Children's Research Institute Behavioral/Procedural Intervention Study Protocol Template

(for behavioral and non-drug interventions that do not fall under FDA regulations—support groups, questionnaires, blood draws, MRIs, etc.)

Version 2.0, 21OCT2019

Sensitivity and Specificity of the Red Reflex Test for Detecting Anterior and Posterior Segment Ophthalmic Pathology in the Pediatric Population

CN IRB Protocol Number: Pro00011173

Principal Investigator: William Madigan, MD

No Funding Sponsor

Protocol Version Number: 1.0

10/21/2019

Table of Contents

Protocol Revision History

Protocol Version with Summary of Changes	Version Date

.

List of Abbreviations

AACO	American Association of Certified Orthoptists
AE	Adverse Event
AAFP	American Academy of Family Physicians
AAO	American Academy of Ophthalmology
AAP	American Academy of Pediatrics
AAPOS	American Association for Pediatric Ophthalmology and Strabismus
CFR	Code of Federal Regulations
CRF	Case Report Form
COC	Certificate of Confidentiality
CLIA	Clinical Laboratory Improvement Amendments
CRI	Clinical Research Institute
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DSMP	Data and Safety Monitoring Plan
eCRF	Electronic Case Report Form
FWA	Federalwide Assurance
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IATA	International Air Transport Association
ICF	Informed Consent Form
IRB	Institutional Review Board
LAR	Legally Authorized Representative

MOP	Manual of Procedures
OHRP	Office for Human Research Protections
PD	Protocol Deviation
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
RE	Reportable Event
RPM	Rotation/Revolutions per Minute
RR	Red Reflex
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
SRC	Scientific Review Committee
UP	Unanticipated Problem
USPSTF	US Preventative Services Task Force

Protocol Summary

Title:	*Sensitivity and Specificity of the Red Reflex Test for Detecting Anterior and Posterior Segment Ophthalmic Pathology in the Pediatric Population*
Brief Summary:	*Early detection of ocular abnormalities is critical for the prevention of visual loss. The American Association for Pediatric Ophthalmology and Strabismus (AAPOS), the American Academy of Ophthalmology (AAO), and the American Association of Certified Orthoptists (AACO) continue to support inclusion of the RRT as part of the ophthalmology screening exam in the general pediatrics clinic. While there is scientific evidence demonstrating that the RRT is a fairly reliable examination technique for identifying anterior segment pathology, studies to date have highlighted significant limitations of the RRT in accurately detecting posterior segment pathology. Delayed diagnosis of such conditions can lead to significant permanent visual impairment, and in rare cases, systemic morbidity for the child.* ***Aims/Objectives:*** *The primary aim of this study is to determine the sensitivity and specificity of the RRT performed in a pediatric ophthalmology clinic, according to standardized practice guidelines, for detecting both anterior and posterior segment pathology in the pediatric population. The secondary aim of this study is to evaluate the impact of pharmacologic dilation on the sensitivity and specificity of the RRT in detecting anterior and posterior segment pathology in the pediatric population. We hypothesize that the sensitivity and specificity of the RRT will be sufficient for detecting anterior segment pathology but will be insufficient for detecting posterior segment ophthalmologic pathology with or without pharmacologic dilation.*
Study Population:	*The study population will include pediatric patients under 17 years old recruited in clinical practice with known anterior or posterior segment pathology as well as patients without anterior or posterior segment pathology. In order to determine sensitivity and*

	specificity of anterior and posterior segment pathology separately, we will split the patients with pathology into two groups, anterior and posterior. *Ophthalmology residents who are blinded to the patient's ophthalmologic diagnosis (or lack thereof) will perform the standardized RRT, which includes both a lights on and lights off examination*
Study Site(s):	*Children's National Health System and Fairfax*
Number of Participants:	*100-200*
Accrual Ceiling:	*200. We estimate that all consented subjects will be able to participate in this routine vision exam.*
Study Duration:	*This study is expected to be completed within one year.*
Subject Duration:	*The subject duration is expected to be one outpatient clinic visit.*
Objective(s):	*The primary aim of this study is to determine the sensitivity and specificity of the RRT performed in a pediatric ophthalmology clinic, according to standardized practice guidelines, for detecting both anterior and posterior segment pathology in the pediatric population. The secondary aim of this study is to evaluate the impact of pharmacologic dilation on the sensitivity and specificity of the RRT in detecting anterior and posterior segment pathology in the pediatric population. We hypothesize that the sensitivity and specificity of the RRT will be sufficient for detecting anterior segment pathology but will be insufficient for detecting posterior segment ophthalmologic pathology with or without pharmacologic dilation.*
Methodology:	*Prospective study*
Outcome Measures:	*Sensitivity and specificity of the RR test to detect*

anterior and posterior ocular abnormalities with and without pharmacologic pupillary dilation.

