PN-ACQ-673

REPORT OF THE REVIEW TEAM FOR THE ZVITAMBO PROJECT

Final Report

A

Table of Contents

EXECUTIVE SUMMARY 2

Recommendations to USAID regarding the management of clinical trials 3

INTRODUCTION 5

SCOPE OF THE REVIEW 5

COMPOSITION OF THE REVIEW TEAM 5

Activity of the review team. 6

ZVITAMBO STUDY DESIGN 7

FINDINGS AND RECOMMENDATIONS 9

1. The public health significance of ZVITAMBO 9
2. The scientific merit of ZVITAMBO 9
3. Reporting mechanisms 10
5. Cost effectiveness of ZVITAMBO management 10
5. Protection of human subjects 11
6. Capacity building 13
7. Ways that ZVITAMBO operations can be strengthened 14
8. The relationship between ZVITAMBO, the Harare City Health Department, the Ministry of Health and the University of Zimbabwe 15

FUNDING RECOMMENDATION 15

RECOMMENDATIONS TO USAID REGARDING THE MANAGEMENT OF CLINICAL TRIALS 16

ANNEX A: SCOPE OF WORK FOR THE REVIEW TEAM FOR THE ZVITAMBO PROJECT 19

ANNEX B: ZVITAMBO STUDY DESIGN 21

EXECUTIVE SUMMARY

ZVITAMBO is a study of postpartum Vitamin A supplementation to newborns and postpartum mothers, whose purpose is to learn if this intervention will reduce infant mortality, reduce transmission of HIV via breastfeeding and reduce postpartum HIV infections of mothers. The team spent five days reviewing the study and meeting with ZVITAMBO staff, USAID/Zimbabwe staff, and Zimbabwean stakeholders. Below are our findings and recommendations.

Conclusions:

1. The ZVITAMBO study is very significant to pressing public health issues.
2. The ZVITAMBO study is scientifically and technically sound.
3. Reporting mechanisms from the field to USAID/Washington and Zimbabwe are not adequate.
4. While we find no suggestion for misuse of funds, the budget process used by JHU and ZVITAMBO preclude a complete understanding of how the need for increased funding arose and what the actual current needs are.
5. ZVITAMBO staff are taking steps to protect human subjects. They have modified their recruitment and follow-up procedures to protect study participants. They have demonstrated a willingness to modify the study in response to changes in WHO policy on HIV and breastfeeding.
6. ZVITAMBO has built both human and infrastructure capacity in the form of substantial training in HIV/AIDS counseling as well as state of the art laboratory facilities.
7. ZVITAMBO enjoys good relations with the Harare Department of Health, the University of Zimbabwe, the Ministry of Health, and UNICEF.

Funding recommendation

It is the conclusion of the review team that the study is of sufficient merit and importance for USAID to continue to provide financial support, especially in light of the substantial investment already made. However, USAID should make this funding contingent on the following recommendations:

1. ZVITAMBO should provide a budgeting of the study that makes clear (in US dollars) the costs of the components of the study (i.e. one-year, two-year and sub studies). In the event that the current request proves insufficient, it will then be possible to determine the source of the increased costs, facilitating selected cuts in the project and/or selected funding of essential elements of the study.
2. ZVITAMBO should modify hospital based recruitment procedure: recruit study subjects using nurse-counselor teams rather than solo recruiters. *This was discussed with ZVITAMBO staff during the review.*

Additional Recommendations

1. USAID/W should request a cost accounting from Johns Hopkins University for the ZVITAMBO project.
2. All communications between ZVITAMBO and USAID/Washington should go through Dr. Frances Davidson in G/PHN/HN. ZVITAMBO should copy USAID on all reports sent to JHU and CIDA.

3. ZVITAMBO should review information given at recruitment, offer test results and appropriate counseling to participants at week-six visits. *This was discussed with ZVITAMBO staff during the review.*
4. ZVITAMBO should extend promotion of the study to ANC settings. *This is being done.*
5. ZVITAMBO should cease use of materials labeled with Ross Lab or any other brand names of infant formula.
6. ZVITAMBO should develop an exit strategy for disposition of laboratory facilities. This equipment will require at least $1000 per year to maintain even if not used.
7. ZVITAMBO should provide additional professional upgrading for counseling. This is a response to a lack of regular access to a supervisor specially qualified to deal with health care professionals under stress.
8. ZVITAMBO should introduce periodic refresher courses so that clinic staff and counselors are equipped to handle new information, as well as to cope more effectively with such matters as possible burn-out.
9. ZVITAMBO should create a top-level management position to provide administrative backup, which would relieve the PI and PM of attending to the details of running the project. Capacity building would be in the scope of work of the Zimbabwean local manager.
10. ZVITAMBO staff should develop a community advisory board comprised of study subjects and community leaders in order to strengthen the study's ties to the community. This is a standard requirement of all NIH-funded HIV research.
11. ZVITAMBO should limit the number of add-on substudies added to the main study.
12. ZVITAMBO should seek technical assistance from Linkages' experts in infant feeding practices. Take advantage of their expertise in the study of infant feeding practices (e.g. Piwoz's work in Zambia).
13. ZVITAMBO should explore avenues for increasing awareness of the benefits of knowing one's HIV status.
14. ZVITAMBO Should redesign/refine substudy C and submit a proposal (with full Budget) for review and funding independent from primary studies.

Recommendations to USAID/Harare and ZVITAMBO

1. ZVITAMBO staff should participate in the regular quarterly coordination meetings of the Mission and Cooperating Agencies in Zimbabwe.
2. USAID/Harare and ZVITAMBO should schedule biannual briefings for the Mission to (1) keep the Mission apprised of research activity progress (2) how ZVITAMBO is contributing to the Mission's response to HIV/AIDS, and (3) how ZVITAMBO is contributing to the national response to HIV/AIDS.
3. USAID/Harare and ZVITAMBO should seek ways to provide input to R4's and other reporting on results of mission investments in HIV/AIDS activities. ZVITAMBO should be showcased as an example of how investment in research moves policies and programs.

Recommendations to USAID regarding the management of clinical trials

1. Research proposals submitted to USAID should be required to include a section detailing how the project will contribute to local research and/or service delivery capacity.
2. Research proposals submitted to USAID for support of research should be required to include a section detailing the project's exit strategy.
3. Investigators and USAID mission personnel should meet regularly to review the progress of

the research, one purpose being to identify ways the research can support the mission's overall strategy.

4. USAID funded clinical trials should be required to establish community advisory groups. USAID funded operations research should be strongly encouraged to do the same.

INTRODUCTION

On March 15, 1999 a team consisting of four members and one technical advisor commenced a 5-day review of ZVITAMBO, a vitamin A intervention trial in Harare, Zimbabwe. The purposes were (1) to perform a midterm review of the ZVITAMBO project; (2) to inform USAID Zimbabwe and USAID Washington (AFR and G bureaus) on its funding decisions regarding the ZVITAMBO study; and (3) to guide future decisions by USAID in supporting clinical trials. This review focused on technical, ethical and public health issues in this continuously evolving field.

SCOPE OF THE REVIEW

The scope of work of the team is attached to the end of this report as Appendix A. The team was asked to address the following questions:

1) What is the public health significance of the ZVITAMBO study? What impact might its results have on maternal and child health, breast feeding, and HIV/AIDS programs in Zimbabwe and/or other countries?

2) Is ZVITAMBO able to test its stated hypotheses?

3) What reporting mechanisms are in place or could be put in place to better inform donors of the progress of the study.

4) Is the ZVITAMBO being managed in a cost-effective manner?

5) Are study procedures adequate for the protection of human subjects?

6) How does ZVITAMBO build capacity within the public health sector to address the HIV/AIDS epidemic and health research in Zimbabwe?

7) Are there ways that ZVITAMBO operations can be strengthened or streamlined?

8) Subsequent to the questions posed in the original scope of work, the team was asked by the Zimbabwe mission to address an additional question: What is the relationship between ZVITAMBO and the ministry of health, the University of Zimbabwe?

COMPOSITION OF THE REVIEW TEAM

The review team was comprised of four individuals with the following areas of expertise:

- Vitamin A research
- Clinical research and services (pediatric, maternal-child health)
- Mother-to-child transmission issues (including infant feeding, HIV counseling and testing)
- Epidemiology and study design
- HIV/AIDS interventions (including socio-cultural issues)
- Gender and HIV research ethics in developing countries.

Team members were:
David Stanton MPH (USAID): Team Leader
Dr. Virginia Vitzthum (USAID)
Dr. Wafai Fawzi (Harvard University)
Dr. E. Maxine Ankra (The Uganda Christian University of Uganda)

Dr. Timothy Quick: Technical Advisor (USAID)

Activity of the review team.

The team spent four days touring the ZVITAMBO offices, laboratory facilities, data processing offices, recruitment sites. The team observed the process of recruitment, follow-up, and accompanied ZVITAMBO staff on home visits. The team also met with key constituents of the study. These included the Vice Chancellor or the University of Zimbabwe, the Director of the Harare City Health Department, and the Director of UNICEF Zimbabwe.

ZVITAMBO STUDY DESIGN

Appendix B contains a full description of the study design, which is summarized below. The ZVITAMBO study is a double blind placebo controlled trial of 14000 mother infant pairs randomized into one of four arms, and studied for one year. All HIV infected women/infants pairs (approximately 4200) plus 4200 uninfected women/infant pairs will be studied for an additional year. The study of HIV+ and HIV- women was designed to blind participants and study staff to the HIV status of women in the study. Major outcomes for the study are infant mortality, the rate and timing of vertical HIV transmission, and post partum infection of mothers.

The study is designed to address the following key questions:

1. Does vitamin A supplementation of infants at birth reduce infant mortality?
2. Does vitamin A supplementation of infants at birth reduce the incidence of HIV infection via breastmilk?
3. Does vitamin A supplementation of mothers postpartum reduce the incidence of postpartum HIV infection?
4. *After WHO modified its stand on HIV and breastfeeding, the investigators elevated the purpose of one of it sub-studies to the level of a key question:* "What is the best way to inform HIV infected mothers about the risks of breastfeeding to their infants and the options that are available to them?"

The four arms of the study are:

1. Both receive vitamin A (50,000 IU for infant and 400,000 IU for mother)
2. Both receive placebo
3. Mother receives vitamin A; baby receives placebo
4. Baby receives vitamin A; mother receives placebo

Sub studies

In addition to the one and two year studies addressing the principal hypotheses, three substudies are proposed.

Substudy A:

One hundred HIV+ women from each study arm (400 total) will be examined to determine the effect of maternal vitamin A supplementation among HIV+ women on plasma and breast milk viral load and lymphocyte subsets.

Substudy B:

Two hundred infants born to HIV+ mothers (and 50 infants born to HIV- mothers) from each study arm (1000 total) will be enrolled into a study of the efficacy of vitamin A supplementation of neonates lactating mothers, or both during the immediate post-partum period on infant vitamin A status.

Substudy C:

270 children will be identified for a study of the acute-phase response and its relationship to serum retinol as an indicator of vitamin A status.

Linkages breastfeeding study:
Negotiations are underway to add an operations research study to be conducted by the HN/POP cooperative agreement Linkages. The purpose of the study will be to develop appropriate methods for helping women make choices surrounding breast-feeding in light of the link between HIV and breastfeeding. This study will probably take place in antenatal clinic settings.

