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Survey of Mounting Media Used for Hair and Fiber Microscopy

ABSTRACT

A survey was conducted to determine what mounting media are currently being used by laboratories to prepare hair and fiber samples on glass microscope slides for examination. Although forensic laboratories represent the majority of respondents, the survey also represents medical, consulting, and research laboratories, as well as museums. The results reveal that laboratories across the continental United States, as well as laboratories outside the continental United States, use a variety of different mounting media for microscopic hair and fiber examinations. Respondents commented on their own experiences with their chosen mounting media, reporting instances of crystallization over time, bubble formation, yellowing, and other characteristics. The results of this paper represent a large-scale survey of microscopists concerning the mounting media types they use to prepare hair and/or fiber samples for forensic examination, providing laboratories an updated and diverse pool of commercially available media from which to consider alternatives. Additionally, any region-specific trends in mounting media usage were identified.

Keywords: Fiber Analysis, Light Microscopy, Microspectrophotometry, Mounting Medium

INTRODUCTION

Currently, Permount™ is being used by multiple forensic laboratories as a semi-permanent mounting medium for hair and fiber samples because of its performance as both a rapid mounting medium and a matrix for long-term storage [1,2,3]. At the FBI Laboratory, hair and fiber samples are mounted in Permount™ on a glass microscope slide and are covered with a glass coverslip for examinations, which may include feature

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comparison, polarized light microscopy, and fluorescence microscopy. Dyed fibers may also be analyzed in the visible (VIS-MSP, 380nm–800nm) region. When information in the VIS-MSP is limited, fibers may be analyzed in the ultraviolet (UV-MSP, 200nm–380nm) region. However, for UV-MSP to be performed, the glass coverslip must be removed, and the textile fiber samples must be extracted from the mounting medium. The samples are then temporarily remounted in glycerol on quartz microscope slides and are covered using quartz coverslips. Since quartz slides and quartz coverslips cost substantially more than glass slides and glass coverslips, and because glycerol is only a temporary mounting medium, both are impractical for use during initial microscopical examinations. Additionally, removing fibers from Permount™ comes with an increased risk of loss or damage to the textile fiber samples in the process. Ideally, forensic hair and fiber examiners would prefer to avoid transferring samples from glass to quartz slides to minimize this risk. The identification of a semi-permanent mounting medium which does not absorb in the UV spectral region would address this concern. In an effort to find such an alternative mounting medium to Permount™, we conducted two surveys – a domestic survey and an international survey. This manuscript reports the results of these surveys.

METHODS

A questionnaire developed in conjunction with FBI Laboratory hair and fiber forensic examiners consisted of seven free-response questions, which inquired about specific information on different aspects of slide preparation by the respondent.

- What type of mounting medium/media do you use for hair and/or fiber microscopy?
- If you have experienced any challenges with the media above, please describe.
- What type of solvent(s) do you use to dissolve solidified media during hair and/or fiber microscopy?
- If you have experienced any challenges with the solvents above, please describe.
- What type of microscope slides and coverslips (brand and thickness) do you use for fiber and hair microscopy?
- If you have experienced any challenges with slides and coverslips the above, please describe.
- How do you package and store your slide preparations?

A template of the questionnaire is provided (Appendix 1).

The questionnaire was made available to participants using an online survey tool between October 2017 and November 2017. Participant recruitment involved working with the President of the American Society of Trace Evidence Examiners (ASTEE) to reach out to the organization's members. Completion of the questionnaire was made available via a

private link (FBI IRB# 615-21). Individual responses were downloaded from the online survey tool and sorted using a combination of manual verification and scripts in R (version 3.4.0) [4] to generate spreadsheets that linked answers to different questions.

RESULTS

This paper focuses on questions pertaining to mounting media used for analysis of hair and textile fiber samples, as well as challenges occurring in these preparations and ways slides are stored. In no way does this report endorse, promote, or advocate the usage of any mounting media described by respondents or discussed in the literature, nor does it generate inferences on suitability of mounting media types or brands for any kind of analysis. All data, including challenges experienced with mounting media, are solely the view of the respondents and do not reflect the opinion of the authors. Note that reported challenges represent anecdotal observations and opinions and are not supported by statistical evidence or empirical data.

