

Review Article

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## Rapid Methods for Histamine Detection in Fishery Products

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### ABSTRACT

Seafood is the most significant perishable commodity. Decomposition of seafood, especially during storage at elevated temperatures (48°C), various amounts of selected biogenic amines is usually produced, depending on the fish species. Most common biogenic amines in seafood associated with spoilage are histamine, tyramine, putrescine and cadaverine which are formed by bacteria that decarboxylates the corresponding free amino acids. Histamine is known as a biogenic amine which is low molecular weight and possesses biological activity. Histamine poisoning also referred to as 'Scombroid fish poisoning'. The levels of histamine have been suggested as rapid fish spoilage indicators. The reason for the monitoring of selected biogenic amines in seafood is twofold: as indices of decomposition and to prevent potential toxicity on human health. Different determination methods have been reported for the analysis of histamine, including Thin-layer chromatography. Gas chromatography, Colorimetric assay, Fluorometric assay, Enzymatic assay, Immunological assay, High-performance liquid chromatography. Simple and rapid method for monitoring histamine levels in fish and fishery products are Biosensors, Eliza, colorimetric methods.

#### Keywords

Histamine,  
Fishery Products,  
Seafood

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### Introduction

There has been a global increase in seafood consumption, especially in countries that are not traditional seafood consumers. However, seafood-related diseases have been frequently reported. During decomposition of seafood, especially during storage at elevated temperatures (48°C), various amounts of selected biogenic amines are usually produced, depending on the fish species.

The most common biogenic amines in seafood associated with spoilage are histamine, tyramine, putrescine and cadaverine. They are formed by bacteria naturally present in decomposed fish that decarboxylates the corresponding free amino acids. Histamine is a compound which is released by cells in response to injury and in allergic and inflammatory reactions, causing contraction of smooth muscle and dilation of capillaries. Histamine is secreted by basophils and mast cells as part of a local immune

response to the presence of invading bodies. Aside from humans, histamine is found in virtually all animals. Histamine and other biogenic amines are present in various amounts in many foods.

Fresh fish at harvest are virtually free of histamine, but post-harvest conditions that allow the growth of spoilage bacteria can result in histamine formation. Among amines, histamine is important from the toxicological point of view as it is the causative agents of scombroid fish poisoning and food intolerance. The reason for the monitoring of selected biogenic amines in seafood is twofold: as indices of decomposition and to prevent potential toxicity on human health. Histamine is known as a biogenic amine which is low molecular weight and possesses biological activity (Tombelli and Mascini, 1998). Histamine poisoning is also referred to as 'Scombroid fish poisoning'. The levels of histamine have been suggested as rapid fish spoilage indicators (Male *et al.*, 1996; Patange *et al.*, 2005; Tombelli and Mascini, 1998). Histamine poisoning probably occurs frequently in Asia, and was reported in extremely high levels in some salted, and dried fermented products. Other countries outside Asia have also reported cases of histamine poisoning (Lehane and Olley, 2000). Histamine exerts its effects by binding to receptors on cellular membranes in the respiratory, cardiovascular, gastrointestinal and haematological, immunological system and the skin in the course of allergic and other actions such as hypotension, flushing, diarrhea, vomiting and headache (Lehane and Olley, 2000).

### **Histamine formation**

The amino acid histidine undergoes decarboxylation. This chemical reaction is catalysed by the enzyme L-histidine decarboxylase. Histamine is a hydrophilic

vasoactive amine and once formed, it is either quickly inactivated or stored. When released at synapses, it is broken down by acetaldehyde dehydrogenase. Histamine can be broken down by the enzymes diamine oxidase and histamine-N-methyl transferase. The histamine toxicity is increased by the presence of other amines, such as putrescine and cadaverine. Figure 1 shows the decarboxylation reaction.

