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Original Research Article

Microsampling-Enabled Stress Biomarker Bioanalysis to Improve Mental Health Access for Mothers of Children with Autism in Rural Agricultural Communities: A Critical Review and Development Framework

Article History:

Name of Author:
Yan Yan[1] and Erlina Binti Abdullah[1]

Affiliation: *[1]Lincoln University College, Petaling Jaya, Selangor, Malaysia*

Corresponding Author:
Ankur Gupta

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Abstract: ***Background***: Mothers who are the primary caregivers of children with autism spectrum disorder (ASD) experience elevated chronic stress, anxiety, and depression. In rural agricultural communities, additional barriers – including long travel distances, seasonal farm work demands, limited childcare, social stigma, and poor internet connectivity – further impede timely access to mental health support. At the same time, patient-centric microsampling techniques coupled with liquid chromatography–tandem mass spectrometry (LC–MS/MS) enable decentralized measurement of stress-related endocrine biomarkers. ***Methods***: We conducted a critical scoping review focusing on three areas: (i) the mental health burden among ASD caregivers, particularly mothers; (ii) rural-specific barriers to mental health care and the potential of tele-mental health interventions; and (iii) the feasibility of microsampling-based bioanalysis of stress biomarkers (specifically hair and dried blood spot samples) for remote monitoring. We synthesized evidence into a practical implementation framework that integrates biomarker monitoring with a stepped-care tele-mental health model. ***Results***: Tele-mental health interventions can reduce geographic and travel-related barriers, but their impact is limited by digital inequities, privacy concerns, and constrained specialist referral capacity in rural areas. Hair cortisol concentration (HCC) provides an integrated measure of chronic stress over weeks to months, whereas dried blood spot (DBS) steroid panels can monitor shorter-term stress fluctuations over days to weeks. Deploying these tools in real-world rural settings requires standardized home-collection kits, stability controls tailored to variable shipping conditions, and validation strategies appropriate for endogenous hormone assays. ***Conclusions***: We propose a bioanalysis-enabled stepped-care access pathway (BESAP) that combines at-home microsampling, targeted LC–MS/MS analysis of stress biomarkers, and tele-mental health triage. This framework is designed to improve screening, triage, and longitudinal outcome monitoring for mothers caring for children with ASD in resource-constrained rural agricultural environments.

Keywords: autism spectrum disorder; caregiver stress; microsampling; tele-mental health; hair cortisol.

INTRODUCTION

Autism spectrum disorder (ASD) is a prevalent neurodevelopmental condition associated with significant support needs across the lifespan. Recent population-based surveillance and global burden data underscore the considerable ongoing health impacts of ASD, reinforcing the importance of family-centered

services and sustained caregiver support. Co-occurring mental health conditions are also common among autistic individuals, which can add complexity to caregiving and heighten family stress. Mothers often serve as primary caregivers for children with ASD and face higher risks of anxiety, depressive symptoms, and caregiver burden compared to other groups. These challenges are not only clinically significant for the mothers' own well-being; they also influence household functioning, parenting capacity, and the ability to engage consistently with interventions for the child. Over time, there may be economic repercussions, such as reduced employment participation and lost income, compounding the overall burden on families.

In rural agricultural communities, caregiver support is constrained by a *stacking* of structural and logistical barriers. These regions tend to have fewer mental health professionals and long waitlists, requiring caregivers to travel one to two hours or more to reach clinics. Long travel distances are exacerbated by limited childcare availability and inflexible work schedules tied to farming. Privacy concerns in tight-knit rural communities may discourage seeking mental health services due to stigma or fear of recognition. Additionally, seasonal peaks in agricultural labor (e.g. planting or harvest seasons) leave caregivers with little free time, and those periods of high stress often coincide with reduced opportunity to seek help. Inconsistent broadband internet access and low bandwidth in many rural areas limit the use of videoconferencing and other data-heavy digital health tools. Tele-mental health services can mitigate distance and transportation barriers by enabling remote access to care. However, simply offering tele-services does not automatically resolve issues of equity or capacity. Effective implementation requires attention to local realities – for example, building workable regional referral networks for severe cases and providing flexible modalities (such as telephone-based counseling or asynchronous messaging) when video calls are not feasible.

This review addresses a translational question at the intersection of mental health services and applied bioanalysis: Can microsampling-enabled stress biomarker monitoring support scalable, measurement-based mental health care for ASD caregivers in rural agricultural settings? We hypothesize that supplementing caregiver self-report measures with low-burden physiological stress indicators could improve triage decisions, enable earlier detection of non-response to interventions, and provide an objective complement to self-reported symptoms – particularly in contexts where in-person follow-up is challenging.

The review is organized around three guiding questions: (1) Which access barriers most strongly limit caregiver mental health support in rural agricultural communities? (2) What can tele-mental health realistically deliver for ASD caregiver support, and where are its limitations? (3) How can microsampling and stress biomarker bioanalysis (via hair and DBS samples) be integrated into stepped-care mental health pathways to improve screening, triage, and outcome monitoring?

REVIEW APPROACH AND SCOPE

We performed a critical narrative review using a scoping approach to map existing evidence and identify implementation-relevant gaps. Our literature searches emphasized recent publications (primarily the last 5 years), while also including foundational studies where needed. We supplemented peer-reviewed literature with recent *grey literature* reports on rural telehealth implementation to capture practical insights. The reporting of this review was guided by the PRISMA-ScR (Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews) checklist[17][18]. Methodological decisions were informed by the JBI (Joanna Briggs Institute) framework for scoping reviews and the PRISMA 2020 guidelines for transparent reporting[19][20].

