**Project Title:** Optimizing viral load suppression in Kenyan children on antiretroviral therapy (Opt4Kids)

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**ABSTRACT**

Among nearly 1 million HIV-infected children receiving antiretroviral treatment (ART), as many as 40% of those living in resource limited settings have not achieved virologic suppression. Kenya, a UNAIDS fast-track and PEPFAR priority country, has an estimated 98,000 children aged 0-14 years living with HIV. Virologic suppression is achieved by only 65% of Kenyan children on ART translating to only 38% of the final UNAIDS 90-90-90 goal for population-level viral suppression. Feasible, scalable and cost-effective approaches to maximizing durability of ART regimens and ensuring viral load (VL) suppression in HIV-infected children are urgently needed. The goal of this pilot study is to determine the feasibility and impact of point-of-care (POC) VL drug resistance mutation (DRM) testing to improve VL suppression in children on ART within a PEPFAR-funded HIV care and treatment program in Kenya.

The proposed research is a randomized, controlled study to pilot the use of POC VL and DRM testing in children aged 1-14 years on ART at sites with on-site access to GeneXpert® technology. Children enrolling at each site will be randomized 1:1 to intervention (POC VL and targeted DRM testing) vs. control (standard-of-care) arms and followed for up to 5 years. The proposed study will be conducted in PEPFAR-supported HIV treatment facilities in western Kenya, a PEPFAR priority country. This pilot study will evaluate two critical components related to viral suppression in children via: 1) POC VL testing (**Aim 1a**) and 2) targeted DRM testing (**Aim 2a**) among children on ART. Our primary outcome is the proportion of children achieving VL suppression 12 months after enrollment for each child, with two secondary outcomes: 1) VL suppression and DRM patterns up to 5 years and, 2) time to viral suppression in those children not suppressed or initiating ART. We will also validate HIV-1 POC VL testing against current in-country gold standard VL testing (**Aim1b**) in order to eventually scale up POC technologies for VL testing at national levels. We will also validate HIV-1 POC DRM testing, also named OLA Simple, against current in-country gold standard, consensus sequencing, DRM testing (**Aim 2b**).

In order to maximize the impact of POC technologies to improve health outcomes for children with HIV, it is critical to explore health care provider, caregiver, and adolescent perspectives on the use of POC VL testing. We will also query caregivers and adolescents to understand how the novel coronavirus disease 2019 (COVID-19) has impacted intervention delivery and participants' overall well-being. Additionally, the costs and cost-effectiveness of implementing POC VL and targeted DRM testing for children on ART will critically inform key clinical and policy stakeholders for future implementation, whether in Kenya or other resource-limited settings. Therefore, we also propose to 1) use qualitative research methods to better understand how our POC VL intervention functions and how policymakers might use tools to scale up POC VL (**Aim 3a**) and 2) determine the cost-effectiveness of POC VL combined with targeted DRM testing in this group, as well as model the potential POC machine placement within a geographic area (**Aim 3b**).

This pilot study helps build the foundation for a future adaptive trial which packages optimized POC VL and DRM monitoring algorithms with socio-behavioral interventions to maximize VL suppression rates in children on ART and facilitate timely switch of ART in resource-limited settings. This pilot study will provide critical information on the impact of POC VL testing on viral suppression among children on ART in a resource-limited setting. It will additionally show current patterns and impact of DRM testing among children undergoing routine VL monitoring. Findings from this pilot will inform the development of an adaptive clinical trial which evaluates the impact of combination interventions, including POC VL and DRM testing at programmatic scale, facility- and community-based care packages, and the cost-effectiveness of implementing each strategy in achieving viral suppression. This proposal directly addresses the urgent need to find interventions to maximize viral suppression among children on ART and achieve the UNAIDS 90-90-90 goals.

**LIST OF ABBREVIATIONS**

**ART**- antiretroviral treatment
**CDC-** Centers for Disease Control and Prevention
**DBS-** Dried Blood Spot
**DRM-** Drug resistance Mutations
**EDTA-** Ethylenediaminetetraacetic acid
**FACES**- Family AIDS Care and Education Services
**FWA**- Federal Wide Assurance
**HIV**- Human Immunodeficiency Virus
**KEMRI**- Kenya Medical Research Institute
**mL**- milliliter
**MOH** – Ministry of Health
**NASCOP**-National AIDS and STI Control Programme
**NIAID**- National Institute of Allergy and Infectious Disease
**NICHD**- National Institute of Child Health and Development
**NIH**- National Institutes of Health
**NGS**- Next-generation sequencing
**NNRTI-** Non-nucleoside/nucleotide reverse transcriptase inhibitor
**OLA-** Oligonucleotide Ligation Assays
**PEPFAR** – President's Emergency Plan For AIDS Relief
**PI-** Protease Inhibitor
**PMTCT** - Prevention of mother to child transmission
**POC**- Point of Care
**RCTP-FACES**- Research Care and Training Program- Family AIDS Care and Education Services
**SOC-** Standard of Care
**UCD** – University of Colorado at Denver
**UCSF-** University of California San Francisco
**UW**- University of Washington
**UNAIDS**- Joint United Nations Programme on HIV/AIDS
**VL**- Viral Load

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# INTRODUCTION

## *A. Specific Aims*

Among nearly 1 million HIV-infected children receiving antiretroviral treatment (ART), as many as 40% of those living in resource limited settings have not achieved virologic suppression. The proposed research is a randomized, controlled study to pilot the use of point of care (POC) viral load (VL), drug resistance mutation (DRM) testing, and clinical decision support in children aged 1-14 years on antiretroviral treatment (ART) at sites with on-site access to GeneXpert® technology. Children enrolling at each site will be randomized 1:1 to intervention (POC VL and targeted DRM testing) vs. control (standard-of-care) arms and followed for up to 5 years. The proposed study will be conducted in PEPFAR-supported HIV treatment facilities in western Kenya, a PEPFAR priority country. We will evaluate two critical components related to viral suppression in children in this pilot study: 1) POC VL testing and 2) targeted DRM testing among children on ART. This pilot study helps build the foundation for a future adaptive trial which packages optimized POC VL and DRM monitoring algorithms with socio-behavioral interventions to maximize **VL suppression rates in children on ART and facilitate timely switch of ART through the following specific aims:**

**Aim 1:** To determine the impact of POC VL on the proportion of children on ART achieving viral suppression and time to viral suppression among those not suppressed or initiating ART (**Aim 1a)** and to validate HIV-1 POC VL testing against current in-country gold standard VL testing (**Aim 1b**).

**Aim 2:** To determine the impact of targeted DRM testing and patterns of DRMs among children on ART without viral suppression, to detect emergency DRM to dolutegravir via next-generation sequencing for genotypic resistance and phenotypic resistance (Aim 2a), and to validate HIV-1 POC DRM testing, also named OLA Simple, against current in-country gold standard, consensus sequencing, DRM testing (**Aim 2b**).

**Aim 3**: To understand how our intervention functions and how policymakers might use tools to scale up POC VL and DRM testing, by qualitatively exploring the barriers to achieving viral suppression and evaluating the feasibility of incorporating POC VL and DRM testing at programmatic scale using different sharing strategies to overcome of these barriers (**Aim 3a**) and to estimate the costs of implementing pediatric POC VL testing and DRM monitoring and the incremental cost-effectiveness per disability adjusted life year (DALY) averted, compared to standard clinical care, and determine the optimal placement of limited POC VL machines and DRM testing (**Aim 3b**).

## *B. Hypotheses*

We hypothesize that 1) viral suppression rates will be higher among children with access to POC VL testing; 2) time to suppression will be shorter compared to children with standard VL testing; 3) we will detect >90% concordance for categorization of plasma VL as <1000 or =>1000 copies/mL for POC (via GeneXpert) vs. gold standard (via Abbott) VL testing; 4) hypothesize that targeted DRM testing will shorten time to viral suppression; 5) pediatric POC VL testing and DRM can be implemented in HIV care clinics in Kenya at reasonable costs and is cost-effective compared to standard-of-care adherence monitoring; and 6) modeling tools to inform placement of POC VL machines in a geographic area will be usable to policymakers.

## *C. Background and Significance*

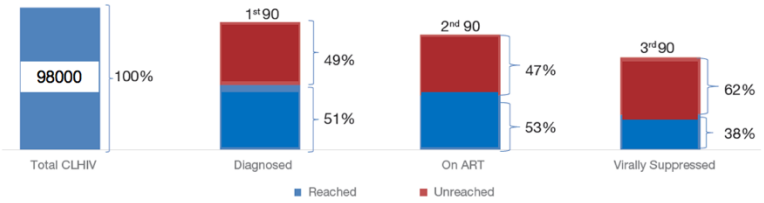
### C.1 Background

**Viral suppression in children lags UNAIDS goals and the optimal approach for achieving VL suppression and maximizing the duration of ART regimens for children in resource-limited settings is unknown.** A recent meta-analysis of 72 studies including over 50,000 children from resource-limited settings showed pediatric viral suppression rates of 60-75%.[1] These rates are much lower than the ≥90% suppression rates in high-income countries, well below the target goal of 90% established by the UNAIDS, and significantly lower than rates among adults in resource-limited settings.[2-4] Kenya, a UNAIDS fast-track and PEPFAR priority country, has an estimated 98,000 children and young adolescents aged 0-14 years living with HIV and 6,600 new pediatric infections annually.[5] Similar to many other PEPFAR countries, Kenya has continued to increase access to pediatric ART, yet viral suppression rates among children remain poor. Based on national survey data, only 65% of children and adolescents on ART achieve VL suppression in Kenya.[6] Kenya's internal gap analysis shows that this translates to achieving only 38% viral suppression of the final 90-90-90 goal (**Figure 1**). Such

dismal viral suppression rates not only contribute to pediatric HIV morbidity and mortality but will undoubtedly slow down global efforts to control the HIV epidemic.

