# The influence of altitude on the volume result of a piston pipette with air cushion

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## 1 Summary

In this study, it was proved that in the calibration of pipettes with air cushion, the altitude of the measuring equipment has an influence on the measurement result.

- If a pipette is adjusted correctly at 1013 hPa ($V_{20} = V_s$), the same pipette can, at an air pressure of approx. 850 hPa (about 1500 m.a.s.l.), dispense an effective volume which is close to the lower limit of the admissible systematic error according to ISO 8655.
- Piston pipettes with air cushion should be adjusted and calibrated at the intended location of operation.

For comparison measurements between laboratories (for round robin tests, for example), the measured result can be corrected to the same altitude as the pilot laboratory. This study shows the accuracy of the correction factor.

- For comparison measurements, the measured value can be corrected. The exact dead volume and the capillary rise must, however, be known. These two values must be taken into consideration in the measurement uncertainty budget.

## 2 Starting point

A working group, formed by members of the Technical Committee on "Mass" of the DKD (German Calibration Service), studied the influences on the measurement uncertainty during the calibration of pipettes. It was found that insufficient data existed about the influence on the measurement uncertainty related to the geographic location of the measuring station. With increasing height above sea level, the air pressure decreases and has an influence on the measurement.

## 3 Application and purpose of the study

With tests, the behavior of the pipettes at different altitudes was to be investigated by calibrating certain pipettes at different altitudes. To limit the influence of the examiner, of the scales, of the pipette, of the water and of the environment, the same pipettes were always calibrated by the same examiners and using the same measuring equipment, but at different altitudes. The measuring equipment was installed and calibrated at the various locations.

## 4 Implementation

The study was carried out by Spaelti TS-AG. Spaelti-TS AG is a testing laboratory for volumes accredited by SAS (Swiss Accreditation Service). Spaelti-TS AG carried out the calibrations and was responsible for the interpretation of the results.

Two examiners were responsible for the measuring equipment, its calibration and the implementation of the calibrations at the following locations:

Gebenstorf 360 m.a.s.l. mean absolute air pressure 969 hPa
Thusis 740 m.a.s.l. mean absolute air pressure 924 hPa
Samedan 1720 m.a.s.l. mean absolute air pressure 817 hPa
Jungfraujoch 3460 m.a.s.l. mean absolute air pressure 657 hPa

The measurements were carried out between 5 April 2011 and 10 May 2011.

**Measuring equipment:**
The measuring equipment consisted of:

Scales Mettler WXTS205DU with evaporation protection
Thermometer Testo
Hygrometer Elpro ECOLOG TH1
Pressure gauge Vacuubrand DVR 2
The measuring range for this measurement equipment is limited to between 10 µl und 10 ml.

The measuring equipment was set up at every location and the installation was successfully qualified. The basis for the qualification was the standard ISO 8655 and the requirements of the guideline DKD-R 8-1 (during the tests, this guideline was still in draft).

**Specimens:**

| Identification | Piston pipette | Volume | Handling | Type of tip | Max. volume of the tip |
|---|---|---|---|---|---|
| SpaH_01 | Variable | 2 to 20 µl | Manually | Crystal | 20 µl |
| SpaH_02 | Variable | 2 to 20 µl | Manually | Yellow | 200 µl |
| SpaH_06 | Variable | 10 to 100 µl | Manually | Yellow | 200 µl |
| SpaH_11 | Variable | 10 to 100 µl | Manually | Yellow | 200 µl |
| SpaH_12 | Fixed | 100 µl | Manually | Yellow | 200 µl |
| SpaH_03 | Variable | 50 to 1000 µl | Electronically | Blue | 1000 µl |
| SpaH_07 | Variable | 50 to 1000 µl | Electronically | Blue | 1000 µl |
| SpaH_08 | Variable | 50 to 1000 µl | Electronically | Blue | 1000 µl |
| SpaH_05 | Variable | 100 to 1000 µl | Manually | Blue | 1000 µl |
| SpaH_09 | Variable | 100 to 1000 µl | Manually | Blue | 1000 µl |
| SpaH_10 | Variable | 100 to 1000 µl | Manually | Blue | 1000 µl |
| SpaH_04 | Variable | 1 to 10 ml | Manually | | 10 ml |

In each case, the tips recommended by the manufacturer were used in their standard version (no filter/no extra-long or extra-short design).
After use at the various locations, the specimens were tested successfully by the respective manufacturers.

**Tests**
The tests were conducted in accordance with the standard ISO 8655 and the guideline DKD-R 8-1 (during the tests, this guideline was still in draft). In particular, the measurement uncertainties according to DKD-R 8-1 were determined.

# 5 Measurement results

The detailed and complete results gained at the various locations are shown graphically in Annex 1.
Explanation of the diagrams:

| **Abbreviation** | **Explanation** |
|---|---|
| $e_s$ | Systematic measurement error |
| $p_L$ | Air pressure |
| $V_{20}$ | Volume at the reference temperature of 20 °C |
| $V_s$ | Selected volume |
| $V_T$ | Volume of the air cushion (dead volume) |
| $U$ | Expanded measurement uncertainty (*k=2*) |

An explanation of the diagrams is shown in Figure 1.

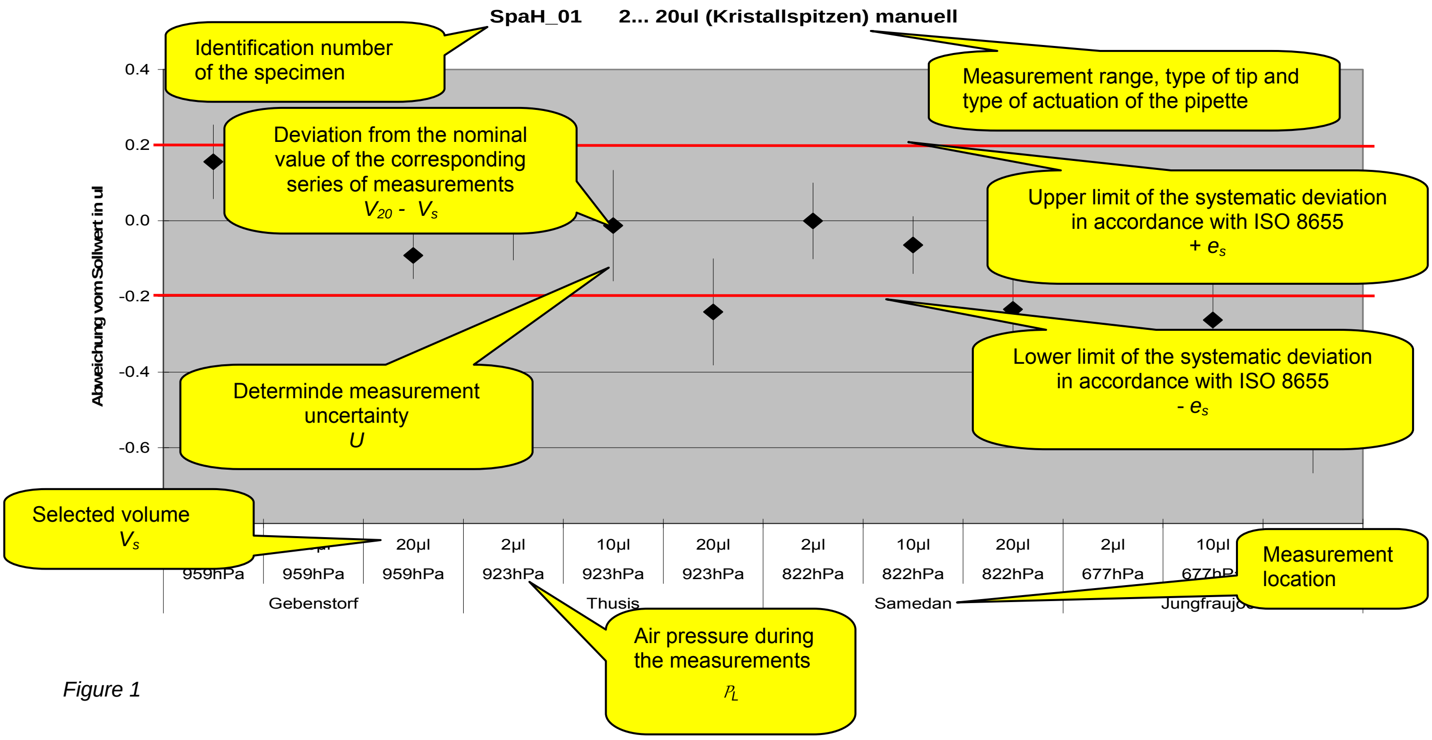



*Figure 1*

**Additional remarks on the measurement results:**
Due to the resolution of the scales, the measuring equipment was not designed for measurements below 10 µl. Nevertheless, measurements were made below 10 µl. For these, a corresponding contribution was included in the measurement uncertainty budget.
At the measuring location *Thusis,* the required humidity of 50 % r.H. was not achieved for technical reasons. The measurements were taken at about 40 % r.H. The measurement uncertainty was adjusted accordingly.

# 6 Analysis of the measurement results

The following observations apply to all pipettes:

6.1 The delivered volume decreases with increasing height above sea level, whereby the decrease in the volume and the decrease in the air pressure behave linearly (Figure 2).

