



The Laboratory Reporter

A publication of the Colorado State Public Health Laboratory

Laboratory Measures Chlorophyll to Determine Water Quality

by James Saunders, Water Quality Control Division, and
Laurie Peterson-Wright, Laboratory Services Division Chemistry Program manager

Rapid Detection
for the
Public's Protection

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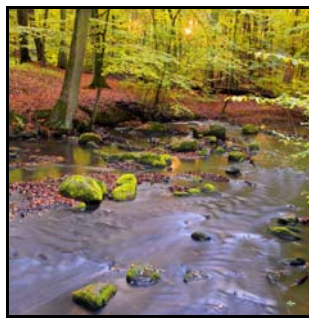
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Measuring chlorophyll provides a convenient and practical measurement of the abundance of algae in lakes and streams. The task of estimating abundance would be very difficult if it required a census of all algae in the community, because the many species represent a broad range of sizes and growth forms (including filaments, individual cells and colonies of different sizes). Samples would have to be examined under a microscope and the algal cells counted, measured and converted to a biomass measure. Clearly, the cost of such an undertaking would be prohibitive for most sampling programs.

Measuring chlorophyll, which all algae contain, assesses abundance with a simple extraction and no microscope.

Algal abundance is commonly used as an indicator of nutrient enrichment (eutrophication). The growth of algae in lakes and streams subject to nutrient enrichment is likely to be stimulating, resulting in an over-abundance of algal biomass, which is quantified by measurement of chlorophyll.



Concern about eutrophication has led to development of "nutrient criteria" as a means of curtailing the abundance of algae. The U.S. Environmental

Protection Agency has produced a set of recommendations defining the amount of chlorophyll consistent with the desired level of water quality and is pressing states to adopt those recommendations or propose alternatives.

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Laboratory Validated as a Reference Laboratory for the Detection of Clostridium botulinum toxin

by Laura Gillim-Ross, PhD, Public Health Microbiology/Serology Supervisor

The Colorado Department of Public Health and Environment laboratory has validated the Laboratory Response Network protocol for the detection of *Clostridium botulinum* toxin.

The department joins only a few other state laboratories as a reference laboratory for the detection of this toxin.

Botulinum toxin is a neurotoxin produced by the anaerobic gram-positive bacilli *Clostridium botulinum*, which causes botulism.

Five categories of botulism have been identified:



CDC/ Dr. V. R. Dowell

- (1) food-borne botulism caused by the ingestion of preformed botulinum toxin in contaminated food;
- (2) wound botulism resulting from infection of a wound with *C. botulinum*;
- (3) infant botulism resulting from colonization of the intestinal tract with *C. botulinum*;

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What are VOCs?

Volatile organic compounds are compounds that have a high vapor pressure and low water solubility.

Many VOCs are human-made chemicals that are used and produced in the manufacture of paints, pharmaceuticals, and refrigerants.

VOCs typically are industrial solvents, such as trichloroethylene; fuel oxygenates, such as methyl tert-butyl ether (MTBE); or by-products produced by chlorination in water treatment, such as chloroform.

VOCs are often components of petroleum fuels, hydraulic fluids, paint thinners, and dry cleaning agents.

VOCs are common ground-water contaminants.

-United States Geological Survey (USGS)



Organic Chemistry Laboratory Attains EPA-Certification to Test for Drinking Water Regulated Parameters

by Pat Ayres, Organic Chemistry lab scientist

The organic chemistry laboratory recently completed the arduous task of recertification, to become state-certified for the analysis of drinking water. This task included the setup and operation of six instruments for the analysis of volatile organic compounds, synthetic organic compounds, pesticides, herbicides and disinfection byproducts. This adds more than 50 drinking water contaminants to the EPA-certified status of the chemistry laboratory. The entire certification effort took six years to complete.

Much of the instrumentation used in the recertification process was obtained from the Centers for Disease Control and Prevention through chemical terrorism response and biomonitoring funding and modified to be capable of contaminant testing under stringent EPA quality-assurance rules.

The organic chemistry laboratory now can accept samples from Colorado public drinking water suppliers for the analysis of all Phase I, II and IV drinking water analytes (sometimes called regulated parameters).

Operating more efficiently today, the laboratory is able to maintain this enhanced testing with one-third the staffing capacity than in the prior certification year 2000.

New Water Testing Being Developed to Protect Aquatic Life

by Mark Angerhofer, Organic Chemistry lab scientist

Nonylphenol, a surfactant component commonly found in many residential and industrial products, is an emerging contaminant of concern because of its endocrine-disrupting properties on aquatic life.

Nonylphenol concentrations have been measured extensively throughout Europe, and discharge limits have been established for wastewater treatment plants. The Colorado Department of Public Health and Environment, along with concerned parties, aim to implement nonylphenol standards to protect aquatic life by July 2010.

