

Testing of tobacco and related products

Practical guidance for countries
in the South-East Asia Region



REGIONAL OFFICE FOR

**World Health
Organization**
South-East Asia

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Preface

Despite being a major public health threat for several decades, tobacco and related products remain largely unregulated or underregulated in most Member States of the World Health Organization (WHO) South-East Asia (SEA) Region. The limited progress in the area of tobacco product regulation can largely be attributed to inadequate testing and analysis of tobacco and other products, primarily due to a lack of requisite resources and/or technical understanding at the disposal of these countries.

Over the years, WHO has repeatedly emphasized the utmost importance of testing and regulation of tobacco and tobacco products as a seminal step towards controlling the ever-growing tobacco epidemic. In the WHO Framework Convention on Tobacco Control (WHO FCTC), Articles 9 and 10 elaborately deal with these aspects of tobacco control. The *Handbook on tobacco product regulation*, published by WHO in 2018, further articulates these resolves in a comprehensive manner.

In continuation with its ongoing commitment to assist its Parties and Member States in meeting their obligations under Articles 9 and 10 of the WHO FCTC, the WHO Regional Office for South-East Asia has come up with this practical guidance document. This document aids countries in the SEA Region to strengthen their tobacco-control policies through initiation and adoption of WHO-approved testing protocols of the contents and emissions of tobacco products. The document also provides guidance on testing betelnut products and electronic nicotine delivery systems (ENDS). Testing of these products in the Region would contribute to the evolving body of knowledge and help regulators to better understand the chemical composition and possible effects of these products. The document aims to support and expedite tobacco testing efforts not only as an inevitable component of a tobacco control strategy but also support and accelerate implementation of the WHO FCTC at the country level.

Executive summary

The importance of laboratory testing of tobacco products is reflected in the World Health Organization Framework Convention on Tobacco Control (WHO FCTC). Article 9 of the WHO FCTC defines obligations for Parties with respect to the testing of tobacco products, while Article 10 deals with the disclosure of information on the contents and emissions of tobacco products. The disclosure of product information encompasses the disclosure of information by manufacturers to regulators and disclosure of information from regulators to the public. Testing of tobacco products is needed to generate the data necessary to support both these forms of disclosure.

This document provides guidance to Member States of the WHO South-East Asia (SEA) Region on practical, step-wise approaches to implementing tobacco testing. The document would be relevant to all Member States of the Region, including those with limited resources and capacities, in the context of establishing laboratories for testing the contents and emissions of various types of tobacco products, areca nut products and electronic nicotine delivery systems (ENDS). Notably, tobacco products can be tested in existing laboratories that employ standard/valid procedures and have all the requisite equipment, as underscored in this guidance document. Such an approach will strengthen the regulatory capacity of Member States as well as provide robust public health evidence based on the toxic and addictive nature of the tested tobacco and related products.

Chapter 1 of this document discusses the relevance of tobacco testing in the context of the SEA Region. Chapter 2 lays down WHO-validated procedures for tobacco testing whereas Chapter 3 describes the working principles of the instruments employed in the testing of tobacco and related products. Chapter 4 recommends specific human resource requirements to perform validated procedures for testing tobacco and related products and shares additional resources for further reading.

List of abbreviations

cDC	US Centers for Disease Control and Prevention
DAD	diode array detector
FID	flame ionization detector
GATS	Global Adult Tobacco Survey
GC	gas chromatography
GSHS	Global School-based Student Health Survey
GYTS	Global Youth Tobacco Survey
HPLC	high performance liquid chromatography
HR	human resources
IC	ion chromatography
LC-MS-MS	liquid chromatography tandem mass spectrometry
MS	mass spectrometry
NAT	N- nitrosoanatabine
NAB	N- nitrosoanabasine
NNAL	(4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol))
NNK	(4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone))
NNN	(N'-nitrosornicotine)
NDIR	non-dispersive infrared detector
SEA	South-East Asia

SIM	selected ion monitoring
SLT	smokeless tobacco
SOP	standard operating procedure
TCD	thermal conductivity detector
TFI	Tobacco Free Initiative
TRPs	tobacco and related products
TSNAs	tobacco specific nitrosamines
UV	ultraviolet
WHO	World Health Organization
WHO FCTC	World Health Organization Framework Convention on Tobacco Control
WHO TobLabNet	WHO Tobacco Laboratory Network

Urgency for tobacco testing in the WHO South-East Asia Region

Tobacco use and exposure to second-hand smoke lead to nearly 9 million deaths globally (8 million attributed to tobacco use and 1 million from exposure to second-hand smoke), and the majority of these deaths happen in low- and middle-income countries. According to the 2019 WHO global report on trends in the prevalence of tobacco use (1, 2), since the year 2000, the WHO South-East Asia (SEA) Region has consistently exhibited the highest rate of tobacco use among adults aged 15 years and over. There were an estimated 1351 million adult tobacco users worldwide in 2015, of whom 421 million were from the SEA Region. Furthermore, there are 303 million global smokeless tobacco (SLT) users aged 15 years and above, with 248 million of these users concentrated in the SEA Region alone, constituting approximately 82% of all SLT users worldwide.

These alarming statistics are clear indicators of the urgent necessity for tobacco product testing, which has thus far been an underutilized tool, to achieve effective regulatory outcomes in the Region. Effective tobacco testing and consequent tobacco product regulation can potentially lead to a paradigm shift in reducing the demand for tobacco and contribute to reducing the morbidity and mortality associated with tobacco use.

