

**Title 2. ADMINISTRATION**  
**DIVISION 3. STATE PROPERTY OPERATIONS**  
**CHAPTER 1. STATE LANDS COMMISSION**  
**ARTICLE 4.7 PERFORMANCE STANDARDS AND COMPLIANCE ASSESSMENT**  
**PROTOCOLS FOR THE DISCHARGE OF BALLAST WATER FOR VESSELS**  
**OPERATING IN CALIFORNIA WATERS**

*Staff has illustrated changes to the original text in the following manner: proposed language is underlined; deletions from the original text are shown in strikeout using a “-”.*

**Section 2291. Purpose, and Applicability. ~~and Date of Implementation.~~**

- (a) The purpose of the regulations in Title 2, Division 3, Chapter 1, Article 4.7 of the California Code of Regulations is to move the state expeditiously toward elimination of the discharge of nonindigenous species into the waters of the state or into waters that may impact the waters of the state, based on the best available technology economically achievable.
- (b) The provisions of Article 4.7 apply to all vessels ~~that discharge ballast water, 300 gross registered tons or more, carrying, or capable of carrying, ballast water in~~ California waters except those vessels that are exempt under Section 71202, Public Resources Code.

Note: Authority cited: Sections 71201.7, 71202 and 71205.3, Public Resources Code. Reference: Sections 71201.7, 71202 and 71205.3, Public Resources Code.

**Section 2292. Definitions.**

Unless the context otherwise requires, the following definitions shall govern the construction of this Article:

- (a) “Ballast Water Capacity” means the total volumetric capacity of any tanks, spaces, or compartments on a vessel used for carrying, loading, or discharging ballast water, including any multi-use tank, space, or compartment designed to allow carriage of ballast water.
- (b) “Ballast Water Sample” means a unit of ballast water collected and assessed for compliance.
- (c) “Ballast Water Treatment System” means a system designed to remove, kill, or inactivate (prior to discharge) organisms in ballast water.

- (d) “Board” means the State Water Resources Control Board.
- (e) “Colony Forming Unit” means a unit of measurement used to estimate the number of viable bacteria cells in a sample.
- (f) “Commission” means the California State Lands Commission.
- (g) “Constructed” means a stage of vessel construction where:
  - (1) the keel is laid; ~~or~~
  - (2) construction identifiable with a specific vessel begins; ~~or~~
  - (3) assembly of the vessel has commenced comprising at least 50 tons ~~tonnes~~ or 1 percent of the estimated mass of all structural material, whichever is less; or
  - (4) the vessel undergoes a major conversion.
- (h) “HDPE” means high density polyethylene
- ~~(g) “Isokinetic Sampling Facility” means a sampling apparatus in which the velocity (or speed) of the sample stream does not change from the pipe being sampled to the sample pipe itself.~~
- ~~(h) “Isokinetic Diameter” assumes a circular main flow pipe and circular sampling pipe of which the diameter is designed to maintain the fluid velocity from the main flow to the sample flow.~~
- (h) (i) “Major Conversion” means a conversion of a vessel that:
  - (1) ~~which~~ changes its ballast water carrying capacity by 15 percent or greater; ~~or~~
  - (2) ~~which~~ changes the vessel type (e.g., from a bulk vessel to a tank vessel); or
  - ~~(3) which in the opinion of the Commission, is projected to prolong its life by ten years or more; or~~
  - (3) ~~(4) which~~ results in modifications to its ballast water system other than component replacement-in-kind.

Conversion of a vessel to comply with the provisions of this Article shall not be deemed to constitute a major conversion under this Section.

- (i) ~~(j)~~ “Sampling Facilities” Collection Device means a device used to collect a ballast water sample from a sampling port. the equipment installed to take the ballast water sample.
- (i) ~~(k)~~ “Sampling Port Point” means that place in the ballast water piping where the sample is taken the flanged opening (“the door”) into the ballast main line; the sampling port is ship-supplied equipment and may be covered by a closed valve or blank plate when not in use.
- ~~(l)~~ “Vessel” means a vessel of 300 gross registered tons or more.

