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1. SCOPE OF APPLICATIONS

1.1 This is applicable to the Nitric Oxide (NO) and Total Oxides of Nitrogen (NO_x) generated in the gas phase of filtered sidestream tobacco smoke on a continuous basis.

1.2 This method describes the collection of vapour phase NO and NO_x in sidestream tobacco smoke using a fishtail assembly and their quantification by a dual channel chemiluminescence analyzer. The method can be applied to both cigarettes and cigars.

2 NORMATIVE REFERENCES

2.1 Health Canada Test Method T-115 – Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, 1999-12-31.

3 DEFINITIONS

3.1 Refer to T-115 for definitions of terms used in this document.

4 METHOD SUMMARY

4.1 A single port smoking machine is configured with a fishtail chimney assembly and calibrated flow-controlled vacuum pumps.

4.2 One cigarette or cigar is smoked beneath the fishtail chamber following a set smoking protocol and the smoke is swept up the chimney by vacuum at the rate of 2 L/minute. The Total Particulate Matter (TPM) of the sidestream smoke is collected on a glass fibre filter disc (pad) at the top of the chimney and a subsample of the filtered sidestream vapour phase is dynamically diluted with N_2 and then pumped to a dual channel chemiluminescence nitrogen oxides analyzer.

4.2.1 The gas stream is split immediately into two channels.

4.2.2 In channel A, the sample stream is reacted with ozone and the resultant chemiluminescent emission is directly proportional to the NO concentration in the sample.

4.2.3 In channel B, the sample stream is chemically reduced first by a catalytic converter and then mixed with ozone in the reaction cell where the resultant chemiluminescent emission is due to NO_x or NO + NO_2 .

4.2.4 The NO_2 concentration is then derived electronically by subtracting the NO signal from the NO_x signal.

4.3 Selective photomultiplier detection monitors the reaction cell gas stream and the NO and NO_x found in the vapour phase of sidestream tobacco smoke are quantified by external standard calibration procedures.

Note: The testing and evaluation of certain products against this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

5 APPARATUS AND EQUIPMENT

5.1 Equipment needed to perform conditioning as specified in T-115.
5.2 Equipment needed to perform marking for butt length as specified in T-115.
5.3 Equipment needed to perform smoking of tobacco products as specified in T-115.
5.4 Dual Channel Chart Recorder.
5.5 Dual Channel Chemiluminescence Nitrogen Oxides Analyzer with vacuum pump.
5.6 V/F Integrator.
5.7 Digital Multimeter.
5.8 Fishtail Chambers – British American Tobacco (BAT).
5.9 2 X 1 L Round bottomed boiling flask 1 L (PEC1 and PEC2).
5.10 Gas Regulator CGA 660 - Inlet 0-3000, Delivery 0-100.
5.11 Gas Regulator CGA 580 - Inlet 0-4000, Delivery 0-100.
5.12 Flow meter FM1 (NO_x Analyzer Make-up).
5.13 Flow meter FM2 (Calibration Gas).
5.14 Flow meter FM3 (Sidestream Sub Sample).
5.15 Flow meter FM4 (Sidestream Makeup).
5.16 Flow meter FM5 (Sidestream Make up gas).
5.17 Flow meter FM6 (Fishtail total flow).
5.18 Glass fibre filter holders 44 mm (threaded, screw cartridge) see Diagram 2.
5.19 Nalgene Tubing 1/4” ID X 3/8” OD.
5.20 Teflon (TFE) Tubing 1/4” (6.35 mm) X 5.8 mm ID.
5.21 Inert Valves (or stop cocks) with three port housing and plug with 90 ^{o} right angled flow (STC1 & STC2).
5.22 Balance capable of measuring to four decimal places.
5.23 Vacuum Pump (VP1) (NO_x Analyzer make-up).
5.24 Vacuum Pump (VP3) (Sidestream Sub-sample).
5.25 Vacuum pump (VP4) (Sidestream sub-sample dilution).
5.26 Vacuum pump (VP6) (Sidestream total flow).
5.27 Flow Meter/High capacity bubble meter to measure sidestream flow
5.28 Filter Cartridges (Silica gel cartridges).
5.29 Barometer.

