



## Measuring CN content in leaf samples using Elementar Vario MICRO Cube

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In devel.

Canadian Airborne Biodiversity Observatory



### ABSTRACT

Here we describe the standardised protocol used by the [Canadian Airborne Biodiversity Observatory](#) (CABO) to measure CN content in leaf samples, using the Elementar Vario MICRO Cube.

### EXTERNAL LINK

[www.caboscience.org](http://www.caboscience.org)

### PROTOCOL STATUS

#### In development

We are still developing and optimizing this protocol

### GUIDELINES

#### Equipment

Elementar Vario MICRO Cube

Mettler-Toledo XPR2 balance

### MATERIALS

NAME	CATALOG #	VENDOR
Tin boat 12 x 4 x 4 mm	D5130	Isomass Scientific Inc.
Tin boat 8 x 8 x 15 mm	D5032	Isomass Scientific Inc.
Acetanilide	00401-5G	Sigma Aldrich
Oxygen 99.993%	OX4.3UH-T	
Helium 99.999%	HE5.OUH-T	

### STEPS MATERIALS

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### SAFETY WARNINGS

## BEFORE STARTING

Leaf samples have been dried 72 hours at 65°C then crushed with a cyclone mill (2mm screen).

Some videos may help you understanding and visualising this protocol:

<https://www.youtube.com/watch?v=ELYHSCX4wGY>

[https://www.youtube.com/watch?v=MJ\\_Zlbidufw](https://www.youtube.com/watch?v=MJ_Zlbidufw)

<https://www.youtube.com/watch?v=XMkG0ysD2as>

<https://www.youtube.com/watch?v=jTSh5k4yQvo>

<https://www.youtube.com/watch?v=3clPeNt8DhE>

### Weighing samples and boat preparation

- With a microbalance, put 7 mg (+/-0.2mg) of sample in a tin boat.



XPR2

Microbalance

Mettler Toledo XPR2 [🔗](#)



Tin boat 8 x 8 x 15 mm

by Isomass Scientific Inc.

Catalog #: [D5032](#)



Tin boat 12 x 4 x 4 mm

by Isomass Scientific Inc.

Catalog #: [D5130](#)

2 With tweezers, gently and carefully close the boat. Using the pressing tool, slowly press the boat to remove any remaining air.

3 Weigh again and note. You will use this in the software before starting the analysis (see step 22).



Prepare all your samples the same way. Prepared samples should be placed in 24-well plates and stored in a dessicator while waiting for the analysis to get started.

4 With a microbalance, put 2 mg (+/-0.2mg) of acetanilide in a tin boat. With tweezers, gently and carefully close the boat. Using the pressing tool, slowly press the boat to remove any remaining air. Weigh.



Tin boat 12 x 4 x 4 mm

by Isomass Scientific Inc.

Catalog #: D5130



Acetanilide

by Sigma Aldrich

Catalog #: 00401-5G



XPR2

Microbalance

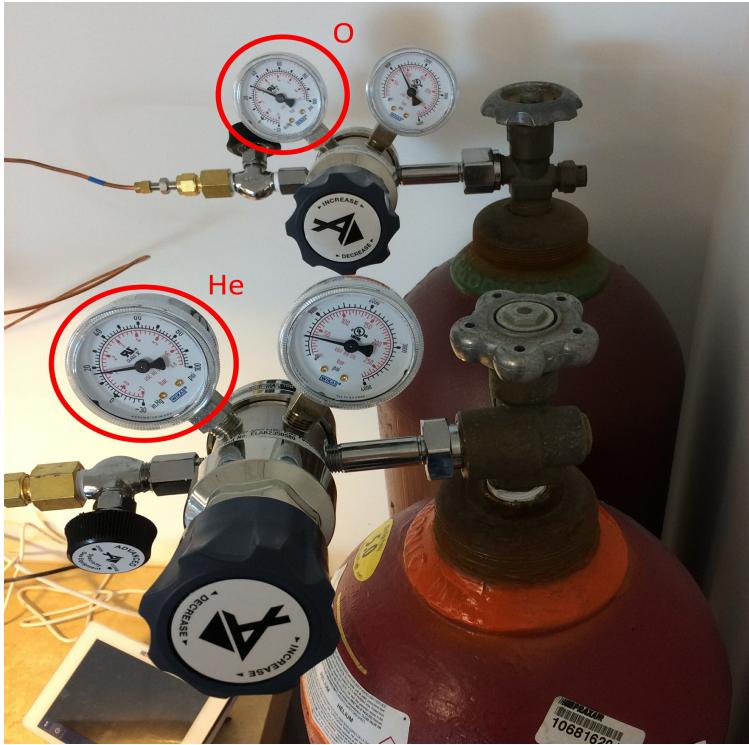
Mettler Toledo XPR2



Calculate 4 acetanilid standards (for instrument conditioning) + 1 acetanilid standard for each 10 samples.

