



# Operations Challenge 2026

## Laboratory Event

### Version 1.0

The 2026 laboratory event will include two different chemical analyses, orthophosphate ( $\text{PO}_4$ ) and total residual chlorine. The challenge will utilize Hach's TNTplus vial chemistry and the powder pillow PermaChem style of testing. Utilizing two-stage and tensette pipets to demonstrate the operator's understanding of determining dilution calculations and performing volumetric dilutions.

The participants will need to determine the  $\text{PO}_4$  concentration from a 1000 mg/L  $\text{PO}_4$ -P stock standard to create two known  $\text{PO}_4$  working standards to analyze. The TNTplus TNT844 reagent set will be used for the  $\text{PO}_4$  testing.

For the total residual chlorine procedure, the operator will need to prepare a 1 mg/L chlorine standard from a known concentration provided by Hach and perform standard additions.

Please note the new reagent set and standard for 2026.

### Introduction

Laboratory results are valuable as a record of plant operations. This data lets the operator know how efficiently the plant is running and helps predict and prevent troubles that may be developing within the various processes. Laboratory results are required as a record of performance for regulatory agencies and are of value to the operators, staff and design engineers for performance optimization, troubleshooting, determination of loadings, and for determining when plant expansions are necessary. For these reasons, laboratory tests should be conducted as carefully and consistently as possible and according to appropriate analytical methods.

Reactive Phosphorus is a highly monitored nutrient for wastewater plants. Many wastewater plants have or will have total phosphorus limits in their permits. The results from the TNT tests can be used to optimize biological phosphorus removal processes and/or estimate chemicals needed to help remove the phosphorus from the final effluent before it is discharged into the receiving waters. The test can be performed at different areas of the plant to maximize the removal efficiency and optimize chemical costs at the plant. These samples are preserved with sulfuric acid and held at  $<6^\circ\text{C}$ . The hold time is 28 days.

Total Residual chlorine is measured to optimize effluent chlorination disinfection processes, which is the most common of the disinfection processes found at facilities in the US. For facilities that use chlorination for disinfection, some states require seasonal chlorination while others require year-round chlorination. Chlorine is added to render the pathogen ineffective at reproduction, mitigate illness, and reduce E. coli that is being discharged to the receiving waters from in the final effluent. All permits require the residual chlorine to be down to a certain level before it is discharged into the receiving waters from the final effluent. This test has a 15-minute hold time and is typically done in the field.

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## Material

- (Hach #-TNT844) (USABB#-202104) - Phosphorus (Reactive and Total) TNTplus Vial Test, HR (1.5-15.0 mg/L PO<sub>4</sub>), 25 Tests
- (Hach #-2105669) (USABB #- 32140) - DPD TOT Chlorine RGT PP 10 ML 100/pk
- (Hach #-1426810) (USABB #- 32480) - Chlorine, 50-75MG/L 16/pk 10ML
- (Hach #-2196800) (USABB #- 47903) - Ampule breaker kit
- (Hach #-2321142) (USABB #- 200544) - Phosphate STD SOLN, 1000MG/L 100ML (used to prepare 100 mg/L PO<sub>4</sub> stock standard)
- (Hach #-27256) (USABB #- 77485) - Water, deionized 4L (demineralized) (or equivalent)
- (Hach #-1970010) – Tensette pipet, 1.0 – 10 mL
- (Hach #-2199796) – Pipet tips, for tensette pipet, 1.0 – 10 mL
- (Hach #-BBP065) (USABB #- 86417) – Two-stage pipet, adjustable volume, 1.0 - 5.0 mL
- (Hach #-BBP068) (USABB #-201593) - Pipet tips, for 1.0 - 5.0 pipet
- (Hach #-BBP078) (USABB #- 86418) – Two-stage pipet, adjustable volume, 0.2 - 1.0 mL
- (Hach #-BBP079) (USABB #-201594) - Pipet tips, for 0.2 - 1.0 mL pipet
- (Hach #-2749220) – Transfer pipets, 20/pk (or equivalent)
- (Hach #-1406042) (USABB #-33300) - Flask, volumetric PP 100ML (4) (or equivalent)
- (Hach #-217242) (USABB #- 38020) - Cylinder, graduated PMP 100ML (4) (or equivalent)
- (Hach #-2497901) - Rack, test tube 16MM 5X12 rows (USABB #-alternate wire tub rack 48975 15-16mm 48 holes, 48985 15-16mm, 60 holes)
- (Hach #-2495402) (USABB #-201380) - Sample cell, 10ML matched 2/pk (extra sample cells needed for multiple sample replicates)
- (Hach #-50041H) (USABB #- 34801) – 50 mL Glass beaker (50071H for 12/pk) (or equivalent)
- (Hach #-108074) (USABB #- 34820) – 150 mL Plastic beakers 12/pk (or equivalent)
- (Hach #-108076) (USABB #- 205152) – 250 mL Plastic beakers 6/pk (or equivalent)
- (Hach #-2097000) (USABB #- 36989) – Kimwipes Laboratory wipes (or equivalent)
- (Hach #-LPV440.99.00012) (USABB #-203706) - DR3900 (or equivalent direct reading spectrophotometer)
- (USABB #-202822) 500 ml Wash bottles
- (USABB #- 39829) 5000 ml Waste Beakers (2)-Trash
- (USABB #- 63050) Multipurpose Trays (4) for used glassware
- (USABB #- 40792) Timer (2)
- (USABB #- 35450) DI Carboy with spigot, 10L
- (USABB #- 73904) 1L Tri Corner beaker (2)
- Calculators, S, M, L, XL nitrile gloves, paper towels
- Bleach

