

# Guidelines for Postmortem Protocol for Ocular Investigation of Sudden Unexplained Infant Death and Suspected Physical Child Abuse

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**Abstract:** Postmortem examination is a cornerstone in identifying the cause of unexplained sudden death in children. Even in cases of suspected or known abuse, an autopsy may help characterize the nature of the abuse, which is particularly important in the forensic autopsy of children in the first 3 to 4 years of life when inflicted neurotrauma is most common. Forensic examinations are vital in cases that might otherwise be diagnosed as sudden infant death syndrome. The ocular autopsy in particular may demonstrate findings that were not appreciated on antemortem clinical examination. This protocol for postmortem examination of the eyes and orbits was developed to promote more consistent documentation of findings, improved clinical and forensic decision making, and more replicable and coherent research outcomes.

**Key Words:** postmortem, autopsy, child abuse, shaken baby syndrome, retinal hemorrhage, orbit

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Postmortem examination is a cornerstone in identifying the cause of unexplained sudden death in children. Even in cases of suspected or known abuse, an autopsy may help characterize the nature of the abuse, which is particularly important in the forensic autopsy of children in the first 3 to 4 years of life when inflicted neurotrauma is most common. Forensic examinations are vital in cases that might otherwise be diagnosed as sudden infant death syndrome (SIDS).

The ocular autopsy may demonstrate findings that were not appreciated on antemortem clinical examination for various reasons. Sometimes, the premortem examination is not performed by an ophthalmologist; in other instances, although the examiner was an ophthalmologist, funduscopy can be limited because of the absence of pharmacologic dilation of the pupils needed to visualize the peripheral retinal edges (ora serrata). This circumstance usually occurs in comatose children where the pupillary reaction is being used as indicator of intracranial pressure. Other reasons for the premortem failure to appreciate retinal pathology include opacities of the ocular media, such as a vitreous hemorrhage, which may obstruct the view, and the situation in which some children die days, weeks, months, or years after the abusive incident. In such cases, a timely ophthalmology examination of the live child at the initial presentation may prove more useful than later autopsy findings in which deterioration or healing obscures the initial injury pathology. Yet, late autopsy according to this protocol may still be useful. In all fatal cases, the autopsy findings should be correlated with the ophthalmology examination in vivo.

In contrast to what may be commonly perceived, postmortem removal of the eyes and orbital contents is not disfiguring as caps are placed under the lids and the orbit filled with gauze or other material in preparation for viewing

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and burial. Embalmers routinely engage in these procedures, and there are existing ophthalmic pathologic protocols to ensure "postmortem cosmesis" after larger ophthalmic and facial autopsy excisions.

To achieve the optimal yield of useful information, it is important that the autopsy is conducted in a goal-oriented fashion with regard to the pathology known to occur in the specific conditions within the differential diagnosis. Elucidation of the ocular findings of inflicted childhood neurotrauma has occurred over the last 30 years since Caffey's first description of what he called "the whiplash shaken infant syndrome."<sup>1,2</sup> Over the ensuing decades, evidence has accumulated to support the concept that repetitive violent acceleration-deceleration forces, with or without blunt head trauma, in the first few years of life result in a characteristic pattern of intracranial, skeletal, and ocular injury that we will refer to herein as the shaken baby syndrome (SBS). The reader is referred elsewhere for reviews and additional information beyond the scope of this article.<sup>3-11</sup>

Kiffney described retinal detachment with bilateral subretinal hemorrhage in 1964.<sup>12</sup> Preretinal and intraretinal hemorrhage, engorgement of retinal veins, and papilledema were reported in 1967 by Gilkes.<sup>13</sup> Mushin showed intraretinal, subhyaloid, and small vitreous hemorrhages in 1971.<sup>14</sup> Harcourt and Hopkins described intraocular hemorrhages as well as the ocular sequelae of such hemorrhages in 1971.<sup>15</sup> Caffey's articles in 1972<sup>1</sup> and 1974<sup>2</sup> mention ocular hemorrhages. Two fatal cases described in his 1974 article had autopsies, but only one had a postmortem ocular examination that revealed "bleeding in the fibrous layer and scattered hemorrhages in the inner nuclear layer."<sup>2</sup> As more clinicians and pathologists became aware of the importance of ophthalmic manifestations in child abuse, additional observations were presented including Purtscher retinopathy<sup>16</sup>; injuries simulating congenital glaucoma<sup>17</sup>; retinal detachment<sup>18</sup>; white centered retinal hemorrhages<sup>19</sup>; and paramacular retinal folds.<sup>20,21</sup> Hemorrhagic macular retinoschisis, first described by Greenwald and coworkers in 1986<sup>22</sup> has only been reported because of inflicted childhood neurotrauma with repetitive acceleration-deceleration forces (SBS) in children younger than 5 years of age<sup>7</sup> except for a single case report of a child victim of alleged head crush injury.<sup>23</sup>

