



Determination of Selenium in Goat and Sheep Sera by ICP-OES with Continuous Flow Hydride Generation Using a Modified Pre-Reduction and Digestion Method

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Background: Selenium deficiency leads to muscle weakness and poor reproductive performance in goats and sheep. Determination of selenium (Se) by ICP-OES is challenging because this trace element is a weak emitter, which makes it difficult to measure low levels in serum samples. One technique to enhance the sensitivity of Se is gas-phase hydride conversion. In addition, the combination procedures of pre-reduction and digestion are used to improve the limit of detection. Our recent in-house Se analytical method is complicated, time-consuming, and suffers from excessive hazardous acids. Therefore, a simplified and effective modified method is necessary to improve diagnostic readiness and strengthen laboratory capacity.

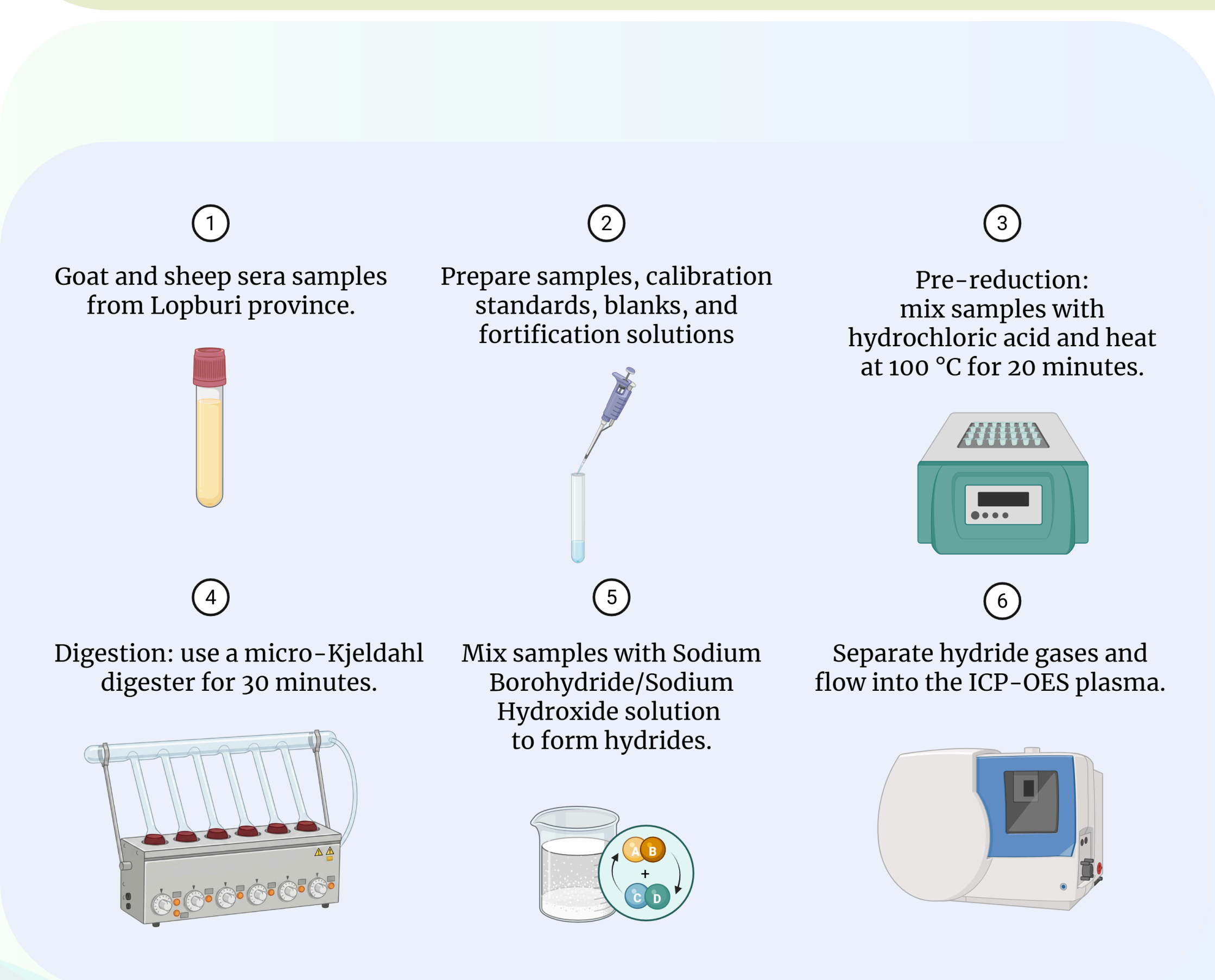
Objectives: This study aimed to determine selenium concentrations in goat and sheep sera, assess method accuracy using percent recoveries of fortified method blanks (FMB), fortified analytical solutions (FAS), and fortified analytical portions (FAP), and evaluate method precision using relative percent difference (RPD) and relative standard deviation (RSD) of replicate samples.

