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Improving the laboratory diagnosis of TB in Ghana: the impact of a quality assurance system

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SUMMARY

SETTING: Greater Accra region, Ghana.

OBJECTIVE: To establish a pilot quality assurance (QA) system in sputum smear microscopy and to evaluate its impact.

DESIGN: Quarterly supporting visits were paid to participating laboratories between 2000 and 2002. Fifteen examined slides were selected randomly from each laboratory during the visits and blindly re-assessed. Feedback was given promptly to the various laboratories. Training and stakeholder workshops were organised whenever necessary.

RESULTS: General improvements in smear preparation and staining as well as the reading ability of the laboratory personnel included in the study were observed. The average marks for specimen quality, staining ability, smear cleanness, thickness, size and evenness increased from 64%, 79%, 69%, 46%, 67% and 60% in the last quarter of 2000 to 81%, 90%, 86%, 79%, 80% and 74%, respectively, 24 months after the establishment of the QA system. Within the same period, the rate of false-positives and -negatives decreased from respectively 14.8% and 20.5% to 0%, and agreements in positivity grade increased from 74% to 95%. The performance of the participating laboratories in keeping the laboratory registers up to date also improved.

CONCLUSION: The QA system needs to be extended to the rest of the country.

KEY WORDS: quality assurance; pilot system; sputum smear microscopy; Ghana

INFECTIOUS DISEASES, including tuberculosis (TB), remain the largest cause of illness and death in the world. In Ghana, the National Tuberculosis Programme (NTP), established in 1994 to control illness and deaths associated with TB, uses the DOTS strategy. Within this framework of TB control, the identification and treatment of infectious TB cases is the priority.

Sputum smear microscopy is the main method of diagnosis in Ghana. The purpose of smear microscopy is three fold: 1) to diagnose patients with infectious TB, 2) to monitor the treatment progress of individual patients, and 3) to document cure at the end of treatment.[1,2] For the diagnosis of cases in Ghana, three repeated sputum specimens are collected from each TB suspect: one on-the-spot, followed the next day by one early morning and another on-the-spot. Pulmonary tuberculosis (PTB) treatment is usually initiated when two of the specimens are smear-positive or one is smear-positive but X-ray and/or clinical findings are suggestive of PTB. For follow-up cases, only one specimen, preferably early morning, is collected at the second (repeated at month 3, if smear is still positive at month 2), fifth and eighth month.[3]

The advantages of smear microscopy are that it is rapid, cost-effective and requires little in the way of equipment. In addition, it detects most infectious cases. The disadvantages, however, are that it is dependent on the quality of the sputum and the staining technique and reading ability of the microscopist. To maintain a reliable laboratory service that will facilitate accurate diagnosis and offset the disadvantages of using smear microscopy, a well functioning quality assurance (QA) system is essential to ensure that information generated by the laboratory is accurate, reliable and reproducible.[4]

The three main components of QA are: 1) quality control/internal quality assurance (IQA), a process by which each laboratory systematically and effectively monitors its own performance; 2) external quality

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assessment (EQA), a process whereby laboratory performance is assessed by external assessors; and 3) quality improvement (QI), which involves continuous monitoring and identification of defects/errors followed by remedial action.[4]

A nationwide evaluation of the public laboratories that were performing smear microscopy was conducted previously (Addo K K, Owusu-Darko K, Dan-Dzide M, et al., country report submitted to the Japan International Cooperation Agency under the Infectious Diseases Project, 2003). The quality of the laboratories was assessed in terms of manpower, technical skills, logistics and biosafety using a structured questionnaire. The study recommended the establishment of a nationwide QA system for smear microscopy to improve TB diagnostic services. However, implementation of a countrywide QA system can be successful only after a pilot system has been initiated. A successful pilot system can then be replicated in other laboratories nationwide.

We report here the results of a study evaluating the impact of setting up a pilot QA system on the performance of peripheral laboratories performing smear microscopy in the Greater Accra Region of Ghana.

