INT J TUBERC LUNG DIS 9(10):1127–1133


# Performance of variations of carbolfuchsin staining of sputum smears for AFB under field conditions

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**SUMMARY**

**SETTING: A field project in Bangladesh.**
**OBJECTIVE: To compare the effectiveness of commonly used carbolfuchsin staining variations.**
**DESIGN: Routine hot Ziehl-Neelsen (ZN) 1% basic fuchsin staining for 15 min in 75 field clinics. Blind reading of duplicate smears stained by ZN 1% vs. 0.3% basic fuchsin applied for 5 min, or by ZN 1% 5 min vs. Kinyoun cold staining. Rechecking of discordant series.**
**RESULTS: For comparable numbers of false positives, sensitivity was significantly lower with Kinyoun than with ZN 1% 5 min (85.6% vs. 93.0%, $P < 0.001$). Sensitivity with ZN 1% 5 min was not significantly higher than with 0.3% 5 min staining (89.9% vs. 86.5%). Routine examination using 1% 15 min ZN identified more positives than any of the study techniques.**
**CONCLUSIONS: Kinyoun cold staining sensitivity was unsatisfactory in field clinics. The sensitivity of the WHO/IUATLD recommended 0.3% fuchsin for 5 min was not significantly different from the original 1% ZN for 5 min, but 1% 15 min hot staining might be superior. A reduced fuchsin concentration together with a short staining time may leave too narrow a margin for error. TB programmes using hot ZN with a concentrated stain or longer staining time should not be urged to change.**
**KEY WORDS:** Ziehl-Neelsen; carbolfuchsin; microscopy; staining

SPUTUM SMEAR MICROSCOPY for acid-fast bacilli (AFB) remains the most important diagnostic method for tuberculosis (TB) in high prevalence countries. Because of its speed, its high specificity even under adverse conditions, and its efficiency in detecting the main sources of transmission, it constitutes one of the pillars of the DOTS strategy.[1] The technical guidelines published by the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) stick to the original Ziehl-Neelsen (ZN) technique with heating of carbolfuchsin stain on the smear (hot method), but deviate from the original stain composition in specifying a 0.3% rather than a 1% concentration of basic fuchsin.[2–4] Cold staining methods, using a concentrated stain or prolonged staining time, are common in industrialised countries when carbolfuchsin staining is still used. They have also been adopted in a number of National Tuberculosis Programmes (NTPs), against the advice of the guidelines[3,5] and despite warnings about the greater risk of error.[6] The effect of restaining on detection of false-negative errors has been interpreted as indicative of poor performance of a routine cold staining method.[7] Other NTPs still apply the original 1% fuchsin concentration, with heating.

Kinyoun described his cold staining method as long ago as 1915.[8] However, this was essentially a bleach concentration method, probably the reason why it yielded more positives than ZN. Furthermore, he used a stain with a high (3.1%) basic fuchsin and phenol concentration (6.25%), which made staining for only 5 min without heating possible. Tan Thiam Hok introduced an even simpler and faster technique which has also been widely accepted, combining 3 min Kinyoun cold staining with combined destaining/counterstaining.[9] Its liability to error has been reported.[10] Devulder contradicted Tan Thiam Hok's claim of higher sensitivity and reported very pale AFB which could be improved by applying heat or by staining for 3 h.[11] In a comparative study organised by the International Union Against Tuberculosis (IUAT), both of his methods visualised more AFB than 1% fuchsin stains and/or a shorter staining time.[12] A WHO comparative study, investigating simpler or faster staining methods, reported three cold staining methods to be clearly inferior to ZN.[13] Several other studies came to the same

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*Article submitted 13 February 2005. Final version accepted 2 April 2005.*

[A version in French of this article is available from the Editorial Office in Paris and from the Union website www.iuatld.org]

conclusion,[14–16] while a few concluded an equivalence based on inadequate numbers or poorly defined ZN techniques.[17–19] More errors with cold staining have also been reported from quality assurance programmes.[20,21]

