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Interlaboratory Evaluation of a Portable Fluorescence Method for the Measurement of Trace Beryllium in the Workplace*

ABSTRACT: Researchers at Los Alamos National Laboratory (LANL) developed a field-portable fluorescence method for the measurement of trace beryllium in workplace samples such as surface dust and air filters. The technology has been privately licensed and is commercially available. In cooperation with the Analytical Subcommittee of the Beryllium Health and Safety Committee, we have carried out a collaborative interlaboratory evaluation of the LANL field-portable fluorescence method. The interlaboratory study was conducted for the purpose of providing performance data that can be used to support standard methods. Mixed cellulose ester (MCE) membrane filters and Whatman 541 filters were spiked with beryllium standard solutions so that the filters spanned the range ≈0.05 – ≈0.5 μg Be per sample. Sets of these filters were then coded (to ensure blind analysis) and sent to participating laboratories, where they were analyzed. Analysis consisted of the following steps: 1. Removal of the filters from transport cassettes and placement of them into 15-mL centrifuge tubes; 2. mechanically-assisted extraction of the filters in 5 mL of 1 % ammonium bifluoride solution (aqueous) for 30 min; 3.-4. filtration and transfer of sample extract aliquots (100 μL) into fluorescence cuvettes; 5. introduction of 1.9 mL of detection solution (to effect reaction of the fluorescence reagent with beryllium in the extracted sample); and 6. measurement of fluorescence at ≈475 nm using a portable fluorometer. This work presents performance data in support of a procedure that is targeted for publication as a National Institute for Occupational Safety and Health (NIOSH) method and as an ASTM International standard.

KEYWORDS: beryllium, field-portable, fluorescence, interlaboratory evaluation, on-site monitoring, trace analysis, workplace

Introduction

Occupational exposure to beryllium can cause insidious and sometimes fatal disease, and new exposure limits for beryllium in air and on surfaces have been established in efforts to reduce exposure risks to potentially affected workers [1]. Advances in sampling and analytical methods for beryllium are needed in order to meet the challenges relating to exposure assessment and risk reduction. Accurate knowledge of the level of beryllium metal present in the workplace environment is crucial for the determination of the health risks posed to workers.

Field-portable techniques for the accurate, expeditious, and cost-effective monitoring of beryllium are desired to enable rapid assessment of potential worker exposures to this toxic metal

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in the occupational environment. These considerations have resulted in efforts to develop field-portable analytical methods for measuring trace concentrations of beryllium on-site in the workplace. Candidate techniques for beryllium field monitoring have included fluorescence [2] and electroanalysis [3].

In the last few years, a field-portable fluorometric method was developed by researchers at Los Alamos National Laboratory (LANL) [4]; this method has recently been licensed and marketed commercially [5]. Owing primarily to the use of a novel fluorophore for Be^{2+} ion [6], hydroxybenzoquinoline sulfonate, the LANL field method offers significantly better limits of detection (LODs) for beryllium than were attainable by using fluorometric reagents investigated earlier. The previous methods [7,8] relied on fluorescence reagents that demonstrate insufficient sensitivity for trace measurements of beryllium, which are now required in workplace settings. More recent investigations have proposed new fluorometric techniques using reagents that enable ultratrace beryllium measurement in the laboratory [4,9].

The objective of the present study was to carry out an interlaboratory evaluation of the on-site fluorometric method for beryllium as it is currently marketed. An aim of this work was to establish estimates of method performance based on a collaborative interlaboratory analysis. These method performance parameters can then be used to support governmental methods such as those published by the National Institute for Occupational Safety and Health (NIOSH) [10]. Also, it is intended that method performance data obtained through this interlaboratory trial will be used as a basis for voluntary consensus standards, such as those published by ASTM International [11].