Tobacco product testing, as a part of tobacco product regulation, is an essential driving force towards a comprehensive tobacco control programme. This will also simultaneously fulfil other obligations of the WHO FCTC in Member States of the SEA Region and facilitate acceleration of the public health gains of tobacco control. Testing and measuring the contents and emissions of tobacco products through laboratory analyses of tobacco products and the regulation of these are in line with Article 9 of the WHO FCTC and are crucial for tobacco control.

As the next step in WHO's commitment to fight the tobacco epidemic, this guidance document explores the basis of testing tobacco and related products, and outlines validated scientific methods available for the analyses of their contents and resultant emissions. It serves as a practical guide to Member States of the Region soliciting scientific information on how to initiate and move forward with the testing of tobacco products using standard and validated methods and making use of available laboratory support.

This chapter further highlights the immediate importance of testing tobacco-related products (TRPs) in the Region by providing data on the prevalence of tobacco consumption in Member States and provides a brief description of the chemicals in the contents and emissions of tobacco products that contribute to their attractiveness, addictiveness and toxicity. The ensuing chapters in this document outline WHO-validated scientific procedures to test tobacco products, the list of chemicals they contain and the equipment required to conduct systematic analysis of tobacco products. They also explain the working principles of relevant analytical instruments employed for tobacco product testing.

1.1 Prevalence of tobacco consumption in Member States of the SEA Region

Data on tobacco consumption have been compiled from the most recent Global Adult Tobacco Survey (GATS), Global Youth Tobacco Survey (GYTS) and Global School-based student Health Survey (GSHS) completed in each country, and the WHO SEA Region 2018 factsheet (3–16) on tobacco prevalence in Member States.

Fig. 1.1 summarizes the prevalence of tobacco consumption in Member States of the Region among the adult population.

Fig. 1.1: Prevalence of tobacco consumption (in %) among adults in Member States of the SEA Region

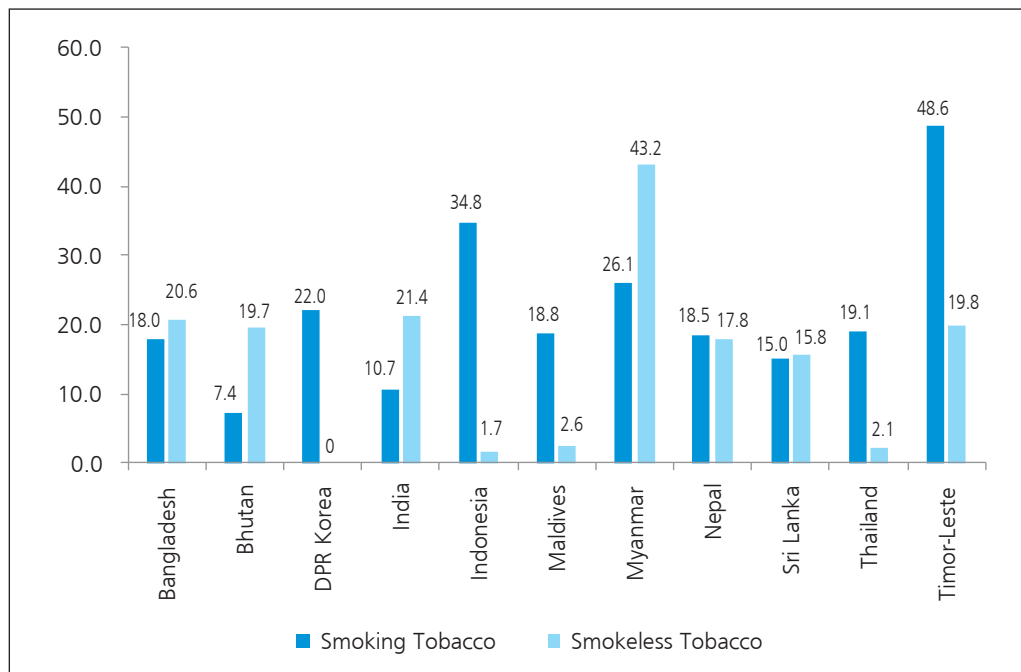
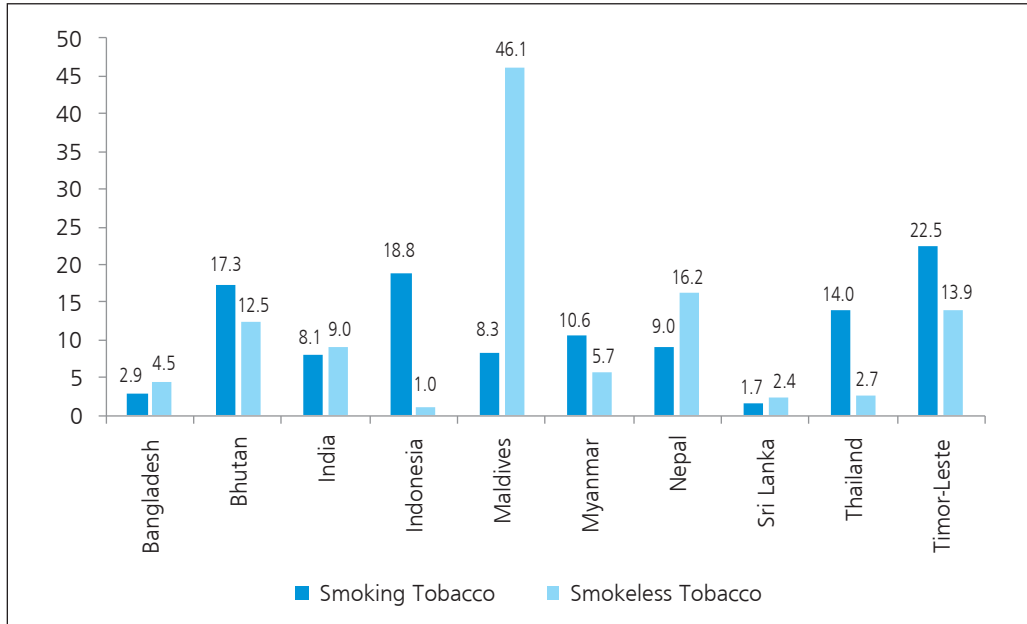