Study Intervention/Procedures:

Ophthalmology residents who are blinded to the patient's ophthalmologic diagnosis (or lack thereof) will perform the standardized RRT, which includes both a lights on and lights off examination. Patients will then be pharmacologically dilated and the RRT repeated. Results will be recorded for each. Pharmacological dilation will be achieved according to standard practices. For all patients, one drop of proparacaine hydrochloride ophthalmic solution will be instilled in each eye to achieve anesthesia. For patients under 1 year old, one drop of Cyclomydril will then be instilled in each eye and followed second drop in each eye 5 minutes later. For children over 1 year of age, pharmacologic dilation will be achieved with 1 drop of Cyclopentolate 1% and 1 drop of Phenylephrine 2.5% in each eye. Photography may be performed to document the presence or absence of anterior and/or posterior segment pathology.

Statistical Analysis:

The baseline demographic data will be presented descriptively. Continuous data will be summarized as mean with standard deviation or median with interquartile range depending on the distribution of the data. Categorical data will be summarized using frequencies with percentage. Summary demographic statistics between patients with and without eye abnormalities will be compared using unpaired t-test (normal) and/or Mann-Whitney U test (skewed) for continuous data, and Chi-square test and/or Fisher's exact test (if any of the expected cell frequencies are <5) for categorical data. Normality assumption will be cheeked by using statistical test (e.g., Shapiro Wilk test) as well as graphical methods (e.g., histogram, q-q plot).

To assess our primary and secondary hypothesis, sensitivity and specificity of the red reflex test (RRT) will be determined along with their 95% confidence interval using contingency tables for both anterior and posterior segment pathology. Positive predictive value (PPV), negative predictive value (NPV), and area under ROC curve (AUC) will also be determined. We

will also compare sensitivity and specificity of RRT for both anterior and posterior pathology between with and without pharmacologic dilation using two-sided McNemar's test for correlated proportions.

All statistical tests will be two-sided and will be performed at the 5% level of significance unless otherwise stated. Stata 15.1 software will be used for all statistical analyses.

*Our sample size calculation is based on our primary hypothesis. Assuming a 75% prevalence of anterior or posterior segment pathology and a sensitivity of 90% in our sample, a total of **59** patients will be required to construct a two-sided 95% sensitivity confidence interval with a width of at most +/-10%. Under the same assumptions, the sample size needed for a two-sided 95% specificity confidence interval of +/-10% around 80% is **280**. We will take the larger one of these two, which is **280**. The power analysis was carried out using PASS 2019 software.*

Section 1: Key Roles

Principal Investigator
William Madigan, MD, Vice-Chair, Ophthalmology
Children's National Health System
111 Michigan Ave NW, Washington, DC 20010
202-476-3015
WMadigan@childrensnational.org

Performance	*Performance Site PI*	*Describe Activities Conducted at*

Site(s) Name(S)	*(First and Last Name)*	*Performance Site by PI and Research Team*
Children's National Health System Fairfax	*William Madigan, MD*	*1. RR exam using direct ophthalmoscope under physiologic and pharmacologically dilated conditions* *2. Automated vision screening using the Spot photoscreener under physiologic and dilated conditions.*
Children's National Medical Center, Sheik Zayed campus	*William Madigan, MD*	*Same*

Section 2: Introduction, Background Information and Scientific Rationale

2.1 Background Information and Relevant Literature

The early detection of ophthalmic pathology is critical for the prevention of vision loss and systemic morbidity in rare cases. The American Academy of Pediatrics (AAP), the American Association for Pediatric Ophthalmology and Strabismus (AAPOS), the American Academy of Ophthalmology (AAO), and the American Association of Certified Orthoptists (AACO) continue to advocate for inclusion of the red reflex test (RRT) as part of the ophthalmologic screening exam in the general pediatrics clinic. However, studies to date have demonstrated that there are potential limitations to the RRT, especially in its ability to accurately and reliably detect posterior segment pathology.

Standard procedure for the RRT currently involves viewing both eyes at arms length in lighted room followed by a darkened room to assess for symmetry of the reflection in terms of brightness and color. If an abnormality is suspected, then the operator may move closer for further inspection. With its current application, the RRT introduces subjectivity in interpretation and likely variation in pupillary reflection based on factors such as, the specific lighting condition, degree of physiologic dilation, and patient movement. Differences in operator experience with the RRT may further compound the shortcomings of this exam technique. These factors unfortunately reduce the accuracy and reliability of the RRT as a screening measure.

