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TRANSMISSION ELECTRON MICROSCOPY ASBESTOS LABORATORIES QUALITY ASSURANCE GUIDELINES

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PREFACE

On October 22, 1986, the Asbestos Hazard Emergency Response Act of 1986 (AHERA) was signed into law. AHERA required the U.S. Environmental Protection Agency (EPA) to develop regulations for the control of asbestos-containing materials in schools and the National Institute of Standards and Technology (NIST) to formulate a laboratory accreditation program for transmission electron microscopy (TEM) laboratories involved in the analysis of air samples for asbestos.

EPA's regulation for schools included new analytical protocols for TEM analysis of air samples, the Interim Transmission Electron Microscopy Analytical Methodologies (40 CFR 763, Appendix A to Subpart E). NIST, in its development of a National Voluntary Laboratory Accreditation Program (NVLAP) for TEM laboratories, determined the test method for accreditation would be EPA's methodology.

NIST accredits laboratories in the NVLAP based on a multistep process. First, laboratories are evaluated by an expert technical assessor in an on-site assessment. The assessor reviews all aspects of the laboratory's operation. In addition, the assessor examines the quality manual and quality assurance system instituted by the laboratory. The quality assurance system and its associated procedures are critical components of routine laboratory operation. Second, NIST issues proficiency testing samples to the laboratory. The laboratory analyzes the samples and returns the results to NIST. Finally, based on the results of the site visit and the proficiency testing, NIST determines whether the laboratory meets all requirements for accreditation.

These EPA guidelines have been produced to assist TEM laboratories in developing and refining their QA programs. The guidelines themselves do not constitute a QA manual. Instead, they provide background information on the role of QA and suggestions on its implementation. Following these guidelines does not guarantee accreditation by NIST. Quality assurance is an ongoing process specific to each laboratory. Therefore, laboratories should continually endeavor to extend and improve their programs beyond the suggestions in these guidelines.

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1.0 INTRODUCTION

These EPA guidelines have been produced to assist laboratories in developing and refining quality assurance programs for analysis of airborne asbestos by transmission electron microscopy (TEM). While much of the information will be useful for TEM analysis of airborne asbestos in general, the guidelines are directed specifically toward the analytical protocol required by the U.S. Environmental Protection Agency (EPA) to determine completion of response actions in schools (Appendix A to Subpart E, 40 CFR 763).

Regulatory language tends to be terse and, by necessity, lacks discussion and explanation. These guidelines explain the purpose of a quality assurance program and provide examples of how a laboratory might establish a program to achieve and maintain specific data quality objectives. The information should assist laboratories in the preparation of quality manuals as required by the National Voluntary Laboratory Accreditation Program (NVLAP) for TEM administered by the National Institute of Standards and Technology (NIST). This document is not concerned with the administrative aspects of quality manuals; these aspects are clearly and adequately described in the NVLAP Airborne Asbestos Handbook (NIST 1989).

The suggested procedures are intended to be compatible with existing regulations, but may not cover all requirements and are not necessarily the only way in which compliance may be achieved. Also, regulations are subject to revision over time. Readers should regard the guidelines as supplementary information to be used in association with regulatory or other requirements, not as a substitute for the requirements themselves. The guidelines will be updated as required to reflect new information and changing requirements.

The remainder of this document is divided into two parts. Part 1 provides general background information. Quality assurance is described from the perspective of the laboratory and the laboratory's client. The statistical concepts of bias and precision are introduced and the objectives of a quality system stated. A "How to Use these Guidelines" section is directed particularly to the new laboratory establishing a QA program for the first time. Part 2 of the

guidelines describes specific procedures designed to achieve quality assurance objectives. The organization of the section follows the progress of a sample through the analytical procedure, beginning with general laboratory methodology and receipt of samples, through control of contamination, re-counting of certain samples, and use of standards, to data recording, calculation and reporting of results.

2.0 HOW TO USE THESE GUIDELINES

As stated earlier, these guidelines alone are not sufficient to develop and implement a QA program. For inexperienced laboratories the following steps are recommended:

- Read Section 3.0 (Background) of these guidelines and consult other references to gain a general understanding of quality assurance. Information sources are listed in Appendix A.

- Read the remainder of the document to get an overview of items that need to be considered in a TEM laboratory.

- Collect all the information relevant to the types of analyses and mandatory or voluntary accreditation programs with which you expect to be involved (e.g., AHERA, NVLAP, State and local regulations, and other accrediting organizations).

- Plan the sections of your quality manual to cover all the items you have identified in the first three steps. Arrange the sections of the manual so that it will be easy to use in the lab. For example, you might organize it according to the analytical process (receipt of samples, preparation, analysis, reporting) or according to personnel responsibilities (laboratory manager, technicians, analysts).

- Prepare each section using material from these guidelines and any other relevant sources. Where appropriate, define data quality objectives and specify how compliance with them is to be measured on a section by section basis. Start describing what you do now. If present procedures are obviously inadequate, decide how to modify them.

- Organize the quality manual so that it can be revised easily. Add new procedures or modify existing ones as the need arises. Ensure that the manual is read, understood, and followed by everyone in the laboratory.

3.0 BACKGROUND

3.1 WHAT IS QUALITY ASSURANCE?

Quality assurance (QA) is a system of activities intended to assure the producer (the laboratory) or the user of reported data (the client) that the data are of known quality. Since almost every activity taking place in a laboratory potentially affects the quality of data produced, QA covers most aspects of laboratory practice including facilities, personnel, and procedures. Quality control (QC) is the subset of QA activities that involves measuring quality on an ongoing basis and taking corrective action when necessary. For example, applying regular proficiency tests to monitor performance falls under the definition of QC, whereas requiring that personnel have certain education qualifications, although part of QA, is not part of QC. These guidelines tend to emphasize QC, not because other aspects of QA are less important, but because QC procedures often require greater explanation.

QA is important to the laboratory because it establishes the quality of its product. Superior quality attracts and keeps clients. In addition, a realistic statement of quality safeguards the laboratory from misinterpretation by clients who might otherwise interpret individual results as being more precise than they really are. QA measurements have important legal implications when the results of a particular analysis or set of analyses are questioned. A knowledgeable client will ask for details of the QA program when selecting a laboratory. Measures of quality are needed by the client in order to design a monitoring program (the quality of individual measurements affects the number of analyses required to achieve a particular objective) and to interpret the results. Decisions made on the basis of laboratory measurements may involve considerable cost and have significant health impacts. Therefore, it is critical for the client to be adequately informed regarding data quality.

3.2 QUALITY ASSURANCE FOR TEM ANALYSIS OF AIRBORNE ASBESTOS

The general principles of QA and QC apply to TEM analysis of airborne asbestos. EPA has defined Good Laboratory Practice (GLP) Standards (40 CFR 792). The standards are not specific for TEM laboratories because they address topics to be considered in any laboratory QA program. According to EPA regulations, laboratories must address these requirements when establishing a QA program. The GLP Standards may be obtained by contacting EPA at US EPA

(TS-799), 401 M Street, S.W., Washington, D.C. 20460 or by phoning (202) 554-1404. Other sources of general information on QA are listed in Appendix A.

There are aspects of TEM analysis that differ from other types of chemical analyses and require special consideration when formulating the details of a QA program. Unlike many analyses that involve a calibration curve approach, TEM is a counting protocol in which individual particles are identified and counted. The data are essentially discrete, rather than continuous. Furthermore, there is often a need to measure low asbestos concentrations for which approximations using continuous statistical methods (for example, the normal distribution) are least satisfactory. The approaches suggested in this document have been developed taking into account these special features of TEM. Since the AHERA TEM protocol is relatively new, there is a lack of data and experience on which to base specific recommendations. Approaches, particularly those involving quantitative measures of quality, are expected to evolve as laboratories experiment and find approaches that work best in their situations. This document will be revised periodically to reflect new developments. These guidelines present one way of monitoring errors in the laboratory. As NIST conducts proficiency testing rounds, additional information will be gathered. A reasonable system for monitoring and reporting errors will be developed using the NIST data.

TEM asbestos laboratories are responsible for estimating the concentration of asbestos structures per unit surface area of a filter. To do so, the laboratory makes two measurements: the area of filter examined and the number of asbestos structures present in that area. The number of asbestos structures present is divided by the area of filter examined to obtain an estimate of the number of asbestos structures per unit surface area of filter. Of course, many laboratories will make additional calculations such as estimating airborne asbestos concentration using data provided by the client. The quality of these additional data depends on factors beyond the control of the laboratory. The factors include the accuracy with which the air volume was measured, filter handling during sampling and transport, and filter contamination from various sources before the filter reached the laboratory. Realization that the laboratory analysis provides only an estimate of the number of asbestos structures per surface area of filter simplifies the approach to laboratory QA and reduces confusion over certain analytical terms. Blanks (filters through which no air has been drawn), regular air samples (filters through which

air has been drawn), standard material, and filters of unknown origin are all treated in the same manner. This argument is not meant to suggest that laboratories should lose sight of the objective of their clients, which in most, but not all, cases is related to asbestos in the sampled air. Rather, the intention is to clarify the distinction between factors affecting quality that can be controlled by the laboratory and those which cannot, so that efforts to improve the quality of data can be properly focused.

Having reduced TEM analysis to the generation of two quantities--the area of filter examined and the number of asbestos structures present in that area--QA is directed to ensuring that these quantities are sufficiently accurate for their intended purpose. Accuracy is usually defined in terms of bias and precision as discussed in the following sections.

3.2.1 Bias

Bias is a systematic error that is either inherent in a method or caused by some artifact or idiosyncrasy of the measurement system. Bias may be positive or negative, and several kinds can exist concurrently. Examples of positive bias include

- use of contaminated reagents that artificially inflate the number of asbestos structures counted; and

- inclusion of nonasbestos structures in the asbestos structure count because an analyst is insufficiently skilled in identifying electron diffraction patterns and consequently overestimates asbestos concentration.

Examples of negative bias include

- etching too much of the filter through improper calibration of the plasma etcher resulting in a loss of asbestos structures; and

- incorrectly rejecting some asbestos structures as too small because of inaccurate magnification calibration and thereby underestimating asbestos concentration.

Although many sources of bias can be identified and steps taken to reduce their contribution, the net bias associated with a final measurement may be difficult to assess because there is sometimes no way to determine the "correct" answer. Assessment of net bias is often by consensus. For example, if five laboratories consistently obtain comparable results on interlaboratory tests and a sixth laboratory consistently obtains lower results, the conclusion is that the sixth laboratory has a consistent negative bias, even though it is possible that the sixth laboratory is least biased and the other five laboratories have a consistent positive bias. Standard materials (See Section 4.9.10) can be used to measure bias for a restricted set of cases. Unlike other types of chemical analyses, asbestos samples cannot be spiked easily in order to assess bias. One source of bias that can be measured and reported in a relatively straightforward manner is the positive bias due to contamination from sources other than the sample received from the client. Contamination may occur during filter manufacture, through the use of reagents that contain asbestos, or through handling in the laboratory. Contamination is measured with laboratory blanks as discussed in Section 4.6.2.

3.2.2 Precision

Precision is the degree of mutual agreement betweeen repeated independent measurements of the same quantity. It is concerned with the scatter of the results. Unlike bias, precision can be readily measured. Repeated measurements on the same sample following the complete preparation and analysis procedure each time provide an overall measure of precision. Repeated measurements that are close together indicate high precision. The coefficient of variation (also called the relative standard deviation) is one measure of precision that has proved convenient for asbestos analysis. The coefficient of variation is defined as the standard deviation divided by the mean. A small coefficient of variation indicates high precision.

Sources of variability, and hence contributors to lowered precision, include

- variability introduced during the sample receiving and preparation steps;
- subsampling of the filter (selection of a small portion for microscopic examination); and

- variability introduced during microscopic examination due to variability in equipment and analyst performance.

Each of these major sources can be broken down into smaller components, such as variability introduced during plasma etching, or day-to-day variability in the performance of a single analyst.

The subsampling step in which a portion of filter is selected for microscopic examination has special significance because it places an upper bound on precision. No matter how meticulously procedures are followed, or how skilled the analyst, repeated measurements will give different results because there are different numbers of asbestos structures on different sections of the filter. Since the laboratory has no control over the spatial distribution of structures on the filter, variability introduced through subsampling cannot be controlled by the laboratory QA program.