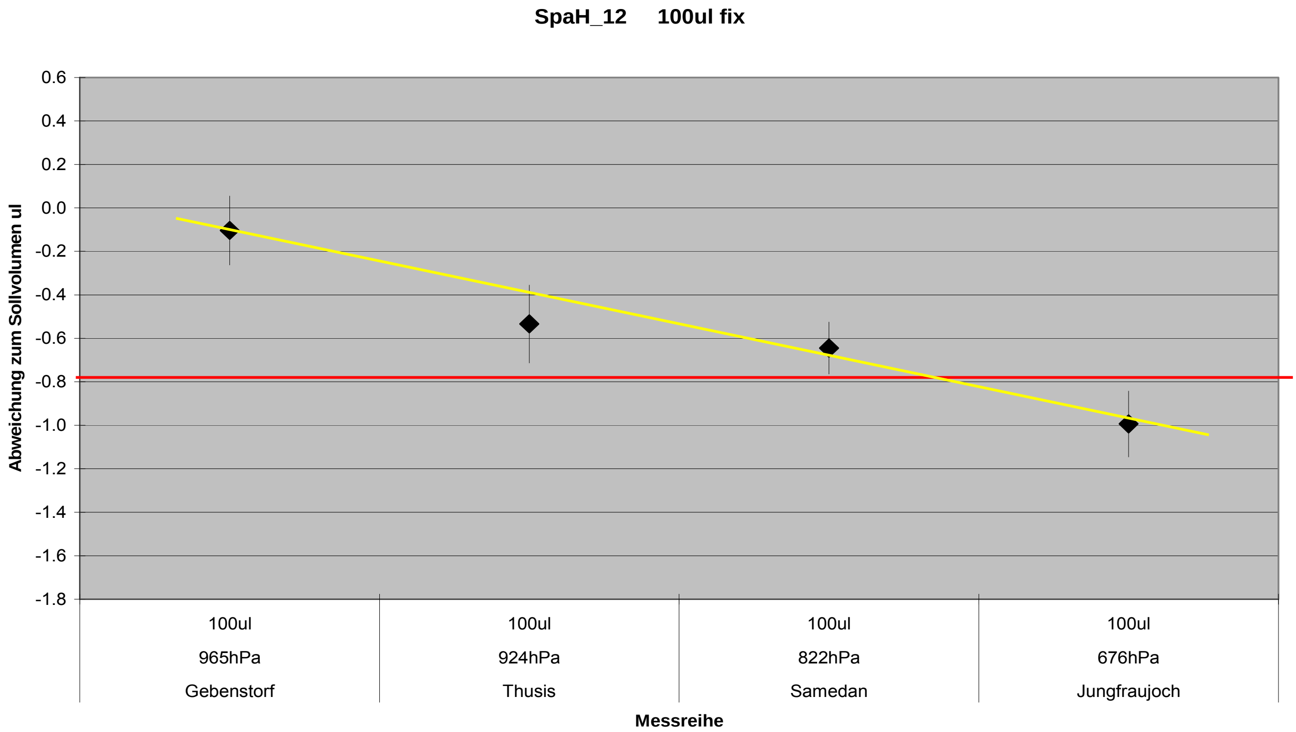



*Figure 2*

6.2 Adjustable pipettes behave differently, depending on the selected volume (Figure 3).

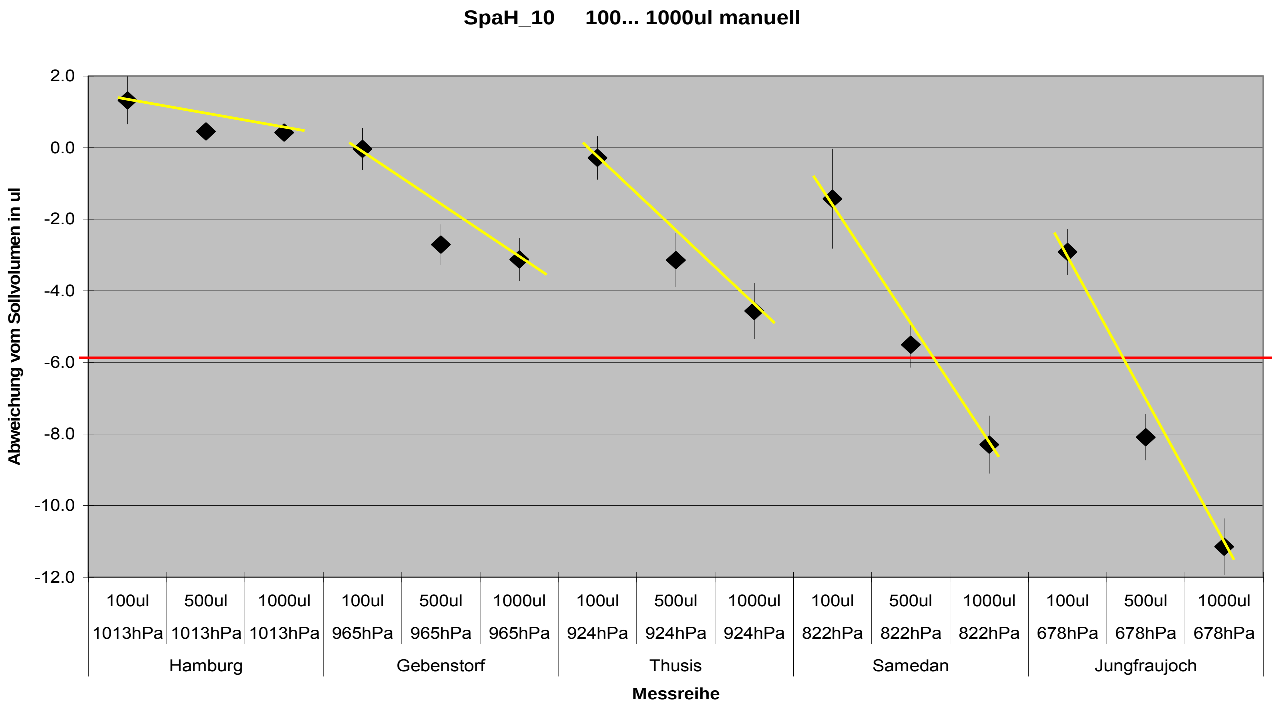



*Figure 3*

6.3 Pipettes with the same nominal volume, but from different manufacturers, have different errors (Figures 4 & 5).

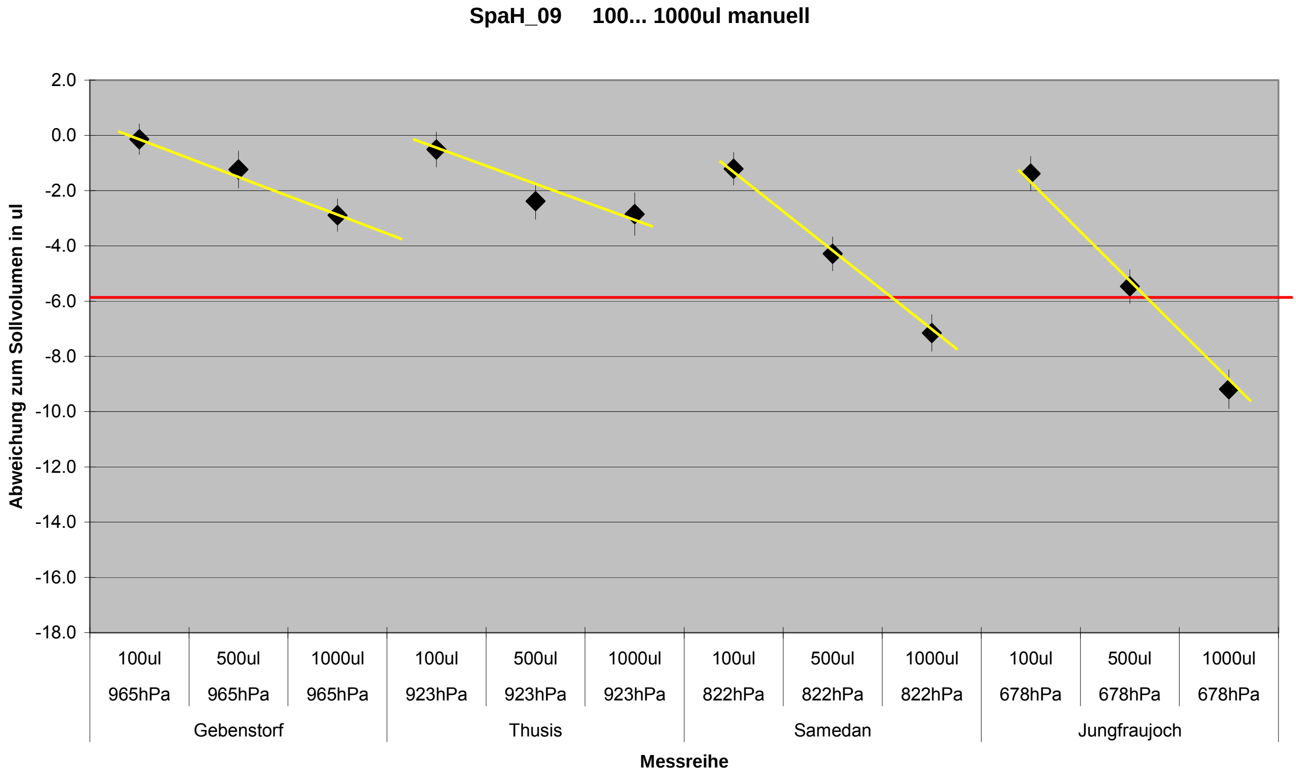



*Figure 4*

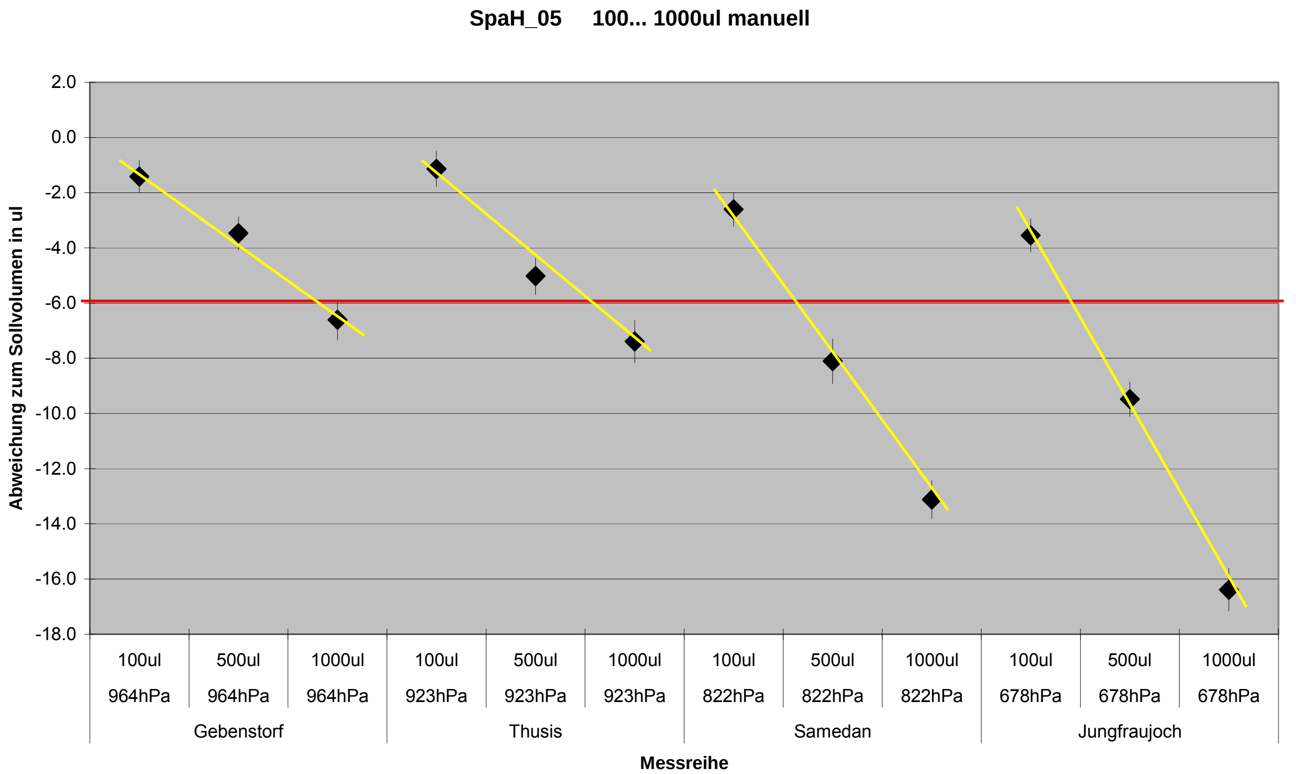


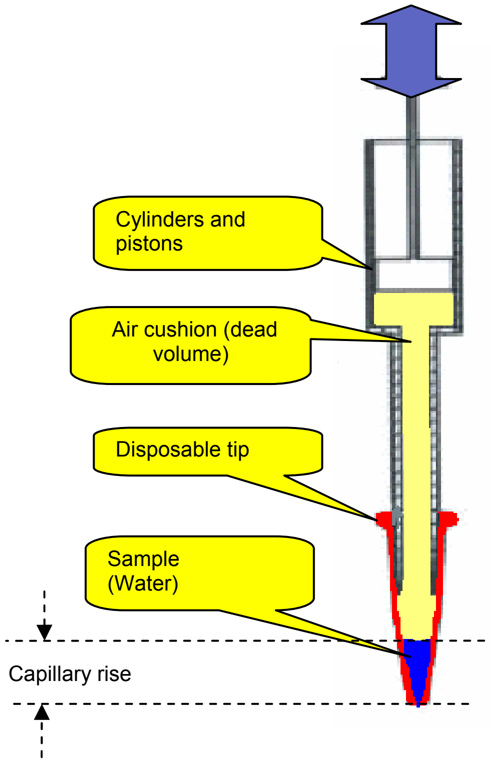

*Figure 5*

## 7 Reason

The volume change at different altitudes is mainly determined by the air cushion in the pipette and by the quantity of the dosed fluid (water).

- **Influence of the air cushion:**
  With decreasing air pressure, the density of the air cushion decreases strongly (a decrease of approx. 10 % per 1000 m difference of altitude).
  The density of the dosed liquid changes only slightly (approximately 0.01 % per 1000 m difference of altitude).
  The size of the air cushion has a significant influence on the delivered volume**.**
- **Influence of the amount of the dosed liquid:**

This influence is visible only if the pipette is of the variable type.
The dosed liquid "hangs" with its weight on the dead volume (air cushion). The greater capillary rise of the dispensed fluid, the worse is the ratio between the density of the air cushion and the density of the dosed liquid.
In Figure 3, this error can be seen from the trend lines. In Hamburg, the trend line is virtually horizontal, at low air pressure (for example at *Jungfraujoch*), the trend line is strongly inclined.




## 8 Correction factor

If the dead volume and the capillary rise of the liquid column in the pipette tip are known, a correction can be calculated by using the formula:

$$\Delta V = -V_T \bullet \rho_W \bullet g \bullet h_W \bullet \left( \frac{1}{\mathrm{P}_{L,X2} - \rho_W \bullet g \bullet h_W} - \frac{1}{\mathrm{P}_{L,X1} - \rho_W \bullet g \bullet h_W} \right)$$