No comparative studies showing an at least equivalent efficacy of the 0.3% fuchsin stain seem to have been undertaken prior to its adoption in the guidelines. The earliest recommendation found for this lower concentration was based on more solid staining of AFB with some types of basic fuchsin, yielding a granular staining at higher concentration.[22] A cleaner smear has also been mentioned as an advantage,[23] possibly because of the poor solubility of some fuchsin brands.[22] The 0.3% 5 min staining seems to have been selected for the IUATLD guide because it was the technique used at the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA (S R Pattyn, personal communication). This is even more remarkable given that the IUAT-initiated comparisons almost simultaneously documented increased numbers of AFB with higher fuchsin concentrations, prolonged staining time and heating, without even considering a 0.3% stain.[12]

A recent study conducted at the Tuberculosis Research Centre (TRC), Chennai, reported the ineffectiveness of a 0.3% compared to a 1% fuchsin stain, but may have been biased.[24,25] Another comparative study including fuchsin concentrations below 1% from the same TRC scientists, using a correct technique, concluded an equivalence of 0.3% and 1% concentrations.[26] The same study also compared a 0.1% fuchsin stain, using which about one third of positives were missed. Yegian et al. also reported poor staining on reducing the concentration of fuchsin to 0.15%.[22]

Because of these controversies between original

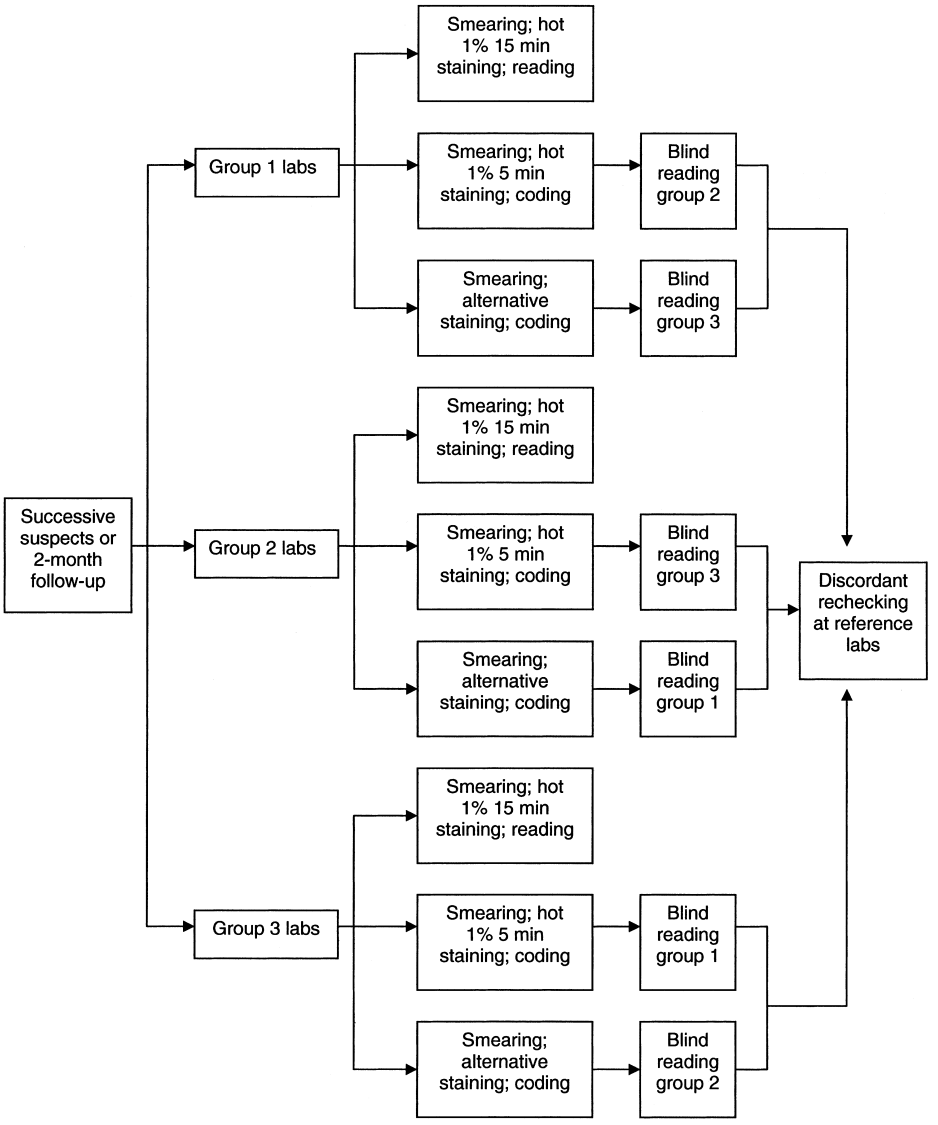


**Figure 1** Flow chart illustrating one phase of the study. Alternative staining: 0.3% 5 min hot staining during Phase 1, or Kinyoun cold staining during Phase 2.

**Table 1** Carbolfuchsin stains and staining techniques used

| | Variation | | | |
|---|---|---|---|---|
| | 1% 15 min ZN | 1% 5 min ZN | 0.3% 5 min ZN | Kinyoun |
| Composition | | | | |
| Basic fuchsin | 10 g | 10 g | 3 g | 25 g |
| Phenol | 50 g | 50 g | 50 g | 50 g |
| Denatured ethanol | 100 ml | 100 ml | 100 ml | 125 ml |
| Distilled water | 850 ml | 850 ml | 850 ml | 625 ml |
| Heating | Once till steam rises | Once till steam rises | Once till steam rises | Not used |
| Time left on the smear | 15 min | 5 min | 5 min | 5 min |

