputum smear-positive TB on a third serial smear examination is in excess of the consensus in both countries. In Moldova, even the second smear was above the critical value.

## DISCUSSION

This study demonstrated that the efficiency of a third serial sputum smear examination was very poor in both Moldova and Uganda. The number of examinations required to identify an additional smear-positive TB case exceeded by far what the responsible authorities in both countries had formulated as an acceptable maximum to make three serial smears a routine requirement. These findings complement recent studies in other countries using a similar approach to determine the effectiveness of serial sputum smear examinations.<sup>12</sup>

The number of examinations required to identify one TB case depends on the prevalence of TB cases among suspects and on the incremental gain from serial smears, which vary in different settings. In rural Tanzania, the average proportion of TB cases found

**Table 2** Proportion of TB cases among suspects, Moldova (2003) and Uganda (1999–2001)

|                  | Moldova  |          |                          |                   | Uganda   |          |                          |                   |
|------------------|----------|----------|--------------------------|-------------------|----------|----------|--------------------------|-------------------|
|                  | TB cases |          | Non-TB cases<br><i>n</i> | Total<br><i>N</i> | TB cases |          | Non-TB cases<br><i>n</i> | Total<br><i>N</i> |
|                  | <i>n</i> | Fraction |                          |                   | <i>n</i> | Fraction |                          |                   |
| Total            | 1141     | 0.091    | 11 384                   | 12 525            | 7 280    | 0.202    | 28 774                   | 36 054            |
| Sex              |          |          |                          |                   |          |          |                          |                   |
| Female           | 251      | 0.052    | 4 560                    | 4 811             | 2 810    | 0.174    | 13 335                   | 16 145            |
| Male             | 890      | 0.115    | 6 823                    | 7 713             | 4 111    | 0.234    | 13 464                   | 17 575            |
| Unknown          | 0        | 0.000    | 1                        | 1                 | 359      | 0.154    | 1 975                    | 2 334             |
| Age group, years |          |          |                          |                   |          |          |                          |                   |
| 0–14             | 6        | 0.011    | 535                      | 541               | 165      | 0.100    | 1 479                    | 1 644             |
| 15–24            | 151      | 0.138    | 941                      | 1 092             | 1 343    | 0.274    | 3 557                    | 4 900             |
| 25–34            | 259      | 0.175    | 1 222                    | 1 481             | 2 236    | 0.235    | 7 277                    | 9 513             |
| 35–44            | 338      | 0.160    | 1 775                    | 2 113             | 1 255    | 0.208    | 4 779                    | 6 034             |
| 45–54            | 256      | 0.087    | 2 675                    | 2 931             | 627      | 0.194    | 2 598                    | 3 225             |
| 55–64            | 80       | 0.041    | 1 856                    | 1 936             | 290      | 0.153    | 1 609                    | 1 899             |
| 65+              | 46       | 0.019    | 2 325                    | 2 371             | 144      | 0.107    | 1 200                    | 1 344             |
| Unknown          | 5        | 0.083    | 55                       | 60                | 1 220    | 0.163    | 6 275                    | 7 495             |

TB = tuberculosis.

**Table 3** Pattern of serial sputum smear examinations among suspects, missed tuberculosis (TB) cases, corrected proportion of TB cases among suspects and incremental yield from serial smears, Moldova (2003) and Uganda (1999–2001)

|                                        | Moldova | Uganda |
|----------------------------------------|---------|--------|
| Observed pattern, <i>n</i>             |         |        |
| Total                                  | 12 525  | 36 054 |
| NNN                                    | 8 424   | 14 736 |
| NN9                                    | 1 579   | 3 325  |
| N99                                    | 1 381   | 10 713 |
| Px                                     | 1 023   | 6 686  |
| NPx                                    | 84      | 487    |
| NNP                                    | 34      | 107    |
| Missed TB cases, %*                    |         |        |
| Second examination                     | 11.5    | 279.7  |
| Third examination                      | 11.9    | 99.2   |
| Expected yield (fraction) <sup>†</sup> |         |        |
| Observed + missed TB cases             | 1.000   | 1.000  |
| Expected from first                    | 0.879   | 0.873  |
| Expected from second                   | 0.082   | 0.100  |
| Expected from third                    | 0.039   | 0.027  |

\* Number of estimated TB cases missed among those who never turned up for the 2nd and 3rd examinations, assuming the same proportion of TB cases as in those who turned up for the 2nd and 3rd examinations, respectively.

