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# The Diagnostic Value of the Diatom Test for Drowning, I. Utility: A Retrospective Analysis of 771 Cases of Drowning in Ontario, Canada

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**ABSTRACT:** The utility and validity of the diatom test for drowning was studied using a retrospective analysis of 771 cases of drowning mostly from Ontario, Canada, over the period 1977 to 1993. In this article (part one), the utility of the test was assessed using an analysis of test outcomes. In the companion article (part two), the validity of the test was assessed by analyzing the relationship between test outcome and characteristics of diatoms in the bone marrow and samples of putative drowning medium. In the present study, freshwater drownings accounted for 738 of the cases and 33 cases were drownings in bathtubs, pools, or toilets. Diatoms were recovered from the femoral bone marrow of 205 cases (28%) of freshwater drowning and four cases (12%) of domestic water drowning. There was a monthly variation in the frequency of positive test outcomes that could not be explained by seasonal differences in the total number of drownings. However, the monthly variation was strongly correlated with the periodic cycle of diatom blooms that occurs in freshwater. Positive diatom tests were characterized by a limited number of distinctive diatom species per case, and a restricted quantity and size range of diatom frustules. These results indicate that the diatom test for drowning will identify approximately one in three victims of freshwater drowning and may be useful in the assessment of deaths occurring in bathtubs. The correlation of the outcome of the diatom test for drowning with diatom blooms provides further evidence for the reliability of the test.

**KEYWORDS:** forensic science, forensic pathology, diatoms, seasonal variation drowning, Ontario, Canada

One of the most controversial issues in forensic pathology is the validity and utility of the diatom test for drowning. Although this diagnostic test for drowning was first implemented in the early 1900s and subsequently investigated by various European scientists, the test has not been extensively applied outside of Britain and continental Europe (for reviews see (1–6)). The restricted use of the diatom test for drowning is largely due to disagreement concerning the reliability of the test and the possibility of false positives thus limiting applicability. Although the literature on the diatom test for drowning contains authoritative contributions of investigators both for (2,5–10) and against (1,11–13) the test, no studies have provided a quantitative analysis on the outcomes of the test in a large series.

The diatom test for drowning is based on the postulate that when diatom-laden water is inhaled in the course of drowning, a subset of the diatoms in the drowning medium enter the circulatory system and embolize to internal organs. If diatoms can be recovered from the tissues of a body found in water this is, *a priori*, evidence of drowning. The postmortem extraction and recovery of diatoms is possible because the silica-based extracellular coat of the diatom (the frustule) is resistant to acid digestion. Therefore, samples of organs can be digested using concentrated acid and the putative diatoms extracted from the digested suspensions using centrifugation. Although some investigators have used portions of lung and other tissues for the diatom test (4,6,7,11,12), marrow extracted from an intact long bone is preferred because diatoms present in this site can be interpreted to have become enlodged in the marrow cavity antemortem. The same is not true for diatoms recovered from lung tissue because postmortem transfer of water into the lung may cause postmortem contamination with diatoms. Among the bones used for the test (femur, sternum, and vertebra), the femur has been the most widely utilized (6,9–11,14).

The opponents of the diatom test for drowning have two main criticisms: That diatoms cannot be recovered from all cases of drowning, and that diatoms can be recovered from the organs of nondrowned individuals. Despite these criticisms, quantitative outcome rates for the diatom test has not been reported for a large series of cases. In addition, although investigators have reported the recovery of diatoms from the organs of nondrowned individuals, the possibility of laboratory contamination was not excluded. On this basis, in part one of this study, we report the analysis of the outcome of the diatom test for drowning in 771 cases of presumed drowning mostly from Ontario, Canada with specific reference to positive and negative outcome rates of the test, the possibility of laboratory contamination, and the characteristic features of a positive test.

We also assessed the reliability of the diatom test for drowning using information about the population dynamics of diatoms. Because there is a well described seasonal variation in diatom content of lakes, rivers, and ponds, we analyzed the incidence of positive diatom tests by the season and month of drowning. It is well known that diatom blooms follow a pattern seasonal periodicity (15–17). In early spring (April), a massive diatom bloom occurs in freshwater. This is followed by a decline in the live diatom population but a persistence of a high levels of dead diatoms in the water in the summer. In the autumn, another diatom bloom occurs and there is a progressive decline in the diatom population

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in the winter months until the next spring bloom. Therefore, diatom population dynamics suggest that the monthly incidence of positive test outcomes should correlate with the cyclic variation in the diatom population. In part two of this investigation, the diatoms present in bone marrow and samples of putative drowning medium are compared, and the relationship between test outcome and diatom content of the putative drowning medium is analyzed.

