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Expert Report DKD-E 8-3

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

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Deutscher Kalibrierdienst (DKD) – German Calibration Service

Since its foundation in 1977, the German Calibration Service has brought together calibration laboratories of industrial enterprises, research institutes, technical authorities, inspection and testing institutes. On 3rd May 2011, the German Calibration Service was reestablished as a *technical body* of PTB and accredited laboratories.

This body is known as *Deutscher Kalibrierdienst* (DKD for short) and is under the direction of PTB. The guidelines and guides developed by DKD represent the state of the art in the respective areas of technical expertise and can be used by the *Deutsche Akkreditierungsstelle GmbH* (the German accreditation body – DAkkS) for the accreditation of calibration laboratories.

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Foreword

DKD expert reports pursue the objective of providing background information and references in connection with other DKD documents, as, e.g., the DKD directives, and to handle special aspects in more detail. They do not replace the original DKD documents, but they do provide extensive supplementary information worth knowing. In the expert reports, the authors' views are expressed, which do not necessarily have to be consistent in all details with the view of the Management Board or the Technical Committees of the DKD.

The DKD expert reports are to present significant aspects from the field of calibration and, by means of publication within the framework of the DKD, they are to be made accessible to the large community of the national and international calibration laboratories.

This expert report has been approved by the DKD Managing Board and was drawn up by the following authors:

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Revision 1 of this expert report differs from the original version in the following aspects:

- The layout of pages 1 to 4 and the information on copyright have been revised.
- Page 22 with information about the publisher has been added.
- The expert report now has a DOI.

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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 and 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.

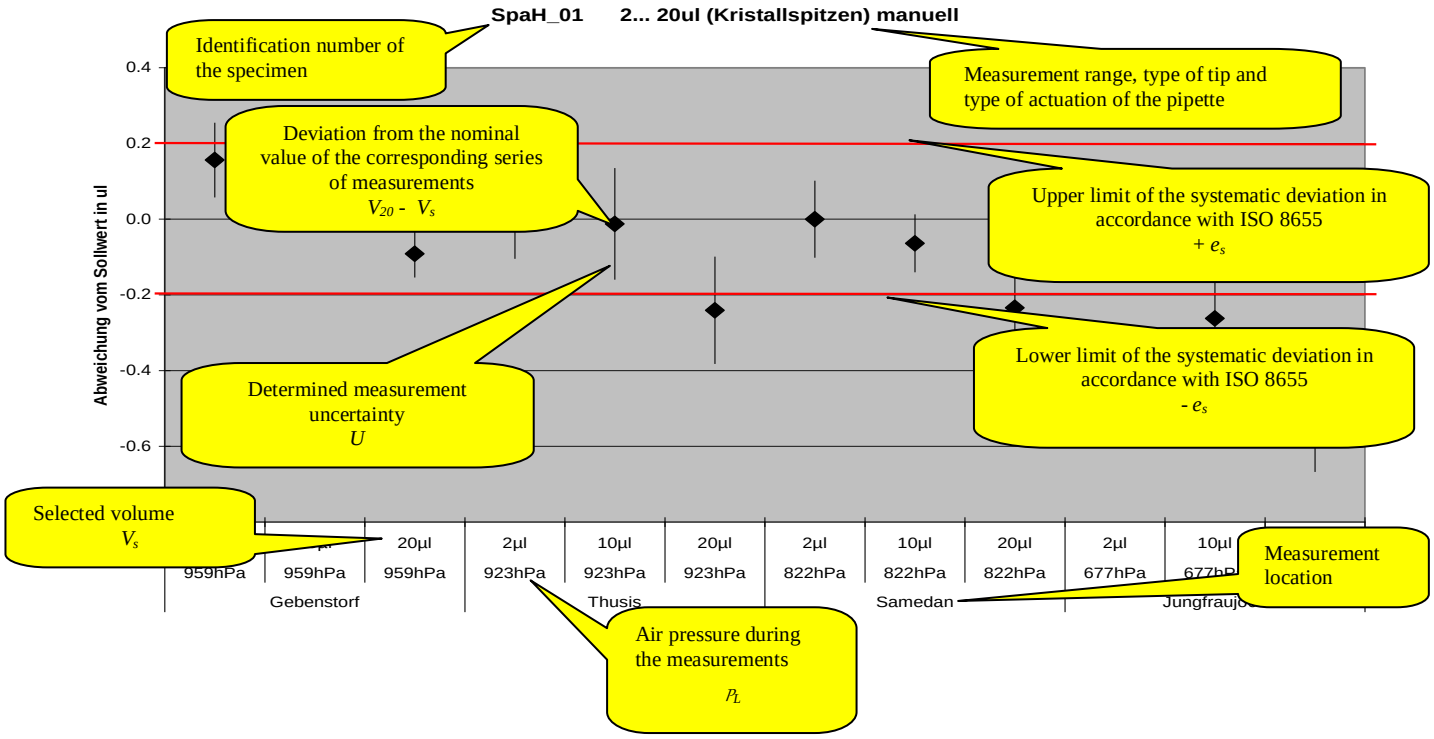


Figure 1: explanation to the diagrams 2 to 5

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 % RH was not achieved for technical reasons. The measurements were taken at about 40 % RH. 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).

