AN EVALUATION OF PHASE CONTRAST MICROSCOPES FOR ASBESTOS COUNTING

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16. Abstract (Limit: 200 words) An evaluation was made of five phase contract microscopes for asbestos (1332214) counting, to determine whether there was a possibility of gross differences in asbestos fiber count results when the same filter was counted on different microscopes by the same personnel. Five trained persons counted the fibers on five filter wedges using five microscopes. The samples were collected by drawing air through a membrane filter by means of a battery powered vacuum pump. The filter was charged into a transparent homogeneous plastic mount, and the fibers sized and counted by the microscopes which were modified for use with phase contrast techniques. For each of the five counters the counts obtained using a Nikon microscope were 45 percent higher than those obtained using the particular Leitz, Zeiss, Olympus, or Bausch and Lomb microscopes in this study. The results indicated that film thickness was uniform and that clumping of fibers did not occur. Taking the average of the three counters as 100 percent (as they had statistically homogeneous counts), counter A's average was 16 percent lower and counter E's average was 27 percent higher than this average. Not only did significant differences occur depending on the equipment used, but also with different trained operators.

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INTRODUCTION

The U.S. Public Health Service (USPHS) through the National Institute for Occupational Safety and Health (NIOSH) has established a sampling and evaluation procedure for determining airborne asbestos dust exposures. The sample is collected by drawing air through a membrane filter by means of a battery powered vacuum pump. The filter is charged into a transparent homogeneous plastic mount, and the fibers are sized and counted by optical microscopes modified for use with phase contrast techniques (commonly referred to as "phase contrast microscopes"). Uniform procedures must be used for sampling and evaluating asbestos dust if reproducible results are to be achieved. Detailed equipment and procedures specified by the National Institute for Occupational Safety and Health are described in NIOSH TR-84, "USPHS/NIOSH Membrane Filter Method for Evaluating Airborne Asbestos Fibers".

Recently questions have arisen concerning the compatibility of asbestos fiber count results from different makes and models of phase contrast microscopes. The NIOSH Proficiency Analytical Testing (PAT) program has indicated that the most frequent contributors to gross differences in asbestos fiber count results are 1) improperly trained personnel and 2) improperly adjusted equipment. In a few cases though, questions remained concerning the basic performance characteristics of the microscopes. This evaluation study was conducted to see if there was a possibility of gross (greater than 20%) differences in asbestos fiber count results from counting the same filter on different microscopes by the same personnel. A description of the evaluation procedures and results is contained in this report.

TESTED EQUIPMENT

The manufacturer of each phase contrast microscope tested with model and accessory equipment is listed below:

Zeiss

#KL14B Binocular, Compact B illuminator with iris, 40X 0.65 N.A. Achromat objective, KPL 10X Huygenian eyepieces, IIZ phase condenser

Nikon

#SKE Trinocular, 6V30W illuminator, DLL 40X 0.65 N.A. objective, KPL10X Zeiss eyepieces, 77040 phase condenser

Leitz

#SM-LUX Binocular, 6V5W illuminator, Phaco 402A1 condenser, 40X 0.65 N.A. objective, KPL10X Zeiss eyepieces

Olympus

#EH Binocular, 6V illuminator, PL 40X 0.65 N.A. objective, Zeiss KPL10X eyepieces, phase turret condenser

Bausch and Lomb

Dynoptic Binocular, PR-27 illuminator, 40X 0.65 Achromat objective, Bausch and Lomb 10X Huygenian eyepieces, phase turret condenser

The above microscopes meet the following equipment specifications as stated in NIOSH TR-84:

1) Microscope body with binocular head.

2) 10X Huygenian eyepieces are recommended. Other eyepieces can be substituted if necessary.

3) Koehler illumination (preferably built in and having provisions for adjusting light intensity).

4) A Porton reticle is recommended. Others such as the Patterson Globe and Circle can be substituted, if necessary.

5) Mechanical stage. Abbe or Zernicke condenser fitted with phase ring (or Heine-type) with a numerical aperture (N.A.) equal to or greater than the N.A. of the objective.

6) 40-45X (N.A. 0.65 to 0.75) positive phase contrast achromatic objective.

7) Phase-ring centering telescope or Bertrand lens.

8) Green filter, if recommended by microscope manufacturer.

9) Stage micrometer with 0.01 mm subdivisions.

