PROCEDURE FOR TKN/TP 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-049 and NU-091). Clean glassware is essential for the accurate determination of TKN and TP.

2. EQUIPMENT AND SUPPLIES

2.1. Equipment:

Equipment ID: Description	Manufacturer	Model	Serial Number
Lancer-2: Dishwasher, equipped with two 64-spindle jet racks.	Lancer	1400 LX	9E033649
Lancer-3: Dishwasher, equipped with two 64-spindle jet racks.	Lancer	1400 LXP	26G02094
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 TP caps)	Fisher	11-815-11A
Black Marker	Sharpie/ Office Depot	203349
Scissors	Westcott/ Office Depot	612855
Bench paper, Versi-Dry lab Soakers	Research Products International Corp (SR)	248891
ORS Nasco Weiler Scratch Brush	Fisher	19-313-462

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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	Storage
3.1.1.	Hydrochloric Acid	HCl	Fisher Chemical (SR)	A144S-212	Reagent grade or higher	Room Temp
3.1.2.	Nitric Acid	HNO_3	Fisher Chemical (SR)	A509P-212	Reagent grade or higher	Room Temp
3.1.4.	Methanol	MeOH	Fisher (SR)	A412-4	Reagent grade or higher	Room temp, Flammable cabinet
3.1.5.	Detojet or Detergent, Decon Contrad* NF Liquid 30-1/3 gal	detergent	Fisher	04-355-9	N/A	Room Temp, corrosive-safe location
3.1.6.	LancerAcid	acid	Fisher (SR)	NC9991290	N/A	Room Temp, corrosive-safe location
or equivalent						
[1]Note: (SR) designates chemical is typically ordered through the in-house stockroom						

Deionized water is replaced daily. All other purchased chemicals are given the expiration date from the CoA and/or chemical container. If there is not an expiration date listed, the expiration date is designated by following the protocol in SOPs NU-041 and NU-042.

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.

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

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gloved finger over the tip of the bottle and invert several times over a sink. Caution - solution heats 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 - solution heats up.

4. PROCEDURE

Note: Lancer-2 (1400 LX) and Lancer-3 (1400LXP) with the high capacity 64-spindle wash racks are both used as washers for the TKN and TP digestion tubes. If one of the dishwashers is out of service for any reason, the 64-spindle racks are interchangeable and can be removed and installed in either washer.

4.1. Empty tubes:

4.1.1. TKN (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. TP (autoclave digestions):

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

4.2. Remove labels from tubes:

4.2.1. TKN:

- Lay tubes out on a cut piece of bench paper.
- Using methanol (3.1.4.) and a scouring pad/sponge, wash ink off tubes.
- Place ink-free tubes back into tray.
- Scrub visible debris with a bottle brush.
- Remove any leftover parafilm, use the methanol and sponge for caked-on residue.

4.2.2. TP:

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

4.3. Rinse the tubes with DI in the sink before putting them in the dishwasher. Attach a hose to the DI faucet and run water over the tops while filling the tubes. Then

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

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4.4.1. Load the tubes into the dishwasher:

- Pull door down. On Lancer-2, the prominent lever at the top of the door must be turned counterclockwise to release the latch. The door then swings down for loading. For Lancer-3, the door must be unlocked from the operator screen before the door can be opened.
- 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.
- Set the tube racks on top of the dishwasher.

Note: Soap and acid levels are checked manually. Push the lower rack in. Check all rails are pushed in all the way. Lancer-3 has reagent level sensors that will alert when the reagents need to be refilled.

4.4.2. Close the door and turn the door lever clockwise until it comes to a firm halt.

4.4.3. 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. 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.
- Make sure the hose does not get kinked during the process.

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 sample receiving room for cleanup purposes. The detergent and acid containers are placed in white plastic trays in front of Lancer-1. Make sure that the exterior surfaces of the containers are wiped clean, before returning the containers in Lancer-2. The outside surface of the dishwasher should be cleaned/polished regularly (once a month).

4.4.4. Check acid level:

- The acid (3.1.6.) is located at the bottom of the dishwasher on the left-hand side. The acid container is in the rear of the sliding shelf.

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- The acid level should be monitored daily. 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 replaced. Section 5.6 describes the procedure for handling the remaining acid in the old container.
- Place the acid container back into the dishwasher.
- Record all maintenance in the maintenance logbook.
- Make an entry in the logbook for every use..

Note: Make sure soap and acid levels are decreasing after normal use. If they are not decreasing, the dishwasher may be malfunctioning.