Fig. 1.2 summarizes the prevalence of tobacco consumption in Member States of the Region among youth aged between 13 and 15 years.

Fig. 1.2: Prevalence of tobacco consumption (in %) among youth (13–15 years) in Member States of the SEA Region



An assessment of the prevalence data amply demonstrates the heavy burden of tobacco consumption in Member States, with Myanmar displaying the highest percentage of smokeless tobacco (SLT) consumption among adults, and Timor-Leste having the highest percentage of adult smokers.

Among youth, Bhutan has the highest percentage of SLT users, while Timor-Leste has the highest percentage of smokers.

However, it is noteworthy that although countries such as India, Bangladesh and Indonesia are not leading the statistics, with their enormous populations, even a relatively low prevalence denotes a very large number of individuals using tobacco in different forms.

1.2 Tobacco ingredients and emission

WHO primarily attributes the poor health outcomes of tobacco consumption to three prime elements (17, 18) of tobacco products: attractiveness, addictiveness and toxicity. These three factors are interrelated, with the first leading to the second and then to the third.

Attractiveness stems from the product characteristics that encourage initiation followed by prolonged use of tobacco products by a large proportion of the global population, particularly the youth. In order to attract non-users and appeal to consumers, a myriad of non-tobacco chemicals/additives, including humectants, fragrances, flavours (anethole, benzaldehyde and menthol), fruit extracts and sugars are added to tobacco products. Several aromatic compounds, mainly isoprenoids, found in SLT products are also known to contribute to their attractiveness.

Apart from direct addition of external agents into tobacco products, other product features, including structural features such as batteries and filter make them easy or pleasurable to use, and create an illusion of harm reduction, which also adds to the overall attractiveness.

Addictiveness is the main consequence of the presence of nicotine, which is a psychoactive drug inherently contained in tobacco products. By acting as a central nervous system (CNS) stimulant, nicotine makes it difficult for users to limit consumption or quit the use of tobacco and related products. Apart from nicotine, several other additives are intentionally added to tobacco products to increase their dependence potential. This may include alkaline agents, such as ammonia or slaked lime, which increase the bioavailability of unprotonated nicotine and areca nut, thereby enhancing the addictive potential of the product.

Further, additives such as eugenol and menthol work to indirectly enhance the addictiveness of tobacco products by numbing the throat, thereby increasing tolerance towards tobacco products, especially in amateur users.

The **toxicity** of tobacco products results from continued exposure to toxic compounds contained in or generated upon the consumption of these products, some of which include cancer-causing chemicals (19).

The following class of chemicals contribute to the toxicity of tobacco products:

- (1) Heavy metal ions, such as lead, arsenic, mercury and nickel
- (2) Aldehydes, which are known carcinogens, produced from the burning of sugars added as sweeteners in tobacco products
- (3) Tobacco-specific nitrosamines (TSNAs), namely NNN and NNK, which are the most potent carcinogens in SLT
- (4) Nicotine
- (5) Polyaromatic hydrocarbons
- (6) Nitrates and nitrites.

WHO-Validated procedures for tobacco testing

in 2005, the WHO Tobacco Free Initiative (TFI) established the WHO Tobacco Laboratory Network (TobLabNet) as a WHO technical advisory body, with the aim of assisting WHO Member States and regions to develop national and regional capacity for testing and research on the contents and emissions of tobacco products, and to assist countries in fulfilling regulatory requirements under the purview of the WHO FCTC.

Thus far, WHO TobLabNet has published ten standard operating procedures (SOPs) (20–29) pertaining to the testing and validation of standardized tobacco industry-independent methods for priority contents and emissions of cigarette tobacco and mainstream cigarette tobacco smoke. Recently, WHO published an information sheet (30) on the WHO TobLabNet methods for measuring priority contents and emissions in tobacco and related products, which further highlights the necessity of measuring the contents and emission of tobacco products and subsequent regulation as an effective means to achieve a comprehensive tobacco control strategy.

This chapter provides the details of all these methods as published by WHO TobLabNet, including a description of the equipment, chemicals and standards required to carry out testing. Table 2.1 lists WHO TobLabNet-validated SOPs for the testing of smoked tobacco products.