The asbestos filters counted were as follows:

Slide #	Asbestos type	Industry
1	chrysotile	friction
2	amosite	insulation
3	chrysotile	textile
4	chrysotile	friction
5	chrysotile	cement

EXPERIMENTAL DESIGN AND TEST PROCEDURES

Statistical Experimental Design

A 5x5x5 factorial experiment was performed with three factors: microscope, counter, and slide at the following levels.

Microscope	Counter	Slide
B & L	A	#1
Nikon	B	#2
Leitz	C	#3
Olympus	D	#4
Zeiss	E	#5

Each counter counted each slide using each microscope, resulting in a total of 125 counts. No duplicate counts were performed. Counting was done in random order under the restriction that the five microscopes were always to be in simultaneous use by the five counters. Such random scheduling can be assumed to preclude the possibility that effects of extraneous factors varying systematically during the course of the experiment (e.g. lighting, learning, etc.) could be mistaken for counter or scope differences. Only the experimental error would be inflated by the random presence of such uncontrolled factors and environmental changes.

The five individuals who did the counting for this experiment cannot be considered to be a random sample from any hypothetical population of counters. Therefore, any statistically significant average differences which may have occurred between counters are not to be used to estimate a variance of systematic counter errors. Instead, counter differences should be investigated individually if possible and interpreted in terms of mechanistic causes.

The experimental design specified that enough fields were to be counted to yield approximately 100 total fibers. However, the actual data for the four high-range slides show total counts ranging from 87 to 315 fibers and numbers of fields counted ranging from 20 to 44. For the low-range slide (#5), 100 fields were always counted and the total counts range from 6 to 19 fibers.

Counts were expressed in terms of fibers per square millimeter of surface counted. The equation is:

Fibers/sq mm = (total fibers/number of fields)/(f), where

f = area (sq mm) of one counting field for the microscope used (see below),

Microscope	f(sq mm/field)
B & L	0.006084
Nikon	0.004225
Leitz	0.007225
Olympus	0.006724
Zeiss	0.006806

STATISTICAL EVALUATION PROCEDURE

The method of analysis of variance (ANOVA) was used to partition the total variability of the 125 counts into component parts accountable to the following sources of variations.

Source of Variation	Degrees of Freedom
Microscopes (M)	4
Counters (C)	4
Slides (S)	4
M x C Interaction	16
M x S Interaction	16
C x S Interaction	16
M x C x S Interaction (used as an estimate of experimental error)	64
Total	124

F-ratios were used as the statistical criteria to test significance of each source of variation. Decisions were made at the 0.05 type-1 error level.

Since results (fibers/sq mm) ranged from 327 to 2935 for slides 1, 2, 3, and 4, but only from 8.9 to 30.8 for slide #5, it was clear that variability of results among counters and scopes was roughly proportional to mean fiber concentrations. Therefore, the response variable for use in the analysis of variance was taken to be:

$$y = \log 10(\text{fibers/sq mm}).$$

Such a $\log_{10}$-transformation was used to minimize interactions over the wide range of concentrations of the five slides. If no transformation had been used, it would have been necessary to perform a separate statistical analysis on each slide.

The wide range of counts on different filters also creates the problem that the standard deviation of y-values for filter 5 is much higher than the standard deviations for filters 1, 2, 3, and 4. The theoretical (Poisson-derived) variance of true y-values (calculated from true counts in different areas of the same filter) is equal to the reciprocal of the average number of fibers counted. Since considerably fewer total fibers were counted for filter 5 than for filters 1, 2, 3, and 4, the assumption of homogeneity of variance (which is required in order for the method of analysis of variance to be strictly valid) is not true with respect to the F-test of overall filter means. However, the invalid F-test for filter means does not concern us because filter means are known to be unequal. F-tests of counter means or of microscope means are valid because each mean has approximately the same standard error, namely

$$\text{S.E.(mean)} = (\sigma^2 /25)^{1/2} \text{, where}$$

$$\sigma^2 \simeq (1/5) \{ (1/857) + (1/1006) + (1/822) + (1/1537) + (1/19) \} = 0.0113$$

The variance $\sigma^2 \simeq 0.0113$ is the theoretical (Poisson-derived) error variance of the y-transform, but in the analysis of variance an empirical estimate was calculated from the data to allow for the possibility that additional (non-Poisson) sources of variation had inflated the experimental error (e.g. clumping of fibers, uneven film thickness, or other cause for non-uniform distribution over the surfaces of slides).