We structured our search strategy around three key concept blocks: **(i)** ASD caregiving AND (stress OR depression OR anxiety OR burden); **(ii)** rural OR agricultural communities AND (telehealth OR tele-mental health); and **(iii)** microsampling OR dried blood spot OR VAMS AND (cortisol OR steroid panel OR hair cortisol) AND LC–MS/MS. We prioritized evidence with clear implications for feasible, low-burden implementation – for example, studies on kit design, remote sampling logistics, biomarker stability under field conditions, and integration into service workflows. Rather than performing a meta-analysis of outcomes, our goal was to synthesize diverse evidence into a development framework that could inform pilot implementations.

3. Mental health burden in mothers caring for children with autism

A consistent finding across many studies is that caregivers of children with ASD — typically mothers — report higher levels of psychological distress and burden than other caregiver groups. Key drivers of this elevated stress include the child's behavioral challenges, chronic sleep disruptions, co-occurring medical needs, financial strain, societal stigma, and reduced social support. Recent research also highlights several potentially modifiable mediators of caregiver stress, such as the availability of emotional support, use of adaptive coping strategies, and tolerance of uncertainty. These factors are important because they can be targets for interventions (e.g.

psychoeducational programs, peer support groups, skills training, or therapy for caregiver coping) that may alleviate overall distress.

There is growing recognition that addressing caregiver mental health is not only about improving the caregiver's well-being, but it can also positively influence outcomes for the child with ASD. When mothers experience high stress or depressive symptoms, it can impair parenting consistency, limit engagement with the child's therapies, and strain family relationships. Conversely, reducing caregiver burden and improving mental health may enhance the caregiver's capacity to support the child's development and could indirectly benefit the child's progress. This reciprocal relationship underscores the need for integrated approaches that support both child and caregiver.

However, many rural caregivers lack access to basic mental health services. In agricultural regions, mothers often have to balance caregiving with farm responsibilities, and they may delay or forgo their own care due to time constraints or lack of local services. Economic pressures can further exacerbate stress: studies have documented that families of children with ASD incur significant indirect costs, including lost work hours and diminished productivity for caregivers. Rural mothers, especially, may have fewer employment opportunities and less workplace flexibility, making them even more vulnerable to these economic stresses. These observations point to an urgent need for innovative strategies to deliver mental health support to caregiver populations who are geographically and socially marginalized.

Table 1 summarizes major categories of access barriers for ASD caregiver mental health support in rural agricultural settings, along with practical design responses identified in the literature and practice. The barriers span workforce shortages, travel and time limitations, technology gaps, privacy concerns, seasonal workload peaks, and limited specialist referral options. Each barrier demands a tailored response that combines service innovation with, potentially, bioanalytical tools (e.g. at-home monitoring) to create a more accessible and sustainable support system.

Table 1. Access barriers and practical design responses for ASD caregiver mental health support in rural agricultural communities.

Barrier category	Example in rural agricultural settings	Design response (service + bioanalysis)
Workforce scarcity	Few psychologists/psychiatrists; long waitlists	Stepped-care triage; brief interventions with referral escalation; CHW-supported navigation where available
Travel distance & time	>1–2 hour travel to clinics; childcare constraints	Tele-mental health as default for services; home sampling kits to reduce clinic visits
Broadband limitations	Unreliable video calls; data caps	Low-bandwidth modalities (telephone, asynchronous messaging); flexible sampling windows for biomarker collection
Privacy & stigma	Fear of being recognized seeking help; small-community dynamics	Discreet packaging of kits; remote sampling; privacy-by-design workflows
Seasonal work peaks	Harvest periods and other labor-intensive seasons	Align scheduling of sessions and sampling with agricultural calendar; offer micro-interventions (10–20 min modules)
Limited referral options	Few local specialists for severe cases	Regional referral networks; telepsychiatry consults; clear escalation protocols for crises

4. Rural barriers to care and potential of tele-mental health

In rural and remote areas, technology-enabled interventions have rapidly expanded the possibilities for mental health care delivery. Within autism services, various telehealth approaches have been tested – including remote caregiver coaching, parent training programs delivered via video, online psychoeducation modules, and other digital health interventions (DHIs). Recent reviews indicate that telehealth-based psychosocial interventions can improve maternal mental health outcomes and aspects of the parent–child relationship for mothers of young children with ASD. Tele-interventions like cognitive behavioral therapy (CBT) or acceptance and commitment therapy (ACT) delivered remotely have shown feasibility and efficacy in small trials. Furthermore, group-based tele-support programs and telehealth-facilitated parent training have been associated with reduced parenting stress and improved family routines.

However, tele-mental health is not a panacea for all challenges. Implementation in rural settings must confront the reality of the digital divide. Many rural families have limited or unreliable internet connectivity – for instance, they may have to rely on costly data plans or experience frequent service outages. Digital literacy can also be a barrier, as

some caregivers may not be comfortable navigating telehealth platforms or troubleshooting technical issues. Privacy is another concern: lacking a private space at home (especially in multigenerational farm households or small homes) can make it difficult for caregivers to participate openly in therapy sessions. Additionally, telehealth by itself cannot solve the scarcity of specialists – if a mother's needs exceed the capabilities of basic tele-therapy, there must be a path to refer her to higher-level care (which might still be distant). In fragmented rural systems, coordinating crisis response or arranging medication management remains challenging even if initial therapy is delivered remotely.

Caregiver-focused interventions can be conceptualized on a spectrum of intensity. Low-intensity options include self-guided web-based resources or short telephone check-ins. Moderate-intensity interventions involve scheduled tele-support groups or remote coaching sessions. High-intensity care may require structured psychotherapy delivered via telehealth (e.g. weekly CBT or ACT sessions by a clinician) and possibly telepsychiatry consultations for medication management. Stepped-care models are particularly appealing for rural contexts because they aim to allocate resources efficiently: caregivers with milder or moderate distress receive scalable supports first, while those who do not improve or who present with severe symptoms are "stepped up" to more intensive interventions. In this way, scarce specialist time can be focused where it is most needed, and others can benefit from less resource-intensive support.

