*Lepr Rev* (2006) **77,** 311–316

*SHORT REPORT*

# Appraisal of skin smear reports of field laboratories

K.V. DESIKAN, K.V.S.M. RAO, M.S. BHARAMBE & P.V. RANGANADHA RAO
*Leprosy Histopathology Centre, Mahatma Gandhi Institute for Medical Sciences, Sevagram 442 102, Via Wardha, Maharashtra, India*

Accepted for publication 25 September 2005

## Introduction

A valuable investigation in leprosy is the examination of skin smears. Being a very simple technique, it can be performed satisfactorily in the field. Unfortunately, it is being given up because the reports from several field laboratories were not found to be dependable, in the sense that they would not match with the clinical diagnosis. There could be many causes for such a mismatching, pertaining to several operational factors and skills of the field staff. This paper restricts itself to the possible defects or deficiencies that could occur in the working of the field laboratories. Such defects could be countered essentially by three steps, (a) training the technician properly, (b) providing minimal facilities, proper reagents and a good microscope, (c) supervision by a periodic cross-checking of the reports. Enough attention has been paid to the first two steps in all the field laboratories of LEPRA-India. Supervision by cross-checking of randomly selected slides is carried by an experienced senior technician in the central laboratory. By these means, a field laboratory is well managed and best utilized. The method of cross-checking and the results of such a procedure are detailed in the paper.

## Materials and methods

Sixteen field laboratories of LEPRA-India are situated in remote small towns, six in Andhra Pradesh, five in Orissa, and one each in Kerala, Karnataka, Bihar, Mahdya Pradesh and Uttar Pradesh. The laboratories have very minimal facilities and equipments. They are manned by 'Smear Technicians' trained in processing and examining skin smears. At the start, the senior technician from central laboratory visited each centre, set up the laboratory and guided the smear technician in all procedures. There were no further visits by the senior technician to the peripheral units. The skin smears are prepared by the paramedical worker in the field. They are fixed and the slides are sent to the field laboratory.

Correspondence to: K. V. Desikan (e-mail: Desikan_wda@sancharnet.in)

0305-7518//064053+06 $1.00  

Staining and examination is done by the field laboratory technician. Zeihl–Neelsen technique by the cold method is adopted. The technician is instructed to examine at least 100 microscope fields in negative smears and 25–50 fields in positive smears. Positivity is graded by the Ridley–Jopling scale.

The procedure adopted for cross-checking is that each month, from the list of slides examined in the field laboratory, every 10th slide is picked up starting from any number between 1 and 5, decided by the Project Officer. The slides are properly packed and sent to central laboratory. Along with the slides the technician also sends Form I, which contains a list of all slides, with the slide number, name of the patient, date of smear done but not the reports by him. Simultaneously, he sends Form II to the 'blind observer' who is not connected with the study. Form II has the same details as in Form I, and in addition, the reports of the field laboratory are included. The senior technician at central laboratory examines all the slides. He enters the results in Form I and sends it to the blind observer. The blind observer transfers these results to Form II and tallies both the reports. The tallied form containing results of the field and central laboratories is sent to the central laboratory and also to the concerned field unit. During a period of 9½ years from June 1991 to December 2000, a total of 36,662 smears were received and cross checked in the central laboratory.

## Results

The findings of the field and central laboratories are shown in Table 1. Out of a total of 5127 smears found positive in the field laboratory; the central laboratory reported 4496 as positive and 631 as negative. While 31,535 smears were found negative in the field laboratory, central laboratory reported 31,962 as negative. The positive predictive value is 87·7% while the negative predictive value is 99·4%.

Out of a total of 36,662, examined in the field, the positive smears formed 14% while in the central laboratory, they were 12·8%. Of the 5127 smears reported as positive in the field laboratories, 631(12·3%) were found negative.

Gradewise distribution of the findings of field and central laboratories is shown in Table 2. There is a concurrence of grades in 33,750 smears (92%) if the negative reports are also included. Taking the central laboratory reports as standard, 1168 smears are under-rated and 1744 smears are over rated.

Considering only the positive findings, concurrence in different grades are shown in Table 3. In the lower grades of 1 + and 2 +, the concurrence is 40·4 and 37·4% only.

**Table 1.** Number of smears cross-checked

| | Central laboratory | | |
|---|---|---|---|
| Field laboratory | Positive | Negative | Total |
| Positive | 4496 | 631 | 5127 |
| Negative | 204 | 31331 | 31535 |
| Total | 4700 | 31962 | 36662 |

Positive predictive value: 87·7%.
Negative predictive value: 99·4%.