RESULTS

The results of the analysis of variance are shown in Table 1. The main effects of both microscopes and counters were highly significant and, of course, a significant main "effect" was also expected for slides. None of the interactions approached statistical significance because, on the scale of the log10-transformation, approximately equal differences among scopes and among counters were obtained, respectively, for all five slides. This implies that systematic factor effects tended to be equal for all five slides when expressed as percentages of respective mean

Table 1

Analysis of Variance of y = log10(fibres/sq.mm)

Source of Variation	Degrees of Freedom	Pooled d.f.	Variance	Pooled Variance	F-Ratio	Probability
Microscopes (M)	4		0.1320		13.2	10 (**)
Counters (C)	4		0.1098		11.0	10 (**)
Slides (S)	4		15.3681		1537.	--
M x C	16	112	0.0056	0.0095	0.56	0.90 (NS)
M x S	16		0.0055		0.55	0.91 (NS)
C x S	16		0.0153		1.53	0.12 (NS)
M x C x S (Error)	64		0.0100			
Total	124					

** = statistically significant at the 0.01 probability level.
(NS) = not significant at the 0.05 probability level.

counts. In fact, there was an almost complete absence of interactions in this experiment as indicated by the size of the pooled variance for the four interaction effects which was equal to 0.0095. This value agrees almost exactly with the expected theoretical (Poisson) variance of 0.0113 derived earlier in this report.

The final step in the statistical analysis was to elucidate the pattern of differences among the five levels of each main effect. This was done by using Duncan's Multiple Range Test to perform multiple pairwise comparisons among main effect means. Results are shown in Table 2 which indicate that: 1) the Nikon microscope produced significantly higher counts than the other four scopes which were statistically homogeneous among themselves, and 2) one counter had significantly lower results and one counter had significantly higher results compared to the remaining three counters whose results were statistically homogeneous among themselves.

Table 2

Results of Duncan's Multiple Range Test of Ratios Among Main-Effect Geometric Means

Microscope	Geometric Mean (% of Grand Mean)		Counter	Geometric Mean (% of Grand Mean)	
Leitz	90.3		A	82.8	
Zeiss	92.5		B	94.5	
Olympus	93.2	Geom. Mean = 92.8%	C	97.7	Geom. Mean = 98.7%
B & L	95.5		D	104.1	
Nikon	134.6		E	125.7	

Values within a bracket do not differ significantly at the 0.05 probability level.

SUMMARY

The following observations about scope differences and counter differences apply to each of the five slides. NOTE: These observations cannot be used as a basis for a general evaluation of the quality of a particular make or model microscope since only one instrument from each manufacturer was used in this study.

1) For each of the five counters, the Nikon microscope tested produced counts which were approximately 45% higher on the average than counts obtained using the particular Leitz, Zeiss, Olympus, or B & L microscopes in the evaluation.

2) The excellent agreement between the empirical error variance, calculated from pooled interaction effects, and the theoretical error variance, based upon the assumption of Poisson-distributed true counts, indicated that each filter had been sampled such that excessive variability among fields in different areas of a slide did not exist. That is, results indicate that film thickness was uniform and that clumping of fibers (non-random coalescence) did not occur.

3) Using any of the five scopes, three counters had statistically homogeneous counts: B, C, and D. Taking the average of these three counters as 100%, counter A's average was 84% (16% lower) and counter E's average was 127% (27% higher).

4) The evaluation study has shown that a significant difference in asbestos fiber counts can result when the same filters are counted on different makes and models of microscopes. Significant differences can also exist between trained counters.

5) It cannot be concluded that all Nikon phase contrast microscopes will consistently yield asbestos fiber counts 45% higher than counts done on other microscopes or that the other four makes of scopes will yield consistently equal counts. A much more extensive study would be required to answer these questions.

6) The authors believe that any laboratory counting asbestos on membrane filters should be part of an interlaboratory collaborative quality program where asbestos filters are exchanged between laboratories. This participation would insure that 1) equipment is properly adjusted and calibrated, 2) counters are properly trained and their counting proficiency is continually evaluated, and 3) comparable results are obtained from each laboratory. Of the counters who participated in this study, only one was a participant in an interlaboratory collaborative quality control program. This type of program is now being conducted by the National Institute for Occupational Safety and Health (NIOSH) for both governmental and private laboratories.

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