Study protocol with statistical analysis plan

Study's Official Title: Validation of Indiana's Early Evaluation Hub System (EAER21)

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IRB PROTOCOL

Validation of Indiana's Early Neurodevelopmental Evaluation Hub Program

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Abbreviations
Ages and Stages Questionnaire, Third Edition (ASQ-3)
Autism spectrum disorder (ASD)
Autism Diagnostic Observation Schedule, Second Edition (ADOS-2)
Child Behavior Checklist (CBCL)
Developmental Delay (DD)
Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)
Early Evaluation (EE)
Modified Checklist for Autism in Toddlers (M-CHAT)
Mullen Scales of Early Learning (MSEL)
Primary care physician (PCP)
Pupillary light reflex (PLR)
Research Electronic Data Capture (REDCap)
Screening Tool for Autism in Toddlers (STAT)
Vineland Adaptive Behavior Scale, Third Edition (VABS-3)

1.0 Background & Rationale

(A) Significance

Autism spectrum disorder (ASD) is a complex, highly heritable neurodevelopmental disability with recent prevalence estimates of 1 in 59 children [1] and lifetime costs exceeding $2.4 million [2]. Families of young children across the nation face significant barriers to accessing timely, local early ASD diagnostic evaluations, ultimately delaying entry into evidence-based interventions, which have the potential to improve outcomes [3] and lower societal costs [4]. As such, federal agencies (e.g., National Institute of Mental Health), advisory committees (e.g., Interagency Autism Coordinating Committee), and investigators and stakeholders in the field of ASD research [5] are collectively calling for studies that evaluate the diagnostic accuracy of streamlined assessment models, determine the accuracy of novel biomarkers for the classification of ASD in population-based samples, and examine whether these tools may be feasibly implemented along with existing clinical assessment within primary care settings to improve diagnostic classification accuracy. To date, researchers have *independently* examined community-based models of early ASD diagnosis [6-9] and measures of underlying biological processes [10] as alternative approaches to identifying children with ASD. However, given the complex, heterogeneous nature of the ASD phenotype, multi-method community-based evaluation approaches that integrate both clinical and biobehavioral tools have the potential to improve the accuracy and timeliness of early ASD diagnosis [5].

Overall Scientific Premise

There is consensus among ASD experts that symptoms emerge in the first year of life and the disorder can be reliably identified during the toddler years [11]. Yet, nationally the average age of ASD diagnosis is not until 4 to 5 years [5], with diagnosis of children from lower income, minority, and rural families lagging an additional year and a half [12-14]. Clinical guidelines for ASD diagnosis support the use of multidisciplinary team assessment [15]; however, a shortage of expert providers leads to long wait times and travel burden for families of young children and significant strain on the public health care system. Most critically, the significant delay between the emergence of ASD symptoms and diagnosis means that young children are missing critical

opportunities for early, evidence-based interventions at the time of optimal neuroplasticity. The current proposal addresses this need by linking a community-based clinical evaluation model with the utilization of biobehavioral markers to test the diagnostic efficacy of these approaches for accurately classifying young children with ASD in a high-risk community-referral sample in the primary care setting.

Scientific Premise for Aim #1

Pediatric advocacy groups [16] have highlighted the challenges around access to early diagnostic services for children at risk for ASD and have called for the evaluation of new models of care delivery [5]. To date, three small studies have investigated whether training community-based clinicians to provide ASD diagnostic services is a feasible endeavor. McClure and colleagues [7] trained local teams of health professionals to perform ASD assessment. Of 33 children evaluated, there was 87% diagnostic agreement with the expert ASD evaluation team and a significant reduction in wait time for evaluations. Warren and colleagues [9] reported on a program in which pediatricians were trained to diagnose young children with ASD in community practice settings. In this pilot study of 21 children ages 22-37 months, there was 71% agreement between pediatrician diagnosis and independent expert ASD evaluation. In follow-up to this work, Swanson and colleagues [8] used the same training model to measure the accuracy of ASD diagnostic evaluation in a sample of 14 children with 86-93% diagnostic agreement between local pediatric providers and independent expert evaluation. Limitations to these important preliminary studies include small sample sizes and limited variability in child diagnosis (including under representation of those ultimately diagnosed with ASD and those with co-occurring intellectual disability) and baseline level of knowledge in community providers. Yet, these studies represent promising preliminary evidence for implementation of innovative models in which local pediatric health professionals provide ASD diagnostic evaluations in community settings. The proposed project builds on this preliminary evidence by testing an existing statewide model of early ASD diagnosis in the primary care setting with a high-risk community-referral sample of children. We will determine the diagnostic validity of this existing model by comparing diagnostic agreement between primary care providers and independent expert ASD evaluation.

