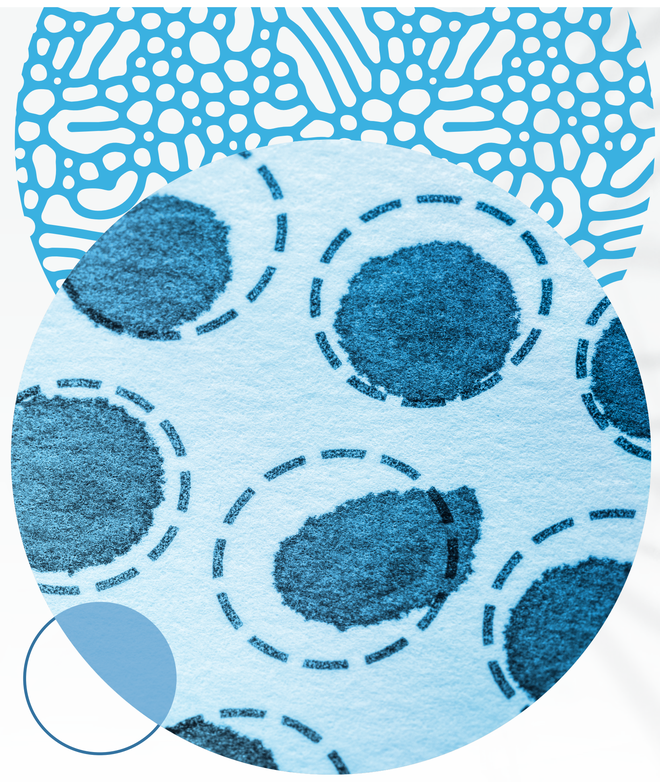
A seven-year review of a dried bloodspot external quality assurance program



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Introduction

The RCPAQAP Dried Blood Spot (DBS) External Quality Assurance (EQA) Program, established in 2019, was developed to provide local quality assurance resources for laboratories performing newborn bloodspot screening (NBS) for inborn errors of metabolism and other conditions.

The program began with five core analytes distributed across six linearly related concentrations. Over the following years, the number of measurands was progressively expanded, reaching fifteen with the addition of galactose in 2025. In 2020, interpretation of 'patient' sample measurand results was incorporated to capture variations in laboratory screening protocols. We aim to outline the program's evolution, summarised in Table 1, including the rationale for adding new analytes and assessing overall performance.

Method

Twelve linearly related samples and six individual patient samples comprising three 75 µL whole blood spots applied to filter paper were distributed annually to enrolled participants. Participant results were reviewed using performance criteria determined by the Inborn Errors Advisory Committee. The 'patient' samples were designed to simulate a screening scenario. Laboratories were asked to classify results by selecting one of several follow-up actions: normal, recollect, reflex second-tier testing, or referral for clinical follow-up.

Results

By 2025, the DBS EQA program included 25 enrolled laboratories across seven countries. Figure 1 shows performance improvements for several analytes, including 11-deoxycortisol, 17-hydroxyprogesterone, 21-deoxycortisol, methionine and thyroid stimulating hormone. However, notable variation persists for phenylalanine and tyrosine, with coefficients of variation (CVs) exceeding 13% (n=11). Figure 2 demonstrates that laboratories consistently identified normal 'patient' sample results, with >95% concordance across all distributions. Most laboratories recognised the need for further investigation when faced with abnormal results. However, the choice of action—whether recollection, clinical referral, or reflex to second-tier testing—varied, reflecting differences in local protocols.

Table 1 Dried Blood Spot EQA Program Expansion: Participants, Analytes, and Key Additions

Year	No. of participants enrolled	No. Analytes offered	Key Additions
2019	5	5	Program began with 5 key measurands: 17-OHP, immunoreactive trypsinogen, phenyalalinine, TSH and tyrosine
2020	9	5	"Patient" sample introduced
2021	11	9	Androstenedione, cortisol, 21-deoxycortisol and 11-deoxycortisol added
2022	12	14	17-OHP split into separate measurands to reflect immunoassay and mass spec methods C8-acylcarnitine, citrulline, methionine, leucine added
2023	15	14	C3-acylcarnitine added. Leucine removed
2024	20	14	
2025	25	15	Galactose added Recording calibrator lot numbers for: TSH and 17-OHP (immunoassay)
2026	TBC	17	Biotinidase and C26:0-lysophosphatidylcholine (LPC) to be added

