

#### 1.0 PURPOSE

To provide methods for verification that absorbance measurements at a single wavelength made using the Thermo Scientific Evolution 220 Spectrophotometer are traceable to a national standard and that the instrument is performing within specified limits.

<u>NOTE:</u> Users typically employ the term 'calibration', but the appropriate terminology for the process of testing that a spectrophotometer is performing within manufacturer specifications is called, 'performance verification' (P.V.).

#### 2.0 DEFINITIONS

- 2.1 <u>Complete Performance Verification (Complete P.V.)</u> the process of testing the spectrophotometer using a full battery of tests including wavelength and photometric accuracy testing at three or more points to verify the instrument is performing within the tolerances of the published specifications for that instrument.
- 2.2 <u>Short Performance Verification (Short P.V.)</u> the process of testing the wavelength and/or photometric accuracy at one point as an interim verification performed between Complete P.V. of the instrument.
  - 2.2.1 <u>NOTE:</u> Short P.V. test (this is synonymous with terms such as "daily verification" or "daily check") performed at a frequency of 24 hours reduces the risk of measurement instruments giving erroneous results between the typically longer cycle of Complete P.V. tests.

#### 3.0 FREQUENCY

- 3.1 Complete P.V.
  - 3.1.1 90 days ±15 days (assumes daily verification testing / Short P.V.)
    - 3.1.1.1 30 days ±15 days is the suggested frequency <u>if the user is not</u> employing a performing Short P.V. method.
  - 3.1.2 After any of the following events:
    - Spectrophotometer installation;
    - Location change;
    - After instrument servicing from Thermo;
    - Instrument is jarred or dropped;
    - Before and after lamp replacement.

<u>Note:</u> External cleaning, replacing a PC, replacing the power cable, or replacing the USB cable that connects the spectrophotometer to a PC <u>are not</u> events that require any type of P.V. testing.

- 3.2 Short P.V.
  - 3.2.1 Frequency is dictated by user policy (e.g. "every 24 hours at minimum") and must be justified by the user based on risk assessment.

#### 4.0 MATERIALS

- 4.1 GEX Part# P4300 Evolution 220 Spectrophotometer
- 4.2 GEX Part# P4330 Evolution 220 dosimetry holder system baseplate with beam tubes
- 4.3 GEX Part# P4334 Film dosimeter holder (receiver only)
- 4.4 GEX Part# P4310 Mercury Lamp Accessory (Method 1)
- 4.5 GEX Part# P4320 Calibration Verification Carousel (Method 1)
- 4.6 GEX Part# P4220 Spectronic Standards Set 2 (Method 2)
- 4.7 GEX Doc# 100-269 Spectrophotometer Performance Verification Forms



GEX Doc# 100-271

#### **5.0 SETUP & DESCRIPTION OF TESTS**

- 5.1 Refer to *GEX Doc# 100-221, Selecting a Performance Verification Method for the Evolution 220 Spectrophotometer* for a detailed description of the available methods, and a discussion on selecting methods for performance verification to match your needs and requirements for your business.
- 5.2 Once a method(s) is selected, follow only the applicable sections below.

#### 6.0 SHORT P.V. METHOD

- 6.1 Short P.V. is an abbreviated test of wavelength and photometric accuracy that each involves a single reference instead of multiple reference points to bracket the range of use.
- 6.2 Short P.V. is executed in the DoseControl software using the Performance Verification module, and includes the following tests:
  - 6.2.1 Wavelength Accuracy at 541.9nm (±0.8nm) using the internal Xenon Lamp of the Evolution 220 as the reference.
  - 6.2.2 Wavelength Repeatability (3 tests) at 541.9nm using the internal Xenon Lamp of the Evolution 220 as the reference, and evaluation of the resulting Standard Deviation (0.5 or less is the specification for the Evolution 220).
  - 6.2.3 Photometric Accuracy using a photometric reference defined by the DoseControl Application Administrator and performed at a wavelength that the Application Administrator configures within the DoseControl software.
- 6.3 For detailed discussion and procedure on configuring and executing Short P.V. ("Daily Check"), refer to Section 6 of *GEX Doc# 100-266, DoseControl Software User Guide.*
- 6.4 Deviation of Short P.V. Method
  - 6.4.1 If a failure occurs, the DoseControl software will prevent the user of the instrument from making dosimeter measurements with DoseControl.
  - 6.4.2 The user will be forced to follow an explicit method in the software to return the instrument to service. For details refer to Section 6.4 of *GEX Doc# 100-266, DoseControl Software User Guide.*
  - 6.4.3 After repeated failure to return the instrument to service used in accordance with the method within the software, refer to Section 10 below for further instruction.