### **Role of bacteria in the histamine formation**

Both groups have different coenzymes associated with them. Gram-positive histamine-producing bacteria are more commonly associated with fermented products like salami, cheese, sauerkraut and wine, while Gram-negative histamine producing bacteria are more common in fish. A wide range of Gram-negative bacteria

Can produce histamine in fish, but the major types are mesophilic enteric and marine bacteria. Certain bacteria produce the enzyme histidine decarboxylase during growth. Time/temperature abuse of certain species of fish can cause consumer illness. Histamine is more commonly the result of high temperature spoilage than of long term, relatively low temperature spoilage. Growth is particularly rapid at temperatures near 90°F (32.2°C). Cooking does not destroy the histamine. They naturally exist on the gills and in the gut of live, salt water fish, with no harm to the fish. Upon death, the defence mechanisms of the fish no longer inhibit bacterial growth, and histamine-forming bacteria start to grow and produce histamine. Scombroidae (tuna, mackerel), Clupeidae (sardine, herring, Engraulidae (anchovy), Mahi-mahi (*Coryphaena hippurus*), Bluefish (*Pomatomus saltatrix*) were the fishes associated with histamine formation. The amount and type of amine formed is therefore strongly influenced by the food composition,

microbial flora and by several parameters which allow bacterial growth during food storage, such as food treatment prior to storage, food additives, temperature, moisture, ripening and packaging. *Morganella morganii*, *Morganella psychrotolerans*, *Photobacterium damsela*, *Photobacterium phosphoreum*, *Raoultella planticola*, *Hafnia alvei* were reported as histamine formers. In the case of fermented sea food, *Staphylococcus* spp. and *Tetragenococcus* spp.

### **Symptoms**

Histamine poisoning, which results from ingestion of food with high levels of histamine, produces a variety of gastrointestinal (nausea, vomiting, diarrhea, abdominal cramps), cutaneous (rash, urticaria, edema), hemodynamic (hypotension) and neurological (flushing, itching, burning, tingling, headache). Blood clot, gastric acid secretion, blood vessel to dilate, bronchoconstriction, adrenaline releasing, swelling and inflammation and the causes of histamine was illustrated in Figure 2.

### **Nature of histidine decarboxylase enzyme**

Once the enzyme histidine decarboxylase is formed, it can continue to produce histamine in the fish even if the bacteria are not active. The enzyme can be active at refrigeration temperatures. The enzyme is remain stable while the fish is frozen and reactivated very rapidly after thawing. Freezing may inactivate the enzyme-forming bacteria. Cooking can inactivate both the enzyme and bacteria.

### **Steps to prevent histamine formation in fishery products**

Rapid chilling as soon as possible to inhibit formation of the enzyme histidine decarboxylation. Good hygienic practice is

required at every step of processing of the fish. Careful handling to avoid damage to muscle tissue is also important in preventing contamination. Good practice at processing and preparation stages further along the supply chain such as cutting and packing is also required.

### **Treatment for histamine poisoning**

Since symptoms generally only last a few hours and the condition is rarely life threatening, antihistamines are usually the only drugs necessary. Adrenaline is often given, as the diagnosis of fish anaphylaxis.

### **Recent outbreaks of histamine fish poisoning**

Between January 2002 and July 2004 there were 12 outbreaks of HFP reported to the Auckland Regional Public Health Service. The majority of outbreaks implicated smoked kahawai (*Arripis trutta*). These outbreaks grossly underestimate the prevalence of Scrombroid Fish poisoning in New Zealand.

RASFF week 29 - EUROPEAN COMMISSION HEALTH and CONSUMER PROTECTION DIRECTORATE-GENERAL reported seafood recalls in 2011. Mostly chilled tuna from Maldives were notified by Italy. The level of histamine reported were 30ppm to 1000 ppm.