Timetable: ZVITAMBO began recruiting subjects in November of 1997 and is scheduled to end recruitment in March 2000 and complete data collection in December 2002.

FINDINGS AND RECOMMENDATIONS

1. The public health significance of ZVITAMBO

The ZVITAMBO project has important public health significance. If vitamin A supplementation were found to reduce infant mortality, as hypothesized, this would be a low cost intervention that could be introduced in Zimbabwe and other countries with high infant mortality rates. Similarly, the study set out to address important questions related to the role of vitamin A, breast feeding, and HIV transmission. Given the very high prevalence of HIV infection among pregnant women in this population, and indeed in many sub-Saharan African countries, the results from this study will be directly relevant to HIV treatment and prevention programs in these settings.

Infant mortality: The annual global death toll for children under five is approximately 12.5 million. Most of these deaths are from preventable causes, and most of these occur in the first months of life. Data from a study of neonatal Vitamin A supplementation in Indonesia suggested that neonatal mortality could be reduced by as much as 60% with vitamin A supplementation of infants at birth. However this study has not been replicated. ZVITAMBO, unlike previous studies, is designed to test this hypothesis and has a large enough sample size to address this question.

HIV transmission via breastfeeding: Annually 600,000 children under 15 years of age are infected with HIV. Mother-to-child transmission accounts for 90% of these cases, and approximately one third of these are infected via breastfeeding. It is theorized that vitamin A will improve the integrity of the gastric mucosal lining and thus provide additional protection against HIV infection. If this theory holds true it will be the first time that vitamin A has been shown to prevent any infection. Other studies have already demonstrated that Vitamin A reduces the severity of some infectious diseases. Currently the only known way to prevent HIV transmission via breastfeeding is to use replacement feeding, which also carries a high mortality risk in developing countries. If vitamin A supplementation reduces breastmilk-associated HIV transmission, this will give a second prevention option to women who have very few options.

Postpartum HIV infection: Women are especially susceptible to HIV infection postpartum for a number of biological and sociological reasons. The process of birth disrupts the integrity of the endometrium and vaginal mucosa, which theoretically raises the women's susceptibility to HIV infection during intercourse. It is hypothesized that vitamin A given postpartum will hasten the repair of the disrupted vaginal mucosa and reduce this susceptibility.

2. The scientific merit of ZVITAMBO

The review team agreed that ZVITAMBO is a scientifically sound study able to test the stated hypotheses. The technical infrastructure and expertise, both local and expatriate are impressive. The investigators have put in place a data collection and management system that is essential to addressing these questions. The investigators have the appropriate sample size based on assumptions that have been well considered. There has been a change in the time line for recruitment, which is now to be completed between December 1999 and March 2000. Follow-up

rates are acceptable.

3. Reporting mechanisms

While ZVITAMBO investigators send biannual and other reports to CIDA and Johns Hopkins University, including DSMC reports, these have not been sent to USAID/Washington and USAID/Harare. Reports to JHU have not been routinely forwarded to USAID/Washington.

> **Recommendation: All reports to CIDA and JHU will be copied to Dr. Francis Davidson at USAID/W, as well as the USAID mission in Harare.**
>
> **Recommendation: ZVITAMBO should participate in the regular quarterly coordination meetings of the Mission and Cooperating agencies in Zimbabwe.**
>
> **Recommendation: ZVITAMBO should try to schedule biannual briefings for the Mission to (1) keep the Mission apprised of research activity progress (2) how ZVITAMBO is contributing to the Mission's response to HIV/AIDS, and (3) how ZVITAMBO is contributing to the national response to HIV/AIDS.**

5. Cost effectiveness of ZVITAMBO management

One of the chief concerns raised by the USAID/Harare mission in regard to the ZVITAMBO project had to do with the cost of the project and the need for additional funding. Why the study required additional funds was unclear and it remains unclear at this time. While we find no suggestion for misuse of funds, the budget process used by JHU and ZVITAMBO preclude a complete understanding of how the need for increased funding arose and what the actual current needs are. In reviewing the documents made available to the team, and in interviewing the investigators, three key events appear to have contributed to cost over runs.

The first event was the decision by the ZVITAMBO data safety monitoring committee to insist on a larger sample of HIV-uninfected women in the two-year study. The original design had called for following 4200 HIV infected women and only 200 uninfected women as controls. It was the opinion of the DSMC that the only way to assure blinding of the participants' HIV status was to enroll equal numbers of women into the study. The PI originally estimated that this would add an additional $200,000 to the cost of the study. It is now recognized by the PI that this was an under estimation and in fact the additional cost exceeded $600,000. However, no revised budget has ever been submitted that breaks out the specific marginal costs of adding 4000 women to the study.

The second event was the insistence by the Ministry of Health that ZVITAMBO buy health insurance for all study participants. This created a 6-month delay, during which no study subjects could be enrolled. Unfortunately, this delay began after most of the study staff had been hired and labs built. As a result, six months of operating expenses had to be paid while staff and labs remained idle. As with the first event, it would be helpful to break out of the specific added costs to the overall study due to this delay.

The third event was the change in breastfeeding policy by WHO, UNAIDS, and UNICEF in 1998. Prior to this, all women in developing countries were recommended to breastfeed without regard to HIV status. The new policy states that women should be informed of the risks and options available to her. The ZVITAMBO investigators felt obligated to offer this information to all women participating in the study. This slowed the process of enrollment and took up additional staff time.

To summarize, while it is logical to presume that these three events could increase the cost of ZVITAMBO, none of the budget documents provided to USAID in the request for additional funding, or to the review team during its visit, show how the budget was actually affected by these events.

> **Recommendation: USAID/W should request a cost accounting from Johns Hopkins University for the ZVITAMBO project.**

5. Protection of human subjects

The ZVITAMBO project faces three significant challenges in protecting human subjects: (1) The recruitment environment, (2) the knowledge and services vacuum of Zimbabwe, and (3) the changing policy environment surrounding breastfeeding and HIV.

The recruitment environment

Women are recruited in their first day post partum. In the primary recruitment site, Harare Hospital, mothers are in eight bed wards that have no curtains or other equipment to offer privacy. The rooms are crowded with other family members. It is in this environment that women are given a group presentation on the study and then invited to have a one-on-one discussion with the recruitment nurse if she elects to participate in the trial. The most troubling finding was the rushed nature of the recruitment process. The time span for recruitment in the postpartum ward can be quite short and the setting is very harried if several women in the ward decide to participate. Nurses are understandably focused on quickly collecting the needed data and blood samples; and there is little time for much reflection and questioning.

> **Recommendation: The team's strong recommendation is to pair the recruiting nurse with a recruiting counselor. A paired counselor could alleviate this burden on the nurse, and provide an important service to potential participants. It is possible that this will also increase the recruitment rate.**
>
> ***This is the team's strongest recommendation.***

The knowledge and services vacuum of Zimbabwe

Zimbabwe has an HIV prevalence of about 30% in antenatal clinics. As such it has one of the worst HIV epidemics in the world. Unfortunately, the government response has not been commensurate with the extent of the epidemic. Zimbabwe had only one VCT center, located far from Harare, at the time of the review (USAID is providing funding for 8 additional sites). Care and support services are scarce for a country with one of the highest HIV prevalences in the world. Public education about HIV/AIDS is rare and the disease is highly stigmatized. When the

review team toured the teaching hospital post partum wards, there was no evidence of any effort to inform women about their personal HIV risk or the risk to their newborn children.

A questionnaire was administered to new recruits by a social scientist recently hired with funds from Linkages. Approximately half of the women who chose to participate in ZVITAMBO indicated that they did so to learn their HIV status. However, only 11% of women participating in the study ask to know their status. This is a complex issue. There are few data on the proportion of Zimbabwean that choose to know their HIV status in research settings or in voluntary counseling and testing settings. It can be argued that in the absence of a supportive environment, people do not choose to know their HIV status.

The review team held extensive discussions with the investigators on this subject. Various suggestions were considered. One of these was to give women their results on the same day the blood sample is drawn. Subjects are screened with a rapid test and confirmed later by ELISA and Western blot. Unfortunately, the investigators have had a high number of false positives with the rapid tests and feel that these results need to be confirmed before results can be given out. A second suggestion was to give subjects the option to know their results at each follow up throughout the project. This cannot be done currently as the DSMC has decided that to offer HIV test results beyond the first follow up visit would be coercive. The investigators agreed that some participants might not remember the details of what they consented to at the time of delivery. Therefore the investigators decided that the recruitment information would be repeated at the first follow-up visit.

> **Recommendation: It was agreed that the information given to subjects at recruitment would be repeated in its entirety at the first follow-up visit as this is the last opportunity to specifically present women with the option of knowing their HIV status. If a woman chooses to know her status, two professional counselors are available at the follow-up clinic site.**

The changing policy environment surrounding breastfeeding and HIV.
The ZVITAMBO investigators have modified their recruitment process to include the provisioning of information about the risk of HIV transmission via breastfeeding. The team found that study participants had limited time at recruitment to decide that they did not want to breastfeed. Therefor the investigators embarked on an effort in antenatal clinics to strengthen HIV/AIDS awareness and promotion of ZVITAMBO. The hope is that future project participants come for delivery with the knowledge of their HIV status and the implications of that status for their breastfeeding decisions. Even when these activities at the antenatal clinics are implemented, these study participants will be recruited at the time of delivery with the usual detailed procedures pertaining to consent, data collection, etc.

> **Recommendation: The investigators are encouraged to continue their efforts to strengthen awareness of the project by potential subjects prior to delivery. More intensified use of the media would contribute toward the much needed information, education and communication campaign in the capital area. ZVITAMBO's efforts in this direction also would help to reduce the stigma associated with AIDS, a factor that limits the uptake by mothers of the test results.**

Use of brand-named equipment.
Protection of subjects must also include preventing the undue influence of those standing to monetarily profit from those subjects. In this case, the use of measuring devices displaying the Ross Laboratories logo is not acceptable. Ross Labs manufactures and promotes infant formula. Brand recognition is critical to this promotion. The use of Ross labeled materials may lead some mothers to believe that this is a brand sanctioned by ZVITAMBO and/or the hospital staff. This is an especially critical and difficult issue for women who are HIV+ and elect not to breastfeed.

> **Recommendation: Either unlabeled devices or those in which the Ross or any other logo is thoroughly eradicated should be used.**

6. Capacity building

ZVITAMBO is the largest research project currently being conducted in Harare, Zimbabwe. It is raising public awareness in a societal context still showing little capacity to cope with the HIV/AIDS epidemic and the burden of maternal and infant morbidity and mortality. The provisioning of free counseling and testing to the study subjects and their partners is a public health service that has been largely out of the reach of most Zimbabweans. The size of the sample makes this aspect of the study a significant public health contribution to the community as a whole.

The project has contributed to strengthening the capacity of Zimbabwe to address research questions related to micronutrients as well as HIV. Significant time and resources have been invested in setting up modern HIV/vitamin A labs. The data management infrastructure that is in place is excellent and should be very valuable for future research activities. In addition to contributions to research capacity, the project has also trained a large number of nurses and other health professionals in counseling related to HIV infection, and these will be a valuable resource as Zimbabwe starts to address the HIV epidemic.

