

**Title: Potential Mechanisms for Intussusception after Rotavirus Vaccine-Pilot Study**

**Cincinnati Children's Hospital Medical Center**

**Principal Investigator:** Mary Allen Staat, MD, MPH

**Co-Investigators:** Steven Black, MD  
Alexander J. Towbin, MD  
Elizabeth P. Schlaudecker, MD, MPH  
Jean A. Tkach, PhD  
Ardythe L. Morrow, PhD  
Monica M. McNeal, MS  
Mekibib Altaye, PhD

**Centers for Disease Control and Prevention (CDC)**

**Co-Principal Investigators:** Karen Broder, MD and Margaret Cortese, MD

**Co-Investigators:** Umesh Parashar, MD, MBBS  
Devindra Sharma, MSN, MPH

**ClinicalTrials.gov ID: NCT02542462**

## Contents

1	KEY ROLES.....	4
2	BACKGROUND AND SCIENTIFIC RATIONALE .....	4
2.1	Rotavirus Infection and the History of Rotavirus Vaccines.	4
2.2	Intestinal Imaging	7
2.3	Potential Modifiers of Rotavirus Infection, Vaccine Response and Shedding and Intussusception	9
3	Purpose and Objectives.....	12
3.1	Purpose	12
3.2	Hypothesis	12
3.3	Primary and Secondary Objectives	12
3.4	Study Outcome Measures	12
4	Research Plan.....	13
4.1	Overview of Study	13
4.2	Selection of Study Population	14
4.3	Inclusion and Exclusion Criteria	14
4.4	Randomization and Study Withdrawal	16
5	STUDY SCHEDULE ( See Appendix 1).....	16
5.1	Recruitment and Enrollment	16
5.2	Parent Education about Rotavirus Vaccines and the Study	17
5.3	Study Visits	18
6	STUDY PROCEDURES .....	20
6.1	Rotavirus Vaccine Randomization and Administration	20
6.2	Breast Milk Collection	20
6.3	Saliva Collection	20
6.4	Stool Collection	20
6.5	Blood Collection	20
6.6	MRI/Ultrasound Imaging	21
6.7	Rotavirus Vaccine Administration	21
6.8	Reactogenicity Assessment	21
6.9	Receipt of other Recommended Vaccines	22
6.10	Study Compensation	23

7	STUDY VACCINE PRODUCT .....	23
7.1	Study Product Description, Storage and Stability	23
7.2	Dosage, Preparation, and Administration of Study Product	23
7.3	Assessment of Subject Compliance With Study Product	24
8	LABORATORY EVALUATIONS AND METHODS .....	24
8.1	Blood-Cytokine and Inflammatory Responses and Maternal Antibody (IgG) .....	24
8.2	Stool-RV1 and RV5 Shedding and Cytokine Responses	25
8.3	Saliva-DNA Genotyping for Secretor Status	25
8.4	Breast Milk-IgA antibody and Secretor Status of the Mother	25
8.5	Future Testing	25
9	IMAGING METHODS .....	26
9.1	MR Imaging	26
9.12	Methods	26
9.13	Image Interpretation	26
9.2	Ultrasound Imaging	26
9.21	Methods	26
9.22	Image Interpretation	26
10	RANDOMIZATION, DATA MANAGEMENT, STATISTICAL PLAN .....	27
10.1	Randomization Plan	27
10.2	Data Management Plan	27
10.3	Statistical Analysis Plan	27
11	ASSESSMENT OF SAFETY .....	29
11.1	Adverse Events	29
11.2	Safety Reporting: For Adverse Events	30
12	POTENTIAL RISKS AND BENEFITS .....	30

## PROTOCOL SUMMARY

<b>Title:</b>	Potential Mechanisms for Intussusception after Rotavirus Vaccine – Pilot Study
<b>Phase:</b>	Phase 4
<b>Population:</b>	Infants 6 weeks to 13 weeks of age
<b>Site:</b>	Cincinnati Children’s Hospital Medical Center
<b>Subject Participation Duration:</b>	Two weeks
<b>Description of Agent Or Intervention:</b>	Single oral dose of either of the licensed and recommended rotavirus vaccines: Rotarix® (RV1) or RotaTeq® (RV5) given alone or with other routinely given vaccines
<b>Primary Objectives:</b>	<ol style="list-style-type: none"> <li>1) to examine the effects of RV1 and RV5 with or without other routine immunizations on gastrointestinal anatomy</li> <li>2) to assess the feasibility of conducting a larger scale study</li> </ol>
<b>Exploratory Objectives:</b>	<ol style="list-style-type: none"> <li>1) to examine the effects of RV1 and RV5 with or without other routine immunizations on gastrointestinal motility</li> <li>2) to examine the effects of RV1 and RV5 with or without other routine immunizations on blood and stool cytokines and inflammatory responses, including clinical reactogenicity events</li> <li>3) to assess patterns and level of vaccine virus shedding after RV1 and RV5</li> <li>4) to provide preliminary data assessing associations with the pattern of rotavirus vaccine strain (RV1 and RV5) shedding in the stool and blood, stool cytokine responses, and the anatomy and/or functional motility of the small intestine; and examine other factors such as maternal antibody, breast milk antibody, and human blood group antigens to see if these factors modify these responses</li> <li>5) to compare MRI and ultrasound imaging</li> </ol>
<b>Description of Study Designs:</b>	Prospective randomized clinical study of the potential effects of the first dose of RV1 or RV5 on gastrointestinal motility and anatomy and blood and stool cytokine responses and assessment of associations with the pattern of the shedding of vaccine strain rotavirus in the stool with these outcomes

## 1 KEY ROLES

### Cincinnati Children's Hospital Medical Center

<b>Principal Investigator:</b>	Mary Allen Staat, MD, MPH
<b>Co-Investigators:</b>	Steven Black, MD Alexander J. Towbin, MD Elizabeth P. Schlaudecker, MD, MPH Jean A. Tkach, PhD
	Ardythe L. Morrow, PhD Monica M. McNeal, MS Mekibib Altaye, PhD
<b>Study Coordinator:</b>	Nancy Back, RN, MPH
<b>Study Manager:</b>	Marilyn Rice, MS
<b>Study Team:</b>	Michelle Roth, RN Krista Doerflein, LPN Brittany Cassell, LPN Jacqui DellaTorre, LPN

### Centers for Disease Control and Prevention (CDC)

<b>Co-Principal Investigators:</b>	Karen Broder, MD and Margaret Cortese, MD
<b>Co-Investigators:</b>	Umesh Parashar, MD, MBBS Devindra Sharma, MSN, MPH
<b>Coordinator:</b>	Oidda Museru, MSN, MPH

This study is funded by a CDC contract with Cincinnati Children's Hospital Medical Center as a Task Order in the CISA Project Contract. The Cincinnati Children's Medical Center PI (Mary Allen Staat) will oversee the overall study and direct activities. CDC personnel will collaborate to develop the protocol, conduct the study, ensure the study is aligned with CDC public health priorities, and analyze the data and disseminate the results. CDC may receive access to coded data. This study will be registered at [ClinicalTrials.gov](http://ClinicalTrials.gov).

## 2 BACKGROUND AND SCIENTIFIC RATIONALE

### 2.1 Rotavirus Infection, the History of Rotavirus Vaccines and Intussusception

Prior to the licensure of rotavirus vaccines, rotaviruses were the most common cause of severe acute gastroenteritis (AGE) in children worldwide with nearly all children infected in the first five years of life<sup>1,2</sup>. In the United States (US), rotaviruses were estimated to cause 55,000 to 70,000 hospitalizations, 205,000 to 272,000 emergency department visits, and 20 to 60 deaths in young children annually before rotavirus vaccines were approved and universally recommended for use in infants<sup>3-6</sup>. Worldwide, rotaviruses were estimated to be responsible each year for 2 million hospitalizations and 352,000 to 592,000 deaths in children younger than 5 years<sup>7-11</sup>. Due to the morbidity in the US and morbidity and mortality around the world in resource poor countries there was a need to develop rotavirus vaccines.

The first rotavirus vaccine licensed in 1998<sup>2,12</sup> was effective but was withdrawn within a year due to its association with intussusception<sup>13,14</sup>. Years later, two new vaccines were licensed in the US, resulting in a dramatic decline in rotavirus disease. While these two vaccines have been found to be effective in other countries, in the resource-poor countries both vaccines have been far less efficacious compared to resource-rich countries. Further, these two new vaccines have now been found to be associated with intussusception, albeit at a lower attributable risk than the original vaccine. Rotavirus experts and those responsible for rotavirus vaccine policy have proposed that the benefits of rotavirus vaccines in preventing rotavirus disease and death, even at a lower efficacy in developing countries, far outweigh the risks of intussusception. CDC's Advisory Committee on Immunization Practices (ACIP) routinely recommends rotavirus vaccine for infants<sup>2</sup>. It is however, important to have a level of certainty about this balance of risk and benefit given that intussusception in resource poor countries has a higher likelihood of a poor outcome since there are delays in evaluation and decreased access to standard treatment which can lead to high surgery rates and death. A better understanding of the potential biological mechanisms of rotavirus vaccines to cause intussusception as well as factors which may modify risk may provide information to lessen the risk for intussusception or a poor outcome if a child gets intussusception. Below, we summarize what is known with regard to rotavirus vaccines, rotavirus infection and intussusception to date.

### **RRV-TV and Intussusception**

On August 31, 1998, the tetravalent rhesus-based rotavirus vaccine (RRV-TV) (RotaShield®, Wyeth-Lederle Vaccines and Pediatrics) was licensed by the Food and Drug Administration (FDA) for routine use among infants<sup>15</sup>. RRV-TV was found to be safe and effective pre-licensure. However, less than a year after licensing, 15 cases of intussusception which occurred shortly after receipt of RRV-TV were reported to the Vaccine Adverse Event Reporting System (VAERS)<sup>16</sup>. Subsequent analyses led by the CDC found an association between the receipt of RRV-TV and the development of intussusception. This led the ACIP to withdraw the recommendation for use of RRV-TV<sup>14,17</sup> and the manufacturer to voluntarily withdraw RRV-TV from the market in the US<sup>14</sup>. The initial publication describing the association reported an increased risk for development of intussusception from 3 to 14 days after receipt of the first dose of vaccine (adjusted odds ratio, 21.7)<sup>18</sup>. A smaller risk also existed after receipt of the second dose of vaccine. Researchers estimated that one case of intussusception attributable to vaccination with RRV-TV would occur for every 4670 to 9474 infants vaccinated. Ecologic studies that followed reported a lower vaccine-attributable risk<sup>19,20</sup>. Further analysis revealed that the risk was age related and substantially increased in those older than 90 days<sup>21,22</sup>. Of note, in pre-licensure studies of this rotavirus vaccine, intussusception was seen in 5 of 10,054 vaccine recipients (0.5 per 1,000) and 1 of 4,633 controls (0.2 per 1,000); a difference that the investigators concluded was not statistically significant<sup>23</sup>. The pathogenic mechanism for this association has not been identified<sup>24</sup>.

### **Current Rotavirus Vaccines**

After RRV-TV was withdrawn from the market, two new rotavirus vaccines were subsequently developed and introduced into the pediatric immunization schedule. Large pre-licensure studies for both vaccine candidates were required to determine if there was an increased risk of intussusception for each vaccine. In these large phase 3 studies, no increased risk for intussusception was identified<sup>25,26</sup>. The ACIP and American Academy of Pediatrics (AAP) recommended routine use of these two rotavirus vaccines in US infants: RotaTeq® (RV5) which was licensed by the FDA in February 2006<sup>27</sup> and Rotarix® (RV1) which was licensed in April 2008<sup>2</sup>. Post-licensure programs to monitor the safety of these two vaccines were established both in the US and abroad, and after years of use, post-marketing studies from different countries have identified a low level of risk of intussusception with both rotavirus vaccines; however, results have varied<sup>28-30</sup>.

### **RV1 and Intussusception**

In a post-licensure study using a self-controlled case series and case-control methods in Mexico and Brazil, cases of children with intussusception were identified through active hospital-based surveillance<sup>30</sup>. In Mexico, there was an increased risk of intussusception 1-7 days after dose one with either method accounting for 1 additional case of intussusception for every 52,000 children vaccinated with RV1. However, no increased risk was found after the first RV1 dose in Brazil. They did, however find a smaller attributable risk (AR) of 1 case of intussusception for every 76,000 children vaccinated after dose 2. In Australia, active surveillance data from a self-controlled case series analysis estimated the AR for intussusception to be 4.3 (95% CI, 0.8–23.3) cases per 100,000 infants vaccinated in the 1–21 days after dose 1 and the 1–7 days after dose 2<sup>29</sup>. Similarly, in US infants, an increased risk for intussusception after RV1 was identified in the Vaccine Safety Datalink (VSD) with an elevated risk during the first 1-7 days after vaccination with dose 1 and dose 2<sup>31</sup>. In this study, there were 207,995 doses of RV1 which included 115,908 first doses administered. The AR after the RV1 series was 5.3 per 100,000 infants vaccinated compared to historical background rates when no rotavirus vaccine was in use<sup>31</sup>. In another US study, preliminary data from an FDA-sponsored study through the Post-licensure Rapid Immunization Safety Monitoring System (PRISM) suggested an increased risk after RV1, although the study was underpowered<sup>32</sup>.

### **RotaTeq (RV5) and Intussusception**

Since RV5 was licensed and recommended for use in the US prior to RV1, there have been more doses given and therefore a larger amount of data for monitoring post-licensure safety. Four studies in the US from 2010 to 2012 found no increased risk of intussusception with RV5<sup>33-36</sup>. However, more recently, in an Australian study, active surveillance data from a self-controlled case series analysis estimated an AR for intussusception in the first 21 days after dose 1 and the first 7 days after dose 2, to be 7.0 (95% CI, 1.5–33.1) cases per 100,000 for RV5<sup>29</sup>. Data from PRISM identified an increased risk of intussusception after RV5. In the first 7 days after dose 1 of RV5 the AR was 1.1 (95% CI 0.3, 2.7) additional intussusception cases per 100,000 infants vaccinated and in the first 21 days after dose 1 of RV5 the AR was 1.5 (95% CI 0.2, 3.2) additional cases of intussusception per 100,000 infants vaccinated<sup>32</sup>.

### **Gaps in Knowledge-Intussusception and Rotavirus Vaccines**

In summary, consistent levels of risk for intussusception have not been observed in these post-licensure studies. This could be due to the low level of risk, small sample sizes for a rare outcome and/or differences in vaccine responses in different populations. Even though there are inconsistencies in these studies, overall there is evidence that RRV-TV, RV1 and RV5 are associated with an increased risk of intussusception. The reasons for the increased risk are unknown and it is not clear if there are product-specific differences that are real or if factors specific to the populations studied differ. Factors such as transplacental maternal antibody and breast milk antibody can influence vaccine-virus replication<sup>37</sup>, and human blood group antigens (FUT-2) can affect susceptibility to rotavirus disease<sup>38</sup>. Other factors such as nutritional status, micronutrient deficiencies and interfering gut flora could also impact viral replication<sup>39</sup>. It is also possible that oral polio vaccine could influence risk for intussusception. In the post-licensure safety studies in Brazil and Mexico<sup>30</sup>, Brazilian children routinely received oral polio vaccine (OPV) while Mexican children are given inactivated polio vaccine as part of their routine immunizations. Since replication of polio virus is highest with dose one, and has been shown to lower the take of RV1<sup>40,41</sup>, this may be one factor that influences risk of intussusception where OPV is used. While OPV is not used in the US, as we examine the association of intussusception and rotavirus in resource poor countries, concomitant use of OPV with rotavirus vaccines will need to continue to be studied. In considering factors associated with the risk of intussusception from rotavirus vaccines, it will be important to examine these potential modifiers whenever possible. Understanding the pathophysiology for such possible associations is important not only in terms of performing risk-benefit analyses for existing vaccines but also to facilitate introduction of new vaccines for which large pre-licensure assessments have not been

done. This information could also be used in determining ways to monitor for intussusception in resource poor countries, which could aid in evaluating new rotavirus vaccines such as those nearing licensure in Viet Nam and India.

