An evaluation of skin snip techniques used in the quantitative assessment of microfilarial densities of *Onchocerca volvulus* *

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Abstract

A study was undertaken to determine whether significant variations occur in the quantitative assessment of onchocercal microfilarial densities when different methods of treating skin snips are used. The results show that teasing of the snip should be avoided because it reduces the sensitivity of the method.

In the diagnosis of onchocerciasis, the skin snip method is to be preferred to scarification, which is unsuitable for precise quantitative studies (1, 2). The teasing of skin snips before microfilarial counts are taken is favoured by some workers (3, 4) whereas others (5–7) prefer unteased snips.

The present study was designed to determine whether the number of microfilariae recovered from teased snips differed significantly from the number recovered from unteased snips and, if so, to recommend a standard procedure for use in quantitative field studies.

Materials and methods

Forty-two men and 9 women aged 24–60 years, who were adjudged on clinical grounds to have onchocerciasis, were enrolled for the study, which was conducted at the endemic diseases clinic of the University College Hospital, Ibadan.

Eight skin snips were taken from each patient, 4 from the lateral aspect of each buttock in the manner described by Duke (4). Each snip measured 1–2 mm and was taken approximately 1 cm away from the site of the previous one to form a diamond shaped grid of about 2 cm × 2 cm on each buttock as recommended by Buck (6).

Each of the 4 snips from each buttock was then allocated at random to one of the following treatment groups:

1. The snip was placed in a drop of 0.58% saline on a slide and teased.
2. The snip was similarly treated but left unteased.
3. The snip was placed in 3 drops of 0.58% saline in a haematocrit tube and teased.
4. The snip was put in saline in a separate haematocrit tube and left unteased.

All the microfilariae emerging from the first 2 preparations were counted under the microscope after 1 h by means of a $^2/_3$ low power objective. Preparations 3 and 4, after incubation for 24 h at 37°C, were centrifuged at 1 000 *g* for 5 min; the deposit from each was then emptied on to a glass slide and the microfilariae counted.

Statistical analysis. The experimental design is a factorial one with 3 factors each at 2 levels, namely:

Nature of snip (N): teased and unteased.

Side of the body (S): right and left.

Time of taking count (T): 1 h and 24 h.

This gives a $2\times2\times2$ factorial arrangement in a randomized block design, each patient constituting a block of experimental units—4 from each buttock. The data were analysed by standard statistical procedures (8, 9).

Results

The main interest in the study is in sources of variation other than those between the patients. Of the three main factors studied only that involving the nature of the skin snip was significant in that there was a significant difference ($P<0.005$) in the mean microfilariae count between teased and unteased skin snips. Among the interactions only N×T was statistically significant ($0.025>P>0.01$).

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Table 1. Two-way table of total count classified by time and nature of snip

	Nature of snip		Total	Mean	Standard error
	teased	unteased			
after 1 hour	6 882	7 617	14 499	71.07	7.27
after 24 hours	6 123	12 259	18 382	90.11	7.27
total	13 005	19 876			
mean	63.75	97.43			
standard error	7.27	7.27			

Table 1 is a two-way arrangement of the total microfilariae count classified by the time of taking the count and the nature of the skin snip. The mean count for the unteased snips was 97.43 compared with 63.75 for the teased snips. The difference between them is statistically significant ($t = 3.28$, $0.005 > P > 0.001$), confirming the observation in the analysis of variance. The difference in total counts between the 1-h and the 24-h slides for the unteased snips was very much larger than the corresponding difference for the teased snips (4642 as against −759), indicating a significant interaction between the nature of the skin snip and the time of taking the count.

Table 2 shows the means, together with their standard errors, of the eight types of determination. The tendency of counts for unteased snips to be higher than those for teased snips is clearly evident in the table.

Discussion

Various studies have been undertaken in an attempt to standardize research techniques in onchocerciasis surveys. The counting of microfilariae from teased or unteased skin snips introduces a variable that could significantly affect quantitative comparative epidemiological studies. Several authors (5, 10, 11) have recommended the use of the corneoscleral punch, which was not available for this study; this instrument makes it unnecessary to weigh snips as advocated by Kershaw et al. (3) and Duke (4), or to take linear measurements as recommended by Lagraulet and Bard (12).

It has been shown that after 1 h over 80% and nearly 90% of the microfilariae have emerged from snips placed in distilled water (5) and in saline (13) respectively. One hour therefore represents both a convenient period of time and one during which, irrespective of the medium used, the numbers of microfilariae that emerge are similar. Where field conditions necessitate the postponement of counts, examining centrifuged deposits of specimens that have been incubated for 24 h is satisfactory.

The results show that at both 1 h and 24 h counts were significantly higher in unteased than in teased skin snips. An obvious explanation for this difference is that teasing destroys a number of microfilariae. The number so destroyed is totally unpredictable, and depends on how thoroughly the teasing is carried out. These results are in agreement with those of Tada et al. (7), who found that higher yields of microfilariae were obtained from unteased snips and those torn into 2 pieces than from snips that had been torn into smaller pieces.

No differences were found between the microfilarial densities on the right and left sides of the body. This agrees with the findings of Duke (4).

Conclusion

This study has shown that teased skin snips gave significantly lower microfilarial densities than unteased snips when the counts are taken after 1 h or

Table 2. Mean and standard error of mean (SEM) for the 8 types of determination

	After 1 hour				After 24 hours			
	right side		left side		right side		left side	
	unteased	teased	unteased	teased	unteased	teased	unteased	teased
mean count	74.7	53.7	74.7	81.3	120.2	68.1	120.2	52.0
SEM [a]	14.55	14.55	14.55	14.55	14.55	14.55	14.55	14.55

[a] Estimated as pooled value from the Error Mean Square in the analysis of variance.

24 h. We therefore recommend that, as a standard procedure for accurate quantitative studies, skin snips should be left unteased before microfilariae are counted.

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