Table 2.1: WHO TobLabNet-validated SOPs for the testing of smoked tobacco products

S. no.	WHO TobLabNet method for smoking products	Name of the method	Parameter(s) or analyte tested and brief description
1	SOP 01 (20)	Standard operating procedure for intense smoking of cigarettes	Parameters required for intense smoking regimens
2	SOP 02 (21)	Standard operating procedure for validation of analytical methods for the contents and emissions of tobacco products	Standard template for establishing WHO TobLabNet SOPs for the measurement of the content and emissions of tobacco products
3	SOP 03 (22)	Standard operating procedure for determination of tobacco-specific nitrosamines in mainstream cigarette smoke under International Organization for Standardization (ISO) and intense smoking conditions	NNN, NNK, NAB and NAT, responsible for toxicity
4	SOP 04 (23)	Standard operating procedure for determination of nicotine in cigarette tobacco filler	Nicotine, responsible for toxicity and addictiveness
5	SOP 05 (24)	Standard operating procedure for determination of benzo[a]pyrene in mainstream cigarette smoke under ISO and intense smoking conditions	B[a]P, responsible for toxicity

S. no.	WHO TobLabNet method for smoking products	Name of the method	Parameter(s) or analyte tested and brief description
6	SOP 06 (25)	Standard operating procedure for determination of humectants in cigarette tobacco filler	Glycerol, propylene glycol and triethylene glycol, responsible for attractiveness
7	SOP 07 (26)	Standard operating procedure for determination of ammonia in cigarette tobacco filler	Ammonia, responsible for addictiveness
8	SOP 08 (27)	Standard operating procedure for determination of aldehydes in mainstream cigarette smoke under ISO and intense smoking conditions	Formaldehyde, acetaldehyde and acrylaldehyde, responsible for toxicity
9	SOP 09 (28)	Standard operating procedure for determination of volatile organics in mainstream cigarette smoke under ISO and intense smoking conditions	Benzene and 1,3-butadiene, responsible for toxicity
10	SOP 10 (29)	Standard operating procedure for determination of nicotine and carbon monoxide in mainstream cigarette smoke under intense smoking conditions	Nicotine and carbon monoxide, responsible for addictiveness and toxicity

Table 2.2 lists all the relevant analytical instruments required for testing different tobacco parameters as per WHO TobLabNet SOPs, with the assumption that the testing laboratories have the set-up and general analytical equipment, including an analytical balance, laboratory benches, testing apparatus, etc.

Table 2.2: Analytical instruments required for testing different tobacco parameters as per WHO TobLabNet SOPs

S. no.	WHO TobLabNet official method for smoking products	Required equipment
1.	SOP 01 (20)	<ul style="list-style-type: none"> • Environment chamber, as per ISO 3402 • Smoking machine, as per ISO 3308
2.	SOP 03 (22)	<ul style="list-style-type: none"> • Environment chamber • Smoking machine, as per ISO 3308 <p>High-performance liquid chromatography–tandem mass spectrometry (HPLC-MS-MS) with electrospray ionization</p>
3.	SOP 04 (23)	<ul style="list-style-type: none"> • Gas chromatography (GC) equipped with a flame ionization detector (FID)
4.	SOP 05 (24)	<ul style="list-style-type: none"> • Environment chamber, as per ISO 3402 • Smoking machine, as per ISO 3308 • Rotary evaporator • Gas chromatography with mass spectrometry (GC-MS)
5.	SOP 06 (25)	<ul style="list-style-type: none"> • GC-FID
6.	SOP 07 (26)	<ul style="list-style-type: none"> • Ion chromatography equipped with a conductivity detector
7.	SOP 08 (27)	<ul style="list-style-type: none"> • Environment chamber, as per ISO 3402 • Smoking machine, as per ISO 3308 • High performance liquid chromatography (HPLC), equipped with ultraviolet (UV)/diode array detector (DAD)
8.	SOP 09 (28)	<ul style="list-style-type: none"> • Gas chromatography–mass spectrometry (GC-MS) with electron ionization mode
9.	SOP 10 (29)	<ul style="list-style-type: none"> • Environment chamber, as per ISO 3402 • Smoking machine, as per ISO 3308 • Non-dispersive infrared (NDIR) analyser integrated with the smoking machine • GC-FID and GC-TCD

Table 2.3 provides the list of chemicals and reference standards required to carry out testing on tobacco products as per WHO TobLabNet SOPs listed in Table 2.2.

Table 2.3: Chemicals and reference standards required to carry out testing on tobacco products

WHO TobLabNet SOP	Name of the chemical	Chemical Abstracts Service (CAS) number
SOP 03 – TSNA _s	3-(1-Nitrosopyrrolidin-2-yl) pyridine (NNN)	16543-55-8
	Deuterium-labelled 3-(1-nitrosopyrrolidin-2-yl) pyridine(NNN-d ₄)	66148-19-4
	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	64091-91-4
	Deuterium-labelled 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK-d ₄)	
	N-Nitrosoanatabine (NAT)	71267-22-6
	Deuterium-labelled 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK-d ₄)	764661-24-7
	Deuterium-labelled N-nitrosoanatabine (NAT-d ₄)	1426174-82-4
	N-Nitrosoanabasine (NAB)	37620-20-5
	Deuterium-labelled N-nitrosoanabasine (NAB-d ₄)	1020719-68-9
	Ammonium acetate, HPLC grade	631-61-8
	Glacial acetic acid	64-19-7
	Acetonitrile, HPLC grade	75-05-8
	Methanol, HPLC grade	67-56-1
	Water, type 1	7732-18-5

