



**Cooperation Centre for Scientific Research
Relative to Tobacco**

CORESTA Guide N° 11

Technical Guide for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products

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**Tobacco and Tobacco Product Analytes
Sub-Group**



CORESTA TECHNICAL GUIDE N° 11

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Technical Guideline for Sample Handling of Smokeless Tobacco
and Smokeless Tobacco Products

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0. INTRODUCTION

This Technical Guideline for the handling of Smokeless Tobacco and Smokeless Tobacco Products (STPs) has been developed by the CORESTA Tobacco and Tobacco Product Analytes Sub-Group to provide guidance to the tobacco industry and independent testing laboratories.

The appropriate storage and preparation of tobacco samples is one of the most important factors regarding the achievement of representative and reproducible test results. A laboratory can only produce high quality results if the integrity of samples is maintained.

The procedures for the representative sampling of tobacco products can be found in CORESTA Recommended Method No. 71 (CRM No. 71; Smokeless Tobacco Products - Sampling) and CORESTA Guide No. 13 (Guidance for Sampling the Tobacco Leaf Supply Chain). Please note the importance of the precautions to be taken when sampling. Careful documentation during sampling is required. All relevant information, such as when and where a sample was taken and under what conditions it was taken should be clearly recorded. This is necessary because variations in sampling procedures can have a marked effect on the results of analysis.

In addition to the sampling procedure, appropriate sample handling (e.g. storage and preparation) prior to analysis is also important to ensure representative analytical results. It is important to ensure that the passage of a sample through the laboratory's analytical system is fully documented and corresponds to each laboratory's relevant Standard Operating Procedure (SOP).

Sub-sampling or splitting of a sample into two or more aliquots may be necessary for some types of products or analytes. Different types of sample pre-treatment may also be necessary to homogenize different types of product before analysis can be performed.

1. FIELD OF APPLICATION

This Technical Guide is applicable for the sample handling of Smokeless Tobacco and Smokeless Tobacco Products before analysis and retention of samples. In Tables 1 & 2, matrices display the recommended sample preparation and sample storage per product type and analyte.

2. TERMS and DEFINITIONS

2.1 Laboratory Sample

A sample intended for laboratory inspection or testing.

2.2 Test Sample

Sample as prepared for testing, taken at random from the laboratory sample and which is representative of each of the increments making up the laboratory sample.

2.3 Test Portion

A group of STPs prepared for a single determination and which is a random sample from the test sample or conditioned sample as appropriate.

3. PURPOSE

The objective of this guideline is to describe the sample handling (e.g. sample storage and preparation) after the sample has reached the laboratory.

It is difficult to recommend a guideline for sample handling that will address every possible situation. The objective of sample handling is to store and prepare the sample for analysis without sample deterioration. For example, the elapsed time between sampling and testing will affect sampling and handling parameters. This guideline will describe the sample storage and preparation of samples that has reached the laboratory.

Variables such as particle size, moisture content and analyte volatility can impact sampling/handling parameters. Other considerations are:

- Amount needed for all analyses - including amounts for retests.
- Containers for storage to ensure sample integrity, such as metal or plastic, clear or amber, Ziploc® bag or gas/air-tight container.
- Freezer or refrigerator storage.
- Sample preparation (i.e., grinding or no grinding).
- Other circumstances may have an impact and require different materials and unique storage conditions.

4. SAMPLING

This guideline describes the sample handling after the sample has reached the laboratory.

It is recommended that after the samples have been collected, they shall be packed securely with adequate protection against damage and sent to the laboratory by the most expeditious means reasonably available. The amount of each sample needed is related to the requested analysis.

The shipping conditions should preferably keep the samples cold.

5. SAMPLE PREPARATION AND STORAGE

5.1 Sample Preparation

After arrival at the laboratory, the sample should be prepared in accordance to **Table 1**. However, if there is specific demand of particle size or preparation in the analytical method, then that preparation should be followed.

It is important to ensure that sample integrity is maintained when being ground. Cross-contamination between samples must be prevented by implementing appropriate cleaning procedures between samples and checks where appropriate. When preparing samples; i.e., grinding, contamination of samples by metals (Cr and Ni) may occur, therefore an appropriate grinder should be selected to prevent such contamination. In addition, when grinding, the temperature should be controlled, especially when grinding several samples with various particle sizes. An increase in temperature in the grinding mill could affect the sample material especially if volatile compounds are present. Some compounds are so volatile that grinding the sample while frozen will be necessary (e.g. carbonyls).

Moist products with small particle size (< 4 mm), like snus and fine cut moist snuff could normally be analyzed without further preparation.

5.2 Recommended Containers

Appropriate storage of samples is very important and storage containers should be selected to prevent deterioration (e.g. degradation, loss of water, uptake of water, or contamination). Depending on short-term or long-term storage of samples, different storage containers could be used, some recommended containers are: Original package, light protected glass jar, or light protected gas tight plastic bags with minimal headspace. If samples are stored in original package as cans, then after opening, the sample from the can should not be reused again at a later occasion. Appropriateness of storage container should be verified by the testing facility before use.

5.3 Sample Storage

After arrival at the laboratory, but before analysis, the sample should be stored in accordance to Table 2. However, if the sample was taken or shipped refrigerated or frozen, then it should be stored in a refrigerator or freezer.

If full scope of analyses must be performed (according to Table 2), enough sample material for the different analyses need to be stored in accordance to recommended conditions for the specific analysis.

General recommendations:

1. Refrigerator:

Smokeless tobacco samples should be stored in a refrigerator preferably in their original unopened packaging or in a composite sample until required for sample preparation or analysis.