### **Intussusception-Still Not Well Understood**

Intussusception is a type of bowel obstruction where one segment of bowel becomes enfolded within another segment. While intussusception can occur at any age, it is most commonly seen among young children, especially infants 4 to 10 months of age. The diagnosis is considered in young children with severe abdominal pain. In the US, the use of ultrasound allows for a rapid, non-invasive diagnosis of intussusception which is sensitive and somewhat specific. Children with findings of intussusception on ultrasound are usually promptly sent for an air or contrast enema for treatment and simultaneous confirmation of the intussusception. In the case that the intussusception cannot be reduced, the child is then taken to surgery for manual reduction with some children requiring partial resection of their intestine due to ischemic damage from the intussusception or because of a pathologic lead point.

The causes of intussusception are not known; however, prevailing theories include: 1) edema/thickening of the bowel wall (resulting from inflammation and lymphoid hyperplasia), 2) prominent mesenteric lymph nodes acting as lead points<sup>42</sup> and 3) alterations in gut motility<sup>43</sup>. A small fraction of cases have an anatomical or pathologic “lead point”, such as a polyp, or Meckel’s diverticulum, that increases a child’s susceptibility to intussusception. However in most children there is no clear explanation. Some studies have suggested infectious agents as causes of intussusception, including adenoviruses<sup>24,44-48</sup>, rotavirus<sup>49</sup>, HHV-6<sup>48</sup>, HHV-7<sup>48</sup>, Epstein-Barr virus<sup>48</sup>, *Escherichia coli*<sup>50</sup>, *Salmonella*<sup>50</sup>, *Shigella*<sup>50</sup>, and *Campylobacter*<sup>50</sup>; however, the implications of these findings are unclear since most studies do not include a comparison group. Evidence of rotavirus infection has been found in 5-37% of infants with intussusception in case series<sup>49</sup>; however, the association between natural rotavirus infection and intussusception has never been confirmed with rigorous epidemiologic studies<sup>11,42,45,51</sup>. Prior to the widespread use of rotavirus vaccines in the US, when rotavirus disease was distinctly seasonal, hospitalizations for intussusception were not found to be seasonal, suggesting that either rotavirus did not play a major role in the etiology of intussusception or that multiple infectious agents with different or less distinct seasonal patterns made the association between rotavirus infection and intussusception difficult to detect<sup>51,52</sup>. Thus, while wild-type rotavirus infection has not been linked to increased risk for intussusception, a small increased risk for intussusception after wild-type rotavirus cannot be ruled out<sup>11</sup>. One study suggested that rotavirus infection induced anatomical changes in the intestine (hyperplasia of Peyer’s patches and mesenteric lymph node enlargement) compatible with those that could cause intussusception<sup>42</sup> however another examining intussusception in RRV-TV recipients did not find these changes<sup>24</sup>, suggesting there may be different pathophysiologic mechanisms that lead to intussusception.

## **2.2 Intestinal Imaging**

### **Imaging Studies in Children with Rotavirus Infection**

Two previous studies have been done in children to explore possible mechanisms for the cause of intussusception in children with wild-type rotavirus infection<sup>42,43</sup>. In the first study, 13 children with confirmed rotavirus infection were age- and sex-matched to healthy children and ultrasound measurements were done to assess ileum wall thickness and mesenteric lymph node diameters at enrollment and one month later; motility was not assessed<sup>42</sup>. The average age at enrollment was comparable (7.2 months for cases and 7.3 months for controls). They found children with rotavirus infection to have significantly thicker ileum wall thickness, greater lymph node size and number of nodal aggregates and greater free fluid compared to controls. While distal ileum loops were visualized in all study subjects, the terminal ileum was only visualized in 46% of patients with rotavirus infection and in 62% of controls. They concluded that these changes suggested a mechanism by which rotavirus could cause intussusception. Since most intussusception occurs at the terminal ileum, it is critical that an

imaging study evaluating risk for intussusception can effectively image this area. With MRI, we will be able to visualize and measure the wall of the terminal ileum for all patients as well as assess motility.

In a second study, investigators enrolled five children with symptomatic rotavirus infection and did imaging with ultrasound and MRI within 5 days of the onset of symptoms and repeated imaging 5-9 weeks later with an age range of 8-20 months (mean age of 14 months)<sup>43</sup>. They found three of the five infants had a significantly thicker ileal wall thickness in the acute period compared to the convalescent period but no differences in the number and size of mesenteric lymph nodes. They found no difference in peristaltic activity between the two time periods using MRI imaging without sedation. The authors did not appear to have a period where the infants were made NPO. A small sample size (4 infants for the MRI) and lack of a specific protocol for feeding may have impacted their ability to assess motility patterns.

While these studies provide useful preliminary data, none were done in the age group in question or with rotavirus vaccine exposure. In addition, radiographic techniques and assessments of motility have improved since 2004 allowing for more standardized measures of normal anatomy and motility (pre-immunization) and more sensitive measures post-immunization with the use of MRI.

### **Intestinal Motility Imaging**

The standard imaging modality for clinical evaluation of intestinal motility is fluoroscopy where opaque contrast material is administered orally or via a nasogastric tube into the bowel lumen, followed by serial radiographs to determine bowel anatomy and successful movement of the contrast through the bowel. A significant drawback is that fluoroscopy results in a relatively large radiation exposure. While abdominal ultrasound avoids radiation exposure, technical challenges of ultrasound limit the ability to assess intestinal motility. Ultrasound can only focus on one bowel loop at any given time, making assessment of the entire bowel quite time intensive. Assessment of a single bowel loop over several minutes in order to qualitatively and quantitatively assess motility is challenging. Any air within the bowel lumen significantly decreases image quality. In addition, ultrasound is very dependent on the skill and experience of the ultrasonographer and there is a high degree of inter-operator variability. Alternatively, MRI is extremely effective in providing detailed information about anatomy and function including motion<sup>53</sup>. MRI uses magnetic fields and radio waves to create detailed images of the organs and tissues; there is no radiation exposure with this procedure. Recently, MRI has been used in the evaluation of in-utero suspected congenital malformations of the intestine<sup>54</sup>, as well as evaluation of ongoing disease activity in pediatric patients with inflammatory bowel disease<sup>55</sup>. Several recent studies have demonstrated the ability of dynamic MRI, using ultrafast acquisitions (< 1 sec), to evaluate intestinal motility<sup>56-61</sup>.

## **2.3 Inflammatory Mediators and Intussusception-Use of Blood and Stool Cytokine Analyses**

While we know that both infectious agents and receipt of vaccines induce immune responses and inflammatory mediators, little is known about specific patterns of inflammatory mediator expression which might be associated with an adverse event from a vaccine such as rotavirus vaccine and intussusception. We know that specific cytokine responses can alter intestinal motility and lymph node enlargement. Therefore we postulate that specific patterns of expression could predispose to intussusception. While limited animal and human studies have examined serum cytokine responses, to our knowledge, no studies of stool cytokines have been undertaken in evaluating rotavirus vaccines or intussusception.

### **Animal Studies**

Early studies in mice demonstrated that lipopolysaccharide (LPS) can induce intussusception<sup>62</sup>. Inflammatory mediators such as tumor necrosis factor, platelet-aggregating factor, and other cytokines

were thought to disrupt gastrointestinal tract motility and contribute to the etiology of intussusception<sup>63-65</sup>. In a more recent model, both homologous murine and heterologous simian rotavirus strains significantly enhanced the rate of LPS-induced intussusception in mice<sup>66</sup>. This occurred despite there being no measured effect of rotavirus infection on gastrointestinal transit. TNF $\alpha$  and IL-6 are also known to significantly affect gastrointestinal motility and have been associated with LPS-induced intussusception in mice<sup>67</sup>. In one study, indomethacin (a non-steroidal anti-inflammatory drug) treatment of mice after LPS, prevented intussusception<sup>67</sup>. However, in another study, no correlation was demonstrated between serum TNF $\alpha$  levels and the development of intussusception in mice<sup>68</sup>.

### Human Studies

Several studies have evaluated serum cytokines in children at the time of acute ileocolic intussusception<sup>69,70</sup>. Levels of IL-6, neopterin, and C-reactive protein (CRP) were significantly elevated in children with intussusception compared to normal laboratory ranges and convalescent samples<sup>69</sup>. Another study demonstrated elevated IL-6 levels in 22 infants with intussusception compared to 20 healthy control infants<sup>70</sup>. Levels were significantly increased in infants requiring surgery compared to those reduced by enema. In another report, in two children with adenovirus and intussusception, the investigator identified an inflammatory neuropathy of a network of nerves in the intestines and proposed that inflammation of the intestine caused disruption of gastrointestinal motility that could lead to intussusception.

### Stool Cytokine Responses

As compared to serum cytokine levels described above, stool cytokine levels are more likely to reflect gut mucosal immune responses to infection or other inflammatory changes. Studies have examined fecal cytokine responses to norovirus<sup>71</sup>, *E. coli*<sup>72,73</sup>, and other etiologic agents of travelers' diarrhea using enzyme immunoassays (EIA)<sup>74</sup>. Dr. Haslam, our co-investigator, enrolled children with symptomatic *C. difficile* (cases), symptomatic controls (without *C. difficile*) and asymptomatic controls with and without *C. difficile*. Stool samples were tested using EIA for fecal IL-8, lactoferrin, and phosphorylated-p38 protein concentrations, as well as the novel quantitative reverse transcriptase polymerase chain reaction (q RT-PCR) to determine IL-8 and chemokine ligand (CXCL)-5 RNA relative transcript abundances<sup>75</sup>. Fecal inflammatory cytokines were found to be useful in distinguishing *C. difficile* colonization from disease and identifying children with *C. difficile* infection likely to have prolonged diarrhea. In addition, fecal cytokines were higher in samples from symptomatic children (with and without *C. difficile*) than in samples from asymptomatic children with *C. difficile*. In a study in adults, persistent diarrhea was also shown to be correlated more with intestinal inflammation than fecal pathogen burden. The RT-PCR method was more sensitive than ELISA for IL-8 measurement, and the mRNA levels measured by RT-PCR correlated with disease severity. Cytokine levels measured by ELISA did not correlate with disease severity, as the cytokines were frequently undetectable via ELISA<sup>76</sup>.

### Cytokines Associated with Lymphocyte Proliferation and Systemic Inflammation

Lymphoid hyperplasia in the terminal ileum is a recognized cause of intussusception<sup>77,78</sup>. Cytokines specifically associated with lymphocyte proliferation and development include IL-2<sup>79</sup>, IL-7<sup>80</sup>, IL-15<sup>81</sup>, and various members of the TNF superfamily<sup>82</sup>. Elevated CRP and IL-6 have also been associated specifically with acute intussusception<sup>69,70</sup>. Measuring these cytokines that drive lymphocyte proliferation, as well as those associated with acute systemic inflammation, may identify inflammatory markers in both stool and serum that may be associated with the pathophysiology seen in intussusception. This project will be the first to investigate novel fecal measures of inflammation and lymphoid hypertrophy during immunization.

## 2.3 Potential Modifiers of Rotavirus Infection, Vaccine Response and Shedding and Intussusception

### **Age-Dependent Susceptibility to Rotavirus Disease and Intussusception**

Severe rotavirus disease occurs most commonly in infants younger than 24 months. The onset of severe rotavirus disease has been reported to coincide with the decline of maternal IgG antibody titers<sup>83</sup>. In addition, we have found a decreased antibody response to the live rotavirus vaccine RV1 in pre-licensure studies in infants with higher levels of transplacental neutralizing antibody to the vaccine strain (unpublished results). Therefore, early protection from rotavirus disease including asymptomatic neonatal rotavirus infections<sup>84,85</sup> may be due, at least partially, to protection by transplacental antibody that may persist for the first months of life<sup>86</sup>. The risk of intussusception increases with age and many felt the risk of RRV-TV-associated intussusception also increased with age<sup>18</sup>. Therefore, in order to best understand potential mechanisms associated with intussusception and rotavirus vaccines, ideally, it would be important to examine responses in children with the broadest age range possible. Since the age recommendations for the first dose of rotavirus vaccine is 6 weeks to 14 weeks, 6 days, the prospective study will be limited to that age group. We have selected the age range of 6 week to 13 weeks (12 weeks, 6 days) in order to provide assurances that the other 2 month old vaccines can be given on schedule.

### **Breast Milk-Rotavirus Susceptibility and Rotavirus Vaccine Response**

The role of breast milk and rotavirus susceptibility and rotavirus vaccine response is unclear. Two studies found that the highest incidence of rotavirus-associated diarrhea is in children aged 4 to 6 months, suggesting that risk for rotavirus infection increases at the age when weaning from breast-feeding began<sup>87,88</sup>. Other studies have shown breast-feeding to be associated with protection against symptomatic rotavirus infections<sup>89-92</sup>. Unfortunately, there are limitations in study design, as other factors such as the presence of maternal antibody or lack of opportunity for exposure were not examined. However, one study specifically supported the role of human milk and factors other than breast milk antibody in protection against rotavirus infection, demonstrating that infants who received human milk with higher concentrations of the glycoprotein lactadherin were more likely to have asymptomatic rotavirus infections compared with those receiving milk with lower concentrations<sup>90</sup>. Since both licensed vaccines are live-attenuated vaccines, there is also a concern that breast milk could play a role in limiting the response to rotavirus vaccination which would impact protection but also the pattern of cytokine response after vaccination<sup>37,93,94</sup>. While sub analyses in studies of RV1 and RV5 did not show a decreased efficacy in breastfed infants, the immune response and protection provided by live oral rotavirus vaccines are less in resource poor countries, where nearly all infants were breastfed, than in developed countries<sup>95,96</sup>. It has been speculated that this could at least in part be due to breast milk antibody and other constituents limiting the replication of the vaccine strains as the levels of antibody are higher in women in resource poor countries. However, more recent data have not found this to be true. To our knowledge there are no data describing differences in vaccine virus shedding, cytokine responses or intussusception in breastfed and non-breastfed infants. In the case-control study evaluating intussusception and RRV-TV, data were re-analyzed to assess risk factors for intussusception unrelated to RRV-TV<sup>97</sup>. Cases were infants who had intussusception between November 1998 and June 1999. Controls were matched by age and hospital of birth. Sociodemographic and feeding practice data were collected through parent and provider interviews. Risk factors for intussusception were identified while controlling for exposure to RRV-TV. Of the 429 cases enrolled, 87% had not received RRV-TV. There were 1763 controls. In addition to finding male sex, Hispanic or black race/ethnicity, and Medicaid enrollment to be factors associated with intussusception, an interaction was found between introduction of solid food and type of formula consumption. Using breast milk as the referent group, infants with introduction of solid food for at least 5 weeks who consumed soy milk-based formula had a lower risk (OR 0.26; 95% CI 0.1-0.7) and infants without introduction of solid food who consumed cow's-milk formula had an increased risk (OR 2.33; 95% CI 1.4-3.9).

Another case control study which studied idiopathic intussusception found that, compared with non-breast-fed infants, breast-fed infants had a relative risk of intussusception of 6.0 (95% CI, 1.8 to 20.4) when breast-feeding at admission was exclusive and of 2.3 (95% CI, 0.8 to 6.6) when it was partial<sup>98</sup>. They concluded that exclusive breast-feeding may be a risk factor for intussusception in infancy.

### **FUT2 Secretor Gene and Rotavirus Susceptibility**

Recently, we and others have reported that, similar to norovirus, risk of rotavirus infection is determined in part by an individual's *FUT2* (fucosyltransferase 2) genotype<sup>38,99,100</sup>. The *FUT2* (secretor) gene is responsible for producing an enzyme that makes carbohydrate histo-blood group antigens (HBGAs) available on the surface of the gut. The major human rotavirus genotypes (P4, P6, and P8) bind to HBGA that are synthesized by *FUT2* gene enzymes, specifically H type 1, Lewis B or A antigens<sup>101,102</sup>. Individuals who are homozygous recessive for the *FUT2* (secretor) gene, and are thus known as “non-secretors,” lack these HBGA on their mucosal surfaces, thereby reducing the ability of rotaviruses to bind and infect intestinal epithelial cells. From a collaborative multi-site surveillance study with the CDC New Vaccine Surveillance Network (CDC NVSN), we identified 56 rotavirus-positive cases of acute gastroenteritis (AGE). None of these rotavirus AGE cases had the non-secretor *FUT2* mutation, compared with 23% of healthy controls ( $p < 0.01$ ). Additional surveillance by the same network identified another 120 rotavirus positive cases, and similarly, found that only 2% of the rotavirus AGE cases were *FUT2* non-secretors compared to 23% of healthy controls ( $p < 0.01$ ).

