

Toward standardisation in clinical diagnostics: Investigations into liquid handling robotics to underpin SI traceable, multiple analyte quantification

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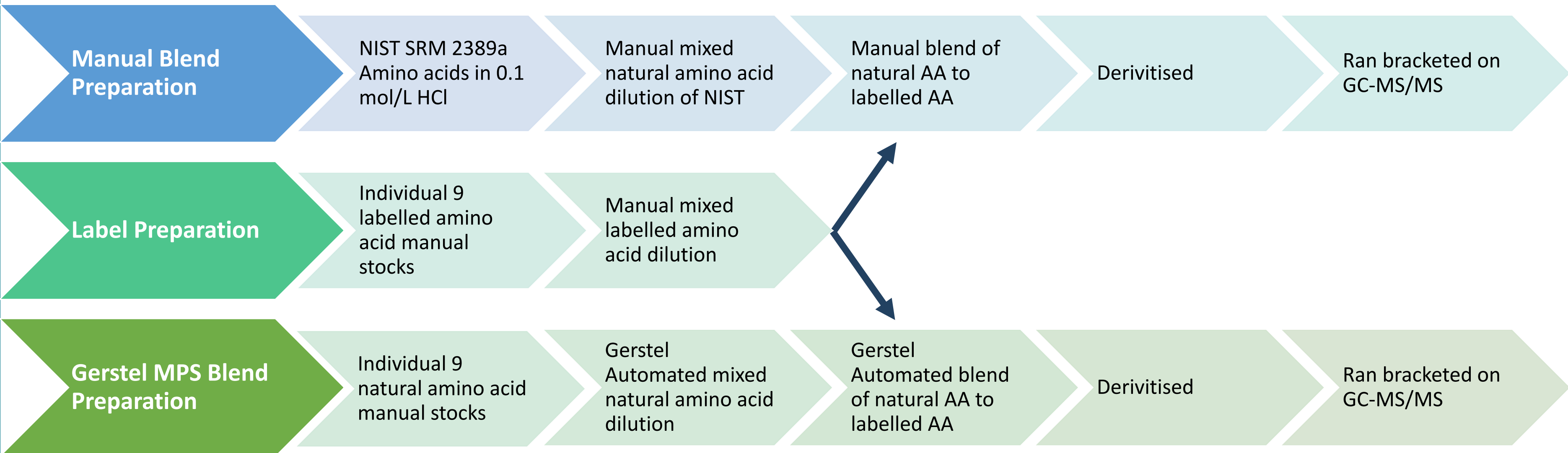
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Introduction

- Certified Reference Materials (CRMs) are needed to support standardisation of clinical diagnostics as required by ISO 15189 and the in-vitro diagnostic regulation (IVDR) EU/2017/746.
- ISO 17034 certification workflows for one or very few analytes usually span several months.
- To increase both the number of analytes and speed of CRM production, novel workflows are required that have the capability of traceably measuring multiple analytes, with high accuracy and low uncertainty.
- Therefore, the automation of sample preparation to underpin the characterisation and monitoring processes by use of liquid handling robots with integrated balances has been investigated. In this vein, this work highlights advances and key challenges.
- Amino acids were used as the model system for evaluating the liquid handling robot by completing a standard comparison against a known CRM (NIST SRM 2389a Amino acids in 0.1 mol/L HCl).