Laboratory QA includes procedures for minimizing variability and hence increasing precision. Almost any action that defines and refines the procedures to be followed (educational qualifications, written standard operating procedures, recording requirements) has the potential to improve precision. Laboratory QC, as a subset of the QA program, is involved in the active on-going assessment of precision and the taking of corrective action when precision falls below a predetermined level. Different types of repeated measurements are used to measure different components of precision. Figure 3-1 provides three examples of the many possibilities. See Section 4.7 for a more detailed discussion. Note that whenever repeated analyses involve a different subsampling of the filter surface, the spatial distribution of structures will contribute variability and provide an upper limit to the precision that can be obtained.

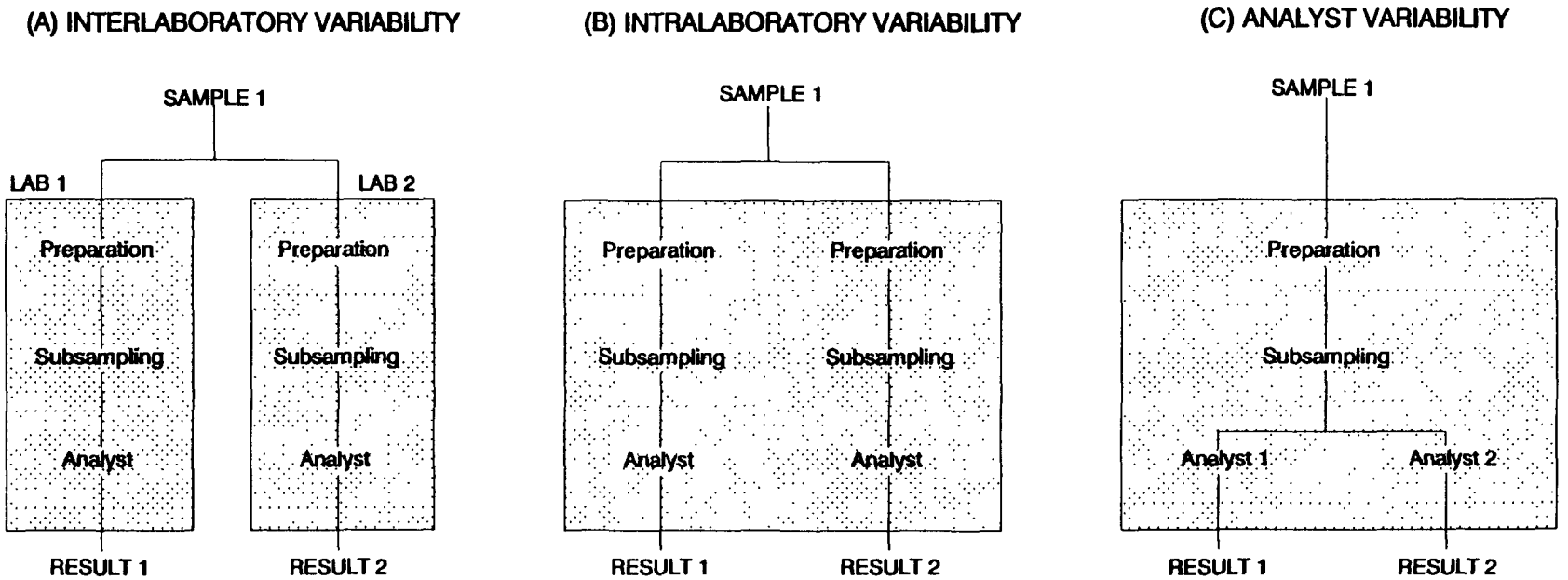

Figure 3-1. Examples of different types of repeated analyses that may be used to assess precision. (A) Measurement of interlaboratory variability by having two laboratories analyze the same sample. (B) Measurement of intralaboratory variability by making two separate preparations of the same sample. (C) Measurement of between analyst variability by having two analysts count the same set of grid openings. Note that the subsampling step contributes to the variability in (A) and (B), but not in (C). Subsampling places an upper limit on the level of precision that can be achieved.

3.3 OBJECTIVES OF A QUALITY SYSTEM

The quality system, the system of procedures that represents the QA program, has three main objectives:

1. To achieve acceptable data quality;
2. To identify and correct problems that are having an undesirable effect on data quality; and
3. To characterize the quality of the data produced.

The fourth and final section of this document discusses specific procedures that are designed to achieve these objectives. To the extent permitted by applicable regulations and requirements, these procedures will vary from laboratory to laboratory depending on individual circumstances. First, each laboratory must define its own acceptable data quality objectives. A data quality objective is a statement of the quality of data needed for a particular purpose. For example, a laboratory might specify that asbestos structure levels measured on blanks should not exceed a certain concentration, or an analyst should correctly identify at least a certain percentage of electron diffraction patterns presented during monthly QA checks. Data quality objectives may change with time as the laboratory gains experience, acquires new equipment, or makes other modifications. Second, the emphasis on particular problem areas may shift as some problems are eliminated (e.g., a supplier of contamination-free filters is located) and others are introduced (e.g., an inexperienced analyst joins the staff). Third, as stated earlier, although the general principles have been widely used by laboratories for many types of analyses, the specific quantitative methods suggested in this document for characterizing TEM data quality are largely untested and are expected to be modified as experience is gained. The procedures described in Section 4.0 should be adapted and incorporated into a Quality Manual written specifically for an individual laboratory (See Section 4.1).

4.0 ESTABLISHING AND MAINTAINING A QUALITY SYSTEM

4.1 QUALITY MANUAL CONCEPTS

There is often some confusion between the terms "quality manual", "quality assurance manual," and "quality assurance records." The term "quality manual" has been used by NVLAP to denote a manual which contains not only a complete quality assurance manual, but also information on various other topics such as staff qualifications and training programs. A quality manual should be an up-to-date document which completely describes all of the procedures used in the TEM laboratory. In the quality manual, a new employee should be able to find all of the procedures used by the laboratory from log-in of the sample to the issuing of the final report. It should also contain examples of all of the forms used by the laboratory in processing of a sample through the system.

The quality assurance section of the quality manual provides a description of the various types of QA activities and samples, how frequently these activities will be performed, along with details of the methods by which QA data are summarized, and a description of when and how corrective actions are to be taken.

Quality assurance records are the data output from calibrations and from the analyses of quality assurance samples. These data are more conveniently maintained and summarized in a separate file, and computer records are often used. The quality assurance records should contain details of the conclusions drawn from the data, and any corrective actions which have been taken. If the corrective actions involve a change of analytical procedure, the new procedure should then appear as a revision of the quality manual.

The quality manual should be a document which progressively evolves along with changes in the size and nature of the analytical laboratory. A quality manual which is appropriate in content for a one-person laboratory will not be satisfactory for a large laboratory with numerous TEM operators and several instruments. In setting up a manual, it must be recognized that frequent revisions and additions will be necessary, and the system of page numbering and format should be chosen to accommodate these changes easily. The system of numbering used in this document (Abbreviated title, Revision #, Date, Section #, Page # of #) is useful. Changed or deleted sections should be archived, not destroyed.

The responsibility for writing and incorporating changes into the quality manual must be delegated to a particular individual. Periodic meetings should be held with administrative and laboratory staff to determine what changes are necessary in the quality manual. An organizational change of senior analytical staff, change in facilities, or the introduction of changes in either the analytical methodology or the sample recording procedures are examples of situations where revisions in the quality manual are warranted.

4.2 LABORATORY METHODOLOGY

All of the analytical procedures used by the laboratory for measurement of asbestos structure concentrations in air by TEM should be fully documented in the quality manual; however, merely inserting a copy of the published methodology in the quality manual is not considered sufficient. Each laboratory implements methods in different ways because different equipment and facilities are used. Also, analytical methodologies are not static; experienced laboratories continue to incorporate significant improvements, which are not part of the published methodologies, into their procedures. Where the analytical methodology is complex, as it is for TEM asbestos analysis, and the methodology is available as a published document, the basic method may be referenced and bound separately, or it may be included in full in the quality manual. In particular, the method described in Appendix A to Subpart E, 40 CFR 763, October 30, 1987, must be practiced by the laboratory, and a copy of this method must be available in the laboratory. However, the quality manual must contain detailed descriptions of the analytical procedures exactly as practiced by the particular laboratory, in order to ensure that all analysts in the laboratory have the required information enabling them to work to a uniform standard.

4.3 PROCEDURES FOR RECEIVING AIR SAMPLES

4.3.1 Documentation

The method of handling documentation of air samples upon receipt by the laboratory must be fully described in the quality manual. For example, procedures and forms should exist for recording of the method of shipping, the date and time of receipt, how many samples were in the shipment, and also for recording of the sample descriptions and data, such

as air volumes, provided by the client. The action to be taken in the event of incomplete sample documentation should be described in the quality manual.

4.3.2 Acceptance/Rejection Criteria

The quality manual should contain a clear description of the criteria used by the laboratory for rejection of samples. There may be cause for rejection of a sample at various points in the receiving and analysis process. For example, if air samples are received with bulk samples in the same container, then in accordance with the AHERA method, they must be rejected. As the sample is processed, it may be discovered that the filter had been damaged in some way, or there may be evidence of air leakage during sampling. Any such defect which could possibly compromise the final result must be noted, and the action taken must be recorded.

4.4 SAMPLE CUSTODY CONSIDERATIONS

Documentation of the chain of custody for samples is very important. Chain of custody involves recording the passage of samples from person to person and place to place and identifying where and under whose responsibility samples are at any particular time. Any gap in knowledge concerning the custody of samples can be used to discredit the results of the analyses. The procedures for recording chain of custody on all samples must be described, and a copy of the chain of custody form used by the laboratory should be included in the manual. Chain of custody records and the samples should be stored securely, and their locations should be specified in the quality manual. The policy of the laboratory concerning breaks in the chain of custody and the actions required in this event must also be specified.

4.5 MEASUREMENT OF GRID OPENING DIMENSIONS

The area of filter examined is one of the two measurements for which the laboratory is responsible. The number of asbestos structures present in that area will be divided by the area to give the estimated concentration of asbestos structures per surface area of filter. The method used by the laboratory to measure the grid opening dimensions must be specified, and the accuracy of the measurement method must be established. One option is to measure a sufficient number of grid openings from a batch of grids and to derive a mean dimension which

is then used for all samples. Another option is to measure grid openings on each of the actual TEM grids examined for a sample, and calculate an area for each sample.

When the first method is used, the area estimate affects the estimated concentration in two ways. If the area estimate is greater than the true average area, all concentrations estimated using that area estimate will suffer a negative bias; if the area estimate is less than the true average area, all concentrations estimated using that area estimate will suffer a positive bias. The area estimate also contributes variability to the estimated concentration because, even if the estimate is an unbiased estimate of the true average area, it will not be exactly equal to the area examined in any particular analysis since grid opening size varies from grid opening to grid opening. In general, the effect of the area estimate on the accuracy of the estimated concentration is expected to be small relative to inaccuracies surrounding the estimate of the number of asbestos structures. The following quality control procedure is suggested to ensure that this is the case.

Select 20 grids at random from each lot of grids (approximately 1,000 grids). Measure the area of at least 20 grid openings on each grid. Calculate the mean of the 400 measurements and use the mean as the estimated area of one grid opening for all calculations using grids from that lot.

When the second method is used, at least one grid opening must be measured on each grid. Additional measurements may be needed if there is significant variability among grid openings within a single grid.

Depending on lot size and the amount of variability within and between grids, the first method may be more practical for routine laboratory operations. Individual measurements on each grid may be called for in special cases where specific documentation is needed. Maximum accuracy is achieved when the area of each grid opening examined in the analysis is measured.

4.6 CONTROL OF CONTAMINATION

4.6.1 General Considerations

Chrysotile asbestos is ubiquitous in the general environment. Therefore, materials used in preparation of TEM specimen grids from air sample filters can be contaminated by

asbestos fibers. Accordingly, the methods used by the laboratory to ensure that results are not affected by contamination must be addressed in detail. The methods used by each laboratory will vary, depending on what has been found satisfactory. Some laboratories specify that all TEM specimen preparation will be performed in a positive pressure clean room. Others have found that reliable data can be produced by less extreme measures. The precise procedures used by the particular laboratory should be fully described. The actions to be taken in the case of a contamination incident must be specified. If a source of contamination is identified, procedures should be modified to minimize the possibility of a recurrence. The modifications should be documented by a revision to the quality manual.