The Laboratory Services Division's organic chemistry lab is developing lab techniques to analyze wastewater influent and effluent for nonylphenol concentrations. Measurement of nonylphenol in wastewater is difficult because of matrix interferences and the low detection limits needed to ensure safe concentrations that preliminary nonylphenol standards propose.

The lab currently is developing a solid-phase extraction gas chromatography/mass spectrometry method that chemically alters nonylphenol and detects concentrations at the parts per billion level. This method aims to be robust, affordable and provide wastewater treatment plants with quality data. A liquid chromatography/dual mass spectrometry method that can detect lower concentrations of nonylphenol for use in research on nonylphenol fate and transport is also being developed.



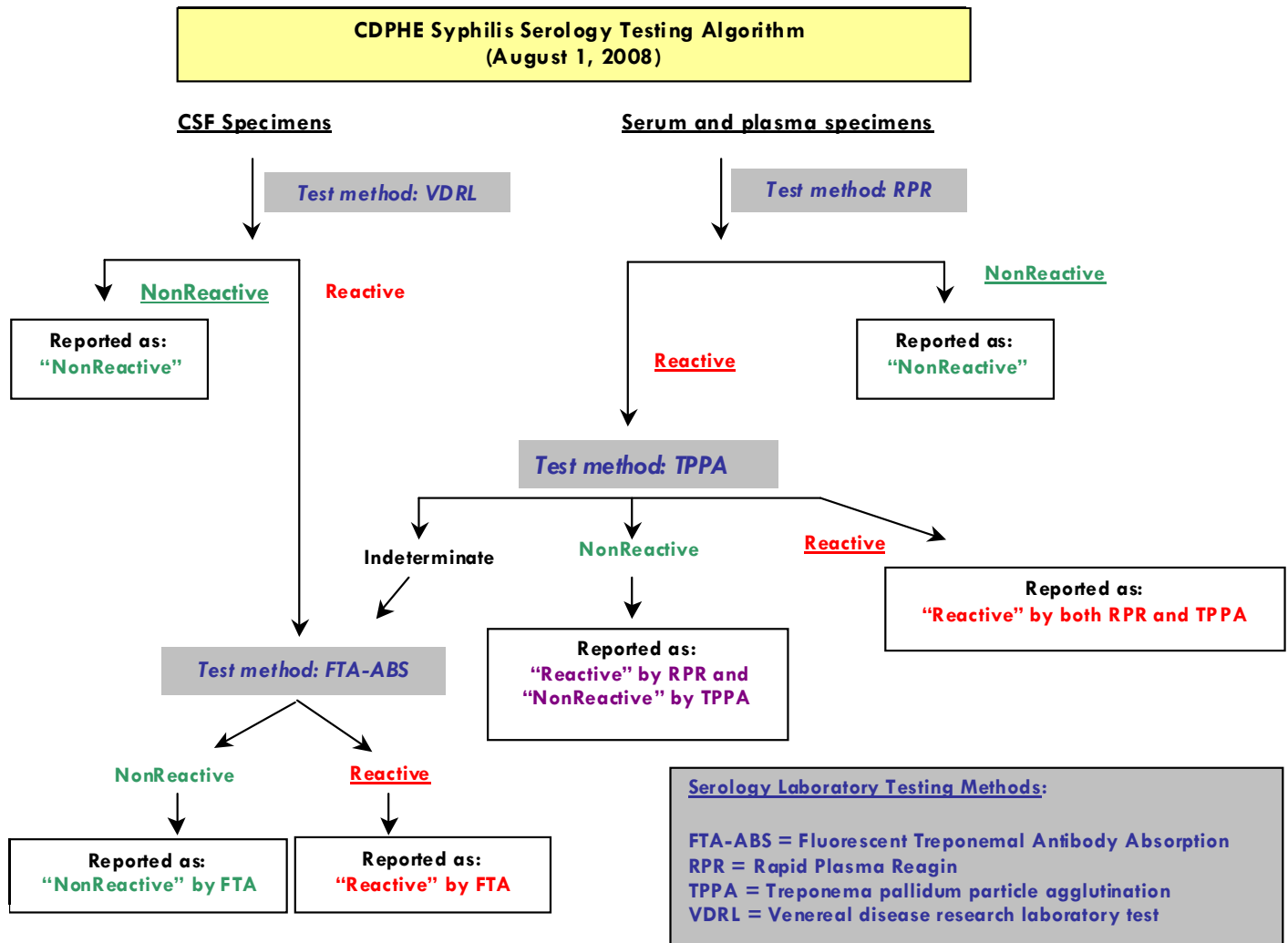
Changes in Syphilis Testing with Implementation of a New Treponemal Assay

by *Laura Gillim-Ross, PhD, Public Health Microbiology/Serology Supervisor*

Beginning Aug. 1, 2008, the Colorado Department of Public Health and Environment serology laboratory will be implementing a change in syphilis serology testing. The fluorescent-treponemal antibody-absorption (FTA-ABS) assay will be replaced with the *Treponema pallidum* particle agglutination (TPPA) assay. Like the FTA-ABS, TPPA is a qualitative treponemal assay for the detection of *Treponema pallidum* antibodies in human serum and plasma. The sensitivity of this assay is comparable to FTA-ABS at the different stages of syphilis infection. Implementation of this change will increase the frequency with which syphilis serology testing is performed in the laboratory, resulting in decreased turn-around time.