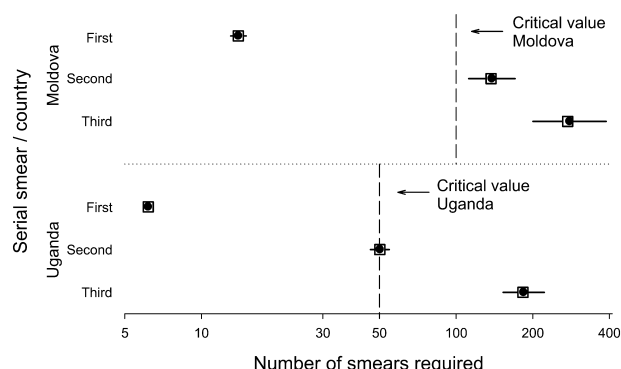
<sup>†</sup> The proportion of TB cases expected if each suspect had a complete set of three examinations.

TB = tuberculosis; NNN = all three smears examined and negative; NN9 = first two smears examined and negative, third not examined; N99 = first smear examined and negative, second and third not examined; Px = first smear-positive; NPx = first smear negative, second positive; NNP = first and second smear negative, third positive.

among suspects was 19%.<sup>3</sup> In a multinational study conducted in Benin, Malawi, Nicaragua and Senegal, the proportion of TB cases found among suspects was 32%, 17%, 5% and 19%, respectively,<sup>4</sup> highlighting the wide variations in different settings. The proportion of TB cases among suspects was more than twice as high in Uganda as in Moldova. This could be partly explained by the high prevalence of TB<sup>9,13</sup> and the lower population coverage by TB laboratories (1/100 000) in Uganda compared to Moldova (1/80 000). The low TB laboratory distribution in Uganda could have caused suspects to have difficulty in accessing diagnostic services, thus leading to delays in diagnosis or TB cases being missed completely. On the other hand, a higher distribution of TB laboratories per population in Moldova may result in better access to services and therefore a lower prevalence of positive results. The difference in proportion of TB cases found in suspects among countries emphasizes the need to conduct locally based studies to determine the usefulness of the three serial smear examinations recommended by the WHO and The Union before excluding smear-positive pulmonary TB.<sup>1,2</sup>

The full potential of sputum smear microscopy can only be determined if technicians are blind to the result of the other examination.<sup>14</sup> This is not the case in routine laboratory work, which was evaluated in the present study. Nevertheless, the findings from this study portray accurately what clinicians might expect from laboratory results in the two countries.

In both settings, only a small fraction of all TB cases

**Figure** Point estimates and 95% credibility interval (CI) for the number of suspects to be examined to identify one additional case of tuberculosis (TB) with serial smears, Moldova and Uganda. Filled circles are means, hollow squares medians with 95% CI shown as solid horizontal lines. Vertical dashed lines indicate critical values for Moldova (100 smear examinations) and Uganda (50 smear examinations) to identify one additional case on a third serial smear examination.

(less than 4%) were identified with the third examination, corresponding to 1–3 weeks of a full-time microscopist to identify one additional case with the third serial smear examination. The number of slides significantly exceeded the critical values set by technical experts and policy makers in the two settings, findings not dissimilar to those in studies conducted elsewhere.<sup>3–8</sup> In Moldova, even the work required to identify one additional TB case with a second smear examination exceeded the predetermined critical value. As no cost-effectiveness evaluation was performed, the opinion of local experts was sought. The availability of resources and the costs per TB case identified varies greatly. It therefore seems most appropriate to determine the appropriate policy locally instead of issuing global blanket recommendations. As the prevalence of TB cases among suspects is also likely to change within a country as the programme expands, a re-evaluation might be deemed appropriate.