4.6.2 Laboratory Blank Measurements

The preparation of unused filters along with actual sample filters provides a means of detecting whether the result of any TEM analysis has been compromised by contamination from sources in the laboratory during preparation. The unused filter material employed for laboratory blanks should be of the same type (mixed cellulose ester (MCE) or polycarbonate (PC)) as that employed for the samples, and it should be selected from a stock of filters maintained by the laboratory for the purposes of its own laboratory blank program. These filters should have a low and well-characterized rate of asbestos contamination. The AHERA TEM protocol specifies one laboratory blank be prepared for each group of samples prepared together, or a minimum of one blank for every 10 samples prepared. At least one of the laboratory blanks will be analyzed for every 25 samples.

The AHERA protocol specifies the minimum number of blanks. The laboratory may exceed these minima. It is recommended that at least one laboratory blank be included with every group of samples processed as a group through the plasma asher and the Jaffe washer in order to provide blank control over every sample. No sample should be passed through the preparation sequence unless it is accompanied by a laboratory blank. If high concentrations are observed on a group of samples, and no blank was processed simultaneously along with the group, there is no evidence available to prove that the results are not a consequence of laboratory contamination. A blank filter incorporated with some earlier group of samples is of little diagnostic value, and re-preparation of the filters would be necessary because the validity of the

high results cannot be demonstrated.

The policy of the laboratory concerning the point at which blank filters are introduced, the number of blank filters to be prepared, and the number of these blank specimens to be examined in the TEM should be described. The AHERA protocol specifies 53 structures/mm^2 as the acceptable upper limit for a single background measurement, for both PC and MCE filters, and the long-term average of these background measurements may not exceed 18 structures/mm^2. The laboratory may apply more stringent standards, and should attempt to achieve the lowest possible laboratory blank values. The quality manual should specify the upper limits of acceptability for blank measurements, along with the actions to be taken if these limits are exceeded.

4.6.3 Filter Lot Blanks

Before air samples are collected, it is important to establish that the particular batch of filters to be employed for air sampling has an acceptably low background contamination. Filters are selected at random from the batch for analysis. These measurements are known as "filter lot blanks." For blank measurements to be truly representative of the filter materials used to collect the actual samples, the laboratory should analyze unused filters from the same lot number. The AHERA protocol requires that the maximum permissible levels of contamination on filter lot blanks are 53 structures/mm^2 with a long-term average not exceeding 18 structures/mm^2.

The relevance of filter lot blanks to the operation of a particular laboratory depends on the range of services offered by the laboratory. If the laboratory supplies filter cassettes to the client, or if the laboratory personnel collect field samples, the responsibility and methods for ensuring that the filters are of sufficiently low background must be clearly defined in the quality manual.

4.6.4 Field Blanks

The AHERA TEM protocol specifies that one closed and two open field blanks shall be submitted along with each group of air sample filters. Closed field blanks are cassettes which have been transported to the sampling site and sent to the laboratory without having been

opened; open field blanks are cassettes which have been transported to the sampling site, opened for a short time without any air having passed through the filter and sent to the laboratory. The sealed blank should not be regarded by the laboratory as equivalent to a laboratory blank, because it has been out of the control of the laboratory. The laboratory should always add laboratory blanks from its own stock of well-characterized filter medium to each preparation series, because if the sealed blank exhibits any contamination, a laboratory blank provides the only way by which a laboratory can confidently demonstrate the integrity of the results.

4.6.5 Reagent, Equipment and Material Blanks

The extent to which reagents, equipment and materials used in the TEM specimen preparation require investigation for the possible presence of asbestos contamination depends on the results of laboratory blanks. If, with the existing reagents, equipment and materials, laboratory blanks consistently meet the required specifications, there is no need to investigate further. Each of the items used in the specimen preparation can potentially be sources of asbestos contamination. The procedures used by the laboratory to establish that these materials do not contribute fiber contamination to the measurement should be described in the quality manual. If contamination is detected on a laboratory blank, then it will be necessary to investigate every item used in the analysis, including the reagents, tools, glassware, plasma asher and vacuum evaporator. It is recommended that records be kept of any changes in the analytical procedure, particularly such events as opening of a new bottle of solvent or the introduction of new glassware.

4.6.6 What to Do When Contamination is Detected

A contamination incident is usually detected by finding asbestos structures on a blank, or on any other sample which was not anticipated to have asbestos structures. The procedures which the laboratory follows in this event should be described. When a laboratory blank is found to be contaminated, all of the samples which were prepared following the previous satisfactory blank measurement must be assumed to have been compromised. An example of these procedures is described in Appendix B.

4.7 QUALITY ASSURANCE REANALYSIS OF SAMPLES

4.7.1 Types of Reanalysis

The precision of TEM asbestos analysis is determined by a statistical evaluation of repeat analyses of samples. The terms "duplicate analysis" and "replicate analysis" have often been used, but the meanings of these terms are ambiguous, and they are not sufficiently descriptive to accommodate the range of reanalysis possibilities. The following combinations are possible for repeat analyses:

(a) Reanalysis of the same grid openings of the specimen grids by the same operator;

(b) Reanalysis of the same grid openings of the specimen grids by another operator who is a staff member of the same laboratory;

(c) Reanalysis of the same grid openings of the specimen grids by another operator who is a staff member of another laboratory operating under a different quality assurance program;

(d) Reanalysis of the same specimen grids by the same operator, without restricting analysis to the same grid openings;

(e) Reanalysis of the same specimen grids by another operator who is a staff member of the same laboratory, without restricting analysis to the same grid openings;

(f) Reanalysis of the same specimen grids by another operator who is a staff member of another laboratory operating under a different quality assurance program, without restricting the analysis to the same grid openings;

(g) Preparation of new specimen grids from the original filter by the original laboratory, followed by analysis of the grids according to protocols (d), (e) or (f); and

(h) Preparation of new specimen grids from the original filter by a different laboratory operating under a different quality assurance program, followed by analysis of the grids according to protocols (d), (e) or (f).

In the case of reanalysis protocols (a), (b) and (c), another and more sophisticated level of control is possible. This is called "verified counting" (Steel and Small 1985; Ogden, et al. 1986). Verified counting is the only method known for determining many of the causes of analysis discrepancies between TEM operators. The use of verified counting allows the

classification of discrepancies into categories based on identification, complex counting rules, stage traverse methods, fiber sizing, etc. Verified counting is performed by having the same grid opening analyzed by more than one operator.

The only requirement for verified counting is simply that each structure in a grid opening be uniquely identifiable, so that cross comparison can be made between operators and verification can be performed on structures for which there is disagreement or those structures found by only one operator. One way of implementing verified counting is using an identical orientation of the same grid opening, using a similar scanning pattern so that asbestos structures are encountered by each operator in approximately the same order. Each asbestos structure detected is then identified and measured, after which a sketch is made of the appearance of the structure and its orientation. The asbestos structure counts obtained and the sketches of each structure made by the different operators are then compared and evaluated to determine which asbestos structures were correctly classified by each operator. It is then possible to specify each operator's performance in terms of true positive counts, false positive counts and false negative counts. Since it is often impractical to define all nonasbestos structures, the false positive and false negative error rates are defined in terms of the total number of asbestos structures. For example, suppose the area examined during verified counting contains 20 asbestos structures and an analyst correctly identifies 18 of the asbestos structures as asbestos and classifies the 2 remaining asbestos structures as nonasbestos. The true positive rate for that analyst is 90% (18/20 x 100), and the false negative rate is 10% (2/20 x 100). If the analyst also incorrectly classifies 3 nonasbestos structures as asbestos, the false positive rate is 15% (3/20 x 100).

The data on precision generated by repeat analyses according to the above protocols have different meanings. Laboratories do not need to apply all of these reanalysis types on a routine basis, but it is important to understand the various possibilities and the meaning of data derived from each.

It is necessary for a laboratory to generate information concerning the precision attained by individual TEM operators and the precision attained by the laboratory overall. It is also necessary to establish that the results are comparable with any consensus standards which exist, and with those generated by other laboratories. Absolute standards are not available to assist in these tasks, and the air filters to be analyzed do not generally exhibit good uniformity

of particle deposition.

The quality manual must show that the laboratory staff understand the types of reanalyses which are possible, and it must contain a description of how the laboratory uses the various kinds of reanalyses to characterize the precision and accuracy of the operators and the laboratory. Complete characterization of the precision and accuracy of the overall analytical method is not a responsibility for the individual laboratory, although some of the data generated from the reanalyses will provide estimates.

4.7.2 Determination of Operator Precision

The number of asbestos structures recorded on each grid opening of a TEM specimen grid is usually not constant. Accordingly, if the performance of an operator is checked by allowing the operator to select different sets of grid openings for each successive recount of the same sample, it is not certain whether any variability in the results is a consequence of operator variability or nonuniformity of particulate deposition on the specimen grid. Therefore operator precision can be measured only by reanalysis of the same grid openings. On a routine basis, these measurements should be made by simple recounts of the same grid openings. If significant discrepancies are detected by these routine QA procedures, the full verified counting procedure, incorporating a sketch of each asbestos structure and using defined grid orientations, should be used to investigate the origin of the discrepancies. It should be recognized that the use of the full verified counting procedure could alert the operator prior to the first examination of the grid that a test is being conducted. The operator is likely to take more care and therefore the precision value derived from verified counting will usually be more optimistic than that normally achieved by the operator under routine conditions. Laboratories can counteract this effect by recording all information needed for verified counts during their routine counts. Then verified counts can subsequently be performed on any previously counted squares.

4.7.3 Determination of Laboratory Precision

Recounts made by different operators on the same grid openings of specimens provide a measurement of the precision of fiber counting in the laboratory. As in the case of determination of individual operator precision, the most realistic data representative of normal

operation are obtained using routine re-counts of identical grid openings. If the analyst conducting the first examination is alerted to the fact that the count is a test, the verified counting procedure will yield an optimistic value for the laboratory precision. Laboratories can counteract this effect in several ways. One way would be to use the full verified counting procedure for all samples so that the operator is not aware of which grid openings are later to be verified by a second examination. This approach, however, is somewhat time consuming. An alternative approach is to select a proportion of the grid openings from routine sample examination and to examine these by the verified counting method in order to generate QA data. In the interpretation of verified counts, it should be recognized that chrysotile can suffer beam damage during the initial TEM examination, and this will compromise the ability of operators in successive examinations to obtain diffraction patterns.

4.7.4 Determination of Intralaboratory Method Precision

As discussed in Section 3.2.2, the distribution of asbestos structures collected on a filter is not within the control of the laboratory, and different areas of the filter will generally yield different results. This variability is an additional component related to the overall method of analysis. Repreparation of several sets of specimen grids from the original air filters and counting of asbestos structures on each of these sets by a different operator provides a measurement of the overall method precision as practiced by the particular laboratory.

4.7.5 Determination of Interlaboratory Method Precision

Interlaboratory measurements on different areas of the collection filter will generally exhibit the most variability. This type of interlaboratory measurement, in which each laboratory prepares its own grids from the original filters, provides information on the precision of the overall method since it replicates the entire protocol. All other precision estimates described here encompass a portion of the total.

4.7.6 Analysis of Proficiency Testing Samples

Proficiency testing samples will be issued to TEM asbestos laboratories as part of NVLAP. All operators in the laboratory will be required to count asbestos structures on these

samples in order for the laboratory to maintain its accredited status. This type of sample will provide an external reference. It is not possible to issue such samples frequently enough to serve as the only interlaboratory proficiency testing standards. Therefore, laboratories should, in addition, have arrangements for interlaboratory sample analyses to be conducted regularly with other organizations.

As described in section 4.7.2 (Determination of Operator Precision), a stringent internal proficiency testing program must also be incorporated into the QA program of a laboratory. A continuing program of this type has been shown to improve the performance of operators. Proficiency testing samples can be generated internally by the laboratory, using either previously-analyzed samples, or samples generated artificially. Three types of proficiency testing sample should be used: one type to test specimen preparation; one to test routine identification and quantification; and a third type to challenge the identification skills of the operators.