Respondent information and locations

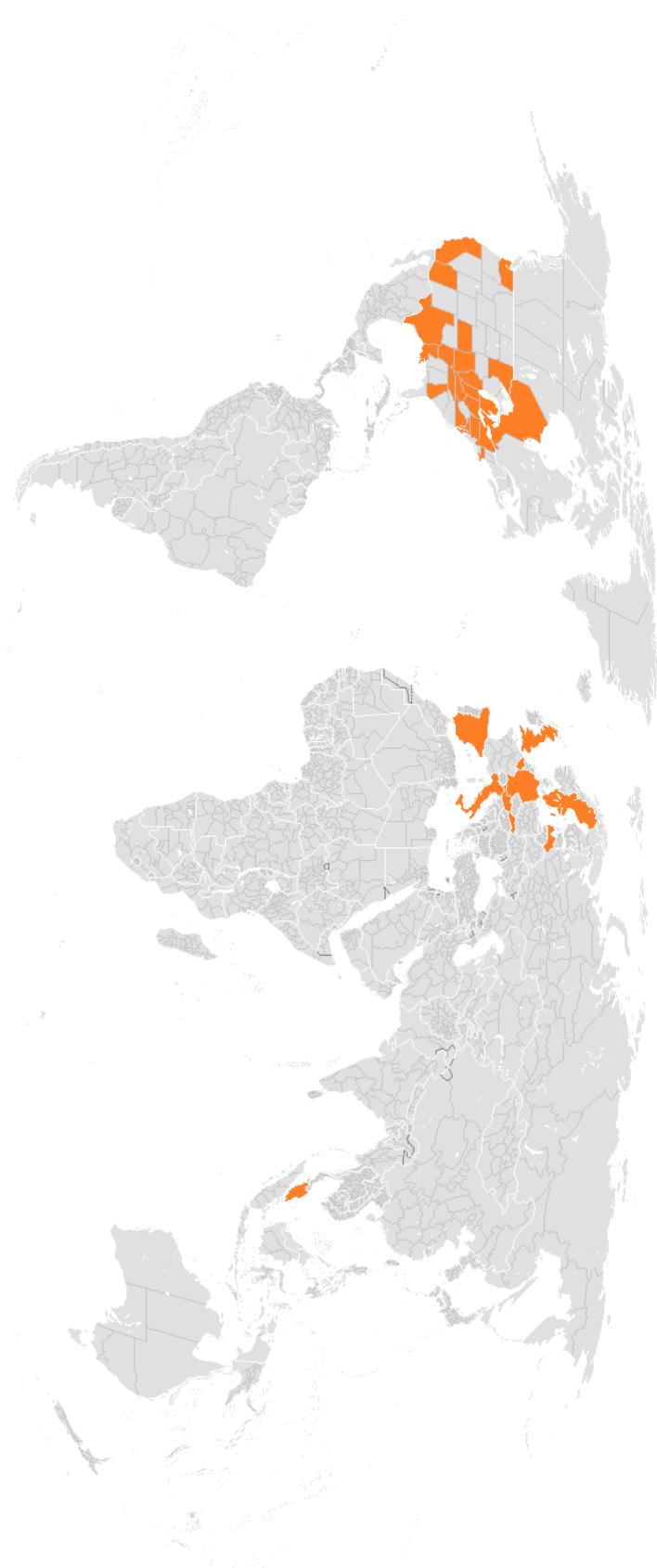
A total of 76 responses were obtained: 57 (75%) from North America, 18 (24%) from Europe, and 1 (1%) from Asia (Figure 1). Only one response was received per facility. Domestic respondents were involved in facilities exercising authority on the federal ($n=25$, 33%), state ($n=27$, 36%), and local ($n=16$, 21%) levels (Figure 2). Private companies are also represented ($n=8$, 11%). Of the 57 domestic responses, laboratories located in the southern United States were represented the most ($n=19$, 34%). The majority of the 19 international respondents exercised federal or nationwide authority ($n=16$, 80%). Note that European countries were considered federal, composed of many regions, each exercising authority on those regions. The majority of all respondents ($n=58$, 76%) conducted forensic analyses, while others were oriented toward safety ($n=5$, 7%), research ($n=5$, 7%), medical diagnostics ($n=2$, 3%), consulting ($n=2$, 3%), other purposes ($n=1$, <1%), and unknown/unspecified ($n=3$, 4%).

Types of mounting media used for hair and/or fiber examination

Overall, 159 responses for mounting media were received from the 76 respondents, representing 39 unique mounting media, including temporary, semi-permanent, and permanent mounting media (Figure 3). The most popular mounting medium was Permount™ ($n=31$, 19%), with usage instances reported only by respondents in American laboratories. Figure 4 shows the color-coded geographic areas where the mounting media usage were reported by survey respondents.

Mounting media that were used by more than two respondent facilities were placed into broad categories (Figure 5). Semi-permanent to permanent mounting media included

Figure 1: General locations of respondents for mounting media questionnaire.



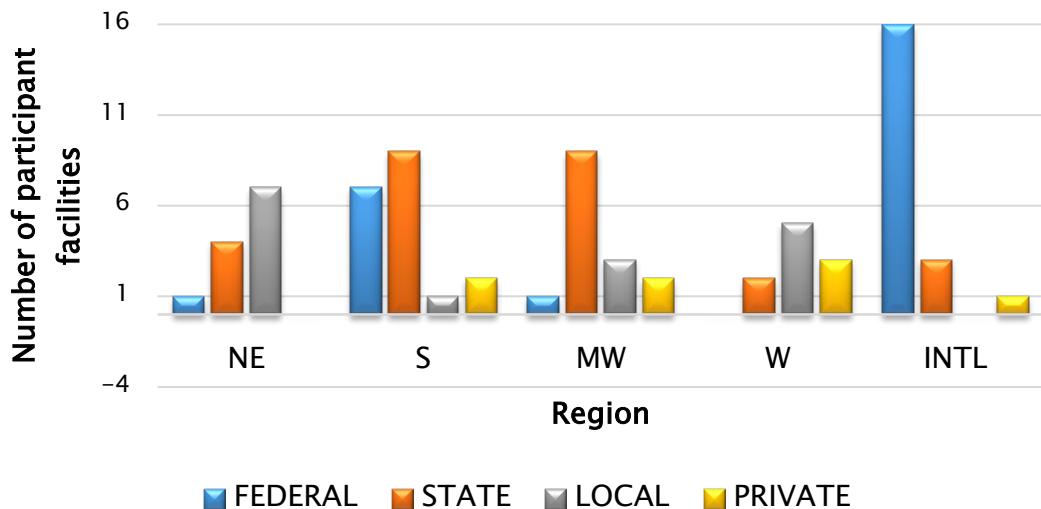


Figure 2: Respondent facility region and level of authority. NE = northeast, S = south, MW = midwest, W = west, and INTL = international regions (including Canada). Note that federal refers to facilities whose organization exerts authority on a national level; state refers to those exerting authority limited to a state level; local refers to those exerting authority on a county or city level.

Aroclor™, Permount™, Entellan™ (both Entellan™ and Entellan™ New™), Meltmount™, and Norland Optical Adhesive™. Temporary mounting media were represented by immersion oil (oil of any type), glycerol (including glycerin), and xylene (including its derivatives). All other mounting media that did not fall into these broad categories and that were reported with a frequency of less than 2% (less than four out of 159 total responses) were placed into the "Other" category. The use of these broad categories is further detailed by region in Appendix 2.

Most facilities examined both hairs and fibers in the same laboratory (Figure 6A). Regardless of the mounting media category, the vast majority of analyses in a given facility used the same mounting medium for both hair and fiber preparations (Figure 6B). Among laboratories which conduct both hair and fiber examinations or hair-only examinations, Permount™ was reported most often. Cargille-Oil was the most used mounting media among laboratories conducting fiber-only examinations.

