GOVERNMENT OF WESTERN AUSTRALIA

Department of **Health**

Department of **Primary Industries and Regional Development**

WESTERN AUSTRALIA SHELLFISH QUALITY ASSURANCE PROGRAM (WASQAP)

SAMPLER MANUAL

2021

WESTERN AUSTRALIA SHELLFISH QUALITY ASSURANCE PROGRAM (WASQAP) Sampler Manual

Prepared by the WA Department of Health and WA Department of Primary Industries and Regional Development

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1.0 Introduction

1.1 Background

The sampler manual is a guidance document containing step by step instructions for sample collection, submission and results interpretation. This document supports and is consistent with the online sampler training content for WA shellfish growers. The online sampler training course for WA is delivered by the University of Tasmania and a link is available here (external site). Successful completion of the online training course provides a Western Australia Shellfish Quality Assurance Program (WASQAP) sampler qualification.

The general information below is from the online UTAS sampler course designed for WASQAP. Instructional videos for the sampling methods listed below are available in the online course content.

1.2 Why shellfish and the waters they are grown in need to be tested

Shellfish produced for human consumption are considered a high-risk food due to the following:

1. They are filter feeders, therefore they ingest what is in the water for food (including phytoplankton, pollution and human waste) causing a potential health risk.
2. Shellfish may accumulate pathogens (such as Hepatitis A virus, norovirus and bacteria) during filter feeding. Due to this, they are considered a public health risk when they are eaten raw or lightly cooked.
3. Algae or microscopic phytoplankton are the main food for shellfish. Some phytoplankton produce harmful toxins that bioaccumulate in shellfish. These toxins remain in the flesh and are not destroyed by cooking.

Effective management of these risks occur through a robust shellfish quality assurance program implemented at a state level (WASQAP) and guided by a national program the Australian Shellfish Quality Assurance Program (ASQAP). The basis of which is ongoing monitoring and involves the regular sampling of harvesting areas to monitor the safety of shellfish. Risks vary according to harvest area classification, and the sampling frequency that each area is required to undertake, reflects this risk. Environmental risks can change; and the annual reviews conducted by the Department of Health (DoH) will assess any changes that may affect a harvest areas classification.

1.3 What is sampled?

The two main types of sampling are microbiological and phytoplankton. Samples are targeted from both water and shellfish sources. Microbiological indicators are **thermotolerant faecal coliforms** and ***E. coli*** bacteria. **Phytoplankton (microalgae) species** and **biotoxins** associated with shellfish poisoning are also targeted for testing.

Microbiological (Water and Shellfish)	Phytoplankton (Water) and Biotoxin (Shellfish)
What? (Thermotolerant coliforms, *E. coli*)	**What?** (Phytoplankton, Biotoxin)
Why? (Indicators, classification, shellfish poisoning, management plans)	**Why?** (Shellfish poisoning)
How? (Collection, storage and transport, laboratory)	**How?** (Collection, storage and transport, laboratory)
When? (Systematic, Adverse, reopening, event)	**When?** (in accordance with the Marine Biotoxin Monitoring and Management Plan (MBMMP)

1.4 Classification status

The classification status and conditions of the harvest area determines the sampling strategy. For example, approved or conditionally approved areas, operators must have in place harvest area management plans. These set out the sampling strategy and schedules and are developed by the operators and approved by the shellfish control authority i.e. Department of Health in WA. (Refer to ASQAP Operational Manual and WASQAP guidance documents for further details on classification and sampling requirements).

The Marine Biotoxin Monitoring and Management Plan (MBMMP) is a generic plan currently applied across all harvest areas in WA. This involves routine scheduled bimonthly (twice a month) water samples and monthly flesh samples in all harvest areas. Details on sampling when toxin trigger levels are reached can be found in the MBMMP 2020.