4.7.7 Interlaboratory Sample Analyses

If a laboratory is operating in isolation under an internal QA program, the results from the laboratory can be strongly biased. This is a consequence of the fact that asbestos analysis has a subjective component, and bias will only be detected when the laboratory results are compared with those of another laboratory for the same samples. A positive or negative bias can occur as a result of improper fiber identification criteria, or as a consequence of improper application of fiber counting criteria. A negative bias can arise if the specimen preparations are too thick, if the fiber identification criteria are excessively rigid, if fiber crystallinity is destroyed due to electron beam damage during examination, or if fibers are lost during the specimen preparation. Thick preparations lead to low image contrast and failure to obtain discernible electron diffraction patterns, leading to reporting of lower results. Excessively rigid fiber identification criteria may not take account of the instrumental limitations resulting in the rejection of many fibers when they are actually asbestos. Over-irradiation of the specimen in the TEM may lead to an inability to identify asbestos fibers in any succeeding analysis. Loss of fibers during preparation can occur as a result of some systematic feature of the specimen preparation procedure used. Any of these situations can exist indefinitely within one laboratory, because each operator uses the same criteria as specified originally by the laboratory supervisor. Moreover,

there may be good agreement between results generated by all of the operators, despite the fact that the results are actually strongly biased (precision but not accuracy).

Each laboratory should establish a relationship with at least one external laboratory for the purposes of conducting interlaboratory analyses. These analyses must be conducted blind, so the operator in the second laboratory is not aware of the result already produced by the first laboratory. The samples should be selected to have suitable fiber loadings so that the results will be diagnostic for any major discrepancies. If major discrepancies are detected, the reasons must be investigated, and verified counting is recommended as a useful tool for this purpose.

The number of samples to be analyzed by an external laboratory and the frequency with which this should be done are dependent on the number of analysts in the laboratory and the volume of samples analyzed. The number of analyses required can be minimized by an agreement between the laboratories to share all of the interlaboratory data. A sample exchange resulting in four interlaboratory results made four times per year is an acceptable minimum number. Filter sectors should be exchanged, so that the entire analytical sequence is tested and TEM specimen grids should also be exchanged for counting of identical grid openings by the two laboratories. If discrepancies are found, verified counting procedures should be instituted between the laboratories in order to resolve them.

The choice of external laboratory is important, because any discrepancies generated (possibly as a result of selection of an inexperienced partner) will form part of the laboratory's QA records. The external laboratory must, however, be totally independent; sample exchanges with a laboratory from the same corporation, the same organization, or one which is associated in any formal manner with the first laboratory, cannot be considered as interlaboratory exchanges.

4.7.8 How to Correct Counting Discrepancies

When discrepancies arise between two operators or two laboratories, they must be resolved before either the operators or laboratories can have total confidence in their results. An example of the resolution of such a discrepancy is discussed in Appendix B.

4.8 INTERPRETATION OF RECOUNT DATA

Repeated analyses of the same sample are intended to identify problems (failure to follow operating procedures in a consistent manner, inability of an analyst to distinguish asbestos structures from nonasbestos, different interpretations of counting rules) and to characterize performance over time both for the laboratory as a whole and for individual analysts. Repeated analyses are expected to give different results. To achieve the first objective it is necessary to decide when the differences are sufficiently large to indicate a problem. Ideally, this is based on accumulated performance data. A repeated analysis that falls outside the usual performance standards calls for investigation and corrective action. Performance data for a new laboratory, or a new protocol such as the AHERA TEM protocol, are not available when a QA system is originally developed. The following discussion provides some suggestions for accumulating and interpreting the necessary data.

There is an important distinction between repeated analyses that include different subsamplings of the filter (for example, (A) and (B) in Figure 3-1) and those that do not (for example, (C) in Figure 3-1). Different subsamplings of the filter introduce a component of variability that cannot be controlled by the laboratory. When a repeated analysis is done using the same grid openings that were examined in the original analysis, no subsampling is involved and differences between results cannot be attributed to the spatial distribution of asbestos structures on the filter. Verified asbestos analysis which does not involve a subsampling step is described in detail in Steel and Small (1985). and will not be discussed further in this section. When a subsampling step is involved, variability, as measured by the coefficient of variation, will depend on the number of asbestos structures counted. This fact complicates QC, especially at low concentrations (Ogden, et al. 1986). Since the AHERA TEM protocol is a clearance protocol, low concentrations are expected and cannot be ignored. The following procedure is designed for low concentrations.

For each type of repeated analysis involving a subsampling step, accumulate data for plotting the coefficient of variation against the mean. The coefficient of variation is given by:

$$CV = (s/m)$$

where $m = \Sigma x_i/n$ is the mean of the results obtained from n analyses of a single sample and $s = (\Sigma(x-m)^2/(n-1))^{\frac{1}{2}}$ is the standard deviation. If n varies from sample to sample, create a separate graph for each n, or distinguish the different values of n by using different colors or symbols on the graph.

Figure 4-1 indicates how the graphs of the coefficient of variation would look if the only source of variability were that introduced by a Poisson distribution describing the spatial distribution of structures on the surface of the filter[1]. The Poisson distribution represents the best case in which asbestos structures are distributed randomly across the surface of the filter. In practice, the distribution of asbestos structures across the surface of the filter may be somewhat patchy and other distributions such as the negative binomial may be more realistic (Javitz and Fowler 1981). If asbestos structures are distributed according to a negative binomial, the coefficient of variation will be greater than the coefficient of variation predicted by the Poisson distribution.

Ideally, if a Poisson distribution were appropriate, 50 percent of the coefficients of variation calculated by a laboratory should lie below the 50th percentile line,[2] 75 percent should lie below the 75th percentile line, and so on. In practice, coefficients of variation calculated by a laboratory from actual samples will be higher because, (1) the true spatial distribution may be more variable than that represented by the Poisson distribution, and (2) there are many other sources of variability in addition to the effect of subsampling. A laboratory might find, for example, that only 35 percent of its values fall below the 50th percentile, only 60 percent of its values fall below the 75th percentile, etc. The discrepancy between the ideal performance and actual performance is a result of additional variability beyond that expected from subsampling a Poisson distribution. There is insufficient experience at present to indicate the

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[1]The graphs were generated by computer simulation. A random sample of size n was generated from a Poisson distribution with a specified mean and the empirical coefficient of variation calculated. The process was repeated 500 times for each mean value.

[2]The theoretical percentiles were estimated from the 500 values of the coefficient of variation generated in the computer simulation. For example, the 75th percentile was determined by finding a value that was greater than 75 percent of the observations and smaller than 25 percent of the observations.

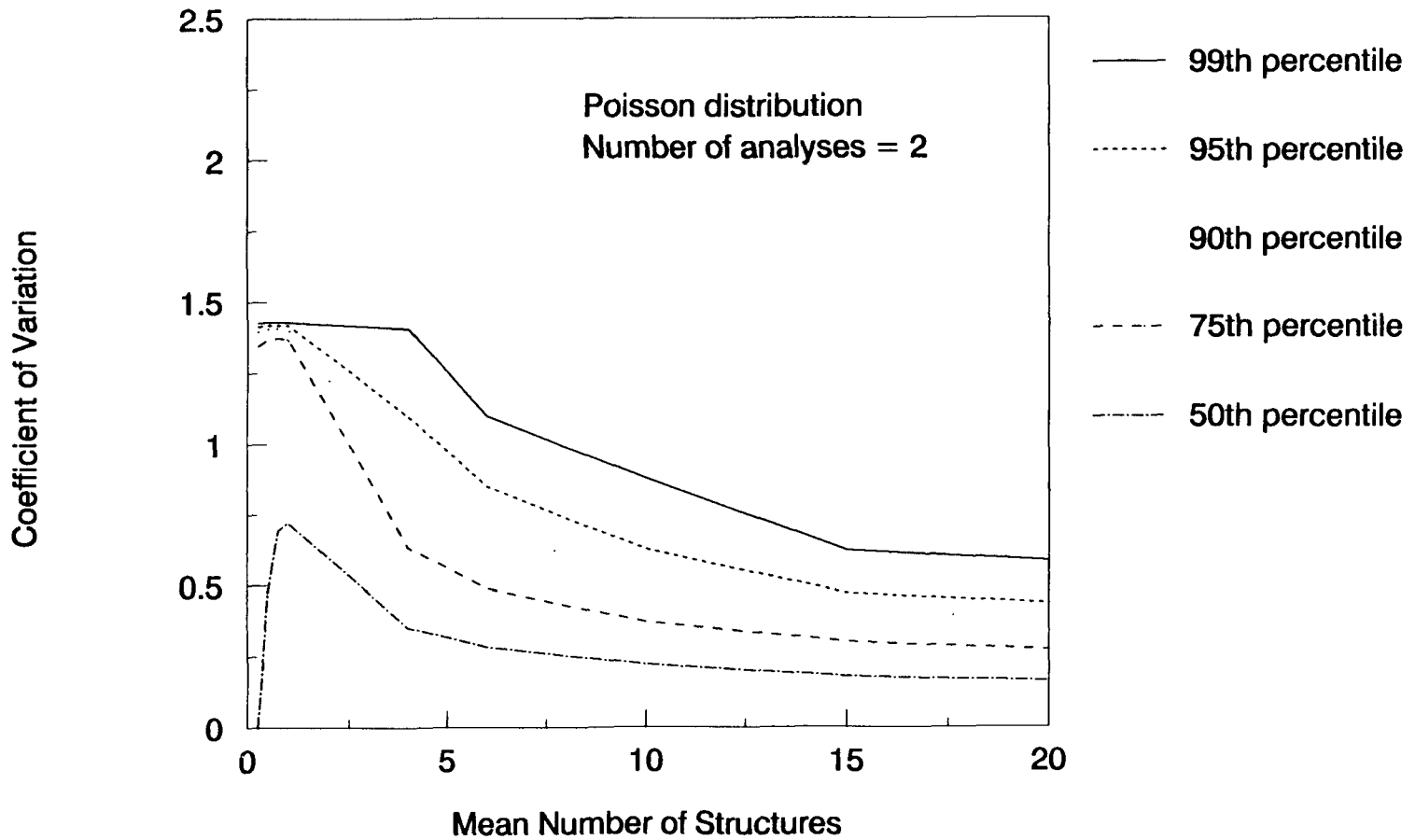

Figure 4-1(a). Percentiles for the calculated coefficient of variation as a function of the mean number of asbestos structures present for different values of the number of repeated analyses (n=2).

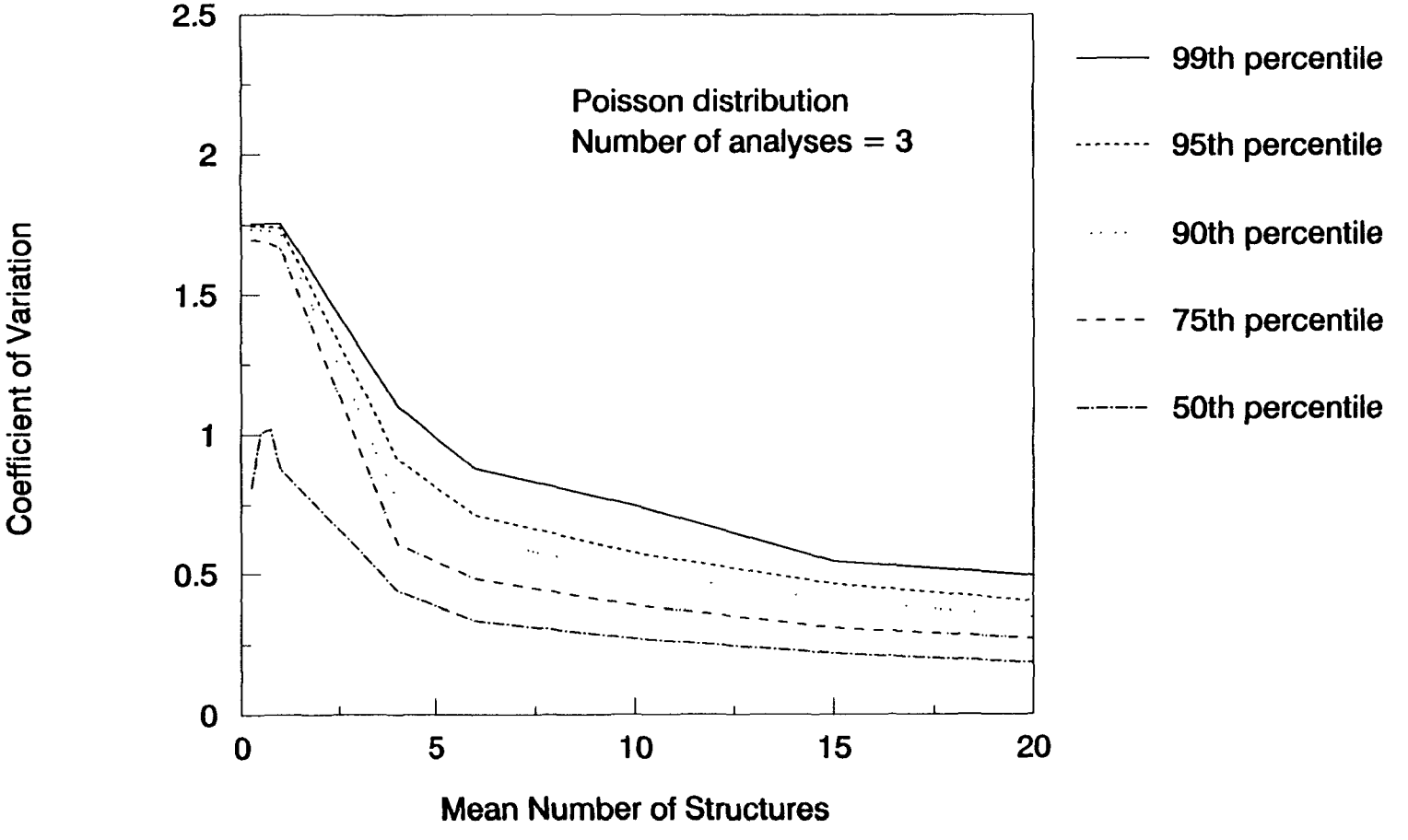

Figure 4-1(b) Percentiles for the calculated coefficient of variation as a function of the mean number of asbestos structures present for different values of the number of repeated analyses (n = 3).