ZVITAMBO has established state of the art laboratory facilities. These facilities will become the property of the University of Zimbabwe at the completion of the study. However the exact details of how this will take place have yet to be established. The equipment purchased by ZVITAMBO is expensive and requires fastidious upkeep even when not in use. The investigators estimate that at least $1000 per year is required to keep the lab equipment in working order.

> **Recommendation: ZVITAMBO investigators are urged to commence planning their exit strategy. Issues to consider are the sustainability of the infrastructure, the value of the facilities to future research and other activities, when all components are maintained as a single package, and willingness to accommodate other researchers who may be in a position to take over when ZVITAMBO is completed.**

A related issue is the $500,000 that USAID/Zimbabwe invested in the trial. In spite of ZVITAMBO contributions to building future research program capacity, the mission doesn't feel that this has been a particularly good investment in support of its HIV/AIDS objectives. The

review team attributes this largely to the failure of ZVITAMBO to keep the mission regularly and fully informed on the progress of the trial and particularly how ZVITAMBO is contributing and building capacity of the national effort to address HIV/AIDS. This will make it difficult to attract additional field support funds for or to use ZVITAMBO as a precedent/model for attracting mission field support for trials in other countries.

Recommendation: ZVITAMBO should engage the Harare mission to provide input to R4's and other reporting on results of mission investments in HIV/AIDS activities. ZVITAMBO should be showcased as an example of how investment in research moves policies and programs.

7. Ways that ZVITAMBO operations can be strengthened

The review team has the following additional recommendations for ZVITAMBO:

Recommendation: Provide additional professional upgrading for counseling. Once the nurse/midwife counselors are trained, there is no apparent further systematic development of skills. Adhoc means have been devised by the counselors to relieve the emotional pressures of their work. This is a response to a lack of regular access to a supervisor specially qualified to deal with health care professionals under stress.

Recommendation: Introduce periodic refresher courses so that clinic staff and counselors are equipped to handle new information, as well as to cope more effectively with such matters as possible burn-out.

Recommendation: Create a top-level management position to provide administrative backup, which would relieve the PI and PM of attending to the details of running the project. Capacity building would be in the scope of work of the Zimbabwean local manager.

Recommendation: While the ZVITAMBO project has excellent links to academic and governmental structures, its links to the community are weak. We recommend that ZVITAMBO establish a community advisory board (CAB) made up of key community members, such as clergy, business, other opinion leaders, as well as study participants who are willing to volunteer their time. Such a committee could assist the investigators in identifying the strengths and weaknesses in the recruitment and follow up and serve as a much needed "ear to the ground" to alert the investigators of public misconceptions about ZVITAMBO. They could also serve as a conduit to the community for the dissemination of study results. Community advisory boards are a requirement by the NIH in all HIV-related clinical trials.

Recommendation: Limit the number of add-on substudies. The team expressed concern about the number of substudies that the investigators could potentially initiate during the life of the study. Each substudy will add to the already heavy workload carried: administrative and supervisor responsibility increase with every

funded project. Timely reporting and closing out of the main components of ZVITAMBO will be hampered if other ideas in development are pursued. As primary funders of the overall study, both CIDA and USAID should be consulted and have an opportunity to review proposals for add-on studies. To the extent possible, add-on substudies should not just budget marginal costs of supplies, equipment, etc., but should "pay the freight" for overall project costs such as personnel and facilities.

8. The relationship between ZVITAMBO, the Harare City Health Department, the Ministry of Health and the University of Zimbabwe

There are apparently excellent links with the MOH and City Health Department. The links with the University are probably acceptable for the purposes of implementing this project. The Vice Chancellor of the University is supportive of the project. However, we did not have a chance to meet with any of the senior administrators at the medical school where the work is actually implemented. The medical school is likely to continue taking the lead in medical research in the country. The investigators indicated that they are interested in seeing that the infrastructure created by the study is used as a base for future studies, an effort that requires a strong commitment from the senior administration at the medical school. In addition, a greater involvement of Zimbabwean investigators in administering and "owning" the project is fundamental to the success of this project and other collaborative activities with US institutions.

A supportive relationship has been built up between ZVITAMBO and the Ministry of Health, University of Zimbabwe, the City Health Department of Harare, and other organizations. This bodes well for the completion and implementation of the study results. In addition to technical infrastructure development, human capacity building also is reported. The project leadership is sensitive to the psychosocial demands placed on the clinic and field staff and the need for emotional support at the individual level. These are being addressed among several ways through weekly meetings for updating clinical and counseling staff, calling in a psychologist to review staff support needs, and leave time for the counselors who work with women seeking to know their HIV status.

FUNDING RECOMMENDATION

It is the conclusion of the review team that the study is of sufficient merit and importance for USAID to continue to provide financial support, especially in light of the substantial investment already made. However, USAID should make this funding contingent on the following recommendations:

1. ZVITAMBO should provide a budgeting of the study that makes clear (in US dollars) the costs of the components of the study (i.e. one-year, two-year and sub studies). In the event that the current request proves insufficient, it will then be possible to determine the source of the increased costs, facilitating selected cuts in the project and/or selected funding of essential elements of the study.

2. ZVITAMBO should modify hospital based recruitment procedure: recruit study subjects using nurse-counselor teams rather than solo recruiters. *This was discussed with ZVITAMBO staff during the review.*

RECOMMENDATIONS TO USAID REGARDING THE MANAGEMENT OF CLINICAL TRIALS

This review of the ZVITAMBO clinical trial made evident, actions that USAID should consider when supporting future clinical trials. Three recommendations arose from this experience: the need for USAID supported trials to contribute to local capacity; the ability of clinical trials to support mission objectives; and the need for community participation in large clinical trials.

Capacity Building

Large-scale clinical trials create an excellent opportunity to increase the local capacity to conduct research and to provide services. This opportunity stems from both the technical and the human capital brought to the host country. The presence of scientists and access to state of the art equipment should be exploited to provide training to local scientists and students in areas such as study design, data collection, laboratory technology, basic science, statistical analysis etc.

> **Recommendation: Research proposals submitted to USAID should be required to include a section detailing how the project will contribute to local research and service delivery capacity.**

> **Recommendation: Research proposals submitted to USAID for support of research should be required to include a section detailing the project's exit strategy.**

Support of Mission Strategic Objectives

Often centrally funded research activities do not attempt to contribute to the strategic objectives of the local mission. This may occur because the research is seen as peripheral to the mission's objectives. However it is very likely that centrally funded research is being conducted in a particular county because the area of study is prominent in that country. For example malaria researchers seek countries with high malaria prevalence. In such cases it is very likely that the mission has a health SO to which the research may contribute significantly.

> **Recommendation: Investigators and USAID mission personnel should meet regularly to review the progress of the research, one purpose being to identify ways the research can support the mission's overall strategy.**

Community Participation

Clinical trials than enroll large numbers of people for many years can have a profound impact on the communities they are studying. This impact can be both positive and negative. Often the populations selected for study have minimal education, and may have only a limited understanding of the purpose of research. This can lead to problems ranging from poor recruitment and retention in the study, to resentment of expatriate and host country investigators, to out right

hostility toward the study. Allowing community members to be represented by an advisory committee has been shown to substantially mitigate these problems while adding little to the management burden of the investigator. Members are unpaid and can be selected to represent a cross section of the community. The National Institutes of Health requires community advisory groups for all its HIV/AIDS clinical trials. The success of this is well established.

Recommendation: USAID funded clinical trials should be required to establish community advisory groups. USAID funded operations research should be strongly encouraged to do the same.

PERSONS MET

USAID

Rose Marie Depp, Mission Director
Roxana Rogers, PHN officer
Patrick Osewe, CDC HIV/AIDS advisor
William Martin, Program Officer

ZVITAMBO

Dr. Jean Humphrey: Principal Investigator
Dr. Peter Illif, ZVITAMBO Co-Investigator. Senior Lecturer, Pediatrics Dept., University of Zimbabwe.
Prof. Kusum Nathoo, ZVITAMBO Co-Investigator. Pediatrics Dept., University of Zimbabwe

ZIMBABWE NATIONAL AIDS CONTROL PROGRAM

Mrs. Margaret Mehlomakulu, Assistant Coordinator

HARARE CITY HEALTH DEPARTMENT

Dr. Agnes Mahomva, ZVITAMBO co-investigator. Medical Officer, City of Harare Health Dept.

MINISTRY OF HEALTH

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ANNEX A: SCOPE OF WORK FOR THE REVIEW TEAM FOR THE ZVITAMBO PROJECT

Scope of Work for the Review Team for the ZVITAMBO Project

The purpose of the review team visit to Zimbabwe is to inform USAID Zimbabwe and USAID Washington on its funding decision regarding the ZVITAMBO study.

The team is asked to answer the following questions:

1) Does the public health significance of the study warrant USAID funding?
   a) What impact will the results of ZVITAMBO (positive or negative) have on MT and HIV/AIDS programs in Zimbabwe and or other countries?
   b) What impact will the results of ZVITAMBO (positive or negative) have on USAID funded programs?
2) Is ZVITAMBO able to test the hypotheses posed by the investigators?
   a) Does study the design fit the stated objectives?
   b) Does the study design have a reasonable chance of successful completion?
   c) What are the costs and benefits of the various sub-studies that are underway or proposed?
3) Is the study being managed in a cost-effective manner?
   a) What mechanisms exist to inform funders of changes in the budget?
   b) What local accounting mechanisms are in place?
4) Do the design and management of the study allow for the ethical recruitment and treatment of participants?
   a) Are subjects able to give informed consent immediately post partum?
   b) Are subjects given appropriate information regarding HIV testing, breast feeding, and HIV prevention?
   c) Do the study design and implementation safeguard the confidentiality of subjects?
   d)
5) Are there ways that the project operations can be strengthened regarding the above questions?

This review will focus technical issues and ethical concerns in this continuously evolving field. The research team will be comprised of four individuals with the following areas of expertise:

- Vitamin A research
- Clinical research and services (pediatric, maternal-child health)
- Mother to child transmission issues (including infant feeding, HIV counseling and testing)
- Epidemiology and study design
- HIV/AIDS interventions (including socio-cultural issues)
- Gender and HIV research ethics in developing countries

ANNEX B: ZVITAMBO STUDY DESIGN

Annex B

Subjects taking vitamin A supplements had higher concentrations of serum retinol compared to those not taking supplements, an association which remained after adjusting for CD4%. Retinol supplementation of cultures with the lowest baseline cytokine levels resulted in increased sIL-2R production, even among lymphocytes obtained from patients with apparently normal vitamin A status. This study demonstrated that vitamin A supplementation can result in apparently better vitamin A status, even among patients who are not severely deficient, and that lymphocytes even from apparently vitamin A sufficient patients may be retinoid responsive.

E. Analysis of archived serum samples from WHO Perinatal HIV Transmission Study:

The University of Zimbabwe was one of two sites for the recently completed Perinatal HIV transmission Study coordinated by WHO/Geneva. Dr. Michael Mbizvo (Dept of OB/GYN) was the study coordinator in Harare. This study enrolled a cohort of 700 consecutively selected HIV seropositive pregnant women making their first antenatal visit to either Harare Maternity Hospital or one of three antenatal clinics. Blood samples were collected and archived at 32 and 36 weeks gestation and cord blood was collected at delivery. Infants were followed for two years when they were tested for HIV status. A 92% rate of follow-up was achieved. Preliminary analysis indicates overall mother-to-child transmission rate was 30-40% (unpublished findings, Dr. Mbizvo).