## Materials and Methods

### *Retrospective Review of Cases*

All cases ($n = 771$) processed for the diatom test for drowning at the Office of the Chief Coroner for Ontario between 1977–1993 were used for this retrospective study. Most cases were presumed drownings recovered from bodies of water in Ontario, Canada. The outcome of the diatom test for drowning was correlated with the site, season, and month of drowning. In addition, we ascertained the potential contribution of laboratory contamination leading to false positives. For this determination, the species of diatoms recovered from 149 consecutive cases of freshwater drowning between 1982–1984 were compared to determine if cross-contamination in the laboratory (e.g., reagents or bandsaw) could contribute to false positive diatom tests.

### *Laboratory Procedure for Diatom Test*

Bone marrow from intact femurs, or rarely, an intact kidney was used for the extraction of diatoms using the method of Timperman with minor modifications (6). Briefly, intact femurs were removed at autopsy, and washed in two changes of distilled water. Femurs were longitudinally sectioned using a band saw, and the bone marrow (~50 g) removed using a clean spatula and placed into a boiling flask. Approximately 50 mL of concentrated analytical grade nitric acid was added and the marrow-acid suspension was simmered on a hot plate for approximately 48 h in a fume hood. The suspension was then cooled to room temperature and centrifuged (250 to 500 g, 20 to 30 min) using a table top centrifuge. The supernatant was discarded and the pellet resuspended in distilled water and recentrifuged. This washing procedure was repeated at least once. After the final supernatant was discarded, the pellet containing nitric acid-resistant material was aspirated using a Pasteur pipette and dropped onto a clean microscope slide. The residue was air dried and mounted using standard mounting medium. Slides were examined using phase contrast microscopy. The diatom test was classed as positive when unequivocal diatom frustules was present on the slide. Positive tests usually yielded less than six individual diatom frustules or conspicuous fragments. All equipment (saw, spatula, and flasks) were washed in diatom-free laboratory grade detergent and exhaustively rinsed in distilled water. All reagents used for the preparation of acid digests of bone marrow were periodically tested to exclude the possibility of contamination with exogenous diatoms.

## Results

### *Analysis of Outcome*

Most of the 771 cases of drowning in Ontario used for this study occurred during the summer, late spring, and early autumn (Fig. 1 and Table 1) correlating with the increased use of naturally-occurring bodies of water. The diatom test for drowning was positive in 205 cases (28%) of presumed freshwater drowning but was rarely positive if drowning occurred in water from a domestic source that lacked diatoms due to water filtration and processing (Table 2). Only four cases (12%) of domestic drownings had a positive diatom test. Drowning medium was available for analysis in two of these cases, and diatoms were found in both cases. The diatoms were derived from an abrasive cleaning agent in one case and from gravel added into the water in the other case.

There was a conspicuous monthly variation in the incidence of a positive outcome of the diatom test (Fig. 2). The diatom test was most likely to be positive in April (~40%), July (~40%), and November (~30%), and least likely to be positive in the winter months. However, the pattern correlated with the well documented monthly variation in diatom population due to cyclic diatom blooms. The principal diatom blooms occur in early spring and autumn with a sharp decline in the live diatom population between

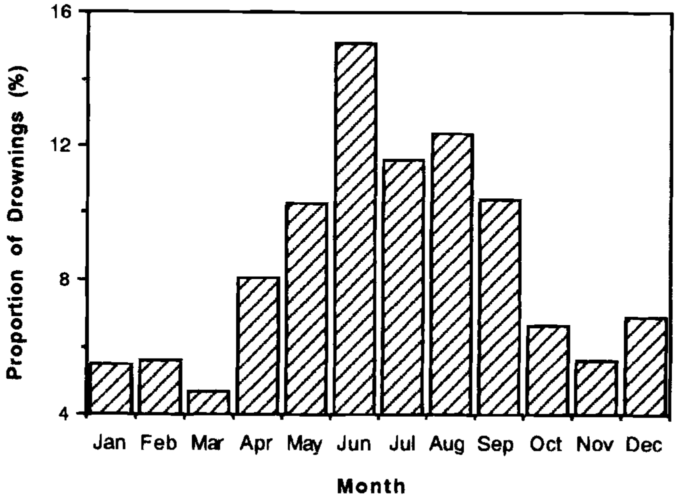


FIG. 1—*Relative proportion of drowning cases in various months for 771 cases of drowning in Ontario, Canada between 1977 and 1993.*