Scientific Premise for Aim #2

Prospective, longitudinal studies of high-risk infants have begun to yield potential risk factors, or *biomarkers,* of infants later diagnosed with ASD. These biomarkers have the potential to elucidate the mechanisms that contribute to the development of ASD, ultimately providing methods for earlier identification of the disorder and potential early intervention targets. Neuroimaging methods, such as structural magnetic resonance imaging (MRI) measures [17-19] and electroencephalography (EEG) [20-22], have been utilized to study early neural markers of ASD. However, it remains unclear whether these are appropriate tools to be used with infants, children, and lower-functioning individuals, because obtaining high-quality data from these groups can be extremely difficult. To date, the most non-invasive, low-cost, and feasible approach to identifying early risk factors for ASD may be the use of eye-tracking technology.

Several brief, easy to administer eye-tracking indices of neuromodulator activity have been shown to be sensitive to ASD. For example, Nyström and colleagues [23] reported that pupillary light reflex (PLR), which is associated with the neurotransmitter acetylcholine (Ach) [24], differed between 9- to 10-month-old high-risk infants later diagnosed with ASD compared to both high-

and low-risk infants that did not, and was associated with ASD symptom severity at 36 months. Anderson and colleagues [25] demonstrated, in two independent samples, that tonic pupil size, an indirect index of locus-coeruleus (LC) / norepinephrine (NE) activation [26], can be used to classify young children with ASD (> 80% correct classification rates) and is associated with level of ASD symptomatology. More recently, we have shown that an indirect measure of central dopaminergic activity, spontaneous eye blink rate [27], is lower in children with ASD [28]. In addition, behavioral eye-tracking measures including the latency of saccadic eye movements and fixation durations have been shown to differ in high-risk infants in both social and non-social paradigms. For example, high-risk infants later diagnosed with ASD become slower at shifting their attention by the end of the first year of life [29, 30] in non-social paradigms and show reduced attention to eyes [31] and decreased attention to social videos [32].

However, it is not yet known whether these biomarkers may accurately distinguish ASD from other children at high-risk for non-ASD neurodevelopmental disabilities. Further, it remains to be determined whether these eye-tracking indices can be successfully measured in the primary care setting. The proposed project builds on early evidence of potential ASD biomarkers by testing whether these data are able to be feasibly acquired in the community-based setting and if these indices can accurately classify children with ASD in a high-risk sample referred for neurodevelopmental evaluation.

Scientific Premise for Aim #3

Converging evidence from studies employing diverse (i.e., genetic, neuroimaging, behavioral) methodologies suggests that ASD is associated with multiple developmental pathways resulting in the emergence of a complex, heterogeneous phenotype. To assist in the diagnosis of ASD and account for this variability, several multivariate approaches using a single neuroimaging modality [33-35] or employing multiple imaging modalities [36] have been used for diagnostic classification. These methods have generally demonstrated positive results; however, as reviewed above, the utility of MRI in the context of early neurodevelopmental evaluation for ASD remains limited. Others have attempted to use multiple behavioral observational and parent/caregiver-report measures to classify ASD [37]. Despite clear group differences, classification of ASD (at 8 months) at the individual level using this approach was only moderately accurate. Recently, Lombardo and colleagues [38] combined neuroimaging and clinical measures to classify language outcomes in children with ASD. These authors showed that fMRI in combination with developmental, adaptive, and ASD symptom severity scores provided good specificity and sensitivity for future language outcome compared to any measure alone. The proposed project aims to adapt this approach by combining novel eye-tracking assays of ASD risk with standardized clinical measures in the context of community-based evaluation to predict diagnostic outcome.