## General Notes

1. The Team Captain tells the Head Judge they are ready to begin, and the Head Judge says "START" to signal the beginning of the event. The Head judge and one other judge will be the timekeepers.
2. The event is complete when all tasks have been completed and Team Captain hands in the worksheets to the Head judge and says the team is finished.
3. To ensure a fair contest and to avoid challenges, judges will not speak to contestants while the event is being performed.
4. The Event Coordinator will settle disputes with input from the event judges.
5. All team members must participate in the event but are not limited to performing only one task.
6. After the event, the Head Table Judge may explain to the Team Captain what was done incorrectly but will NOT reveal penalty points.
7. Team members may ask judges questions before the beginning of the event, but the Judge(s) may choose not to answer the question, depending on the question asked. Questions related to specific steps in the procedure will not be answered.
8. 2 Pencils, 3 Sharpies, and 2 Calculators will be provided for each set-up. Teams may bring their own to substitute if they wish.
9. All bench sheets must be completed in their entirety. Any blanks will counted as incorrect with an associated penalty.
10. The DR 3900 and carboys cannot be moved but all other lab items may be moved during 2-minute set up. All trash needs to end in trash cans including gloves.
11. Note: Anytime you pipette out of the solution B or the unknown Phosphate bottle you need to use a fresh pipette so there is no cross contamination. If you don't change out the pipette tip a severe penalty will be assessed.

## Setup

Teams will have two minutes before beginning of the event to organize items on the tables. Any item with the exception of the DR3900 and carboys may be moved. The Head Judge will time the setup. At the end of setup time, the judge will say "TIME", team members must remove their hands from the table. No labeling of any glassware will be permitted during set up. Pipette tips will not be allowed to be placed on pipettes. Pillow packets and TNT tubes need to stay in boxes until the event starts. See "General Notes" - above for instructions.

## Pipetting Guidelines

Inaccuracies in the pipetted volumes will immediately affect the accuracy of measurement results. Errors in pipetted volumes are frequently attributed to poorly adjusted pipettes. This error may occur after long use, over winding, or mechanical stress. Regular Quality Assurance with standard solutions facilitates its early detection. Another possible cause is incorrect handling of the pipette. This is usually indicated by wide differences in the values obtained from double determinations.

### Standard Pipetting Technique

#### With Two-Stage piston pipettes:

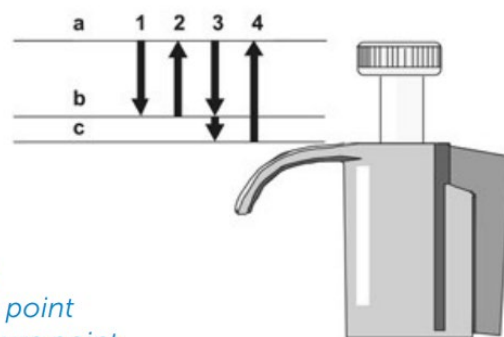
1. Turn the push button cap to align the volume to the desired volume.
2. Press the push button to the first pressure point.
3. Dip the pipette tip under the surface of the liquid (2 – 3 mm) and slowly release the push button. Take the tip out of the liquid and touch it against the inside wall of the vessel to remove excess liquid.
4. Place the tip against the inside wall of the vessel and press lightly and evenly on the push button until the first pressure point is reached. Hold the pipette in this position. After about one second, press the push button until the second pressure point is reached. This completely empties the tip.
5. Allow the push button to return to its resting position.

