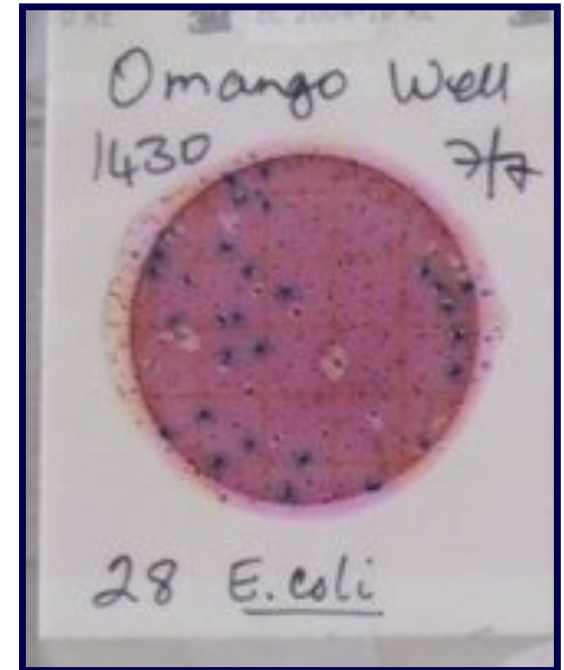


Technical Notes on Water Quality Testing for *E.coli*, Turbidity, Total Dissolved Solids & Residual Chlorine



Susan Murcott

D-Lab WASH, Week 8



E. coli Definition

- Escherichia coli (including *E. coli* 0157:H7 and non-0157 serotypes, all members of the Enterobacteriaceae family) are gram-negative bacteria that are rod-shaped, have the ability to survive in aerobic and anerobic environments (termed a facultative anaerobe), and may or may not produce flagella and pili (thin hair-like projections) depending on their environmental needs.
- *E. coli* strains are found worldwide and live in significant numbers in human and other warm-blooded animals as part of the normal bacterial population of the large [intestine](#)

Gram Negative Bacteria

- Microbiologist to classify **bacteria** into two groups (**gram-positive** or **gram-negative**).
- Gram-negative bacteria are bacteria that do not retain the crystal violet stain used in the gram-staining method of bacterial differentiation.
- **Gram-negative bacteria** are found everywhere, in virtually all environments on Earth that support life.
- The **gram-negative bacteria** include the model organism *Escherichia coli*, as well as many pathogenic **bacteria**, such as *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Yersinia pestis*.

WHO - Guideline for Microbial Water Quality (4th Ed. GDWQ)

“*E.coli* or thermotolerant coliform bacteria must not be detected in any 100 milliliter sample for all water directly intended for:

- Drinking;
- Treated water entering the distribution system;
- Treated water in the distribution system.”

From Table 7.7 p. 143. See also Table 5.2. p. 97

Criteria of an "Ideal" Fecal Contamination Indicator

- Applicable to all types of water (and other relevant samples).
- Present in feces, sewage and fecally contaminated samples when pathogens are present; numbers correlate with amount of fecal contamination; outnumber pathogens.
- No "aftergrowth" or "regrowth" in the environment.
- Survive/persist $>$ than or $=$ to pathogens.
- Easily detected/quantified by simple lab tests in a short time.
- Constant characteristics.
- Harmless to humans and other animals.
- Numbers in water (food, etc..) are associated with risks of enteric illness in consumers (dose-response relationship).

(From Mark Sobsey's lecture 4, slide 82/89 – see Week 7, Oct. 16, 2019 LMOD folder)

There are many methods for testing for *E.coli*.

Three of the most common are:

1. Multiple Tube Fermentation
2. Membrane Filtration
3. QuantiTray



←
Multiple
Tube
Fermentation

←
QuantiTray

(both are Most Probable
Number Methods)

Quanti-Tray[®] - a Most Probable Number Method



STEP 1
Collect 100 ml
sample and
add sample
and reagent
into tray



STEP 2
Seal tray in
sealer



•Step 3:
Incubate tray at
35 degrees C for
18 – 24 hours

STEP 4
Count # of
positive large and
small wells and
refer to MPN
statistical table for
translating into
coliform count

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Membrane Filtration Set-up
(in Millipore's portable system)