*FUT2* non-secretors are approximately one-quarter of the population and appear to be largely protected not only against rotavirus but also against norovirus, while the majority of the population with *FUT2* secretor genotype appears to convey risk. Thus, the question arises, why is the *FUT2* genotype maintained – what is the benefit? Several lines of investigation suggest that the carbohydrates synthesized by the *FUT2* enzyme have an anti-inflammatory impact on the intestinal tract. The *FUT2* secretor genotype and oligosaccharide made by *FUT2* enzyme favors microbial diversity<sup>103</sup>, and the growth of beneficial, immune-regulatory intestinal microbiota<sup>104-106</sup>. Conversely, the lack of these carbohydrates in the *FUT2* non-secretor genotype is a risk factor for inflammatory bowel disease (IBD), and functional changes in the microbiota that are accompanied by sub-clinical levels of inflammation in the local intestinal mucosa<sup>103</sup>.

### **Other Routine Immunizations**

Young infants receive multiple immunizations that are associated with elevated blood cytokines and other inflammatory markers. Routine childhood immunizations have been found to be associated with pro-inflammatory cytokine responses, including  $\text{INF-}\gamma$ <sup>23,24</sup>, IL-5<sup>25</sup>, IL-6<sup>26</sup>, and IL-8<sup>27</sup>. These routine immunizations may therefore contribute to or interfere with the inflammatory response following rotavirus immunization, potentially altering the risk for intussusception.

Therefore it will be important that in two arms of the study infants will receive routine 2 month old immunizations with one of the two recommended rotavirus vaccines (RV5 or RV1) to evaluate the contribution of blood cytokine responses associated with the addition of routine immunizations to rotavirus vaccine and whether there are stool cytokine responses that are associated with routine immunizations with rotavirus vaccine given concurrently. This will provide two arms that reflect “real world” immunization schedule, allowing for the determination of any changes in the outcomes from receiving all the recommended vaccines. The outcomes found in these two arms will be compared to the RV1 and RV5 alone arms.

### 3 Purpose and Objectives

#### 3.1 Purpose

The purpose of this project is to conduct a pilot study to explore potential biological mechanisms for an association between rotavirus vaccines and intussusception.

#### 3.2 Hypothesis

Our hypothesis is that rotavirus vaccines may increase the risk for intussusception by inducing cytokines or other inflammatory responses that affect the anatomy or functional motility of the small intestine and that there will be a relationship between the pattern of the shedding of vaccine strain rotavirus and the anatomy and or functional motility of the small intestine. We also hypothesize that potential factors such as maternal antibody, human blood group antigens and breast milk in breast-fed infants could modify rotavirus vaccine virus shedding and/or inflammation following vaccination.

#### 3.3 Primary and Exploratory Objectives

Primary Objectives:

- 1) to examine the effects of RV1 and RV5 with or without other routine immunizations on gastrointestinal anatomy
- 2) to assess the feasibility of conducting a larger scale study

Exploratory Objectives:

- 1) to examine the effects of RV1 and RV5 with or without other routine immunizations on gastrointestinal motility
- 2) to examine the effects of RV1 and RV5 with or without other routine immunizations on blood and stool cytokines and inflammatory responses, including clinical reactogenicity events
- 3) to assess patterns and level of vaccine virus shedding after RV1 and RV5
- 4) to provide preliminary data assessing associations with the pattern of rotavirus vaccine strain (RV1 and RV5) shedding in the stool and blood, stool cytokine responses, and the anatomy and/or functional motility of the small intestine; and examine other factors such as maternal antibody, breast milk antibody, and human blood group antigens to see if these factors modify these responses
- 5) to compare MRI and ultrasound imaging

#### 3.4 Study Outcome Measures

##### **Intestinal Anatomy and Motility-Use of Magnetic Resonance Imaging (MRI) and Ultrasound (US)**

We have selected magnetic resonance imaging (MRI) for our main imaging study outcome. Ultrasound will also be done and the results obtained will be compared to the MRI results. This will be done because ultrasound is the standard clinical mode of imaging for intussusception and is more readily available in both the US and abroad compared to MRI. In addition, ultrasound was used in previous studies. While ultrasound is a useful modality to assess the presence or absence of intussusception clinically, it has several limitations which make it difficult to assess the bowel serially. Some of the limitations of ultrasound include its lack of standard planes of imaging, lack of reproducibility, operator dependence, and inability to accurately assess motility and to image the entire abdomen. Alternatively, MRI is ideally suited to evaluate both intestinal anatomy and motility to assess for changes pre- and post-immunization with rotavirus vaccines and this is why MRI will be our main imaging study outcome.

##### **Inflammatory Mediators-Blood and Stool Cytokines**

We will measure both blood and stool cytokines pre- and post-rotavirus vaccination. The cytokines chosen include those shown in past studies to be associated with intussusception and others were chosen because of the association with lymphoid hyperplasia. For blood, we will test C-reactive protein as it is

readily available and used clinically. For both blood and stool, we will measure: IL-2, IL-6, IL-7, IL-8, IL-15, INF- $\gamma$  and TNF- $\alpha$ .

### **Rotavirus Vaccine Virus Shedding**

We will use RT-PCR to identify rotavirus vaccine strains, to examine the duration of shedding and to quantitate rotavirus shedding 5 days post-vaccination. We will then assess the correlation of the amount of virus shedding with intestinal anatomy and motility as well as cytokine and inflammatory responses. The Ct values will be examined and categorized as none, low or high for analysis. We chose 14 days to assess the duration of shedding since studies have shown that most vaccine-associated events occur in the first 14 days and shedding becomes less frequent after 14 days. Vaccine virus shedding (qualitative) will be done on all stools collected. In addition, since we will be collecting reactogenicity data for Days 0-14, stool from other days could be evaluated for stool cytokines should the infant have an adverse event either before or beyond the Visit 2 stool evaluation.

### **Potential Modifiers of Rotavirus Infection, Vaccine Response and Shedding and Intussusception Maternal Serum Antibody**

In order to examine the possible role of maternal antibody to rotavirus, we will test the initial blood sample for maternal antibody to see if maternal antibody affects shedding of vaccine virus, blood and stool cytokine responses and intestinal motility and anatomy. In addition, we will measure the infant's IgA antibody to assure that the child has not been infected with rotavirus.

### **Breast Milk Antibody**

We will obtain a breast milk sample from breastfeeding mothers of infants enrolled in the study in order to examine the potential effect of rotavirus IgA in breast milk on vaccine virus shedding, cytokine responses, and intestinal anatomy and motility. The analysis will be exploratory given the small sample size.

### **Secretor Status**

Thus, in an exploratory analysis, we will examine whether there is an association with secretor status and inflammatory response.

## **4 Research Plan**

### **4.1 Overview of Study**

This study is a prospective clinical study designed to 1) examine the effects of RV1 and RV5 alone or with other routine immunizations on gastrointestinal anatomy 2) to assess the feasibility of conducting a larger scale study. Exploratory objectives include 1) to examine the effects of RV1 and RV5 with or without other routine immunizations on gastrointestinal motility 2) to examine the effects of RV1 and RV5 with or without other routine immunizations on blood and stool cytokines and inflammatory responses, including clinical reactogenicity events 3) to assess patterns and level of vaccine virus shedding after RV1 and RV5 4) to provide preliminary data assessing associations with the pattern of rotavirus vaccine strain (RV1 and RV5) shedding in the stool and blood, stool cytokine responses, and the anatomy and/or functional motility of the small intestine; and examine other factors such as maternal antibody, breast milk antibody, and human blood group antigens to see if these factors modify these responses and 5) to compare MRI and ultrasound imaging.

Healthy infants 6-13 weeks of age will be randomized (1:1:1:1) to receive either, RV1 alone, RV5 alone, RV1 with ACIP routinely recommended immunizations (Diphtheria, Tetanus and Pertussis (DTaP), *Haemophilus influenzae* type b (Hib), pneumococcal conjugate (PCV13), Hepatitis B (HBV) and

inactivated polio (IPV)) or RV5 with routine immunizations. Imaging study personnel and parents will be blinded to the rotavirus vaccine type; parents will be informed about the rotavirus vaccine type at the completion of the study. Up to 150 infants will be enrolled (see inclusion/exclusion below). The age-group of infants 6-13 weeks of age was chosen so enrolled infants can receive their first dose of rotavirus vaccine at the AAP/ACIP recommended age of 2 months (minimum age of 6 weeks and maximum age of 14 weeks and 6 days)<sup>2</sup> and, to assure that other ACIP recommended vaccines can be administered at the recommended age.

Recruitment and enrollment will occur prior to the first study visit. There will be three scheduled study visits after recruitment/enrollment. . Infants will be randomized to either RV1, RV1 plus other immunizations, RV5 alone, or RV5 plus other immunizations. Visit 1 (day 0) will include blood, saliva, stool and breast milk collection . The MRI and ultrasound will be performed prior to vaccination. Children will receive the immunizations to which they are randomized. Imaging personnel and parents will be blinded to rotavirus vaccine type; they will be informed about other vaccines administered. A second MRI and ultrasound will be performed at Visit 2 (day 5) and blood and stool samples collected. Parents will be unblinded at the completion of Visit 3 (day 14). Arrangements will be made to get remaining doses of same rotavirus vaccine. Daily stool samples will be collected at home during the 15 day study period (on vaccination day 0 and for next 14 days). A memory aid will be completed to collect reactogenicity data on days 0 and for the next 14 days. Remaining stools and reactogenicity data from parents will be collected at Visit 3. **See Appendix 1 for detailed description of visits and timeline.**

We will assess blood and stool cytokine responses and intestinal anatomy and motility after rotavirus vaccination by comparing pre-vaccination with post-vaccination responses in the study infants. Cytokines and intestinal anatomy and motility will be assessed at baseline (Visit 1, the day of vaccine receipt) and 5 days after vaccination (Visit 2). For both blood and stool, the following cytokines will be tested: IL-2, IL-6, IL-7, IL-8, IL-15, INF- $\gamma$  and TNF- $\alpha$ . Additional biomarkers may be studied. The 5 day period post-vaccination was chosen because this time period corresponds with the peak period for intestinal replication of rotavirus vaccine virus for both vaccines<sup>107</sup>.

In order to protect the health of infants, we will provide procedures to facilitate and assure that infants enrolled in the study will receive the routinely recommended vaccines for 2 month old infants which are DTaP, Hib, PCV13, HBV and IPV that will also allow them to get their 4 month vaccines on schedule<sup>108</sup>.

## 4.2 Selection of Study Population

Normal healthy full term infants ( $\geq 37$  weeks gestational age) will be recruited. Subjects will be recruited without regard to race, gender, or ethnicity. Up to 150 subjects will be recruited from established patient populations at CCHMC. These include our CCHMC Pediatric Primary Care Clinic, employees at CCHMC, and our CCHMC study database. Parental permission /informed consent will be obtained from the parents/legal guardians before any procedures are performed. Their parents/legal guardians will be interviewed to assure that the infants meet all the eligibility criteria and will be available for the duration of the study.

Geographically, our proposed study population will be from our primary service area at CCHMC. We do not anticipate targeted recruitment based on minority status, but no minority will be excluded from the study.

## 4.3 Inclusion and Exclusion Criteria

### Subject Inclusion Criteria

Infants who meet the following criteria will be eligible to participate in this study:

1. healthy infant 6 to 13 weeks (12 weeks and 6 days) of age at day of rotavirus vaccine administration
2. free of obvious health problems as established by medical history and confirmed with infant's primary physician prior to Visit 1
3. parent/legal guardian willing to have infant feed from a bottle for contrast
4. parent/legal guardian willing and capable of signing informed consent
5. parent/legal guardian and infant expected to be available for entire study
6. parent/legal guardian can be reached by telephone
7. parent/legal guardian expresses willingness to complete study procedures and receive 2 month immunizations, according to recommended schedule

### Subject Exclusion Criteria

Infants will not be eligible if any of the below conditions apply:

1. gestational age of <37 weeks
2. infant unable to fast for 4 hours prior to MRI procedure
3. receipt of any vaccine except initial HBV (must have at least 28 days between HBV and Visit 1 to be included)
4. history of severe allergic reaction to HBV vaccine
5. contraindications for any of the routine vaccines
  - a. Severe Combined Immune Deficiency
  - b. history of intussusception
6. precautions for either RV1 or RV5 (may interfere with study outcomes)
  - a. altered immunocompetence
    - i. infants with primary and acquired immunodeficiency states, cellular immunodeficiency, hypogammaglobulinemic and dysgammaglobulinemic states
    - ii. infants with blood dyscrasias, leukemia, lymphomas, or other malignant neoplasms affecting the bone marrow or lymphatic system
    - iii. infants on immunosuppressive therapy (including high-dose systemic corticosteroids)
    - iv. infants who are HIV-exposed or infected
  - b. acute gastroenteritis
  - c. moderate or severe acute illness with or without fever
  - d. pre-existing chronic gastrointestinal diseases (e.g., congenital malabsorption syndromes, Hirschsprung's disease, or short-gut syndrome)
  - e. infants with spina bifida or bladder exstrophy (latex rubber is contained in the RV1 oral applicator)
7. sensitivity to latex (latex rubber is contained in the RV1 oral applicator)
8. febrile illness within previous 14 days (axillary temperature of 100.4° F or higher)
9. history of vomiting (forceful expulsion of partially digested milk/food) within 24 hours prior to Visit 1 and/or diarrhea (3 watery stools) within 14 days of Visit 1
10. receipt of any steroids, immunoglobulins, other blood products/transfusion
11. receipt of non-steroidal anti-inflammatory drugs in previous 72 hours (may affect cytokine response)
12. receipt of an antipyretic medication (acetaminophen or ibuprofen) within 72 hours prior to the first dose of rotavirus vaccine or is already planning to administer a prophylactic antipyretic medication on the day of and the day following vaccination (this exclusion does not apply if the caretaker indicates he/she might administer antipyretics after vaccination to reduce a fever)
13. is enrolled or plans to enroll in another clinical trial while participating in this study (observational studies are allowed)
14. any condition which, in the opinion of the investigators, may post a health risk to the subject or interfere with the MRI or vaccine evaluation

15. currently receiving medication for gastroesophageal reflux (GERD) or any other gastrointestinal condition including colic
16. infant who is a relative of any research study personnel
17. allergy to barium
18. failed newborn hearing screening

#### **4.4 Randomization and Study Withdrawal**

##### **Randomization Procedures**

The randomization code will be prepared by statisticians in the Epidemiology and Biostatistics Core at CCHMC. The randomization code will link to the vaccine assignment. The subject will be assigned a randomization code. Randomization will take place prior to the vaccine administration, but after the parent/legal guardian has given permission/informed consent, signed the IRB-approved consent form and met all of the inclusion and none of the exclusion criteria. Infants will be randomized 1:1:1:1 to one of four groups: 1) RV1 alone, 2) RV5 alone, 3) RV1 with routine immunizations (DTaP, Hib, PCV13, HBV and IPV or 4) RV5 with routine immunizations.

##### **Reasons for Withdrawal**

The study personnel may withdraw a subject from the study at any time for the following reasons:

1. unable to comply with the study protocol
2. receipt of blood or blood products (including immunoglobulin) during the study period
3. malignancy or confirmed or suspected immunodeficiency such as HIV infection or severe combined immunodeficiency
4. receipt of or history of any medications or treatments that affect the immune system, such as immune globulin, interferon, immunomodulators, cytotoxic drugs, or other drugs known to be frequently associated with significant major organ toxicity since enrollment
5. receipt of long-term ( $\geq 2$  weeks) potentially immunosuppressive corticosteroid medication during the study period
6. parent/legal guardian request
7. infants who vomit within 15 minutes of rotavirus vaccination will not be withdrawn but will be replaced for analysis purposes
8. allergic reaction to barium

##### **Handling of Withdrawals**

Every effort will be made to undertake protocol-specific safety follow-up procedures for subjects who are withdrawn by their parents/legal guardians. The subject's parents/legal guardians will be encouraged to participate in the final follow-up contact to occur 14 days after the initial visit.