2.0 Objective(s)

The overall objective of this proposal is to test an innovative method of ASD diagnosis for young children in the primary care setting, which integrates clinical assessment by general pediatric providers with utilization of eye-tracking biomarkers to improve diagnostic accuracy. Our rationale for this project is that providing evidence for a novel, integrative model of ASD assessment in local communities has the potential to reduce barriers to care, resulting in earlier entry into evidence-based interventions, thereby improving outcomes and decreasing strain on the public health system. Three specific aims guide the proposed research:

Aim #1: ***Evaluate the diagnostic validity of the Early Evaluation (EE) Hub model of ASD diagnosis in the community-based primary care setting***. A team at Indiana University School of Medicine has implemented a community-based early ASD evaluation program at primary-care practices across the state of Indiana. The EE Hubs will refer a consecutive sample of children, ages 14-48 months, for comprehensive expert ASD evaluation. Diagnostic validity will be measured by comparing agreement between the EE Hub and expert ASD-specialist on categorical diagnosis to estimate indices of diagnostic accuracy.

Aim #2: ***Determine whether biobehavioral markers can reliably differentiate young children with and without ASD in a high-risk community-referral sample.*** A series of eye-tracking measures (pupil dilation, pupillary light reflex, blink rate) will provide indirect measures of neuromodulator activity (i.e., norepinephrine, acetylcholine, and dopamine, respectively) and social (looking time) and non-social (saccadic latency) attentional processes. Differences in metrics between children with and without ASD will be compared to validate potential biomarkers.

Aim #3: ***Determine whether a combination of clinical and biobehavioral measures can be used to accurately predict ASD diagnostic outcome in a high-risk sample of young children evaluated in the primary care setting.*** We will evaluate the predictive efficacy of clinical measures and biobehavioral markers for accurate ASD diagnostic classification, and further determine whether a multimodal combination of clinical and biobehavioral measures may potentially provide a more promising method for ASD diagnostic classification in the primary care setting. Classifier analysis for diagnostic outcome will be completed.

3.0 Outcome Measures/Endpoints

Standardized Clinical Measures

Toddler ASD Interview for Caregivers: Standard caregiver interview questions to assess for DSM-5 [39] [6]ASD criteria in toddlers. To be administered and ratings completed by the expert ASD specialist following the clinical interview. Additional ratings based on observation of the child throughout the evaluation will be completed by the expert ASD specialist.

Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) [40]: The ADOS-2 is a semi-structured standardized measure of social communication, play, and restricted and repetitive behaviors for individuals, ages 12 months and up, with varying developmental and language levels. Algorithm scores are compared with cutoff scores to yield a diagnostic classification (i.e., Autism, Autism Spectrum, or Non-spectrum; or Range of Concern for the Toddler Module). The comparison score allows for examination of an individual's overall level of ASD-related symptoms in relation to those of other same-aged children diagnosed with ASD who have similar language skills.

Mullen Scales of Early Learning (MSEL) [41]: The MSEL is a brief and reliable measure of cognitive ability in children from birth to 68 months of age, and includes five scales: Gross Motor, Visual Reception, Expressive Language, and Receptive Language. T-scores for each scale and an Early Learning Composite standard score are produced.

Vineland Adaptive Behavior Scales, Third Edition (VABS-3) [42]: The VABS-3 is a parent/caregiver interview that assesses a child's adaptive skills across the domains of Communication, Daily Living Skills, Socialization, Motor Skills, and Maladaptive Behavior.

Subdomain scaled scores, domain standard scores, and an Adaptive Behavior Composite standard score are derived from the measure.
Sensory Processing Assessment [43]: An abbreviated version of the Sensory Processing Assessment will be used to measure behavioral sensory responses to tactile, auditory, and/or visual information during play.