There is ample evidence in the literature validating the RRT as a useful screening tool for pediatric anterior segment ocular pathology, with consistently high sensitivity and specificity. However, studies describing its utility in detecting posterior segment disease are lacking. Two recent studies have attempted to address this issue. Sun and colleagues used the RRT to screen over 7000 newborns followed by retinal imaging using a Retcam-3 with pharmacologic pupillary dilation. They found that for anterior segment disease, the RRT had a sensitivity of 99.6% (95% CI 97.1%-100%) compared to only 4.1% (95% CI 3.3%-5.1%, $\chi 2 = 1521.382$, $\varphi = 0.836$, $P < .001$) for posterior segment pathology. In a similar study, Ludwig and colleagues detected posterior segment pathology in 49 of 194 eyes examined using the Retcam-3 with pharmacologic dilation (sensitivity = 100%); however, no abnormalities were detected in this same set of eyes with the RRT performed by a pediatrician in the newborn nursery (sensitivity = 0%).

Based on these results, there is insufficient evidence that the RRT is an effective screening exam for detecting posterior segment pathology - even with physiologic dilation during the lights off portion of the RRT. It has been established that pupillary size and viewing angle certainly do impact the quality of a posterior segment exam. In a study by Li and colleagues, the rate of tumor detection in a model eye was optimized when viewing at an oblique angle through a maximally dilated pupil of 8 mm. Even under the darkest lighting conditions, physiologic mydriasis will be limited, and especially with introduction of the direct ophthalmoscope light for the RRT. The average infant scotopic pupil size is 3.8 mm ± 0.9 mm in diameter. When light is shone from ophthalmoscope, pupils usually constrict by 2 mm. Pharmacologic dilation, however, is not currently included as part of the practice pattern guidelines outlining the RRT due to its perceived associated potential risks and side effects.

Based on the above, additional investigation is needed to further clarify whether the sensitivity and specificity of the RRT for detecting posterior segment pathology are sufficient for its use as a screening tool, or if alternatively, this current standard practice simply offers a false sense of security. Evaluating the impact of pharmacologic dilation on the sensitivity and specificity of the RRT may help identify a potential avenue for improving the validity of the RRT as an ophthalmology screening tool.

2.2 Scientific Rationale

Question: Is the RRT a sensitive or specific screening tool for detecting anterior or posterior segment pathology under physiologic conditions or following pupillary dilation in the pediatric population?

Hypothesis: The RRT is not a sensitive or specific screening tool for detecting anterior or posterior segment pathology in the pediatric population even following pharmacologic pupillary dilation.

Visual acuity and disease are difficult to assess in the pediatric population and especially younger children due to normal developmental changes as well as

challenges in patient cooperation or symptom reporting. However, assessing visual and anatomic abnormalities in this age group remains of paramount importance to avoid visual morbidity, and rarely systemic complications, from undetected ophthalmic pathology. The RRT is currently recommended by the Joint Committee as an initial screening test for anterior and posterior segment ocular abnormalities in the pediatric population. The recommendations suggest that dim room lighting aids in the accuracy of the RRT. However, limited available evidence to date has demonstrated that the RRT is not a sensitive or specific test for detecting posterior segment pathology. Furthermore, there are no studies to date specifically examining the effect of dim lighting conditions or pupillary dilation on the sensitivity and specificity of the RRT.

This study aims to evaluate the sensitivity and specificity of the RRT for detecting anterior or posterior segment pathology in both ambient and dark lighting conditions, before and after pharmacologic dilation.

2.3 Potential Risks

There is minimal immediate or long-term risk in this study. The interventions used are included in a standard ophthalmologic exam. Patient's may experience mild discomfort from the light of the equipment (direct ophthalmoscope) shone into their eyes. Additionally, patients have a risk in breach of confidentiality from usage of PHI data. However, all steps in accordance with CNHS policy will be performed in order to ensure patient confidentiality. These minimal risks are worth the potential benefit of understanding how to perform ophthalmologic screening in the pediatric medical home.

2.4 Potential Benefits

Study benefits to subjects include, but are not limited to, careful evaluation of known anterior or posterior segment ophthalmologic disease.

Study benefits to the greater community include, but are not limited to, helping improve vision screening guidelines for the early detection and treatment of eye disease.

Section 3: Objectives and Endpoints

3.1 Primary Objective(s)

The primary outcomes of this study will be the sensitivity and specificity of the RRT in detecting anterior segment pathology, as well as the sensitivity and specificity of the RRT in detecting posterior segment pathology.