#### Instrument preparation

5 Check gaz pressure on manometers. Helium should be 1.2 bar, oxygen should be 2 bar.



Oxygen 99.993%

Catalog #: [OX4.3UH-T](#)

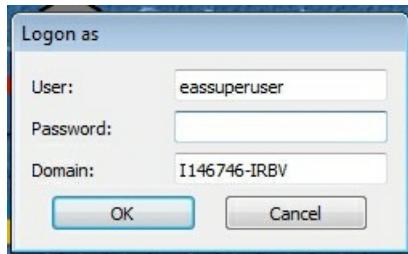


Helium 99.999%

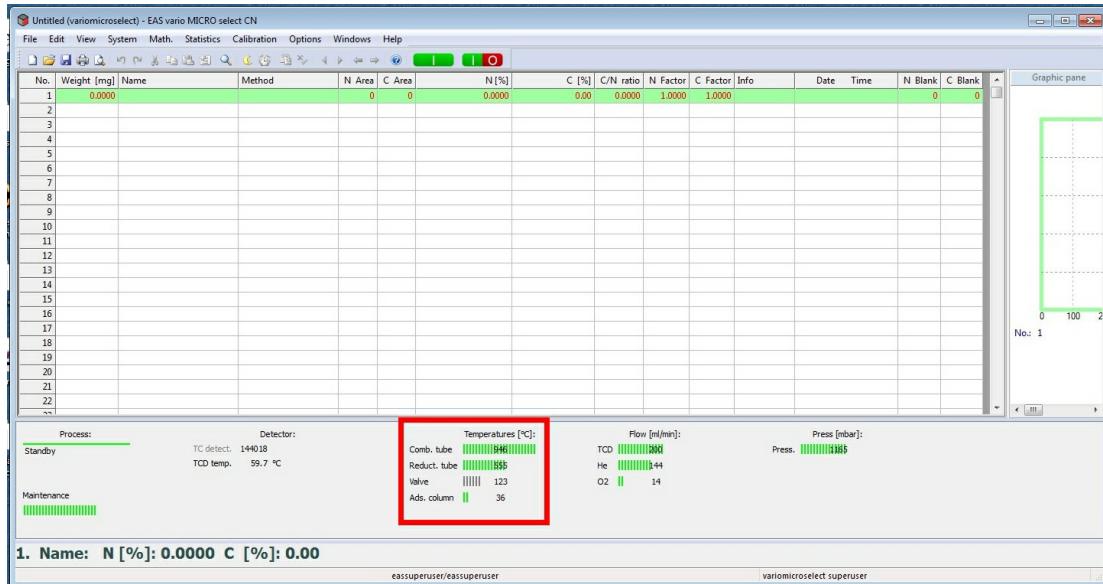
Catalog #: [HE5.0UH-T](#)

- 6 Launch **Variomicroselect software**. There is no password. Click **OK**.





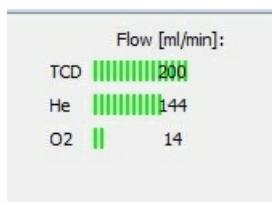
- 7 On main screen, check if combustion tube temperature is 950°C and reduction tube is 550°C.



