

Mass Spectrometry for Steroid Profiling in Suspected Congenital Adrenal Hyperplasia: A Laboratory Perspective

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Abstract

Immunoassay-based 17-hydroxyprogesterone measurement, still the most widely used first-line test for suspected congenital adrenal hyperplasia (CAH), carries a well-documented false-positive rate driven by cross-reactivity with structurally related steroids, particularly in the preterm and low-birth-weight neonates who make up a disproportionate share of positive newborn screening results. This perspective sets out why liquid chromatography-tandem mass spectrometry (LC-MS/MS) steroid profiling, measuring 17-hydroxyprogesterone alongside several other steroids in the same biosynthetic pathway, offers a materially more specific second-tier test, and addresses the practical barriers -- capital cost, specialist staffing, and the smaller sample volume tolerance of the typical dried blood spot screening specimen -- that have kept many laboratories from adopting it despite broad professional endorsement of the second-tier approach.

Keywords: Congenital adrenal hyperplasia; 17-hydroxyprogesterone; Mass spectrometry; Second-tier testing; Newborn screening

Why This Perspective Addresses a Laboratory, Rather Than Purely Clinical, Audience

Much of the published literature on CAH newborn screening is written primarily for a paediatric endocrinology or clinical genetics audience, and this perspective deliberately centres the laboratory operational viewpoint instead, since the adoption barriers this perspective addresses, capital cost, specialist mass spectrometry staffing, and dried blood spot volume constraints, are primarily laboratory operational questions rather than clinical ones.

A laboratory director or newborn screening programme manager considering second-tier adoption needs, in our view, a different emphasis than a clinical audience: less detail on the downstream clinical management of confirmed CAH, well covered elsewhere in the endocrinology literature, and more detail on the specific operational and economic trade-offs between in-house adoption and regional consolidation that

determine whether and how a given programme can practically move from first-tier-only to second-tier screening.^[6]

We have accordingly structured this perspective around those operational and economic considerations specifically, while still summarising the underlying clinical and analytical rationale briefly enough to situate the practical discussion that follows within its proper clinical context.

The First-Tier Problem

Newborn screening for congenital adrenal hyperplasia relies on first-tier immunoassay measurement of 17-hydroxyprogesterone (17-OHP) from a dried blood spot, chosen for its suitability for high-throughput screening rather than for its analytical specificity, and cross-reactivity with structurally related steroids -- particularly sulfated steroid precursors present at higher concentration in preterm and low-birth-weight infants -- produces a well-documented excess of false-positive results concentrated in exactly this population.^{[1][2]} Published positive predictive values for first-tier 17-OHP immunoassay screening alone are frequently below 5% once preterm infants are included, meaning the substantial majority of infants recalled for further testing do not have CAH.

This high false-positive burden is not evenly distributed across the screened newborn population: term, appropriately grown infants show a materially lower false-positive rate than preterm or low-birth-weight infants, whose immature adrenal steroid metabolism produces elevated concentrations of several sulfated steroid precursors that cross-react with the first-tier 17-OHP immunoassay to a degree not seen in term infants, meaning the population most burdened by false-positive recall is precisely the population, already vulnerable due to prematurity or low birth weight, least well served by additional avoidable clinical investigation and parental anxiety.^{[2][9]}

The consequences of a false-positive first-tier result are not trivial: affected families are typically recalled urgently for confirmatory testing, often before definitive results are available, generating substantial anxiety during a period already stressful for parents of a preterm or unwell newborn, and the downstream confirmatory testing burden on paediatric endocrinology services scales directly with the first-tier false-positive rate a given screening programme tolerates.

The Case for Second-Tier Mass Spectrometry

LC-MS/MS steroid profiling measures 17-OHP alongside several other steroids in the same adrenal biosynthetic pathway -- typically including 21-deoxycortisol, androstenedione, and cortisol -- from the same dried blood spot, and calculating a steroid ratio or ordinal combination of these measurements distinguishes true CAH from the cross-reactivity-driven false positives far more specifically than 17-OHP measured alone.^[3] Published second-tier LC-MS/MS protocols report positive predictive values above 80% in populations where first-tier immunoassay alone achieved single-digit percentages, a difference large enough to materially reduce unnecessary infant recall, parental anxiety, and downstream confirmatory testing burden on paediatric endocrinology services.^{[4][5]}