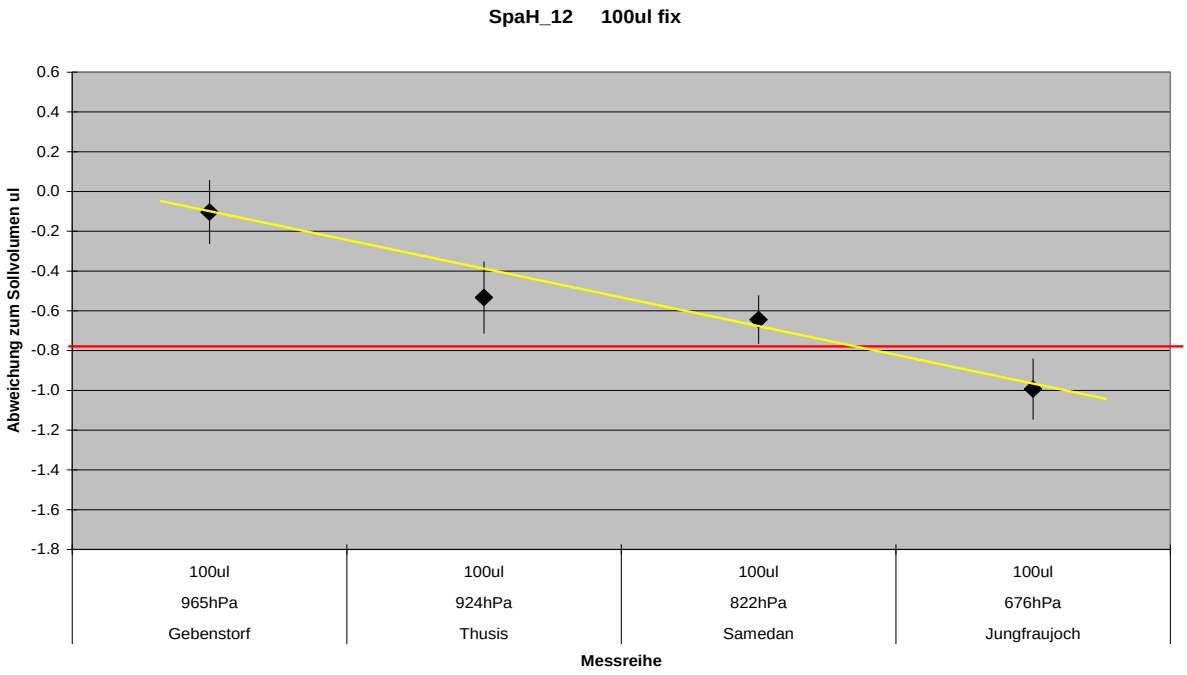


Figure 2: delivered volume as a function of height

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

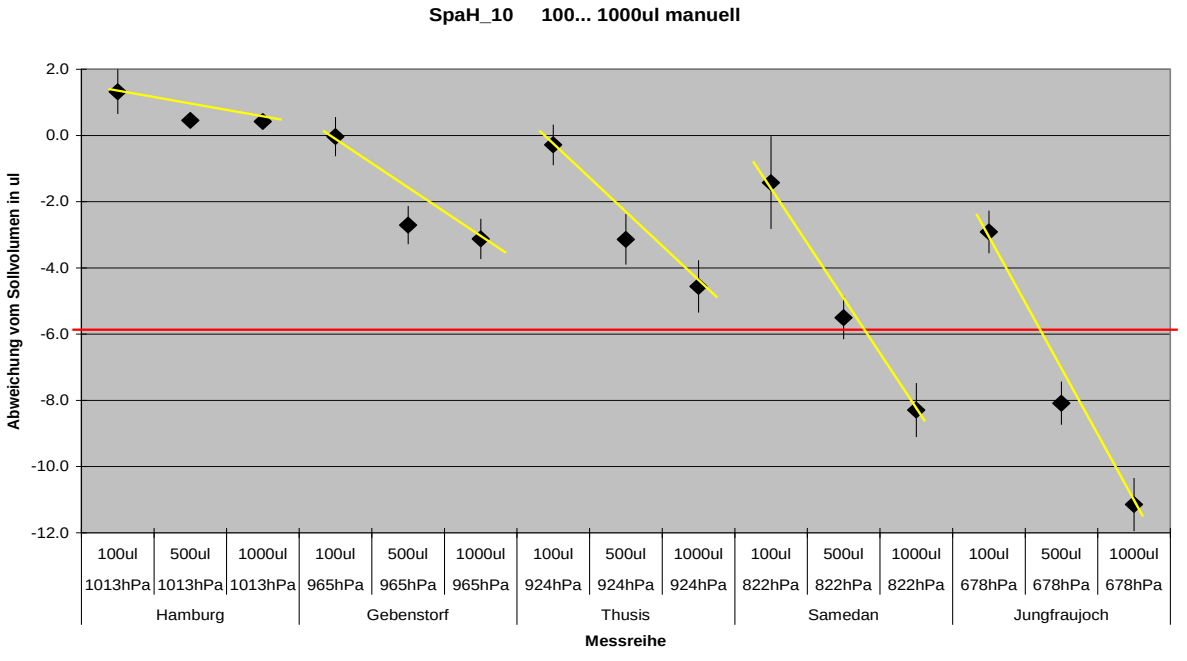


Figure 3: adjustable pipettes as a function of the selected volume

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

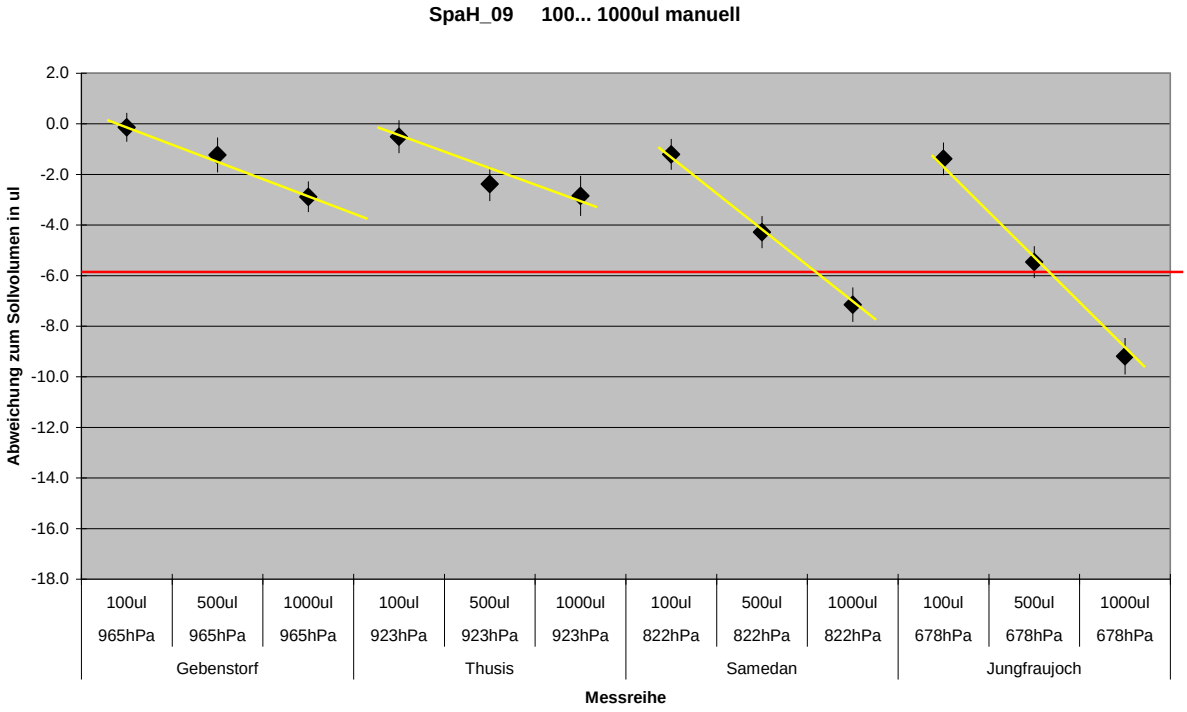


Figure 4: errors of pipettes with same norminal volume, but from different manufacturers