6 REAGENTS AND SUPPLIES

Note: All reagents shall be, at the least, recognized as analytical grade in quality.

6.1 Compressed Primary Standard Gas Mixture – 127 ppm NO balance nitrogen (certified).
6.2 Compressed Primary Standard Gas Mixture – 70 ppm NO balance nitrogen (certified).
6.3 Compressed Primary Standard Gas Mixture – 35 ppm NO balance nitrogen (certified).

6.4 Compressed Primary Standard Gas Mixture – 9 ppm NO balance nitrogen (certified).
6.5 Nitrogen N_2 (Zero Gas) UHP.
6.6 Methanol.
6.7 Reagent Alcohol.
6.8 Chart Paper.
6.9 Tweezers.
6.10 Stop watch.
6.11 Paper towels.
6.12 Glass fibre filter discs (pads) 44 mm in diameter, with no more than 5 % acrylic type binder.

7 PREPARATION OF GLASSWARE

7.1 The BAT fishtail chamber is rinsed three times with reagent alcohol and once with methanol and then air-dried.

7.2 All Teflon three way stopcocks are rinsed with reagent alcohol to remove the tar and once with methanol and allowed to air dry.

7.3 The piston and chamber of the single port smoking machine is cleaned and rinsed with methanol and then allowed to air dry. The piston is then reassembled, free of any lubricants.

8 SAMPLING

8.1 The sampling of tobacco products for the purpose of testing shall be as specified in T-115.

9 TOBACCO PRODUCT PREPARATION

9.1 Product shall be conditioned as specified in T-115.

9.2 Cigarettes and cigars shall be marked for butt length as specified in T-115.

9.3 Cigarettes to be smoked under intense smoking conditions shall be prepared as specified in T-115.

10 SMOKING MACHINE PREPARATION

10.1 Ambient Conditions

10.1.1 The ambient conditions for calibration shall be between 21 and 23 °C.

10.2 Machine Conditions

10.2.1 The machine conditions shall be as those specified in T-115.

11 SAMPLE GENERATION

11.1 Allow the smoking machine to warm up on the automatic cycling for several minutes and ensure that the piston is oil-free.

11.2 The smoking machine operation shall be carried out as specified in T-115.

11.2.1 Because the analysis is dynamic and ongoing, it is important to ensure that the sidestream tobacco smoke generated was characteristic of the test sample. This can be accomplished if the mainstream and sidestream TPM are determined (i.e. net difference in the weight of the pad before and after smoking). The mainstream and sidestream TPM data and puff count information can then be used to characterize the vapour phase samples and monitor the smoking process.

11.3 Set-up of Sidestream Flow Detection Equipment

11.3.1 The NO/NO_x Analyzer is pumped down (left on) overnight, then the ozone generator and molycon catalytic converter are activated.

11.3.2 The NO/NO_x Analyzer inlet filter is changed once per day.

11.3.3 The sidestream NO/NO_x equipment and tubing are assembled as per **Appendix: Diagram 1.**

12 INSTRUMENT ANALYSIS

12.1 Performance Test of NO/NO_x Analyzer

12.1.1 The NO/NO_x Analyzer electronic output is synchronized to both the two pen chart recorder and to the V/F Integrator to be in the range of 10 mV (ppm).

12.1.2 Various NO/NO_x Analyzer instrument tests are performed prior to any smoking or calibration.

12.1.3 Test T1, T2, and T3 outputs and measure on the analogue scale. This should remain constant day to day.

12.1.4 Switch the Range PPM to Optic and Electric test modes. Allow to stabilize at least 15-20 minutes before reading. These outputs should be consistent in the range of 40 – 80 % on a day to day basis but with some variation expected.

12.1.5 Switch the Range PPM back to 10 for the rest of the experiment.

12.2 Zero the NO/NO_x Analyzer with Nitrogen

Note: This is performed in vacuum mode.

12.2.1 Connect the nitrogen (zero gas) to FM2 to deliver 1.8 L/minute to the inlet port of the pressure equalization chamber (PEC1).

12.2.2 There are two outlets from PEC1, one to the exhaust and the other is connected directly to STC2.

12.2.3 Set the N_2 regulator to deliver 10 psi.

12.2.4 STC2 is directed to deliver flow to the NO/NO_x Analyzer.

12.2.5 Allow to equilibrate for 30 minutes.

12.2.6 Adjust the Zero pots on the NO/NO_x Analyzer to give an average of 0.000 over 60 seconds reading NO and NO_x on the digital multimeter.

