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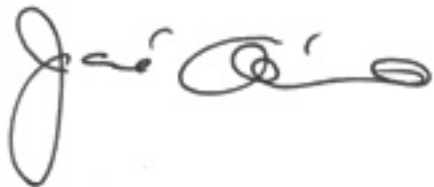
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Executive Summary:

This research project aims to improve the **scientific validity** of the **probative information** resulting from applying elemental analysis methods in the examination of trace evidence materials. The first part of the project coordinated a series of five (5) inter-laboratory exercises (also called round-robin exercises in this report) involving a large group of experienced examiners from several established operational forensic laboratories using mature forensic techniques and certified reference materials to demonstrate that the quality of the chemical information derived from a variety of analytical methods is extremely good, regardless of the method used. The focus of the inter-laboratory exercises was on the characterization of glass evidence (RR1-RR4) but the soil matrix was also analyzed during one of the inter-laboratory trials (RR5). Many studies have been conducted over the last three decades illustrating the **discrimination power** of elemental analysis for glass (and other types of trace evidence), particularly when LA-ICP-MS was used. The technique of μ XRF is preferred in the US forensic laboratory community with approximately 40 forensic laboratories

employing the technique currently but, until this project, there was no standardization of the μ XRF technique nor was there a systematic evaluation of the performance of μ XRF for forensic glass examination by a large number of users. More than 30 experienced forensic examiners, academics and other interested scientists representing 23 organizations (Elemental Analysis Working Group (EAWG)) worked together to enhance the understanding of the selection of a **match criteria** (determination of match/no match based on objective and statistically valid reasoning) for univariate and multivariate comparisons. Two of the round robin exercises were specifically designed to evaluate the performance of the different match criteria currently in use by forensic scientists in an attempt to elucidate the best-performing match criteria for both μ XRF data comparisons and LA-ICP-MS data comparisons. The results of these exercises are reported in chapters 2 and 3 of this report. There is also growing evidence that the emerging analytical technique of Laser Induced Breakdown Spectroscopy (LIBS) can produce similar information to both μ XRF and LA-ICP-MS for the analysis of materials while also provide significant advantages in the form of ease of use, cost and speed of analysis and therefore also evaluated during this phase of the project.

The forensic utility of LA-ICP-OES is also expected to provide similar advantages (in comparison to LA-ICP-MS) and was also be evaluated during the second part of the project which also resulted in a publication submitted to a scientific journal (Appendix B of this report). Finally, a number of interested EAWG members also completed an interlaboratory exercise (RR5) focused on the bulk analysis of soils for forensic application. This exercise resulted in the submission of a publication to a scientific journal and is referenced in the report.

The major accomplishments of this project were: two (2) ASTM methods of analysis (submitted as appendix C and appendix D in this report) and five (5) publications related to the project research goals (see complete list of publication and presentation references as appendix E of this report).

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Chapter 1. Introduction and Review of the Current Literature

Many research groups have worked to improve the value of trace evidence analyses including the application of mature analytical techniques such as solution inductively coupled plasma optical emission spectroscopy (ICP-OES) [1,3-6,8-9], micro X-ray fluorescence (μ XRF) [2], laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) [7,10-17] and, more recently, laser induced breakdown spectroscopy (LIBS) [18-20] for the elemental analysis of the materials glass and paint. Efforts in our research group have resulted in the optimization of both solution ICP-MS analysis of glass [21] and LA-ICP-MS analysis of glass [22,23], paint [16] and soils [24], including a recent review of the literature summarizing the utility of elemental analysis methods for glass analysis [25] and the approval of a “standard method” for the analysis of glass by solution digestion ICP-MS by the ASTM in 2004 [26]. While there have been several reports in the literature successfully using ICP-AES to discriminate glass samples (this is the current method used by the FBI laboratory for casework analysis) and there are many successful reports of coupling laser ablation to ICP-MS instruments, there are, as yet, no publications to our knowledge describing the utility of coupling laser ablation to an ICP-AES instrument to determine the utility of LA-ICP-AES for forensic glass analysis. One of the aims of this research was to evaluate the utility of coupling LA-ICP-AES for forensic glass characterization and comparison. The results of this research are described in the next chapter (chapter 2) of this report.

Several authors and researchers have proposed criteria to use to determine whether or not two samples of glass can be found to be indistinguishable from each other including for the comparison of refractive index values and chemical composition measurements. Experienced glass examiners have recently compiled a list of the possible criteria examiners can choose as follows:

1. **Fixed Difference** – Fixed differences in refractive index or density beyond which a conclusion of two distinct sources is made have been used by many glass examiners for at least 25 years, based on the

suggestion of Miller [27]. Miller suggests a “positive opinion of nonidentity” if flat glass specimens fail to match within the limits of 0.0002 for n_D , 0.0004 for n_C or n_F , or 0.00010 g/cm³ for density. It is not stated to be a standard or rule but rather “judgement” is to be used in each case. These numbers are not derived from the variability of the broken glass object in question. Instead, they are suggested limits based on an assumption of no measurable variation in modern flat glass and assumed operator and equipment errors. Miller suggests his numbers are only good for modern flat glass. Wider variations, particularly in refractive index, have been measured in headlamp lenses, containers, and in float and tempered flat glasses. A limitation of the fixed difference approach, is that for other parameters, such as element concentrations, fixed difference match criteria have not been established nor recommended. In fact, fixed differences of element concentrations are not appropriate as match criteria, because the measurement precision and source variability both vary as a function of concentration [28]. The fixed difference approach is included here for discussion primarily because of its historical significance. Its use in evaluating glass evidence is *not recommended*.

2. **Fixed sigma (σ)** – In this approach, for each measured parameter, the mean of multiple determinations from a questioned source is compared to the mean of multiple measurements of a known source using as a match criterion, a fixed number of standard deviations, calculated from the measurements (at least three measurements for each questioned and known fragment are required). The match criterion most often used is $\pm 2\sigma$, because that corresponds approximately to a 95% confidence level for errors of false exclusion. Rules based on 1σ , 2.6σ , and 3σ are also discussed in the literature and used by some examiners. A small match criterion, such as 1σ has been advocated by some, because it is more conservative in the sense of minimizing the frequency of incorrect associations (although this significantly

increases the number of false exclusion to as much as 32% of the comparisons). Two assumptions that must be met in order to apply the fixed sigma approach are that the measured variables follow Normal distributions and that the limited number of measurements provides an accurate measure of the population variance. A statistical disadvantage of the fixed sigma approach is that the probability of an incorrect exclusion is also a function of the number of replicates. This drawback is addressed in the use of the Students t-test. One advantage of the fixed sigma approach is that it is easily performed by simple observation of the data, without the need for computer calculated statistics (not necessarily a major obstacle in the typical forensic laboratory). The fixed-sigma approach is also not recommended for the reasons mentioned above.

3. **Range overlap** – For each variable, the range of measured values for a questioned sample is compared to the range of measured values for a known sample. If the ranges overlap, the two samples are determined to be indistinguishable. Advantages of this approach are that no assumptions are required concerning the distribution of measured values over the sample population and that it is simple to compare ranges for multivariate data without complicated statistical calculations. For Normally distributed data, the range overlap method results in approximately the same match criteria as a 2σ to 3σ overlap, depending upon the number of replicates. Range overlap has been used most frequently for comparison of element concentrations [29-30]. The range overlap method can be used for one vs. many and for many vs. many comparisons. The disadvantages of range overlap include the same disadvantages as a fixed sigma approach and when a small number of measurements are reported, the range of values may not adequately characterize the population variance for a given glass object.

