MANUAL PIPETTING: A MULTI-SECTOR STUDY WITH DRY-VERIFICATION TECHNOLOGY (ATMOS-1)

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Abstract
This study evaluated pipetting accuracy, precision, and leakage across six Estonian laboratories—including medical diagnostics, university research, and authority labs—using the Atmos-1 device, an advanced, rapid, and environmentally independent pipette verification tool. The results revealed substantial variability in pipetting performance among the different sectors. Authority and medical laboratories, which operate under strict regulatory frameworks and frequent calibration routines, demonstrated consistently higher accuracy and precision in pipetting. In contrast, university research laboratories exhibited the greatest frequency of unacceptable deviations, primarily due to inconsistent or reactive pipette verification practices. Notably, the smallest volume pipettes (10–20 µl), used mainly in academic settings, were most prone to significant inaccuracies. Leakage issues were common but often resolved by replacing incompatible pipette tips, highlighting the impact of procurement decisions on laboratory performance. In clinical and authority settings, the consequences of pipetting inaccuracies are minimized by the use of automated analysers, whereas in research environments, manual pipetting errors can directly compromise experimental validity and scientific outcomes. These findings underscore the importance of regular pipette maintenance, comprehensive staff training, and careful selection of compatible consumables. Implementing routine pipette verification, particularly in research institutions, and prioritizing quality in procurement strategies can significantly enhance data reliability and overall research quality. The Atmos-1 device proved to be a valuable tool for enabling rapid, user-friendly, and effective pipette assessment, supporting improved laboratory practices across diverse sectors.
Keywords: Atmos-1, pipetting, quality assurance, accuracy, leakage.

Introduction

Modern biology and chemistry laboratories, including those for veterinary clinical diagnostics, require precise liquid handling and making micropipette accuracy vital for reproducible results. Accurate and precise measurements begin with correct and proper sampling, labelling and sample transport (delivery) to laboratories. These preanalytical activities will be followed by in-lab work as aliquoting, preparation of necessary solutions, dilutions and finally actual analytical step. For these activities the correctness is often not controlled as it is taken as self-evident. However, this introduces unexpected risks. To mitigate these risks, it is critical to verify measuring devices (such as pipettes) and containers (Blues et al., 2004; Huber, 2025). Traditional pipette calibration methods, gravimetric and photometric methods (Lochner, 1998; Lochner et al., 1996; Pati et al., 2021), are labour-intensive and therefore often outsourced, leaving gaps in regular quality assurance. The Atmos-1 device we used in our study for pipette verification is a gamechanger; it employs advanced pressure sensors and microelectronics to assess precision rapidly, without liquid, in under a minute (Unipix, 2025). Atmos is independent of environmental conditions and can also detect leaks, ensuring optimal pipette performance prior to critical experiments. This is especially important while the user experience may vary or to be insufficient in emerging testing situation (Karus A. & Karus V., 2024), but also in development, validation, and verification of new tests (Carle et al., 2004; Cremonesi et al., 2017; Karus et al., 2017). Problems in volume measurement accuracy and precision can present unexpected obstacles, raising doubts in reliability of scientific findings, adding it to already known challenges (Batista et al., 2007; Guan et al., 2023; Karus A. & Karus V., 2018). The aim of this study was to analyze pipetting practices across different sectors involved in biochemistry and molecular biology using the novel dry measurement technology Atmos-1, to identify potential gaps in pipetting performance, provide evidence of prevalent issues, suggest solutions, and ultimately enhance the quality of pipetting and research data.

Materials and Methods

The single-channel pipette accuracy tester Atmos-1 version 2024-V4.2 was used for measuring pipette accuracy, precision and leakage in six institutions: three medical diagnostics laboratories, research laboratories of two universities and one authority laboratory in Estonia in years 2024 and 2025 on voluntary basis. In every institution, the introductory training was conducted by author on-site for one to three responsible specialists (Yamamoto et al., 2014). Following the training, the Atmos-1 was left in institution for internal evaluation for up to two months. Results were recorded in tester internal memory and downloaded using ReadATMOS software. The selection of pipettes, their volume and number were identified by laboratory personnel according to their everyday use of pipettes and the selection has no impact of authors' influence. Measurements conducted during the initial user training, as well as all measurements with fewer than three repetitions, were excluded from analyses. Results were grouped by user and pipette volume. If leakage was observed, changing pipette tips (to another producer) was recommended and thereafter the testing was subsequently repeated. Accuracy was calculated as relative deviation of the

