PROCEDURE FOR TKN/TP/LLTP GLASSWARE CLEANING

1. SCOPE AND APPLICATION

This SOP describes the procedure used to clean all glassware used for the Total Kjeldahl Nitrogen (TKN) and Total Phosphorus (TP) digestion procedures (DEP SOPs NU-048, NU-049, NU-091, and NU-097). Clean glassware is essential for the accurate determination of TKN and TP.

2. EQUIPMENT AND SUPPLIES

2.1. Equipment:

Equipment	Manufacturer	Model	Serial Number
Dishwasher (Old), equipped with two 24-spindle jet racks	Lancer	1400 UP	5E06Y268
Dishwasher (New), equipped with two 64-spindle jet racks.	Lancer	1400 LX	9E033649
or equivalent			

2.2. Supplies:

Equipment	Manufacturer/Vendor[1]	Model/Catalog Number
Beakers (200-400 mL)	Fisher (SR)	02-540G; 02-540K; 02-540M; 02-555-20E; 20-540R
Parafilm	Fisher (SR)	13-374-12
Buchner funnel	Fisher (SR)	FB966F
Waste container: Thermo Scientific™ Nalgene™ HDPE, Jerry Can with Closure	ThermoFisher	22400015
Scouring pads	Grainger	1CJ70
Nalgene Teflon methanol squeeze bottle	Thermo Scientific	03-409-12E
2 L Nalgene containers (for LLTP caps)	Fisher	11-815-11A
Scrub brush (for soil tubes)	Fisher	03-572-5
Black Marker	Sharpie/ Office Depot	203349
Scissors	Westcott/ Office Depot	612855

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Bench paper, Versi-Dry lab Soakers	Research Products International Corp (SR)	248891
or equivalent		
[1]Note: (SR) designates chemical is typically ordered through the in-house stockroom		

3. REAGENTS AND CHEMICALS

3.1. Chemicals:

	Chemical	Alias	Manufacturer[1]	Catalog Number	Purity	Expiration	Storage
3.1.1.	Hydrochloric Acid	HCl	Fisher Chemical (SR)	A144S-212	Reagent grade or higher	See COA	Room Temp
3.1.2.	Nitric Acid	HNO_3	Fisher Chemical (SR)	A509P-212	Reagent grade or higher	See COA	Room Temp
3.1.3.	DI water	H_2O	In house	N/A	N/A	N/A	Room Temp
3.1.4.	Methanol	MeOH	Fisher (SR)	A412-4	Reagent grade or higher	N/A	Room temp, Flammable cabinet
3.1.5.	Detojet or Detergent, Decon* Contrad* NF Liquid 30-1/3 gal	detergent	Fisher (SR) *Has to be pre-ordered with the Stockroom	04-355-9	N/A	N/A	Room Temp, corrosive-safe location
3.1.6.	LancerAcid	acid	Fisher (SR)	NC9991290	N/A	N/A	Room Temp, corrosive-safe location
or equivalent							
[1]Note: (SR) designates chemical is typically ordered through the in-house stockroom							

3.2. Reagents:

3.2.1. Dilution definition: 1:2 is defined as 1 part of acid (by volume) to 2 parts of the final total volume.

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3.2.2. **Dilute Reagent Grade Hydrochloric Acid (1:2)** - Fill a labeled (1:2 HCl) nalgene Teflon wash bottle halfway with DI water (3.1.3.). Under a hood, fill up the rest of the wash bottle with concentrated HCl (3.1.1.). Put a gloved finger over the tip of the bottle and invert several times over a sink. Caution - may heat up.

3.2.3. **Dilute Reagent Grade Nitric Acid (1:2)** - Fill a labeled (1:2 HNO_3) nalgene Teflon wash bottle halfway with DI water (3.1.3.). Under a hood, fill up the rest of the wash bottle with concentrated HNO_3 (3.1.2.). Put a gloved finger over the tip of the bottle and invert several times over a sink. Caution - may heat up.

4. PROCEDURE

Note: The new washer (1400 LX) with the high capacity 64-spindle wash racks is used **only** for washing TKN and LLTP tubes. The old washer (1400 UP) with the 24-spindle is used as a backup washer for the TKN and LLTP digestion tubes. However, in the event that one of the dishwashers is out of service for any reason, the 24- and 64-spindle racks are interchangeable and can be removed and installed in either washer.

4.1. Empty tubes:

4.1.1. TKN and TP (mercury and copper digestions):

- Dump waste from tubes into a hazardous waste bottle using a Buchner funnel to separate the beads.
- Set beads aside.

4.1.2. Low-level TP (LLTP) (autoclave digestions):

- Remove caps from the tubes and place them in a plastic holding rack.
- Pour waste from tubes down the sink flushing with plenty of tap water.