4. **Student's t-test** – The Student's t-test, with various modifications, is the most widely used statistical method for comparison of the means of two sample populations [31-33]. In this test, the match criteria is selected such that the probability of an error of false exclusions is fixed at an acceptable level, usually 5%. An assumption required for use of the t-test is that the measured variable has Normal distributions in the two populations being compared. Equal variances are also required for use of the simple t-test. If variances are not equal, then a pooled standard deviation can be used and the Welch's modification equation is used (Microsoft Excel has options for either of these cases and can easily calculate the t-test). Variations of the t-test can be used to test hypotheses of a single population in the cases of one measurement vs. many as well as many vs. many. Single-variate versions of the t-test treat each variable independently in making source comparisons. Independence of measured variables must then be assumed or shown in order to assess the significance of samples that are indistinguishable for multiple variables. The multivariate version of the t-test is known as Hotelling's T^2 and this approach has been recommended by the National Academy of Sciences (NAS) committee on bullet lead analysis for the evaluation of multivariate data derived from the composition of bullet lead, for example [34]. Our research group (and other workers) have used both the successive use of the uni-variate t-test for multiple elements with much success [9,17-18] as well as the use of Hotelling's T^2 for multivariate glass comparisons [33, 35].

The creation of a database of elemental data for over 700 different glass samples has provided background for the interpretation of the significance of trace elemental "matches" [36]. These databases, some of which were created at FIU over the course of the last 10 years can be used as the basis to determine the **significance of a match**, once a match has been determined. Forensic

examiners can then use this information to assist in the interpretation of the value of a “match” by trace elemental content for a given case so that the opinion is ***not overstated or understated***. Glass examiners have also reported the use of LA-ICP-MS in real cases [37-39] and testified in Frye hearings in Miami Florida in 2004 [40] whereby the judge allowed this method for use in a hit and run accident fatality case. While LA-ICP-MS has been accepted as a powerful technique to discriminate between different glass samples through the comparison of the elemental data generated, it is an expensive and sophisticated technique that is out of the reach of many forensic laboratories. This project included the use of a large number of experienced uXRF users in a series of inter-laboratory exercises for the evaluation of glass evidence (round-robin 1-4) and for the elemental analysis of soil samples (round robin 5). The results of the first 4 round robin exercises are summarized in the chapters 3 and 4 of this report and include the conclusions derived from performance tests for the different match criteria used in the round robin exercises. The results from the soil sample round robin exercise are summarized in chapter 5 of this report. Finally, the Elemental Analysis Working Group (EAWG) collaborated on an ASTM method of analysis for the use of uXRF for glass evidence analysis and for the use of LA-ICP-MS for glass evidence analysis. These ASTM methods have been submitted to the E30 Committee on Forensic Sciences for evaluation and voting and are included at the end of this report in the appendix.

Some ICP laboratories still detected differences on the tin content, even after applying wider match criteria. Although SRM NIST 1831 was not produced by the float glass process, ICP methods detected a slightly different composition on the original surfaces versus the cross section of the glass. Original surfaces were only present on the full thickness fragments. Nevertheless, in casework, tin is typically monitored to detect the float versus the non-float side of a glass and is not typically included as part of the elements used for comparison between samples.

The results in Table 10 demonstrate that the differences detected between the SRM NIST 1831 fragments submitted for the interlaboratory tests were due to a combination of the heterogeneity between surface and bulk composition on SRM NIST 1831 and the selection of match criteria used for comparisons.

First, the use of wider match criteria, such as $\pm 4s$ with minimum 3%RSD, reduced the number of false exclusions. Using ANOVA, 18 out of 28 possible comparison pairs were excluded (64%); using $\pm 4s$ criterion, the number of exclusions was reduced to 13 out of 28 possible comparison pairs (46 %), whereas using the wider match criteria the number of exclusions were limited to 7 out of 28 possible pairs (25 %).

Second, when using wider criteria (*i.e.* $\pm 4s$ criteria with a minimum of 3 %RSD) significant differences are still detected between one of the original surfaces (S2) and the rest of the fragments, while no significant differences are detected between the rest of the fragments regardless of their size.

The results revealed that one of the original surfaces of the SRM NIST 1831 is depleted in Sr, Zr, Hf and Pb which causes a significant heterogeneity for microsampling techniques like LA-ICP-MS.

Although this study implies that fragment size does not affect comparison of the elemental composition of glass by LA-ICP-MS, caution should be taken when using full thickness fragments to avoid possible differences in the composition of original flat surfaces.

The effects of expanding the match criteria on type I and type II errors was further studied by the working group and is reported in a separate publication [89].

Conclusions

This study allowed for a direct comparison between three of the most sensitive methods currently available for the forensic elemental analysis of glass samples (LA-ICP-MS, solution ICP-MS and μ -XRF). The methods were compared in terms of analytical performance and discrimination capability.

ICP-based methods (ICP-MS and LA-ICP-MS) are the most sensitive methods, with limits of detection on the order of sub-ppm in the solid material. Advantages of these methods are that they are fairly standardized among participant laboratories, they are currently used in forensic laboratories and they have been accepted in court. A standardized ASTM method already exists for the digestion and analysis by ICP-MS (ASTM E2330) [34] and the EAWG is currently working on developing a standardized method for LA-ICP-MS. Both methods are fairly mature with several publications previously reporting the evaluation of their capabilities and limitations. In addition, laser ablation sampling has unique advantages over digestion-based methods, such as reducing the sample consumption from milligrams to just few a hundred nanograms, reducing the time for analysis and eliminating the use of hazardous digestion reagents. Interlaboratory comparisons of glass reference standard materials demonstrated that ICP-methods provide accurate and precise quantitative data with deviations lower than 10% for nearly all elements measured in the studies.

Important findings from LA-ICP-MS methods include: a) the detection and report of heterogeneity of Ce and La close to the rim on FGS standards (< 250 μ m) and b) the awareness that possible differences between surface and bulk composition in compared glasses may lead to false exclusions if sampling and data interpretation are not carefully evaluated.

XRF methods provided consistent data among participants after normalization with a reference standard material such as SRM NIST 1831. The EAWG is also

using the experience gained from these interlaboratory tests to work towards the standardization of a μ -XRF method for the elemental analysis of glass. Limits of detection are 2-3 orders of magnitude higher than ICP-based methods; therefore, the number of trace elements typically detected in glass samples is more limited. Nevertheless, good performance was also observed among XRF laboratories. The measurement of LODs provided a better understanding of the capabilities of the technique and permitted a means of quantitatively comparing the performance of different instrument configurations. Relevant observations derived from the studies include: a) the use of normalized data to a glass standard such as SRM NIST 1831 provide a means to account for differences among instrumental configurations and to conduct interlaboratory comparisons, b) the use of a glass standard as a “control” glass is recommended to check method performance prior to analysis, and c) the use of K and Q fragments with similar size and shape is necessary to improve precision and thus increase discrimination power.

Mock case samples allowed an inter-method comparison of the capabilities to associate samples that originated from the same source and to discriminate among samples that were manufactured in the same plant line at different time periods. Excellent agreement between laboratories was achieved in both blind tests with 100% correct conclusions. The interlaboratory tests also provided an excellent opportunity for participants to fine-tune their methods and protocols and cross-validate their methodology. The study revealed that a wide variety of match criteria are currently employed by forensic laboratories to conduct statistical comparisons of elemental composition data. Extensive discussions between the group members led to the design of additional interlaboratory tests to address the interpretation of evidence and the systematic selection of match criteria for elemental comparisons of glasses, based on simultaneously minimizing the frequency of both false exclusions and false inclusions. Results of these studies will be presented in a separate publication [89].

Chapter 3. Forensic analysis of glass by μ -XRF, ICP-MS, LA-ICP-MS and LA-ICP-OES; Evaluation of the performance of different criteria for comparing elemental composition

Introduction

Elemental composition when combined with optical and physical properties has been shown to provide excellent discrimination between glass samples originating from different manufacturing sources [91-103]. With the increasing use of methods of elemental comparisons of glass fragments by forensic laboratories, the need for consistent analytical protocols and interpretive criteria has been recognized [104]. The Elemental Analysis Working Group (EAWG), made up of forensic glass examiners and research scientists, was formed to develop robust analytical protocols and to assess the accuracy of various criteria used for source comparison. The tests concerning development of analytical protocols have been addressed in a separate paper [105]. Analytical protocols using μ -XRF and ICP-based methods that provide quantitative data for 6 to 18 elements or intensity ratios were assessed. In order to make a decision as to whether the elemental compositions of two fragments are analytically indistinguishable, a match criterion must first be selected. In this study, if the known and the questioned glasses are significantly different by the selected criterion in at least one of the monitored element concentrations or intensity ratios, then it can be concluded that the two samples did not originate from the same source. Conversely, a lack of significant differences in the elemental compositions suggests that the samples could have originated from the same source. This paper describes the results of interlaboratory tests that measured the error rates obtained when using a variety of selected match criteria on glasses having similar elemental compositions.