We are currently planning to analyze the 32-week gestation specimens for retinol concentration to determine if maternal vitamin A deficiency during pregnancy, as indicated by low serum retinol concentration is a risk factor for mother-to-child HIV transmissionThis study will be an important replication of the study reported from Malawi by Semba, et al, which demonstrated a dose response relationship between increasing maternal serum retinol during pregnancy and decreasing rate of mother to child transmission.[7] If all 32-week samples from HIV-positive women are analyzed, we will have power to detect a difference of 3 μg/dl or greater, between mean serum retinol concentrations between transmitters and nontransmitters, assuming a SD = 15 μg/dl. The difference observed in Malawi was 6 μg/dl.

IV. Study Design and Methods

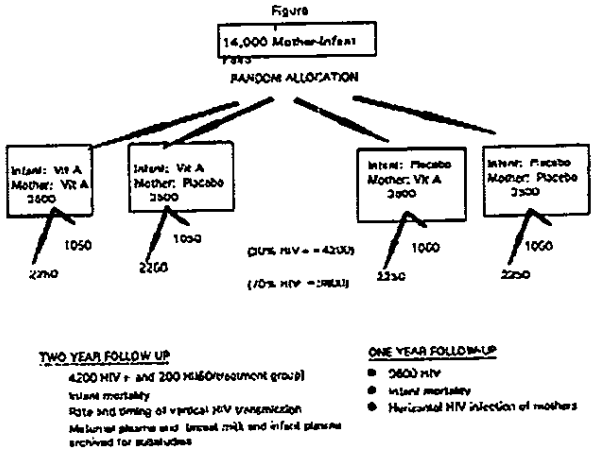
A. Introduction:

We propose to determine the efficacy of a single oral dose of vitamin A given to mothers, their infants or both mother and infant during the immediate post-partum period on infant mortality, HIV transmission during breast feeding, and horizontal HIV infection of mothers by sexual partners during the first year post-partum. In Zimbabwe, where the study will be carried out, there is no policy or programs for vitamin A supplementation of post-partum women or neonates. The study will be

a randomized, double-masked, placebo-controlled trial of 14,000 mother infant pairs who will be randomized to one of four treatment arms:

	Vitamin A Dose	
Treatment	Infant	Mother
I	50,000 IU	400,000 IU
II	50,000 IU	placebo
III	placebo	400,000 IU
IV	placebo	placebo

Figure 1 illustrates the study design:



Two year study: Approximately 30% of mothers are expected to be HIV+ at delivery. All HIV+ mothers and their infants (~4500 mother-infant pairs) plus 200 HIV seronegative mother-infant pairs (50 from each treatment group) will be re-examined at 6 weeks, and then during 3 monthly clinic visits for 2 years. Inclusion of the HIV seronegative pairs in the two year follow-up clinic will keep the mothers and the clinic staff blinded to the mother's HIV status. Maternal breast milk and plasma and infant plasma samples will be obtained and archived at each visit. Infants born to HIV+ mothers will be tested for HIV by ELISA at 24 months, positives confirmed by Western Blot, and for those confirmed to be infected, archived pre-prepared plasma samples will be tested by qualitative PCR from approximately 3 time points per child to estimate time of infection. Major outcomes will be vertical HIV transmission rate and timing and infant mortality.

One year study: HIV-negative mother-infant pairs not selected for the two year follow-up will be re-examined at 6 weeks and then during 3 monthly clinic visits for one year.

Mothers will be tested for HIV infection by ELISA at 6 and 12 months and those positive confirmed with a second ELISA. Major outcomes will be horizontal HIV infection of mothers and infant mortality. We are currently seeking permission from ethical review boards to also collect a blood sample from all infants in the one year study at 12 months of age. This will enable us to calculate the rate of mother to child transmission among the approximately 550 women we estimate will sero-convert during the post-partum year, and to identify risk factors for this transmission.

Substudies:

Substudy A. Effect of maternal vitamin A supplementation among HIV+ women on plasma and breast milk viral load and lymphocyte subsets:

Between baseline and the 6 week follow-up, 400 HIV+ women (100 from each treatment group) will be randomly selected by computer and this list sent to the laboratory. For these samples, CD4/CD8 will be determined from fresh plasma samples at each visit, and archived plasma and breast milk samples obtained at each time point will be assayed for viral load and retinol concentration. AGP and CRP will also be measured in plasma samples.

Substudy B. Efficacy of vitamin A supplementation of neonates, lactating mothers, or both during the immediate post-partum period on infant vitamin A status:

The infants of the 400 women selected for substudy A will be recruited to substudy B. In addition, from the Two-Year study, 400 infants born to HIV+ women will be randomly selected from each of the four treatment arms. These 800 infants born to HIV+ women, plus the 200 infants born to HIV- women selected for the Two-Year study (50 from each treatment arm) will be asked to participate in substudy B. This substudy of 1000 infants will determine the impact of the interventions on infant vitamin A status throughout the first year of life. Serum retinol will be measured on baseline serum samples from these infants and the modified relative dose response test will be carried out on these infants at 6 weeks, 6 months and 12 months; AGP and CRP will be measured concurrently. Inclusion of the larger number of infants born to HIV+ mothers in this substudy will allow stratified analysis for infants who are exposed but not infected (~70% of infants born to infected mothers or 140 per treatment arm), and infants who become infected themselves (~30% or 60 per treatment arm).

Substudy C. The acute phase response (APR) and its relationship to serum retinol as an indicator of vitamin A status:

A total of 270 children will be identified for this substudy. Beginning in the 13th month of the trial, 780 children will come to the clinic for their 12 month visit each month. Of these, 70% (546) will be HIV seronegative born to HIV seronegative mothers in the One Year Study (uninfected infants). For a 6 week period, we will request parental permission to obtain a blood sample from the 12 month old children graduating from the One Year Study. These children will be reexamined

in their homes one week later to identify 30 children who developed fever, and 30 children who developed diarrhea during that week. These 60 children will be asked to enroll in the APR substudy. An estimated 30% (234) of the 780 12 month old children attending clinic each month will be in the Two Year Study, born to HIV+ mothers. For an 11 month period, all these infants will be screened by ICL for HIV sero-status at 12 months, and reexamined in their homes one week later to identify 30 HIV+ children (infected) who developed fever, 30 HIV+ children who developed diarrhea during that week and 30 children with each symptom who are HIV seronegative (exposed, uninfected). All children enrolled in the substudy, will be reexamined 5 times over the succeeding 3 weeks when a 3 ml blood sample will be drawn for measurement of AGP, CRP, neopterin, b2-microglobulin, soluble CD8 and serum retinol. Their 12 month clinic samples will be assayed for the same outcomes to determine prodromal concentrations. Children, rather than mothers, are being studied because their much higher rates of infection will facilitate obtaining the required sample size. However, we expect findings will be relevant in interpreting serum retinol concentrations during all human infection.

B. Location:

The University of Zimbabwe (UZ) was historically the University College of Rhodesia and Nyasaland. It was incorporated by Royal Charter in 1953 and became the University of Rhodesia in 1971. The Medical School opened in 1963. Following national independence, the school became the University of Zimbabwe and it now has an student intake of 10,000. It has 10 faculties, most of which are housed at the main campus; most clinical departments of the School of Medicine are housed at Parirenyatwa Teaching Hospital, and operate both there and at Harare Hospital.

The Department of Paediatrics and Child Health is staffed by 11 faculty, three technologists, and three research nurses. It provides most of the clinical paediatric service for Harare and the northern two-thirds of the country, there being only three MOH paediatricians in post. Many of the department's research activities have been directed in concert with the MOH and focussed on development of effective management of common childhood diseases, for example Shigella dysentery and acute respiratory infection. There has been considerable emphasis on infectious disease research. Other active research areas include the extent and nature of the problem of "AIDs orphans" and alternative solutions and the epidemiology of perinatal mortality and morbidity.

The Department of Obstetrics and Gynaecology (OB/GYN) serves as the principle national resource for information, advice, research, training and specialist consultation on scientific, clinical, and technical aspects of reproductive health in Zimbabwe. The department operates and administers Harare Maternity Hospital (HMH), a referral maternity teaching hospital which attends to 20,000 deliveries annually. Low risk deliveries are attended in a system of primary care clinics

managed by the City Health Department (CHD) with technical direction from the Departments of OB/GYN and PEDs. Together, HMH and the CHD clinics constitute the Greater Harare Maternity Unit (GHMU) and have more than 35,000 deliveries each year. The GHMU is made up of two main divisions, the University division (staffed by 14 faculty, 6 research midwives, and 3 medical laboratory technologists) and the Government division (staffed by 34 physicians and a large complement of nursing staff). The department reviews Zimbabwe's programs and services which address reproductive health issues including vertical and sexual transmission of HIV. In this respect, the department maintains close links with the Maternal and Child Health Department (MCH) in the Ministry of Health and with the Zimbabwe National Family Planning Council (ZNFPC). These links facilitate the department's mandated and on-going role of translating scientific research findings into health care policy and programs.

The Faculty of Science is made up of ten Departments offering bachelor, masters and doctoral degrees to a student population of 1000. The faculty is actively engaged in scientific research which has led to a substantial number of publications in internationally renowned publications. Consequently, the faculty has attracted a sizeable amount of foreign funding for the establishment of infrastructure. One of its 10 departments, the Department of Biochemistry, is actively engaged in biochemical, biotechnology, food science, and nutrition research activities.

Women and infants will be enrolled from Harare Hospital and four antenatal clinics of the CHD (Highfield, Rutsanana, Glen View, and Mabvuku) and one semi-rural clinic of Mashonaland East Province, Epworth.

Follow up visits will be carried out in a clinic being rented from CHD located in Mbare. This clinic will have 11 consultation rooms, a private room for the project counselor and offices for the nursing supervisors. Administrative and data management offices are housed at 18 Van Praagh Avenue, Milton Park.

C. Eligibility Criteria:

Mothers and their infants will be eligible to participate in the trial if they delivered at one of the recruitment sites, if the mother has no life-threatening conditions (defined as Mother in ICU, imminent or definite eclampsia recorded in medical notes, maternal temperature >39°C); if the infant weighed at least 1500 grams at birth (IMR at Harare Hospital drops dramatically from ~40% among infants 1250-1499 grams to 14% among infants 1500-1749 grams); and if the infant does not have another life threatening condition (defined as infant being NPO).

D. Enrollment:

A total of 14,000 mother-infant pairs will be recruited. Enrollment will reflect the usual distribution of births in Harare: approximately 60% occur at Harare Hospital and 40% in City Health Service Clinics. Therefore, of the 14,000 mother-infant pairs, approximately 8000 will be recruited from the hospital and 6000 from the

CHS clinics. Enrollment will take place over an 18 month period, Monday-Friday (360 working days). Thus, approximately 22 mothers will be recruited each working day from the hospital (22x360~8000). This is about half of the total number of women delivering each day at Harare Hospital. Accordingly, each morning, all mothers delivering in the past 24 hours will be listed in order of the time of birth. Beginning with a random start, every other woman will be selected to determine if they meet eligibility criteria for the study. For women who are not eligible, or do not agree to participate, the next eligible woman on the list will be invited. In the five clinics, all eligible women delivering Monday-Friday will be invited to enroll to achieve the required sample during the 18 month period of enrollment. Based on previous experience with the WHO perinatal transmission study, we expect the majority of eligible women will agree to participate. However, we will monitor recruitment rates carefully, and if recruitment is inadequate in the hospital we will increase the proportion of deliveries invited to enroll and if inadequate in the clinics, we will begin enrolling on Saturdays.

