

UK-NRL

TITLE: STANDARD OPERATING PROCEDURE (SOP) FOR THE TRANSPORT, RECEPTION AND SHORT TERM STORAGE OF BIVALVE MOLLUSCS, TUNICATES AND ECHINODERMS PRIOR TO TESTING FOR MARINE BIOTOXINS IN THE UK'S STATUTORY BIOTOXIN MONITORING PROGRAMMES

Production Summary

Author:	UK-NRL
Reviewed by:	UK-NRL
Date:	XX February 2020
Issue Authorisation	UK-NRL for Marine Biotoxins ¹

Distribution of copies

Authorised Recipient	Location
NRL Representative	UK-NRL
Cefas Representative	CEFAS
AFBI Representative	AFBI
FSA Representative	FSA, London
FSS Representative	FSS, Aberdeen
FSANI Representative	FSANI, Belfast

History of procedure

Issue	Date issued	Changes
1	May 02 2006	
2	June 2010	Use of Coleman boxes detailed Storage conditions updated
		Conditions under which specific cool boxes should be used.
3	February 2015	Use of updated Coleman boxes
4	February 2020	EU Regulation reference updated Addition of Igloo profile 16 cool box to list of suitable transport boxes
		Table 1 short term storage criteria updated

¹ United Kingdom National Reference Laboratory for Marine Biotoxins (AFBI, Northern Ireland)

1

INTRODUCTION

The SOP applies to bivalve molluscs, echinoderms, tunicates and gastropods to be tested for marine biotoxins listed in EU Regulations **2017/625 and 2019/627** within the UK statutory biotoxins monitoring programme. It is based on recommendations of the UK-NRL Network Working Group on transport/storage for statutory biotoxin monitoring (6 and 7th October 2004). The SOP aims to standardise storage conditions of shellfish samples during transport to the test laboratory and upon receipt at the laboratory and to standardise short term sample storage requirements prior to testing.

The SOP sets out time and temperature criteria to ensure as far as possible, animals taken for analysis arrive at the laboratories in the best possible condition, and the possibility of the total toxin content changing during transport and storage is minimised.

To aid the regulation of temperature control, where the time from harvest to receipt at the laboratory exceeds 12 hours, the UK-NRL recommends the use of Biotherm™ boxes for transport of shellfish (UK-NRL Transport Trials Report, 2005). Given the records on performance across the Scottish monitoring programme, and when used in accordance with specifications given in this SOP, the Coleman 16Q box is considered a suitable alternative. As of February 2020 the Colman 16Q boxes are no longer commercially available and an alternative product is now required. Cefas have carried out an alternative sample transport box assessment and demonstrated that the Igloo profile 16 (small 15 litre) cool box meets the required performance criteria when used in accordance with specifications given in this SOP (see literature reference page 11).

For geographical locations close to the monitoring laboratories, providing that the laboratories can supply evidence that the samples arrived within 12 hours of harvest and that the samples comply with the criteria set out in this SOP, the use of other coolboxes is considered an adequate alternative.

Relevant instructions from this SOP should be included in sample collection documents issued to parties responsible for collection and packing of samples at the harvesting point.

2 **EQUIPMENT**

Use of Biotherm™ boxes

Freezer at $\leq -10^{\circ}\text{C}$

Biotherm™ 10L or 25L boxes, and validated summer kit (supplied by Intelsium DGP Group, <http://www.intelsium.com/product/temperature-controlled-packaging-and-cold-chain-solutions/biotherm>).

Prior to shellfish collection the cool packs provided with the summer kit (3 per Biotherm box) should be chilled in a freezer for a minimum of 24 hours.

Use of Coleman 16Q coolboxes

Freezer at $\leq -10^{\circ}\text{C}$

Coleman 16Q box (either model no 6216/6215 or model no 5877)

Cool packs previously chilled in a freezer for a minimum of 24 hours. A sufficient number of coolpacks is required to ensure that in each coolbox, the sample(s) can be placed between 2 complete layers of coolpacks.

Flexible non-cross linked polyethylene foam spacers

Use of Igloo profile 16 coolboxes

Freezer at $\leq -10^{\circ}\text{C}$

Igloo profile 16 coolbox (small 15litre)

Cool packs previously chilled in a freezer for a minimum of 24 hours.

Sufficient numbers of coolpacks are required to ensure that in each coolbox, the sample(s) can be placed between 2 complete layers of coolpacks.

Flexible non-cross linked polyethylene foam spacers

Other coolboxes

Freezer at $\leq -10^{\circ}\text{C}$

Coolbox

Cool packs previously chilled in a freezer for a minimum of 24 hours. A sufficient number of coolpacks is required to ensure that in each coolbox, the sample(s) can be placed between 2 complete layers of coolpacks.