Subjects who are withdrawn may be replaced if deemed necessary by the study team to meet study enrollment goals.

## **5 STUDY SCHEDULE ( See Appendix 1)**

### **5.1 Recruitment and Enrollment**

Recruitment will be done by our study personnel through the Pediatric Primary Care (PPC) Clinic at CCHMC and through study advertisement at CCHMC targeted towards our employee e-mail system and database of past research participants who have agreed to be e-mailed for future studies. Any written communication provided to the potential subjects' parents/legal guardians about the study will be submitted to the IRB for their approval before study initiation.

We routinely conduct studies through the PPC and our research personnel are present in the clinic. We will review the patients scheduled and will identify those who are age eligible for the study. The medical

record for those age-eligible will be screened to identify children who would not be eligible for the study (exclusion criteria). Parents of potentially eligible infants will be approached to discuss participation in the study. If interested, informed consent will be obtained before giving instructions and scheduling Visit 1. After consent is obtained, infant will be randomized into one of four study groups.

For recruitment of infants of CCHMC employees and through our research database, the interested parent will call our study line. Relatives of study personnel will be excluded from participation as is standard procedure. Study personnel will discuss the study by phone. Subjects will be screened by medical history from the parent. If eligible and interested, a meeting time will be arranged prior to Visit 1 to obtain informed consent. The infant's primary care provider will be contacted to determine if the infant is healthy and to inform the primary care provider of the study after informed consent and prior to Visit 1. Parents will be informed that their decision to participate or not to participate in the study will not have any effect on them as an employee.

Because the infants to be enrolled will be healthy and will be receiving a licensed vaccine that is approved for use in this population, no additional screening will be necessary. No baseline laboratory screening will be performed prior to enrollment. Screening records will be kept to document the reason why a subject was screened but failed entrance criteria. If a parent chooses not to agree to the study, we will ask for a reason and it will be documented.

Infants must be 6 weeks to 13 weeks of age (12 weeks, 6 days) for Visit 1, the day of immunization. Inclusion and exclusion criteria will be reviewed. All medications taken within the 30 days prior to enrollment will be recorded. Demographic and contact information will be obtained. The parent/legal guardian will be given containers for stool and breast milk collection at home. Instructions will be given to the parent with the date, time and location for Visit 1 and the need for their infant to be NPO for 3 hours prior to appointment and to return with a stool collected the day of Visit 1 (note for MRI must be 4 hours NPO, but will ask to be 3 hours NPO since it will take ~one hour from arrival until feed for MRI).

## **5.2 Parent Education about Rotavirus Vaccines and the Study**

Parents approached for this study will be educated about the two licensed rotavirus vaccines (RV1 and RV5) and informed that the AAP and ACIP routinely recommend rotavirus vaccines for infants and do not express a preference for RV1 or RV5<sup>2</sup>. We will explain that their infant may be randomized to a vaccine type different from the vaccine given at their primary care practice. The parent will also be informed that RV1 is a two dose vaccine series and RV5 is a three dose vaccine series and that if they choose to have their infant participate in the study, their infant will need to complete the series with the same vaccine type (all RV1 or all RV5). RV1 is to be administered orally in a 2-dose series, with doses administered at ages 2 and 4 months. RV5 is to be administered orally in a 3-dose series, with doses administered at ages 2, 4, and 6 months. The minimum age for dose 1 of rotavirus vaccine is 6 weeks; the maximum age for dose 1 is 14 weeks and 6 days. The minimum interval between doses of rotavirus vaccine is 4 weeks and there is no maximum interval set. All doses should be administered by age 8 months and 0 days. The parent of infants who are randomized to receive rotavirus vaccines without other childhood vaccines will be informed that since the purpose of the study is to study the effects of rotavirus vaccine, their infant will wait to receive the rotavirus vaccine until two weeks or more after other routinely administered immunizations or will not receive their other recommended vaccines until two weeks or more after their rotavirus vaccine. If the child is receiving routine immunizations after the rotavirus vaccine, every effort will be made for the infant to receive these vaccines within the recommended ACIP schedule for vaccines routinely recommended at 2 months of age (DTaP, Hib, Prevnar, Hepatitis B and IPV vaccine)<sup>108</sup>. The parents of infants who are randomized to receive rotavirus vaccines with other recommended vaccines will be informed of this for their planning and provided with a

letter for their pediatrician. These infants will still be recommended to have a 2-month well child check-up, but will be asked not to receive 2-month vaccines at this visit. Children receiving other childhood vaccines as part of the study will receive the following vaccines Pentacel (DTaP, Hib and IPV combination), Prevnar (PCV13) and HBV, in addition to the rotavirus vaccine. If one of these is not available we will replace it with another equivalent licensed vaccine. All vaccines administered as part of the study will be documented for the parent and child's health care provider. This information will be faxed to the provider. The vaccines administered will be documented in EPIC, in IMPACT (the Ohio State Registry) and in letter form with copies of the EPIC documentation to the parent and provider of the enrolled child.

## 5.3 Study Visits

### Enrollment/Recruitment Visit

- review inclusion/exclusion criteria and medical history
- obtain informed consent
- obtain a release to get medical information from the primary care provider
- collect contact information including the primary care provider of infant and the date of 2 month check up if known
- if refuses study, record refusal reason on screening log
- sample collection instructions
- distribute sample collection supplies
- review MRI and ultrasound prep
- randomize to study group and inform parent of result of all routine vaccines or rotavirus vaccine alone (not specific rotavirus vaccine type)
- schedule visit 1  
give written instructions for Visit 1 to parent (Appendix 5) **Reminder Call 1 (day prior to Visit 1)**
- review sample collection instructions
- review MRI/ultrasound prep  
review time, date and location of Visit 1 **Visit 1 (Day 0-Vaccine Administration)**
- contact information will be reviewed and updated if necessary
- inclusion and exclusion criteria will be reviewed to determine if child still meets criteria for study
- temperature will be recorded prior to immunization; axillary temperature must be <100.4° F prior to administration of vaccines
- breast milk specimen brought from home if mother is breast feeding or can be collected at the visit if needed
- saliva specimen collected for human blood group antigen testing
- baseline stool specimen (brought from home) before receiving the rotavirus vaccine
- MRI to assess intestinal anatomy and motility
- Ultrasound of abdomen to assess intestinal anatomy
- baseline blood specimen for cytokine testing and maternal antibody testing (IgG)
- The Vaccine Information Sheet (VIS) will be provided to parents/guardians for all vaccines administered.
- The study product administrator, who is licensed to administer the vaccine (e.g., MA, LPN, RN, NP, PA, or MD), will orally administer the rotavirus vaccine as indicated in the randomization code
  - if the infant vomits after receiving the vaccine, in accordance with AAP and ACIP recommendations, the vaccine will not be readministered but time of vaccine administration and vomiting will both be documented.

- For infants randomized to receive other vaccines as part of the study, the study product administrator, who is licensed to administer the vaccine (e.g., MA, LPN, RN, NP, PA, or MD), will administer the vaccines, according to standard practice: Pentacel (DTaP, Hib and IPV combination), Prevnar (PCV13) and HBV, in addition to the rotavirus vaccine. If one of these is not available we will replace it with another equivalent licensed vaccine.
- Memory aide will be reviewed with the parent and the parent will demonstrate ability to take temperature and will be instructed in the collection of reactogenicity, adverse event, fever and concomitant medication administration data for 14 days after vaccination. Parent/guardian will be given a thermometer to use for daily axillary temperature recording
- distribution of supplies for daily stool collection for 14 days with instructions for storage and return
- written instructions (Appendix 6) will be given to the parent with the date, time and location for Visit 2 and need for infant to be NPO (nothing to eat or drink) for 3 hours prior to appointment and to return with daily stools collected
- payment for time and travel will be provided for the study procedures
- study personnel will complete the case report form CRF

### **Reminder Call 2 (Day 3 +2 days)**

- review sample collection instructions
- review MRI/ultrasound prep
- review time, date and location of Visit 2
- review memory aide with parent/guardian

### **Visit 2 (Day 5 +/- 1 day) Post-Vaccination)**

- contact information will be reviewed and updated if necessary
- inclusion and exclusion criteria will be reviewed to determine if child still meets criteria for study (no vaccines given since last visit)
- MRI to assess intestinal anatomy and motility
- ultrasound to assess intestinal anatomy
- submission of daily stool specimens collected
- blood collected for cytokine testing
- memory aide will be reviewed with the parent and the parent will demonstrate ability to take temperature and will be instructed in the collection of reactogenicity, adverse event, fever and concomitant medication administration data for remaining study period
- distribution of supplies for daily stool collection with instructions for storage and return
- payment for time and travel for study procedures
- written instructions (Appendix 7) given to the parent with the date, time and location for Visit 3 and need to return with the collected daily stool specimens and memory aide
- study personnel will complete the Case Report Form (CRF)

### **Reminder Call 3 (Day 11 +/-2 days)**

- review sample collection instructions
- review time, date and location of Visit 3
- review memory aide with parent/guardian

### **Visit 3 (Day 14 +/- 4 days)**

- inclusion and exclusion criteria will be reviewed to determine if child still meets criteria for study (has not had any vaccines since Visit 1)
- submission of all remaining daily stool specimens collected from the infant
- memory aide reviewed with the parent and collected
- payment for time and travel provided for the study procedures
- ensure parent has written plan on how to receive next dose(s) of rotavirus vaccine
- administer parent/guardian satisfaction survey

## 6 STUDY PROCEDURES

### 6.1 Rotavirus Vaccine Randomization and Administration

Infants will be randomized to receive 1) RV1 alone, 2) RV5 alone, 3) RV1 with routine immunizations (DTaP, Hib, PCV13, HBV and IPV or 4) RV5 with routine immunizations prior to Visit 1. They will receive the vaccination(s) after the MRI/Ultrasound is completed at Visit 1.

### 6.2 Breast Milk Collection

Breastfeeding mothers will be asked to collect one breast milk sample to bring to Visit 1 to assess anti-rotavirus IgA antibody ELISA and the secretor status of the mother. Approximately 30 mL of breast milk will be collected at home prior to the study visit. Collection via hand expression or breast pump is acceptable. The breast milk sample should be collected up to 24 hours prior to the clinic visit and stored and transported with an ice pack. Once delivered to the lab, the whey will be separated from the sample, and the breast milk will be frozen at a minimum of -20°C. If unable to collect this sample at Visit 1, it can be collected at Visit 2 or 3.

### 6.3 Saliva Collection

At Visit 1, a saliva specimen will be collected for secretor genotyping by placing sterile cotton-tipped swabs in the infant's mouth, saturating it with saliva and placing it in the Oragene (DNA Genotek) solution. Once mixed with Oragene, the DNA in saliva is stabilized and can be stored indefinitely at room temperature. If unable to collect this sample at Visit 1, it can be collected at Visit 2 or 3.

### 6.4 Stool Collection

Parents will be asked to collect bulk stool samples daily (Day 0-Day 14) to assess rotavirus vaccine virus shedding patterns and stool cytokines. Cytokines will be tested for stools from Visit 1 and Visit 2 (stool collected on the days of the MRI). Vaccine virus shedding (qualitative) will be done on stools from Visit 1 through Visit 2 (stool collected on the day of the MRI). If the Visit 2 stool is positive then the additional stools will be tested to determine the duration of shedding. If the Visit 2 stool is positive, a quantitative PCR will be done on that stool.

Only bulk specimens will be collected. Stools may be collected in diapers and placed in collection bag. Rectal swabs will not be used for stool collection because we have found that they are not a reliable source for detection of rotavirus<sup>109</sup>. Specimens will be refrigerated or frozen within 8 hours of collection. Parents who collect the specimen at home will be asked to freeze the specimen within 8 hours of collection. Once delivered to the lab, bulk stool and extracts will be frozen at a minimum of -20°C.

### 6.5 Blood Collection

Experienced nurses will obtain a venous blood specimen (approximately 2-3 mL of blood) by venipuncture (preferably) or by heel stick if venipuncture unsuccessful at Visit 1 (pre-vaccination and Visit 2 (post-vaccination) for cytokine and biomarker assays.

## 6.6 MRI/Ultrasound Imaging

Using MRI, a non-invasive imaging method that does not use radiation, we will 1) assess intestinal wall thickness, 2) lymph nodes and 3) intestinal motility patterns that may be associated with an increased risk for intussusception. The MRI will be done at Visit 1 just prior to the ultrasound and rotavirus vaccination and similarly at Visit 2 to ascertain for any changes in these three parameters between the two exams. A trained MRI technician will conduct the procedures in accordance with standard practice.

At recruitment/enrollment, parents will be informed that to be included in the study, they will be asked to keep their infant NPO for approximately 3 hours prior to Visit 1 and Visit 2 appointment time. Parents will have a reminder call the day prior to each visit to confirm the study time and remind them to keep the infant NPO. Research staff will meet the parent at the Welcome Center and escort the parent and child to the CCHMC Imaging Research Center (IRC). The infant will not be sedated for the MRI; rather, the infant will be fed and swaddled<sup>110,111</sup>. All images will be obtained on a 1.5T Philips MRI scanner located in the IRC after appropriate metal screening and ear protection (ear plugs and MiniMuffs™ (Natus Medical Incorporated, San Carlos, California)).

The infants will not receive intravenous contrast. However, they will receive enteric contrast by mouth to optimally distend the small bowel and therefore improve image quality. Thirty minutes prior to imaging, each subject will be fed 20 mL/kg of a 3:1 mixture of VoLumen (Bracco Diagnostics) slowly over about 25 minutes. The infants will then be imaged supine in an appropriately sized coil such as a cardiac coil. The approximate time in the MRI to obtain the images needed will be 30-60 minutes. Once started the MRI procedure may be stopped if the baby becomes upset. Once calm, the scan can be repeated as necessary. If a clinically concerning finding is found on the images, the radiologist will notify the PI who will contact the parents and primary care physician.

After completion of the MRI, an ultrasound of the abdomen will be done. The non-invasive ultrasound will be done to 1) assess intestinal wall thickness and 2) lymph nodes that may be associated with an increased risk for intussusception. The ultrasound will be done at Visit 1 just after the MRI and prior to rotavirus vaccination and similarly at Visit 2 to ascertain for any changes in these two parameters between the two exams. A trained ultrasound technician will conduct the procedures in accordance with standard practice. The ultrasound will take up to 30 minutes to complete.

## 6.7 Rotavirus Vaccine Administration

No restrictions are placed on the infant's feeding before or after receipt of rotavirus vaccine (except as needed for the MRI). Documentation of whether the child is being breastfed and/or type of formula will be collected. While the efficacy of the rotavirus vaccine series is similar among breastfed and nonbreastfed infants, breastfeeding could potentially be related to outcome so we will document feeding practice at each visit and daily on the memory aid. Even though rotavirus vaccine can be administered to infants with minor acute illness (e.g., mild gastroenteritis or mild upper-respiratory tract infection, with or without fever), both minor and major acute illnesses will be exclusion criteria, as these conditions could interfere with our outcome measures (gastrointestinal motility and anatomy and cytokine responses).