| **Symbol** | **Explanation** |
|---|---|
| $\Delta V$ | Volume change which results during the calibration at a location $X_1$ compared to a location $X_2$ |
| $V_T$ | Volume of the air cushion |
| $g$ | Acceleration of gravity |
| $h_W$ | Capillary rise of the liquid column in the pipette tip |
| $P_L$ | Air density |
| $\rho_W$ | Density of the water used as test liquid |
| $X_1$ | Location 1 (in this document: 0 m.a.s.l. with air pressure 1013.25 hPa) |
| $X_2$ | Location 2 (In this document: the respective point of measurement at different altitudes) |

The capillary rise of the liquid depends on the tip used and on the pipette used. It can be measured easily with calipers.

The dead volume is determined by the design of the pipette and of the tip used. It must be calculated by the designer of the respective pipette. For this study, only insufficient data were available. Therefore, for the following diagrams the following values were used:

| Pipette | Tip | Dead volume | Capillary rise at | Capillary rise at | Capillary rise at |
|---|---|---|---|---|---|
| 2… 20 µl | Crystal (20 µl) | 360 µl | 26 mm / 20 µl | 19 mm / 10 µl | 8 mm / 2 µl |
| 2… 20 µl | Yellow (200 µl) | 320 µl | 15 mm / 20 µl | 10 mm / 10 µl | 5 mm / 2 µl |
| 10…100 µl | Yellow (200 µl) | 410 µl | 29 mm / 100 µl | 21 mm / 50 µl | 29 mm / 10 µl |
| 100 µl Fix | Yellow (200 µl) | 410 µl | 30 mm / 100 µl | | |
| 100… 1000 µl | Blue (1000 µl) | 2700 µl | 50 mm / 1000 µl | 34 mm / 500 µl | 19 mm / 100 µl |
| 1… 10 ml | (10 ml) | 17800 µl | 130 mm / 10 ml | 89 mm / 5 ml | 40 mm / 1 ml |

The diagrams (Figures 6 to 10) show the measured values (lower end of the wide column) and the calculated change of volume (upper end of the wide column). With this correction, the measured values obtained at the various locations can be compared more exactly.

