Microbiology Laboratory Analyst Training

Kentucky Division of Water

Laboratory Certification Program

To Protect and Enhance Kentucky's Environment

Field Tests

Sampling | pH | Total Residual Chlorine | Temperature | Conductivity

Sampling Procedure Overview
Total Coliform Rule

- Sample with clean hands and relatively clean clothes to avoid contamination
- Choose an acceptable State approved sampling location. Some things to keep in mind include:
  - Surrounding foliage
  - Weather (any precipitation should be avoided)
  - Try to avoid taps with inside threads
  - Remove any aerators or hoses
  - Use a tap with separate cold water and hot water knobs (sample only cold water)
- Allow tap to flush for several minutes before collecting a sample then reduce tap flow to about the width of a pencil

Sampling procedure continued…

- Use a pre-sterilized sample bottle that can hold 100 mL plus headspace
  - Many micro sample bottles come with a dechlorination preservative in them – either in liquid or solid form. DO NOT pour or rinse this out.
  - Do not overfill or overflow sample bottle.
    - If a laboratory receives a sample that is over the 100mL line, either pour into another sterile bottle or pipette excess off (after homogenization). This procedure should be outlined in your laboratory's SOP
- While filling sample bottle, hold the lid in your hand. Do not place in pocket or on counter in order to avoid contamination.
- Place sample in cooler and immediately begin icing (do not freeze!).
- Deliver to laboratory as soon as possible to avoid hold time issues
  - Ensure Chain-of-custody is completely and accurately filled out
  - Hold time: 30 hours from time of collection to time placed in incubator
- If a sample is positive for *total coliforms* and/or *E. coli*, repeat samples must be taken as follows:
  - One from original location
  - One upstream from original location
  - One downstream from original location
  - Note: Upstream and downstream samples must be taken from State approved sampling locations

pH

- Analyze pH for: laboratory prepared media, commercially received media, and TSB used for sterility checks
- Glassware is checked with pH test strips

Quality Control (for metered readings)

- QCS: Quarterly; 95-105% recovery

- Sample duplicate: 1 every 20 samples; 20% Relative Percent Difference (RPD)*

- Annual PT

* Refer to Definitions & Equations slide
1 – EPA Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance, 5th Edition, EPA 815-R-05-004 (January 2005)

pH continued…

- Follow manufacturer instructions for calibration setup and analysis

- Calibrate pH meter with every power on; Slope should be between 95-105%
  - If the meter does not display the slope, **calibration verification** can be used to assess calibration
    - Perform maintenance and/or corrective action if calibration cannot be verified (outside slope range or verification recovery range)
  - use at least two standards that bracket expected pH range (KYDOW strongly recommends that you use three standards if your meter allows)
  - **Calibration Verification**: Daily; Reading should be ±0.1 S.U. of the expected value
    - Record calibration information & calibration verification results on micro QC forms

Total Residual Chlorine (TRC) / Free Chlorine

SM 4500 Cl G; DWCM Chapter V

- Chlorine is analyzed at time of sampling as required by 40 CFR 141.132
  - Results for free or total chlorine* are recorded on the Bacteriological Analysis Report Form (BARF) and submitted as part of data package to KYDOW
    - **Free chlorine** is required for all disinfectants except chloramine
    - **Total Chlorine** is required when the disinfectant is chloramine

- Total Residual Chlorine is also analyzed on **Reagent Grade Water**
  - Monthly; <0.1 mg/L

- Follow meter manufacturer instructions for calibration and analysis.

Quality Control

- Method Blank: Daily; <Reporting Limit*

- Calibration Verification: Daily; 90-110% recovery

- QCS: Quarterly; 90-110% recovery

- Sample duplicate: 1 every 20 samples; 20% RPD*

- Perform maintenance and/or corrective action if calibration cannot be verified

* Refer to Definitions & Equations slide
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Temperature

- In the microbiology lab, record temperature of:
  - **Incubator** twice per day in use, separated by at least four hours.
  - **Freezers** once per day
  - **Autoclave** once per day in use
    - **Spore strips or ampules** are used monthly as a bioindicator of autoclave performance. When in use, the ampule incubator temperature should be recorded daily.
- **For more quality control details about the equipment above and temperature monitoring devices, refer to *General Laboratory Practices Module***

- Measure in °C
- Temperature of samples are taken in field and recorded on chain of custody or in bench log
  - QC: Sample duplicate: 1 every 20 *samples;* 20% RPD*
- Can use a 'liquid in glass' or electronic thermometer (usually part of pH probe and meter)
  - Liquid in glass thermometer should be verified annually

* Refer to Definitions & Equations slide
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Conductivity

- Follow meter manufacturer instructions for calibration and analysis.
  - Calibrate meter at least **monthly**. For meters that cannot be calibrated with a standard, the cell constant should be determined monthly using a method in SM 2510.
  - Calibration Verification: Daily; 90-110% recovery
    - Record calibration information & calibration verification results on micro QC forms
- Conductivity is analyzed on **Reagent Grade Water**
  - Monthly; >0.5 megohms resistance or <2micromhos/cm at 25°C
  - In-line units that cannot be calibrated should not be used for monthly reagent grade water conductivity checks.

Quality Control

- QCS: Quarterly; 90-110% recovery

- Duplicate: 1 every 20 samples; 20% RPD*

* Refer to Definitions & Equations slide
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Definitions & Equations

- RPD – Relative Percent Difference –
  - %RPD = $\dfrac{|(\text{sample result} - \text{duplicate result})|}{(\text{sample result} + \text{duplicate result})/2} \times 100$

- %Recovery = (analyzed value / expected concentration) x 100

- Free vs Total Chlorine
  - Free Chlorine – the amount of chlorine in an aqueous environment that is available for sanitization. Forms **combined chlorine** with contaminants during the sanitization process.
  - Total Chlorine – Free Chlorine + Combined Chlorine = Total Chlorine

- Reporting Limit – Method/Minimum Reporting Limit (MRL) – the lowest concentration of standard used for calibration and quantitation

1 – EPA Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance, 5th Edition, EPA 815-R-05-004 (January 2005)
2 – *Standard Methods For the Examination of Water and Wastewater* 22nd Edition ; SM 1020 B & SM 4500-Cl