TRADE FORWARD
SOUTHERN AFRICA

List of European Union Testing Requirements for Farmed Bivalve and Gastropod Molluscs

Produced by Imani Development in Partnership with Development Alternatives Incorporated (DAI) and made possible by the Foreign, Commonwealth and Development Office (FCDO) of the United Kingdom

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Trade Forward Southern Africa (TFSA) is a programme under the Foreign, Commonwealth & Development Office (FCDO) of the UK Government, promoting trade in the SACU+M region.

Development Alternatives Incorporated (DAI) is the lead contractor for TFSA, covering the core implementation team and all sub-activities. Imani Development is an implementing partner of DAI for the suite of aquaculture activities and other matters.

This report has been developed from the work in Phase 2 of the TFSA aquaculture pilot. Etienne Hinrichsen (specialist in African aquaculture development and planning), and Ian Goulding (specialist in aquaculture export regulation) provided the required technical expertise.

It is important to note that this report contains information pertaining to the current situation as of April 2022. Additionally, it should be noted that the guidelines, regulations, legal instruments, and reference materials that have been used to inform this report are subject to amendment and even repeal, from time to time. For this reason, readers and users of this guideline document should consult the official instruments to check for any such amendments.

CONTENT

ACRONYMS

AAS	Atomic Absorption Spectrometry
AbHV	Abalone Herpes-like Virus
As	Arsenic
ASP	Amnesic Shellfish Poisoning
AZA	Azaspiracid
Cd	Cadmium
CEN	European Committee for Standardisation
DAI	Development Alternatives Incorporated
DSP	Diarrhetic Shellfish Poison
DTX	Dinophysistoxin
EC	European Commission
E.coli	*Escherichia coli*
EU	European Union
EURL	European Union Reference Laboratory
EURL-MN	European Union Reference Laboratory for Metals and Nitrogenous Compounds
FCDO	Foreign, Commonwealth and Development Office
GC - HRMS	Gas Chromatography - High Resolution Mass Spectrometry
GC-ICP-MS	Gas Chromatography - Inductively Coupled Plasma - Mass Spectrometry
GC-MS	Gas Chromatography - Mass Spectrometry
Hg	Mercury
HPLC	High-Performance Liquid Chromatography
ISH	*In Situ* Hybridisation
ISO	International Standards Organisation
LC	Liquid Chromatography
MPN	Most Probable Number
MRL	Maximum Residue Level/Limit
MS	Mass Spectrometry
NSAID	Non-Steroidal Anti-Inflammatory Drug
NSI	National Standard Institution
OA	Okadaic Acid
OMA	Official Methods of Analysis
OsHv	Oyster Herpes Virus
OIE	World Organisation for Animal Health
Pb	Lead
PCB	Polychlorinated biphenyl
PCR	Polymerase Chain Reaction
PSP	Paralytic Shellfish Poison
PTX	Pectenotoxin
RFTM	Ray's Fluid Thioglycolate Medium
RFLP	Restriction Fragment Length Polymorphism
TEM	Transmission Electron Microscopy
UV	Ultraviolet
VMP	Veterinary Medicinal Product
YTX	Yessotoxin

1 Introduction

South African and Namibian farmers have expressed a strong interest to export molluscs (mussels, oysters, and abalone) to the European Union (EU). This document contains a list of all the testing requirements associated with compliance to the EU legal framework for the import of farmed bivalve and gastropod molluscs.

The list in this document contains all the testing requirements for the farmed mollusc species in South Africa and Namibia (i.e., abalone, mussels, and oysters), as well as the associated testing requirements for phytoplankton, biotoxins, microbiological aspects, food safety aspects, drug residues, metals, aquatic animal health aspects (such as histology, and Polymerase Chain Reaction (PCR) for parasite identification). The list details the test parameter, test methodology and the EU legal reference.

2 Methodology and Sources

In developing this document, the relevant EU legislation via the EURLEX website (https://eur-lex.europa.eu/) was consulted. In all cases the consolidated legal instruments (incorporating amendments) were used. Legal references therefore refer to the original instrument, and not to subsequent amendments. The EU Commission disclaimer on use of consolidated documents therefore applies[1].