4.2. Remove labels from tubes:

4.2.1. TKN and TP (mercury and copper digestions):

- Lay tubes out on a cut piece of bench paper.
- Using methanol (3.1.4.) and a scouring pad, wash ink off tubes.
- Place ink-free tubes back into tray.
- Scrub and rinse out any excess soil/particulates remaining in the tubes before placing them in the dishwasher (use scrub brush located by the sink).

4.2.2. LLTP:

- Peel off label from tube.
- Place tubes back into the rack.

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4.3. Cleaning using the Lancer Dishwasher (if dishwasher is not available skip to section 4.4.):

4.3.1. Load the tubes into the dishwasher:

- Pull door down. On the new dishwasher, the prominent lever at the top of the door must be turned counterclockwise to release the latch. The door then swings down for loading.
- Pull out the top rack, then put one tube upside down on each prong. The top rack is loaded first, to prevent breaking tubes on the lower rack. Then push the rack in.
- Pull out the bottom rack, then put one tube upside down on each prong. For the old dishwasher skip over the middle section (only has four prongs in the row).
- Set the tube racks on top of the dishwasher.

4.3.2. There is no need to add salt to the old washer. The salt is optional and serves as a water softener. There is no provision for adding salt in the new washer.

Note: Soap and acid levels are checked manually. The dishwasher alarm/warning will display when the soap and acid are approximately one inch from depletion and should be changed then.

4.3.3. Push the lower rack in. Check all rails are pushed in all the way.

4.3.4. Push the door in until you hear it snap shut (old washer). For the new washer, close the door and turn the door lever clockwise until it comes to a firm halt.

4.3.5. Check dishwasher detergent and acid.

Note: Always wear gloves when handling dishwasher soap and acid.

- The detergent (3.1.5.) is located at the bottom of the dishwasher on the right-hand side. The 2-gallon container is placed in a white plastic tray to protect the floor. On the new dishwasher, the detergent and acid containers are placed in a slide-out shelf on the bottom left of the front panel.
- Never let soap get lower than an inch from the bottom of the plastic container.
- To add soap, take out plastic container and set on the floor. If removal of the hose from the container is necessary, place it where contamination won't occur.
- Pump desired amount into container. Place hose back on the plastic container.
- Put soap container back into the dishwasher.

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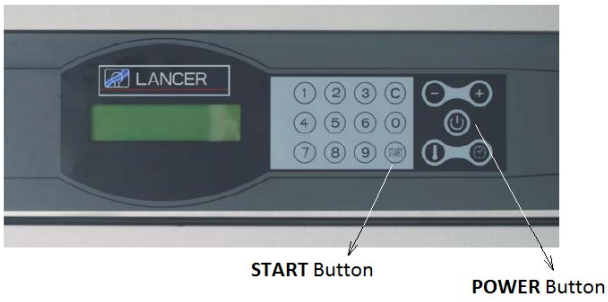
Note: Any spillage that might occur during the refilling of the container must be cleaned up immediately. A mop and bucket are available in the room for cleanup purposes. The detergent and acid containers are placed in white plastic trays in front of the old washer. Make sure that the exterior surfaces of the containers are wiped clean, before returning the containers in the new washer. The outside surface of the dishwasher should be cleaned/polished regularly (every 2 weeks).

4.3.6. Check acid level:

- The acid (3.1.6.) is located at the bottom of the dishwasher on the left-hand side. On the new washer, the acid container is in the rear of the sliding shelf.
- The acid level should be monitored daily. For the new washer this is done together with the soap level. Never let the acid get lower than an inch from the bottom of the plastic container.
- When the level is within one inch from the bottom, the container should be refilled. Old washer uses only a very small amount of acid, and the gallon sized acid reservoir lasts for about a year under normal use.
- Place the acid container back into the dishwasher.
- Record all maintenance in the maintenance logbook.

Note: Both dishwashers are equipped with filters that separate the drainage compartment from the main compartment. These filters must be removed and scrubbed regularly as glass and adhesive will collect on the surface of it. It should be cleaned weekly or as needed.

4.3.7. Check to make sure the dishwasher control panel is set correctly before starting operation. For regular TP and TKN tubes, choose Program 2 on the old washer and Program 3 on the new washer using the numeric keypad. For the low-volume TKN tubes (see DEP SOP NU-097), choose Program 5. Press and hold the START button until the dishwasher activates. A diagram of the control panel on the new dishwasher is shown below.



Old Washer: Program **2** **New** Washer: Program **3**

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4.3.8. Tubes can be removed and placed in their appropriate trays (blue for TKN, white for LLTP), after the display screen reads "End of Cycle". ****CAUTION* TUBES MAY BE HOT****

4.3.9. The dishwasher will power off upon opening the door after a completed wash cycle.

4.4. Washing tubes manually (if using dishwasher skip to section 4.5):

4.4.1. Remove ink or labels from the tubes. Empty waste in the appropriate container and rinse three times with DI water.