WHO TobLabNet SOP	Name of the chemical	Chemical Abstracts Service (CAS) number
SOP 04 – Nicotine	n-hexane, GC grade	110-54-3
	–(–)Nicotine or nicotine salicylate with not less than 98% purity	54-11-5/29790-52-1
	Sodium hydroxide	1310-73-2
	n-Heptadecane or quinaldine or isoquinoline or quinoline, purity not less than 98%	629-78-7/91-63-4/119-65-3/91-22-5
	Helium gas	7440-59-7
	Hydrogen and air	1333-74-0
SOP 05 – B[a]P	Cyclohexane	110-82-7
	B[a]P	50-32-8
	B[a]P-d12	63466-71-7
SOP 06 – Humectants	Methanol, HPLC grade	67-56-1
	Glycerol	56-81-5
	Triethylene glycol	112-27-6
	Propylene glycol	57-55-6
	1,3-Butanediol	107-88-0
	Helium gas	7440-59-7
	Hydrogen and air	1333-74-0
SOP 07 – Ammonia	Methanesulfonic acid HPLC grade	75-75-2
	Concentrated sulfuric acid	7664-93-9
	Ammonium sulfate	7783-20-2
	Water, type 1	7732-18-5

WHO TobLabNet SOP	Name of the chemical	Chemical Abstracts Service (CAS) number
SOP 08 – Aldehydes	2,4-Dinitrophenylhydrazine hydrochloride of more than 98% purity	55907-61-4
	Formaldehyde-DNPH solution	1081-15-8
	Acetaldehyde-DNPH solution	1019-57-4
	Acrolein-DNPH solution	888-54-0
	Acetonitrile, HPLC grade	75-05-8
	Phosphoric acid, 85% purity	7664-38-2
	Carbon disulfide	75-15-0
	Anhydrous methanol, analytical grade	67-56-1
	Ethanol, >98% purity	64-17-5
	Carboxen-572 particles, 20/45 mesh size	
SOP 09 – Volatile organics	Benzene, analytical grade	71-43-2
	Carbon disulphide	75-15-0
	Anhydrous methanol, analytical grade	67-56-1
	Carboxen-572 particles, 20/45 mesh size	
	Benzene-d6, purity >99%	1076-43-3
	1,3-Butadiene standard solution (2 mg/mL)	106-99-0

WHO TobLabNet SOP	Name of the chemical	Chemical Abstracts Service (CAS) number
SOP 10 – Nicotine and carbon monoxide	Helium gas	7440-59-7
	Hydrogen and air	1333-74-0
	Propan-2-ol	67-63-0
	(-) Nicotine/nicotine salicylate of purity not less than 98%	54-11-5/29790-52-1
	n-Heptadecane or quinaldine or isoquinoline or quinoline, purity not less than 98%	629-78-7/91-63-4/119-65-3/91-22-5
	Standard carbon monoxide gas mixtures	

2.1 Determination of pH and moisture content

The pH of a tobacco product (cigarette filler/SLT) is of paramount importance in determining the amount of unprotonated or free nicotine bioavailable to the consumer. Since the unionized form of nicotine obtained at basic pH values is more readily absorbed by the human body, the pH of a tobacco product plays a crucial role in deciding its efficacy, which provides a good basis for why it should be measured. Thus, it is a useful and necessary parameter for researchers/regulators, as it can help to inform policy.

Additionally, determination of the moisture content is also imperative so that the concentration of nicotine and other constituents can be reported both on a dry and wet weight basis, as variation in moisture can potentially affect the concentration of other parameters. Table 2.4 provides the details of instruments required to test the pH and moisture content of tobacco products.

Table 2.4: Instruments required to test the pH and moisture content of tobacco products

Name of the parameter	Instrument required
pH measurement	<ul style="list-style-type: none"> pH meter
Moisture content	<ul style="list-style-type: none"> Drying oven Analytical weighing balance

The United States Centers for Disease Control and Prevention (US CDC; WHO TobLabNet member) has several scientific methods (29, 31) for testing the contents and emissions of tobacco products. Table 2.5 summarizes these methods, including instrumentation and chemicals required to carry out testing.

Table 2.5: Instruments and chemicals required for testing the contents and emissions of tobacco products

Tobacco parameter	Instrumentation required	Applicable to	Chemicals required
pH and nicotine (total and unprotonated)	GC-MS with selected ion monitoring (SIM) mode and pH meter	Smokeless tobacco, unburned tobacco and e-cigarettes	Nicotine (purity 99.97%); isopropanol; quinoline (purity 98%) as internal standard; sodium hydroxide, and methyl tert-butyl ether (MTBE), HPLC grade (purity 99.8%)
Tobacco-specific nitrosamines	Liquid chromatography tandem mass spectrometry (LC-MS-MS)	Smokeless tobacco, unburned tobacco, cigarette filler and leaf tobacco	Ammonium acetate, HPLC grade; water (LC-MS grade); acetonitrile, HPLC grade; methanol, HPLC grade; TSNA standards (NAB, NAT, NNN, NNK, NNAL); ¹³ C labelled TSNA internal standards