Interestingly, when looking at the type of sample analyzed with respect to individual mounting media, there is a clear prevalence of one mounting medium for both hair and fiber analyses (Figure 7). Permount™ accounted for 11% of both hair and fiber responses. Approximately 50% of Entellan™ users also reported using Permount™ as well. In the facilities which use both Entellan™ and Permount, Entellan™ was reported to be used for fiber examinations, whereas Permount™ was used for both hair and fiber examinations. Note that Entellan™ is the second most commonly used semi-permanent mounting medium.

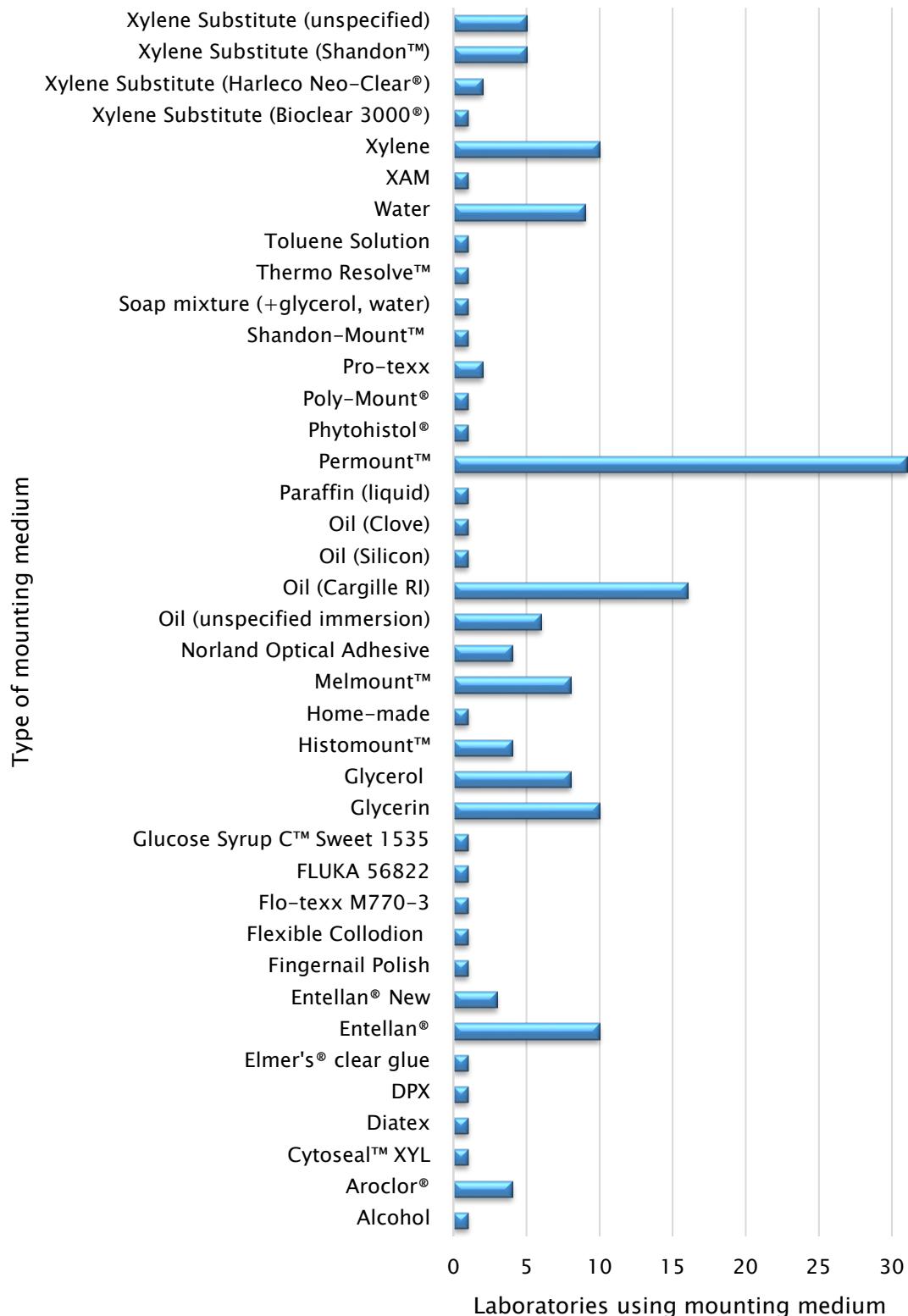


Figure 3: Mounting media used by survey respondents.