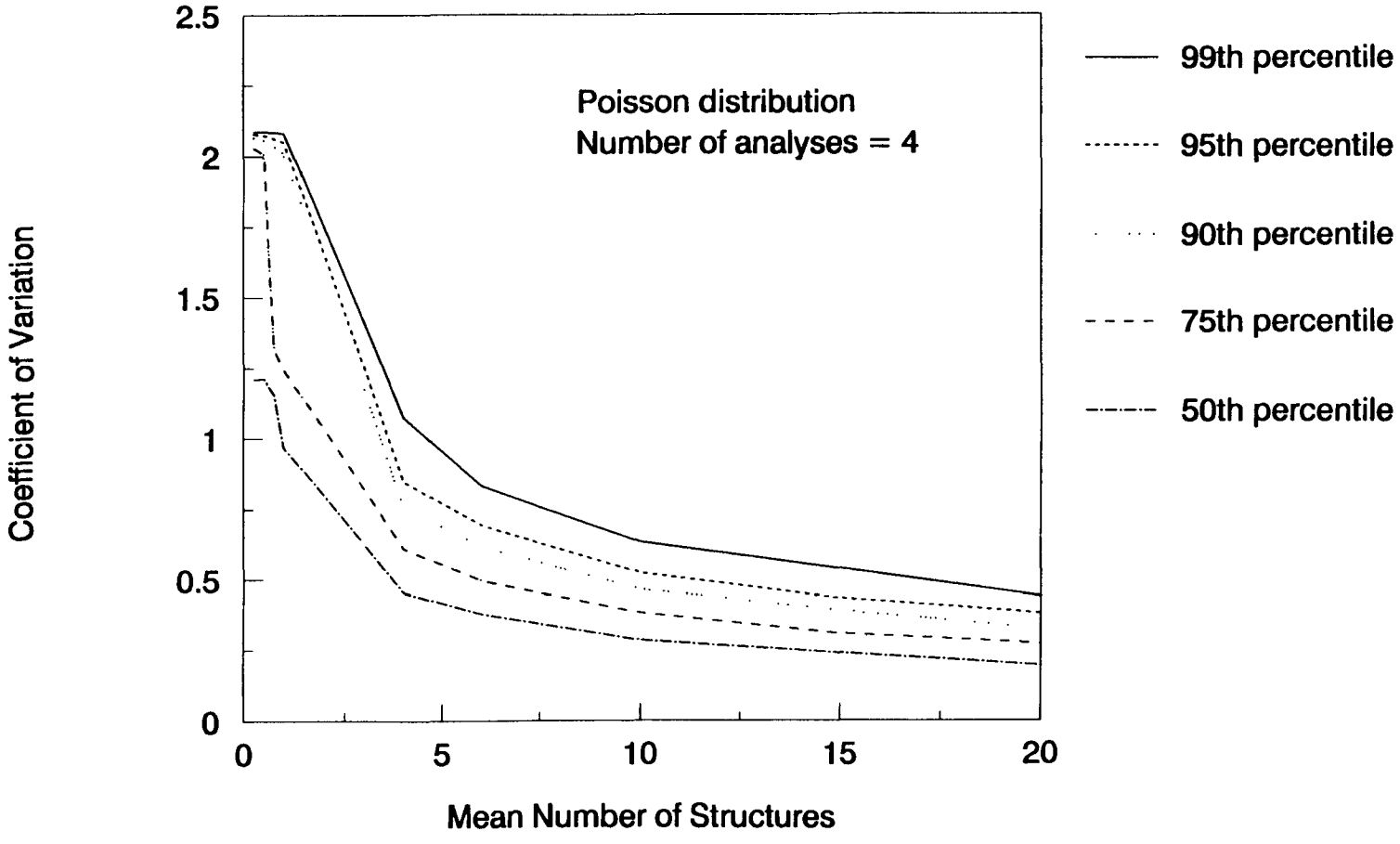

Figure 4-1(c) Percentiles for the calculated coefficient of variation as a function of the mean number of asbestos structures present for different values of the number of repeated analyses (n = 4).

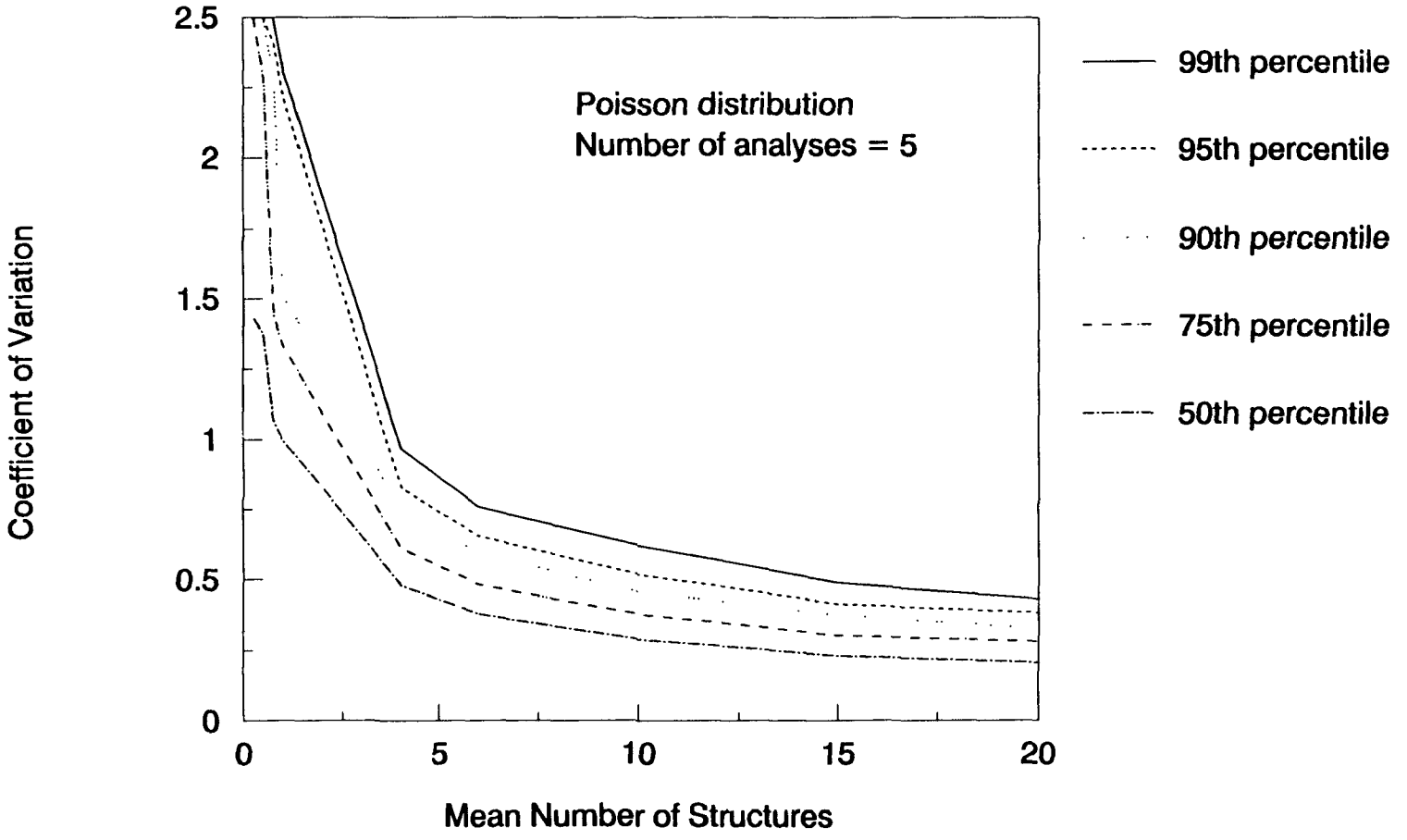

Figure 4-1(d) Percentiles for the calculated coefficient of variation as a function of the mean number of asbestos structures present for different values of the number of repeated analyses (n = 5).

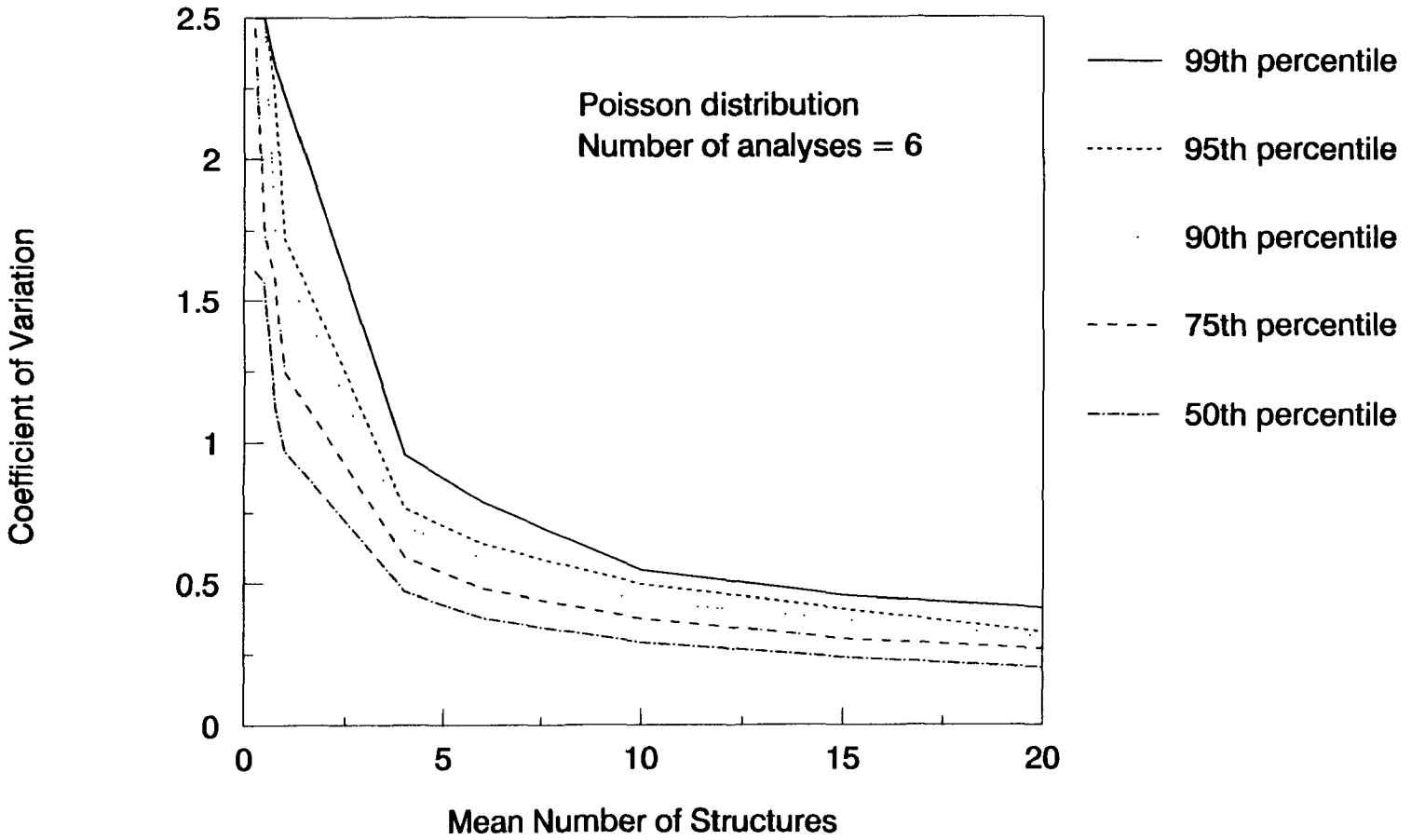

Figure 4-1(e) Percentiles for the calculated coefficient of variation as a function of the mean number of asbestos structures present for different values of the number of repeated analyses (n = 6).

typical magnitude of the additional variability. As data are accumulated, achievable guidelines will emerge, and laboratories should strive to improve beyond them.