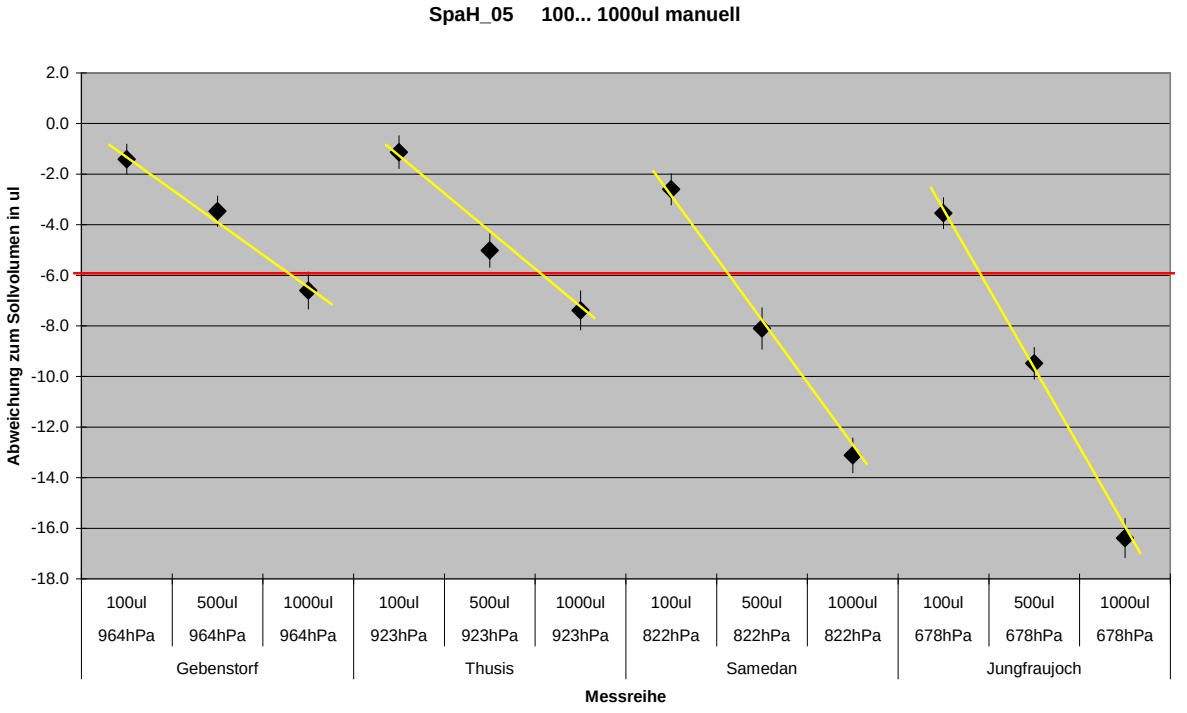
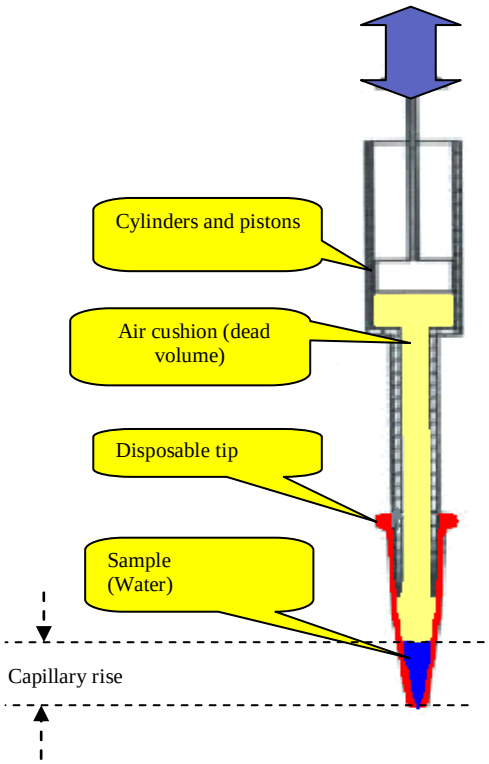


Figure 5: errors of pipettes with same nominal volume, but from different manufacturers

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 the 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**
ΔV	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
ρ_W	Density of the water used as test liquid
X1	Location 1 (in this document: 0 m.a.s.l. with air pressure 1013.25 hPa)
X2	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.