#### Push button

*a: Rest position*

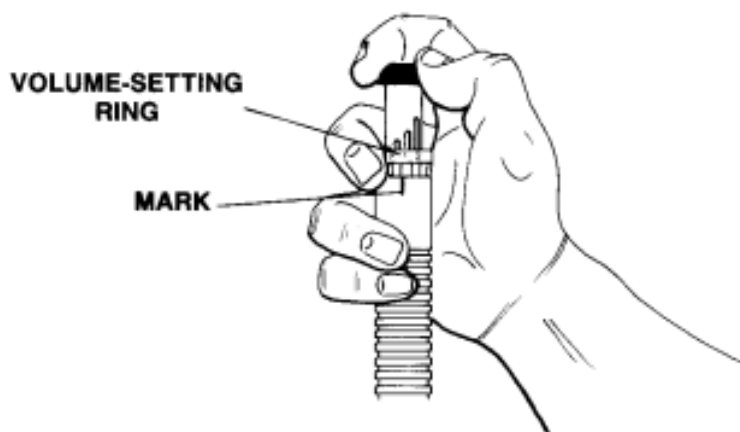
*b: First pressure point*

*c: Second pressure point*



#### With Tensette Pipets:

1. Turn the turret cap to align the desired volume on the volume-setting ring with the mark on the housing assembly.
2. Press down on the turret cap with the thumb, using a smooth motion, until the turret reaches the stop.
3. Dip the pipet tip under the surface of the liquid (2-3 mm), and slowly allow the turret to return to the extended position. Take the tip out of the liquid and touch it against the inside wall of the vessel to remove excess liquid.
4. Use the thumb and forefinger to twist the turret cap to the next higher position on the volume-setting ring to ensure full blowout. The "F" position provides a full blowout for the largest volume setting.
5. Place the tip against the inside wall of the vessel and press lightly and evenly on the turret cap until the turret reaches the stop.
6. Allow the turret to return to its resting position.



## Tips for Maximum Accuracy

- Make sure that the correct volume was selected and that the volume scale clicked into place properly. The volume shown in the display must be completely visible.
- For every new sample use a new pipette tip.
- Make sure to use the original tips designed for either the tensette or two-stage piston pipette.
- Use light pressure to fit a new tip to the cone, turning the tip slightly as you do so.
- Make sure that there are no foreign bodies between the tip and the cone.
- Make yourself familiar with the pipette. If you are not sure of the two pressure points, test it on air before inserting the tip into the sample.
- Insert the pipette tip into the sample no deeper than the pointed part of the tip about 2-3 mm.
- Hold the pipette almost vertically (no more than 10° from the vertical) while the liquid is being drawn into the tip.
- Move the piston up or down slowly and evenly.
- Do not allow the push button to spring back into its original position. Never allow liquid to come into contact with the cone!
- Move the piston down just once when pipetting the sample.