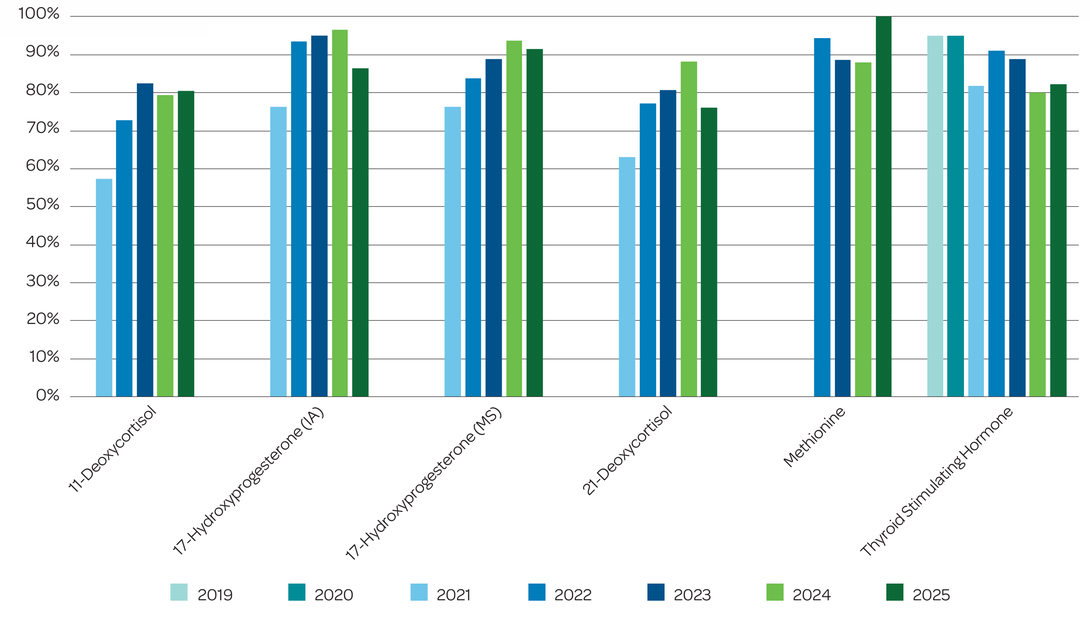

Figure 1 Percentage of laboratory results within Analytical Performance Specifications (APS) across program years

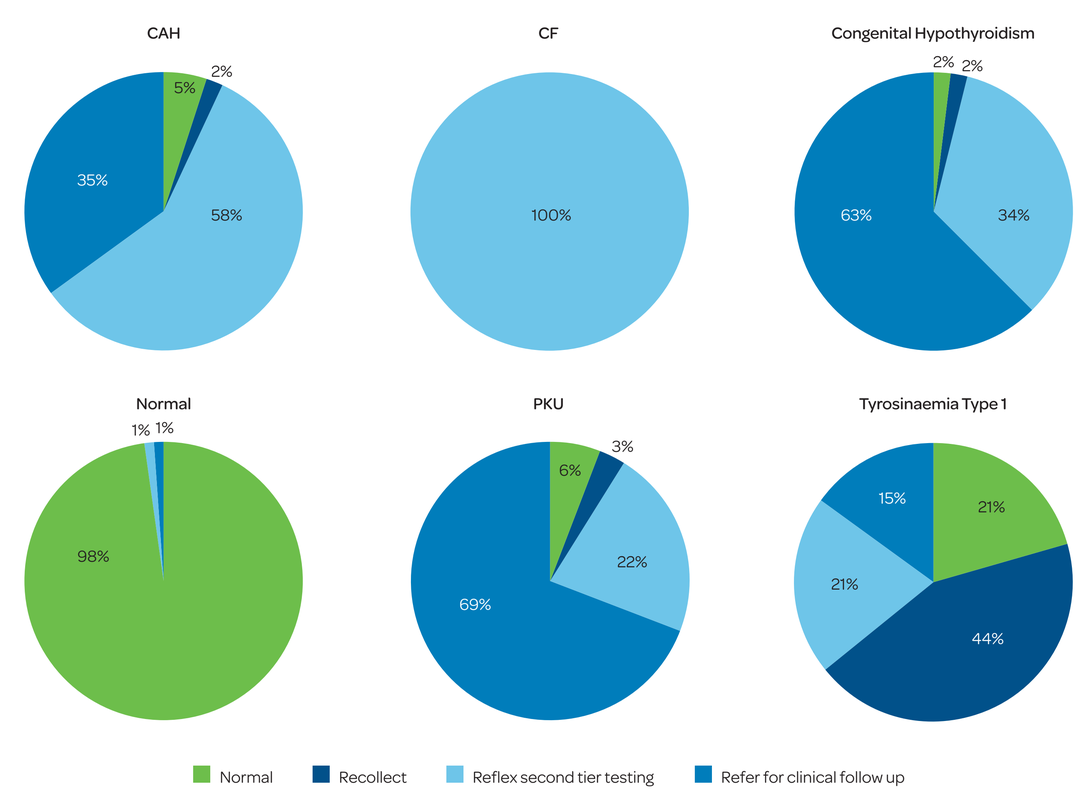

Figure 2 Laboratories' Interpretation of 'Patient ' samples designed to mimic a screening scenario

Discussion and Conclusions

The regular addition of new analytes reflects the evolving clinical landscape, supporting laboratories as they address emerging screening needs. The program's growth in scope and reach across 25 laboratories in seven countries demonstrates expanding impact and engagement.

High concordance rates in 'normal' patient sample interpretations indicate a strong baseline of agreement among participants. Nevertheless, differences in approaches to abnormal results—recollection, clinical referral, or second-tier testing—highlight variations in laboratory protocols.

Persistent analyte variability in phenylalanine and tyrosine suggests further methodological harmonisation may be needed.

Future enhancements will include biotinidase testing for biotinidase deficiency screening and C26:0 LPC, to support screening for X-linked adrenoleukodystrophy (X-ALD).

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