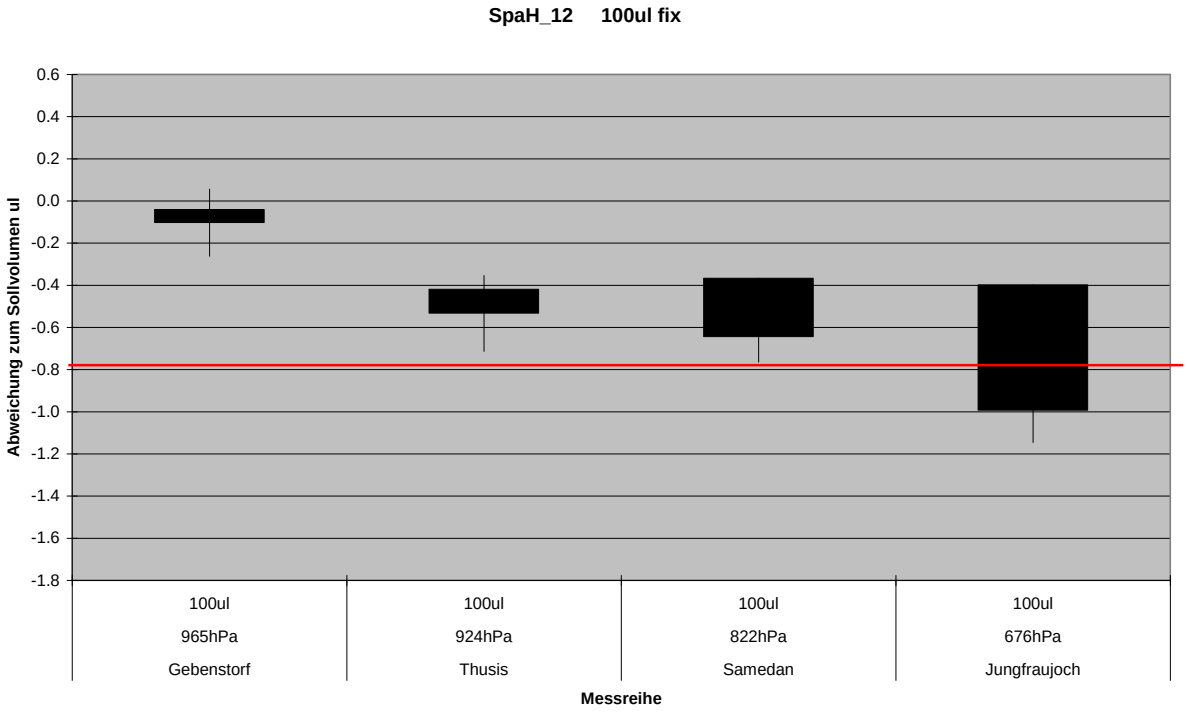


Figure 6: description for the diagrams 7 to 10

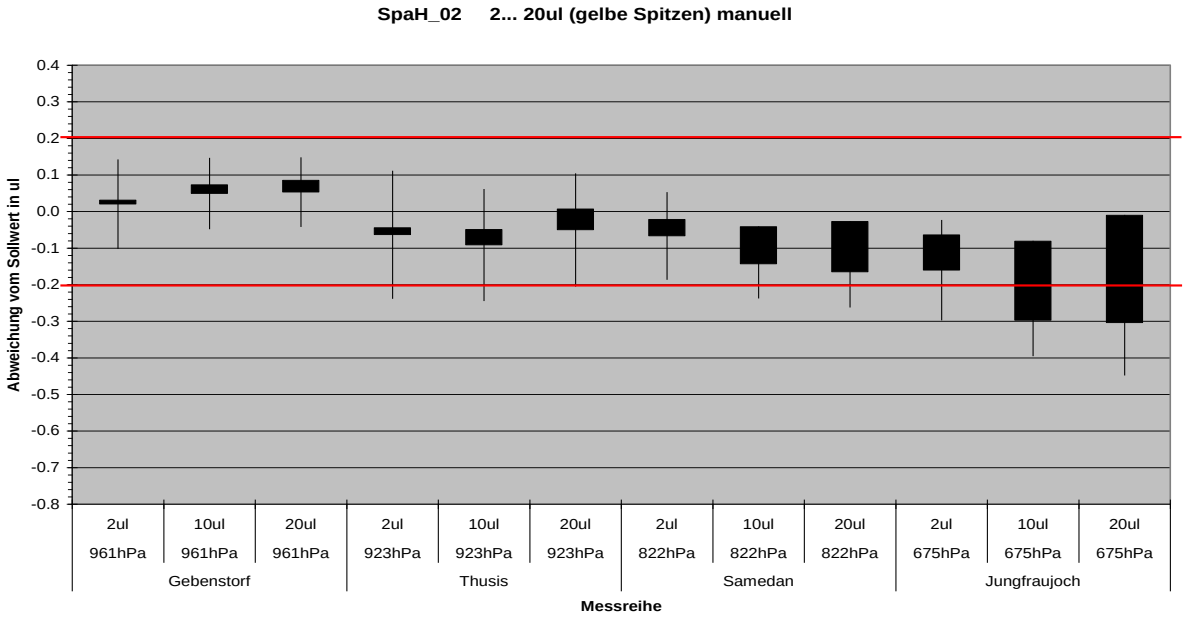


Figure 7: results for 2 to 20 µl, manually

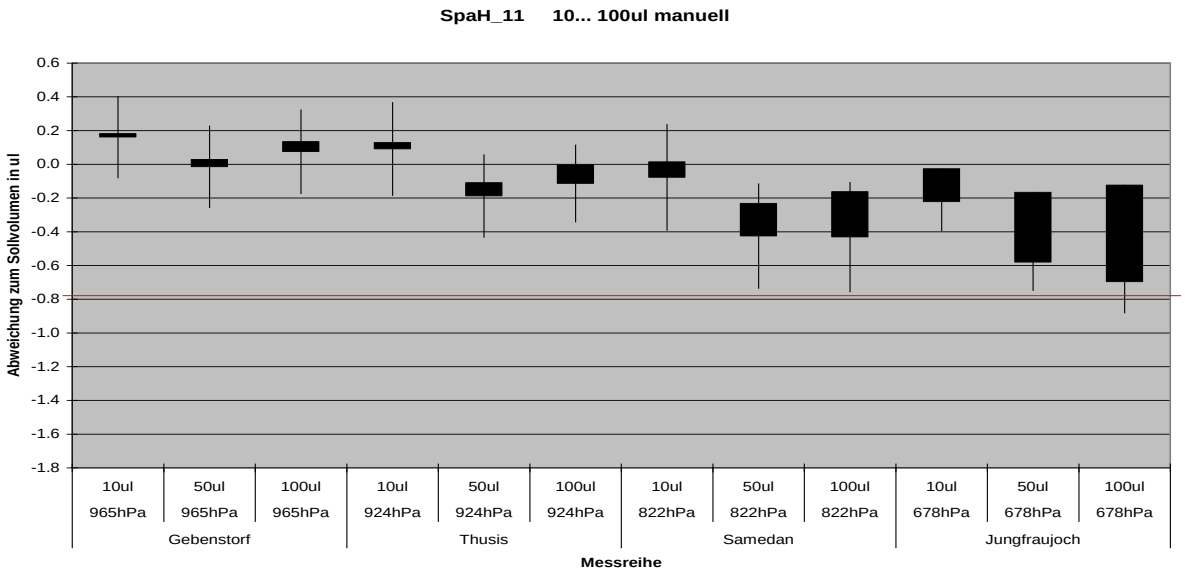


Figure 8: results for 10 to 100 µl, manually

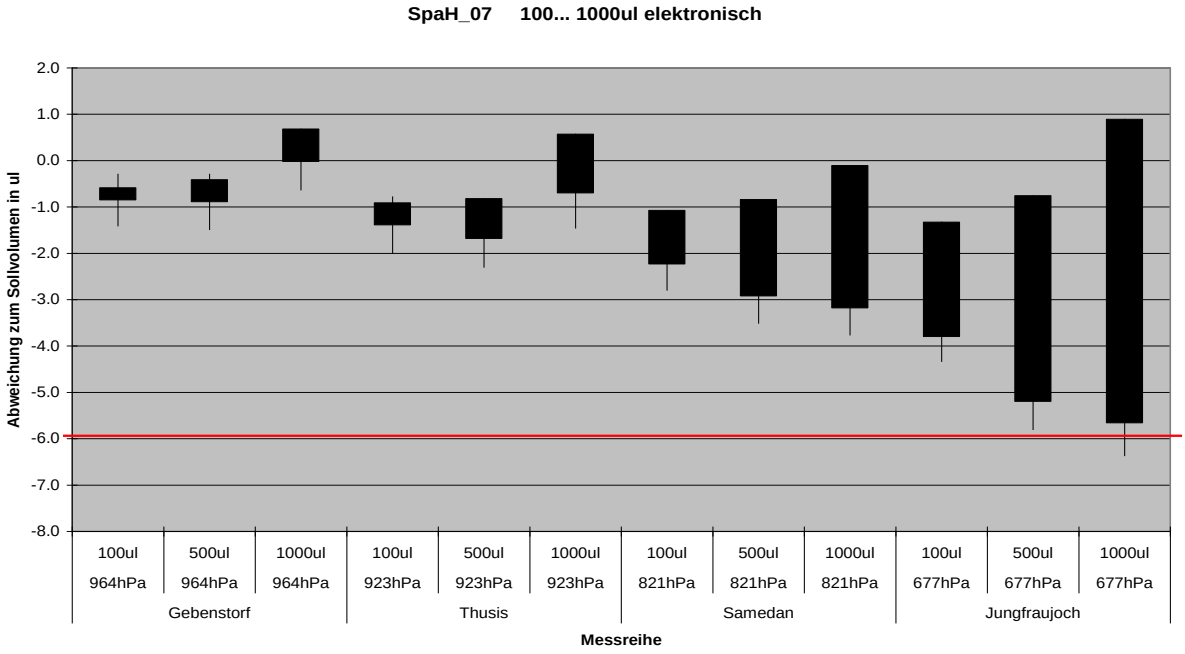


Figure 9: results for 100 to 1000 µl, electronically

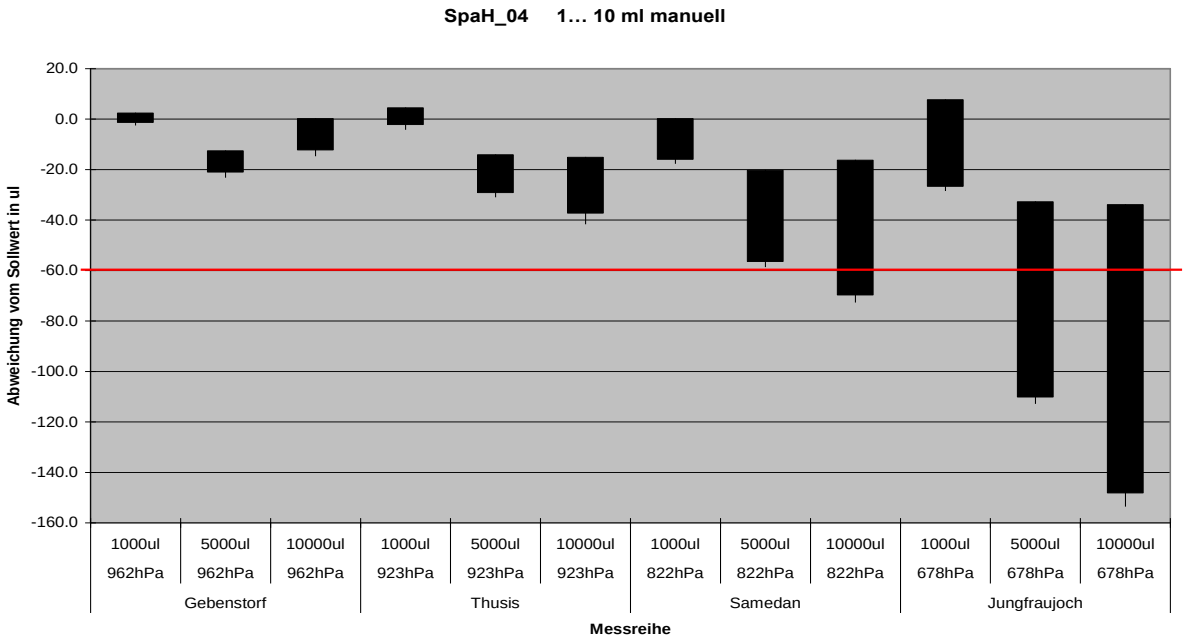


Figure 10: results for 1 to 10 µl, manually