A persistent gap in current practice is the lack of objective, low-burden outcome monitoring that can be obtained remotely. Care providers often rely solely on self-report questionnaires to gauge caregiver progress (e.g. depression scales, stress surveys). Self-report is indispensable, yet it can be influenced by factors such as momentary mood, social desirability, and survey fatigue. In agricultural communities, external stressors like weather or market conditions can fluctuate seasonally, affecting self-reported mood independently of any intervention. Applied bioanalysis offers the potential to complement self-report with physiological measures of stress. In theory, tracking a caregiver's biological stress markers (like cortisol levels) over time could help identify when stress remains high despite self-reported improvements (or vice versa), enabling more informed and timely adjustments to the care plan.

5. Tele-mental health in rural caregiver support: evidence and limits

Real-world implementation of tele-mental health for caregiver support must address several practical challenges. Engagement and retention are critical issues – enrolling caregivers into a tele-program is only the first step, and keeping them engaged over weeks or months is hard. Rural caregivers might drop out due to technology frustrations, competing responsibilities (especially during planting or harvest season), or because the format does not meet their needs. Providing technical orientation, offering sessions at flexible times (e.g. evenings after farm work), and periodically checking in can help maintain engagement. Also, offering a mix of formats (for example, allowing telephone participation if video fails, or asynchronous text-based coaching for those with unpredictable schedules) can increase accessibility.

Another limitation of tele-mental health is crisis management. If a caregiver shows signs of severe depression or expresses suicidal ideation, a remote provider may have difficulty ensuring their safety without local support. Rural tele-mental health programs, therefore, need clear protocols for emergencies – such as collaborations with local clinics or community health workers (CHWs) who can perform in-person checks or facilitate referrals to emergency services. Training CHWs or other local personnel to support the tele-mental health initiative (for example, by helping deliver materials, assisting with technology, or acting as a liaison) can strengthen the overall system.

Measurement-based care – the practice of systematically monitoring outcomes and using the data to drive decisions – has proven effective in many areas of mental health. In rural caregiver support, implementing measurement-based care via telehealth will require creative solutions. This is the context in which microsampling-enabled biomarker monitoring may play a role, as discussed in the next sections. Before introducing the bioanalytical tools, however, we emphasize that any biomarker strategy should be seen as *augmenting*, not replacing, the subjective experience of the caregiver. Psychological well-being is multidimensional, and cortisol or other biomarkers provide only one perspective. Still, as we outline below, combining multiple sources of data (self-report, behavioral indicators, and physiological markers) could create a richer picture of caregiver health and help tailor interventions more precisely.

6. Stress biomarkers and microsampling: an applied bioanalysis toolkit for rural settings

6.1 Why focus on cortisol and related steroid panels?

Cortisol is a core hormone of the hypothalamic–pituitary–adrenal (HPA) axis and is widely used as an indicator of physiological stress. However, how cortisol reflects stress can depend greatly on *how* and *where* it is measured. Different biological sample matrices offer distinct "time windows" of assessment, each with unique confounding factors. Therefore, selecting the appropriate matrix is crucial for matching the measurement to the clinical decision context. For example, if our goal is to stratify baseline chronic stress risk over months, a hair sample might be ideal; if we need to monitor short-term response over days or weeks, a DBS or saliva sample might be more informative.

Mass spectrometry-based assays (like LC–MS/MS) offer higher analytical specificity for steroid hormones than most immunoassays. This specificity is particularly important for endogenous steroids such as cortisol and cortisone, where immunoassays can suffer from cross-reactivity or interference by structurally similar molecules. For instance, LC–MS/MS can distinguish cortisol from cortisone and even quantify both simultaneously, providing additional insight (the cortisol:cortisone ratio can reflect certain metabolic or endocrine conditions). In summary, a targeted steroid panel via LC–MS/MS can yield a more nuanced and accurate profile of the caregiver's stress hormone levels than a single cortisol measurement by immunoassay.

6.2 Microsampling matrices suited to decentralized collection

Microsampling refers to techniques that allow collection of small quantities of biological samples (blood, saliva, etc.) with minimal invasiveness, often in a home setting. Embracing microsampling in caregiver support programs can reduce participant burden, improve feasibility of repeated measurements, and extend monitoring to geographically remote individuals.

Two sample matrices stand out for chronic stress monitoring in a decentralized context: hair and dried blood spots (DBS) (including volumetric absorptive microsamples, VAMS).

- **Hair (for HCC and related steroids):** Hair cortisol concentration (HCC) is interpreted as an integrated measure of systemic cortisol exposure over long periods (typically weeks to months, depending on hair length and growth rate). This makes it well-suited for tracking longer-term stress patterns and baselines. Key considerations for hair analysis include individual variability in hair growth rates, effects of hair cosmetic treatments (dyes, bleach) on cortisol levels, ethnic differences in hair characteristics, the specific segment of hair analyzed (e.g. the 3 cm proximal segment to represent ~3 months), and the potential decline of hormone signals during prolonged storage of hair samples. LC–MS/MS methods for hair can measure not only cortisol but also cortisone and other steroids, improving specificity and enabling ratio-based interpretations (cortisol vs. cortisone) that might give insights into HPA axis dynamics. However, hair analysis requires meticulous sample preparation (washing, grinding, extraction) and benefits from standardized reporting conventions (e.g. expressing results per unit hair weight or length).
- **DBS/VAMS (for capillary blood microsamples):** Dried blood spots provide a minimally invasive way to capture short- to intermediate-term endocrine profiles, including cortisol, cortisone, and possibly DHEA or other relevant steroids. A caregiver can self-collect a few drops of blood from a fingerstick onto a filter card (DBS) or a volumetric absorptive tip (VAMS) and mail it to the lab. Advantages of DBS include the simplicity of shipping (dried spots are stable for days and can often be mailed in an envelope without refrigeration) and the feasibility of doing repeated samples, for example weekly, during a caregiver intervention[50][53]. These features are ideal for monitoring short-term changes or the impact of a new support program. Key challenges with DBS/VAMS include the **hematocrit effect** (variations in blood hematocrit can affect the volume of blood absorbed and the distribution of analytes in the spot), variability in spot quality/volume if the user does not follow the procedure exactly, and potential analyte degradation if samples are exposed to high temperatures or humidity during transport[54][55]. As shown in **Table 2**, hair and DBS complement each other: hair captures cumulative stress exposure over months, while DBS captures more acute fluctuations over days to weeks.