RASFF notifications on food poisoning in 2012 (EUROPEAN COMMISSION HEALTH and CONSUMER PROTECTION DIRECTORATE-GENERAL) – reported recalls of frozen and chilled tuna steaks having histamine at a level upto 5000 ppm from various countries like Vietnam, Srilanka, Spain, Indonesia. India- Hyderabad: Histamine (which causes allergy and food intolerance) forming in fish species like tuna, Indian mackerel and sardine — recently listed

by Food Safety Authority of India under the category of “fish species having potential to cause histamine poisoning” — is making its way into homes due to improper refrigeration and preservation in cities in Telangana including Hyderabad and in Visakhapatnam in Andhra Pradesh. Published on Aug- 24 2016 (DECCAN CHRONICLE) According to FSSAI experts, at room temperatures the histamine concentration rapidly increases. Transport to Hyderabad from Visakhapatnam and other coastal areas is not done properly in several cases. Even the ice used by fish stalls and units contain *E-coli* as inferior/impure quality of water is used to freeze into ice. Pathogens *E-coli* and *Klebsiella* convert histidine present in fish tissue to histamine.

### **Limits of Histamine in fish**

The Australia New Zealand Food Standards (ANZFS) code allows 100mg/kg histamine as the maximum permitted level.

Commission Regulation (EC) No 2073/2005- Fishery products - 100 mg/kg, 200 mg/kg, Fishery products which have undergone enzyme maturation treatment in brine - 200 mg/kg 400 mg/kg.

Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission 2010 - A maximum average level of not more than 100 ppm is considered satisfactory.

USFDA - FDA and EPA Safety Levels in Regulations 50 ppm defect action level, because histamine is generally not uniformly distributed in a decomposed fish.

INDIA - No sample of fresh and frozen mackerel shall contain histamine content exceeding 20 mg/kg No sample of canned mackerel shall contain histamine content exceeding 20 mg/100 g.

Food Safety and Standards Authority of India has proposed the limit for histamine as 100 mgkg<sup>-1</sup> for acceptable limit and 200 mgkg<sup>-1</sup> for rejection limit for dried/ salted fishery products (FSSAI, 2016).

### **Histamine detection**

A simple and rapid method for histamine analysis is now required all over the world. Different determination methods have been reported for the analysis of histamine, including Thin-layer chromatography. Gas chromatography, Colorimetric assay, Fluorometric assay, Enzymatic assay, Immunological assay, High-performance liquid chromatography, Concerning the determination of histamine, it is a very serious problem to separate histamine completely from a very large amount of interference compounds such as histidine or carnosine. Most methods of analysis need a careful and tedious pretreatment to eliminate potential interference substances, which is time consuming and prolongs the analytical process. A simple and rapid method for monitoring histamine levels in fish and fishery products is therefore needed to avoid delay in the analysis in order to ensure safety of fish products.

### **Rapid methods for histamine detection**

#### **Biosensors**

A biosensor is a quantitative analytical instrumental technique containing a sensing element of biological origin, integrated with a physico-chemical transducer. A device that uses specific biochemical reactions mediated by isolated enzymes immunosystems tissues organelles whole cells Detect chemical compounds usually by electrical, thermal or optical signals. Figure 3 shows the principle of biosensor.

Commercially available biosensors for fishery products are

BIOFISH 300

BIOFISH 700

### BIOFISH 300

A compact analytical device for the quantification of histamine in tuna and mackerel in a precise, simple and fast way. Combine high specificity and selectivity of the specific enzyme with an amperometric transduction of this biological signal, easily detectable and quantifiable. Detection device. Dimensions: 26cm x 22cm x 26cm. 4.6 kg.

The Biofish-300 HIS method is a simple, reliable, and specific enzymatic biosensor for the detection of histamine. This technology is highly specific and selective and allows quantification of histamine in fishery products (fresh/frozen and processed) in a short time frame (2-3 min) (Fig. 4).

Histamine in raw tuna, raw mackerel, raw sardine, raw anchovy, boiled tuna, canned tuna in water, canned tuna in oil, canned mackerel in tomato sauce, canned pickled sardine, and canned salted anchovy was analyzed using a water-based extract.

### BIOFISH 700

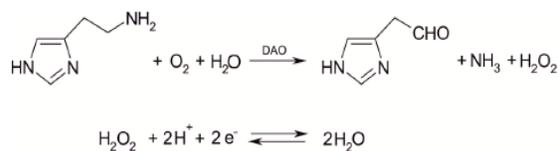
This is a portable, pocket biosensor that uses screen-printed electrodes to monitor parameters of interest in the quality of fish and/or seafood in less than one minute (Fig. 5).