The incidence of retinal hemorrhages due to inflicted childhood neurotrauma, in particular SBS, ranges from 49% to 100% with the higher prevalence reported from autopsy series.<sup>7,24-29</sup> The hemorrhages may involve any or all of the retinal layers although preretinal and intraretinal hemorrhage, especially in the most superficial layers, is more common than vitreous and subretinal hemorrhage.<sup>21,30-32</sup> Nevertheless, subretinal hemorrhage is surprisingly common in some studies and may result in disruption of the overlying retinal pigmented epithelium (RPE).<sup>28,31</sup> Subinternal limiting membrane (ILM) blood may occur with or without macular folds.<sup>7,22,25,28,29</sup> Vitreous hemorrhage may be due to blood that has broken through the ILM from a traumatic retinoschisis.<sup>7,30</sup> Although difficult to identify in the living child,

choroidal hemorrhage or engorgement has been observed in 30% to 50% of eyes of victims of SBS at autopsy.<sup>28,30</sup>

Several series of postmortem ocular examination in victims of abuse have been reported. Rao and coworkers presented the ocular pathology of 14 fatally abused children in 1988.<sup>32</sup> These eyes were compared with 16 nonabused controls that had no ocular hemorrhages except for a single patient with traumatic head injury. Seventy-seven pairs of eyes, including the 14 described by Rao were studied by Riffenburgh and Sathyavagiswaran in 1991.<sup>29,33</sup> They found that retinal hemorrhage is the most common form of eye damage in child abuse and is more likely to occur in infants who have been shaken than after blunt trauma to the head. Accordingly, retinal involvement is seen more frequently in younger children. Munger and coworkers in 1993<sup>28</sup> reported neuropathologic and ophthalmologic observations of 12 abused children. Hemorrhages in the superficial retinal layers and subretinal space predominated. Retinal hemorrhages were found in all 12 cases, and intracranial hemorrhage was found in all but one case. Cerebral edema occurred in 10 cases. The intraretinal and periretinal hemorrhages were most prevalent at the posterior pole. Five cases had retinal folds. There was a low incidence of optic disc edema and choroidal hemorrhage. An autopsy study by Budenz and coworkers highlighted the importance of ocular and optic nerve hemorrhages in 13 abused infants with intracranial injury.<sup>31</sup> A prospective postmortem study reported in 1994 examined the eyes of 169 children, both abused and nonabused.<sup>26</sup> Retinal hemorrhages were found much more frequently in inflicted head injury than accidental injury or natural disease. Most other causes of retinal hemorrhages in this age group such as leukemia and coagulation abnormalities can be readily recognized on clinical retinal examination and diagnostic investigations. In addition, retinal hemorrhages in these diseases are less extensive, without schisis or retinal folds. Randomly oriented retinal folds or a circumferential fold at the ora may be fixation artifact, whereas a circumlinear paramacular fold (often with vitreous adherent to the apex) is most commonly a sign of SBS.

Although the first descriptions of the Battered Child syndrome and SBS were published decades ago, there is still confusion of terminology and reference standards. The term "retinal hemorrhages" for example is often used in the medical literature as a generic term without specification of the hemorrhage type, severity or pattern of distribution.<sup>7</sup> Even though hemorrhages most often involve the posterior pole, extension to the ora serrata may be of particular significance.<sup>7,24,28,30</sup> Before sectioning of the eye for histology and after the eyes have been fixed and sectioned it is advantageous to photograph and document the gross distribution pattern of the hemorrhages.<sup>7,34</sup> One group proposed a grading scheme based on microscope grids<sup>24</sup> which may be useful in quantifying the amount of hemorrhage in a given histologic preparation.