#### 7.0 COMPLETE P.V. - METHOD 1

- Method 1 requires the user to remove the GEX Dosimeter Holder System from the sample compartment of the Evolution 220 spectrophotometer.
- Method 1 involves the completion of almost all available automated testing available with the Thermo Insight software Performance Verification module, and includes the use of the GEX Part# P4310, Mercury Lamp Accessory and the GEX Part# P4320, Calibration Verification Carousel (CVC).
  - 7.1 Test Preparation
    - 7.1.1 Turn off the power to the Evolution 220 Spectrophotometer.
    - 7.1.2 Carefully remove all GEX hardware from the sample compartment of the Evolution 220.
    - 7.1.3 Install and execute P.V. testing using the *Part# P4320, CVC* in accordance with the instructions given in *GEX* Doc #100-158, P4320 Calibration Validation Carousel (CVC) Product Specification and Usage (PSU).
    - 7.1.4 A .pdf file with the results appears on the screen upon completion of the testing. Print, Sign and Date the report.
    - 7.1.5 Install and execute P.V. testing using the *GEX Part# P4310, Mercury Lamp* in accordance with the instructions given in *GEX Doc #100-157, Mercury Lamp Accessory Product Specification and Usage (PSU)*



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- 7.1.6 The Mercury Lamp P.V. test results appear on the screen, in a PDF file, upon completion of the testing. Print, Sign and Date the report.
- 7.1.7 Have a reviewer sign both reports.
- 7.1.8 For deviations (failure) of any test refer to section 10 below.

#### 8.0 COMPLETE P.V. - METHOD 2

- Method 2 allows the user to keep the GEX Dosimeter Holder system installed at all times.
- Method 2 includes two parts executed separately:
  - Part 1: Manual execution of photometric accuracy testing using the GEX Part# P4220, Spectronic Standards Set 2.
  - Part 2: Using Thermo Insight with the *GEX Part# P4310, Mercury Lamp Accessory* for wavelength testing.

#### Part 1 – Photometric Accuracy Testing using Spectronic Standards Set 2

- 8.1 <u>Method 2: Part 1 Preparation</u>
  - 8.1.1 The objective of this testing is to determine if the measured photometric and wavelength results on the user's Evolution 220 lie within an acceptable range.
  - 8.1.2 The acceptable range is derived by adding/subtracting (±) the summation of the instrument specification, and the reference standard's uncertainty to the certified value for a given reference standard.
  - 8.1.3 First, the user needs to enter pertinent information from the calibration certificate for the Spectronic Standards into *GEX Doc# 100-269(b)*, *Evolution 220 Performance Verification Form Method 2*.
    - 8.1.3.1 The form calculates the upper and lower limits for each test by calculating the allowable variability from the sum of the uncertainty of each standard, plus the instrument specification for each test. The specifications and summation method are given in the Thermo Scientific Spectronic Standards Set 2 User Guide
    - 8.1.3.2 <u>Note:</u> If preferred, this information can be entered in advance by a senior person, and the form protected so that a technician can simply enter measurement results later. The acceptance criteria is the same for any Evolution 220, so only one form is required to be pre-filled for each Spectronic Standards Set 2 on site. The form should be reviewed and updated each time the standards set is recertified.
  - 8.1.4 First, complete all of the information in cells D2 through D8.

Relevant					
Performance Specifica-	Spectrophotometer	Photometric Accuracy	Photometric Repeatability	Wavelength Accuracy	Stray Light
tions for Thermo	Evolution* 201/220/260Bio	1A: ±0.006 A 2A: ±0.010 A Measured at 440 nm	±0.0002 A	±0.8 nm	≤0.05%T (220nm)
Scientific Spectropho- tometers	Evolution* 300	1A: ±0.004 A 2A: ±0.004 A 3A: ±0.006 A	±0.0025 A	±0.30 nm	<0.0065%T (220nm)

To establish pass/fail criteria for testing spectrophotometers, add the uncertainty printed on the calibration report for the standard to the specification for the instrument shown in the table above.

