



**Kingdom of Bahrain
Ministry of Health
Public Health Directorate**

Policies of Tobacco Analysis in Public health Laboratory

2015

Preface

The purpose of this manual is to provide an insight in to the policies and procedures followed by the Public Health Laboratory in analysis of tobacco products. This manual is meant for use as a guide in tobacco products sampling and its receiving and processing in the laboratory.

The samples are analyzed as per the worksheet(s) which accompanies it through various stages in the laboratory. These worksheets are retained as records for future reference, for up to 2 years.

Issued under the authority of:

Dr. Mariam Al-Mulla Harmas
Director,
Public Health Directorate

Amjad Ghanem Zaed Mohamed
Chief,
Public Health Laboratory

Edited by:

Dr. Abbas Ali AbdulWahab Salman
Quality Assurance Officer,

December 2015

INTRODUCTION

Tobacco smoke is the most common environmental pollutant causing a variety of health related hazards. The effect of route of tobacco smoke exposure of breastfed and non-breastfed infants on urinary cotinine levels. Tobacco smoke is shown to have negative consequences on infant's health especially during the first year of life. The aim of establishing this laboratory is To investigate tobacco smoke exposure. The appropriate storage and preparation of tobacco samples is one of the most important factors with regard to the achievement of representative and reproducible test results. A laboratory can only produce high quality results if the integrity of samples is maintained.

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, (i.e., storage and preparation) prior to analysis are 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.

Note: Training in use of the smoking machine and other analytical equipment is important for successful operation. People not experienced in operating smoking machines or in using the analytical methods for measuring tobacco product emissions and contents should be trained.

TPM: Total particulate matter ISO regimen: Parameters used to smoke tobacco products.

Tobacco products: Products entirely or partly made of leaf tobacco as the raw material that are manufactured to be used for smoking, sucking, chewing or snuffing (Article 1(f) of the WHO FCTC).

Laboratory sample: Sample intended for testing in a laboratory, consisting of a single type of product delivered to the laboratory at one time or within a specified period.

TERMS AND DEFINITIONS

ISO regimen: Parameters used to smoke tobacco products that include a 35-ml puff volume, a 60s puff interval, 2-s puff duration and no blocking of the filter ventilation holes.

Intense Regimen – Parameters used to smoke tobacco products which include 55-ml puff volume, 30-s puff interval, 2-s puff duration and 100% blocking of the filter ventilation holes.

Tobacco products: Products entirely or partly made of leaf tobacco as the raw material that are manufactured to be used for smoking, sucking, chewing or snuffing (Article 1(f) of the WHO FCTC).

Laboratory sample: Sample intended for testing in a laboratory, consisting of a single type of product delivered to the laboratory at one time or within a specified period.

Test sample: Product to be tested, taken at random from the laboratory sample. The number of products taken shall be representative of the laboratory sample.

Test portion: Random sample from the test sample to be used for a single determination. The number of products taken shall be representative of the test sample.

METHOD SUMMARY

All samples are conditioned and marked according to ISO standard procedures. Ventilation holes are blocked 100 %. Cigarettes are smoked according to ISO standard procedures with the exception of puff volume and puff frequency.

1.0 SMOKING LABORATORY IN PUBLIC HEALTH DIRECTORATE:

Public health laboratory has established a new unit for tobacco analysis. The unit for the time being capable of execution the following test:

- 1- Determination of moisture in tobacco product using Karl fisher tiltrotor.
- 2- Determination TPM (total particulate matter).
- 3- Physical tests (cigarettes diameter, weight, circumference and pressure drop).
- 4- Determination of CO and CO₂ in cigarettes.
- 5- Determination of Nicotine and Tar in tobacco products.
- 6- Determination of Pesticides in tobacco products.

The inspectors of Tobacco control unit are intended to fill the request form of tobacco sampling analysis and sent to sealed samples to the public health reception as analysis request.

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

Note: The inspectors should not ask for any more tests unless otherwise approved by the Public Health Laboratory chief.

All the laboratory results should be recorded in the worksheet as in the Appendix,2 and 3. The results were documented in OFWSA system under tobacco analysis section.