Note: Authority cited: Sections 71201.7 and 71205.3, Public Resources Code.  
Reference: Sections 71200, 71201.7 and 71205.3, Public Resources Code.

### **Section 2293. Interim Performance Standards for Ballast Water Discharges.**

Subject to the Implementation Schedule in Section 2294, before discharging ballast water in the waters ~~subject to the jurisdiction of California~~ of the State, the master, owner, operator, or person in charge of a vessel to which this section applies shall conduct ballast water ~~treatment~~ management so that ballast water discharged will contain:

- (a) No detectable living organisms that are greater than 50 micrometers in minimum dimension;
- (b) ~~Less than~~ 0.01 or fewer living organisms per milliliter that are equal to or less than 50 micrometers in minimum dimension and equal to or greater ~~more than~~ 10 micrometers in minimum dimension; and
- (c) For living organisms that are less than 10 micrometers in minimum dimension:
  - (1) ~~less than~~ 1,000 or fewer bacteria per 100 milliliters;
  - (2) ~~less than~~ 10,000 or fewer viruses per 100 milliliters;
  - (3) concentrations of microbes that are less than:
    - (A) 126 colony forming units per 100 milliliters of *Escherichia coli*;
    - (B) 33 colony forming units per 100 milliliters of intestinal enterococci; and

- (C) 1 colony forming unit per 100 milliliters or 1 colony forming unit per gram of wet weight of zoological samples of Toxicogenic *Vibrio cholerae* (serotypes 01 and 0139).

Note: Authority cited: Sections 71201.7 and 71205.3, Public Resources Code.  
Reference: Sections 71201.7 and 71205.3, Public Resources Code.

### **Section 2294. Implementation Schedule for Interim Performance Standards for Ballast Water Discharges.**

Sections 2293, and 2297, 2297.1, 2297.2, and 2297.3 apply to vessels in accordance with the following schedule:

- (a) Upon first arrival at a California port for new vessels constructed on or after January 1, 2020; or
- (b) As of the first scheduled drydocking on or after January 1, 2020, for all other vessels.
- ~~(a) Beginning January 1, 2010, for vessels constructed on or after that date with a ballast water capacity of less than or equal to 5,000 metric tons.~~
- ~~(b) Beginning January 1, 2012, for vessels constructed on or after that date with a ballast water capacity greater than 5,000 metric tons.~~
- ~~(c) Beginning January 1, 2014, for vessels constructed before January 1, 2010, with a ballast water capacity of 1,500 metric tons or more but not more than 5,000 metric tons.~~
- ~~(d) Beginning January 1, 2016, for vessels constructed before January 1, 2010, with a ballast water capacity of less than 1,500 metric tons, and for vessels constructed before January 1, 2012, with a ballast water capacity greater than 5,000 metric tons.~~

Note: Authority cited: Sections 71201.7 and 71205.3, Public Resources Code.  
Reference: Sections 71201.7 and 71205.3, Public Resources Code.

### **Section 2295. Implementation Schedule for Final Performance Standards for Ballast Water Discharges.**

Beginning January 1, ~~2020~~2030, before discharging ballast water into waters of the state, the master, owner, operator, or person in charge of a vessel to which this section applies shall conduct ballast water ~~treatment~~ management so that ballast water discharged will contain zero detectable living organisms for all organism size classes.

Note: Authority cited: Sections 71201.7 and 71205.3, Public Resources Code.  
Reference: Sections 71201.7 and 71205.3, Public Resources Code.

### **~~Section 2296. Delay of Application for Vessels Participating in Promising Technology Evaluations.~~**

~~If an owner or operator of a vessel applies to install an experimental ballast water treatment system and the Commission approves that application on or before January 1, 2008, the Commission shall deem the system to be in compliance with any future treatment standard adopted, for a period not to exceed five years from the date that the interim performance standards would apply to that vessel.~~

- ~~(a) — The Commission may rescind its approval of the system at any time if the Commission, in consultation with the Board and the United States Coast Guard, after an opportunity for administrative appeal with the executive officer of the Commission, determines that the system has not been operated in accordance with conditions in the agreed upon application package, or that there exists a serious deficiency in performance, human safety, or environmental soundness relative to anticipated performance, or that the applicant has failed to provide the Commission with required test results and evaluations.~~