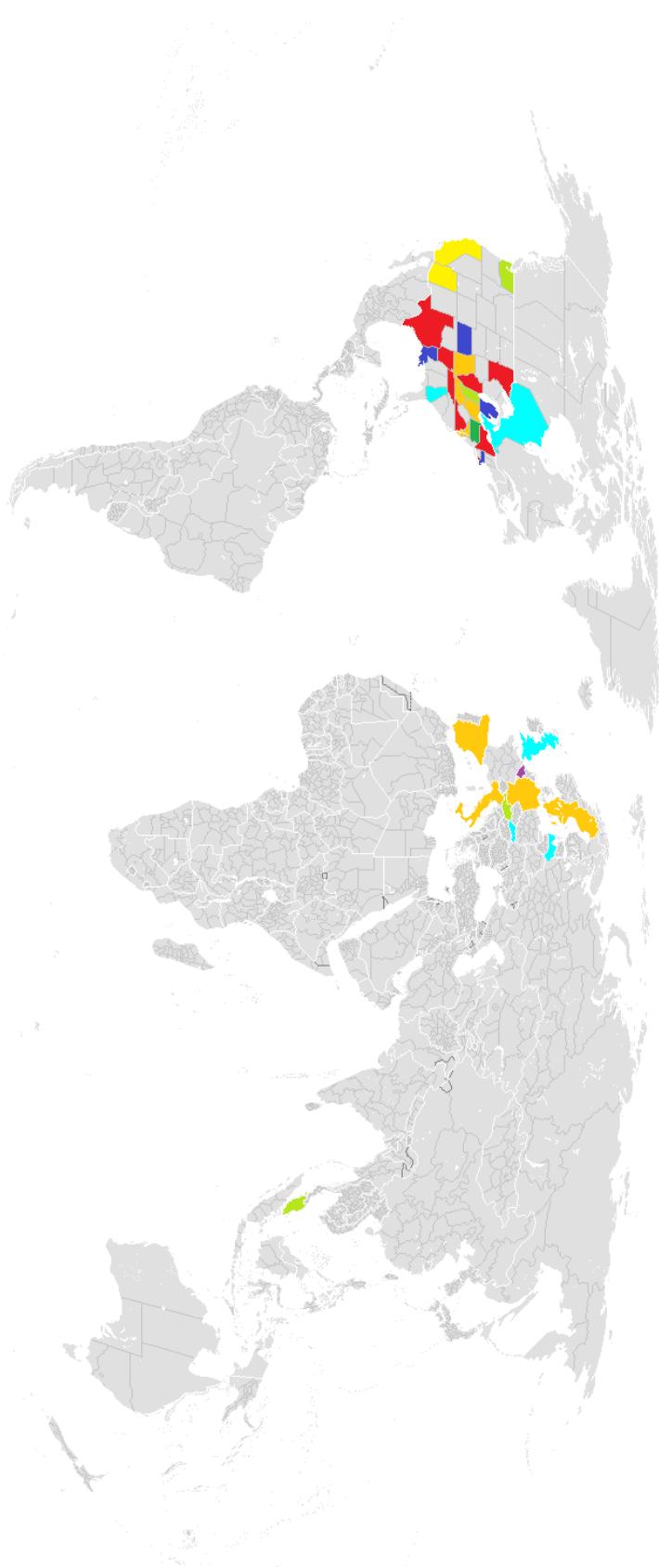


Figure 4: Overall media usage distribution. Orange = water; red = Permount™; yellow = xylene or xylene substitute reagents; aqua = Entellan New™; indigo = Cargille™ reagents (including Norland Optical Adhesive and Meltmount™); purple = Histomount™; green = use of other mounting media or an even representation of mounting media. Note that Cargille™ reagents were used together.

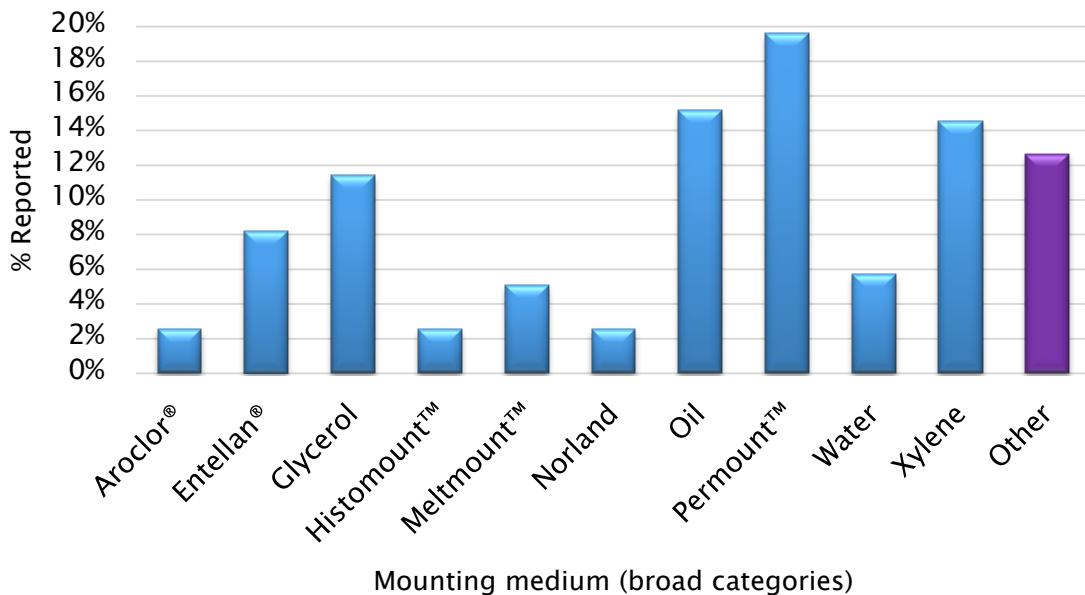


Figure 5: Percentage of reported uses of each mounting media type, grouped into broad categories. Other = mounting media reported with a frequency of less than 2% (less than four out of 159 total responses).

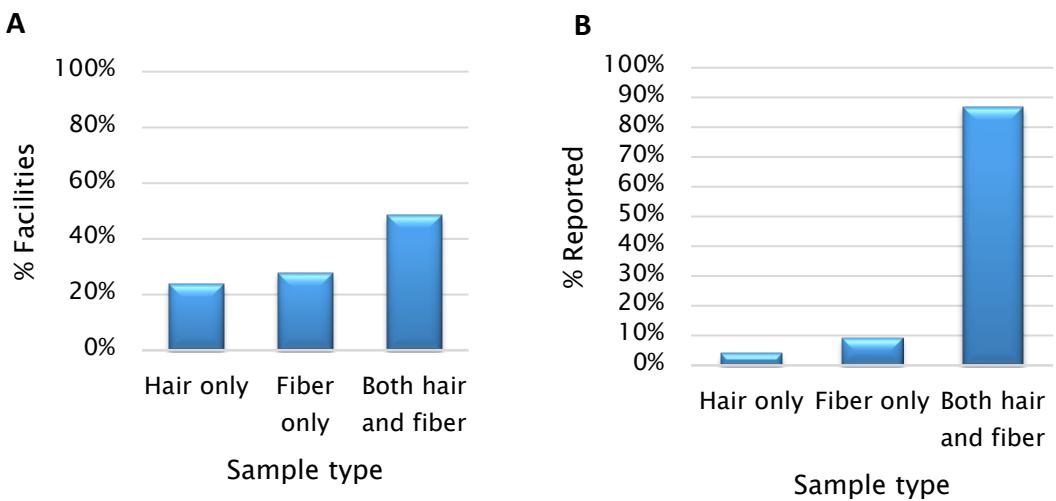


Figure 6: Hair and fiber analysis by facility and mounting media. (A) Frequency of participant facilities that examine hair-only, fiber-only, or both hair and fiber samples, based on responses ($n=76$). (B) Total number of responses pertaining to analysis of hair, fiber, or both hair and fiber ($n=139$) conducted with individual mounting medium regardless of specific mounting media type.