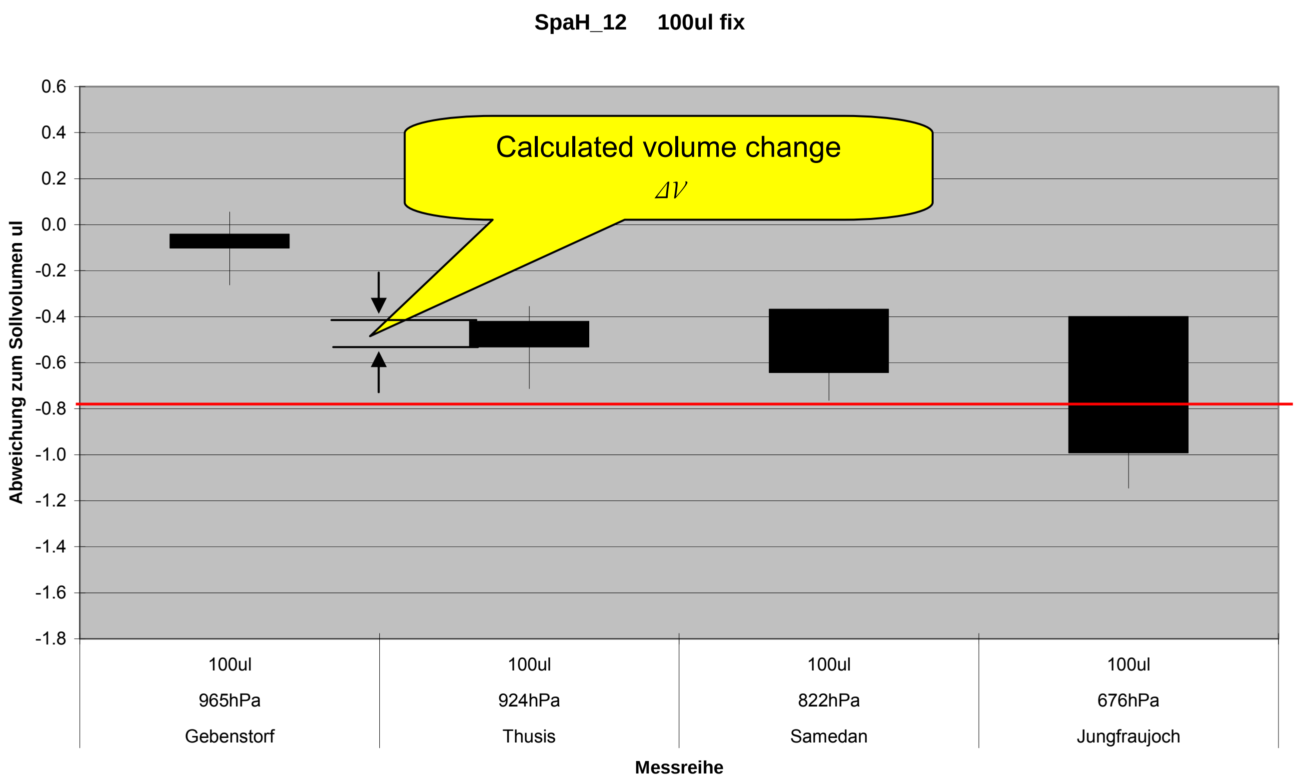



*Figure 6*

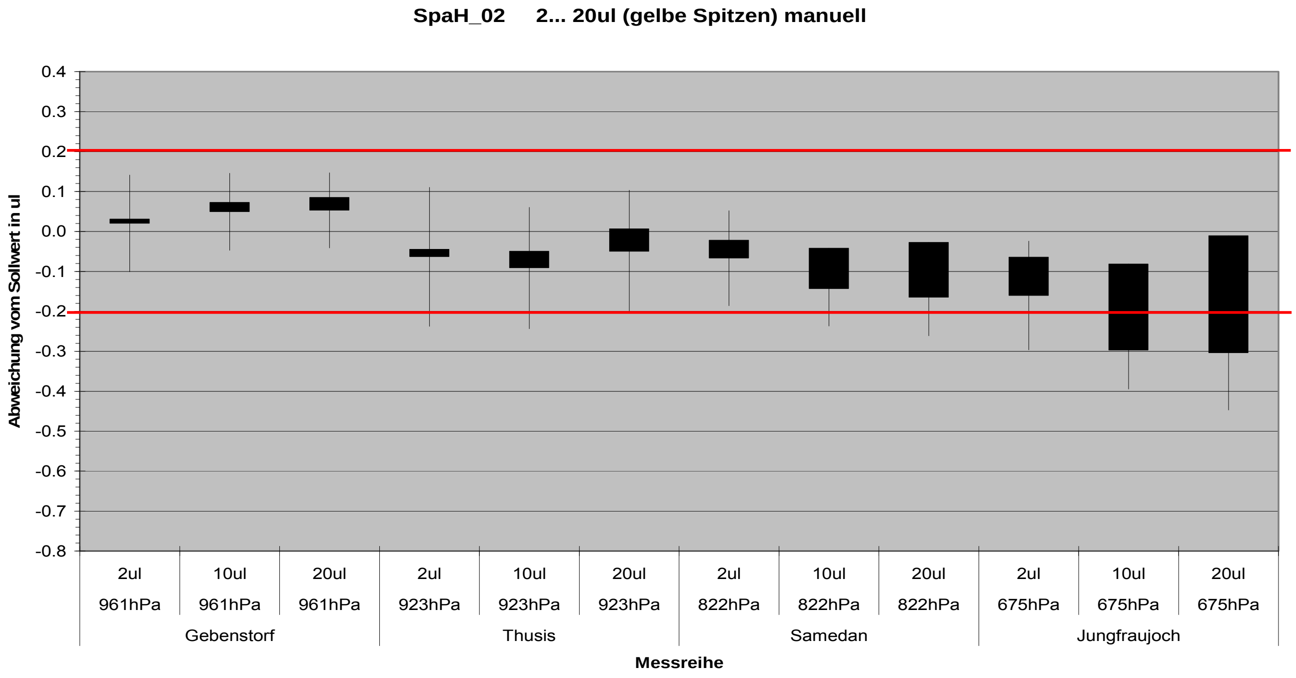



*Figure 7*

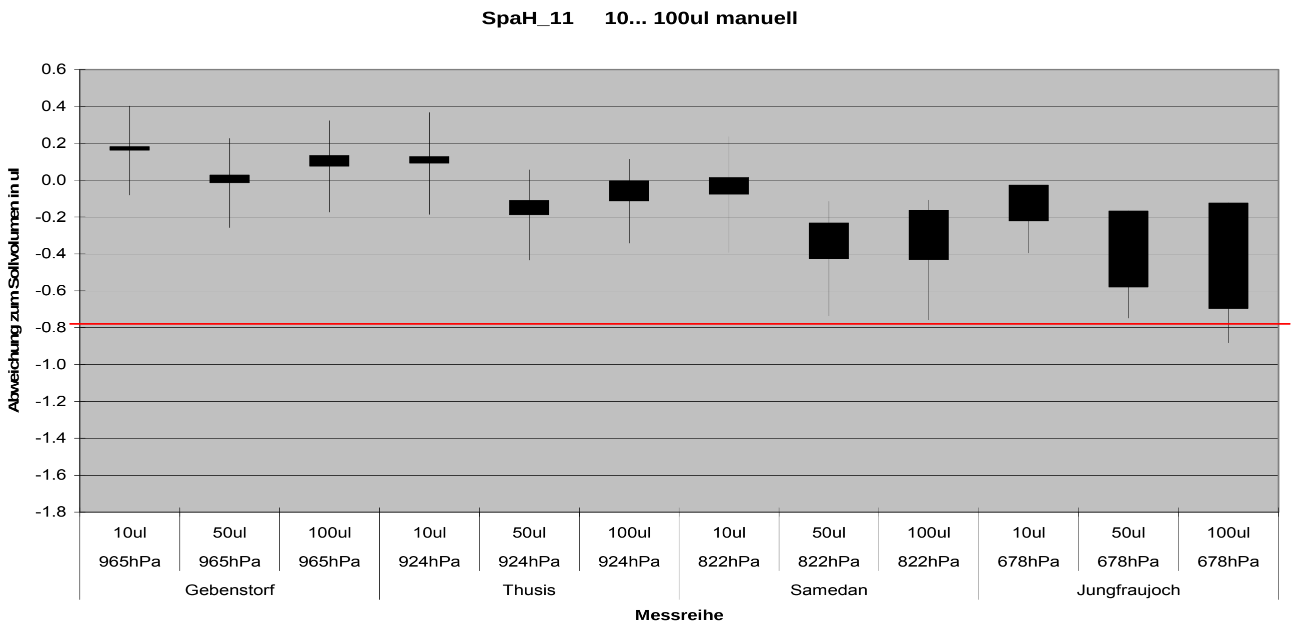



*Figure 8*

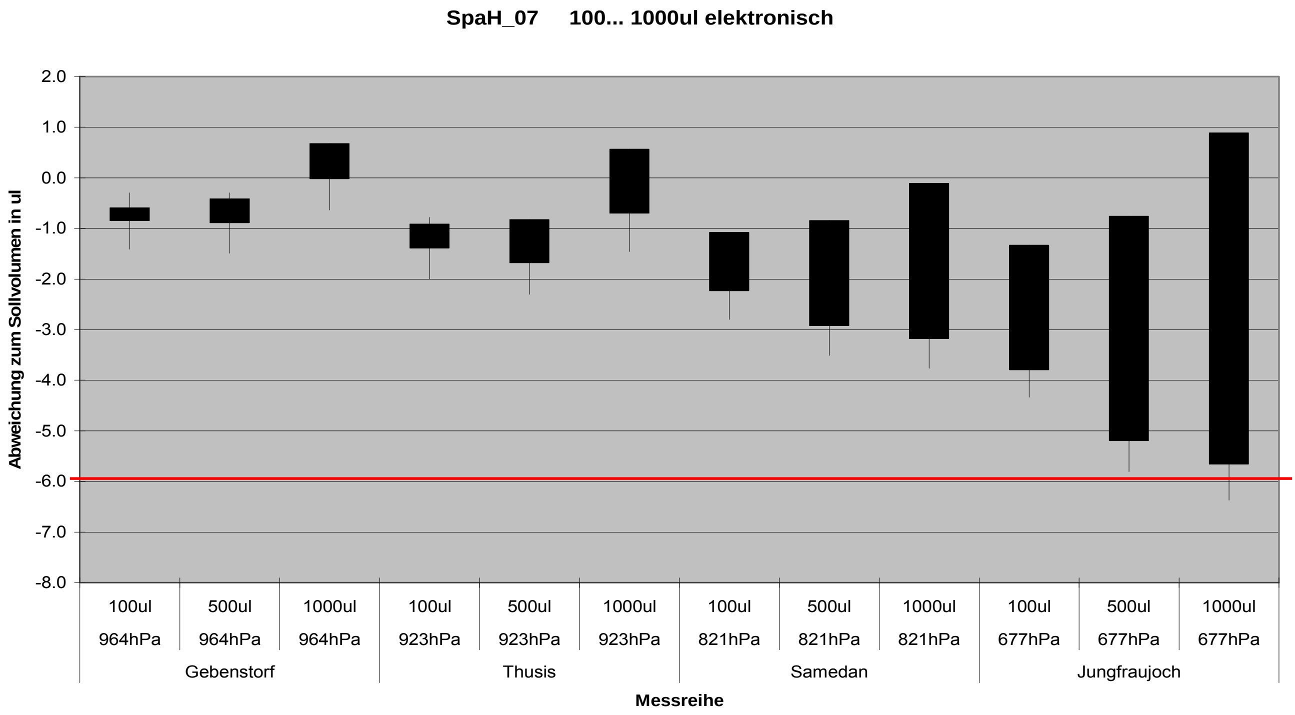



*Figure 9*

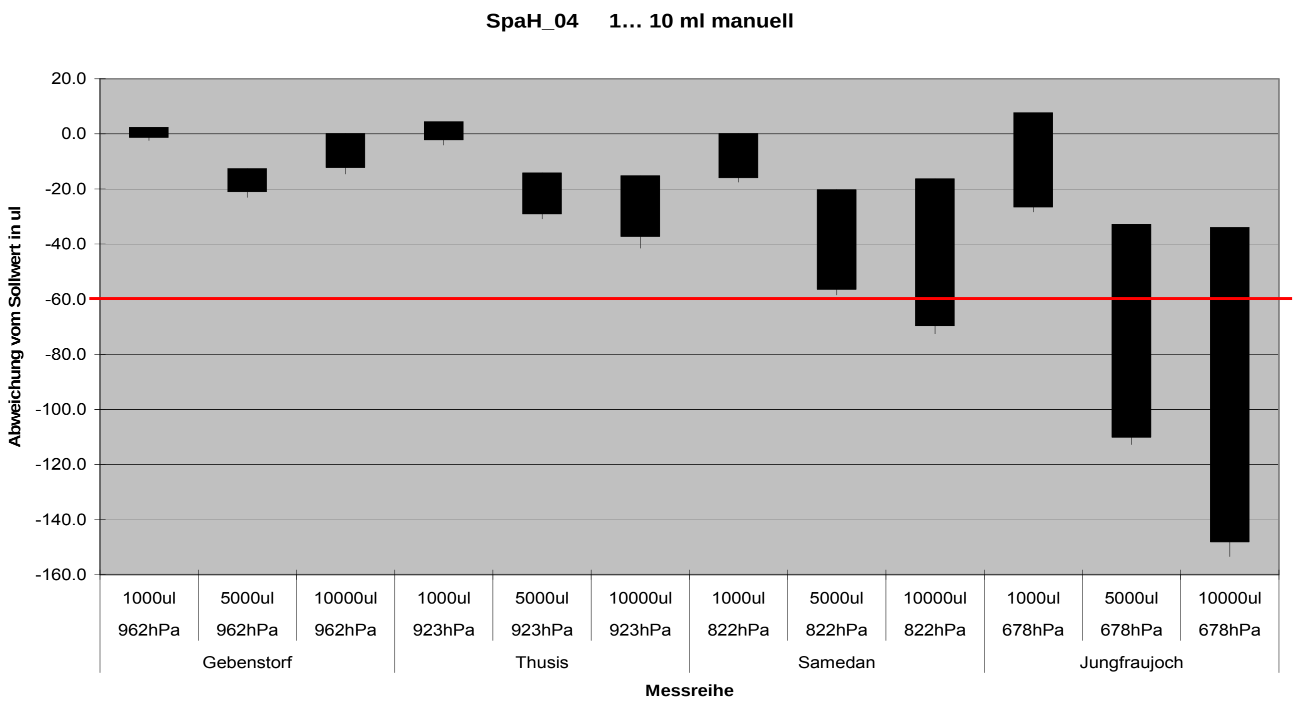



*Figure 10*

# 9 Summary

• The altitude above sea level of the measuring location has a significant influence on the measured result. If a pipette is adjusted correctly at 1013 hPa, the same pipette will dispense, at an air pressure of 850 hPa (about 1500 m.a.s.l.), an effective volume which is close to the lower limit of the admissible systematic error according to ISO 8655.
• Pipettes with air cushion should be adjusted and calibrated at the intended location of operation.
• Electronic pipettes can apply the correction factor in such a way that the pipette balances the error out according to the air pressure or to the altitude above sea level. For this purpose, the user must feed in either the air pressure or the altitude above sea level.