~~Note: Authority cited: Sections 71201.7, 71204.7 and 71205.3, Public Resources Code. Reference: Sections 71201.7, 71204.7 and 71205.3, Public Resources Code.~~

### **Section 2296. Functionality Monitoring, Calibration, and Efficacy Testing of Shipboard Ballast Water Treatment Systems.**

Subject to the implementation schedule in Section 2294, the master, owner, operator, or person in charge of a vessel arriving at a California port employing a shipboard ballast water treatment system to manage ballast water prior to a discharge in California waters, shall monitor the functionality and calibration of the system.

- (a) Monitoring, calibration, and functionality of shipboard ballast water treatment systems
- (1) Monitoring of specific parameters that are relevant and applicable to the installed ballast water treatment system is required at least once per month. Specific parameters are listed in Appendix J of the U.S. Environmental Protection Agency's Vessel General Permit (December 19, 2013). Appendix J is hereby incorporated by reference.

- (2) To ensure proper functioning, all applicable sensors and other ballast water treatment system control equipment must be calibrated no less frequently than recommended by the sensor or other equipment manufacturer or the ballast water treatment system manufacturer, if available.
  - (3) Before discharging treated ballast water into the waters of the state, vessels shall have conducted testing of the biological performance of the installed ballast water treatment technology at least once within the previous twenty-four months. The test of biological performance shall be at least equivalent to the sampling and analysis requirements in Section 2297.1 and 2297.2.
- (b) Printed or electronic records, signed by the responsible party, of all functionality monitoring, calibration results, and biological analyses shall be kept on board the vessel for a minimum of two (2) years and be available for review by Commission staff upon request.

Note: Authority cited: Sections 71201.7 and 71205, Public Resources Code.  
Reference: Sections 71201.7 and 71205(g), Public Resources Code.

### **~~Section 2297.1. Ballast Water Treatment Technology Reporting Requirements.~~**

#### ~~(a) Ballast Water Treatment Technology Annual Reporting Form~~

~~(1) The following form "Ballast Water Treatment Technology Annual Reporting Form (Revised July 1, 2010)" is hereby incorporated by reference into this section and shall be used to comply with the provisions of Public Resources Code section 71205(g) by the master, owner, operator, agent, or person in charge of a vessel that has a ballast water treatment system installed on board and has discharged treated ballast in waters of the state.~~

~~(2) The "Ballast Water Treatment Technology Annual Reporting Form" shall be submitted to the Commission in written or electronic form within 60 days of receiving a written or electronic request from the Commission.~~

#### ~~(b) Ballast Water Treatment Supplemental Reporting Form~~

~~(1) The following form "Ballast Water Treatment Supplemental Reporting Form (Revised July 1, 2020)" is hereby incorporated by reference, and shall be used to comply with the provisions of Public Resources Code Section 71205(g) by the master, owner, operator, agent, or person in charge of a vessel that has a ballast water treatment system installed on board and has discharged treated ballast in waters of the state.~~

- ~~(2) The “Ballast Water Treatment Supplemental Reporting Form shall be submitted to the Commission in written or electronic form if that vessel discharged treated ballast water into the waters of the state.~~

~~Note: Authority cited: Sections 71201.7 and 71205, Public Resources Code.  
Reference: Sections 71201.7 and 71205(g), Public Resources Code.~~

**Section 2296.1 The Collection of Ballast Water Samples for the Rapid Assessment of Non-Compliance with California’s Performance Standards for the Discharge of Ballast Water.**

- (a) Rapid assessment of ballast water samples to assess non-compliance.
- (1) Upon inspection of the required documents in section 2296, Commission staff may take one or more ballast water samples for the rapid assessment of non-compliance with section 2293.
  - (2) Samples will be analyzed using a commercially available handheld device capable of providing a rapid assessment of the abundance or activity of organisms equal to or less than 50 µm in minimum dimension and equal to or more than 10 µm in minimum dimension.
  - (3) The sample volume shall be based on the rapid assessment tool’s specific manufacturer’s requirements.
  - (4) Samples shall be processed onboard a vessel at the time of collection.
  - (5) A minimum of three replicate measurements shall be performed per analysis.