METHODS

Study area

This study covers the Greater Accra Region, where the national capital, Accra, is located. The region is made up of five districts, with a population of over 4 million. The region was chosen based on its proximity to the National Tuberculosis Reference Laboratory (NTBRL). The study was conducted in the Accra and Tema districts of the region, where all 12 of the public TB diagnostic centres are situated, including the Korle-Bu Teaching Hospital (KBTH). The other districts are without diagnostic centres.

On-site evaluation or supporting visits

Quarterly visits to all participating laboratories were carried out between October 2000 and September 2002. Data were collected during the visits using a structured questionnaire about the laboratory situation in terms of availability of written standard operating procedures (SOPs), level of laboratory training of personnel, performance of IQA, availability of functional microscopes and other materials needed for TB microscopy, adequacy of consumables and reagents, prompt reporting of results, and observation of biosafety practices. The laboratory register was also checked to verify that all information had been recorded accurately and completely. Slide boxes were checked to see whether positive and negative slides on which xylene had been used to remove excess oil were stored away from direct sunlight and in the same order as they were listed in the laboratory register. Sufficient time was allotted for all the visits and corrective action and on-site training were offered where necessary.

Blinded re-checking

Slide sampling

Using a national average slide positivity rate of 20%, 15 examined slides per quarter per laboratory were randomly selected and blindly re-assessed in our laboratory. This was based on the 'Lot Quality Assurance System statistical sampling' method.[5] Minimal sensitivity was set at 80%. The slides were selected using the laboratory register by one of the study personnel during the quarterly visits. The total number of diagnostic and follow-up slides examined in the last quarter in each laboratory was divided by 15 to obtain the sample size. For example, if 120 slides were used in the last quarter, the number was divided by 15 to get 8, which means every eighth slide was sampled.

In some of the laboratories that had low slide positivity, the starting position for sampling was changed whilst maintaining the sampling interval to include some or all positive slides for re-checking.[4] When a slide was missing, the next slide identified in the laboratory register was substituted, irrespective of the result. In the private laboratory where fewer than 15 slides had been worked on in two quarters, all available slides were selected for re-assessment.

Slide evaluation

On arrival at the NTBRL, the slides were evaluated blind by different study personnel who acted as the first assessor. Each slide was evaluated according to the International Union Against Tuberculosis and Lung Disease/World Health Organization recommended six parameters of smear preparation:[2] 1) specimen quality—indicated by the presence of dust cells/macrophages and/or other white blood cells; a smear of good quality should have >25 leucocytes/field at a total magnification of 100×; 2) staining—to check whether smear is understained (acid-fast bacilli [AFB] in faint red colour) or overstained (red colour background); AFB covered with dark colour are also an indication of excessive counterstaining; 3) cleanness—to check whether the smear is free from stain deposit, dirt (artefacts) and crystals produced by overheating of the stain, debris, etc; 4) thickness—to check whether the whole depth of the smear is focused sharply in each field; 5) evenness—to check whether smear is evenly spread in a repeated, small, coil-like pattern on the slide, not too thick and not too thin; 6) size—to check whether the smear is between the appropriate size of 1 × 2 and 2 × 3 cm. All the centres used the Ziehl-Neelsen (ZN) technique: 0.3% carbolfuchsin for 5 min, 20% sulphuric acid as a decolouriser for 5 min and 0.3% methylene blue as a counterstain for 1 min.

Slide reading

The slides were re-read by the first assessor and results were compared with those of the participating laboratory. Interpretation of the results was based on the following: 1) overall agreement—consistency in smear-negative and smear-positive results; 2) false-positive—the result by the assessor was negative but misread by the microscopist as positive; 3) high false-positive—a negative smear was misread by the microscopist as 1+ to 3+; 4) low false-positive—a negative smear was misread by the microscopist as a scanty positive (1–9 AFB/100 fields); 5) false-negative—the result by the assessor was positive but was misread as negative by the microscopist; 6) high false-negative—a high-positive smear (1+ to 3+) was misread as negative; 7) low false-negative—a scanty positive smear was misread as negative by the microscopist; and 8) quantification error—a difference of more than one grade between the assessor and the microscopist in grading a positive smear.