Several match criteria have been used to compare the elemental compositions of two or more glass fragments to determine if they could have been from the same broken glass source [102, 106-113]. The match criteria evaluated in this study are briefly described below.

Spectral overlay is typically one of the first steps used in comparison of μ -XRF spectra. Reproducible differences in the elemental peaks present or marked differences in their relative peak heights indicate that the samples have different sources. The benefits of this technique are its simplicity and the ability to recognize the presence of unusual elements, such as Mo or Er, which may be present at measurable concentrations. It is, however, somewhat subjective in that the ability to differentiate among visually similar spectra depends upon the similarity of background shapes, the signal to noise levels, and the experience of the person making the comparison. One disadvantage to spectral overlap is that its use does not provide a measure of the statistical significance when the result is that two samples are indistinguishable.

Range overlap is a relatively simple assessment that consists of determining whether or not the overall range of values of a measured parameter from one sample overlaps the range of values of that parameter in another sample. If the two measured ranges for all of the measured variables overlap, it may be concluded that the two samples could have originated from the same source. Advantages of range overlap are that there is no need for the assumption that the data are normally distributed and it is easy to understand for a layperson and to explain to a jury. On the other hand, the statistical significance of any observed overlap may be difficult to determine since the distributions of the observed measurements may not be known, particularly when the number of measurements is small [114].

The other criteria for comparison of elemental compositions that were evaluated in this study can conveniently be grouped into two general approaches. The first consists of tests of the hypothesis of equality of means of two sets of measurements. Examples of this approach are Student's t-test and its multivariate version, Hotelling's T^2 test. One advantage of the t-test comparison is that the statement regarding a match can be supported with a significance or probability value. Forensic practitioners have utilized various significance levels for the t-test. When using a t-test successively for each of a number of measured elements, a probability adjustment, such as the Bonferroni correction,

can be applied to limit a cumulative increase in the family-wise error rate for multivariate comparisons.

Hotelling's T^2 -test is a multivariate equivalent of Student's t-test [114-116]. It has the limitation that the number of measurements must be at least two greater than the number of variables. For example, if the concentrations of 18 elements are determined during the analysis, then Hotelling's T^2 -test requires a total of at least 20 replicate measurements on the two samples. This is not always practical, depending on the analytical technique used and/or the sample size.

The second approach is designed to more closely reflect the common forensic situation where many measurements can be made on one sample (the known sample, K, typically a large fragment of a broken glass object from a known source) but only a few measurements can be made on the other sample (the questioned sample, Q, usually a small fragment recovered from a crime scene or suspect). The measurements from the broken object are used to characterize an acceptance interval for each element or intensity ratio using the mean and some measure of dispersion of measurements about that mean obtained from multiple measurements. The smaller number of measurements made from each recovered glass fragment are used to find mean values for that fragment which can then be compared to the acceptance interval. The glass samples are considered indistinguishable only when mean concentrations of each of the elements or element intensity ratios of the questioned sample fall within the matching known sample acceptance intervals. Conversely, if one or more of the measured values for the Q sample fall outside of their acceptance intervals, then K can be excluded as the source of that Q sample. When the data are normally distributed about the mean, a confidence interval and a corresponding significance level can be assigned. Intervals of ± 2 , 3, 4, 5 and 6 standard deviations about the mean were examined in this study. Of course this approach does not apply to all situations. If the Q sample consists of a sufficient number and size of fragments that can reasonably be assumed to be from one source, it too can be characterized like the broken object and their acceptance intervals compared to see if they overlap.

For LA-ICP-MS measurements, the standard deviations of concentration values determined for a given piece of glass are sometimes so small that an unacceptably high rate of false exclusions can occur. To correct for this effect, it has been suggested that the acceptance interval should be set to the appropriate multiple times the greater of either the actual standard deviation or a value equal to 3% to 5% of the mean concentration for each element in the known sample [112].

Regardless of which match criterion is used, there are two types of errors that can occur, either incorrect exclusion of samples from the same source (type 1) or incorrect association of samples from different sources (type 2). Two comprehensive studies have recently been reported for the evaluation of error rates for several match criteria for univariate refractive index data [117] and for multi-elemental concentration data [112].

The aim of the research presented here is to determine directly the error rates associated with various match criteria using data obtained by several of the elemental techniques currently being used for the forensic analysis of glass. This study involved elemental analysis of glass fragments via four interlaboratory tests with each part of the study designed based on the previous interlaboratory test. The data for these studies was obtained by the participants using analytical instrumentation in current use in their laboratories. The development of standardized analytical protocols was described in a previous paper [105]. The blind testing of glass fragments under several case scenarios was conducted in the last three collaborative tests and these results are described in this paper. Topics discussed include blind test results for individual laboratories using their testing and interpretation criteria, evaluation of several match criteria by determination of the number of type 1 and type 2 errors that result when each criterion is applied to the data provided by the participants, and a homogeneity study of one of the glass sheets used in these studies. These interlaboratory tests were designed to determine which match criteria are appropriate for the interpretation of the data generated from the elemental analysis of glass and whether results obtained using these criteria are dependent on the technique

used to acquire the data. This study represents an effort towards the standardization of the match criteria and sampling strategies used by glass examiners, and represents another step towards assessing the significance of glass associations.

Materials and Methods

Instrumentation and measurement parameters

Several different instruments were used for the interlaboratory tests. The μ XRF, ICP-MS and LA-ICP-MS instruments and analytical parameters used in the first two tests in this study were previously reported [105]. The instrumental parameters for LA-ICP-MS and LA-ICP-OES used by new participants that joined the group for the third and/or fourth tests are shown in Table 1 of this paper. The element list employed by the other participants was described in the first part of this paper. Typical intensity ratios reported by μ -XRF users included Ca/Mg, Ti/Ca, Ca/Fe, Sr/Zr, Ca/K, and several other minor elements, when detectable. Participants using ICP-based methods reported between 10 and 18 element concentrations from the following list: Li, Mg, Al, K, Ca, Fe, Ti, Mn, Rb, Sr, Zr, Sn, Ba, La, Ce, Nd, Hf, and Pb.

The test samples provided to each participant were accompanied by instructions for analysis and reporting depending on their analytical method. The protocol of analysis was standardized for each analytical method to facilitate interlaboratory comparisons [105]. However, each laboratory was allowed some latitude in setting instrumental parameters according to their own optimized method. As the protocols were refined in later tests, several of the individual laboratories made minor changes to their protocols from those shown in the tables.

Standards and Samples

The standard reference materials SRM NIST 612 and SRM NIST 1831 (National Institute of Standards and Technology, Gaithersburg, MD, USA) were provided to each participant for the interlaboratory tests. The SRM NIST 1831 standard was

provided as full thickness fragments of 5 to 12 mm across to be used for evaluation of the analytical performance of each laboratory and also to normalize the μ -XRF data across participants. The matrix-matched float glass standards FGS 1 and FGS 2 (Bundeskriminalamt, Wiesbaden, Germany) were included in interlaboratory test 2.

Glass test samples were submitted as mock casework items. Those samples were selected from a collection of glass samples from different sources collected and analyzed by Florida International University researchers between 1998 and 2010. The glass samples were washed with methanol, nitric acid (0.8 M) and deionized water and examined microscopically to assure full thickness and/or original surfaces were present when required. Once the samples were dried, they were carefully broken and grouped by size to insure that all participants had a series of fragments of similar size and shape. Each sample was prepared in a separate clean area to avoid potential cross contamination.

