

# Veribarr - The Science Behind

## INTRODUCTION

Even a small virus can bring havoc by causing disease. The first line of defense is the mucosal surfaces; the largest site of host-pathogen interaction in any animal systems. QuantiDoc specializes in measuring the defense activity in mucosal barriers with VERIBARR<sup>™</sup>, safeguarding the frontline barriers against disease.

The slimy, 0.07mm hair-thin mucosal barrier is part of the immune system. This tissue is sensitive and vulnerable to treatments, feeds, environment and breeding. These actions influence the basics in sustainable fish production, fish health and welfare.

## OUR FOCUS

QuantiDoc specializes on the cellular response patterns and cell changes of mucosal barriers. A barrier can have many or few mucous cells but essentially if there are no mucosal cells, there is no protective mucosal barrier.

QuantiDoc is alone in having a verified, standardized, unbiased, quantitative way to measure and express host health in universally applicable units. Therefore results from one trial can be directly compared with those from another years, site, tissue or treatment. We can answer the key questions; what is normal – and how much is normal?

## VERIBARR<sup>™</sup> - A STANDARDIZED METHOD

Veribarr<sup>™</sup> is the registered brand name for the commercial application of mucosal mapping to **verify barriers** with the calibrated semi-automated digitization.

Comparison	Histological quantification of mucous cells	Veribarr <sup>™</sup> on mucous cells (design-based stereology, 3D from 2D)
Length or area	1-2 mm running length	1-2 cm <sup>2</sup> surface area
Unit of measure	Relative to existing structures	Universally applicable
Orientation of section	Very important	Not important
Standardization	- No standardized units - Not directly comparable across treatment and organs	- Standardized reporting - Comparable across treatment and organs
Qualitative or quantitative	Qualitative and quantitative	Quantitative
Method	Manual	Semi-automated
Bias	Biased unless random rules applied	Unbiased

(Table modified from Dang *et al.* 2020, Table 4).

Veribarr<sup>™</sup> has many advantages over traditional histology (Dang *et al.*, 2020). The stereological Veribarr<sup>™</sup> method is a mathematical technique where meaningful unbiased quantitative descriptions of 3D structures are derived from measurements made on 2D images like biopsies. Such methods are “best practice” for validation of human pharmaceutical products.

Standardization is essential. After fixing a biopsy from a standardized site, we slice the embedded samples in *tangential* sections and apply widely available PAS-AB to clearly stain the glycoproteinaceous mucous cells. Veribarr™ analyses one (1) section per tissue, gives statistically reproducible results and is *industrially scalable*.

**MUCOMASTER AND MACHINE LEARNING**

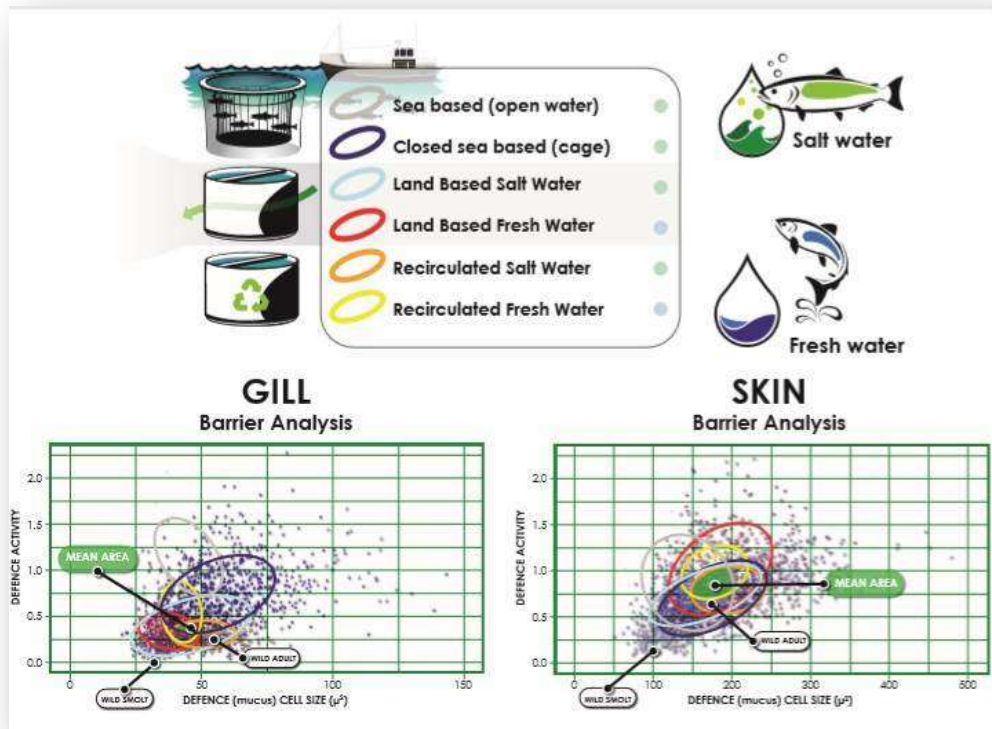
High resolution digital scans of mucosal tissues are used for the design-based stereological analysis by our semi-automated hardware, the MucoMaster. Machine learning has been applied to train our proprietary software on millions of mucous cell pictures from our samples, with an aim to fully automate the digital analysis. We measure how big the cells are at their equator (cell size), how much of the mucous barrier is filled with these cells (volumetric density) and how actively the mucosal layer produces these mucous cells (defense activity). This is all unbiased mathematics. No subjective categorization is used.