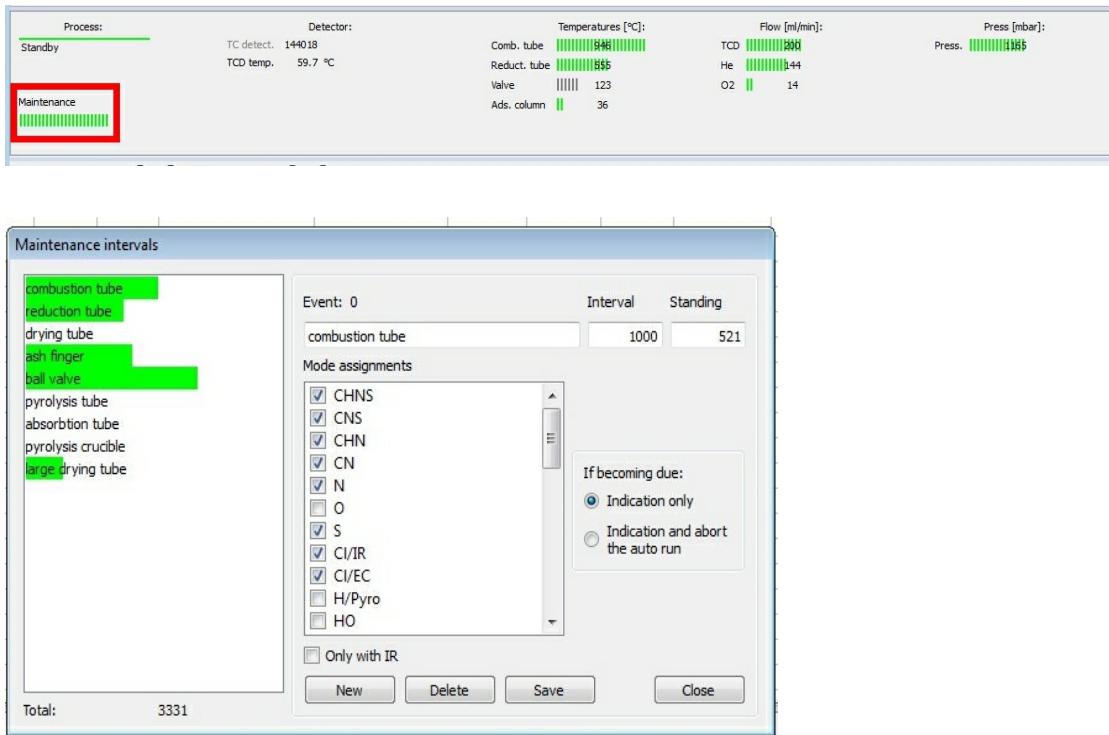
If it is in blue and flashing, it means it is heating up and will reach correct temperatures after few minutes.

If not, you can modify by clicking on Options> Settings>Parameters... and change manually the temperatures.

- 8 On main screen, check the meter flow: TCD should be 200 mL/min; helium should be 200 mL/min; oxygen should be 13 mL/min.



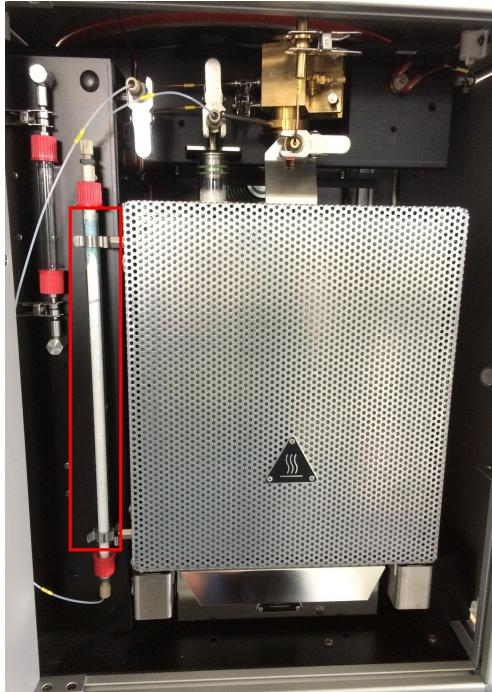
- 9 On main screen, have a look on general components by clicking on Maintenance.



If some components are in orange, see with the lab manager if you need to perform a maintenance.

10

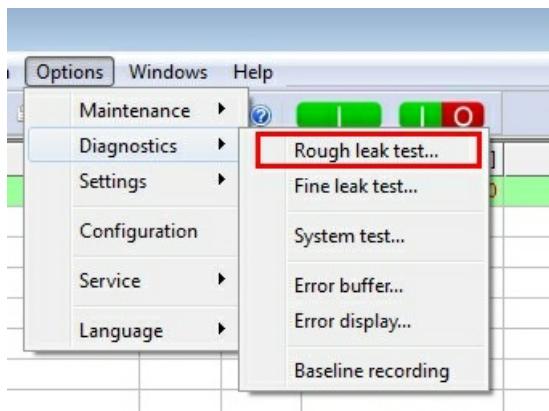
Open the front door of the Vario MICRO Cube. If the drying tube is mostly white, close the front door and go ahead with next step.



If the drying tube is 2/3 blue, contact the lab manager for a maintenance.