Table 2. Candidate matrices for stress biomarker monitoring in remote caregiver support programs.

Matrix	Candidate biomarkers	Time window	Key advantages	Key limitations / controls	Best-fit use case
Hair	Cortisol, cortisone, other steroids	Weeks–months	Captures chronic exposure; stable in shipping; non-invasive	Hair treatments and ethnicity influence levels; segment standardization needed; signal decline over storage	Baseline risk stratification; tracking chronic change across seasons
DBS/VAMS	Cortisol, cortisone, DHEA(S); steroid ratios	Days–weeks	Home sampling feasible; mail-friendly; allows repeated measures	Hematocrit effect; spot quality variability; temperature stability concerns	Short-cycle monitoring during interventions; triage support
Saliva	Cortisol (e.g. CAR) and diurnal slope	Minutes–hours	Non-invasive; captures diurnal dynamics	Strict timing adherence needed; risk of sample contamination; requires cold-chain for storage if prolonged	Mechanistic sub-studies; circadian rhythm assessments

Matrix	Candidate biomarkers	Time window	Key advantages	Key limitations / controls	Best-fit use case
Urine	Free cortisol, cortisone, metabolites	Hours–day	Integrates secretion over collection interval	Collection burden; hydration influences concentrations	Targeted use when 24-hour collection is feasible

Figure 1 illustrates the relative time scales captured by different sampling matrices, from the very short-term measures to longer-term integrative measures. Saliva and urine reflect acute changes over minutes to ~1 day, DBS/VAMS cover intermediate periods on the order of days to weeks, and hair reflects cumulative hormone exposure over several weeks to months.

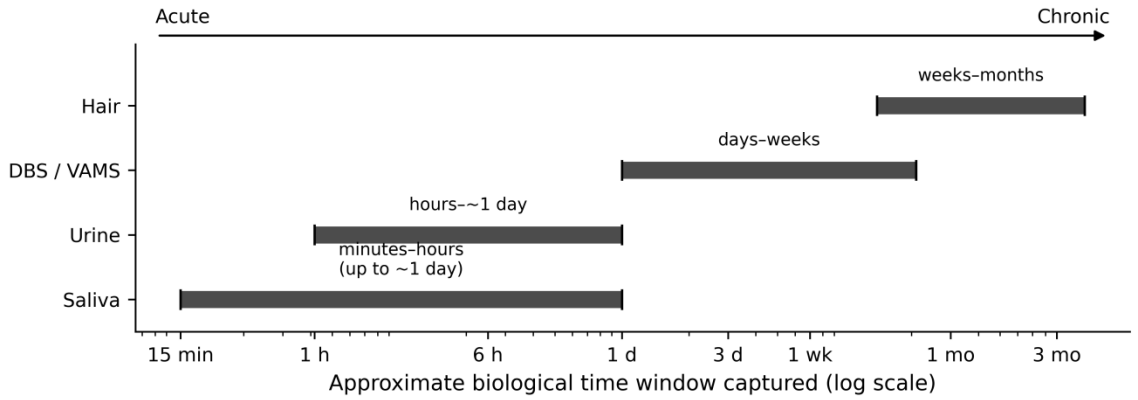


Figure 1. Approximate time windows of different stress biomarker sampling matrices, ranging from short-term to long-term measures. *Saliva and urine capture acute changes (minutes to hours, up to ~1 day). DBS/VAMS cover intermediate durations (days to weeks). Hair reflects integrated exposure over weeks to months.*

6.3 Bioanalytical workflows and validation considerations

When deploying LC–MS/MS assays for cortisol and related steroids in hair or DBS, robust validation is essential to ensure data quality and reliability, especially since these are non-traditional matrices for clinical monitoring. Validating assays for endogenous analytes like cortisol poses unique challenges because a true zero concentration cannot be obtained easily (everyone has some level of cortisol). Laboratories often must use surrogate matrices or "stripped" matrices (from which the analyte is removed) to prepare calibration standards. Approaches like standard addition (spiking known quantities into the real sample) can also help confirm accuracy in the native matrix.

Key validation parameters include: selectivity (ensuring the assay distinguishes cortisol and other targets from interfering compounds), recovery and matrix effects (checking that extraction from hair or DBS is efficient and that ionization in the mass spectrometer isn't suppressed or enhanced by matrix components), precision and accuracy (both within-run and between-run reproducibility using quality control samples), carryover (making sure a high sample doesn't contaminate the next), and stability (demonstrating that samples remain stable under expected shipping and storage conditions).

Dried matrix sampling introduces additional pre-analytical variability that must be managed. For DBS, factors such as the volume of blood applied, the uniformity of spot drying, ambient humidity, and the location of the punch taken from the DBS card can all influence measured concentrations. Best practices to mitigate these issues include providing standardized collection kits with clear pictorial instructions, using volumetric devices (like VAMS) that absorb a fixed volume of blood to reduce volume uncertainty, including humidity indicators and desiccants in kits to ensure proper drying, and implementing a laboratory QC triage step where DBS cards are visually inspected or even measured for spot adequacy before analysis.

Automation can also be a boon: as caregiver monitoring programs grow, incorporating automated sample preparation (e.g. robotic punching of DBS cards, automated extraction) and data processing can increase throughput and consistency while reducing human error. This is particularly relevant if a program scales up from a pilot study to a larger community implementation involving dozens or hundreds of caregivers.