Coliform Indicator Tests – 4 Common Methods

- Multiple Tube Fermentation (Standard Method #9221A): statistical
- Presence/Absence (Standard Method 9221D)
 - *Colilert 10 ml tubes
 - *H₂S Bacteria
- Colony-Count Methods: Enumerative = counting (Standard Method #9223)
 - Petrifilm (3M)
 - Membrane Filtration (Millipore, other)
- Most Probable Number (MPN): statistical
 - QuantiTray with Colilert (IDEXX)

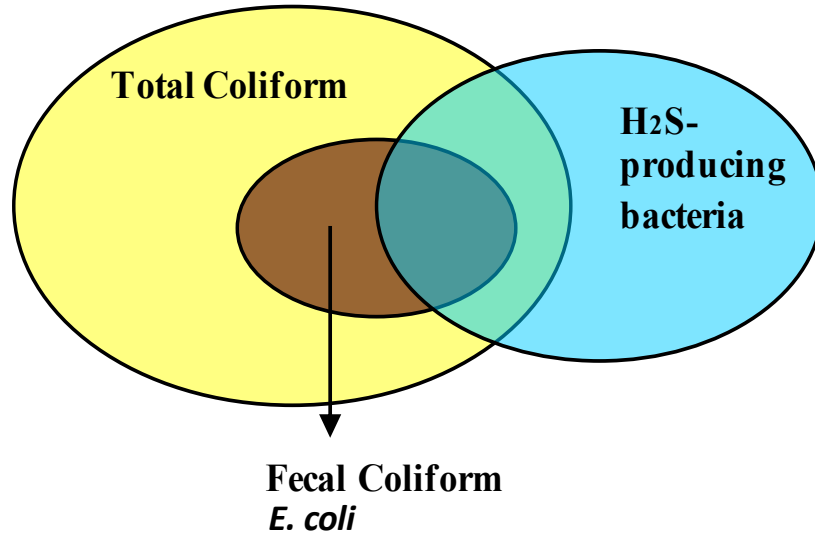


Illustration of the relationships between TC,
FC and H₂S bacteria. Low, 2002

TC = Total Coliform

FC = Fecal Coliform

H₂S = Hydrogen sulfide-producing bacteria

FC: Able to grow at 44.2°C

**EC: Some *E. coli* able to grow
at 37°C**

TC: Multiply at 37°C

Hydrogen Sulfide Bacteria Presence/Absence Results



Yellow = negative

Black = positive

EC-Kit

Combines tests for:

E. coli

&

total coliform

and includes a:

presence/absence test

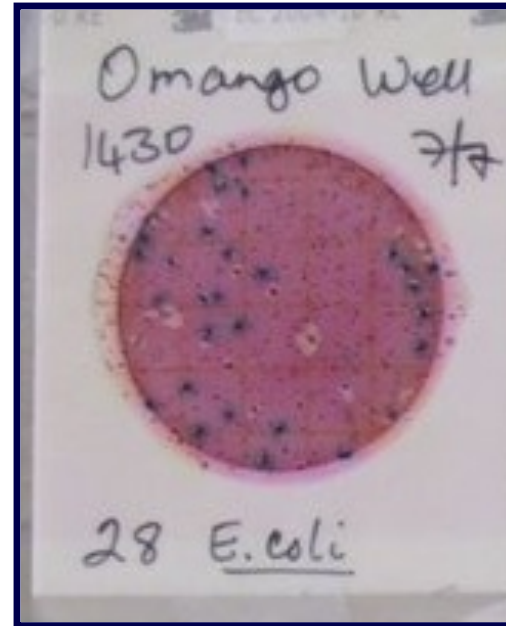
plus a colony-count test



2 EC-Kit Tests



Colilert Test (10 ml pre-dispensed tube)



Petrifilm

Colilert® - Interpreting Results



Tube on left has no coliform. The yellow tubes have coliform bacteria.

Enzyme Substrate Coliform Method

- Enzymes are proteins that catalyze (i.e., increase the rates of) chemical reactions.
- The enzyme substrate coliform method uses hydrolyzable chromogenic substrates for detection of the enzymes of total coliform and *E.coli* bacteria.

