Western and Central Pacific Fisheries Commission

Voluntary HSBI Regional Guides

TOOLS FOR HIGH SEAS BOARDING AND INSPECTIONS

HSBI DNA Sampling Guide

Document History

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1.1	31 October	For WCPFC22 Consideration	HSBI WG	HSBI WG Chair
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PURPOSE STATEMENT

1. This document provides guidance to Authorised inspectors conducting tissue sampling and subsequent DNA sequencing and analysis (hereafter, broadly referred to as "DNA sampling") as part of WCPFC High Seas Boarding and Inspections (HSBI[1]). For CCMs wishing to use DNA sampling for HSBI, the development of this Guide also intends to:
   - support the establishment of a robust DNA sampling process by CCMs at the CCM's level to verify species identification of individual specimens in support of HSBI
   - assist CCMs in ensuring that DNA data are credible and admissible using appropriate methods and procedures throughout the entire DNA sampling process
   - support the establishment of minimum practices at the CCM's level which are necessary to ensure that DNA sampling produce accurate, precise analytical findings, and findings are conveyed in an unbiased, objective manner

[1] HSBI, refers to boarding, inspection, and related activities on the high seas within the Convention Area conducted pursuant to CMM 2006-08 Western and Central Pacific Fisheries Commission Boarding and Inspection Procedures or any successor CMM.

- provide guidance to CCMs on tools that can be used for gathering and preserving tissue samples during HSBI and the minimum standards for DNA sequencing and analysis, where the results or findings are intended to be admissible as evidence in support of potential court or administrative proceedings

2. This Guide sets out the minimum standards in the application of DNA sampling during a HSBI and the post analysis process, which includes:
   - tissue sampling
   - tissue sample handling, preservation, sealing and storage
   - tissue sample transfer/shipping
   - DNA extraction, sequencing, and analysis in accordance with accredited procedures
   - transmission of DNA results
   - DNA sequence, extracted DNA, and tissue sample retention and accessibility for flag CCM testing.

3. The application of this Guide will be voluntary and apply to authorised HSBI activities within the WCPFC convention area.

4. This guide can be modified in response to new information, technical innovations, and perspectives. It is expected that this guide will continue to evolve as the field develops.

Application of DNA sampling in WCPFC HSBI activities.

5. The aim of HSBI is to check whether a vessel is operating in compliance with the WCPFC Convention and all applicable WCPFC Conservation and Management Measure (CMM) obligations.

6. Inspectors conducting HSBI activities can detect and confirm species on board at the time of inspection. Sometimes, a visual inspection of morphological characteristics may be all that is needed to obtain a species identification.

7. The application of molecular genetics offers a powerful tool to complement the work of Authorised inspectors conducting HSBI activities, including the conclusive identification of specimens at the species level.

8. Genetic analysis can be a useful method for species identification when species identity cannot be determined on a purely morphological basis, for example if the morphological characteristics are unfamiliar to the inspector, similar, or are absent (e.g. processing of specimens retained onboard).

9. Genetic analysis through DNA sequencing of fish for identification can support investigations to verify a vessel's reported catch, through providing additional proof and the ability to confirm the identity of the species in question. Examples include, to determine between:
   - Pacific and Southern bluefin tunas
   - small-sized bigeye and yellowfin tunas, and

- different bycatch species that are prohibited for retention.

10. DNA sequencing results can be used to corroborate other forms of evidence such as vessel logbooks and photographs taken by the Authorised inspectors. This can be used, among other tools and sources of information, to support risk assessments to prioritise the vessel for further inspection, investigation and prosecution as determined by the flag CCM.

11. The use of DNA sampling during HSBI can assist the flag CCM, or the inspecting CCM, where field-based tools are applied, with assessing compliance with vessel licensing, catch and reporting obligations, including to:
    - confirm species identification
    - verify that only species which a vessel is authorised to catch are being retained and declared
    - verify catch reporting and catch log data
    - verify, or provide rapid screening for retention of protected species.

HSBI DNA SAMPLING Minimum Standards

Methods of tissue sampling (not limited to)

Laboratory-based analysis		Field-based analysis
Muscle Biopsy	Fin Biopsy	Other
DNA biopsy sampling involves taking a tissue sample from a single fish.	Tissue sample is collected from a single fish through cutting off a section of the fin.	Rapid field-based DNA testing (ie. environmental/tissue samples) designed for risk assessment and screening purposes only.

EVIDENTIARY PROCEDURES for DNA Sampling

12. The general principles and procedures for DNA sampling for evidentiary purposes in fisheries monitoring and investigations:

a) Documenting and recording tissue sampling

13. Tissue sampling should be documented using a recording device, including photographs and videos. To the extent practicable, the entire sampling process (and any field-based DNA testing, if conducted) should be recorded (preferably with video) for evidentiary purposes. Additional notations with details of suspected infringements and time stamps for relevant tissue sampling and analysis results should be provided with the video files.

14. Tissue sampling should be conducted by authorised inspectors, with witnesses' present (master or crew), especially if not documented with a recording device (and prioritise that witnesses from the fishing vessel are present.