Each laboratory should develop its own quality objective or standard based on its performance record. For example, after collecting data for several months, a laboratory could derive its own 95th percentile line which would lie somewhat above the ideal 95th percentile shown in the figures. Any repeated analysis falling above the laboratory's 95th percentile line would receive special scrutiny. If over a period of time, or within a particular batch of samples, more than 5 percent of repeated analyses fell above the laboratory's 95th percentile line, a systematic effort to pinpoint and correct the decrease in performance would be undertaken. Note that one reason for an apparent decrease in performance could be due to an unusually clumped distribution of asbestos structures on the filter surface, a factor over which the laboratory has no control. This should not be used as an explanation for every repeated analysis that falls outside the designated range, but it should be kept in mind when investigating a problem.

Figure 4-1 shows that the coefficient of variation depends on the number of repeated analyses (n) used in its calculation and it is particularly sensitive to (n) when both (n) and the average structure count are small. Keeping the number of repeated analyses as constant as possible and preferably greater than 3 simplifies interpretation of the results. While this may be possible in some cases (e.g., a within laboratory program involving four analysts), it may be impractical in others (e.g., an interlaboratory program with a variable number of participants). The behavior of the coefficient of variation is more erratic at low structure counts. This difficulty can be reduced by increasing the number of grid openings counted on QC samples with low structure concentrations. The same number of grid openings must be counted for each repeated analysis, otherwise the coefficient of variation will not be estimated correctly.

The relative contributions of different parts of the process to total variability can be assessed by comparing the coefficient of variation graphs for different types of repeated analyses. For example, the percentile lines for intralaboratory samples of the type illustrated in Figure 4-1 (A) should lie above the corresponding percentile lines for repeated analyses of a single grid in which different grid openings are counted each time. If the difference between the percentile lines is large, the sample preparation step is introducing a large amount

of variability into the process and may be a future target of attempts to reduce total variability. (Note that the subsampling step is involved in both types of repeated analysis, therefore any consistent differences between the percentile lines in this example cannot be attributed to the spatial distribution of structures on the filter.) The coefficient of variation graphs also provide a means of indicating to the client the amount of uncertainty associated with an individual data value. This is discussed in Section 4.13.

Although the coefficient of variation is a familiar and useful way of summarizing information on variability it does not capture all the relevant information that can emerge from repeated analyses. For example, the coefficient of variation is not sensitive to an analyst in an intralaboratory program who consistently obtains slightly lower counts than other analysts. Laboratories are encouraged to develop additional and alternative procedures to display and interpret the results of repeated analyses.

4.9 USE OF STANDARD SAMPLES

4.9.1 Calibration of Plasma Asher Performance

In the preparation of TEM specimen grids from MCE filters, the filter is collapsed and a plasma etching treatment is used to remove a thin layer of the MCE polymer from the surface. This is done to remove polymer which may have engulfed asbestos fibers during the filter collapsing procedure. However, since the performance of plasma ashers cannot be accurately specified, it is necessary for each laboratory to determine satisfactory operating conditions for its particular unit. The operating conditions can be specified approximately in terms of a performance standard. An uncollapsed 0.45 μm pore size MCE filter is placed in the plasma asher, and conditions are established such that the filter completely oxidizes in approximately 15 minutes. The laboratory then should develop calibration data to show that, under these operating conditions, the time period selected for the etching treatment produces satisfactory recovery of fibers and an acceptable level of background structure in the final replica. In other words, the laboratory needs to carry out tests to determine that the etching time and operating conditions produce the proper etching results. It has been found for one manufacture of MCE filters that an etching period of approximately 8 minutes on the collapsed filter under

these conditions provides satisfactory samples. An alternate approach, specified in the AHERA TEM method, is to establish conditions in the plasma asher such that 10% of the filter thickness is etched away.

This performance standard, however, is very approximate. The thickness and precise mixture of polymers used in filters varies with the manufacturer, and etching rates of the different makes of filter may vary. Laboratories must perform their own experiments to establish that their procedure for plasma etching of the collapsed filters does not generate excessive background detail on the replica, or cause asbestos structures to fail to transfer to the final TEM specimen grids. These data should form part of the QA records, and the procedures used for obtaining the data should be fully documented in the quality manual. The performance of any particular plasma asher varies with aging of the electronic components; hence a need exists for the calibration to be repeated on a regular basis. The
policy of the laboratory for performing this calibration should be documented in the quality manual.

4.9.2 Calibration of TEM Magnification

The magnification of the TEM can change. Particularly after any maintenance of the TEM, and also at regular intervals, the magnification of the TEM at which fiber measurements are made must be determined, both on the fluorescent screen and on a photograph, to ensure the measurement of fiber dimensions continues to be accurate. If the required measurements of grid opening dimensions are performed in the TEM, it will also be necessary to calibrate the lower magnification used for this measurement. The measurements must form part of the QA records, and the procedures used for making the measurements must be specified in the quality manual. Carbon replicas of ruled gratings are normally used to calibrate the magnification of the TEM. A replica with 2160 lines/mm is suitable for calibration of the high magnification used for fiber counting. For calibration of the low magnification used for grid opening dimension measurement, a replica of approximately 600 lines/mm is suitable. These can be obtained from electron microscopy accessory suppliers. The calibration standards used must be specified in the quality manual.

4.9.3 Calibration of the Diffraction Camera Constant

The interpretation of electron diffraction patterns requires that the camera constant for the diffraction pattern be known precisely. Calibration of the camera constant can be achieved using any known crystalline material. A thin polycrystalline film of gold is a suitable choice. The procedures for determination of a camera constant for the fluorescent screen, and for a photograph, must be clearly described in the quality manual. These procedures must take into account specimen eccentricity, objective lens excitation, and the effects of lens distortions on the camera constant. The procedures for correction of measurements for these distortions must also be described in the quality manual. References that may be consulted for electron diffraction calibration include Lee (1978), Andrews et al. (1967), and Hirsch et al. (1965).

4.9.4 Calibration of Electron Beam Dose

Chrysotile asbestos is easily damaged by excessive irradiation in an electron beam. During such an irradiation, the morphological appearance of a single fibril of chrysotile changes. Even if an electron diffraction pattern was visible initially, it rapidly disappears. This change is irreversible, and since the electron diffraction pattern is the primary identification criterion for chrysotile, degradation of fibers in this way may cause low values to be reported. It is most important, therefore, that the electron beam dose administered to a fiber be calibrated in some way, so that excessive irradiation can be avoided. It is not required that this calibration be an actual measurement; but the operating conditions must be specified such that excessive irradiation does not occur. A demonstration that the electron diffraction pattern obtained from a single fibril of chrysotile can still be seen after an irradiation period of 15 seconds or more constitutes adequate calibration. The operating conditions normally used by the laboratory to achieve this condition and the requirement to confirm these conditions must be specified in the quality manual.

4.9.5 Calibration of Operators Using SRM 1876a

NIST Standard Reference Material (SRM) 1876a is a portion of a filter which has been examined by a number of microscopists to determine the fiber density. This material can be used to prepare specimen grids which have a well-characterized fiber density.

These grids should be used for training of new operators, and also to provide an external reference for the accuracy of fiber counting. SRM 1876a is a very simple material, and the fibrous structures consist mainly of single fibers. This sample, therefore, is limited in its usefulness for testing of operators' ability to apply the fiber identification and counting criteria correctly. SRM 1876a should not be relied on as the only reference sample. It has limited complexity and is available only as a polycarbonate filter.

4.9.6 Quality of TEM Specimen Preparation

The quality of the TEM specimen grids prepared by a laboratory seriously affects the results obtained. Although some aspects of the quality of the TEM specimens are necessarily subjective, others can be put on a more quantitative basis. The aspects of TEM specimen quality which must be addressed in the quality manual include the following:

(a) The TEM specimen must not be too thick. If the carbon replica is too thick or if substrates are used and the combined replicas and substrates are too thick, the contrast of small fibers will be reduced, and operators will more readily overlook them during scanning. The substrates used and the carbon replica may also be too thick to permit satisfactory electron diffraction patterns to be obtained. On typical TEM specimens prepared by the laboratory, more than 90% of the single fibrils of reference chrysotile should exhibit satisfactory electron diffraction patterns. Data which demonstrate that this can be routinely achieved by the laboratory must form part of the QA records.

(b) Undissolved filter polymer should not remain on the TEM specimens after solvent extraction. This has the same consequences as those discussed in (a), and the specific procedures used by the laboratory to solve this problem when it occurs should be described.

(c) In MCE preparations, there should be no evidence of residual filter structure due to incomplete collapsing of the spongy structure of the membrane filter. Residual filter structure results in a replica containing confusing detail which acts as camouflage for fibers. Operators readily overlook short fibers when they are presented against a highly detailed and contrast background structure. Criteria for satisfactory preparations must be specified.

(d) In MCE filter preparations, the collapsed filter should not be either over- or under-etched during the plasma etching step. The degree of etching can be estimated by tilting the specimen in the TEM to an angle of 45°, and observing the structure generated by the etching treatment. Criteria for satisfactory preparations must be specified.

(e) For PC filter preparations, there should not be evidence that the filter was over-heated during the carbon evaporation. This leads to a "connected pore" appearance in the final TEM specimen, which may conceal fibers. Criteria for satisfactory preparations must be specified.

(f) There should be no evidence that significant numbers of particles have been lost from the TEM specimens. Holes in the carbon replica are clues that this has happened, and if these form a significant proportion of the features in a TEM specimen, the preparations are unsatisfactory. The policy of the laboratory in assessing suitability in this regard must be described in the quality manual.

(g) Sample filters which are over-loaded by particulate will generally yield TEM specimens on which a large proportion of the grid openings will exhibit broken carbon replicas. Since breakage normally occurs in regions of the specimen where particles are closely spaced, the intact areas tend to represent regions of the original filter which were more lightly loaded. Counting of such a specimen may yield results which are biased downwards. The criteria used by the laboratory for rejection of specimens should be described.

(h) Where a large proportion of the area of each grid opening is obscured by particulate, asbestos structures may be obscured. A count on such a specimen may yield results which are biased downwards. As for (g), the criteria used by the laboratory for rejection of such specimens should be included in the quality manual.

(i) The quality manual should address the procedures followed by a laboratory if a replica shows any other major defects, such as regions of folding, or unexplained debris on blank preparations.

4.9.7 Quality of Electron Diffraction Patterns

Chrysotile is identified on the basis of an electron diffraction pattern. It is therefore important that the procedures used by the laboratory yield patterns on which the relevant properties can be seen or measured. For example, the spots should be sharply defined, and a camera constant which is sufficiently large so that spot positions on recorded diffraction patterns can be accurately measured should be used. The area of the specimen from which the

diffraction pattern is obtained should be sufficiently small so that interferences from adjacent particles do not compromise the interpretation. The precise procedures used to obtain satisfactory patterns should be documented in the quality manual.

4.9.8 Calibration of Electron Beam Spot Size and EDXA System

The AHERA TEM protocol specifies that the TEM must be capable of producing an electron beam with a diameter less than 250 nm. This ensures that adequate spatial resolution is available during acquisition of EDXA spectra from small fibers. The quality manual must contain a description of how the beam diameter can be measured (Williams, 1986; 1984).

