



# Auto-Block Plus Tester V.1

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*Works for me*

This protocol is published without a DOI.

PDI Test

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USDA-ARS

SUBMIT TO PLOS ONE

## ABSTRACT

Previous Auto-Block Digester protocols developed by Aprel Ellison and Paul Shumaker

## PROTOCOL CITATION

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<https://protocols.io/view/auto-block-plus-tester-bs7wnhpe>



## KEYWORDS

Auto-Block, Plus, Digester

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## GUIDELINES

### Standards

A **blank** is just an empty digestion tube (at the start) which undergoes the digestion procedure; this allows us to see any contamination which occurs from the digestion process upon analysis.

**Standard materials** are materials that have certified concentrations to check the efficiency of the digestion procedure. Weigh out 0.15g of desired standard into digestion tube (run in duplicate). We have several standard materials options at the Center depending on the sample type (all certification datasheets are located here ([P:\Teams\Analytical\\_Team\Expert\\_Group\\_Matrix\Method\\_Data\Auto-Block\\_Digester\Standard\\_Materials](P:\Teams\Analytical_Team\Expert_Group_Matrix\Method_Data\Auto-Block_Digester\Standard_Materials)) and hard copies are stored in the "Chemical Methods" binder in lab 1):

- Manure Samples with Trace metal analysis required:
- Sewage Sludge 2 (location: Szogi lab, ask Paul or Dr. Szogi if you need some)
- EnviroMAT Sewage Sludge (location: Szogi lab, ask Paul or Dr. Szogi if you need some)
- Manure Samples with no Trace metal analysis required:
- Apple Leaves (location: Lab 1 chemical cabinet)
- Peach Leaves (location: Lab 1 chemical cabinet)
- Soil Samples with or without manure amendments:
- Sewage Sludge Amended Soil (*label on bottle says Sewage Sludge*) (location: Lab 1 Building 1 refrigerator)
- Metals on Soil/Sediment #4 (location: refrigerator in lab 3 building 1)
- Plant Samples:
- Apple Leaves (location: Lab 1 chemical cabinet)
- Peach Leaves (location: Lab 1 chemical cabinet)

### QA/QC

Depending on sample type, digest duplicate samples of one of the standard materials on site

Place two empty tubes as blank digestions at the end

- A blank is an empty tube which undergoes the digestion procedure, this allows us to see any contamination which occurs from the digestion process upon analysis

If auto fill-to-volume over shoots the 50mL mark, do not evaporate using the auto-block. Do the following:

- Using a pipette, remove liquid from digestion tube until the liquid reaches the 50mL mark
- Pipette the removed sample on to scale to weigh. (now you know how much over 50mL the sample is)
- Repeat this procedure for 6 tubes (2 at beginning, 2 middle, & 2 end)
- Average the amount of mL's removed and add that to 50 to determine the total volume
- Apply the total volume to all calculations
- I.E. (You removed (2mL, 2.1mL, 1.7mL, 1.8mL, 1.9mL, 2mL) from digestion tubes to reach the 50mL mark. The average of those 6 tubes are 1.92mL. Add 1.92mL + 50mL = 51.92mL. Total volume to use in calculations is now 51.92

### Instrument Log Book

Kept in the notebook labeled "Auto-block". For each run, record the following:

- Date and time
- Users initials
- Project/Experiment name
- Number of samples digested
- Indicate if any tubes did not auto-fill
- Indicate if the auto fill to volume step worked properly (meaning were all tubes filled to the 50mL mark  $\pm$  1mL.
- Indicate if a new bottle of acid or peroxide was used
- Any observations (good or bad) such as heavy fumes inside, acid spills in the acrylic trays, a spotless process, etc.