Our review of bioanalytical literature and guidelines emphasizes that *laboratory readiness* is just as important as the

field feasibility of sampling. A lab supporting a BESAP program should ideally demonstrate that its methods meet accepted validation criteria (such as those from the FDA, EMA, or ICH for bioanalytical method validation) and that they have conducted *stability studies* that simulate real-world conditions – for example, testing cortisol stability in hair stored at room temperature for months, or cortisol stability in DBS cards mailed during summer vs. winter.

6.4 Interpretation of biomarkers: supportive, not diagnostic

It is crucial to frame the role of stress biomarkers properly: these measurements are supportive indicators and not diagnostic tests for conditions like depression or anxiety. Many factors can influence cortisol readings, including time of day, acute illnesses, medications (especially glucocorticoids like prednisone), and individual physiological differences. Therefore, any interpretation of a caregiver's biomarker results should be contextualized with at least four pieces of information: (i) the caregiver's self-reported symptoms (e.g. standardized stress or depression scale scores); (ii) current psychosocial stressors or life events (for rural mothers this might include things like the harvest season, financial strain, etc.); (iii) any relevant medications (particularly steroid medications that would alter cortisol levels); and (iv) the within-person change over time relative to their own baseline.

Studies of ASD caregivers suggest that combining physiological markers with self-report can yield a more comprehensive understanding of chronic stress. For instance, some research has measured both hair cortisol and salivary cortisol patterns (like the cortisol awakening response, CAR) alongside questionnaires, finding that together they can better characterize the caregiver's stress profile than either alone. In our proposed model, we envision biomarker tracking as a way to flag sustained high stress (or blunted cortisol patterns) that might indicate a need to *step up* care, even if the caregiver hasn't verbally reported worse symptoms. Figure 2 provides a schematic of how these measurements could integrate into a stepped-care service pathway tailored for rural ASD caregiver support.

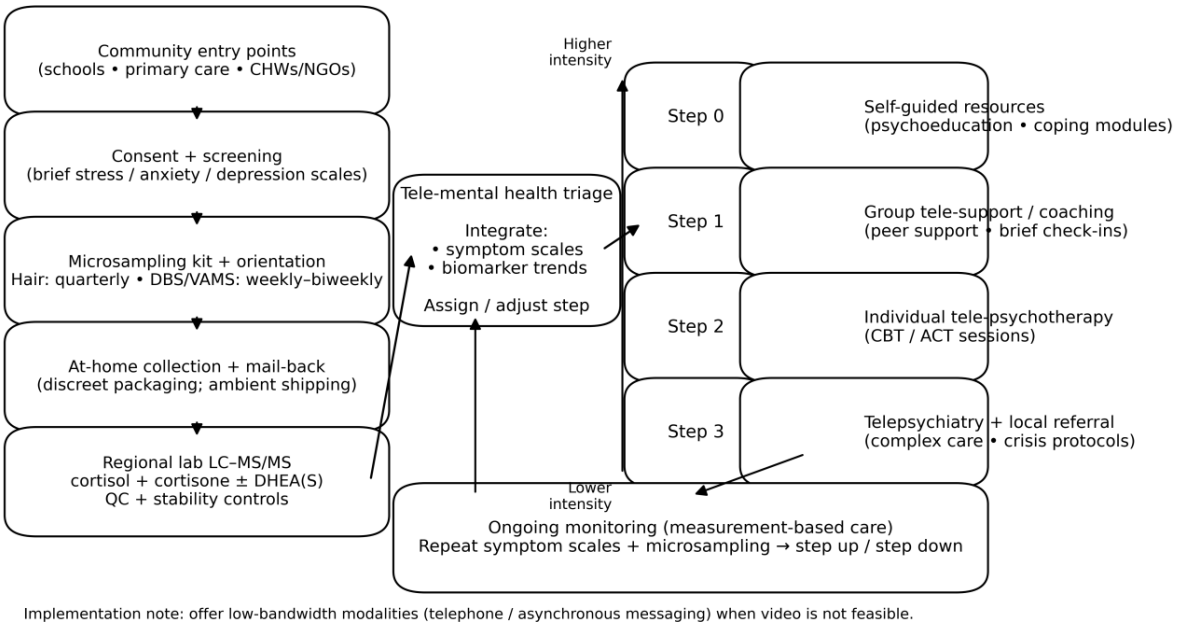


Figure 2. Bioanalysis-Enabled Stepped-care Access Pathway (BESAP) for autism caregiver mental health in rural agricultural communities. *In this model, community entry points (e.g. schools, clinics, community health workers) lead to initial screening (with consent and brief mental health scales). Caregivers who screen positive for elevated stress or distress then receive remote kit distribution and training for microsample collection. Caregivers self-collect samples at home (e.g. hair samples quarterly for long-term trends; DBS weekly or biweekly during active support periods) and mail them to a regional lab for LC–MS/MS analysis of cortisol, cortisone, and optionally DHEA(S). Lab results are returned to the care team and incorporated into telehealth triage. Based on self-report and biomarker trends, the caregiver is placed in a stepped-care sequence: Step 0 – self-guided digital resources; Step 1 – group tele-support or coaching; Step 2 – individual telehealth psychotherapy (e.g. CBT or ACT); Step 3 – psychiatric consultation with local referral for complex cases. Outcomes are monitored and fed back in an iterative loop, allowing escalation or de-escalation of care intensity as needed.*

7. Development framework: implementing BESAP in real-world rural systems

7.1 Service design principles

The BESAP framework is designed with feasibility in mind for low-resource rural settings. It intentionally combines: **(i)** tele-mental health modalities, **(ii)** CHW-supported navigation where available, and **(iii)** low-burden biomarker monitoring for measurement-based care. By leveraging stepped-care, the model seeks to improve efficiency—higher-intensity resources (therapist time, psychiatric consultation) are allocated to caregivers who demonstrate persistent or severe distress, while those with milder issues receive scalable, lower-intensity support in earlier steps.