Enzyme Substrate Coliform Test

- When the enzyme substrate coliform technique is used, the total coliform group definition is based on systematic bacteriology (not on method)
- ***All bacteria possessing the enzyme β -D-galactosidase, which cleaves the chromogenic substrate, resulting in the release of the chromogen.***
- ***E.coli are defined as bacteria giving a positive total coliform response and possessing the enzyme β -D-galactosidase, which cleaves a fluorogenic substrate, resulting in the release of the fluorogen.***
- From: SM 9223 (22nd Edition)
- <http://www.standardmethods.org/store/ProductView.cfm?ProductID=313>

Colilert®

- **Colilert®** uses the Defined Substrate Technology (DST®)
- DST® contains two carbon sources:
 - ONPG (ortho-nitro-phenol-beta D-galactopyranoside) - for total coliform detection
 - MUG (4-methyl-umbelliferone-beta-glucuronidase) - for *E.coli* detection
- DST® does not contain any organic sources of nitrogen
- The test can be used in either a multiple-tube or a presence-absence (100-mL sample) format.
- The Colilert 10 milliliter pre-dispensed Presence/Absence test detects *E.coli* down to 10 coliform forming units (CFU) per 100ml, below which is considered low risk.

Colilert®

- **Total coliform** produces the enzyme beta-galactosidase, which hydrolyzes the substrate ortho-nitrophenol-beta D-galactopyranoside (ONPG) to yellow nitrophenol.
- This gives a **yellow color change** to the sample if the test is positive.
- **E.coli** can be detected at the same time by incorporating a fluorogenic substrate, (4-methyl-umbelliferone-beta-glucuronide (MUG), which produces a fluorescent end product after interaction with the enzyme beta-glucuronidase found in E.coli, but not in other coliforms.
- **Blue fluorescence** is detected with a long-wave ultraviolet light.

Glucoronidase- based Tests

- MUG in Colilert (-> *E.coli* fluoresces blue)
- BCIG in the Petrifilm (-> blue colonies with gas bubbles are *E.coli*)

Petrifilm's 2 Food Sources

The two main food sources (substrates) that are metabolized by *E.coli* to produce the Petrifilm test results are:

Lactose ----> Gas Bubble

Galactosidase of all the Coliform Group

BCIG ----> **BCI** + G

Glucuronidase of *E. coli*



Petrifilm™:

- Petrifilm uses sample-ready plates to quantify the level of *E. coli* and total coliform
- Produced by the 3M™ Company.
- Simple, only requires 1 mL of sample
- Developed for detection of coliform in food products (e.g. meats, dairy and juice products).
- Regulated in the U.S. by the Association of Official Analytical Chemist (AOAC), APHA, US Grade A Pasteurized Milk Ordinance (NCIMS) and the Canada Health Protection Branch
- It has not been approved for use in drinking water treatment

Petrifilm™ contains

- Violet Red Bile (VRB) nutrients (a cold water-soluble gelling agent coating),
- BCIG (5-bromo-4-chloro-3 indolyl-beta D Glucuronide), an indicator of glucuronidase activity (the same enzyme that hydrolyzes MUG in the Colilert test and which is produced by *E.coli*, but not by other coliform bacteria),
- Tetrazolium: an indicator that facilitates colony enumeration (gram negative bacteria reduce tetrazolium to a red color to enhance colony visualization),
- A top film on the grid plate that traps gas produced by lactose fermenting *E.coli* and coliforms

EC-Kit - Interpreting Results



Petrifilm: *E. coli* will grow into blue colonies with gas bubbles. The blue color comes from the enzyme glucuronidase hydrolyzing BCIG to release the blue BCI. The gas bubbles come from fermentation of lactose to produce hydrogen and carbon dioxide gases. Red colonies with gas bubbles are coliform bacteria other than *E. coli*. Red colonies without a gas bubble are other Gram-negative bacteria. A blue colony with gas indicates the presence of *E. coli*, and such water is considered high public health risk.

Determining Risk Levels – *E.coli*

EC-Kit Results – <i>E.coli</i>		WHO Risk Level Categories – <i>E.coli</i>	
Colilert <i>E. coli</i> Result (Metcalf, 2006)	Petrifilm <i>E. coli</i> Result (Metcalf, 2006)	Risk Level (WHO, 1997)	<i>E.coli</i> in sample (coliform forming unit per 100 mL) (WHO, 1997)
Absent (clear, no fluorescence)	0	Conformity	< 1
Absent (clear, no fluorescence)	0	Low	1-10
Present (yellow, blue fluorescence)	0	Intermediate	10-100
Present (yellow, blue fluorescence)	1-10 (blue with gas bubbles count)	High	100-1000
Present (yellow, blue fluorescence)	> 10 (blue with gas bubbles count)	Very High	> 1000