The estimate of the number of slides that need to be examined to identify a TB case with the third smear examination may have been limited by the fact that no quality assurance system for sputum microscopy existed in the settings where data were gathered. Quality control could have increased, decreased or not affected the estimate.

In Moldova and Uganda, approximately 96% and 97%, respectively, of pulmonary TB cases were diagnosed using two sputum smears. A full-time microscopist would require on average 11 days (8–15) and 8 days (7–9), respectively, in Moldova and Uganda to diagnose one additional case of TB with a third smear. The results of this study emphasize the importance of national studies to adapt policy to available resources.

It will be essential for both countries to implement an external quality assurance system for microscopy.<sup>15</sup>

Such a system has been shown to improve the quality of sputum smear microscopy. Combined with a reduced workload in terms of the number of smears that have to be examined, more diligence might be devoted to the remaining smear examinations if fortified by external quality assurance.

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### RÉSUMÉ

**CONTEXTE :** Respectivement 24 et 30 laboratoires de microscopie de la tuberculose (TB) en Moldavie et en Ouganda.

**OBJECTIF :** Estimer la charge de travail nécessaire pour identifier un cas supplémentaire de TB grâce à un troisième examen en série des frottis d'expectoration.

**MÉTHODES :** Etude rétrospective dans les registres de laboratoire pour déterminer la prévalence et le rendement supplémentaire de cas de TB provenant de l'examen d'un troisième frottis d'expectoration en série parmi les suspects en Moldavie et en Ouganda, la réciproque du produit de ces deux fractions fournissant le nombre d'exams requis pour identifier un cas supplémentaire de TB.

**RÉSULTATS :** En Moldavie, 9% (1141/12 525) et en

Ouganda 20% (7280/36 054) des suspects ont répondu à la définition de cas de TB, c'est à dire au moins un frottis d'expectoration positif. Le rendement supplémentaire du troisième examen a été de 4% en Moldavie et de 3% en Ouganda. Pour détecter un cas supplémentaire de TB sur un troisième frottis, 273 exams (IC95% 200–389) sont nécessaires en Moldavie et 175 (IC95% 153–222) en Ouganda. Ceci correspond respectivement à une moyenne de 11 jours (8–16) et de 7 jours (6–9) pour diagnostiquer un cas supplémentaire de TB.

**CONCLUSION :** Dans les deux pays, le troisième examen de frottis d'expectoration en série s'est avéré inefficace pour le diagnostic d'une TB à bacilloscopie positive des expectorations.

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**RESUMEN**

**MARCO DE REFERENCIA:** Veinticuatro laboratorios de microscopia en Moldavia y 30 laboratorios en Uganda.

**OBJETIVO:** Estimar la carga de trabajo implicada en la detección de un caso adicional de tuberculosis (TB) con el examen de la tercera muestra seriada de esputo.

**MÉTODOS:** Estudio retrospectivo de los registros de laboratorio, con el fin de determinar dos datos: la prevalencia y el incremento del rendimiento diagnóstico de casos de TB, con el examen de la tercera muestra seriada de esputo, en los presuntos casos de TB en Moldavia y Uganda. El inverso del producto de estos dos datos aporta el número de exámenes necesario a fin de detectar un caso adicional de TB.

**RESULTADOS:** En Moldavia 9% (1141/12 525) y en

Uganda 20% (7280/36 054) de las personas con presunción diagnóstica cumplieron con la definición de caso, es decir como mínimo una baciloscopia positiva. El incremento del rendimiento diagnóstico con la última muestra fue del 4% en Moldavia y del 3% en Uganda. De esta manera, la detección de un caso adicional de TB con la tercera muestra de esputo precisó 273 exámenes (IC95% 200–389) en Moldavia y 175 (IC95% 153–222) en Uganda. Esto corresponde a un promedio de 11 días de trabajo (8 a 16) en Moldavia y a 7 días (6 a 9) en Uganda con el fin de diagnosticar un caso adicional de TB.

**CONCLUSIÓN:** En ambos países, la tercera muestra seriada de esputo fue ineficiente en el diagnóstico de casos de TB con baciloscopia positiva.

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