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## Topic: Aquatic Sciences

# EVALUATION OF FOUR STAINING METHODS FOR THE DETECTION OF STAPHYLOCOCCI IN FISH TISSUE SECTIONS

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### Abstract

This study was carried out for the evaluation of four different tissue Gram-staining methods for the determination of the presence and location of staphylococci in the infected gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*) and sharpsnout sea bream (*Diplodus puntazzo*) tissues. The distinguishing staining abilities of these methods targeting tissue cells, hemosiderin and other pigments and Gram-positive bacteria were analyzed. Their advantages and disadvantages were evaluated for being used in the staphylococcal infections of fishes.

### Introduction

Turkish marine aquaculture sector has shown a great development both in the number of species cultured and in the production amount in the last 20 years. Bacterial fish diseases are among the limiting factors for this development. Histopathological methods are used for the determination of the effects of microbial agent to the fish tissues. Besides they are useful tools for the identification of the causative agent of the disease.

### Materials and Methods

In this study, hearth, kidney, spleen, liver and eye tissues of gilthead seabream, European seabass and sharpsnout seabream samples cultured in Turkey infected with staphylococci were fixed in formaldehyde solution, processed with routine methods and embedded in paraffin. Later, 5µm sections were stained with modified Brown&Hopp, Brown&Brenn, modified Brown&Brenn and Hucker&Twort tissue Gram-staining methods separately.

### Results

Modified Brown&Hopp method stained tissue cells in red and purple, hemosiderin and other pigments in black and Gram-positive bacteria in deep purple and black. Brown&Brenn and modified Brown&Brenn methods stained tissue cells in pale yellow, hemosiderin pigments and Gram-positive bacteria in black. Hucker&Twort method stained tissue cells in pale turquoise, hemosiderin pigments in reddish brown and Gram-positive bacteria in deep blue. When they are located close to each other, it is hard to distinguish the hemosiderin pigments and Gram-positive bacterial cells in the first three methods, but Hucker&Twort staining is found to be suitable in such studies.

### Innovation

With these methods, the presence and location of Gram-positive bacterial cells were demonstrated in staphylococcal infections of cultured fishes. Despite they are not species-specific staining methods, the use of these methods, which were previously used in human health, in moribund fish tissues were evaluated and a new approach is offered to the fish health specialists when studying a Gram-positive infection case in fishes.

**Keywords:** Fish Histopathology, staining methods, staphylococcal infections of fishes

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