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Laboratory Examination in Congenital Adrenal Hyperplasia: A Literature Review

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ABSTRACT
Congenital adrenal hyperplasia (CAH) is a group of inherited genetic disorders that affect the adrenal glands, which produce essential hormones such as cortisol, aldosterone, and sex steroids. The most common form of CAH is caused by a 21-hydroxylase enzyme deficiency resulting from mutations in the CYP21A2 gene. This deficiency disrupts normal cortisol and aldosterone production, leading to excessive androgen synthesis and a range of clinical manifestations, including ambiguous genitalia in females and adrenal crisis in both sexes. Current biomarkers, such as 17-hydroxyprogesterone (17-OHP), androstenedione, and testosterone, have limitations, and further research on 11-oxygenated androgens—which are more specific for CAH—is needed. Non-invasive methods, such as measuring steroids in saliva and urine, are increasingly accessible and useful, particularly for children showing signs of hyperandrogenism.

Keyword: Androgen, Andrenal, Biomarker, Screening, Confirmatory

INTRODUCTION

Congenital adrenal hypertrophy or Congenital Adrenal Hyperplasia (CAH) is a group of inherited genetic disorders that affect the adrenal glands, which are responsible for producing essential hormones such as cortisol, aldosterone, and sex steroids. The most common form of CAH is caused by a deficiency of the enzyme 21-hydroxylase, resulting from a mutation in the CYP21A2 gene. Deficiencies in this enzyme interfere with the normal production of cortisol and aldosterone, leading to excessive androgen production and a range of clinical manifestations, including ambiguous genitalia in females and adrenal crises in both sexes (Omona & Ssanyu, 2025).

CAH is divided into two main types (Kelestimur & Unluhizarci, 2021). The first is classical CAH, which is diagnosed from birth and characterized by significant hormonal imbalance that can cause life-threatening adrenal crises and atypical genital development in females. The second is non-classical CAH, a milder form that often appears in childhood or adulthood, presenting with androgen-related symptoms such as early puberty or irregular menstrual cycles in females, while many males may remain asymptomatic (Paparella et al., 2026).

Globally, the prevalence of CAH varies, ranging from 1 in 5,000 to 1 in 15,000 live births in the United States and Europe, with higher frequencies reported among certain populations such as the Yupik people of Alaska. Advances in neonatal screening have significantly improved early detection and management, thereby enhancing prognosis by identifying the disorder promptly (Lipkin et al., 2020; Zhang et al., 2023). Epidemiological data in Indonesia

indicate a relatively high prevalence of CAH, although follow-up treatment remains a major challenge. Screening results from five cities reported a positive rate of approximately 0.71%. The need for a comprehensive national newborn screening program in Indonesia is evident, emphasizing the importance of government support and increased public awareness to improve diagnosis and treatment.[2]

Despite global progress in neonatal screening programs, the diagnosis and management of Congenital Adrenal Hyperplasia (CAH) remain challenging, particularly in developing countries such as Indonesia. Current reliance on conventional biomarkers—including 17-hydroxyprogesterone (17-OHP), androstenedione, and testosterone—has several limitations, including variability influenced by stress, circadian rhythm, and glucocorticoid therapy, as well as a lack of specificity for CAH. These biomarkers are not exclusively produced by the adrenal glands, leading to potential misdiagnosis or delayed diagnosis. Furthermore, false-positive and false-negative results in neonatal screening pose significant risks, ranging from unnecessary parental anxiety to missed life-threatening adrenal crises. The identification of more specific biomarkers, such as 11-oxygenated androgens, and the development of non-invasive methods utilizing saliva and urine specimens offer promising alternatives; however, their clinical application in Indonesia remains understudied. Therefore, a comprehensive review of current laboratory examination methods for CAH is urgently required to synthesize the latest evidence, evaluate the diagnostic accuracy of emerging biomarkers, and provide practical recommendations for clinicians and laboratory personnel. This review aims to improve early detection, accurate diagnosis, and optimal management of CAH, thereby reducing morbidity and mortality associated with the disorder.