• For comparison measurements, the measured value can be corrected. For this purpose, however, the exact dead volume and the capillary rise must be known. These two values must be taken into account in the measurement uncertainty budget.

# 10 Acknowledgments

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Rainer Feldmann, BRAND GMBH + CO KG, D-97877 Wertheim, Germany
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Barbara Werner, Zentrum für Messen und Kalibrieren GmbH, D-06766 Bitterfeld-Wolfen, Germany
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Krankenhaus Thusis, Alte Strasse 37, CH-7430 Thusis, Switzerland
Spital Oberengadin, Via Nuova 3, CH-7503 Samedan, Switzerland
High Altitude Research Stations *Jungfraujoch* and *Gornergrat*, Siedlerstrasse 5, CH-3012 Bern, Switzerland

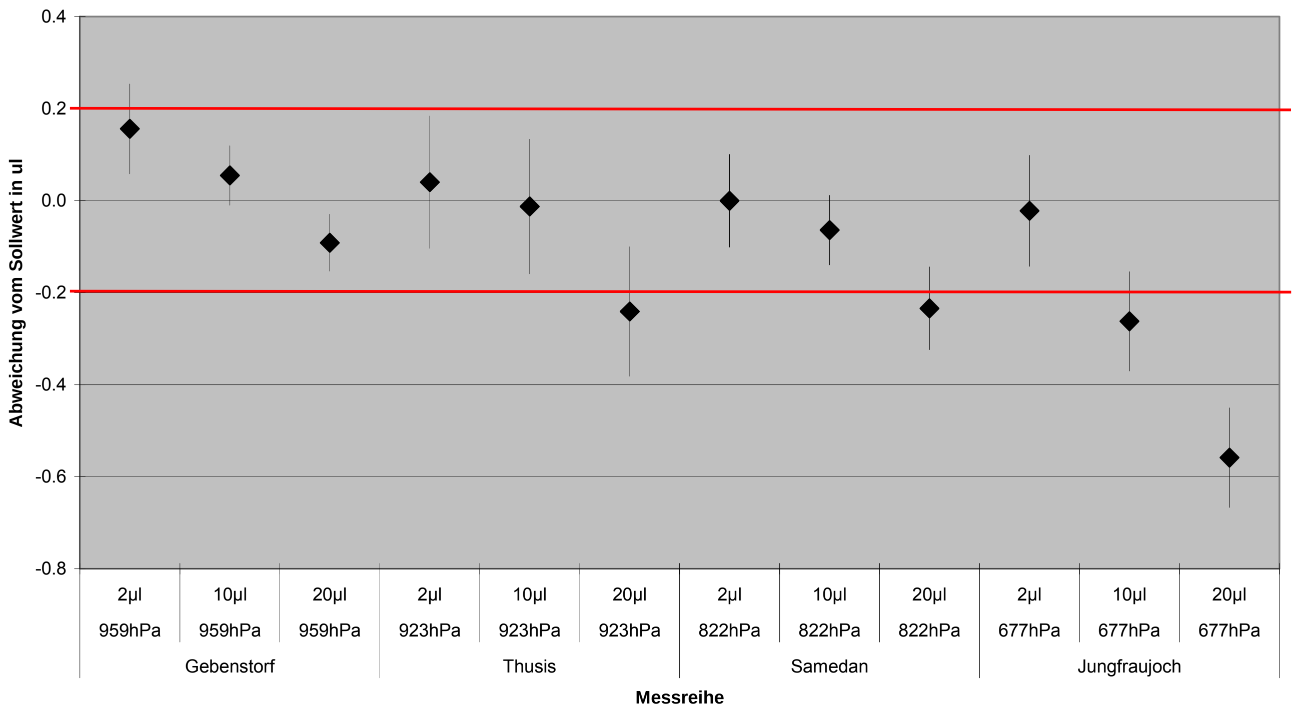
# 11 Annexes / References

Appendix 1: Measurement results of the individual measurements in diagrams (12 diagrams)

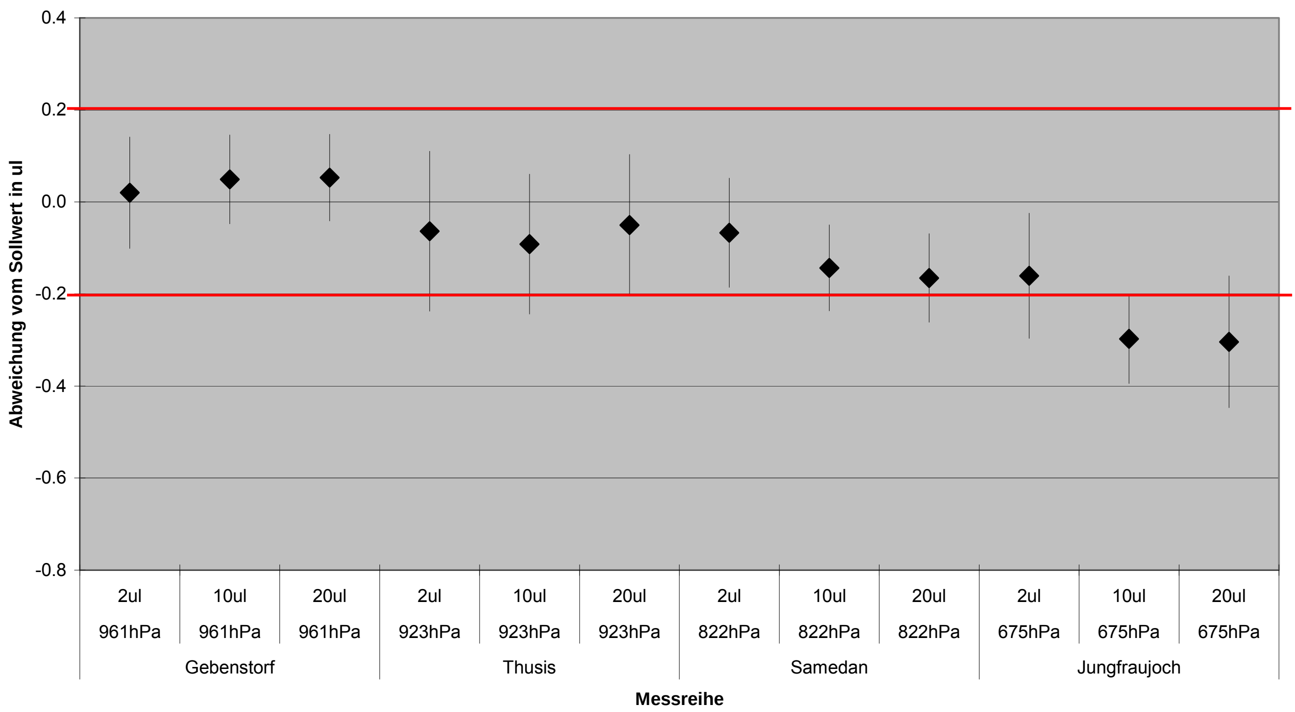
[1] ISO 8655:2002; Piston-operated volumetric apparatus
[2] Guideline DKD-R 8-1; Calibration of piston pipettes