Newspaper or other insulating material

At the laboratory

Refrigerator at 2°C to 8°C (monitored)

Calibrated thermometer or calibrated temperature probe

3. PREPARATION OF SAMPLES FOR TRANSPORT

3.1 Instructions from this section (sample preparation for transport) should be issued to authorities responsible for sample harvest and transport.

3.2 Laboratories providing evidence that samples from specific sites are received within 12h of harvest may use alternative coolboxes for sample transport from those sites. An evaluation period should be in place to ensure samples do not regularly exceed 12h delivery. The temperature upon receipt should also be recorded for further evaluation.

If more than 12h routinely elapses from sample collection to receipt at the laboratory, Biotherm™, Coleman 16Q or Igloo profile 16 boxes must be used.

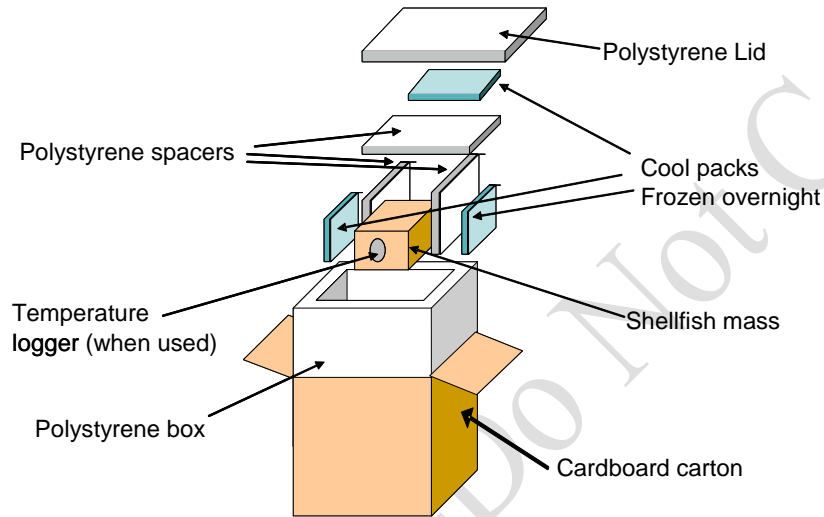
USING BIOTHERM™, COLEMAN 16Q or Igloo profile 16 BOXES

3.3 Shellfish that have organoleptic characteristics associated with freshness must be selected and placed inside a polythene bag (allow air space above the sample before tying the bag), which is then put inside the boxes along with pre-frozen cool packs (see Fig. 1 for Biotherm™ and Fig. 2 for Coleman 16Q and Igloo profile 16) with the spacers placed to prevent the sample coming into direct contact with coolpacks.

3.4 The pre-printed sample information sheet should be completed and placed inside a separate bag and secured within the box.

3.5 Once correctly assembled secure the box lid with adhesive tape to prevent leakage and send *via* the delivery option requested by the test laboratory.

Fig 1



- Top 2 layers of foam
- Top layer of 3 ice packs
- Layer of foam
- Sample in polythene bag
- Layer of foam
- Bottom layer of 3 ice packs
(Please note that for the new version* of the Coleman 16Q, 4 ice packs should be placed on the bottom layer)

Fig 2: Packing Coleman 16Q and Igloo profile 16 coolboxes

**New version, model no 5877
Original version, model no 6216/6215*

COOLBOXES

- 3.6** Shellfish that have organoleptic characteristics associated with freshness must be selected and placed inside a polythene bag which, in turn is wrapped in newspaper (or other insulating material) to prevent the sample from coming into direct contact with the ice packs and freeze. Position the wrapped up sample inside the coolbox between 2 layers of pre-frozen cool packs.
- 3.7** The pre-printed sample information sheet should be completed and placed inside a separate bag and secured within the box
- 3.8** The coolbox lid should be secured in place with tape and delivered to the test laboratory to arrive no later than 12h after harvesting.

4 DELIVERY AND RECEIPT

- 4.1** The sample should be delivered to the monitoring laboratory as soon as practical after collection.
- i. If the time between harvest and receipt at the test laboratory is anticipated to be greater than 12 hours, samples must be transported using Biotherm™, Coleman 16Q or Igloo profile 16 coolboxes.
 - ii. If the time between harvest and receipt at the test laboratory is anticipated to be less than 12 hours, alternative coolboxes may be used.
- 4.2** Laboratory staff should check that samples arriving in alternative coolboxes are from those sites where use of these coolboxes is approved.
- 4.3** Samples from sites where Biotherm™, Coleman 16Q or Igloo profile 16 coolboxes have not been used and application of alternative coolboxes has not been approved should not be processed unless specific instructions to the contrary have been given by the competent authority.
- 4.4** Defective or materially damaged boxes should be withdrawn from circulation.
- 4.5** Samples should be received and logged according to each laboratory's relevant SOP recording the temperature of the sample mass and either the

date and time of harvesting and receipt at the laboratory or the time elapsed between collection and receipt.