Adrenal steroidogenesis occurs through three main pathways: glucocorticoids, mineralocorticoids, and sex steroids, as illustrated in Figure 2.1. Glucocorticoids (mainly cortisol), androgens, and estrogens are produced in the zona fasciculata and zona reticularis, while aldosterone is synthesized in the zona glomerulosa. The hypothalamic–pituitary–adrenal (HPA) axis regulates these processes through a feedback mechanism involving circulating cortisol levels. Cortisol exerts negative feedback on the secretion of corticotropin-releasing factor (CRF) and adrenocorticotropic hormone (ACTH). A decrease in cortisol levels leads to increased ACTH production, which in turn stimulates excessive synthesis of adrenal products in pathways unaffected by enzyme deficiency, while precursor molecules accumulate in the pathways inhibited by the deficiency.

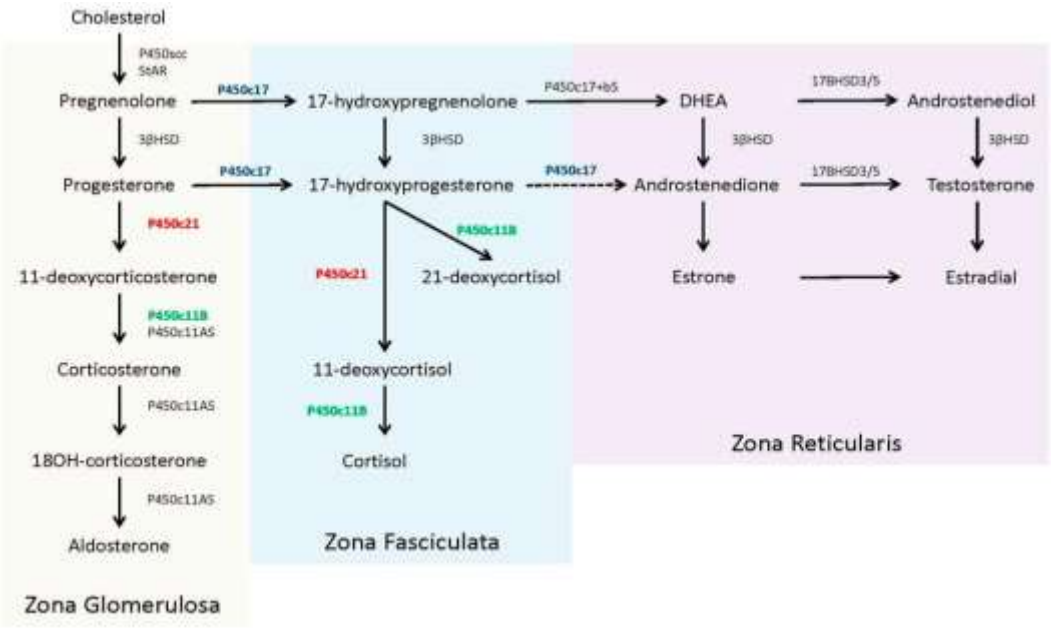

Figure 1. Normal Pathways of Steroid Production (Steroidogenesis)
Source: Held4

The clinical manifestations of the various forms of Congenital Adrenal Hyperplasia (CAH) are determined by the deficient and overproduced hormones, as detailed in Table 2.1. In the case of 21-hydroxylase deficiency (21-OHD CAH), 17-hydroxyprogesterone (17-OHP) accumulates because of inhibition in the 21-hydroxylation step. This excess 17-OHP is then directed to the androgen synthesis pathway, where the enzyme 17,20-lyase (also known as 17,20-desmolase) converts it to Δ4-androstenedione, which is subsequently converted into active androgens. The most severe form of CAH, known as salt-wasting CAH (SW-CAH), is characterized by a deficiency of mineralocorticoids. In contrast, in non-classical CAH (NC-CAH), the enzyme defect is only partial, and salt-wasting is absent in milder forms of the disease (Podgórski, Podgórska, & Sumińska, 2023).

