SECTION XI: IMMUNE (LYMPHOCYTE PROLIFERATION) SUB-STUDY

Project Viva has received funding to investigate how the development of children's immune systems influences the development of asthma and allergies. There were 486 mother-child pairs identified as eligible for this sub-study based on a positive lymphocyte proliferation response during analysis of the V3 cord blood samples.

The Immune Sub-Study protocols take place at the time of the participant's three-year visit (V7). Optimally, any mother-child pair who is eligible for the Immune Sub-Study is asked to participate in a full in-person V7 with a few additional protocols. These are: collection of a dust sample, an additional health question, and an additional blood analysis. Because eligibility for the Immune Sub-Study is independent of a participant's "in-person" versus "mail-only" V7 status, the eligible participants are tracked separately. If a participant is unable to participate in a full V7, a modified "Immune Visit" is possible. In this case, the participant and her child are asked to participate in the Immune Sub-Study protocols, as well as, height and weight.

Because the Immune Sub-Study visit mirrors the V7 in-person and blood visits so closely, this section will focus primarily on the additional Immune Sub-Study protocols. Research Assistants should refer to Visit Seven Blood ***(Section V)*** *and Visit Seven In-Person Visit* ***(Section VI)*** *for detailed accounts of the shared protocols.*

In conjunction with the HVMA pediatric clinics, Project Viva has worked to minimize the number of needle sticks any child must endure. As a courtesy to participants who receive pediatric care at an HVMA center, and as an incentive to have the child's blood drawn for Project Viva, the 1st tube of blood drawn from these child participants is used for blood analyses that typically occur as a standard part of the HVMA 3-year, well child, check-up: lead count and complete blood count (CBC). The results of these analyses are then forwarded to the child's HVMA pediatrician. Alternatively, the Research Assistant may meet the participant at the time of the scheduled, routine 3-year check-up lab visit. At this time, the Research Assistant will arrange to have an HVMA phlebotomist draw the extra study blood sample, as well. Project Viva participants who do not receive pediatric care at an HVMA center and who agree to participate in the blood visit, will have their blood drawn separately from any pediatric blood work that may be required (i.e. the child may need to have more than one needle stick).

It is important to note that some women have increased sensitivity about participating, or having their child participate, in the blood visit. This is the first time that Project Viva is requesting a blood sample from children. Additionally, it is the first time that Project Viva is requesting a blood sample that is "free-standing." In other words, it is not an additional tube taken from a medically necessary lab visit, as happened during Visits 1 and 2, for every participant. Furthermore, contact is made regarding a blood appointment prior to attempting to schedule the V7 in-person visit. For these reasons, and because the blood samples are an integral source of data for study analyses, it is critical that the Research Assistants and Phlebotomists who participate in the blood visit take every measure to assuage participant's concerns, while still encouraging full participation.

A. Initiation of Immune Sub-Study

Whenever possible, the Immune Sub-Study protocols (including blood collection) occur during one visit. For the sake of efficiency a Research Assistant and Phlebotomist travel together to conduct these visits. Unless the participant refuses, all Immune Sub-Study visits occur in the participants' homes in order to procure the dust sample.

1) Contact Logs

The Data Manager prints the contact logs on a weekly basis. The immune logs are marked with a "411" in red in the blood column to signify their immune status and distributed to the appointed Research Assistant(s). The contact logs are cued to print once the child has turned 2 years and 9 months old. When the contact log prints, both the blood and in-person visit are initialized in the Adept, Data Management System. **(See Appendix M: Visit 7 Contact Logs).**

In addition to basic contact information, the contact log reflects the first name of the mother and the child, the child's date of birth, and the most recent Toddler Enrollment Status (TEN). The contact log also reflects the child's pediatrician, when known. Finally, the log offers a space for the Research Assistant to fill in the date of the child's scheduled 3-year pediatric check-up, as well as, to indicate if the child lives at an address different from that of his/her mother. When contacting a participant on the telephone, the Research Assistant should remember to confirm and when necessary, update, the information on the contact log.

2) Enrollment Status

The original Diet-Asthma consent includes the standard 3-year child blood draw in the procedures section. As such, differing levels of "blood consent" exist for children prior to their V7 windows opening.

When calling to schedule a blood visit, the Research Assistant should be mindful of the level of enrollment already obtained for the child. The TEN status is printed in the top right hand corner of the V7 contact log. All eligible participants, even those who had refused consent for the child blood draw in the past, are approached at this time.