Comparison of Major Bacterial Indicator Test Kit Options, including EC-Kit

	1	2	3	4	5	6	7
Attributes							EC Kit
Capital cost			≈\$2,000- \$3600		see below		\$10 \$25 (depends on size)
Recurrent (cost per test or set of tests)							\$2.50/test set
Portability			Yes, but still heavy!		Yes		Yes
Easy to use			Trained technicians	Yes	Some Skill		Yes
Test Duration							24 hrs
Summary			 (Not E.coli)				

Turbidity Tube and Hach Turbidimeter



Turbidity

- Suspended and colloidal particles of clay, silt, organic and inorganic matter, minerals, plankton and microscopic organisms which impede the passage of light through water. • Turbidity > 5 NTU is noticeable to the consumer
- Effective coagulation or filtration should remove turbidity

Turbidity Measurement & Units

Turbidity Measurement

- Jackson Candle Units – an historic method, however the lowest value measured was 25 Jackson Turbidity Units (JTU), whereas treated water usually falls in the range 0 – 1 JTU, so it couldn't detect pure water.
- Nephelometers are turbidimeters which measure intensity of light scattered at 90° to the incident beam. Commonly used today. • Because there is no standard type of turbidity, an arbitrary standard is used in electronic nephelometers, composed of an aqueous suspension of formazin polymer.
- Unit of measurement = nephelometric turbidity unit (NTU)
- A simpler, field-research option: Turbidity Tube = a method of turbidity measure using a visual observation, recorded as “turbidity units (TU).
- Turbidity Tube (units = Turbidity Units (TU)
- Portable Turbidimeter (Hach 2100P) units = Nephelometric Turbidity
- Units (NTU)

Total Dissolved Solids (TDS)

- TDS is comprised of inorganic salts (principally calcium, magnesium, potassium, sodium, bicarbonates, chlorides and sulfates) and small amounts of organic matter that are dissolved in water.
- TDS in drinking-water originates from natural sources, sewage, urban runoff and industrial wastewater. Salts used for road de-icing in some countries may also contribute to the TDS content of drinking-water.
- Concentrations of TDS in water vary considerably in different geological regions owing to differences in the solubilities of minerals.
- Ref: TDS: GDWQ 4th Edition – Chemical Fact Sheets (p.423)

TDS (WHO)

	TDS (mg/l)
Excellent	< 300
Good	300 – 600
Fair	600 – 900
Poor	900 – 1,200
Unacceptable	➤ 1,200

WHO/SDE/WSH/03.04/16

Total Dissolved Solids in Drinking-water

Background document for development of WHO *Guidelines for Drinking-water Quality* 2nd Ed. Vol. 2. 1996

Common Forms of Chlorine

- Most common forms of chlorine:
- Dry (calcium hypochlorite – $[\text{Ca}(\text{OCl}_2)]$)
- Liquid (sodium hypochlorite – (NaOCl) =bleach)
- Chlorine gas (Cl_2)

Chlorination Chemistry

- When chlorine is added to water, it disinfects the water by damaging the cell structure of bacteria, thereby destroying them. The amount of chlorine needed to do this is the **chlorine demand**.
- **Chlorine demand** varies with the amount of impurities in the water.
- The purpose of chlorination is to satisfy the chlorine demand, with some excess left over. The excess chlorine is **residual chlorine**.

Residual (Free) Chlorine Guideline Values

- WHO:
 - “For effective disinfection, there should be a residual concentration of free chlorine of greater than or equal to 0.5 mg/l after at least 30 minutes contact time at pH <8.0. (WHO, 2004)
- Centers for Disease Control – “Safe Water System” of Household Disinfection.
 - At 30 minutes after addition of sodium hypochlorite, there should be no more than 2.0 mg/L of free chlorine residual present.
 - At 24 hours after the addition of sodium hypochlorite to containers that are used by families to store water, there should be a minimum of 0.2 mg/L of free chlorine residual present.

Summary Residual Chlorine Guidelines

- ≥ 0.5 mg/L and ≤ 2.0 mg/L after 30 minutes
- ≥ 0.2 mg/L after 24 hours

- More than this will taste bad
- Less will not guarantee that the supply is adequately protected.