## 6.8 Reactogenicity Assessment

Reactogenicity will be assessed for both groups for fourteen days after vaccine receipt (Days 0-14) and recorded by the parent/legal guardian on a provided memory aid. The events will include fever, fussiness and gastrointestinal symptoms such as vomiting and diarrhea (number of stools per day and looser than normal). Parents will be instructed not to take temperature after bundling and how to distinguish vomiting from normal spitting. Data will be collected using review of memory cards with the parent. We have found this to be accurate, simple and acceptable to the parents of young infants. The following reactogenicity signs/symptoms will be collected:

- Diarrhea: defined as 3 or more episodes of looser-than-normal stools in 1 day
- Vomiting: 1 episode of vomiting (forceful expulsion of partially digested milk/food) in 1 day
- Fever: axillary temperature  $\geq 99.5$  degrees F (measured once daily or more frequently if perceived fever)
- Hematochezia: maroon stools or any evidence of blood in the stool-black and tarry stools or frank red blood
- Irritability

The following grading system will be used in evaluating the fever and systemic events during Days1-14 following vaccination:

Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever (axillary)	$\geq 99.5^{\circ}\text{F}$ to $\leq 100.4^{\circ}\text{F}$	$>100.4^{\circ}\text{F}$ to $\leq 102.2$	$>102.2^{\circ}\text{F}$ and higher
Diarrhea	3 looser than normal stools/ day	4-5 loose stools/ day	$> 6$ loose stools/ day
Vomiting	1 episode of vomiting/ day	2 episodes of vomiting/ day	$\geq 3$ episodes of vomiting/ day
Irritability	Crying more than usual	Some interference with normal activity	Significant interference with normal activity
Hematochezia	Any stools that are black and tarry; maroon in color; or, frank red blood-each episode will be reported to infant's medical provider immediately		

## 6.9 Receipt of other Recommended Vaccines

At the final visit, Day 14, arrangements will be made to facilitate receipt of initial ACIP recommended vaccines for 2 month olds<sup>108</sup> if they have not already received them prior to Visit 1. See tables below to reference the recommended ages and time intervals between vaccine doses:

Study Visit 1	Study Visit 3	Primary MD Visit (not part of study)
<b>RV Immunization</b>	<b>"2 month" Immunizations</b>	<b>"4 month" Immunizations*</b>
6 weeks	8 weeks	12 weeks
7 weeks	9 weeks	13 weeks
8 weeks	10 weeks	14 weeks
9 weeks	11 weeks	15 weeks
10 weeks	12 weeks	16 weeks
11 weeks	13 weeks	17 weeks
12 weeks	14 weeks	18 weeks

\*minimum of 4 week interval between immunizations

Recommended and minimum ages and intervals between vaccine doses				
Vaccine and dose number	Recommended age for this dose	Minimum age for this dose	Recommended interval to next dose	Minimum interval to next dose
HepB-1	Birth	Birth	1-4 months	4 weeks
HepB-2	1-2 months	4 weeks	2-17 months	8 weeks
DTaP-1	2 months	6 weeks	2 months	4 weeks
DTaP-2	4 months	10 weeks	2 months	4 weeks
Hib-1	2 months	6 weeks	2 months	4 weeks
Hib-2	4 months	10 weeks	2 months	4 weeks
IPV-1	2 months	6 weeks	2 months	4 weeks
IPV-2	4 months	10 weeks	2-14 months	4 weeks
PCV13-1	2 months	6 weeks	8 weeks	4 weeks

PCV13-2	4 months	10 weeks	8 weeks	4 weeks
---------	----------	----------	---------	---------

### Assurance of Rotavirus Vaccine Series Completion with Same Brand

The ACIP recommends that the rotavirus vaccine series be completed with the same product whenever possible (all RV1 or all RV5). We have established procedures to make every effort for children in the study to complete the rotavirus vaccine series with the same brand used for the first dose in the study. We will provide the parent and their infant's health care provider with written and electronic/fax documentation respectively of the dates, types, route, dose and manufacturer of vaccines given and will enter these into the CCHMC electronic medical record (immunization section and encounter note) as well as the Ohio State Registry IMPACT system. We will offer the parents to return to receive the same brand dose if they do not receive their care at CCHMC and their primary care provider does not offer the same brand. For children receiving their care at CCHMC, we will provide their primary care provider with the same brand dose. These additional doses of rotavirus vaccines will not be considered a study procedure.<sup>7</sup>

**6.10 Study Compensation** Parents will be compensated for their time, effort and travel while in the study. The compensation provided will be based on the standard amounts decided on by CCHMC for research subjects who undergo the procedures outlined in this study and the time involved in the clinic visits and collection of samples and reactogenicity data at home. Payments will be given in the form of a debit card (ClinCard). We will load payment onto the ClinCard after each clinic visit that is completed.

- \$150 at 1<sup>st</sup> Clinic Visit
- \$150 at 2<sup>nd</sup> Clinic Visit
- \$100 at 3<sup>rd</sup> Clinic Visit
- \$50 if asked to come in for an unscheduled visit

If parent comes in for a clinic visit and the child is not eligible, they will receive \$25. If unable to complete the study, they will be paid for each study visit completed.

## STUDY VACCINE PRODUCT

### 7.1 Study Product Description, Storage and Stability

See the following links for study product package inserts. Since the focus of the study is rotavirus vaccine, we have provided details about dosage preparation and administration of RV1 and RV5. Other standard vaccines will be administered according to standard practice.

<https://www.gsksource.com/gskprm/htdocs/.../ROTARIX-PI-PIL.PDF>

[https://www.merck.com/product/usa/pi.../r/rotateq/rotateq\\_pi.pdf](https://www.merck.com/product/usa/pi.../r/rotateq/rotateq_pi.pdf)

### 7.2 Dosage, Preparation, and Administration of Study Product

#### RotaTeq®

Each 2-mL dose is supplied in a container consisting of a squeezable plastic, latex-free dosing tube with a twist-off cap, allowing for direct oral administration (RotaTeq® 2009).

To administer the vaccine:

1. Tear open the pouch and remove the dosing tube.
2. Clear the fluid from the dispensing tip by holding the tube vertically and tapping the cap.
3. Open the dosing tube in 2 easy motions:
  - a. Puncture the dispensing tip by screwing the cap *clockwise* until it becomes tight.
  - b. Remove the cap by turning it *counterclockwise*
4. Administer dose by gently squeezing the liquid into the infant's mouth toward the inner cheek until the dosing tube is empty. (A residual drop may remain in the tip of the tube.)

If for any reason an incomplete dose is administered (e.g., infant spits or regurgitates the vaccine), a replacement dose will not be administered. The infant should continue to receive any remaining doses in the recommended series. There are no restrictions on the infant's consumption of food or liquid, including breast milk, either before or after vaccination with RotaTeq® (RotaTeq® 2009).

### **Rotarix®**

Each 1-mL dose consists of a vial of lyophilized vaccine to be reconstituted with a liquid diluent in a prefilled oral applicator, allowing for direct oral administration (Rotarix® 2009).

To administer the vaccine:

1. Remove the vial cap and push the transfer adapter onto the vial (lyophilized vaccine).
2. Shake the diluent in the oral applicator (white, turbid suspension). Connect the oral applicator to the transfer adapter.
3. Push the plunger of the oral applicator to transfer the diluent into the vial. The suspension will appear white and turbid.
4. Withdraw the vaccine into oral applicator.
5. Twist and remove the oral applicator.
6. Administer Rotarix® orally.

In the event that the infant spits out or regurgitates most of the vaccine dose, a replacement dose will not be administered. There are no restrictions on the infant's food or liquid consumption, including breast milk, either before or after vaccination with Rotarix® (Rotarix® 2009).

## **7.3 Assessment of Subject Compliance With Study Product**

The study vaccines, RotaTeq® and Rotarix®, will be administered by the study product administrator, who is licensed to administer the vaccine (e.g., LPN [licensed practical nurse], RN [registered nurse], NP [nurse practitioner], PA [physician assistant], or medical doctor [MD]), in the clinic. Study personnel will record the administration time on the CRF and include whether it was a complete dose or a partial dose due to regurgitation.

## **8 LABORATORY EVALUATIONS AND METHODS**

### **8.1 Blood-Cytokine and Inflammatory Responses and Antibody Testing (IgG and IgA)**

Blood specimens from Visit 1 will be used for assessment of anti-rotavirus IgG antibody (maternal) and anti-rotavirus IgA antibody to rotavirus and measurement of serum cytokine levels and on the day of the second MRI (Visit 2) for measurement of serum cytokine levels and anti-rotavirus IgA. Blood for the antibody assays will be drawn into a serum separator tube by venipuncture (2-3 mL). After collection, the tubes will be kept at room temperature until the blood has clotted. The tubes will be centrifuged at room temperature at approximately 1000-1300g (2,400-2,700 RPM) for 10-15 minutes to ensure the sample is sufficiently separated. The serum will be removed using sterile glass or plastic pipets and aliquoted into

appropriately labeled cryovials (approximately 3 x 0.5 mL). The serum aliquots will be stored at -20 °C or colder until testing. The cytokines of interest will be assayed in a multiplex Luminex assay using the serum samples (Nefertiti C. DuPont et al 2005). The following cytokines will be measured: IL-2, IL-6, IL-7, IL-8, IL-15, INF- $\gamma$  and TNF- $\alpha$ . In addition, a C-reactive protein (pre- and post-vaccination) will also be done. Additional cytokines or biomarkers may be done should funding become available.

Maternal antibodies to rotavirus will be measured using an anti-rotavirus IgG ELISA assay developed and qualified in the Laboratory for Specialized Clinical Studies (LSCS). The antibody enzyme immunoassay will be used to detect and quantify rotavirus IgG antibody concentrations in human serum<sup>112</sup>. Each assay will include a serially diluted 8-point standard curve generated by a reference standard consisting of a human serum pool arbitrarily assigned as having 1000 Units/mL. The curve will be modeled using the 4-parameter logistic fit regression function. Samples will be tested at 4 different concentrations. Dilution-corrected titers will be reported for the sample using the quantifiable range of the standard curve. Serum anti-rotavirus IgA will be determined using the same assay but using an anti-human IgA conjugate.

## 8.2 Stool-RV1 and RV5 Shedding and Cytokine Responses

Stool specimens will be utilized for both assessment of rotavirus vaccine shedding patterns and measurement of stool cytokines. For stool cytokine testing, stool specimens from Visit 1 and Visit 2 will be used. For assessment of rotavirus vaccine virus shedding patterns, all collected stools will be analyzed. Cytokine testing will be done on Visit 1 and Visit 2 specimens only. All stool samples will be kept frozen at -20 °C or colder until testing. For determination of rotavirus shedding and cytokine responses, stools will be thawed and nucleic acids will be extracted. For cytokine determination, PCR will be performed using Taqman gene expression assays from Life Technology for each of the cytokines of interest. The following cytokines will be done: IL-2, IL-6, IL-7, IL-8, IL-15, INF- $\gamma$  and TNF- $\alpha$ . Additional biomarkers or cytokines may be done should funding become available.

## 8.3 Saliva-DNA Genotyping for Secretor Status

Extracted DNA from the saliva will be tested to determine the child's secretor status by genotyping. Assay plates can accommodate up to 96 subjects each. We anticipate a yield of 25-30 ug of DNA per child using Oragene solution, which is more than sufficient for the purposes of this analysis. Secretor genotype will be determined at CCHMC through testing for known single nucleotide polymorphisms of the secretor gene (*FUT2*), 428G>A<sup>114</sup>.

## 8.4 Breast Milk-IgA Antibody and Secretor Status of the Mother

Breast milk samples will be used to assess anti-rotavirus IgA antibody and secretor status of the mother. The anti-rotavirus IgA assay is a validated, standard ELISA assay that has been used for both RV1 and RV5 clinical trials by our lab (LSCS)<sup>112</sup>. We will also evaluate the mother's secretor status from breast milk using the genotyping method described above<sup>114</sup>.

## 8.5 Future Testing

The informed consent form includes information about specimen storage and future use of the stored specimens. Samples will be retained for possible future testing with a linkage to identifiers. The samples will be stored locally at the site and local site investigators will have access to the samples. However, before the use of these samples by any investigator(s), request for use of these samples will require project approval by the CDC. The stool, saliva, serum, breast milk and DNA samples will be banked for future testing beyond the defined scope of this project; the consent forms will specifically include consent for banking samples for future use. Potential testing that could be done if funding were available includes (but is not limited to) testing of additional biomarkers or cytokines in the stool and blood as well as examining changes to the stool microbiome pre- and post-vaccination.

## 9 IMAGING METHODS

### 9.1 MR Imaging

#### 9.12 Methods

Anatomic imaging will be acquired with axial and coronal T2-weighted sequences. Motility will be evaluated using dynamic high spatial resolution 2D balanced steady state free precession (bSSFP) imaging. Coronal and axial balanced steady state images will be acquired during free breathing with an acquired temporal resolution of approximately 0.6s/image over 85 seconds. Diffusion weighted imaging will also be acquired using multiple b-values in order to evaluate any changes in diffusion restriction within the bowel wall or mesenteric lymph nodes between the two MRI exams.

#### 9.13 Image Interpretation

**Anatomic imaging** will be assessed by a pediatric radiologist with expertise in abdominal imaging (Dr. Towbin). The well-distended distal/terminal ileum bowel wall thickness will be measured in 3 locations, and a mean wall thickness value will be obtained on each exam. The largest visible right lower quadrant mesenteric lymph nodes will be measured in short axis on each exam. Any secondary signs of inflammation (perienteric fat stranding, engorged vasa recta, free fluid) will be documented on each exam. After completion of the primary anatomic review by Dr. Towbin, a second pediatric radiologist with expertise in abdominal imaging will perform the same review on a random subset of subjects in order to determine inter-reader reliability. The second reviewer will be blinded to the initial assessment as well as to the time point of imaging.

**Motility imaging** will be assessed as follows: First, a quantitative assessment will be performed. A well-distended segment of distal/terminal ileum will be chosen by the first reviewer (Dr. Towbin). Novel, semi-automated software, will be utilized to assess the coronal cine sequences in this investigation. No pre-market submission to the FDA has been made for the software nor are there any current plans to do so in the future. The semi-automated method computes a cross sectional area across time and the creates a best fit curve to determine the frequency or frequencies of contraction. After reviewer 1 identifies the bowel segment of interest, a post processing assistant will draw a region of interest on the bowel lumen on every image of the sequence. The application then creates a map of luminal cross-sectional area over time. The area versus time waveforms can be submitted to secondary analysis from which contraction frequency and other quantitative metrics of interest can be extracted.

Next a qualitative assessment will be performed. The research assistant will create a movie file for each subject, randomize the timepoints, and then insert the movie file into a PowerPoint presentation. The two reviewers, along with a third reviewer (a pediatric gastroenterologist with expertise in gut motility) will review the images to determine which movie has faster motility, and for each movie, if the motility is organized or disorganized. Finally, each of the three reviewers will assess the focal segment of the distal/terminal ileum in the same manner.

### 9.2 Ultrasound Imaging

#### 9.21 Methods

Anatomic imaging of the terminal ileum will be done by ultrasound by an ultrasound technologist.

#### 9.22 Image Interpretation

**Anatomic imaging** will be assessed by a pediatric radiologist with expertise in abdominal imaging (Dr. Towbin). The well-distended distal/terminal ileum bowel and cecum wall thickness will be measured in 3

locations, and a mean wall thickness value will be obtained on each exam. The largest right lower quadrant mesenteric lymph nodes will be measured in short axis on each exam. Any secondary signs of inflammation (perienteric fat stranding, engorged vasa recta, free fluid) will be documented on each exam.

After completion of the primary anatomic review, a second pediatric radiologist with expertise in abdominal imaging will perform the same review on a random subset of subjects in order to determine inter-reader reliability. The second reviewer will be blinded to the initial assessment as well as to the time point of imaging.

## **10 RANDOMIZATION, DATA MANAGEMENT, STATISTICAL PLAN**

### **10.1 Randomization Plan**

Upon meeting the inclusion and exclusion criteria, subjects will be randomized to one of the four vaccine groups: 1) RV1 alone, 2) RV5 alone, 3) RV1 with routine immunizations (DTaP, Hib, PCV13, HBV and IPV) or 4) RV5 with routine immunizations. This will be a simple randomization using SAS<sup>®</sup> PROC PLAN, which uses a random number generator to make assignments. Study statisticians will generate the assignments in advance of any enrollment. After obtaining informed consent, an unblinded research personnel will contact the statistician to obtain a specific subject's assignment. Parents and all study radiologists will be blinded as to which arm the child is randomized to until completion of the study. Parents will learn which rotavirus the child received at the completion of Visit 3. Parents will be informed about the specific other vaccines used in the study. If there is an urgent medical need to unblind during the study, then this can be done by calling the on call study staff.

### **10.2 Data Management Plan**

The Data Management Center (DMC) of the Division of Biostatistics and Epidemiology (DBE) will provide full data management support for this study. In collaboration with the study team and the CDC, case report forms will be developed. The DMC will develop the data capture system using a web-based data collection system, REDCap, as the primary source of data entry and storage. REDCap is a software toolset and workflow methodology for electronic collection and management of research and clinical trial data developed by Vanderbilt University, with collaboration from a consortium of institutional partners including the University of Cincinnati Academic Health Center. REDCap provides HIPAA-compliant and 21 CFR Part 11-ready audit trails for tracking page views, data manipulation and export procedures. A copy of coded data with the unique study ID number but without personal identifiers will be sent to CDC upon request; patient identifiers will be kept at CCHMC; no identifying information will be included on any data sent to the CDC.