Failure to describe retinal hemorrhage in detail may lead to statements claiming that a particular condition "can result in retinal hemorrhages" even though the hemorrhages

may look dramatically different from the ones seen in inflicted childhood neurotrauma with repeated acceleration-deceleration forces with or without impact (SBS). The description of ocular findings in the published literature was often vague or absent as was the case even in Kempe's 1962 landmark article defining the Battered Child syndrome.<sup>35</sup> One child suffered from "a defect in vision." No description of the ocular injuries was given. Accurate, uniform descriptions of postmortem ocular findings could be helpful in resolving such uncertainties. This will be facilitated by standardization of the forensic postmortem ocular protocol, as has been proposed in the United Kingdom for ocular necropsy in general<sup>36</sup> and the forensic autopsy.<sup>37</sup>

Appreciation of the importance of posterior optic nerve and orbital findings in shaken babies has been a more recent development. In 1986, Lambert and coworkers were the first to describe optic nerve sheath hemorrhages which were subarachnoid and subdural.<sup>38</sup> Optic nerve sheath hemorrhage occurs in 65% to 100% of victims.<sup>25,26,28,31,32</sup> Elner and coworkers<sup>25</sup> examined the eyes of 10 abused children. This was the first report describing intrascleral hemorrhage at the optic nerve-globe junction. Other authors have suggested that intrascleral hemorrhage in SBS may be caused by acceleration-deceleration trauma which results in tears of the intrascleral ciliary vessels forming the circle of Zinn-Haller.<sup>25-26,32,39,40</sup> Immunohistochemical staining studies of postmortem specimens from abused children with markers such as beta-amyloid precursor protein also identify this site as a prime area for optic nerve damage.<sup>41,42</sup> There seems to be a positive correlation between the finding of retinal hemorrhages, intrascleral hemorrhage at the optic nerve-sclera junction and retrobulbar optic nerve sheath hemorrhage.<sup>26,31</sup> The most common site for optic nerve sheath hemorrhage is the immediate retrobulbar portion of the optic nerve,<sup>9</sup> with most hemorrhage located in the subdural space.<sup>28,31</sup> Blood can be found in the orbital optic nerve sheath despite the absence of blood in the canalicular portion of the optic nerve sheath.<sup>26,31,32,38</sup>

Other reported orbital findings include the presence of intradural hemorrhage (within the dural tissue proper) of the optic nerve sheath, and hemorrhage into the orbital fat, and sheaths of the orbital portion of cranial nerves and extraocular muscles.<sup>7,28,31</sup> Most recently, Marshall and coworkers presented a series of postmortem ocular examinations which underscores the value of evaluation of abnormalities in the orbit as well as the globe.<sup>43</sup> In a study of orbit pathology of abused and accidentally head injured children it was found that the combination of optic nerve sheath and orbital hemorrhage was significantly ( $P < 0.01$ ) more common in abusive head trauma.<sup>44</sup> In inflicted neurotrauma the hemorrhage was significantly more common in the subdural, subarachnoid, intradural and levels of the optic nerve sheath and occupied a larger portion along the length of the orbit. Hemorrhage was also observed significantly more often in the orbital fat, extraocular muscle sheaths, and cranial nerve sheaths. Orbital injury was observed in accidental trauma only when there was direct trauma to the orbit or severe

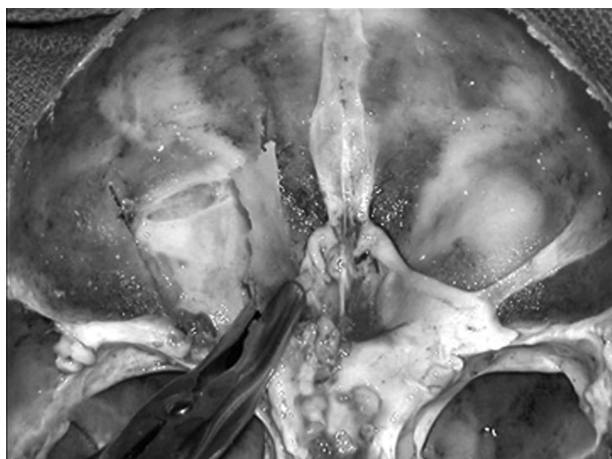
multiple repetitive acceleration-deceleration injury. When orbital hemorrhage did occur in accidentally injured children without direct orbital fracture, the amount of hemorrhage in the orbit and dura was minimal, being visible only histologically. Extraocular muscle hemorrhage occurred only in the abused children.