The project consisted of four interlaboratory tests conducted by the EAWG members. The first and second tests were designed to develop and characterize the μ -XRF and LA-ICP-MS analytical protocols. The results of these tests are detailed in the first part of this paper. The second, third, and fourth tests contained sample fragments that each participant analyzed and compared to determine which ones could be distinguished as having come from different sources. In every case, participants made three measurements on each of three fragments provided for each sample. Previous analytical results obtained at FIU for the samples included in these tests are shown in Table 2. Due to space limitations, the data in Table 2 are shown without analytical uncertainties. The information is given to show the general differences between the samples and show which elements are most likely to be distinguishable among samples by the various techniques. Specific information concerning the samples for each test is as follows.

Second interlaboratory test

The purpose of this test was to serve as a proficiency test during the development of the analytical protocols. The samples for this test consisted of fragments of architectural float glass manufactured by Cardinal Glass Industries (WI, USA) that were packaged and labeled as known (K1) and questioned samples (Q1 and Q2). Glass samples labeled K1 and Q1 were sampled from a 4 x 4 cm glass fragment collected from a glass pane sampled at the Cardinal manufacturing plant on April 1, 2001. Glass samples labeled Q2 originated from the same float line as those labeled sample K1, but they were manufactured 2 years and 8 months before glasses K1 and Q1 (August 12, 1998). Three full-thickness fragments, from 2 to 7 mm across, were provided as sample K1. The Q1 and Q2 samples each consisted of three fragments, at least two of which were full thickness fragments from approximately 1 to 4 mm across.

The participants were not told of the sources of the samples for this blind test. They were given only the results of preliminary tests (color, microscopic examination and refractive index) that showed no significant differences between K1 and items Q1 and Q2. For this test, participants were told to group the three fragments together for each of the samples. All comparisons were to be made using ten measurements on the K sample and nine measurements on each Q sample (three replicates on each of three fragments). Fifteen participants reported analytical results for this test. One laboratory performed acid digestion followed by ICP-MS, seven participants conducted the analysis using LA-ICP-MS, and seven laboratories used μ -XRF.

Third interlaboratory test

The glass samples for this test were selected to study the capabilities of the techniques to discriminate glass produced in the same manufacturing plant at different time intervals (*i.e.* manufactured years apart, months apart and weeks apart). The samples, labeled K1, K2, Q1, Q2, and Q3 were architectural float glass manufactured between April 15, 1998 and August 31, 2001 at the same Cardinal Glass Industries plant as used in the second test (see Table 2). They

were sampled from a 2 x 2.5 cm glass fragment originally sampled from a glass ribbon at the manufacturing plant. Samples labeled as K1 and K2 consisted of fragments that were 2 to 7 mm in size and those labeled as Q1, Q2, and Q3 consisted of fragments that were approximately 1 to 4 mm in size. Each sample contained three fragments.

Each participant was asked to conduct elemental analysis in order to compare K1 and K2 with each of the questioned items. The participants were informed that preliminary screening analysis (color and refractive index) showed no significant differences between K1 and K2 and any of the questioned items, Q1, Q2 and Q3. Participants received no other information concerning the sources of the samples. Participants were instructed to make three measurements on each of the fragments. As in test 2, participants were again told to group the data from the three fragments together for each of the samples when making interpretations. Fourteen participants reported analytical results for this test. One participant performed acid digestion followed by ICP-MS, six conducted the analysis using LA-ICP-MS, and seven used μ -XRF.

Fourth interlaboratory test

The set of glass samples for this test was selected primarily to study the capabilities of the techniques to associate glass that originated from the same source and also to discriminate glass produced in the same manufacturing plant at different time intervals. This set of glass fragments, consisted of 2 known samples and 3 questioned samples. Samples K1, K2, Q2 and Q3 originated from the same source. The glass fragments originated from two pieces, one 4.0 x 4.3 cm and the other 5.0 x 4.5 cm, which were once part of a single piece of glass. The glass was manufactured at a Pilkington plant (CA, USA) on 03/03/10. The glass items labeled as Q1 originated from glass manufactured in the same plant approximately two weeks before the other samples (02/18/10). Known samples, K1 and K2 consisted of three full thickness fragments. Questioned samples Q1, Q2, and Q3 were each three small irregular fragments of

approximately 0.5 to 1 mm in size, smaller than the fragments used in the prior tests.

Participants were told that preliminary analysis (color and refractive index) showed no significant differences between K1 and K2 and all of the questioned items (Q1, Q2 and Q3) and were given no other information about the sources of the samples. Each participant was instructed to conduct elemental analysis in order to compare each of the questioned items with K1 and K2 to determine if any of the questioned items could have originated from either K1 or K2. For this test, each Q fragment was to be considered separately, rather than grouping the three fragments as in the previous tests. Additionally, the Q fragments in this test were smaller than in previous tests, making this test more difficult. Seventeen participants submitted results for this test. One laboratory performed acid digestion followed by ICP-MS, eight conducted the analysis using LA-ICP-MS, one used LA-ICP-OES, and seven used μ -XRF.

Data analysis

Statistical analyses were performed using SYSTAT for Windows (v.8.0, SPSS Science, IL, USA), JMP (v.5.0.1 SAS, NC, USA), Excel 2003 (v9.0.2719, Microsoft Corp., Redmond, WA, USA), Plot for mac OSX (v.0.997, Berlin, Germany), Mathematica (v. 5.2.0.0, IL, USA) and R (v2.13.2).

Results and Discussion

It has been well established that major, minor, and trace element profiles can provide excellent discrimination among glass sources [91-103]. In order to assess the extent to which this discrimination can be made, participants in three interlaboratory tests provided elemental data measured in their laboratories using several analytical instrumental methods. The μ -XRF users provided fluorescence peak intensity ratios, typically reporting between six and eight ratios for each sample. The ICP-MS and ICP-OES users reported the measured concentrations of up to 18 elements. Analytical data were received from 24 participants in 18 laboratories. The pool of participants used a suite of different instruments, brands, configurations and analytical parameters that represent

instrumental techniques currently used by the forensic community. The data were utilized to assess the ability of the participants to correctly associate glass fragments from the same source and to distinguish between fragments from different sources. Throughout this study, a K and Q pair of samples was considered to be indistinguishable when every measured parameter, either element concentration or intensity ratio, for the two samples could not be distinguished using the pertinent match criterion. For the purposes of error rate analysis, the “correct” result was that two samples were considered indistinguishable only when they came from the same small panel of glass in the FIU collection. Two samples produced on the same float line at different times were considered as different sources in assessing the accuracy of conclusions. This approach was taken because in most cases, the question of forensic interest is whether or not two fragments can be associated with the same window, rather than made in the same manufacturing plant.

Results as reported by each participant laboratory using their selected match criteria

Second interlaboratory test

This test was organized like a traditional proficiency test with one K sample and two Q samples. All 15 of the responding participants correctly reported that samples K1 and Q1 were indistinguishable and K1 and Q2 were distinguishable. Results for each analyst with corresponding match criteria and the number of element concentrations or intensity ratios measured are shown in Table 3. As indicated, the participants used several different match criteria to reach their conclusions. However, it is noteworthy that all methods gave correct results in this rather simple test. This result was anticipated, since, as shown in Table 2, the concentrations of Ti, Mn, Fe, and Rb are quite different between K1 and Q2. With these results, the protocols for both μ -XRF and LA-ICP-MS were considered robust and further, more difficult tests were designed.

Third interlaboratory test

The purpose of this study was to evaluate the capabilities of each method to discriminate samples manufactured at the same plant at different time intervals. Samples with similar refractive indices but distinctive elemental compositions were selected for this exercise. Samples were manufactured at the same plant on dates that were weeks, months, and years apart from each other. The dates of manufacture and the elemental profile of each of the samples as recorded in the FIU glass database are shown in Table 2. The mean concentrations shown were obtained following the ASTM method for acid digestion and solution-based ICP-MS analysis (E330-04). The values shown are reported in parts per million ($\mu\text{g g}^{-1}$). Samples manufactured only weeks or months apart have small, but significant differences in their elemental composition, e.g., K1 and Q1. However, most of the differentiating elements are present at low trace levels and it was therefore expected that only the more sensitive methods might detect some of the differences in compositions. The comparison results reported by the participants and the respective match criteria used to arrive at those conclusions are summarized in Table 4.