**To optimize management of children on ART and provide early intervention for those failing to achieve virologic suppression, access to timely laboratory monitoring is required. POC VL testing can drastically improve time-sensitive clinical decision-making, and potentially increase viral suppression.** Laboratory technologies have become a critical component of clinical decision-making, including in resource-limited settings. Starting in 2013, the WHO has recommended monitoring both adults and children on ART with routine VL testing.[7] Kenya, has adopted these recommendations.[8] Kenya recommends obtaining viral loads in children six months after ART initiation and annually thereafter for those with VL <1000 copies/mL or repeated three months after adherence intervention for those who remain viremic.[8] While these recommendations are being scaled-up on the ground, several limitations exist to laboratory-based VL testing. A CDC report shows between 2014-2015, only 38% of individuals on ART in Kenya had evidence of routine VL monitoring defined as more than one VL documented since initiation of ART.[9] Currently in Kenya, laboratory-based VL testing is conducted in centralized facilities that require highly trained staff and specialized, expensive equipment. Given the need to transfer the samples to these facilities, the turn-around time to results reaching health care providers and patients is prolonged (often greater than 4-6 weeks), significantly delaying the time to clinical decision-making.[9-11] Our pilot work shows that a third of children in Kenya do not have follow up viral loads and 77% with virologic failure are not suppressed up to one year after initial VL.[12] Failure to follow National Guidelines on timing of follow up VL and clinicians reluctance to order resource-intensive VL tests may contribute to this poor uptake. Delaying interventions to address treatment failure, such as improving adherence or switching treatment, increases the risk of clinical deterioration and multi-drug resistance. POC testing, done at or near the site of care, with the results available to the ordering provider and patient within hours or days to expedite clinical decision-making, has the potential to dramatically overcome the limitations of centralized laboratory-based testing.[13] In the field of HIV, rapid HIV antibody and POC CD4 count tests are reliable and have shown to improve clinical decision-making.[14-22] With VL assessments becoming integral to routine HIV care, POC VL testing has the potential to facilitate drastic improvement in viral suppression rates and longevity of ART regimens, especially among children. Furthermore, POC VL-based care models are predicted to be even more cost-effective than laboratory-based VL testing.[23]

**Figure 1. Kenya Pediatric 90-90-90 Targets and Achievements**




**The WHO and others have expressed concerns that HIV DRM will undermine the attainment of the global targets for HIV.[24,25] Understanding current patterns of DRM among children failing ART is critical to determining the optimal management of these children.** Among children not achieving viral suppression, small cohort reports in East Africa indicate between 68-77% may have DRMs while on NNRTI-based regimens, while much fewer (<10%) have drug resistance on PI-based regimens.[26-28] This problem is likely to be worsening, with more ART exposure through earlier initiation and combination ART now used for prevention of mother to child transmission (PMTCT).[29] Among newly diagnosed children <2 years of age in South Africa, 60% of those with PMTCT exposure and 24% with no reported PMTCT exposure demonstrated major non-nucleos(t)ide-reverse transcriptase inhibitor (NNRTI) resistance.[30] The first-ever Kenya national pediatric HIV DRM surveillance study conducted in 2013, showed detectable viremia in more than 30% of children on ART for 12-36 months. More than 90% of these children had at least one DRM detected; 77% had both nucleos(t)ide-reverse transcriptase inhibitor (NRTI) and NNRTI DRMs, while none had major resistance to protease inhibitors (PIs).[31] Notably, this study was conducted prior to Kenya's roll-out of lifelong ART for all pregnant women and before guidelines changed to treat children <3 years of age with PI-based regimens as 1st line ART. The current levels and pattern of DRMs among children failing 1st line ART, now including PIs, and 2nd/ 3rd line ART in Kenya are not known. If current DRM remains at 90% or is even higher due to more pre-treatment antiretroviral exposure, it is highly likely that the current management strategies of children with elevated VL will be unsuccessful, since no amount of adherence intensification will overcome these multiple DRMs. Understanding DRM patterns in children on ART in Kenya may provide evidence for revision of algorithms used to manage children with virologic failure that can be rigorously tested in the future.

Current challenges in DRM monitoring in Kenya include pre-consultation with centralized committees, extremely delayed turn-around times, and testing only for those failing 2nd or 3rd line regimens**. A POC DRM assay could significantly overcome these barriers and extend longevity of 1st and 2nd line regimens.** While the causes of treatment failure in individuals on ART are multifactorial, from health systems[32-34] to sociobehavioral factors,[7,14,35] this proposal begins to address the biomedical issue of DRMs.[36] We believe that rapid return of results via POC DRM testing will facilitate earlier and more appropriate clinical decision-making, acting as "cues to action" as postulated in the Health Belief Model.[37] Collaborators on this proposal, Drs. Frenkel and Lutz, have developed exactly that—a POC DRM assay named OLA Simple.

**Oligonucleotide Ligation Assays (OLA) Simple is an extremely promising POC technology.** OLA Simple is the POC version of the "parent" lab-based OLA technology. These OLA tests overcome a major limitation of other past, failed single point mutation technologies which did not tolerate nearby polymorphisms.[38] They hybridize well below melt temperatures, which allows tolerance to unknown variable polymorphisms. The parent, lab-based OLA has been tested in studies of pretreatment DRM in Kenya using DNA from peripheral blood mononuclear cells (PBMC). DRMs that arise during treatment failure often occur at very low levels in PBMC DNA, but at higher levels in plasma RNA; thus, the Lutz lab has needed to adapt OLA Simple to test DRMs in plasma RNA. Therefore, this will be the first study to evaluate the performance of a POC DRM test using plasma RNA in treatment failure in children. Lastly, the OLA Simple has only been previously tested for codons for nucleotide reverse transcriptase inhibitors (NRTIs) and NNRTIs; the OLA Simple kit we plan to use now includes codons for protease inhibitor (PI) mutations, which has yet to be validated. The lab has developed probes for integrase strand transfer inhibitor (INSTI) DRMs (manuscript under review), so while we anticipate very few children will be on INSTI-containing ART in our study as noted above, if we find that a significant portion of the children in our study are on INSTI-containing ART, we can easily adapt the OLA Simple kits to include the relevant INSTI probes. No study to-date examines the use of a POC DRM technology to determine the minimum number of mutant codons needed to detect clinically meaningful DRMs for pretreatment or acquired DRMs in a RLS.

**OLA Simple requires validation prior to clinical use.** While the parent, lab-based OLA, the predecessor to OLA Simple, has undergone validation and is Clinical Laboratory Improvement Amendments (CLIA)-certified,[38,39] validation of OLA Simple must occur prior to regulatory approval for its use clinically. OLA Simple has significant technology differences from lab-based OLA, including reformulation of reagents and probes, different enzymes concentrations, dry reagents, and assay timing. These changes are substantial enough that they require rigorous validation prior to clinical use. Initial testing of the OLA Simple was conducted in Seattle with a small set of sixty dried blood spot samples collected from HIV-infected infants in South Africa and against previously-sequenced samples of HIV, with 95% sensitivity, and 100% specificity.[56,57] In addition, a digital lab assistant "app" now exists for OLA Simple that provides step by step tutorial for running tests; 40 untrained users in Seattle piloted OLA Simple using cloned plasmids to test the automated instructions and collection of results on a tablet with 97% accuracy with no prior instructions about the test (manuscript in preparation). However, OLA Simple has not been evaluated for technician performance using clinical specimens in a RLS where its use is intended. While the above incremental advances help in optimizing OLA Simple kits, they are not sufficient to be used for making treatment decisions in patients. The next step in validation for clinical use is field-testing OLA Simple and validating its performance—a WHO pre-qualification requirement for approval of diagnostic technologies.[40] Without the type of validation of OLA Simple we propose here, it would be unethical to begin using OLA Simple for clinical decision-making.

**Monitoring dolutegravir (DTG) resistance.** A rapid transition to more effective ART regimens, specifically DTG, holds promise for improving VS in CLHIV, but on its own, cannot address the multifactorial challenges that reduce ART adherence in CLHIV and result in VF.[23] Despite its higher barrier to resistance, DTG resistance can develop and will undermine the durability of first- and second-line regimens in LMIC.[24,25] While ART-naïve individuals treated with DTG, including tenofovir + lamivudine + DTG (or TLD), rarely select DRMs, ART-experienced individuals with some pre-existing resistance prior to switch to DTG may be at higher risk for developing additional DRMs, including to the tenofovir or lamivudine components of TLD. Further, it is not clear if minority variant populations, those HIV-1 subpopulations that are not detected by current consensus sequencing techniques due to being <10% of the population of viruses circulating, play a role in fostering DRM. Losing our ability to use DTG more widely, including in children and for potent viral suppression among pregnant and postpartum women, will be a tremendous loss for the global HIV community. Therefore, proactive DRM monitoring of individuals on DTG are needed urgently.