Tobacco parameter	Instrumentation required	Applicable to	Chemicals required
Toxic metals	Inductively coupled plasma-mass spectrometer (ICP-MS)	Cigarettes, smokeless and leaf tobacco	Isopropanol (ultra-high purity); 30–35% hydrogen peroxide; standards for arsenic, beryllium, cadmium, chromium, cobalt, lead, manganese, nickel and uranium; deionized water; hydrofluoric acid; ICP-MS tuning solution; internal standards of iridium, rhodium, tellurium, and thulium; ultra-pure nitric acid
Flavours in tobacco	GC-MS	Cigarette filler and smokeless tobacco	Flavour standards (eucalyptol, camphor, menthol, methyl salicylate, pulegone, ethyl salicylate, cinnamaldehyde, eugenol, diphenyl ether and coumarin); 4'-(methylenedioxy)-acetophenone (MDA) to be used as internal standard
Mercury	Mercury analyser	Smokeless tobacco	Deionized water; L-cysteine; certified mercury standard

2.2 Areca nut: growing public health concern in the SEA Region

Apart from smoked and smokeless tobacco products, some countries in the SEA Region face the challenge of extensive consumption of areca nut (32–34) in several forms, including betel quid (*paan* or *pan*), *gutka*, *khaini*, *mawa*, *pan masala* and *zarda* or flavoured betel nut. These are mostly consumed as part of several types of smokeless

tobacco products but also as stand-alone products in some countries. Areca nut is derived from *Areca catechu* L., a palm tree that grows in South and South-East Asia and the Pacific islands.

Areca nut, besides being addictive, is a recognized Group 1 human carcinogen (35, 36), and the prime trigger for oral submucous fibrosis, a potentially malignant disorder most rampant in the Region. Thus, the growing prevalence of areca nut use is a major cause of public health concern.

Areca nut: composition

The main constituents of areca nut include carbohydrates, fats, proteins, crude fibre, polyphenols, alkaloids (37) and minerals. Arecoline, arecaidine, guvacine and guvacoline are the four alkaloids conclusively identified in areca nut, with arecoline, a pyridine-based alkaloid, being the most toxic constituent. Upon consumption, arecoline is known to undergo N-nitrosation in the oral cavity to give rise to N-nitrosamines, which are known human carcinogens.

Copper found in areca nut has also been designated as a causal link in the pathogenesis of oral submucous fibrosis.

Testing of areca nut

Qualitative estimation of areca nut in test samples can be done by the means of Fourier transform infrared spectroscopy (FT-IR). Quantitative determination of arecoline can be carried out by employing the high performance thin layer chromatography (HPTLC) technique. The method uses methanol as an extracting solvent for arecoline from the test sample, which is further analysed through HPTLC.

2.3 Electronic cigarettes

Electronic nicotine delivery systems (ENDS), of which electronic cigarettes are a common type, are devices that heat a liquid to create an aerosol that is inhaled by the user. The liquid is devoid of tobacco but contains nicotine, flavourants and other chemicals that may be toxic to human health. In recent years, several variants of these products have gained immense popularity, especially among adolescents and young adults in some jurisdictions, raising concerns regarding the impact of exposure to the aerosols or emissions of these products.

For nicotine-containing products, the potential to keep present and future generations dependent on nicotine is a major public health concern and the long-term impact of these products are as yet unknown. Therefore, testing of these products will add to the body of knowledge and help regulators to better understand their chemical composition and possible effects of these products on the users.

Analysis of electronic cigarettes

Nicotine. Nicotine in e-liquids can be tested by solvent extraction, followed by GC-FID or GC-MS analysis in SIM mode.

Flavours. The flavours in the e-liquid can be tested by employing GC-MS instrumentation, as per the US CDC method.

2.4 Checklist of instruments required to initiate testing of tobacco and related products

Table 2.6 gives the list of instruments required to test the contents and emissions of tobacco and related products (TRPs). Most of these instruments can be easily found in drug- or food-testing laboratories. Consequently, Parties and Member States interested in commencing or taking forward their endeavours at testing tobacco and related products but restricted by the unavailability of the required instrumentation are recommended to explore a possible collaboration with such laboratories in their countries as a viable recourse.

Table 2.6: *Instruments required to test the contents and emissions of tobacco and related products*

Smoked tobacco products (as per WHO TobLabNet methods)	
Name of the instrument	Name of the parameter to be tested
Smoking machine	TSNAs, B[a]P, aldehydes, nicotine and carbon monoxide
Environmental chamber	TSNAs, B[a]P, aldehydes, nicotine and carbon monoxide
GC-MS/GC-FID	Nicotine, humectants, tobacco flavours, volatile organics, B[a]P

Smoked tobacco products (as per WHO TobLabNet methods)	
Name of the instrument	Name of the parameter to be tested
HPLC-MS-MS	TSNAs
HPLC with DAD	Aldehydes
Smokeless tobacco products	
GC-MS	Nicotine, added flavours
LC-MS-MS	TSNAs
pH meter	pH
Drying oven	Moisture
ICP-MS	Metal
FTIR	Areca nut
Electronic cigarettes	
GC-MS/GC-FID	Nicotine in e-liquids
GC-MS	E-liquid flavours

Instrumentation required for testing tobacco and related products

This chapter describes the working principles of the instruments employed in testing of tobacco and related products.

3.1 Gas chromatography

Gas chromatography (GC) (38, 39) is the most ubiquitous analytical technique for separation, identification and quantitation of components of a mixture in gas phase. Like basic chromatographic techniques, GC separates a mixture based on the differential affinity of its components within a stationary and carrier gas system. In GC, the carrier gas is usually an inert gas, such as helium or nitrogen, while the stationary phase is a microscopic layer of liquid or solid support on either packed columns or open tubular columns, also known as capillary columns. The sample in gas phase is carried by the carrier gas through the stationary phase, and the separation of compounds is achieved on the basis of different strengths of interaction or affinity of the compounds with the stationary phase.