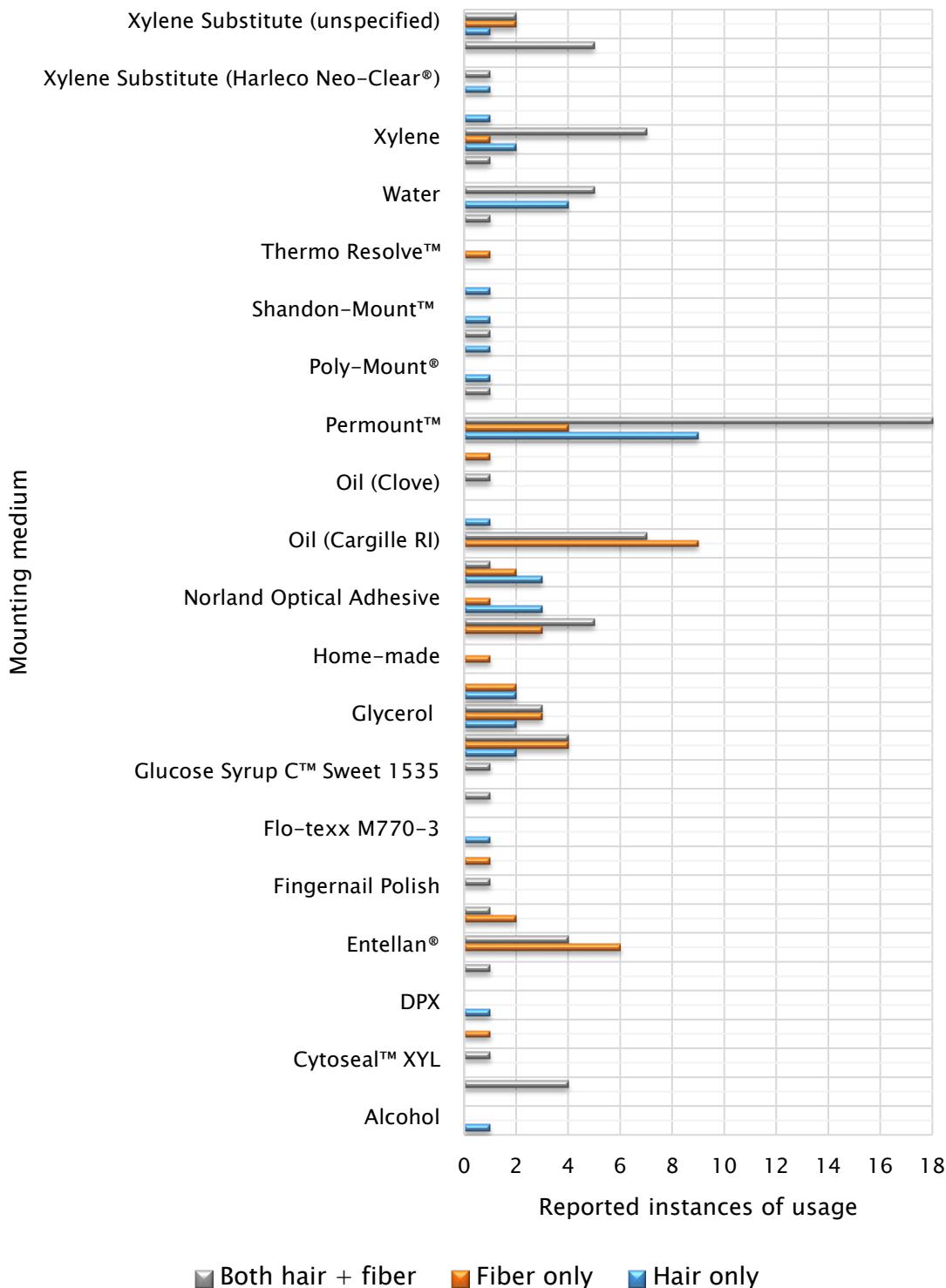


Figure 7: Breakdown of sample analysis per individual mounting medium. Note that Permound™ has the highest number of instances ($n=18$, 11%) in which it is used by respondent facilities to examine both hair and fiber samples.

Challenges experienced by respondents when using mounting media

Respondents reported their experiences and personal observations with using their chosen mounting media. A total of 31 reported challenges within 15 categories were identified from the survey results (Figure 8A). To simplify them by type, the challenges are grouped into six broad categories (Figure 8B). Media integrity combines crystallization, oxidation, dryness, and permanence, while sample integrity considers sample degradation, sample interaction with media, and sample incompatibility with media. The ultraviolet category combines UV absorbance and fluorescence, occurring when the mounting medium itself emits light when excited by UV, causing high background when samples are viewed using fluorescent microscopy. Refractive index covers both change in refractive index or an inherently higher than desired refractive index. Yellowing was maintained as a distinct category, and “other” entails various challenges too small to be a category on their own, relating to application of mounting media, fluidity, and dismounting difficulties.

Mounting media were given a point for each challenge experienced by respondents, resulting in an accumulated challenge score (Figure 9). Only mounting media reported with a challenge score frequency of more than 4% were considered. Note that reported challenges represent anecdotal observations and opinions and are not supported by statistical evidence or empirical data. Even though Permount™ is represented in all broad categories, it is important to distinguish the types of challenges contributing to the overall score. Permount™ users observed more long-term challenges that include yellowing, crystallization, and oxidation of the media overtime as well as sample degradation, whereas Entellan™ users complained of more immediate issues that affect refractive index and UV transmission upon initial analysis, as well as fiber interaction. Entellan™ has not been reported to be as challenge-free as Aroclor™, whose users reported no issues in this survey. Note that Permount™ has been widely used for more than a few decades and as a result has the highest challenge count, unlike others that were evaluated and used in facilities relatively recently. If given more time and increased usage, other mounting media may experience similar challenges to those encountered by Permount™ users.

The most common issues reported are those affecting mounting medium integrity (Figure 10). Seven participants reported observing yellowing of the mounting media. Most often, the yellowing is accompanied by crystallization (also termed “crazing”) of the media (reported by five respondents). Although semi-permanent and permanent media are designed to solidify quickly and allow the specimen to set, in crystallization, the media may polymerize in an undesirable way, representing another commonly reported challenge.