## C.2 Innovation

Our proposal contains two key innovations: (**1**) we will utilize novel, emerging technologies, namely POC VL testing and POC DRM assay (OLA Simple), for ART monitoring among children, and (**2**) we are proposing a more aggressive VL monitoring algorithm than current standard of care (SOC). First, POC diagnostic and laboratory monitoring technologies are improving delivery of healthcare in resource-limited settings.[40] For example, POC testing for early infant diagnosis of HIV is increasingly being scaled up, yet POC VL testing for HIV treatment monitoring lags behind.[41-44] POC VL testing has the potential to dramatically improve HIV care in small, rural or high-volume urban facilities. Several studies have now demonstrated high analytical performance of POC VL testing.[45-48] However, a more comprehensive evaluation of its clinical end-point, viral suppression, is critically needed to guide its programmatic adoption.[49] Our study proposes to accomplish precisely this by scaling up POC VL testing at the facility level. Second, our proposed POC VL monitoring plan is more aggressive than current practice, and if successful in promoting better viral suppression, may lend support to implementing similar VL monitoring algorithms among children. **Coupling POC DRM monitoring with a POC VL testing strategy brings together two powerful innovations that could revolutionize HIV treatment monitoring.**

## *D. Preliminary Studies/Progress Report*

**Pediatric viral suppression at FACES:** Drs. Abuogi, multiple-PI on this proposal, Oyaro, co-investigator, and colleagues have assessed prevalence of treatment failure amongst children undergoing routine VL testing at FACES sites between June 2014–May 2015.[12,50-52] Among 1190 children and young adolescents 0-14 years of age undergoing routine VL testing, 748 (63%) were virologically suppressed. In multivariable analysis, unsuppressed children were more likely to be male (adjusted odds ratio (aOR) 2.1, 95% Confidence Interval (CI) 2.1-3.6) and have had an ART regimen change (aOR 2.0, CI 1.0-3.7) than suppressed children. A third of children with virologic failure never had a follow-up viral load. Among those that did, VL suppression was only achieved by 23% versus 93% among those suppressed at baseline (p<0.0001). Mean time for a follow-up VL in children with virologic failure was 11.3 months (95% CI 9.5-13.2).

**Table 2. Patterns of HIV DRMs among children and adolescents in Kenya, 2013[31]**

| **Specific mutations with VL ≥ 1000 copies/mL** | **N (%) (n=136)** |
|---|---|
| **NRTI, any** | 108 (79%) |
| M184V | 103 (75%) |
| K65R | 6 (4%) |
| 1 TAM | 13 (10%) |
| 2 TAMs | 7 (5%) |
| 3 TAMs | 15 (11%) |
| **L74V** | 36 (27%) |
| **NNRTI, any** | 117 (86%) |
| K103N | 46 (34%) |
| Y181C | 43 (32%) |
| G190A/C | 39 (29%) |
| **PIs, any** | 0 |
| **Any mutations** | **121 (89%)** |

**National drug resistance surveillance for children in Kenya:** In 2013, Dr. Irene Mukui, site-PI on this proposal, led the National AIDS and STI Control Programme's (NASCOP) cross-sectional, nationally-representative survey of acquired HIV DRMs among adults and children on ART for at least 12 months in Kenya.[31] Virologic failure was defined as a VL ≥1000 copies/mL per national guidelines and 90% of the children were on a NNRTI-containing regimen. Overall, 31% of the 461 children had virologic failure, as compared to only 11% of the adults. Of the children with virologic failure, 89% had at least one DRM detected. Seventy-nine percent had DRMs to NRTIs, 86% to NNRTIs, 77% to both NRTIs and NNRTIs, and 0% to PIs (though PI use was infrequent in this cohort). The most common DRMs were M184V and L74V for NRTIs and K103N, Y181C, and G190A for NNRTIs (**Table 2**). In addition to conducting this survey, Dr. Mukui was involved in training and capacity building of providers in handling DRM testing results and results dissemination.

## *E. Conceptual Framework*

Based on socioecological frameworks that encompass factors from individual to societal levels, we conceptualize the causes of treatment failure in children on ART under three broad, overlapping, and multifactorial themes of: 1) biomedical; 2) health system; and 3) socio-behavioral factors (**Figure 2**).[53] Biomedical causes of treatment failure may include transmitted or acquired resistance, complex drug regimens that are difficult for caregivers to dispense, or failure to provide the appropriate weight-based dosing for younger children.[54,55] Health system barriers include unresponsive centralized laboratory systems, undertrained health care workers, or drug stock outs.[1,4,54] Socio-behavioral factors, such as adherence, disclosure, and stigma are critical to treatment success but complex for children who are dependent on caregivers. It is clear that a multifactorial approach is ultimately necessary to achieve optimal viral suppression rates in this population. Nonetheless, our current pilot proposal begins to address the biomedical and health systems components of treatment failure in children and provides the foundation for our larger vision of addressing the barriers to optimal viral suppression in this population.

As conceptualized in the Health Belief Model, we believe that timely feedback on viral suppression and DRM to both patients and providers, will act as "cues to action" driving their perceived severity and threat of their HIV disease progressing and their likelihood of taking positive actions, such as improving adherence or switching to more optimal ART regimens, to improve HIV disease status.[37] Thus, our fundamental hypothesis is that earlier and more frequent VL monitoring will facilitate earlier and more appropriate clinical decision-making.

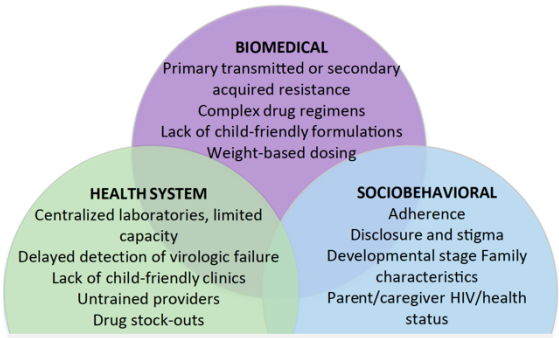



**Figure 2: Conceptual framework for treatment failure in children and young adolescents.** Three inter-related themes of biomedical, health systems, and sociobehavioral issues drive treatment failure in children and young adolescents.

# RESEARCH METHODS

## *A. Outcome Measure(s)*

Study Aim 1: Our primary outcome for **Aim 1a** is rates of viral suppression (defined as VL <1000 copies/mL) at 12 months after enrollment for each child at the study facilities. Our secondary outcomes for Aim 1 are VL suppression and DRM patterns up to 5 years and time to viral suppression among those children without viral suppression at their 1st POC VL testing or newly initiating ART after POC VL testing implementation. We will also examine a set of process outcomes in this study, such as the feasibility of POC VL testing by describing the uptake, which we define as the proportion of children undergoing VL testing within each group at the scheduled intervals. We will also examine the turn-around time for the VL testing results, retention-in-care, proportion of children switching ART, time to switch to 2nd or 3rd line ART, etc. for each arm. We hypothesize viral suppression rates will be higher among children with access to POC VL testing and time to viral suppression shorter compared to children with standard VL testing in the control arm one year after POC VL testing implementation. In **Aim 1b**, we intend to validate HIV-1 POC VL testing against current in-country gold standard VL testing by generating concordance/discordance rates and Cohen's kappa to determine assay agreement. We will also conduct sensitivity analyses to the concordance rates at lower thresholds of viral load cut offs for viral suppression (down to <40 copies/mL, the lower limit of quantification for the POC VL testing).

Study Aim 2: In our **Aim 2a**, we intend to evaluate the impact of targeted HIV DRM testing on viral suppression in the intervention arm and for all children newly initiating ART in both arms. We will describe the proportion of children tested for DRMs with significant mutations within each class of HIV drugs, e.g. NRTIs, NNRTIs, and PIs. We will also explore how sociodemographic, behavioral, clinical, and facility factors may be contributing to the DRM patterns we observe. We hypothesize that targeted DRM testing will shorten time to viral suppression among children on ART without viral suppression, high levels of antiretroviral DRMs in this population, and both individual factors, such as prior exposure to antiretrovirals, and facility-level factors, such as facility type, will predict the presence of DRMs. The same descriptive and analytic approaches will be used for data generated from the next-generation sequencing genotypic resistance and phenotypic resistance, especially to monitor emerging DRMs to DTG. In **Aim 2b,** we intend to determine and validate the test performance of a novel POC DRM assay, OLA Simple, against current in-country gold standard VL testing by generating concordance/discordance rates and Cohen's kappa to determine assay agreement.

Study Aim 3: In our **Aim 3a**, we intend to understand how our intervention functions and how policymakers might use tools to scale up POC VL and DRM testing by conducting focus group discussions (FGDs) and in-depth interviews with key informants. In these FGDs, we will assess policymakers' views on using systems engineering to inform decision-making. We will seek diverse opinions about the feasibility, acceptability, and scalability of systems engineering tool utilization, to understand how such tools would modify, support, enhance, or interfere with decision-making. In the KIIs, we will interrogate factors which acts as both facilitators and barriers to children achieving viral suppression and focus specifically on how POC VL and DRM testing may improve viral suppression. Furthermore, due to the on-going coronavirus disease 2019 (COVID-19) pandemic, we will query participants through study visit questionnaires and in-depth interviews to understand how the novel coronavirus disease 2019 (COVID-19) has impacted intervention delivery and participants' overall well-being. In addition, we will query particular logistical aspects of optimally operationalizing POC VL testing, e.g. how caregivers prefer to learn of the results or where facility staff see the most need for POC VL testing. These logistical aspects, such as the preferred approach to delivering results, result counseling content and methods, and provider reaction to results and additional capacity building needs for the providers and health facilities, need to be explored further in order to optimize implementation and scale up of POC testing. To understand how policymakers might use tools to scale up POC VL and DRM testing, we will use KIIs to understand how usable the model built in Aim 3b; we will cover domains of learnability, efficiency, memorability, error recovery, and satisfaction. In addition to qualitatively assessing these domains, we will

additionally collect a quantitative survey from the same participants to assess learnability, efficiency, memorability, error recovery, and satisfaction. For **Aim 3b,** we will estimate three main outcomes 1) the incremental cost-effectiveness of POC VL testing alone compared to standard laboratory testing, and 2) the effectiveness threshold needed to reach in order for a combination intervention scenario, which goes beyond POC VL testing alone (e.g. combines financial incentives for remaining virally suppressed), to be considered more cost-effective than POC VL testing alone, and 3) the optimal placement of POC machines in Kenya to minimize turnaround time. For the first two outcomes, we will calculate ICER as the ratio of the difference in costs divided by the difference in effects across simulations for the intervention compared to standard-of-care over a 10 year horizon. Consistent with guidelines, we will discount costs and health benefits at 3% annually, and consider ICERs below Kenya's per capita GDP to be cost-effective.[56] We will perform extensive sensitivity analyses to identify influential assumptions. For the third outcome, we will create a *queuing model* – used in industrial engineering to model waiting times – to identify optimal placement of POC machines in Kenya. We will model the reduction in turnaround time and waiting time associated with placement of POC machines in a hub-and-spoke model, where select "hub" facilities (sites with a POC machine) and "spoke" (sites that send samples to a hub) facilities are chosen. We will also model a platform sharing model, in which POC machines rotate between different facilities within a sharing network. At different budget levels, we will identify the optimal number, placement, and network of POC machines.