Where additional certainty was required, data was drawn from the relevant EU reference laboratory, as follows:

- AECOSAN: Agencia Española de Consumo, Seguridad Alimentaria y Nutrición
  www.aesan.gob.es/en/CRLMB/web/home.html
- ANSES: Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
  https://eurl-veterinaryresidues.anses.fr/
- IFREMER: L'Institut français de recherche pour l'exploitation de la mer
  www.eurl-mollusc.eu/
- DTU Danmarks Tekniske Universitet
  www.eurl-mn.eu/

[1] *"This text is meant purely as a documentation tool and has no legal effect. The Union's institutions do not assume any liability for its contents. The authentic versions of the relevant acts, including their preambles, are those published in the Official Journal of the European Union and available in EUR-Lex".*

The output is delivered in sections each relating to a group of test parameters as follows:

a) Classification and area controls for live bivalve molluscs - biotoxins / phytoplankton

b) Environmental contaminants

c) Residue monitoring for aquaculture products

d) Chemical and microbiological standards for potable water

e) Other food safety requirements applicable to molluscs

f) Aquatic animal health requirements

g) Radioactive contamination

3 Classification and Area Controls for Live Bivalve Molluscs - Biotoxins / Phytoplankton

Test Parameter	Official Test Method	Test Method Legal Reference	Monitoring Requirement / Additional Information
Escherichia coli (E.coli)	The reference method for analysis of *E. coli* in live bivalve molluscs shall be the detection and 'most probable number' (MPN) technique specified in ISO 16649-3. Alternative methods may be used if they are validated against this reference method in accordance with the criteria in ISO 16140.	Commission Implementing Regulation (EU) 2019/627/ Annex IV	Commission Implementing Regulation (EU) 2019/627 Title V, Chapter 1 Articles: 53; 54; 55: Defines requirements for Class A, B and C. based on numbers of *E. coli.* **NB:** This Title applies to live bivalve molluscs. It also applies to live echinoderms, live tunicates, and live marine gastropods. This Title does not apply to live marine gastropods and live *Holothuroidea* that are not filter feeders.
Phytoplankton	Enumeration of phytoplankton species of interest using inverted microscopy (Utermöhl technique).	Annex V to Directive 2000/60/EC, Section 1.3.6 - EU Standard EN 15204:2006	Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000, establishing a framework for Community action in the field of water policy.

			Regulation (EU) 2017/625 Article 59/60 Monitoring of classified production and relaying areas, requires phytoplankton monitoring and chemical contaminants, microbiological quality (could be other indicators). Annex V to Directive 2000/60/EC, Section 1.3.6 EU Standard EN 15972:2011 Water Quality - Guidance on quantitative and qualitative investigations of marine phytoplankton.
Saxitoxin causing Paralytic Shellfish Poisoning (PSP)	The PSP toxin content of the whole body or any part edible separately of bivalve molluscs shall be determined using AOAC official method OMA 2005.06, as published in AOAC International Journal 88(6), 1714-1732 (Lawrence method), the mouse bioassay or any other internationally recognised validated method.	Annex V to Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission	Regulation (EC) 853/2004 of the European Parliament and of the Council of 29 April 2004, laying down specific hygiene rules for food of animal origin. Annex III to Regulation (EC) No 853/2004, Section VII, Chapter V – Health Standards for Live Bivalve Molluscs:

		Regulation (EC) 2074/2005 as regards official controls.	Such products must not contain marine biotoxins in total quantities (measured in the whole body or any part edible separately) that exceed the following limits: • For paralytic shellfish poison (PSP), 800 micrograms of saxitoxin equivalent per kilogram. • For amnesic shellfish poison (ASP), 20 milligrams of domoic acid per kilogram. • For okadaic acid, dinophysistoxins and pectenotoxins together, 160 micrograms of okadaic acid equivalents per kilogram. • For yessotoxins, 1 milligram of yessotoxin equivalent per kilogram. • For azaspiracids, 160 micrograms of azaspiracid equivalents per kilogram. Note Chapter IX - Specific Requirements for *Pectinidae*, Marine Gastropods and
Domoic Acid causing Amnesic Shellfish Poisoning (ASP)	The ASP toxin content of the entire body or any part edible separately of bivalve molluscs shall be determined using the high-performance liquid chromatography with ultraviolet detection (HPLC/UV) method or any other internationally recognised validated method.		
(a) Okadaic acid group toxins causing Diarrhetic Shellfish Poison (DSP): okadaic acid (OA), dinophysistoxin (DTX1 and DTX2), including their esters (DTX3).	The reference method for the detection of marine toxins as referred to in points (c), (d) and (e) in Chapter V(2) of Section VII of Annex III to Regulation (EC) 853/2004 shall be the EU reference laboratory liquid chromatography-mass spectrometry/mass spectrometry (EURL LC-MS/MS) method.		

(b) Pectenotoxin (PTX) group toxins: PTX1 and PTX2. **(c) Yessotoxin (YTX) group toxins: YTX, 45 OH YTX, homo YTX and 45 OH homo YTX.** **(d) Azaspiracid (AZA) group toxins: AZA1, AZA2 and AZA3.**			Echinoderms which are not filter feeders harvested outside classified production areas.

4 Environmental Contaminants

Test Parameter	Official Test Method	Test Method Legal Reference	Monitoring Requirement / Additional Information
Metals: **Lead (Pb)** **Cadmium (Cd)** **Mercury (Hg)**	Performance criteria for methods of analysis for lead, cadmium, mercury, inorganic tin and inorganic arsenic set out in	Commission Regulation (EC) 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the control of the	Commission Regulation (EC) 1881/2006 of 19 December 2006 setting maximum

Arsenic (As)	Commission Regulation (EC) 333/2007 Annex, Part C , Table 5. Where no specific methods for the determination of contaminants in foodstuffs are prescribed at European Union level, laboratories may select any validated method of analysis for the respective matrix provided that the selected method meets the specific performance criteria set out in Tables 5, 6 and 7. EURL-MN validated methods available form: www.eurl-mn.eu/library/list-of-methods. EN 14084:2003 - Foodstuffs - Determination of trace elements - Determination of lead, cadmium, zinc, copper, and iron by atomic absorption	levels of trace elements and processing contaminants in foodstuffs. Annex V to Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls.	levels for certain contaminants in foodstuffs. Commission Regulation (EU) 2015/1005 of 25 June 2015 amending Regulation (EC) 1881/2006 as regards maximum levels of lead in certain foodstuffs.

	spectrometry (AAS) after microwave digestion. EN 17266:2019 - Foodstuffs - Determination elements and their chemical species - Determination of organomercury in seafood by elemental mercury analysis. EN 16801:2016 - Foodstuffs - Determination of elements and their chemical species - Determination of methylmercury in foodstuffs of marine origin by isotope dilution Gas Chromatography - Inductively Coupled Plasma - Mass Spectrometry (GC-ICP-MS).		
Polychlorinated biphenyl (PCB)	Screening - Bioanalytical and Gas Chromatography - Mass Spectrometry (GC-MS) methods. Confirmatory methods - Gas Chromatography - High Resolution Mass Spectrometry (GC-HRMS). For confirming	Annex 5 and 6 of Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) 589/201.	Section 5, Annex of Commission Regulation (EC) 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

	compliance or non-compliance with the maximum level, also GC-MS/MS can be used. See: www.crl-freiburg.eu/dioxin/methods.html		

5 Residue Monitoring for Aquaculture Products

Test Parameter	Official Test Method	Test Method Legal Reference	Monitoring Requirement / Additional Information
Group A: **(1) Stilbenes, stilbene derivatives, and their salts and esters.** **(3) Steroids.** **(6) Compounds included in Annex IV to Council Regulation (EEC)**	See above for environmental contaminants. See Commission Regulation (EC) 401/2006 for mycotoxins. See Commission Decision 2002/657/EC of 14 August 2002 for remaining parameters.	Commission Decision 2002/657/EC of 14 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results sets performance criteria for testing of veterinary drugs and other residues required under 2017/625 (previously under Directive 93/23). Includes a list of suitable confirmatory methods for organic residues or contaminants (Table 1 in Annex).	Article 19 of Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection product. General requirement for monitoring under Article 19 - Residue monitoring requirements.