The underlying biochemical rationale for measuring multiple steroids rather than 17-OHP alone is that the specific cross-reactive precursors responsible for first-tier false positives sit at a different point in the adrenal steroid biosynthetic pathway than the enzymatic block characteristic of classic 21-hydroxylase

deficiency CAH, so a ratio or ordinal combination incorporating a downstream product such as 21-deoxycortisol, which rises specifically in genuine 21-hydroxylase deficiency but not in simple cross-reactivity-driven false positives, discriminates the two scenarios far more reliably than 17-OHP concentration alone can.^{[3][6]}

Published second-tier protocols vary somewhat in the exact combination of steroids measured and the specific ratio or algorithm applied to combine them, reflecting differences in mass spectrometry platform, local population characteristics, and individual programme validation data, but share the same underlying principle of using a multi-steroid biochemical signature rather than a single analyte threshold to distinguish true CAH from cross-reactivity-driven elevation.^{[4][5]}

Approach	Reported PPV	Key Limitation
First-tier 17-OHP alone (preterm)	<5%	High cross-reactivity
First-tier 17-OHP alone (term)	Moderate	Still suboptimal
Second-tier LC-MS/MS profiling	>80%	Cost, staffing, DBS volume

Table 1: Reported Positive Predictive Value by Screening Approach for Congenital Adrenal Hyperplasia.

Practical Barriers to Adoption

Despite this evidence and broad professional guideline endorsement of second-tier steroid profiling as best practice, adoption remains uneven, and the barriers are practical rather than evidentiary. Capital cost and the specialist mass spectrometry expertise required for method development and ongoing operation exceed what many newborn screening laboratories, particularly smaller national or regional programmes, can justify or staff internally.^[6]

The dried blood spot specimen itself imposes a further practical constraint: the small residual punch volume available after first-tier testing limits how many second-tier analytes can be reliably measured, and a programme designing its second-tier panel must balance analytical completeness against the sample volume actually available without requesting a repeat specimen, which reintroduces the delay a rapid second-tier test is meant to avoid.^[7] Regional consolidation -- several smaller screening programmes referring second-tier testing to a single regional mass spectrometry reference laboratory rather than each developing in-house capability -- has been adopted successfully in a number of settings and offers a practical route to the specificity benefit without every individual programme bearing the full capital and staffing cost.^{[8][9]}

A Laboratory Perspective

From an operational standpoint, the specificity gain from second-tier LC-MS/MS steroid profiling is well enough established that the remaining question for most programmes is not whether to adopt it but how to reach adequate throughput and staffing to sustain it reliably; a programme too small to justify or staff an in-house service is better served referring to a consolidated regional facility than either declining second-tier testing altogether or running an under-resourced in-house service without the volume needed to maintain method competence and turnaround.^[10] We consider consolidated regional provision, rather than universal in-house adoption, the more realistic path to broad second-tier coverage over the next several years.

Background on Congenital Adrenal Hyperplasia and Newborn Screening Rationale

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency, the form of CAH targeted by newborn screening programmes worldwide, is a potentially life-threatening condition in its classic salt-wasting form, where affected infants can develop adrenal crisis, electrolyte disturbance, and shock within the first days to weeks of life without timely diagnosis and treatment, providing the clinical rationale for population-wide newborn screening rather than relying on clinical presentation alone.^[1]

The screening programme's overall value depends on detecting genuine cases promptly while minimising the harm, parental anxiety, unnecessary clinical investigation, and specialist appointment burden, caused by false-positive results, and the balance between these two goals is precisely what distinguishes a well-designed screening algorithm, incorporating appropriately specific second-tier testing, from a poorly designed one relying on first-tier immunoassay alone.^[9]

This perspective's central argument, that second-tier LC-MS/MS steroid profiling materially improves this balance and that practical barriers to adoption, while real, are addressable through approaches such as regional consolidation, rests on this underlying premise that both false-negative and false-positive results carry genuine costs that a well-designed screening algorithm should actively work to minimise together, rather than treating sensitivity alone as the only criterion that matters.

Method Development and Validation Considerations

Establishing an in-house second-tier LC-MS/MS steroid profiling service, where a programme's volume justifies this approach rather than regional referral, requires a substantial method development and validation effort distinct from, and generally more demanding than, validating a routine immunoassay platform.