Identification of asbestos fibers, particularly amphibole asbestos fibers, requires the use of an energy dispersive x-ray analysis (EDXA) system. It is important that the energy (horizontal) scale of the x-ray spectrum be calibrated to ensure that the x-ray peaks appear at the correct energy values. This calibration must be performed frequently and is easily accomplished using a standard specimen referred to below. Two variables may require adjustment on the x-ray system: the amplifier gain and the zero point. In order to perform this calibration, a standard specimen is required which yields a low and a high energy peak. An evaporated film of aluminum on a carbon film, supported on a copper TEM grid is a suitable specimen. This specimen gives rise to a low energy aluminum peak and a high energy copper peak.

If misidentification of amphibole fibers is to be avoided, the intensity scale of the EDXA system must be calibrated carefully, using known standard materials. Calibrations are required for the intensities of the K (alpha) lines for sodium, magnesium, aluminum, silicon, potassium, calcium, titanium, manganese and iron. NIST's SRM 2063 provides a means of calibrating the EDXA unit for magnesium, silicon, calcium and iron. Calibrations are also required for sodium, aluminum, potassium, titanium and manganese in order to confidently discriminate asbestos fibers from nonasbestos mineral species.

It is important to recognize that changes in detector efficiency can occur, and periodic recalibration is required. The intervals for recalibration should be specified in the quality manual.

4.9.9 Preparation and Use of In-House Reference Standards

The TEM operator should have available a series of mineral reference standards consisting of TEM specimen grids with different kinds of mineral fiber on them. These mineral fibers should include the common asbestos varieties and also other nonasbestos minerals which have been incorrectly identified as asbestos (e.g., halloysite, palygorskite, vermiculite, talc). Small samples of minerals can be obtained from some museums and also from the U.S. Geological Survey. The laboratory should have a written procedure for use of such reference standards in its training program, and for regular checking of any fiber identification procedures which are used.

Suitable reference specimen grids may be prepared using well-characterized minerals. It is often not sufficient to accept identification of mineral standards from geological collections, since many of the classifications have been done using less specific techniques than are required for reference standards. Before incorporating a new mineral reference standard into the set used by the laboratory, sufficient data or analysis should be documented to establish the suitability of the material as a reference standard. This documentation should form part of the quality manual.

4.9.10 Use of Samples and Proficiency Testing Samples as Operator Training Materials

Maintenance of proficiency in established operators must be a continuing operation, and proof of proficiency in new operators must be demonstrated before they are allowed to report results independently. Reference samples and proficiency testing samples play a vital role in both activities. The quality manual should contain details on how the laboratory maintains and demonstrates proficiency in its overall analytical program, from the initial preparation of the filter to reporting of the result. Although the NVLAP will regularly issue proficiency testing samples, each laboratory should also have an internal proficiency development and maintenance program based on samples from other sources.

Proficiency must be maintained and demonstrated in three separate areas: specimen preparation, fiber identification, and asbestos structure counting. These three areas require different types of proficiency testing samples. For specimen preparation, the key factors to test for are listed in Section 4.9.6. Proficiency testing filters for this purpose can be

prepared by filtration of liquid suspensions of any well-characterized particulate or asbestos dispersion. TEM specimen grids which exhibit any of the defects discussed in Section 4.9.6 should be cause to suspend preparation of samples and to review the preparation techniques in detail. Any modifications in the preparation procedures which are made should be documented and added to the quality manual as revisions. Proficiency in fiber identification can be demonstrated only by periodic submission of specimens containing a series of reference materials. Proficiency in asbestos structure counting can be maintained by periodic analysis of specimens, some of which contain only simple structures such as fibers and bundles, and others which contain a high proportion of complex structures. It is possible to accumulate a library of such training samples from those analyzed routinely.

4.10 DATA RECORDING

The system used for recording of data must be fully described in the quality manual. It should incorporate elements concerning sample identification, filter type and specimen preparation, grid selection, fiber identification, asbestos structure dimension measurement, micrograph labeling, spectrum labeling, TEM magnification, grid opening dimensions, and any file structure and numbering system which is used for computer data entry.

4.10.1 Sample Identification

The data recording system must provide a clear tracking for each sample through the analytical system. If the laboratory assigns sample numbers, the cross-referencing system should be fully documented in the quality manual. At various points during TEM analyses, there are opportunities for loss of sample identification and interchange of samples. The system used should record, for example, which samples were prepared as a single group, and which blank samples would be representative of the preparation conditions under which the group of samples were prepared.

4.10.2 Filter Type and Specimen Preparation

The specimen preparation procedure used depends on whether the sample was collected on a PC or an MCE filter; therefore, the filter type must form part of the data records.

If special procedures were employed during specimen preparation, details of the variances from the standard procedure should be recorded. For example, if the filter was found to be overloaded, and the TEM grids were prepared by an indirect transfer procedure, all details of the procedure must form part of the sample preparation records. The relevant details for this example would be the area of filter ashed, the volume of liquid in which the ash from the filter was dispersed, the volume of this dispersion which was filtered for each of the secondary filters prepared, the area of each secondary filter, and the type of secondary filter.

Minor variances from the AHERA protocol, such as redesign of the Jaffe washer, or use of a different solvent for dissolution, may still allow the analysis to conform to the requirements of AHERA. The use of such variances, however, must be supported by sufficient analytical work and documentation to demonstrate equivalence with the standard procedures. Major variances, such as the indirect protocol used as the example above, do not conform to the requirements of AHERA. All variances normally used in the laboratory must be fully described in the quality manual, and when they are used they must form part of the sample preparation records.

4.10.3 Grid and Grid Opening Selection

There is often reason to believe that the particulate on a collection filter is restricted to localized areas of the filter. Accordingly, it is important to record the point in a filter count at which another grid is examined. It is also necessary as part of the NVLAP to be able to relocate, at a later time, all of the individual grid openings on which the reported results are based. The data recording system must therefore accommodate some means of identifying each particular grid and grid opening for which data are reported. The simplest way to achieve this is to use finder TEM grids, in which each grid opening can be uniquely identified by a letter or number incorporated into the design of the grid. Alternatively, if the grid design incorporates a center marker which is asymmetric, grid openings can be relocated using a grid map. For verified counting activities, the data recording system must permit the orientation of the grid as it is placed in the TEM specimen holder to be recorded.

The precise openings to be examined should be selected randomly, although care should be taken to ensure that those selected appear representative of the overall specimen. This

can be judged by an examination of the grid at low magnification. If the sample appears to be nonhomogeneous, a representative grouping of grid openings should be selected for examination. It is advantageous to avoid selection of grid openings near the edge of the grid, since the specimen holder profile may obstruct the EDXA detector's view of particles on these openings.

4.10.4 Fiber Identification

In a TEM examination, not all fibers can be unequivocally identified as asbestos or nonasbestos. QA interpretation is greatly assisted if the basis for each fiber identification is recorded. For example, during an initial examination of a specimen containing chrysotile, the first operator may damage chrysotile fibers to the extent that the second operator, no matter how experienced, cannot obtain a high proportion of electron
diffraction patterns. In other types of samples, the specimen preparation may be too thick to allow electron diffraction patterns from chrysotile to be discerned, or the crystal structure of the chrysotile may have been damaged by acid treatment or heating before air sampling took place. None of these situations reflect on the competence of the microscopist, but the failure to observe a high proportion of fibers which exhibit satisfactory electron diffraction patterns often provides a clue to other, and perhaps more serious QA problems associated with the analyses.

The quality manual must have a complete description of the techniques used by the laboratory for identification of asbestos fibers on the basis of their morphology, electron diffraction patterns and EDXA spectra. This description should include or refer to data showing that the techniques used by the laboratory reliably discriminate asbestos fibers from nonasbestos fibers. If, in the system of fiber identification used by the laboratory, ambiguities of fiber identification exist (e.g., the inability to discriminate chrysotile from antigorite on the basis of the EDXA spectrum alone), these ambiguities should be described in the quality manual.

4.10.5 Measurement of the Dimensions of Asbestos Structures

TEM analyses performed by the AHERA method do not require the dimensions of asbestos structures to be reported, other than a discrimination of fibers with lengths between 0.5 μm and 5 μm, and those longer than 5 μm. Other analytical methods may require the dimensions of each asbestos structure to be reported. The data recording system must allow for

these dimensions to be recorded for each fibrous structure detected.

4.10.6 Labeling of Micrographs and EDXA Spectra

For the purposes of the AHERA TEM method, it is necessary to photographically record some electron diffraction patterns, and also to record EDXA spectra. The quality manual should contain a description of the system used to identify micrographs and spectra, as they relate to specific fibrous structures in the fiber counting data.

4.10.7 Reporting of Grid Opening Dimensions

The data record for each sample must contain the value of the grid opening dimensions to be used in the calculation of structure concentration.

4.10.8 Structure of Sample Data File

The majority of laboratories use computers to manipulate the large amounts of data generated by TEM analysis of air samples for asbestos. The structure of the data file used must be fully described in the quality manual.

4.10.9 Checking of Analytical Data

Proof-reading of data is recommended to guard against major errors which may be introduced by simple keyboard entry errors. The methods in use by the laboratory to carry out a second check on the accuracy of raw data should be described in the quality manual.

4.11 ELECTRON DIFFRACTION PATTERN MEASUREMENTS

Measurements on electron diffraction patterns must be made very accurately if a confident identification is to be achieved. The method by which these measurements are made must be included in the quality manual. For those patterns which are recorded photographically, the method by which the camera constant, d-spacing and angles between the diffraction maxima are measured must be described, along with estimates of error for each of these measurements. For example, in the case of chrysotile, it is necessary to observe the spacing of the layer lines and the separation between the (002) reflections, in addition to qualitative

aspects of the pattern such as the streaking of the (110) and (130) reflections. Although observation of a layered ED pattern with a layer line spacing of
0.53 nm does not constitute conclusive identification for amphibole, this observation can often be made on amphibole asbestos fibers and contributes to the identification process
when supported by morphological and EDXA data.

It is not economically feasible to record every pattern photograpically and to measure each pattern from the photograph. The manual must therefore contain a description of the procedures used for visual identification of ED patterns. Visual observation of ED can be achieved with least beam damage if small camera lengths are used and observations of the pattern are made through the binoculars. The tilted screen introduces an elliptical distortion into the image. However, with experience, an operator can recognize the essential features of the ED patterns semi-quantitatively even when the image is distorted in this way. There must be some arrangement to allow estimation of significant measurements directly on the fluorescent screen of the TEM. For chrysotile, it is necessary to measure the separation of the 0.73 nm (002) maxima and the separation of the 0.53 nm layer lines. With some care, these measurements can be made with sufficient accuracy directly on the screen. For those patterns which are recorded photographically, the method by which the camera constant, d-spacings and angles between the diffraction maxima are measured must be described, along with estimates of error for each of these measurements.

4.12 MEASUREMENTS ON EDXA SPECTRA

Identification of chrysotile can usually be achieved by electron diffraction, combined with observations of morphology. However, if the morphology is not distinct, which may be the case in some complex structures, the EDXA spectrum may be the only identification aid available. The area of the magnesium peak relative to the silicon peak allows discrimination between talc and chrysotile. For the amphiboles, the relative areas of the relevant peaks are the only means of discriminating between the amphiboles, and it is therefore important that these peak areas be measured accurately. The method by which statistically-valid peak areas are obtained must be described in the quality manual. Where a fiber is identified on the basis of the EDXA spectrum, the peak areas obtained must be quoted in the data records, or a spectrum

must be stored such that the peak areas can be derived.

4.13 CALCULATIONS AND REPORTING OF RESULTS

As discussed in Section 3.0, the laboratory is responsible for estimating the concentration of asbestos structures on the surface of the filter. This estimate is calculated by dividing the number of asbestos structures counted by an estimate of the area of filter examined. The number of significant digits reported should be consistent with the precision of the measurement. Structure concentrations per square millimeter based on a count of less than 50 asbestos structures should be reported to no more than two significant digits, although an extra digit should be carried to avoid rounding errors when calculations are being made. If no asbestos structures are counted in the area of filter examined, the reported estimated concentration is zero structures per area of filter. Although this estimate may be imprecise, zero is the proper estimate of concentration. Substituting some other number for zero biases the estimated concentration and distorts interpretation of sets of measurements. To be useful to the client, the estimated concentration should be accompanied by indicators of data quality. These include an estimate of the positive bias introduced by contamination, and an indication of precision as discussed below.