measured volume from the set value, expressed as a percentage ($\frac{|\bar{x}-V|}{V} \times 100\%$, where $\bar{x}$ – mean of measured pipetting volume and V – set volume on pipette) with good performance defined within 1% and acceptable accuracy as within 2%. Unacceptable failure in accuracy was defined as a deviation greater than 10% and is reported separately. These limits are recommended by manufacturer. Precision was calculated as the coefficient of variation (CV) in percentage. The leakage limit of 1% is set by the instrument software (Unipix, 2025) and was used as fixed recommendation for result acceptance. Institutions are anonymized. Thus, simple descriptive statistics were used to compare different pipette types. Discriminant analysis was performed only at the highest level of generalization to evaluate differences in pipetting quality between user groups (research, medical diagnostics and authority/control laboratories). Because the number of studied pipettes differed across these groups, the Tukey-Kramer test was applied to accommodate the unequal sample sizes, as there were sufficient measurements available (Tukey, 1949; Kramer, 1956). As the ATMOS1 pipetting accuracy tester is indicated for controlling the pipettes in-between required calibration intervals, we did not limit and did not record the measurements done with pipettes recently arrived from regulated periodic calibrations.

Results and Discussion

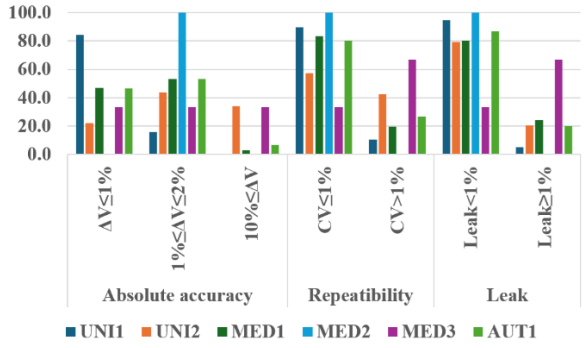
There are no earlier publications available about real-laboratory situation usage of no-liquid pipette controlling devices of ATMOS-1 or analogues. The introductory training was essential for ensuring an understanding of the basics of techniques, tips and tricks, and best practices required to obtain comparable and reliable results. Data recorded in Atmos-1 memory revealed the diversity of the pipettes in use across institutions. Table 1 provides a summary of pipettes in use in the participating laboratories. The number of other pipette sizes (300 µl and 50 µl) was insufficient for meaningful summary and thus are not shown. Study results demonstrate substantial variation in the accuracy of analysed pipettes. The number of pipettes decreased with smaller volume sizes, and it is notable that smallest volume pipets were used only in university laboratories. Pipette performance data were also analysed on the institutional level and are presented in Figure 1 and summarized in Table 2. The mean deviation values differed across the three institutional groups ($n_1 = 91$, $n_2 = 75$, $n_3 = 15$). A one-way analysis of variance (ANOVA) indicated a statistically significant overall effect of laboratory group on the measured variable ($p < 0.01$). Post hoc pairwise comparisons were performed using the Tukey–Kramer test to account for unequal sample sizes. The Tukey–Kramer analysis demonstrated that all pairwise differences between all groups were statistically significant. Specifically, UNI sector labs differed significantly from MED (medical diagnostic laboratories) ($p < 0.01$) and AUT (authority laboratory) ($p < 0.01$), while MED also differed significantly from AUT ($p < 0.01$). These results indicate that all groups are significantly distinct from one another. Superior pipette performance, and particularly accuracy, in authority and medical laboratories likely reflects regulated testing environments, where pipettes are routinely controlled and calibrated annually or semi-annually according to established rules. In two participated medical laboratories the verification is done locally by gravimetric method. In authority and one medical laboratory, calibration and verification are conducted by certified service providers. In academic (university research) laboratories, pipette calibration and verification are generally not regulated on university level. Practices vary by institute or laboratory, and verification is often only performed when there are reasonable doubts about accuracy.