### Other histamine biosensors

#### Diamine oxidase screen printed electrode

The electrochemical signal of the DAO-nPt/GPH/chitosan/CSPE biosensor is principally associated with the oxidation process of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which

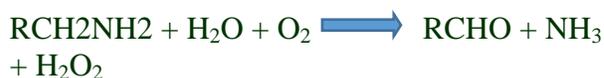
is the enzymatic product of interaction between DAO and histamine.



The presence of –COOH and –OH groups facilitates the immobilization of DAO on the biosensor surface (nPt/GPH/chitosan) by means of electrostatic, hydrophobic, van der Waals, hydrogen bonding interactions. The sensitive layer of biosensor is stable and the cross-linking process is not necessary, resulting in an increasing of the biosensor sensitivity. Sensitivity to histamine 0.0631 μM.

### Amine oxidase – based flow biosensor

Principle: Specific enzyme copper containing amine oxidase catalyzes the oxidative deamination of biogenic amines to the corresponding aldehydes and ammonia accompanied by two electron reduction of molecular oxygen to hydrogen peroxide.



Enzymatic method using a free-state enzyme, such as measuring the oxygen uptake by an oxygen electrode and *Aspergillus niger* AO±I (Ohashi *et al.*, 1994), which is a recent application of the classical amine oxidase activity assay (Machol\_an, 1968), is relatively simple, but requires large quantities of purified enzyme. Amperometric biosensor based on the detection of oxygen uptake or hydrogen peroxide release with pea seedling (Toul and Machol\_an, 1975; Machol\_an and Slanina, 1991) or pig kidney amine oxidase (Male *et al.*, 1996; Bouvrette *et al.*, 1997) immobilized on a nylon net via glutaraldehyde, and bovine serum and A.

niger amine oxidases immobilized on a collagen membrane (Karube *et al.*, 1980) are more advantageous.

However, these methods require relatively large volume of the reaction mixture that can accommodate the electrode. Also an equilibration of the electrode with the reaction mixture before each assay is necessary. Recently, very advanced flow biosensor with immobilized pig kidney amine oxidase based on a chemiluminescent detection of hydrogen peroxide has been described (Alam *et al.*, 1995). This biosensor overcomes most of previous problems, however requires special instrumentation that is not generally available. More applicable seems to be an amperometric biosensor based on a carbon paste with immobilized pea seedling amine oxidase and peroxidase that could be eventually applied as a postcolumn detector (Wimmerov\_a and Machol\_an, 1996)

#### **Amperometric biosensor for tiger prawn**

An enzyme based histamine biosensor that can operate at a lower potential. Response range 0- 300 ppm. The decrease in the operation potential was achieved by the electrochemical oxidization of the product imidazole acetaldehyde, which was produced from the enzymic reaction of diamine oxidase on histamine.

The biosensor also utilized a photocuring technique for the immobilization of the diamine oxidase enzyme where it was directly entrapped in a photocured membrane and deposited onto a carbon paste screen-printed electrode (SPE).

Histamine was then determined using an amperometric method. The biosensor was used to evaluate histamine in tiger prawns and for the monitoring of histamine release during prawn spoilage.

#### **Colorimetric assay**

Principle: A saline extraction of histamine, followed by a centrifugation, a n-butanol extraction and an evaporation before the colorimetric reaction with *p*-phenyldiazonium sulfonate. Assay proposed by Patange *et al.*, (2004). Limit of quantitation is 10 mg/kg. The reaction between purified histamine and copper which form a visible red complex.

#### **Histamine colorimetric assay**

1. Enzymatic assay kit
2. Hista strip
3. Agra strip

#### **Histamine enzymatic assay kit: BIOO scientific**

A very rapid (10 minutes) and robust enzyme-based assay which does not require chemical derivatization or expensive instrumentation. Detection Limit: Meat/Seafood- 6 ppm, Fish Meal- 10 ppm.