## Procedures

### Set up for Practice – (Step 2-4 below will be done by the event coordinators at competitions)

#### Using TNT844 (0.5-5.0 mg/L P; 1.5-15.0 mg/L PO<sub>4</sub>-P)

1. Prepare two PO<sub>4</sub> standards at concentrations between 2 and 14 mg/L PO<sub>4</sub>.
  - a. The stock standard of 1000 mg/L PO<sub>4</sub>-P (3067 mg/L PO<sub>4</sub>) will be used to prepare the secondary standard.
  - b. Prepare a secondary standard solution of 100 mg/L PO<sub>4</sub> by taking 3.26 mLs of the 3067 mg/L PO<sub>4</sub> standard and dilute to 100 mLs in a 100-mL volumetric flask
  - c. Prepare two working PO<sub>4</sub> standards between the concentration ranges of 2 and 14 mg/L PO<sub>4</sub> from the 100 mg/L PO<sub>4</sub> secondary stock standard. Analyze these working standards with TNT844
  - d. During the challenge you will be given two concentrations to prepare within the 2 - 14 mg/L PO<sub>4</sub> concentration range and be evaluated on your accuracy (% recovery) and precision (%RSD) to these known concentrations
2. Fill a 10L carboy container with chlorinated tap water.
  - a. Open the faucet and let it run for 5 mins.
  - b. Fill the container with tap water and label it.
  - c. The amount of chlorine in the tap water shouldn't matter if it contains some chlorine. If tap water doesn't contain chlorine, add a couple drops of bleach. The coordinator will need to run a quick chlorine test using the Hach equipment on the tap water during the setup of the event to ensure there is trace chlorine in the tap water.
3. Fill a 10L carboy container with deionized water and label it.
  - a. This can be purchased or from a demineralized system. Distilled water can be used in place of demineralized water. What's most important is that it should be chlorine-free and phosphate-free.

4. Each station should contain:
- a. 1x DR3900 or equivalent Hach Spectrophotometer
  - b. 1x 0.1-1.0 mL two-stage pipet and tips.
  - c. 1x 1-5 mL two-stage pipet and tips
  - d. 2x 10L carboy 1 DI and 1 Tap Water prelabeled
  - e. 2x 1L Tri corner beaker with pre marked paper-tape label
  - f. 2x 500 mL Wash bottle with pre marked paper-tape label
  - g. 4x 250 mL Plastic beaker with unmarked paper-tape label
  - h. 3x 150 mL Plastic beaker with unmarked paper-tape label
  - i. 4x 100 mL Plastic volumetric flask
  - j. 2x Boxes of laboratory wipes or paper towels
  - k. 2x Felt tipped permanent marker
  - l. 2x Calculators
  - m. 1x Pack of transfer pipets
  - n. 1x "Unknown" Phosphate sample prelabeled
  - o. 1x Test tube rack
  - p. 1x TNT844 reagent set
  - q. 1x Phosphate bench sheet
  - r. 1x 1-10 mL Tensette pipet and tips
  - s. 1x 50 mL Glass beaker with unmarked paper-tape label
  - t. 4x 10 mL Square glass sample cells
  - u. 1x Voluette ampule breaker
  - v. 1x Box of chlorine standard ampules
  - w. 1x Bag of 10 mL total chlorine powder pillows
  - x. 1x Chlorine bench sheet.

Please refer to the pictures as examples of how these items can be arranged. There will be 2 tables to spread out the items. 1 table will have the spectrophotometer. The table dimensions are 96" L x 30" W x 36" H

### Labeling

- 1) Each 500 mL wash bottle (f) should be labeled "DI" and filled with the 4L Deionized water (d). This will be labeled by the coordinator.
- 2) Each 1L tri corner (e) should be labeled "Waste". This will be labeled by the coordinator.
- 3) The remaining items below will need to be labeled by the teams during the event not during the 2-minute setup.
- 4) For phosphate testing, the 250 mL beaker (g) should be labeled "100 mg/L PO<sub>4</sub> STD"
- 5) For phosphate testing, (2) 250 mL beakers (g) should be labeled "Working STD 1" and "Working STD 2"
- 6) For phosphate testing, the 150 mL beaker (h) should be labeled "DI", rinsed 3 X with DI and filled with the 10L Carboy Deionized water (d).
- 7) For phosphate testing, (3) 100 ml volumetric flasks (i) should be labeled "100 mg/L PO<sub>4</sub>", "Working STD 1" and "Working STD 2"
- 8) For chlorine testing, the 250 mL beaker (g) should be labeled "Tap" and filled with the 10L Carboy Tap water.
- 9) For chlorine testing, one 150 mL beaker (h) should be labeled "DI" and filled from the 10L Carboy Deionized water (d).

- 10) For chlorine testing, the other 150 mL beaker (h) should be labeled “STD”
- 11) For chlorine testing, the 50 mL glass beaker (s) should be labeled “Cl<sub>2</sub>”
- 12) For chlorine testing, the 100 mL volumetric flask (i) should be labeled 1 mg/L Cl<sub>2</sub>

### Phosphate standard preparation

1. During the challenge, teams will receive a 1000 mg/L PO<sub>4</sub>-P (3067 mg/L PO<sub>4</sub>) stock standard.
2. From this stock standard you will prepare a 100 mg/L PO<sub>4</sub> secondary standard.
3. From the 100 mg/L secondary standard teams will prepare two working standards for analysis.
4. The concentration of the two working standards will be between 2 – 14 mg/L and will be given to the teams at the start of the competition.