Discordant slides

Discrepancies between the results of the first assessor and the microscopist at the participating laboratory were resolved by a second assessor. Because stained bacilli can fade in certain conditions (e.g., exposure to sunlight and high humidity with high temperatures),[6] all false-positive slides and slides with quantification errors were re-stained and re-read by decolourising the slides with 20% sulphuric acid and re-staining with ZN.

Feedback/follow-up visits

Prompt feedback was given to the participating laboratories and results of blinded re-checking were discussed with the personnel. Discordant slides were returned to the original laboratory and re-read by the microscopists. This gave them the opportunity to re-examine the slides and correct earlier errors.[7] Other aspects of poor performance were also investigated on-site by identifying the possible causes and finding remedies.

Training and stakeholder workshops

During the follow-up visits, laboratory personnel were trained on-site. Formal training workshops were also periodically organised at the NTBRL. A stakeholder workshop was held quarterly to discuss study progress, including problems encountered during the visits to laboratories and any remedial actions needed.

Data analysis

All data were analysed using SPSS (SPSS Inc, Chicago, IL, USA) and Microsoft Excel (Microsoft, Palisade Corp, Newfield, NY, USA).

RESULTS

The establishment of the QA system in the Greater Accra Region has led to the recognition by the laboratory personnel that they have a crucial role to play in TB control. The number of laboratories initially identified as performing smear microscopy was nine. Within 3 months of the study, this number increased to 12, with the inclusion of a private laboratory. At the start of the study, none of the participating laboratories was practising IQA, but this changed 6 months into the study. NTP-approved SOPs were pasted in all the laboratories for easy reference, staining reagent containers were properly labelled with the date of preparation and expiration, new batches of stains prepared by the regional technologist and distributed to the participating laboratories were tested against their performance with control slides, and carbolfuchsin and methylene blue were filtered regularly to remove crystals. Microscopes were maintained by cleaning objective lenses with lens paper or soft cloth and covered with plastic cases or stored in cleaned, dry and stable lockers at the end of each work day. In laboratories with high humidity, a 15–20 watt bulb was installed in the lockers to prevent fungal growth. A rubber blower was used in most facilities to remove dust on microscope eyepieces, condensers and stages before storage. Objective lenses (×100) were replaced in three centres and a new microscope was given to a centre where the microscope could not be repaired. Biosafety measures, such as proper handling of sputum and cleaning of work benches with phenol-based disinfectants, were employed. At first it was not possible to obtain data from any of the participating laboratories due to poor documentation practices. However, this has changed, as laboratory registers are now filled out completely and accurately.

Slide labelling has also improved, as lead pencils are used to label frosted slides and diamond pencils to label non-frosted slides. Examined slides are now cleaned with xylene and properly stored for re-checking in all laboratories.

The on-site and two formal training courses conducted for 24 participating laboratory personnel were beneficial, as there has been a general improvement in smear preparation techniques and in the reading abil-

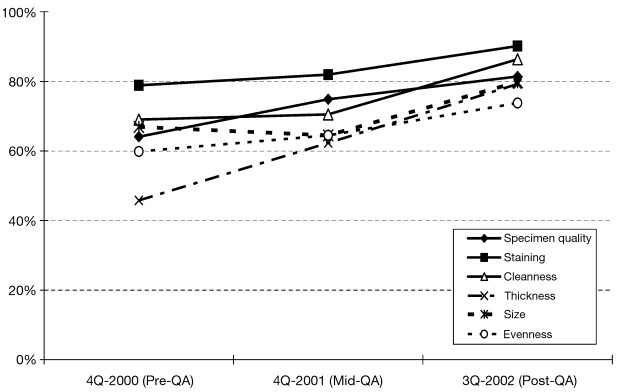

Figure 1 Assessment of smear preparations. 4Q = fourth quarter; QA = quality assessment; 3Q = third quarter.