3) Scheduling Appointments

While it is good practice to check the schedule daily for any cancellations or changes, the Immune Sub-Study schedule should be tentatively "set" one week in advance. Appointments should not be added to a "set" schedule until approval has been received from both the staff overseeing the visit and the Research Assistant who is responsible for registering participants through the HVMA laboratory system.

The Immune Sub-Study appointment scripts **(Appendix O: Age 3 411 Blood Appointment Script, Age 4 Blood, Dust & In-person Appointment Script, Age 4 Blood, Dust & Child Measurement Appointment Script)** offer the Research Assistant detailed guidance. It is important to consistently attempt contacting participants and to rely on help from the tracking protocol, for hard to reach participants **(Section: XII).**

In addition to reacquainting the participant with Project Viva (because V5 and V6 are mail-only, this is the first time she will have spoken with someone about the study in at least one year and often times it will have been much longer!), the appointment script aids the Research Assistant in outlining the importance of participating in the child and adult portion of the blood visit.

If the participant is willing to consent A mutually agreeable appointment **home visit** time is determined and recorded in the Lotus Notes Calendar **(See Appendix K: Blood and 411 Scheduling Guidelines).** The appointment time and other pertinent information should also be recorded on the V7 contact log. While on the phone, the Research Assistant confirms the participant's contact information, briefly describes the blood draw procedure, and if a home visit has been scheduled, gets directions and then gives the name and phone number of the phlebotomist who will oversee the appointment.

If the participant is <u>not</u> willing to consent to any portion of the Immune Sub-Study, the Research Assistant should indicate the consent refusal on the V7 contact log. In addition the Research Assistant should complete both the Immune Enrollment Form (IENC) and the Maternal Blood Enrollment Form (BEN), indicating "refused consent." The Child Genetics Form (CGEN) should also be completed with "Ineligible".

If the participant wishes to disenroll entirely from the study (See Appendix J: Disenrollment), the Research Assistant should fill out a Disenrollment Form (DISN). The DISN should be attached to the contact log and given to the V7 In-Person Research Associate. **If an unenrolled participant is contacted and expresses that she is not interested in participating in Diet-Asthma (Appendix J: TEN of 3 for Unenrolled Participants),** the Research Assistant should fill out a Toddler Enrollment Form (TEN) with a status of TEN=3 "No Consent." The TEN should similarly be attached to the contact log and given to the V7 In-Person Research Associate. The Principal Investigator will review these contact logs with the Research Associate. When he deems the situation appropriate, he will call the participant to discuss possible continuation in the study.

If the Research Assistant is unable to schedule an appointment before the blood window closes the contact log should be filed in the "V7 Contact Log" hanging file in the gray filing cabinet. Before doing so, the Research Assistant should verify that she has carefully detailed all attempts at contact, as well as, pursued all avenues of

contact using the tracking protocol **(Section XII)**. The contact log will be redistributed to a V7 in-person Research Assistant who will further attempt to reach the participant for a combined in-person/blood visit.

B. Preparing for Immune Sub-Study Visit

1) Preparing Blood Visit Folder

After an appointment has been scheduled, the appointed Research Assistant should personalize a generic blood folder **(Appendix M: Compiling 411 Materials)**. Labels for the child and adult blood tubes should be added. If a final disposition has yet to be reached, 2 copies of the Child Genetics and 2 copies of the Maternal Genetics Consents, as well as, the associated Enrollment Forms should also be added. **(Appendix M: Compiling 411 Materials)**. Finally, if the child receives pediatric care at an HVMA center, a form letter from the HVMA Central Pediatric Department **(Appendix M: HVMA Pediatric Letter to Participant)** should be added. This letter is given to the mother at the time of the blood visit. It reflects HVMA's endorsement of Project Viva and assures the participant that every effort will be made to use the Viva lead and CBC results in lieu of the standard 3-year blood draw that occurs at an HVMA well child visit. The contact log is then given to the phlebotomist doing the visit. The personalized blood folder is then placed in the "Blood Folders To Go" hanging file.

2) Pre-registration

Any child participant who is scheduled for any type of blood visit must be pre-registered with HVMA. Pre-registration serves several purposes. First, it allows billing to charge the insurance company for the analyses used by the HVMA pediatric staff. It also helps the laboratory track charges owed (by Viva) for services rendered. Finally, it also allows an "order" to be given to the HVMA laboratory system, thus allowing for HVMA lab blood draws and lab analyses. The appointed Research Assistant overseeing registration should check for all newly scheduled (it is not necessary to re-register a rescheduled appointment) blood appointments on a weekly basis and tend to their pre-registration. In order to register an individual, the participant's information is sent to a designated employee in HVMA Central Registration, who registers all non-HVMA participants into the HVMA system and assigns them a Medical Record Number (MRN). All participant MRNs and Primary Care Providers Names (if applicable) are then faxed back to the RA to complete blood lab orders for the child participants. Registration is usually completed within 48 hours. **(Appendix M: HVMA Pre-Registration for Child Blood Visits).**

3) Confirming the Appointment

Each night the Immune Sub-Study Research Assistant or Phlebotomist should call and confirm the next day's appointments. Cancellations and reschedules should be tracked on the contact log.