Performance Evaluation Samples

Performance evaluation material samples (PEMs) consisted of beryllium (in solution and diluted from standard beryllium nitrate solutions using deionized water) pipetted onto mixed-cellulose ester (MCE) membrane filters (Millipore, Billerica, MA) and Whatman® 541 cellulose fiber filters (SKC, Inc., Eighty-Four, PA). The filters were fortified at known levels between ≈0.05 and ≈0.5 µg Be per sample; the volume of the spiking aliquot was 0.1 mL. Also included were blanks of each sample medium ("spiked" with pure deionized water). After spiking by using micropipettes, the spiked filters were then allowed to dry in air at ambient temperature.

To ensure consistency with an ASTM International standard practice pertaining to interlaboratory testing [12], PEMs consisting of blanks plus sampling media spiked at four loading levels (0.050, 0.10, 0.20, and 0.40 µg Be per sample) were prepared. These PEMs were prepared with beryllium loadings targeted to bracket new action levels of 0.2 µg per 100-cm^2 sampling area for surface wipe samples [1] and 0.2 µg m^{-3} for 8-h time-weighted average (TWA) air filter samples [13]. The PEMs were prepared at a contract laboratory (Environmental Resource Associates, Arvada, CO; Lot no. 0809-04-04) under the oversight of LANL. PEMs were subsequently repackaged by the CDC/NIOSH Quality Assurance Coordinator to ensure blind analyses by the participating laboratories.

Interlaboratory Evaluation

Participating laboratories consisted of a subset of prospective participants that were identified by members of the Analytical Subcommittee of the Beryllium Health and Safety Committee [14]. PEMs were mailed to each volunteering laboratory by the coordinating laboratory

(CDC/NIOSH, Cincinnati, OH). Each participating laboratory, along with associated PEM samples, was assigned a numerical code in order to ensure anonymity.

It was requested that the participating laboratories prepare and analyze the PEMs in accordance with the marketed procedure and kit [5]. Briefly, the analysis procedure consisted of the following steps (schematized in Fig. 1):

1. Removal of the filter samples from transport cassettes and placement of them into 15-mL plastic centrifuge tubes
2. Mechanically-assisted extraction of the filters in 5 mL of 1 % ammonium bifluoride solution (aqueous) for 30 min (in 15-mL centrifuge tubes mounted in a mechanical shaker)
3. Filtration of the extracted solutions through plastic syringe microfilters
4. Transfer of sample extract aliquots (100 μL) into fluorescence cuvettes using mechanical pipettes
5. Introduction of 1.9 mL of fluorescent dye detection solution to effect reaction of the fluorescence reagent with beryllium in the extracted sample
6. Measurement of fluorescence at ~475 nm using a portable fluorometer

The participating laboratories were asked to report analysis results in units of mass of beryllium (in μg) per PEM sample. (This required comparison of results for unknowns with calibration standards, along with consideration of appropriate dilution and correction factors, to convert fluorescence intensity to mass [5].)