3.2 Secondary Objective(s)

The secondary outcomes of this study will be the sensitivity and specificity of the RRT in detecting anterior segment or posterior segmented pathology following pharmacologic dilation.

3.3 Primary Outcome Measure(s)

Number of eyes with posterior segment pathology correctly identified as having posterior segment pathology and incorrectly identified as not having pathology. Number of healthy eyes correctly identified as being healthy.

3.4 Secondary Outcome Measure(s)

Number of eyes with anterior segment pathology correctly identified as having anterior segment pathology and incorrectly identified as not having pathology. Number of healthy eyes correctly identified as being healthy.

Section 4: Study Design

Prospective study

Section 5: Study Enrollment and Withdrawal

5.1 Study Population, Recruitment and Retention

Pediatric patients will be recruited through clinical practice at the designated study sites. Participants with known anterior segment or posterior segment pathology will be eligible for inclusion. Patients who have a history of disease, treatments and/or surgical procedures affecting the ability for normal pupillary reaction are excluded. An equal number of age matched participants with a normal anterior and posterior segment exam will be recruited from routine outpatient clinic visits. Informed consent will be obtained and will include an option to be considered for medical photography. Participants may opt out of photography and still be included in the study. For Spanish speaking participants, a telephone interpreter will be used for study recruitment and informed consent. A Spanish informed consent (developed by a Spanish medical translator from our English written consent) will be provided. Current estimated number of subjects required for the study is 200 total including 100 controls. Patient charts will be accessed from date of birth to patient's current age using either Cerner or Epic depending on the study site location. Examiners will include ophthalmology residents with one completed year of ophthalmology training. They will be blinded to participants' ophthalmologic history.

5.2 Inclusion Criteria

There is no difference in criteria for screening and enrollment. Patients with general eyelid pathology, ptosis, corneal pathology, astigmatism, cataract, vitreous pathology, vitreous detachment, vitreous hemorrhage, retinal pathology, retinopathy of prematurity, retinal detachment, retinal tear, retinal hemorrhage, retinoblastoma, other intraocular tumors overlying or outside of the central visual axis, retinal pigmented epithelial cell pathology, choroidal pathology, optic nerve pathology, retinal artery or vein pathology may be included.

5.3 Exclusion Criteria

Patients who have a history of disease, treatments and/or surgical procedures affecting the ability for normal pupillary reaction are excluded.

5.4 Vulnerable Subjects

Subjects 3 years old and under will be recruited because this study is evaluating a routine examination in this population. This study has no more than minimal risk to the patient.

5.5 Recruitment

Subjects will be recruited through clinical practice at Children's National Health System and its Fairfax outpatient site. Subjects will be identified by the PI, Dr. William Madigan, Vice-Chair, Department of Ophthalmology, in a review of medical records after a HIPAA authorization waiver for recruitment is granted. Subjects will be approached at a clinical care visit or an IRB approved study information letter will be sent to the participant's legally authorized representative (LAR). If the LAR expresses interest in the study, a copy of the IRB approved consent will be provided to the LAR. The informed consent will be signed by the LAR and assent will be obtained from the patient at an in-person visit before any research procedures or interventions are done.

The privacy of patients received for screening will be protected by password file encryption on Children's National Health System computers/hardware and will be viewable only to the PI, treating physicians and study staff. All study data that have been de-identified and requiring transport will be done in an encrypted flash memory drive.

5.6 Retention

There are no procedures in place to ensure retention as this study will be completed in one outpatient clinic visit for each subject.

5.7 End of Participation Criteria and Procedures

Participants are free to withdraw from participation in the study at any time upon request. An investigator may terminate subject study participation if:

- *Any adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.*

- *The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.*

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination will be provided by the suspending or terminating party to participants, site investigators, the funding agency and regulatory authorities (e.g. OHRP). If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant study termination or suspension include, but are not limited to:

- *Determination of unexpected, significant, or unacceptable risk to participants*
- *Insufficient study team or site participant compliance to protocol requirements*
- *Data that are not sufficiently complete and/or evaluable*
- *Determination of futility*

The study may resume once any concerns about safety, protocol compliance, data quality or funding are addressed and satisfy the sponsor, IRB and OHRP.

Section 6: Study Procedures

6.1 Informed Consent/Assent and HIPAA Authorization

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study. It continues throughout the individual's study participation. Consent and assent forms will be IRB-approved. The participant/LAR will be asked to read and review the consent document(s). Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families/LAR in a language they understand. An IRB approved short form consent and a translator will be used when consenting participants who do not speak English. Illiterate subjects will have the consent form read to them aloud. Participant/parental consent will then be documented by having participants make their "mark" on the consent document in the presence of a non-study team member witness to avoid any possible coercion. The investigator will explain the research study to the participant/LAR/parent and answer any questions that may arise. All participants/parents will receive a verbal lay explanation of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants/LARs/parents will have the opportunity to carefully

review the written consent form and to ask questions prior to signing. Consent of at least one parent/LAR will be documented on the consent form (or as determined by the IRB). Assent for subjects aged 0 – 3 years old will be obtained and documented on the parental/LAR consent form.