The laboratory results were interpreted according to GSO standards limit as in Appendix 4 in this manual.

2.0 CHECKING OF SAMPLING AND ANALYSIS FORMS AND ITS COMPLIANCE:

1- Check that the forms are completed properly and bearing the following information:

- Sample description; brand name, country of origin etc.
- Reason(s) for sampling.
- Names and signatures of collecting and submitting officials with date and time.
- Other relevant information if necessary.

The information of the form must be checked against the sample label. In case of any discrepancy the inspector must be asked to make the required changes and also attest them.

2- Check that the quantity of the sample is adequate to carry out the requested CJ tests. In case of fitness analysis a minimum of 3 units or its multiple are required as indicated.

3- Check that the condition of the sample is satisfactory upon arrival at the laboratory. Frozen samples must arrive solidly frozen, chilled at 1 - 4 C and must not exceed 7C at any time. In case of evident temperature abuse the sample must be rejected. For suspected abuse make a note on the form so that the sample may be retested in case it turns out to be unsatisfactory.

4- Check the sample container for any physical defects such as damaged packages, pin holes, loose lids, leakage etc. Defective containers must be rejected outright.

3.0 PROCEDURE FOR REGISTRATION OF SAMPLES

The Sampling and Analysis Forms after numbering and assigning of test codes, cat. No. and Sub. Cat. No. are sent to the laboratory clerk for registration in the computer system. Code numbers for different categories/sub-categories are maintained in the computer. The details of the Sampling and Analysis forms shall be entered in the computer. The samples will be identified in the computer by registration numbers. Priority shall be given to registration of samples received from ports of entry.

4.0 PROCEDURE FOR PRELIMINARY ANALYSIS OF SAMPLES

Check sample labels for their compliance with details of sample description. Check all Unit Head, Chemistry Unit heads, and In-charge Sample Management has to examine the sample. A report duly signed by at least two committee members is forwarded to the Head of the Laboratory for taking appropriate action.

5.0 PROCEDURE FOR REQUEST OF REPEAT SAMPLES

The Head of Unit (s) shall make a re-sampling request to In-charge sample Management by filling an Internal Resample Request Form (Annex). The In-charge Sample Management, upon receipt of request, will initiate another request by filling a Re-sampling Request Form (Annex) and forwarding it to the appropriate authority. Upon receipt of samples, they must be checked against the details of re-sampling Request Form, and relevant details should be entered in both forms.

The samples shall be delivered to the sample In-charge of the respective unit-obtaining signature for the receipt of samples.

In case of non-receipt of samples within 2 days of re-sampling request, a reminder will be sent on the 3rd day. In case of non-receipt of samples by 4th day, In-charge sample Management shall inform the concerned Unit head for taking appropriate action.

WORKSHEET- CHEMICAL ANALYSIS
 TOBACCO ANALYSIS (OTHER TOBACCO PRODUCT)

TG # -----

PHL # -----

Type of Sample -----

Chemist Code -----/----- Sign -----/----- Date of Analysis ____/____/____			
Result: SAT. UNSAT. F.A.		Sr. Chemist -----	
Remarks ----- -----			
Chemist Comments ----- -----			
----- Date Analysis completed ____/____/____			
Titration: Karl Fischer		Spectroscopy	HPLC
Furnace			GC-FID
Conditioning Chamber	(22°C) Other	Moisture 60 %	Time 24H 48H 72H
Moisture Test: Karl Fischer;	Sample weight	% (W/W)	
Moisture (M)		Total Ash (TA)	
Sample + dish weight, g (W1)			
Dish weight, g (W2)			
Dried/Ash sample + dish weight, g (W3)			
Formula	$(W1 - W3) (100) / (W1 - W2)$	$(W3 - W2) (10000) / [(W1 - W2) * TS]$	
% w/w =			
Nicotine Content & Glycerin (BY GC-FID)			
Sample weight, g (W)			
% w/w = $(W1 - W2) * D * 104 / [W * TS]$			
Total Nicotine (Content % W/W)			
Glycerin (mg/Kg)			
Acid-Insoluble Ash (AIA)			
Sample weight used for TA determination, g (W)			
Acid-insoluble ash, g (W1)			
% w/w = $W1 * (10^4) / [W * TS]$			
Sugar %			
	<i>Fructose</i>	<i>Glucose</i>	<i>Sucrose</i>
	<i>Maltose</i>	<i>Galactose</i>	
Sample [Wt., g] [Vol., ml] (W)			
Total volume/ aliquot = (D, ml)			
Reading (R)			
% = $R * D / W$			
Acid (Benzoic and Sorbic) and its Salts (Sodium, Na and Potassium, K)			
Acid/Salts	Benzoic	Sorbic	Na-Benzoate
K-Sorbate			
Sample [weight, g] [volume, ml] (W)			
Total volume / aliquot = (D, ml)			
Reading (R)			
Blank (B)			
$mg/[kg][L] = (R - B) * D * F * T / W$			