In order to study a sample with an unknown concentration, a reference standard with known concentration is injected into the instrument, wherein the standard sample peak retention time and area are compared to the test sample to determine the concentration. Therefore, in this technique, the quantitation of an unknown sample is performed by comparing the peak area of an analyte in resultant chromatograms of samples to its standard.

3.2 Types of GC detection systems

The detector provides a quantitative measurement of the components of the analyte that exit the column in combination with the carrier gas. The detector serves to

convert the detected property changes into an electrical signal that is recorded as a chromatogram.

Based on the analytical requirements, structural attributes of the analyte and desired selectivity, there are several kinds of detectors that can be used in conjunction with GC. Given below is a list of detectors most commonly used with a GC instrument:

- (a) Flame ionization detector (FID)
- (b) Mass spectrometry (MS)
- (c) Electron capture detector (ECD)
- (d) Thermal conductivity detector (TCD)
- (e) Nitrogen phosphorus detector (NPD)

3.2.1 Gas chromatography-flame ionization detector (GC-FID)

In a gas chromatograph equipped with FID, the effluent from the column is directed at an air–hydrogen flame and ignited. Organic compounds burning in the flame produce ions and electrons, which conduct electricity through the flame. The current resulting from the pyrolysis of sample components is measured by a high-impedance picoammeter. The resultant current is proportional to the rate of ion formation, which depends on the sample concentration and is detected by a suitable electrometer and displayed on an analogue output.

FID is a preferred choice for sensitive gas chromatographic separations of volatile organic compounds, owing to excellent response linearity, low noise and high sensitivity.

3.2.2 Gas chromatography-mass spectrometry (GC-MS)

GC-MS combines the attributes of gas chromatography with mass spectrometry to separate and identify different constituents of an organic mixture with a high degree of precision and accuracy. In a GC-MS assembly, the eluate exiting from the GC column is passed through a heated transfer line into the inlet of the mass spectrometer. Once the components reach the MS, they are ionized and fragmented by employing an appropriate ionization technique. Ionized molecules are then accelerated through the instrument's mass analyser and are separated based on their mass-to-charge (m/z) ratios, appearing as a distinct peak in the final spectrum. The mass spectrometer scans the masses continuously throughout the separation.

3.2.3 High-performance liquid chromatography

High-performance liquid chromatography (HPLC) is an advanced form of column chromatography that, instead of relying on gravitational elution, pumps a sample mixture in a solvent at high pressure through a column packed with chromatographic material. The working principle of HPLC is the same as conventional column chromatography, wherein the mobile phase flows through the stationary phase and carries the components of the mixture with it, which get separated by virtue of differential affinity towards the stationary and mobile phases. Further, a detector monitors the separated components as they elute from the high-pressure column. The detector sends information to a computer, which generates the chromatogram.

A wide range of HPLC detectors are available; however, the choice of detector is based on the selectivity and sensitivity of the desired analyte. UV-visible/diode array detectors (DAD) are most widely used with HPLC systems, because of associated sensitivity, superior linearity and easy operability. The eluate is exposed to a beam of electromagnetic radiation, and these detectors make use of the spectral absorption properties of the analytes for identification.

3.2.4. Tandem mass spectrometry

Tandem mass spectrometry (MS/MS) consists of two scanning mass analysers separated by a collision cell. In this technique, precursor ions of selected m/z ratio in the first analyser are further reacted with an inert gas in the collision cell, resulting in additional fragmentation. These daughter product ions are then resolved in the third quadrupole for analysis. The resultant unique product ion spectrum provides confirmation of the target analyte.

An MS/MS detector coupled with a GC or LC system serves as a confirmatory procedure, which can provide additional information on analysis of organic compounds when singular GC/MS or LC/MS analysis fails, because even if the matrix contains another compound with the same mass as the parent ion of interest, it is highly unlikely that the interfering ion would also yield the same daughter ion as that of the analyte under consideration. Thus, it is a more sensitive analytical technique that provides a robust method to purify and quantify organic compounds in complex matrices and allows for detection of trace levels of organic contamination.

3.2.5 Ion chromatography

Ion chromatography (IC) is a chromatographic technique that can be used for the qualitative and quantitative determination of cations, anions and neutral molecules, such as ammonia or carbohydrates. IC achieves separation of the components of a mixture based on their interactions with a stationary phase or a resin and a mobile phase, and the eluent and column type are chosen on the basis of the sample to be analysed.

In this technique, the test sample is mixed with an appropriate eluent, and passed through the charged stationary phase of the column. Analyte constituents move through the column at different speeds, depending on their affinity for the specific resin, resulting in their separation from each other based upon their charge and size. As the eluent passes through the column, ions with a weaker affinity for the resin move faster through the column and are eluted out first, while ions with a stronger affinity for the column are retained for a longer duration. The charged eluent further elutes the retained ions, which go through the detector placed at the end of the column, which in turn analyses the output by producing a chromatogram.

The most commonly used detector with an IC machine is an electrical conductivity detector, which plots conductivity versus time. Each ion in the sample produces a distinct peak on the graph, the height of which is dependent on the relative ion concentration in the injected solution. These measurements can then be used to determine the concentrations of analytes in a test sample.