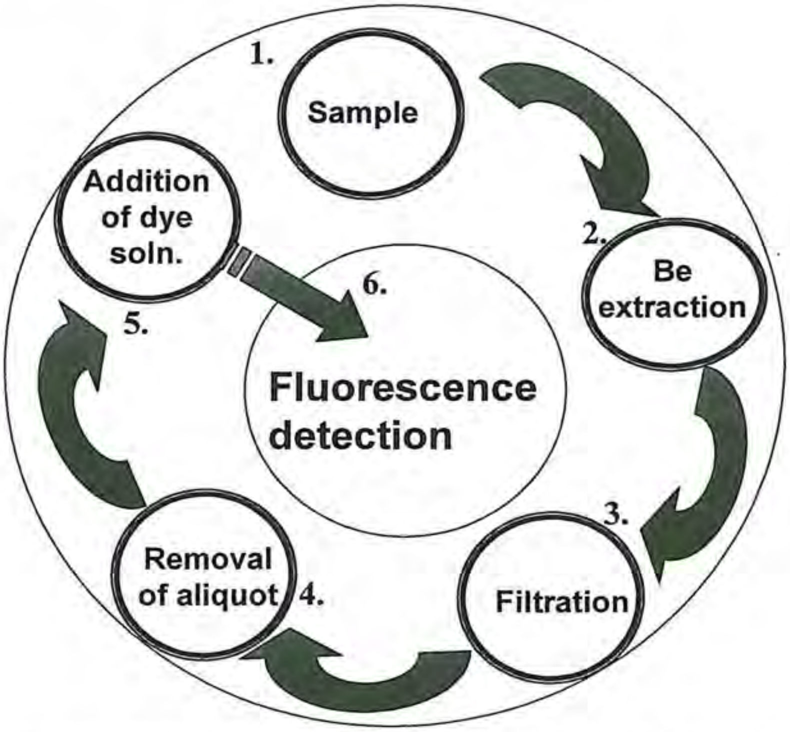

FIG. 1—*Scheme for field-based preparation and fluorescence analysis of workplace samples for determination of beryllium content.*

Precision, Bias, and Statistical Analysis

ASTM International voluntary consensus standard test methods require estimates of measurement uncertainty, and this can be in the form of precision and bias data [15]. Precision estimates are preferably obtained through data from interlaboratory evaluations. Bias of a test procedure must be estimated by evaluating the performance of the test method in question against a reference method, or from determinations of recoveries from the analysis of reference material samples, or both.

In this investigation, interlaboratory precision of analytical results from the volunteer laboratories was examined using statistics from overall interlaboratory analysis results. For purposes of satisfying ASTM International standard criteria, the analysis was done using statistics described in ASTM Standard E 691 [12], which requires a minimum of six participating laboratories. This standard practice also recommends a minimum of four samples for each type of matrix, with duplicate analyses of each of the four samples. Thus, each laboratory received a total of ten PEM samples (five for each filter matrix) for analysis by the field-portable fluorescence method for beryllium. An analogous interlaboratory validation study has been carried out previously in order to evaluate field-portable electroanalytical procedures for on-site determination of lead in environmental samples [16].

Repeatability and reproducibility were calculated for each of the four beryllium levels in the PEMs analyzed by the participating laboratories. Repeatability is an estimate of within-laboratory variability, while reproducibility is an estimate of the variability of both within- and between-laboratory results. Repeatability was calculated by averaging the squares of the standard deviations of within-laboratory results for each beryllium level; hence the average within-laboratory variance is given by the repeatability variance, $(S_r)^2$. Reproducibility variance is expressed by:

$$(S_R)^2 = (S_r)^2 + (S_L)^2$$

where S_L is the sample standard deviation of the mean value estimated from the average of reported interlaboratory test results for a given PEM. Relative standard deviations (RSDs) for repeatability and reproducibility (RSD_r and RSD_R, respectively) are then computed by dividing the standard deviations S_r and S_R by the mean interlaboratory test result for a particular PEM. The RSDs calculated can then be compared with the minimum precision that is desired (e.g., RSD=0.20 [17]) for the test method under evaluation.

Estimates of analytical bias, B, were computed by simply dividing the difference between the measurand and the reference value by the reference value:

$$B = (\mu_i - R_i) / R_i$$

Here, μ_i and R_i are the mean and reference beryllium contents, respectively, for the i^{th} beryllium loading level in each PEM sample.

Results of the Interlaboratory Evaluation

Of the candidate volunteer laboratories that were identified by members of the Analytical Subcommittee of the Beryllium Health and Safety Committee, PEM filter samples were sent to eleven prospective laboratories. Analysis results were subsequently reported from eight laboratories, thereby exceeding the minimum number (for evaluation of an ASTM International test method) of six participants. Hence, for purposes of this round-robin evaluation, recruitment of a sufficient number of volunteers was achieved.

Analytical results reported by the eight individual laboratories that participated in the interlaboratory exercise are summarized in Table 1 for MCE filters and in Table 2 for Whatman 541 filters. For six of the eight laboratories, duplicate analyses were reported for PEM samples at each beryllium loading level for different sample aliquots run using (a) different portable fluorescence spectrometers, or (b) different fluorescence intensity integration times, or (c) both. Overall means were computed based on the pooled means for the average of the two results reported by each laboratory for each sample (excepting the two laboratories that reported a single result). Data from blank measurements were all near to or below the reported LOD of the analytical method (≈0.01 µg Be per sample) [5].