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 ($V_{20} = V_s$), 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.

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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

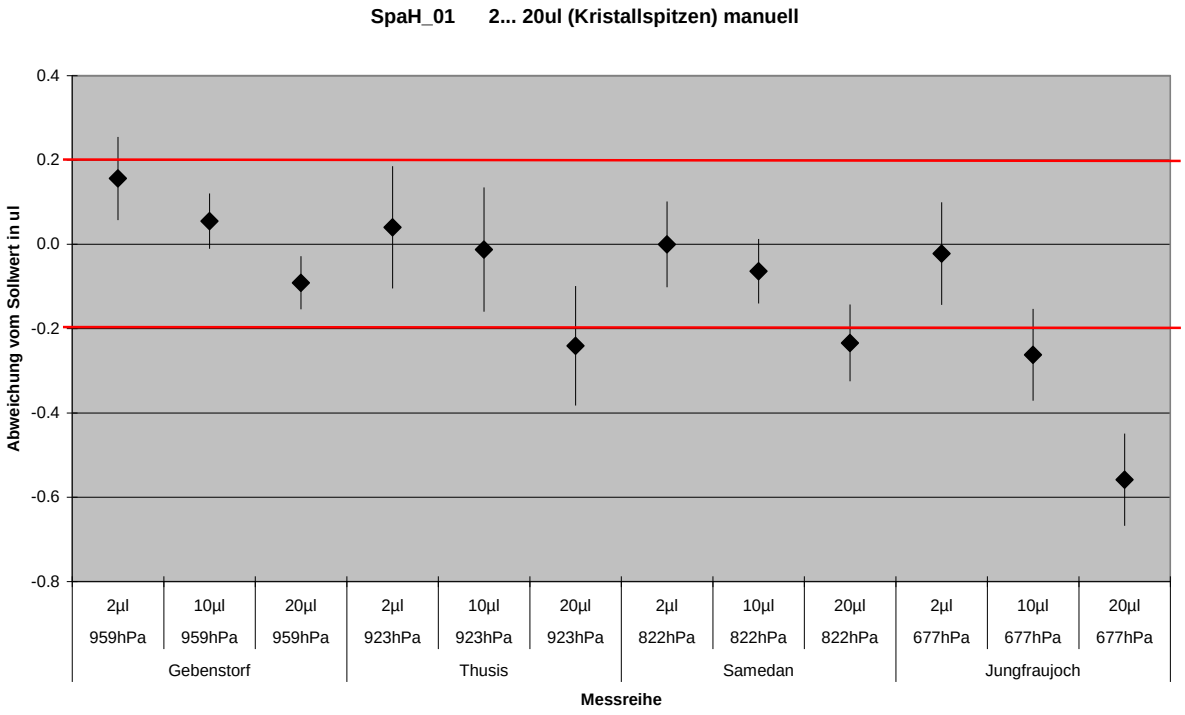
Appendix 1: Measurement results of the individual measurements in diagrams (12 diagrams)

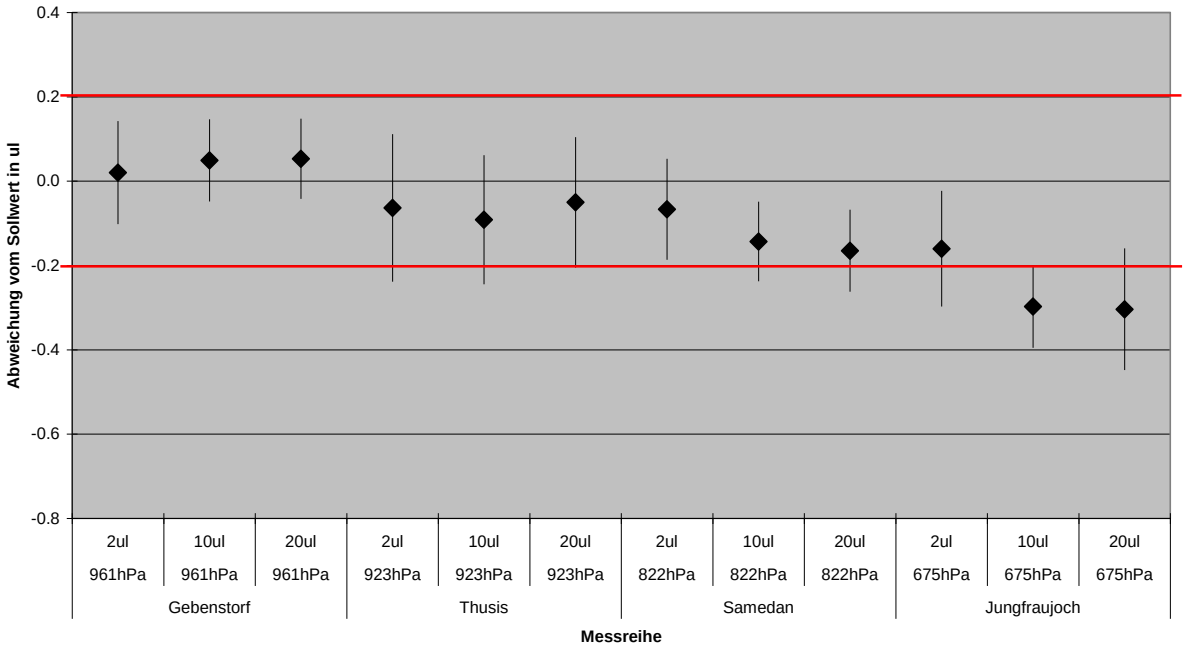
[1] ISO 8655:2002; Piston-operated volumetric apparatus