### Phosphate Standard Solutions

1. Prepare a 100 mg/L PO<sub>4</sub> secondary stock solution.
  - a. Rinse a 100 mL volumetric flask 3x with Deionized water.
  - b. Fill the 100 mL volumetric flask ~1/2 full with Deionized water.
  - c. Pipet 3.26 mLs of the 3067 mg/L PO<sub>4</sub> stock standard into the 100-mL flask
  - d. Add deionized water to the volumetric flask until the meniscus aligns with the 100 mL graduation.
  - e. Cap the volumetric flask and invert it 3 times to mix.
  - f. Transfer a small volume of the standard to the “100 mg/L PO<sub>4</sub> STD” labeled beaker to rinse.
  - g. Transfer the remaining volume of the standard to the “100 mg/L PO<sub>4</sub> STD” labeled beaker.
2. Calculate the volume of the secondary standard that would be needed to prepare working standard 1 and 2 to a final volume of 100mL.
  - a.  $C_1 \times V_1 = C_2 \times V_2$
  - b.  $C_1 = 100 \text{ mg/L PO}_4$
  - c.  $V_1 =$  To be determined at challenge
  - d.  $C_2 =$  To be determined at challenge
  - e.  $V_2 = 100 \text{ mL}$
  - f. Add deionized water to the volumetric flask until the meniscus aligns with the 100 mL graduation.
  - g. Cap the volumetric flask and invert it 3 times to mix.
  - h. Transfer a small volume of the standard to the “Working STD 1” and Working STD 2” labeled beakers to rinse.
  - i. Transfer the remaining volume of the standards to the “Working STD 1” and Working STD 2” labeled beakers.
3. Analyze the prepared working PO<sub>4</sub> standard solutions 1 and 2, follow the method instructions from TNT844, and method 10209 for reactive phosphorus. Analyze three replicates of each working standard solution and measure the concentration in the DR3900 after a 3-minute reaction time.
  - a. Using a two-stage pipet with a fresh tip, add 0.5 mL of the standard to each of three test vials.

- b. Mix Solution B by inverting 5 times at a 90-degree angle.
- c. Using a two-stage pipet with a fresh tip, add 0.2 mL of Solution B to each of the three test vials. Ensure a new pipet tip is used for each test vial.
- d. Immediately tighten the cap on the Solution B (part of TNT844 set (-q)) container.
- e. Put a grey DosiCap C (part of the TNT844 set (p)) on each of the three test vials.
- f. Tighten the cap on each of the vials and invert them 3 times. All samples need to be placed in test tube rack before timer is set for 3 minutes.
- g. Start a timer for 3 minutes.
- h. When the timer expires, invert each of the vials 3 times.
- i. Clean the surface of the vials using a laboratory wipe.
- j. Insert the vials into the DR3900.
5. Calculate the average of the replicates.
  - a. Add the three results together and divide the sum by 3.
  - b. Bonus time will be awarded based on accuracy to the nominal concentration of two working standard solutions.

### Chlorine Standard Solution

Prepare and run a 1 mg/L  $\text{Cl}_2$  chlorine standard from ampules.