Table High and low positive/negative readings by microscopist per quarter

Year	False positive readings		False negative readings		Total slides selected (n = 180)*		Total false slides	
	High	Low	High	Low	Positive	Negative	False Positive	False Negative
2001								
First quarter	3	5	7	9	95	85	8	16
Second quarter	3	4	5	7	88	92	7	12
Third quarter	2	4	4	5	90	90	6	9
Fourth quarter	2	3	3	5	96	84	5	8
2002								
First quarter	1	2	2	3	89	91	3	5
Second quarter	0	1	0	1	94	86	1	1
Third quarter	0	0	0	0	87	93	0	0

* n = total number of slides selected per quarter (15 slides/centre × 12).

ity of the microscopists. In the last quarter of 2000 (Pre-QA), the average scores for specimen quality, staining ability, smear cleanness, thickness, size and evenness were 64%, 79%, 69%, 46%, 67% and 60%, respectively; these rose to 81%, 90%, 86%, 79%, 80% and 74%, respectively, at the third quarter of 2002, 2 years after the establishment of the QA system (Post-QA) (Figure 1). Within the same period, the rate of false-positives/negatives decreased from 14.8% and 20.5%, respectively, to 0% for both. Although false readings were observed, most of them were low false-positive/negative (Table). The overall smear-positive/negative agreements and agreement in positive grading also increased from 85% to 100% and from 74% to 95%, respectively (Figure 2).

DISCUSSION

Smear microscopy is the most cost-effective method of diagnosing PTB suspects reporting to health institutions. It is used to identify infectious cases of disease, assess response to treatment and monitor cure rates.[1,2] However, in most diagnostic centres in Ghana, smear microscopy is usually performed by personnel with minimal training. Functional QA of their work is therefore essential. QA is a system designed to continuously improve the reliability, efficiency and use of microscopy as a diagnostic and monitoring tool. This is very important for the management of TB cases, as suspects are not treated unless diagnosed in the laboratory. In the current study, the number of centres performing smear microscopy, including private laboratories, increased by 25% within one quarter. This was a very important development, as the increase in diagnostic centres led to increases in case detection in the study areas from 43.5% to 53%,[8] and the workload of other centres, especially the teaching hospital, was reduced. IQA practices, such as testing of new stains and regular filtering, contributed to the reduction in false-positive/negative results. Safety measures for handling sputum specimens observed in all the laboratories, such as: 1) sputum collection in the open air instead of in the washroom; 2) frequent hand washing; 3) careful opening of sputum containers to avoid splashes, which can create aerosols; and 4) the use of the rough end of wooden applicators instead of wire loops (which helps prevent the formation of aerosols that could occur during the flaming of the loop) to make smears, have aided in ensuring the safety of laboratory personnel and patients.

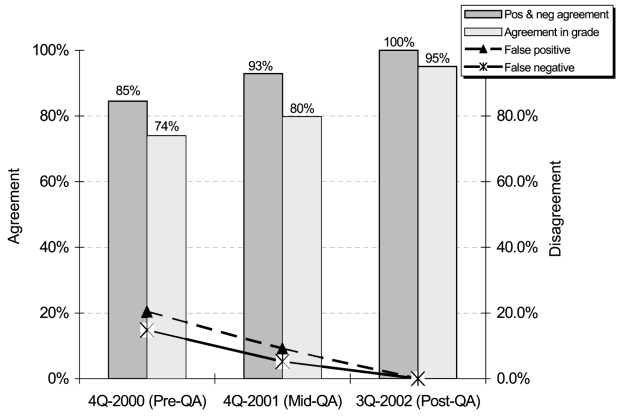

Figure 2 Assessment of reading ability. 4Q = fourth quarter; QA = quality assessment; 3Q = third quarter; Pos = positive; neg = negative.

The improvement in slide labelling and storage has facilitated the random selection of examined slides for re-assessment.

Prompt reporting of smear results by microscopists to requesting officers has also facilitated early treatment for newly diagnosed cases and rapid decision making as to whether to continue or to change the patient's drug regimen in follow-up cases.