[2] Guideline DKD-R 8-1; Calibration of piston pipettes

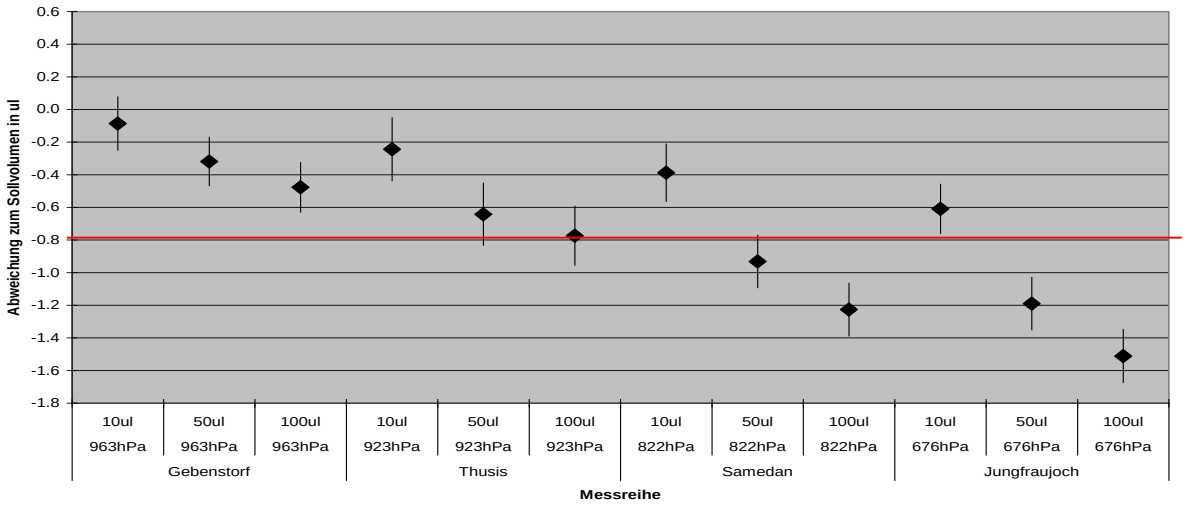
Annex 1:
Measurement results

The influence of altitude on the volume of a piston pipette with air cushion, 31 December 2011.

