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# Better safe than sorry: Verification of spectrophotometers for accurate and reliable measuring results

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## Executive Summary

As a rule, high-quality spectrophotometers will produce accurate and precise measuring results; but is this still the case after longer periods of use? Environmental influences, wear and tear, damage and dirt may impair instruments and lead to incorrect results. Regular checks can identify possible impairments and thus avoid unnecessary repetition of work and associated costs. Proper performance verification of spectrophotometers is carried out using certified and traceable reference materials, typically filter sets. It can be performed by the user or, alternatively, by an appropriately qualified service provider.


## Introduction

Spectrophotometers are used to measure the absorbance of samples at specific wavelengths. A beam of light passes through the sample, and the resulting reduction in light intensity is measured by a detector. In the field of chemical/biochemical and clinical analytics this method is primarily used to determine concentrations of samples in solution. This allows quality control of prior steps, as well as the use of defined amounts of sample in subsequent applications. Many laboratories are subject to regulations (e.g. Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP) or ISO 9001) or they operate in accordance with their own standard operating procedures (SOPs). These guidelines mainly serve the traceability of products and processes. This includes regular verification of equipment employed in measurement and testing procedures, which includes spectrophotometers.

Even laboratories which do not work in accordance within these regulations have good reasons to have their spectrophotometers tested. The instruments are exposed to environmental influences, and parts are subject to wear and tear. In addition, they may have been damaged, e.g. during transport, or dirt may have entered during everyday use. Measurement errors can impair downstream applications and may lead to non-reproducible or false results. Subsequent repetitions of measurements and applications can cause considerable expenditure in the form of additional work and materials used. Proper verifications help avoid these consequences by recognizing and rectifying sources of error.

## Verification of spectrophotometers

If not prescribed by regulatory provisions, the nature of the verification, its scope, and the interval between verification may be determined by the user. In the case of a spectrophotometer, regular checks of wavelength accuracy, as well as photometric accuracy, are of particular importance. Further verifiable parameters include resolution and stray light.

A proper verification relies on certified, traceable reference materials. Traceable reference materials can be traced back through regular comparative measurements to a standard at a metrological institute via a continuous, uninterrupted chain. Reference materials customary for checking photometers include glass and liquid filters, which can be used to test the photometric properties of the instrument. Certain commercial suppliers, for example Hellma® Analytics, offer DAkkS (Deutsche Akkreditierungsstelle GmbH - German Accreditation Body) certified testing materials. Certification of these materials is performed in a calibration laboratory accredited to DIN EN ISO 17025 and certified by a DAkkS calibration certificate. Within the scope of monitoring of testing materials, reference materials are also subject to regular testing and re-certification.

During verification of a spectrophotometer, the measurement values of an instrument are compared to the nominal value of the reference standard, and deviations are noted and documented. The deviations must remain within defined limits which are based on the nominal value of the reference standard, but which also take into consideration the technical data of the instrument. If deviations fall outside the defined limits, an adjustment of the instrument is required. Calibration may be performed manually, or it may run in an automated manner within the instrument's software. Control charts constitute a helpful tool to assist the manual procedure; they largely consist of a template within a spreadsheet software such as Excel®. Using this approach, analysis occurs automatically after entering the measured values. This further enables display of results from several calibrations in their correct chronological sequence, which simplifies subsequent monitoring of precision and accuracy.

It is possible for users to carry out verifications of their photometers themselves if the respective certified reference materials are available, as well as having verifications performed by a qualified service company (e.g. the manufacturer's service technician).

A very important prerequisite for accurate sample measurement, as well as for testing the instrument, is regular cleaning. The cuvette shaft is especially vulnerable to contamination by sample material, which should be removed per manufacturer's instructions.

## Solutions by Eppendorf

**Photometric self test**
The Eppendorf BioPhotometer® D30 and the Eppendorf BioSpectrometer feature an integrated self test which can be activated by the user at defined intervals. During the course of the self test the instrument independently tests the functionality of the photometric unit (light source, detector, accuracy and precision of one wavelength in the UV-range). This "small testing routine" provides quick and simple verification. In case the instrument does not pass the self test, the Eppendorf Service should be contacted.