ZN = Ziehl-Neelsen.

technique, guidelines and routine NTP practices, we compared the effectiveness of 1) original ZN hot vs. Kinyoun cold staining and 2) hot staining with 0.3% vs. 1.0% basic fuchsin concentration routinely applied in a field setting.

## METHODS

The study was done in two phases. A flow chart illustrating the various steps for one phase is shown in Figure 1. Stain formulation and staining method details are summarised in Table 1.

All Damien Foundation Bangladesh field clinics continued to perform routine AFB smears according to NTP guidelines. Briefly, the specimen was spread evenly over a 2 × 1 cm area of a new slide, using the broken end of a single-use bamboo sliver, left to dry, and fixed over the flame of a spirit lamp. Hot 1% fuchsin stain was applied for 15 min (1% 15 min ZN). At the same time, a group of clinics prepared two more duplicate smears for 60 successive patients (suspects or 2-month follow-up), coded and stained them. One smear was stained hot with 1% fuchsin for 5 min (1% 5 min ZN), the other with the method under comparison (the same hot staining but using a 0.3% fuchsin stain during Phase 1 [0.3% 5 min ZN]; or 5 min 3% fuchsin cold staining during Phase 2 [Kinyoun]). Reading of these coded smears was done in two other groups of clinics blinded to the result and staining method. To compensate for reader bias, equal numbers of slides stained by each of the methods were read in each of the clinics. All microscopes used were Olympus CH series (Olympus, Tokyo, Japan), and in good condition. Routine readings covered 100 oil immersion fields (OIF) before being declared negative.

Supervisors assured blinding and overall coordination. Study smears of series with discordant results were sent to project reference laboratories for rechecking before and after restaining with 1% 15 min ZN. Using these readings as gold standard, errors were assigned using the internationally recommended classification,[27] but excluding readings below 1 AFB/100 OIF. Routine smears from the same sputum sample were considered for identification of discordant smears, but they were not rechecked. Destaining and counterstaining were always done using 20% sulfuric acid for 5 min and 0.1% methylene blue for 1 min.