4.4.2. Fill the tubes with about an inch of 1:2 HCL (3.2.1.). Fill up the rest of the tube with DI water, parafilm and label as 1:2 HCL. Allow to soak for at least eight hours.

4.4.3. Rinse the tubes three times with DI water.

4.4.4. Fill the tubes with about an inch of 1:2 HNO_3 (3.2.2.). Fill up the rest of the tube with DI water, parafilm and label as 1:2 HNO_3. Allow to soak for at least eight hours.

4.5. Rinse the tubes at least 10 times with DI water.

4.6. Fill the tubes with DI water, cover with Parafilm and label "clean, with DI water".

4.7. Place the clean tubes in the designated area.

4.8. Cleaning Teflon beads TKN and TP (mercury and copper digestions):

- Place the used beads in a beaker.
- Rinse three times with DI water.
- Add sufficient 1:2 HCl to cover the beads. Parafilm and label "1:2 HCl".
- Soak for at least eight hours.
- Pour off the dilute HCl down the sink and rinse the beads 6 times with DI water.
- Add sufficient 1:2 HNO_3 to cover the beads. Parafilm and label "1:2 HNO_3".
- Soak for at least eight hours.
- Pour off the dilute HNO_3 into the sink and rinse the beads eight to ten times with DI water. Be sure to rinse thoroughly.

4.9. Store the beads in a clean and dry, closed container.

4.10. Cleaning Teflon Caps (LLTP):

- Transfer caps from the wire holding rack into a plastic container designated for LLTP caps.
- Rinse caps 3 to 4 times with DI water.
- Fill the container half-way up with DI water. Under the hood fill the container the rest of the way with concentrated HCl (3.1.1.).

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- Close the container and label 1:2 HCL. Invert several times and let soak for 8 hours.
- Pour off the dilute HCl down the sink flushing with plenty of DI water.
- Rinse caps at least 6 times with DI water to remove all traces of residual acid.
- Caps are now ready for use. Store all unused caps in a clean plastic container under DI water. Label the container 'clean caps in DI water'.

5. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

5.1. Discard sample wastes in sample waste containers. The TKN sample waste is acidic and contains copper or mercury (sediment digestions only). Copper containing wastes should be discarded in the TKN copper waste drum, while mercury wastes should be disposed of in a hazardous waste container labeled as "Acidic Metals Waste".

5.2. Proper safety equipment such as lab coats, safety glasses, and gloves must be worn at all times during this procedure.

5.3. Read MSDS information on the chemicals used in this procedure prior to use.

5.4. Review the Laboratory Health and Safety Plan (accessible on the intranet at https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents).

5.5. Please note that as the Sodium Hydroxide detergent is depleted to under 25% of its initial volume in the drum, the transfer hose will not remain stable during solution transfer from the drum to the dishwasher cannister. At this point, secure the hose to the cannister with tape to avoid potential spills and exposure.

5.6. Note that the Lancer dishwasher acid will never fully deplete but leave about an inch-high volume in its canister. This acid should be collected, accumulated, and labeled as "Salvage." This acid may be used again after enough has been collected. Emptied acid containers should be rinsed thoroughly before being discarded as trash waste. If there is acid salvaged that is believed to be contaminated or unusable by any means, label as "Corrosive Waste" and notify the laboratory safety officer of its presence.

6. REFERENCES

6.1. EPA methods 365.1 Rev. 2.0 (1993), 365.4 (1974), and 351.2 Rev. 2.0 (1993).

6.2. Lancer Dishwasher Manuals.

6.3. Quality Manual, Bureau of Laboratories, FDEP.

6.4. Laboratory Health and Safety Plan.

6.5. DEP SOP NU-048, Preparation of Sediments for Total Kjeldahl Nitrogen and Total Phosphorus Analyses.

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6.6. DEP SOP NU-049, Total Phosphorus (TP) Autoclave Sample Preparation.

6.7. DEP SOP NU-091, Preparation of Waters for Total Kjeldahl Nitrogen Analysis (Copper Catalyst).

6.8. DEP SOP NU-097, Preparation of Waters for Total Kjeldahl Nitrogen Analysis (Copper Catalyst, Low Volume)

Appendix of Significant Changes

09/14/2009	Revisions were made to sections 2.0 and 4.0 to reflect the use of a second Lancer dishwasher in addition to the original, in the lab.
09/13/2010	Revisions were made to section 4 to clarify and add more detail to the procedure.
09/08/2011	The section referring to the removal of labels from Biology tubes is not performed by the Nutrients group anymore and was removed.
12/12/2014	Revisions were made to section 5 to ensure safe transfer of the detergent.
12/07/2018	Revisions were made to sections 4 and 5. Clarification of the acid recycling/disposal process and the dishwasher filter cleaning.
02/07/2022	Reorganized equipment, supplies, chemicals, and reagents in table format.

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