#### **Hista strip**

A new laboratory-free test: A dipstick approach, providing a visual indication of histamine levels without any additional equipment, solvents, or processes. Very rapid (4 minute test). Simple extraction. No equipment or instruments required. Ideal for ANY testing environment, even in restaurant kitchens. when it comes into contact with histamine in seafood, milk, cheese or wine. When the strips are dipped into samples containing histamine, the pad rapidly changes colour to indicate the presence of the analyte. The colour change is proportional to the amount of histamine present in the sample. Detection capabilities using the strips are well below global action levels. The equipment-free and visual nature of the test allows convenient rapid testing in and out of the

laboratory. The equipment-free and visual nature of the test allows convenient rapid testing in and out of the laboratory.

### **Agra strip**

A lateral flow immune chromatographic assay that determines a semiquantitative level for the presence of histamine. The Agra Strip Histamine Test has been validated for fresh fish, canned fish, salted fish, fish meal.

The AgraStrip Histamine Test uses the unique FLORIDA Technology. Gives highest precision in different kinds of fish samples. Even under difficult light conditions or in complete darkness, the test signals can be read quite easily by visual evaluation (Fig. 6).

In contrast to gold and latex beads used in traditional rapid immunoassays. AgraStrip Histamine uses a fluorescence dye to label the antibody. The combination of FLORIDA and the highly specific immune reagents shows sensitivity as high as 5 ppm and allows for the flexible adjustment of the cut-off.

The cut-off of the AgraStrip Histamine is set to 50 ppm. By using the acylation reagent, histamine is quantitatively derivatized into N-acyl histamine. The amount of fluorescence-labeled antibody bound to the solid phase histamine is inversely proportional to the histamine concentration of the sample.

### **Interpretation of results in Agrastrip**

2 lines < 50 ppm

1 line: Control line visible: > 50 ppm

**Test line visible:** An invalid result and has to be clarified by trouble shooting

**No line:** An invalid result and has to be clarified by trouble shooting

### **ELISA**

The histamine assay is an immune competitive assay which uses XL665-labeled histamine and an anti-histamine Cryptate-labeled antibody. The assay has a two-step protocol acylation and detection. Figure 7 shows the principle of histamine ELISA (Fig. 7).

### **Commercially available histamine ELISA**

Agra Quant  
RIDASCREEN  
Alert  
Reveal  
Histasure

### **Agra Quant**

Reliable enzyme-linked immune sorbent assays (ELISA) in quantitative format. validated for the analysis of histamine in fresh fish, fish meal. Limit of detection: Agra Quant Histamine- 0.15 ppb, AgraQuant Histamine Rapid- 1ppm.

### **RIDASCREEN**

Simple - 3 pipetting steps, hot water extraction, Short assay time 15 min.

Limits of detection	Measurement range
Fish 0.75 mg/kg	Fish 5 - 100 mg/kg
Fish meal 3.75 mg/kg	Fish meal 25 - 500 mg/kg

### **Alert**

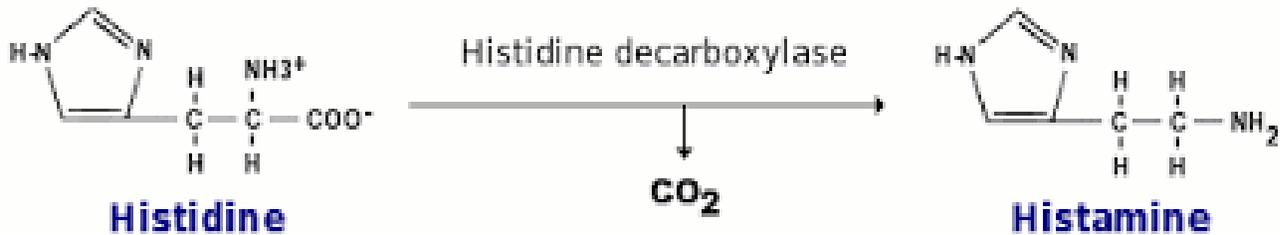
A competitive direct ELISA intended for the screening of histamine in scombroid species of fish, such as tuna, bluefish and mahi-mahi, and in fishmeal.