## *B. Description of Population to be Enrolled*

The study population will be recruited from HIV-infected children and young adolescents age 1 to 14 years newly initiating or already receiving ART at the study facilities (n= approx. 700 children in total). Infants <1 year of age will not be included in the study as they frequently require more than 6 months of ART to suppress their initially high VL, and thus require specialized interpretation and management of VL results.[57-59]

## *C. Study Design and Research Methods*

### Overall Study Design

We propose an open-label randomized, controlled study to pilot the use of POC VL testing and targeted DRM testing among HIV-positive children on ART age 1-14 years over a 12-month period in high-volume HIV treatment facilities in Kisumu, Kenya. We have chosen the study facilities to leverage existing POC technologies, as they are already equipped with GeneXpert® IV systems. Currently, these GeneXpert® systems are primarily being used for tuberculosis management. While validated for HIV VL monitoring, the technology remains to be tested for optimal integration into routine HIV clinical care.[13,60] At each facility, eligible children will be randomized 1:1 to either receive the intervention testing, consisting of quarterly POC VL testing and targeted DRM testing, or standard-of-care (SOC) testing based on the existing Kenyan national guidelines. We will follow the viral outcomes up to 24 months after enrollment for each child and compare VL suppression rates, defined as VL <1000 copies/mL by the Kenyan national guidelines, among intervention vs. control arms, accounting for pre-intervention VL suppression rates (**Figure 4**). Of note, after the 12-month study visit, the study will enter an observational-only phase to continue to monitor for viremia and DRMs, especially among children on DTG-containing ART. During this observational-only phase of the project, participants will continue to receive testing, treatment, and other procedures as part of routine care, according to the standard-of-care then; study staff will not be providing any interventions, but only collect blood samples and administer questionnaires every 6 months up to 5 years of follow-up.

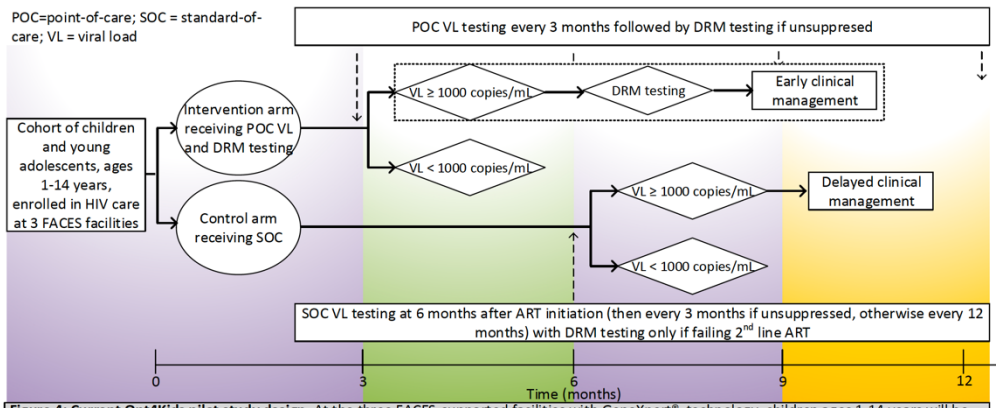



**Figure 4: Current Opt4Kids pilot study design.** At the three FACES-supported facilities with GeneXpert® technology, children ages 1-14 years will be randomized 1:1 to either receive the intervention (POC VL testing every 3 months followed by DRM testing if unsuppressed) or SOC. The primary outcome is VL suppression rates 12 months after POC VL testing implementation.

## Study Setting

The proposed study will occur in western Kenya in Kisumu County. Adult HIV prevalence in Kisumu is 19.9%, 3.4 times higher than the national prevalence.[61] Kisumu County accounts for the second highest incidence of pediatric HIV infections, with 8,600 children estimated to be living with HIV and over 500 annual HIV-related deaths in children.[61] The study will be implemented at low-resource, high-volume, high-HIV burden primary health care facilities and compare viral suppression rates in children randomized to intervention vs. control arms. These facilities are among the more than 70 health facilities in Kisumu County supported by the Family AIDS Care and Education Services (FACES), a PEPFAR/CDC-funded HIV prevention, care and treatment program run through a collaboration between the University of California, San Francisco (UCSF), the Kenya Medical Research Institute (KEMRI), and the Kenya Ministry of Health (CDC NU2GGH001947). FACES's unique model of technical support in direct collaboration with the local Ministry of Health (MOH) and CDC Kenya for provision of HIV care will allow implementation of the study interventions within government health facilities with strong technical support from FACES and study staff, thus promoting local ownership.[62]

**Table 1. Preferred 1st and 2nd line antiretroviral therapy regimens for children and adolescents in Kenya[8]**

| Age | Preferred 1st Line | Preferred 2nd Line |
|---|---|---|
| **< 3 years** | ABC+3TC+LPV/r | National referral for DRM testing/consultation |
| **3-15 years** | | |
| **<35 kilograms** | ABC+3TC+EFV | AZT-3TC-LPV/r |
| **≥ 35 kilograms** | TDF+3TC+EFV | AZT+3TC+LPV/r or ATZ/r |

Using a family model of care, FACES implements comprehensive pediatric HIV care and treatment services in accordance with the Kenyan MOH ART Guidelines. The latest guidelines, released in 2016, support initiation of ART for all individuals living with HIV, regardless of CD4 cell count or disease severity.[8] Recommended 1st and 2nd line ART regimens are outlined in **Table 1**. Routine VL monitoring is offered through a laboratory network connected to a national, centralized laboratory and is recommended for children after six months of continuous ART and annually thereafter for those with VL <1000 copies/mL. Management of children with VL ≥ 1000 copies/mL includes adherence intensification and repeat of VL testing in three months followed by possible switch of ART for those who are still not virologically suppressed. Only those children on 2nd or 3rd line ART who continue to have viremia after adherence optimization, undergo DRM testing at a national reference laboratory. This testing process requires initial consultation with the Kenyan MOH HIV ART treatment committee, who then approves centralized DRM testing, receives the results, and eventually guides the local provider on management of the child failing their 2nd or 3rd line ART.

## Study Collaborators

The proposed intervention builds upon long-standing and strong collaborations among the co-investigators and collaborating institutions. FACES serves as a platform for multiple clinical and implementation science research studies. Drs. Abuogi, Patel, and Oyaro have jointly led a series of implementation science projects and pediatric HIV studies with FACES in Kenya. Dr. Patel is an infectious diseases/HIV physician who conducts implementation science research in optimizing concomitant ART and hormonal contraceptive use for HIV-positive a and adolescents (NIH/NIAID K23AI20855, PI Dr. Patel) and was recently awarded a CFAR pilot grant to study POC VL testing for pregnant women in Kenya for which Drs. Abuogi, Oyaro, and Thomas are collaborators. She has worked in Kenya since 2013, collaborating with FACES on crucial operations research, which has impacted the care of adolescents and women. Dr. Abuogi is a pediatrician, who previously served as FACES Deputy Director Clinical, and alongside Dr. Oyaro, has spearheaded FACES efforts to improve pediatric HIV prevention and treatment services through programmatic interventions and key operational research since 2008. Key studies by Dr. Abuogi include the ongoing MOTIVATE Study (Mother and Infant Visit Adherence and Treatment Engagement Study) (NICHD R01HD080477-01, co-PI Dr. Abuogi), a cluster community randomized trial in Kenya to evaluate the impact of community mentor mothers versus text messaging to optimize adherence and retention for women and infants on lifelong ART. Dr. Abuogi, in collaboration with FACES, has also studied the uptake of routine viral load testing among children and evaluated of risk factors for treatment failure in children on ART. Dr. Oyaro, a medical doctor and Country Director of FACES and current Chief Executive Officer of RCTP-FACES NGO, has led FACES to expand HIV care and treatment for children and served as co-investigator and/or site PI on a series of implementation science studies within FACES. He is currently Co-PI for the LIVING study, which is studying the feasibility of Lopinavir/Ritonavir pellets amongst HIV-infected children (NCT02346487). Dr. Irene Mukui was the NASCOP HIV Care and Treatment Manager for five years and has been intensely involved in implementation science research in Kenya including treatment outcomes for children, sustainability of the national HIV response, and HIV drug resistance. She has led and participated in development of key national and global HIV strategies and policy. Drs. Frenkel and Lutz, developed the POC DRM assay, OLA Simple. Dr. Frenkel's lab will lead the next-generation sequencing for genotypic resistance and Dr. Gotlieb's lab will lead phenotypic resistance testing. Dr. Anjuli Wagner has led several investigations using systems sciences to improve the HIV care cascade in Kenya and other settings.