Females with classical 21-hydroxylase (21-OHD) deficiency or 11β-hydroxylase deficiency are born with virilized external genitalia due to excess adrenal androgen exposure during a critical period of sexual differentiation. This results in clitoral enlargement, partial fusion of the labial folds, and a genital appearance resembling male external anatomy. Virilization is classified into five Prader stages, ranging from clitoromegaly without labial fusion to a fully penis-like clitoris with scrotum-like labia. Despite these external changes, internal female reproductive organs develop normally, preserving fertility potential (Andina, 2023).

Without appropriate treatment, children with CAH experience continued androgen exposure, leading to progressive genital virilization, premature development of pubic and axillary hair, acne, and accelerated linear growth. Premature puberty and short adult stature are common due to early epiphyseal closure. Adequate treatment typically normalizes secondary sexual characteristics, though females may continue to exhibit mild hyperandrogenic signs such as acne or hirsutism. Male children may experience short stature and fertility difficulties (Kufoof et al., 2025).

Severe 21-hydroxylase deficiency results in insufficient aldosterone secretion, causing salt loss, cortisol deficiency, and androgen excess. Infants with this condition are at risk of dehydration, hypotension, and adrenal crises, which can occur early in life. Diagnosis can be challenging in males with normal genitalia, making newborn screening particularly important.

Simple-virilizing CAH (SV-CAH) is characterized by prenatal virilization and postnatal masculinization with rapid growth and premature epiphyseal closure but without mineralocorticoid deficiency. Diagnosis in females is often straightforward due to ambiguous genitalia, whereas in males it usually depends on newborn screening results or physical signs such as hyperpigmentation (Witchel & Miller, 2021).

Non-classical CAH tends to appear postnatally with mild to moderate symptoms, including premature pubic hair development, acne, and polycystic ovary syndrome (PCOS)-like features, without genital ambiguity at birth. This condition may cause premature baldness and infertility in males, with diagnosis frequently occurring later in life. Infants who test positive for CAH in newborn screening should be referred to a pediatric endocrinologist for evaluation and undergo a cosyntropin stimulation test if necessary. In cases where symptoms arise after infancy, CAH screening should be performed by measuring early morning serum 17-OHP levels using LC–MS/MS. For individuals with borderline 17-OHP values, a complete adrenocortical steroid profile should be obtained following cosyntropin stimulation to distinguish 21-OHD from other enzyme deficiencies. Genotyping is recommended only when adrenocortical results remain unclear, when cosyntropin stimulation cannot be performed accurately due to glucocorticoid therapy, or for genetic counseling purposes (Speiser et al., 2018).

Newborns with positive screening results should be monitored by primary healthcare providers to reassess moderately elevated 17-OHP levels with follow-up testing. If abnormalities persist, consultation with a pediatric endocrinologist is essential. Second-tier screening using LC–MS/MS is effective for identifying various forms of CAH, including 11β-hydroxylase deficiency. Ideally, a cosyntropin stimulation test should be performed 24–48 hours after birth, measuring both cortisol and 17-OHP. In individuals presenting with symptoms beyond infancy, morning serum samples should be analyzed via LC–MS/MS. For menstruating females, testing should be conducted during the early follicular phase. If initial 17-OHP results are inconclusive, cosyntropin stimulation and genotyping may be used to confirm the diagnosis of CAH (Bizzarri et al., 2025).

METHOD

The research method used in this article aims to comprehensively examine laboratory examinations related to Congenital Adrenal Hyperplasia (CAH), particularly in the areas of screening, diagnostic confirmation, and the development of new biomarkers. Data were collected through a systematic search of scientific literature from various reliable sources, including international and national journals, medical textbooks, clinical guidelines, and research reports relevant to CAH, steroidogenic enzyme deficiencies, and laboratory examination methods such as 17-hydroxyprogesterone, androstenedione, 11-oxygenated androgens, and the use of LC–MS/MS. The selected literature was assessed based on its relevance to the topic, novelty of information, and scientific validity. Data analysis was carried

out descriptively and qualitatively by comparing findings across studies to obtain a comprehensive understanding of the role of laboratory examinations in neonatal screening, confirmatory diagnosis, and monitoring of CAH, as well as identifying the limitations of conventional biomarkers and the potential development of non-invasive diagnostic methods in the future.