A team of 8 specially trained nurses (3 in the hospital, 5 in the clinics) will be devoted full-time to the recruitment process. This number of nurses will permit a nurse to spend one hour or more, on average, with each woman. For mothers who agree to participate, written consent will be obtained following the appropriate consent form. A standardized questionnaire and chart review will be completed to obtain infant sex, birth date, weight, and length, maternal parity and age, method of delivery and infant presentation, gestational age and 5-minute Apgar score. The mother's address will be carefully recorded with a map drawn to facilitate locating it in the event a home visit is necessary. Blood will be collected from the mother by venipuncture and from the infant by heel stick. Colostrum will be collected by manual expression.

E. Treatment regimens

Capsule packets containing two oval-shaped capsules for the mother and one nippled capsule for the infant will be prepared by a team in Baltimore. Four types of packets will be prepared, corresponding to the 4 treatment arms:

I. 50,000 IU vitamin A nippled + 2-200,000 IU vitamin A oval;
II. 50,000 IU vitamin A nippled + 2-placebo oval;
III. placebo nippled + 2-200,000 IU vitamin oval
IV. placebo + 2-placebo oval.

Each pair of capsules will be packed in dark coloured zip-lock plastic bags to protect them from light and humidity. Vitamin A capsules contain vitamin E added as an antioxident (10 IU vitamin E per 50,000 IU vitamin A); placebo capsules contain oil with vitamin E only. A unique 6-digit ID number (00001 – 14000 + a check digit) will be printed on two stickers affixed to the plastic bag. The ID numbers will be randomly assigned to one of the four treatment arms. Assignment will be blocked by 12 to assure allocation to treatment will be balanced over time. Capsule packets will be arranged in numerical order in separate boxes. Sealed

copies of the allocation lists will be kept in sealed envelopes in Baltimore, by CIDA, the Data Safety and Monitoring Committee (DSMC), and by the Zimbabwe MOH.

After the enrollment nurse completes the questionnaire and collection of blood and milk specimens, she will select the next capsule packet from the appropriate birth weight strata box. The 6-digit number on this bag will be the assigned unique study ID for the mother-child pair. The nurse will observe the mother swallow the two oval capsules, and then remove one of the ID stickers from the capsule bag and place it in the appropriate place on the enrollment form, documenting receipt of the capsules by the mother. The nurse will snip the nippled capsule with a scissors, and squeeze the contents into the infant's mouth. After the infant dose is administered, the second ID sticker will be peeled off the plastic bag and placed in the appropriate place on the enrollment form documenting receipt of the capsule by the infant.

F. Justification of the dosages to be used and monitoring for acute toxicity to the maternal dose:

The WHO recommended dose for neonates, especially for those not being breast fed is 50,000 IU.[10] This dose has been shown to be safe[54] and, in one study, highly efficacious in reducing infant mortality.[11] Therefore, 50,000 IU will be administered to neonates in the trial.

For women of reproductive age, in whom pregnancy can not be definitively ruled out, the safe recommended dose is no more than 10,000 IU vitamin A daily to avoid the possible risk of teratogenicity.[53] Accordingly, on a population level, only small daily doses can be given to women during their reproductive years. The exception is women in the immediate post-partum period (up to 8 weeks post-partum) - a biologic window of opportunity when the risk of pregnancy is nil [75]. The WHO currently recommends that a 200,000 IU dose be used prophylactically for post partum women,[53] a recommendation based primarily on the availability of 200,000 IU capsules in the community. We will, however, give 400,000 IU because we believe it will be safe and optimize benefit. Four trials have reported health impacts of maternal post-partum dosing.[8,76-78] One of these trials administered a 200,000 IU dose,[77] two gave 300,000 IU,[8,78] and one gave 400,000 IU.[76] None reported any side effects, which makes sense when considered theoretically. Preschool children have been safely given 200,000 IU for many years. A 50 Kg woman's body weight, her plasma volume to accommodate the absorbed dose, and her liver size to process and store the dose is ~3-4 times greater than that of a preschool child. Therefore, it is reasonable to assume that a dose twice that given to preschool children can be easily tolerated and utilized by adult women. Why might a dose greater than 200,000 IU provide greater benefit to women? While the trial mentioned above which gave 200,000 IU did report an increase in breast milk retinol concentration, it was not placebo-controlled. In all the remaining efficacy trials a larger dose produced the observed benefits. Considered theoretically, the demands of the mammary gland over the

first year of lactation are about 400,000 IU (assuming 50 ug/dl, the concentration observed in well nourished women [40], 750 ml/day for first 6 months, 600 ml/day for second 6 months) in addition to the needs of the mother herself (~950,000 IU). Thus estimated total needs of a woman in her post partum year are ~1,350,000 IU, or 3.5 times that of the supplement dose we propose.

Pilot Safety Study:
Because there are no data collected in a rigorous fashion documenting women's tolerance of large doses of vitamin A, we will carefully monitor acute side effects among 1500 women and infants enrolled during a two month period prior to beginning the main trial. The protocol for this safety study is attached in the Appendix.

G. Selection of mother-infant pairs for Two Year Follow-Up and for the Substudy:

The baseline blood samples will be tested by the Immunochemical Laboratory Dipstick HIV1+2 Test (ICL) HIV-1/HIV-2 dipstick test (McDonald Scientific, Harare, Zimbabwe). This test was developed by PATH and evaluated by Zimbabwe AIDs Prevention Programme (ZAPP), McDonald, and PATH in Harare. The sensitivity and specificity of a single ICL test against 3 ELISA's (two Abbott HIV-1&2 3rd generation recombinant ELISA and one Behring Enzygnost HIV-1&2 2nd generation ELISA) were 99.6% and 95.7%, respectively. All ICL positive specimens will be tested by ELISA (Cambridge Biotech) and those confirmed positive will be provisionally assumed infected. Thus, of the ~9800 women who will test ELISA negative, approximately 421 will test ICL false positive and not be confirmed by ELISA. However, of the 4200 women who will test ELISA positive, only ~17 will be missed by the screening and test ICL false negative. These 17 women will be identified when they test ELISA positive at 6 months and their baseline blood is retested by ELISA. Baseline lymphocyte subset enumeration (CD4/CD8) will be carried out for all women who test ICL+ since flow cytometry must be carried out on fresh whole blood before ELISA results would be available. Women who are ICL and ELISA positive will be confirmed by Western Blot at 6weeks. The Western Blot will distinguish women who are HIV-1 infected, HIV-2 infected and HIV-1/HIV-2 co-infected. In the WHO perinatal transmission study, 7.6% of antenatal women were co-infected with HIV-1 and HIV-2, and none had isolated HIV-2 infection. However, this data is now 2 years old and the current epidemiology of these two infections is unknown. We are particularly concerned about misclassification of women with isolated HIV-2 infection since this infection is much less virulent and is only rarely transmitted either horizontally or vertically.

HIV status data will be immediately sent from the laboratory to the Data Shop. ID number and HIV status of the mother will be entered into a specially written computer program. The treatment code will be on one computer but no one in Harare will have access to it. Special programs will be written to randomly select:

1. 200 HIV-mothers and their infants, (50 from each treatment arm). Since there

will be a total of ~2250 HIV- women per treatment group, approximately 1 in every 45 HIV- women and their infants assigned to each treatment group will be selected. These 200 mother-infant pairs plus all HIV+ mothers and their infants will be invited to join the Two Year Study.

2. 400 HIV+ women (100 from each treatment arm) for substudy A. From these women, CD4/CD8 will be determined from fresh whole blood samples obtained at each visit, and viral load and retinol will be assayed in archived plasma and breast milk samples collected at each time point. AGP and CRP will also be measured in all plasma samples.

3. 800 infants born to HIV+ women (the infants of the 400 women selected for substudy A plus 200 additional infants born to HIV+ women from each treatment arm) for substudy A. A list merging these 800 infants with the 200 infants of HIV- women selected for the two-year Study (a total of 1000 infants) will be produced. These 1000 infants will be invited to join substudy B.The infants will be assessed by serum retinol at baseline and by the modified relative dose response test (MRDR) and serum retinol concentration at 6 weeks, 6 months, and 12 months for the vitamin A status substudy.

H. Follow-up

Mothers and infants will be requested to return to the clinic when the infant is 6 weeks. Before leaving the hospital at the time of delivery, they will be given an appointment card, listing the day and time of their 6 week clinic appointment, and a map showing the location of the clinic. At the 6 week clinic visit, the mother-infant pairs selected for the Two Year Study will be invited to enroll in this special follow-up and re-consented following the appropriate form. Infants selected for substudy B will be re-consented. Mothers and infants who are ill at the time of this study visit will be treated and/referred to other health institutions. Acute diarrhea and respiratory illness among infants will be managed according to WHO guidelines.[79] Children requiring oral rehydration will be managed in the clinic while children requiring intravenous rehydration and/or intravenous antibiotic therapy will be admitted to Harare Hospital. Similarly, children requiring in-patient management of other illnesses will be admitted. Acute maternal illnesses will be evaluated in the follow-up clinic and managed according to WHO guidelines;[80] women who require in-patient therapy will be referred to Harare Hospital where a full component of therapies and services are available.

H.1. 6 Week Follow-up:

At the first clinic follow-up visit, all infants will be registered by a study nurse supervisor. She will note which mother-infant pairs have been selected for the Two Year study or substudy B and refer these mothers to a nurse who will complete the informed consent process. Consenting mothers and their infants, will under go a physical examination by a trained nurse which will include clinical signs of HIV/AIDS, measurement of infant

weight, length and head circumference, history of maternal and infant illness requiring medical care since birth, a 1-week morbidity history, and a history of the frequency of breast feeding and use of complementary foods.

Blood specimens will be obtained from the mother and infant, and a breast milk sample will be obtained from the mother. Only nurses with extensive experience and training in drawing blood from infants will be assigned this task. Remaining mothers and their infants will be continued in the One Year Study. Follow-up of One-Year study mothers and infants will include measurement of weight, length, and head circumference and history of maternal and infant illness requiring medical care.

H.2 Visits 3, 6, 9, 12, 15, 18, 21, and 24 months:

All mother-infant pairs will continue to return to the clinic when the infant is 3, 6, 9, and 12 months of age. At 12 months, those in the One Year Study will be thanked for their participation and graduated from the study. The Two Year Study pairs will continue coming through the second year. The following tables list the laboratory follow-up of HIV- and HIV + women and infants, selected and not selected for the Substudy:

H.3 Laboratory Follow-up:

One Year Study (n=9600 mothers (M) and infants (I)).

