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An American National Standard

Standard Guide for Forensic Examination of Hair by Microscopy[1]

This standard is issued under the fixed designation E3316; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide covers procedures used by forensic laboratory personnel in the forensic examination of hair by microscopy, including microscopical comparisons and classification of hair samples.

1.2 This guide addresses instrument setup, hair collection, sample handling techniques, and the use of various microscopes in the examination and comparison of hair.

1.3 This guide addresses the benefit of following microscopical examinations with DNA analysis.

1.4 This standard is intended for use by competent forensic science practitioners with the requisite formal education, discipline-specific training (see Practice E2917), and demonstrated proficiency to perform forensic casework.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E620 Practice for Reporting Opinions of Scientific or Technical Experts

E1459 Guide for Physical Evidence Labeling and Related Documentation

E1492 Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Science Laboratory

E1732 Terminology Relating to Forensic Science

E2917 Practice for Forensic Science Practitioner Training, Continuing Education, and Professional Development Programs

2.2 *Other Standards:*

ISO 17025 Testing and calibration laboratories[3]

3. Terminology

3.1 *Definitions*—For definitions of terms used in this guide, refer to Terminology E1732.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *anagen, n*—the active growth phase of a hair follicle in the hair growth cycle.

3.2.1.1 *Discussion*—The root from a pulled anagen hair is elongated and is usually fully pigmented (**1**).[4]

3.2.2 *ancestral group, n*—a biogeographic designation of human populations (for example, Asian, African, European) whose hair can share similar morphological and microscopic traits.

3.2.2.1 *Discussion*—The racial terms Caucasoid, Mongoloid, and Negroid should not be used as these terms are no longer acceptable in the field of anthropology (the field from which these designations originated) (**2**).

3.2.3 *association, inclusion, n*—the result of a comparison between two hair samples in which the characteristics of the questioned hair are present in the known sample without any exclusionary differences, and therefore the donor of the known hair sample can be included as a possible source of the questioned hair.

3.2.3.1 *Discussion*—A microscopical association of hair cannot identify the definitive source of a questioned hair to the exclusion of all others, and the number of individuals who could be included as a possible donor of a specific hair is unknown and cannot be reliably estimated.

3.2.4 *buckling, n*—an abrupt change in the shape and orientation of a hair shaft with or without a slight twist.

[1] This test method is under the jurisdiction of ASTM Committee E30 on Forensic Sciences and is the direct responsibility of Subcommittee E30.01 on Criminalistics.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

[4] The boldface numbers in parentheses refer to the list of references at the end of this standard.

3.2.5 *catagen, n*—the transitional phase of the hair follicle between the active growth phase (anagen) and the resting phase (telogen) in the hair growth cycle (**1**).

3.2.6 *classification, n*—the systematic arrangement of hairs into categories (for example, human, animal, somatic origin, ancestry) based on shared traits.

3.2.7 *cortex, n*—the primary anatomical region of a hair between the cuticle region and the medullary region composed of elongated and fusiform cells.

3.2.8 *cortical fusi, n*—small air spaces that form between the cortical cells in the hair shaft and under transmitted light appear as tiny, dark structures.

3.2.9 *cortical texture, n*—the relief or definition of the margins of the cortical cells when viewed using transmitted light microscopy.

3.2.10 *cross-sectional shape, n*—the shape of a hair shaft when cut at a right angle to its longitudinal axis.

3.2.10.1 *Discussion*—When viewed longitudinally with transparent light, the apparent cross-sectional shape is determined by slowly focusing through the hair (optical cross-sectioning). When viewed longitudinally between crossed polars, the cross-sectional shape can be determined by observing the interference colors.

3.2.11 *cuticle, n*—the outermost region of a hair composed of layers of overlapping scales.

3.2.11.1 *Discussion*—The dimension of the cuticle as measured from its outer margin to the cortex is often described in relative terms (for example, thin, medium, thick).

3.2.12 *cuticle, cracked, n*—a cuticle with linear breaks that are perpendicular to the length of the shaft.

3.2.13 *cuticle, looped, n*—a feature in which the distal edges of the cuticular scales are curved away from or cupped toward the hair shaft.

3.2.14 *cuticle, serrated, n*—a cuticle in which the outer margin has a notched appearance like a saw blade.

3.2.15 *decompositional changes, n*—alteration in the root or the proximal end of a hair that can include discoloration, postmortem root banding, or a tapered or brush-like appearance as well as fungal tunneling along the length of the shaft.