The instrument used in this study was a literature review matrix developed by the researcher to systematically extract and organize data from the selected articles. This matrix included categories such as the authors' names, publication year, study objectives, laboratory methods used, biomarker types, sensitivity and specificity values, key findings, and study limitations. Since this is a literature review, traditional validity and reliability tests (commonly used in primary research) were not applicable. However, to ensure the quality and trustworthiness of the synthesized data, source triangulation was applied by comparing findings across multiple studies with different designs and populations. Additionally, only articles from reputable, peer-reviewed sources were included. The review followed the principles of systematic review methodology to minimize selection bias.

Data collection was conducted through a comprehensive search of electronic databases, including PubMed, Scopus, Web of Science, ScienceDirect, and Google Scholar, between June and December 2025. The search strategy was developed based on preliminary readings on CAH to ensure that all relevant aspects of laboratory examination—from neonatal screening to confirmatory testing and biomarker development—were adequately covered. Reference lists of the included articles were also hand-searched (snowballing technique) to identify additional relevant studies. All identified articles were screened based on titles and abstracts, followed by a full-text assessment against the inclusion criteria.

The data analysis procedure followed the stages of thematic analysis commonly used in literature reviews: data reduction, data display, and conclusion drawing. First, the data reduction process involved extracting relevant information from each selected article into the review matrix, focusing on laboratory methods, diagnostic accuracy, biomarker performance, and clinical recommendations. Second, data display consisted of organizing the extracted data into thematic categories corresponding to the main topics of this review: (1) neonatal screening methods and challenges, (2) confirmatory diagnostic procedures, (3) conventional biomarkers and their limitations, and (4) emerging biomarkers, including 11-oxygenated androgens and non-invasive testing methods (saliva and urine samples). Third, conclusion drawing involved synthesizing findings across studies to identify patterns, consistencies, and contradictions in the literature, and to formulate evidence-based recommendations for laboratory practice in CAH diagnosis and monitoring.

No statistical software was used to analyze the data, as this study employed a qualitative literature review approach. However, Microsoft Excel was utilized to organize the review matrix and facilitate data management. The synthesis was conducted narratively, comparing and contrasting findings from different studies to provide a comprehensive overview of the current state of laboratory examination in CAH and to identify research gaps for future investigation.

RESULTS AND DISCUSSION

Laboratory Examination on CAH

Congenital adrenal hyperplasia is a genetic disorder characterized by a deficiency in the production of adrenal steroid hormones, primarily due to a mutation in the CYP21A2 gene that encodes the enzyme 21-hydroxylase. Such deficiencies lead to the accumulation of steroid precursors, especially 17-hydroxyprogesterone (17-OHP), and result in excessive androgen production. Evaluation of CAH biochemistry is essential for diagnosis, treatment monitoring, and management of potential complications (Claahsen-Van Der Grinten et al., 2022).

Screening or Screening Inspection

Neonatal screening for 21-hydroxylase (21OH) deficiency, the most common form of CAH, is usually done by measuring the concentration of 17-hydroxyprogesterone (17OHP) in dried blood spots on filter paper. More specific second-level screening, using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), can efficiently measure steroid panels and has successfully diagnosed 11β-hydroxylase (11βOH) deficiency. However, the primary focus of neonatal screening remains on detecting 21OH deficiency.10 In certain situations, such as in premature, stressed, or sick infants, falsely elevated concentrations of 17OHP can be observed. Stratification of 17OHP measurements based on gestational age helps to improve the specificity of diagnosis. Examinations that measure 21-deoxycortisol, an elevated marker of 21OH deficiency, can improve the specificity of neonatal screening.