There were three pairs of samples that were produced over three years apart; K1/Q2, K1/Q3, and K2/Q1. Based on the results of pre-distribution analysis shown in Table 2, these sample pairs have differences in elemental compositions that were expected to be recognized using sensitive analytical methods.

All fourteen respondents correctly reported that items K2 and Q1, manufactured 3 years and 4 months apart and items K1 and Q2, manufactured 3 years and 3 months apart were distinguishable. Thirteen of the fourteen respondents correctly reported that items K1 and Q3, samples that were manufactured 3 years and 1 month apart were distinguishable. The participant that used solution-based ICP-MS reported an inconclusive result due to some uncontrolled problems during the digestion of sample Q3. Thus, each of the participants that completed the analysis was able to correctly discriminate between samples that were manufactured approximately 3 years apart in the same manufacturing plant, despite their indistinguishable refractive indices and physical properties.

The match criteria that were used by the participating forensic glass examiners included spectral overlay, range overlap, several different forms of confidence intervals, the t-test, and ANOVA. These criteria were used in various forms either individually or in combinations.

Test 3 also contained three pairs of samples that were produced several weeks to months apart; K1/Q1, K2/Q2, and K2/Q3. The results of pre-distribution analysis shown in Table 2 indicate that these sample pairs have very similar elemental compositions with relatively small differences in the concentrations of some elements. It was expected that these differences could only be detected by those techniques that have good precision of the measurements and low limits of detection.

Only four of the fourteen respondents reported that item K1 was distinguishable from item Q1. These four respondents used LA-ICP-MS methods to arrive at that conclusion. As reported in Table 3, these samples were manufactured at the same plant 2 weeks apart and therefore their elemental compositions are very similar. The discriminating elements reported by the few laboratories that found significant differences between K1 and Q1 were Ba (by three of the four laboratories using LA-ICP-MS) and Mn, K, Zr, Fe, Sr, Sn or Rb. Of the latter, the only other element that was common to two of the laboratories was Zr. Table 2 shows that these elements were present in those samples at concentrations ranging from <2 to 30 ug g^{-1} and therefore only sensitive methods with excellent precision between measurements would be able to detect those differences in concentration. For example, the reported Ba concentration difference between the two samples is approximately 2.5 ug g^{-1} . Iron, the trace element having the largest difference in concentration, was reported as significantly different in the two samples by only one of the LA-ICP-MS laboratories. This result is consistent with the fact that not only the differences in mean element concentrations, but also the variations of the measured data within a sample, are important in defining the ability of a method to distinguish two different sources of glass.

Thirteen of the fourteen respondents reported that item K2 was distinguishable

from item Q2. The only respondent that could not distinguish between item K2 and item Q2 used μ -XRF. However, after discussion of the results, this respondent re-examined their data and found significant differences in the Fe/Mn peak intensity ratios that were missed during the test. These samples were manufactured at the same plant 1 month apart and their elemental compositions are similar, but significant differences were detected for some elements, in particular Mn and Ti for μ -XRF measurements and between 6 to 12 elements for the ICP measurements.

Five of the fourteen respondents reported that item K2 was distinguishable from item Q3. None of the seven μ -XRF users were able to differentiate these two samples. The participants that were able to detect differences between these samples used LA-ICP-MS techniques. Two of the ICP-MS users did not differentiate this pair. Lab ICP-A was unable to differentiate the samples probably as a consequence of their smaller number of elements measured, and their match criteria that allows one element to differ and still call the results indistinguishable. Lab ICP-F, who used solution-based ICP-MS, reported an inconclusive result due to problems with the digestion of sample Q3. Samples K2 and Q3 were manufactured at the same plant 3 months apart. However, the elemental compositions of K2 and Q3 are more similar than those of K2 and Q2, which were manufactured only one month apart. The concentrations of discriminating elements were present at levels below 30 ug g^{-1} in the glass.

This third interlaboratory test allowed the study of the ability of the different analytical methods to discriminate among samples that shared very similar composition. All techniques were able to differentiate samples manufactured three years apart in the same plant, regardless of the match criteria employed by each respondent. Samples manufactured weeks to months apart could only be differentiated in some instances by the more sensitive analytical techniques.

Fourth interlaboratory test

The EAWG members felt that the results of the third interlaboratory test were encouraging, particularly in the excellent ability of the ICP-MS methods to

discriminate glass sources produced over fairly short time periods. However, the high degree of source discrimination could lead to the incorrect source exclusion of glass fragments that came from the same source. To address this, a fourth interlaboratory test was designed and carried out. The set of samples for this test was selected primarily with the aim of studying type 1 errors, although one sample was also included to evaluate type 2 errors on samples produced in the same manufacturing plant at different times. There were seventeen participants in this test, including one who used an additional technique, LA-ICP-OES.

Samples K1, K2, Q2 and Q3 all originated from glass manufactured at the Pilkington plant on 03/03/10. Sample Q1 was manufactured at the Pilkington plant on 02/18/2010. As shown in Table 2, the pre-distribution analysis indicated that the composition of Q1 is significantly different from that of the other samples. To simulate typical casework, known samples K1 and K2 consisted of three small full thickness fragments, while questioned samples were each three small irregular fragments of approximately 0.5-1 mm in size. The participants were instructed to make a comparison and reach an opinion concerning possible source for each Q fragment separately, rather than grouping them as was done in the prior tests. The participant (ICP-F) who used solution-based ICP-MS was unable to analyze the small Q fragments individually due to the sample size requirements in the ASTM method, and thus combined the three fragments for digestion and analysis. The results of comparisons made by the participants in the fourth interlaboratory test are shown in Table 5 for μ -XRF methods and Table 6 for ICP methods.

Sixteen of seventeen respondents correctly reported that all of the Q1 fragments were distinguishable from items K1 and K2 (see Tables 5 and 6). Although these samples were manufactured only 2 weeks apart on the same float line, significant differences exist in composition for Fe, Al and Ti and several trace elements that were readily detected by all participant methods. One of the ICP participants, designated as ICP-L, does not conduct glass comparisons on a routine basis at their laboratory and therefore only reported their measured concentration data

and did not make a decision of association or exclusion. Their data were only utilized for comparison of match criteria in the next part of this study.

All of the K1, K2, Q2, and Q3 fragments came from the same pieces of a single glass sheet, so they should have been associated by the participants. Using their selected match criteria, all seven respondents that used μ -XRF correctly reported that each fragment labeled as item Q2 or Q3 were indistinguishable from both K1 and K2. Therefore, all participants who used μ -XRF were correct in both their distinguishable and indistinguishable conclusions in this fourth interlaboratory test. It is noteworthy that by the completion of this fourth interlaboratory test most μ -XRF participants agreed on the selection of match criteria for their comparisons, based on previous results and discussion from the interlaboratory tests. All participants used spectral overlay as a preliminary assessment of similarity followed by a $\pm 3s$ criterion for comparison of intensity ratios with the exception of one laboratory that used range overlap.

As shown in Table 6, of the 88 reported comparisons for these four samples made by the ten participants using ICP-based methods, there were 16 incorrect discriminations of fragment pairs. Labs A, E, H, and K correctly found each of the Q2 and Q3 fragments to be indistinguishable from both K1 and K2. Lab C had only one incorrect result for a K1/Q3 comparison. Lab F, the one that used solution-based ICP-MS had one incorrect result, but it was out of only four comparisons because the limited fragment size forced grouping of the fragments for each sample for digestion. The majority of incorrect exclusions were made by Lab D with six and Lab J with eight. These two participants used the t-test with Bonferroni correction for their match criterion.