2.0 Sampling

2.1 General requirements for submitting samples

Safety is a priority, therefore before following these methods ensure that it is safe to do so. Please adhere to health and safety requirements and do not attempt to collect samples if conditions are hazardous.

Planning

- Contact the accredited laboratory to arrange sample delivery
- Confirm connections from remote and /or to interstate locations
- Collate sample submission forms
- Ensure you are familiar with standard methods and have all required equipment

Sampling

- Sample quality is important
- Samples should be kept refrigerated or cool
- Shells should be cleaned if submitting whole shellfish
- Sample delivery should be timed to minimise delays in delivery to the laboratory

Submission

Label samples clearly with the following information (refer to appendix 1 for sampling program information sheet).

- Location that includes WASQAP station ID
- Time and date
- Observations (e.g. weather)
- Ambient conditions – water temperature, etc
- Sample submission forms should be filled in and arrive with the sample to be tested
- Samples that do not meet standards maybe rejected

2.2 Bacteriological Water sampling procedure

Important points

- Hands must be clean to avoid cross contamination
- Care must be taken not to touch inside of the lid or the rim of the bottle
- Take bacterial samples before taking other samples
- Label sample bottle before sampling
- Discard any damaged or contaminated sample bottles
- Samples should be received ideally within 6 hours and less than 24 hours from collection
- Cool chain should be maintained, at less than 5°C to prevent bacterial growth and false results

Equipment required

• Esky
• Ice packs
• Sample Labels
• Sterile sample collection bottles 250ml
• Water proof pen to fill in labels and sample form
• Specific Laboratory submission form

Method

1. Locate sample site on map
2. Take a labelled sterile 250ml sample bottle. Keep the lid on the bottle until ready to collect the sample
3. Hold the sterile bottle in one hand near the base, and carefully remove and hold the screw cap with the other hand. Be careful not to touch the inside of the screw-cap when sampling.

OR
Place the sampling bottle into the sampling pole, making sure it is securely clamped into position. Carefully remove and hold the screw cap with your free hand. Extend the pole out into the water.
4. Plunge the sample bottle neck downwards approximately 30 cm below the water surface, moving the bottle away from your body. This sample depth should be adhered to consistently.
5. Turn the bottle neck slightly upwards to allow air to exit, which allows the bottle to fill up. Move the bottle into the current, remembering to keep your hand away from the mouth of the bottle at all times. The current should fill the bottle to the rim. If there is no current, move the bottle horizontally through the water until it is full.
6. Once the bottle is full remove it from the water.
7. Tip enough of the water from the bottle to leave an air space of about 1-2 cm from the rim of the bottle. This air space is necessary to facilitate mixing of the sample by the laboratory.

8. Carefully replace the screw-cap immediately and tightly.
9. Place the bottle in the esky on ice packs and record time of sample on bottle and sampling program information form (send details to DoH electronically see Appendix 1) and send to laboratory promptly.

2.3 Bacteriological Shellfish flesh sampling procedure

Important points

- Hands must be clean to avoid cross contamination

- A sample is one dozen (12) mature whole shellfish (un-shucked), if shellfish are small, sample size should be increased to ensure that a minimum of 100g of flesh is available for testing
- Clean shells of detritus
- Samples must be refrigerated or kept cool (e.g. esky with ice pack), especially if delivery is delayed
- Sample submission forms should be filled in prior to arrival at the laboratory and arrive with the sample to be tested
- Samples should arrive at the laboratory with minimal delay from the time of sampling <24 hr

Equipment required

- Esky
- Ice packs
- Sample jars sample labels
- Water proof pen to fill in labels and sample form
- Specific Laboratory submission form

Method

1. Collect one dozen mature shellfish per sample site.
2. Clean shellfish of detritus.
3. Place shellfish in a zip lock plastic bag and ensure the bag is properly closed.
4. Fill in details for sampling noting species (mussel/oyster/akoya/ clam) growing area and site codes, sampler name, date, harvest time, time into esky (with ice packs, salinity, water temperature and weather details, send details to DoH electronically see appendix 1 and use designated laboratory request form.
5. Place the bag of shellfish and the label in another zip lock plastic bag and ensure it is properly closed.
6. Shellfish need to be kept cool in transit less than 4°C, using ice packs in an esky. Deliver to the laboratory as soon as possible within 24 hours.