4) Before Leaving the Office

If the participant has agreed to a home visit, the phlebotomist should verify that she has all needed materials before leaving the office. The participant's folder is housed in the "V7 Blood Folders To Go" hanging file. The folder may be picked up the night before the appointment with supervisor approval, provided it is housed in a locked area and is not kept inside of a car.

The Phlebotomist should also gather all needed blood supplies (e.g. tubes and ice packs) before leaving the office. **(Appendix M: Compiling 411 Blood Materials).** Importantly, the Immune Sub-Study two different coolers (one kept at room temperature and the other kept cool with ice-packs) are needed to accommodate the types of blood samples drawn for the Immune Sub-Study.

The Phlebotomist is responsible for gathering the participant's folder and all necessary equipment for the measurements and dust sample **(Appendix P: 411 Appointment Checklist).** Additionally, the phlebotomist should bring incentive money and directions to the participant's house **(Appendix N: Reimbursement Forms)**.

C. Immune Sub-Study Protocol

a.) Travel

The staff overseeing the visit should arrive to the participant's home five minutes prior to the appointment time, allowing plenty of extra travel time, accounting for traffic and weather, in order to ensure timeliness. If the running late, or lost, one staff member should use the cell phone provided to contact the participant and/or the Viva office.

Mileage should be recorded on the mileage reimbursement form (by the staff member who is driving) before getting out of the car at each appointment. All parking and toll receipts should be saved and attached directly to the back of the mileage reimbursement form **(Appendix N: Reimbursement Form).**

If the participant does not answer the door, staff should use the cell phone to call the participant. If no one answers, staff should wait a minimum of fifteen minutes before leaving the residence. If the participant fails to keep the appointment, this should be recorded on the contact log. After two missed home visits, no further appointments should be made for a home visit unless otherwise advised by the Project Manager.

b.) In the Home

Prior to entering the home, study staff should introduce themselves to the participant **(Appendix P: Age 3 Immune Script-mom and child, Age 4 Immune Script-mom and child, Age 4 Immune Script-TEN=2, Age 4 Immune Script-Unenrolled).** If at any time a staff member does not feel safe, she should excuse herself from the home. After driving a comfortable distance, she should use the cell phone to report to her supervisor. Any interactions that occur outside of the standard protocol should be recorded on the contact log. Staff should maintain the utmost respect and be as non-intrusive as possible while in the home.

c.) Conducting the Visit

Using the script provided, the phlebotomist should review the Maternal Blood Consent form, the Diet-Asthma consent form and the Immune Sub-Study Consent Form answering any questions the participant may have. **(Appendix P: Age 3 Immune Script-mom and child, Age 4 Immune Script-mom and child, Age 4 Immune Script-TEN=2, Age 4 Immune Script-Unenrolled).** Once a final consent disposition has been established for both the child and the mother, the phlebotomist should make sure that both copies, of each of the consent forms, have been signed by the Research Assistant AND the mother. One copy of each form is left with the participant. The other copy is retained for study records.

Blood should NEVER be drawn without a signed consent. The phlebotomist should next complete the Blood Enrollment Form (BEN).

If the child receives care at an HVMA pediatric clinic, the phlebotomist should distribute the form letter from the HVMA pediatric staff **(Appendix M: HVMA Pediatric Letter to Participants).** At this point, the phlebotomist should set out the supplies needed for the blood draw. The phlebotomist should work in a safe, well-lit area. The kitchen table is often a good space for working.

Using the last page of the script, the phlebotomist should now review the details of the blood draw. **(See Appendix P: Age 3 Immune Script-mom and child, Age 4 Immune Script-mom and child, Age 4 Immune Script-**

TEN=2, Age 4 Immune Script-Unenrolled). It is best to ask the participant if she has a preference as to whether she or the child should have their blood drawn first. If there is no preference than it is best to draw the mom's blood first so that the child can observe.