12.3 Span the NO/NO_x Analyzer

Note: This is performed in vacuum mode.

12.3.1 Connect the 9 ppm NO primary standard to FM2 to deliver 1.8 L/minute to inlet port of PEC1.

12.3.2 Set the gas regulator to deliver 10 psi.

12.3.3 The outlet line from PEC1 is connected directly to STC2 which delivers flow to the NO/NO_x Analyzer.

12.3.4 Allow to equilibrate for 15 minutes.

12.3.5 Adjust the Span pots on the NO/NO_x Analyzer to give an average of 9.000 (see calibration gas) over 60 seconds on the digital multimeter. (Adjust the span pots to give a corresponding voltage to concentration i.e. NO=9.1 NOx=9.3).

12.3.6 Record Span settings and adjust attenuation on plotter if nessesary. **Do not adjust Span or Zero from here on.**

12.3.7 Optional: The above procedure can be repeated with a 5 ppm NO standard gas or another standard NO gas that fall in the linear range.

12.4 Confirm sidestream setup

Note: This section is confirmed in the positive displacement mode.

12.4.1 Disconnect VP1 from the NO/NO_x Analyzer and allow the NO/NO_x Analyzer to vent directly to exhaust.

12.4.2 Join the inlet ports of VP3 and VP4 using a Y connector.

12.4.3 Connect the outlet from PEC1 directly to the Y connector.

12.4.4 Connect the Nitrogen to FM2 to deliver 1.8 L/minute to the inlet port of PEC1. Set the gas regulator to deliver 10 psi.

12.4.5 Nitrogen is pumped through both VP3/FM3 and VP4/FM4 and the flow is merged and directed to the NO/NO_x Analyzer. FM3 is calibrated to 80 cc/minute and FM4 to 1420 cc/minute (total 1.5 L/minute).

12.4.6 Allow Nitrogen to equilibrate for 20-30 minutes maximum. Read voltage over 60 seconds for NO/NO_x. Do not adjust the zero pots.

12.4.7 Results should be consistent with that obtained in section 12.2.6.

12.4.8 Repeat the above procedure (12.4.1 – 12.4.6) with 9ppm standard gas and record the voltage over 60 seconds.

12.4.9 Results should be consistent with that obtained in section 12.3.5.

12.5 Calibrate Sidestream Fishtail Chimney Assembly

Note: This section is confirmed in the positive displacement mode.

12.5.1 Activate vacuum pump VP6 and adjust flow meter FM6 to deliver 2.0 L/minute. Check flow using high capacity bubble meter. STC1 is directed to room air.

12.5.2 Connect the 127 ppm NO Standard gas to FM2 to deliver 2.5 L/minute to the inlet port of PEC1.

12.5.3 Set the gas regulator to deliver 10 psi.

12.5.4 The outlet line from PEC1 is connected directly to the calibration gas port of STC1. The common port of STC1 is connected to FM6/VP6 which has been calibrated to 2 L/minute.

12.5.5 Connect Nitrogen (set to deliver 10psi) to FM5 which is set to deliver 1.5 L/minute to PEC2.

12.5.6 Connect the outlet line/port of PEC2 to VP4/FM4. Set FM4 to 1.42 L/minute. The purpose of the gas is to dilute the sidestream sub-sample.

12.5.7 Read the baseline from the V/F Intergrator.

12.5.8 Take 90 seconds sidestream samples by rotating STC1 between the 127 ppm NO standard gas and room air.

12.5.9 Note the voltage on the digital multimeter and compare to the expected (as a guide):

$$\frac{127 \text{ ppm} \times 0.080 \text{ L}}{1.5 \text{ L}} = 6.7 \text{ mV}.$$

12.5.10 Add baseline to expected voltages to give actual target values.

12.5.11 Record the counts and adjust the V/F Integrator threshold pots. Repeat until total counts are reproducible and consistent between the NO and NO_x within 1-2 % from one trial to the next.