One major obstacle of this study was the issue of rotation or transfer of staff, where eight laboratory personnel trained in smear microscopy were put on other benches, while two were transferred to other laboratories outside the study area. This was evident in the fourth quarter of 2001 (mid-QA), when the smear size level dropped from the fourth quarter of 2000 level of 67% to 64% (Figure 2). Investigations

during follow-up visits revealed that the smears in two of the participating laboratories were being prepared by two new technicians who had been transferred to the laboratories. This indicates a need to train all laboratory personnel in the country in smear microscopy.

There are many different methods for conducting QA in smear microscopy. None of these methods had been studied in Ghana to ascertain their feasibility. The present study was intended to serve as a model for future nationwide QA implementation. Our findings clearly indicate that supporting visits, which act as motivation for laboratory personnel, on-site and formal training, blinded rechecking of examined slides and timely feedback lead to improvements in TB laboratory services. We therefore recommend that this system be extended to the rest of the country.

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RÉSUMÉ

CONTEXTE : Région Greater-Accra du Ghana.
OBJECTIF : Mettre en place un système pilote d'assurance qualité (AQ) en microscopie des frottis de crachat et évaluer son impact.
SCHÉMA : Des visites d'assistance trimestrielles ont été effectuées aux laboratoires participants entre 2000 et 2002. Quinze lames déjà examinées ont été sélectionnées au hasard dans chaque laboratoire au cours des visites et ont été évaluées à l'aveugle. Les résultats ont été envoyés immédiatement aux laboratoires. Des ateliers de formation et des partenaires ont été organisés selon les besoins.
RÉSULTATS : Une amélioration générale dans la préparation et la coloration des frottis ainsi que la capacité de lecture du personnel du laboratoire inclus dans l'étude a été remarquée. Ainsi, au dernier trimestre de 2000, les points moyens pour la qualité du spécimen, la qualité de la coloration, la propreté, l'épaisseur, la dimension et la régularité des frottis étaient respectivement de 64%, 79%, 69%, 46%, 67% et 60%, mais ces points ont augmenté respectivement à 81%, 90%, 86%, 79%, 80% et 74%, 24 mois après la mise en œuvre du système AQ. Pendant la même période, les taux des faux positifs et faux négatifs ont diminué de 14,8% et 20,5% respectivement à 0%, et la concordance du degré de positivité a augmenté de 74% à 95%. La performance des laboratoires participants sur le plan de la documentation administrative a aussi connu une amélioration, puisque les registres des laboratoires ont été remplis complètement et avec précision.
CONCLUSION : Il y a un besoin d'expansion du système AQ pour couvrir le reste du pays.

RESUMEN

MARCO DE REFERENCIA : La región del Gran Accra en Ghana.
OBJETIVO : Establecer un sistema experimental de control de la calidad (AQ) para el examen microscópico del esputo y evaluar su repercusión práctica.
MÉTODOS : Se realizaron visitas trimestrales de apoyo a los laboratorios participantes entre 2000 y 2002. En cada visita, se escogieron aleatoriamente 15 frotis examinados por cada laboratorio y se llevó a cabo una segunda lectura en forma anónima. El resultado de la reevaluación se transmitió prontamente a los diversos laboratorios y se organizaron talleres de adiestramiento para el personal de laboratorio y de discusión con las partes interesadas, cuando se consideró necesario.
RESULTADOS : Se observó una mejoría global en la preparación de los frotis y las coloraciones y en la capacidad de lectura del personal de los laboratorios participantes. En el último trimestre de 2000 la calificación promedio fue del 64% para la calidad de la muestra, del 79% para la coloración, del 69% para la limpieza del

frotis, del 46% para su espesor, del 67% para su tamaño y del 60% para su homogeneidad. Las calificaciones mejoraron respectivamente al 81%, 90%, 86%, 79%, 80% y al 74%, 24 meses después de la instauración del sistema de AQ. En el mismo lapso, las tasas de falsos positivos del 14,8% y de falsos negativos del 20,5% disminuyeron al 0% y la concordancia en el grado de positividad aumentó del 74% al 95%. Asimismo, se observó una mejoría en la documentación de los laboratorios, pues los registros se llenaron integralmente y de manera exacta.
CONCLUSIÓN: Es necesario extender el sistema de AQ de la baciloscopia al resto del país.