15. Authorised inspectors should record in the HSBI report, including but not limited to the following information related to DNA sampling:
    - tissue sampling information:
        - sample identification number
        - location of fish sampled (e.g. blast freezer, hold #)
        - description (processed state of fish)

  - comments, including the reason for or background of the tissue sampling
- the master of the vessel must be provided with an interim copy of the report which includes details of any tissue sampling. The master must also be given to opportunity to include any objection or comment to be included in the final report.

b) Collection and preservation of tissue samples

16. Authorised inspectors should:
- photograph and video record tissue sampling (see chain-of-custody, below)
- take tissue samples, to the extent practicable, from the commercially least valuable part of the fish, such as the tail.
- take at least two tissue samples and ideally three samples from the same fish, one for the inspecting CCM and one for the flag CCM (if requested) and one for the laboratory to keep as a backup and confirm in case of diverging results].
- collect, label, preserve and seal each tissue sample separately, at the sampling site.
- label each tissue sample with the following minimum details on the sample labels:
  - Date
  - Unique sample reference number
  - Vessel name
  - Collector name
  - Witness name, role and signature
- affix the waterproof sample labels directly to the sample vials or collection bags.
- secure each sample container or evidence bag containing the tissue sample, with a tamper-evident seal. The seal should be signed by at least one authorised inspector and the vessel master, or a crew member designated by the master.
- ensure both vials and associated evidence bags can be traced back to the same sampled fish.
- photograph the sealed container or evidence bag showing these signatures.
- keep the tissue samples in a freezer. If a freezer is not available samples should be stored in a cool, dark environment, as long as they are not deteriorated.

c) Preventing cross-contamination of tissue samples

17. Protective measures are necessary to prevent cross-contamination of samples. The following should be used for each individual sample:
- Use new or unopened sampling tools.
- Wear single-use disposable gloves.

d) Maintaining a chain of custody for tissue samples between the sample site and the testing laboratory

18. From the beginning to the end of the DNA sampling process, it is crucial to be able to demonstrate every single step undertaken to ensure traceability and continuity of the sample. The integrity of tissue samples and, later, extracted DNA samples, must be maintained as they pass from one person to another.

19. The ‘chain of custody’ is a continuous record of the life of the sample from the moment it was sampled to the moment it is analysed. Every step must be recorded and verified to ensure the

sample is not tampered with, changed or lost. It is the Authorised inspector's responsibility to ensure chain of custody of the tissue sample by ensuring:

- the tissue samples are stored in a tamper-evident sealed bag or envelope, preferably stamped with a unique serial number.
- the chain of custody record is maintained.
- the tissue samples are sent to an accredited laboratory for testing as outlined in the CCM's DNA sampling procedures.

e) Transmission of DNA sampling results to the flag CCM

20. Authorised inspectors should note in the full inspection report, that DNA sampling occurred.

21. The inspecting CCM should update without delay, but no later than 30days, the flag CCM of the sample arriving in port and advise them when testing results are expected to be available, if not already shared with flag CCM.

22. Timing of DNA analysis and results will vary depending on circumstances, such as:
    - time for tissue sample to return to port
    - time to facilitate arrangements to deliver the tissue sample to an accredited laboratory
    - time to deliver the tissue sample to an accredited laboratory
    - time for extraction, sequencing, and analysis of the DNA by an accredited laboratory.

23. Once the finalised DNA sampling results are received by the relevant authority of the inspection vessel, they should be provided, together with the inspection report, to the flag CCM within 5 business days. In addition to the results, the credentials from the testing laboratory and recognised standards as outlined in the CCMs Sampling Procedures including the DNA extraction, analysis method information and reference sequence database used should be attached.

CCM DNA sampling and analysis procedures

24. CCMs wishing to use DNA testing for HSBI evidentiary purposes should share their DNA Sampling for Evidentiary Purposes Procedures with the Secretariat for posting on the HSBI page on the WCPFC website.

25. The CCM's DNA Sampling for Evidentiary Purposes Procedures should include:
    - DNA sampling method for HSBI activities
    - DNA sampling procedures for HSBI activities
    - Chain of custody form
    - Details of testing Laboratory and credentials and recognised standards, these could include:
        - ISO 17025 / 9001 – *this accreditation supports laboratories in maintaining complex processes of testing and calibration to the highest standards and demonstrates to external clients that the laboratory outputs are valid and reliable.*
        - Quality Management Systems (QMS)

- Society for Wildlife Forensic Science (SWFS) Standards and Guidelines for Wildlife Forensic Analysis – *the minimum standards and additional guidelines for wildlife forensic analysts in the sub discipline of DNA*
- Genetic reference database – *used for species assignment for WCPFC catch and compliance. This should include the GenBank Accession number of the reference sequence used for positive species identification*.
- Sample retention and accessibility – tissue and DNA extracts should be retained, for up to 2 years to allow for future testing by the flag CCM, if requested.
- If requested, the DNA sample should be sent to the flag CCM within 30 days after DNA extraction, sequencing and analysis by the laboratory.
- The flag CCM retains the right to conduct DNA sampling to verify the sampling results submitted by the inspection vessels. If the flag CCM conducts DNA sampling, it should observe the same standards as set out in this guide and follow similar methods to the original testing as much as possible (including assay and genetic reference database used for species identification).

Accessibility of DNA Sampling and Multi-language information

26. To assist the DNA sampling process during HSBI activities, it may be beneficial for the CCM's DNA sampling procedures to be translated into languages that are in use on fishing vessels and/or as pictographs to bridge any language barriers.

27. The following supporting documentation could be considered for translation into flag CCM languages by the Authorized inspector CCMs:
    - HSBI multi-language cards
    - DNA sampling for Evidentiary Purposes procedures provided online.
    - DNA sampling for Evidentiary Purposes procedures potentially given/shown to master of vessel prior to DNA sampling by HSBI Authorised inspectors.

28. In addition, flag CCMs should also consider providing information about DNA sampling procedures that may be used during HSBI Inspections to their fishing vessels in a language(s) used by their vessels.

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