#### FIGURE 1: Excerpts taken from the Thermo Scientific "Spectronic Standards 2 Kit" information document

8.1.5 Next, configure the spreadsheet with information from the Calibration Certificate for Photometric Accuracy.



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- 8.1.5.1 There are nominal 50%T, 30%T, 10%T, and 3%T Standards for Photometric Accuracy testing. Each standard is labeled with a serial number ID that is referenced on the Calibration Certificate.
- 8.1.5.2 Each standard is certified at multiple wavelengths. This procedures tests at 465nm (at the bottom) and 590nm (at the top) to bracket the B3 wavelength of measurement of 552nm. If measuring other dosimeters, adjust this procedure as necessary using the values at other certified wavelengths.
- 8.1.5.3 Enter the ID, Certified Value, and Uncertainty of each of these standards at 465nm (from the standard's Calibration Certificate) into the specified spreadsheet cells according to the table below:

Standard Type	Standard ID	Certified Value	Uncertainty
50%T	F5	G5	H5
30%T	F6	G6	H6
10%T	F7	G7	H7
3%T	F8	G8	H8

8.1.6 When completed, the information should look like this example below:

Photometric Performance at 465nm (values are in absorbance units)							
Standard ID	Certified Value	Uncertainty	Evo220 Spec	Lower Limit	Upper Limit	Value As Found	Pass/Fail
SA0799-1	0.2889	0.0026	0.0060	0.2803	0.2975		FAIL
SA0799-2	0.4765	0.0026	0.0060	0.4679	0.4851		FAIL
SA0799-3	1.0334	0.0031	0.0060	1.0243	1.0425		FAIL
SA0799-4	1.5356	0.0070	0.0060	1.5226	1.5486		FAIL

8.1.7 Enter the ID, Certified Value, and Uncertainty of each of these standards at 590nm (from the standard's Calibration Certificate) into the specified spreadsheet cells according to the table below:

Standard Type	Standard ID	Certified Value	Uncertainty
50%T	F11	G11	H11
30%T	F12	G12	H12
10%T	F13	G13	H13
3%T	F14	G14	H14

8.1.8 When completed, the information should look like this example below:

Photometric Performance at 590nm (values are in absorbance units)							
Standard ID	Certified Value	Uncertainty	Evo220 Spec	Lower Limit	Upper Limit	Value As Found	Pass/Fail
SA0799-1	0.3004	0.0026	0.0060	0.2918	0.3090		FAIL
SA0799-2	0.5017	0.0026	0.0060	0.4931	0.5103		FAIL
SA0799-3	1.0968	0.0031	0.0060	1.0877	1.1059		FAIL
SA0799-4	1.6306	0.0070	0.0060	1.6176	1.6436		FAIL

8.1.9 The preparation of the form for recording the results is complete.

#### 8.2 Method 2: Part 1 Execution

- 8.2.1 Before beginning any testing ensure the following:
  - 8.2.1.1 The Evolution 220 has been powered on with no dosimeter holder or sample in the sample compartment.
  - 8.2.1.2 The sample compartment front panel is attached, and the lid is closed.

<u>NOTE:</u> The sample compartment lid must be closed for each measurement after inserting the sample during this procedure because the acceptance criteria is derived from specifications established by Thermo with the lid closed.

8.2.2 Insert the GEX Part# P4334 WINdose dosimeter holder <u>receiver</u> into the baseplate and ensure that it is fully and correctly seated. See Figure 2 below.





#### FIGURE 2: P4334 WINdose Dosimeter holder receiver installed in baseplate

- 8.2.3 Open form GEX Doc# 100-269(b), Evolution 220 Performance Verification Form Method 2 in MS Excel.
- 8.2.4 Verify the instrument status in the bottom left has the green check mark.
- 8.2.5 Select the Fixed menu button to create a measurement session using a single (fixed) wavelength setting.



FIGURE 3: Thermo Insight 2 menu screen

- 8.2.6 Verify the P4334 dosimeter holder receiver is empty and close the sample compartment lid.
- 8.2.7 Select the "Settings" button on the lower left of the screen and then select the instrument tab.
- 8.2.8 Enter the wavelength value of 465.0 nm. Ensure the other settings match Figure 4 below.



FIGURE 4

8.2.9 Select the "Measure Fixed" button on the lower-left of the screen. Press the "Zero" button on the top-left.



8.2.10 The instrument makes a few sounds. When the instrument status on the bottom-left again has the green check mark, the instrument is ready.