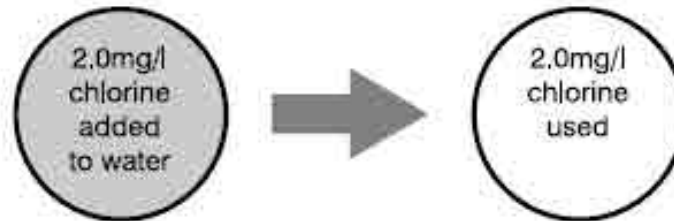
Example

If a water supply has a chlorine demand of 2.0 mg/L

If exactly 2.0 mg of chlorine is added per litre of water, then the chlorine demand will *just* be met and there will be no chlorine residual.

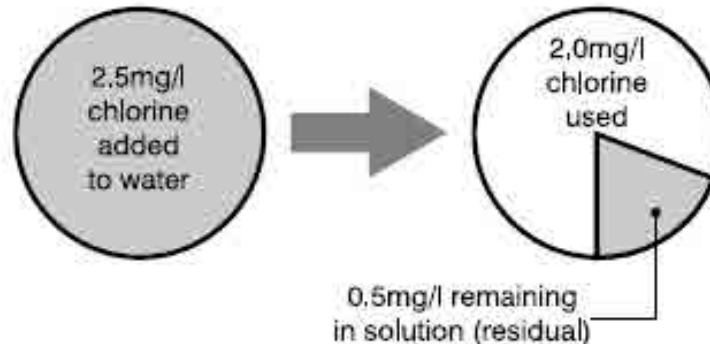
If 2.5 mg/l of chlorine is added, then the chlorine demand will be met and exceeded, so that a residual of 0.5 mg/l will be left in the water when it goes into supply.

+ 2.0 mg/L Cl



No chlorine residual

+ 2.5 mg/L Cl



0.5 mg/L chlorine residual

Chlorination Chemistry

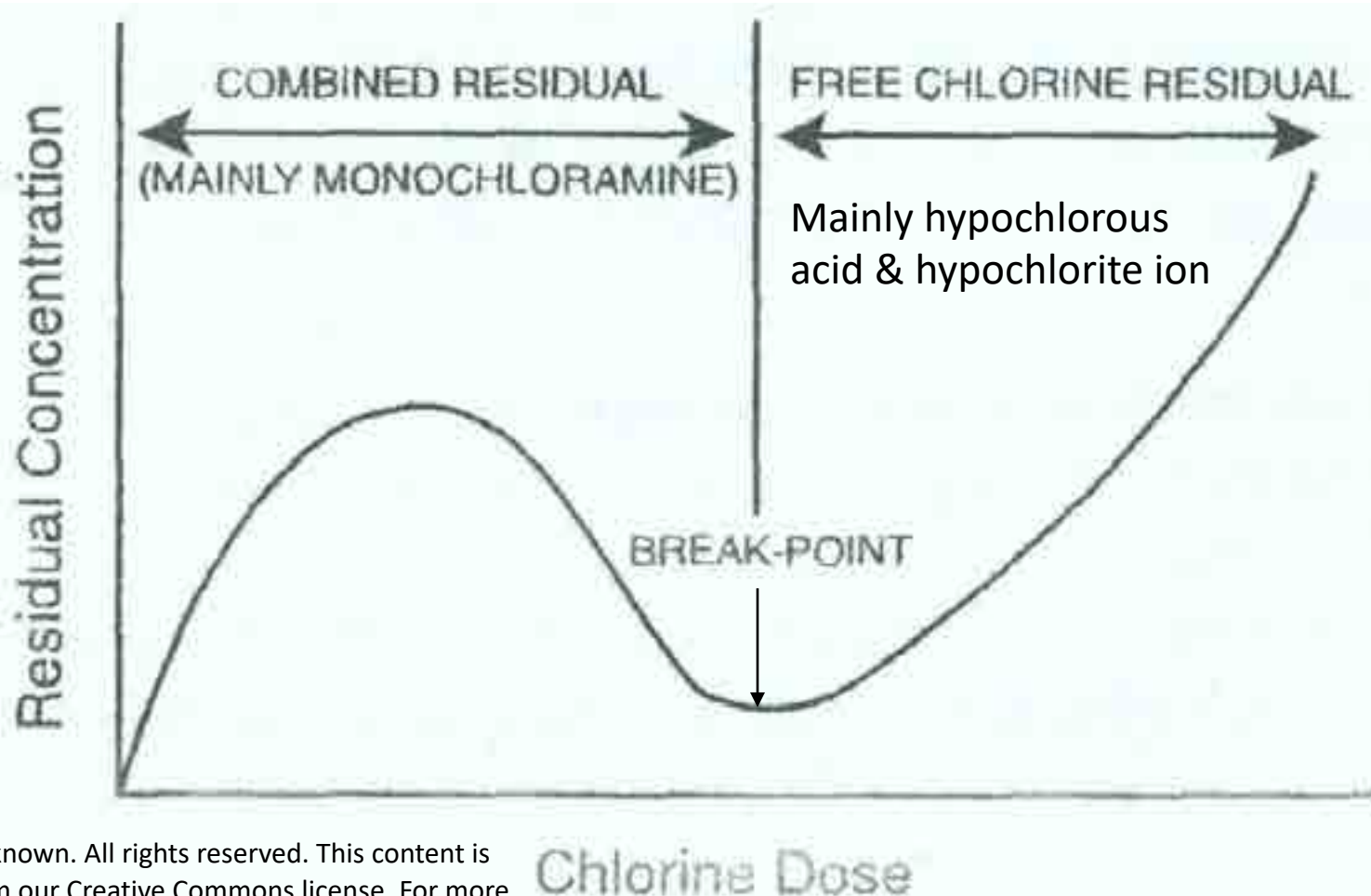
- Free residual chlorine (a.k.a. free available chlorine) = quantity of hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) in the water. The relative distribution of these two species is very important, because the efficacy of HOCl is about 40 – 80 x greater than OCl^- .
- Ammonia, commonly present in natural waters, will react with HOCl or OCl^- to form monochloramine, dichloramine and trichloramine, depending on factors such as temperature and pH.