3.2.16 *exclusion/elimination, n*—the result of a comparison between two hair samples in which exclusionary differences are observed in the characteristics of the questioned hair that are not present in the known hair sample, and therefore the donor of the known sample can be excluded as a possible source of the questioned hair.

3.2.16.1 *Discussion*—This result is reached in a comparative hair examination when exclusionary differences (for example, color, characteristics indicative of ancestry) are noted in the macroscopic or microscopic characteristics between the questioned and known hairs. In these circumstances, the source of the known hairs, as represented by the known sample, is eliminated as a possible source of the questioned hair.

3.2.17 *exclusion with limitations, n*—the result of a comparison between two hair samples in which the characteristics of the questioned hair differ from those present in the known hair sample, and therefore the donor of the known sample cannot be included as a possible source of the questioned hair.

3.2.17.1 *Discussion*—This result is reached in a comparative hair examination when differences are noted in the macroscopic or microscopic characteristics between the questioned and known hairs; however, the differences are insufficient for an absolute exclusion of a person as a possible source. This could be due to the natural variation that occurs in hairs as a biological specimen, the effect that time or environment can have upon a hair, or the reference sample does not capture the complete variation of the individual's hair.

3.2.18 *exclusionary difference, n*—a difference in a feature or property between compared items that is substantial enough to determine that they did not originate from the same source.

3.2.19 *follicular tag, n*—tissue from a hair follicle that is still attached to the root end of a hair which has been forcibly removed.

3.2.20 *fungal tunneling, n*—air pockets in a hair shaft caused by fungal growth.

3.2.21 *imbricate, n*—a term that describes a scale pattern in which the scales overlap and the edges have an irregular wavy pattern; this pattern is typical of human hair.

3.2.22 *inconclusive, n*—the result of a comparison between two hair samples in which similarities and differences were observed in the characteristics of the provided standard and the questioned hair to the extent that the known source of the standard could not be included or excluded as a possible source of the questioned hair.

3.2.23 *individualization, n*—a term indicating an individual can be discriminated to the exclusion of all other sources.

3.2.23.1 *Discussion*—Hairs cannot be individualized by means of microscopical hair comparison.

3.2.24 *inner cuticle margin, n*—the border between the cortex and the visible cuticle.

3.2.25 *keratin, n*—a class of sulfur-containing fibrous proteins that forms the foundation of outgrowth tissue from the epidermis, such as hair, nails, feathers, and horns (**1**).

3.2.26 *medulla, n*—the core of the hair shaft that is composed of vacuoles and cells that can be air- or fluid-filled.

3.2.26.1 *Discussion*—The medulla (if present) occurs in a continuous, discontinuous, or fragmented pattern along the length of a hair and appears translucent or opaque.

3.2.27 *monilethrix, n*—a hair disorder that results in periodic nodes or beading along the length of the hair with intervening, tapering constrictions that are not medullated.

3.2.28 *ovoid bodies, n*—oval-shaped, heavily-pigmented inclusions usually found in the hair cortex.

3.2.29 *pigment aggregation, n*—clusters of pigment granules.

3.2.30 *pigment density, n*—the relative abundance of pigment granules in the hair cortex as described along a continuum (for example, sparse, medium, heavy).

3.2.31 *pigment distribution, n*—the pattern or arrangement of the pigment granules in the hair shaft, such as uniform, peripheral, one-sided, variable, or central.

3.2.32 *pigment granules, n*—small particles in hair composed of melanin that impart color.

3.2.32.1 *Discussion*—Melanin is a natural pigment of which two forms, eumelanin (brown to black) and phaeomelanin (reddish brown to yellow), determine the color of human and animal hair.

3.2.33 *pili annulati, n*—a hair disorder causing hairs to appear ringed or banded due to the alternating light and dark bands in the hair shaft; the dark bands are a manifestation of abnormal air spaces in the cortex.

3.2.34 *pili torti, n*—a hair disorder characterized by the hair shaft being flattened and twisted 180 degrees numerous times along its axis; it is usually found at irregular intervals along the shaft.

3.2.35 *postmortem root banding, n*—the appearance of an opaque band near the root/proximal end of a hair potentially observed in anagen or catagen hairs that have been removed from a decomposing body; the possibility of other conditions causing the same or similar characteristics cannot be eliminated (**3**).

3.2.36 *root, n*—the structure that anchors a hair to a follicle and from which cells divide and produce the hair shaft.

3.2.36.1 *Discussion*—The portion of follicular tissue surrounding a root structure is the sheath.

3.2.37 *sample, known, n*—a sample for which the identity of the donor is established and which is used for comparison purposes.

3.2.38 *sample, limited, n*—a sample of known hairs that is insufficient in quality or quantity to reflect a representative range of characteristics or traits.