- 4.6** Samples arriving in Biotherm™ boxes or alternative coolboxes should be within a 2 – 10°C window at the time of receipt, with the exception of those samples harvested from warm waters and transported to the laboratory within 4 hours of harvest (i.e. insufficient time for coolpacks to reduce temperature) and those samples (for example processed scallops) already in a frozen state when collected by the local authority.
- 4.7** Evidence suggesting that the Biotherm™, Coleman 16Q or Igloo profile 16 coolboxes are no longer performing satisfactorily (i.e. sample temperature regularly exceeds 2-10°C) should immediately be brought to the attention of the UK-NRL and the FSA, FSS and FSANI.
- 4.8** Any defects in the manner in which the Biotherm™ boxes/coolboxes have been packaged (insufficient cool packs, improper placement of cool packs relative to the sample mass) will be recorded. Persistent evidence of poor packaging should be brought to the attention of the responsible Local Authority / collection centre.
- 4.9** Samples can be further processed providing they have organoleptic characteristics associated with freshness, including, and where appropriate, shells free of excessive dirt, an adequate response to percussion and normal amounts of intravalvular liquid. Samples which do not exhibit these organoleptic characteristics should not be analysed.
- 4.10** Any animals that are frozen (unless already in a frozen state when collected), gaping or are severely damaged should not be included in homogenates.
- 4.11** Samples which exceed the specified transport period and temperature requirements may be analysed with the minimum of delay providing the organoleptic properties described above are met. The collection date and the date of receipt at the laboratory as well as the recorded temperature must be noted alongside the reported analysis result.

5 SHORT TERM STORAGE AT THE LABORATORY

5.1 Whole shellfish, shellfish homogenates and shellfish extracts should not be frozen prior to official control analysis. Shellfish in a frozen state when sampled may be stored frozen until analysis unless they have already started to thaw when received at the laboratory.

5.2 Shellfish should be extracted and tested within the laboratory as soon as practical upon receipt. In cases where sample storage is unavoidable storage criteria which apply are defined on Table 1. Storage details should be recorded.

Table 1: Storage criteria for whole shellfish, homogenates and extracts prior to official control analysis

Matrix	Conditions to apply upon storage
Whole shellfish	<p>Whole shellfish which meet the criteria of section 4.1 may be stored so that no longer than 72 hours elapses between sample harvest and sample extraction. For example:</p> <ul style="list-style-type: none"> a) a sample which takes 24 hours to reach the laboratory may be stored for a further 48 hours in the laboratory b) a sample which takes 48 hours to reach the laboratory may be stored for only 24 hours in the laboratory. <p>Unless the shellfish were already in a frozen state when collected, shellfish should be stored at 2-8°C. Frozen shellfish that have not yet started to thaw may be stored at ≤-10°C.</p>
Shellfish homogenates	Should not be stored prior to extraction

100% Methanol extracts for LCMSMS analysis of lipophilic toxins	May be stored at -20°C for a maximum of 28 days
50 % (v/v) aqueous methanol for ASP analysis	May be stored at 2-8°C for a maximum of 5 days
HCl extract (0.1M pH 2.5-3.5), acetic acid extract and C18 extracts	May be stored at 2-8°C for a maximum of 5 days in total from the day of extraction. In the event C18 extracts require storage prior to periodate and/or peroxide oxidation, the cleaned extracts may be stored with or without any pH adjustment. Care should however be taken to ensure this is clearly marked.
Oxidised extracts for PSP analysis	Oxidised extracts must not be stored.

LITERATURE

Recommendations of the first meeting of the UK-NRL Network Working Group on transport/storage for statutory biotoxins monitoring. Oct 2004

Regulations (EC) No 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure application of food and feed law; and Regulation (EC) No 2019/627 of the European Parliament and of the Council 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 2017/625.

Effect of storage on Amnesic shellfish poison (ASP) toxins in king scallops (*Pecten Maximus*), Smith E.A. et al. Harmful Algae 5 (2006), 9-19.

Validation report for Coleman Excursion transport box (version 2)

Assessment of alternative sample transport boxes for the purpose of the E.coli and toxin monitoring programmes – summary report: Cefas 29/01/20

Public Version-Do Not Copy