These false exclusion results raised a flag for further discussion by the EAWG members concerning the appropriate match criteria for ICP-based methods. Past experience of ICP-MS and ICP-OES users was that false exclusions rarely occur when an appropriate number of elements are used with reasonable match criteria. The observed rate of false exclusions, particularly for Labs D and J were unexpectedly high. It was felt that this high false exclusion rate was a result of participants using match criteria that were too narrow when considering the

relatively large number of elements measured. Therefore, further data analysis was conducted to assess the error rates for a number of match criteria with the aim of finding an optimum match criterion that would simultaneously minimize both type 1 and type 2 errors. The results of these studies are discussed in the following section.

Evaluation of performance of different match criteria

In order to evaluate how the choice of match criterion affects error rates, the data provided by each participant were used to assess the error rates for the following criteria for the μ -XRF methods: range overlap, t-tests ($p=0.05$, 0.01 and Bonferroni correction to 0.05), confidence intervals ($\pm 2s$, $3s$, $4s$), Hotelling T^2 , and for the ICP-based methods: these plus modified confidence intervals ($\pm 2s$, $3s$, $4s$, $5s$, $6s$ with minimum 3% RSD). The calculations of error rates were performed for data collected for the second, third and fourth interlaboratory tests. The data from each of the individual Q fragments were used when making the comparison to the known sample for the purpose of this error rate analysis. Therefore, each K/Q comparison was made between nine or more measurements from the K sample and three measurements from the Q sample. The result of a comparison was declared as indistinguishable when the values for all measured variables met the match criterion, otherwise the samples were deemed to be distinguishable.

The second test had one K/Q pair that originated from the same source (K1 vs. Q1), which allowed the evaluation of false exclusions, or type 1 errors and one pair of samples that originated from different sources (K1 vs. Q2), which allowed the evaluation of false inclusions or type 2 errors. The third test had five glass items, 2 known samples and 3 questioned samples, all of them originating from the same plant manufactured on different dates. Because this test did not have pairs of samples that originated from the same source, it did not have the possibility for type 1 errors. There were six sample pair comparisons that could result in false associations, or type 2 errors; (K1/Q1, K1/Q2, K1/Q3, K2/Q1, K2/Q2, and K2/Q3). The fourth test had five glass items, two known and 3 questioned samples. Two K/Q comparison pairs allowed the evaluation of type 2

errors (K1/Q1 and K2/Q1) and 4 K/Q sample pairs (K1/Q2, K1/Q3, K2/Q2, and K2/Q3) were used to evaluate the rate of type 1 errors. For each sample pair, the number of errors was determined three times for the individual fragments of each Q sample and summed across all participants reporting results for that sample pair. All reported sample pairs were used to calculate the number of incorrect associations using each of the tested match criteria.

Error rates for μ -XRF data

The summary results of error rate analyses obtained using μ -XRF data for different match criteria expressed as the percentages of incorrect associations or exclusions are shown in Table 7. The number of comparisons used to calculate each percentage is given in the footnote to the table. False inclusions, or type 2 errors, were determined for the data from all three interlaboratory tests. The rate of false inclusions on this test was very low regardless of the match criteria employed for μ -XRF data. For the 68 sample pair comparisons made for the second and fourth interlaboratory tests, only one pair resulted in a type 2 error. This error only occurred for the t-test at $p=0.01$, the t-test with Bonferroni correction, and the 4s test for the second test. The sample pairs used for the evaluation of type 2 error rates on the second and fourth interlaboratory tests were manufactured in the same plant more than 2 years apart and 2 weeks apart, respectively. Their elemental composition was fairly distinctive and significant differences were detectable using μ -XRF methods.

As expected, the type 2 error rates on the third interlaboratory test are larger than for the other tests because the samples for this test were manufactured on the same float line and, in some cases, at relatively short date intervals. As a result, these samples have only minor differences in elemental composition. The t-test at the 0.05 and 0.01 levels resulted in the lowest numbers of type 2 errors in this set, 6 % and 15 %, respectively.

Type 1 error rates (false exclusions) were determined for the second and fourth tests. At least one false exclusion was observed for all of the match criteria except for 4s in the fourth test. The number of type 1 errors when using the

narrower match criteria of the t-tests and the 2s test are generally quite high. The range, 3s, 4s, and Hotelling's T^2 tests, with their wider match criteria, result in more acceptable type 1 error rates. The high number of type 1 errors is somewhat surprising considering that μ -XRF measurements have repeatability values of 10% or greater for elements present at lower concentrations, such as Ti, Sr, and Zr. The most likely reason as to why all pairs of samples from the same source are not correctly associated is that the irregular shapes and small sizes of the Q fragments result in biases in measured intensities when compared to the data from the larger, multiple K fragments. It is also significant that the conclusions reported by individual participants in the interlaboratory tests were all correct. There are several possible reasons for the better performance by the participants than that indicated by the various match criteria. First, the participants used spectral overlay as a pretest prior to comparison of analytical data. As a result, they may have removed some elements that were present at close to limits of quantitation from further quantitative comparison. Some of the false exclusion errors seen here result from comparisons of ratios involving these barely detectable elements which are more prone to sample size and orientation errors than elements present at higher concentrations. The second reason for the lower number of type 1 error rates for individual participant is that they grouped the data for Q fragments in Test 2, which improved the error rates compared with treating fragments individually.

For a compromise between type 1 and type 2 error rates, the optimum match criteria were 3s, range overlap, and Hotellings T^2 . As shown in Table 7, 3s and Hotellings T^2 criteria had higher rates of false inclusions than range overlap for the data from the third test, which employed samples having very similar elemental compositions. However, the range overlap criterion had a significantly greater false exclusion rate than the 3s or Hotellings T^2 criteria for the small irregular shaped fragments encountered in the fourth test. One advantage of μ -XRF data is that the typical number of variables (6-8 ratios) allows the fulfillment of the requirement of Hotellings T^2 to have more replicate measurements than variables (i.e., at least 5 to 7 replicate measurements for the known sample and

at least 3 for each questioned sample). However, in instances with small Q fragments such as debris cases, it may not be practical, or even possible, to collect the required number of replicate measurements on each fragment unless the position of the x-ray beam remains stationary between measurements. Spectral overlay was not included in the tested match criteria because it is a qualitative comparison. However, based on the experience of EAWG members and the results reported by the participants in the interlaboratory tests, spectral overlay is one of the best match criteria. A protocol for μ -XRF that has been submitted to ASTM for consideration as a standard test method recommends the use of spectral overlay followed by either a 3s or range overlap match criterion using element intensity ratios. Although the results of this study cannot be applied directly to other manufacturers or even other dates for the float lines studied, they should be generally applicable. That is, the μ -XRF methods are capable of detecting differences in composition of flat glass from the same line within a float glass plant when they are produced over time periods of weeks to months apart.

Error rates for ICP data

The summary of error rates obtained for ICP data for different match criteria are shown in Table 8. Because of the good precision of most ICP data, additional broader match criteria were included in this study (5s, 6s and modified confidence intervals with minimum of 3% RSD)[15]. False inclusions or type 2 errors were estimated for the three tests. The only type 2 error that was made for the samples of the second and fourth interlaboratory tests was from the t-test with Bonferroni correction for one fragment from one participant in the second test. The samples used for the evaluation of type 2 error rates on these sets were manufactured in the same plant more than 2 years apart or 2 weeks apart, respectively. However, differences in their elemental composition as measured by ICP methods are detectable by any of the match criteria.

The type 2 error rate in the third interlaboratory test was expected to be larger than the other tests because the samples were purposely selected to be closer in

manufacture date and also very similar in elemental compositions. In spite of this, the false inclusion rate was very low. All the K/Q pairs that showed false inclusions came from the pair of samples manufactured only 2 weeks apart, demonstrating that the sensitivity and precision of ICP data allows for the discrimination of samples manufactured at the same plant during short time intervals. Confidence intervals greater than 5s provided the largest number of type 2 errors in this set.