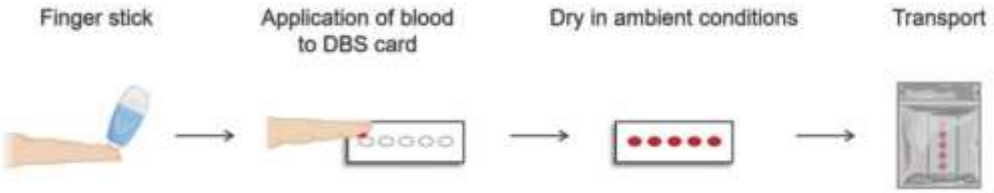

Figure 2. Dried *Blood Spot Examination*
Source: Garza11

LC-MS/MS is recommended as a second-level test to increase the positive presumption value on newborn screening for CAH. However, its use in screening programs requires further evaluation, especially since some studies still report high levels of false negatives with two-level screening. If the neonal examination results are positive for 21OH deficiency during screening or show clinical signs of CAH (e.g., ambiguous genitals), confirmatory testing is essential. Although initial LC-MS/MS steroid panels can be diagnostic, cosyntropin stimulation testing is often required to confirm diagnosis.12 These tests are based on an increase in adrenal hormone characteristics prior to enzymatic blockage. It is important to know that 17OHP concentrations can also increase in other types of CAH, such as 11βOH deficiency or P450 Ocsidoreductase (POR), yang requires additional diagnostic considerations (Kelestimur & Unluhizarci, 2021).

On newborn screening screenings for 21-OHD, it was evident that false positives and false negatives present significant challenges. False positive results often appear due to several key factors. First, physiological changes immediately after birth can lead to increased levels of 17-OHP, a marker used in screening. These levels can be higher due to stress at birth, resulting in false positive results if specimens are collected too early. In addition, premature babies

whose adrenal glands are not fully developed have high levels of 17-OHP due to a lack of enzymes. Stress-inducing conditions such as respiratory distress or maternal preeclampsia may also increase 17-OHP levels.

Early specimen collection in the first two days of life can miss 21-OHD cases because 17-OHP levels often increase later. Doing a second screening can help identify those missed cases. Immature adrenal function in premature infants characterized by lower levels of 17-OHP can produce false negatives due to varying enzyme activity at different stages of fetal development. Increased fetal exposure to maternal cortisol can suppress fetal ACTH hormones and steroidogenesis, also leading to lower 17-OHP levels and delayed diagnosis (Feng et al., 2023). Glucocorticoid treatment given to the mother can temporarily lower 17-OHP levels which has an impact on screening results.

Confirmation Examination of CAH Diagnosis

A confirmatory diagnosis of Congenital Adrenal Hyperplasia (CAH) often involves comprehensive serum and urine steroid profiling. In 21-hydroxylase (21-OH) deficiency, cosyntropin stimulation testing remains the gold standard, helping to identify the characteristic hormonal increase that occurs before the enzymatic block. Urinary steroid profiling, which encompasses all steroid types, provides an alternative means of distinguishing the various forms of CAH. Traditional biomarkers such as 17-hydroxyprogesterone (17-OHP) and androstenedione show high variability, primarily due to acute elevations under stress or reductions following glucocorticoid administration. These biomarkers are also not exclusively produced by the adrenal glands, making them susceptible to interference from external factors such as timing, dosage, and the type of glucocorticoids used (Mizdrak, Tičinović Kurir, & Božić, 2021). A study involving 24-hour serial sampling of 17-OHP and androstenedione in adults with classical CAH demonstrated that these biomarkers exhibit circadian variations and are influenced by both the timing and the type of glucocorticoid therapy (Besci et al., 2022; Nella et al., 2016).