2377/90 of 26 June 1990. **GROUP B - Veterinary drugs and contaminants.** **(1) Antibacterial substances, including sulphonomides, quinolones.** **(a) Anthelmintics.** **(3) Other substances and environmental contaminants.** **(a) Organochlorine compounds including PCBs.** **(d) Chemical elements.** **(d) Mycotoxins.**		1. Methods used for sampling and for laboratory analyses, tests and diagnoses during official controls and other official activities shall comply with EU rules establishing those methods or the performance criteria for those methods. 2. In the absence of the EU rules as referred to in paragraph 1, and in the context of official controls and other official activities, official laboratories shall use one of the following methods according to the suitability for their specific analytical, testing, and diagnostic needs: (a) Available methods complying with relevant internationally recognised rules or protocols including those that the European Committee for Standardisation (CEN) has accepted; or relevant methods developed or recommended by the EU reference	**NB:** 96/23 repealed but under Article 150 of 2017/625 there are transitional measures related to the repeal of Directive 96/23/EC that read: "*Competent authorities shall continue to perform the official controls necessary to detect the presence of the substances and groups of residues listed in Annex I to Directive 96/23/EC, in accordance with Annexes II, III and IV to that Directive, instead of the corresponding provisions of this Regulation, until 14 December 2022 or an earlier date to be determined in the delegated act adopted in accordance with paragraph 3 of this Article*." Under Directive 96/23 Annex 1 monitoring is required for: GROUP A - Substances having anabolic effect and unauthorized substances:

(e) Dyes.		laboratories and validated in accordance with internationally accepted scientific protocols. (b) In the absence of the suitable rules or protocols, as referred to in point (a), methods which comply with relevant rules established at national level, or, if no such rules exist, relevant methods developed or recommended by national reference laboratories and validated in accordance with internationally accepted scientific protocols; or relevant methods developed and validated with inter or intra-laboratory methods validation studies in accordance with internationally accepted scientific protocols. Additional guidelines are published at: https://ec.europa.eu/food/safety/chemical_safety/vet_med_residues_en	**(1) Stilbenes, stilbene derivatives, and their salts and esters.** (2) Antithyroid agents. **(3) Steroids.** (4) Resorcylic acid lactones including zeranol. (5) Beta-agonists. (**6) Compounds included in Annex IV to Council Regulation (EEC) 2377/90 of 26 June 1990.** GROUP B - Veterinary drugs (1) and contaminants: **(1) Antibacterial substances, including sulphonomides, quinolones.** (2) Other veterinary drugs. (**a) Anthelmintics.** (b) Anticoccidials, including nitroimidazoles. (c) Carbamates and pyrethroids. (d) Sedatives.

		For mycotoxins: Commission Regulation (EC) 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs sets performance criteria.	(e) Non-steroidal anti-inflammatory drugs (NSAIDs). (f) Other pharmacologically active substances. (**3) Other substances and environmental contaminants.** **(a) Organochlorine compounds including PCBs.** (b) Organophosphorus compounds. **(d) Chemical elements.** **(d) Mycotoxins - Commission Regulation (EC) 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs.** **(e) Dyes** (f) Others NB the list is conditioned for each sector by ANNEX II. The residue or substance group to be detected by type of animal, their feeding stuffs, including drinking water,

			and primary animal product. **Parameters in bold above are required for aquaculture products.** Commission Regulation (EU) 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. **NB:** List of permitted and non-permitted substances and their MRLs. Note none approved for molluscs. Regulation (EU) 2019/6, which enters into application on 28 January 2022, replaces the legal framework for veterinary medicinal products (VMPs) established by Directive 2001/82/EC and Regulation (EC) No 726/2004. Article 118 - List of substances not authorised for use in food animals due to being reserved for use in human medicine.