**Section 22976.2 Protocols for the Collection of Ballast Water Samples to Assess Compliance with California’s Performance Standards for the Discharge of Ballast Water.**

~~Subject to the implementation schedule in Section 2294 and taking into account the following considerations, a vessel to which this section applies shall install sampling facilities to enable collection of ballast water samples in order to assess compliance with Section 2293. If the result of a rapid assessment test on a vessel subject to Section 2293 reveals that the sampled ballast water discharge exceeds the compliance threshold, the California State Lands Commission shall collect ballast water samples to assess compliance.~~

- (a) Sampling port for the collection and disposal of ballast water samples.

- (1) Subject to the implementation schedule in Section 2294, ballast water shall not be discharged into waters of the state from a vessel subject to this section unless that vessel has installed at least one sampling port to enable collection and disposal of ballast water samples to assess compliance with Section 2293.
  - (A) The sample port design shall conform to United States Coast Guard requirements in Code of Federal Regulations Title 46 CFR Subpart 162.060-28(f), hereby incorporated by reference.
  - (B) A sample port shall be available to allow for sampling at each point of overboard ballast water discharge, or as close to the discharge point as is practical.
- (b) Collection of ballast water samples for use in determining vessel compliance with Section 2293.
  - (1) For all samples:
    - (A) Samples shall be collected by Commission staff, or designated representatives, to assess vessel compliance with the ballast water discharge performance standards set forth in Section 2293;
    - (B) All samples shall be collected from the sampling port, as required by Section 2297(b);
    - (C) Samples shall be collected so that they are representative of the discharge at the time of collection.
    - (D) Temperature of the sample should be maintained at the ambient temperature (plus or minus 5 degrees Celsius) of the discharged ballast water; and
    - (E) Storage containers shall be cleaned and rinsed following standard laboratory techniques;
  - (2) For organisms greater than 50 micrometers (µm) in minimum dimension:
    - (A) A total of 3 cubic meters (m<sup>3</sup>) of ballast water to be discharged shall be sampled;



- (B) Samples shall be concentrated at the time of collection using a 35  $\mu\text{m}$  (no greater than 50  $\mu\text{m}$  in diagonal dimension) plankton net or mesh. Samples may be further concentrated using a 35  $\mu\text{m}$  (no greater than 50  $\mu\text{m}$  in diagonal dimension) mesh sieve in order to reach a final sample volume of between 1 – 4 liters (L), depending on the concentration of the sample. Sterilized artificial seawater, filtered seawater, or freshwater, as appropriate, should be added to maintain oxygen levels for the living organisms to be counted; and
- (C) Samples shall be stored in a clean flask or carboy with a capacity not more than 4 L.
- (3) For organisms equal to or less than 50  $\mu\text{m}$  in minimum dimension and equal to or greater than 10  $\mu\text{m}$  in minimum dimension

  - (A) A minimum of 3 L of ballast water to be discharged shall be collected.
  - (B) Samples shall be stored in clean, opaque HDPE bottles.
- (4) For Bacteria

  - (A) 1 L of ballast water to be discharged shall be collected.
  - (B) Sample shall be collected and stored in sterile glass or HDPE plastic bottles at least 1 L in volume.
- (5) *Escherichia coli*

  - (A) 1 L of ballast water to be discharged shall be collected.
  - (B) Samples shall be collected and stored in sterile glass or HDPE plastic bottles at least 1 L in volume.
- (6) Intestinal enterococci

  - (A) 1L of ballast water to be discharged shall be collected.
  - (B) Samples shall be collected and stored in sterile glass or HDPE plastic bottles at least 1 L in volume.
- (7) Toxigenic *Vibrio cholera* serotypes O1 and O139

(A) 1 L of discharged ballast water shall be collected.

(B) Samples shall be collected in sterile glass or HDPE plastic bottles at least 1 L in volume.