3.2.39 *sample, representative, n*—a collection of hairs from a specific somatic region that reflects the range of characteristics of a person's hair in that area.

3.2.40 *scales, n*—overlapping, plate-like structures composed of keratin that form the cuticle.

3.2.41 *shaft, n*—the portion of the hair emerging from the hair follicle.

3.2.42 *shaft form, n*—the shape of the hair both longitudinally (for example, curly, straight) and cross-sectionally (for example, round, flattened).

3.2.43 *shaft thickness, n*—the diameter of the hair.

3.2.43.1 *Discussion*—This is expressed either numerically or in relative terms, such as fine, medium, or coarse.

3.2.44 *shouldering, n*—a variation of the hair form along the shaft, resulting in an irregular and often asymmetrical change of cross-sectional shape.

3.2.45 *somatic region, n*—an area of the body, such as head, pubic, or leg; synonymous with "body area".

3.2.46 *telogen, n*—the resting phase of the hair follicle in the hair growth cycle.

3.2.46.1 *Discussion*—During this phase, the hair has stopped growing and the root becomes keratinized and bulbous (club-like) in shape (**1**).

3.2.47 *trichonodosis, n*—a condition characterized by apparent or actual knotting of the hair.

3.2.48 *trichoptilosis, n*—a condition characterized by longitudinal splitting or fraying of the hair shaft.

3.2.49 *trichorrhexis invaginata, n*—a genetic disease characterized by a segment of bulbous, dilated hair enfolded into a concave hair terminal, recalling the appearance of a bamboo node; if the hair breaks at the bulbous end, the hair has a "golf-tee" shaped end.

3.2.50 *trichorrhexis nodosa, n*—a condition characterized by the formation of nodes; the hair is weaker at the node and subject to breakage.

3.2.51 *trichoschisis, n*—a condition in which the hair readily breaks or splits along transverse cracks.

4. Significance and Use

4.1 A microscopical hair examination is conducted to determine if the item is a hair; from a human; from a particular somatic region; characteristic of a broad geographically-assigned ancestral group; characteristic of a particular growth phase; damaged; symptomatic of disease, condition, or disorder; forcibly removed; chemically altered (for example, dyed or bleached); suitable for microscopical comparison; suitable for DNA analysis; and similar to or different from a known sample (**4-9**).

4.2 Most often, hairs from the head and pubic regions of the body are used for microscopical comparisons. There is usually more interpersonal variability in the characteristics of head and pubic hairs than in the hairs from other somatic regions. Head hairs usually show more interpersonal variation than pubic hairs. Hairs from other somatic regions may also be compared, but these comparisons are usually limited and less frequently conducted. Accordingly, this guide primarily considers human head and pubic hair comparisons.

4.3 Microscopical hair comparisons are not a means of individualization (**10**). This limitation is to be stated in any communication (for example, reports, testimony) when an association is reported.

4.4 Additional analyses can be performed on hairs that have been chemically altered (for example, dyed hair) or have trace materials on the surface (for example, glitter). Such techniques are beyond the scope of this document.

5. Summary of Guide

5.1 This guide includes a summary of techniques for collecting hair samples, a description of the instrumentation used in the microscopical examination of hair, a description of the microscopical examination procedure, a section on interfacing with subsequent DNA analysis of hair, and examples of the types of microscopical hair examination results. Interpretation and significance will be addressed in more depth in a separate document for trace evidence.

6. Sample Collection

6.1 *Questioned Sample:*

6.1.1 Loose hairs are collected from an object by picking them off individually. Embedded hairs or hairs adhering to a

person or object are inspected prior to removal and the location from which these hairs are recovered is documented. Use caution when interpreting the significance of the location from which the hair was recovered since hairs could be redistributed on an item or among items that are packaged together. Take care not to contaminate, crush, or break the hairs.

6.1.2 Hairs are collected from clothing, bedding, or other large surfaces using adhesive lifts, scraping, or vacuuming. Be aware that the adhesive from the lifting material can interfere with the examination of surface treatments that might be present on the hairs. Vacuuming or adhesive lifting can be useful in collecting hairs from larger areas or specific areas such as carpets, vehicle seats, etc.

6.1.3 A new comb is used when retrieving evidence from a person's head or pubic region. Place a clean piece of paper, to catch loose hairs and debris, under the area to be combed and include the paper in the evidence package with the comb.

6.1.4 Since hairs can be very small, package them in a manner that prevents their loss (for example, in a paper fold, on a sticky note, on a gel lifter).