The phlebotomist should draw two 10mL blood tubes from the ***adult participant in the following order: 10 mL purple top and then 10 mL green top.*** The phlebotomist should draw three tubes, totaling 14mL, from the ***child in the following order: 2mL purple top, 6mL green top (kept at room temperature, and the 6mL green top.***

The Phlebotomist is responsible for accurately labeling and storing all tubes of blood. The IDs for the child's blood tubes are highlighted fluorescent yellow for the chilled tubes and highlighted blue for the room temperature tube. Each label should have "Viva" written in red in the upper right hand corner and the date of birth written by the phlebotomist in the bottom right corner. The adult participant labels are kept white and the ID is highlighted purple (event 07 color). The phlebotomist should fill in the visit number, event date, and initials, after the draw is complete. The blood tubes should be labeled, put into a biohazard bag, and placed in a cooler (with a foam cushion between the tubes and the ice pack) immediately after the draw is completed. The room temperature tube should also be placed in the room temperature cooler immediately after the draw.

The Phlebotomist should demonstrate sound clinical judgment if any problems arise during any part of the visit. The information provided in **Appendix L: Blood Draw Risk Management** is intended for use as guidelines and are not conclusive.

In the event of an emergency, the phlebotomist should **call 911** and then call her supervisor to report the events. All visit information should be recorded on the V7 Contact Log. This is especially true for any happenings outside of standard protocol.

A dust sample should only be collected if consent has been received. A detailed protocol for dust sample collection can be found in Appendix P. (Dust Collection Protocol, Dust Collection- 1 minute trainer)

Each participant will receive a cash incentive for participating in the visit. A sum of $15.00, for the child blood draw, and $10.00, for the adult blood draw, is paid in cash at the end of the visit. No additional incentive is received for participating in the other parts of the visit (measurements and dust). The staff member should ask the participant to sign a receipt stating that she received the money. Additionally, the staff member should enter the amount given on an Incentive Tracking Log. **(See Appendix N: V7 Incentive Log, V7 Incentive Receipt).**

Staff should be sure to throw away any trash and gather all forms and materials before leaving the home. After answering any outstanding questions, staff should give the participant a business card, with contact information.

2) Transporting the Blood

The 2mL child purple-top tubes of blood should be given directly to the Kenmore HVMA laboratory technician. This can be done directly after the visit if the blood has been drawn at the Kenmore HVMA. Otherwise, the phlebotomist should deliver these tubes to the technician as soon as she returns to Kenmore.

The remaining tubes are directly delivered to the Channing Laboratory. The 6mL green top that is left at room temperature is given to the Lab Tech. The mom tubes and the child chilled tube are given to a Lab Tech or left in the refrigerator and the blood log is taped to the front. The only day the tubes are not delivered directly to Channing is Sunday. On Sundays, the phlebotomist leaves the chilled tubes in the refrigerator in the HVMA Kenmore Lab. The Child room temperature tube is left in the room temperature cooler at the Phlebotomists desk. The Channing tubes are then transported to Channing on Monday morning before 10am for processing.

Every day of the week, a Research Assistant or Phlebotomist gathers all tubes of blood from the Viva bin in the Kenmore laboratory's refrigerator and delivers them to the Channing Lab between the hours of 9:00 a.m. and 2:30 p.m. Any blood that is received after 2:30 should be held at Kenmore and delivered to Channing the following morning.

All blood transport information is recorded on a Blood Delivery Log (See Section E below). The Research Assistant or Phlebotomist responsible for transport should be sure to carefully fill this form out in full.

D. Immune Sub-Study Forms

After completing the Immune Sub-Study visit, the participant's forms should be cleaned and coded. The folder(s) should be placed in the green hanging file labeled "Immune Sub-Study." The Blood Delivery Log should be completed and placed in the hanging file located in the filing cabinet (or taken directly to the Channing with the tubes).

Information for the majority of the forms used at the Immune Sub-Study visit can be found in the Blood and V7 In-Person **(Section V & VI)** protocols. The staff overseeing the visit should be careful to complete all necessary paperwork **(See Appendix P: 411 Appt Checklist)**

The additional forms that are used at the Immune Sub-Study visit are:

1) Immune Sub-Study Consent Form

This form serves to enroll the child participant into the Immune Sub-Study. Procedures specific to this study include: Measurement of the child's height and weight, dust sample, immune blood sample. The Research Assistant should describe the participation and then ask the participant to carefully read the informed consent. After answering any questions that the participant may have, she should sign two copies of the consent, and ask the participant to sign both copies as well. One copy is sent home with the participant for her records and one copy is kept for the study.