Months Post-partum

Test	0	6wk	3	6	9	12
ICL	M			M		M
ELISA	M[1]I			M[2]		M[2]
Plasma arch.	M,I			M		M,I[3]
Milk arch	M			M[4]		M[4]
CD4/CD8	M					

[1] Women who are false positive by ICL test.
[2] Women who seroconvert at 6 or 12 months will require a second ELISA for confirmation: also includes women who are false positive by ICL.
[3] Permission currently being sought to collect infant blood at 12 months
[4] Flow cytometry will be run on women who sero-convert at 6 or 12 months

Two Year Study (n=4,400 mothers (M) and infants (I)).

Months Post-partum

Test	0	6wk	3	6	9	12	15	18	21	24
ICL	M									I[4]
ELISA	M[1]			M[3]		M[3]				I[5]
Western Blot		M[2]								
PCR[6]	I		I	I	I	I	I	I	I	I
CD4/CD8	M[1]									

Plasma arch.	M,I	M,I	M,I	M,I	M,I	M,I	M,I	M,I	M,I	M,I
Milk archived	M	M	M	M	M	M	M	M	M	M

[1] For women who are ICL+ only.
[2] For women who are ICL+ and ELISA+ at baseline only.
[3] For HIV- women only.
[4] Only infants born to HIV+ mothers.
[5] Only ELISA+ infants.
[6] Samples from all infants of HIV+ women will be prepared for PCR at every time point (HIV-DNA will be extracted); only 3 samples/HIV+ infant will under go amplification and detection.

Amplification and detection will be carried out on archived samples, which have been prepared for qualitative PCR (i.e, HIV-DNA has been extracted) only from ELISA/WB+ infants to determine the timing of transmission. For the ~50% infants infected in-utero, 2 PCR's will be done (3 month and birth); for the ~40% infants infected either in late pregnancy, at delivery, or during early breast feeding, 3 PCR's will be done (3 month, birth, 6 week). For the remaining infants infected during breast feeding, 2-7 PCR's will be done (3 month and 6 months, plus up to 5 additional post-6 month samples). Thus, it is estimated that samples from an average of 3 time points for each ELISA/WB+ infant will undergo amplification/detection.

Additional laboratory studies for Substudies:

Substudy A: Effect of vitamin A on plasma and breast milk viral load (n=400 mothers).

Months Post-partum

Test	0	6wk	3	6	9	12	15	18	21	24
Milk viral load	x	x	x	x		x		x		x
Plas viral load	x	x	x	x		x		x		x
CD4/CD8	*[1]	x	x	x		x		x		x
AGP/CRP	x	x	x	x		x		x		x
Milk retinol	x	x	x	x		x		x		x
Plasma retinol	x	x	x	x		x		x		x

[1]CD4/CD8 will have been done on these 400 women drawn from the Two Year Study.

Substudy B: Effect of maternal and/or neonatal vitamin A supplementation on infant vitamin A status (n=1000 infants)

Months Post-partum

Test	0	6wk	3	6	9	12
Plasma retinol	x	x		x		x
MRDR test		x		x		x
AGP/CRP	x	x		x		x

Substudy C. The acute phase response (APR) and its relationship to serum retinol as an indicator of vitamin A status:

Weeks after 12 mth

Test	12 months	1	1.5	2	2.5	3
Serum Retinol	x	x	x	x	x	x
AGP/CRP. Neopterin, b2-microglb., sol.						
CD8	x	x	x	x	x	x

H.4 Follow-up visits for mothers and infants failing to keep clinic appointments

Based on other studies carried out in this community, we estimate that ~30% of women will fail to keep their clinic appointment at each time point. These mother-infant pairs will be followed up by a team of 12 nurses dedicated to this purpose. The catchment area will be divided into 12 regions each including approximately 1/12 of the total study population. When the enrollment form is entered into the data base, a member of the data management team will note the home address of the mother, determine which of the 12 regions she lives in, and assign a study region code to the mother. Each day, at the close of the clinic, the data management center will

produce a list of "no-shows" for each study region. The list will contain the ID number, name of mother, father, and infant, address, visit missed (e.g. 6 month), and the tissue specimens needed (e.g., for a mother-infant pair in the Two Year study missing the 6 month visit, blood and milk specimens are required from the mother, and a blood specimen from the infant). One home visit nurse will be assigned to each region. Each morning, she will be given the list of no-shows for her region from the clinic of the day before. The nurse will gather the data collection forms to be completed, the blood taking equipment and labeled specimen tubes, an infant Salter scale, simple medications, thermometer, and a photocopy of the map and directions to the child's house recorded on the enrollment form. Study vehicles will transport the nurses (3-4 nurses per vehicle) and drop them in their respective study regions. It is expected that over the 3-1/2 years of the study, each home visit nurse will become well-acquainted with the community leaders and other key informants in her region, who are likely to be aware of families who have permanently or temporarily moved and facilitate locating them. The home visit nursing supervisor will begin work earlier in the morning to organize the supplies and forms needed by each home visit nurse. She will check in forms and specimens as nurses return from the field, and arrange transport to the data management center and laboratory. She will also relieve home visit nurses who are ill or on leave, or assist nurses with particularly heavy assignments to assure that the visits can all be completed on the assigned day. During study months 7 –30(when the patient load is greatest), 6 junior nurses will be added to the staff to assist the home visit team.

I. Data Safety and Monitoring Committee

An outside DSMC will be selected to monitor the trial. Specifically, the DSMC will be charged with reviewing interim data analyses at selected time points (to be determined at their first meeting). Their mandate will be twofold. First they will identify any unexpected adverse effects of either neonatal or maternal vitamin A supplementation among several subgroups of the study population (eg: HIV seropositive and negative; normal and low birth weight infants). Second, they will determine if positive effects of vitamin A supplementation on HIV transmission and infant mortality have occurred which are sufficiently strong that the study should be terminated early. Prior to enrollment of the first subject, the DSMC will meet with the team of investigators to agree on which data will be reviewed and what the criteria will be for termination of the study.

The members of the DSMC will be carefully selected by the investigators, with consultation with donors. Four members are proposed who will represent the following areas of expertise: vitamin A supplementation and mortality; vertical HIV transmission; longitudinal data analysis of large clinical trials, and a knowlegable representative of the Government of Zimbabwe with appropriate authority who can serve as an advocate for the study population. The members will not be affilatiated with any of the collaborating institutions or donors.

J. Laboratory Methods

a). Facilities

1. HIV Laboratory in the Department of Obstetrics and Gynecology: Dr. Michael Mbizvo heads this laboratory of 394 sq ft, which is housed in the Medical School, Parirenyatwa Hospital campus. Serologic studies including HIV ELISA and western blot, and flow cytometry are conducted in this laboratory. Major equipment includes an MSE Mistral refrigerated centrifuge, Becton Dickinson Facscount flow cytometer, Abbott IMX Analyzer, and Labsystems plate washer/reader. PCR technology is currently being set up. A smaller laboratory of 295 sq ft. maintained at Harare Hospital, is equipped with specimen processing equipment (centrifuge) and other small equipment (autoclave, spectrophotometer, incubator, AVL blood gas analyzer). The two laboratories are currently staffed by 6 technical staff. The equipment requested in this proposal will be placed in these two laboratories, as well as additional space being negotiated. All ICL, ELISA, western blot, qualitative and quantitative PCR, and flow cytometry assays of this project will be carried out in this laboratory.

2. Vitamin A Laboratory in Department of Biochemistry: A vitamin A laboratory is being established by Dr. Malaba within the Molecular Biology laboratory of the Biochemistry Department with funding from Canadian Micronutrient Initiatives. A Shumadzu HPLC and spectrophotometer have been ordered. Dr. Malaba is already an expert in the assays for retinol, retinol binding protein, and 3,4 didehydroretinol (used for the MRDR test) from her years at The Institute for Nutrition Research, University of Oslo, Norway. All retinol, MRDR, and acute phase reactant assays will be carried out in this laboratory.

b). Specimen Collection and processing:

Mothers: Venipuncture blood will be obtained using needles and syringes. **At baseline**, 5 ml will be collected into an EDTA tube, and 7 ml into a red top tube. Blood will be held at room temperature in a cool box until transported to the laboratory within 4 hours. From the red top tube 3.5ml serum will be obtained following centrifugation, 0.5-1.0ml will be transferred into an Eppendorf tube and tested by ICL; samples which are ICL+ will be tested by ELISA; samples ICL- will be discarded. The remaining 2.0-2.5 ml serum will be archived. From the EDTA tube, 0.5 ml whole blood will be transferred to an Eppendorf tube and held until the results of the ICL test are known. For women whose serum tests ICL+, the companion whole blood sample will be run by flow cytometry for determination of CD4/CD8 lymphocyte counts. For women whose serum tests ICL- the companion whole blood will be discarded. The remaining blood in the EDTA tube will be spun to obtain 2.0-2.5 ml plasma which will be archived. **6 and 12 months**, 5 ml blood will be collected into an EDTA from all women in the One Year Study, plasma

obtained by centrifugation, tested for HIV-1 by ICL (with ICL+ samples confirmed by ELISA) and the remaining plasma archived. **At 6 weeks, and 6 and 12 months,** both plasma (5 ml in EDTA tube) and serum (7 ml in heparinized tube) will be obtained from mothers in the Two Year Study. Plasma will be tested for viral load in those HIV+, retinol, and acute phase proteins for those in Substudy A, and the remainder archived. Serum will be tested for HIV-1 antibodies by ELISA in those HIV-, and the remainder archived. Both plasma and serum must be collected from all mothers in the Two Year Study because the clinic nurses will be masked to the mothers' HIV status. **At 3 and 9 months,** plasma (5 ml blood in an EDTA tube) will be obtained from mothers in the Two Year Study and archived.

Milk and colostrum specimens will be collected through manual expression into a 50 ml centrifuge tube at all visits in the Two Year Study. In the laboratory, milk will be gently homoginized and 3 capillary tubes will be filled with whole milk and spun for creamatocrit. An additional 2.5ml whole milk will be archived. The remaining milk will be ultracentrifuged and the resulting supernatant archived.

Infants: **At baseline (birth),** 1.0 ml blood will be obtained from all infants by heel stick into an EDTA microtainer tube. Blood will be processed using the Roche Whole Blood Specimen Preparation Kit (US:87253) and the pellet stored at -70C. Plasma will be archived at -70° C. 1.0ml blood will be collected into a clot microtainer tube, spun and the serum archived. **At 6 weeks,** 0.5 ml capillary blood will be obtained from infants enrolled in the Two Year Study. Blood will be processed using the Roche Whole Blood Specimen Preparation Kit (US:87253) and the pellet stored at -70°C. Plasma will be archived at -70° C. From infant selected for Substudy B, retinol and acute phase reactants will be analyzed in archived samples. From infants becoming HIV infected, selected archived prepared samples will be assayed by PCR. At **all subsequent visits,** 3.0 ml blood will be obtained by venipuncture using butterfly needles and syringes from infants in the Two Year Study into an EDTA tube. Blood will be processed using the Roche Whole Blood Specimen Preparation Kit (US:87253) and the pellet stored at -70C. Plasma will be archived at -70 C. From selected infants, retinol, acute phase reactants, and PCR will be carried out on archived samples. At **6 weeks and 6 and 12 months,** the infants selected for Substudy B (n=1000) will be tested by the Modified Relative Dose Response test. Upon arrival to the clinic, children will be given a single oral dose of 5.3 umol 3,4-didehydroretinyl acetate (DRA). Five hours later, 2 ml blood will be drawn from the antecubital vein, and plasma obtained and frozen at -70 C.

c). <u>Assays:</u>
<u>Immunochemical Laboratory Dipstick HIV1+2 Test (ICL)</u> (McDonald Scientific, Harare, Zimbabwe) will be used to screen all maternal blood at

baseline. This test was developed by PATH with USAID assistance, and evaluated by Zimbabwe AIDs Prevention Programm (ZAPP), McDonald, and PATH in Harare. The sensitivity and specificity of a single ICL test against 3 ELISA's (two Abbott HIV-1&2 3rd generation recombinant ELISA and one Behring Enzygnost HIV-1&2 2nd generation ELISA) was 99.6% and 95.7%, respectively.