REDCap is hosted on a network specially designed to support the rigorous security and compliance requirements of basic, clinical and translational research projects. Administered by the Division of Biomedical Informatics (BMI), this network features multiple firewalls as well as a central facility for managing hosted systems and users. The result is another layer of access control and audit capability on top of what REDCap already provides.

### **10.3 Data Analysis Plan**

Primary objectives - Statistical Analysis.

#### **a) Descriptive statistics:**

Descriptive statistics will be calculated for all demographic, anthropometric, reactogenicity, serum, image and fecal variables. For continuous variables, five-number (mean, SD, minimum, median, maximum) summaries will be produced. For class (categorical) variables, frequencies and percentages will be reported. Descriptive statistics will be calculated for the entire study population, as well as for each vaccine group at each time point. Investigators will be advised by study statisticians about potential outliers, or data entry issues, based on these summary statistics. To determine whether continuous serum, image and fecal outcomes meet the assumption of a Gaussian (normal) distribution, the Shapiro-Wilk test will be used. Where a significant departure from normality exists, an appropriate transformation of the data will be employed prior to inferential analyses. If transformations are not successful, non-parametric analyses will be used.

**b) Inferential statistics**

The primary analysis for this study is the comparison of anatomical outcome as measured by MRI between (RV1, RV5 with and without other vaccines: 4 groups) and within (pre and post) study groups. The primary anatomical outcome will be the terminal ileum thickness measured in mm. The change in this outcome before and after vaccination will be compared for each study group (within group test) using a paired t-test, while the between group difference at pre, post and on the difference between pre and post (change score) will be initially analyzed using general linear model (GLM) where the ileum thickness (at pre, post and change score) is modeled as a function of group (4-groups). Depending on the result of the F-test from the GLM analysis, this will be followed by pairwise comparison between groups (6-comparisons). This will be examined using a two sample t-test. If the difference between groups with and without other vaccines for a given vaccine (RV1 or RV5) is not significant we might combine the categories and conduct a comparison test for the outcome between the two vaccine types. If the primary outcome is not normal before or after transformation we will use the non-parametric Kruskal-Wallis test to compare the groups. Although the primary between group comparisons is based on the pre-post change score, we are conducting additional analysis based on the pre and post data to get more insight about the distribution of the primary outcome variable.

We will also examine the distribution of socio-demographic and other potential covariates among the groups using a GLM analysis. If some covariates are differentially distributed among the groups, those covariates will be controlled for in the primary analysis described above. Potential modifiers such as breast milk or secretor status will be examined by modeling the primary outcome as a function of group, potential modifier and the interaction between group and modifier. In this case the parameter of interest is the coefficient of the interaction term, however it is possible that the interaction test might not be sensitive due to sample size. Regardless we will examine and report the observed change between groups for each potential modifier as a subgroup. For example we will report difference between groups in the primary outcome for secretors and non-secretors separately.

To assess how vaccine shedding is related with the primary outcome by looking at the correlation between the imaging outcome and the fecal shedding for each group (using sample obtained close in time with the imaging). This will be examined using Pearson or Spearman correlation coefficients based on the normality of the data. The resulting correlation coefficient from each group can be compared with each other (pairwise) using Fisher's transformation and compare the resulting z-scores. If the correlation are not different we may then combine the groups and run the correlation across group to get an insight on the relationship between shedding and imaging parameter.

For all the other imaging parameters (cecum thickness, wave pattern and amplitude) similar analysis described for the primary outcome (ileum thickness) will be conducted. For Lymph node presence a logistic regression instead of GLM, and for the lymph node number, and wave frequency a Poisson regression will be used.

For the cytokines outcome (IL2, IL6, IL7, IL8, IL15, TNF) similar analysis described for the primary imaging outcome including examination of modifying variables and potential subsequent stratified analysis will be conducted.

### **Power**

Since sample-sizes were predetermined and since this is a pilot study, no power calculations were conducted. Effect sizes will be reported from the results, to aid in the development of future studies.

### **Feasibility Analysis**

The proportion of children meeting the outlined feasibility benchmarks (Appendix 3) will be determined.

## **11 ASSESSMENT OF SAFETY**

### **11.1 Adverse Events**

RV1 and RV5 are recommended routinely for infants in the US and is consistent with normal standard of care. The risks associated with vaccination are not greater than those encountered in the performance of routine medical care.

#### **RV1 Adverse Events**

Anticipated common ( $\geq 5\%$ ) solicited adverse events related to the administration of the RV1 rotavirus vaccine include (as per Package Insert) fussiness/irritability, cough/runny nose, fever, loss of appetite, and vomiting<sup>115</sup>. There is also a small risk for intussusception as shown in recent studies<sup>31,32</sup>.

#### **RV5 Adverse Events**

Anticipated common adverse events related to the administration of the RV5 rotavirus vaccine include (as per Package Insert) include diarrhea, vomiting, irritability, otitis media, nasopharyngitis, and bronchospasm<sup>116</sup>. There is also a small risk for intussusception as shown in recent studies<sup>31,32</sup>.

Other vaccines – All vaccines used will be licensed and recommended for routine use by the ACIP.

Possible risks are explained in the Vaccine Information Statements.

(<http://www.cdc.gov/vaccines/hcp/vis/>).

Events meeting Vaccine Adverse Event Reporting System (VAERS) reporting requirements will be reported as indicated below.

As previously mentioned, there are minimal risks associated with venipuncture for obtaining research related labs. Foreseeable adverse events associated with venipuncture area: mild, temporary discomfort at the venipuncture site, bruising, and phlebitis.

#### **MRI /Ultrasound Adverse Events**

Subjects may have some discomfort due to the noise from the MRI scanner. Earplugs and headphones and/or MiniMuffs will be used to decrease sound levels to  $<+99\text{dB(dBA)}$ . Infants may

become fussy/irritable from being NPO 4 hours prior to the MRI (NPO three hours prior to visit and one hour for intake and preparation for the MRI).

Low density barium sulfate (VoLumen) will be used as an oral contrast agent for the MRI enterography. Barium sulfate is an inert substance and has an excellent safety profile with well over 60 years of use in patients. Barium is biologically inert and is not absorbed or metabolized by the body and is excreted unchanged. The risk of an allergic reaction is 1 in 1 million and the risk of a fatal reaction is 1 in 10 million (**Appendix 3**-Package Insert). There are no known risks from ultrasound.

## 11.2 Safety Reporting: For Adverse Events

The investigator must report the following events to VAERS with a notification to the CDC Project Manager by e-mail, including a copy of the report (VAERS form). In addition, these events will be reported on the VAERS/AESI case report form:

- Any adverse event listed by the vaccine manufacturer as a contraindication to further doses of the vaccine such as a severe allergic reaction
- Any adverse event listed in the VAERS Table of Reportable Events Following Vaccination found using the following link:  
[https://vaers.hhs.gov/.../VAERS\\_Table\\_of\\_Reportable\\_Events\\_Following\\_Vaccination.pdf](https://vaers.hhs.gov/.../VAERS_Table_of_Reportable_Events_Following_Vaccination.pdf)
- In the judgment of the PI, any unexpected reaction to the vaccine will report to VAERS as well as the CDC Project Manager.  
The PI and the CDC project manager will report adverse events to their respective IRBs in accordance with institutional policies

## 12 POTENTIAL RISKS AND BENEFITS

### Potential Benefits

There is not likely to be a direct benefit to the infants participating in this study. The information learned from this research study may benefit other children receiving rotavirus vaccines and increase our knowledge about why some babies get intussusception.

### Potential Risks, Discomforts, Inconveniences and Precautions

This study poses no greater than minimal risk to the subjects. As defined in 45 US Code of Federal Regulations (CFR) 46.102 (i), “Minimal risk means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.” The risks associated with rotavirus vaccination and other recommended vaccines are the same as those encountered in the performance of routine medical care and are described in section 11.1.

Subjects will have their saliva specimen collected using a sponge provided by an Oragene collection kit. The sponge may cause temporary dryness of the mouth that should not last longer than 5-10 minutes. Subject will also be asked to provide stool specimens by collection of soiled diapers.

There are minimal risks associated with venipuncture for obtaining research related labs. Foreseeable adverse events associated with venipuncture area: mild, temporary discomfort at the venipuncture site, bruising, and phlebitis.

The MRI uses a large magnet and a computer to take detailed pictures of the intestine. There is no radiation in an MRI scan. There are no known risks to an intestinal MRI in babies when appropriate procedures are used. MRI is routinely used in neonates and infants for certain conditions such as brain

anomalies and gastroschisis. Subjects may have some discomfort due to the noise from the MRI scanner. Earplugs and headphones and/or MiniMuffs will be used to decrease sound levels to <+99dB(dBA). Infants may become fussy/irritable from being NPO prior to the MRI. There is a very small chance of identifying clinically significant findings from MRI/Ultrasound imaging. If this occurs, the parents and the primary care physician will be notified.

Low density barium sulfate (VoLumen) will be used as an oral contrast agent for the MR enterography. Barium sulfate is an inert substance and has an excellent safety profile with well over 60 years of use in patients. Barium is biologically inert and is not absorbed or metabolized by the body and is excreted unchanged. The risk of an allergic reaction is 1 in 1 million and the risk of a fatal reaction is 1 in 10 million (Appendix package insert). There are no known risks for ultrasound imaging.

The primary goals of this study are to evaluate potential mechanisms for intussusception by randomizing children to receive either of the two licensed rotavirus vaccines. No serious (or severe) adverse events are anticipated using these licensed products but we will collect adverse event data as outline in section 11.

There is a potential risk of loss of confidentiality of data.

## APPENDICES

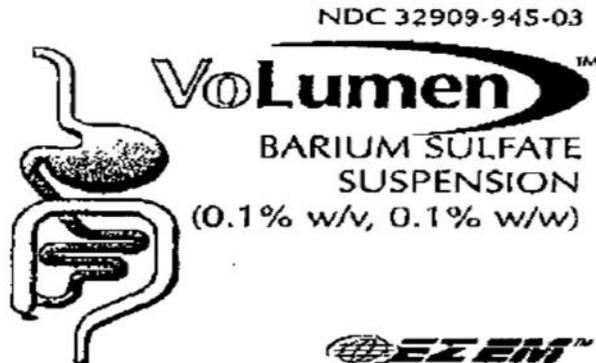
### APPENDIX 1. STUDY VISITS AND PROCEDURES

		Study Days															
	Recruitment/ Enrollment	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Visit	Recruitment		1					2									3
Age	<11wks		6 wks to- 12 wks, 6 days														
Informed Consent	X																
Demographics	X		X					X									X
Medical History	X		X					X									X
Inclusion/Exclusion Criteria	X	X	X					X									X
Explain & Distribute Memory Aide			X					X									
Axillary Temperature			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Reactogenicity			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Stool Instructions	X	X						X						X			
Supplies Distributed	X							X									
Review MRI Prep	X	X				X											
Schedule Appointment	X		X					X									
Reminder Call		X				X								X			
Infant Stool Collection			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomized	X		X														
MRI			X					X									
Ultrasound			X					X									
Stool Submission			X					X									X
Memory Aid Review			X					X						X			X
Blood Obtained			X					X									
Breast Milk Sample			X														
Saliva Sample			X														
Vaccination			<b>One of Four Groups</b>														
Case Report Form	X		X					X						X			X
Vaccination Record			X														X
Parent Satisfaction																	X

**APPENDIX 2. FEASIBILITY BENCHMARKS**

<b>Item</b>	<b>Goal</b>
Willingness to enroll	10% of subjects who are screened and found eligible will enroll in the study
Reason for non-enrollment	90% of those not enrolled will be asked for reason for refusal
Temperature monitoring	At least 70% of parents will check and record their baby's axillary temperature on at least 70% of the study days
Reactogenicity	At least 70% of the parents will complete the reactogenicity assessment for 70% of the days
Study visits	At least 70% of subjects will complete all study visits on time (per protocol) and remain in the study until completion
Blood draws	At least 70% of the blood draws will be successfully completed per protocol
MRI imaging	At least 70% of subjects will successfully complete both MRIs
Stool collection	At least 70% of parents will collect 70% of the 14 stool samples
Breast milk collection	At least 50% of breastfeeding moms will collect a breast milk sample
Saliva collection	At least 70% of subjects will successfully have saliva obtained
Receipt of 2 month vaccinations	90% of infants will receive remaining immunizations within ACIP guidelines
Remaining rotavirus doses	75% of infants will receive the second RV immunization with the same RV vaccine given as the first dose

APPENDIX 3.  
VoLumen Package Insert



**DESCRIPTION:** VoLumen is a barium sulfate suspension 0.1% w/v, 0.1% w/w for oral administration. Each 100 mL contains 0.1 g barium sulfate. Barium sulfate, due to its high molecular density is opaque to x-rays and therefore, acts as a positive contrast agent for radiographic studies. The active ingredient is barium sulfate and its structural formula is BaSO<sub>4</sub>. Barium sulfate occurs as a fine, white, odorless, tasteless, bulky powder which is free from grittiness. Its aqueous suspensions are neutral to litmus. It is practically insoluble in water, solutions of acids and alkalis, and organic solvents. **Inactive Ingredients:** citric acid, natural gum, benzoic acid, sodium citrate, natural and artificial blueberry flavor, potassium sorbate, purified water, saccharin sodium, simethicone emulsion, sodium benzoate, and sorbitol.

**CLINICAL PHARMACOLOGY:** Barium sulfate, due to its high molecular density is opaque to x-rays and, therefore, acts as a positive contrast agent for radiographic studies. Barium sulfate is biologically inert and, therefore, is not absorbed or metabolized by the body, and is eliminated from the GI tract unchanged.

**INDICATIONS AND USAGE:** For use in Computed Tomography to opacify the GI tract.

**CONTRAINDICATIONS:** This product should not be used in patients with known or suspected gastrointestinal perforation or hypersensitivity to barium sulfate or any component of this barium sulfate formulation.

**WARNINGS:** Rarely, severe allergic reactions of an anaphylactoid nature, have been reported following administration of barium sulfate contrast agents. Appropriately trained personnel and facilities should be available for emergency treatment of severe reactions and should remain available for at least 30 to 60 minutes following administration, since delayed reactions can occur.

**PRECAUTIONS: General:** Diagnostic procedures which involve the use of radiopaque contrast agents should be carried out under the direction of personnel with the requisite training and with a thorough knowledge of the particular procedure to be performed. A history of bronchial asthma, atopy, as evidenced by hay fever and eczema, or a previous reaction to a contrast agent, warrant special attention. Caution should be exercised with the use of radiopaque media in severely debilitated patients and in those with marked hypertension or advanced cardiac disease. Ingestion of barium is not recommended in patients with a history of food aspiration. If barium studies are required in these patients or in patients in whom integrity of the swallowing mechanism is unknown, proceed with caution. If barium is aspirated into the larynx, further administration should be immediately discontinued.

**Information for Patients:** Before administration of this product, patients receiving barium sulfate diagnostic agents should be instructed to:

1. Inform their physician if they are pregnant.
2. Inform their physician if they are allergic to any drugs or food, or if they have had any prior reactions to barium sulfate products or other contrast agents used in x-ray procedures (see **PRECAUTIONS-General**).
3. Inform their physician about any other medications they are currently taking.
4. Seek immediate medical attention if they experience an allergic reaction after using this product.

**Drug Interactions:** The presence of barium sulfate formulations in the GI tract may alter the absorption of therapeutic agents taken concomitantly. In order to minimize any potential change in absorption, the separate administration of barium sulfate from that of other agents should be considered.

**Usage in Pregnancy:** Radiation is known to cause harm to the unborn fetus exposed in utero. Therefore, radiographic procedures should only be used when, in the judgement of the physician, its use is deemed essential to the welfare of the pregnant patient.