After infancy, screening for 21-OH deficiency depends on early morning (before 8:00 a.m.) measurements of 17-OHP. Concentrations of 17-OHP greater than 30 nmol/L (1,000 ng/dL) are diagnostic for 21-OH deficiency, while random concentrations exceeding 303 nmol/L (10,000 ng/dL) are typically observed in the classical form of CAH. Conversely, 17-OHP levels below 6 nmol/L (200 ng/dL) generally rule out non-classical CAH (NCCAH), especially when measured during the follicular phase in women of reproductive age (Loli, Menotti, di Filippo, & Giustina, 2025). Cosyntropin stimulation testing remains the preferred diagnostic tool for non-classical forms of CAH (Nakhleh et al., 2023). In CAH, dehydroepiandrosterone (DHEA) levels are typically low, whereas 17-OHP and androstenedione are elevated and diverted to alternative 11-oxygenated androgen synthesis pathways. These lesser-known adrenal metabolites—such as 11-ketotestosterone—are bioactive and prominent in CAH. Elevated 11-oxygenated androgen levels have been observed in patients with 21-hydroxylase-deficient CAH undergoing replacement therapy compared with age- and sex-matched controls. Increased levels of these metabolites, particularly those produced by the adrenal glands, correlate with poor long-term disease control and are associated with comorbidities such as increased adrenal volume, testicular adrenal rest tumors (TART), menstrual abnormalities, and hirsutism (Mallappa & Merke, 2022).

The procedure for testing dried blood spot (DBS) samples involves collecting blood via heel prick on filter paper when the newborn is 36–72 hours old. Once received by the laboratory, a 3.2 mm punch from the card is used for first-tier 17-OHP immunoassay analysis. If the result is ≥20.0 nmol/L, a second 3.2 mm punch is analyzed using second-tier LC–MS/MS steroid profiling. The LC–MS/MS analysis procedure begins with the preparation of chemical reagents, including steroid standards and internal standards such as carbon-labeled and deuterated compounds. Hormone-free plasma is used as the calibration matrix, and solvents such as methanol and acetonitrile are obtained from certified suppliers. A mixed steroid stock solution is prepared, diluted, and stored at –80°C for calibration (Weisser et al., 2016).

For sample preparation, 200 µL of plasma or control material is mixed with internal standard, followed by the addition of methanol and ddH_2O, and then centrifuged. The supernatant is processed through solid-phase extraction (SPE) plates, washed, and dried before injection into the LC–MS/MS instrument. Analysis is performed using the Waters UPLC system coupled with a TQ-S mass spectrometer, with separation achieved through a BEH C8 column. Gradient elution and multiple reaction monitoring (MRM) modes are used for steroid detection. Method validation ensures accuracy, precision, and recovery through calibration curves, matrix effect assessments, and carryover evaluations, in accordance with CLSI guidelines (Wille, Coucke, De Baere, & Peters, 2017).

Research on bone maturation and bone density in CAH patients indicates that elevated 17-OHP levels are commonly associated with accelerated bone aging in children, although studies in adults have produced inconsistent findings regarding bone mineral density. Dehydroepiandrosterone sulfate (DHEAS), a marker of adrenarche, has limited clinical utility for CAH monitoring. In adults, persistently high 17-OHP concentrations are linked to higher body mass index (BMI), menstrual irregularities, and hyperandrogenism, though data on ovulatory function remain limited.

Adrenal nodules are more frequently observed in untreated CAH patients and tend to regress with improved biochemical control. High levels of 11-oxygenated androgens have been associated with TART; however, studies have reported varying results regarding their usefulness for monitoring such tumors. Recent investigations into 11-ketotestosterone and 11-hydroxyandrostenedione have also yielded mixed findings, suggesting potential clinical value that warrants further study. Overall, neonatal screening strategies and advanced biochemical testing are essential for the early detection and effective management of CAH, with LC–MS/MS analysis and cosyntropin stimulation testing playing pivotal roles in confirming the diagnosis of both classical and non-classical forms of the disorder.

CONCLUSION

Congenital adrenal hypertrophy or Congenital Adrenal Hyperplasia (CAH) is a group of congenital genetic disorders that affect the adrenal glands, which function to produce essential hormones such as cortisol, aldosterone, and sex steroids. The most common form of CAH is caused by a deficiency of the enzyme 21-hydroxylase resulting from mutations in the CYP21A2 gene. Deficiency of this enzyme disrupts the normal production of cortisol and aldosterone, leading to excessive androgen synthesis and various clinical manifestations, including ambiguous genitalia in females and adrenal crises in both sexes.

