



## Pathology Biology Section – 2011

### G16 Diagnosis Of Drowning: The Contribution Of Microbiological Investigations

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After attending this presentation, attendees will gain knowledge of the effectiveness of marine bacteria and/or bacterial indicators of water fecal pollution as a new marker of drowning.

This presentation will impact the forensic science community by introducing an alternative test that could be useful in drowning diagnosis.

To investigate the effectiveness of marine bacteria and/or bacterial indicators of water fecal pollution as a new marker of drowning, an experimental protocol was performed to identify bacteria on samples of drowned victims recovered from umor vitreo (UV) and blood of different anatomic sites such as: right ventricular blood (RV), left ventricular blood (LV) and peripheral blood (P). The study, performed in 2008 and 2009, was performed on ten victims: six drowned victims (two cases in sea water, three in rivers or lake; one in rainwater collection tank) (study group), and four subjects who died from causes other than drowning (three cases of heart attack and one case of death by vehicle collision) (control group). From all groups at least 0.5 ml of each sample were obtained and the tests were calibrated by considering the water fecal pollution rates. Selective culture media were used to detect bacterial growth. Each samples of control or study groups (RV, LV, P and UV) or water samples (all 100 ml) were incubated in Tryptic soy broth (TSB) for 48 h at 37°C and 5% CO<sub>2</sub>. After incubation, evaluation of bacterial

growth was assessed by plating 100 ml of each sample onto: Todd Hewitt and Marine agars, selective for marine bacteria, m-FC agar, selective for fecal coliforms (FC) and KF Streptococcus agar, selective for fecal streptococci (FS). The plates were incubated for 24 h at 44°C and for 48 h at 37°C and 5% CO<sub>2</sub> to determine FC and FS growth, respectively. The presence of FC was indicated by the development of blue colonies, whereas the presence of FS was indicated by the development of red colonies. The absence of blue and red colonies indicated a negative result, i.e., no blood fecal pollution. The presence of marine bacteria was evaluated through the observation of their growth on selective culture media. Results showed that in the samples of drowned victims in sea water there is growth of marine bacteria, as evidenced by the presence of colonies on TH4% and MA culture media for LV and P blood samples, for the case 1, and for P and UV samples for the case 2. Moreover, the case 2 showed growth of FS and FC bacterial colonies. Regarding drowned victims in rivers or lake water, the analysis of case 4 showed the presence of marine bacteria from RV blood sample; on the other hand the case 5 resulted positive to marine bacteria and fecal streptococci. Surprisingly, case 3 was negative for marine bacteria and fecal streptococci. All anatomic sites of case 6, drowned victim in rainwater collection tank, resulted positive to all the bacterial species considered. Bacteriological analysis of RV, LV, P and UV samples of the control group evidenced a total absence of bacteria. This result showed the reliability of the microbiological test. All the water samples obtained from locations where corpses were found showed a bacterial presence according to samples obtained from the related victims. Applied method is sensitive since a very few bacteria aspirated at follow drowning can be evidenced. Positive results obtained for various anatomic sites (RV, LV, P, and UV) can be an internal control of the sampling procedure to avoid the possibility of bacterial contamination during blood and umor vitreo sampling. Notably, umor vitreo as a new sample for the microbiological test of drowning diagnosis was used.

**Drowning Diagnosis, Microbiological Test, Umor Vitreo**