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.

4.4.5. Check to make sure the dishwasher control panel is set correctly before starting operation. For regular TP and TKN tubes, select Program 3 on Lancer-2 using the numeric keypad and the "Chemistry" Program on Lancer-3. Press and hold the START button until the dishwasher activates. A diagram of the control panel on Lancer-2 and Lancer-3 is shown below.

Program 3 (1400 LX):

- Prewash with hot water with the detergent
- Wash with hot water with the detergent
- Acid rinse with the neutralizant acid
- Rinse with demineralized water
- Final rinse at 80° with demineralized water
- Drying Max
- Cooling

Program "Chemistry" (1400 LXP):

- Prewash with hot water with the detergent
- Wash with hot water with the detergent
- Acid rinse with the neutralizant acid
- Rinse with demineralized water
- Final rinse at 80° with demineralized water
- Drying Max
- Cooling



Lancer-2: Program 3



Lancer-3: Program "Chemistry"

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4.4.6. Tubes can be removed and placed in their trays after the display screen reads “End of Cycle”. ***CAUTION* TUBES MAY BE HOT*** Lancer-3 will alarm for 30 seconds after a cycle is completed.

4.4.7. The dishwasher will power off upon opening the door after a completed wash cycle. To open the door of the Lancer-3, you must unlock the door using the operator screen.

4.5. Washing tubes manually (if using dishwasher skip to section 4.6):

- Remove ink or labels from the tubes. Empty waste in the appropriate container (see Section 5.1) and rinse three times with DI water.
- 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.
- Pour off the dilute HCL down the sink flushing with plenty of DI water and rinse the tubes three times with DI water.
- 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.
- Pour off the dilute HNO_3 down the sink flushing with plenty of DI water and rinse the tubes at least 10 times with DI water.

4.6. Cover clean tubes with Parafilm.

4.7. Place the clean tubes in the designated area.

4.8. Cleaning Teflon beads (TKN):

- 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 (TP):

- Rinse caps 3 to 4 times with DI water.

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- 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.).
- 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. Label the container 'clean caps'.

5. MAINTENANCE/TROUBLESHOOTING

5.1. In addition to the regular-use entries, record all maintenance, troubleshooting, and service visits in the instrument's maintenance logbook.

5.2. Monthly cleaning procedure –

- Using the designated cleaning reservoirs, fill each reservoir with **HOT DI** (use the sink in C307 and a cart if the hot tap water isn't working upstairs).
- Carefully replace the acid and detergent containers with the hot DI reservoirs.
- Start Program 11 (Lancer-2) "Monthly Cleaning" (Lancer-3).
- Once the program is complete, put the acid and detergent containers back in the reagent drawer, making sure to check the reagent line labels.
- Discard the remaining hot DI down the sink and put the empty reservoirs in the designated area.
- Using the stainless-steel polish, clean the outside of the dishwashers.
- Clean up the dishwasher room, organize the acid containers, etc.

6. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

6.1. Discard sample wastes in sample waste containers. The TKN sample waste is acidic and contains copper. Copper containing wastes should be discarded in the TKN copper waste drum.

6.2. Proper safety equipment such as lab coats, safety glasses, and gloves must always be worn during this procedure.

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

6.4. Review the Laboratory Health and Safety Plan (accessible on the intranet at https://floridadep.sharepoint.com/dear/SitePages/Lab%20Health%20and%20Safety.aspx).

6.5. 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

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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.

6.6. 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.

7. REFERENCES

7.1. EPA methods 365.1 Rev. 2.0 (1993) and 351.2 Rev. 2.0 (1993).

7.2. Lancer Dishwasher Manuals.

7.3. Quality Manual, Bureau of Laboratories, FDEP.

7.4. Laboratory Health and Safety Plan.

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

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

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.
06/27/2023	Removed information in regard to Sediment Total Kjeldahl Nitrogen and Total Phosphorus analysis (SOP NU-048) as well as Low Volume Total Kjeldahl Nitrogen Analysis (SOP NU-097).
10/01/2024	Dishwashers given unique equipment IDs. Aliases of "old" and "new" dishwashers changed to "Lancer-1" and "Lancer-2", respectively.
10/23/2026	Added new dishwasher "Lancer-3" and removed all references to the old "Lancer-1". Added Maintenance/Troubleshooting section. Modified 4.3 to mention sink cleaning of the tubes prior to loading into the washer.

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