Tele-mental health components in BESAP can draw from the growing evidence base of caregiver interventions delivered remotely. This includes programs focused on stress reduction techniques, mindfulness or ACT interventions tailored for parents, and online peer support communities. A core principle is flexibility: not all rural caregivers will benefit from the same modality. Some may prefer structured sessions via video, others might engage more with a mobile app or text-messaging check-ins due to scheduling issues. The BESAP design emphasizes meeting caregivers where they are technologically and logistically.

Equity considerations are embedded throughout the framework. This means proactively addressing digital inequities by, for example, providing options for phone-based participation and ensuring materials are accessible to those with limited tech literacy. It also means being mindful of language, cultural norms, and stigma. In some communities, mental health struggles are not openly discussed; framing biomarker monitoring as a general "health check" and tele-support as "parent coaching" might reduce barriers to acceptance.

Finally, the service design assumes partnerships with existing rural institutions. Local schools, extension services, or faith-based organizations could serve as trusted entry points for identifying and reaching mothers in need. These partners can also assist with follow-up – for instance, a local nurse might help a caregiver who missed a tele-session or ensure a replacement kit is delivered if a sample was unusable. By blending high-tech approaches (remote assays, telehealth) with community-based support, BESAP aims to be both innovative and locally grounded.

7.2 Bioanalytical implementation: sample logistics and quality systems

Operationalizing the biomarker component of BESAP in a rural context requires careful planning of logistics and quality controls. At minimum, a BESAP kit needs to include all materials for hair and DBS collection along with user-friendly instructions. For example, a hair collection kit might contain a small pair of scissors, a ruler or marked paper for measuring a standard hair length, and a foil or pouch to protect the hair sample. A DBS kit would include items such as sterile lancets for fingerstick, alcohol swabs, gauze, a filter paper card or VAMS device, a drying container, and a sealable bag with a desiccant pouch and humidity indicator card.

Clear, pictorial instructions are essential, given the varying literacy levels and language preferences in rural populations. The instructions should demonstrate how to cut a hair sample as close to the scalp as possible (for consistency, perhaps from the posterior vertex) and how to perform a fingerstick and apply blood to the card without over- or under-spotting. Including a simple log sheet for the caregiver to record sample collection date, time of day, and any notable events (like "very little sleep last night" or "felt sick") can assist with later interpretation.

Once samples are collected, shipping logistics come into play. Ideally, kits provide prepaid mailers so that caregivers can send samples back without incurring cost or needing to travel. Given rural mail variability, ensuring that samples can withstand a range of conditions is crucial. Hair is quite stable at room temperature, but DBS can be sensitive to humidity and heat. Therefore, BESAP kits include desiccant packs and a humidity indicator that changes color if moisture exposure is excessive. If a kit is returned with the humidity indicator showing high exposure, the lab knows to be cautious (or can decide if re-sampling is needed).

At the laboratory end, a clear chain-of-custody and accessioning process is needed. When samples arrive, they should be logged, and each undergoes a quick **QC triage**. For hair, this might mean checking that enough hair was provided and it appears to be from the scalp (not shed hairs). For DBS, this involves examining the spots for sufficient size and uniformity, and noting any obvious issues like clotted or smeared samples. Any sample failing this initial QC could trigger sending the caregiver a request for a repeat sample (with troubleshooting tips).

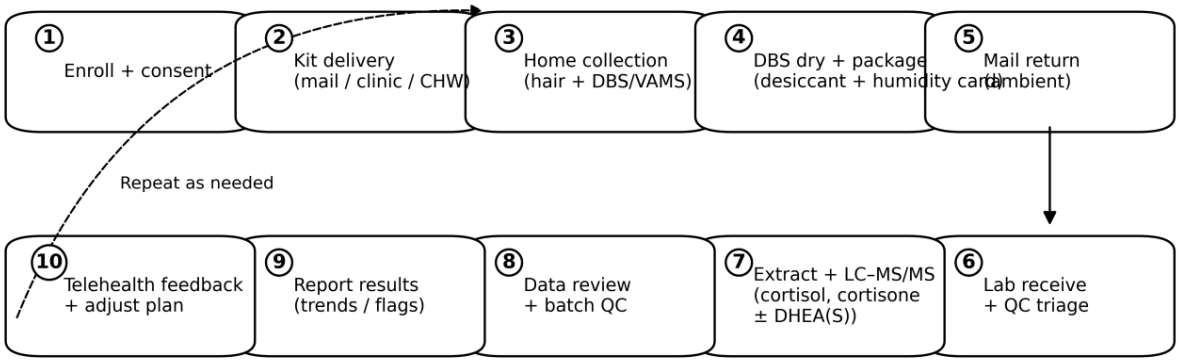
Analytically, the lab will run the LC–MS/MS assays with appropriate controls. This includes stable isotope-labeled internal standards in each sample to correct for any extraction losses or instrument variability. Quality control (QC) samples at low, medium, and high concentrations should be analyzed in each batch to ensure the assay is performing within validation parameters. Incurred sample reanalysis (repeating a subset of actual study samples) can be done to confirm reproducibility. Importantly, for a programmatic context, labs might implement batch processing – for example, hold all samples from a given month and analyze together – to reduce cost. This is acceptable as long as

stability data support that holding time.

For DBS, special controls include steps to address hematocrit-related bias. Some labs may use software that estimates spot volume or corrects for hematocrit by analyzing the blood spot image. Others might exclusively use volumetric devices (like those that absorb 20 µL reliably) to circumvent the issue. If an assay can't fully correct for hematocrit, the program can adopt a pragmatic workaround: focus on within-person changes rather than absolute values, under the assumption that an individual's hematocrit is relatively stable over time.