Method development must establish robust extraction and derivatisation protocols suited to the limited dried blood spot punch volume available, define the specific steroid panel and combination algorithm to be used locally, drawing on but

typically adapting published protocols to local population characteristics and instrument platform, and establish population-specific cutoffs for each steroid and ratio, since published cutoffs from other programmes' populations and platforms do not necessarily transfer directly without local validation.^{[3][8]}

Ongoing validation additionally requires ongoing participation in an external quality assessment scheme specific to steroid profiling by mass spectrometry, where available, and regular competency assessment of the specialist staff operating the platform, both of which represent an ongoing resource commitment beyond the initial method development effort, and both of which are more straightforward to sustain at a higher-volume regional reference laboratory than at an individual smaller programme attempting to maintain its own in-house service at low volume.

Interaction with Clinical Decision-Making at the Point of Recall

The laboratory-focused discussion throughout this perspective should not obscure the fact that a second-tier result, however generated, ultimately feeds into a clinical recall decision, and it is worth briefly addressing how second-tier results are best communicated to support that decision appropriately.

Rather than simply reporting a binary pass or fail second-tier result, several well-regarded programmes report the full multi-steroid profile and the resulting ratio or algorithm output alongside an interpretive comment indicating the relative likelihood of true CAH versus cross-reactivity-driven elevation, giving the receiving clinical team more context than a binary result alone would provide for triaging the urgency of any resulting recall and confirmatory assessment.^[4]

This richer reporting approach does require that receiving clinical teams, typically paediatric or neonatal teams at the birth hospital alongside paediatric endocrinology, are familiar with how to interpret a multi-steroid profile report rather than a simple positive or negative result, underscoring the value of clear, consistent reporting templates and accompanying clinician education when a programme first transitions from first-tier-only to second-tier reporting.

Analytical Considerations Specific to Dried Blood Spot Steroid Profiling

Measuring multiple steroids simultaneously from a dried blood spot specimen, rather than from a conventional serum sample, introduces several analyte-specific technical considerations that a programme developing an LC-MS/MS steroid profiling method must address directly.

Steroid extraction efficiency from a dried blood spot differs from serum extraction, and the small, fixed punch volume available constrains how much extraction and clean-up can be performed before analysis, requiring a method sensitive enough to reliably quantify each target steroid, including those present at

comparatively low concentration, from this limited sample matrix.^{[7][8]}

Haematocrit variation between dried blood spot specimens, a well-recognised source of quantitative variability in dried blood spot analysis generally, also affects steroid profiling specifically, and a well-validated method should account for or demonstrate robustness to the haematocrit range expected in the screened newborn population, a validation step that adds further complexity relative to a conventional serum-based assay where haematocrit is not a comparable analytical concern.

These dried-blood-spot-specific analytical considerations, on top of the general LC-MS/MS method development requirements described above, reinforce why second-tier steroid profiling validation is a substantially more demanding undertaking than validating an immunoassay platform, and why regional consolidation, concentrating this validation effort at fewer, higher-volume reference laboratories, is an efficient response to that demand rather than simply a cost-cutting measure.

Instrument Platform and Vendor Considerations

A programme pursuing in-house adoption must also choose among available LC-MS/MS instrument platforms and, where applicable, commercial second-tier steroid profiling kits versus a fully laboratory-developed approach, a choice that carries its own set of trade-offs worth briefly noting.

Commercial kit-based approaches, where available for CAH second-tier steroid profiling, generally offer a faster path to validated, ready-to-use method implementation than a fully laboratory-developed approach, at the cost of less flexibility to adapt the specific steroid panel or algorithm to local population characteristics and generally higher per-sample reagent cost than an optimised in-house method.^[8]

A fully laboratory-developed approach, building directly on published protocols such as those cited throughout this perspective, offers greater flexibility and generally lower per-sample cost once validated, but requires the specialist method development expertise discussed under Method Development and Validation Considerations above, and represents a larger upfront validation burden than adopting a commercial kit where one is available and suitable for the programme's population.

We would suggest that a programme's choice between these two approaches should be driven primarily by its available specialist staffing expertise and expected long-term testing volume rather than by upfront cost alone, since a commercial kit approach may represent the more realistic near-term path for a programme without extensive in-house method development expertise, even where a laboratory-developed approach would offer better long-term economics at higher volume.

Staffing and Expertise Requirements

Beyond the capital cost noted in Practical Barriers above, the specialist staffing requirement for LC-MS/MS steroid profiling deserves fuller discussion, since staffing, rather than capital cost alone, is frequently the more binding constraint for a smaller programme considering in-house adoption.

