Microscopic Counts of Mycobacteria
Conversion Factors for the Pinhead Method [1, 2]

John H. Hanks[3]

One merit of the pinhead method ([1]) of transfering microsamples of bacteria to glass slides is the rapidity with which large numbers of samples can be placed on each slide. The system assures identical staining, differentiation, and rinsing of eight samples per slide. Nakayama *et al.* ([2]) reported the standard deviation between volumes per pinhead to be 7.4 per cent and concluded that the method is more practical and convenient than other available procedures. With precautions to avoid atypical or defective films (see 1, PROCEDURE), the degree of reproducibility is further improved.

A unique feature is that conversion factors can readily be calculated for any microscope, thus eliminating all the problems of calibration. The method of counting and the derivation of conversion factors have already been described ([1]).

With any given microscope, the number of fields observed per diameter of some films may be one less (or one more) than the average. For example, with a system and a microscope that usually yields 30 fields/film diameter, the number observed will sometimes be 31 or 29 fields. If one regards the average diameter as 30 fields and uses the corresponding conversion factor, the estimates are somewhat less accurate than when a conversion table is used that shows the factors for 29, 30 and 31 fields. Furthermore, I find that some persons are not adept at calculating conversion factors for different situations or different microscopes.

The accompanying Table of Conversion Factors, therefore, has been prepared to eliminate inconvenience or errors in calculation. To convert the number of bacilli observed/film diameter to bac./ml., three points of information are essential:

1. The number of fields observed/diameter.
2. The number of bacilli counted/diameter.
3. The corresponding Conversion Factor.

PROCEDURE

1. Select the Conversion Factor that corresponds to the number of fields and counted/diameter.

2. Multiply the number of bacilli seen/diameter by this factor.

Examples:

Fields counted/ diameter	Conversion Factor		If bacilli seen/diam. = 750		Bac/ml. $\times 10^6$
25	.175	×	750	=	131
30	.21	×	750	=	158

The Conversion Factors employed in the examples above assume a 1:5 dilution of sample, which is usual in standardizing lepromin. In other types of experimental work, we correct the estimated bac./ml. for the actual dilution of sample.

The Conversion Factors shown apply only to pinheads that transfer 0.00056 ml./film. The recommended pins ([1]) are widely available. We can supply a modest

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[3]J. H. Hanks, Ph.D., Microbiologist, Johns Hopkins-Leonard Wood Memorial Leprosy Research Laboratory, School of Hygiene and Public Health, 615 N. Wolfe Street, Baltimore, Maryland 21205.

number of pins that have been selected for standard dimensions, or can assist in locating and standardizing such pins. In the event that there is reason to adopt a different volume of sample/film, a new constant can be calculated, as shown in the footnote to Table 1.

TABLE 1. *Conversion Factors.*

A	(B)	(C)	D
Fields counted /diameter	Fields/ film Area: πr^2	Diameters/ film (B/A)	Conversion Factor ($\times 10^6$)
20	314f	15.7	0.140
21	347	16.5	0.147
22	380	17.3	0.154
23	415	18.0	0.161
24	452	18.8	0.168
25	490	19.6	0.175
26	530	20.4	0.182
27	572	21.2	0.189
28	615	22.0	0.196
29	660	22.8	0.203
30	706	23.5	0.210
31	754	24.3	0.217
32	804	25.1	0.224
33	855	25.9	0.231
34	908	26.7	0.238
35	962	27.5	0.245

Note: Columns (B) and (C) are included only to show the steps in calculations.
(C) = the number of diameters that would have to be counted to observe an entire film.
D = (C) × the constant 8,925 (see below)

Derivation of the Constant:

$$\text{Number of pinheads/ml.} = \frac{1}{0.00056 \text{ ml.}} = 1{,}785$$

$$\text{Dilution of sample} \times \frac{5}{8{,}925}$$

Derivation of a Conversion Factor:

Example: 1 bac./25 fields (C = 19.6) $\times \frac{8{,}925}{19.6}$

174,930

Moving decimal so that the base gives $\times 10^6$ 0.175

SUMMARY

The pinhead method of enumerating mycobacteria and other particles permits the preparation of approximately 30 films per minute, and does not require calibration of the diameters or areas of microscopic fields. This paper provides a table of conversion factors that yields the millions of bacteria per milliliter, irrespective of the number of microscopic fields counted per film diameter.

RESUMEN

El método de cabeza de alfiler para la enumeración de las mycobacterias y de otras partículas permite le preparación de 30 películas por minuto, aproximadamente, y no exige calibrar los diámetros o áreas de campos microscópicos. Este trabajo trae una tabla con factores de conversión que producen millones de bacterias por milímetro, independientemente del número de campos microscópicos contados por diámetro de película.

RÉSUMÉ

La méthode de la tête d'épingle (pinhead method) pour énumérer des mycobactéries et d'autres particules permet la préparation d'environ 30 frottis par minute, et n'exige pas la calibration des diamètres ou des surfaces des champs microscopiques. Cet article fournit une table des facteurs de conversion, qui livre les millions de bactéries par millilitre, quel que soit le nombre de champs microscopiques comptés par diamètre de frottis.

REFERENCES

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