All stains for the field were freshly prepared at the project's reference laboratories (Table 1), routinely using NTP-provided chemicals, including basic fuchsin powder manufactured by Loba, Bombay, India. Reference laboratories used New Fuchsin, Merck, Germany, for restaining carbolfuchsin. The Kinyoun cold staining method was performed according to the original publication,[8] resulting in about 3.1% fuchsin and 6.25% phenol in the stain.

Data were registered and analysed using Epi Info 6.04d (CDC, Atlanta, GA). Pearson's $\chi^2$ test was used for comparison of proportions; for paired data we used McNemar's $\chi^2$ test.

## RESULTS

Table 2 gives an overview of total smears examined and AFB detected by study phase and technique. In total, 4621 series of results were available for Phase 1, and another 4367 for Phase 2. AFB were detected by at least one technique in 618 Phase 1 and in 603 Phase 2 series, of which respectively 201 and 173 pairs were reread at the reference laboratories.

**Table 2** Series of smears examined and AFB positives (any quantification), by study phase and technique

| *n* | Phase 1* | Phase 2† |
|---|---|---|
| Total series examined | 4621 | 4367 |
| 1% 15 min ZN‡ positives | 521 | 530 |
| 1% 5 min ZN§ positives | 499 | 503 |
| 0.3% 5 min ZN¶ positives | 478 | NA |
| Kinyoun# positives | NA | 457 |
| Positives by any technique | 618 | 603 |

* Phase 1: first study phase comparing 1% 5 min ZN and 0.3% 5 min ZN.
† Phase 2: second study phase comparing 1% 5 min ZN and Kinyoun.
‡ 1% 15 min ZN: routine smears stained for 15 min using a hot 1% basic fuchsin stain.
§ 1% 5 min ZN: study smears stained for 5 min using a hot 1% basic fuchsin stain.
¶ 0.3% 5 min ZN: study smears stained for 5 min using a hot 0.3% basic fuchsin stain.
# Kinyoun: study smears stained for 5 min using a cold 3% basic fuchsin stain.
AFB = acid-fast bacilli; ZN = Ziehl-Neelsen; NA = not applicable.

**Table 3** Cross-tabulation of quantified results for each study method, by study phase. Results <1 AFB/100 OIF are shown, but were not counted among 'Any positive/scanty'

| | <1/100 | Scanty | 1+ | 2+ | 3+ | Any positive/ scanty | Negative | Total |
|---|---|---|---|---|---|---|---|---|
| 0.3% 5 min ZN | | | | | | | | |
| Study phase 1 | | | | | | | | |
| 1% 5 min ZN | | | | | | | | |
| <1/100 | 1 | 1 | 0 | 0 | 0 | 1 | 12 | 14 |
| Scanty | 0 | 24 | 16 | 3 | 0 | 43 | 51 | 94 |
| 1+ | 1 | 21 | 57 | 17 | 3 | 98 | 18 | 117 |
| 2+ | 0 | 7 | 40 | 64 | 23 | 134 | 6 | 140 |
| 3+ | 0 | 1 | 4 | 31 | 96 | 132 | 2 | 134 |
| Any positive/scanty | 1 | 53 | 117 | 115 | 122 | 408 | 77 | 485 |
| Negative | 9 | 41 | 9 | 7 | 2 | 59 | 4054 | 4122 |
| Total | 11 | 95 | 126 | 122 | 124 | 467 | 4143 | 4621 |
| Kinyoun | | | | | | | | |
| Study phase 2 | | | | | | | | |
| 1% 5 min ZN | | | | | | | | |
| <1/100 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 12 |
| Scanty | 2 | 21 | 15 | 0 | 0 | 36 | 44 | 82 |
| 1+ | 0 | 30 | 51 | 17 | 0 | 98 | 26 | 124 |
| 2+ | 0 | 7 | 49 | 69 | 14 | 139 | 6 | 145 |
| 3+ | 0 | 0 | 17 | 54 | 69 | 140 | 0 | 140 |
| Any positive/scanty | 2 | 58 | 132 | 140 | 83 | 413 | 76 | 491 |
| Negative | 0 | 28 | 11 | 1 | 2 | 42 | 3822 | 3864 |
| Total | 2 | 86 | 143 | 141 | 85 | 455 | 3910 | 4367 |

AFB = acid-fast bacilli; OIF = oil immersion field; ZN = Ziehl-Neelsen.