Operating an LC-MS/MS platform for steroid profiling requires staff with specific expertise in mass spectrometry method operation, troubleshooting, and ongoing quality assurance, expertise that is not interchangeable with the immunoassay platform expertise most newborn screening laboratory staff already hold, and that typically requires either recruiting specifically for this expertise or providing substantial additional training to existing staff.^[6]

This specialist staffing requirement is particularly acute for a smaller programme, where the testing volume may not justify a dedicated, full-time mass spectrometry specialist role, creating a structural tension between the volume needed to justify dedicated specialist staffing and the volume a smaller programme's population actually generates, a tension that regional consolidation, discussed further below, is specifically designed to resolve.

Quality Assurance and Ongoing Monitoring

Sustaining a second-tier LC-MS/MS steroid profiling service, whether in-house or through regional consolidation, requires ongoing quality assurance distinct from the initial validation effort already discussed, and we note the key elements of this ongoing monitoring here for completeness.

Regular internal quality control material analysis alongside patient and screening specimens, periodic review of the positive predictive value actually being achieved locally against the published benchmarks cited throughout this perspective, and participation in an external quality assessment scheme specific to steroid profiling, where available, together provide the ongoing assurance that a second-tier service continues to perform as validated over time rather than only at the point of initial method development.^[8]

A programme operating through regional consolidation should additionally establish a clear mechanism for receiving and reviewing this ongoing quality assurance information from its reference laboratory partner, rather than treating consolidation as a one-time referral arrangement requiring no ongoing quality oversight from the referring programme's own side of the relationship.

Turnaround Time Considerations

Turnaround time is a particularly important consideration for CAH newborn screening specifically, given the risk of life-threatening adrenal crisis in affected infants with the classic salt-wasting form, and any second-tier testing approach must be evaluated against this time-sensitivity as well as against the positive predictive value improvement emphasised elsewhere in this perspective.

In-house second-tier LC-MS/MS testing, where a programme has sufficient volume to sustain it, generally achieves the shortest total turnaround time, since the second-tier result can be generated from the same specimen without any additional transport step, typically within a day or two of the first-tier result flagging a possible abnormality.^[5]

Regional consolidation introduces an additional transport step that, as noted above, several programmes have addressed through dedicated, prioritised courier arrangements, but that nonetheless typically adds at least a day to total turnaround time relative to an in-house service, a trade-off that most adopting programmes have judged acceptable given that even this longer turnaround remains substantially faster than the alternative of proceeding directly to serum-based confirmatory testing without any second-tier screening step at all.^[10]

A programme evaluating whether to pursue in-house adoption versus regional consolidation should explicitly weigh this turnaround-time difference against the volume, cost, and staffing considerations discussed throughout this perspective, since for a programme with a sufficiently high proportion of at-risk preterm births, the turnaround-time advantage of in-house testing may tip the balance toward in-house adoption despite its higher cost, while a programme with a lower-risk population profile may reasonably accept the modest additional delay of regional consolidation in exchange for its substantially lower cost and staffing burden.

Economic Considerations for Regional Consolidation

The regional consolidation approach introduced in Practical Barriers above warrants fuller economic discussion, since the case for consolidation rests specifically on matching testing volume to the fixed costs, capital and specialist staffing, that LC-MS/MS steroid profiling requires.

A regional reference laboratory serving several smaller screening programmes can spread the fixed capital and staffing cost of an LC-MS/MS platform across a substantially larger combined testing volume than any individual smaller programme could generate alone, improving the economics of adoption meaningfully without requiring any individual programme to bear the full cost independently.^{[8][9]}

This consolidated model does introduce its own practical requirements, principally a reliable, rapid specimen transport arrangement between each referring programme and the regional reference laboratory, since any transport delay directly erodes the turnaround-time benefit that a rapid second-tier test is

intended to provide over waiting for definitive confirmatory testing through an alternative pathway; several successfully operating consolidated models have addressed this through dedicated courier arrangements specifically for second-tier specimens, prioritised ahead of routine inter-laboratory transport.^[10]

Governance Considerations for Programmes Choosing Between Models

Drawing together the considerations discussed throughout this perspective, we propose the following governance framework for a screening programme deciding between in-house adoption, regional consolidation, or continued reliance on first-tier testing alone.