12.5.12 Do multiple 10 minute samplings; record count and determine average total counts per 10 minute sample. This figure is inserted into the calculation equation at a latter stage.

12.5.13 The NO/NO_x Analyzer is now calibrated for operation.

12.5.14 Repeat the above procedure (12.5.1 – 12.5.12) with the 70ppm NO Standard gas recording multiple 10 minute samplings without adjusting the voltages.

12.5.15 Close gas cylinders and disconnect the line from PEC1 to STC1. Be sure the room and the sidestream apparatus are clear of calibration gas before starting smoking procedure.

13 SMOKING PROCEDURE

13.1.1 Re-assemble configuration as per Diagram 1.

13.1.2 Fit a conditioned pad into the mainstream pad holder and sidestream pad holder with the rough side towards the incoming smoke.

13.1.3 Raise chimney level to highest position (loading position).

13.1.4 Attach the weighed pad holders to the smoking machine (sidestream and mainstream).

13.1.5 Turn the **Puff Counter** switch to **ON** and switch to automation.

13.1.6 Gently insert the cigarette into the cigarette holder to a depth greater than 9 mm. Withdraw the cigarette until the 9 mm mark is just visible.

13.1.7 Turn on the sidestream pumps (VP3, VP4 and VP6) at the beginning of the lighting procedure at t minus 30 seconds.

13.1.8 Light the cigarette and initiate the puff count according to the following schedule. Be sure to light the cigarette on the first puff.

13.1.8.1 Normal lighting procedure is 15 seconds warm-up followed by five seconds ignition. (Three seconds prior to puff plus the two seconds puff).

13.1.9 Lower the fishtail assembly over the cigarette to a position of 6 mm above a plate that is beneath the cigarette. Do not allow the cigarette to touch the chimney. This is to create a uniform flow of air around the cigarette and up the fishtail chimney.

13.1.10 Sidestream smoke pattern should be similar to **Figure 1**.

13.1.11 The test cigarette is smoked to the previously marked standard butt length. Remove the butt with a pair of tweezers from beneath the BAT fishtail chamber and extinguish in a beaker of water.

13.1.12 All vacuum pumps (VP3, VP4 and VP6) continue to draw any residual smoke within the sidestream chamber until the V/F Integrator counter stops. Record the time.

13.1.13 At the end of the smoking process raise the chimney and remove the pad holders.

13.1.13.1 Re-weigh the sidestream pad holder and record the “after smoking” weights of the sidestream pad holders.

13.1.13.2 Re-weigh the mainstream pad holder and record the “after smoking” weights of the mainstream pad holders.

13.1.13.3 Record the puff count.

13.1.13.4 Record the total number of counts for each channel, the NO and NO_x and the total time to the end of smoking (counting) on the run sheet.

14 CALCULATIONS

14.1 The total counts for each channel for each test brand are summarized and the vapour phase concentrations of NO and NO_x in [µg/cigarette] in sidestream tobacco smoke are calculated.

$$\text{NO µmole/cigarette} = \frac{TC_{(NO)}}{AC_{(NO)}} \times \frac{127}{TS} \times \frac{273}{273 + {}^{o}C} \times \frac{10}{22.4} \times \frac{BP}{SBP} \times DF.$$

$$\text{NO µg/cigarette} = \frac{\text{µ mole}}{\text{cigarette}} \times \frac{\text{µg}}{\text{µ mole}}.$$

and

$$NO_x \text{ µmole/cigarette} = \frac{TC_{(NOx)}}{AC_{(NOx)}} \times \frac{127}{TS} \times \frac{273}{273 + {}^{o}C} \times \frac{10}{22.4} \times \frac{BP}{SBP} \times DF.$$

$$NO_x \text{ µg/cigarette} = \frac{\text{µ mole}}{\text{cigarette}} \times \frac{\text{µg}}{\text{µ mole}}.$$

where:

- $TC_{(NO)}$ is the total continuous counts during smoking of the NO channel.
- $TC_{(NOx)}$ is the total continuous counts during smoking of the NO_x channel.
- TS is the time to end of Smoking (minutes).
- DF is the dynamic dilution factor. Dilution factor is 1/sss/(sss+ss). where sss = sidestream sub-sample; ss = sidestream flow (L/minute).
- 127 is the Calibration gas 127 ppm (µL/L).
- $AC_{(NO)}$ is the average continuous count per NO channel for the calibration gas for 10 minutes.
- $AC_{(NOx)}$ is the average continuous count per NO_x channel for the calibration gas for 10 minutes.
- ^{o}C is the room temperature.
- 22.4 µL/µmole.
- BP is the barometric pressure (inches Hg).
- SBP is the barometric pressure at standard temperature and pressure (29.92 inches Hg).