Double exact matching isotope dilution mass spectrometry methodology

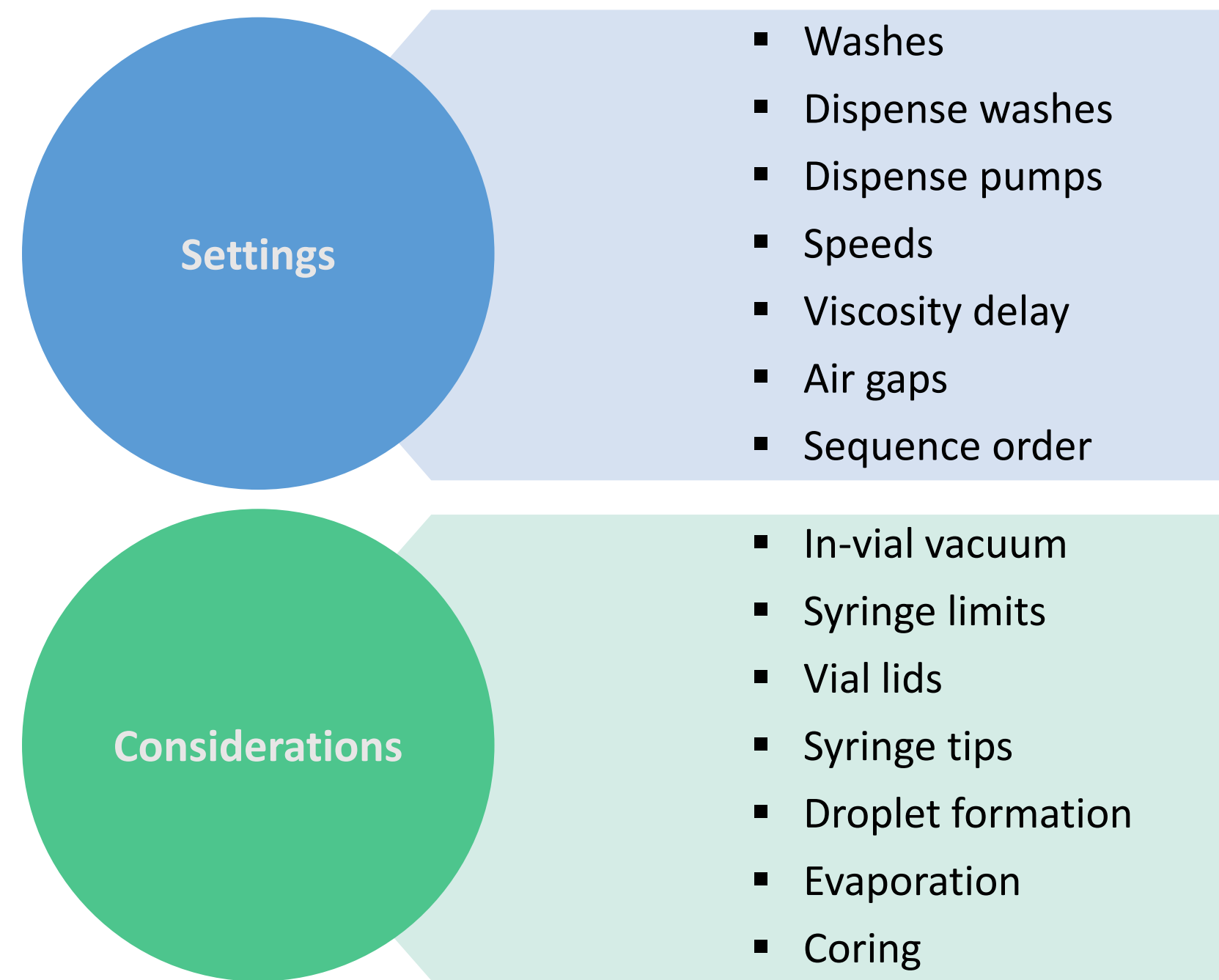


The Liquid Handling Robot

- 5 decimal place balance with de-ioniser
- Vortex mixer
- Access to 6 syringes per run
- Large solvent reservoir
- Large sample wash bottles
- Large waste bottle
- Pipette tool
- Two decapper tools
- 20 mL, 10 mL, 4 mL & 2 mL vials
- Future flexibility