## Study methods

**Aims 1 & 2**

POC VL testing: In this study, we propose the use of POC VL testing to optimize viral suppression rates in children and young adolescents. Several POC VL tests have undergone analytical performance evaluations and are becoming increasingly available in resource-limited settings.[40,41,46-48,63] Of such POC VL tests, the GeneXpert® HIV-1 VL Assay developed by Cepheid is a leading test that has been validated and found feasible and reliable in rural African communities.[63] It is an in-vitro test designed for rapid quantification of HIV-1 in human plasma from infected individuals over a range of 40 to 10 million copies/mL.[64] The lower limit of quantification for the GeneXpert® VL assay is 40 copies/mL, with the lower limit of detection as low as 15 copies/mL. This assay uses reverse transcriptase polymerase chain reaction (PCR) technology to detect HIV in plasma, achieving a high sensitivity and specificity for quantification of HIV. The GeneXpert® HIV VL assay utilizes the existing GeneXpert® Instrument Systems. These systems consist of the instrument, personal computer, and preloaded software, and are already in place at the intervention facilities for detection of *Mycobacterium tuberculosis*. CDC Kenya has supported the purchase and maintenance of these GeneXpert® IV systems at some of these facilities and continue to provide ongoing training to MOH laboratory staff on its use. For facilities that do not have GeneXpert®, a hub and spoke lab networking model will be used to scale up POC VL. The sites with GeneXpert® technology will serve as the hub and near-by sites will transport their samples to the hub with quick turnaround times. Operationalizing such a model will foreshadow real-life implementation of such systems, where a hub and spoke model could be more feasible and sustainable. As such, the proposed study leverages the existing GeneXpert® technology already being supported by the Kenya MOH and CDC Kenya. In addition, the GeneXpert® VL assay kit is self-contained, including the necessary PCR reagents and supplies. The only additional tool required is a centrifuge to separate the plasma from whole blood which is available at all the intervention facilities in addition to trained laboratory staff capable of performing such procedures. This assay requires <1 minute of hands-on time with an average run time of 90 minutes reported from cartridge insertion to VL report generation. The GeneXpert® platform does not require batching tests and since the VL assay can be run with other tests simultaneously on the same machine, study use of the machine should not interfere with routine clinical use of the GeneXpert® machines. Ultimately, while the analytical performance of GeneXpert® HIV VL assay has been established, its clinical effect and optimal use remain to be determined.[32-34] As POC VL testing becomes increasingly popular, rigorous evaluations, which include clinical outcomes, of such testing is necessary.

For those assigned to the intervention arm, our proposed POC VL testing will be done earlier than SOC, for those children newly initiating ART, and more frequently than SOC for children already on ART. For children newly initiating ART, we will conduct a POC VL test and DRM test pre-ART initiation, then POC VL only 3 months post-ART initiation, and every 3 months thereafter for up to 24 months. For children already on ART, we will conduct a POC VL test 3 months after their last VL test (or at the time of their next visit, if no VL testing has been done before), and every 3 months thereafter for up to 24 months. A blood sample will be collected by a study nurse during study visits every 3 months, which will be coordinated with their routine clinical visits; children on ART return to the clinic monthly for routine visits. Approximately 4mL (<1 tablespoon) of whole blood will be collected in EDTA plasma tubes from participating children for analysis of POC VL, potential DRM testing, and storage for any future studies. If phlebotomy of 4mL is difficult to achieve, we will aim to collect a minimum of 2mL of blood, using most of the sample for POC VL testing, and then prepare dried blood spots (DBS) for potential DRM testing if needed. All samples can be stored at room temperatures up to 25° Celsius, and in a cooler box if study facility temperatures rise above 25° Celsius, for up to 6 hours after sample collection. The samples will be processed within 6 hours of collection for plasma POC VL testing by existing lab staff already trained on GeneXpert® at intervention sites only after obtaining approval from the study nurses. Remaining plasma will be placed in aliquots and transported to the KEMRI-CDC laboratory in coolers for DRM testing or stored for any future testing. The study staff will return POC VL results to the ordering providers and caregivers within 24 hours of sample collection via text messages or phone (and paper results for providers). A generic message that lab results are available and a phone number to call free of charge for results will be sent to caregivers. Results will be given after verifying the caregiver and child's identity followed by appropriate counseling on viral load results. Name of the participant will not be included in the text messaging.

For those assigned to the control arm, VL testing will be conducted per national guidelines (as outlined above) through laboratory networking and transport to a centralized laboratory as the SOC. Children in the SOC arm who are newly initiating ART will have blood collected prior to ART initiation for future VL and DRM testing to be conducted at completion of study follow up. These results will be returned to clinical providers and patients/caregivers after the study. Additionally, in order to have comparable outcomes, POC VL will be conducted on ALL SOC participants at 12 and 24 months of follow up. Study nurses will prompt providers to order follow-up VL tests for those who qualify to ensure uptake of VL testing in both study arms.

We will batch test all stored samples for gold standard VL testing towards the end of the study period for both the arms (n= up to 700 samples), and generate concordance/discordance rates and Cohen's kappa to determine assay agreement. We will also conduct sensitivity analyses to the concordance rates at lower thresholds of viral load cut offs for viral suppression (down to <40 copies/mL, the lower limit of quantification for the POC VL testing). To conduct the in-country

gold standard HIV-1 VL testing (or quantification), we will build on our existing partnership with the KEMRI-CDC HIV Research Laboratory, led by Dr. Maxwell Majiwa, a collaborator on this proposal. The KEMRI-CDC facilities are located immediately outside of Kisumu. The KEMRI-CDC HIV Research laboratory utilizes state of the art HIV-1 quantification technologies by using Abbot m2000sp/RT, Roche CAP/CTM, and Roche C8800 systems (cite). Overall, the two main assay platforms, the Abbot vs. the Roche platforms, both perform extremely well, with subtle difference in accuracy and precision of each platform (cite). As we get closer to the time of running our samples for this validation, we will determine which specific platform to use depending on logistical aspects, such as current usage of the platforms. These platforms can run the assays with 0.5-1mL of plasma, which will already be stored in cryovials of this volume at the same facility. For each sample analyzed, we will receive printed reports noting exact quantification or below or above the limits of quantification.

HIV DRM testing: We will conduct targeted HIV DRM testing at the Kisumu-based KEMRI-CDC HIV Research laboratory using consensus sequencing on all plasma samples which lack viral suppression, defined as VL $\geq$ 1000 copies/mL per national guidelines. "Targeted" DRM testing will include DRM testing for each child on the first detection of such a VL and in children newly initiating ART. Subsequent samples from the same child on the same ART regimen will not be routinely retested, since costs of repeat DRM testing outweigh detection of new DRMs. Since approximately 65% of children achieve viral suppression currently in the FACES-supported facilities, including the study facilities, i.e. 35% have viremia VL $\geq$ 1000 copies/mL, we anticipate conducting DRM testing in at least 100 samples in participants in the intervention arm over the course of this pilot study. Such plasma samples, once processed from whole blood within 6 hours of collection, will be transported from the local health facility to the KEMRI-CDC laboratory the same day.

To conduct the HIV DRM testing, we are partnering with KEMRI-CDC, which has extensive facilities, including the HIV Research Laboratory led by Dr. Maxwell Majiwa, a collaborator on this proposal, located immediately outside of Kisumu. The KEMRI-CDC HIV Research laboratory utilizes Applied Biosystems (ABI) 3130*xl* Genetic Analyzers to conduct its HIV drug resistance testing on 0.5-1mL of plasma. These analyzers have all steps automated, from polymer loading to sample injection to data analysis. This laboratory utilizes an optimized in-house assay to detect reverse transcriptase- and PI-based mutations. This validated, assay is broadly sensitive in genotyping HIV-1 subgroups, detecting all mutations classified in the IAS-USA mutations list, and more sensitive than commercially available assays for mixed viral populations.[65] The KEMRI-CDC in-house assay can run samples from plasma or DBS; the ability to also use DBS is a key asset for our study, in case adequate volume of plasma cannot be obtained from our younger study population. DBS samples will be prepared by spotting 100 µL of whole blood onto each of the preprinted circles on the DBS paper, then dried overnight at the local health facility at ambient temperature, and then transport wrapped DBS cards the next day.

At the KEMRI-CDC laboratory, the samples will be batched together daily, or at most weekly as this laboratory experiences a high volume of testing for other clinical and research projects. Results will be returned to the study staff within 24 hours of assay results, noting that the assay can take up to 24 hours to run. The DRM results report contains a list of the major and minor DRM genotypes as well as phenotypic interpretations, based on the scoring systems generated by the Stanford Genotypic Resistance Interpretation Algorithm. Study staff will then forward the DRM results to the ordering providers within 24 hours of their receipt and enter them into the patient file. Providers will use the results to guide patient management. Providers at the study facilities will undergo training on interpretation of the DRM testing results and study staff will work closely with facility leadership to assist providers in managing children with DRM including making any potential switches in accordance with national guidelines. As noted above, Dr. Mukui has extensive background from the 2013 Kenya DRM survey in training and helping to build capacity for local HIV providers in understanding and interpreting DRM testing results. She will lead the standard operating procedures related to DRM testing results sharing with the facility providers and actively be involved in clinical decision-making as needed. We anticipate the turn-around time for the DRM testing to be 1-2 weeks at most; remarkably faster than the months-long process currently in place as SOC in Kenya. We anticipated that very few children in the control arm will undergo DRM testing as DRM testing is not currently recommended for children on 1$^{st}$ or 2$^{nd}$ line ART per national guidelines.