These guidelines incorporate a rigorous reproducible "best practice" protocol for postmortem ocular examination in sudden unexplained death in children younger than 5 years and in those where physical child abuse, in particular the SBS, is suspected. Future research, such as the possible use of staining for beta-amyloid precursor protein,<sup>41</sup> may demand additional investigations and revision of this protocol. The dating of hemorrhagic ocular injuries using hemosiderin assessment remains somewhat controversial and imprecise and is therefore not addressed here. The goal of this proposed protocol is to facilitate uniform description of postmortem ocular and orbital findings that can serve as a basis for scientific investigation and comparison, and help to ensure that all potentially useful findings are considered in arriving at a diagnosis.

## PROTOCOL

1. Review the enabling legislation in your jurisdiction for authority to remove eyes at autopsy.
    - 1.1. If child abuse is suspected, the law is likely to identify the authorizing authority. If SIDS is under consideration, is also very likely that the authorizing authority will be specified.
    - 1.2. If required, informed consent for removal, retention and ultimate disposal of eye tissues should be obtained from the custodial caretaker.
  2. Before removing the globes and orbits observe and document:
    - 2.1. the lids for evidence of abrasion, laceration or ecchymoses,
    - 2.2. the surface of the eye for any subconjunctival hemorrhage,
    - 2.3. the anterior chamber for hyphema or other evidence of trauma.
- Documentation should include diagrams and color photography of the eyelids, periorbital facial structures, conjunctiva, and visible portions of the globe (cornea, iris, and pupil). This should be part of full photographic documentation of the body, including all aspects of the head and neck.
3. After removal of the brain, remove the eyes using a combined anterior and posterior approach.
    - 3.1. Using small cartilage cutters or small scissors, remove the orbital roof completely (Fig. 1). Include the bone overlying the optic nerve canal so that the entire nerve can be removed intact to the point of the intracranial transection created during the removal of the brain. The orbital roof should peel away from the underlying periosteum easily. However, this separation may be more difficult over the





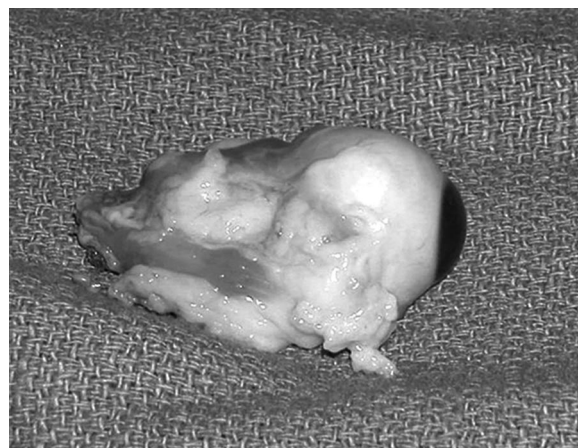
**FIGURE 1.** Using scissors or a rongeur (shown here) the roof of the left orbit is removed in pieces.

canal. The goal of this step is to leave the orbital cone of tissues en bloc with out disruption.

- 3.2. Using blunt dissection, separate the periosteum that encases the orbital contents from the lateral, medial and inferior orbit walls. Sharp dissection will only be required to cut the attachments of the periosteum to the inferior orbital fissure (Fig. 2), to disinsert the apical attachments of the extraocular muscles, to disinsert the inferior oblique muscle attachment to the anterior medial orbital wall, to transect the superior oblique muscle at the trochlea, to sever the apical entry points of the nerves and vessels entering the orbit, and to remove the intracanalicular optic nerve.
- 3.3. Insert an eyelid speculum to separate the lids. Be sure not to allow the eyelids to evert.



**FIGURE 2.** As the left orbit and globe are lifted from the orbit en bloc the attachments of the periosteum to the inferior orbital fissure are identified and cut with a scalpel.