2.4 Phytoplankton water sampling procedure

Important points

A tube sampler is used to take a representative sample for deeper water >2m and a bucket to sample water < 2m deep to get one litre subsamples. It is best to sample at high tide or on the incoming tide. Avoid sampling at low tide. Bottles are used to collect a subsample for quantitative count of phytoplankton cell numbers.

Equipment required

- Integrated tube sample pipe of 2m length and 25-40mm diameter with bung or foot valve
- Bucket
- 1L Sample bottles

- Labels
- Pencil or waterproof pen
- Lugols iodine
- Disposable gloves
- Plastic transfer pipette
- Specific Laboratory submission form

Method - Integrated tube and bucket

1. Integrated sample collects water at three depths between 2m and 5m depending on the location of the shellfish in the water column and the depth of the water. A 40mm internal diameter pipe of 2m length can be used to repeat sample at several depths (see pic below).

2. Remove the bung from the bottom end of the tube/pipe sampler. If using one-way foot valve as pictured, open the valve using the rope. Lower the weighted end valve end carefully using the rope (so as not to disturb any layers of phytoplankton in the water column). The pipe must remain vertical in order to take an even sample of the whole water column.

3. Either replace bung securely in top and pull up or if using a one-way valve, use the rope to pull up. This closes off the one-way valve automatically and raise pipe from the water (see picture below) The depth integrated water sample is poured into a large bucket and then well mixed by hand and immediately a IL sub-sample is collected.

4. At those sites where the water depth is less than 2m and the water column is well mixed, a bucket with a rope attached should be used to collect the water sample. Avoid stirring up the sediment before sample collection. Collect at high or incoming tide. Mix the contents of the bucket and immediately fill the sample bottle to the neck by immersing the bottle in the bucket (see picture below). Do not allow the contents of the bucket to settle before filling the bottle.


5. Leave a small airspace at the top of the sample bottle and ensure the cap is screwed on (see picture above).

6. All samples are to be labelled clearly and accompanied with the laboratory sample form and delivered to the analytical laboratory without concentration. Send details to DoH electronically see Appendix 1.

7. Place the bottle in the esky and deliver to the laboratory in less than 24 hours, if delivery time is longer than 24 hours preserve the sample with a few drops of Lugols iodine with a disposable pipette. The sample should resemble a weak tea colour. Use gloves when handling Lugols as the iodine stains.

2.5 Biotoxin Sampling of Shellfish flesh procedure

Considerations for Shellfish Meat Biotoxin sampling

Flesh samples are collected on the same day as the phytoplankton water sample and are either frozen pending the phytoplankton result or submitted as one of the monthly routine samples. If the phytoplankton counts exceed the phytoplankton alert levels (PAL) listed in the MBMMP the shellfish flesh sample (if not already submitted) is submitted for biotoxin testing. If the flesh samples exceed the maximum levels (in the Australia New Zealand Food Standards Code Schedule 19 Maximum levels of contaminants and natural toxicants) the harvest area is closed immediately. If phytoplankton counts do not exceed alert levels the shellfish samples are kept frozen and are held in rotation for a minimum of six weeks.

Voluntary closure at trigger threshold levels can occur in the absence of immediate biotoxin sampling. Reopening of a harvest area closed for biotoxins requires two clear samples one week apart. Sample turnaround time should be considered with respect to meeting harvest re-opening schedules.