6.2 *Known Sample:*

6.2.1 Known hairs are collected from specific somatic regions of relevant people for comparison to questioned hairs. These hairs are collected as soon as possible relative to the occurrence of the crime. Hairs collected a period of time after the occurrence of the crime can appear different than they did at the time of the crime since hairs can change due to a variety of factors (for example, age, health, environment, cosmetic treatments).

6.2.2 Obtain at least 25 full-length hairs with roots for examination and comparison.

6.2.2.1 Because the majority of pulled hairs are in the anagen stage, a separate combing procedure is used to obtain hairs in the telogen stage (**7**, **11**). The regions being sampled are repeatedly combed or brushed over a large sheet of clean paper. The known pulled hairs and combed hairs should be packaged separately.

6.2.2.2 When applicable, pick, tape-lift with a low adhesive tape (for example, sticky-note), or comb for foreign hairs or other trace debris (for example, fibers, paint, glass) prior to combing for known hairs.

6.2.3 Different hairs from the same somatic region of a person can exhibit variation in microscopic characteristics and features. Therefore, it is important to obtain a sufficient number of hairs from the somatic region of interest in order to attempt to adequately represent the range of variation in all characteristics present. If the variation is large (for example, color, length), it becomes necessary to obtain more hairs.

6.2.3.1 A known head hair sample is collected from the five different areas of the head (top, front, back including nape, and both sides). Known head hairs are obtained by a combination of pulling and combing from the sampled region. In an attempt to obtain the full range of characteristics within an individual's head hair, it is recommended to sample at least 25 hairs from the head (**7**, **11**).

6.2.3.2 A known pubic hair sample or a sample from any other somatic region is recommended to consist of at least 25 hairs obtained by a combination of pulling and combing from the sampled region.

6.2.4 A comparison can still be performed with less than the recommended number of hairs, but this can increase the chance of a false exclusion.

7. Microscopes

7.1 *Stereomicroscope:*

7.1.1 Because of the increased working distance and ability to observe a sample in three dimensions, a stereomicroscope with a magnification range of 10× to 60× or greater is used for the initial examination of unmounted and mounted hairs.

7.2 *Transmitted Light Microscope:*

7.2.1 It is necessary to examine the microscopic characteristics of hairs with a high-quality compound transmitted light microscope with a range of objectives permitting observations from approximately 40× to 400×. Quality objectives are important, but highly corrected plan apochromats are not necessary (**12**).

7.2.2 A polarized light microscope can enhance the ability to see certain features and determine the cross-sectional shapes of the hairs.

7.2.3 Equip the microscope with a high-intensity light source, suitable for photomicrography, and a daylight correction filter (if necessary).

7.3 *Comparison Microscope:*

7.3.1 A comparison microscope, consisting of two transmitted light microscopes joined by an optical bridge, is used for microscopical hair comparisons.

7.3.2 Balance both sides of a comparison microscope for light intensity and color and check the magnification.

7.4 *Microscope Maintenance:*

7.4.1 Be familiar with the instruction manual and the manufacturer's maintenance recommendations for each microscope used in hair examination.

7.4.2 The following maintenance procedures are performed on microscopes routinely used to ensure their precision, reliability, and performance:

7.4.2.1 Dust, oil, and dirt is cleaned from the optics in accordance with the manufacturer's recommendations.

7.4.2.2 The external surfaces are cleaned.

7.4.2.3 The optical alignment is checked and realigned, if necessary, to establish Köhler or modified Köhler illumination.

7.4.2.4 When not in use, cover with a dust cover.

7.4.2.5 If the microscope cannot be cleaned or aligned, discontinue use until the microscope is repaired.

7.4.2.6 All service and repairs are recorded in a log; however, routine cleaning and aligning of the microscope need not be recorded in the log.

7.5 *Calibration of the Eyepiece Reticle:*

7.5.1 If measurements are to be made, a microscope with an eyepiece reticle calibrated to a stage micrometer is used. The steps for calibrating the eyepiece reticle with a stage micrometer are listed below.

7.5.1.1 Place a stage micrometer with a linear scale of known dimensional divisions on the stage of the microscope.

7.5.1.2 Focus on the dividing lines of the stage micrometer.

7.5.1.3 Align the scale in the eyepiece reticle with the scale on the micrometer.

7.5.1.4 Determine the number of reticle divisions that equal a defined increment of the stage micrometer.

7.5.1.5 Divide the number of stage micrometer divisions (in microns) by the number of ocular micrometer divisions to obtain microns per ocular division.