Since the breakthrough in 2010, this method has been used both scientifically and industrially over a wide range of studies. The company QuantiDoc AS (**quantification** and **documentation**) was established in 2014 and some of the scientific articles are available through the website <https://www.quantidoc.no/the-science.html> .

**VERIBARR GRID DATABASE**

Veribarr™ documents how production systems challenge fish to be in balance with their environment when it is full of potential pathogens. QuantiDoc has through a decade of operation and research built a database of healthy fish comprising nearly 10.000 points covering 3 tissues in 9 species in 7 countries. This undisputed resource allows us to compare any sample taken from fish farming with scientifically proved robust fish – both farmed and wild.

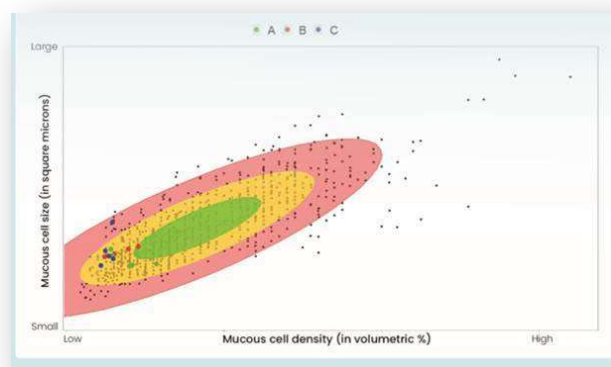
Veribarr Grid is our comprehensive database of numbers supplemented by annotated high resolution digital images backing up each and every data point. The Veribarr Grid allows comparison of even single samples against “normal” values for each system.



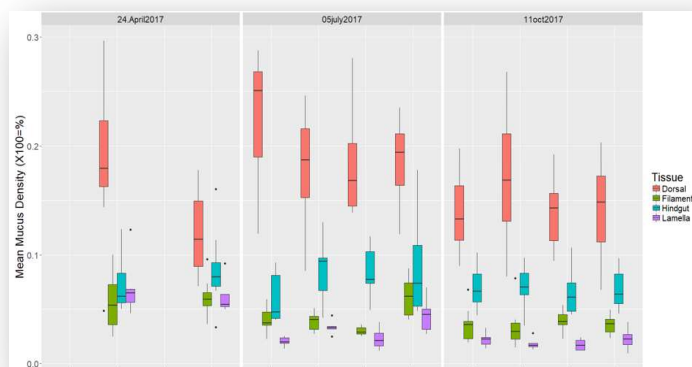
**VERIBARR AT A GLANCE**

With Veribarr™ technology, any attempt to improve the barrier status can be compared against Veribarr Grid to show whether it is effective or efficient. Regular monitoring can be used to define when the weakening of immune barriers occurs - and forensically improve production routines, saving time, labor and other operational expenses.

In the anonymized illustration of real data below, the colored dots of A, B and C are results of different dates from the same farm. The small black dots are results from the database for the same tissue from the same weight class of *clinically healthy fish* as those in the farm, where the green zone is where most healthy fish lie. All colored samples reveal that the farm’s fish have moderate vulnerability to pathogens in the surrounding seawater because the mucous cells are in low density and reacting to a chronic situation.