**Table 2.** Distribution of results of field and central laboratories according to grades

| Field laboratory | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Central laboratory | | | | | | | | |
| B1 | NEG | 1 + | 2 + | 3 + | 4 + | 5 + | 6 + | Total |
| NEG | 31331 | 393 | 173 | 61 | 2 | 1 | 1 | 31962 |
| 1 + | 114 | 433 | 216 | 77 | 21 | 3 | 0 | 864 |
| 2 + | 43 | 139 | 382 | 200 | 56 | 5 | 1 | 826 |
| 3 + | 42 | 87 | 210 | 610 | 274 | 27 | 2 | 1252 |
| 4 + | 3 | 20 | 39 | 243 | 677 | 158 | 10 | 1150 |
| 5 + | 2 | 0 | 2 | 21 | 182 | 289 | 63 | 559 |
| 6 + | 0 | 0 | 0 | 0 | 0 | 21 | 28 | 49 |
| Total | 31535 | 1072 | 1022 | 1212 | 1212 | 504 | 105 | 36662 |

In the higher grades of 3 + and above, the concurrence is slightly higher. Grading by Ridley's method is subjective and could vary from person to person or by same person after a time. Hence for practical purposes, a variation by two grades has to be considered. It is seen in Table 4 that the variation by 2 grades or more is only 1·9%.

As mentioned above, 12·3% of the smears were found negative out of the smears reported as positive by the field laboratories. These may be false positives. Or, the bacilli which were in small numbers might have faded and been missed by the senior technician who is examining after a lapse of time. To look into this possibility, the 5127 smears reported as positive in the field were further analysed, dividing into those that were cross-checked within 30 days of their examination in the field and those that were checked after 30 days. Table 5 shows the variation in the reports in these two categories. The variation was found to be 7·15% in smears examined within 30 days and 13·0% in smears examined after 30 days. It is therefore evident that a delay in examination of stained smears could adversely affect the findings.

## Discussion

Skin smear examination helps in diagnosis, classification, mode of treatment, and assessment of therapy. Unfortunately, it has been found that the quality of bacteriological examination in most of the leprosy control units is unsatisfactory and forms the 'weakest link; in the MDT programme.[6] If it is so, it is necessary to find out the causes for such an unsatisfactory performance of the peripheral laboratories. Bhatia[3] has proposed methods to improve the

**Table 3.** Concurrence of different grades of positivity

| Grades | Field laboratory report | Concurrence | Percent |
|---|---|---|---|
| 1 + | 1072 | 433 | 40·4 |
| 2 + | 1022 | 382 | 37·4 |
| 3 + | 1212 | 610 | 50·3 |
| 4 + | 1212 | 677 | 55·9 |
| 5 & 6 + | 609 | 317 | 52·1 |

**Table 4.** Variation by number of grades of positivity

| Variation | Number | Percent |
|---|---|---|
| By 1 grade | 2213 | 6·0 |
| By 2 Grade | 533 | 1·5 |
| By 3 Grade | 153 | 0·4 |
| By 4 Grade | 9 | 0·02 |
| By 5 Grade | 4 | 0·01 |

quality of bacteriological examination. Georgiev and McDougall[7] have indicated some radical changes in the operational methodology, so that the smear reports become dependable and acceptable. On the same count, Poricha[9] has suggested some modifications in smear examination and has made a strong plea to revive this investigation. The present report proves that by very minimal supervision, and periodic cross-checking it is possible to achieve good quality and dependable skin smear reports at the peripheral field laboratories.

It was found that out of 36,662 smears cross checked, there was a good concurrence of field laboratory reports with those of the reference laboratory, giving a positive predictive value of 87·7% and negative predictive value of 99·4%. There was a very small degree of under-rating or over-rating. The field laboratories have reported a slightly higher rate of positivity.

While the above findings show a significant correlation, a comparison of positivity by Ridley–Jopling scale showed a low concurrence in all grades. This semi-quantitation of bacilli in the smears is highly subjective, and so there could be wide inter-observer and intra-observer variation. It would therefore be reasonable to accept the views of Sayer *et al.*[10] that a variation of $-1$ or $+1$ in the grading is not particularly significant. Abraham and Cariappa[1] have also observed that a variation of $1+$ is to be expected between the laboratories in a field programme. As such a correlation by 2 grades of positivity should be considered for practical purposes. It was found that a variation by 2 grades or more was only 1·9%. Variation by more that 2 grades was negligible.

A noteworthy finding in the present study was that the field laboratories showed a higher positivity rate of 12·3% over the central laboratory. If the findings of the senior technician in the central laboratory are to be taken as the gold standard, the higher figures from field laboratories should be considered as false positives. On the other hand, the findings at the field laboratories may be true and the bacilli might have been missed at the central laboratory due to fading of the stains. Fading may due to exposure to sunlight and examination after a long interval. It was found that checking at the central laboratory within 30 days of its examination gave a variation of 7·15%, while after 30 days, it was 13·0%, a very significant difference indeed ($P < 0{\cdot}01$). Collecting and transferring slides to a distant laboratory cause

**Table 5.** Variation by time lag of examination of smears reported positive at field laboratories

| Time lags | Total Smears | Smear with variation | % Variation | *P*-value | Result |
|---|---|---|---|---|---|
| $<30$ days | 573 | 41 | 7·15 | $<0{\cdot}01$ | Highly significant |
| $\geq 30$ days | 4554 | 591 | 31·0 | | |

an unavoidable delay; but it should be reduced as much as possible. Sayer *et al.*[10] also tried to find out other causes in the field that could affect the results of reading. They found that air drying of smears in direct sunlight and staining of the smears by a delay up to 3 weeks did not produce any significant difference in the result. However, they found that there was a difference by reading the stained slides after storing for a long time, similar to our results.