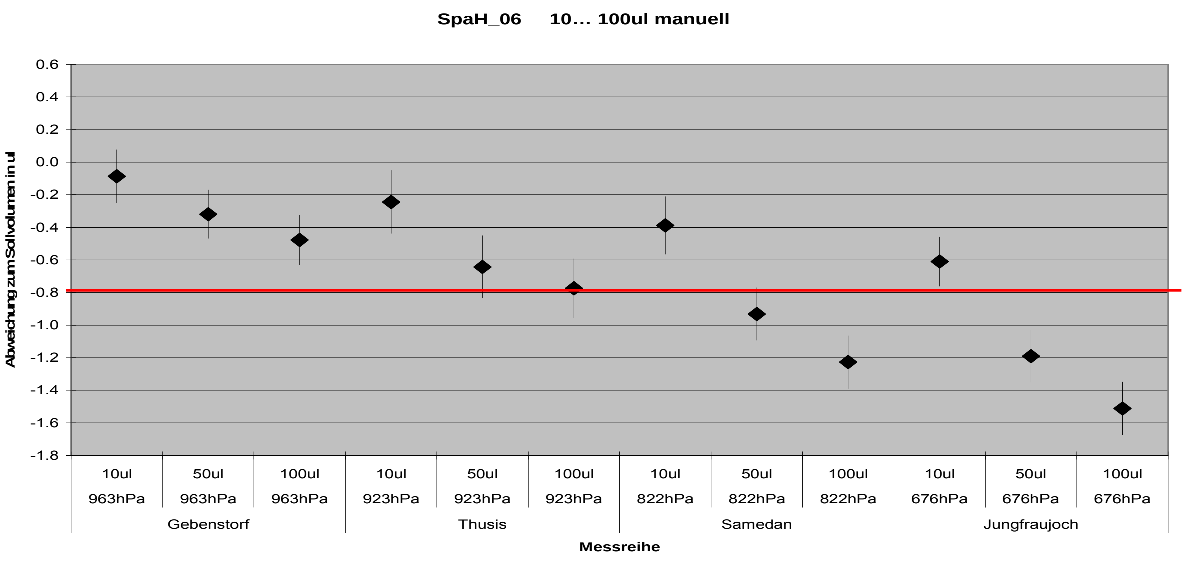
Annex 1:
Measurement results

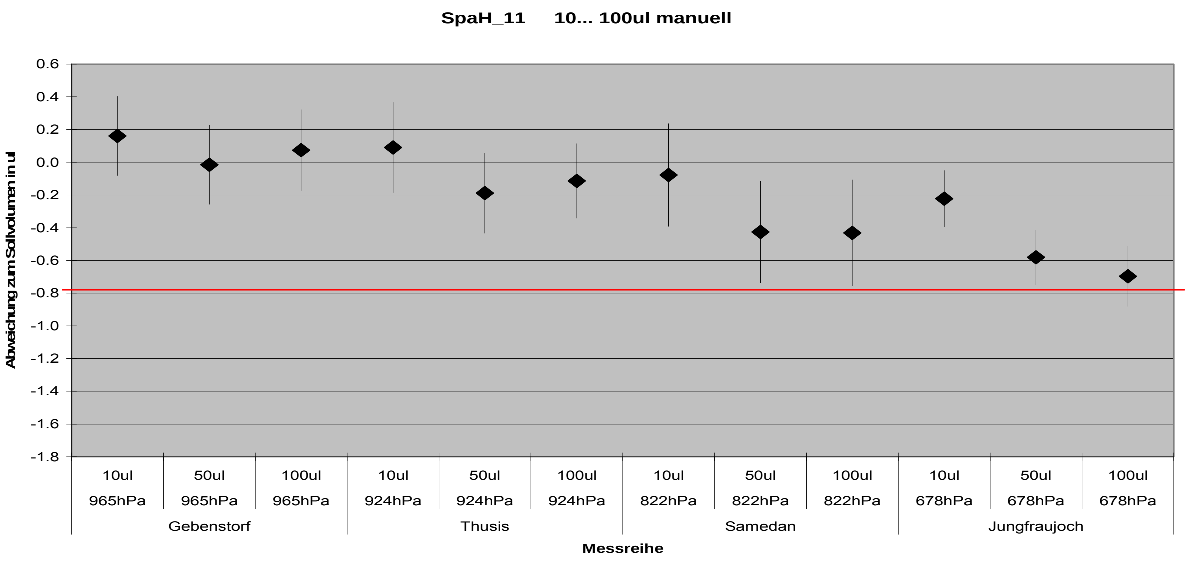
**SpaH_01 2... 20ul (Kristallspitzen) manuell**

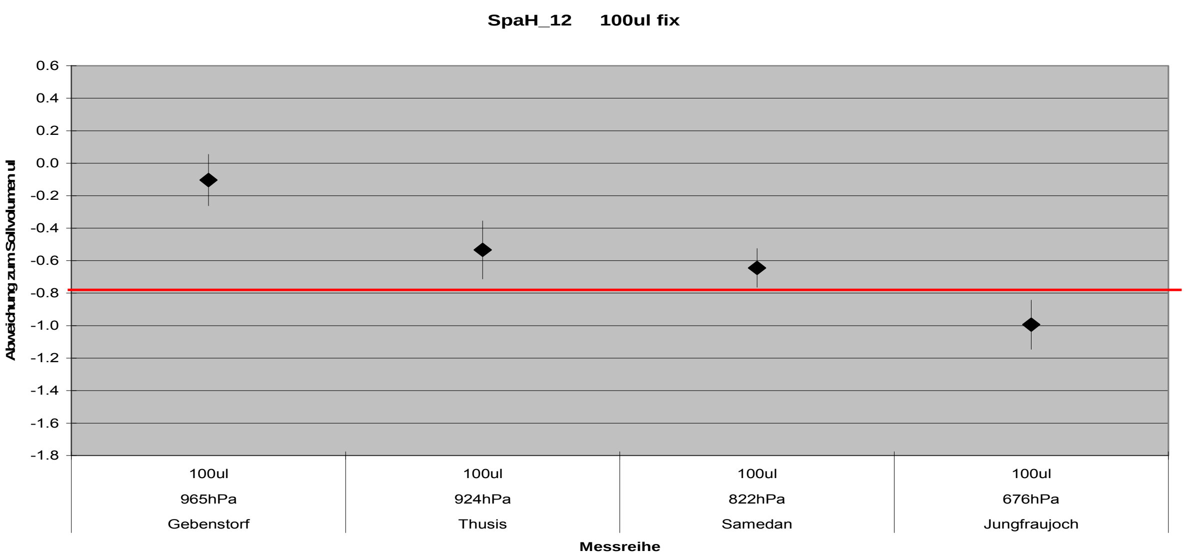


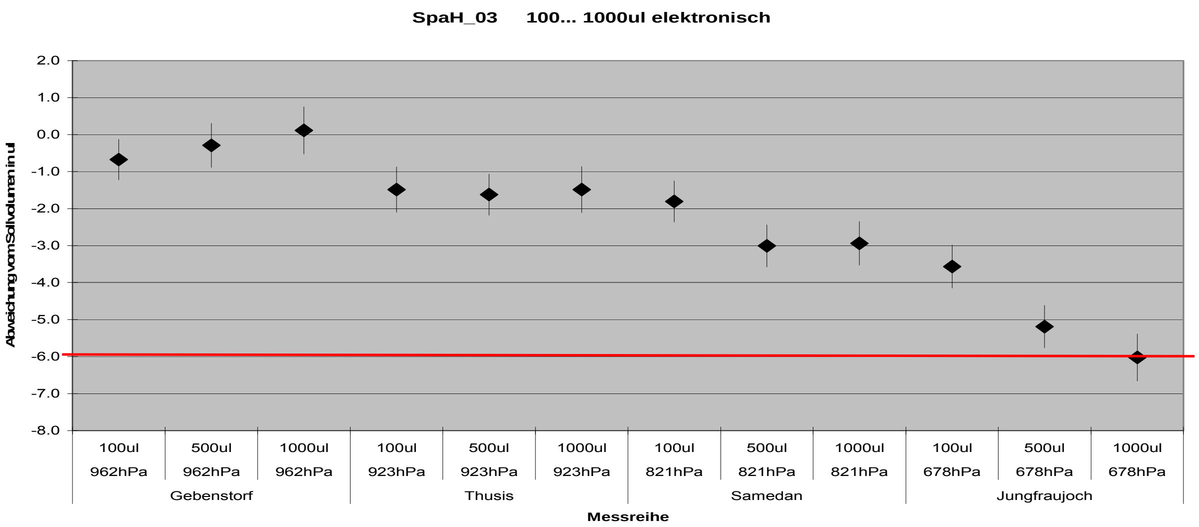

**SpaH_02 2… 20ul (gelbe Spitzen) manuell**




SpaH_06 10... 100ul manuell
Abweichung zum Sollvolumen in ul
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
10ul 963hPa
50ul 963hPa
100ul 963hPa
Gebenstorf
10ul 923hPa
50ul 923hPa
100ul 923hPa
Thusis
10ul 822hPa
50ul 822hPa
100ul 822hPa
Samedan
10ul 676hPa
50ul 676hPa
100ul 676hPa
Jungfraujoch
Messreihe