A cross-tabulation of quantified results by study phase is shown in Table 3. Results with <1 AFB/100 OIF are shown separately. As they were considered unreliable and not sufficiently reproducible, they were excluded from further evaluation. In Phase 1, this left 485 positive or scanty results for 1% 5 min ZN and 467 for 0.3% 5 min ZN. In Phase 2, there were 491 positive or scanty results for 1% 5 min ZN and 455 for Kinyoun. Any technique mainly missed smears that were scanty or 1+ with the alternative one, but quantifications were consistently higher with 1% 5 min ZN: respectively 274/485 (56%) and 285/491 (58%) were 2+ or 3+ compared to 246/467 (53%) for 0.3% 5 min ZN and 226/455 (50%) for Kinyoun.

Table 4 shows errors and sensitivities for Phase 1 before and after restaining, after 0.3% 5 min ZN and/or 1% 5 min ZN staining. Smears that were false-positive on rechecking and negative by any other technique were excluded from the sensitivity denominator, leaving 533 series confirmed as positive by at least one technique. Few false-positives were declared for either stain concentration (0 vs. 2 high false-positive [HFP] and 7 vs. 5 low false positive [LFP] after restaining for 0.3% and 1.0% fuchsin, respectively). Quantification errors (QE) were also very low for both. High and low false-negatives (HFN, LFN) were far more common, especially after restaining (respectively 55 and 42 for 0.3% 5 min ZN and 1.0% 5 min ZN). The sensitivities of the two techniques were not significantly different (86.5% vs. 89.9%, $P = 0.11$).

Table 5 shows errors and sensitivities declared for Phase 2, analysed as for Phase 1, leaving 526 series for the sensitivity denominator. Very few false-positives were declared, and numbers were almost identical for

**Table 4** Errors and sensitivities for Phase 1* smears, determined by rechecking before and after restaining (533 confirmed positive series, at least 1 AFB/100 fields)

| Technique | HFP *n* | LFP *n* | QE *n* | HFN *n* | LFN *n* | Sensitivity % | $P_{sens}$ |
|---|---|---|---|---|---|---|---|
| Errors declared before restaining | | | | | | | |
| 0.3% 5 min ZN | 0 | 14 | 2 | 6 | 36 | | |
| 1% 5 min ZN | 3 | 11 | 3 | 9 | 22 | | |
| Errors declared after restaining | | | | | | | |
| 0.3% 5 min ZN | 0 | 7 | 4 | 13 | 42 | 86.5 | 0.11 |
| 1% 5 min ZN | 2 | 5 | 2 | 10 | 32 | 89.9 | |

* Phase 1: 0.3% 5 min ZN; 1% 5 min ZN: see Table 2 legend.
AFB = acid-fast bacilli; HFP = high false-positive error; LFP = low false-positive error; QE = quantification error; HFN = high false-negative error; LFN = low false-negative error; $P_{sens}$ = significance of the difference in sensitivity; ZN = Ziehl-Neelsen.

