



Bellingham + Stanley

Technical Bulletin

Bulletin No: R022

Title: *Refractometer Calibration Fluids – AG Range*

Introduction

AG Calibration fluids manufactured by Bellingham + Stanley Ltd., A UKAS accredited Calibration Laboratory No. 0834, can be used to check, verify and re-calibrate all makes of critical angle refractometers:

- Manual Hand-held refractometers such as the B+S Eclipse range
- Optical-mechanical instruments such as the B+S Abbe 60 models
- Digital automatic refractometers such as the B+S RFM models

Physicochemical properties and toxicity

B+S AG fluids are aqueous-based fluids that are non-flammable, non-toxic thus making them suitable for use in food testing/QA laboratories. AG fluids, unlike sucrose solutions, are chemically and biologically stable – B+S guarantees a shelf life of 12-months for un-opened samples (see recommended user protocol below).

Because they are aqueous solutions, prolonged exposure to the atmosphere can result in some evaporation or moisture uptake (some of the fluids are mildly humectant and moisture uptake depends upon relative humidity). However, correct handling and storage can prevent this. The fluid will remain stable for the duration of a calibration sequence (several minutes), as evidenced by a steady reading on the instrument.

Refractive Index (Brix)

AG fluids have refractive indices ranging 1.33-1.40. This is equivalent to a °Brix range 0 – 40. Standard products are currently available and are featured in the Calibration Section of the web site.

Temperature Control and Temperature Compensation

AG fluids do not contain sucrose. Therefore, the RI-temperature relationship is different to that of pure sucrose solutions. This means that standard sucrose-based automatic temperature compensation (available in some mechanical and most electronic instruments) is not appropriate for AG fluids.

However, B+S will provide a data sheet, which gives the relationship between RI (Brix) and temperature both graphically and numerically. Therefore, an RI or Brix value can be selected/calculated at the particular instrument operating temperature.

Accuracy/Uncertainty

The reported expanded uncertainty of measurement is stated as the standard uncertainty of measurement in units of °Brix (equivalent to weight % sucrose in water) multiplied by the coverage factor $k=2$, which for a normal distribution corresponds to a coverage probability of approximately 95%. The standard uncertainty of measurement has been determined in accordance with publication EA-4/02.

Protocol for using AG calibration fluids

It is important that the fluids are not exposed to the atmosphere for prolonged periods in order to avoid loss or uptake of moisture. Re-sealing of the bottle and avoidance of any contamination (dirty pipettes) are clearly important.

Many companies now operate a *Quality Management System*, which usually demands strict control in the supply and use of calibration materials. To ensure AG fluids are reliable at the time of use, B+S recommends that companies consider a 'use and discard' policy as part of their calibration protocol. For this reason, B+S supplies AG fluids in 5 ml bottles and multi-bottle packs – ample quantity to accomplish a calibration test. In this way, the calibration fluid will always be supplied from a newly opened bottle, thus ensuring the B+S certified specification and shelf life are valid at the time of use.

If AG fluid is purchased in larger quantity, the user must ensure the bottle is always sealed during storage and contamination is avoided by use of clean (unused) pipettes and by minimising exposure of the liquid to the atmosphere. B+S recommends that a competent person be assigned responsibility for the storage and handling of calibration fluids and fluids are re-assigned a shelf life according to the particular use pattern.

To ensure accurate results when using AG fluids, it is important that the B+S calibration procedure is followed.

Calibration Procedure (*Suitable for RFM300 or RFM800 Series*)

Consult Instrument Manual

1. Ensure the refractometer is in a stable condition at the desired operating temperature.
2. Check the instrument 'zero' with distilled water before proceeding with the AG fluid. Adjust 'zero', as necessary.
3. Clean the prism (and presser or upper prism, if fitted) with suitable fluids – typically water and/or ethanol.
4. Apply a small volume of calibration fluid with a fresh disposable pipette, sufficient to cover the prism to a depth as recommended in the instrument instruction manual. Discard the pipette.
5. Lower the presser or upper prism (if fitted).
6. Allow a short time for the sample to stabilise at the instrument (prism) set temperature. Take successive readings until a steady reading is obtained. A period of about 90 seconds is recommended by B+S to ensure a steady state condition.
7. Adjust the instrument reading using the 'span' software sequence. Ensure the correct value for the calibration fluid is used by reference to the RI (Brix)-Temperature relationship available on the B+S website.
8. Take a further reading to check that the value has not changed during the calibration sequence.
9. Discard the AG Fluid after each calibration routine (may be more than one instrument)

Verification Procedure (*Suitable for Manual Hand Held or Abbe Instruments*)

Consult Instrument Manual

1. Ensure the refractometer is in a stable condition at the desired operating temperature.
2. Check the instrument 'zero' with distilled water before proceeding with the AG fluid. Adjust 'zero', as necessary.

3. Clean the prism (and flap or upper prism, if fitted) with suitable fluids – typically water and/or ethanol.
4. Apply a small volume of calibration fluid with a fresh disposable pipette, sufficient to cover the prism to a depth as recommended in the instrument instruction manual. Discard the pipette.
5. Lower the flap or upper prism (if fitted).
6. Allow a short time for the sample to stabilise at the instrument (prism) set temperature. Take successive readings until a steady reading is obtained. A period of about 90 seconds is recommended by B+S to ensure a steady state condition.
7. Check that the reading is within the stated accuracies of the fluid and the instrument. Ensure the correct value for the calibration fluid is used by reference to the RI (Brix)-Temperature relationship available on the B+S website.
8. Discard the AG Fluid after each verification routine (may be more than one instrument)

Some users verify the calibration by applying an intermediate sample to make sure that the linearity of the instrument is within the instrument specification. Note: It is not required to 'adjust' the instrument during the intermediate verification.

NOTE:

Refractometer Calibration Fluids – AG Range cannot be used on instruments that do not have temperature control or have “fixed” temperature compensation (ICUMSA) such as the RFM110, RFM130, RFM190, RFM710, RFM730, RFM740, Digital Hand Held and Eclipse ATC Hand Held instruments.

New RFM712, RFM732 & RFM742 have special software to allow easy use of AG Fluids for verification and calibration.