**Reference filter sets**
Eppendorf offers certified UV-/Vis reference filter sets for each type of Eppendorf BioPhotometer and Eppendorf BioSpectrometer® for verification of photometric accuracy and wavelength accuracy (Figure 1). The filters are traceable to NIST® (National Institute of Standards and Technology) and carry a certificate which is valid for two years. The software of Eppendorf instruments has a program for performing this verification. In addition to being displayed on the screen (Figure 2), data can be printed for documentation purposes, or exported as a PDF file. The Userguide No. 10 [1] and the Operating Manual [2] describe this process in detail.

Companies that are obliged to use reference materials which have been certified by an accredited body can test their instruments using, for example, the DAkkS certified filters by Hellma Analytics in combination with Eppendorf Control Charts. Their use is described in Short Protocols [3,4].
If deviations from defined limit values are detected during verification, the Eppendorf Service should be contacted.


**Figure 1:** Eppendorf reference filter set for checking photometric and wavelength accuracy of the Eppendorf BioSpectrometers basic and kinetic.

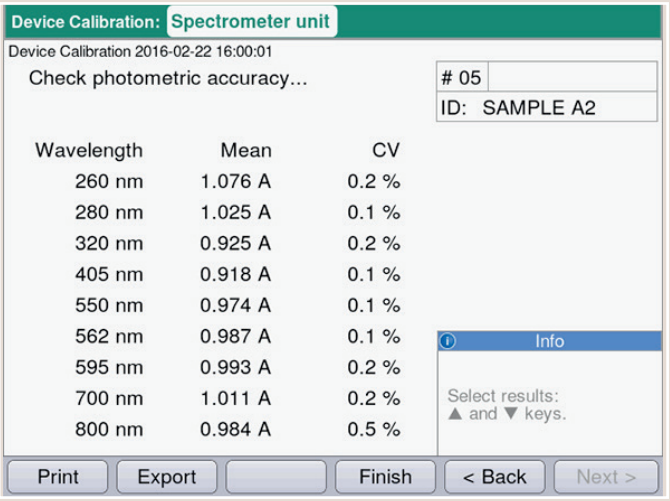


**Figure 2:** Eppendorf BioSpectrometer: Display of data during calibration with the Eppendorf Reference Filter Set.

## Eppendorf Service

Eppendorf offers a variety of performance plans for photometry (www.eppendorf.com → Service and Support → Photometry Service) which include preventive maintenance as well as certification services (Figure 3).

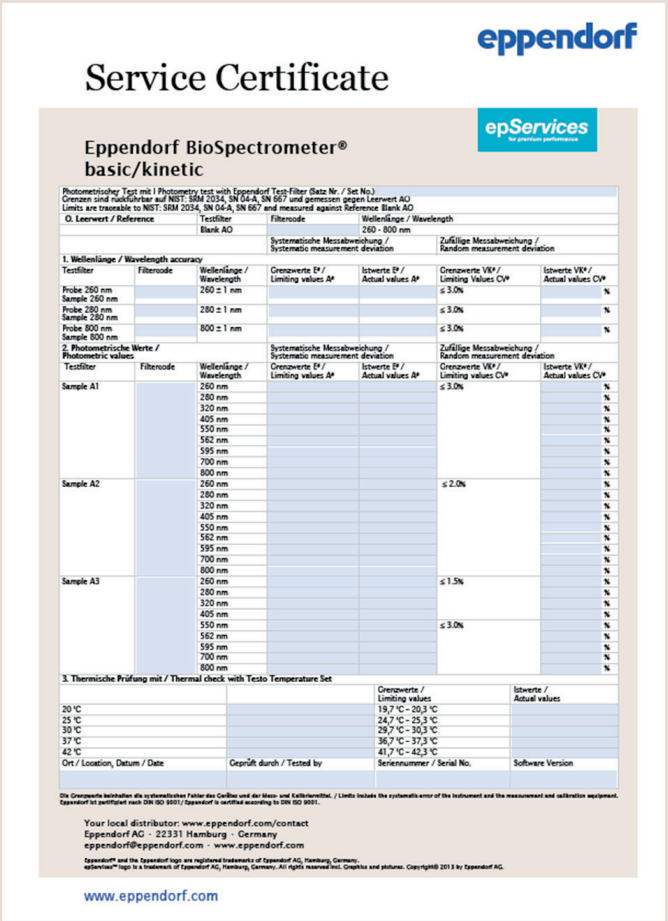


**Figure 3:** Example of a certificate for the Eppendorf BioSpectrometer basic/kinetic, issued after verification by the Eppendorf Service.