Important points

- Hands must be clean to avoid cross contaminating samples

- For leases utilising longlines samples should be representative of the depths being harvested
- All species in an affected harvest area should be tested for biotoxins
- Samples are sent fresh or frozen depending on transit time to the laboratory

Equipment required

- Esky
- Ice packs
- Sample jars
- Ziplock plastic bags
- Sample labels
- Waterproof pen or pencil
- Laboratory submission form

Method

1. Collect one dozen mature shellfish (or at least 100g) of each species that represent those bivalve shellfish that are most likely to be impacted by a bloom
2. Shuck shellfish and drain excess water
3. Place shellfish in sample jar
4. Label, noting species, growing area, lease, sampler name, date, time and purpose (biotoxins)
5. Place jar of shellfish in zip lock plastic bag and ensure closure. Send details to DoH electronically see Appendix 1 and use designated laboratory request form
6. Contact laboratory and ensure delivery times and processing turnaround times for results
7. Place bag on ice packs and delivery within 24hours
8. If delivery time is extended due to interstate processing, then frozen samples should be submitted
9. Appendix 2 provides an example of a biotoxin Laboratory report detailing the different biotoxins and calculation methods

N.B. All microbiological, phytoplankton and biotoxin sample results must be received by the Laboratory in a reasonable time as a delay in the results could cause closure of a harvesting area pending the results.

2.6 Chemical Sampling of Shellfish

Considerations for sampling

Shellfish sampled should have been in the harvest area for a minimum of six months. A sample of mature shellfish of all species cultured are taken. Ensure 100g of each species is available for testing. If repeat testing required a larger flesh sample may need to be submitted. Please check flesh quantity required by testing laboratory before sending and if repeat testing is likely.

Important points

- Ensure your hands are clean

- Collect shellfish from current harvest areas that have been on the lease for at least six months
- Annual or triennial chemical testing required for all harvesting areas
- Check with laboratory test suitability
- Samples usually sent shucked and frozen, check with laboratory

Equipment

- Esky
- Ice packs
- Ziplock bags
- Sample labels
- Water proof pen or pencil
- Laboratory submission form

Method

1. General site sampling - collect shellfish from a mix of sites representing the current harvest areas
2. Specific site sampling - collect shellfish from a mix of baskets in the general vicinity of the sample site or closest proximity to contamination risk
3. Clean shells of detritus
4. Place in zip lock bag whole or in jars if shucked
5. Label noting growing area, sampler name, date, species
6. Place bag or jar of shellfish in another ziplock bag and ensure it is closed
7. Freeze the sample
8. Place bag on ice packs in esky and deliver frozen to laboratory within 24 hours send details to DoH electronically see Appendix 1 and use designated laboratory request form
9. Contact laboratory sample is arriving

N.B. Total arsenic may initially be tested as it is assumed that 10% of total arsenic is present in the inorganic form. Where the result is ≥10mg/kg then separate testing for inorganic arsenic would be required.

Appendix 1 Sampling Program Information Sheet

WESTERN AUSTRALIAN SHELLFISH QUALITY ASSURANCE PROGRAM SAMPLING PROGRAM INFORMATION FORM	
Rainfall (24hr)..... wind direction..... **Rainfall (48hr)..... wind speed.....** **Water Temp. ..... Salinity .....** **Date.....**	**Adverse Conditions** **True ☐ False ☐** **Other comments on conditions e.g. water clarity, fish kills, pollution spills, discolouration, blooms etc.**
Tide: Incoming ☐ Tide: Outgoing ☐	

NAME OF HARVESTING AREA:

Sample Site ID Number	**Microbial Testing**		**Phytoplankton Testing**	**Biotoxin Testing**	**Chemical Testing**
	Water Time Taken	**Flesh Time Taken**	**Water Time Taken**	**Flesh Time Taken**	**Flesh time taken**

LABORATORY: Samples Taken by:

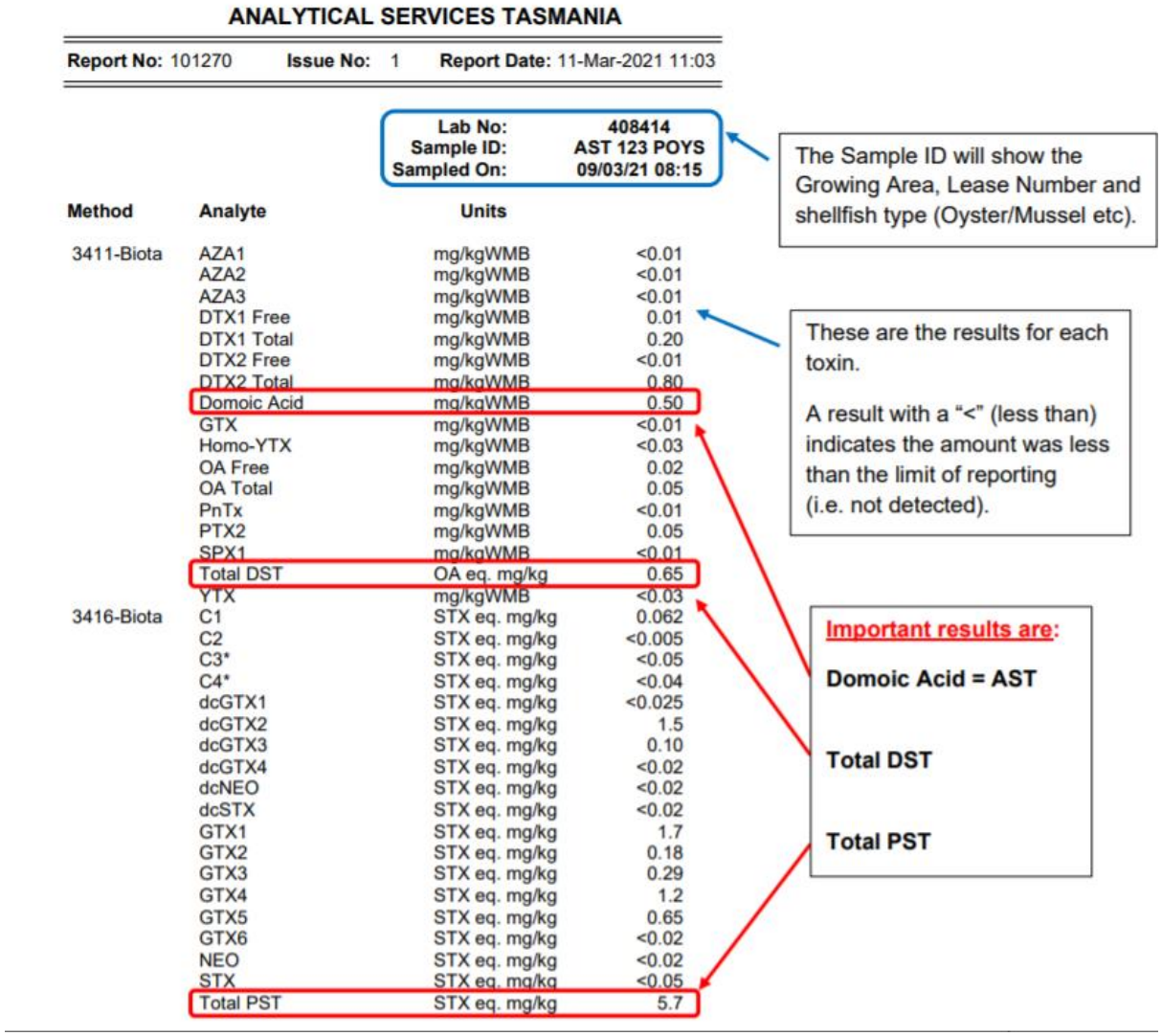
……

Please send this by email to : Food Unit, Department of Health or alternatively scan and send to

tracey.stamp@health.wa.gov.au and scott.whiddon@health.wa.gov.au or foodsafety@health.wa.gov.au