Table 1

Pipette accuracies and precisions among different pipette sizes (absolute numbers)

		Absolute accuracy			Repeatability (precision)		Leak	
V (µl)	n	ΔV≤1%	1%≤ΔV≤2%	10%≤ΔV	CV ≤ 1 %	CV > 1%	Leak < 1 %	Leak ≥ 1%
1000	66	**39**	24	3	**48**	18	47	19
200	56	19	29	8	42	14	41	15
100	22	9	11	2	17	5	21	2
20	20	5	13	2	16	4	20	**0**
10	17	0	5	**12**	7	10	17	**0**
Total	181	72	82	27	130	51	146	36

Figure 1
Accuracy, repeatability and leakage rate data of pipettes in different institutions



It is also important to note that absolute accuracy deviations have a lower impact in medical and authority laboratories because manual pipetting is mainly limited to sample transfer and aliquoting. Analytical measurements are largely performed with fully automated analysers, reducing the risk of generating erroneous results; the primary concern is obtaining invalid results due to insufficient sample volume (AlShahrany & Ahmed, 2025). In contrast, in research laboratories, pipetting errors can have a greater effect, as deviation in pipetted volume may introduce systematic error. However, bigger volume pipettes tend to expose better accuracy (Lochner et al., 1996). Failures in precision (reproducibility) increase result bias, which can undermine scientific findings, especially when small differences in measured biological values are significant.

Leakage in pipettes can result from a lack of hermeticity, but is most often caused by incompatibility between the pipette and pipette tips (Art et al., 2016; Huber, 2025). Leakage problems were frequently resolved (but not always, with at least six known unresolved cases) by replacing pipette tips as already decades ago was demonstrated by Joyce & Tyler (1973).

This common issue may be partly due to purchasing practices that prioritize price, resulting in suboptimal compatibility between pipettes and tips.

Table 2
Accuracies and precisions of pipettes in different laboratory sectors (research, medical, and authority laboratories) (%)

		Absolute accuracy			Repeatability (precision)		Leak	
Sector	n	ΔV≤1%	1% ≤ΔV≤2%	10%≤ΔV	CV ≤ 1 %	CV > 1%	Leak < 1 %	Leak ≥ 1%
UNI	92	35.2	38.5	**26.4**	64.8	**35.2**	83.5	16.5
MED	74	44.0	52.0	**4.0**	78.7	21.3	76.0	25.3
AUT	15	**46.7**	**53.3**	**0.0**	80.0	20.0	86.7	**13.3**

Conclusions

1. Atmos-1 proved as reliable and easy-to-use pipette accuracy tester in all types of laboratories.
2. There is a substantial variation in pipette accuracy across sectors. Pipette accuracy and precision varied widely between laboratories, with university research labs showing the highest rates of unacceptable deviations and medical/authority labs demonstrating better accuracy and precision.
3. Authority and medical laboratories, which follow regular calibration and verification protocols, consistently maintained pipette accuracy within acceptable limits, highlighting the importance of routine, regulated quality control.
4. The smallest volume pipettes (10–20 µl), predominantly used in university labs, had the highest rates of unacceptable deviations, emphasizing the need for special attention to small-volume pipetting.
5. Pipette leakage is commonly due to tip incompatibility. Most leakage issues were resolved by switching to compatible pipette tips, suggesting that procurement practices focusing solely on cost can undermine pipetting reliability through poor tip compatibility.

Acknowledgements

The author expresses his deep gratitude to Surgitech AS for providing the ATMOS-1 instrument for the study and for approving its use in real-life laboratory conditions.

References

Al Shahrany, A. & Ahmed, K. M. (2025). Detailed improved uncertainty analysis for micropipette calibration in the range below 10 µL. *Measurement: Sensors. Supplement,* 38, Article 101528. https://doi.org/10.1016/j.measen.2024.101528

Art, M., Dufey, V., Gast, U., Gligor, I., Koch, L., & Kubasch, R. (2016). *The Tip of the Iceberg: How Pipette Tips Influence Results.* Application note No. 354, Eppendorf. https://www.pipettes.com/pub/media/pdf/Eppendorf_Liquid-Handling_How-Pipette-Tips-Influence-Results.pdf

Batista, E., Pinto, L., Filipe, E., & van der Veen, A. M. H. (2007). Calibration of Micropipettes: Test Methods and Uncertainty Analysis. *Measurement, 40*(3), 338–342. https://doi.org/10.1016/j.measurement.2006.05. 012

Blues, J., Davis, B., & Buckley, M. (2004). *Measurement Good Practice Guide No. 69, the Calibration and Use of Piston Pipettes.* United Kingdom, National Physical Laboratory. https://pipette.com/mm5/pdfs/knowledgebase/UKAS MGP Guide 69_Calibration and Use of Piston Pipettes.pdf