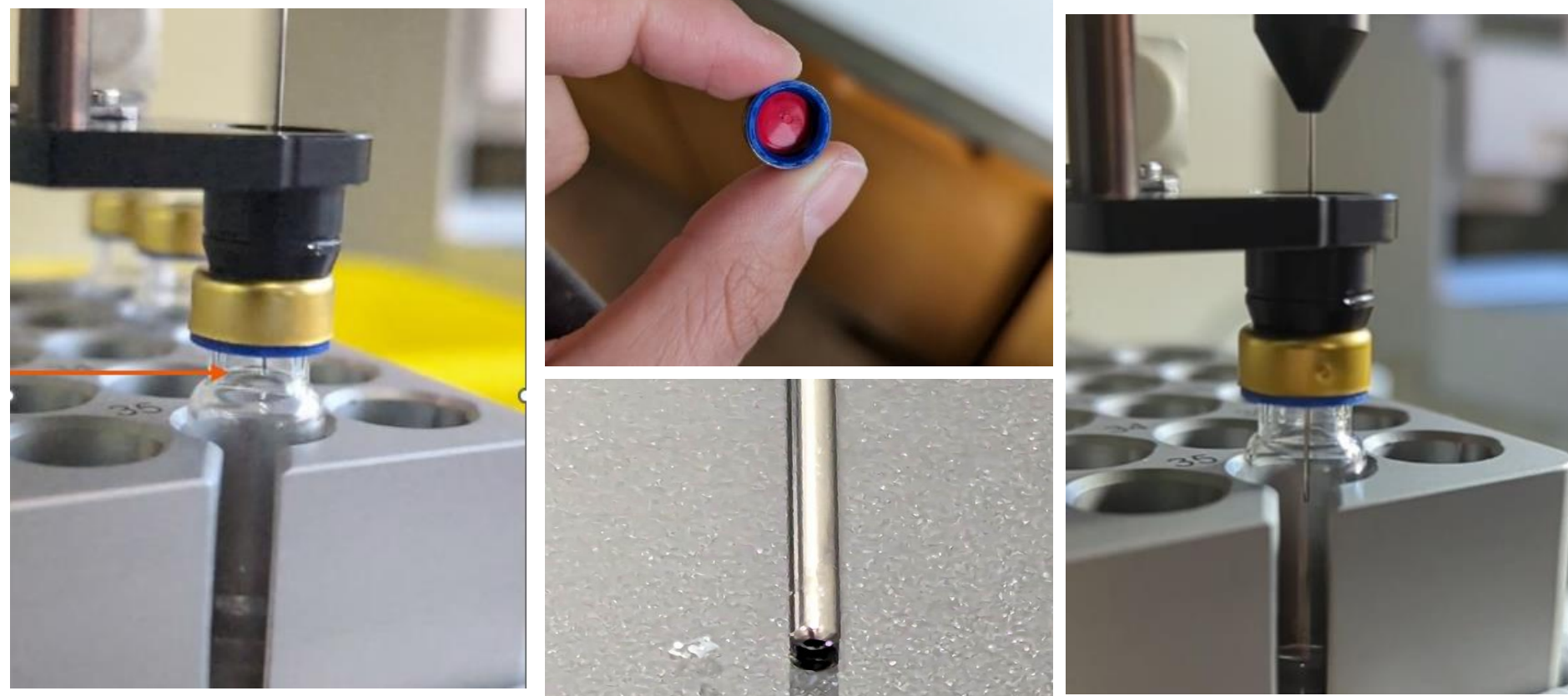


Robot optimisation



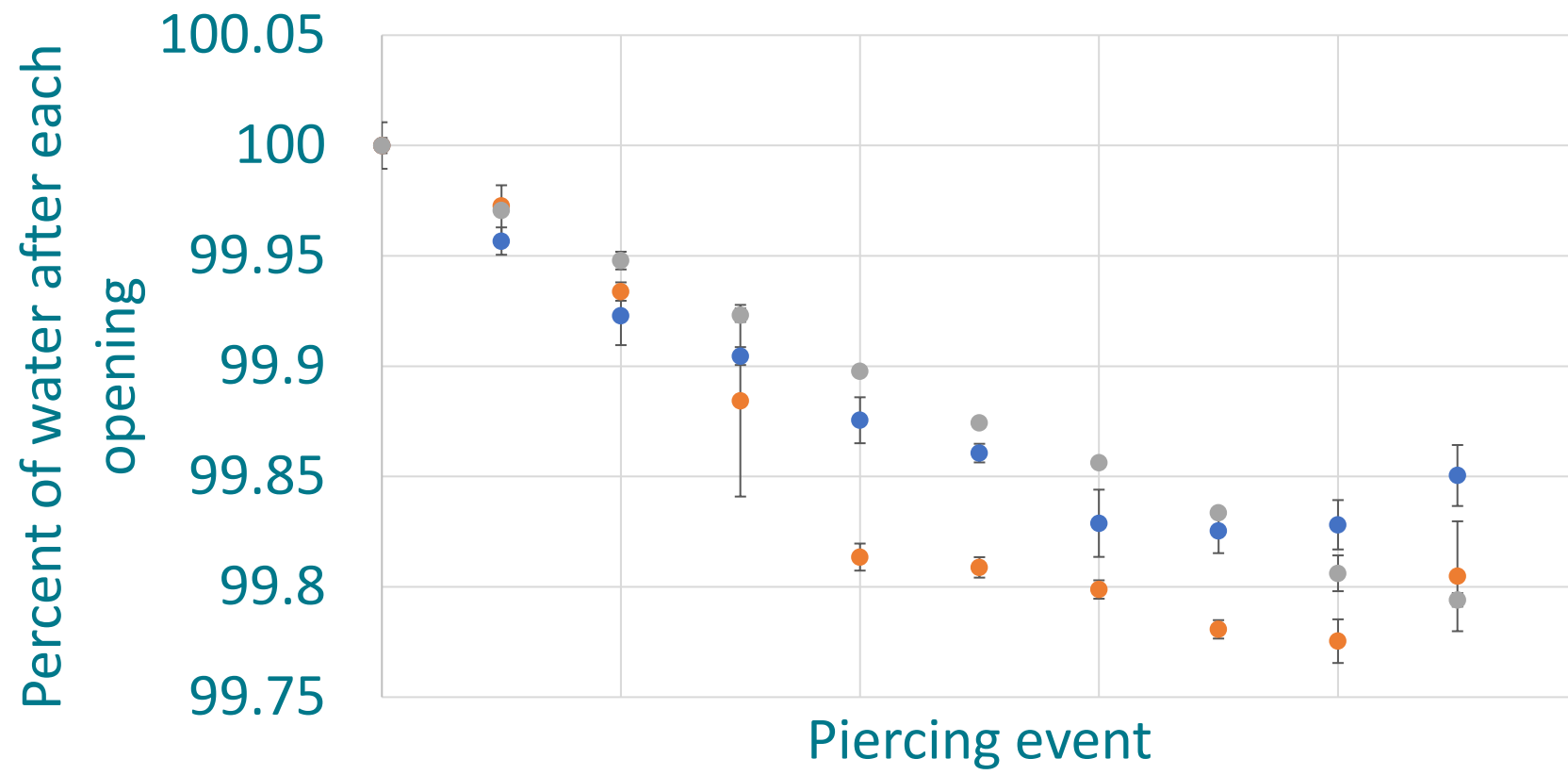
Septa Piercing

Using a syringe piercing through a septa caused many inconsistent results due to droplets that were caught in the septa and accounted for in the weight but not in the mass fraction. The tip of the syringe, air gaps and speeds were all optimised, but there was still variable data.



The left shows a droplet forming when using the 100 µL blunt tip syringe and the droplet being caught in the septa (middle, top). The right picture shows the lack of a droplet when optimising speeds on a 250 µL cone ended tip, however this result was variable. Pictures on the left and right are stills from videos filming at 8x slow speed. Picture in the middle bottom shows coring.

Evaporation of 100 mg of water from 10 mL vial after piercing the septa with a syringe



The evaporation from the pierced vial was also variable. The colours show three different vials being pierced repeatedly. The error bars are equal to +/- the standard deviation of three weight replicates (n=3)

Decapping

Decapping shows much more consistent evaporation and less variability issues, however vial choice and sequence order becomes important to minimise the percent of evaporation.

Evaporation of water from 10 mL vial after opening the lid, waiting 2 minutes and closing the lid



Error bars are equal to +/- the standard deviation of analytical replicates (n=3)