Figure 4 shows an overview of the different possible ways of testing an Eppendorf BioPhotometer and Eppendorf BioSpectrometer. The time intervals shown are examples only.

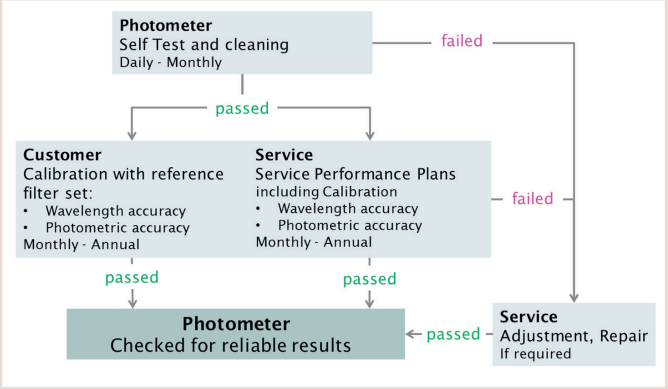


**Figure 4:** Schematic diagram of a possible verification flow for an Eppendorf BioPhotometer and Eppendorf BioSpectrometer

## Conclusion

In order to ensure reliable photometric measurements, photometers should be checked at regular intervals, even if this is not explicitly prescribed by regulations. Quick and simple verifications of basic functions can be carried out by activating the instrument's integrated self test. Proper performance testing is carried out using certified reference materials, either by the users themselves or by a service technician. Additionally, regular inspections and preventive maintenance by Eppendorf Service are recommended. If one of the tests mentioned above is not passed, Eppendorf Service must be contacted.
Finally, regular verifications can save time and money, bearing in mind that in the case of error series of measurements, and possibly even subsequent applications, have to be repeated.

## References

[1] Martin Armbrecht-Ihle, Lars Borrmann. Evaluating the functionality of Eppendorf BioPhotometer® and Eppendorf BioSpectrometer®. Eppendorf Userguide 10; www.eppendorf.com
[2] Operating Manuals Eppendorf BioSpectrometer®, Eppendorf BioPhotometer® D30. www.eppendorf.com
[3] Weiss N. Verification of the Eppendorf BioSpectrometer® with Hellma® Filter F1 using a control chart. Eppendorf Short Protocol 019
[4] Weiss N. Verification of the Eppendorf BioSpectrometer® with Hellma® Filters F2, F3, F4 using a control chart. Eppendorf Short Protocol 020

**Ordering information**

| Description | Order no. international | Order no. North America |
|---|---|---|
| **Eppendorf BioPhotometer® D30**<br>Reference Filter Set | 6133 928.004 | 6133928004 |
| **Eppendorf BioSpectrometer®**<br>Reference Filter Set | 6135 928.001 | 6135928001 |
| **Eppendorf BioSpectrometer® fluorescence**<br>Reference Filter Set | 6137 928.009 | 6137928009 |

## About Eppendorf

Eppendorf is a leading life science company that develops and sells instruments, consumables, and services for liquid-, sample-, and cell handling in laboratories worldwide. Its product range includes pipettes and automated pipetting systems, dispensers, centrifuges, mixers, spectrometers, and DNA amplification equipment as well as ultra-low temperature freezers, fermentors, bioreactors, $CO_2$ incubators, shakers, and cell manipulation systems. Associated consumables like pipette tips, test tubes, microtiter plates, and disposable bioreactors complement the instruments for highest quality workflow solutions. Eppendorf was founded in Hamburg, Germany in 1945 and has more than 3,000 employees worldwide. The company has subsidiaries in 25 countries and is represented in all other markets by distributors.

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