## MATERIALS TEXT

Environmental Express (phone: 843-881-6560; website: [www.envexp.com](http://www.envexp.com))

- HEPA Air Filter (P/N SC868)
- 50mL digestion tubes with screw cap, 500 pack (P/N SC475)
- FilterMate, 0.45µm PTFE Filter, 100 pack (SC0409)
- FilterMate, 2µm PTFE Filter, 100 pack (SC0408)
- Disposable Reflux caps for SC475 tubes, 1000 pack (SC506)

Reagents Incorporated (phone: 800-732-8484; website: [www.reagents.com](http://www.reagents.com); personal contact: Stephanie Harris email [sharris@reagents.com](mailto:sharris@reagents.com))

- ACS grade HNO<sub>3</sub> (P/N 5-10163-1, 5-10163-2, 5-10163-3, or 5-10163-4 depending on size and quantity you want)
- Trace Metal Grade HNO<sub>3</sub> (P/N NX0407-1, NX0407-1/cs, NX0407-2, or NX0407-2/cs depending on the size and quantity you want)
- ACS grade 30% H<sub>2</sub>O<sub>2</sub> (P/N 1-21460-1, 1-21460-3, 1-21460-4, 1-21460-5, or 1-21460-6 depending on the size and quantity you want)

## Reagents

- Reagent 1 = H<sub>2</sub>O
  - Reagent 2 = HNO<sub>3</sub>
  - Reagent 4 = H<sub>2</sub>O<sub>2</sub>
  - Reagent 5 = Trace HNO<sub>3</sub>
- No mixing of reagents is necessary. The transfer lines for the HNO<sub>3</sub>&H<sub>2</sub>O<sub>2</sub> will connect directly to the bottle these are purchased in. No need to transfer these too additional bottles.
- **H<sub>2</sub>O** is Milli-Q H<sub>2</sub>O
- **HNO<sub>3</sub>** For most auto-block digestions, use ACS Grade HNO<sub>3</sub>. If trace metal analysis is to be completed, use trace metal grade HNO<sub>3</sub> (additional acid is stored in the chemical shed)

In this context Digestion is defined as destroying the organic matter components and making soluble the elements of interest (Peters, 2003)

Trace Metal Grade HNO<sub>3</sub> is HNO<sub>3</sub> that is pure enough to be used for Trace Metal Analysis. This has lower maximum impurities than ACS Grade Acid. We use Omnitrace Nitric Acid supplied by "Reagents" part number "NX0407-2" or use the following link, [Omintrace HNO<sub>3</sub>](#) if you would like to purchase for your use.

- **H<sub>2</sub>O<sub>2</sub>** is located in the fridge in lab 1

## Data Sheets

[P:\Teams\Analytical\\_Team\Expert Group Matrix\Method Data\Auto-Block Digester\Auto-Block Digester Data Sheet](#)

## SAFETY WARNINGS

- Wear lab coat, gloves, and safety glasses while working with samples.
- Wear lab coat, heavy gloves, and safety glasses and work in the hood while filling HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> reservoirs
- Wear lab coat, gloves, and safety glasses and work over the sink while filtering
- Protocol revision 2/8/16 after near-miss:

If the reagent lines need to be disconnected from the auto-block at the port entering the auto-block (i.e. to move the auto-block), always disconnect the reagent lines from the bottle first. Remove each reagent line straw from the reagent bottle and place into a 1-L graduated cylinder. After all reagent lines are in the graduated cylinder, add about 500 mL of H<sub>2</sub>O to graduated cylinder to flush reagent lines. In the software, under the manual tab, flush each reagent line with 50mL of H<sub>2</sub>O (see "flushing the reagent lines with H<sub>2</sub>O" below). This will need to be done for every reagent line except the current H<sub>2</sub>O line (2 Nitric acid lines and 1 Hydrogen Peroxide line). This procedure will eliminate the hydraulic head in each reagent line and prevent the siphoning effect that could spill the reagent contents.

Flushing the Reagent lines with H<sub>2</sub>O:

- select reagent 2 "HNO<sub>3</sub>" on the Reagent Selection at the top left of the screen by clicking on it with the mouse
- enter "50" into the Injection Amount under injection control
- Hit "Inject" button underneath the Injection Amount

Repeat this procedure until ALL reagent lines have been flushed (2 nitric acid and 1 hydroxide peroxide lines).

