

Transitioning from laboratory-developed tests to a single commercial reagent kit in a national newborn screening program: impact on analytical performance and harmonization.

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Study overview

Study type	Multi-center national verification study
Country	England, United Kingdom
Setting	13 NHS Newborn Screening (NBS) laboratories
Revvity product	NeoBase™ 2 (NB2) Non-Derivatized MSMS Kit
Study design	22,049 residual NBS specimens analyzed with NeoBase™ 2 kit compared with historical data from 131,128 NBS specimens analyzed with laboratory developed test (LDT)
Publication year	2026

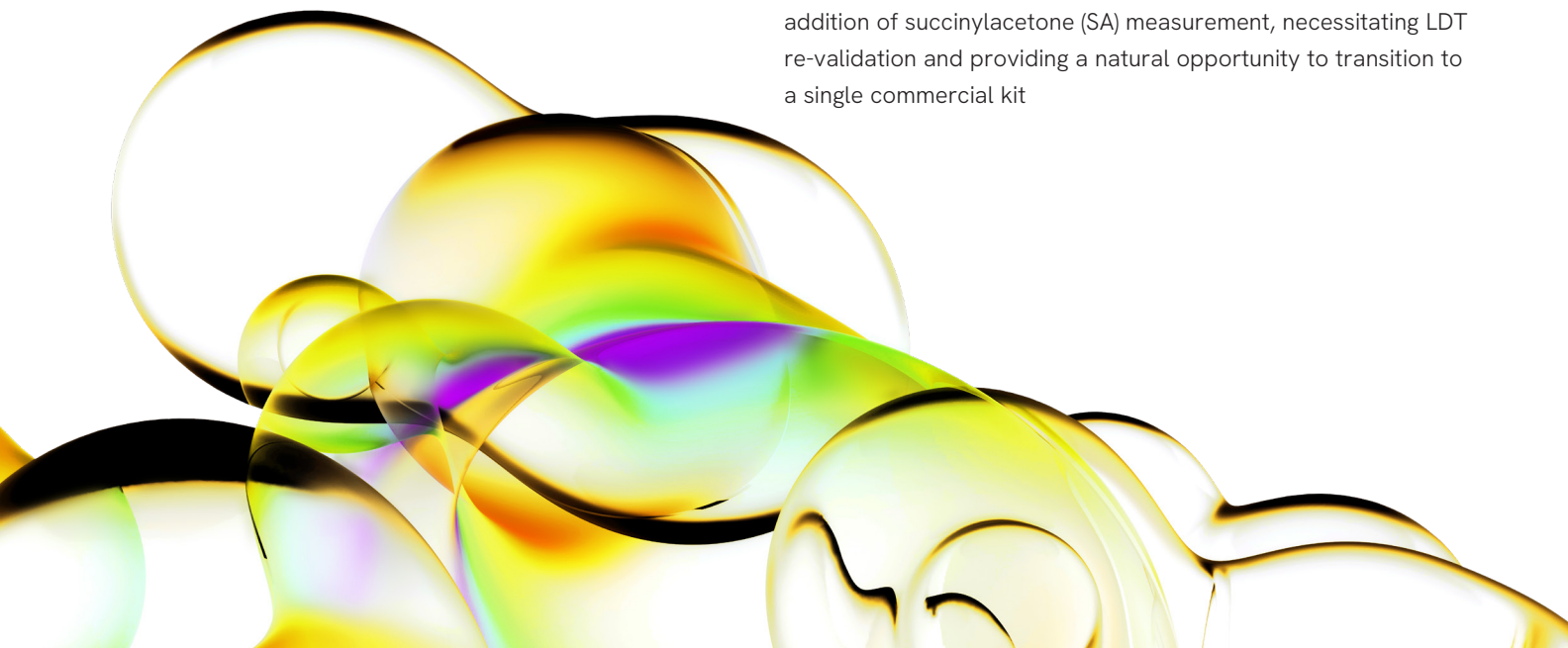
Background and rationale

Newborn screening (NBS) in England is a national program screening all babies for nine disorders, with six inherited metabolic diseases (IMDs) detected by flow injection analysis tandem mass spectrometry (FIA-MS/MS). Until recently, **10 of 13 laboratories** used laboratory-developed tests (LDTs) and 3 used commercial assays, creating persistent challenges in **inter-laboratory harmonization**.

Two key drivers prompted change:

- **Regulatory pressure:** The transition toward IVD Regulation (IVDR) in Europe and anticipated MHRA regulatory reform in the UK highlighted the growing disadvantages of LDTs

Program expansion: The 2024 English government approval of **hereditary tyrosinemia type 1 (HT1)** screening required addition of succinylacetone (SA) measurement, necessitating LDT re-validation and providing a natural opportunity to transition to a single commercial kit



The NeoBase™ 2 (NB2) kit was selected as the single commercial reagent kit for all 13 English NBS laboratories, with the dual aim of improving analytical performance and harmonization across the program.

Study objectives

1. Verify analytical performance of the NB2 kit across all 13 English NBS laboratories
2. Compare population distributions obtained using the NB2 kit with historic LDT data
3. Determine whether transition to a single commercial kit improved inter-laboratory harmonization
4. Establish evidence-based screening cut-off values (COVs) for all analytes, including SA

Methods

Instrumentation: Each of the 13 laboratories verified the NB2 kit on two instruments — a total of 26 instruments across three MS platforms:

Platform	Models	n Instruments
Waters (Milford, MA, USA)	TQD, Xevo TQD, TQ-S micro	17
Sciex (Framingham, MA, USA)	API 4500, 5000, 5500, 6500	7
Shimadzu (Kyoto, Japan)	8050	2

NeoBase™ 2 Non-derivatized MSMS kit is only validated for newborn screening with following instruments: Waters TQD, Waters Xevo TQD and Sciex 4500MD.