SpaH_11 10... 100ul manuell
Abweichung zum Sollvolumen in ul
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
10ul 965hPa
50ul 965hPa
100ul 965hPa
Gebenstorf
10ul 924hPa
50ul 924hPa
100ul 924hPa
Thusis
10ul 822hPa
50ul 822hPa
100ul 822hPa
Samedan
10ul 678hPa
50ul 678hPa
100ul 678hPa
Jungfraujoch
Messreihe


SpaH_12 100ul fix
Abweichung zum Sollvolumen ul
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
100ul 965hPa
Gebenstorf
100ul 924hPa
Thusis
100ul 822hPa
Samedan
100ul 676hPa
Jungfraujoch
Messreihe


SpaH_03 100... 1000ul elektronisch
Abweichung vom Sollvolumen in ul
2.0
1.0
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
-6.0
-7.0
-8.0
100ul 962hPa
500ul 962hPa
1000ul 962hPa
100ul 923hPa
500ul 923hPa
1000ul 923hPa
100ul 821hPa
500ul 821hPa
1000ul 821hPa
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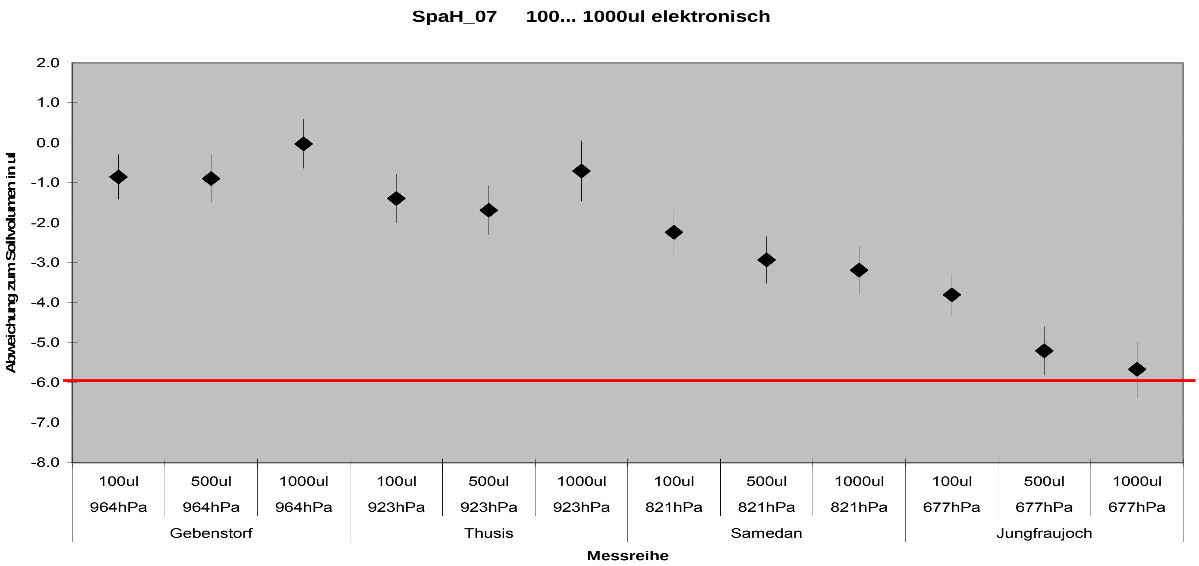
Gebenstorf
Thusis
Samedan
Jungfraujoch
Messreihe


SpaH_07 100... 1000ul elektronisch
Abweichung zum Sollvolumen in ul
2.0
1.0
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
-6.0
-7.0
-8.0
100ul 964hPa
500ul 964hPa
1000ul 964hPa
100ul 923hPa
500ul 923hPa
1000ul 923hPa
100ul 821hPa
500ul 821hPa
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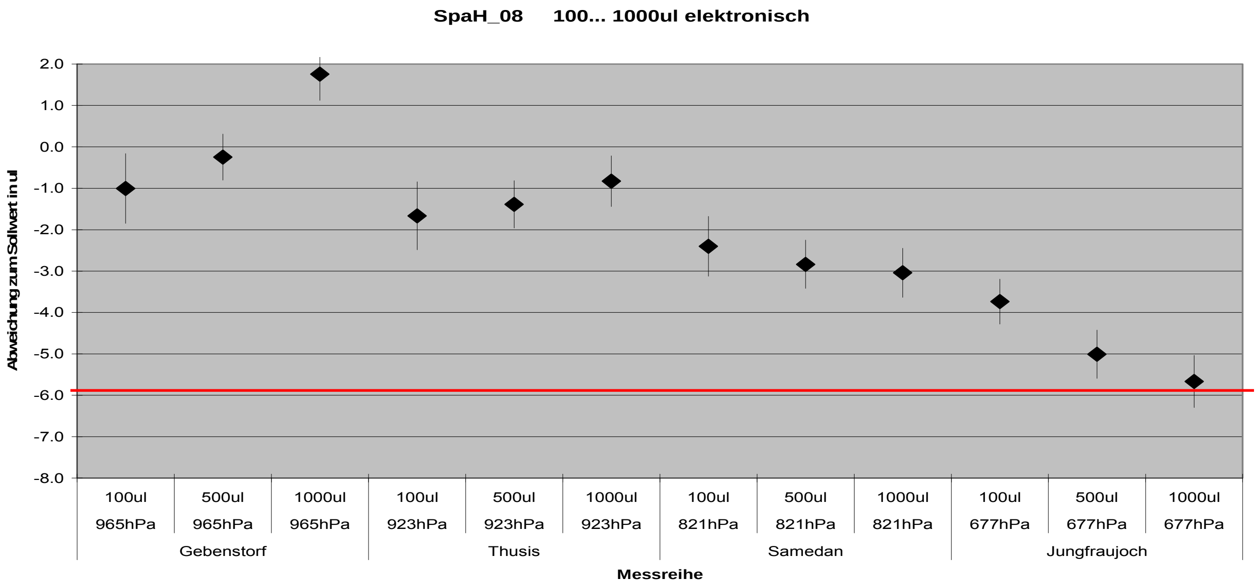
SpaH_08 100... 1000ul elektronisch
Abweichung zum Sollwert in ul
2.0
1.0
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
-6.0
-7.0
-8.0
100ul 965hPa
500ul 965hPa
1000ul 965hPa
100ul 923hPa
500ul 923hPa
1000ul 923hPa
100ul 821hPa
500ul 821hPa
1000ul 821hPa
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500ul 677hPa
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Gebenstorf
Thusis
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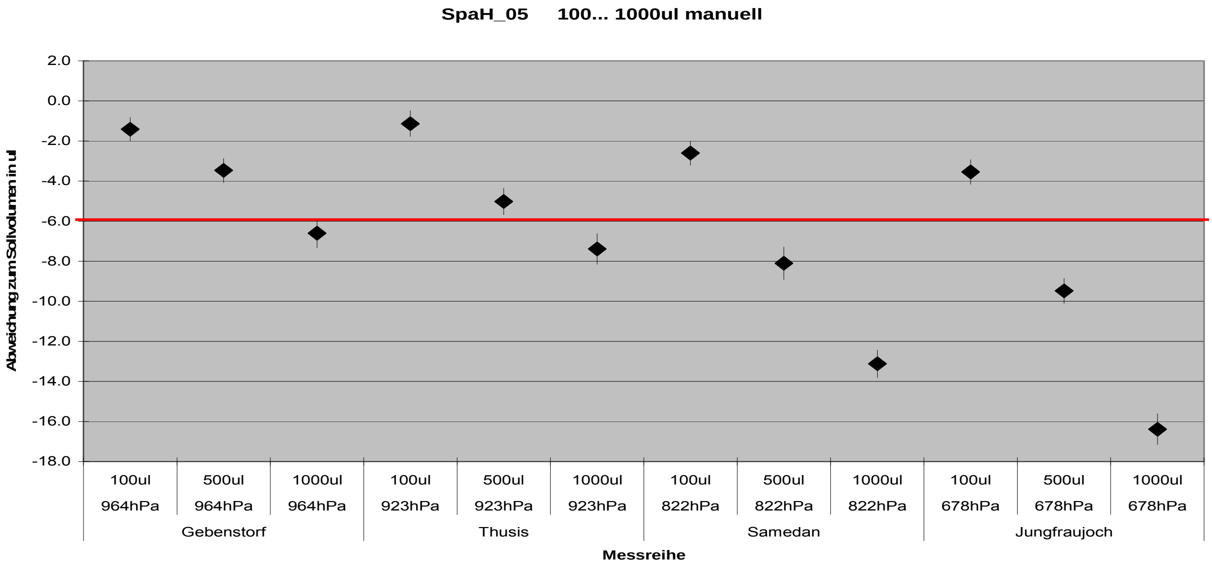
Jungfraujoch
Messreihe