Participants/parents will be given the opportunity to discuss the study with their surrogates and other care providers prior to agreeing to participate. The participant/parent and/or LAR will sign the informed consent document(s) prior to any procedures being done specifically for the study. The participants/LAR may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document(s) will be given to the participant/LAR/parent for their records. The rights and welfare of the participants will be protected by emphasizing that the quality of their medical care will not be adversely affected if they decline to participate in this study.

6.2 Screening Process

Screening Visit Day -30 to the day of the first in-person outpatient clinic visit

- *Informed consent discussion*
- *Eligibility confirmation*
- *Medical history (diagnoses and medications)*
- *Demographics (date of birth)*

6.3 Study Interventions and Follow-Up

Patients will undergo a routine red reflex examination using a direct ophthalmoscope in a lighted and darkened exam room before and after pharmacologic dilation.

The study will only take place over one outpatient clinic visit, and there will be no follow-up other than continued medical care.

6.4 Description of Study Procedures/Evaluations

- *Medical history—ocular history from prenatal care and on will be obtained through the medical record or in person.*
- *Medication history—Current medications taken required.*
- *Physical examinations—Subject's red reflex and pupillary size will be examined using a direct ophthalmoscope in bright and dim room lighting conditions as well as before and after pharmacologic dilation. Automated vision screening using the Spot photoscreener will be performed before and after dilation.*
- *The results of the red reflex exam will be discussed with the patient during the clinic visit.*
- *Photographs of the subject's red reflex may be taken and edited to ensure anonymity. These photographs will allow for digital analysis of the patients red reflex. These photographs will be kept on password*

protected computers at Children's National Health System and on password protected encrypted flash drives. These photographs may be used for dissemination of study results such as in conference abstracts and medical journals. Once the study is complete, original photographs may be destroyed.

6.5 Study Team Training and Intervention Reliability

The study PI will be present and ensure the blinded examiner perform the red reflex test according to the current guideline recommendations.

6.6 Concomitant Interventions and Procedures

There are no concomitant interventions or procedures in this study. Any additional studies as part of the patient's care which may interfere with the red reflex test will be performed after the red reflex test.

Section 7: Safety Assessments and Reporting

The intervention in this study is less than minimal risk. The highest level of patient safety will be ensured as part of routine clinical care.

7.1 Adverse Events (AEs)

There are no AEs anticipated in this study.

7.2 Serious Adverse Events (SAEs)

There are no SAEs anticipated in this study.

7.3 Unanticipated Problems (UPs)

OHRP considers unanticipated problems involving risks to participants or others to include any incident, experience, or outcome that meets all of the following criteria:

- *Unexpected in terms of nature, severity, or frequency given the research procedures described in the study documents (e.g., consent, protocol) the participant population; AND*
- *Related or possibly related to participation in the research. "Possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research; AND*
- *Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic or social harm) than was previously known or recognized.*

At each study visit, the study team will ask the participant/LAR if any AE/SAE/UPs have occurred since the last study contact. All adverse events will be captured on the adverse events CRF. Information collected includes event term, onset date, severity, relationship to study intervention (assessed and documented by an authorized study team member), and date of event

resolution/stabilization. All events occurring while on study must be documented, regardless of relationship to the research intervention(s). All events which meet the definition of a serious adverse event or unanticipated problem that occur during the study visit will be followed until resolved or stable.

Any medical condition that is present before the first study intervention will be considered a baseline condition; it will not be reported as an AE.

Serious Adverse Event and Unexpected Problem Reporting
All suspected adverse reactions to study interventions (including comparators) that are both serious AND unexpected should be reported to the IRB. The reporting time frames are as follows:

- *Report the death of a Children's National subject enrolled in an interventional study if the death is unexpected (not due to disease progression) and related or possibly related to the research within one (1) business day of learning of the event. A follow-up report must be submitted within two (2) business days.*
- *Report all other unanticipated problems to the IRB within seven (7) days.*

If any needed information is missing or unknown at the time of initial reporting, the study team will actively try to obtain it. The study team will maintain records of efforts to obtain additional follow-up information. Any additional relevant information to a previously submitted report will be submitted to oversight bodies as soon as the information is available.