## Leak test

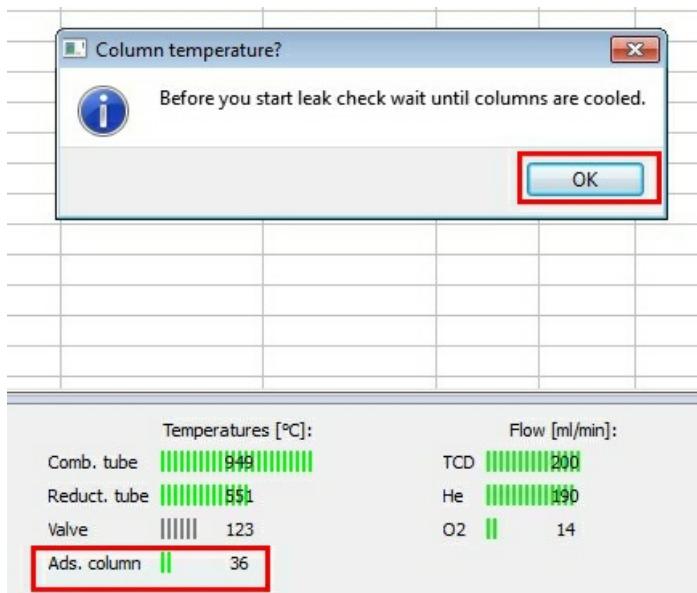
11 Click on Option -> Diagnostics -> Rough leak test



12 Click Yes

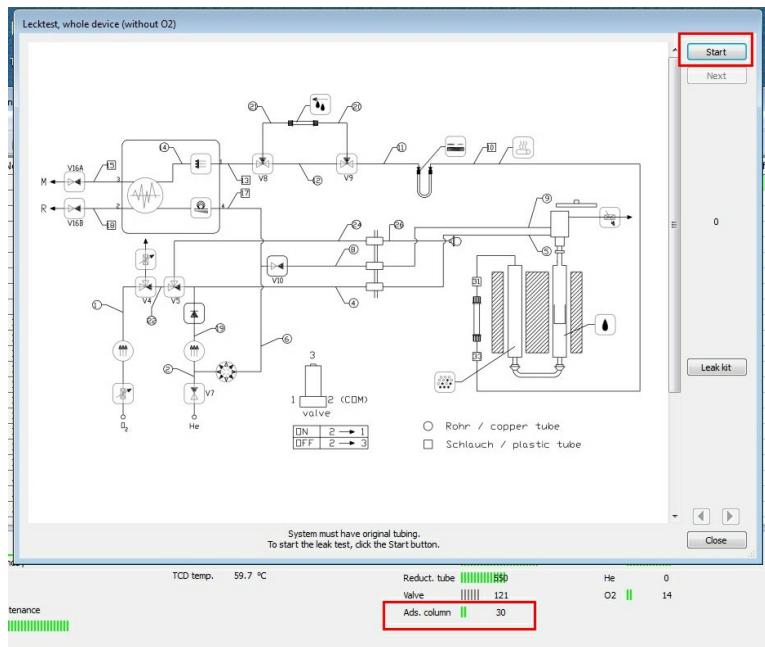


13 A window will open, saying to wait until columns are cooled. Press OK.



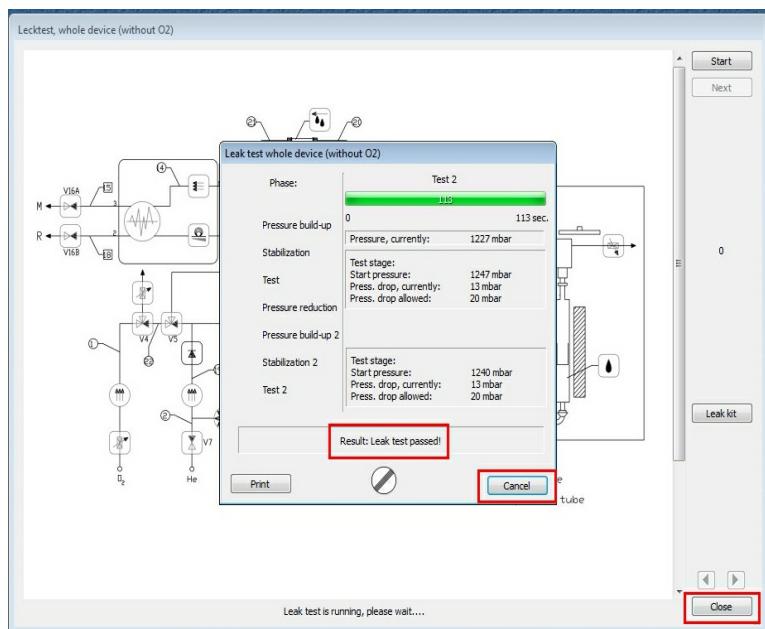
14 Wait for few minutes until the ads. column temperature is below 30°C and then press Start.