WORKSHEET- CHEMICAL ANALYSIS
 TOBACCO ANALYSIS (CIGARETTES)

TG # -----

PHL # -----

Type of Sample -----

Chemist Code -----/-----	Sign -----/-----	Date of Analysis ____/____/____
Result: SAT. UNSAT. F.A. Sr. Chemist -----		
Remarks ----- -----		
Chemist Comments ----- -----		
----- Date Analysis completed ____/____/____		

P10	PV 10	DTL (drop through Laser)	Tobacco Machine
Titration: Karl Fischer		Spectroscopy	Co/Co2 Analyzer C24
Furnace		HPLC	GC-FID

Conditioning Chamber	(22°C) Other	Moisture 60 %	Time 24H 48H 72H
Tobacco passing through a 425 micron sieve (on dry weight basis)			%
Circumference Size (mm)			
Draw Resistance (%)			
Pressure Drop (mm WG)			
Moisture Test: Karl Fischer;	Sample weight	% (W/W)	

	Moisture (M)	Total Ash (TA)
Sample + dish weight, g (W1)		
Dish weight, g (W2)		
Dried/Ash sample + dish weight, g (W3)		
Formula	$(W1 - W3) (100) / (W1 - W2)$	$(W3 - W2) (10000) / [(W1 - W2) * TS]$
% w/w =		

Carbon Monoxide (Co/Co2 Analyzer C24)

Tobacco machine setting	# of cigarette			
Filter paper weight before smoking g (W1)				
Filter paper weight after smoking g (W2)				
Total Particular Matter (TPM)				
Co content % w/w / Per = cigarette				

Nicotine Content & Glycerin (BY GC-FID)

Sample weight, g (W)	
% w/w = $(W1 - W2) * D * 104 / [W * TS]$	
Total Nicotine (Content % Per cigarette) /W	
Tar content % (TPM- (water % + Nicotine %)	
Glycerin (mg/Kg)	

Acid-Insoluble Ash (AIA)

Sample weight used for TA determination, g (W)	
Acid-insoluble ash, g (W1)	
% w/w = $W1 * (10^4) / [W * TS]$	

WORKSHEET- CHEMICAL ANALYSIS
 TOBACCO ANALYSIS (CIGARETTES)

TG # -----

PHL # -----

Type of Sample -----

Chemist Code -----/-----	Sign -----/-----	Date of Analysis ____/____/____		
Result:	SAT.	UNSAT.	F.A.	Sr. Chemist -----
Remarks ----- -----				
Chemist Comments ----- -----				
Date Analysis completed ____/____/____				

P10	PV 10	DTL (drop through Laser)	Tobacco Machine
Titration: Karl Fischer		Spectroscopy	Co/Co2 Analyzer C24
Furnace		HPLC	GC-FID

Conditioning Chamber	(22°C) Other	Moisture 60 %	Time 24H 48H 72H
Tobacco passing through a 425 micron sieve (on dry weight basis)			%
Circumference Size (mm)			
Draw Resistance (%)			
Pressure Drop (mm WG)			
Moisture Test: Karl Fischer;	Sample weight	% (W/W)	

	Moisture (M)	Total Ash (TA)
Sample + dish weight, g (W1)		
Dish weight, g (W2)		
Dried/Ash sample + dish weight, g (W3)		
Formula	$(W1 - W3) (100) / (W1 - W2)$	$(W3 - W2) (10000) / [(W1 - W2) * TS]$
% w/w =		