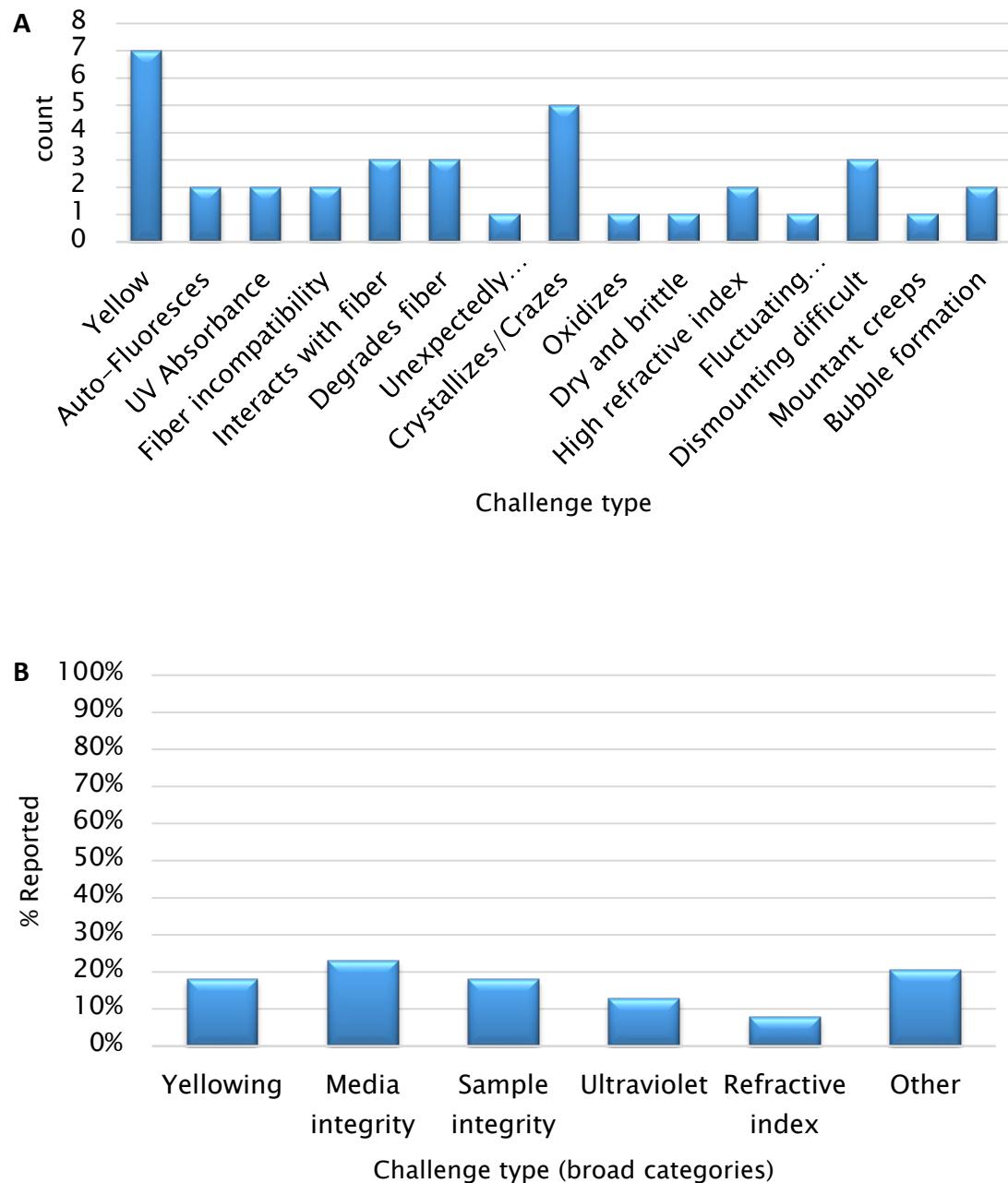


Figure 8: Mounting media challenges. Reported types of challenges observed by respondents when using a particular mounting medium. See first paragraph under "Challenges experienced by respondents when using mounting media" for specific challenges included in each broad category.