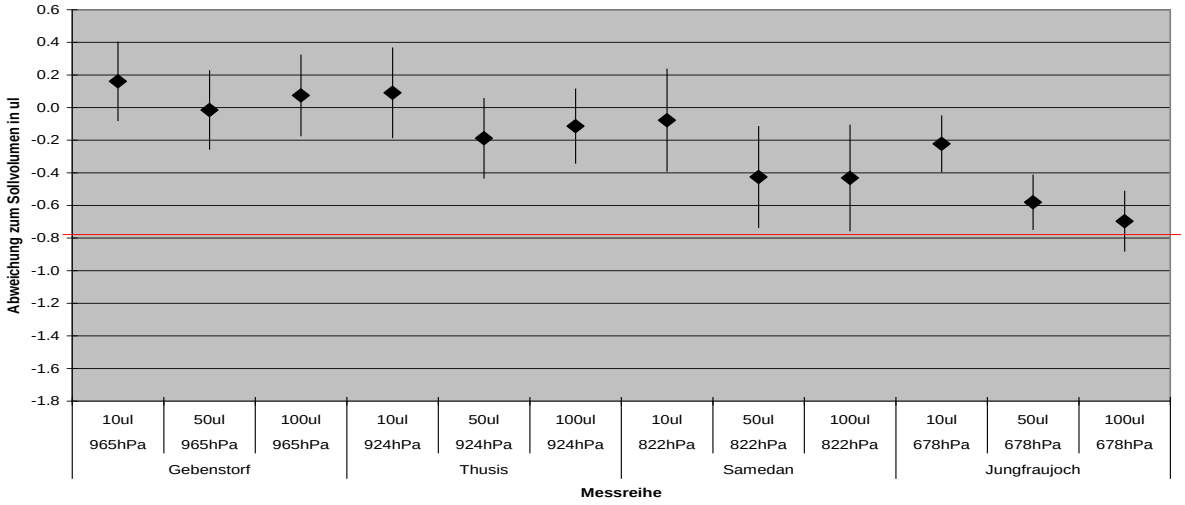




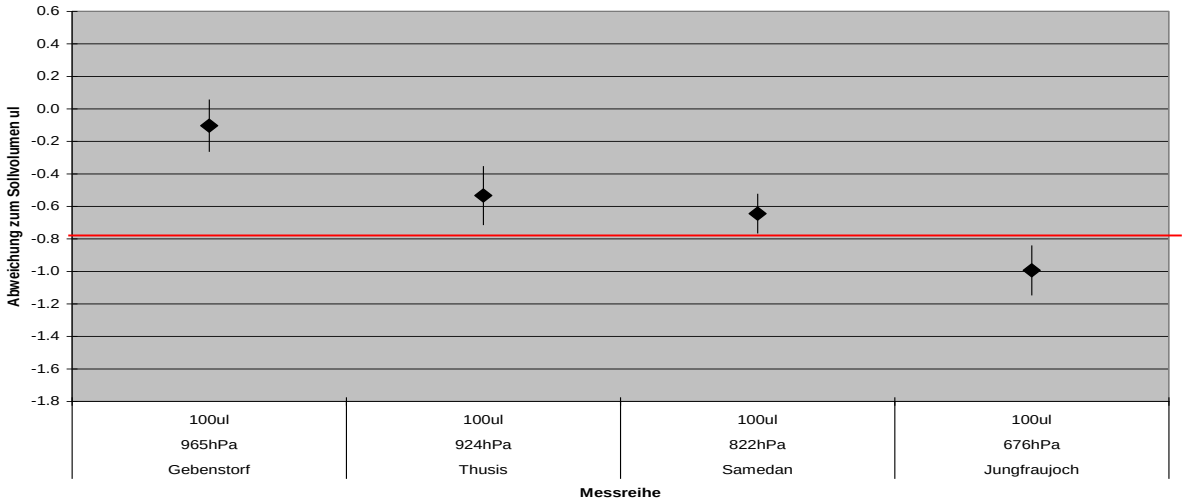
SpaH_06 10... 100ul manuell



SpaH_11 10... 100ul manuell



SpaH_12 100ul fix



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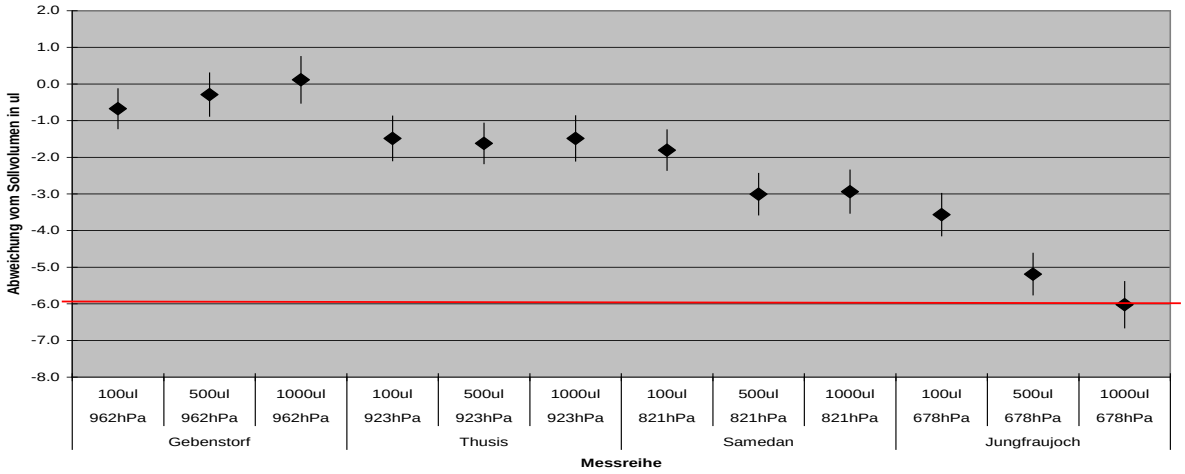
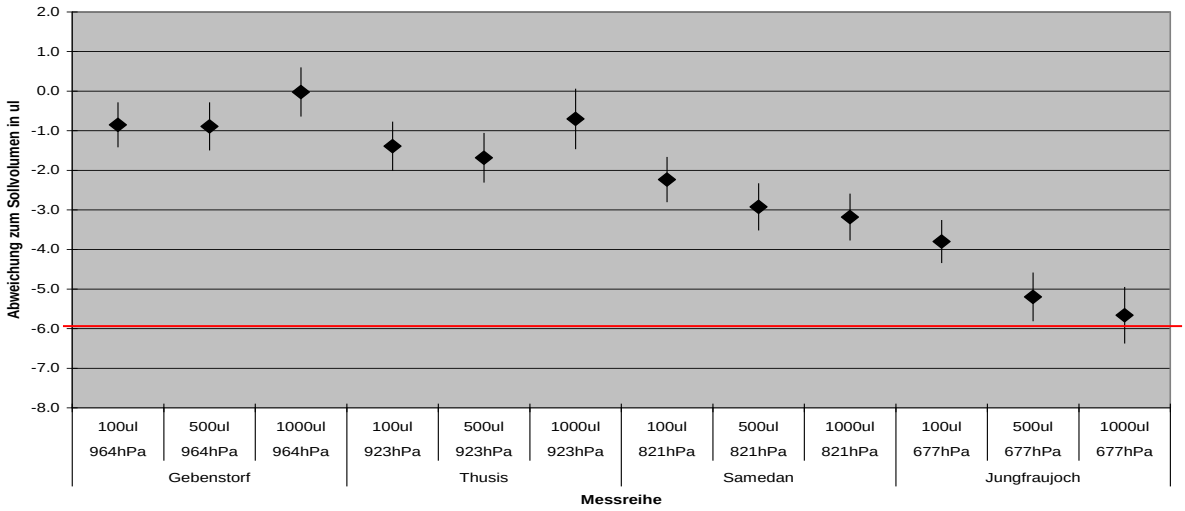
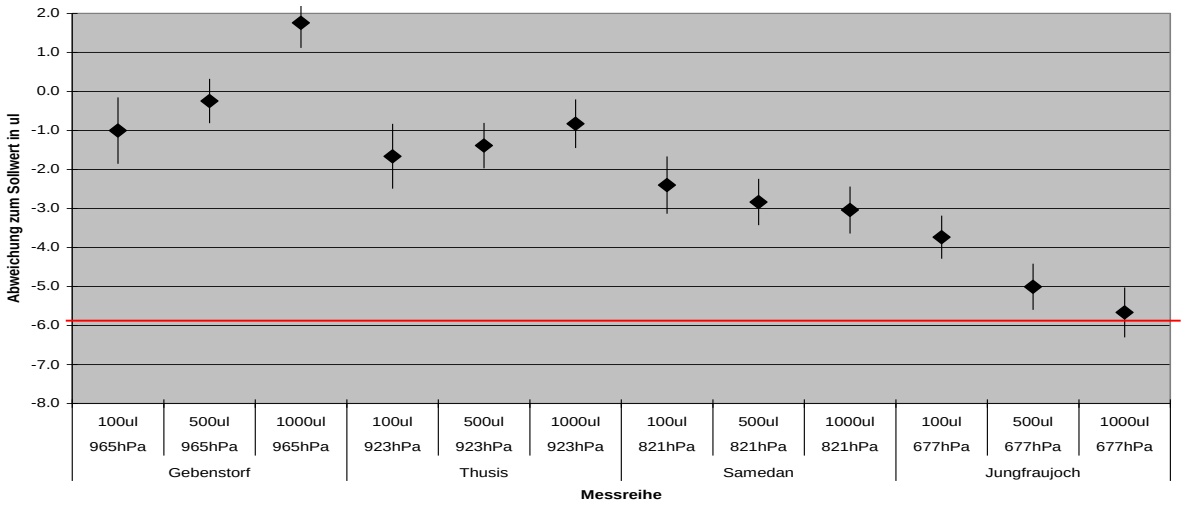