**Table 5** Errors and sensitivity for Phase 2 smears, as determined by rechecking before and after restaining (526 confirmed positive series, at least 1 AFB/100 fields)

| Technique | HFP *n* | LFP *n* | QE *n* | HFN *n* | LFN *n* | Sensitivity % | $P_{sens}$ |
|---|---|---|---|---|---|---|---|
| Errors declared before restaining | | | | | | | |
| Kinyoun | 3 | 10 | 1 | 6 | 35 | | |
| 1% 5 min ZN | 3 | 12 | 0 | 3 | 13 | | |
| Errors declared after restaining | | | | | | | |
| Kinyoun | 1 | 4 | 6 | 11 | 54 | 85.6 | 0.001 |
| 1% 5 min ZN | 0 | 2 | 0 | 5 | 21 | 93.0 | |

Phase 2: Kinyoun; 1% 5 min ZN: see Table 2 legend.
AFB = acid-fast bacilli; HFP = high false-positive error; LFP = low false-positive error; QE = quantification error; HFN = high false-negative error; LFN = low false-negative error; $P_{sens}$ = significance of the difference in sensitivity; ZN = Ziehl-Neelsen.

both techniques. QEs were seen with the cold technique only, and mainly after restaining. The total false-negatives detected after hot restaining were more than double for the Kinyoun technique (65 vs. 26 of all 526 series with any positive result). Kinyoun sensitivity was also significantly lower (85.6% vs. 93.0%, $P < 0.001$).

Figure 2 compares the sensitivity of the various study techniques, considering any positive or scanty result in a series, including those found only by routine smear. Routine smears (1% 15 min ZN) were not reread, and for this reason no corrections for false-positives in rechecking were applied for any technique. There were 595 series with a positive result during Phase 1 and 583 during Phase 2. Averaging the sensitivities for Phase 1 and 2, of a total of 1178 positives, 1039 and 976 were identified by 1% 15 min ZN and 1% 5 min ZN, respectively, yielding sensitivities of 88% and 83% ($P = 0.0001$). Of Phase 1 positives, 467 were also positive by 0.3% 5 min ZN (78% sensitivity), and of Phase 2 positives, 455 were Kinyoun-positive (78%). Compared to the average 1% 15 min ZN or 1% 5 min ZN, the sensitivities of 0.3% 5 min ZN and Kinyoun were also significantly lower ($P < 0.001$).

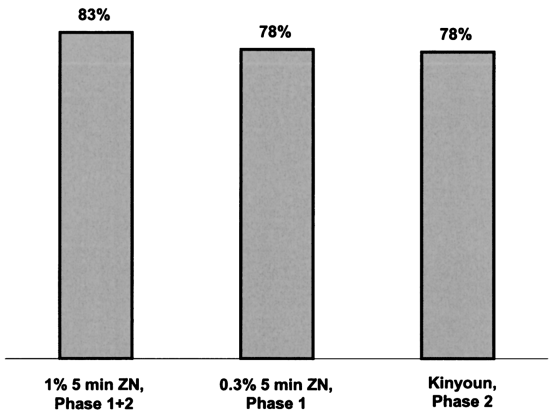


**Figure 2** Overall sensitivity of study techniques, on total yield of positives, including those detected in the routine smear only (at least 1 AFB/100 fields). Phase 1, Phase 2, 1% 15 min ZN, 1% 5 min ZN, 0.3% 5 min ZN and Kinyoun: see Table 2 legend. ZN = Ziehl-Neelsen; AFB = acid-fast bacilli.

## DISCUSSION

Our study aimed to evaluate the current WHO/IUATLD guidelines for staining of AFB, i.e., use of a 0.3% fuchsin stain with the smear heated three times for 5 min. They follow the original ZN method, except for the basic fuchsin concentration (about 1% in the original method). For study smears, timing was kept as prescribed in the WHO/IUATLD guidelines, or in the original Kinyoun paper. Although this comparison was not the primary aim of the study, we kept the time with carbolfuchsin for routine smears to 15 min, according to the project guidelines. Heating was applied only once for routine as well as study smears, according to project and NTP guidelines, which were still in accordance with the earlier edition of the IUATLD guide.[28] Moreover, in our experience this continues to be standard practice in most NTPs.