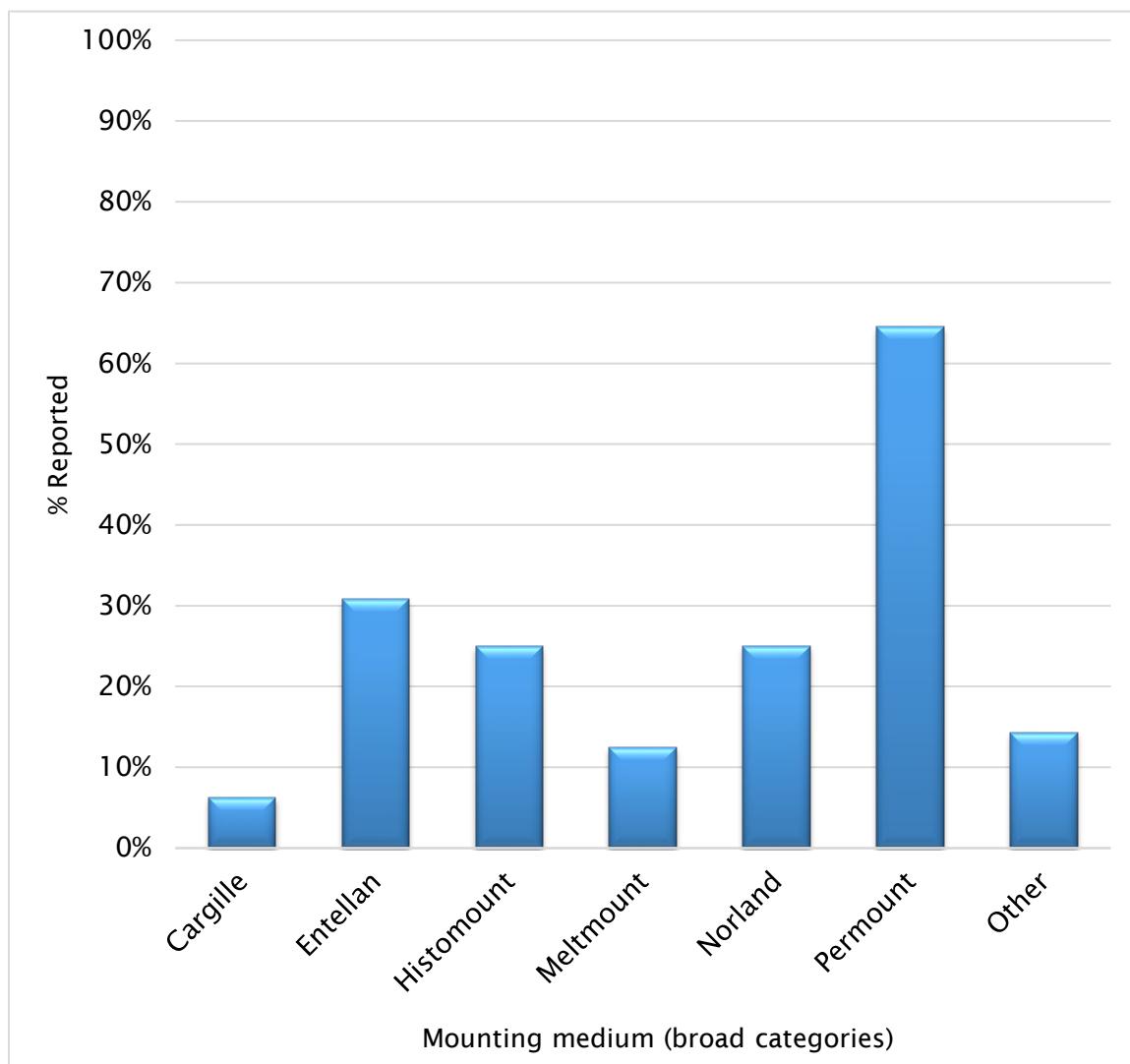


Figure 9: Reported challenges by mounting media type. Total cumulative scores for each mounting medium type derived from respondent feedback and descriptions from all facilities.

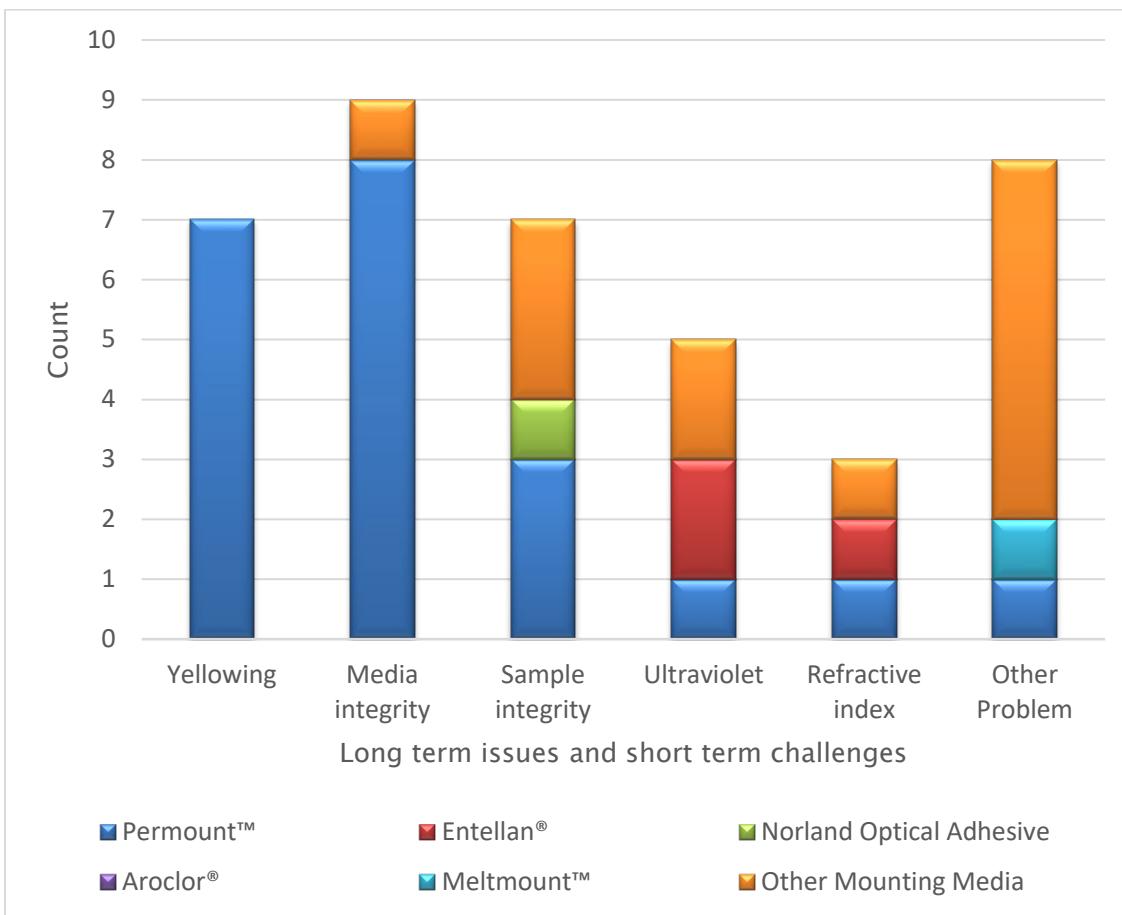


Figure 10: Reported challenge score of individual mounting media. Reported challenges represent anecdotal observations and opinions and are not supported by statistical evidence or empirical data. No follow-up interviews were conducted to gain more insight into the reported challenges.

Storage of slide preparations

Lastly, participant data on storage practices was collected. The majority of respondents reported using cardboard or plastic mailers/boxes to store slides or to return slides to submitting agencies, customers, and/or donors (n=53, 72%). Others include storage and mailing of slides in paper towels (n=7, 9%). All instances of single or double-sided tape usage occurred specifically for the storage of hairs but not for fibers (n=7, 9%). Some respondents used other semi-permanent mounting media listed in this paper such as nail polish to seal the edges of coverslips, which were analyzed using glycerol or water-based reagents (n=4, 5%). This information may be important as storage conditions will always influence the integrity and longevity to the sample preparation over time.