Results for repeatability and reproducibility for the two PEM filter matrices, determined in accordance with ASTM E 691 [12], are summarized in Table 3. Bias estimates for each PEM sample containing beryllium are presented in Table 4; overall mean values μ_i used in estimations of bias were taken from Tables 1 and 2 (for MCE and Whatman filter PEM samples, respectively).

TABLE 1—*Results from measurement of beryllium content in MCE filters, as reported by laboratories participating in the interlaboratory evaluation. Reference values for beryllium loadings on the PEM filter samples are given in parentheses in the column headings.*

Laboratory Number (n = 8)	Low (0.05 µg Be)	Medium Low (0.10 µg Be)	Medium High (0.20 µg Be)	High (0.40 µg Be)
001	0.0512; 0.0504	0.104; 0.105	0.203; 0.207	0.468; 0.484
003	0.060; 0.050	0.11; 0.10	0.21; 0.20	0.43; 0.40
005	0.052; 0.063	0.103; 0.125	0.222; 0.273	0.459; 0.503
006	0.050	0.10	0.21	0.41
007	0.0505; 0.0490	0.103; 0.103	0.210; 0.198	0.406; 0.396
009	0.051; 0.041	0.103; 0.092	0.208; 0.199	0.421; 0.421
010	0.053; 0.053	0.104; 0.107	0.197; 0.194	0.412; 0.415
011	0.053	0.105	0.203	0.404
Overall mean ± sample standard deviation	0.052 ± 0.0038	0.10 ± 0.0048	0.21 ± 0.016	0.43 ± 0.032
Relative Standard deviation	0.073	0.048	0.076	0.074

TABLE 2—*Results from measurement of beryllium content in Whatman 541 filters, as reported by laboratories participating in the interlaboratory evaluation. Reference values for beryllium loadings on the PEM filter samples are given in parentheses in the column headings.*

Laboratory Number (n = 8)	Low (0.05 µg Be)	Medium Low (0.10 µg Be)	Medium High (0.20 µg Be)	High (0.40 µg Be)
001	0.0528; 0.0519	0.103; 0.104	0.198; 0.203	0.399; 0.406
003	0.060; 0.050	0.11; 0.10	0.22; 0.20	0.42; 0.40
005	0.055; 0.063	0.114; 0.145	0.198; 0.251	0.437; 0.492
006	0.050	0.11	0.21	0.40
007	0.0530; 0.0500	0.103; 0.099	0.203; 0.198	0.401; 0.393
009	0.056; 0.052	0.101; 0.093	0.207; 0.198	0.409; 0.410
010	0.053; 0.057	0.106; 0.104	0.205; 0.209	0.403; 0.412
011	0.056	0.104	0.207	0.409
Overall mean ± sample standard deviation	0.054 ± 0.0028	0.11 ± 0.0099	0.21 ± 0.0078	0.41 ± 0.022
Relative Standard Deviation	0.052	0.090	0.037	0.054

TABLE 3—*Repeatability and reproducibility for beryllium measurements from performance evaluation MCE and Whatman 541 filters, as computed using values reported by laboratories (n = 8) participating in the interlaboratory evaluation.*

Beryllium Level	Average (µg Be)	S_r	S_R	RSD_r	RSD_R
MCE Filters					
Low	0.052	0.0034	0.0051	0.065	0.098
Medium Low	0.10	0.0052	0.0071	0.052	0.071
Medium High	0.21	0.012	0.020	0.057	0.095
High	0.43	0.0080	0.033	0.019	0.077
Whatman 541 Filters					
Low	0.054	0.0027	0.0039	0.050	0.072
Medium Low	0.11	0.0068	0.012	0.062	0.11
Medium High	0.21	0.012	0.014	0.057	0.067
High	0.41	0.012	0.025	0.029	0.061