Type 1 error rates, or false exclusions, were determined for the second and fourth tests. Some false exclusions were observed for the majority of the match criteria, with lower rates provided by broader match criteria ($> 4s$). Failure to associate samples with the same origin was observed in the second test only for 2 out of 7 participant laboratories and in all cases the differences were found only for one out of the 16-18 elements monitored. Repeatability between measurements in the discriminating element was lower than 2% RSD. The larger number of type 1 errors on the fourth test is attributed mainly to the atypical heterogeneity discovered in the samples, which is discussed in more detail in the following section.

The best performance for a compromise between type 1 and type 2 error rates is found for 4s and modified 4s interval. Using these broader match criteria reduced significantly the Type 1 errors without sacrificing the capability to discriminate samples (type 2 errors). Hotelling's T^2 is not as practical for ICP data as for the μ -XRF data due to the larger number of variables measured (16-18 elements). However, Hotelling's T^2 could be applied in cases where the questioned sample is large enough to allow the requisite number of replicate measurements. Since that was not the case in these studies, no statement can be made as to the error rates that might result when using Hotelling's T^2 with ICP data.

The need to widen the match criteria for ICP measurements is a consequence of the high precision of the measurements (typically less than 2% RSD). Using the broader criteria, the ICP methods were still able to correctly discriminate between

samples with similar elemental profiles that originated from the same plant and were manufactured more than 2 weeks apart. It should be noted here that the significant factor affecting changes in composition of float glass is not time, per se, but rather changes in the compositions of raw materials and internal processes within the manufacturing plant that occur over time. Again, the results of this study cannot be applied directly to other manufacturers or even other dates for the float lines studied. However, they should be generally applicable in that the ICP-based methods, when applied to many major, minor, and trace elements, are capable of detecting differences in composition of flat glass originating from one plant over time periods of weeks to months.

Homogeneity study: Pilkington and Cardinal plants

The samples selected for the fourth interlaboratory test originated from a Pilkington glass manufacturing plant that experienced changes in the formulation of the glass due to market requirements. Figure 1 shows the variation of concentration of iron in glass samples collected over a 2-month period. Error bars represent the variation (as standard deviation) obtained from 5 replicates of a single sample measured by LA-ICP-MS. Drastic concentration changes in iron content were observed in glass manufactured between February 25, 2010 and March 19, 2010. Nevertheless, the plant reported that their “transition period”, where the glass was not released to the market, was between March 14, 2010 and April 16, 2010.

Samples selected for the interlaboratory test were manufactured approximately two weeks and one month before the transition period, respectively. Due to the unexpectedly high rates of false exclusions found in the fourth interlaboratory test by ICP-based methods, it was suspected that one cause for the occurrence of this type of error could be an atypical heterogeneity at the micro-scale of the samples submitted for analysis.

To test this hypothesis, homogeneity studies were conducted on the original source samples from the Pilkington plant. In addition, a set of glass samples from another plant was included for comparison purposes. The Cardinal sample

manufactured on August 17, 2001, which was used as K1 for the third interlaboratory test, was selected for this study because in that interlaboratory test none of the participants reported false exclusion errors in their findings.

The homogeneity study was designed to compare the variation: a) between fragments from the same source (i.e. 6-7 fragments from the same source, 3 replicates each) and b) within fragments (spatial variation, i.e. float side, non-float side, and different areas through a cross section of the glass)

Variation between fragments from the same source (non-float sides only)

Six fragments were randomly selected from each sample, 3 replicates were conducted on each of the non-float original surfaces of the fragments for a total of 18 measurements per sample.

Comparisons between the six fragments versus each other were conducted using ANOVA with Tukeys post-hoc test. The results show more heterogeneity in samples from the Pilkington plant than in the samples from the Cardinal plant. Significant differences for 7 out of 18 elements monitored (Mn, Al, K, Ca, Ti, Fe and Sr) were observed between fragments from the Pilkington glass manufactured on February 18, 2010 and for 3 out of the 18 elements monitored (Al, Ca and Hf) for the Pilkington sample manufactured on March 3, 2010. In contrast, no significant differences were observed between any of the fragments sampled from the Cardinal glass using the same criterion.

In order to simulate the statistical treatment given to the data during the fourth interlaboratory test, the comparison between fragments was also conducted by randomly choosing 3 of the fragments to act as the Known sample (K) and the remaining fragments as independent Questioned samples (Q), with 3 measurement replicates each. Only non-float surfaces were analyzed during this experiment.

In general, the Cardinal glass sample showed evidence of uniform distribution of elemental composition among non-float surfaces. No significant differences were detected between fragments using different match criteria (except t-test $p=0.05$).

On the other hand, the Pilkington samples showed more heterogeneity, as evidenced by more false exclusions than the Cardinal sample. The false exclusions were reduced to zero for the three Pilkington subsets when applying broader match criteria, such as $\pm 4s$ and modified $\pm 4s$ (minimum 3-5% RSD).

Variation between original surfaces and across the thickness of the fragment

Figure 2 shows the sampling scheme used to study spatial variation within a single fragment. Five replicate analyses were conducted on each of the sampling sites (original surfaces such as float versus non-float side, and fracture surfaces as different areas across the thickness of the fragment).

Significant differences in composition were found between float and non-float surfaces on all tested samples (Cardinal and Pilkington) using all match criteria. Differences between the surfaces were detected not only for the content of Sn, but also for other elements, such as Al, Ca, Sr, Zr, Ti and Fe.

These the relevance of sampling. Whenever possible, sampling from fracture surfaces is preferred over original surfaces. Otherwise, if analyzing original surfaces, either all non-float surfaces or all float surfaces should be used for the comparisons of known and questioned sources. An easy way to detect if the analysis is being done on the float side is to monitor the content of Sn, which will typically be 1-2 orders of magnitude larger on the float side of the glass. This observation can be done in-situ during the analysis and the sample can be easily turned to the non-float side if needed.

The study of elemental variability across the thickness of the interior portion of glass fragments also revealed more heterogeneity in the Pilkington samples. Significant differences were detected, depending on the match criteria, particularly close to the non-float surface ($<200\mu\text{m}$). This variability was detected regardless of the match criteria applied to sample PK030310, which was manufactured close to the time of the reported transition in Fe formulation. This sample was the one selected for the fourth interlaboratory test to examine false exclusion errors.

Significant differences across the thickness of the sample were also detected for the Cardinal sample, depending on the match criteria. Nevertheless, no significant differences were detected using $\pm 4s$ or modified $\pm 4s$ match criteria. Figure 3 shows the variability of the iron content observed between different sampling areas of the fragments for one of the Pilkington samples (PK03010) and the Cardinal sample.

The results of this study demonstrate that the heterogeneity between fragments is more pronounced for the Pilkington samples than for the Cardinal samples. Due to the shape and small fragment size chosen for the Q samples on the fourth interlaboratory test, it is likely that participants received, Q samples from different areas across the thickness of the fragment and encountered heterogeneous compositions. This could have contributed to the elevated number of false exclusions obtained with sensitive ICP-based methods.

Consequently, based on post distribution experiments and various statistical evaluations of the data, the rate of false exclusions found in the fourth test for ICP-based methods is attributable to several factors: a) the limited number of replicates for questioned samples, a common casework concern, b) unusual heterogeneity of the samples of Pilkington glass, and c) match criteria too sensitive for methods achieving very high precision between replicates.

In the fourth interlaboratory test, participants were asked to compare the known fragments to each of the individual questioned fragments (instead of grouping all questioned fragments). This approach was selected to be a more realistic simulation of a case where small, irregular fragments recovered from surface debris must be treated as individual fragments. Due to the small size of the fragments submitted for analysis, only 3 replicates were requested per questioned fragment. As a result, for the sensitive ICP-based methods that have high precision, only some of the fragments were correctly identified as indistinguishable from the known sources. The precision and sensitivity of μ -XRF techniques, in combination with the selected match criteria, were shown to be appropriate for these types of samples.