DISCUSSION

Studies seeking alternative mounting media to improve certain aspects of microscopical examination of biological and non-biological samples have been conducted [1-3,5-9]. These studies were conducted because microscopists sought improvement in protocols and to address safety and environmental practices. Among these studies, few have focused on evaluating mounting media specifically for forensic microscopic hair and fiber examinations. In 1982, Cook and Norton [5] recommended using the mounting medium XAM for forensic examination of textile fibers after evaluating several commercially available candidate mounting media. XAM was used by one of the respondents to our survey. Nine years later, Roe et al. [6] conducted a similar study and evaluated 31 mounting media for use in forensic hair examination. Roe et al. found Hystomount, DPX, DePeX, and Styrolite to be suited for purpose, but these authors ultimately recommended Histoclear because of its non-toxicity and its ability to dissolve in xylene-based mounting media. Histoclear was not reported as being used by any of the respondents in our survey. In 1995, Grieve and Deck [7] evaluated six mounting media to find a replacement for xylene-based permanent mounting media for forensic examinations of textile fibers. They identified Phytohistol®, a water-soluble mounting medium, as the best alternative. In addition, the authors reported that Phytohistol® did not exhibit any absorbance in the UV spectral range. In our survey results, no respondents reported using Phytohistol® as a mounting medium. More recently, in 2007, Wiggins and Drummond [8] evaluated mounting media based on regulations requiring microscopists to use less hazardous mounting media for forensic examinations of textile fibers. These authors identified Entellan® New as the best alternative, which was reported by three of the respondents to our survey.

Because of the limited scope of these studies, a larger-scale inquiry across laboratories was undertaken. Furthermore, since the majority of previous studies were done well over two decades ago, discovering the newer mounting media that other laboratories may be

using gives examiners an updated pool of commercially available media for future consideration.

It is clear from the survey data there are multiple types of mounting media used in both the United States and Europe (Appendix 2). The observed diversity in mounting media types is not likely the result of diversity in the respondent facilities' fields given that the majority (76%) of the respondents conduct forensic analyses. For example, had there been more academic or biomedical respondents than forensic examiner respondents, additional mounting media types may have been reported because these fields may require different procedures and reagents depending on unique project needs.

CONCLUSION

Overall, the survey results demonstrate surprising diversity in the types of mounting media used by respondents across the globe. In a follow-up study, a subset of relevant mounting media presented in this manuscript, as well as those identified via vendor inquiries and a scientific literature review, will be selected and evaluated for absorbance in the UV region on glass microscope slides and glass coverslips, in addition to other characteristics.

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Appendix 1: Template of questionnaire.

The Counterterrorism and Forensic Science Research Unit at the FBI Laboratory is conducting a nation-wide survey of mounting media used by forensic examiners for hair and fiber microscopy. We greatly appreciate your participation. Thank you for taking a few minutes to answer the following questions.

Respondent Information:

Company:

State/Province:

When asked about reagents and items, please provide specific brand names and catalog numbers.

When asked about your challenge with reagents and items, leave blank if no challenge were experienced.

What type of mounting medium/media do you use for hair and/or fiber microscopy?

hair _____

fiber _____

If you have experienced any challenges with the media above, please describe.

hair _____

fiber _____

What type of solvent(s) do you use to dissolve solidified media during hair and/or fiber microscopy?

hair _____

fiber _____

If you have experienced any challenges with the solvents above, please describe.

hair _____

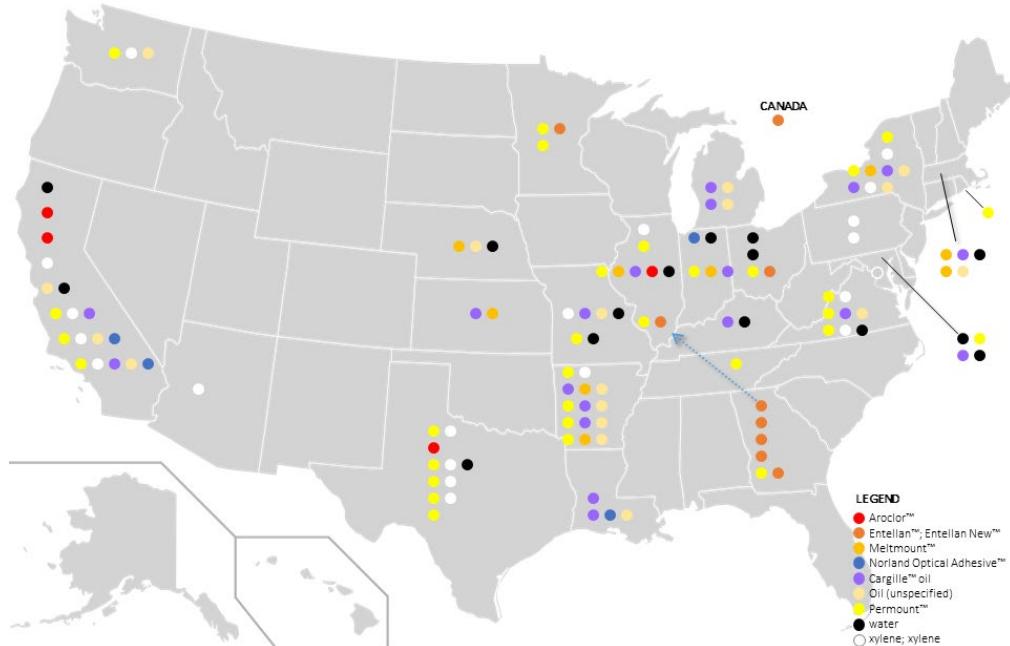
fiber _____

What type of microscope slides and coverslips (brand and thickness) do you use for fiber and hair microscopy?

If you have experienced any challenges with slides and coverslips the above, please describe.

How do you package and store your slide preparations?

Appendix 2: Overall media usage distribution. Map detailing the usage of mounting media in United States & Canada (A), and Europe (B). Tags represent more commonly used mounting media. Less commonly used media, such as Polymount™, are not represented here. Due to the anonymous nature of the survey, specific locations cannot be displayed.

A**B**