A programme should first estimate its own expected second-tier testing volume, derived from its historical first-tier positive rate and birth cohort size, and compare this volume against the throughput typically needed to sustain in-house LC-MS/MS competency and cost-effectiveness, drawing on the published programme experience cited throughout this perspective as a benchmark.^[6]

Where estimated volume falls well below this threshold, the programme should actively investigate regional consolidation options, including identifying candidate reference laboratories already operating a validated second-tier service and assessing the feasibility of a reliable, prioritised specimen transport arrangement, rather than defaulting to continued first-tier-only screening simply because in-house adoption is not individually justified.

Where no regional consolidation option is practically available, a programme should document this gap explicitly to its own governing health authority as a barrier to achieving current professional guideline-endorsed best practice, since making this gap visible is itself a useful step toward the kind of inter-programme coordination that regional consolidation ultimately requires.

Impact on Confirmatory Testing and Paediatric Endocrinology Services

The downstream impact of adopting second-tier LC-MS/MS steroid profiling extends beyond the screening laboratory itself to the paediatric endocrinology services that manage infants recalled for confirmatory assessment, and this downstream impact deserves explicit mention as part of the overall case for adoption.

A programme relying on first-tier immunoassay screening alone, with its correspondingly low positive predictive value, directs a large volume of recalled infants to paediatric endocrinology confirmatory assessment who do not, in fact, have CAH, consuming specialist appointment capacity and generating family anxiety for a result that second-tier testing would, in the large majority of cases, have resolved at the screening laboratory stage itself without requiring specialist referral at all.^[4]

Programmes that have adopted second-tier steroid profiling report a substantial reduction in confirmatory referral volume to paediatric endocrinology, freeing specialist appointment capacity for infants genuinely requiring assessment and reducing the family-level anxiety burden associated with an urgent but ultimately unnecessary specialist referral, a benefit that, while harder to quantify in purely economic terms than the laboratory testing cost itself, represents a meaningful component of the overall case for adoption.

Patient and Family Communication Considerations

The communication burden on families during the newborn screening recall process deserves separate comment, since this burden is directly proportional to the first-tier false-positive rate a screening programme tolerates and is therefore materially affected by the first-tier-versus-second-tier choice this perspective addresses.

A family receiving notification of a positive first-tier CAH screening result, with no second-tier context available to distinguish a likely true positive from a likely cross-reactivity-driven false positive, must navigate the recall and confirmatory testing process under the full weight of uncertainty about whether their infant has a potentially life-threatening condition, a burden that falls disproportionately on families of preterm or low-birth-weight infants given the elevated false-positive rate in this group.^[2]

Where second-tier testing is available and has already substantially narrowed this uncertainty before the family is contacted, communication can be calibrated accordingly, distinguishing a result still requiring urgent assessment from one that, while technically still being confirmed, carries a second-tier result pattern more consistent with cross-reactivity than with genuine CAH, allowing a materially less alarming initial communication for this larger group of families than a first-tier-only result alone would support.

Comparison with Previously Published Programme Experiences

Several published accounts of programmes transitioning from first-tier-only to second-tier LC-MS/MS screening report broadly consistent findings, and briefly summarising this comparative experience situates this perspective's recommendations within the wider field's accumulated practical experience.

Programmes transitioning to second-tier testing have generally reported the positive predictive value improvement described above being realised in practice, not only in the validation study setting, within the first one to two years of routine second-tier operation, with the specific magnitude of improvement varying somewhat by local population characteristics, particularly the proportion of preterm infants screened, and by the exact steroid panel and algorithm adopted locally.^{[5][7]}

Programmes that adopted a regional consolidation model rather than in-house adoption have generally reported a longer total turnaround time than programmes with in-house capability, reflecting the additional specimen transport step, but have nonetheless generally achieved a turnaround time still substantially faster than relying on serum-based confirmatory testing alone, supporting regional consolidation as a workable, if not fully optimal, intermediate approach for programmes unable to justify in-house adoption.

Applicability to Other Newborn Screening Conditions Using Second-Tier Testing

CAH is not the only condition on most newborn screening panels where a second-tier test improves specificity over a first-tier screen, and briefly situating this perspective's recommendations relative to that broader second-tier screening landscape is useful context.

Several other conditions on contemporary expanded newborn screening panels, including certain inherited metabolic disorders detected by first-tier tandem mass spectrometry acylcarnitine profiling, already routinely incorporate a second-tier test, often itself a more targeted mass spectrometry or molecular assay, to improve specificity before recall, meaning the second-tier testing principle advocated in this perspective for CAH specifically is already well established elsewhere within the same newborn screening programmes.^[1]

This existing precedent is, in our view, a further practical argument for CAH second-tier adoption: a programme that has already built the infrastructure, expertise, and regional consolidation or referral relationships needed to support second-tier testing for other conditions on its panel is better positioned to extend that same infrastructure to CAH steroid profiling than a programme building second-tier testing capability for the first time specifically for CAH.