TABLE 4—*Bias estimates for beryllium measurements from performance evaluation MCE and Whatman 541 filters, computed using mean values from Tables 1–3. Reference values for beryllium loadings on the filters are given in parentheses.*

PEM Matrix	Low (0.05 µg Be)	Medium Low (0.10 µg Be)	Medium High (0.20 µg Be)	High (0.40 µg Be)
MCE filters	0.040	0.0	0.050	0.075
Whatman 541 filters	0.080	0.10	0.050	0.025

Discussion

Results shown in Tables 1 and 2 give estimates of interlaboratory precision (as measured by the relative standard deviation, RSD) that are similar for both MCE and Whatman 541 filters. For each loading level there are no statistically significant differences between the mean beryllium contents measured in the two different sampling media (*t*-tests for independent means; n = 8). Also, for both media (Tables 1–3), there is no apparent trend of precision changing as a function of beryllium loading. It is noted that no outlier tests were conducted on the data which were reported by the participating laboratories; all results were included and treated statistically, despite the possibility of statistical outliers. The highest intralaboratory RSD encountered is 0.065, and all interlaboratory RSDs are 0.11 or less (Tables 1–3). Ordinarily, interlaboratory precision estimates of 0.15 and below are regarded as acceptable for PEMs such as these, that is, consisting of liquid spikes on sampling media [16].

The results summarized in Table 3 show that figures for within-laboratory precision RSD_r spanned the range ≈0.02 – ≈0.07, while data for between-laboratory precision RSD_R were slightly greater, ranging from ≈0.06 – ≈0.11. These precision estimates compare very favorably with precision estimates from interlaboratory results for PEMs consisting of MCE filters spiked with beryllium in liquid form at similar levels (Beryllium Proficiency Analytical Testing [BePAT] program, American Industrial Hygiene Association [AIHA], 2003) [18]. For AIHA BePAT PEM samples, interlaboratory RSDs of ≈0.06 – ≈0.15 (n = 25) were computed from five different loading levels ranging from ≈0.15 – ≈0.6 µg Be per filter. The AIHA BePAT samples were prepared and analyzed by laboratories using reference analytical methods involving concentrated acid digestion and atomic spectrometric analysis, for example NIOSH Method 7102 [19]. Thus, it is shown that, for filter samples, the interlaboratory precision of the field-portable fluorescence method is at least as good as that of fixed-site laboratory methods.

Bias estimates were negligible or positive for all beryllium loadings for both PEMs (Table 4), and ranged from 0.0–0.10. In terms of recovery, mean values determined for beryllium loadings for all of the PEM samples (e.g., see Tables 1 and 2) were within ±10 % of the reference values. Typically, recoveries of 100 % ±15 % are regarded as acceptable for meeting the requirements of quantitative analytical methods [20].

A limitation of this study is that this collaborative interlaboratory evaluation did not utilize real aerosol samples generated from beryllium-containing materials. Generally, it is desirable to evaluate methods using performance evaluation samples that are as realistic as possible. But because of the serious health hazards and high costs associated with the generation of beryllium aerosols, it was not deemed feasible to prepare PEMs from beryllium-containing aerosols for this study. It would also be of interest to evaluate the portable fluorescence method on-site in the field, but such an effort is outside the scope of this investigation.

In summary, the results of the interlaboratory evaluation of the field-portable extraction and fluorescence method for beryllium indicate that the method is effective for the quantitative measurement of soluble forms of trace beryllium in MCE and Whatman 541 filter samples. Estimates of within-laboratory and between-laboratory precision compared favorably with interlaboratory precision estimates from a beryllium proficiency testing program, and bias estimates were 10 % or below for each performance evaluation sample tested. Performance data obtained here represent the minimum that is required for NIOSH methods and ASTM International standards. It is intended that future studies will address real-world sample matrices and on-site evaluations of the portable fluorescence method.

Disclaimer

Mention of company names or products does not constitute endorsement by the Centers for Disease Control and Prevention.

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