Chlorination Chemistry

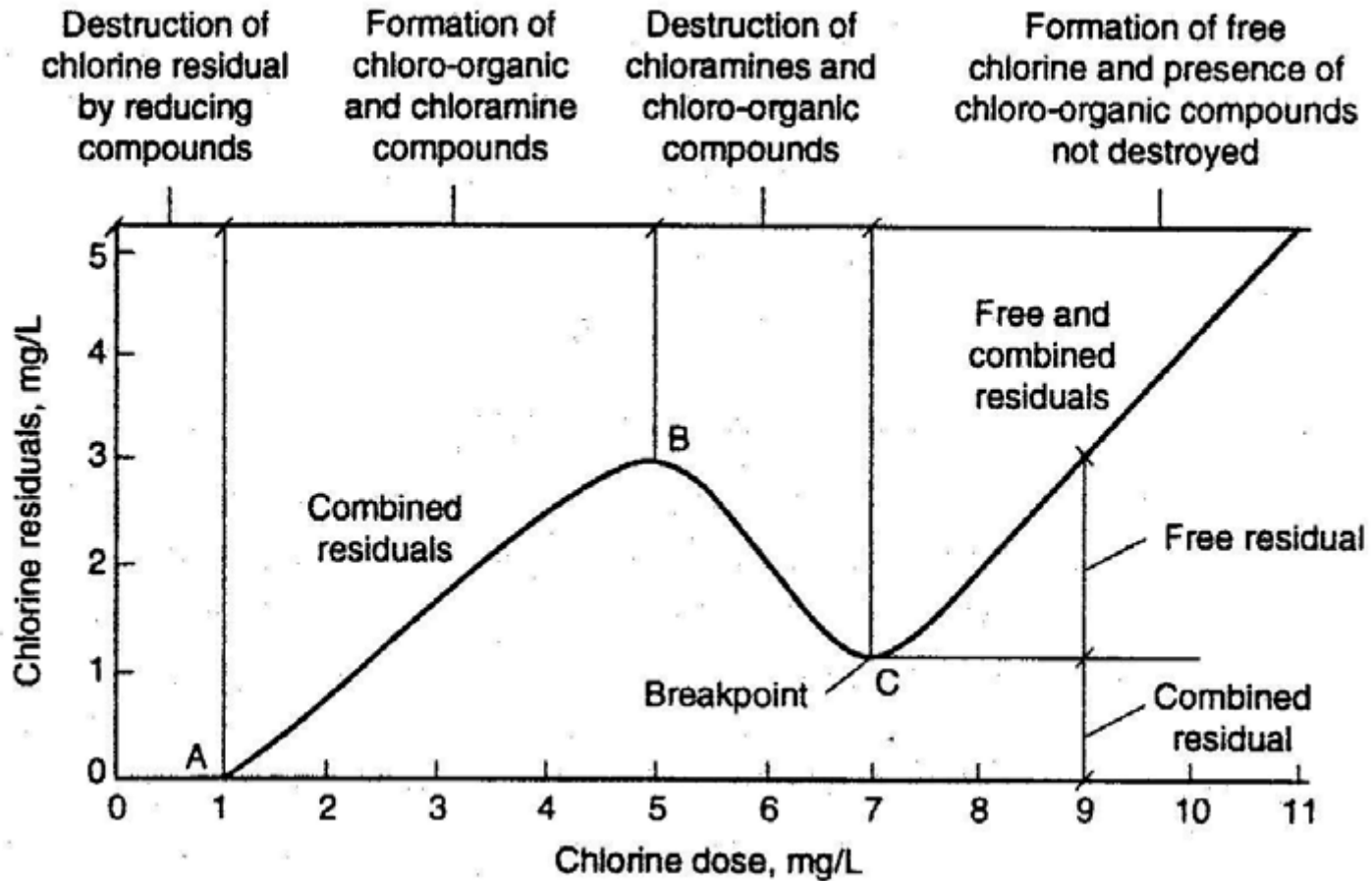
- Chlorination of water to the extent that all ammonia is converted to either trichloramine or oxidized to nitrate or other gases is referred to as **“breakpoint chlorination.”**
- Before breakpoint, “combined” chlorine predominates (monochloramine + dichloramine)
- After breakpoint, free residual chlorine predominates (hypochlorous acid + hypochlorite)
- Combined Residual Chlorine (ppm) + Free Residual Chlorine = Total Residual Chlorine