Study Limitations of the Underlying Evidence Base

As a perspective piece rather than a primary research report, this article's conclusions rest on the published literature cited throughout rather than on new primary data, and several limitations of that underlying evidence base are worth noting explicitly.

Published positive predictive value estimates for both first-tier and second-tier approaches vary considerably across studies depending on population characteristics, particularly the proportion of preterm infants included, and the specific cutoffs and algorithms used locally, meaning the specific percentage figures cited in this perspective should be understood as illustrative of the general pattern reported in the literature rather than as precise, universally applicable values.^[2]

Most published second-tier LC-MS/MS steroid profiling validation studies have been conducted at larger, well-resourced screening programmes or reference laboratories, and the practical experience of smaller programmes considering regional

consolidation specifically, while discussed in several of the programme-experience reports cited above, is less extensively documented in the peer-reviewed literature than the analytical validation evidence for the second-tier approach itself.

Equity Considerations in Access to Second-Tier Testing

The uneven adoption of second-tier LC-MS/MS steroid profiling described throughout this perspective raises a direct equity concern worth stating explicitly: an infant's access to the more specific, less anxiety-inducing second-tier testing pathway currently depends substantially on which screening programme and jurisdiction they happen to be born into, rather than on any clinical characteristic of the infant themselves.^[1]

This geographic inequity in testing pathway quality is, in our view, a further argument for actively pursuing regional consolidation models specifically in jurisdictions or regions where no individual programme can justify in-house adoption, since consolidation offers a practical route to equalising access to the better-performing second-tier pathway without requiring every individual programme to independently reach the volume threshold needed for in-house justification.

We would encourage national and regional newborn screening oversight bodies to treat second-tier CAH testing access as an equity metric worth actively monitoring and reporting, alongside the more commonly reported first-tier screening coverage and timeliness metrics, given the material difference in family experience and confirmatory testing burden that second-tier access produces as documented throughout this perspective.

Future Directions

Continued publication of real-world programme experience with regional consolidation models, including the specific logistical and turnaround-time trade-offs involved, would help smaller programmes currently relying on first-tier testing alone make a more informed decision about whether and how to pursue second-tier adoption through this route.

Further standardisation of the specific steroid panel and algorithm used for second-tier profiling across programmes and platforms, building on the existing but still somewhat heterogeneous published protocols, would also support broader adoption by reducing the local method development burden individual programmes currently face when establishing a second-tier service, whether in-house or as part of a consolidated regional arrangement.

Practical Checklist for Programmes Considering Adoption

As a closing practical summary, we offer the following checklist for a newborn screening programme director assessing whether and how to pursue second-tier LC-MS/MS steroid profiling adoption.

Quantify the programme's current first-tier false-positive burden, ideally stratified by gestational age and birth weight category given the disproportionate burden in preterm and low-birth-weight infants documented throughout this perspective, to establish a clear local baseline against which any future second-tier adoption can be measured.

Estimate the programme's expected second-tier testing volume and compare it against the throughput generally needed to justify in-house adoption, drawing on the published programme experience cited throughout this perspective, to determine whether in-house adoption or regional consolidation is the more realistic near-term path.

Where regional consolidation is the realistic path, identify candidate reference laboratories and establish the specimen transport, result reporting, and governance arrangements needed to support a reliable, appropriately prioritised second-tier referral pathway, rather than treating consolidation as a vague future aspiration without concrete implementation steps.

Where in-house adoption is the realistic path, budget explicitly for the method development, validation, and ongoing specialist staffing requirements described throughout this perspective, recognising that these requirements exceed those typically associated with maintaining a routine immunoassay platform.

Conclusion

Second-tier LC-MS/MS steroid profiling substantially improves positive predictive value over first-tier 17-hydroxyprogesterone immunoassay screening for congenital adrenal hyperplasia, particularly in preterm and low-birth-weight infants where first-tier false-positive rates are highest. Capital cost, specialist staffing, and dried blood spot volume constraints remain the practical barriers to adoption, and regional consolidation of second-tier testing offers a realistic route to broader coverage for programmes unable to justify an in-house service.

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