15 QUALITY CONTROL

15.1 Typical Graph

15.1.1 See **Figure 1.**

15.2 Recoveries and Levels of Contamination

Each analytical run of test cigarettes should also include the following:

15.2.1 A Laboratory Reagent Blank (LRB) to evaluate the extent of any interferences due to glassware, trapping reagents, glass fibre filter pads, and analyzer effects. In this case it would be the Zero gas.

15.2.2 A Laboratory Fortified Blank (LFB) to evaluate the extent of potential analyte loss. The 9 ppm NO standard gas is the LFB.

15.2.3 Test a Reference Sample such as 1R4F to determine the inter-experimental reproducibility of the entire method of analysis.

15.3 Method Detection Limit (MDL) and Limit of Quantitation (LOQ)

15.3.1 Method Detection Limit (MDL)

15.3.1.1 The method detection limit is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The MDL is then calculated as three times the standard deviation of these determinations.

15.3.2 Limit of Quantitation (LOQ)

15.3.2.1 The limit of quantification is determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The LOQ is then calculated as 10 times the standard deviation of these determinations.

15.4 Stability of Reagents and Supplies

15.4.1 All primary standard gas mixtures are maintained at room temperature (22 °C).

15.4.2 All samples are analyzed on a continuous basis as smoked.

16 MODIFICATIONS FOR INTENSE SMOKING

16.1 No modifications are necessary under intense smoking conditions.

17 REFERENCES

17.1 Rickert, W.S., Robinson, J.C., and Collishaw, N.E., 1987. Decay of Cigarette Smoke NO_x an Ambient Air Under Controlled Conditions, *Environmental International* 13, p. 399-407.

17.2 Norman, V., Ibrig, A.M., Larson, T.M., and Moss, B.L., 1983. The Effect of Some Nitrogenous Blend Components on NO/NO_x and HCN Levels in Mainstream and Sidestream Smoke, *Beitrige zur Tabakforschung International* 12 , No. 2, p. 55-62.

17.3 Neurath, G. and Dunger, M., 1972. IARC (International Agency for Research on Cancer) *Science Publication*, No.3, p.134-136.