Existing biomarkers such as 17-hydroxyprogesterone (17-OHP), androstenedione, and testosterone have limitations, and further research on 11-oxygenated androgens—which are more specific to CAH—is urgently needed. Non-invasive methods, such as steroid measurement in saliva and urine, are increasingly available and beneficial, particularly for children presenting with signs of hyperandrogenism. Future research should focus on validating the clinical utility of 11-oxygenated androgens as biomarkers for monitoring treatment efficacy and long-term outcomes in CAH patients across different age groups. Additionally, large-scale multicenter studies are necessary to establish normative reference ranges for non-invasive specimens (saliva and urine) in diverse populations. These efforts would facilitate the integration of non-invasive testing into routine clinical practice and improve early detection and management of CAH, particularly in resource-limited settings such as Indonesia.

REFERENCES

Andina, Indri. (2023). An overview of the female reproductive system: A narrative literature review. *Sriwijaya Journal of Obstetrics and Gynecology*, *1*(2), 73–80.

Besci, Özge, Erbaş, İbrahim Mert, Küme, Tuncay, Acinikli, Kübra Yüksek, Abacı, Ayhan, Böber, Ece, & Demir, Korcan. (2022). A 4-hour Profile of 17-hydroxyprogesterone in Salt-wasting Congenital Adrenal Hyperplasia: Is the Serial Monitoring Strategy Worth the Effort? *Journal of Clinical Research in Pediatric Endocrinology*, *14*(2), 145.

Bizzarri, Carla, Chioma, Laura, Bottaro, Giorgia, Paone, Laura, Todisco, Tommaso, Chiarito, Mariangela, Surace, Cecilia, Porzio, Ottavia, D'Alessandro, Annamaria, & Ravà, Lucilla. (2025). Diagnostic cut-offs of 17-hydroxyprogesterone by LC-MS/MS in children with non-classical congenital adrenal hyperplasia. *Journal of Endocrinological Investigation*, *48*(7), 1623–1633.

Claahsen-Van Der Grinten, Hedi L., Speiser, Phyllis W., Ahmed, S. Faisal, Arlt, Wiebke, Auchus, Richard J., Falhammar, Henrik, Flück, Christa E., Guasti, Leonardo, Huebner, Angela, & Kortmann, Barbara B. M. (2022). Congenital adrenal hyperplasia—current insights in pathophysiology, diagnostics, and management. *Endocrine Reviews*, *43*(1), 91–159.

Feng, Disheng, Wang, Zixuan, Li, Hang, Shi, Xianzhe, Zou, Lin, Kong, Hongwei, Xu, Zhiliang, Yu, Chaowen, Hu, Chunxiu, & Xu, Guowang. (2023). Steroid profiling for the diagnosis of congenital adrenal hyperplasia by microbore ultra-performance liquid chromatography–tandem mass spectrometry. *Clinica Chimica Acta*, *543*, 117304.

Kelestimur, Fahrettin, & Unluhizarci, Kursad. (2021). Congenital Adrenal Hyperplasia (CAH): Definition and Enzymatic Defects in Various Forms. In *Fertility and Reproductive Outcomes in Different Forms of Congenital Adrenal Hyperplasia* (pp. 1–18). Springer.

Kufoof, Tamara, Saad, Randa K., Al-Ghawanmeh, Redab, Sawaqed, Seri, Hamdan, Zaid, Qolaghasi, Zaid, Alswiti, Adnan, Sharkas, Layan, & Sharkas, Osama. (2025). Association between short stature and behavioral and emotional difficulties among children in Jordan: a cross-sectional study. *Frontiers in Endocrinology*, *16*, 1630919.

Lipkin, Paul H., Macias, Michelle M., Norwood, Kenneth W., Brei, Timothy J., Davidson, Lynn F., Davis, Beth Ellen, Ellerbeck, Kathryn A., Houtrow, Amy J., Hyman, Susan

L., & Kuo, Dennis Z. (2020). Promoting optimal development: identifying infants and young children with developmental disorders through developmental surveillance and screening. *Pediatrics*, *145*(1).

Loli, Paola, Menotti, Sara, di Filippo, Luigi, & Giustina, Andrea. (2025). Non-classical congenital adrenal hyperplasia: current insights into clinical implications, diagnosis and treatment. *Endocrine*, *90*(1), 1–16.