Biomarker Measures
An SR Research EyeLink Portable Duo remote eye-tracking system will be used to measure eye movements and pupil diameter. Four separate paradigms will be administered totaling 8-10 minutes in duration. Eye-tracking measures will include pupil dilation, percentage looking time, and latency to initiate saccadic eye movements.

Caregiver Report Measures
EAE Hub Satisfaction Questionnaire: Brief caregiver report questionnaire to assess caregiver satisfaction with Early Evaluation Hub clinical diagnostic evaluation visit.
Demographic and Background History Questionnaire: Brief caregiver-report questionnaire that collects information on child's race and ethnicity, family income, caregiver education level, and child medical history.
ASD Risk Questionnaire: Brief caregiver-report questionnaire to assess for and quantify potential environmental risk factors for ASD.
Child Behavior Checklist (CBCL1.5-5) [44]: The CBCL obtains caregiver ratings for 99 problem behaviors in children ages 1.5 through 5 years. Items are scored on the following syndrome scales: Emotionally Reactive, Anxious/Depressed, Somatic Complaints, Withdrawn, Attention Problems, Aggressive Behavior, and Sleep Problems. Items are also scored on the following DSM-oriented scales: Affective Problems, Anxiety Problems, Pervasive Developmental Problems, Attention Deficit/Hyperactivity Problems, Stress Problems, Autism Spectrum Problems, and Oppositional Defiant Problems.
Intervention Service Questionnaire: Brief caregiver report questionnaire that gathers information on interventions and services a child may be enrolled in, intervention duration and intensity, caregiver perceived progress, and barriers faced in service enrollment.

See Section 5.0 for study procedures (including how measures will be collected).

4.0 Eligibility Criteria

Inclusion Criteria

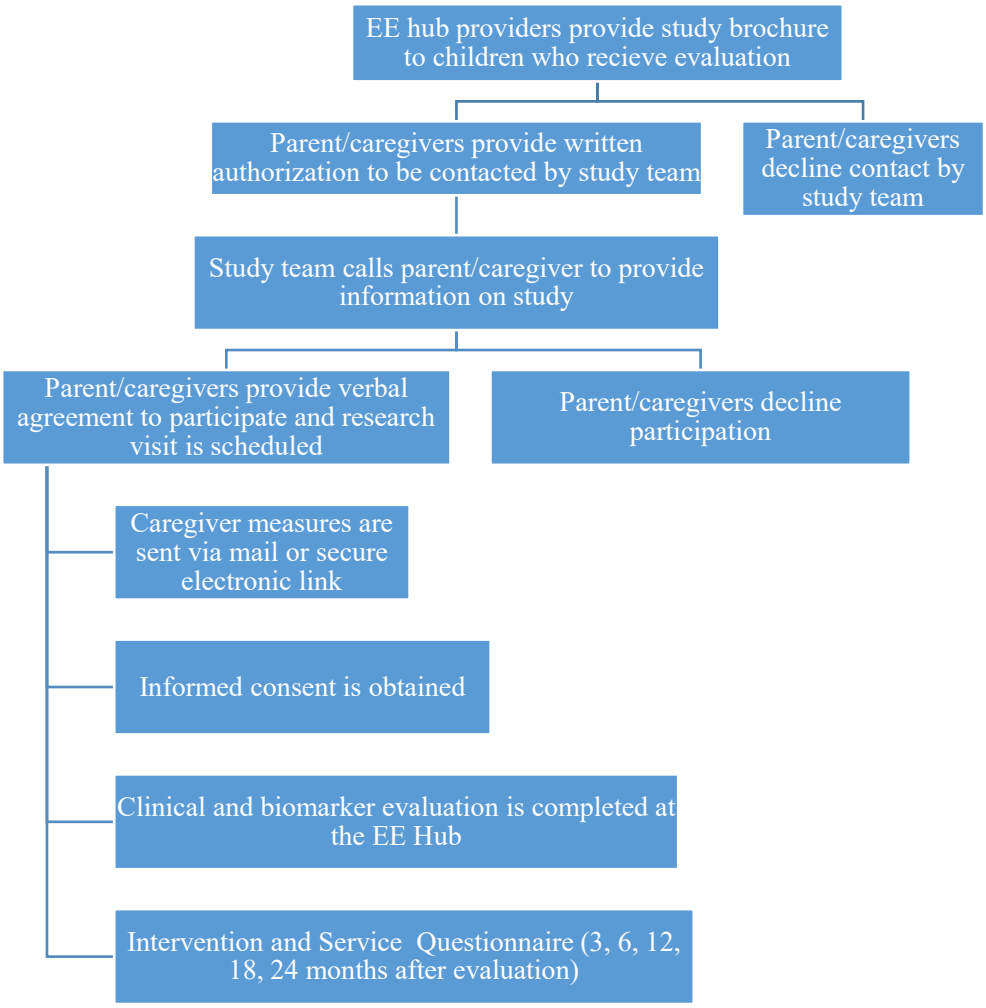
- The target sample of participants will be children, ages 14-48 months referred to the EE Hubs due to a screen-positive result on the MCHAT-R/F and/or parent/caregiver or pediatric provider concerns about development.