CD4/CD8: Lymphocyte phenotyping will be carried out using commercial, dual-label monoclonal antibodies and standard flow cytometric analysis following whole blood lysis.

HIV IgG ELISA and Western Blot: Sera will be analyzed for IgG-specific anti-HIV vital proteins by a commercial ELISA kit (Cambridge Biotech, product 96040, 96043, 6055) and confirmed by Western blot (Cambridge Biotech, Cambridge, MA, product 8048G).

Qualitative PCR for HIV-1 DNA will be measured by the Roche HIV-1 test (Roche Diagnostics, Branchburg, N.J.). Blood samples from all infants in the Two Year Study will be prepared at each time point using the Roche Whole Blood Specimen Preparation Kit. Samples from ELISA/WB+ infants will be tested by the Roche HIV-1 Amplification and Detection kits.

Quantitative PCR for HIV-1 RNA: Viral load in plasma and breast milk will be measured by the HIV-1 RNA quantitation kit (Amplicor HIV monitor, Roche Diagnostic Systems, Branchburg, NJ).

Drs. Ruff and Quinn recently completed preliminary studies of milk viral load. These studies were undertaken to evaluate the feasibility of measuring viral load in breast milk supernatants using the HIV-1 RNA quantitation kit (Roche Diagnostic Systems, Branchburg, NJ). Briefly, 400 μl milk supernatant specimens were ultracentrifuged, extracted, added to a master mix containing Bio-SK 431/462 primers, dNTPS, AmpErase and *rTth*, and amplified using a 9600 Thermocylcer. HIV RNA was detected in 9/11 colostrum specimens and 0/4 later milk specimens from HIV seropositive women. Colostrum contained from 0 to 29,833 copies/ml with a median value of 3,315 copies/ml. None of the 4 milk specimens from HIV seronegative women contained detectable HIV copies. These preliminary studies demonstrate our ability to measure viral load in breast milk samples. Dr. Zijenah has been trained in this application of the assay in Dr. Quinn's laboratory and Ms. Kuda Mutasa (laboratory Manager) underwent training in Harare by Dr. Quinn's technician.

Retinol: Plasma retinol and 3,4 didehydroretinol will be analyzed by HPLC (Shimadzu LC10AD system) using retinyl acetate as an internal standard, by the method of Tanumihardjo.[81] Breast milk samples will be saponified with potassium hydroxide, extracted with hexane:ether (75:25), redissolved in

50:50 methanol:methylene dichloride and injected onto the HPLC system; 3,4 didehydroretinol will be used as the internal standard for breast milk vitamin A.

Alpha-1-acid glycoprotein (AGP): AGP will be measured by an ELISA technique by the method of Filteau[82].

C-reactive protein (CRP): CRP will be measured by an ELISA technique by the method of Gillespie.[83]

Immunologic acute phase reactants will be measured by commercially available kits:
Neopterin: (DRG International, Mountainside, NJ)
b-2-microglobulin: (Pharmacia, Piscataway, NJ)
soluble CD8: (Tcell Sciences, Cambridge, MA)

K. Data Management

At enrollment, the enrollment nurse will select the next capsule packet, as described in Section E. The ID number on this packet is the unique Study ID number assigned to the mother-infant pair. This number will be recorded on each data form and specimen to facilitate linkage of the data. Specimens will be further identified by a two one-digit visit number, and a one digit code for specimen type (i.e maternal or infant plasma, serum, cells, whole breast milk, or breast milk supernatant). To minimize transcription errors, all required stickers will be computer generated after it has been determined whether the mother-infant pair will be in the One Year Study or the Two Year Study, and whether they have been selected for one of the substudies. The mother will also be given a laminated study identification which she can present when coming to the clinic to hasten registration.

During the period of enrollment (study months 1-18), enrollment forms completed in Harare Hospital will be checked for completeness, legibility, and accuracy by an appointed senior enrollment nurse. They will be collected from a central point by a data entry clerk at the end of each day and taken to the Data Entry Shop. In the clinics, the enrollment nurse will double check all forms for completeness, legibility, and accuracy. One driver will be responsible for driving to each of the three clinics at the end of each working day, collecting the forms completed that day, and taking them to the Data Entry Shop. For follow-up forms, the nurse supervisor will gather all forms completed each day in the clinic, and check them for completeness, legibility, and accuracy before the clinic nursing staff leave for the day. Checked forms will then be delivered to the Data Entry Shop. Home visit nurses will turn in their completed forms to their supervisor, who will check them for completeness, legibility, and accuracy before the home visit nurses leave for the day, before turning the forms into the Data Entry Shop. Laboratory results will be recorded on specially designed forms and delivered to the Data Entry Shop.

Upon arrival in the Data Entry Shop, all forms will be logged in on a computerized file by a data entry clerk: ID, form ID, date the form was completed, and date of arrival in the data entry shop will be recorded. All study instruments are designed using Teleform software and will be scanned using Visual dBase software. Related files will be linked and logical and range errors checked with specially written programs, and errors will be corrected accordingly.

L. Sample Size and Power

A total of 14,000 mothers and their infants will be enrolled over an 18 month period from Harare Hospital and 3 antenatal clinics of the City Health Department. Based on a 92% follow-up rate achieved at 24 months in the WHO perinatal transmission study carried out in this same population by some of the same investigators, sample size calculations were based on achieving a retention rate of 90% for both the Two Year and One Year studies.

Sample size calculations were also based on the following assumptions:

1 The prevalence of HIV infection among women enrolled in the study will be 30% at baseline (4,200 of the 14,000 women, or 1,050 women per treatment arm, will be HIV+ at delivery).

2. Among HIV+ mothers receiving placebo, the mother-to-child HIV transmission rate will be 30% by 24 months, and 10% during breast feeding (among 2,100 HIV+ placebo treated mothers, 630 will transmit the virus to their infants at some time before 24 months, and 210 of these transmissions will be during lactation).

3. Among HIV- mothers receiving placebo, the seroconversion rate during the first year post-partum will be 6% (among the 4,900 women who are HIV- at delivery and receive placebo, 294 will seroconvert within the post-partum year).

4. The infant mortality rate in the treatment arm in which both mother and infant are treated with placebo will be 60/1000 (among the 3,500 infants whose mothers and they both received placebo, 210 will die during infancy).

5. An overall type I error of 5%.

6. A type II error of 20%.

7. Use of 2-tailed tests.

Following are power calculations for each primary aim to be achieved by the main trial:

1. Vertical transmission during lactation. Because our intervention is being

administered post-partum, it has the potential to affect transmission only during lactation. However, transmission during lactation alone can not be measured precisely with current technology: among infants who seroconvert between birth and 3 months, it is not possible to distinguish between those who acquired the infection in late pregnancy or delivery from those who were infected by early breast milk. Therefore, sample size calculations are based on detecting a difference between vitamin A and placebo-supplemented mothers in total post-partum transmission. This results in a loss of power. It is conservatively estimated that 10% of all mother-to child transmission occurs during breast feeding. If we assume that 50% of infants who are ELISA/WB+ at 24 months have already seroconverted at birth, detection of a 30% reduction in transmission during lactation would require an adequate sample to detect a 20% reduction in total post-partum transmission. *With these assumptions, we will be able to detect a 31% reduction in transmission during lactation, or a 21% reduction in total post-partum transmission.*

2. Horizontal HIV transmission A total of 9,800 HIV- women will be enrolled, 4,900 will be treated with vitamin A and 4,900 with placebo. If 90% are retained in each group (4,410) we will be able to detect a *25% reduction in seroconversion during the post partum year* due to vitamin A treatment (265 (6%) verses 198 (4.5%)).

3. Infant Mortality A total of 3,500 infants will be enrolled into each treatment If 90% (3,150) are retained at one year, we will be able to detect a *30% reduction in infant mortality* from the currently estimate rate of 60/1000.

Power calculations for substudies:

1. Maternal plasma and breast milk viral load and CD4/CD8.

A total of 200 HIV+ vitamin A treated and 200 HIV+ placebo treated women will be enrolled into the viral load substudy. Estimating that the baseline viral load of maternal plasma and breast milk will be ~$10^{4.5}$ and that the estimated SD will be 10^5 as reported by Henrard[84] we will be able to detect a mean reduction of $\geq$0.5 log. Our own preliminary data from 19 HIV seropositive women of reproductive age in Baltimore (see Section III.A), whose CD4 counts ranged from 160-600, agrees closely with the data of Henrard: mean baseline plasma viral load was 2.1 x 10^4 with a SD of 2.6 x 10^4. In this range, an increase of 0.5 log represents about a tripling of the concentration. Though Roche Systems publicly claims that increases of less than 5 fold are of no probable clinical significance, they have presented data showing evidence of clinical effects with as little as a 3-fold increase (personal communication, Dr. T. Quinn). Because the women in our study will have just completed a pregnancy , they will be well enough to be fertile and carry a pregnancy. Therefore, we estimate that the distribution of their plasma and breast milk viral load at baseline will be in the range of the data

reported above. Thus, we estimate we will be able to detect these smallest differences of possible clinical significance with 200 women per group. Finally, this sample size estimate is very conservative: because viral load data are is skewed to high the analyses will be carried out on the log values.

2 Infant vitamin A status

50 infants born to HIV- mothers from each treatment arm will be enrolled and 200 infants born to HIV+ mothers from each treatment arm will be enrolled. If at least 25% of the infants born to women in each treatment arm become HIV infected themselves by 24 months, there will be at least 50 infected infants from each treatment arm enrolled in this substudy (many more exposed but not infected will also be included). This sample size will be sufficient to detect a difference of $\geq$0.015 in the mean MRDR ratio (about ½ SD) between treatment group-HIV status specific groups of infants. Thus, we will be able to determine the affect of each treatment arm on infant vitamin A status among infants born to HIV- mothers, born to HIV+ mothers but are not infected, and those who do acquire the infection. Because HIV infection and the additional acute opportunistic infections associated with HIV infection are likely to increase vitamin A requirements, the affect of supplementation regimens on infant vitamin A status are likely to be different for these different groups of infants.