**Nursing Mothers:** Barium sulfate products may be used during lactation.



TX1272-2

**ADVERSE REACTIONS:** Adverse reactions, such as nausea, vomiting, diarrhea and abdominal cramping, accompanying the use of barium sulfate formulations are infrequent and usually mild. Severe reactions (approximately 1 in 1,000,000) and fatalities (approximately 1 in 10,000,000) have occurred. Procedural complications are rare, but may include aspiration pneumonitis, granuloma formation, intravasation, embolization and peritonitis following intestinal perforation, vasovagal and syncopal episodes, and fatalities.

**ALLERGIC REACTIONS:** Due to the increased likelihood of allergic reactions in atopic patients, it is important that a complete history of known and suspected allergies as well as allergic-like symptoms, e.g., rhinitis, bronchial asthma, eczema and urticaria, be obtained prior to any medical procedure utilizing these products. A mild allergic reaction would most likely include generalized pruritus, erythema or urticaria (approximately 1 in 250,000). Such reactions will generally respond to an antihistamine such as 50 mg of diphenhydramine or its equivalent. In the rarer, more serious reactions (approximately 1 in 1,000,000) laryngeal edema, bronchospasm or hypotension could develop. Severe reactions which may require emergency measures are often characterized by peripheral vasodilation, hypotension, reflex tachycardia, dyspnea, agitation, confusion and cyanosis, progressing to unconsciousness. Treatment should be initiated immediately with 0.3 to 0.5 cc of 1:1000 epinephrine subcutaneously. If bronchospasm predominates, 0.25 to 0.50 grams of intravenous aminophylline should be given slowly. Appropriate vasopressors might be required. Adrenocorticosteroids, even if given intravenously, exert no significant effect on the acute allergic reactions for a few hours. The administration of these agents should not be regarded as emergency measures for the treatment of allergic reactions. Apprehensive patients may develop weakness, pallor, tinnitus, diaphoresis and bradycardia following the administration of any diagnostic agent. Such reactions are usually non-allergic in nature and are best treated by having the patient lie flat for an additional 10 to 30 minutes under observation.

**OVERDOSAGE:** On rare occasions following repeated administration, severe stomach cramps, nausea, vomiting, diarrhea or constipation may occur. These indicated responses can be present in both fluoroscopic and CT procedures. These are transitory in nature and are not considered serious. Symptoms may be treated according to currently accepted standards of medical care.

**DOSAGE AND ADMINISTRATION:** The volume and concentration of the CT barium sulfate suspension to be administered will depend on the degree and extent of contrast required in the area(s) under examination and on the equipment and technique employed.

**For Oral Administration: GI Tract Marking:** The patient should begin drinking VoLumen approximately 20 - 30 minutes prior to the scheduled procedure. It is recommended that the patient consume multiple bottles, about 900 mL to 1,350 mL total volume prior to the exam or use as directed by physician. For improved gastric marking have patient consume the final 200 mL immediately prior to scan. Bowel marking is consistent due to the uniformity of the 0.1% concentration of BaSO<sub>4</sub>. Bowel lumen marking can be improved by increasing the volume of VoLumen consumed (see below). Other dosing regimens may be followed as applicable. In patients where marking is problematic, such as obesity and delayed transit, improved marking may be possible by increasing the total volume of VoLumen administered up to 1,800 mL or four (4) bottles.

**STORAGE:** Store product to protect from freezing and excessive heat (above 40°C).

**HOW SUPPLIED:** VoLumen™ is supplied in the following quantity: 450 mL bottles, Cat. No. 9450, NDC 32909-945-03

**Rx Only (USA)**

**SHAKE WELL PRIOR TO USE**

Patent Pending



Manufactured by  
E-Z-EM Canada Inc.,  
a subsidiary of E-Z-EM, Inc.  
Lake Success, NY 11042

Tel: 1-516-333-8230 1-800 544-4624

rev. 05/06 TX1272-2 ©2006 E-Z-EM, Inc.



#### APPENDIX 4. REFERENCES

1. Prevention of rotavirus disease: guidelines for use of rotavirus vaccine. American Academy of Pediatrics. *Pediatrics* 1998;102:1483-91.
2. Cortese MM, Parashar UD, Centers for Disease C, Prevention. Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2009;58:1-25.
3. Charles MD, Holman RC, Curns AT, Parashar UD, Glass RI, Bresee JS. Hospitalizations associated with rotavirus gastroenteritis in the United States, 1993-2002. *Pediatr Infect Dis J* 2006;25:489-93.
4. Ho MS, Glass RI, Pinsky PF, Anderson LJ. Rotavirus as a cause of diarrheal morbidity and mortality in the United States. *J Infect Dis* 1988;158:1112-6.
5. Kilgore PE, Holman RC, Clarke MJ, Glass RI. Trends of diarrheal disease--associated mortality in US children, 1968 through 1991. *JAMA* 1995;274:1143-8.
6. Malek MA, Curns AT, Holman RC, et al. Diarrhea- and rotavirus-associated hospitalizations among children less than 5 years of age: United States, 1997 and 2000. *Pediatrics* 2006;117:1887-92.
7. Parashar UD, Burton A, Lanata C, et al. Global mortality associated with rotavirus disease among children in 2004. *J Infect Dis* 2009;200 Suppl 1:S9-S15.
8. Parashar UD, Gibson CJ, Bresee JS, Glass RI. Rotavirus and severe childhood diarrhea. *Emerg Infect Dis* 2006;12:304-6.
9. Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Dis* 2003;9:565-72.
10. Tate JE, Burton AH, Boschi-Pinto C, et al. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis* 2012;12:136-41.
11. Tate JE, Patel MM, Cortese MM, et al. Remaining issues and challenges for rotavirus vaccine in preventing global childhood diarrheal morbidity and mortality. *Expert Rev Vaccines* 2012;11:211-20.
12. FDA licenses rotavirus vaccine for infants. *Am J Health Syst Pharm* 1998;55:2226, 8.
13. Intussusception among recipients of rotavirus vaccine--US, 1998-1999. *Ann Pharmacother* 1999;33:1020-1.
14. Centers for Disease C, Prevention. Withdrawal of rotavirus vaccine recommendation. *MMWR Morb Mortal Wkly Rep* 1999;48:1007.
15. Rotavirus vaccine for the prevention of rotavirus gastroenteritis among children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1999;48:1-20.
16. Centers for Disease C, Prevention. Intussusception among recipients of rotavirus vaccine--United States, 1998-1999. *MMWR Morb Mortal Wkly Rep* 1999;48:577-81.
17. Abramson JS, Baker CJ, Fisher MC, et al. Possible association of intussusception with rotavirus vaccination. American Academy of Pediatrics. Committee on Infectious Diseases. *Pediatrics* 1999;104:575.
18. Murphy TV, Gargiullo PM, Massoudi MS, et al. Intussusception among infants given an oral rotavirus vaccine. *N Engl J Med* 2001;344:564-72.
19. Murphy BR, Morens DM, Simonsen L, Chanock RM, La Montagne JR, Kapikian AZ. Reappraisal of the association of intussusception with the licensed live rotavirus vaccine challenges initial conclusions. *J Infect Dis* 2003;187:1301-8.
20. Simonsen L, Morens D, Elixhauser A, Gerber M, Van Raden M, Blackwelder W. Effect of rotavirus vaccination programme on trends in admission of infants to hospital for intussusception. *Lancet* 2001;358:1224-9.

21. Simonsen L, Viboud C, Elixhauser A, Taylor RJ, Kapikian AZ. More on RotaShield and intussusception: the role of age at the time of vaccination. *J Infect Dis* 2005;192 Suppl 1:S36-43.
22. Rothman KJ, Young-Xu Y, Arellano F. Age dependence of the relation between reassortant rotavirus vaccine (RotaShield) and intussusception. *J Infect Dis* 2006;193:898; author reply -9.
23. Rennels MB, Parashar UD, Holman RC, Le CT, Chang HG, Glass RI. Lack of an apparent association between intussusception and wild or vaccine rotavirus infection. *Pediatr Infect Dis J* 1998;17:924-5.
24. Lynch M, Shieh WJ, Bresee JS, et al. Intussusception after administration of the rhesus tetravalent rotavirus vaccine (Rotashield): the search for a pathogenic mechanism. *Pediatrics* 2006;117:e827-32.
25. Vesikari T, Matson DO, Dennehy P, et al. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *N Engl J Med* 2006;354:23-33.
26. Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *N Engl J Med* 2006;354:11-22.
27. Parashar UD, Alexander JP, Glass RI, Advisory Committee on Immunization Practices CfDC, Prevention. Prevention of rotavirus gastroenteritis among infants and children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2006;55:1-13.
28. World Health Organization. Statement on Rotarix and Rotateq vaccines and intussusception. 2010. 2010. (Accessed July 2, 2014, at [http://www.who.int/vaccine\\_safety/committee/topics/rotavirus/rotarix\\_and\\_rotateq/intussusception\\_sep2010/en/](http://www.who.int/vaccine_safety/committee/topics/rotavirus/rotarix_and_rotateq/intussusception_sep2010/en/).)
29. Carlin JB, Macartney KK, Lee KJ, et al. Intussusception risk and disease prevention associated with rotavirus vaccines in Australia's National Immunization Program. *Clin Infect Dis* 2013;57:1427-34.
30. Patel MM, Lopez-Collada VR, Bulhoes MM, et al. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. *N Engl J Med* 2011;364:2283-92.
31. Weintraub ES, Baggs J, Duffy J, et al. Risk of intussusception after monovalent rotavirus vaccination. *N Engl J Med* 2014;370:513-9.
32. Yih WK, Lieu TA, Kulldorff M, et al. Intussusception risk after rotavirus vaccination in U.S. infants. *N Engl J Med* 2014;370:503-12.
33. Haber P, Patel M, Izurieta HS, et al. Postlicensure monitoring of intussusception after RotaTeq vaccination in the United States, February 1, 2006, to September 25, 2007. *Pediatrics* 2008;121:1206-12.
34. Shui IM, Baggs J, Patel M, et al. Risk of intussusception following administration of a pentavalent rotavirus vaccine in US infants. *JAMA* 2012;307:598-604.
35. Belongia EA, Irving SA, Shui IM, et al. Real-time surveillance to assess risk of intussusception and other adverse events after pentavalent, bovine-derived rotavirus vaccine. *Pediatr Infect Dis J* 2010;29:1-5.
36. Loughlin J, Mast TC, Doherty MC, Wang FT, Wong J, Seeger JD. Postmarketing evaluation of the short-term safety of the pentavalent rotavirus vaccine. *Pediatr Infect Dis J* 2012;31:292-6.
37. Moon SS, Wang Y, Shane AL, et al. Inhibitory effect of breast milk on infectivity of live oral rotavirus vaccines. *Pediatr Infect Dis J* 2010;29:919-23.
38. Van Trang N, Vu HT, Le NT, Huang P, Jiang X, Anh DD. Association between norovirus and rotavirus infection and histo-blood group antigen types in Vietnamese children. *J Clin Microbiol* 2014;52:1366-74.
39. Patel M, Shane AL, Parashar UD, Jiang B, Gentsch JR, Glass RI. Oral rotavirus vaccines: how well will they work where they are needed most? *J Infect Dis* 2009;200 Suppl 1:S39-48.
40. Steele AD, De Vos B, Tumbo J, et al. Co-administration study in South African infants of a live-attenuated oral human rotavirus vaccine (RIX4414) and poliovirus vaccines. *Vaccine* 2010;28:6542-8.
41. Zaman K, Sack DA, Yunus M, et al. Successful co-administration of a human rotavirus and oral poliovirus vaccines in Bangladeshi infants in a 2-dose schedule at 12 and 16 weeks of age. *Vaccine* 2009;27:1333-9.

42. Robinson CG, Hernanz-Schulman M, Zhu Y, Griffin MR, Gruber W, Edwards KM. Evaluation of anatomic changes in young children with natural rotavirus infection: is intussusception biologically plausible? *J Infect Dis* 2004;189:1382-7.
43. Bass D, Cordoba E, Dekker C, Schuind A, Cassady C. Intestinal imaging of children with acute rotavirus gastroenteritis. *J Pediatr Gastroenterol Nutr* 2004;39:270-4.
44. Okimoto S, Hyodo S, Yamamoto M, Nakamura K, Kobayashi M. Association of viral isolates from stool samples with intussusception in children. *Int J Infect Dis* 2011;15:e641-5.
45. Bines JE, Liem NT, Justice FA, et al. Risk factors for intussusception in infants in Vietnam and Australia: adenovirus implicated, but not rotavirus. *J Pediatr* 2006;149:452-60.
46. Selvaraj G, Kirkwood C, Bines J, Buttery J. Molecular epidemiology of adenovirus isolates from patients diagnosed with intussusception in Melbourne, Australia. *J Clin Microbiol* 2006;44:3371-3.
47. Guarner J, de Leon-Bojorge B, Lopez-Corella E, et al. Intestinal intussusception associated with adenovirus infection in Mexican children. *Am J Clin Pathol* 2003;120:845-50.
48. Hsu HY, Kao CL, Huang LM, et al. Viral etiology of intussusception in Taiwanese childhood. *Pediatr Infect Dis J* 1998;17:893-8.
49. Konno T, Suzuki H, Kutsuzawa T, et al. Human rotavirus infection in infants and young children with intussusception. *J Med Virol* 1978;2:265-69.
50. Nylund CM, Denson LA, Noel JM. Bacterial enteritis as a risk factor for childhood intussusception: a retrospective cohort study. *J Pediatr* 2010;156:761-5.
51. Chang EJ, Zangwill KM, Lee H, Ward JI. Lack of association between rotavirus infection and intussusception: implications for use of attenuated rotavirus vaccines. *Pediatr Infect Dis J* 2002;21:97-102.
52. Velazquez FR, Luna G, Cedillo R, Torres J, Munoz O. Natural rotavirus infection is not associated to intussusception in Mexican children. *Pediatr Infect Dis J* 2004;23:S173-8.
53. Darge K, Anupindi SA, Jaramillo D. MR imaging of the abdomen and pelvis in infants, children, and adolescents. *Radiology* 2011;261:12-29.
54. Brugger PC, Prayer D. Fetal abdominal magnetic resonance imaging. *European journal of radiology* 2006;57:278-93.
55. Chalian M, Ozturk A, Oliva-Hemker M, Pryde S, Huisman TA. MR enterography findings of inflammatory bowel disease in pediatric patients. *AJR American journal of roentgenology* 2011;196:W810-6.
56. Froehlich JM, Patak MA, von Weymarn C, Juli CF, Zollikofer CL, Wentz KU. Small bowel motility assessment with magnetic resonance imaging. *Journal of magnetic resonance imaging : JMRI* 2005;21:370-5.
57. Froehlich JM, Daenzer M, von Weymarn C, Erturk SM, Zollikofer CL, Patak MA. Aperistaltic effect of hyoscine N-butylbromide versus glucagon on the small bowel assessed by magnetic resonance imaging. *European radiology* 2009;19:1387-93.
58. Ailiani AC, Neuberger T, Bresseur JG, et al. Quantitative analysis of peristaltic and segmental motion in vivo in the rat small intestine using dynamic MRI. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine* 2009;62:116-26.
59. Wakamiya M, Furukawa A, Kanasaki S, Murata K. Assessment of small bowel motility function with cine-MRI using balanced steady-state free precession sequence. *Journal of magnetic resonance imaging : JMRI* 2011;33:1235-40.
60. Shen SH, Guo WY, Hung JH. Two-dimensional fast imaging employing steady-state acquisition (FIESTA) cine acquisition of fetal non-central nervous system abnormalities. *Journal of magnetic resonance imaging : JMRI* 2007;26:672-7.
61. Patak MA, Froehlich JM, von Weymarn C, Breitenstein S, Zollikofer CL, Wentz KU. Non-invasive measurement of small-bowel motility by MRI after abdominal surgery. *Gut* 2007;56:1023-5.