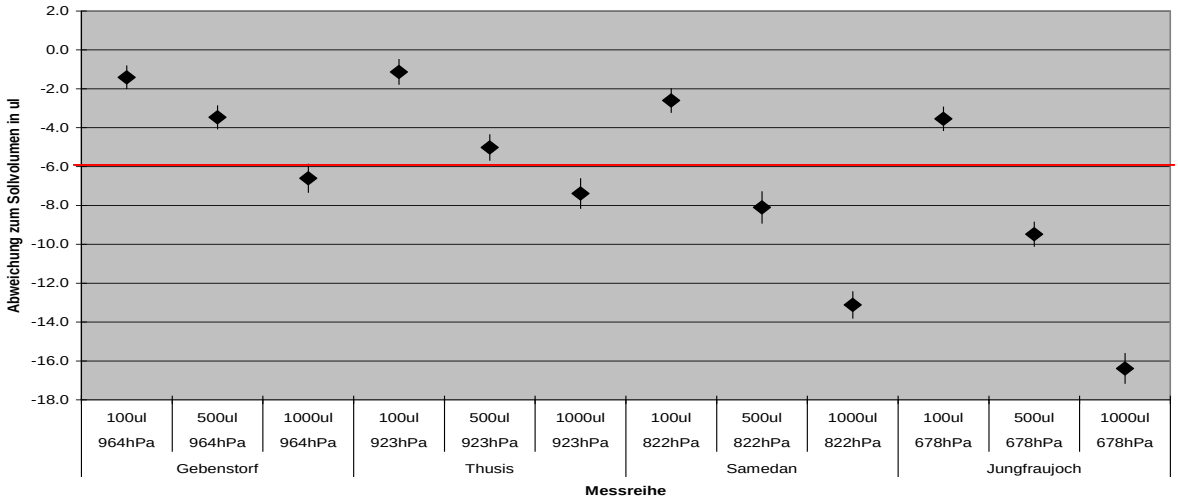
SpaH_07 100... 1000ul elektronisch



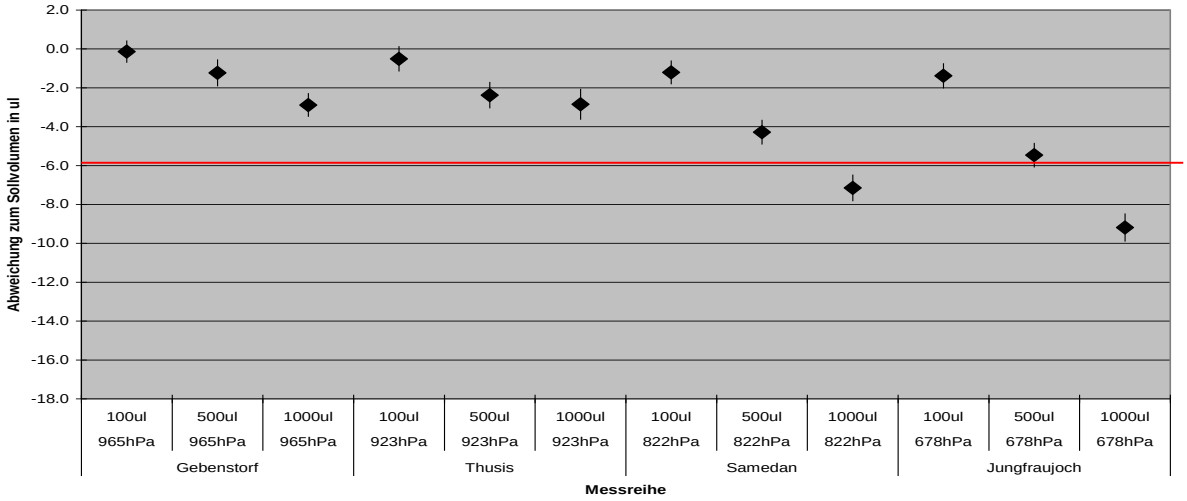
SpaH_08 100... 1000ul elektronisch



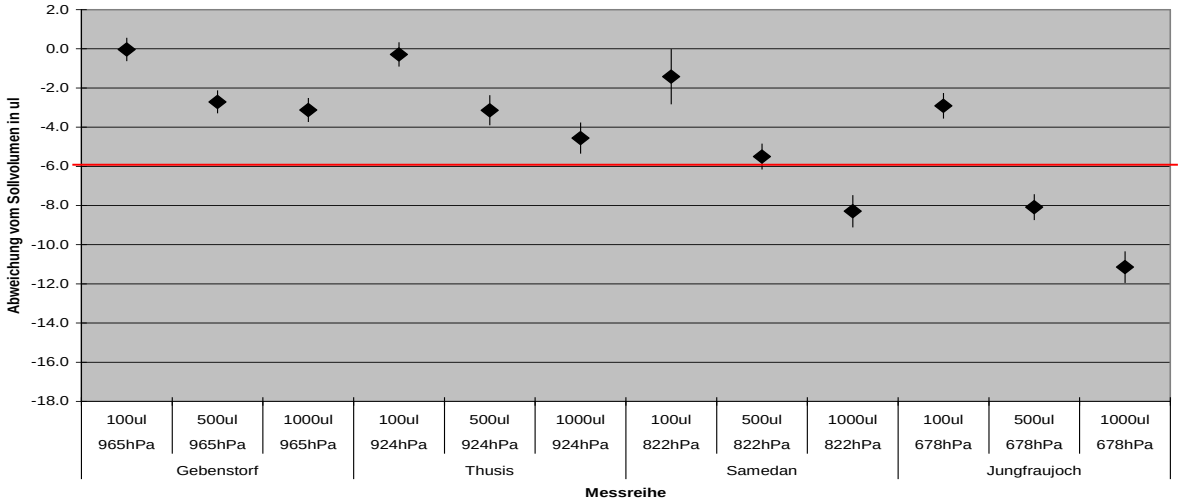
SpaH_05 100... 1000ul manuell



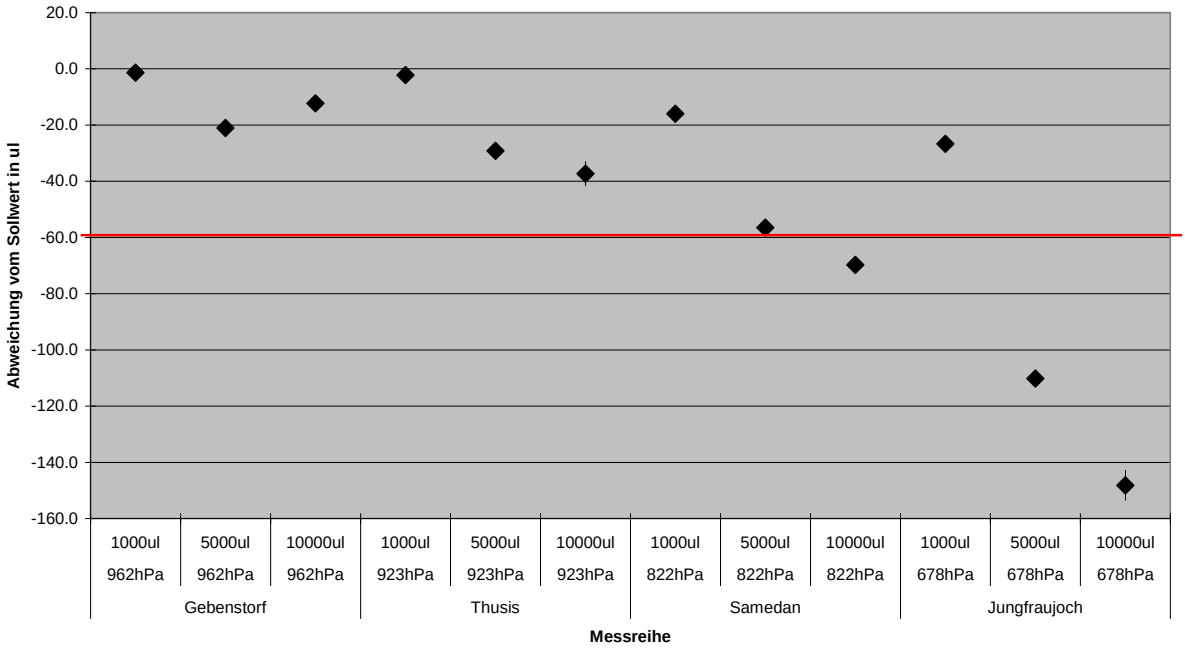
SpaH_09 100... 1000ul manuell

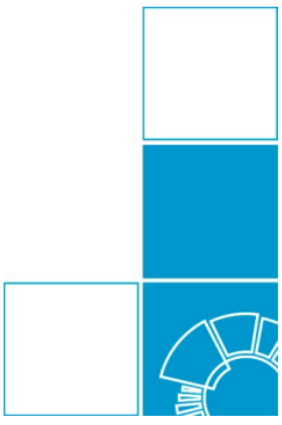


SpaH_10 100... 1000ul manuell



SpaH_04 1… 10 ml manuell





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