The tests provide visible results that clearly show whether a sample contains more or less of a specific food allergen or toxin than the control provided. Testing time - 20min.

**Table.1** Different types of ELISA and their sensitivity

Test	sensitivity	Quantitative/Qualitative	Test time
ALERT	2.5 ppm	Qualitative blue colour	35min
Veratox	2.5 ppm	Quantitative 650nm	35 min
Histamarine	0.5 ppm	Quantitative 405- 414 nm	1 hr
EIA (fish)	2.5ppm	Quantitative 405 nm	90 min
EIA (raw, canned fish)	5ppm	Qualitative Yellow colour	35 min
Histameter	0-50 ppm	Qualitative	1hr
Histaquant	0-500ppm	Quantitative	1- ½ hr
RADIOSCREEN HistaminR1602	2.5 ppm	Quantitative 450nm	2hr
RidaQuick Histamin	20ppm	Quantitative	15min

**Fig.1** Decarboxylation reaction



**Fig.2** Causes of histamine in humans

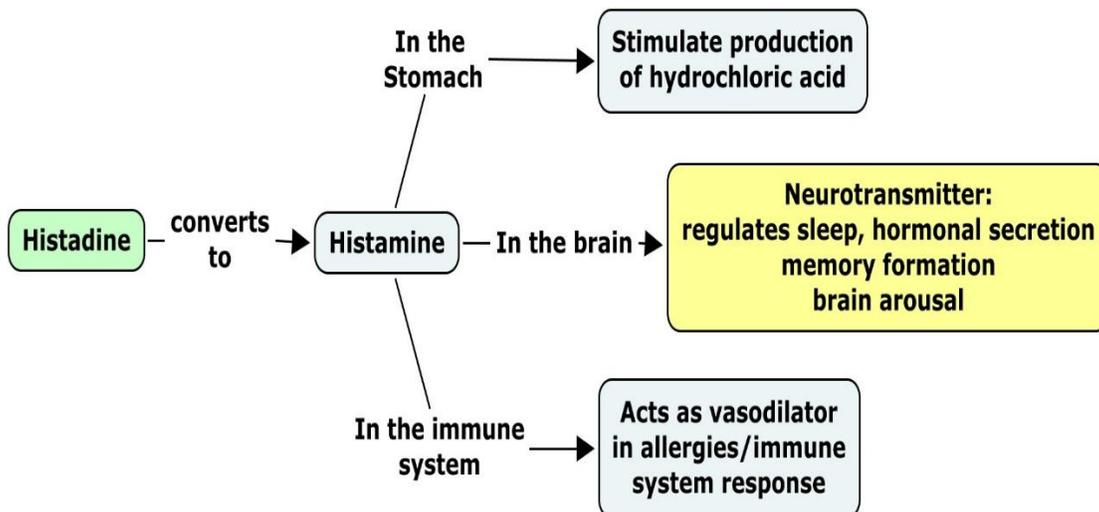