2) Immune Sub-Study Enrollment Form (IENC)

After reviewing the Immune Sub-Study Consent, the Immune Enrollment Form should be completed. This form reflects the child's enrollment status. All eligible children should have an Immune Enrollment Form completed, regardless of final disposition. There are 12 possible dispositions on this form. Option 1, "Enrolled, Full Consent" is the only option that permits the blood draw to occur. Option 2, "Enrolled, No Blood Draw" is used when a participant has refused consent for her child's blood to be drawn. Option 4 "Enrolled- Partial" is used when something other than blood has been explicitly declined. In this case the Research Assistant should cross the procedure out on the consent and then specify the procedure refused on the space provided next to Option 4. Option 3, "None-Participant refused consent," Option 5- "Not Approached-Long Distance," Option 7 "Not Approached- Refused Enrollment in Diet-Asthma," Option 8- "Passive Refusal," Option 9- "Traced but could not contact," Option 10- "Unable to Trace," and Option 11, "Study Ended" are used to distinguish reasons the Immune Sub-Study did not occur. Staff should refer to the IENC QxQ forms for more specific guidance in accurately reflecting a participant's enrollment status.

3) Immune Blood Form (IBL)

This form is very similar to the child blood form. **(See Section V. Visit 7 Blood)** An additional question has been added to obtain health status during the past week.

4) Immune Child Anthropometry Form (IAT8)

This form is used to gain height and weight measurements at the time of the immune blood draw. If a V7 in-person anthropometry form has been completed within 6 months of the immune visit, then no Immune Anthropometry is completed and the form is set to missing with the reason "V7 within 6 months".

5) Immune Dust Collection Form (IDC)

This form records the outcome of the dust collection and is filled out regardless of whether consent for the dust sample has been given. If any dust sample has been

collected, the entire form should be filled out. If dust was not collected, Question A1 and A2 only should be filled out. The reason that the dust was not collected should be checked off in the space provided.

6) Dust Delivery Log (DDL)

This form is similar to the Blood Delivery Log. **(See Section V. Visit 7 Blood)** This form tracks the dust samples that have been dropped off at the Channing lab, for each study participant. The phlebotomist (or Research Assistant, when applicable) is responsible for filling out the form in its entirety. Line C should be completed at the time of transfer.

E. V7 Blood Follow-Up

1) Thank You letters

On a weekly basis, the appointed Research Assistant should send a thank-you letter to participants who took part in a Blood Visit **(See Appendix N: Preparing and Sending Blood Thank You Letters).** These letters are printed onto Viva cards and then hand signed and addressed. The Research Assistant should indicate directly on the contact log that a blood thank you was mailed and then file the contact log in the "V7 Contact Logs" green hanging file.

2) Obtaining and Sending Blood Results

Each week the Channing Laboratory faxes a copy of all Blood Delivery Logs received in the previous week to Viva's V7 Blood Research Associate. These logs are used by Viva to identify the number of lab results expected. The appointed Research Assistant uses Adept to look up the names associated with the IDs on the faxed delivery logs and then references the Pre-Registration Forms to locate the corresponding medical record number. This information is sent, via email, to the director of the Kenmore Laboratory.

The Director of the Kenmore Laboratory is responsible for generating results for all Viva lead and CBC analyses for the participants indicated in the email. The results will be printed and returned to the V7 Blood Research Associate within one week. Upon receipt, the V7 Blood Research Associate will compare the results to Viva guidelines and determine if there is any "abnormal" or concerning result. Any abnormal results are given to the Principal Investigator, who will either contact the participant directly to discuss any necessary medical follow-up or write an explanatory note on the blood results letter. **(Appendix N: Blood Results Letter- Not Normal).**

A copy of the results for any child participant who receives pediatric care at an HVMA medical center is sent to the Central Pediatric Department. From here, a HVMA clinician enters the lab results into the HVMA medical database. In this way, the results are made available to HVMA pediatricians for review at the 3-year well-child check-up. **(Appendix N: Obtaining and Sending Blood Results).**

Results, for any child participant who receives pediatric care with a clinician outside of the HVMA practice, are sent directly to the parents in a form letter **(See Appendix N: Results Letter-Normal, Results Letter- Not Normal)**. If the results are out of the normal range, the Principal Investigator provides a short written explanation.

All pre-registration forms are marked with a red "R" to signify the receipt of results from the HVMA laboratory. The pre-registration forms are filed in the "Registration" binder. An ID label is placed on each individual's lab results. These results are entered into Adept and then filed in the "Results" binder.

Please see **Appendix N: Obtaining and Sending Blood Results** for detailed instructions.