US Environmental Protection Agency
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Microbiology Laboratory
Environmental Science Center, Ft. Meade, MD

Standard Operating Procedure for
Use and Maintenance of Spectrophotometers

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Title	Use and Maintenance of Spectrophotometers
Revisions Made	• Minor editorial changes for clarification purposes.

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Scope	This protocol describes the procedure for verifying the performance of spectrophotometers.
Application	These procedures are applicable for determining the absorbance and percent transmittance of microbial suspensions as an indicator of concentration.

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1. Definitions	Additional abbreviations/definitions are provided in the text. 1. Percent transmittance (%T). The ratio of the intensity of the light that has passed through the sample to the intensity of the light when it entered the sample ($T = I_{out} / I_{in}$). The transmittance is displayed as a percentage on an analog scale. 2. Absorbance (Abs), also referred to as optical density (OD). The measure of the quantity of light that a sample neither transmits nor reflects and is proportional to the concentration of a substance in a solution. The absorbance scale is logarithmic. In turbid bacterial cultures, the scatter of light is measured; this scatter is what the spectrophotometer interprets as absorbance. NOTE: To convert between the absorbance and transmittance scales, use the equation: A = -log (%T/100). Values for both A and %T are unitless as both are derived from a ratio between the incident light and the transmitted light.
2. Health and Safety	1. Follow procedures specified in SOP MB-01, Laboratory Biosafety. The Study Director and/or lead analyst should consult the Safety Data Sheet for specific hazards associated with products. 2. Cap cuvettes containing microbial suspensions prior to removal from the BSC. The use of a cuvette rack for transporting capped cuvettes within the laboratory is recommended. For transporting microbial suspensions in capped cuvettes between laboratories, place the cultures in secondary containment.
3. Personnel Qualifications and Training	Refer to OPP Microbiology Laboratory SOP ADM-04, Personnel Training.
4. Instrument Calibration	Use an ISO 17025 accredited vendor to certify the spectrophotometers on an as-needed basis.
5. Sample Handling and Storage	1. For sample readings, aliquot microbial suspensions into cuvettes in the biological safety cabinet (BSC) and cap prior to removal from the BSC. Cuvettes should remain capped at all times once removed from the BSC. 2. Fill all blank and sample cuvettes at least half-full to a level just above the window shoulder (23 mm). Once filled, cap the cuvette. Avoid touching the cuvettes below the shoulder.

	3. Check that no air bubbles are present in the cuvette; gently tap to remove if necessary. Using a clean, dry, and lint free towel, gently wipe the cuvette sides oriented in the optical path (indicated with an arrow – see 11.2) and firmly position the cuvette in the sample holder. 4. Remove cuvettes containing microbial suspensions from the cell holder once a reading has been taken.
6. Quality Control	1. For quality control purposes, document the required information on the appropriate form(s) (see section 14).
7. Interferences	1. The enclosure, the cell compartment, and all accessories must be kept clean. If it becomes dirty, wipe it clean with a mild soap solution and a soft cloth.
8. Non-conforming Data	1. Management of non-conforming data will be consistent with SOP ADM-07, Non-Conformance Reports. 2. Upon use, if any function does not pass or if any error messages are displayed, refer to section 17, Troubleshooting in the Operating Instructions (see 15.1) and, if necessary, contact the manufacturer for assistance. Record corrective actions on the Systems Check Record Form under "Comments." Remove the spectrophotometer from service if troubleshooting does not resolve the issue.
9. Data Management	1. Archive data consistent with SOP ADM-03, Records and Archives.
10. Cautions	1. Maintain an ambient temperature of 10-35°C for proper instrument operation. Leave at least a 15 cm clearance at the top and on all sides for air circulation. Keep the air vents under the instrument clean and free of materials that might obstruct the air flow. 2. Allow the instrument to warm up for a minimum of 5 minutes before taking any sample readings (for instrument verification, allow the instrument to warm up for at least 1 hour). 3. During system startup and initialization, the analyst must remain present during the entire system check of the instrument and must observe the check marks in order to verify that the instrument has passed its system checks. 4. Do not switch the instrument off and on in rapid succession; always wait at least 5 seconds before switching the instrument on again.