Mallappa, Ashwini, & Merke, Deborah P. (2022). Management challenges and therapeutic advances in congenital adrenal hyperplasia. *Nature Reviews Endocrinology*, *18*(6), 337–352.

Mizdrak, Maja, Tičinović Kurir, Tina, & Božić, Joško. (2021). The role of biomarkers in adrenocortical carcinoma: a review of current evidence and future perspectives. *Biomedicines*, *9*(2), 174.

Nakhleh, Afif, Saiegh, Leonard, Shehadeh, Naim, Weintrob, Naomi, Sheikh-Ahmad, Mohammad, Supino-Rosin, Lia, Alboim, Sandra, Gendelman, Raya, & Zloczower, Moshe. (2023). Screening for non-classic congenital adrenal hyperplasia in women: new insights using different immunoassays. *Frontiers in Endocrinology*, *13*, 1048663.

Nella, Aikaterini A., Mallappa, Ashwini, Perritt, Ashley F., Gounden, Verena, Kumar, Parag, Sinaii, Ninet, Daley, Lori Ann, Ling, Alexander, Liu, Chia Ying, & Soldin, Steven J. (2016). A phase 2 study of continuous subcutaneous hydrocortisone infusion in adults with congenital adrenal hyperplasia. *The Journal of Clinical Endocrinology & Metabolism*, *101*(12), 4690–4698.

Omona, Kizito, & Ssanyu, Balamaga Samuel. (2025). Biology and Pathology of Cortisol in Sexual Dysfunctions. In *Handbook of the Biology and Pathology of Mental Disorders* (pp. 2629–2652). Springer.

Paparella, Roberto, Panvino, Fabiola, Pucarelli, Ida, Niceta, Marcello, Spaziani, Matteo, Spalice, Alberto, Pisani, Francesco, Ardizzone, Ignazio, & Tarani, Luigi. (2026). Beyond endocrine features in non-classical congenital adrenal hyperplasia: a narrative review of psychoneuro-social perspectives in pediatric and adolescent patients. *European Journal of Pediatrics*, *185*(1), 11.

Podgórski, Rafał, Podgórska, Dominika, & Sumińska, Marta. (2023). A recent overview of non-classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency: pathophysiology, recognition, and management. *Pediatria Polska-Polish Journal of Paediatrics*, *98*(4), 307–319.

Speiser, Phyllis W., Arlt, Wiebke, Auchus, Richard J., Baskin, Laurence S., Conway, Gerard S., Merke, Deborah P., Meyer-Bahlburg, Heino F. L., Miller, Walter L., Murad, M. Hassan, & Oberfield, Sharon E. (2018). Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an endocrine society clinical practice guideline. *The Journal of Clinical Endocrinology & Metabolism*, *103*(11), 4043–4088.

Weisser, Johan Juhl, Hansen, Cecilie Hurup, Poulsen, Rikke, Larsen, Lizette Weber, Cornett, Claus, & Styrishave, Bjarne. (2016). Two simple cleanup methods combined with LC-MS/MS for quantification of steroid hormones in in vivo and in vitro assays. *Analytical and Bioanalytical Chemistry*, *408*(18), 4883–4895.

Wille, Sarah M. R., Coucke, Wim, De Baere, Thierry, & Peters, Frank T. (2017). Update of standard practices for new method validation in forensic toxicology. *Current*

Pharmaceutical Design, *23*(36), 5442–5454.

Witchel, Selma F., & Miller, Walter L. (2021). Ambiguous genitalia in the newborn. In *Endocrine Emergencies: Recognition and Treatment* (pp. 223–238). Springer.

Zhang, Yingying, Wang, Jingyi, Zhao, Jianxin, Huang, Guoying, Liu, Kaibo, Pan, Wei, Sun, Luming, Li, Jun, Xu, Wenli, & He, Chunhua. (2023). Current status and challenges in prenatal and neonatal screening, diagnosis, and management of congenital heart disease in China. *The Lancet Child & Adolescent Health*, *7*(7), 479–489.