TABLE 1—*Outcome of the diatom test for drowning by season.*

| Season | Number of Drownings* | Number of Positive Tests† |
|---|---|---|
| Winter | 138/771 (18%) | 26/138 (19%) |
| Spring | 172/771 (22%) | 43/172 (25%) |
| Summer | 293/771 (38%) | 93/293 (32%) |
| Autumn | 168/771 (22%) | 43/168 (25%) |

*Indicates the number and proportion of drowning compared with the total series.

†Indicates the number and proportion of positive diatoms tests for all cases in that season.

TABLE 2—*Outcome of the diatom test for drowning by site of drowning.*

| Site of Drowning | Total | Outcome | |
|---|---|---|---|
| | | Positive | Negative |
| Naturally-occurring body of water* | 738 | 205 (28%) | 533 (72%) |
| Bathtub, pool, or toilet | 33 | 4 (12%) | 29 (88%) |
| All locations | 771 | 209 (27%) | 562 (73%) |

*Lake, river, ditch, or pond.

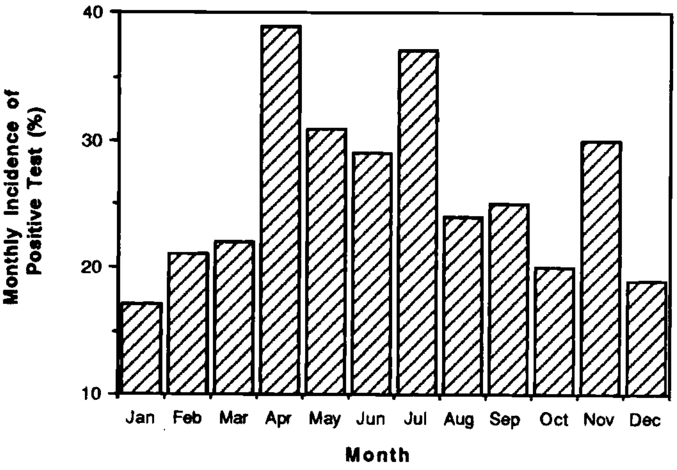


FIG. 2—*Monthly incidence of positive diatom tests in 771 cases of presumed drowning in Ontario, Canada between 1977 and 1993. Reported values are the proportion of drownings in a given month that had a positive diatom test.*

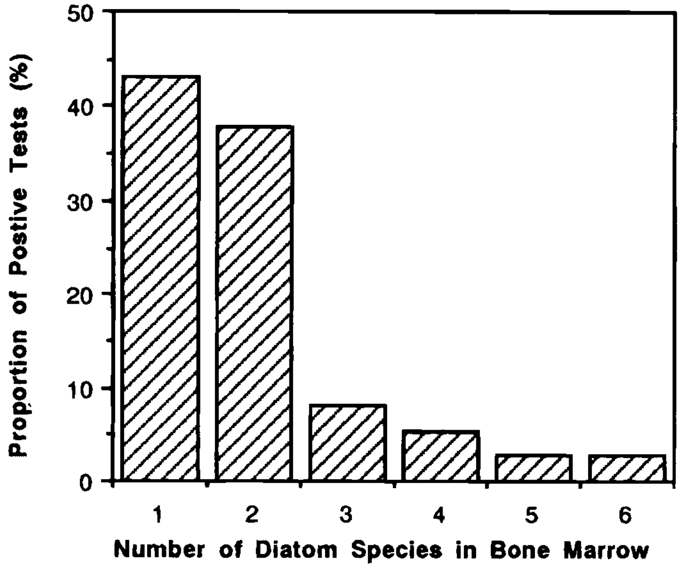


FIG. 3—*Multiplicity of diatom species recovered from bone marrow in 37 positive diatom tests in Ontario, Canada.*

the blooms. The high incidence of positive diatom tests in the summer months was presumably due to persistence of the insoluble frustules of the dead diatoms in water. Although the monthly variation of test incidence correlated with diatom blooms, a basal concentration of diatoms in freshwater must have been maintained because seasonal variations deviated by only 13% from Winter (19%) to Summer (32%) (Table 1).