6 Chemical and Microbiological Standards for Potable Water

Test Parameter	Official Test Method	Test Method Legal Reference	Monitoring Requirement / Additional Information
Potable water: ***E. coli*** ***Enterococci*** **Colony count** ***Clostridium perfrngens***	The methods of analysis for microbiological parameters are: (a) *E. coli* and coliform bacteria (EN ISO 9308-1 or EN ISO 9308-2). (b) Intestinal *Enterococci* (EN ISO 7899-2); (c) Colony count or heterotrophic plate counts at 22 °C (EN ISO 6222). (d) *Clostridium perfringens* including spores (EN ISO 14189).	Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption.	Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption. Annex I - Minimum requirements for parametric values used to assess the quality of water intended for human consumption. See also Annex iii - Specifications for the analysis of parameters. Microbiological parameters. Chemical and indicator parameters for which performance characteristics are specified sets performance limits.
Chemical parameters listed in Table 1 Part A of Annex to Directive (EU) 2020/2184.	Minimum performance characteristic "uncertainty of measurement" set out in Table 1 Part B.		

7 Other Food Safety Requirements Applicable to Molluscs

Test Parameter	Official Test Method	Test Method Legal Reference	Monitoring Requirement / Additional Information
Food Safety Criteria			
Listeria monocytogenes	EN/ISO 11290-1.	Chapter 1, Annex 1, Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.	Ready-to-eat foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes. Before the food has left the immediate control of the food business operator, who has produced it.
Salmonella	EN ISO 6579-1. Horizontal method for the detection, enumeration and serotyping of *Salmonella*.		Cooked crustaceans and molluscan shellfish and live bivalve molluscs and live echinoderms, tunicates, and gastropods. Products placed on the market during their shelf-life.
E. coli	(MPN) technique specified in ISO 16649-3.		Live bivalve molluscs and live echinoderms, tunicates, and gastropods.

			Products placed on the market during their shelf-life. Each sample unit comprises a minimum number of individual animals according to EN/ISO 6887-3.
Process Hygiene Criteria			
E. coli	ISO TS 16649-3.	Chapter 2, Annex 1 Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.	**NB:** Applicable only to shelled and shucked products of cooked crustaceans and molluscan shellfish.
Coagulase-positive *Staphylococci*	EN/ISO 6888- 1 or 2.		
Additives: e.g. Sulfur dioxide, - sulfites, Polyphoshates.	Various methods available: HPLC after extraction or Monier Williams.	Commission Decision 2002/657/EC of 14 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.	List of permitted additives in Annex II of Regulation (EC) No 1333/2008. Database at: https://ec.europa.eu/food/safety/food_improvement_agents/additives/database_en

8 Aquatic Animal Health Requirements

Test Parameter	Official Test Method	Test Method Legal Reference	Monitoring Requirement / Additional Information
Marteilia refringens	• Histopathology. • Cytology.	Commission Delegated Regulation (EU) 2020/689 of 17 December 2019	Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March

	• *In situ* hybridization *M. refringens* - Probe M2A / M3AS, Le Roux et al. 2001 (ITS1). • Conventional PCR *M. refringens,* Le Roux et al. 2001, Primers M2A & M3AS also named Pr4 & Pr5 (ITS1 - 412bp). Type discrimination possible by Restriction fragment length polymorphism (RFLP). • Taqman real-time PCR *M. refringens / Bonamia sp.,* Canier et al. 2020 (18S). • Taqman real-time PCR *M. refringens* type M / type O, EURL unpublished (ITS1). • Histopathology and PCR for surveillance. • Tissue imprints and PCR for presumptive diagnostic. • PCR and sequencing for confirmatory diagnostic.	supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for surveillance, eradication programmes, and disease-free status for certain listed and emerging diseases. www.eurl-mollusc.eu/Diagnostic-manual www.eurl-mollusc.eu/SOPs Commission Implementing Decision 2015/155 of 11 September 2015 lays down rules for the application of Directive 2006/88/EC as regards requirements for surveillance and diagnostic methods (*B. ostreae* and *M. refringens*).	2016 on transmissible animal diseases and amending and repealing certain acts in animal health ('*Animal Health Law*') will enter into force in April 2021**.** Title II - Aquatic Animals and Products of Animal Origin from Aquatic Animals addresses aquatic animal health controls, registration, approval, movements, disease prevention, certification, and obligation not to spread listed diseases. Listed diseases in Annex 2 under aquatic animal health: Infection with *Bonamia exitiosa*. Infection with *Perkinsus marinus*. Infection with *Microcytos mackini.* Infection with *Marteilia refringens*. Infection with *Bonamia ostreae.*