⌚ 00:03:00



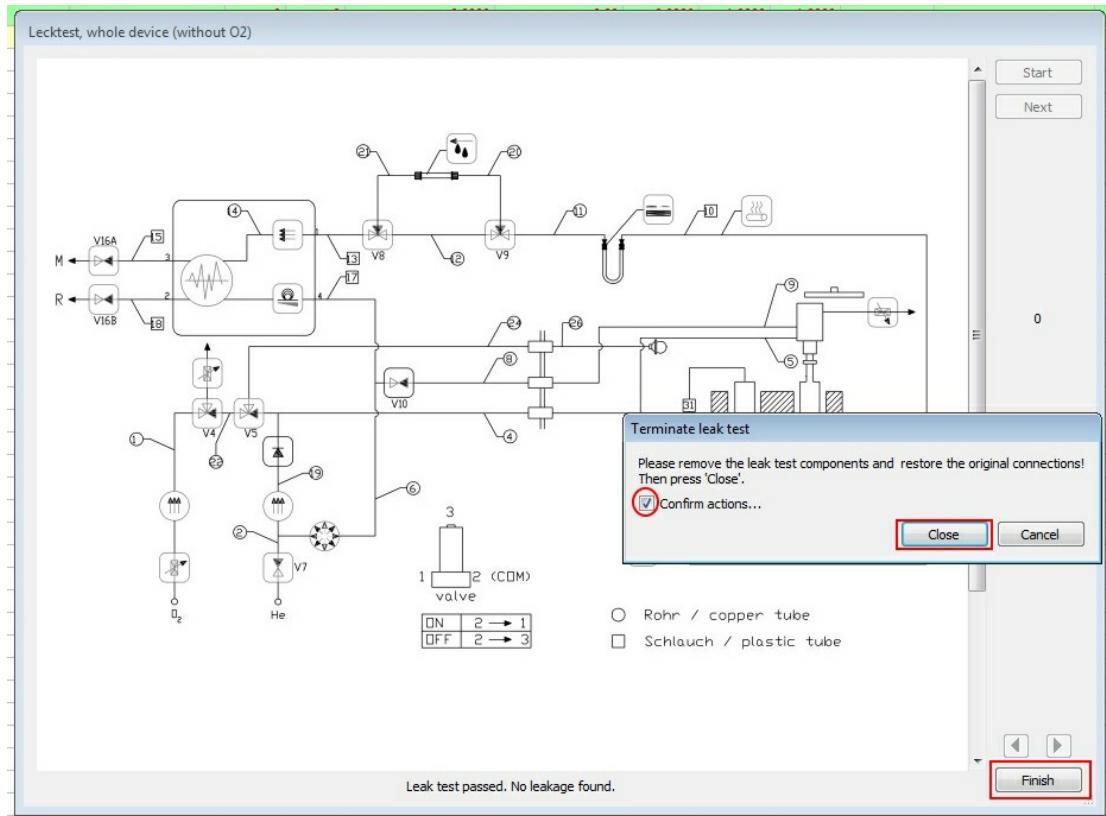
- 15 After 10 minutes, the window will show «Leak test passed» or «Leak test failed». Click **Cancel**.

⌚ 00:10:00



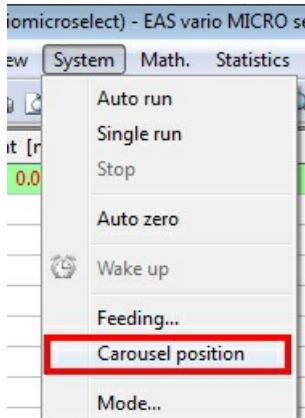
If the leak test has failed, see the lab manager.

- 16 Select **Confirm actions...** Click on **Close** and then **Finish**.

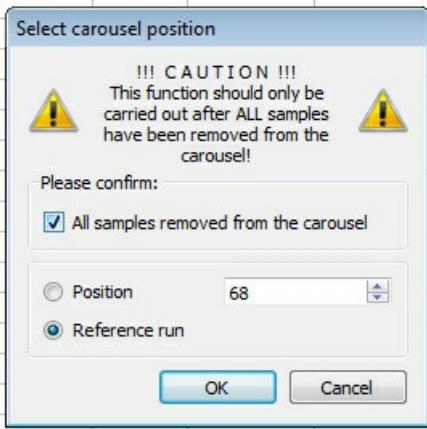


Set carousel position

17 Go to System --> Carousel position



18 Click on All samples removed from the carousel and choose Reference run



### Conditioning the instrument

- 19 On main screen, write in the table, on rows 1 to 6:

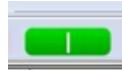
Weight (mg): 100

Name: MT

Method: Blank without O<sub>2</sub>

No.	Weight [mg]	Name	Method	N Area	C Area	N [%]	C [%]	C/N ratio	N Factor	C Factor	Info	Date	Time	N. Blank	C Blk
1	100.0000	MT	Blank without O <sub>2</sub>	2 207	1 105	0.0607	0.04	0.6385	1.0000	1.0000	Cu	2019-02-20	10:03	0	E
2	100.0000	MT	Blank without O <sub>2</sub>	29	9	0.0009	0.00	3.627	1.0000	1.0000	Nu,Cu	2019-02-20	10:09	0	
3	100.0000	MT	Blank without O <sub>2</sub>	4	55	0.0001	0.00	16.0118	1.0000	1.0000	Nu,Cu	2019-02-20	10:14	0	
4	100.0000	MT	Blank without O <sub>2</sub>	0	0	0.0000	0.00	0.0000	1.0000	1.0000				0	
5	100.0000	MT	Blank without O <sub>2</sub>	0	0	0.0000	0.00	0.0000	1.0000	1.0000				0	
6	100.0000	MT	Blank without O <sub>2</sub>	0	0	0.0000	0.00	0.0000	1.0000	1.0000				0	
7	2.0900	RunIn	2mgChem70s	0	0	0.0000	0.00	0.0000	1.0000	1.0000				0	
8	2.0190	RunIn	2mgChem70s	0	0	0.0000	0.00	0.0000	1.0000	1.0000				0	
9	100.0000	Blank	Blank with O <sub>2</sub>	0	0	0.0000	0.00	0.0000	1.0000	1.0000				0	
10	100.0000	Blank	Blank with O <sub>2</sub>	0	0	0.0000	0.00	0.0000	1.0000	1.0000				0	
11	2.0090	acetanilid	2mgChem70s	0	0	0.0000	0.00	0.0000	1.0000	1.0000				0	
12	0.0000	acetanilid	2mgChem70s	0	0	0.0000	0.00	0.0000	1.0000	1.0000				0	
13	7.0520	24051085	Soil120	0	0	0.0000	0.00	0.0000	1.0000	1.0000				0	
14	7.0430	24051261	Soil120	0	0	0.0000	0.00	0.0000	1.0000	1.0000				0	
15	7.0180	24058387	Soil120	0	0	0.0000	0.00	0.0000	1.0000	1.0000				0	
16	100.0000	Blank	Blank with O <sub>2</sub>	0	0	0.0000	0.00	0.0000	1.0000	1.0000				0	
17	0.0000			0	0	0.0000	0.00	0.0000	1.0000	1.0000				0	
18															
19															
20															

- 20 Press **green button** to start the analysis.



00:36:00



The results (N Area and C Area) should be below 100 after few analysis.  
If it is not the case, contact the lab manager.

- 21 On row 7 and 8, write:

Weight (mg): put the weight of 2 acetanilid boats, prepared in step 4.

Name: RunIn

Method: 2mgChem70s

Untitled (variomicroselect) - EAS vario MICRO select CN

No.	Weight [mg]	Name	Method
① 1	100.0000	MT	Blank without O2
② 2	100.0000	MT	Blank without O2
③ 3	100.0000	MT	Blank without O2
● 4	100.0000	MT	Blank without O2
● 5	100.0000	MT	Blank without O2
● 6	100.0000	MT	Blank without O2
● 7	2.0900	RunIn	2mgChem70s
● 8	2.0190	RunIn	2mgChem70s
● 9	100.0000	Blank	Blank with O2
● 10	100.0000	Blank	Blank with O2
● 11	2.0090	acetanilid	2mgChem70s
■ 12	0.0000	acetanilid	2mgChem70s

22 On row 9 and 10, write:

Weight (mg): 100

Name: Blank

Method: Blank with O2

● 9	100.0000	Blank	Blank with O2
● 10	100.0000	Blank	Blank with O2

23 On row 11 and 12, write:

Weight (mg): put the weight of 2 acetanilid boats, prepared in step 4.

Name: acetanilid

Method: 2mgChem70s

● 11	2.0090	acetanilid	2mgChem70s
■ 12	0.0000	acetanilid	2mgChem70s

24 On rows 13 to 23, write:

Weight (mg): put the weight of prepared boats (see steps 1-3).