APPENDICES

Figure 1 : Graph of Vapour Phase NO and NOx in Sidestream Tobacco Smoke

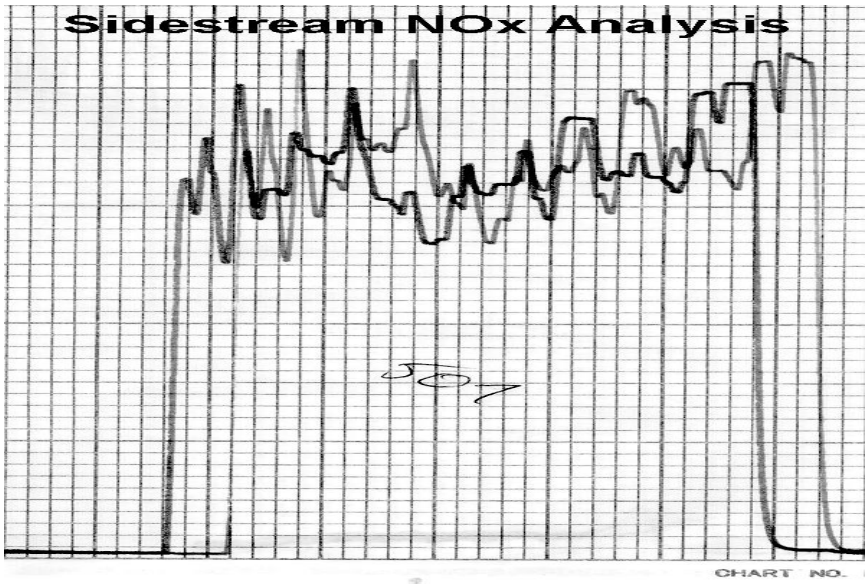


Diagram 1: Sidestream Smoking/ Calibration Mode

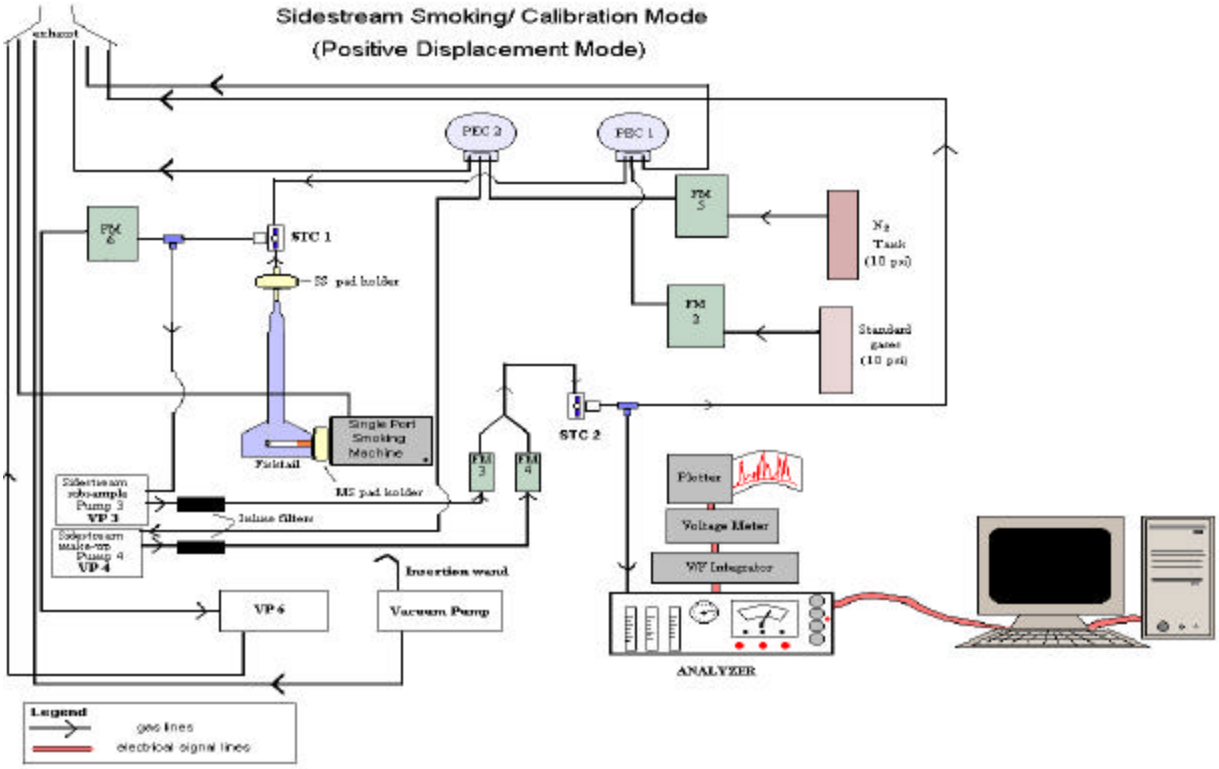
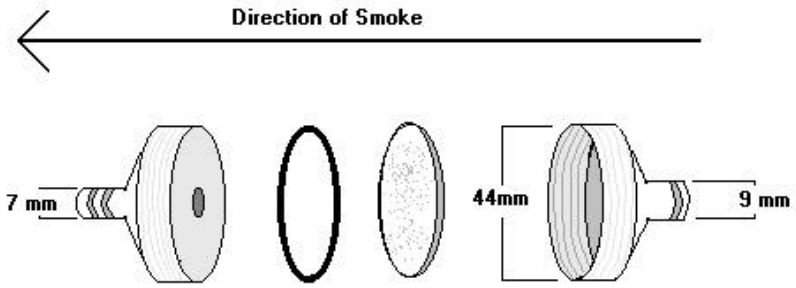


Diagram 2: Glass fibre filter holder: Threaded screw cartridge



Pad Holder Setup (Measurements are inner diameters only)