We preferred field conditions to test the robustness of the standard technique. It was felt that this might bring deficiencies or weaknesses to light more readily than in a research centre. The study was performed during the winter, when the workload is lower and climatic conditions lead less rapidly to fading. To avoid confusion with stains, the study was done in two phases, with hot-cold comparison (Phase 2) during the coldest time of the year, when temperatures are around 15°C.

To equalise for reader bias, all 75 clinics in the project areas participated, with blinding by exchange of smears. Routine conditions were largely maintained by using NTP-supplied basic fuchsin, choosing the least busy period of the year and avoiding remuneration. As culture was not available, positivity by any technique, confirmed by rechecking of discordant smears before and after restaining with a certified fuchsin brand, was used as gold standard.

An analysis of rechecking errors, and considering only positives detected by one of the techniques studied, showed few false-positives for both study phases, with hardly any difference between the study techniques. The 0.3% 5 min ZN technique showed more false negatives and a lower sensitivity than 1% 5 min ZN (55, 86.5% vs. 42, 89.9%), without reaching statistical significance. However, the Kinyoun tech-

nique yielded significantly more false-negatives than 1% 5 min ZN (65 vs. 26), with a highly significant sensitivity difference (85.6% vs. 93.0%).

The same sputum samples were examined routinely, using the same 1% hot basic fuchsin staining, but for 15 min, but these smears were not part of the study and were not rechecked in case of discordance, so they could not be included in the main analysis. As many more positives seemed to have been detected in routine, we compared the crude sensitivities of all staining techniques applied, using as the denominator all positive series by any technique without any correction for false positives. Moreover, for 1% 5 min ZN and 1% 15 min ZN, the two study phases were added up. This yielded slightly different results, with a surprisingly clear and highly significant superiority of the routine 1% 15 min ZN technique (sensitivity 88%, not shown in Figure 2), while there was no difference between 0.3% 5 min ZN and Kinyoun (sensitivity 78%). With 83% sensitivity, the performance of the 1% 5 min ZN technique was average, but was significantly better than both former techniques.

This study has some limitations. Technician quality was shown earlier to be the most important factor in explaining differences in performance of AFB smear examination.[29] We aimed at average quality involving a big number of centres, but because of the blinding requirement, pairs stained by different techniques could not be examined by the same reader. Allocation of about equal numbers of smears stained by each technique to each clinic may have minimised this possible bias.

Reader bias may also have been the most important factor explaining the clear superiority of the routine technique (1% 15 min ZN). Study smears had to be examined after routine work, without incentives, and these examinations may have been less thorough. However, our ranking of sensitivities remains in accordance with the results of staining studies, showing a positive effect of stain concentration, heating and staining time on the numbers of AFB visualised. It is therefore likely that staining quality does explain part of the differences, but that the difference is amplified by the more superficial reading for (part of) the study smears.

Finally, this setting should be considered as optimal, with experienced staff, well-equipped smear laboratories, centrally prepared stains, and a long-standing emphasis on AFB microscopy. The percentage of error reported from regular rechecking has been below 1% HFP and 2% FN for years. It remains possible that the differences observed with these staining variations would be more pronounced in less ideal conditions, i.e., in case of poor stain preparation, poor smearing, superficial reading or deficient microscopes.

## CONCLUSIONS

Applied in the field, Kinyoun cold staining is inferior to hot ZN. Under these conditions, the IUATLD/WHO-recommended ZN technique might also perform less well than 1% fuchsin 5–15 min hot staining, as its low fuchsin concentration and shorter staining time may leave too small a margin for error when other factors are unfavourable. Further evaluations of the staining guidelines in various field programmes are required. Meanwhile, NTPs using hot ZN with the original higher fuchsin concentration or prolonged staining time should not be urged to change.

### *Acknowledgements*

We sincerely thank the field staff of Damien Foundation Bangladesh for their enthusiasm, not only when dealing with patients but also when participating in numerous operational research projects. We thank the Bangladesh NTP for its continued support and for its permission to conduct these studies.