Appendix 2 Interpretation of laboratory results

Reading the biotoxin reports can be difficult due to the range of toxins groups tested by laboratories. Analytical services Tasmania has provided an interpretation guide for their laboratory reports. See example below



ANALYTICAL SERVICES TASMANIA

Report No: 101270 **Issue No:** 1 **Report Date:** 11-Mar-2021 11:03

		Lab No:	**408414**
		Sample ID:	**AST 123 POYS**
		Sampled On:	**09/03/21 08:15**
Method	**Analyte**	**Units**	
3411-Biota	AZA1	mg/kgWMB	<0.01
	AZA2	mg/kgWMB	<0.01
	AZA3	mg/kgWMB	<0.01
	DTX1 Free	mg/kgWMB	0.01
	DTX1 Total	mg/kgWMB	0.20
	DTX2 Free	mg/kgWMB	<0.01
	DTX2 Total	mg/kgWMB	0.80
	Domoic Acid	mg/kgWMB	0.50
	GTX	mg/kgWMB	<0.01
	Homo-YTX	mg/kgWMB	<0.03
	OA Free	mg/kgWMB	0.02
	OA Total	mg/kgWMB	0.05
	PnTx	mg/kgWMB	<0.01
	PTX2	mg/kgWMB	0.05
	SPX1	mg/kgWMB	<0.01
	Total DST	OA eq. mg/kg	0.65
	YTX	mg/kgWMB	<0.03
3416-Biota	C1	STX eq. mg/kg	0.062
	C2	STX eq. mg/kg	<0.005
	C3*	STX eq. mg/kg	<0.05
	C4*	STX eq. mg/kg	<0.04
	dcGTX1	STX eq. mg/kg	<0.025
	dcGTX2	STX eq. mg/kg	1.5
	dcGTX3	STX eq. mg/kg	0.10
	dcGTX4	STX eq. mg/kg	<0.02
	dcNEO	STX eq. mg/kg	<0.02
	dcSTX	STX eq. mg/kg	<0.02
	GTX1	STX eq. mg/kg	1.7
	GTX2	STX eq. mg/kg	0.18
	GTX3	STX eq. mg/kg	0.29
	GTX4	STX eq. mg/kg	1.2
	GTX5	STX eq. mg/kg	0.65
	GTX6	STX eq. mg/kg	<0.02
	NEO	STX eq. mg/kg	<0.02
	STX	STX eq. mg/kg	<0.05
	Total PST	STX eq. mg/kg	5.7

Toxin abbreviation tables supplied by Analytical services Tasmania

Toxin Abbreviations

PST **Paralytic Shellfish Toxins**

- STX Saxitoxin
- Neo Neosaxitoxin
- GTX Gonyautoxins
- dc Decarbamoyl analogue
- C toxins N-sulfocarbamoylgonyautoxins

Total PST is reported as the sum of each of the toxins, corrected for toxicity.

* A star (*) appearing after a toxin (eg. C3* and C4*) in the results designates that the toxin is not covered by NATA accreditation as there are no authentic standards available for purchase at this time.

AST **Amnesic Shellfish Toxins**

- Domoic Acid

DST **Diarrhetic Shellfish Toxins**

- OA Okadaic Acid
- DTX Dinophysistoxin
- PTX Pectenotoxin
- Free/Total Total includes ALL forms (ie. esters)

Total DST is reported as "OA eq. mg/kg" and is calculated as follows:
OA Total + DTX1 Total + 0.5*DTX2 Total

Other Lipophilic Toxins

- AZA Azaspiracid
- GYM Gymnodimine
- PnTx Pinnatoxin
- SPX Spirolide
- YTX Yessotoxin

NST **Neurotoxin Shellfish Toxins**

- PbTx Brevetoxin (not routinely tested)

Results are reported on a Wet Matter Basis (WMB)