DISCLAIMER:

Developed by: William Brigman 2/06/2015  
Revised: Brigman 1/18/2017  
Certified by: MBV 2/11/2015 Re-certified MBV 2-8-2016  
Experts: William Brigman / Paul Shumaker  
Previous Auto-Block Digester protocols developed by Aprel Ellison and Paul Shumaker

**Citation in publication:** The K, Ca, Mg, Na, Cu, and Zn (add additional elements here) were determined using nitric acid digestion with peroxide (EPA 3050B) using a block digester (Peters, 2003) and inductively coupled plasma analysis (Standard Method 3125; APHA, 1998.).

**Reference List:**

Peters, J. 2003. Unit III 5.5: Digestion and dissolution methods for P, K, Ca, Mg, and trace elements. In J. Peters (ed.) Recommended methods of manure analysis (A3769). Univ. of Wisconsin-Extension publication, Madison, WI.

APHA. 1998. Standard methods for the examination of water and wastewater. Am. Public Health Assoc., Washington, DC.

BEFORE STARTING

NOTE 1:

This method protocol was developed for an operator to work during normal working hours. It was first tested to digest manure type samples for ICP (Liquid manure or Solid Manure). It has been used successfully for plant samples, sludges and soils ICP's. The original Wisconsin digestion method for ICP # 5.5 and the adapted program codes for Autoblock by Vanotti are in a PDF (Wisconsinliquids.ahb for Liquid samples or Wisconsin solids.ahb for Solid samples).

NOTE 2:

Our protocol chemistry is that of EPA 3050B (nitric acid and peroxide). If other chemistry needs to be used, this method protocol needs to be revised, tested with Dr. Vanotti approval, and certified.

**\*\*Prior to digestion be sure to check and make sure there is sufficient Milli-Q water, nitric acid, and hydrogen peroxide to complete the digestion. The following volume of each is needed for a complete set of digestions (54 tubes including blanks and standards):**

- 270mL Nitric Acid
- 270mL Hydrogen Peroxide
- At least 2,500mL of Milli-Q water

**THIS IS A TWO-DAY METHOD**

DAY 1

- 1 Pipette 5mL (liquids) or weigh 0.15g (solids) into digestion tubes (always include at the end of the samples 2 blanks and 2 standards— see “**standard section**” for more detail)(Up to 50 samples + 2 standards + 2 blanks = 54 total)
- 2 Number the digestion tubes accordingly.
- 3 Place disposable reflux caps on all digestion tubes.
- 4 Place digestion tubes in auto-block from front to back and then left to right (filling the first column completely before moving to the second column).
- 5 Check that the compressed air is on if it is not already on:

- The pressure gauge should be between 20-30psi
  - If off, open by turning the knob on the counter top behind the auto-block computer.
- 6 Turn the auto-block on with the switch on the front top left-hand corner of the instrument.
  - 7 Turn the auxiliary fan on (fan above auto-block) with the switch located on the power cord. This fan should always remain on until everything is complete.
  - 8 Double check to make sure the fume hood is on. The fume hood should remain on all the time.
  - 9 Logon to the computer next to the auto-block (Username: Autoblock, Password: envexp)
  - 10 Open the Auto-block program from the shortcut on the desktop titled "AutoBlock Plus"
  - 11 A warning will come up indicated the motors will be sent to the "home" position, hit "OK"
  - 12 A warning about Service attention requirement may pop up (depending on if anything is overdue). Press "OK".
  - 13 Go to the "Service tab" in the software and complete any service/maintenance that is due and update the counters in the software if it has been completed. If you have any question contact William Brigman.
  - 14 Go to the "Manual Screen" by hitting the Manual Screen button.
  - 15 Another warning will come up indicating the motors will be sent to the "home" position will appear, hit "OK."
  - 16 Hit "Initialize" button in the bottom left corner.
  - 17 Once again a warning about the motors being sent to the "home" position will appear, hit "OK."
  - 18 Hit "Move to Drain" button in the bottom left corner (the dispensing arm will move over the drain)