**FIGURE 3.** The final en bloc specimen of globe and orbital contents.

- 3.4. Approaching the globe from the front, use small scissors and forceps to cut through the conjunctiva for 360 degrees posterior to the outside edge of the cornea (limbus). Use care to avoid damage to the lids.
- 3.5. Using the scissors, carefully blunt dissect around the globe between the fascia which underlies the conjunctiva (Tenon fascia) and the sclera. The tip of the scissors will then become visible from the superior intracranial view of the orbit as it exerts pressure on the periosteal cone from within. Using a scalpel or scissors, incise the periosteum over the tip of the scissors from the orbital side. Then extend this incision for 360 degrees taking care not to cut the lids from the posterior approach.
- 3.6. Remove the globe and orbital contents en bloc along with the intracanalicular optic nerve (Fig. 3).
4. Fix the en bloc ocular and orbital tissues in neutral buffered formalin. The volume of formalin should be at least 10 times that of the removed specimen. Adequate fixation takes 2 to 3 days. Longer fixation is not harmful, although the sclera becomes progressively harder and more difficult to section from processed tissue blocks.
5. Step section the entire en bloc specimen from the distal optic nerve to within 0.5 to 0.7 cm of the posterior aspect of the globe. Avoid disrupting the globe.
  - 5.1. Before sectioning, it may be helpful to transilluminate the globe through the cornea, taking care not to damage its surface, and note the position of any posterior chamber shadows (indicating extensive hemorrhages, tumors etc.). Retroilluminate the iris and pupil (fiber optic light on the posterior sclera) to demonstrate lens displacement, cataracts, and iris stromal defects.
  - 5.2. On gross inspection of the sections, document and color photograph hemorrhages, in particular hemorrhage inside and outside of the optic nerve sheath (if present).

- 5.3. After hematoxylin and eosin staining, histologic examination should in particular note the presence of hemorrhage in the orbital soft tissues, in the optic nerve subdural space, in the optic nerve subarachnoid space, within the optic nerve dura proper, inside the sheaths of cranial nerves and extraocular muscles, and within the extraocular muscle tissue.
  6. Cut the globe slowly with a new sharp 60 mm blade or razor blade while holding the globe firmly but not squeezing. Look for gross evidence of blood in the vitreous. There are 4 methods for sectioning the globe.
    - 6.1. Method #1: Alternative Section
      - 6.1.1. Note the position of the ora serrata by transillumination and mark it on the sclera with a pencil. The section will be just anterior to this line in the coronal plane through the pars plana to avoid detaching the retina.
      - 6.1.2. One calotte will be the anterior chamber, iris and lens. The posterior chamber including the entire retina will be in the other calotte.
    - 6.2. Method #2: Coronal Section
      - 6.2.1. Cut approximately at the equator of the globe in the coronal plane.
      - 6.2.2. One calotte will contain the anterior segment and the anterior aspects of the posterior chamber including the ora serrata. The remainder of the retina, including the macula, will be in the posterior calotte.
    - 6.3. Method #3: Pupil-Optic Nerve method (Axial Section)
      - 6.3.1. Place the cornea facing down. Start the section posteriorly, superior to the optic nerve stump in the axial plane (ie, parallel to the visual axis). The superior and inferior aspects are identified by the long posterior ciliary vessels, the superior oblique insertion and the inferior oblique insertion. More force may be needed to cut through the lens.
      - 6.3.2. The macula will be in the inferior calotte.
    - 6.4. Method #4: Pupil-Optic Nerve method (Sagittal Section)
      - 6.4.1. Place the cornea facing down. Start the section posteriorly, nasal to the optic nerve stump in the sagittal plane. Nasal (medial) and temporal (lateral) aspects can be identified by first identifying the superior-inferior orientation (see 6.3.1). More force may be needed to cut through the lens.
      - 6.4.2. The macula will be in the temporal calotte.
  7. Photograph the entire inner surface of the eye, including the lens and in particular the retina, in color with dissecting microscope (or equivalent) magnification. Placing the eye under 70% alcohol (ethanol) or normal saline will eliminate obstructing illumination highlights during photography. Ensure that your photographs are properly developed (if film) and in clear focus at high resolution before proceeding with further dissection. For this reason, digital photography is preferred.
- Ensure that all drawings, photographs and notes are carefully labeled and preserved as hard copy, at all stages of the procedure, to preserve evidence at forensic standard (some jurisdictions require specific "forensic evidence" procedures).
8. Document gross (macroscopic) retinal observations:
    - 8.1. The retina may have lifted from the choroid and may commonly have artifactual folds radiating from the optic disk or in random patterns, and circumferentially at the ora (Lange fold). The presence of blood or exudate under the retina indicates that the detachment is not an artifact.
    - 8.2. A complete or partial circular fold in the posterior pole, often with a crater-like appearance, is not artifact and raises the possibility of traumatic paramacular folds with or without traumatic retinoschisis due to vitreous traction as seen in SBS.
      - 8.2.1. Note presence of hemorrhage within a retinoschisis cavity
      - 8.2.2. Note if there is hypopigmentation underlying the folds.
    - 8.3. Count the number of hemorrhages if present and feasible.
    - 8.4. Describe the hemorrhage location and patterns of distribution of hemorrhages (eg, paravascular, peripapillary, posterior pole, macular (within vascular arcades), midperipheral beyond vascular arcades, peripheral (at ora serrata))
  9. Sections of globe for microscopic examination:
    - 9.1. Microscopic sections of the eye are usually taken in the pupil-optic nerve plane (see 6.3 and 6.4).
    - 9.2. The macula, and in particular the fovea, must always be examined.
    - 9.3. The optic nerve must always be included. A pupil-optic nerve section is necessary to adequately assess optic nerve sheath hemorrhage and edema as well as intrascleral hemorrhages at the junction of the optic nerve to the sclera.
    - 9.4. If only a few hemorrhages are present be sure one or more are included in the plane of section.
    - 9.5. If traumatic retinoschisis (eg, posterior pole circular folds) is suspected, the lesion(s) should be included in the sections.
    - 9.6. Sections of the anterior chamber are also required and will be included automatically if a pupil-optic nerve method is used. If another method of globe division is used (see 6.1 and 6.2) then separate sections of the calotte containing the anterior chamber are necessary.
  10. Histologic Examination
    - 10.1. Stain with hematoxylin and eosin. Other stains (eg, Prussian Blue for iron in hemosiderin) are often useful indicators, and may be performed for