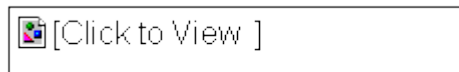
(8) Viruses

Due to the lack of effective, scientifically sound protocols, this section does not establish compliance assessment sampling requirements for the virus standards.

~~(a) Technical specifications for design of in-line sampling facilities:~~

~~(1) The sampling facility shall not damage and/or induce substantial incidental mortality to organisms to be collected in ballast water.~~

~~(2) The isokinetic sample port diameter shall be determined according to the equation:~~



~~Image 1 (1.03" X .42") Available for Offline Print~~

~~where  $D_{iso}$  and  $D_m$  are the diameters of the sample port opening and the main flow in the discharge line, respectively; and  $Q_{iso}$  and  $Q_m$  represent the respective volumetric flow rates through the two pipes. Sample port size shall be based on the combination of maximum sample flow rate and minimum ballast flow rate that yields the largest isokinetic diameter.~~

~~(3) The opening of the sampling pipe shall be chamfered to provide a smooth and gradual transition between the inside and outside pipe diameters.~~

~~(4) The length of the straight sample pipe facing into the flow can vary, but shall not be less than one diameter of the sampling pipe. The sampling port shall be oriented such that the opening is facing upstream and its lead length is parallel to the direction of flow and concentric to the discharge pipe, which may require sampling pipes to be "L" shaped with an upstream facing leg if installed along a straight section of discharge pipe.~~

~~(5) The design of the sample facility shall allow for the servicing and/or cleaning of the sampling facility without impacting the safety of the vessel. The sampling pipe should be retrievable either manually or mechanically, or it should be in a system which can be isolated.~~

- ~~(6) — The sample facility and all associated parts of the sampler that come into contact or near proximity with the ballast piping shall be constructed of galvanically compatible materials and generally corrosion resistant.~~
- ~~(7) — When control of the sample flow rate is required, appropriate valves shall be used that do not result in organism mortality due to sharp velocity transitions. Ball, gate or butterfly valves shall not be used.~~
- ~~(8) — If a pump must be used to sample the discharge side of a tank, an appropriate sampling pump shall be used to minimize organism mortality.~~
- ~~(9) — The Master of the vessel must maintain positive control (e.g. tamper evident lockout seals) over the ballast water sampling facility when compliance verification or scientific sampling is not being conducted.~~
- ~~(a) — Technical specifications for installation of a sample point in the ballast water discharge line:
  - ~~(1) — The sampling point shall be safely accessible to Commission staff, and shall not be in a confined space.~~
  - ~~(2) — The sampling point shall be installed in a straight part of the discharge line, downstream of the last treatment process, as near to the ballast water overboard discharge as practicable.~~
  - ~~(3) — The sample shall be removed from the main pipeline at a location where the flowing stream at the sample point is representative of the contents of the stream. The sample facility should be placed at a point where the flow in the main pipe is fully mixed and fully developed.~~
  - ~~(4) — As many sample points shall be provided as necessary to draw a ballast water sample during typical deballasting of the vessel.~~
  - ~~(5) — In cases where the ballast system design does not enable sampling from the discharge line, other arrangements for a sampling point may be made on a vessel specific basis with prior approval of Commission staff.~~~~
- ~~(b) — Existing sampling facilities~~

~~Vessels may use existing sampling facilities, installed prior to January 1, 2010, to fulfill the requirements of this Section with prior approval of Commission staff.~~

Note: Authority cited: Sections 71201.7, 71205.3, ~~and 71206,~~ and 71207, Public Resources Code. Reference: Sections 71201.7, 71205.3, ~~and 71206,~~ and 71207, Public Resources Code.

**Section 2296.3 Protocols for the Analysis of Ballast Water Samples Collected to Assess Compliance with California's Performance Standards for the Discharge of Ballast Water.**

Commission staff, or designated representatives, may use any combination of the following methods for the analysis of collected ballast water samples to assess compliance with sections 2294 or 2295.

(a) Analysis of collected ballast water samples.

(1) Organisms greater than 50  $\mu\text{m}$  in minimum dimension

(A) Holding Time – Sample processing should be completed no more than six hours after collection to prevent sampling-induced organism mortality.