62. Nissan A, Zhang JM, Lin Z, Haskel Y, Freund HR, Hanani M. The contribution of inflammatory mediators and nitric oxide to lipopolysaccharide-induced intussusception in mice. *J Surg Res* 1997;69:205-7.
63. Pons L, Droy-Lefaix MT, Bueno L. Role of platelet-activating factor (PAF) and prostaglandins in colonic motor and secretory disturbances induced by *Escherichia coli* endotoxin in conscious rats. *Prostaglandins* 1994;47:123-36.
64. Montuschi P, Tringali G, Parente L, Preziosi P, Navarra P. Interleukin-1 beta- and tumour-necrosis-factor-induced inhibition of rat gastric fundus motility in vitro. *Pharmacol Res* 1994;30:25-33.
65. Khan I, Collins SM. Expression of cytokines in the longitudinal muscle myenteric plexus of the inflamed intestine of rat. *Gastroenterology* 1994;107:691-700.
66. Warfield KL, Blutt SE, Crawford SE, Kang G, Conner ME. Rotavirus infection enhances lipopolysaccharide-induced intussusception in a mouse model. *J Virol* 2006;80:12377-86.
67. Sonmez K, Karabulut R, Turkyilmaz Z, et al. Association of tumor necrosis factor, interleukin-6 and cyclooxygenase pathway with lipopolysaccharide-induced intussusception. *Eur J Pediatr Surg* 2008;18:103-6.
68. Badriyyah M, Mazeh H, Brocke S, Osmanova V, Freund HR, Hanani M. Prevention of lipopolysaccharide-induced intussusception in mice by the COX2 inhibitor rofecoxib. *Pediatr Surg Int* 2008;24:333-6.
69. Willetts IE, Kite P, Barclay GR, et al. Endotoxin, cytokines and lipid peroxides in children with intussusception. *Br J Surg* 2001;88:878-83.
70. Karabulut B, Erdogan D, Bostanci I, Onde U, Karakoc AE. Are interleukin-6, body mass index and atopy crucial in infantile intussusception? *Indian J Pediatr* 2010;77:1257-60.
71. Ko G, Jiang ZD, Okhuysen PC, DuPont HL. Fecal cytokines and markers of intestinal inflammation in international travelers with diarrhea due to Noroviruses. *J Med Virol* 2006;78:825-8.
72. Steiner TS, Lima AA, Nataro JP, Guerrant RL. Enteroaggregative *Escherichia coli* produce intestinal inflammation and growth impairment and cause interleukin-8 release from intestinal epithelial cells. *J Infect Dis* 1998;177:88-96.
73. Greenberg DE, Jiang ZD, Steffen R, Verenker MP, DuPont HL. Markers of inflammation in bacterial diarrhea among travelers, with a focus on enteroaggregative *Escherichia coli* pathogenicity. *J Infect Dis* 2002;185:944-9.
74. Jiang ZD, Greenberg D, Nataro JP, Steffen R, DuPont HL. Rate of occurrence and pathogenic effect of enteroaggregative *Escherichia coli* virulence factors in international travelers. *J Clin Microbiol* 2002;40:4185-90.
75. El Feghaly RE, Stauber JL, Tarr PI, Haslam DB. Intestinal inflammatory biomarkers and outcome in pediatric *Clostridium difficile* infections. *J Pediatr* 2013;163:1697-704 e2.
76. El Feghaly RE, Stauber JL, Deych E, Gonzalez C, Tarr PI, Haslam DB. Markers of intestinal inflammation, not bacterial burden, correlate with clinical outcomes in *Clostridium difficile* infection. *Clin Infect Dis* 2013;56:1713-21.
77. Hasegawa T, Ueda S, Tazuke Y, et al. Colonoscopic diagnosis of lymphoid hyperplasia causing recurrent intussusception: report of a case. *Surg Today* 1998;28:301-4.
78. Shteyer E, Koplewitz BZ, Gross E, Granot E. Medical treatment of recurrent intussusception associated with intestinal lymphoid hyperplasia. *Pediatrics* 2003;111:682-5.
79. Nelson BH. Interleukin-2 signaling and the maintenance of self-tolerance. *Curr Dir Autoimmun* 2002;5:92-112.
80. Hofmann SR, Ettinger R, Zhou YJ, et al. Cytokines and their role in lymphoid development, differentiation and homeostasis. *Curr Opin Allergy Clin Immunol* 2002;2:495-506.
81. Porter BO, Malek TR. IL-2Rbeta/IL-7Ralpha doubly deficient mice recapitulate the thymic and intraepithelial lymphocyte (IEL) developmental defects of gammac<sup>-/-</sup> mice: roles for both IL-2 and IL-15 in CD8alpha<sup>+</sup> IEL development. *J Immunol* 1999;163:5906-12.

82. Chen Q, Ghilardi N, Wang H, et al. Development of Th1-type immune responses requires the type I cytokine receptor TCCR. *Nature* 2000;407:916-20.
83. Zheng BJ, Lo SK, Tam JS, Lo M, Yeung CY, Ng MH. Prospective study of community-acquired rotavirus infection. *J Clin Microbiol* 1989;27:2083-90.
84. Perez-Schael I, Daoud G, White L, et al. Rotavirus shedding by newborn children. *J Med Virol* 1984;14:127-36.
85. Bishop RF, Barnes GL, Cipriani E, Lund JS. Clinical immunity after neonatal rotavirus infection. A prospective longitudinal study in young children. *N Engl J Med* 1983;309:72-6.
86. Bernstein DI, Smith VE, Sander DS, Pax KA, Schiff GM, Ward RL. Evaluation of WC3 rotavirus vaccine and correlates of protection in healthy infants. *J Infect Dis* 1990;162:1055-62.
87. Oyejide CO, Fagbami AH. An epidemiological study of rotavirus diarrhoea in a cohort of Nigerian infants: II. Incidence of diarrhoea in the first two years of life. *Int J Epidemiol* 1988;17:908-12.
88. Raul Velazquez F, Calva JJ, Lourdes Guerrero M, et al. Cohort study of rotavirus serotype patterns in symptomatic and asymptomatic infections in Mexican children. *Pediatr Infect Dis J* 1993;12:54-61.
89. Naficy AB, Abu-Elyazeed R, Holmes JL, et al. Epidemiology of rotavirus diarrhea in Egyptian children and implications for disease control. *Am J Epidemiol* 1999;150:770-7.
90. Newburg DS, Peterson JA, Ruiz-Palacios GM, et al. Role of human-milk lactadherin in protection against symptomatic rotavirus infection. *Lancet* 1998;351:1160-4.
91. Dennehy PH, Cortese MM, Begue RE, et al. A case-control study to determine risk factors for hospitalization for rotavirus gastroenteritis in U.S. children. *Pediatr Infect Dis J* 2006;25:1123-31.
92. Clemens JD, Ward RL, Rao MR, et al. Seroepidemiologic evaluation of antibodies to rotavirus as correlates of the risk of clinically significant rotavirus diarrhea in rural Bangladesh. *J Infect Dis* 1992;165:161-5.
93. Chan J, Nirwati H, Triasih R, et al. Maternal antibodies to rotavirus: could they interfere with live rotavirus vaccines in developing countries? *Vaccine* 2011;29:1242-7.
94. Asensi MT, Martinez-Costa C, Buesa J. Anti-rotavirus antibodies in human milk: quantification and neutralizing activity. *J Pediatr Gastroenterol Nutr* 2006;42:560-7.
95. Jiang B, Patel M, Parashar U. Rotavirus vaccines for global use: what are the remaining issues and challenges? *Hum Vaccin* 2010;6:425-7.
96. Jiang V, Jiang B, Tate J, Parashar UD, Patel MM. Performance of rotavirus vaccines in developed and developing countries. *Hum Vaccin* 2010;6:532-42.
97. Johnson B, Gargiullo P, Murphy TV, Parashar UD, Patel MM. Sociodemographic and dietary risk factors for natural infant intussusception in the United States. *J Pediatr Gastroenterol Nutr* 2010;51:458-63.
98. Pisacane A, Caracciolo G, de Luca U, et al. Infant feeding and idiopathic intussusception. *J Pediatr* 1993;123:593-5.
99. Payne DC, Baggs J, Zerr DM, et al. Protective association between rotavirus vaccination and childhood seizures in the year following vaccination in US children. *Clin Infect Dis* 2014;58:173-7.
100. Imbert-Marcille BM, Barbe L, Dupe M, et al. A FUT2 gene common polymorphism determines resistance to rotavirus A of the P[8] genotype. *J Infect Dis* 2014;209:1227-30.
101. Huang P, Xia M, Tan M, et al. Spike protein VP8\* of human rotavirus recognizes histo-blood group antigens in a type-specific manner. *J Virol* 2012;86:4833-43.
102. Liu Y, Huang P, Tan M, et al. Rotavirus VP8\*: phylogeny, host range, and interaction with histo-blood group antigens. *J Virol* 2012;86:9899-910.
103. Tong M, McHardy I, Ruegger P, et al. Reprogramming of gut microbiome energy metabolism by the FUT2 Crohn's disease risk polymorphism. *ISME J* 2014.
104. Wacklin P, Makivuokko H, Alakulppi N, et al. Secretor genotype (FUT2 gene) is strongly associated with the composition of Bifidobacteria in the human intestine. *PLoS One* 2011;6:e20113.

105. Rausch P, Rehman A, Kunzel S, et al. Colonic mucosa-associated microbiota is influenced by an interaction of Crohn disease and FUT2 (Secretor) genotype. *Proc Natl Acad Sci U S A* 2011;108:19030-5.
106. Yu ZT, Chen C, Kling DE, et al. The principal fucosylated oligosaccharides of human milk exhibit prebiotic properties on cultured infant microbiota. *Glycobiology* 2013;23:169-77.
107. Haber P, Patel M, Pan Y, et al. Intussusception after rotavirus vaccines reported to US VAERS, 2006-2012. *Pediatrics* 2013;131:1042-9.
108. CDC ACIP Immunization Schedule. at <http://www.cdc.gov/vaccines/schedules/hcp/child-adolescent.html>.)
109. Stockman LJ, Staat MA, Holloway M, et al. Optimum diagnostic assay and clinical specimen for routine rotavirus surveillance. *J Clin Microbiol* 2008;46:1842-3.
110. Mathur AM, Neil JJ, McKinstry RC, Inder TE. Transport, monitoring, and successful brain MR imaging in unsedated neonates. *Pediatr Radiol* 2008;38:260-4.
111. Windram J, Grosse-Wortmann L, Shariat M, Greer ML, Crawford MW, Yoo SJ. Cardiovascular MRI without sedation or general anesthesia using a feed-and-sleep technique in neonates and infants. *Pediatr Radiol* 2012;42:183-7.
112. Ward RL, Bernstein DI, Shukla R, et al. Protection of adults rechallenged with a human rotavirus. *J Infect Dis* 1990;161:440-5.
113. Gautam R, Esona MD, Mijatovic-Rustempasic S, Ian Tam K, Gentsch JR, Bowen MD. Real-time RT-PCR assays to differentiate wild-type group A rotavirus strains from Rotarix and RotaTeq vaccine strains in stool samples. *Hum Vaccin Immunother* 2013;10.
114. Morrow AL, Meinen-Derr J, Huang P, et al. Fucosyltransferase 2 non-secretor and low secretor status predicts severe outcomes in premature infants. *J Pediatr* 2011;158:745-51.
115. Food and Drug Administration. Product-approval information-licensing action [package insert] Rotarix (rotavirus vaccine, live, oral), GlaxoSmithKline Biologicals. Rockville, MD: US Department of Health and Human Services, Food and Drug Administration.. 2011. at <http://www.fda.gov/downloads/biologicsbloodvaccines/vaccines/approvedproducts/ucm133539.pdf>.)
116. Food and Drug Administration. Product-approval information-licensing action [package insert] RotaTeq (rotavirus vaccine, live, oral, pentavalent), Merck & Co. Rockville, MD: US Department of Health and Human Services, Food and Drug Administration. 2011. at <http://www.fda.gov/downloads/biologicsbloodvaccines/vaccines/approvedproducts/ucm142288.pdf>.)

## APPENDIX 5. First Visit Instruction

**Thank you so much for participating in our study. We will be calling you in the next few days to schedule your baby's study visits. Below are the instructions for the first visit. We will call you before the visit to go over all of this with you. Do not hesitate to call us with any questions between now and your scheduled visit.**

### Day before first study visit

- We will call you the day before your first scheduled visit. If your contact information changes please call us at **513-636-2479/513-303-8800** so we have the most up to date information. You may also email us at **IDstudies@cchmc.org**
- Put the cold pack you were given at enrollment in your freezer so it will be ready to use
- If you are breastfeeding, collect about an ounce of breastmilk within 24 hours of your appointment and keep in refrigerator.
- We want you to bring a fresh refrigerated (not frozen) stool sample with you to the first visit. Save a poopy diaper within 48 hours before the visit. Ideally we would like a diaper within 24 hours of the visit, but all babies do not have bowel movements every day! If baby has another poopy diaper after you have saved one, you should throw away the first and bring the most recent diaper to the visit. Place diaper in clear labeled bag and put in silver bag you were given at enrollment. Label with date and time of collection and keep in refrigerator.

### Day of first study visit

- Do not feed baby for 3 hours before your scheduled visit time
- Put the cold pack in the silver bag with the stool sample and bring with you, along with the breast milk sample, if breastfeeding
- Bring the baby's social security number (if available), pacifier, empty bottle/nipple, formula or breast milk and other items you may need. The visit will last approximately 3.5 hours
- We will meet you at your appointment time in front of the gift shop, main hospital near the location C welcome desk. If you are driving, park near the C elevators in the garage. If you are dropped off or coming by bus, enter thru C location doors in the main entrance circle on Burnet Avenue
- Any questions, do not hesitate to call us at **513-636-2479 or 513-303-8800** or email us **IDstudies@cchmc.org**

**APPENDIX 6.  
Second Visit Instruction**

**Thank you so much for completing your first study visit. We will be calling you the day before your next visit to remind you of the appointment. Below are the instructions for what to do between now and your next visit. Do not hesitate to call us with any questions between now and your scheduled visit.**

**Beginning today until next visit**

- Fill out the memory aid everyday as instructed starting today. Take the temperature under your baby's arm every evening and record on the memory aid. Remember to document any medications or doctor visits on the memory aid.
- Save all poopy diapers in the dated labeled clear bags and keep all in silver bag in your refrigerator.
- We will call you the day before your next scheduled visit. If your contact information changes please call us at **513-636-2479/513-303-8800** so we have the most up to date information. You may also email us at [IDstudies@cchmc.org](mailto:IDstudies@cchmc.org)
- Put the cold pack you were given at enrollment back in your freezer so it will be ready to use

**Day of second study visit**

- Do not feed baby for 3 hours before your scheduled visit time
- Put the cold pack in the silver bag with all of the poopy diapers and bring with you, along with your memory aid
- Bring the baby's pacifier, empty bottle/nipple, formula or breast milk and other items you may need. The visit will last approximately 3.5 hours
- We will meet you at your appointment time in front of the gift shop, main hospital near the location C welcome desk. If you are driving, park near the C elevators in the garage. If you are dropped off or coming by bus, enter thru C location doors in the main entrance circle on Burnet Avenue
- Any questions, do not hesitate to call us at **513-636-2479 or 513-303-8800** or email us at [IDstudies@cchmc.org](mailto:IDstudies@cchmc.org)

**APPENDIX 7.  
Third Visit Instruction**

**Thank you so much for completing your second study visit. We will be calling you the day before your next visit to remind you of the appointment. Below are the instructions for what to do between now and your next visit. Do not hesitate to call us with any questions between now and your scheduled visit.**

**Beginning today until next visit**

- Continue to fill out the memory aid everyday as instructed. Take the temperature under your baby's arm every evening and record on the memory aid. Remember to document any medications or doctor visits on the memory aid.
- Save all poopy diapers in the dated labeled clear bags and keep all in silver bag in **your freezer**.
- We will call you the day before your next scheduled visit. If your contact information changes please call us at **513-636-2479/513-303-8800** so we have the most up to date information. You may also email us at **IDstudies@cchmc.org**
- Put the cold pack you were given at enrollment back in your freezer so it will be ready to use

**Day of third study visit**

- Put the cold pack in the silver bag with all of the poopy diapers and bring with you, along with your memory aid. **We must have both in order to complete the visit.**
- If your baby is a Pediatric Primary Care Clinic (PPC) patient, we will meet you at the PPC. IF baby is not a PPC patient the appointment will be scheduled at the T1 research clinic and we will meet you at the gift shop.
- The study visit will take about 15 minutes. If scheduled in the PPC this will be followed by your child's regularly scheduled well check up with PPC staff.
- Any questions, do not hesitate to call us at **513-636-2479 or 513-303-8800** or email us at **IDstudies@cchmc.org**