Methods: Nineteen goat sera and nineteen sheep sera were collected from the same origin in Lopburi province in October, 2025. All samples, calibration standards, blanks, and fortification solutions were prepared in 1% nitric acid. In the pre-reduction step, samples were mixed with a small volume of concentrated hydrochloric acid and heated in a dry block heater at 100 °C for 20 minutes prior to digestion. Then, a micro-Kjeldahl digester was used for 30 minutes. After that, the samples were mixed in the chemifold assembly with the Sodium Borohydride/Sodium Hydroxide solution to form the hydrides. The hydride gases were separated from the liquid and continuously flowed into the ICP-OES plasma. All quantitative Se measurements were made against linear through-zero external calibration curves from 10, 25, 50, 75, 100 µg/L. The limit of detection (LOD), limit of quantitation (LOQ), recoveries, RPD, and RSD were calculated using Microsoft Excel®. Demonstrated Se levels in goat and sheep sera compared to the reference range values (goat 62-158 µg/L, sheep 55-170 µg/L). Likewise, the method performance parameters were evaluated by method criteria acceptance.

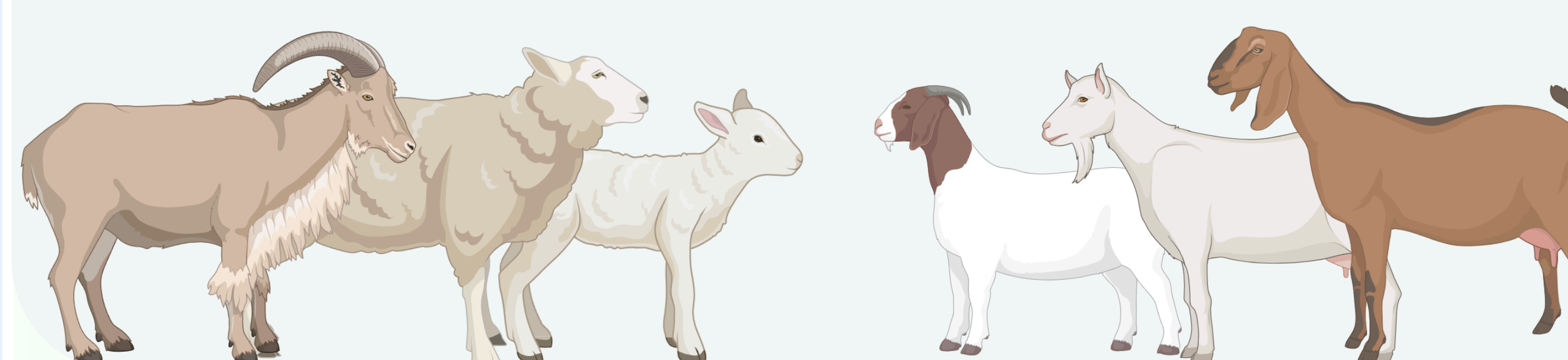
Results and Discussion: LOD of this modified method was 2 µg/L, and LOQ was 9 µg/L. Linearity was confirmed across the concentration range of 10 to 100 µg/L with a correlation coefficient (r) of 0.9987. Percent recoveries of FMB, FAS, and FAP for mid-level fortified solutions in goat matrix serum were 96%, 91%, and 81%, respectively. Percent recoveries of FMB, FAS, and FAP for mid-level fortified solutions in sheep matrix serum were 96%, 92%, and 83%, respectively. The average RPDs of representative duplicate goat and sheep sera were 4.9% and 9.5%, respectively. The average RSDs of ICP-OES triplicate measurements of goat and sheep pooled sera were 3.5% and 2.8%, respectively. All accuracy and precision parameters met the predefined acceptance criteria. The method demonstrated consistent robustness across different animal matrices, remaining stable for both goat and sheep sera.