(B) Concentration – The sample shall be concentrated using a 35  $\mu\text{m}$  (no greater than 50  $\mu\text{m}$  in diagonal dimension) mesh sieve in a manner that will not harm the organisms.

(C) Sample Analysis – The entire sample shall be counted using a dissecting microscope at a magnification of 10x – 40x. Organisms shall be observed for movement. If an observed organism is not moving, it will be gently touched with a dissecting needle or probe to elicit movement and observed for 10 to 20 seconds for any sign of movement or vital organ activity.

(2) Organisms equal to or less than 50  $\mu\text{m}$  in minimum dimension and equal to or more than 10  $\mu\text{m}$  in minimum dimension.

One or both of the following methods will be used to assess vessel compliance with section 2293.

(A) Fluorescein Diacetate (FDA) Method

(1) Holding Time - Sample processing must be completed no more than six hours after collection.

- (2) Concentration – The collected sample shall be concentrated to 1 L on a sieve with less than or equal to 10 micrometer ( $\mu\text{m}$ ) mesh on the diagonal.
- (3) Sample Analysis – Commission staff will homogenize samples before taking each subsample for analysis. Commission staff will take a 1 milliliter (mL) sub-sample, place it in a microfuge tube and stain with 5 microliters ( $\mu\text{l}$ ) of 1 millimolar (mM) FDA and 10  $\mu\text{l}$  of 250 micromolar ( $\mu\text{M}$ ) 5-chloromethylfluorescein diacetate (CMFDA) for a final concentration of 5 and 2.5  $\mu\text{M}$ , respectively. Stained samples shall be incubated in darkness at room temperature for 10 minutes. The sample shall then be loaded into a Sedgewick Rafter Counting Chamber and examined using epifluorescence microscopy using a Fluorescein Isothiocyanate (FITC) narrow pass filter cube. Samples shall be examined for a maximum of 20 minutes. A photomicrograph should be taken to create a visual record of viable cells. A minimum of 4 subsamples shall be analyzed in this fashion.

(B) Most Probable Number (MPN) method

Sample Analysis shall be conducted using the Golden Bear Facility's SOP GBF-BC-8: Standard Operating Procedures for Most Probable Number (MPN) Determination of Viable Phytoplankton Cells  $\geq 10$  to  $\leq 50$   $\mu\text{m}$ , Chlorophyll-based, hereby incorporated by reference.

(3) Culturable, Aerobic, and Heterotrophic Bacteria (except those listed in Section 2293 (c)(3)).

(A) Holding Time - Sample analysis shall begin no later than six hours after sample collection.

(B) The sample analysis shall be conducted using either of the following methods:

(1) Homogenize the sample to ensure organisms are well mixed before taking each subsample. Prepare a 10-fold dilution

series using sterile Phosphate Buffered Saline or sterile ambient water such that concentrations of the original water are 100, 10<sup>-1</sup>, and 10<sup>-2</sup>. Spread 100 µl of sample onto each of two general-purpose media for culturable aerobic heterotrophic bacteria (media type is dependent on the salinity of the water at the time of collection. For marine water, use 1 marine agar (2216 Marine Agar) and 1 nutrient agar (R2A agar modified with sodium chloride according to the salinity of the sample); for freshwater, use Plate Count Agar and Nutrient broth (plus agar (15 g/L). Use at least 3 replicate plates (100 mm wide) for each media type and dilution. The total number of plates (3 dilutions x 2 agars x 3 replicates) should equal at least 18. Plates should be incubated in the dark at 20 °C and monitored to ensure overgrowth does not occur. Colonies should be monitored and counted after 5 days (or after 3-5 days, if colony overgrowth appears imminent on all plates) and recorded as colony forming units (CFUs) per 100 mL of sample water; or

(2) IDEXX SimPlate for HPC, as specified by manufacturer's recommendations.

(4) *Escherichia coli*

(A) Holding Time - Sample analysis shall begin no later than six hours after sample collection.