purposes of exclusion even when hemorrhages are not seen macroscopically or microscopically.

- 10.2. Artfactual separation of the sensory retina from the RPE is common and recognizable by the absence of hemorrhage, infiltration or fluid in the intervening space between the sensory retina and RPE, and by adherence of RPE pigment granules to the extreme outer edge of retinal photoreceptors. Artfactual and autolytic separation of layers of retina may also occur. If present, describe any fluid or debris under the retina (or in intraretinal splits) as this indicates a true retinal detachment or schisis.
- 10.3. Describe retinal hemorrhages using the terms pre-retinal, subretinal, and intraretinal (noting layers of the retina affected).
- 10.4. Describe retinal folds noting if the vitreous is still attached to the apex of the fold and whether underlying retinal pigment epithelium is disrupted.
- 10.5. Describe the contents of any suspected retinoschisis cavity and the status of the underlying retina. Remember that the fovea should be the thinnest part of the retina. If it is not, suspect shearing/schisis.
- 10.6. Describe hemorrhages at the periphery of the retina.
- 10.7. Carefully examine the optic nerve for hemorrhage or edema in its parenchyma, or sheath. Also look for intrascleral hemorrhage at the optic nerve-scleral junction.
- 10.8. When examining the anterior chamber, look for hemorrhage or damage to the iridocorneal angle (recession) indicating blunt trauma.
- 10.9. Describe and photograph all abnormalities.
- 10.10. The above is in addition to a standard detailed description of all of the structures of the eye.
11. Report your findings including all details mentioned above with pertinent positives and pertinent negatives. If your findings are suspicious for child abuse, be sure to notify referring physicians and ensure that they (or you) make a child abuse report, where required by law, if this has not yet been done.

## CONCLUSION

This protocol is offered as a vehicle for standardization, in the hope that it will result in more consistent documentation of findings, improved clinical and forensic decision making, and more replicable and coherent research outcomes. We depart from the autopsy protocol of many centers by adding orbital tissue removal and examination for which there is now evidence suggesting that important abnormalities may be found, in particular in those child victims of abusive head trauma characterized by repeated acceleration-deceleration forces.

Standardization and consistency of approach are important in the context of the examination of the eyes of infants where child abuse is a possible cause. Our guidelines are

intended as a suggested means of obtaining the important positive and negative ophthalmic pathologic data required for the investigation of possible child abuse. Following these guidelines will also ensure that the important primary pathologic data are preserved in good condition for subsequent examination by experts appointed by any appropriate party in an investigation.

As for the families involved, careful attention to the detail in this protocol will ensure that their child can be viewed after the autopsy procedure, and that all removed tissues are documented and stored appropriately, so that eventually they may, if desired by the family, be reunited with the body for appropriate disposal, as may be allowed under prevailing law.

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