Exclusion Criteria

- Children must have a parent/caregiver(s) who speaks English to them such that 1) they are exposed to spoken language in English or 2) the child speaks and/or understands English. Given age and disability of children in the study, some children may not yet have developed functional receptive or expressive language. However, children who do not meet the above criteria will not be included in this research due to the significant language demands of study procedures and some assessments not being validated in languages other than English.

5.0 Study Design

This study uses a quantitative experimental design to address the specific aims. Please see the following diagram for a description of design.



6.0 Enrollment/Randomization

Subjects will be identified by the EE Hubs. Parent/caregiver(s) of a consecutive sample of children (ages 14-48 months) will be given a study information brochure and asked if they are interested in learning more about the study. If interested, parent/caregiver(s) will fill out a contact information sheet, which will be sent to the research coordinator. The research coordinator will contact the parent/caregiver(s) by phone/email to provide additional information about the study. If the parent/caregiver(s) agree to participate, the research coordinator will schedule the research visit. Written informed consent and authorization (to share evaluation results between EE Hub, child's primary care, and research team will be obtained at the time of the appointment.

Blinded Evaluation Process: Because the family will have received an initial diagnosis for their child during the evaluation at the EE Hub, there is risk for the family to share this information with the blinded ASD specialist, thereby introducing bias into the final diagnostic decision. In order to mitigate this bias, families will be informed on several occasions (i.e., at the time of referral, during the consenting process, and by the blinded ASD-specialist) that they are not to share their child's EE Hub diagnosis with the ASD specialist.

7.0 Study Procedures

Children (ages 14-48 months) are referred to the EE hub by their primary care provider when there is concern about a neurodevelopmental disability (e.g., parent/PCP concern or failed developmental screener; i.e., MCHAT-R/F). Children are evaluated by a pediatric health professional at the EE Hub with a standard battery of developmental history, physical exam, and behavioral measures; a diagnosis is made when appropriate and the parent/caregiver(s) receive the diagnosis and next step recommendations. For the purpose of this study, the pediatric health professional will complete a diagnostic certainty form indicating whether or not they provided a diagnosis of autism spectrum disorder and how certain they are about the diagnostic outcome [1-5 Likert scale; 1=completely certain; 5=not at all certain].

The EE Hubs will refer a consecutive sample of children for follow-up evaluation by study personnel (diagnostic evaluation overseen by PI/ASD Specialist: McNally Keehn). The EE Hub pediatric health professional will provide the parent/caregiver(s) with information about the study via a study brochure. If the parent/caregiver(s) is interested in participating, the parent will complete and sign the contact information sheet and it will be sent to the study team. The research coordinator will contact the parent/caregiver(s) by phone to provide additional information about the study. If the parent/caregiver(s) agrees to participate, the research coordinator will schedule a follow-up evaluation, at no cost to the child's family, at the local EE Hub site. This follow-up visit will occur within 16 weeks of the original EE Hub evaluation. During this call, the parent/caregiver(s) will provide verbal consent to have a battery of caregiver-report questionnaires sent to them by mail or via secure electronic link (i.e., Redcap) to return prior to or at the time of the research visit. This process will reduce face-to-face time required for the research visit.