(B) Sample Analysis shall be conducted using one of the following methods:

(1) U.S. EPA Method 1603

Incorporate as reference U.S. EPA Method 1603: Escherichia coli (E. coli) in Water by Membrane Filtration Using Modified membrane-Thermotolerant Escherichia coli Agar (Modified mTEC);

(2) The Enzyme Coliform Substrate Test, Standard Method 9223B

Incorporate as reference Standard Method 9223B: The Enzyme Coliform Substrate Test; or

(3) IDEXX Colilert kit (Westbrook, ME).

(5) Intestinal enterococci

(A) Holding Time - Sample analysis shall begin no later than six hours after sample collection.

(B) Samples shall be analyzed using U.S. EPA Method 1600.

Incorporate as reference U.S. EPA Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl- $\beta$ -D-Glucoside Agar (mEI)

(6) Toxigenic *Vibrio cholera* serotypes O1 and O139

(A) Holding Time – Sample analysis shall begin no later than six hours after sample collection.

(B) Sample Analysis - 1 mL, 10 mL, and 100 mL water samples shall each be passed through separate 0.45  $\mu$ m membrane filters, the filters transferred onto Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar plates, and the plates incubated at 35  $\pm$  2°C for 24 hours. Sucrose-positive (yellow) colonies shall be purified, and inoculated with 2.5% yeast extract and nalidixic acid and fixed after incubation overnight. Viable *Vibrio cholerae* O1 and O139 cells are detected using a direct-fluorescent antibody kit (New Horizons Diagnostics, Columbia, MD) for serogroups O1 and O139 using monoclonal antibodies tagged with fluorescein isothiocyanate (FITC) under an epifluorescence microscope. If cells are positive for serogroups O1 or O139, then a DNA colony blot hybridization method should be used to determine whether the corresponding isolates carry the *ctxA* gene.

Incorporate by reference for the detection and analysis of *Vibrio cholerae* samples: “Huq. et al. 2006. Detection, isolation, and identification of *Vibrio cholerae* from the environment.”

(7) Viruses

Due to the lack of effective, scientifically sound protocols, this section does not establish analysis requirements for the compliance assessment of the virus standards.

(b) Chain of Custody

- (1) Written records shall accurately trace the custody of each sample through all phases of the analysis process.
- (2) Every sample shall be accompanied by a sample tag and a chain-of-custody record, completed using indelible ink, that includes all of the following:
  - (1) The name and signature of all persons who collected the sample;
  - (2) The sample identification number;
  - (3) The date and time the sample was collected;
  - (4) The location on the vessel where the sample was collected;
  - (5) The name and signature of all persons who provide transport to the laboratory; and
  - (6) The name and signatures of all persons who handle the sample in the laboratory.
- (3) Samples shall be sealed to protect the sample's integrity at all times after collection and prior to analysis.
- (4) The container used to transport samples shall be sealed or locked to readily detect any evidence of tampering.

(c) Use of Laboratories

- (1) All laboratories contracted for sample analyses of organisms in sections 2296(a)(A)-(B) shall have a Quality Assurance Project Plan (QAPP), approved by Commission staff, that includes, at a minimum, descriptions of:
  - (1) Procedures for measurement and data acquisition, including maintenance and calibration of equipment, data management and



documentation, records management, and disclosure of any non-standard techniques or equipment to be used;

(2) Analysis assessment and oversight; and

(3) Data validation

(2) All laboratories contracted for sample analyses of organisms in sections 2296(a)(C)-(F) shall be certified by the State Water Resource Control Board's Environmental Laboratory Accreditation Program (ELAP).

(3) For each sample analysis, laboratories must provide a report to the Commission that documents all laboratory results and procedures and shall include:

(A) A statement of results of analyses;

(B) Descriptions of analytical methods used; and

(C) Detection limits for each sample result.

(4) The director or person in charge of the laboratory shall supervise all analytical work and shall sign all reports submitted to the Commission.

(5) Samples shall be handled so they follow all chain of custody procedures as detailed in Section 2297.3(b).

Note: Authority cited: Sections 71201.7, 71205.3, 71206, and 71207, Public Resources Code. Reference: Sections 71201.7, 71205.3, 71206, and 71207, Public Resources Code.