Name: Sample name (cover of sample bottle)

Method: 9mgPlant90s

25 On row 24, write:

Weight (mg): 100

Name: Blank

Method: Blank with O2



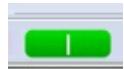
Many series can be run in the same day. Between 2 series of 10 samples, put 2 blanks (Blank with O2) and 1 acetanilid (2mgChem70s). The last row should be a blank.

26

Put prepared boats in the carousel. Numbers from carousel holes are corresponding to the software rows. Leave carousel hole empty when corresponding to a blank. Put back the carousel cover.

27

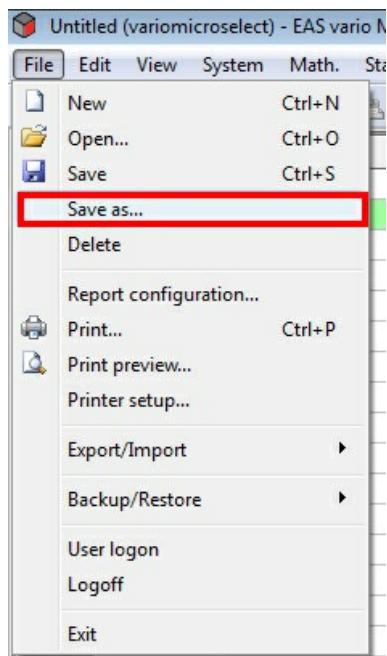
Press **green button** to start the analysis.



Saving data & shut down the instrument

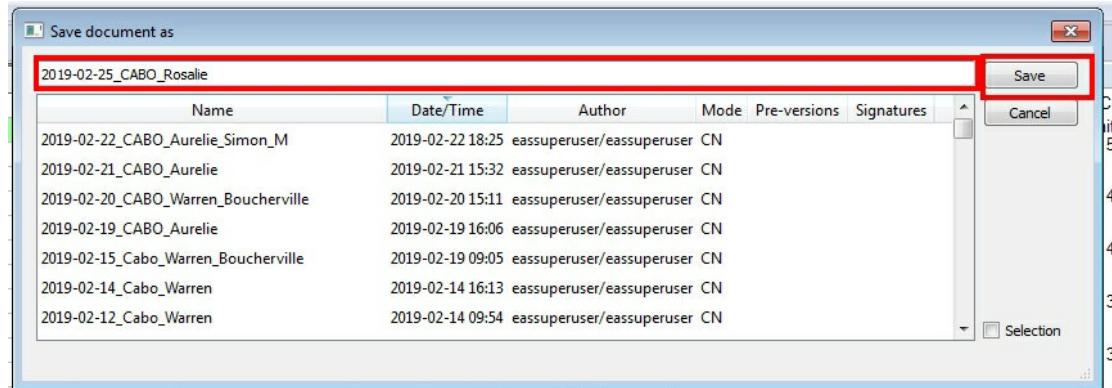
28

Go to **File --> Save as**



29

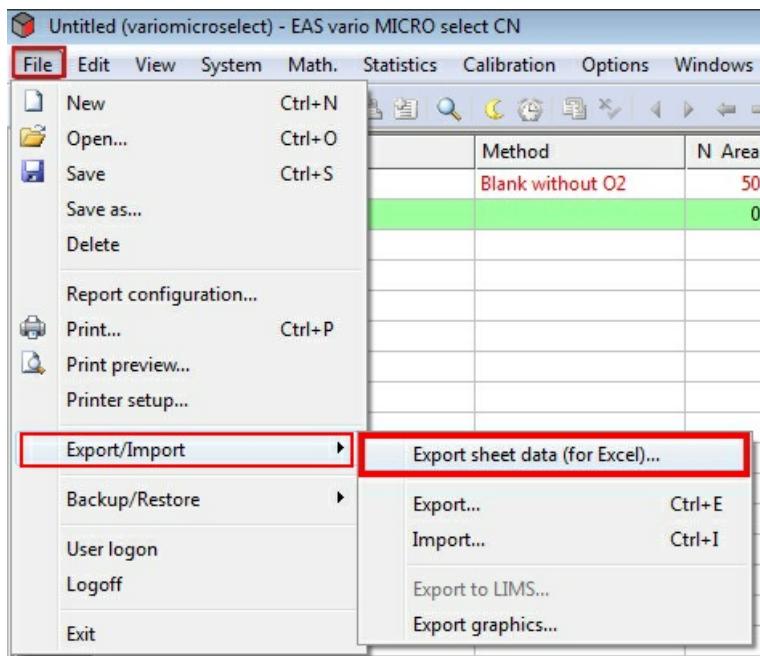
Write the date (YYYY-MM-DD) and name of the project or student and press **Save**.