Breakpoint Chlorination Curve

Combined Residual + Free Residual = Total Residual Chlorine

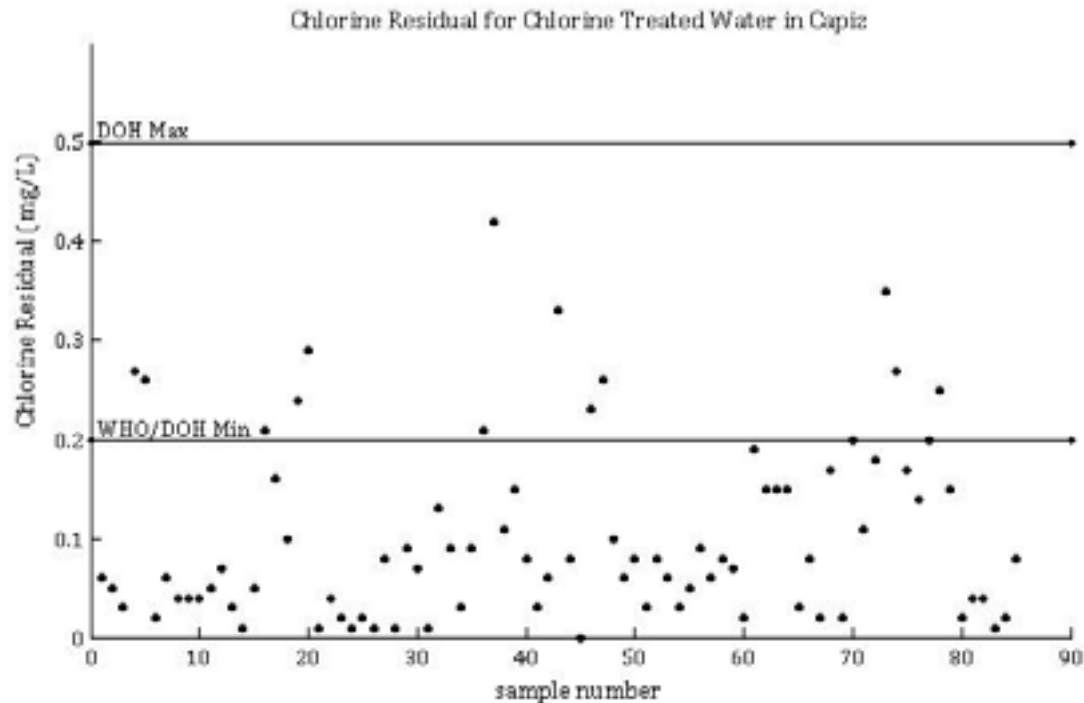


Breakpoint Chlorination Curve



Why Do We Care About Free (Residual) Chlorine?