Finally, data management and reporting are key. The lab should ideally have a system to flag out-of-range results or significant changes and rapidly communicate these to the clinical team. For example, if a mother's hair cortisol comes back extremely high (relative to typical levels), the lab report could highlight this so that the clinician can follow up promptly. Since the goal is not to diagnose but to inform care, results might be reported in a simplified way – e.g. "cortisol trend increasing" or "cortisol within expected range" – along with the numeric values, to facilitate understanding for both providers and caregivers.

Figure 3 outlines an example *home-to-result* workflow, illustrating each step from enrollment through to utilizing the results in a feedback loop with the caregiver.



DBS = dried blood spot; VAMS = volumetric absorptive microsampling; QC = quality control; CHW = community health worker.

Figure 3. Home-to-result workflow for hair and DBS stress biomarker monitoring in a rural tele-mental health program. *Ten key steps are shown: (1) caregiver enrollment and consent; (2) kit delivery (via mail, local clinic, or CHW); (3) at-home sample collection (hair cut near scalp; fingerstick for DBS); (4) proper drying of DBS and secure packaging of samples with desiccant and humidity indicator; (5) mailing samples to the laboratory at ambient conditions; (6) lab accessioning and QC triage (checking DBS spot quality and hair sample adequacy); (7) extraction and LC–MS/MS assay of cortisol, cortisone, and possibly DHEA(S); (8) data review and quality checks, including matrix-specific considerations; (9) reporting of results (focusing on trends or significant changes) to the care team; (10) a telehealth feedback session with the caregiver to discuss results and adjust the care plan as needed.*

7.3 Digital equity, privacy, and ethical considerations

BESAP programs must proactively address issues of digital equity, privacy, and ethics to be successful and trustworthy. For digital equity, as noted, it is important to offer low-bandwidth alternatives and offline options. If a caregiver cannot stream video, she should still be able to receive help via regular phone calls or SMS texts. If internet data is expensive, the program might partner with a sponsor to provide data vouchers or leverage zero-rated health messaging services. Providing devices (like a basic tablet or smartphone) to caregivers who lack one could also be considered in grant-funded pilots to bridge the technology gap.

Privacy is a double concern in these contexts: not only the confidentiality of health data but also the *perception* of participating in a program. To protect privacy, BESAP materials (like kit packages) are designed to be discreet, with no explicit labels that reveal they are for mental health purposes. Data governance protocols should define clearly who has access to the biomarker results and telehealth session notes. For example, results might be accessible only to the core clinical team and the caregiver herself, not to other local staff, unless explicit consent is given.

Another ethical aspect is how to handle potentially sensitive findings. What if a biomarker suggests very high stress

levels? The program should have guidelines on how to communicate this to the caregiver in a supportive, non-alarming way. It should also clarify that these biomarkers are not tests of "effort" or "parenting" – some caregivers might fear being judged by their cortisol readings. Emphasizing that the goal is to personalize support and *not* to pass judgment is important for maintaining trust.

Finally, any research or pilot implementation of BESAP should engage with community stakeholders from the planning stage. Community advisory boards or focus groups can provide input on the acceptability of procedures (like collecting hair, which might have cultural sensitivities for some) and help tailor the approach to local norms. Ethical oversight by an institutional review board (IRB) or equivalent is needed if data will be collected for research, given that we are dealing with personal health information and possibly vulnerable populations (mothers under stress).

8. Implications for local development and sustainability

Implementing a program like BESAP could have broader implications beyond individual caregiver wellbeing. Reducing caregiver psychological distress may in turn improve caregivers' ability to work or engage in community activities. In rural farming communities, this can translate into more stable labor participation and potentially improved agricultural productivity or household income. For example, if a mother is less overwhelmed by stress, she might be better able to manage farm tasks or pursue part-time work, thereby improving the family's economic resilience during critical seasons.

There are also health system sustainability considerations. High caregiver stress is associated with greater healthcare utilization – both for the caregiver (e.g. more clinic visits for stress-related issues) and potentially for the child (if caregiver strain impacts management of the child's needs). By intervening early and proactively (with stepped care and monitoring), BESAP might reduce crisis visits or the need for more expensive interventions down the line. Over time, this could result in cost savings for health services, or at least a more efficient use of limited mental health resources (e.g. focusing specialist time on those in greatest need).

From a community development perspective, a BESAP program could create new roles or jobs locally. For instance, one could employ community members as care navigators or kit coordinators who handle distribution and collection of sampling kits, or as lay counselors supporting tele-mental health follow-ups. Strengthening regional laboratory capacity for bioanalysis is another potential benefit – a regional lab handling BESAP samples might also serve other public health needs, thus building local technical infrastructure.

It is important, however, not to overstate these hypothetical benefits without evidence. We suggest that any pilot of BESAP incorporate an **evaluation of economic outcomes** (as outlined in Table 4) to quantify things like changes in work attendance for caregivers or any observable cost offsets (e.g. reduced travel costs for families, fewer emergency visits). These data would be crucial for convincing policymakers and funders to sustain and scale the program.

Table 4 proposes example outcome measures to evaluate a BESAP pilot across multiple domains: reach/equity, effectiveness (caregiver outcomes), bioanalytical performance, adoption/feasibility, maintenance (sustainability), and broader economic signals. Collecting data in each of these domains can provide a comprehensive picture of the program's impact and inform necessary adjustments.

Table 4. Suggested evaluation outcomes for a BESAP pilot program in rural agricultural communities.