3.3 Smoking machine coupled with NDIR

Tobacco smoke is a complex aerosol (40, 41) consisting of suspended semi-liquid droplets in the gaseous phase. It contains a myriad chemical compounds, which can be collected for analytical purposes. This task can be achieved by employing a smoking machine, which studies tobacco emission in a systematic fashion for quantitative laboratory testing of smoking tobacco products by reliable artificial smoking of cigarettes or other smoking products. Modern, commercially available smoking machines are well equipped to control smoking parameters such as puff volume, duration and frequency, and vary in the number of cigarettes that can be held or smoked simultaneously or alternately.

There are several ways to trap cigarette smoke generated by machine puffing for further analysis. For examples, mainstream smoke from the cigarette sample may be trapped onto a glass fibre filter pad, wherein the change in weight of the pad

determines the total particulate matter (TPM). Extraction of chemicals trapped on the pad with an appropriate solvent allows for further analysis of specific tobacco parameters by employing additional analytical machinery such as GC or HPLC.

As another alternative, cigarette emissions may be collected as a condensate, known as cigarette smoke condensate (CSC) in a liquid trap or smoke may not be collected at all and used directly as whole smoke to carry out toxicological assessment of tobacco smoke.

Non-dispersive infrared detector (NDIR)

NDIR analysers use infrared radiation to allow for highly sensitive quantification of carbon oxides, including carbon monoxide, carbon dioxide and other gaseous components such as methane, nitrogen oxides and sulfur dioxide.

In this technique, the sample is irradiated with an infrared beam, as a result of which the gas in the test analyte absorbs IR at a particular frequency, based on its chemical and physical attributes. The detector, in turn, measures the amount of radiation absorbed by the sample at the necessary wavelength and determines the volumetric concentration of carbon oxides (CO and CO₂) in the sample.

Types of smoking machines

The two most common and commercially popular smoking machines, which yield high accuracy and reproducibility, are linear and rotary smoking machines; however, different smoking machine designs cater to specific analytical requirements.

- (a) **Rotary machine:** in this kind of machine, cigarette ports are arranged horizontally on the outside of a rotating ring, and cigarettes are smoked consecutively. Mainstream smoke generated from all the ports is funnelled into a central trapping system, and the machine draws a puff whenever a port holding a cigarette is linked to the trap. This design is ideally suited to smoking a large number of cigarettes per unit time in order to generate large amounts of smoke for emission studies.
- (b) **Linear machine:** this design features an in-line arrangement of separate traps for each port with a single cigarette and puffing of cigarettes is performed concurrently for all ports. Linear smoking machines are better suited for replicate analysis on individual traps and provide more flexibility for the testing of smoking regimens that better mimic more human-like puffing profiles.

3.4 Inductively coupled plasma-mass spectrometry

Inductively coupled plasma-mass spectrometry (ICP-MS) is an ideal, robust technique for elemental analysis of tobacco products with high sensitivity and low detection limits (of the order of part per quadrillion [ppq]). The technology integrates a high temperature plasma source with a mass spectrometer and allows for synchronous multi-element detection.

In this technique, a test sample is passed through a high temperature plasma source, which induces sample ionization. The ionized sample is further directed to an MS, which distinguishes and separates ions based on their mass/charge ratios, which are further quantified by a detector.

3.5 Environmental chamber

An environmental chamber (EC) is an enclosure employed to control and maintain environmental conditions, such as temperature and humidity. In tobacco testing, an EC is used to condition tobacco products to the recommended environmental parameters prior to or during testing.

4

Information on requirement of human resources and additional reading material

Human resources (HR) play an integral role in reaching the prescribed goals of any organization. Since the success of any endeavour is directly influenced by the competence of its HR, it is crucial that once Parties develop an understanding of the technicalities associated with the testing of tobacco contents and emissions as well as other related products, and are well-equipped to commence analyses of products popular in their region, they must also recruit well-trained staff to perform the task.

The following are the recommended job expectations and requirements for HR to perform validated procedures:

- (1) Compliance with Article 5.3 of the WHO FCTC (42) to protect tobacco control policies from commercial and other vested interests of the tobacco industry in accordance with national law;
- (2) Understanding of Good Laboratory Practices and Good Documentation Practices;
- (3) Understanding of quality control and quality assurance measures and the development of quality management systems, especially in an analytical testing laboratory setting;
- (4) A doctoral degree, with a basic degree in Organic/Inorganic/Analytical Chemistry;
- (5) Hands-on experience of working with GC, HPLC, MS, IC;
- (6) Prior experience of tobacco and tobacco product testing preferred.

4.1 Additional reading material to aid testing of tobacco products

In addition to information described in this guidance document, Parties and Member States striving to initiate or advance their testing and regulation measures for tobacco and related products can access a plethora of additional resources published by WHO in recent years.

Table 4.1 provides a list of supplementary material to aid Parties and countries to reach their TRP testing and regulation goals.

Table 4.1: *Supplementary material*

S. no.	Resource material
1.	Information sheet on WHO TobLabNet methods for measuring priority contents and emissions in tobacco and related products, World Health Organization, 2020 (30)
2.	WHO study group on tobacco product regulation. Report on the scientific basis of tobacco product regulation: seventh report of a WHO Study Group, World Health Organization, 2019 (43)
3.	Consultation on tobacco addictiveness reduction measures, World Health Organization, 2018 (17)
4.	Tobacco product regulation, building laboratory testing capacity, World Health Organization, 2018 (44)
5.	Case studies for regulatory approaches to tobacco products: menthol in tobacco products, World Health Organization, 2018 (45)

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