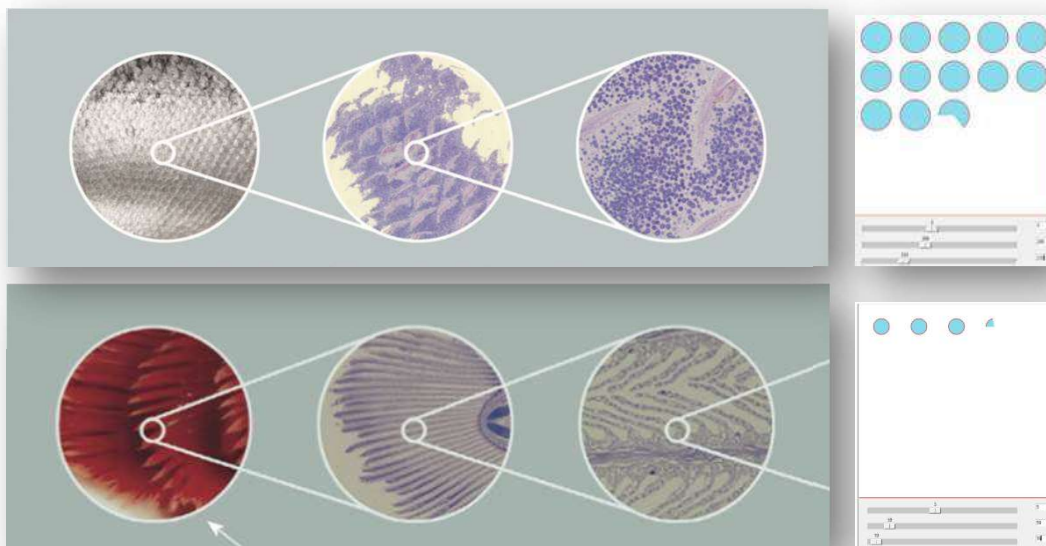
Unbiased numbers from VERIBARR™ analysis can become plots, figures, statistical validation or even standardized visual representations of the sheet of protective mucosa.



For more scientific purposes, anonymized boxplots using real data for 4 tissues show how diet and time in the farm system affect the amount of mucous cells in the skin, gills and hindgut (volumetric density in % of epithelium filled with mucous cells). The two gill segments of filament and lamellae are almost always distinct and the breathing surface of the lamellae has usually the lowest amount of mucus cells when the fish is

healthy. Handling and other activities can reduce the skin's ability to protect the fish but diet can still play an important role.

The alternative form of presentation of the VERIBARR results is our **DiceApp** which shows real mucous cell sizes and densities within a standard 10000 square microns of epithelia (from diet trial on salmon skin and from treatment trial on salmon gills). The DiceApp compensates for patchiness and gives the 3 measures of size, volumetric density and defense activity in one illustration. Sometimes the DiceApp is the easiest way to show where challenges are affecting fish health, fish welfare and production management.



### **CUSTOMER BENEFITS**

Veribarr™ is an operational tool to understand how the fish immune system reacts to production routines, environment and other factors in fish farming systems. The outcome of applying Veribarr™ is to specify challenges and control health and growth within the biomass. Since the slimy barriers of skin, gills and guts are made of living cells, these are always in negotiation with the microbiome and with the environment. If negotiations break down, disease and mortality ensue.

Veribarr™ gives an unbiased support to operational decisions across species, time and systems. As an example, Veribarr™ can distinguish between the effects of single additives to either fishmeal, algal or plant-based diets. Further, Veribarr™ can show whether skin has been depleted by repetitive handling and can no longer deliver the mucosal protection; whether the diet has challenged the foundation of robust health or whether pathogens have already triggered an immune response.

The sensitivity of Veribarr™ can detect significant changes to the gills in response to water quality, particles and pathogens – even when the gills appear to look fine.

## **VERIBARR™ - HISTORY AND DEVELOPMENT**

The basic challenge was to define a healthy fish with relevant and operational criteria. In 2009, anecdotal evidence from the salmon industry indicated that certain feeds produced more mucous on the skin resulting in fewer parasites and fewer treatments being carried out. Traditional histological methods did not explain the changes in mucous production observed anecdotally.

In 2010, Professor Karin Pittman of the University of Bergen was approached by the fish farming industry to develop a robust quantitative and comparative method to measure the mucous-producing cells. A unique technique was developed that was scalable for industrial application, using only 1 section per tissue per fish and generating reliable, reproducible and statistically solid results (Pittman et al., 2011).

Market acceptance of the analytical results was *immediate* as it provided fundamental information on the health of the fish. Maintaining fish in balance with their environment, by keeping the mucosal barriers in balance, has saved millions in treatments and operational expenses.

## **CLOSING REMARKS**

QuantiDoc AS has its base in the scientific environment of the University of Bergen and the Norwegian Center of Expertise in Seafood Innovation at Marineholmen, Bergen, Norway. The mission of the company is to participate in the value chain of a *sustainable* fish farming industry providing high quality proteins to the consumer.

Fish health, fish welfare and sustainable growth are our underlying motives.

**Quantifying Robustness** has become our mantra.

Our competence and our operational experience are at your disposal. For discussion or consultation, you are welcome to contact Professor Karin Pittman on tel. +47 9173 6482 or email [kp@quantidoc.no](mailto:kp@quantidoc.no)

For further references see [www.quantidoc.com](http://www.quantidoc.com)

We also recommend downloading free copies of our book “The Robust Fish” from our website <https://www.quantidoc.no/home.html>

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