Outcome domain	Example measures	Suggested framework
Reach & equity	Enrollment rate; broadband/internet availability; retention rate; subgroup participation (e.g. by socioeconomic status)	[32] (RE-AIM Reach)
Effectiveness	Pre-post changes in caregiver stress, depression/anxiety scales; caregiver burden; sleep quality; quality of life	[8,9,35]
Bioanalytical performance	Percentage of samples adequate for analysis; QC pass rate; turnaround time for results; instances of analyte instability or degradation	[71,65,81]
Adoption & feasibility	Clinic/CHW workload implications; tele-visit completion rate; kit delivery logistics success (on-time, etc.)	[29,30] (implementation outcomes)
Maintenance	Program continuation at 6–12 months; cost per engaged caregiver; ongoing participation rate	[32] (long-term RE-AIM)

Outcome domain	Example measures	Suggested framework
Economic signals	Changes in caregivers' work attendance or productivity; health service utilization; cost offset (e.g. reduced travel or hospitalizations)	[12–14]

9. Research agenda

This review and framework development exercise point to several next steps for research:

- **Biomarker panel optimization:** Determine the minimal but most informative set of biomarkers for caregiver stress monitoring. For example, is measuring cortisol and cortisone sufficient, or does adding DHEA-S (dehydroepiandrosterone sulfate) significantly enhance the utility? The panel should align with intervention goals and be feasible for rural labs to validate.
- **Pilot comparative studies:** Conduct feasibility pilots to compare different monitoring approaches. One pilot could use hair cortisol alone for monitoring, while another uses combined hair + DBS. Key outcomes would be sensitivity to change (can we detect improvement or worsening in stress with each approach?), caregiver acceptability (which method do mothers prefer or adhere to better?), and operational complexity.
- **Digital equity and context adaptation:** Research ways to embed digital equity safeguards into telehealth programs from the outset. This might involve testing offline delivery of content (like providing pre-loaded tablets with psychoeducational videos for those with no internet) or designing intervention schedules around farming calendars (e.g. pausing or simplifying program tasks during harvest time). Implementation science frameworks like CFIR (Consolidated Framework for Implementation Research) can guide how to adapt BESAP to different local contexts.
- **Economic evaluation:** Alongside effectiveness, study the cost-effectiveness and long-term sustainability of the BESAP approach. This likely requires a cluster-randomized trial or a stepped-wedge design in which some communities get the program earlier than others. Important metrics would include healthcare utilization differences, cost per quality-adjusted life year (QALY) if possible, and any changes in caregivers' economic participation. Using frameworks such as RE-AIM (Reach, Effectiveness, Adoption, Implementation, Maintenance) and outcome definitions from implementation science will ensure that both implementation process and outcomes are rigorously documented.
- **Longitudinal outcomes:** It will be valuable to track whether improvements in caregiver stress (as measured by both questionnaires and biomarkers) translate into longer-term gains. Do reduced stress levels sustain beyond the active intervention period? Are there ripple effects, such as improved child outcomes or better family relationships? Longitudinal mixed-methods studies could provide insight here, combining follow-up measurements with qualitative interviews of participants about their experiences.

10. Limitations

Our work is a critical narrative review and the proposal of a development framework, not a formal systematic review or a randomized trial. Thus, it has several limitations. The literature base we synthesized is heterogeneous, covering various settings (high-income vs. low-income countries), different types of interventions (from psychological therapies to tech-based interventions), and different biomarker protocols. We did not perform a quantitative meta-analysis, so we cannot make definitive statements about effect sizes for any intervention. Moreover, many of the ideas presented (such as integrating biomarker monitoring with telehealth in this specific population) have not yet been tested empirically. They represent extrapolations from related fields or logical combinations of findings.

Another limitation is the inherent variability of stress biomarkers. Factors outside of caregiver stress – like comorbid medical conditions, individual differences in steroid metabolism, or even hair treatment habits – can influence results. We caution that biomarkers should not be over-interpreted or used in isolation. There is a risk of *false reassurance* (e.g. a normal cortisol level in a very distressed individual) or *unnecessary alarm* (e.g. an elevated cortisol due to poor sleep one night might not mean the intervention is failing). These possibilities underscore why we emphasize using biomarkers only as an adjunct to established measures and clinical judgment.

Finally, in terms of scope, our focus was primarily on mothers of children with ASD in rural agricultural settings. While many findings likely generalize to other caregivers or other rural communities, readers should be careful in extending the conclusions. For instance, fathers or other family-member caregivers might have different stress profiles or help-seeking behaviors that warrant tailored approaches. Similarly, non-agricultural rural communities (e.g. fishing communities or mining communities) might face some different stressors or resource dynamics. Future work could explore how BESAP-like models might be adapted for those contexts.

CONCLUSIONS

Mothers caring for children with ASD – particularly in rural agricultural communities – face a compounded set of challenges in accessing consistent mental health support. Geographic isolation, scarcity of providers, demanding farm responsibilities, and socio-cultural barriers all contribute to a gap in care. Tele-mental health offers a promising route to bridge distance and bring services into the home, but it must be implemented with an awareness of local constraints, ensuring that technology and outreach strategies are equitable and culturally appropriate.

Microsampling-enabled stress biomarker bioanalysis (using hair and DBS samples) represents a practical toolkit to support a **measurement-based care** approach in these hard-to-reach settings. By objectively tracking physiological indicators of stress over time, alongside traditional self-reports, care providers may gain a more complete picture of a caregiver's well-being. This can inform more timely triage (who needs more help now?) and provide validation of intervention effects (is the chosen support working, as reflected biologically?).

The proposed BESAP framework integrates these elements – tele-mental health, community support, and biomarker monitoring – into a program design that is intended to be feasible in resource-constrained environments. It emphasizes stepping up care intensity as needed, maintaining engagement through accessible means, and continuously learning from data to refine the approach. While this framework is yet to be tested, it offers a roadmap for development and pilot testing.

In sum, leveraging applied bioanalysis in the context of rural mental health care could help transform the way we support caregivers of children with ASD. With careful implementation and community collaboration, such innovations hold the potential to foster healthier, more resilient caregivers and, by extension, more resilient rural families and communities.

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