Carle, B. A., Rodrigues, G. W., & Curtis, R. H. (2009). *Transferability of Pipet Calibrations and Proficiency of Operators: Prerequisites for Method Validations and Method Transfers*. American Laboratory. https:/www.americanlaboratory.com/913-Technical-Articles/1411-Transferability-of-Pipet-Calibrations-and-Proficiency-of-Operators-Prerequisites-for-Method-Validations-and-Method-Transfers/

Cremonesi, P., Pisani, L. F., Lecchi, C., Ceciliani, F., Martino, P., Bonastre, A. S., …, & Castiglioni, B. (2014). Development of 23 individual TaqMan® real-time PCR assays for identifying common foodborne pathogens using a single set of amplification conditions. *Food Microbiology,* 43, 35–40. https://doi.org/10.1016/j.fm.2014.04.007

Guan, X. L., Chang, D. P. S., Mok, Z. X., & Lee, B. (2023). Assessing variations in manual pipetting: An under-investigated requirement of good laboratory practice. *Journal of Mass Spectrometry and Advances in the Clinical Lab,* 30, 25-29. https://doi.org/10.1016/j.jmsacl.2023.09.001

Huber, M. (2025). Good pipetting practice – A science based approach for quality management of pipetting. Measurement: Sensors. *Supplement,* 38, Article 101553. https://doi.org/10.1016/j.measen.2024.101553

Joyce, D. N. & Tyler, J. P. (1973). Accuracy, precision and temperature dependence of disposable tip pipettes. *Med Lab Technol, 30*(4), 331-334. Article 4803941.

Karus, A., Ceciliani, F., Bonastre, A. S., & Karus, V. (2017). Development of simple multiplex real-time PCR assays for foodborne pathogens detection and identification on lightcycler. *Macedonian Veterinary Review, 40*(1), 53-58. https://doi.org/10.1515/macvetrev-2017-0010

Karus, A. & Karus, V. (2018). Food research opportunities and challenges: methods in food safety and functional food development: a review. *Research for Rural Development,* 1, 211−214. https://doi.org/10.22616/rrd.24.2018.033

Karus, A. & Karus, V. (2024). Robustness of Mycobacterium Bovis Determination Using Fast and Simple qPCR Assay. *Veterinarija ir Zootechnika, 82*(1), 13-18, https://vetzoo.lsmuni.lt/data/vols/2024/8201/lt/karus.pdf

Kramer, C. Y. (1956). Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics, 12*(3), 307–310. https://doi.org/10.2307/3001469

Lochner, K. H. (1998). Pipettenprüfung mit gravimetrischer und photometrischer Methode: ein Vergleich [Pipette Check with Gravimetric and Photometric Method: A Comparison]. *LaboratoriumsMedizin/Journal of Laboratory Medizine, 22*(7-8), 401-406. https://doi.org/10.1515/labm.1998.22.7-8.401

Lochner, K. H., Ballweg, T., & Fahrenkrog, H.-H. (1996). Untersuchungen zur Messgenauigkeit von Kolbenhubpipetten mit Luftpolster [Investigations into the measurement accuracy of piston-stroke pipettes with air cushion]. *Lab Med, 20*(7/8), 430–440. https://doi.org/10.1515/labm.1996.20.7-8.430

Pati, S., Mandal, G., Singh, N., Kumar, M., & Sharma, D. C. (2021). A Pilot Study on the Comparison of the Methods for Uncertainty Analysis of Micropipette Calibration. *MAPAN-Journal of Metrology Society of India, 36*(2), 343–347. https://doi.org/10.1007/s12647-021-00459-9

Tukey, J. W. (1949). Comparing individual means in the analysis of variance. *Biometrics, 5*(2), 99–114. https://doi.org/10.2307/3001913

UniPix. (2025*). ATMOS Pipette Accuracy Tester. Operation manual.* https://www.unipix-atmos.com/atmos/downloads/?file=files/unipix-lab/daten/Technical%20Documentation/atmos_manual.pdf&cid=1350

Yamamoto, T., Yamamoto, T., Yoshida, K., Fuchinoue, S., Hayama, Y., Hidano, A., & Tsutsui, T. (2014). Effectiveness of a short training session for improving pipetting accuracy. *Accred. Qual. Assur,* 19, 459–463. https://doi.org/10.1007/s00769-014-1083-2