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**RÉSUMÉ**

**LOCALISATION : Un projet de terrain au Bangladesh.**
**OBJECTIF : Comparer l'efficacité de différentes stratégies communément utilisées de coloration à la carbolfuchsine.**
**MÉTHODES : Coloration en routine par Ziehl-Neelsen (ZN) à chaud avec de la fuchsine basique à 1% pendant 15 min dans 75 centres de santé de terrain. Lecture à l'aveugle de doubles frottis colorés au ZN à 1% en comparaison à une coloration à 0,3% pour 5 min, ou par ZN 1% pour 5 min comparé à la coloration à froid de Kinyoun. Contrôle des séries discordantes.**
**RÉSULTATS : Pour des nombres comparables de faux positifs, la sensibilité avec la coloration de Kinyoun était significativement plus basse qu'avec le ZN 1% pendant 5 min (85,6% contre 93,0%, $P < 0,001$). La sensibilité avec le ZN 1% pendant 5 min n'était pas significativement plus élevée que celle avec la coloration à 0,3% pendant 5 min (89,9% contre 86,5%). L'examen de routine utilisant le ZN 1% pendant 15 min a identifié plus de positifs que n'importe quelle autre technique étudiée.**
**CONCLUSIONS : La sensibilité de la coloration à froid de Kinyoun n'était pas satisfaisante au niveau des centres de santé de terrain. La sensibilité des examens avec la fuchsine 0,3% pendant 5 min telle que recommandée par l'OMS/UICTMR n'était pas significativement différente de celle avec le ZN original à 1% pendant 5 min, mais celle de la coloration à chaud à 1% pendant 15 min pourrait être supérieure. Une concentration réduite de fuchsine associée à un temps trop court de coloration peut ne laisser qu'une trop courte marge pour éviter l'erreur. Les programmes de lutte contre la tuberculose utilisant le ZN à chaud avec une coloration concentrée ou un plus long temps de coloration ne devraient pas être poussés à modifier leur technique.**

**RESUMEN**

**MARCO DE REFERENCIA : Un estudio de terreno en Bangladesh.**
**OBJETIVO : Comparar la eficacia real de las variantes de la tinción con carbofucsina utilizadas corrientemente.**
**MÉTODO : En 75 consultorios de terreno, se practicó la tinción sistemática de Ziehl-Neelsen (ZN) con fuscina básica al 1% durante 15 min. En otros consultorios se prepararon frotis en duplicado para teñirlos alternativamente con ZN con fuscina básica al 1% o al 0,3% (durante 5 min) o con la tinción de Kinyoun en frío. La lectura se llevó a cabo manteniendo el anonimato sobre el tipo de tinción. Las series con lectura discordante se analizaron de nuevo.**
**RESULTADOS : La sensibilidad de la tinción de Kinyoun, para un número comparable de falsos positivos, fue significativamente inferior a la de ZN al 1% durante 5 min (85,6% contra 93,0% ; $P < 0,001$). La sensibilidad de la tinción de ZN al 1% durante 5 min no fue significativamente superior que la tinción al 0,3% durante 5 min (89,9% contra 86,5%). Con la tinción sistemática de ZN al 1% durante 15 min se obtuvieron más frotis positivos que con todas las demás técnicas estudiadas.**
**CONCLUSIONES : La sensibilidad de la tinción de Kinyoun en frío en los consultorios de terreno no fue satisfactoria. La sensibilidad de la tinción recomendada por la OMS/UICTER, con fuscina al 0,3% durante 5 min, no presentó diferencia significativa con la técnica original de ZN al 1% durante 5 min ; sin embargo, al 1% durante 15 min en calor, esta tinción podría ser superior. La reducción de la concentración de fuscina asociada al acortamiento del tiempo de tinción deja quizá un margen de error muy estrecho. No se debería instar a los programas de tuberculosis para que modifiquen su técnica sistemática de tinción de ZN en calor con fuscina más concentrada o con tiempos de tinción más prolongados.**