N114 W19320 Clinton Dr., Unit 5 Germantown, WI 53022, U.S.A.

Sentry 200/300 Series
General Calibration Manual

Purpose and Frequency of Calibration

General calibration of the Sentry 200/300 Instrument is the process of evaluating and adjusting the instrument for general purposes. Proper calibration of Sentry 200/300 Series instruments allows for a safe working environment and valid data for future reference. It provides intra and inter-laboratory consistency of testing results.

What are we evaluating/adjusting?

Calibration provides three important parameters. One is the baseline reading of the fluorescein solution (Low Polarization Standard). When adjusted, this testing/adjustment provides a consistent instrument baseline. Second parameter is the reading of the Medium Polarization Standard. Difference between low and medium polarization standards tells us the condition of optics and the reading span or "sensitivity" of the instrument. If this value is out of range, some of the parts might not be aligned any more or the instrument needs a new photon counter. Third parameter is the consistency of readings. This is done by measuring each standard several times and calculating variability. If the variability is off the prescribed range, the instrument will need a repair.

Some diagnostic tests require separate scaling to assure results consistency between historic data and also inter-laboratory consistency. Separate instructions are provided on how to adjust the scale for each individual test.

When to calibrate an instrument?

The SENTRY® 200/300 needs to be calibrated before its first use (done in the factory), every four to six months, or when there is evidence of a performance problem. If the instrument has not been used for a prolonged period of time and the results are not optimal, contact our technical support at support@ellielab.com. Calibration is done on-site, by the laboratory personnel. If it is necessary to

perform an institutional calibration, due to the regulation, please contact us for information about the available calibration services in your country.

Preparation

Equipment

- Disposable borosilicate test tubes, Size 10x75 mm VWR Cat. No. 47729-568, or Size 12x75 mm, VWR Cat. No. 47729-570, depending on the instrument setup. Test tubes are for one time use;
- 1000 µL pipette;
- Pipette tips;
- Vortex mixer

Materials

- FPCAL KIT Content:
    - Sentry Low Polarization Standard (LOW) (2 ml)

      The Sentry Low Polarization Standard is used to check and adjust the baseline measurement of the instrument. This is a simple fluorescein solution adjusted to give polarization value at around 25 mP on the reference Sentry 200 instrument. It comes lyophilized.

    - Sentry Medium Polarization Standard (MED) (2 ml)

      The Sentry Medium Polarization Standard is used to check instruments measurement range. Most tests have high polarization readings above 190 mP and this standard is verifying instruments sensitivity in this range. It comes lyophilized.

    - 0.01M Phosphate Buffer (PB), pH 7.2-7.4 (8 ml)

Environmental Conditions

All testing is performed in standard laboratory conditions at room temperature 18-25˚C. Record temperature and humidity as instructed on the Sentry Calibration Sheet.

Warnings

- Use clean glass test tubes for each standard to avoid contamination;
- Do not use scratched or defective glass test tubes;
- Do not handle the lower portion of the glass test tube. Fingerprints can distort the FP value;
- Avoid practices that may contaminate reagents;
- Do not open the instrument flip lid while the instrument is performing measurement.

Preliminary Steps

Reconstitute standards by adding 2 ml of 0.01M PB pH 7.2-7.4 (included in the kit) into each standards vial. Close caps and vortex for 30 seconds, then incubate for 30 minutes at room temperature to reconstitute. Vortex for 3-5 seconds again after incubation.

Transfer 1 ml of each reconstituted standard to 10x75 or 12x75 mm tube. Add 1 ml of 0.01M PB into a third tube, to serve as a blank. Label each tube. Keep tubes away from sunlight or excessive laboratory light. Remaining standards can be used during a one month period. Keep them refrigerated.

Calibration Procedure

Calibration Using SentryTools™ with Microsoft® Excel® Software

SentryTools™ software comes with a template for running calibration of Sentry 200/300 instruments. Some older versions of the software do not have the template included. Please contact our tech support at support@ellielab.com.

1. Insert the tube with 0.01M PB in the Sentry tube shaft;
2. Close the flip lid;
3. Mark 10 Rows in the PB II column of the Low Polarization template;
4. Click on the 'Run' icon or simply press F3 on the keyboard and the reading will start;
5. After finishing the readings, the values will be displayed on the Excel file;
6. Remove the tube with 0.01M PB from the Sentry tube shaft and insert the tube with Low Polarization Standard.
7. Close the Chamber.
8. Mark 10 Rows in the Standard II column of the Low Polarization template.
9. Click on the 'Run' icon or simply press F3 on the keyboard and the reading will start.
10. Please DO NOT open the Sentry Chamber while the instrument is performing measurement.
11. After finishing the readings, the mP values for Low polarization standard will be displayed in the Low Polarization template.
12. If the mP values for Low polarization standard are out of range (25±3 mP), change the G factor in the G factor cell. Every one-thousandth of a point change (0.001) in the G- Factor value inversely adjusts the instruments mP values by ca. 0.5 mP.
13. When the G factor is adjusted, press enter and the value will be implemented in all mP formulas in Low, Medium Polarization Template and Brucella FPA template.
14. Repeat the procedure from 1 to 9 for the Medium polarization standard in the Medium Polarization template.
15. After finishing the readings, the mP values for Medium polarization standard will be displayed in the Medium Polarization template.

Results Interpretation

Results for Sentry Low Polarization Standard:

- Average value of the readings for Low Polarization Standard should be 25±3 mP units. If not, change the G-factor in the Excel® sheet. Do not change the G-Factor on the instrument.

- Standard deviation for the last five readings should be ≤1.2. If it is higher, contact our tech support.

Results for Sentry Medium Polarization Standard:

- Average value of the readings for Sentry Medium Polarization standard after adjustment of G-factor with Low Polarization Standard should be between 190 and 270 mP. If it is not, contact our tech support. Lower mP means that the instrument is losing alignment, or some components are aging. Higher mP than 270 in the current generation of instruments is unlikely.
- Standard deviation of the readings for Sentry Medium Polarization Standard should be ≤ 1.2. If not, contact our tech support.

What is the G-Factor

G-Factor is a formula adjustment factor, commonly used in fluorescence polarization.

General formula for FP is (V-gH)/(V+gH) * 1000

Where:

- V is the “Vertical” or “Parallel” fluorescence intensity
- H is “Horizontal” of “Perpendicular” fluorescence intensity and
- “g” is the G-Factor.

The G-Factor changes the mP of the reading to adjust for different temperatures or instrument factor, in different labs. In short it allows for consistent results. The G-Factor does not influence the test result, as the entire range of the instrument is changing as the G-Factor is changed.

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