Serum, plasma and cerebral spinal fluid are acceptable specimens for syphilis serology testing. Please refer to the department's syphilis serology testing algorithm. Serum and plasma received by the laboratory will be screened by rapid plasma reagin, and reactive specimens will be confirmed by TPPA. Cerebral spinal fluid specimens will be screened by a venereal disease research laboratory test, and reactive specimens will be confirmed by FTA-ABS. TPPA alone may be requested for confirmation.

Please contact Dr. Laura Gillim-Ross (303-692-3484) if you have questions regarding testing or results interpretation.





Laboratory Measures Chlorophyll to Determine Water Quality

(Continued from page 1)

Colorado is in the process of evaluating chlorophyll criteria for protection of water quality in lakes throughout the state. The database for Colorado includes almost 4,000 chlorophyll measurements taken from 84 lakes. These data will become part of the hearing record when the Water Quality Control Division submits a proposal for a rule-making hearing in 2010.



“Eutrophication occurs when lakes or slow moving streams receive excess nutrients that stimulates excessive plant growth.

This enhanced plant growth, often called an algal bloom, reduces dissolved oxygen in the water when dead plant material decomposes and can cause other organisms to die.”

-United States Geological Survey (USGS)

The Water Quality Control and Laboratory Services divisions partnered in the summer of 2008 to compare analytical methodologies. Samplers filter volumes of 100 milliliters to 3 liters of water. The chlorophyll is collected on a glass fiber filter. Chlorophyll is highly susceptible to degradation as the cells die and decompose. To minimize degradation, the filter is folded, wrapped in foil and frozen.

Several methods for chlorophyll analysis are available. The state laboratory follows the Standard Methods procedure and extracts the chlorophyll pigment in acetone and analyzes the extract spectrophotometrically. The state laboratory results were compared to the previous method used: a hot methanol extraction followed by spectrophotometric measurements. The two data sets correlated well. The state laboratory will analyze another 60-90 samples this summer to support the rule-making hearing.

Laboratory Validated as a Reference Laboratory for the Detection of *Clostridium botulinum* toxin

(Continued from page 1)



Gram-stain of *C. botulinum* bacterial cells showing a tennis racket shape due to spore formation and terminal swelling of the cell.

CDC/Dr. George Lombard

- (4) non-infant botulism found in children and adults with intestinal colonization with *C. botulinum*; and
- (5) inhalation botulism resulting from inhalation of aerosolized botulinum.

The laboratory recently identified botulinum toxin associated with a case of infant botulism and was able to provide results to the submitting hospital within 24 hours of receipt of specimens. If a case of botulism is suspected, medical providers must first receive consent from the department epidemiologists before the laboratory can perform testing.

Contact Dr. Laura Gillim-Ross (303-692-3484) with any questions regarding testing or results interpretation.



Laboratory Services Division Helps Crack Salmonella Case

by Jan Stapleman, NEWSLink editor

Reprinted with permission from the August 2008 issue of NEWSLink, the Colorado Department of Public Health and Environment's employee newsletter

A three-month investigation into a nationwide outbreak of Salmonellosis finally nailed a source of the illness when scientists from the Laboratory Services Division found that a jalapeno pepper obtained from an ill Montezuma County resident matched the DNA "fingerprint" of the Salmonella Saintpaul strain responsible for the outbreak.

"Specifically, in Colorado, we now have a direct link between the disease in humans and a food product," said **Hugh Maguire**, Microbiology Program manager. The Laboratory Services Division was the first to establish that link.



The outbreak, which has sickened more than 1,300 people nationwide, was first attributed to tomatoes. But over time, the investigation by the U.S. Food and Drug Administration, the Centers for Disease Control and Prevention, and multiple state health departments found links to jalapeno and serrano peppers as well.

The pepper that broke the case in Colorado was recovered from a Colorado man's home by Montezuma County Nursing Services early the week of July 21 and submitted to **Shaun Cosgrove**, an epidemiologist with Disease Control and Environmental Epidemiology.