When examined by a different person, another cause of variation can also be the uneven distribution of bacilli in the smears. Bhatia *et al.*[4] have shown that even in a highly positive smear, the bacilli are not evenly distributed. In such smears, selection of fields by different observers can lead to different results. Gupte *et al.*[8] have calculated that a smear of 1 cm diameter would have nearly 4000 oil immersion fields. By examining 100 fields, only 2·5% of the fields are covered. Added to this, the uneven distribution of bacilli is another limiting factor. Despite these limitations, they found a fair degree of concurrence between different readings.

Quality control by cross checking was also conducted by Vettom and Pritze.[11] They were generally satisfied by the level of concurrence of the results. Out of 50 participating laboratory technicians, the concurrent reading was satisfactory or good by 32 technicians. De Rijk *et al.*[5] have also carried out quality control by cross-checking of smears from 12 districts of Tanzania. Taking an over all assessment of the districts, it was found that there was 79% concurrence, with 18% under-reading and 3% over-reading. Awofeso,[2] on the other hand, found that the performance of the field laboratories in four provinces of Nigeria was extremely poor. He has ascribed the reasons to be an inadequate training and lack of support and supervision to the technicians, as also shortage of laboratory reagents.

It may be true that at present, the reports from the field laboratories are not dependable. In order to make the best use of this important investigation, every effort must be made to find out and correct the deficiencies. First and foremost, the technician should be properly trained. He should be provided with a good microscope and proper stains. Then with a minimum supervision, a high quality of standardized reports can be obtained.

In conclusion, it can be stated that by following the above suggestions and taking necessary corrective measures, satisfactory and dependable reports can be obtained from very simple field laboratories. This paper deals mainly with the performance of field laboratories. The methods suggested for good performance are simple, feasible and cost effective. For a smear report being undependable and not matching with the clinical diagnosis, there can be several operational reasons. If sites of smears are not properly selected, it would lead to wrong reports. There can be defects in the preparation of smears if the paramedical worker is not properly trained. One cannot ignore the fact that the clinical diagnosis can itself be undependable because it is often made by a poorly trained doctor for which the smear report cannot be blamed. There can be several other operational reasons for the disparity of reports. These are beyond the scope of this paper. All the same, the importance of skin smear examination cannot be under-rated. It is for the programme officers to find out ways and means of utilizing this useful investigation to the extent possible instead of abandoning it all together.

## References

[1] Abraham B, Cariappa A. Inter and intra-laboratory variation in reporting of skin smears in leprosy. *Int J Lepr*, 1991; **59**: 76–81.

[2] Awofeso N. Inventory of skin smear practices in 6 leprosy control programmes in Nigeria. *Lepr Rev*, 1993; **64**: 150–156.

[3] Bhatia VN. Skin smear examination in relation to multi-drug therapy. *Ind J Lepr*, 1987; **59**: 75–79.
[4] Bhatia VN, Vanaja G, Rao S, Elango TV. Some observations on skin smear examination. *Ind J Lepr*, 1990; **62**: 338–345.
[5] De Rijk AJ, Nilsson T, Chonde M. Quality control of skin smear service in leprosy programmes; preliminary experience with inter-observer comparison in routine service. *Lepr Rev*, 1985; **59**: 177–191.
[6] Director General of Health Services, Govt of India. (1986) *Report of Independent Evaluation*, p. 27.
[7] Georgiev GD, McDougall AC. The bacteriological examination of slit-skin smears in leprosy control programmes using multi drug therapy. *Ind J Lepr*, 1987; **59**: 373–385.
[8] Gupte MD, Raj CA, Kannan S, Desikan KV. Reliability of direct skin smear microscopy in leprosy. *Ind J Lepr*, 1988; **60**: 566–571.
[9] Poricha D. A plea to revive skin smear examination. *Int J Lepr*, 2001; **69**: 116–119.
[10] Sayer J, Gent R, Jasudasan K. Are bacterial counts on slit-skin smears in leprosy affected by preparing slides under field conditions?. *Lepr Rev*, 1987; **58**: 271–278.
[11] Vettom I, Pritze S. Reliability of skin smear results: experience with quality control of skin smears in different routine services in leprosy control programmes. *Lepr Rev*, 1989; **60**: 187–196.