Carbon Monoxide (Co/Co2 Analyzer C24)			
Tobacco machine setting	# of cigarette		
Filter paper weight before smoking g (W1)			
Filter paper weight after smoking g (W2)			
Total Particular Matter (TPM)			
Co content % w/w / Per = cigarette			

Nicotine Content & Glycerin (BY GC-FID)	
Sample weight, g (W)	
% w/w = $(W1 - W2) * D * 104 / [W * TS]$	
Total Nicotine (Content % Per cigarette) /W	
Tar content % (TPM- (water % + Nicotine %)	
Glycerin (mg/Kg)	

Acid-Insoluble Ash (AIA)	
Sample weight used for TA determination, g (W)	
Acid-insoluble ash, g (W1)	
% w/w = $W1 * (10^4) / [W * TS]$	

PUBLIC HEALTH LABORATORY
RE-SAMPLE REQUEST FORM

TO : _____	DATE: _____
------------	-------------

SAMPLE DETAILS

SAMPLE FORM NO.: _____	DATE: _____	
SOURCE: _____		
DESCRIPTION: _____		
BRAND: _____	ORIGIN: _____	
NET WEIGHT: _____	PROD. DATE: _____	EXP. DATE: _____
QUANTITY REQUIRED _____		
ANY OTHER DETAILS : _____ _____		

INCHARGE, SAMPLE ROOM : _____

FOLLOW-UP ACTION

REMINDER: _____		
DATE OF RECEIPT: _____	S.F.NO. _____	QUANTITY: _____
DATE OF ISSUE : _____	RECEIVED BY: _____	
REMARKS : _____		
INCHARGE, SAMPLE ROOM : _____		

Tobacco & Tobacco products GSO limit

COMMODITIES: Tobacco	RECOMMENDED ANALYSIS TESTS	Maximum accepted limit	Reference
3001 Cigarettes	Fine tobacco content passing through a 425 micron sieve (on dry weight basis)	5 %	GSO 597/2009
	Total ash content (on dry weight basis)	25 %	
	Moisture content	10-16 %	
	Acid-insoluble ash content (silica) (on dry weight basis)	3 %	
	Nicotine content (on dry weight basis)	0.6 % per cigarette	
	Width of tobacco pieces used (minimum)	0.1 mm	
	Circumference of the cigarette	15 - 30 mm.	
	The draw resistance of complete cigarette	160 mm	
	Additives content	20 %	
	Tar	10.0 mg/cigarette.	
	carbon monoxide	12 mg/cigarette	
	DDT	1.5 ppm	
	TDE	1.0 ppm	
	Toxaphene	3.0 ppm	
	Endrin	0.1 ppm	
	Aldrin	0.1 ppm	
	Formathion	0.5 ppm	
	Chlordane	0.3 ppm	
	Dialdrin	0.1 ppm	
	Heptachlor	0.1 ppm	
	Heptachlor epoxide	0.1 ppm	
	Dicamba	0.5 ppm	
	Permethrin	3.0 ppm	
2,4 D	5.0 ppm		
2, 4,5 T	0.5 ppm		
3002 Almeassel Tobacco	Total Sugar	53 % (at 22 % moisture)	GSO 1415/2011
	Sand	5 %	
	Pure glycerin	10 %	
	Moisture content	17%	
	Nicotine content	0.5 %	
	Total ash	12 %	
	Acid insoluble ash	5 %	
	Nitrogen content	0.5%	
	Oils and aromatic essences	ND	
	color	ND	
	Sodium benzoate	2.5 g/ Kg	
	DDT	1.5 ppm	