All SAEs and UPs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or stable. Other supporting documentation of the event may be requested by oversight bodies and should be provided as soon as possible.

Section 8: Statistical Considerations and Analysis

8.1 Statistical and Analytical Plans *(SAP)*

Statistical analysis of the data will be formed at the conclusion of the study. Study examiners will be blinded until the results have been completed.

8.2 Statistical Hypotheses

Our hypothesis is that the sensitivity and specificity of the RRT in detecting posterior ocular abnormalities in children will be poor in lighted or dim room lighting conditions or following pharmacologic dilation.

The null hypothesis is that the sensitivity and specificity of the RRT for detecting posterior ocular abnormalities in children will improve significantly under dim room lighting conditions and/or following pharmacologic dilation.

8.3 Analysis Datasets

The baseline demographic data will be presented descriptively. Continuous data will be summarized as mean with standard deviation or median with interquartile range depending on the distribution of the data. Categorical data will be summarized using frequencies with percentage. Summary demographic statistics between patients with and without eye abnormalities will be compared using unpaired t-test (normal) and/or Mann-Whitney U test (skewed) for continuous data, and Chi-square test and/or Fisher's exact test (if any of the expected cell frequencies are <5) for categorical data. Normality assumption will be cheeked by using statistical test (e.g., Shapiro Wilk test) as well as graphical methods (e.g., histogram, q-q plot).

8.4 Description of Statistical Methods

To assess our primary and secondary hypothesis, sensitivity and specificity of the red reflex test (RRT) will be determined along with their 95% confidence interval using contingency tables for both anterior and posterior segment pathology. Positive predictive value (PPV), negative predictive value (NPV), and area under ROC curve (AUC) will also be determined. We will also compare sensitivity and specificity of RRT for both anterior and posterior pathology between with and without pharmacologic dilation using two-sided McNemar's test for correlated proportions.

All statistical tests will be two-sided and will be performed at the 5% level of significance unless otherwise stated. Stata 15.1 software will be used for all statistical analyses.

8.5 Sample Size

*Our sample size calculation is based on our primary hypothesis. Assuming a 75% prevalence a of anterior or posterior segment pathology and a sensitivity of 90% in our sample, a total of **59** patients will be required to construct a two-sided 95% sensitivity confidence interval with a width of at most +/-10%. Under the same assumptions, the sample size needed for a two-sided 95% specificity confidence interval of +/-10% around 80% is **280**. We will take the larger one of these two, which is **280**. The power analysis was carried out using PASS 2019 software.*

8.6 Measures to Minimize Bias

Blinding

Examiners performing the red reflex test will be blinded to all subjects and results until all study data have been collected.

Breaking the Study Blind

Breaking the study blind for red reflex performers will be at the conclusion of the study data collection period. Intentional and unintentional breaking of the blind should be reported to the PI.

Section 9: Data Quality and Oversight

9.1 Study Team Quality Assurance and Quality Control

Study data will be historical in nature, and therefore will be accurate to the patient's current diagnosis. All further evaluation will be reported by the red reflex examiner and this original data will be used in the analysis. Protocol deviations will be reported immediately or witnessed in person by the PI/co-investigator/study coordinator. Documents to be reviewed include clinic notes. The PI/co-investigator/study coordinator are responsible for reviewing the data each time the red reflex test is performed. The PI/co-investigator/study coordinator is responsible for addressing data quality issues.

9.2 Data Safety and Monitoring Plan

N/A

Section 10: Ethical Considerations

10.1 Ethical Standard

The study team will ensure that this study is conducted in full conformity with the Regulations for the Protection of Human Subjects of Research codified in 45 Part 46 of the Code of Federal Regulations, Children's National Policies and Procedures and Good Clinical Practices.

10.2 Institutional Review Board (IRB)

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Children's National IRB for review and approval. Approval of both the protocol and the consent form will be obtained before any participant is consented. Any change to the protocol, consent, recruitment materials and participant information sheets or letters will require IRB approval before implementation and use. The IRB will determine whether previously consented participants need to be re-consented and whether consent of more than one parent is required for minors.

The IRB will be notified of study team updates via an amendment. DSMB Reports will be submitted at the time of the continuing review or with another applicable IRB transaction.

Other study events (e.g., protocol deviations, data monitoring reports) will be submitted per the Children's National IRB Reportable Events Module.

10.3 Maintaining Subject Privacy

All patients will be taken to a private area for consenting and results discussions. No appointment reminders left on answering machines. All evaluations will be conducted in a private clinical room.

10.4 Maintaining Study Data Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, the sponsor and their agents. This confidentiality is extended to cover the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The sponsor representatives and regulatory authorities (e.g., IRB, OHRP) may inspect all documents and records required to be maintained by the investigator. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each study site for internal use during the study. At the end of the study, all research records will be stored in a secure location for the time period stated by the institutional regulations.