Fig.3 Principle of biosensors

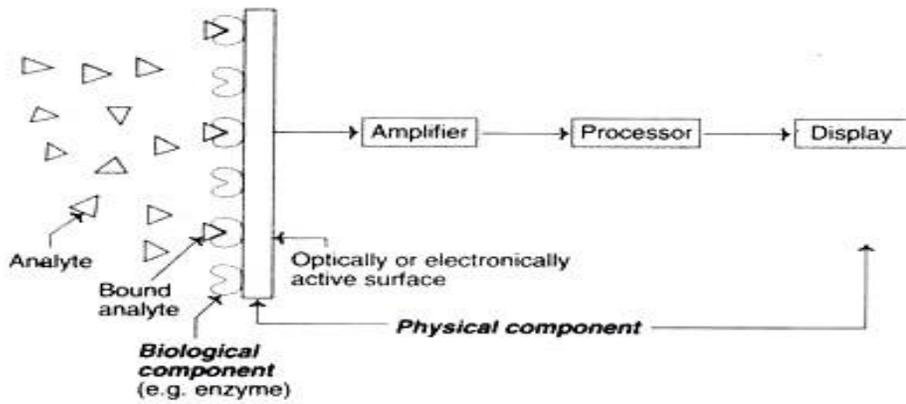


Fig.4 BIOFISH 300 BIOFISH 700



Fig.5 The steps involved in HistaStrip

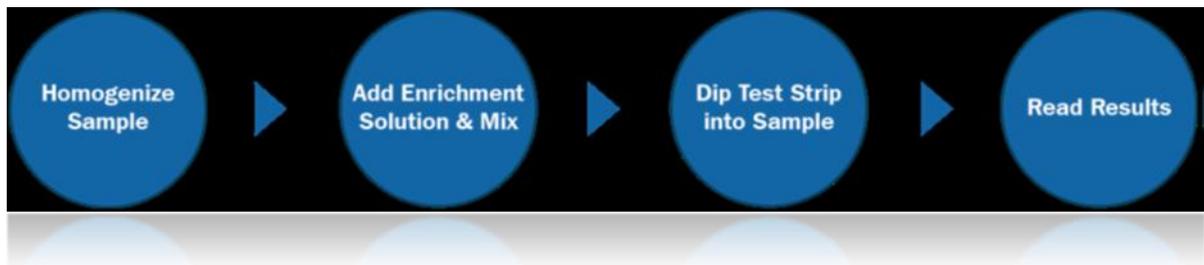


Fig.6 The results of Agrastrip

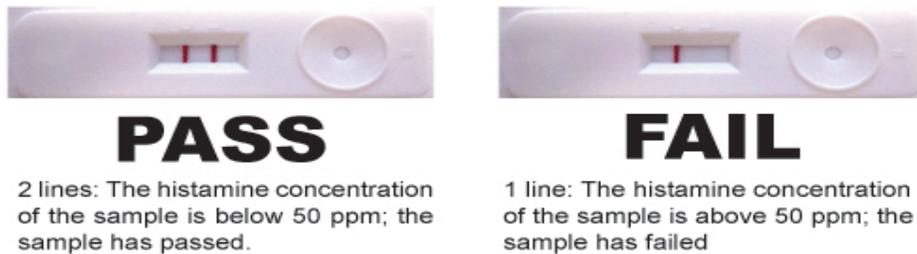


Fig.7 The principle of histamine ELISA

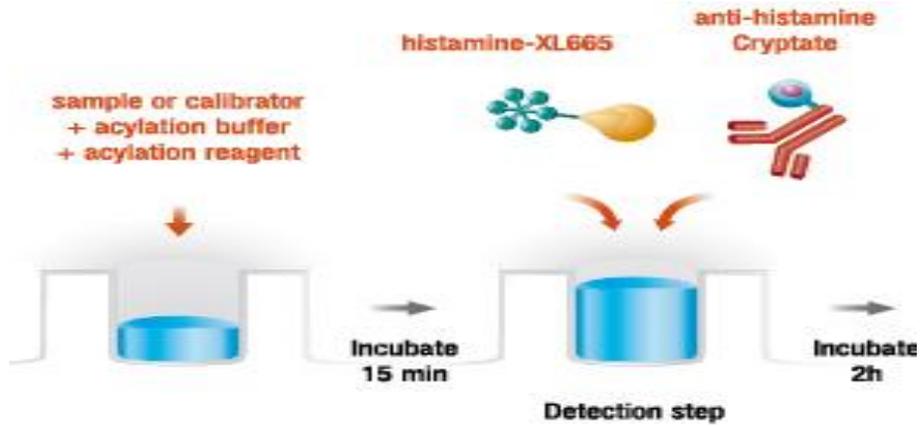


Fig.8 Shows the result of Reveal test kit

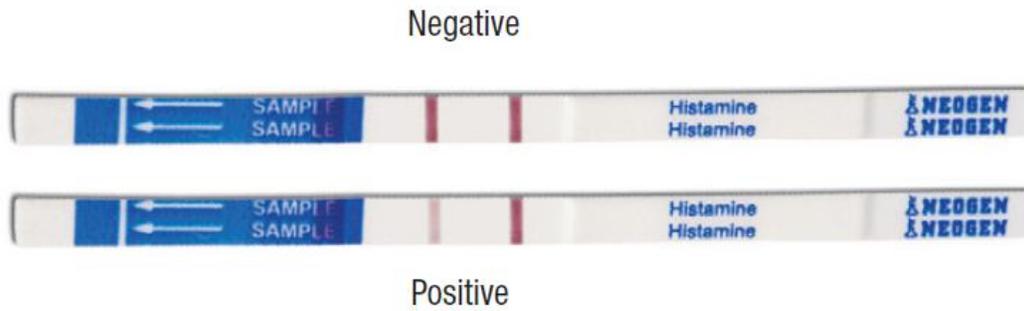
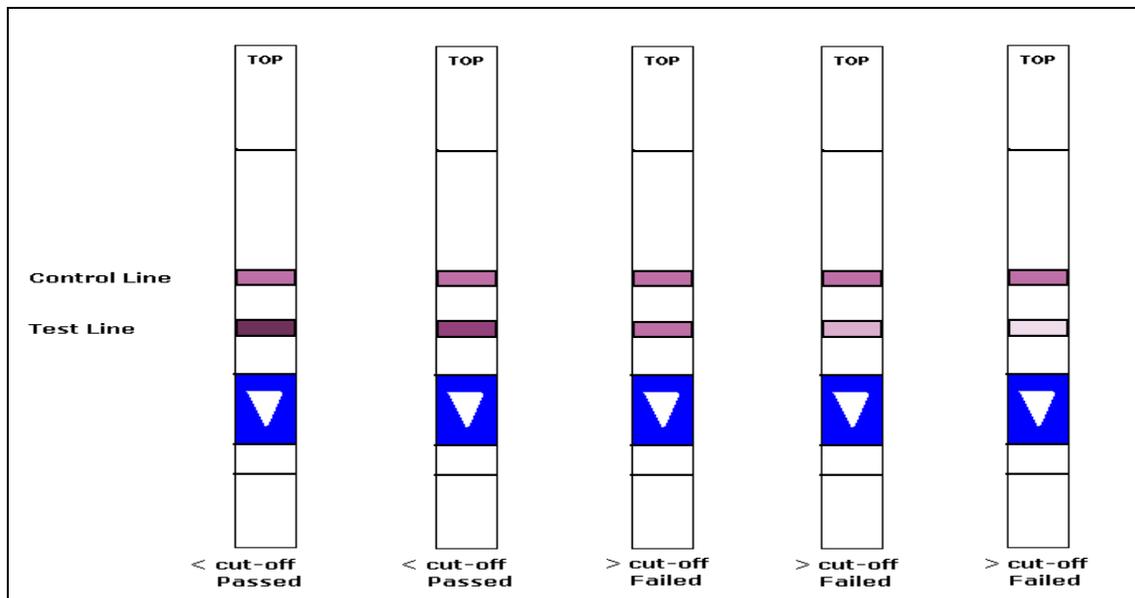


Fig.9 The results of Histasure test kit



## Reveal for histamine

A single-step lateral flow assay based on a competitive immunoassay format intended for the visual screening of histamine in scrombroid species of fish, such as tuna and mahi-mahi. Sensitivity: 50 ppm Testing time- 5 minutes and the results of reveal illustrated on Figure 8 shows the result of reveal test kit.

## Histasure

Histasure ELIZA fast track, Sensitivity: 0.44 ppm, Total assay time: acylation 5 min, ELIZA 20min Sample type- fresh fish, canned fish and Figure 9 shows the result of Histasure.

Conclusions of the study are as follows

Biosensors are the best methods of all to detect histamine in fishery products.

BIOFISH 700 - portable sensitive biosensor which can be used at markets and fields.

Compared to other methods biosensors have short time analysis.

Histamine ELISA has higher sensitivity (0.5ppb) than other methods.

Strips takes lesser time to give results.

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