3. The acute phase response (APR) and its relationship to serum retinol as an indicator of vitamin A status

30 infants will be enrolled from each HIV diagnosis category (unexposed, exposed-not infected, exposed-infected) for each of three morbidity statuses (well, diarrhea, fever). Thus, a total of 270 12-month old children will be enrolled. Of the three acute phase reactants being assessed, the change in AGP during the acute phase is of smallest magnitude. Therefore, sample size for this substudy were based on ability to detect differences in AGP. Our samples sizes will be sufficient to detect a difference of $\geq$ 0.025 g/L in mean AGP concentrations between well and sick children, and a difference of $\geq$0.5 g/L between any HIV diagnostic-morbidity status specific group and another. We will be very interested in being able to detect small differences in the change of AGP from baseline to the lowest point during infection, between children whose serum retinol drops by at least 10% during an infection and those whose serum retinol does not fall by at least 10%. If we assume that 25% of the clinically sick children do not experience a 10% fall in serum retinol and that 25% of the clinically well children do experience a $\geq$10% fall in serum retinol, we will be able to detect a mean difference of 0.22 g/L. In other words, we will be able to distinguish between children whose serum retinol is reflecting both vitamin A status and the presence of infection from those whose serum retinol is not being biased by infection and is more closely reflecting vitamin A status.

M. Statistical Analyses

1. Determination of end-points:

Infected in-utero: Infants who are ELISA/WB+ at 24 months and PCR+ at birth. In infants who die or are lost to follow-up before 24 months, two positive PCR tests, one of which was at birth.

Infected during breast feeding: Data from several centers have revealed that in the absence of breast-feeding, almost all infants who acquired HIV from their mothers were PCR + by 3 months. Therefore, infants who are ELISA/WB+ at 24 months, PCR- at 3 months, with at least 1 positive PCR test between 3 months and 24 months will be assumed to have acquired the infection during breast feeding. In infants who die or are lost to follow up before 24 months, PCR- at 3 months and at least 2 positive PCR tests after 3 months will be assumed to have been infected during breast feeding.

Infected either late in pregnancy, during delivery, or during early breast feeding: Infants who are ELISA/WB+ at 24 months, PCR- at birth and PCR+ at 6 weeks and 3 months OR PCR - at birth, PCR- at 6 weeks, and PCR+ at 3 months will be classified as becoming infected at some time late in pregnancy to early in breast feeding; with current technology a more precise estimate of time of infection can not be made for these infants. In infants who die or are lost to follow up before 24 months, PCR- at birth, PCR+ at 6 weeks and 3 months, or PCR+ at 3 months and at least one time point after 3 months.
Uninfected: ELISA/WB - at 24 months. In infants who die or are lost to follow up before 24 months, PCR- at least two time points after 3 months.

Indeterminant: Infants not meeting the above criteria.

Infant mortality: The clinic and home nurses will be responsible for identifying deaths among the infants in the study. The home visit nurses will actively search for any mother-infant pair who fails to keep a regular clinic appointment, identifying deaths among the cohort in the process. The home visit nurses will be expected to become well-acquainted with the community leaders and other key informants in their areas, who are likely to be aware of deaths in the community and inform the home visit nurses. For deaths which occur in a hospital, the cause of death as assigned by the primary care physician in the medical record will be documented. For deaths which occur outside a hospital, a simple verbal autopsy will be administered by the home visit nurse. The primary purpose of the verbal autopsy will be to distinguish AIDs-related deaths from non-AIDs related and to identify deaths due to accidents (motor vehicle, drowning, etc).

Maternal seroconversion: Mothers will be considered to have seroconverted during the post partum year if they were ICL negative at delivery, and ICL and ELISA positive at 6 or 12 months. For women sero-converting at 6 months, their diagnosis will be confirmed by Western Blot specific for HIV-1 at 12 months. In addition, the baseline sample at delivery (which was ICL-) must be confirmed negative by ELISA.

2. Descriptive analyses:

The demographic and clinical characteristic of the women and infants will be analyzed for the entire study population, and by treatment group to examine success of randomization. Characteristics to be examined include, but are not limited to: maternal age, socioeconomic status, nutritional status of the mother (mid-upper arm circumference at delivery, and BMI at subsequent visits and infant (birth weight, length, and head circumference), maternal CD4 lymphocyte count for HIV+ women, and infant 5-minute APGAR score, gestational age at birth, and gender.

3. Efficacy of maternal vitamin A supplementation in reducing post-partum vertical HIV transmission:

The percent efficacy of maternal vitamin A supplementation on vertical transmission will be defined as:

$$\frac{\text{Incidence of HIV among infants of placebo mothers} - \text{Incidence of HIV among infants of vitamin A mothers}}{\text{Incidence of HIV among infants of placebo mothers}} \times 100$$

Confidence interval (95%) will be computed for the efficacy using binomial approximations. In addition, logistic regression techniques will be used to examine the effect of maternal vitamin A supplementation on vertical transmission while controlling for selected variables. Co-factors which might affect efficacy and which might be used in the analyses include, but are not limited to: maternal CD4 lymphocyte count and serum retinol concentration at delivery, maternal age and nutritional status, socioeconomic status, infant gender, gestational age, and nutritional status at birth. Finally, whether the infant was supplemented with vitamin A or received placebo will be included as a co-factor to determine if infant supplementation reduces susceptibility to infection and further effects risk of vertical transmission

In addition, the effect of maternal vitamin A supplementation on the timing of infection of the infant will be examined using survival analysis techniques to compare the time free of HIV for infants in the maternal supplemented and maternal placebo-treated groups. These analyses will controlled for the same selected variables listed above and account for losses to follow-up. Kaplan-Meirer estimation of survival curves will be used for descriptive analyses, with Cox proportional hazards regression models to be used for estimation purpose

4. Efficacy of maternal vitamin A supplementation in reducing post-partum horizontal HIV transmission:

The percent efficacy of maternal vitamin A supplementation on horizontal transmission (infection of women who are HIV- at delivery during the post partum year) will be defined as:

$$\frac{\text{Incidence of HIV among placebo women - Incidence of HIV among vitamin A women}}{\text{Incidence of HIV among placebo women}} \times 100$$

Confidence interval (95%) will be computed for the efficacy using binomial approximations. In addition, logistic regression techniques will be used to examine the effect of maternal vitamin A supplementation on horizontal transmission while controlling for selected variables. Co-factors which might affect efficacy and which might be used in the analyses include, but are not limited to: maternal age and nutritional status, socioeconomic status, use of condoms, frequency of sexual intercourse during late pregnancy, and time of first post-partum sexual intercourse.

5. Efficacy of vitamin A supplementation of neonates, mothers, or both on reducing the infant mortality rate:

The relative risks of infant mortality among groups in which the infant alone is vitamin A supplemented, the mother alone is vitamin A supplemented, or both the infant and mother are supplemented, respectively, compared with the rate in the group in which neither the infant or mother is supplemented will be defined as follows:

$$\frac{\text{Infant mortality rate in infant-vitamin A supplemented, mother-placebo supplemented group}}{\text{Infant mortality rate in infant-placebo supplemented, mother-placebo supplemented group}}$$

$$\frac{\text{Infant mortality rate in infant-placebo supplemented, mother-vitamin A supplemented group}}{\text{Infant mortality rate in infant-placebo supplemented, mother-placebo supplemented group}}$$

$$\frac{\text{Infant mortality rate in infant-vitamin A supplemented, mother-vitamin A supplemented group}}{\text{Infant mortality rate in infant-placebo supplemented, mother-placebo supplemented group}}$$

Confidence intervals (95%) will be computed for each relative risk. Logistic regression techniques will be used to examine the effect of neonatal and/or maternal vitamin A supplementation on infant mortality while controlling for selected variables which are also known to affect infant mortality. These

variables will include, but not be limited to: infant birth weight and gestational age, 5-minute APGAR score, maternal and infant HIV status at delivery, maternal age, and parity, maternal and paternal years of education, and socioeconomic status.

In addition, the effect of maternal and/or neonatal vitamin A supplementation on the timing of infant death will be examined using survival analysis techniques to compare the time of survival for infants in each of the four treatment arm. These analyses will controlled for the same selected variables listed above and account for losses to follow-up. Kaplan-Meirer estimation of survival curves will be used for descriptive analyses, with Cox proportional hazards regression models to be used for estimation purposes.

6. Effect of maternal vitamin A supplementation on the quantity of HIV in plasma and breast milk, and on CD4 counts:

For the 400 HIV+ women selected for Substudy A, we will compare the mean concentrations of HIV-RNA (copies/ml) in maternal breast milk and plasma at each time point throughout the two years post-partum among women in the maternal vitamin A supplemented and placebo-treated groups. We will also compare the mean CD4 counts at these time points. T-tests will be used to test the level of significance in any observed differences between groups.

7. Effect of maternal and infant vitamin A supplementation on infant vitamin A status throughout the first year of life:

For infants selected for Substudy B, we will initially compare mean MRDR ratios throughout the first year of life for each HIV status (unexposed, exposed-not infected, infected) - treatment group specific group; thus, the analysis will be an ANOVA comparing 12 groups. For HIV-status-treatment regimen groups in which there is no differences between infant MRDR ratios, we will group data to increase our power to detect smaller differences between mean ratios, and to detect difference in the proportion of infants in each group with abnormal (>0.06) ratios. Logistic and/or linear regression models will be used to examine the effect of selected co-factors on infant vitamin A status. Variables which may be included in these analyses include, but are not limited to infant birth weight and gestational age, change in weight from birth to the time vitamin A status is being evaluated, and gender.

8. Association between acute phase reactants and serum retinol concentrations:

For children selected for Substudy C, changes in concentrations of acute phase reactants and serum retinol during episodes of infection will be examined. Mean concentrations of acute phase reactants during their peak

of the acute phase response will be compared between sick infant's whose serum retinol fell by at least 10% during the episode of infection with sick infant's and well infant's whose serum retinol remained stable over the period of follow-up. Observed differences will be tested for statistical significance by t-test. Linear regression models will be used to examine the effect of concurrent HIV infection or HIV exposure on serum retinol during the course of an infection.

N Limitations

Maternal and neonatal vitamin A supplementation administered in the immediate post-partum period will not prevent vertical HIV transmission during pregnancy and delivery, when the majority of transmission is thought to occur. Though it is estimated that only 1/3 of all vertical transmission occurs during lactation, the reality of this risk may result in erosion of breast feeding promotion throughout Africa which may jeopardize the health and survival of the 70% of infants born to HIV negative women in these countries. If vitamin A supplementation at delivery reduces vertical transmission during lactation, governments may elect to promote maternal vitamin A supplementation with continued breast feeding promotion which would result not only in reducing the number of HIV infected infants, but also infant deaths due to alternative infant feeding practices. In addition, pregnancy-linked interventions are logistically more complex and more expensive, and likely to be infeasible for many African countries. Therefore, we thought it critical to examine the efficacy of supplementation at delivery (a time when the vast majority of women come into contact with some member of the health care system, and when the WHO already recommends maternal vitamin A supplementation for prophylacting against vitamin A deficiency) on lactational transmission.

For infants who become PCR+ between birth and 3 months, we will not be able to distinguish those who acquired the infection during late pregnancy or at delivery from those who acquired it from early breast milk. For this reason, however, sample sizes have been inflated to be able to detect differences in over-all post partum transmission. In addition, if there are differences in vertical transmission rates between birth and 3 months between mothers who received vitamin A and those who received placebo, we will attribute the excess transmission to the lactation period since the supplement is being given post-partum.

V. Human Subjects

A. Subject Population:

The study will be conducted in the peri-urban area of Harare, Zimbabwe among women delivering at Harare Hospital and 5 maternity clinics in the community and their infants. During the immediate post-partum period, newly delivered mothers who meet enrollment criteria will be approached by one of the enrollment nurse.