Free Chlorine Residual for Capiz = Roxas City, Panitan, Ivisan and Panay



Ref: Patrick, J.M., Murcott, Punsalan, J. Coupling microbiological testing and sanitary surveys in drinking water quality programs: Results from Capiz Province, Philippines. Journal of Water, Sanitation, Hygiene for Development 01.2.2011

Chlorine Residual Results - Philippines

Figure shows the results of the 85 free chlorine residual tests completed in January 2010 for Roxas City, Panitan, Ivisan and Panay

Two horizontal lines at the 0.2 and 0.5mg/L chlorine residual concentration levels showing the WHO/DOH minimum level and DOH maximum level for chlorine residual after 30min of contact time, at any point in the distribution system.

Only 17.6% of the samples met the WHO and DOH standards

Results show that, of the 50 samples collected in Roxas City, only 10 (20%) of these met the WHO and DOH standards.

Five of the 15 samples (33%) collected in Panitan met the standards.

None of the samples collected in Ivisan and Panay met the standards.

Ref: Water Quality Testing and Water Use Assessment in Capiz Province, Philippines. By: Patty Chuang, John Millspaugh, Jessica Molly Patrick, Stephanie Trottier, Master of Engineering Report, Civil and Environmental Engineering, MIT, June 2010.

Portable Colorimeter and Digital Titrator for Chlorine Residual and Total Chlorine Tests



Colorimeter (Hach – DPD Pocket Colorimeter II)



DPD Titrator (Hach Digital Titrator)

Quality Assurance / Quality Control

Murcott Notes of T. Voelker Lab Training (12/02) and Standard Methods, 2012 (22nd Ed.)

1. Operator Error: Reagents added in the wrong order, volume errors, pressing the wrong button, etc.

New operators should check themselves on each new procedure by analyzing several standards, of known concentrations.

2. Technical Problems:

- * Instrument malfunction,
- * Reagents decay, e.g. left in the sun or heat
- * Etc.

You need to measure standards once a day or 10% of measurements

3. Contamination:

- * Maybe some analyte got into the sample you are measuring, i.e. "carryover" = a high measurement followed by low measurement
- * Reagents can get contaminated
- * Contamination can be added if a little dust falls into the sample
- * Dirty pipettes
- * Etc.

Measure blanks. Blanks are standards that are "zero." Measure blanks several times every day, or at the beginning and end of each day, especially after a high measurement. Or, analyze a minimum of 5% of sample load as blanks.

4. Interferences = "matrix effects" = interferences from constituents in your water sample.

- * Negative interference – measuring less than you should;
- * Positive interference – measuring more than you should.

You need to have an understanding of what causes interferences with your method, so you need to know your method!

One uses "standard additions" to test for matrix effects

- measure sample
- measure sample to which known concentration of analyte has been added.

Do this with every new matrix.

5. Precision – one must understand the precision of the method

Measure duplicates = a split sample collected at the same time from the same location. Measure these periodically.

5% or more of your samples should have duplicates. that are quality control charts of your measurement of standards, standard additions, and blanks.

6. Spatial/Temporal Variability in Field Measurements

Get several separate samples from same location and/or come back a few times and see what happens

Key Terms

- Accuracy: the closeness of a measured value to the true value.
- Analyte: the sample one intends to measure.
- Bias: consistent deviation of measured values from the true value, caused by systematic errors in a procedure.
- Calibration check standard: standard used to determine the state of calibration of an instrument between periodic recalibrations.
- Duplicate: one of two replicates
- Precision: measure of the degree of agreement among replicate analyses of a sample, usually expressed as the standard deviation.
- Replicate: Measurements which are repeated to assess sources of random error in your measurement. There are different kinds of replicates. Measuring the same sample or standard several times tells you about random error due to your technique. Taking several different samples at the same time and location also tells you about other kinds of variability (contamination in different sample bottles, fluctuations on short time and distance scales in a water that is not well mixed, etc.)

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