The research data will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by research staff will be secured and password protected. At the end of the study, all study databases will be archived at Children's National.

Certificate of Confidentiality
To further protect study participants, a Certificate of Confidentiality has been obtained from the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding; whether at the federal, state, or local level. The certificate protects researchers and institutions from being compelled to disclose information that would identify research participants,

10.5 Study Support and Conflicts of Interest

There is no external funding, support or conflicts of interest regarding this study.

Section 11: Data Handling and Record Keeping

11.1 Data Management Responsibilities

All data will be handled by the study staff. The study team will enter the data from source sheets electronically into Microsoft Excel. The analysis and interpretation of study data will be performed electronically. Storage and maintenance of data will be on password protected computers at Children's

National Health System or on flash drives with password protection and encryption. Data collected will accurate, consistent, complete, and reliable and in accordance with good clinical practices.

11.2 Data Capture Methods

Data collection is the responsibility of the trial staff. The PI is responsible for ensuring the accuracy, completeness, legibility, timeliness and completeness of the data reported.

Sites will maintain all relevant source data. Source data include all information and original records of clinical findings, observations, or other activities necessary for the reconstruction and evaluation of the trial. Examples of original source documentation include electronic medical records, laboratory reports, memoranda, subject diaries, subject questionnaires and recorded data from automated instruments.

Source documents should be neat and legible. When making changes or corrections, the original entry should be crossed out with a single line, initialed and dated.

Paper copies of the electronic CRF (eCRF) will be provided for use as source documents. Study data will be recorded for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents. Any discrepancies between the eCRF and source documentation should be explained via a CRF comment or a note to file.

Research data will be entered into Microsoft Excel. Data will be password protected, secure and de-identified and Health Insurance Portability and Accountability Act (HIPAA) compliant.

Only Institutional Review Board (IRB) approved research team members who have current HIPAA and Collaborative Institutional Training Initiative (CITI) Good Clinical Practice (GCP) and human subjects protection training will be authorized to extract data from source documents and enter it into the protect Excel document.

Data should be entered directly from the source documents in to Excel within 10 days of collection.

11.3 Study Record Retention Policy

Study data will be maintained for up to 2 years after the study period. After, all clinical data will be archived on Children's National Health System computers and the study data will be permanently destroyed. Physical data will be shredded and electronic data will be permanently deleted.

Section 12: Publication Policy

Publication rights and authorship will consist of the study staff and examiners that fulfill the criterion for authorship as described by the publisher.

Section 13: References

1. *AAP. Red Reflex Examination in Neonates, Infants, and Children. Pediatrics. Dec 2008; 122:6.*
2. *Swati S. Deshmukh M.E., A.D. Gawande, A.B.Deshmukh. Detection and Correction of Red Eye in Digital Photograph. IJARCET. Jun 2013; 2:6.*
3. *Kolb H. Facts and Figures Concerning the Human Retina. 2005 May 1 [Updated 2007 Jul 5]. In: Kolb H, Fernandez E, Nelson R, editors. Webvision: The Organization of the Retina and Visual System [Internet]. Salt Lake City (UT): University of Utah Health Sciences Center; 1995-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK11556/*
4. *Van Buren JM. The retinal ganglion cell layer. Springfield (IL): Charles C. Thomas; 1963.*
5. *Digvijay Singh, Rohit Saxena, Pradeep Sharma, Vimla Menon. Understanding your Direct Ophthalmoscope. Delhi Journal of Ophthalmology. 2013 Sep 25. http://www.djo.org.in/articles/21/3/understanding-your-direct-ophthalmoscope.html*
6. *Harry A. Quigley, MD; Andrew E. Brown; John D. Morrison, MD. The Size and Shape of the Optic Disc in Normal Human Eyes. Arch Ophthalmol. 1990;108(1):51-57. doi:10.1001/archopht.1990.01070030057028.*
7. *AAP, AAPOS, AACO, and AAO Hoskins Center for Quality Eye Care. Procedures for the Evaluation of the Visual System by Pediatricians: Clinical Report – 2016. https://www.aao.org/clinical-statement/procedures-evaluation-of-visual-system-by-pediatri.*
8. *John D. Roarty, MD; John L. Keltner, MD. Normal Pupil Size and Anisocoria in Newborn Infants. Arch Ophthalmol. 1990;108(1):94-95. doi:10.1001/archopht.1990.01070030100037.*
9. *Larry D.RoeM.D.David L.GuytonM.D. The red reflex from retinoscopy's point of view. Reflections on a small area. Survey of Ophthalmology. Volume 28, Issue 4, January–February 1984, Pages 345-348.*
10. *George T. Timberlake, Ph.D., Michael Kennedy, M.D. The Direct Ophthalmoscope How it Works and How to Use It. http://web.media.mit.edu/~raskar/Eye/TheDirectOphthalmoscope.pdf.*