### *Quality Control Analysis*

To determine if in-house cross-contamination of diatom tests resulted in a significant false positive rate, 149 consecutive diatom tests were reviewed from the period 1982–1984. During that period, 49 tests (33%) revealed diatoms in femoral bone marrow. Although many positive tests did not occur sequentially, 26 (53%) positive tests did occur directly after at least one positive test. Reassessment of these slides revealed that the combination of diatom species in the positive tests were distinctive in all but two of the sequential tests. In these two cases, the same diatom species was expected because both individuals drowned at the same time in the same body of water.

### *Characterization of Positive Tests*

Positive diatom test could be characterized on the basis of the number, size, and type of diatom frustules (Table 3). In most cases, no more than three distinctive species of diatom were found in any given sample (Fig. 3), and the individual diatoms or fragments of diatom frustules were <30 μm in maximal dimension. The total number of frustules or fragments rarely exceeded ten and were more typically less than six in number. In many cases, water from the putative drowning site was available for analysis and the diatom species matched those in the water sample (see companion paper).

TABLE 3—*Characteristics of diatoms extracted from bone marrow in drowning.*

| Feature | Common Pattern |
|---|---|
| Size | <30 μm |
| Types of species | <3 species |
| Number of Frustules | <10 |

## Discussion

The study was designed to assess quantitatively the utility of the diatom test for drowning and to determine if temporal variation in positive test outcomes correlated with the population cycle of diatoms in freshwater. The main findings of the study are that: (i) the presence of diatom frustules in the femoral bone marrow can be used to diagnose drowning in ~30% of freshwater drownings; and (ii) the monthly variation of test outcome correlated with diatom population dynamics.

The relationship of test outcome with diatom population dynamics is important corroborating evidence for the validity of the test because it is predictable that the diatom content of the water would influence test outcome. The correspondence of the high incidence of positive test outcomes with peak months of diatom blooms is consistent with the water concentration of diatoms being a key factor in test outcome. However, because diatom frustules persist is freshwater throughout the year, drownings in months with suboptimal water concentration of diatoms still provide occasional positive test outcomes, although at a lower overall rate. In addition, the data support the reliability of the diatom test for drowning because: (i) the high false negative rate suggests false positives are rare; (ii) domestic water drownings had negative diatom tests unless the water contained diatoms derived from some external source (e.g., abrasive cleansers); (iii) drowning in water with no or few diatoms (e.g., domestic water) gave negative test outcomes; and (iv) paired drownings in the same body of water had the same diatom species in the bone marrow. In addition, the size of diatoms extracted from the femoral bone marrow in cases of drowning was in keeping with embolism in the circulation. Evaluated together, these data provide evidence in support of the utility and reliability of the diatom test for drowning. The data suggest that a positive test outcome is useful in determining if drowning was the cause of death or a contributing factor in death. Conversely, a negative outcome of the test does not exclude drowning as a cause of death since many factors determine test outcome including the concentration of diatoms in the drowning medium.

Several important factors may explain why the diatom frustules are detected at a relatively low rate (28%) in the bone marrow of cases of drowning. One of technical factors include the possibility that not all diatoms present in a sample are extracted using the chemical digestion methods used in this study. In addition, several biological factors may significantly influence the outcome of the diatom test in individual cases. For example, the presence of atherosclerotic heart disease in some victims of drowning may contribute to a rapid death and therefore restrict the amount of water inhaled into the lungs, and secondarily the number of diatoms available for embolism to the bone marrow. In addition, because the femoral bone marrow receives a relatively small proportion of the circulating volume of blood, the number of diatoms that will be deposited in the marrow is comparatively small relative to large blood filtering organs such as the liver and kidneys. Despite these factors, the single most important variable in determining the outcome of the diatom test for drowning is the concentration of diatoms in the drowning medium (companion paper).

On the basis of our experience with the diatom test, there are four circumstances in which application of the diatom test for drowning can provide useful information: (i) confirmation of drowning; (ii) decomposed bodies recovered from water; (iii) bodies recovered from bathtubs; and (iv) selected suspicious deaths found on land with no definitive anatomic cause of death. In the latter category, we have recently used the diatom test to detect a case of presumed homicidal drowning in which the body had been set afire postmortem near a dumpster. In this case, fluid from the maxillary sinus (representative of the drowning medium) and the femoral bone marrow contained the same diatom species. We routinely apply the test in cases of bodies found in water in which decomposition obscures the characteristic postmortem features of drowning. In addition, a minimally explored area of application of the test is in the investigation of drowning in bathtubs. Although tap water contains a very low concentration of diatoms or no diatoms (18), bathtub drownings can give a positive test if diatoms have been added to the water. These diatoms are most frequently derived from residue in the bathtub due to previous application of abrasive cleansers containing diatoms. In our limited experience with such cases, the diatom frustules present in the bone marrow are distinctive and usually be distinguished from typical freshwater diatoms. This is likely due to the origin of the diatoms in commercial cleaning agents because these are derived from diatomaceous earth which contains distinctive types of fossilized diatoms (2).

