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5. Laboratory requirements for bacterial diseases

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5.1. Introduction

In disease management, bacterial analyses are required to identify and implement curative or preventive solutions. Isolation of the pathogenic agent(s) and their *in vitro* sensitivity profile to antibacterials need to be provided fast in order to implement early treatment. The identification of the bacterial agent by different methods will allow the application or development of preventive approaches (vaccination strategies, batch certification, epidemiology and surveillance) in the long-term.

Depending on the production system and the life stage of the sampled fish, identification of bacteria for microbiota management may be required or bacterial strain identification for diagnosis and/or differential diagnosis may be needed. Bacterial disease diagnosis is still based on conventional bacteriological methods that can be performed in small laboratories, but recent modern analytical methods have been applied in bacteriology opening new fields of investigation (microbiota analysis, mass spectrometry, sequencing, and more recently infra-red) but these require heavy investments and therefore platforms and laboratory networks.

Different analytical methods are used for bacterial disease diagnosis and bacteria identification:

Different analytical methods are used for bacterial diagnostics:

- Subculture and strain isolation on artificial media.
- *In vitro* susceptibility testing to antimicrobials.
- Detection of the antigen by serum agglutination tests.
- Strain identification by biochemical tests.
- Strain identification by mass spectrometry and infra-red.
- Detection of bacterial nucleic acid using molecular methods.
- Whole genome sequencing and typing of the bacteria.

The first three methods are widely applied for presumptive diagnostics and treatment recommendation. Nowadays, identification of bacteria by mass spectrometry is substituting biochemical profile identification in routine procedures, being faster and accurate. It requires, however, a validated dataset of reference pathogens to be able to correctly identify the

pathogens in the diagnostic samples. The latest methods of identification are mainly applied for antigen selection in vaccination strategies and for research purposes.

5.2. General management requirement

The general management requirements needed for bacteriology laboratories are the same as those reported for virology laboratories.

Additional information is available in the manual prepared by (Sutton and Singer, 2011).

5.3. The minimum requirement for bacteriological laboratory

A bacterial laboratory should consist of a central room for bacterial analysis with an area for sample reception and a cleaning room, as well as a separate area for media preparation. The one-way organization is recommended. The minimum material requirement should include:

- 1 light microscope (x400 – X1000 immersion),
- 2 thermostatic chambers at a temperature of 20-25°C,
- 1 burner,
- 1 antibiotic disc dispenser and a spectrometer for inoculum density,
- 1 fridge and an autoclave,
- 1 low freezer (-80°C) for bacterial strain conservation and
- 1 microwave oven to melt media.

For media preparation, a balance, a pH meter and a heating magnetic plate will be needed. A biological safety cabinet is not mandatory but it is essential. Basic media for fish bacterial pathogen detection include TSA – Marine agar – TCBS – Blood agar and Mueller-Hinton 2. Additional media may be required for fastidious bacteria such as transport and isolation media for *Tenacibaculum*. Similarly, broth media may be necessary to reactivate some fastidious bacterial strains.

Additional equipment is required for quantitative bacteriology including pipetting systems of different volumes, sterile seawater, an agitator and a bacterial colony counter.

5.4. Biosafety requirements

Few bacterial fish pathogens are zoonotic but for most of them, the zoonotic diseases they might cause are rarely severe. However, a zoonotic infection caused by *Mycobacterium* spp. can be difficult and take a long time to treat in humans. Some human cases of infection with *Vibrio* strains such as *Vibrio vulnificus* or *Vibrio cholerae* have been reported to be harmful to immunocompromised individuals.

For these reasons, even if a biosafety level 1 is generally accepted for laboratories working with fish bacterial pathogens, it should be born in mind that a zoonotic risk remains and that adequate biosafety procedures should be maintained.

Additional information on biosafety is available on the OIE and WHO websites.

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