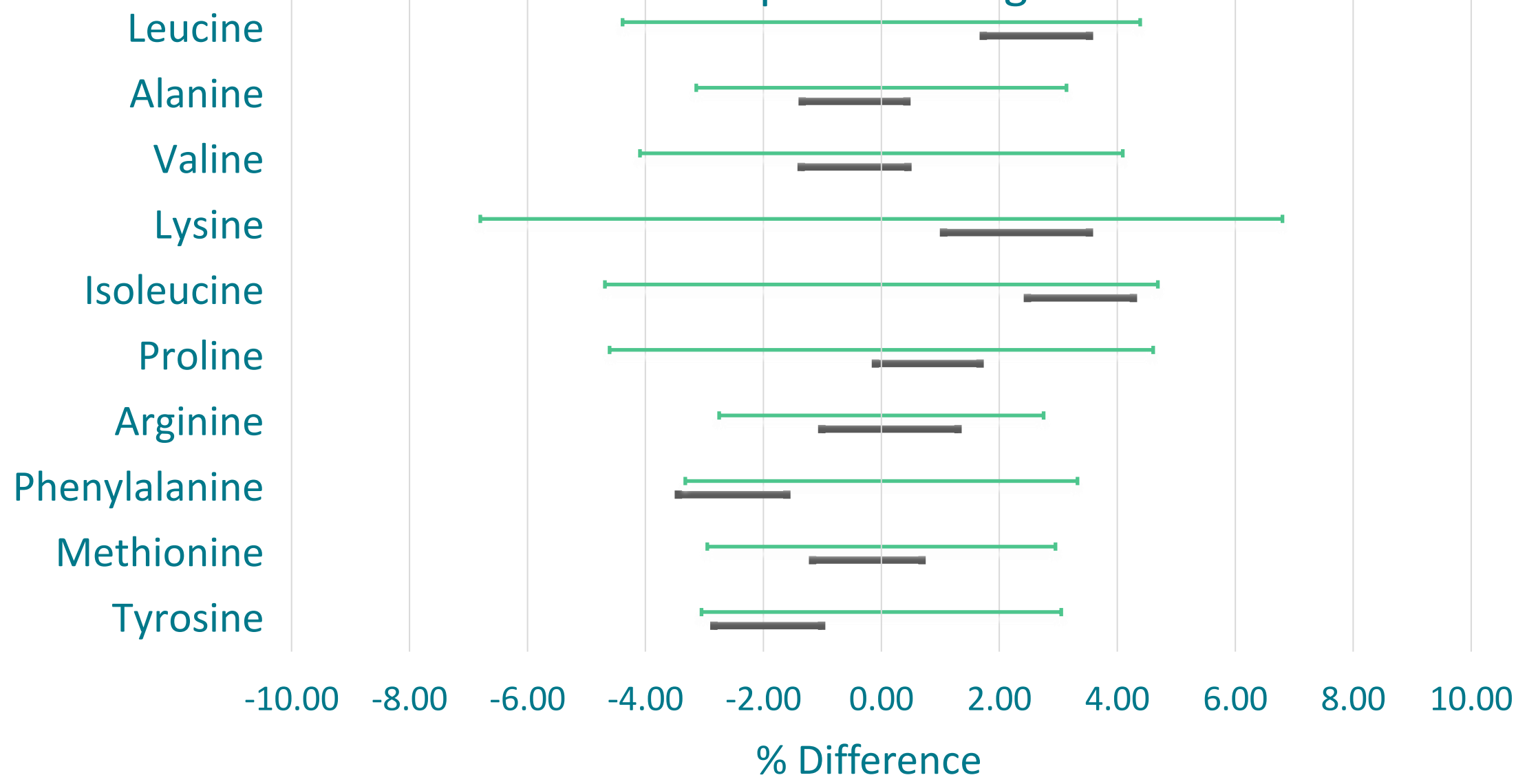
Results

After optimisation and use of decappers, the robot prepared a ten amino acid calibrator which value assigned the NIST SRM 2389a material successfully.

The certified value of the NIST material is compared to the measured value. The Green error bars are equal to +/- the expanded uncertainty of the NIST certified value (k=2). The black error bars are equal to +/- the expanded uncertainty of the measured value which are all < 1.6% expanded (k=2).

All the values calculated by using the robotic workflow are encompassed by the uncertainty of the NIST material.

NIST SRM 2389a certified value vs measured value using robotic liquid handling



Conclusions

- Industrial and clinical demands involving multi-analyte applications have driven the need for metrology institutes to underpin traceability through development of traceable methods and reference materials for multiple analytes using cost and time effective workflows - automation may be the key to achieve this.
- During optimisation of an automated method, using weighing to test accuracy of volume additions is a good first step, but there could be other sources of bias. Settings such as speeds, pumps, washes, sequence order and air gaps all need consideration. Physical set up such as syringe tip, lids and vial sizes need to be considered, as well as wash solvents. Furthermore, variability due to things like droplet formation, evaporation, coring, and in vial vacuum effects can be observed.
- The mass fraction of the NIST SRM 2389A was accurately determined within the uncertainty of the certified value for the ten amino acids chosen in this model system when using a decapper and a mixed calibrator prepared by the multipurpose sampler robot.
- This work will continue by developing automation based higher order reference methods for estradiol in plasma and therapeutic drug monitoring in blood with the Euramet MetPart project "Manufacturing of commutable calibrators and quality control materials for standardisation and post-market surveillance of IVD tests" (23IND02 COMET) as well as being used to assign values to patient samples for commutability assessments.