Analytes measured: PHE, TYR, LEU, MET, C5, C5DC, C8, C10 carnitines, and SA

Key verification parameters assessed:

- Linearity ($R^2 > 0.99$, $n = 6$ DBS multi-level materials)
- Imprecision: intra- and inter-laboratory ($CV \leq 15\%$; $\leq 20\%$ at LLOQ)
- Recovery (relative to Revvity multi-level DBS materials)
- Carry-over
- On-board stability
- Inter-lot variation
- Instrument-specific relative response factors (RRFs)
- Population distributions vs. historic LDT data ($n \approx 1,000$ residual specimens per instrument)

Key results

Analytical performance

Parameter	Finding
Linearity	Demonstrated across clinically relevant ranges for all analytes on most instruments
Intra-laboratory imprecision	Mean CV 8–13% across all analytes and concentrations
Inter-laboratory imprecision	$CV \leq 15\%$ for amino acids and most acylcarnitines; C5DC 14–19%
SA imprecision (low level)	CV 20.8%, limited by instrument-dependent background interference
Recovery (all analytes except SA & C10)	Mean $100 \pm 20\%$, acceptable
SA recovery	Mean 53% (range 31–72%), consistently low across all instruments
C10 recovery	Mean 112% (range 97–130%), consistent over-recovery
Carry-over	Negligible for all analytes on all instruments
On-board stability	Acceptable for all analytes except SA in methanol reconstitution (20.1% bias)

Relative response factors (RRFs)

- RRFs ranged from 0.72 to 1.61 across the nine analytes and 26 instruments
- 13% of RRFs adjusted measured concentrations by >20% inconsistent with the theoretical principles of stable isotope dilution MS (SID-MS)
- Widest RRF ranges observed for C10 carnitine (0.98–1.61), SA (0.72–1.34), and C5DC (0.77–1.55)
- Harmonization was only achievable after application of instrument-specific RRFs, highlighting that harmonization ≠ standardization

Harmonization: The NB2 kit vs. LDTs

NB2 demonstrated reduced inter-laboratory variability for all existing analytes compared with LDTs:

Analyte	Ratio of Between-lab SDs (MAD)	Interpretation
PHE	0.80	✓ Improved
TYR	0.78	✓ Improved
LEU	1.11	⚠ Marginal increase
MET	0.88	✓ Improved
C5	0.47	✓ Substantially improved
C5DC	0.81	✓ Improved
C8	0.33	✓ Substantially improved
C10	0.43	✓ Substantially improved

Note: Ratios < 1.0 indicate reduced inter-laboratory variation with the NB2 kit vs. LDTs. All acylcarnitines showed the greatest gains.

The NB2 kit showed a negative bias relative to LDTs across all analytes (range: –1.4% for TYR to –39.6% for C5DC at the median). Following expert multidisciplinary review, existing LDT-derived COVs were retained for PKU, MCADD, GA1, MSUD, HCU and IVA, as classical presentations would continue to be reliably detected.

Succinylacetone (SA) - Key analytical challenge

- SA measurement is complicated by instrument-dependent background interference from the methionine stable isotope label (MET SIL)
- Background signal ranged from ~0.2 µmol/L (Waters Xevo TQD MS/MS) to ~0.7 µmol/L (Sciex 6500)
- This interference effectively defines the instrument-specific LLOQ for SA
- International SA COVs range from 0.3–7.0 µmol/L, direct adoption of published thresholds is not appropriate without reference to local instrument performance
- Based on UK population data (P99 range: 0.21–0.99 µmol/L across 26 instruments) and international NB2 experience, the English program set:
 - Clinical COV: 1.7 µmol/L
 - Analytical COV: 1.3 µmol/L

Conclusions

- Transition to the NeoBase 2 kit across all 13 English NBS laboratories achieved improved inter-laboratory harmonization for all analytes compared with heterogeneous LDTs
- Harmonization required application of instrument-specific RRFs, demonstrating that harmonization through correction factors does not equate to true analytical standardization
- SA measurement remains an instrument-dependent analytical challenge; laboratories must establish local LLOQs and evidence-based COVs rather than adopting published thresholds
- The study underscores the international need for traceable, matrix-matched certified reference materials and robust calibration hierarchies

Relevance to Revvity

Relevance	Detail
Product validated	NeoBase 2 (NB2) Non-Derivatized MSMS Kit
Scale of validation	First national, multi-center, multi-instrument evaluation of the NB2 kit in a single integrated NBS program
Program expansion	The NB2 kit enabled addition of HT1/SA screening to the existing 8-analyte panel
Harmonization benefit	The NB2 kit reduced inter-laboratory variation vs. LDTs across all analytes — supporting the case for commercial kit adoption
Revvity support acknowledged	Authors explicitly acknowledge the Revvity team for technical support during implementation
Regulatory context	Study supports the transition away from LDTs in the context of evolving IVDR/MHRA regulatory frameworks

NeoBase™ 2 Non-derivatized MSMS kit is a CE marked *in vitro* diagnostic medical device, compliant with IVD Regulation (EU) 2017/746, certified by Notified Body number 0537, Eurofins Electric & Electronics Finland Oy.

This material is a summary of information published in “Carling RS, Barclay ZJ, Ward SC, et al. International Journal of Neonatal Screening 2026; 12: 41” and not claims made by Revvity. The instrumentation and methods used in the study may not have be validated with the NeoBase™ 2 Non-derivatized MSMS kit.