SpaH_05 100... 1000ul manuell
Abweichung zum Sollvolumen in ul
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-14.0
-16.0
-18.0
100ul 964hPa
500ul 964hPa
1000ul 964hPa
100ul 923hPa
500ul 923hPa
1000ul 923hPa
100ul 822hPa
500ul 822hPa
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1000ul 678hPa
Gebenstorf
Thusis
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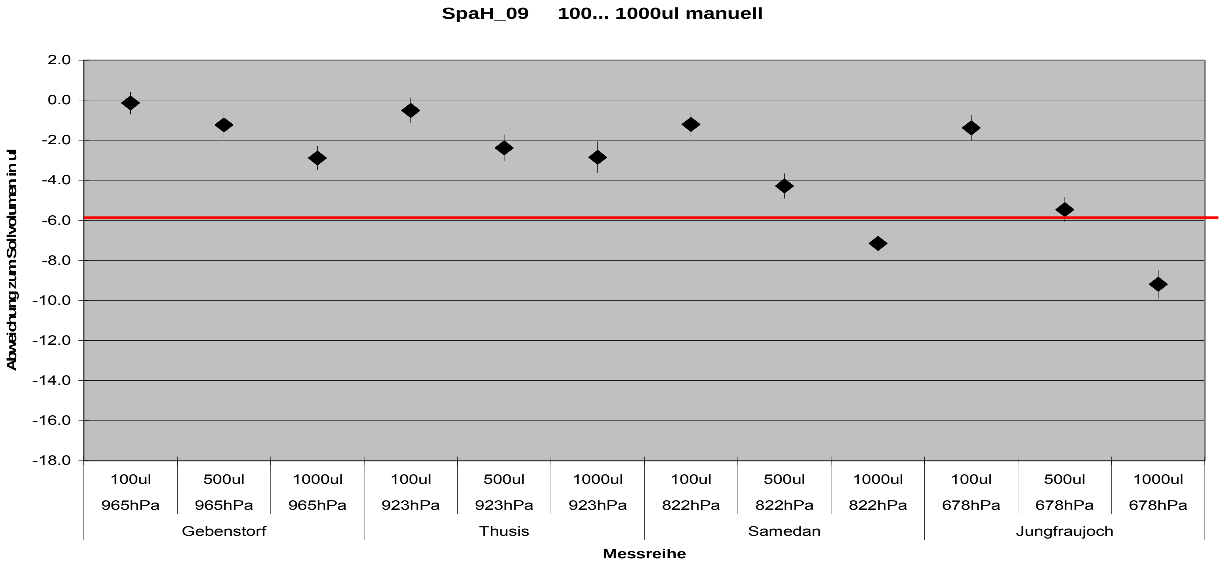
Jungfraujoch
Messreihe


SpaH_09 100... 1000ul manuell
Abweichung zum Sollvolumen in ul
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-14.0
-16.0
-18.0
100ul 965hPa
500ul 965hPa
1000ul 965hPa
100ul 923hPa
500ul 923hPa
1000ul 923hPa
100ul 822hPa
500ul 822hPa
1000ul 822hPa
100ul 678hPa
500ul 678hPa
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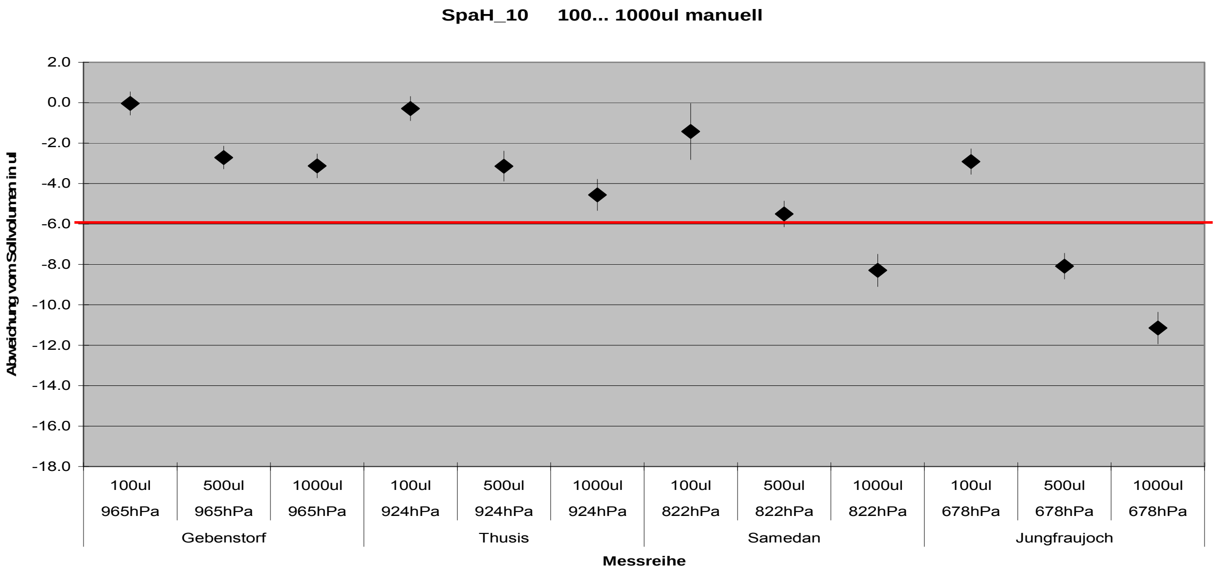
Gebenstorf
Thusis
Samedan
Jungfraujoch
Messreihe


SpaH_10 100... 1000ul manuell
Abweichung vom Sollvolumen in ul
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-14.0
-16.0
-18.0
100ul 965hPa
500ul 965hPa
1000ul 965hPa
100ul 924hPa
500ul 924hPa
1000ul 924hPa
100ul 822hPa
500ul 822hPa
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Gebenstorf
Thusis
Samedan
Jungfraujoch
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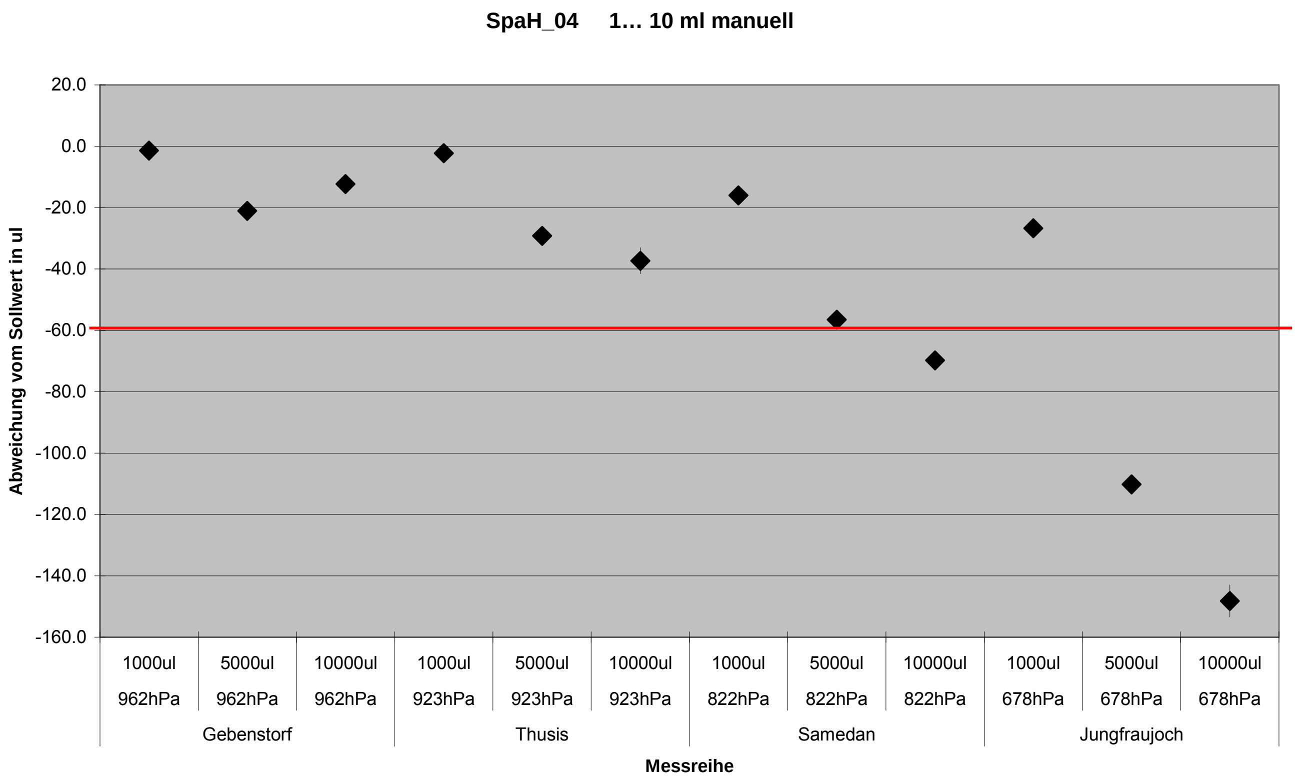
SpaH_04 1… 10 ml manuell
Abweichung vom Sollwert in ul
20.0
0.0
-20.0
-40.0
-60.0
-80.0
-100.0
-120.0
-140.0
-160.0
1000ul 962hPa
5000ul 962hPa
10000ul 962hPa
1000ul 923hPa
5000ul 923hPa
10000ul 923hPa
1000ul 822hPa
5000ul 822hPa
10000ul 822hPa
1000ul 678hPa
5000ul 678hPa
10000ul 678hPa
Gebenstorf
Thusis
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Jungfraujoch
Messreihe