To further analyze emerging DRM for DTG, we will follow participants longitudinally after they reach the study primary endpoint (of 12 months after enrollment) and follow their routine care VL patterns while on DTG via an observational extension of this project for up to 5 years. If VL$\geq$200 copies/mL is detected by reviewing routine care records, study staff will contact the participant for either additional blood collection or permission to use any stored sample for next-generation sequencing (NGS) and phenotyping. All samples with VL ≥200 copies/ml will be tested by NGS using the Illumina MiSeq platform using Primer ID in Dr. Frenkel's lab at Seattle Children's Research Institute in Seattle, WA (the children's hospital affiliated with the University of Washington). This NGS method will allow for detection of low-frequency drug resistance mutations (minority variants), which may be present at baseline and/or at time of viremia. Sequences will be analyzed for HIV drug resistance mutations using the Stanford HIV drug resistance database (HIVdb) genotypic resistance interpretation system, and drug resistance mutations will be reported at detection thresholds of 1%, 2%, 5%, 10%, and 20%. The results

will be returned to the facility staff. For phenotyping resistance patterns, Dr. Geoff Gottlieb's lab at the University of Washington will perform phenotypic resistance testing for those samples with relevant genotypic mutations. His lab requires two 2-mL cryovials of plasma, which will be collected at the time of blood collection for NGS sample collection. Neither validated NGS nor phenotypic resistance testing is currently available in Kenya, thus, requiring shipment of samples to the U.S. During this observational-only phase of the project, participants will continue to receive testing, treatment, and other procedures as part of routine care, according to the standard-of-care then; study staff will not be providing any interventions, but only collect blood samples and administer questionnaires every 6 months up to 5 years of follow-up. However, if any irregularities in routine care are noted in regards to treatment or monitoring by study staff during this observational-only phase, study staff will alert the health facilities for possible routine care interventions.

OLA Simple-based DRM testing: We will evaluate the ability of OLA Simple to identify DRMs by comparing its results against DRM testing results from CS from study samples, as well as from the de-identified stored samples at the Kenya National HIV Reference Lab (NHRL), a key partner and stakeholder in future scale up of POC HIV DRM testing in Kenya. We will also evaluate the usability of OLA Simple when performed in the field in a clinical setting, bringing OLA Simple one step closer to being implemented as a POC DRM test. To increase efficiency, the OLA Simple testing will be conducted retrospectively, once the majority of samples to be tested have been collected, with direct observation from the Lutz team. Nonetheless, to gain better insight into technician performance without direct supervision from the developers, we will also conduct a limited, prospective one-month long OLA testing towards the end of the participant follow-up. We will also partner with NHRL to cross-validate samples that have already undergone CS for clinical purposes and where leftover specimens are now stored and available for analysis at NHRL. These specimens are collected as part of routine clinical care from individuals experiencing viral failure, and are used for routine surveillance and evaluation of new test kits within the Kenya Ministry of Health. For NHRL, the use of their de-identified, stored specimens falls under the existing NASCOP-MOH routine lab validation procedures. Of note, results of the OLA Simple testing will not be returned to patients or providers, as this technology has not yet been approved for clinical use. The following procedures will be followed:

a. Approximately 500uL of plasma from the samples genotyped by CS will be tested by staff at each facility trained in the use of OLA Simple, with oversight and assistance by the Lutz team over a 3-week period. The facility staff performing OLA Simple and the Lutz team will be "blinded" to CS results. Results from OLA Simple will be interpreted as presence or absence of resistance using test strip lines for each codon tested, based on a visual guide for test interpretation and automated analysis from captured test images. For OLA Simple tests that show INDETERMINATE results for one or more codons, samples will be re-tested for that codon in two separate kits; if the two new results concur, that result will be used, but if the two new sets do not concur the result will be reported as a test failure for that codon. (Specimens with indeterminate or discordant results will be further analyzed as noted in "d" below.)
b. The OLA Simple results (MUTANT, WILD-TYPE, or INDETERMINATE at each codon) will be compared to sequencing results to calculate test sensitivity, specificity, indeterminate rate, and other parameters.
c. OLA Simple will target specific NRTI, NNRTI, and PI DRMs, including: (1) NRTI M184V/I, K65R; (2) NNRTI K103N, Y181C, G190A, V106M; and (3) PI V82A, I50V/C, I54V/M/L, I84V/A mutations (based on published analysis of DRMs among Kenyan adults in the Frenkel laboratory using the Illumina platform[38]).
d. Prior studies have shown that OLA Simple may be more sensitive than CS; specifically, OLA Simple can detect DRMs at ~10% of a patient's viral population while CS detects DRMs at about ~20%. To address this sensitivity gap, any sample negative for DRMs by CS but positive by OLA Simple will be tested by the more sensitive Illumina sequencing (detects DRM down to 1% of viral population, requiring as few as 2000 copies of HIV RNA). Illumina sequencing will be performed in Dr. Frenkel's laboratory in Seattle. To avoid shipping biohazardous material, we will ship non-infectious leftover RNA from OLA Simple testing in Kisumu.

Clinical decision support for early management of children on ART: Overall, providers will be instructed to follow current Kenya National Guidelines for the management of any child with VL $\geq$ 1000 copies/mL which includes assessment of barriers to adherence and other potential factors related to virologic failure. For the intervention arm, standardized protocols and clinical decision support flow sheets will be developed to facilitate clinical management and closely record decision-making steps. Both caregivers and providers will receive POC VL results within 24 hours of the study team receiving the results. Providers will perform routine counseling and adherence intensification, then reassess VL results three months from initial VL in the intervention arm only. If DRM testing is performed, study staff will assist providers in interpreting the DRM results and assessing the need to switch to 2$^{nd}$ or 3$^{rd}$ line ART. If a provider determines a child should be switched to 2$^{nd}$ or 3$^{rd}$ line ART based on DRM testing or POC VL results, the child and caregiver will be called back within one week of VL/DRM results for counseling, adherence support, and regimen change. To facilitate caregiver response, we will sensitize patients/families on implications of VL results and provide standardized results counseling. Special consideration will be given to children newly initiating ART as they may not achieve virologic suppression after only 3 months of ART. In such cases, providers will be trained to monitor trends in VL over the first six months on ART and to consider intervention (e.g.

switch to 2nd or 3rd line) only after child has completed a minimum of 6 months on ART, unless significant primary transmitted mutations are found.

Children in the control arm will receive the SOC, which includes clinical management consistent with national guidelines including assessment of adherence and multi-disciplinary team review. Providers will follow their standard protocol for notification and follow up of children with high viral loads.

### Aim 3a: Qualitative interviews

Qualitative evaluation: We will conduct the key informant interviews (KIIs) with five subgroups of key informants (**Table 2**), with 10-20 KIIs conducted within each group with a total of 50-100 persons interviewed, some of whom will be interviewed serially up to four times during the study period, and up to 20 who have participated in Aim 3 will also fill a brief quantitative survey. We will additionally conduct up to 4 focus group discussions (FGDs) (with 6-10 participants each) with policymakers at the county or national level.

**Table 2: Groups and timings of key informant interviews**

| Groups | Enrollment or 3 month visit | 12 month visit | 24 month visit | One Time (towards the end of study period) |
|---|---|---|---|---|
| Group 1: Caregivers | X | X | | |
| Group 2: Adolescents | X | X | | |
| Group 3: Providers, lab staff and other facility staff discussing POC VL and DRM testing questions | | | | X |
| Group 4: Policy makers and others discussing POC VL and DRM testing questions | | | | X |
| Group 5: Providers and policy makers discussing modeling of POC VL scale up | | | | X |

*Focus group discussion guide*: Our FGD guide will be developed from an implementation science framework focusing on feasibility, acceptability, and scalability, seeking to understand how utilizing systems engineering tools would modify, support, enhance, or interfere with decision-making.

Interview guide- Our first KII guide will be developed from a socioecological model of pediatric viral suppression, which include individual, interpersonal, organizational, and structural/policy factors that influence pediatric viral suppression (**Figure 5**). In these KIIs, we will interrogate factors which acts as both facilitators and barriers to children achieving viral suppression and focus specifically on how POC VL testing and OLA Simple may improve viral suppression. We will also query participants through study visit questionnaires and in-depth interviews to understand how the novel coronavirus disease 2019 (COVID-19) has impacted intervention delivery and participants' overall well-being. In addition, we will query particular logistical aspects of optimally operationalizing POC VL testing, e.g. how caregivers prefer to learn of the results or where facility staff see the most need for POC VL testing. These logistical aspects, such as the preferred approach to delivering results, result counseling content and methods, and provider reaction to results and additional capacity building needs for the providers and health facilities, need to be explored further in order to optimize implementation and scale up of POC testing. Our second KII guide will have three phases: in phase I, each participant will be asked first to describe how they might make a decision about where to place POC VL testing machines in their geographic area. Prompts will include factors like cost, equity, coverage, competing disease priorities, and inclusion of other decision-makers. In phase