4.13.1 Bias

Laboratory contamination may add to the number of asbestos structures present on the filter. Section 4.6 described how to minimize contamination. Laboratory blanks provide an upper bound for the magnitude of laboratory contamination. They cannot provide an actual estimate of laboratory contamination because it is not usually possible to distinguish laboratory contamination from contamination introduced during filter manufacture. Note that the laboratory cannot account for initial contamination levels on filters that the client obtains from a source other than the laboratory, nor can the laboratory control contamination or particulate loss introduced during handling and transport from the field. The laboratory can only report there are an estimated x structures per square millimeter on the filter and up to y structures per square millimeter could have been introduced during the laboratory analysis.

The value of y is tied closely to the idea of detection limit. The detection limit from the laboratory's perspective is the smallest measurement that is unlikely (probability less than a specified value) to be due entirely to contamination from the laboratory. This number should be derived from laboratory blank data. A typical approach is to use the 99th percentile, i.e., the concentration per surface area of filter below which 99 percent of measurements on laboratory blanks fall. (EPA has determined a detection limit of 70 structures per square millimeter (s/mm^2) for the AHERA TEM protocol for the purposes of applying the first step of the AHERA clearance test. This level is based on experience with laboratory blank measurements and is thought to reflect contamination during manufacture. Individual laboratories may achieve detection limits that are higher or lower than this value.) The method of deriving the detection limit should be stated clearly in the Quality Manual and in the report to the client.

4.13.2 Precision

Section 4.8 described measuring precision in terms of the coefficient of variation as a function of the mean. The average coefficient of variation for repeated analyses of samples of a particular concentration (where the repeated analysis consists of all preparation and analysis steps) may be reported along with each reported concentration. Alternatively, the laboratory might choose to provide the client with a graph showing the coefficient of variation as a function of the mean.

Confidence intervals are often used to indicate precision. The method used to construct a 95 percent confidence interval, for example, should result in an interval that contains the true asbestos structure concentration 95 percent of the time. A narrow confidence interval indicates high precision. A wide confidence interval indicates low precision.

EPA is developing a method for constructing confidence intervals for TEM results using laboratory-specific coefficients of variation. This research effort is needed because commonly used confidence intervals based on the normal distribution are inappropriate for TEM data especially when only a small number of structures are counted. Until a satisfactory alternative is developed, it is suggested that confidence intervals, if reported, be based on the Poisson distribution. The report should clearly state that the Poisson distribution is an optimistic

assumption that ignores most sources of variability (including laboratory performance) and that the true confidence interval is likely to be larger (possibly much larger). For many clients, the laboratory's graph of the coefficient of variation as a function of the mean will be more informative than a confidence interval for each concentration.

As stated above, samples on which no asbestos structures have been counted should be reported as 0 s/mm^2. This does not necessarily imply, of course, that there are no asbestos structures on the filter, any more than an estimate of 105 s/mm^2, implies that there are exactly 105 s/mm^2 present. The information provided on precision should make this clear to the client. Although the coefficient of variation is not defined at zero, the graph of coefficient of variation plotted against the mean will indicate the tendency for the coefficient of variation to increase as the mean decreases. If confidence intervals are used, the confidence interval should be provided for observations of zero just as it is provided for any other estimate. All measures of precision should be clearly described in the Quality Manual and in the report to the client.

The coefficient of variation or a confidence interval is appropriate for characterizing individual estimates of concentration, and is particularly useful for clients planning air monitoring programs for purposes other than AHERA clearance. However, clients who are applying the AHERA TEM clearance test to their results do not need to use this information as part of the clearance test calculation. The AHERA TEM clearance test is based on an aggregate of at least five individual estimates and the test already takes into account the variability of the individual data values.

4.13.3 Other Calculations

Although the laboratory's control extends only to estimating the concentration of asbestos structures on the surface of the filter, most clients are interested in estimating airborne asbestos concentrations. Consequently, many laboratories routinely make additional calculations using data supplied by the client. The estimated airborne asbestos concentration (ignoring contribution from contamination) is given by:

$$C = (n/a) \times (A/V)$$

where:

C is the estimated airborne asbestos concentration in structures per cubic centimeter,

n is the number of asbestos structures counted,

a is the estimated area of filter surface examined in square millimeters,

A is the collection area of the filter in square millimeters, and

V is the volume of air sampled in cubic centimeters.

Estimates of n and a are provided by the laboratory. Estimates of A and V are provided by the client.

The analytical sensitivity (S), the airborne asbestos concentration corresponding to the counting of a single asbestos structure, is given by:

$$S = (1/a) \times (A/V).$$

The analytical sensitivity can be regarded as a conversion factor between structure count and estimated airborne asbestos concentration. It indicates the coarseness of the measurement method. Note that the analytical sensitivity is determined completely by the values of a, A, and V which are selected by the laboratory (a) or supplied by the client (A and V). The analytical sensitivity does not depend on the concentration of asbestos structures. The AHERA TEM protocol requires an analytical sensitivity of 0.005 s/cm^3 or smaller.

The detection limit and confidence intervals expressed in terms of s/mm2 may be converted to s/cm^3 by multiplying by (A/V). The detection limit is harder to interpret on this scale because contamination is not associated with a volume of air. If the detection limit is expressed as 0.01 s/cm^3 , for example, one could say "The estimated airborne asbestos concentration is 0.06 s/cm^3 , however, up to 0.01 s/cm^3 of this could be due to contamination." Coefficients of variation are unaffected by the change of scale. (The AHERA clearance protocol does not address the question of correcting for contamination because the same correction applied to two sets of samples will not affect a comparison between the sets. Correction for

contamination only becomes an issue when the absolute airborne asbestos concentration is of concern.)

Strictly speaking, the laboratory analyzes individual samples without knowledge of their origin or application and reports the results individually. Some laboratories provide additional consulting services such as applying the AHERA clearance test to determine completion of an asbestos abatement project. These activities are beyond the scope of this document.

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APPENDIX A: SOURCES OF INFORMATION ON QUALITY ASSURANCE

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APPENDIX B: EXAMPLES OF QUALITY ASSURANCE PROBLEMS AND CORRECTIVE ACTIONS

Asbestos analysis by TEM is very complex, and it is therefore not practical to write a complete fault-tracing guide. The following are examples of situations which may arise, and possible solutions are discussed. The relevance of some of the quality assurance practices will be clarified by these examples.

EXAMPLE B1: OBSERVATION OF ASBESTOS STRUCTURES IN A LABORATORY BLANK

During a series of routine AHERA analyses of MCE filters, a laboratory blank is found to be contaminated by asbestos structures containing chrysotile. Two of the five samples identified as originating from inside the work-site have approximately the same level of chrysotile structures as the laboratory blank, and the other three samples have no asbestos structures. The average value of the five samples exceeds 70 s/mm^2.

Before any investigation is made into the possible source of contamination, the data should be reviewed to establish that the asbestos structures have been properly identified by an experienced operator. If satisfactory blank analyses had been consistently obtained before preparation of this particular group of samples, any change in the procedures or reagents which may have occurred immediately prior to preparation of this group of samples should be examined critically.

If no changes in the procedure had been made prior to the preparation of these samples, the reliability of the laboratory blank should be investigated. Was the filter from the same lot number as the other five samples? How well-characterized is the average blank value for filters from this lot number? If no data are available, it cannot be determined whether the contamination was already present on the unused filters or whether the contamination could have arisen during the specimen preparation. This observation emphasizes the importance of well-characterized filter material for use as laboratory blanks. The laboratory should maintain a stock of well-characterized filter material, and should always incorporate a laboratory blank.

The next question concerning these results is whether the samples were at any point processed individually through the collapsing procedure, the plasma etching, or the carbon evaporation. If they were individually prepared on separate slides, it is possible that three of the

microscope slides were contaminated before use, or that contamination was transferred to two of the samples and also to the blank sample by one of these procedures. For this reason, it is good practice to prepare groups of samples on the same slide, along with a laboratory blank on the same slide. By preparing samples in this way, all of the samples experience exactly the same conditions, and the laboratory blank is a control for the whole group of samples. Assuming that all samples were prepared on the same slide, and all processed simultaneously through the plasma etching and carbon evaporation steps, it can be stated that the contamination most probably arose from another source.

The order in which the sample filters were handled should be inspected. Was the order such that the laboratory blank was prepared after handling the two positive samples? If this was the case, is it possible that contamination could have been transferred from the two samples to the laboratory blank by tools such as scalpels, tweezers or punches? This would be unlikely if the two high samples were the first two handled, followed by three low samples and then by a contaminated laboratory blank. If contamination from the handling tools can be rejected on this basis, the filter dissolution should be investigated. Was the Jaffe washer thoroughly washed and filled with fresh solvent prior to its use for this set of samples? In order to eliminate this question entirely, it is good practice to use a freshly washed Jaffe washer and new solvent for each batch of grids. Assuming that this was done, the precise method for cutting of collapsed and coated filter portions should be examined. Were the tweezers and scalpel cleaned between handling of each of the samples? What was the order in which the collapsed filter portions were cut and placed in the Jaffe washer?

If no answer is found, there may be sporadic contamination from the reagents or other materials used. All reagents and other materials which have contacted the filters or specimens should be checked for contamination. If no source of contamination specific to this particular group of samples is identified, it would be necessary to review all of the data from samples which have been prepared since the last satisfactory laboratory blank was prepared. If a source of contamination is identified, then procedures must be modified to eliminate this source, and a series of laboratory blanks prepared to demonstrate that satisfactory performance can be obtained. Preparation of actual sample filters should not be recommenced until satisfactory laboratory blanks can be consistently obtained.

When incidents such as that described above occur, it is important to document all of the measurements and actions in the quality assurance records. It is sometimes found that no source of contamination can be confidently identified, and after demonstrating that satisfactory blanks can be obtained, analysis of actual samples is recommenced. If a contamination incident reoccurs, the quality assurance records may provide valuable clues as to the source, particularly if a source was not identified on a previous occasion.

EXAMPLE B2: THE ASBESTOS STRUCTURE COUNTS BY TWO OPERATORS DISAGREE

As part of the routine quality assurance recounting, it may be found that two operators in the laboratory obtain significantly different asbestos structure counts for a particular specimen. If the two operators were instructed to count asbestos structures on the same set of grid openings, and the discrepancy is a significant one, there may be several reasons:

(a) The grids or grid openings examined by the two operators were not actually the same, due to mislabeling or simple selection of the incorrect openings;

(b) The operators have not used identical fiber identification criteria, resulting in some structures being classified incorrectly;

(c) The operators have not counted structures in the same way. One operator may have perceived structures differently, resulting in a different numerical structure count;

(d) If the asbestos structures were chrysotile, the first operator may have destroyed the crystallinity of the fibers so that they could not be identified by electron diffraction by the second operator; and

(e) The two operators overlooked different proportions of asbestos structures during the scanning of the specimens.

A major disagreement of this type is very serious, because the structures on a particular grid opening are the same for both operators, and ideally the structure counts obtained by the two operators should be identical. Before any action is taken, it is important to establish that a disagreement really exists, and that the problem is not simply one of incorrect selection

or labeling of the grid openings to be examined. This can be established by a review of the specimens, conducted simultaneously by both operators. If an incorrect grid opening selection has been made by one of the operators, the sample should be reexamined so that counts are made on identical sets of grid openings. In addition, the reasons for the incorrect selection should be investigated and appropriate corrective actions taken to minimize the possibility of a recurrence.

If it is certain that the grid openings examined by the two operators were identical, the data should be examined carefully to determine whether there are any consistent differences. If the operator reporting the higher result has reported more short fibers for which the identifications are indisputable, it may indicate that the other operator is not scanning correctly and requires additional training. It could also be that one operator is applying the fiber counting criteria differently. Whatever the reason may be, two operators presented with the same image on the TEM, and with access to the same identification procedures, are reporting different results. Under these conditions, the analysis must be considered out of control and no further analysis can be conducted by these two operators until the problem has been resolved using the verified counting technique. The problem is considered to have been resolved when both operators have demonstrated competence within the criteria (greater than or equal to 80% true positive counts, less than 10% false positive and less than 20% false negative counts) before actual sample counting is allowed to proceed.

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