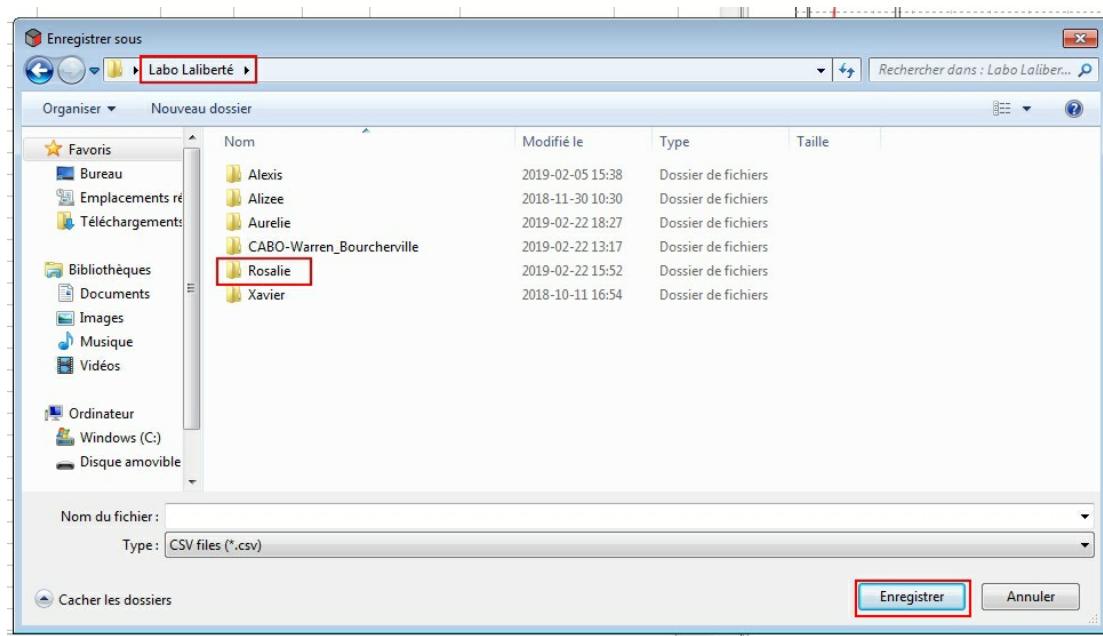
30

Export data:

Go to **File --> Export/Import --> Export sheet data (for Excel)...**



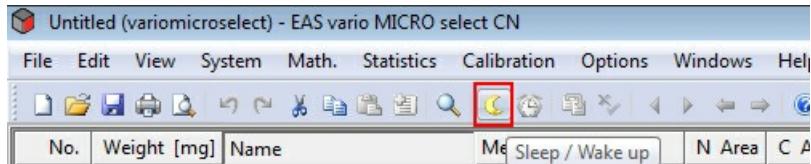
- 31 And then select Labo Laliberté and the project/student. Press **Enregistrer**.



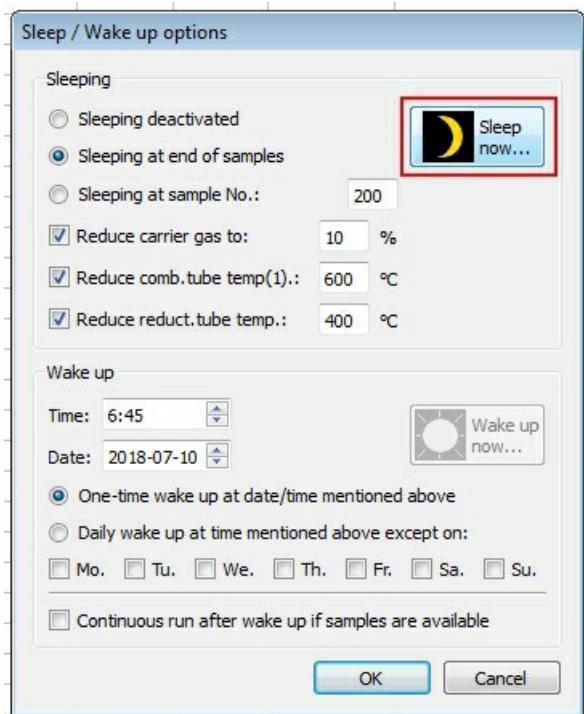
- 32 Make sure the instrument is on sleeping mode before leaving the lab. Have a look at the bottom of the screen.



To put the instrument on sleeping mode, click on the moon on main screen



and on **Sleep now...**



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