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Instrument Status 🧶		Mike Pageau:



- 8.2.11 Carefully remove the Standard ID XXXXX-1 labeled 50% from the Spectronic Standards case
- 8.2.12 Insert it into the Evolution 220. <u>The glass filters are designed to fit into the holder in only one direction; with the glass facing to the right.</u>



- 8.2.13 Close the lid of the sample compartment.
- 8.2.14 Press the "Measure" button on the upper-left of the screen.



FIGURE 8



#### 8.2.15 The software confirms the sample ID with the user.

Confirm	n sample list		
Num	ber of samples: 1		Load Samples
			Save Samples
	Sample ID	Description	
▶ 1	Sample1		
C	ontinue		Cancel

#### FIGURE 9

#### 8.2.16 Type an informative ID value to identify the measurement later. Select continue.

Confirm sample list		
Number of samples: 1	Load Samples Save Samples	
Sample ID 	Description	
	a Fixed − □ File Help	×
Continue	Image: Stop         Frint         650 mm         0.291 Abs           Image: Overlay data         0.6         50% T @ 465nm         50% T @ 465nm           Advanced calculations         0.4         0.4         50% T @ 465nm           Image: Overlay data         0.4         0.4         0.4           Image: Overlay data         0.4         0.4         0.2           Image: Overlay data         0.4         0.2         0.2	
- - -	-0.2         -0.2         -464         466         468           Wavelength (nm)         Wavelength (nm)         Wavelength (nm)         Wavelength (nm)           Settings         # Sample ID         User Name         Date and Time         465nm (Abs)           Reports         50% T @ 465nm Mike Pageau         5/22/2019 12:50 PM         0.291	
	Instrument Status 🦛 Mike Page	eau:

FIGURES 10 and 11

- 8.2.17 Repeat steps 6.11.8 through 6.11.13 for the standards XXXXXX-2 (30%T), XXXXXX (10%T), and XXXXXX-4 (3%T).
- 8.2.18 The list of measurements will build. When complete with all four measurements, transcribe the absorbance values in cells L4 through L7 of *GEX Doc# 100-269, Spectrophotometer Performance Verification Form* (the 'Test Form').
- 8.2.19 If desired, print the report of the raw data to attach with the Test Form (see images below for instruction).





- 8.2.20 Repeat all the steps in section 6.11 with a 590.0 nm wavelength setting and record the results in L10 through L13 of the Test Form.
- 8.3 Method 2: Part 1 Review and Approval
  - 8.3.1 Review all entries in the Test form for formatting completeness.
  - 8.3.2 If any of the values fall outside the limits, the form will report "FAIL". Otherwise, all tests will display "PASS".
  - 8.3.3 Print and sign the form. Obtain the reviewer's signature.
- 8.4 <u>Method 2: Part 2 Wavelength Accuracy Testing using Mercury Lamp</u>
  - 8.4.1 Install the Mercury Lamp in accordance with the instructions given in *GEX Doc* #100-157, P4310 Mercury Lamp Accessory Product Specification and Usage (PSU).
  - 8.4.2 A .pdf file with the results appears on the screen upon completion of the testing. Print, sign and date the reports for both the CVC and Mercury Lamp verification tests.
- 8.5 <u>Method 2: Completion</u>
  - 8.5.1 Have a reviewer sign and date the results.
  - 8.5.2 For deviations (failure) of any test refer to Section 10 below.

#### 9.0 COMPLETE P.V. - METHOD 3

- Method 3 allows the user to keep the GEX Dosimeter Holder system installed at all times.
- Method 3 is completely manual procedure for photometric and wavelength accuracy testing using only the GEX Part# P4220, Spectronic Standards Set 2.
  - 9.1 For method 3 use GEX Doc #100-269(c), Evolution 220 Performance Verification Form Method 3.



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- 9.2 First, execute the same procedure as Method 2: Part 1 (sections 8.1 through 8.3 above) but add the following steps between 8.2 and 8.3 and use the form "C" form noted above.
- 9.3 <u>Wavelength Accuracy Testing using Spectronic Standards Set 2</u>
  - 9.3.1 Test Preparation
    - 9.3.1.1 Enter the ID, Certified Value and Uncertainty of the wavelength standard at the nominal 525nm wavelength (from the standard's Calibration Certificate) into the cells F20:F22.
    - 9.3.1.2 Enter the ID, Certified Value and Uncertainty of the wavelength standard at the nominal 782nm wavelength (from the standard's Calibration Certificate) into the cells K20:K22.
  - 9.3.2 Open the Thermo Insight software and select the "Scan" button.