Biotoxin report result from Symbio below

Note: Pectenotoxin 2 are tested for EU export markets and not required under WASQAP. There is no extra cost for this test

Analytical Results

Compound/Analyte	Method	LOR	Units
Total PSP Screen	04_089 - PSP Screen by HPLC-FLD	0.025	mg STX eq./kg
Domoic acid	04_091 - ASP/DSP/Lipophilic toxins in shellfish by LCMSMS	1	mg/kg
Total DSP	04_091 - ASP/DSP/Lipophilic toxins in shellfish by LCMSMS	0.015	mg OA eq./kg
Pectenotoxin 2	04_091 - ASP/DSP/Lipophilic toxins in shellfish by LCMSMS	0.025	mg PTX2 eq./kg

Appendix 3 Laboratory contact list

Analysis	Product	Laboratory	Address	Phone
E.coli	flesh	Pathwest Food Hygiene Laboratory	2nd Floor J Block Hospital Ave Nedlands 6009	08 64572165
E.coli	flesh	Symbio Laboratories-Perth	2/2-4 Mallaig Way Canning Vale 6155	1300 703 166
E.coli	flesh	Agrifood Technology	38 Clark Court, Bibra Lake 6163	08 9418 5333
Thermotolerant coliforms	water	Pathwest Water Examination Laboratory	2nd Floor J Block Hospital Ave Nedlands 6009	08 64572583
Thermotolerant coliforms	water	ALS Environmental	26 Rigali Way Wangara Perth 6065	08 9406 1301
Thermotolerant coliforms	water	Agrifood Technology	38 Clark Court, Bibra Lake 6163	08 9418 5333
Thermotolerant coliforms	water	Analytical Reference Laboratory -Perth	46-48 Banksia Road, Welshpool 6106	(08) 6253 4444
Thermotolerant coliforms	water	Pro Micro	46-48 Banksia Road, Welshpool 6106	(08) 9401 5699
Biotoxins PST, AST,DST's	flesh	Symbio Laboratories - Sydney	2 Sirius Rd Lane Cove West Sydney NSW 2066	1300703166
PST,AST, DSTs	flesh	Analytical Services Tasmania	18 St Johns Ave New Town Tas 7008	61653300
Phytoplankton ID and enumeration of toxin producing species	water	Dalcon Environmental	Building 38, 3 Baron-Hay Ct South Perth 6151	08 9368 3616

Heavy metals:inorganic arsenic, copper, zinc, cadmium, lead, mercury, organochlorine, organophosphate, pesticides and polychlorinated biphenyls.	flesh	Murdoch University Marine and Freshwater Research Laboratory	96 South St Murdoch	08 9360 2907
		National Measurement Institute Perth	26 Dick Perry Ave Kensington	08 9368 8420
		ChemCentre	Level 2 Building 500 South entrance drive Curtin University	08 9442 9800
		Symbio Laboratories - Brisbane	52 Brandl St Eight Mile Plains Qld 4113	1300703166
Cadmium, lead, mercury, arsenic	flesh	Analytical Reference Laboratory -Perth	46-48 Banksia Road, Welshpool 6106	08 6253 4444

Important to note that current acceptable methods for analysis and the thresholds for testing are outlined in the latest ASQAP manual. The ASQAP manual complies with the Food Standards Australia New Zealand Food Standards Code and Export Orders as they relate to bivalve molluscs. Standard 1.4.1 Contaminants and natural toxicants and Schedule 19 lists the Maximum levels of contaminants and natural toxicants for shellfish.

Please check with the laboratory that they are NATA certified for the method they are using and that the level of test sensitivity or limit of reporting is suitable for WASQAP *e.g. threshold for flesh testing for microbiological testing needs to be below 2.3 E.coli per gram of flesh and for thermotolerant coliforms <1 CFU/100ml in water samples. Not all laboratories have correct sensitivity for the required test.*

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