- 19 On the Fan Control (bottom left) move the gray button to the "On" position
  - You should see the "Blower Overpressure" light turn green (bottom right of the screen)
- 20 On the Rack Position, located in the bottom middle of the screen, click on "Fill" to move the rack to the fill position.
- 21 Make sure all indicating lights in software are green and not red.
  - Red means there is something wrong. Contact William Brigman or Paul Shumaker to resolve issue.
- 22 Purge the dispensing line with H<sub>2</sub>O:
  - 22.1 Select reagent 1 "H<sub>2</sub>O" on the Reagent Selection at the top left of the screen by clicking on it with the mouse
  - 22.2 Enter "20" into the Injection Amount under injection control
  - 22.3 Hit "Inject" button underneath the Injection Amount
  - 22.4 Make sure the arm is over the drain and no cells are selected yet
- 23 Purge and prep lines with HNO<sub>3</sub>:
  - 23.1 Select reagent 2 "HNO<sub>3</sub>" on the Reagent Selection at the top left of the screen by clicking on it with the mouse
  - 23.2 Enter "5" into the Injection Amount under injection control
  - 23.3 Hit "Inject" button underneath the Injection Amount
  - 23.4 Make sure the arm is over the drain and no cells are selected yet.
- 24 Select all cells (to green) that are filled with digestion tubes in the "Cell Position Selection" portion of the software.

- 25 Add 5mL  $\text{HNO}_3$  to each digestion tube (samples, blanks, & standards)
  - 25.1 Enter 5.0 into the injection amount box
  - 25.2 Hit "Inject" button underneath the Injection Amount
  - 25.3 Make sure all highlighted cells have digestion tubes or  $\text{HNO}_3$  will be injected into the block.
- 26 Wait for Auto-block to dispense  $\text{HNO}_3$  into all of the highlighted cells.
- 27 Un-highlight all cells in the "Cell Position Selection" portion of the software.
- 28 Hit the "move to drain" button in the bottom left corner of the software (dispensing arm will move over the drain).
- 29 Purge the line with water to clean out excess acid in the line:
  - 29.1 Select reagent 1 "H<sub>2</sub>O" on the Reagent Selection at the top left of the screen by clicking on it with the mouse
  - 29.2 Enter "20" into the Injection Amount under injection control
  - 29.3 Hit "Inject" button underneath the amount
  - 29.4 Make sure the dispensing arm is over the drain and no cells are highlighted
- 30 Allow samples to sit in the digester with  $\text{HNO}_3$  added until the following morning with both fans running.

- 31 Open the "Operator Test" tab in the software.
- 32 Click "Select Method" – a window will open with a list of all the methods.
- 33 Scroll down and select one of the following methods "Wisconsin Liquids" or "Wisconsin Solids" by double clicking on method.
- 34 Once a method is selected, the window will close and goes back to the software. Make sure the correct method is showing in the top left box under "Selected Method File".
- 35 Enter operator's name.
- 36 Highlight the cells that are needed for the digestion.
- 37 Press "Start Method"
- 38 A pop up window will appear asking for pre-test requirements (One will ask to ensure reagent bottles are filled, the other will ask to ensure door is closed)
  - 38.1 Check to make sure and then put a check in each box and press "ok"
- 39 The digestion is now running – Periodically check on the digestion. The digestion time is dependent upon the number of samples. A full digestion takes 6-7 hours once started
- 40 After the run is complete, the software will have "completed" at the bottom and all the digestion tubes should be filled about 48mL. The internal fan will turn off once the temperature of the block falls below 40 degrees.
- 41 With gloves on, remove all the disposable reflux caps and discard.
- 42 Take digestion tubes out of block, cap, and shake.
- 43 Using a squirt bottle filled with DI water, finish filling the digestion tubes (by hand) to the 50mL mark.



- 44 Once all samples are removed from the block and capped, the auxiliary fan can be turned off using the switch or either left on if another run is immediately following.
- 45 The samples now must be filtered using a 0.45 or 2µm filter and plunger. **BE SURE TO WEAR LAB COAT, GLOVES, AND EYE PROTECTION AND WORK OVER THE SINK FOR THIS BECAUSE THE SAMPLES ARE HIGHLY ACIDIC AND CORROSIVE.**
- 46 The filtered samples are now ready for analysis and have a 6% HNO<sub>3</sub> concentration.
- 47 Leave the system on until the internal fan shuts off, then turn off the auxiliary fan with external switch on power cord and turn off the auto-block using switch on front of instrument unless another run is immediately following.