Bonamia ostreae and ***Bonamia exitiosa***	• Histopathology • Cytology • *In situ* hybridization *Bonamia sp* - Probe BO/BOAS. • Conventionnal PCR *Bonamia sp.,* Cochennec et al. 2000, primers BO/BOAS (18S - 304bp). Species discrimination possible by RFLP. • SYBR Green PCR *B. ostreae / B. exitiosa*, Ramilo et al. 2013, primers BOSTRE-F/R BEXIT F/R (ITS -18S). • Taqman real-time PCR *M. refringens / Bonamia sp*., Canier et al. 2020 (18S). • Taqman real-time PCR *B.ostreae/ B.exitiosa*, EURL. • Tissue imprints, histopathology, and PCR for surveillance. • Tissue imprints and PCR for presumptive diagnostic. • PCR & sequencing and transmission electron microscopy for confirmatory diagnostic.		Commission Delegated Regulation (EU) 2020/689 of 17 December 2019 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council provides rules for surveillance, eradication programmes, and disease-free status for category C (*M. refringens, B. exitiosa, B. ostreae*) and emerging diseases. Several national reference laboratories and pathology laboratories are currently building or developing their Quality Management System based on ISO 17025 Standard (general requirements for the competence of testing and calibration laboratories). Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in animal health (‘*Animal Health Law*’).

Perkinsus marinus	• Histopathology. • Ray's Fluid Thioglycolate Medium (RFTM) culture. • *In situ* hybrization *P. marinus,* Probe PmarLSU-181DIG, Moss et al. 2006 (LSU). • SYBR Green *PCR P. Marinus,* Audemard et al. 2004, Primers PmarITS-70F & PmarITS600R (ITS-509bp). • RFTM culture of tissue for surveillance. • PCR technique for presumptive diagnostic. • *In situ* hybridization for confirmatory diagnostic.		
Mikrocytos mackini	• Histopathology. • *In situ* hybrization *M. mackini*, Probe MACKINI-1 F&R, Meyer et al. 2005 (18S). • Conventional PCR *M. mackin*i, Carnegie et al. 2003, Primers MIKROCYTOS-F&R (18S-546 bp).		

	• Taqman Real Time PCR *M. mackini,* Polinski et al., 2015 (ITS2). • Histopathology and tissue imprints in some cases for surveillance. • Histopathology, imprints, PCR, and *in situ* hybridisation for presumptive diagnostic. • PCR, *in situ* hybridisation, sequencing and transmission electron microscopy for confirmatory diagnostic.		
Abalone Herpes-like Virus (AbHV). (OIE - exotic).	Direct detection methods developed, to date, for detection and identification of AbHV include microscopic methods (examination of tissue sections for typical lesions and electron microscopy for detection of herpesvirus particles), conventional and real-time PCR, and *in situ* hybridisation.	EURL Diagnostic methods for shellfish diseases: www.eurl-mollusc.eu/Main-activities/Tutorials See also OIE Manual of Diagnostic Tests for Aquatic Animals Chapter 2.4.1. Infection with Abalone Herpesvirus: www.oie.int/en/what-we-do/standards/codes-and-manuals/aquatic-manual-online-access/?id=169&L=1&htmfile=chapitre_abalone_herpesvirus.htm	Notifiable disease.