Informed consent will occur at the start of the research visit. The parent/caregiver (or current legal guardian) will sign the HIPAA authorization form allowing the EE Hub to share evaluation data (MCHAT or MCHAT-R/F, ASQ-3, and STAT scores), diagnosis, and diagnostic certainty ratings with the study personnel and for the study personnel to provide research evaluation data back to the participant's primary care physician and EE Hub physician. The research visit will last for a duration of 3 hours, during which an ASD specialist (and/or other study personnel under the direction of an ASD specialist) will: 1) conduct a semi-structured clinical interview to gather developmental history, DSM-5 symptoms of ASD, and related medical/ neurodevelopmental information, 2) administer the Autism Diagnostic Observation Schedule-2 and Mullen Scales of Early Learning to the child, 3) administer the Vineland Adaptive Behavior Scales (Vineland-II) to the parent/caregiver of the child, 4) request that the parent/ caregiver complete symptom/behavior rating scales and a brief demographic questionnaire if not completed prior to the visit (as described above). The ASD specialist will also complete the same

diagnostic certainty form (as above), 5) complete a brief sensory processing assessment (SPA), and 6) collect a brief sample of eye-tracking data (~15mins; see below).

Eye-tracking data will be collected using a remote eye-tracking system (SR Research, Eyelink Portable Duo), which is placed in front and below the stimulus presentation monitor. Eye movements and pupil diameter will be collected while participants view a series of pictures and movies. The modular system is completely non-invasive with no hardware on or touching participants (only a small sticker is placed on their forehead). A camera monitors eye movements while a second scene camera monitors head movements, allowing eye-tracking to take place without a chin rest or bite bar. Children will be asked to sit on a chair or on their caregiver's lap and will face a computer monitor. After the sticker is applied to participant's forehead and brief calibration completed, visual stimuli will be presented on a laptop computer monitor that is placed at approximately 60cm from the participant. Brightness will be at a comfortable viewing level. The eye tracking portion of the visit will last approximately 15 minutes. All study procedures and data collection will occur at the EE Hub sites.

Participants will be compensated with a gift card in the amount of $25 per hour of completed research testing (up to a maximum of $75). The average length of the evaluation is estimated to be 3 hours. This hourly amount includes any travel related expenses. If participants choose to withdraw from the study, they will be compensated for the length of their participation (at the above rate of $25 per hour).

Results of the evaluation will be provided to the child's parent/caregiver(s) verbally following the evaluation. A brief report summarizing results of the evaluation and research diagnosis will be provided to the child's parent/caregiver(s), primary care physician, and the EE Hub physician (as appropriate with authorization). In cases where the diagnosis differs between the EE Hub and ASD specialist, the ASD specialist will contact the child's primary care provider and EE Hub provider (when written authorization is provided) by phone, fax, and/or secure email to discuss difference in diagnosis and to help the provider identify next steps in the child's care. The child's family will be offered further consultation by a neurodevelopmental specialist through Riley Hospital for Children neurodevelopmental subspecialists if desired/indicated (provided and billed as a medical service).

Caregivers will be sent a secure electronic link (i.e., Redcap) to complete the Intervention and Service Questionnaire at 3, 6, 12, 18, and 24 months following their research evaluation. If there is no response within 1 week of survey deployment, the research team will reach out by phone to ask caregivers if they would prefer to complete the survey verbally by phone. This survey is estimated to take less than 30 minutes to complete. Caregivers will be reimbursed with a $15 gift card for every time point a survey is completed.