1. Rinse all of the beakers 3x with tap water, volumetric flask 3x with tap water, and 10-mL glass sample cells 3x with tap water followed by three rinses with deionized water for the beakers, volumetric flasks and 10-ml glass sample cells to remove any chlorine demand or chlorine contamination.
2. Calculate the volume of the standard that would be needed to prepare a 100 mL final volume of 1 mg/L  $\text{Cl}_2$  solution.
  - a.  $C_1 \times V_1 = C_2 \times V_2$
  - b.  $C_1$  = the lot-specific concentration from the label on the box of ampules.
  - c.  $V_1$  = the volume of the sample used to perform the dilution. What is being calculated for.
  - d.  $C_2 = 1 \text{ mg/L } \text{Cl}_2$
  - e.  $V_2 = 100 \text{ mL}$
3. Ready the chlorine standard for dilution
  - a. Remove a chlorine standard voluette ampule from the Styrofoam box.
  - b. Use the ampule breaker to open the ampule.
  - c. Transfer a small volume of the standard to the "Cl2" labeled glass beaker to rinse.
  - d. Transfer the remaining volume of the standard from the ampule to the glass beaker.
4. Prepare a 1 mg/L  $\text{Cl}_2$  solution.
  - a. Fill the volumetric flask ~ ½ full of deionized water.
  - b. Using a two-stage pipet with a fresh tip, add the volume of standard that was calculated in the previous step.
  - c. Add Deionized water to the volumetric flask until the meniscus aligns with the 100 mL graduation.
  - d. Cap the volumetric flask and invert it 3 times to mix.
  - e. Transfer a small volume of the standard to the "STD" labeled beaker to rinse.
  - f. Transfer the remaining volume of the standard to the "STD" labeled beaker.
5. Analyze the prepared chlorine standard following method 8167 using the total DPD powder pillows. Analyze three replicates measuring the concentration on the DR3900 with program 80.

(The reaction time for the total chlorine colorimetric reaction is 3 minutes; however, this reaction time can be skipped since we're using free chlorine standard.)

- a. Start program number 80 on the DR3900.
  - b. Using a Tensette Pipet with a fresh tip, fill each of the four sample cells with 10 mL of the prepared chlorine standard.
  - c. Add the contents of one powder pillow per sample cell to three of the four sample cells. The one without reagent added will be used as the sample blank.
  - d. Swirl each of the sample cells to mix for 20 seconds.
  - e. Clean the surface of the sample blank with a laboratory wipe, insert it into the instrument, and press the Zero button to take a blank measurement.
  - f. Clean the surface of each cell with a laboratory wipe.
  - g. Insert each sample into the instrument close the door
  - h. Press read.
  - i. After each measurement, the sample cells can be emptied to waste and rinsed again 3X with deionized water.
6. Calculate the average of the replicates.
    - a. Add the result of the three results together and divide the sum by 3.
    - b. Bonus time will be awarded based on accuracy to the nominal concentration of 1 mg/L  $\text{Cl}_2$  standard.

### Chlorine Standard Additions

Perform standard additions on DI water.

1. Using a tensette pipet with a fresh tip, fill each of the four sample cells with 10 mL of DI water.
2. Using a two-stage pipet with a fresh tip, add 0.1 mL of the chlorine standard from the "CL2" beaker to one of the cells, 0.2 mL to another cell, and 0.3 mL to another cell. One of the cells will not have any standard added.
3. Add the contents of one powder pillow to each of the sample cells (3 cells).
4. Swirl each of the sample cells to mix for 20 seconds.
5. Clean the surface of the sample that did not have standard added with a laboratory wipe, insert it into the instrument, and press the "zero" button to take the blank measurement.
6. Clean the surface of each of the remaining cells with a laboratory wipe.
7. Insert each sample into the instrument and press read.
8. After each measurement, the sample cells can be emptied to waste.
9. Record the results for each measurement on the bench sheet.

## Safety Procedures

Safety is of major concern during laboratory operations. As such, "good housekeeping" practices for laboratory operations have been incorporated into the procedure.

1. Safety glasses must be worn at all times. Prescription glasses may be worn in lieu of safety

glasses.

2. Approved laboratory gloves must be worn at all times. You may bring your own gloves or wear gloves that we provide. Please note if you have a latex allergy, we encourage you to provide your own gloves.
3. Good housekeeping is required. Competitors will be required to wipe counters, instruments, and work areas dry with paper towels where they have spilled or splashed any liquids during the competition. The entire surface does not need to be wiped down but all visible water must be dried or penalties will be assessed.
4. Competitors must place used graduated cylinders, volumetric flasks and used beakers in trays provided. (Note: all liquid including phosphate tubes, **vials**, beakers, volumetric flasks, and graduated cylinders must be emptied in waste receptacle prior to going into the tray)
5. Sample bottles must have lids in place (screwed on) and placed back in bottle carriers.
6. All trash must be disposed of in waste receptacles along with used gloves. All gloves are considered “contaminated” and must be removed and disposed in waste receptacles prior to turning in paperwork.
7. No competitor is allowed to bring in any outside items to the lab event except for nonscientific calculators, pens, sharpies and **gloves**.
8. Penalties for safety-related issues can also be found on the judge’s penalty sheets.