7.5.1.6 Repeat this procedure for each objective used.

7.6 *Magnification Check:*

7.6.1 The magnification of the comparison microscope is checked using a known reference material to ensure uniform magnification between the left and right fields of view. If the magnification is not the same, request matching objectives from the manufacturer.

7.7 *Color Balance:*

7.7.1 The color balance of the comparison microscope is checked to ensure uniform color between left and right fields of view. If the color balance is not acceptable, discontinue use and correct the problem. The color balance can be checked by the following procedure:

7.7.1.1 Cut a uniformly-colored semi-transparent sample (that is, fibers) in half and mount on two separate slides.

7.7.1.2 Place one slide on the left stage and the other slide on the right stage of the comparison microscope.

7.7.1.3 Compare the color of the images.

7.7.1.4 If the color is balanced, the sample images and the background color on both sides will appear to be the same.

7.7.1.5 If the color is not balanced, correct the problem or contact the microscope manufacturer for instructions on how to balance the microscope for color.

8. Evidence Handling and Sample Preparation

8.1 *Evidence Handling:*

8.1.1 Evidence is handled in accordance with ASTM standards (Practice E1492, Guide E1459) and any other appropriate accreditation standards.

8.1.2 Be cognizant of potential biasing effects resulting from task-irrelevant information and make efforts to minimize the availability of such information (**2**, **13**, **14**).

8.1.3 Blood or debris on a hair sample can be of evidential value. Consult with technically trained personnel (for example, DNA examiner) to determine the value and appropriate technique for the collection and preservation of biological material for future examination. When the adhering material is determined not to be of evidential value, the hair is washed or cleaned prior to mounting. A small amount of blood or debris on a hair does not interfere with the microscopical examination. Washed hairs should be dried prior to mounting.

8.1.4 Hair exhibiting thermal or mechanical damage is more brittle and is to be handled minimally and with care.

8.2 *Preliminary Examination:*

8.2.1 Macroscopical and stereomicroscopical examinations are useful for observing hair characteristics such as color, length, shape, and texture, as well as possible adhering debris. Generally, stereomicroscopy is used to initially assess the suitability of hairs for comparison, determine the presence of other trace materials, and evaluate which hairs have roots suitable for nuclear DNA analysis (**5**, **6**).

8.2.2 Prior to mounting, some internal properties of hairs can also be examined using a transmitted-light stereomicroscope.

8.3 *Mounting:*

8.3.1 A colorless, non-yellowing, non-reactive mounting medium with a refractive index close to that of a hair (1.54) is used to view hairs in transmitted light (**5**, **8**, **15**). The examination of surface particulates and biological material, compatibility with DNA analysis, ease of artifact isolation, and storage needs will determine the mounting medium selected.

8.3.1.1 Hairs that are to be compared are mounted in the same type of mounting medium.

8.3.2 One hair or multiple hairs from the same source are mounted on a glass microscope slide with a cover slip so that each mounted hair is clearly visible. Each slide is labeled as to the source of the hair(s).

9. Hair Characteristics and Other Determinations

9.1 *Human or Other Animal Origin:*

9.1.1 Human hair can typically be distinguished from other animal hair by examining the following features: scale pattern, shaft form, medulla width, medulla appearance/form, pigment distribution, color banding, and root shape (**5**, **6**, **16**).

9.2 *Somatic Origin:*

9.2.1 Somatic origin types include head, pubic, facial, limb, trunk, and eye hairs. The somatic origin of human hair can usually be established by considering features such as length, hair diameter, cross-sectional shape, shaft form, medullary morphology, cortical texture, appearance of the distal end, and appearance of the root (**5**, **11**).

9.3 *Characteristics of Ancestry:*

9.3.1 Features used to classify a hair as having characteristics common among particular ancestral groups (for example, European, Asian, African) are cross-sectional shape, color, pigment aggregation, pigment distribution, hair diameter, and cuticle thickness (**2**, **5**, **11**).

9.3.2 There is a possibility of mixed ancestral characteristics and atypical features.

9.3.3 Classifications of ancestry are based on characteristics that are often observed in hairs from individuals of specific ancestral groups and do not always correspond with outward appearance or how an individual identifies his or her ancestry.

9.4 *Human Hair Characteristics:*

9.4.1 The following is a non-comprehensive list of characteristics used for the classification and comparison of hairs.

9.4.2 Macroscopic characteristics observed in hairs include the following:

9.4.2.1 *Color (in reflected light)*—white, blonde, red, brown, black, etc.

9.4.2.2 *Structure*—shaft form (straight, arced, wavy, curly, twisted, tightly-coiled, crimped, buckling, shouldering, undulating); shaft length range; overall shaft thickness (fine, medium, coarse); etc.