8.0 Study Calendar

Day 1:	Study team receives written authorization to contact parent/caregiver of child who received evaluation in EE Hub
Within 2-14 days*:	Study team contacts parent/caregiver by phone to provide information

	and conduct initial eligibility screening; follow-up evaluation scheduled for children of parent/caregivers who provide verbal agreement; caregiver report questionnaires sent to caregiver for completion (by mail or secure electronic link) prior to the research visit
Within 16 weeks*:	Follow-up evaluation is conducted at EE Hub by ASD specialist and study team
Within 18 weeks*:	Written research evaluation report is provided to child's parent/caregiver, EE Hub physician, and PCP
16 wks + 3 months:	3-month Intervention and Service Questionnaire sent to caregivers.
16 wks + 6 months:	6-month Intervention and Service Questionnaire sent to caregivers.
16 wks + 12 months:	12-month Intervention and Service Questionnaire sent to caregivers.
16 wks + 18 months:	18-month Intervention and Service Questionnaire sent to caregivers.
16 wks + 24 months:	24-month Intervention and Service Questionnaire sent to caregivers.

*Times are relative to Day 1.

9.0 Reportable Events

AE's or unanticipated problems will be reported by the PI to the IU IRB (following the KC Reportable Events procedures; https://research.iu.edu/training/guides/human-subjects/kuali-coeus-irb/submit-reportable-event.html) within 5 business days of the study team becoming aware of the event.

Monitoring of AE's or unanticipated problems will begin at the time of informed consent. AE's or unanticipated problems applicable to this study may include:

1. Adverse event assessed by the principal investigator (PI) as (1) unexpected, (2) related or possibly related to study participation, AND (3) suggests that the research places subject(s) or others at greater risk of harm than was previously known.
2. Unanticipated adverse device effects.
3. Subject complaints that indicate an unexpected risk and/or that affect the rights and welfare of human subjects.
4. Incidents that may compromise information security, subject privacy, and/or confidentiality (e.g., subject data breach).

All other AE's and unanticipated problems as outlined in IU's policy (found at: https://research.iu.edu/compliance/human-subjects/guidance/reportable.html) will be reported to the appropriate monitoring board(s) as specified.

10.0 Data Safety Monitoring

The proposed study is not a clinical trial; however, all of the procedures will be reviewed and approved by the Indiana University IRB. Any changes in risk levels associated with any of the study approaches will be monitored and will be reported to the IRB. Further, unexpected adverse situations will immediately be reported to the IRB. The PI's will review human subjects protection issues regularly to monitor any adverse situations that have arisen.

11.0 Study Withdrawal/Discontinuation

Participants may choose to withdraw from the study at any time. Deciding not to participate, or deciding to leave the study later, will not result in any penalty or loss of benefits to which the participant is entitled, and will not affect the participants relationship with Indiana University School of Medicine, Riley Hospital for Children, or Indiana University Health. Participants may terminate participation at any time during participation in the study procedures. Participants will be instructed to contact the PI should they wish to withdraw from participation. They will be compensated for the amount of time that they participated in the study (as outlined in reimbursement procedures).

12.0 Statistical Considerations

Statistical plan was developed in consultation with Patrick Monahan, PHD of IUSM Department of Biostatistics for NIH R21 proposal; for this funding mechanism, sample size was estimated at 120 participants; we are requesting IRB approval for a sample size of 300 participants to account for potential future funding for this project.

A sample of 120 children (with estimated distribution: ASD = 60, Non-ASD = 60) will provide the logistic regression models with 83% power for two-sided Wald tests to detect an odds ratio of 1.94, using alpha of 0.05. This calculation assumes that the proportion of ASD at a covariate mean value, versus 1 SD from the covariate mean value, equals 0.34 versus 0.50, for an odds ratio of 1.94. Most of the predictors are continuous variables. Under the worst case scenario for power, in which the predictor is binary and the prevalence of a predictor in one outcome category is 0.50 (e.g., without ASD), 60 in each group will provide 81% power to detect an odds ratio of 3.0 (i.e., when the predictor prevalence in the ASD outcome category is 0.75) using a two-sided Wald test and alpha of 0.05. No more than 5 independent variables will be allowed in the final models, according to the rule of thumb to have at least 10 persons per parameter (i.e., per each 1 *df* independent variable plus intercept) for the smallest sized category of the dependent variable.