Note 1: Samples for Carbonyls and NDMA must be kept in unopened packaging.

2. Freezer:

If smokeless tobacco samples are to be kept for longer than three weeks prior to any sample preparation or analysis it is recommended that they are stored in a freezer until needed.

Note 2: Samples for Carbonyls shall only be stored 1 week in refrigerator.

Note 3: Samples removed from the freezer should be placed unopened in the refrigerator for approximately 24 hours to ensure water has sufficient time to fully equilibrate throughout the sample. At the time of analysis, samples should be allowed to equilibrate to room temperature for a minimum of 2 hours before being opened for weighing. Do not use an opened can for re-analysis of carbonyls.

Note 4: Products containing relatively volatile analytes should be processed or stored as quickly as possible.

Note 5: Dry samples can take up water in ambient conditions.

As mentioned above in purpose, it is difficult to recommend a preparation/storage scheme that will address the various characteristics of all smokeless tobacco and smokeless tobacco products. Therefore, samples should be tested as soon as possible after being prepared.

Table 1. Summary of sample preparation required for various smokeless tobacco products

STP	Description	Pool / Composite	Grind	Non-Tobacco Components
(Pouched) Snus	Finely ground tobacco mixed with salts, water, aromatic substances. Product is pasteurized and kept cool to avoid fermentation. Moisture content up to 50%. Examples – CRP 1, CRP 1.1	No	No	Include ⁴
Moist Snuff	Air-cured or fire-cured tobacco fermented and processed into fine particles, fine cut, or strips, long cut. Moisture content up to 50 %. Examples - CRP 2; CRP 2.1	No	No	n/a
Dry Snuff	Inhaled up the nostril. Tobacco is fire-cured, then fermented and processed into a dry, powder. Moisture ≤ 10 %. Examples - CRP 3; CRP 3.1.	No	No	n/a
Loose Leaf	Chewing tobacco. Air-cured tobacco cut or granulated and loosely packed to form small strips of shredded tobacco. Most brands are sweetened and flavored (e.g. with liquorice). Examples – CRP 4, CRP 4.1	Yes ¹	Yes ¹	n/a
Hard Snuff	Heat-treated, ground and mixed with flavoring, packed into pouches or pressed into lozenges.	No ²	No ²	n/a
Plug	Chewing tobacco. Consists of Burley and bright tobacco pressed into cakes and cut before packing. Moisture <15 %.	Yes ³	Yes ³	n/a
Twist	Chewing tobacco. Created by twisting the cured and flavored leaves into a shape that resembles a pig-tail.	Yes ³	Yes ³	n/a

1 - CRP 4.1 (loose leaf) has been subjected to an additional manufacturing step and does not require pooling or grinding.

2 - Hard snuff should be pooled and ground if it is not dissolvable in the extraction solvent used for analysis.

3 - Samples with large moisture content that require grinding may be cryoground (frozen with liquid nitrogen or carbon dioxide prior to grinding).

4 - Pouched material is included with test sample. Pouched samples are analyzed in multiples of their entirety.

Table 2. Storage recommendation

Analyte family	Species	Moist Products ¹	Dry Products ²	Composite sample
Analytes with stability challenges				
Carbonyls	Acetaldehyde, Formaldehyde	C1	C1	Not recommended
Acrylamide	Acrylamide	C3	C12	OK
Hydrogen ion	pH	C3	C12	OK
Nitrogen Compounds	Nitrite	C3	C3	OK
Mycotoxins	Ochratoxin A	R6	R6	OK
Analytes stable in fridge				
Alkaloids, minor	Anatabine,	C12	C12	OK
Humectants	Glycerol, Propylene Glycol	C12	C12	OK
Oven volatiles	Moisture	C12	C12	OK
NDMA	NDMA	C12	C12	Not recommended
Nitrogen Compounds	Ammonia	C12	C12	OK
Sugars	Glucose, Fructose	C12	C12	OK
TSNA	NAB, NAT, NNK, NNN	C12	C12	OK
Analytes stable in room conditions				
Alkaloids	Nicotine	R12	R12	OK
Alkaloids, minor	Anabasine, Nornicotine	R12	R12	OK
Chloride	Chloride	R12	R12	OK
Elements	As, Cd, Cr, Hg, Ni, Pb, Se	R12	R12	OK
Nitrogen Compounds	Nitrate	R12	R12	OK
PAH's	BaP	R12	R12	OK
Water activity	Water activity	R12	R12	OK

1 - CRP1.1, CRP2.1, CRP4.1 Snus, Moist snuff and Chewing tobacco containing around 20-50 percent water

2 - CRP3.1 Dry snuff, containing around 10 percent water

Key to table

- C1:** Should be analyzed within a week. Store in refrigerator (1 °C to 8 °C) for up to 1 week. If stored longer than 1 week, store in freezer until analysis (< -18 °C). Avoid freezing/thawing.
- C3:** Refrigerator (> 1 °C to < 8 °C), 3 weeks. Maximum storage time 3 weeks (otherwise store in freezer).
- C12:** Refrigerator (> 1 °C to < 8 °C), 12 weeks. Maximum storage time 12 weeks (otherwise store in freezer).
- R6:** Room conditions 6 weeks. Maximum storage time 6 weeks (otherwise store in refrigerator not more than three weeks).
- R12:** Room conditions 12 weeks. Maximum storage time 12 weeks (otherwise store in freezer).

6. BIBLIOGRAPHY

- [1] CORESTA Recommended Method No 71 (2011): Smokeless Tobacco Products - Sampling
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- [4] ISO 4874:2000 Tobacco - Sampling of batches of raw material - General principles
- [5] ISO 8243:2013 Cigarettes - Sampling