	5. Lenses are located on both sides of the sampling module. Wipe lenses with a soft lint-free and oil-free cloth to clean. Do not use organic solvents such as acetone to clean the lenses.
11. Special Apparatus and Materials	1. Beckman DU Series 730 Life Science Spectrophotometer: wavelength range 190-1100 nm. a. The Beckman DU 730 operates in both the visible and UV range. Use cuvettes made of silica or quartz in the UV region (190-360 nm). Use glass or plastic cuvettes in the visible region (e.g., 650 nm). To accurately measure turbid samples like bacterial cultures, a turbidity cell holder (part number A23623) is installed. 2. Cuvettes. a. Semi-micro plastic disposable cuvettes: 12.5×12.5×45 mm, capacity of 1.5-3 mL, 10 mm light path. A triangular arrow at the top of each cuvette indicates the optical path orientation. b. Optical glass cuvettes. 10 mm light path; used for spectrophotometer standards.
12. Procedure and Analysis	
12.1 In-house Verification of Spectrophotometers	a. Check the spectrophotometer (when in use) on a quarterly basis using spectrophotometer standards (e.g., In-Spec Spectrophotometer standards and background solution) aliquoted into optical glass cuvettes. b. Use four spectrophotometer reference standards: a 0.3, 0.5, and 0.8 absorbance standard, and a 20% transmittance standard. c. Warm the instrument for at least 1 hour prior to verification (refer to 15.3). d. Do not shake, mix, or agitate the standards. Discard any used or unused aliquoted standards. e. Zero the instrument using the background solution to blank the system at 650 nm. f. Wipe both sides of the cuvette before and after putting the background solution in the cuvette.

	g. Exchange the contents in the sample cuvette with one reference standard solution. Be sure to rinse the cuvette several times with the standard. h. Begin with the least concentrated standard solution. Position the cuvette using the same orientation for each measurement. After reading the least concentrated standard solution, exchange the contents of the cuvette with the next standard following the same procedure as in 12.1g. i. Compare the data generated by the instrument with the certified values provided with each standard. The acceptable tolerance for each instrument is as follows: *Tolerance = Uncertainty + Instrument's Photometric Accuracy* Readings must be within the certified value for each standard (rounded to the nearest hundredth) ± the tolerance. j. The photometric accuracy for the instrument is ±0.005 A at 0.0 to 0.5 A, 1% at 0.5 to 2.0 A. k. Clean optical glass cuvettes using alcohol and de-ionized water after use.
12.2 Instrument Self-Check	a. Close the cell compartment and turn on the instrument. b. Each time the instrument is powered up, a series of diagnostic tests is performed automatically to ensure operation of major system components. This procedure, which takes two minutes, checks the system, lamps, wavelength calibration, filter adjustment and voltage. The instrument also contains programs for checking photometric accuracy, photometric noise, stray light, wavelength accuracy, lamp history, and printer check. c. After each portion of the self-check, a check mark will appear next to each diagnostic test. NOTE: Analyst must be present during the instrument's self-check (see section 10.3). d. When the power-up diagnostics are complete, the Main Menu appears. If any function does not pass or if any error messages appear during the self-check, refer to the "Troubleshooting" section of the manual (see ref. 15.1).

	e. Record results of the system's self-check on the appropriate form (see section 14).
12.3 Using the Spectrophotometer	a. From the Main Menu, select **User Programs** on the touch screen, select the desired program (e.g., **Fixed Wavelength**) and touch **Start** to run the program. b. Touch λ to enter the desired wavelength (e.g., 650 nm). c. Before taking measurements, blank the instrument using a cuvette with a "blank" solution, such as the growth medium or other appropriate background liquid. d. Insert the blank cuvette into the cell holder and close the cell compartment. Touch **Blank** to take the blank reading. When done, the instrument enables the **Read** button. e. Remove the blank and insert a sample cuvette into the cell holder and close the cell compartment. Touch **Read** to take the sample reading. f. To toggle between absorbance and percent transmittance readings, touch the **Abs/%Trans** button. g. For multiple sample readings, it is advisable to take a blank reading between samples. h. Refer to the manual (see ref. 15.1) when using other programs.
13. Data Analysis/ Calculations	1. None
14. Forms and Data Sheets	Test Sheets. Test sheets are stored separately from the SOP under the following file names: Spectrophotometer Systems Check Record EQ-04-09_F1.docx Spectrophotometer Calibration Verification EQ-04-09_F2.docx
15. References	1. Operating Instructions: Beckman DU Series 700 Spectrophotometer User's Guide. Beckman Instructions A24014-AB, July 2007. 2. Scanning Procedures for Photometric Standards (2012).