Oyster Herpesvirus Type 1 (OsHv1).	• PCR (nested, simple, competitive). • Histology (only for screening). • DNA sequencing (confirmatory). • *In situ* hybridization (confirmatory). • Transmission electron microscopy (confirmatory). • In case of suspicion in larvae: all dead and moribund larvae should be collected for DNA extraction and PCR according to Renault et al. 2000. • In case of suspicion in juveniles: tests should preferably be performed on moribund individuals. 30 individuals should be analysed in pools of five animals. DNA extraction and PCR according to Renault et al. 2000. • In case of suspicion in adults: OsHv-1 has not been associated with mortality of adults. However, adults may be asymptomatic carriers. *In situ* hybridization can be used to test presence in connective tissue of adults.	Reference Methods for OsHv1: www.eurl-mollusc.eu/Main-activities/Tutorials/Herpes-virus-OsHV-1	Not currently a listed disease in the EU legislation. OsHv1 - Not listed by the OIE Manual of Diagnostic Tests for Aquatic Animals (2009 version) nor by the Aquatic Animal Health Code (2009 version).

9 Radioactive Contamination

In case of a nuclear accident or other radiological emergency, maximum permitted levels of radioactive contamination apply as described in Annex I of Council Regulation 2016/52 of 15 January 2016 laying down maximum permitted levels of radioactive contamination of food and feed following a nuclear accident or any other case of radiological emergency.

See: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016R0052&from=EN.

10 Laboratories in South Africa and Namibia

The laboratory of the National Standards Institution (NSI) in Namibia performs the required tests for the Namibian shellfish farming sector. Those for which the laboratory is not accredited are outsourced to South African laboratories or send abroad.

In South Africa, several government and private sector laboratories have different capabilities and functions related to meeting the testing requirements. These laboratories are listed in the table on the following page. **Kindly note that this information may change over time.**

Tests	Laboratory	Telephone	Address
Veterinary drug residues	Mérieux NutriSciences	021 683 8436	7 Warrington Road Claremont, Cape Town
Biotoxins	AssureCloud	021 658 2740 021 492 6652	3 Hermes Street Paarden Eiland, Cape Town
	Central Analytical Facility	021 938 9344	Stellenbosch University 16 De Beer Street, Stellenbosch
Heavy metals	AssureCloud	021 658 2740 021 492 6652	3 Hermes Street Paarden Eiland, Cape Town
	Mérieux NutriSciences	021 683 8436	7 Warrington Road Claremont, Cape Town
Dioxins Dioxin-like PCBs Non-dioxin-like PCBs	Mérieux NutriSciences	021 683 8436	7 Warrington Road Claremont, Cape Town
Radionuclides	Nuclear Energy Corporation OF South Africa	012 305 5243 012 305 5728	Elias Motsoaledi Street & Church Street West, Ext Pelindaba, Pretoria
	Mérieux NutriSciences	021 683 8436	7 Warrington Road Claremont, Cape Town
Pesticides	South African Bureau of Standards	012 428 6648 / 6446	1 Dr Lategan Road Groenkloof, Pretoria
	Mérieux NutriSciences	021 683 8436	7 Warrington Road Claremont, Cape Town

Tests	Laboratory	Telephone	Address
E. coli	Mérieux NutriSciences	021 683 8436	7 Warrington Road Claremont, Cape Town
Pathogenic *vibrio*	South African Bureau of Standards	012 428 6648 / 6446	1 Dr Lategan Road Groenkloof, Pretoria
Salmonella	South African Bureau of Standards	012 428 6648 / 6446	1 Dr Lategan Road Groenkloof, Pretoria
	Mérieux NutriSciences	021 683 8436	7 Warrington Road Claremont, Cape Town
Mycotoxins	Mérieux NutriSciences	021 683 8436	7 Warrington Road Claremont, Cape Town
Furans	Mérieux NutriSciences	021 683 8436	7 Warrington Road Claremont, Cape Town
Dyes	Mérieux NutriSciences	021 683 8436	7 Warrington Road Claremont, Cape Town
Polycyclic Aromatic Hydrocarbons	Mérieux NutriSciences	021 683 8436	7 Warrington Road Claremont, Cape Town

11 Conclusion

This list of all the testing requirements associated with compliance to the EU legal framework for the import of farmed bivalve and gastropod molluscs, provides an extensive reference work that should aid the South African and Namibian aquaculture sectors. This list will require updating from time to time to ensure that it remains current.