Aim #1: Evaluate the diagnostic validity of the EE Hub model of ASD diagnosis in the community-based primary care setting: Diagnostic validity will be measured by comparing agreement between the EE Hub and expert ASD-specialist on categorical diagnosis (ASD; non-ASD) to estimate diagnostic accuracy indices of sensitivity, specificity, positive predictive value, and negative predictive value. The kappa statistic for chance-corrected agreement, and its 95% confidence interval, will also be calculated.
Aim #2: Determine whether biobehavioral markers can reliably differentiate young children with and without ASD: Differences in metrics between children with and without ASD will be

compared to validate potential biomarkers. Logistic regression with diagnosis as the dependent variable and eye-tracking measures as the independent variables will be used to estimate diagnostic accuracy indices (sensitivity, specificity, positive predictive value, and negative predictive value) at various cut-points on the continuous scale of eye-tracking measures, and to estimate the area under the receiver operator curve (AUROC) with the c-statistic. Bivariate logistic regression will be used to test each eye-tracking measure separately to determine its individual diagnostic efficacy; then multivariable logistic regression will be used, with eye-tracking measures simultaneously entered into the model, to test the joint effect of all eye-tracking measures on their combined diagnostic efficacy with respect to the diagnostic accuracy indices, as well as to determine which eye-tracking measures contribute most to diagnostic efficacy while adjusting for the other measures. Eye-tracking measures that do not contribute significantly to diagnostic efficacy will be deleted from the multivariable model.

Aim #3: Determine whether a combination of clinical and biobehavioral measures can be used to accurately predict ASD diagnostic outcome: We will evaluate the predictive efficacy of clinical measures and biobehavioral markers for accurate ASD diagnostic classification, and further determine whether a multivariable combination of clinical and biobehavioral measures may potentially provide a more promising method for ASD diagnostic classification of young children in the primary care setting. Classifier analysis for diagnostic outcome (ASD versus non-ASD as determined by the expert ASD-specialist) will be completed using the multivariable logistic regression procedure described above, except the model will contain clinical measures in addition to eye-tracking measures.

13.0 Statistical Data Management

Primary data will be collected via paper (i.e., standardized clinical assessment protocols, as well as data collection instruments provided in KC Attachments), and direct data capture from the SR Research Eyelink Portable Duo device and stored electronically in REDCap and files on IUSM and Purdue University Servers. The storage location will be backed up automatically daily. Quality assurance steps will include: testing of database by study team prior to moving to production mode, random checks for data accuracy, and extraction and cleaning of data that will be used for analysis to be completed by qualified and experienced study team individuals.

14.0 Privacy/Confidentiality Issues

Individuals with access to study data and information will be limited to the PI and the PI's specific designees. A number or a letter string will refer to all subjects. No personal identifying information will be stored with responses. All records will be labeled using the number identification of a particular subject only. There will be dissociation of participant coding and specific identifier information. The key to this coding will be kept separate in a password protected document separate from other data and/or under two locks in a devoted cabinet. Electronic data will be stored on a computer protected by a password, HIPAA compliant firewalls, and backed up on the server. Paper documents will be kept in a cabinet in a locked office of Dr. Rebecca McNally Keehn. At termination of the study, paper copies of data will be maintained or shredded and scanned copies kept on a secure server for the requisite time period. Names and any identifying information will not be used in any presentations or publications that may result from this study.

15.0 Follow-up and Record Retention

The duration of the study is 5 years unless an amendment to extend the study is approved by the IRB. Record retention policies of the IU IRB will be followed as specified in this policy (accessed here: https://research.iu.edu/policies/human-subjects-irb/research-data-management.html):

All data and records produced or collected in connection with this research project will be retained for a minimum of three (3) years from the date of submission of the final expenditure report to the funding agency or the date of study closure with the IRB, whichever is longer. Signed HIPAA authorization forms will be retained for a *minimum* of six (6) years from the date it was obtained.

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17.0 Appendix

See KC IRB documents.