	TDE	1.0 ppm	
	Toxaphene	3.0 ppm	
	Endrin	0.1 ppm	
	Aldrin	0.1 ppm	
	Formathion	0.5 ppm	
	Chlordane	0.3 ppm	
	Dialdrin	0.1 ppm	
	Heptachlor	0.1 ppm	
	Heptachlor epoxide	0.1 ppm	
	Dicamba	0.5 ppm	
	Permethrin	3.0 ppm	
	2,4 D	5.0 ppm	
	2, 4,5 T	0.5 ppm	
3003 Almeassel tobacco fruit flavored	Total Sugar	53 % (at 22 % moisture)	GSO 1749/2011
	Sand	5 %	
	Pure glycerin	10 %	
	Moisture content	25 %	
	Nicotine content	0.5 %	
	Total ash	12 %	
	Acid insoluble ash	5 %	
	Nitrogen content	0.5%	
	Oils and aromatic essences	ND	
	color	ND	
	Sodium benzoate	2.5 g/ Kg	
	Potassium sorbet	2.5 g/ Kg	
	Mix of sodium benzoate & potassium sorbet	5 g/ Kg	
	DDT	1.5 ppm	
	TDE	1.0 ppm	
	Toxaphene	3.0 ppm	
	Endrin	0.1 ppm	
	Aldrin	0.1 ppm	
	Formathion	0.5 ppm	
	Chlordane	0.3 ppm	
	Dialdrin	0.1 ppm	
	Heptachlor	0.1 ppm	
	Heptachlor epoxide	0.1 ppm	
	Dicamba	0.5 ppm	
	Permethrin	3.0 ppm	
	2,4 D	5.0 ppm	
	2, 4,5 T	0.5 ppm	
3004 Mixture of Tobacco pipe	Sand	3 %	GSO 2050/2010
	Fine tobacco content passing through a 600 micron sieve (Moisture 13 %)	5 %	
	Total Sugar	11 %	
	Sodom Benzoate or potassium sorbate	200 gm/100 Kg	
	Pure glycerin	10 %	
	DDT	1.5 ppm	
	TDE	1.0 ppm	

	Toxaphene	3.0 ppm	
	Endrin	0.1 ppm	
	Aldrin	0.1 ppm	
	Formathion	0.5 ppm	
	Chlordane	0.3 ppm	
	Dialdrin	0.1 ppm	
	Heptachlor	0.1 ppm	
	Heptachlor epoxide	0.1 ppm	
	Dicamba	0.5 ppm	
	Permethrin	3.0 ppm	
	2,4 D	5.0 ppm	
	2, 4,5 T	0.5 ppm	
	Total aflatoxins	20 µg/kg.	
3005 Sijaritus	Sand	3 % (of tobacco used in manufacturing of moisture 13 %)	GSO 2051/2010
	Moisture	13 %	
	Pure glycerin	3 %	
	DDT	1.5 ppm	
	TDE	1.0 ppm	
	Toxaphene	3.0 ppm	
	Endrin	0.1 ppm	
	Aldrin	0.1 ppm	
	Formathion	0.5 ppm	
	Chlordane	0.3 ppm	
	Dialdrin	0.1 ppm	
	Heptachlor	0.1 ppm	
	Heptachlor epoxide	0.1 ppm	
	Dicamba	0.5 ppm	
	Permethrin	3.0 ppm	
	2,4 D	5.0 ppm	
	2, 4,5 T	0.5 ppm	
	Total aflatoxins	20 µg/kg.	
3006 Smuff tobacco	Sand	2 %	GSO 2048/2010
	Moisture	17 %	
	Pure glycerin		
	Sodium Carbonate and/or Sodium Bicarbonate	20 %	
	Sodium benzoate or Potassium Sorbate	2g / kg of tobacco	
	Sodium chloride	5 parts/ 100 parts of tobacco	
	Sesame oil	6 parts/ 100 parts of tobacco	
	DDT	1.5 ppm	
	TDE	1.0 ppm	
	Toxaphene	3.0 ppm	
	Endrin	0.1 ppm	
	Aldrin	0.1 ppm	
	Formathion	0.5 ppm	

	Chlordane	0.3 ppm	
	Dialdrin	0.1 ppm	
	Heptachlor	0.1 ppm	
	Heptachlor epoxide	0.1 ppm	
	Dicamba	0.5 ppm	
	Permethrin	3.0 ppm	
	2,4 D	5.0 ppm	
	2, 4,5 T	0.5 ppm	
	Total aflatoxins	20 µg/kg.	