The concentration ranges of Se in goat and sheep sera were <9 – 67.73 µg/L and 26.64 – 58.16 µg/L, respectively. For goat sera, only 16% (3/19) of the samples were within the normal range, leaving 84% (16/19) interpreted as selenium deficiency. Furthermore, 95% (18/19) of sheep were determined to have selenium deficiency, while only 5% (1/19) reached a normal level. These findings revealed a significant health concern for this studied group. The practical application of this modified method could detect a high prevalence of selenium deficiency in a field sample of goats and sheep.

Conclusion: This study successfully established a modified pre-reduction and digestion procedure for Se determination by ICP-OES assembled with hydride generation chemifold. This method not only increases analytical sensitivity but also overcomes the limitations of the hazardous traditional method. The new modified method proved to be a reliable and efficient tool for Se diagnostic preparedness and was crucial for identifying livestock health status. As evidenced by this investigation in these small ruminants, there is an urgent need for nutritional intervention and improved selenium supplementation strategies.



Parameter	Method value	Criteria or recommendation
correlation coefficient (r)	0.9987	$r \geq 0.995$ (EPA 200.7)
concentration range	10 - 100 µg/L	-
LOD	2 µg/L	≤ 5 µg/L (ICH Q2 (R2))
LOQ	9 µg/L	≤ 10 µg/L (CLSI EP17)
FMB recovery	96 %	90 - 110 % (EPA 200.7)
FAS recovery	91 - 92 %	80 - 120 % (EPA 200.7)
FAP recovery	81 - 83 %	70 - 130 % (EPA 200.8)
% RSD	2.8 - 3.5 %	$\leq 15\%$ (AOAC Appendix F)
% RPD	4.9 - 9.5 %	$\leq 20\%$ (EPA 200.8 adapted)