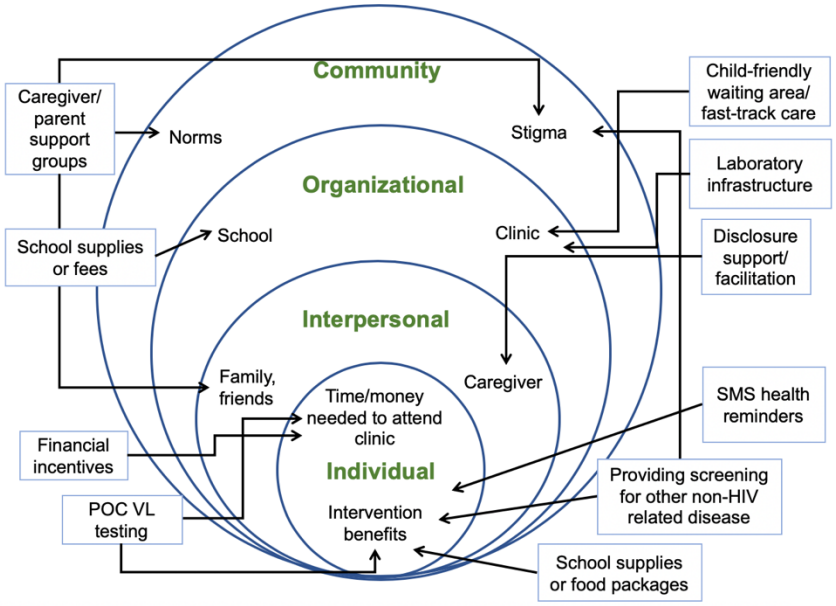


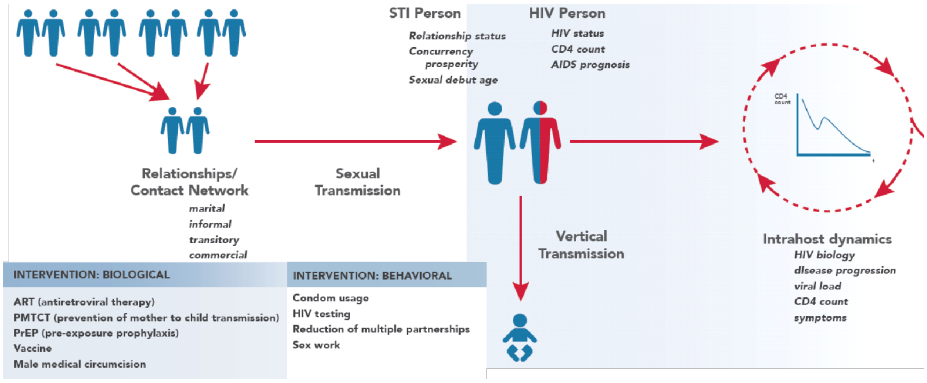

**Figure 1: Socioecological model of pediatric viral suppression**

II, each participant will be asked to look at a computer screen with the Excel-based tool from Aim 3b, be read a short set of standardized instructions, and asked to complete a series of tasks within the tool from Aim 3b. Within the tool, they will be asked to: change 1) the overall budget available to purchase POC VL machines, and 2) the maximum waiting time allowable for each sample. During this phase, policymakers will be asked to narrate their thoughts aloud, borrowing methodological approaches from cognitive interviewing, a technique used in psychology to assess comprehension. They will be asked to reflect on their experience of interacting with the tool and the interpretability of the model results. During phase III, the tool will be removed and they will be asked to describe how satisfied they felt while using the tool and how they could envision this tool being used to make decisions related to placement of POC VL machines. Of note, each interview guide for the appropriate group will be adapted for that group, e.g. the language and content of the interview guides for the adolescents may vary markedly from that we use to interview a policy maker. Following the qualitative interview, participants will complete a brief quantitative survey assessing learnability, efficiency, memorability, error recovery, and satisfaction.

**Aim 3b: Modeling**

Costing and cost-effectiveness: Costing- We will use activity-based micro-costing, staff interviews, and time and motion studies, to estimate the annual cost of HIV monitoring per child living with HIV in the control (SOC) and intervention (POC VL + DRM testing and OLA Simple) arms. An experienced research assistant will conduct time and motion studies to estimate the nurse and clinician times necessary to complete the clinic visits for both arms, and to estimate the average number of patients seen in the clinic each day. We will assess the time required to complete each step of the HIV care visit (VL testing and DRM, adherence counseling). Observing multiple visits by various staff members will allow estimation of the average time taken for each step; time needed for research activities (e.g. administering informed consent) will be removed from intervention time to provide an estimate of the intervention, if implemented as a government program. For the OLA Simple testing, we will only conduct the micro-costing activities during the prospective portion of its use, as this time frame best mimics real-life use of the test and optimizes efficiency once the facility staff have gained experience conducting the tests. We will use standardized cost menus to collect site costs, including start-up costs, clinic space, human resources, supplies, and VL test costs. When data are not available from our cohort, we will utilize data from population-based studies from Kenya and sub-Saharan Africa. Additional cost data may be obtained from health facilities, published government information on labor costs, and health economics literature. Analyses will follow the guidelines for costing HIV interventions[66,67], and will reflect the provider perspective. We will also collect data on patient out-of-pocket costs to assess if POC VL testing saves participants time and expense, reflecting the societal perspective.

Mathematical model- We will parameterize a model with cost data from our microcosting activities and viral suppression data from Opt4Kids and project HIV infections, HIV-related deaths, and DALYs associated with various POC VL testing interventions. We will use EMOD-HIV, an open-source agent-based transmission model to project the cost and clinical impact of implementing POC VL testing alone and as part of a CIS in Western Kenya.[68,69] EMOD includes geographic patterns of (1) age-specific demographics (fertility, mortality, migration); (2) vertical transmission, (3) a detailed, user-configurable care continuum for HIV treatment and prevention interventions that can be configured to reflect differences in treatment access and health seeking behavior among children and adolescents, including heterogeneities in access and retention in care (e.g., age and sex differences in diagnosis and linkage), and (4) a detailed within-host model of HIV progression and treatment to simulate the health and transmission effects of pediatric ART (**Figure 6**).[68,70-72]




Queuing model design: Operational research models are meant to simulate the behavior of a system and use data to inform decision-making. We will use *queuing models*, a specific kind of model commonly used in industrial engineering to model waiting lines or "queues". Queuing models represent queuing systems (i.e., waiting lines) using mathematical formulas to describe the system performance, such as average waiting time and expected queue length in the system. A queuing model has the following components; an input source generating the customer arrival process, the queue capacity, the queue discipline (e.g., first-come-first-served) and the service mechanism (e.g., number of servers, service time distribution). The queuing model example input and output parameters are delineated in Table 4, which will be collected from a representative sample of 12 clinics; we will include 5 health facilities where *Opt4Kids* is taking place and at 7 additional facilities selected to reflect a range of pediatric patient volumes, facility sizes, and distances to the central laboratory. To further model the parameters for a wider range of facilities in Kenya, In conjunction with our colleagues at the Kenyan Ministry of Health, we

will identify 100 facilities in Kisumu (out of ~260) that are representative of the distribution of facility sizes, locations, patient volumes, staffing and level of care, using the existing Kenyan Master Health Facility List. Lastly, we will create the Excel-based queuing model. Our objective is to identify conditions (number of POC machines in the region and the structure of hub-and-spoke or platform sharing networks) that minimize the total expected waiting time and service costs. The model will simulate various hub-and-spoke and platform sharing network configurations under a user's specification (e.g. options on entering maximum travel distance or maximum number of spoke per hub or facilities in a sharing network). For each type of facility, defined by a unique set of input parameters on sample arrival distribution and service mechanism (i.e., the type of queuing model), the model will estimate average turnaround time (including travel time) and the expected number of samples processed at each facility under a particular network structure. Using these estimates, the model will select the optimal network structure with the minimum expected cost.

## Start-up activities, including training

Start-up activities will include laboratory strengthening and training in conjunction with the MOH to ensure high quality and accurate VL testing using the existing GeneXpert® equipment at the study facilities. Health care providers and laboratory staff at study facilities will undergo training on POC VL testing (including sample collection, lab procedures, and documentation), all relevant study logistics, review of National ART Guidelines and interpretation of DRM testing results. Study staff, including study coordinator, study nurses, and data clerks/manager will be trained on recruitment, consenting, and all study protocols. Study staff, led by Dr. Mukui, will work with the intervention facilities to develop standard procedures for clinical management of children with viremia following national guidelines.

## Recruitment and randomization

Caregivers of HIV-infected children already on ART or newly initiating ART at one of the study facilities will be referred by clinic staff during a routine clinic visit to participate in the study. Since the majority of children are seen on a monthly basis (maximum of three months for older children), we anticipate recruiting the majority of eligible participants in 3-4 months; historically, in FACES-supported research studies we have been able to recruit over 80-90% of the eligible participants. As of December 2016, FACES had enrolled 3,727 HIV positive children on ART at all its health facilities. At the proposed study facilities, 683 children were actively enrolled in care on ART as of December 2016, with approximately two children newly initiating ART per month at each site. We therefore anticipate that by time of study recruitment targeted for April 2018, we should have more than 700 eligible children to recruit at the intervention facilities even with some attrition, which tends to be approximately 10% in one year due to loss to follow-up or death, prior to study start. We will encourage referral of eligible participants by providing site staff a small incentive of around $1USD for referral of children who are enrolled. The FACES retention model combines multiple interventions to maintain retention in care including text message reminders, phone calls, home visits, and defaulter tracing which study nurses will supplement with additional phone tracing as needed. We anticipate these efforts will minimize losses to follow up and allow us to maintain sufficient participants to estimate our study outcomes well and minimize any bias due to missing outcomes.