According to Maguire, the detective work at the Laboratory Services Division involved employees in three separate labs within the division:

Skip Gossack, a scientist with the environmental microbiology lab, detected the Salmonella bacteria by a polymerase chain reaction test. He then grew the bacteria in culture and handed it off to the public health microbiology lab for further testing.

At the public health microbiology lab, laboratory scientist **Melissa Jett Nucci** typed the strain and determined that it was Salmonella Saintpaul.

Jett Nucci transferred the Salmonella isolate to **Dave Heltzel** in the molecular science lab, where he and fellow scientist **Mary Kate Cichon** began pulsed field gel electrophoresis (PFGE) testing to determine its DNA fingerprint.

The PFGE test takes several days, so while lab staff waited for those results, **Justin Nucci**, also a scientist in the molecular science lab, performed another genetic typing test called multi-locus VNTR analysis (MLVA). Nucci, one of only a handful of scientists who are authorized by the CDC to perform MLVA testing, confirmed a matching fingerprint at 7 p.m. on Friday, July 25. He called Maguire, who was on his way home from the Mesa County Lab in Grand Junction, and told him about the match.



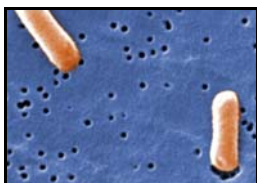
From left, **Dave Heltzel**, **Mary Kate Cichon**, **Justin Nucci** and **Melissa Jett Nucci** of the Laboratory Services Division linked a jalapeno pepper that sickened a Colorado man to the nationwide outbreak of illness from Salmonella Saintpaul. The team was the first to establish a link between the disease in humans and a food product. Not shown is **Skip Gossack**, who also played a role in the testing.



Meanwhile, the PFGE test produced the same results on the following Monday morning. Cosgrove notified the FDA through its Emergency Operations Center, which was set up to respond to the outbreak crisis.

"The important thing is that when the chips are down, these guys will stay with it until they get an answer," Maguire said of the lab employees who put in long hours to crack the case.

On July 30, the FDA confirmed that Salmonella Saintpaul was present in irrigation water and on produce at a farm in Mexico. The investigation is continuing.



Rod-shaped *Salmonella* bacteria. CDC/Janice Haney Carr



LABORATORY SERVICES DIVISION

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Denver, CO 80230
Phone 303-692-3090
Order Line 303-692-3074
Online ordering for testing supplies:
coloradostatelab.us

24-hour Emergency Line 877-518-5608

E-mail: cdphe.lab@state.co.us

NEW Website: coloradostatelab.us

Business Hours: 8 a.m. - 5 p.m.
Monday through Friday
Closed holidays.



Mission

The mission of the Laboratory Services Division is to protect the health, safety, and environment of all Coloradans by providing accurate and timely laboratory analyses and information.

Testing Services Include:

Drinking water testing (lead, nitrites and more)
Newborn Screening (38 disorders)
Tuberculosis, Parasite Identification, Confirmation of food poisoning bacteria (and more)
Human Immunodeficiency Virus (HIV)
Influenza (and other respiratory viruses)
Preparedness (Anthrax, Plague, and more)
Food and Milk Bacterial Contaminants
Visit our website for more information:
coloradostatelab.us

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Calendar of Training Events

More information and registration at: www.peopleware.net/1885a
Training below offered at the State Public Health Lab unless otherwise noted

New Testing Strategies for HIV Detection

September 9th—11 a.m. - 12 p.m.

Preparing Your Laboratory for Safety (Clinical Lab Safety 101 Series)

September 10th—1 p.m. - 2 p.m.

Protecting Your Employees (Clinical Lab Safety 101 Series)

September 24th—1 p.m. - 2 p.m.

Trends in Laboratory Diagnosis of Leishmaniasis

September 30th—11 a.m. - 12 p.m.

Non- Enterobacteriaceae: Practical Strategies for Identification

October 7th—11 a.m. - 12 p.m.

Chemical Hygiene (Clinical Lab Safety 101 Series)

October 8th—1 p.m. - 2 p.m.

Bloodborne Pathogens (Clinical Lab Safety 101 Series)

October 22nd—1 p.m. - 2 p.m.

Additional Important Lab Safety Topics (Clinical Lab Safety 101 Series)

November 5th—1 p.m. - 2 p.m.

Training That Won't Put Your Staff to Sleep (Clinical Lab Safety 101 Series)

November 19th—1 p.m. - 2 p.m.

Updates in Diagnostic Detection of Free-Living Amoeba

December 16th—11 a.m. - 12 p.m.

Ordering Testing Supplies from the Lab

The Laboratory Services Division is now offering two convenient methods for ordering supplies for testing. A new online ordering system was implemented on July 1, 2008. Visit our new web address at: coloradostatelab.us and click on the "Order from the Lab" link. If your access to the Internet is limited, you may use our supply order line at: 303-692-3074.