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tome	Rate				
My Data Votions	Live Display	Performance Verification			
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#### FIGURE 14

- 9.3.3 Select the "Settings" button on the lower left of the screen (see image below).
- 9.3.4 Select the "Peak Pick" tab and use the exact settings shown in the image below.

📸 Scan	-		×
File Help			
Measure Reset	Measurement Instrument Accessories Samples Peak Pick		
	Peak Pick     ✓     Max number of valleys:     3     €       Sort valleys by:     Depth     ✓       Sensitivity     ●     ✓       ●     ✓     ✓		
Home Measure Scan	50% Wavelength Range ☑ Full 900.00 - 340.00		
Reports	Start:         End:           900.00         ♀         340.00         ♀           Use threshold		
Instrument Status	Auto Threshold:	Mike Pag	eau .

#### FIGURE 15

- 9.3.5 Select the "Measure Scan" button in the lower-left of the screen; the screen will change.
- 9.3.6 Push the "Baseline" button to take a zero-absorbance scan of the empty sample compartment over the range of wavelengths.





#### FIGURE 16

- 9.3.7 Open the sample compartment lid. Insert the Wavelength Accuracy test standard XXXXX-5 with the lambda ( $\lambda$ ) symbol label on the top of the filter.
- 9.3.8 Close the sample compartment lid.
- 9.3.9 Push the "Measure" button to begin the scan.
- 9.3.10 When the scan has completed, the software will identify the values of each valley which are the required results.



#### FIGURE 17

9.3.11 Enter the value of the lowest wavelength valley (in the example above this "522.496 nm") into cell F27 of the Test Form.



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- 9.3.12 Enter the value of the highest wavelength valley (in the example above this "777.765 nm") into cell L27 of the Test Form.
- 9.3.13 Testing is complete. Print the raw data results using the same method as described in 8.2.19.
- 9.3.14 For deviations (failure) of any test refer to section 10 below.

#### 10.0 DEVIATION SHORT OR COMPLETE P.V.

- 10.1 If any P.V. testing described in this document or otherwise fails, re-oot the instrument, and then repeat the P.V. procedure to determine if the failure is repeatable.
- 10.2 If the test passes the second time, then repeat the testing a 3<sup>rd</sup> time to confirm that the instrument is passing. Retain all failed and passing test results together.
- 10.3 If the second test confirms the failing result, the instrument will require service.
  - 10.3.1 First, attempt self-service by calibrating the wavelength accuracy against the Xenon lamp or the Mercury Lamp accessory. See *GEX Doc# 100-156, P4300 Evolution 220 Spectrophotometer Product Specifications and Usage (PSU).*
  - 10.3.2 Then repeat the P.V. testing.
  - 10.3.3 After repeated failure, contact GEX Customer Service at <u>cs@gexcorp.com</u> for assistance or contact Thermo Scientific directly to arrange service for the instrument.

#### 11.0 ASSOCIATED DOCUMENTS

- GEX Doc #100-113, Spectronic Standards Set 2 Product Specifications and Usage (PSU)
- GEX Doc #100-156, Evolution 220 Spectrophotometer Product Specifications and Usage (PSU)
- GEX Doc #100-157, P4310 Mercury Lamp Accessory Product Specification and Usage (PSU)
- GEX Doc #100-158, P4320 Calibration Validation Carousel (CVC) Product Specification and Usage (PSU)
- GEX Doc #100-159, Evolution 220 Dosimeter Holder System Product Specification and Usage (PSU)
- GEX Doc #100-221, Selecting a Performance Verification Method for the Evolution 220 Spectrophotometer
- GEX Doc #100-269, Spectrophotometer Performance Verification Form
- Thermo Scientific Evolution 220 Spectrophotometer User Guide (Performance Verification section)
- Thermo Scientific Mercury Lamp User Guide
- Thermo Scientific Calibration Verification Carousel (CVC) User Guide
- Thermo Scientific Spectronic Standards Set 2 User Guide

#### 12.0 REVISION HISTORY

DATE	CHANGE DESCRIPTION	REVISION
05/30/19	Initial release.	А

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