11. *Lim LS, Chong GH, Tan PT, et al. Distribution and Determinants of Eye Size and Shape in Newborn Children: A Magnetic Resonance Imaging Analysis. Investigative Ophthalmology & Visual Science July 2013, Vol.54, 4791-4797. doi:10.1167/iovs.12-10713.*
12. *Trattler WB, Kaiser PK, Friedman NJ. Review of Ophthalmology: Expert Consult. Saunders; 2 edition (March 6, 2012)*
13. *Sun M, Ma A, Li F, et al. Sensitivity and Specificity of Red Reflex Test in Newborn Eye Screening. J Pediatr. 2016 Dec;179:192-196.e4.*
14. *COMMITTEE ON PRACTICE AND AMBULATORY MEDICINE, SECTION ON OPHTHALMOLOGY, AMERICAN ASSOCIATION OF CERTIFIED ORTHOPTISTS, AMERICAN ASSOCIATION FOR PEDIATRIC OPHTHALMOLOGY AND STRABISMUS, AMERICAN ACADEMY OF OPHTHALMOLOGY. Visual System Assessment in Infants, Children, and Young Adults by Pediatricians. Pediatrics 2016;137.*
15. *Sean P. Donahue, MD, PhD, FAAP, Cynthia N Baker, MD, FAAP, COMMITTEE ON PRACTICE AND AMBULATORY MEDICINE, SECTION ON OPHTHALMOLOGY, AMERICAN ASSOCIATION OF CERTIFIED ORTHOPTISTS, AMERICAN ASSOCIATION FOR PEDIATRIC OPHTHALMOLOGY AND STRABISMUS, AMERICAN ACADEMY OF OPHTHALMOLOGY. Procedures for the Evaluation of the Visual System by Pediatricians. Pediatrics 2016;137.*
16. *Jennifer Li, MD, David K. Coats, MD, Derrick Fung, MD, E. O'Brian Smith, PhD, and Evelyn Paysse, MD. The Detection of Simulated Retinoblastoma by Using Red-Reflex Testing. Pediatrics 2010;126:e202–e207.*
17. *Mirhadi Mussavi, MD; Khairollah Asadollahi, MD, PhD; Farhad Janbaz, MD; Esmaiil Mansoori, MD; Nasser Abbasi, PharmD. The Evaluation of Red Reflex Sensitivity and Specificity Test among Neonates in Different Conditions. Iran J Pediatr Dec 2014; Vol 24 (No 6), Pp: 697-702.*
18. *Christopher B. Estopinal, BS, Sean P. Donahue, MD, PhD, and Gary N. Gitschlag, MD. Retinoblastoma in a Child After Normal Autorefraction and Traditional Vision Screening. Pediatrics 2011;128:e1285–e1288.*
19. *David H. Abramson, MD; Carol L. Shields, MD; Francis L. Munier, MD; Guillermo L. Chantada, MD, PhD. Treatment of Retinoblastoma in 2015 Agreement and Disagreement. JAMA Ophthalmol. 2015;133(11):1341-1347.*
20. *Daniel E. Jonas, MD, MPH; Halle R. Amick, MSPH; Ina F.Wallace, PhD; Cynthia Feltner,MD, MPH;*
    1. *Emily B. Vander Schaaf,MD, MPH; Callie L. Brown, MD, MPH; Claire Baker. Vision Screening in Children Aged 6Months to 5 Years Evidence Report and*

Systematic Review for the US Preventive Services Task Force. JAMA. 2017;318(9):845-858.

21. *US Preventive Services Task Force. Vision Screening in Children Aged 6Months to 5 Years US Preventive Services Task Force Recommendation Statement. JAMA. 2017;318(9):836-844.*
22. *US Preventive Services Task Force. Vision Screening for Children 1 to 5 Years of Age: US Preventive Services Task Force Recommendation Statement. Pediatrics 2011;127:340–346.*
23. *Cassie A. Ludwig, MD, MS; Natalia F. Callaway, MD, MS; Mark S. Blumenkranz, MD; Douglas R. Fredrick, MD; Darius M. Moshfeghi, MD. Validity of the Red Reflex Exam in the Newborn Eye Screening Test Cohort. Ophthalmic Surg Lasers Imaging Retina. 2018;49:103-110.*