References

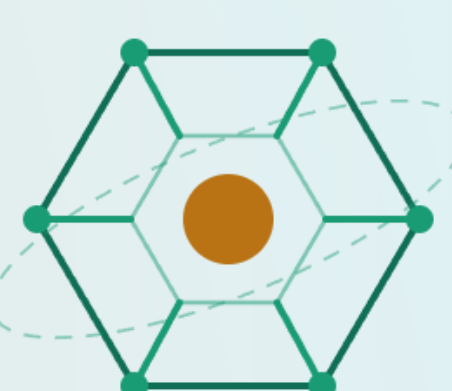
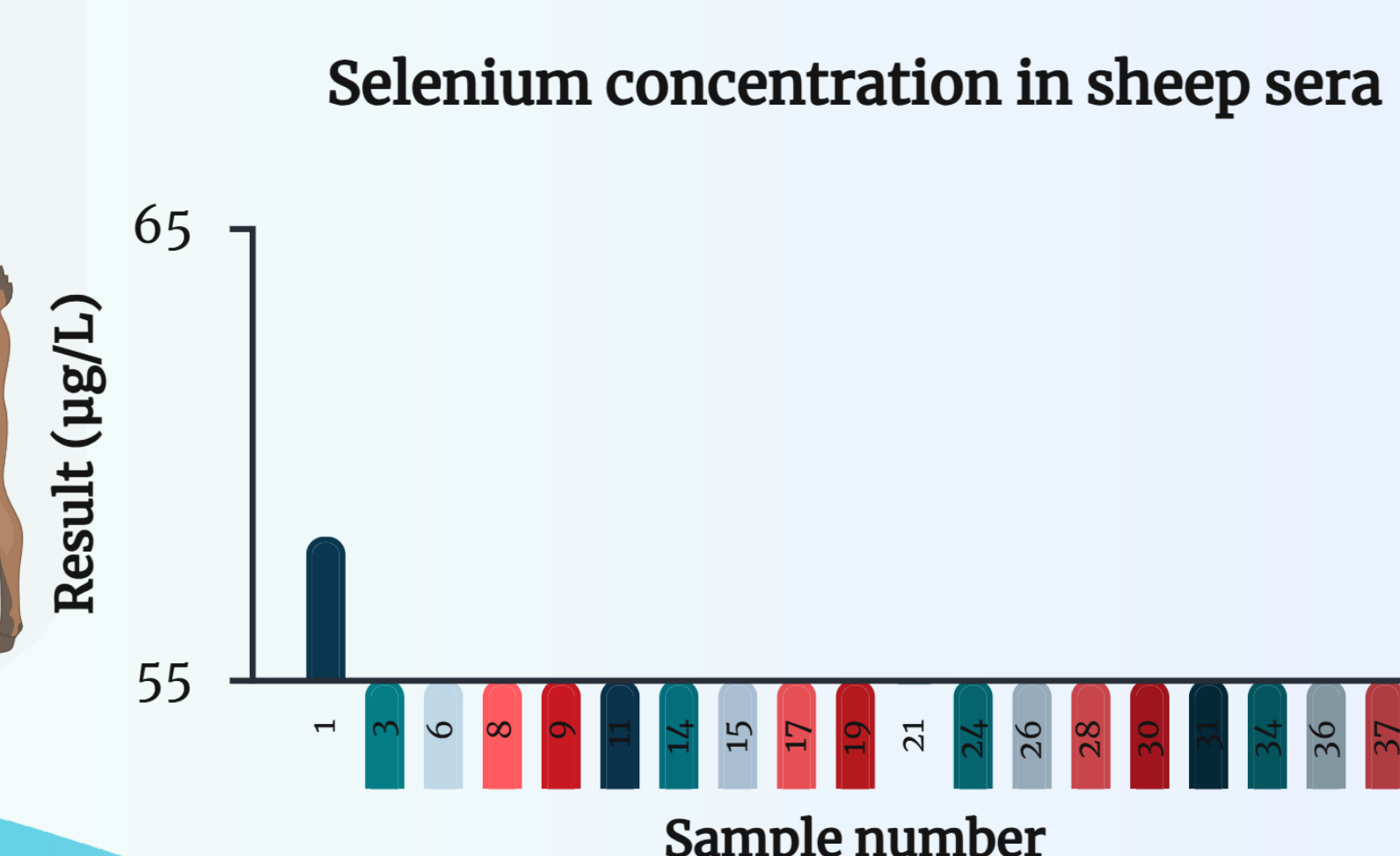
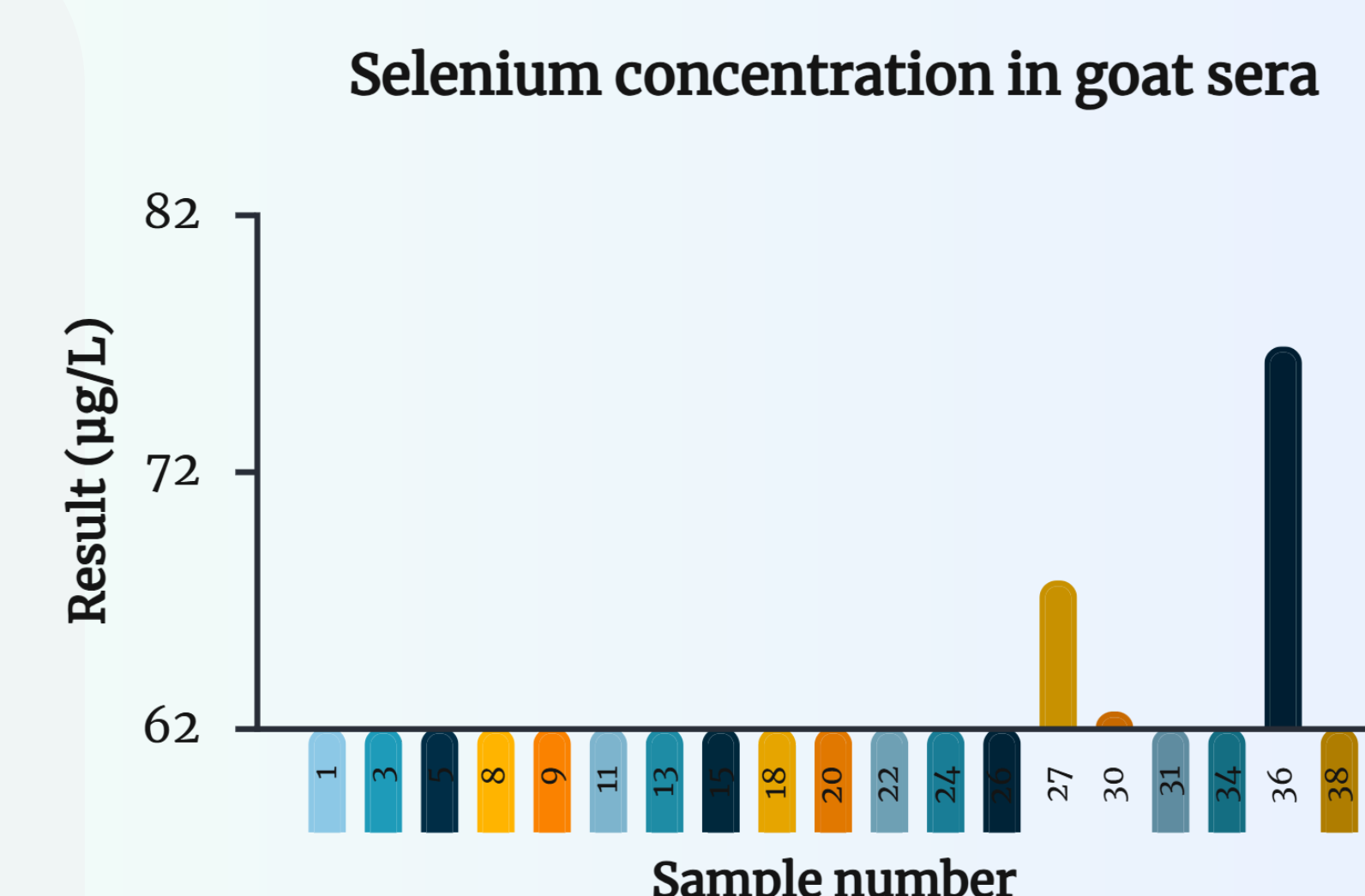
Bosnak, C. P., & Davidowski, L. (2004). Continuous flow hydride generation using the Optima ICP (Field Application Report No. 007062_01). PerkinElmer Life and Analytical Sciences.

Laboklin Laboratory for Clinical Diagnostics. (2022). Reference values: Cattle, sheep, goat, pig [Reference poster]. Laboklin GmbH & Co. KG. <https://www.laboklin.com>

Neubauer, K., & Magarini, R. (2021). Determination of arsenic and selenium in drinking water by ICP-OES with continuous flow hydride generation using a single pre-reduction procedure (Application Note No. 338778). PerkinElmer, Inc. <https://www.perkinelmer.com>

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