The heterogeneity observed on the Pilkington samples, both within a fragment and between fragments originating from a single source is atypical of what has been observed in the float glass encountered in several manufacturing plant studies analyzed by solution ICP-MS and LA-ICPMS at FIU over the last decade. Heterogeneity of these samples is also inconsistent with previous within-sheet homogeneity studies conducted at FIU, the BKA, and the FBI. Nevertheless, as with any commercial product, the variability of its elemental composition is dependent on market requirements and the manufacturing history of the specific plant. For these reasons, samples such as those encountered in this fourth interlaboratory test may be present in a real case and should be taken into account during the selection of match criteria and interpretation of the data.

Conclusions

Based on results obtained in the interlaboratory tests, it is concluded that the match criteria for comparison of elemental composition of glass fragments should be carefully selected based on the technique used for analysis as well as the number of replicates that are conducted to characterize the variability of the known and questioned samples.

For μ -XRF analysis, the following observations are derived from the studies. Spectral overlay, $\pm 3s$, range overlap, and Hotelling's T^2 performed well in terms of both false exclusions and inclusions. Excellent consistency of reported comparison results among participants was achieved for all the interlaboratory tests, not only for comparison conclusions but also for the elements reported to be responsible for discrimination. Participants who used μ -XRF methods were able to detect significant differences between fragments of glass that were manufactured in the same plant within short periods of time. That period of time is dependent on the variability of the formulation of the glass within a plant. For instance, participants who used μ -XRF were able to detect differences in samples manufactured a month apart at the Cardinal plant. Differences were not detected between samples manufactured at this plant 2 weeks apart and 3 months apart when their elemental compositions were extremely similar. However, all participants were able to detect significant differences between

samples manufactured 2 weeks apart at the Pilkington plant. Users of μ -XRF must take into consideration that small, irregularly shaped fragments may result in false exclusions when they are compared to larger fragments, particularly when they are thin enough that high energy x-rays penetrate completely through them.

For ICP-based methods (digestion ICP-MS, LA-ICP-MS and LA-ICP-OES), the following conclusions are derived from the studies. Most participants reported precisions between replicates of 2% RSD or less. This good analytical precision may be one of the factors that contribute to higher false exclusion rates when sensitive match criteria such as the t-test or a low multiple of standard deviations are used. Due to the sensitivity of the method, the capability of multi-elemental analysis of trace elements, the typical high precision, and the concerns for heterogeneity, the use of broader match criteria such as $\pm 4s$ is recommended, either with or without minimum precision values depending on the reproducibility within replicates. These match criteria still allow detection of significant differences between samples manufactured in the same plant over short time intervals, even for samples with quite similar elemental profiles. The performance of these match criteria is in agreement with recent published data [34]. As with μ -XRF methods, the time interval over which samples cannot be distinguished depends upon the variability of the formulation of the glass within a plant. For instance, ICP participants were able to detect differences in samples from the same float line at the Cardinal plant manufactured a month apart and some participants detected differences of samples manufactured 2 weeks apart.

In terms of interpretation of elemental comparisons of glass, it can be concluded from the study that glass samples that are manufactured in different plants, or even at the same plant years apart, are clearly differentiated by elemental composition when μ -XRF or ICP-based methods are used for analysis. Samples produced in the same plant over time intervals of weeks to months may also be differentiated. This level of differentiation can be used to add significance to an association, when one is found, and to assist in assigning recovered fragments to a source when selecting among several potential sources.

Chapter 4. Overall Conclusions

Elemental analysis is a very powerful tool for the identification, characterization and/or differentiation of many man-made materials that could become a critical piece of information in a forensic investigation. The proper assessment of the value of an elemental profile depends on a) a full knowledge of the capabilities and limitations of the analytical technique(s) used for the acquisition of the measurements and b) an understanding of any effect that the nature of the material, its composition and/or its manufacture could have in the overall estimation of the discrimination potential.

In the case of glass, LA-ICP-MS and LIBS methods have been previously developed and optimized hence the main focus of this research was to evaluate the significance of its elemental composition in forensic comparisons. In order to accomplish this, both laser ablation methods were compared to other techniques such as digestion-ICP-MS and uXRF through a series of inter-laboratory studies conducted by 31 forensic examiners representing 23 different laboratories in the US and outside the US (Mexico, Canada and Germany).

The first part of this research describes a series of interlaboratory studies that allowed for a direct comparison between four of the most sensitive methods currently available for the forensic elemental analysis of glass samples (LA-ICP-MS, solution ICP-MS, LIBS and μ -XRF). Members of the Elemental Analysis Working Group (EAWG) completed four (4) interlaboratory studies designed and administered by FIU that were specifically on the evaluation of glass evidence. One additional interlaboratory trial was designed to evaluate the bulk elemental analysis of soil evidence. This project enabled the standardization of two commonly used elemental analysis methods (using LA-ICP-MS and uXRF) for glass analysis, the evaluation of the performance of several forensic laboratories and the evaluation of the use of various different "match criteria" for the interpretation of the elemental data generated.

Some of the important findings from the use of the LA-ICP-MS methods for glass analysis include:

- a) The detection and reporting of heterogeneity of Ce and La close to the rim on FGS standards (< 250 μm)
- b) The observation that possible differences between surface and bulk composition in compared glasses may lead to false exclusions if sampling and data interpretation are not carefully considered.
- c) As a result of the sensitivity of the method, the capability of multi-elemental analysis of trace elements, the typical high precision, and the concerns for heterogeneity, the use of broader match criteria such as $\pm 4s$ is recommended, either with or without minimum precision values depending on the reproducibility within replicates.
- d) These wide match criteria still allow detection of significant differences between samples manufactured in the same plant over short time intervals, even for samples with quite similar elemental profiles.
- e) The time interval after which samples cannot be distinguished depends upon the variability of the formulation of the glass within a plant. For instance, ICP participants were able to detect differences in samples from the same float line at the Cardinal plant manufactured a month apart and some participants detected differences of samples manufactured 2 weeks apart.

Some important outcomes from the use of μ -XRF methods include:

- a) The use of normalized data to a glass standard such as SRM NIST 1831 provide a means to account for differences among instrumental configurations and to permitted the comparison of interlaboratory data.
- b) The use of a glass standard as a “control” glass is recommended to check method performance prior to analysis.
- c) Spectral overlay, $\pm 3s$, range overlap, and Hotellings T2 performed well in terms of both false exclusions and false inclusions.
- d) Participants who used μ -XRF methods were able to detect significant differences between fragments of glass that were manufactured in the same plant within short periods of time.
- e) The period of time after which samples cannot be distinguished is dependent on the variability of the formulation of the glass within a plant. Users of μ -XRF

must take into consideration that small, irregularly shaped fragments may result in false exclusions when they are compared to larger fragments, particularly when they are thin enough that high energy x-rays penetrate completely through them

Although LIBS is not as mature an analytical method as the other techniques evaluated in this work, the results suggest that LIBS offers potential for the forensic analysis of glass samples. LIBS has analytical capabilities close to those obtained by LA-ICP-MS and better than uXRF. Further interlaboratory optimization and validation of the analytical protocols is believed to be key to improve the agreement of results between laboratories.

In summary, based on results obtained in the interlaboratory tests, it is concluded that the match criteria for comparison of elemental composition of glass fragments should be carefully selected based on the technique used for analysis as well as the number of replicates that are conducted to characterize the variability of the known and questioned samples.

It can be concluded from the glass study that glass samples that are manufactured in different plants, or even at the same plant years apart, are clearly differentiated by elemental composition when μ -XRF or ICP-based methods are used for analysis. Samples produced in the same plant over time intervals of weeks to months may also be differentiated. This level of differentiation can be used to add significance to an association, when one is found, and to assist in assigning recovered fragments to a source when selecting among several potential sources.

The inter-laboratory experience demonstrated to be a very efficient method to validate forensic methods and to assess the significance of the evidence and therefore they are recommended in the future for other matrices such as soil, paint, ink and paper.

Appendixes

Appendix A - References

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