This study does not address the issue of whether diatom frustules are ever present in the femoral bone marrow of non drowned people dying of other causes (i.e., determination of the frequency of false positives in a negative control population) (3,4,6,11,12). On a practical basis, false positive outcomes of the test does not seem to be a quantitatively significant problem because the positive outcome rate for the test is relatively low. However, the medicolegal consequence of a false positive test outcome indicates the importance of determining if frustules are present in non drowned people. Studies in several different laboratories over many years have produced contradictory results and do not resolve this issue (3,4,6,11,12). Critical evaluation of these past studies suggest that laboratory contamination, rather than the presence of diatoms in the non drowned people, may explain why diatoms are purported to be found in normal tissues. In this study of positive tests, the diatoms in the bone marrow were specifically restricted in number, size, and type. In a previous study, the absolute number and multiplicity of diatom taxa extracted from drowned and non drowned people exceeded that found in this study (11). In addition, some of the diatoms were up to 300 μm in size. These features are in contrast to the present findings and suggest the possibility of the introduction of diatoms from some exogenous source. In addition, experimental studies on the presence of diatoms in the organs of drowned and non drowned animals are difficult to interpret. In one study, both control and drowned animals had diatoms in organs and few, if any, diatoms of the type used in the experimental drowning medium could be extracted from organs of the drowned animals (12). However, the organs of both drowned and non drowned animals contained diatoms representing several different species. These experimental studies do not differentiate between the presence of diatoms in the non drowned tissues or systematic introduction of exogenous diatoms during tissue processing. In another study of diatom recovery in human tissues in Denmark (11), it was reported that some salt-water diatom species could be recovered from drowned and non drowned people, and atmospheric air. However, the salinity of the lakes in Denmark predicted that there should be a predominance of freshwater diatom species. On this basis, the data also can not be used to discriminate between the artefacutal introduction of diatoms or their presence in tissues during life. Clearly, previous work has not proved that diatoms can enter the tissues of non drowned people during life (4). Additional studies are clearly indicated to resolve this matter.

On the basis of the present and previous analyses, the presence of diatom frustules in extracts of bone marrow currently can be used as proof of drowning only if: (i) the source of diatoms can be determined to be the drowning medium; or (ii) the laboratory performing the test can demonstrate the failure to detect diatom frustules in negative control cases. This has important implications for processing and interpreting individual tests. Specifically, the test must be performed with maximal care to avoid contamination and negative controls may be required to exclude the possibility of the introduction of exogenous diatoms during processing. The demonstration of diatoms in the bone marrow extracts must not be equated with a true positive test result unless the size, species distribution, and number of frustules corresponds to the typical positive test results. If possible, diatom species should be compared with water samples from the putative drowning site to determine if the same species are represented. This utility and significance of this analysis is reported in part two of this investigation. In selected circumstances when drowning medium is not available and the body is not decomposed, diatoms isolated from fluid from the sinuses, watery stomach contents, or lungs may be used as samples that are potentially representative of the drowning medium.

Although the diatom test is an important adjunct to the diagnosis of drowning, the test is best applied as part of a multi-faceted death investigation into the circumstance of death. Because the diatom test is relatively insensitive (28% positive rate) as a sole criterion for the diagnosis of drowning, results should be interpreted in the context of the postmortem findings and scene investigation because a negative test result does not exclude drowning. However, the presence of diatoms in the femoral bone marrow may be the only positive observation that supports the diagnosis of drowning which may otherwise be a diagnosis of exclusion.

In summary, we present evidence for the validity and utility of diatom test for drowning. The data indicate that the test will identify approximately one in three victims of freshwater drowning and may provide useful information in the assessment of obscure deaths and bodies found in bathtubs. The correlation of the outcome of the diatom test for drowning with diatom blooms provides quantitative validation of the test. Although the test can be useful in assessing death by drowning, strict quality control must be applied in performing the test and the results must be cautiously interpreted if the maximal medicolegal benefits are to be achieved.

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