The University of Washington's (UW) International Clinical Research Center staff has extensive experience running high-profile randomized clinical trials, including in the design and implementation of randomization. In order to assure balance by arm within site and age group (1-9 or 10-14 years of age), children will be randomized 1:1 to the intervention vs. control arms in varying sized blocks stratified by facility and age. This means that a randomized allocation list will be made for each age group within each facility. Each list will be composed of blocks (e.g. 8, 10, or 12) with equal numbers of participants assigned to intervention and control arms with random order of assignment within each block. The resulting allocation lists will bear no indication of where each block starts or stops, preventing guessing the next allocation even in an open-label study. Study nurses will ensure fidelity to the arm allocations by flagging participant charts with the study arm and limiting access of POC VL testing to the intervention arm.

**Inclusion criteria:**

- Children age 1-14 years living with HIV (documented HIV positive or HIV viral load)
- On antiretroviral medication (ART) per Kenya National Guidelines OR
- Newly initiating first line ART

**Table 3: Study timeline by study activities**

| Timeline (quarters) | Pre-award | Q 1 | Q 2 | Q 3 | Q 4 | Q 5 | Q 6 | Q 7 | Q 8 |
|---|---|---|---|---|---|---|---|---|---|
| Study preparations, IRB | | | | | | | | | |
| Recruitment and enrollment | | | | | | | | | |
| Participant follow up | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Data analyses | | | | | | | | | |
| SMART protocol and procedures development | | | | | | | | | |
| Manuscripts, R01 application | | | | | | | | | |

## Study Timeline

This study will be conducted over 2 years. Considerable budgetary resources are required to complete POC VL and DRM testing in quarters 2-6, necessitating a two-year project period to meet budget limits. While this is an ambitious timeline, based on our prior experience conducting research in this setting and the need to urgently address suboptimal viral suppression in children on ART, we believe it is achievable. The pre-award and initial two quarters of the study period will be used for protocol development, obtaining ethical approval from relevant institutional review boards, start-up activities, including hiring study staff, trainings, laboratory infrastructure preparation, data collection tool development, and study activation visits. Recruitment will occur rapidly over 3-4 months with continuous follow-up thereafter for a minimum of 12 months and up to 24 months after enrollment for each child at the facility. The final two quarters of the study period will be used for data cleaning, analyses, manuscript(s) writing, and results dissemination.

## Procedures

**Blood sample collection:** For children in the intervention arm: blood samples will be collected every 3 months for POC VL testing and DRM testing per protocol for the first 12 months. For children in the control arm: blood samples will be collected per standard of care by non-study clinic staff per routine clinic schedule after study enrollment. Newly initiating children will undergo baseline VL and DRM testing as outlined above. After either intervention or control arm participants reach the primary endpoint of 12 months after enrollment, for the observational-only phase of this project, they may be followed every 6 months up to 5 years by routine clinic staff for VL monitoring; if VL≥200, then study staff will request routine staff to share any leftover or stored plasma sample to obtain NGS genotyping and phenotyping. If no stored samples exist (for example, at the VL testing facility) or not in sufficient quantities, then the participant will be asked to return to the clinic for additional sample collection for NGS genotyping and phenotyping. Phlebotomy required for POC VL testing and DRM testing is also required for routine lab and viral load monitoring already a part of HIV care in Kenya. However, this will be more frequent than routine lab monitoring. Participants may experience some additional pain and anxiety due to this. This risk will be minimized by utilizing trained study nurses with experience collecting blood in children. Additionally, if the required 4ml of blood is not obtained, we will utilize dried blood spots which require less blood to reduce the need to perform additional phlebotomy. Once the testing for the study is completed, remaining samples will be stored in Kenya and later batch transported to the UW ICRC Laboratory Repository for storage for future studies.

**Blood sample storage and transfer:** Existing standards and best practices will be followed for blood sample storage and transfer. Greater details are found in the laboratory standard operating procedure.

## Data Collection

Our overall data collection strategy leverages the existing electronic medical record systems already running at the study facilities, and supplements data collection by study staff for important gaps in the existing records (**Table 4**). FACES uses standard MOH encounter forms and registers to capture HIV care and treatment data, which are then entered into electronic medical record systems by data clerks daily after clinic visits. These encounter forms support program activities and facilitate thorough history taking, guide medical care, and allow for rigorous research. Data entry into the electronic medical records is performed by FACES-supported and trained MOH data clerks and includes pre-programmed data quality checks. In addition, FACES conducts robust data quality checks daily and audits weekly for each data clerk and provider. We will, therefore, leverage this existing resource for some data collection at the study facilities. Over the last decade, FACES has been able to implement a robust electronic medical record system; several research studies have taken advantage of the robust data and published pioneering and impactful findings.[73,74] Because recording of VL results from centralized laboratories back into the routine HIV encounter forms is sometimes incomplete, we will supplement VL outcome information from the NASCOP online VL database and facility laboratory VL log books. We will also supplement the electronic medical records data with additional data that our study team will collect, targeting important data gaps in the electronic records particularly pertinent to sociobehavioral aspects of our study (e.g. adherence). Study data will be abstracted by trained research data clerks using direct, electronic data entry via tablets into standardized data collections forms developed in REDCap, a data collection and management system hosted by the UW Institute of Translational Health Sciences. REDCap meets all required standards for protection of personal health information.

**Table 4: Example characteristics to be captured by existing electronic medical records[1] or planned study data tools[2]**

| Sociodemographics | Behavioral | Clinical | Laboratory | Process |
|---|---|---|---|---|
| ▪ Child's age at diagnosis[1]<br>▪ Number of siblings and HIV status of each[2]<br>▪ Caregiver HIV status[1,2]<br>▪ Socioeconomic status of family[2]<br>▪ Food security[2]<br>▪ Ethnic, religious background[2] | ▪ Disclosure of HIV status[1,2]<br>▪ Adherence (e.g. pill count,[1] refill pick-up from pharmacy logs,[2] validated adherence scale[2]) | ▪ Date of HIV diagnosis[1]<br>▪ Health status (e.g. WHO clinical stage, weight-for-age, etc.)[1]<br>▪ Start of ART, regimens used, dosing, side effects[1]<br>▪ Exposure to any antiretrovirals prior to HIV diagnosis[1,2]<br>▪ Development and treatment of any opportunistic infections[1] | ▪ Basic labs[1] (e.g. hematology, chemistry)<br>▪ HIV labs[1] (e.g. CD4/8 cell counts, VL testing) | ▪ Turn-around time for VL and DRM testing results[2]<br>▪ Clinical decision-making during study[2] |

We will program in-built data verification and quality tools within our REDCap database. In addition, the data team will conduct daily quality checks for accuracy and completeness of data. The data team will also generate random and routine quality checks for appropriateness and quality of data. Errors detected will be verified against the source documents and an audit trail of corrections will be maintained. The data will be de-identified with a unique, database-specific identifier for confidentiality of personal health information. Data entered into mobile devices, such as tablets, will be synchronized with the REDCap database when internet connection is next available. The REDCap database will undergo daily backups at the UW and the data manager will download the data files on a regular basis and store on HIPAA-compliant and password-protected locations. Proper documentation and storage of the metadata and any files relevant to data management will be handled with utmost care. Only certain study staff will have access to the data files containing personal health information, and only de-identified data will be shared amongst the researchers.

All participants in the KIIs and FGDs will undergo informed consent with additional assent obtained from the participating adolescents (ages 13-14 whose caregivers will provide the consent). The interviewers will audio record the KIIs and FGDs with a digital recorder and take field notes. The interviews or FGDs will last 30-60 or 60-120 minutes, respectively, and occur in the preferred language of the participants, and participants will be provided approximately 6-10 USD as reimbursements for their time. The location of the interviews will vary depending on the groups being interviewed. For example, the interviews for caregivers and adolescents will occur in or near the health facilities on days that they are attending the facility for their routine clinical care visit. The interviews for the facility staff will likely take place in or near the health facilities too and scheduled at times most convenient for the staff. The interviews with policy makers and other stakeholders will take place at locations and times most convenient for them. The FGDs will likely be conducted virtually, if the COVID-19 pandemic precautions persist during study implementation of this activity, either on phone or on a virtual platform like Zoom.

For the costing and modeling studies, in addition to the staff interviews, we will use standardized cost and time menus to collect site costs and times, including start-up costs, clinic space, human resources, supplies, VL test costs, and timing of client arrival at a facility and VL sample collection, processing, and return. When data are not available from our cohort, we will utilize data from population-based studies from Kenya and sub-Saharan Africa.

CS, NGS, phenotyping, and OLA simple-based DRM testing: Scanned copies of the DRM testing results from CS, NGS, and phenotyping will be directly uploaded into a data form in REDCap by the study staff after receipt of each result. Similarly, the DRM testing results from OLA Simple will be entered into a data form and a picture of the resulted assay uploaded into REDCap. The data forms will be linked to the same participant by a unique study ID. Study investigators, namely Drs. Patel and Lutz, will conduct the interviews and surveys in-person or by phone/Skype, if needed. The interviews will be digitally audio-recorded when feasible. Study investigators will conduct the costing studies, including time/motion studies and interviews with clinical staff.

## *E. Potential Scientific Problems*

First, though we will strive to conduct the POC VL testing while the patients are still at the facility, so that the patients and providers have immediate feedback, because we are leveraging existing MOH resources, i.e. the GeneXpert® platforms, to conduct this pilot we cannot guarantee this. Thus, the ultimate utility of a POC test is diminished. However, our proposed work will undoubtedly reduce the current turn-around times of 4-6 weeks for centralized laboratory VL testing. Further, return of results to caregivers and providers via text messaging or phone will facilitate more rapid interventions for those with viremia than current practice. Likewise, as already noted, our current DRM testing process uses a central laboratory. Currently, point of care DRM testing technology is under development but is not available for use in this study. Second, GeneXpert® is arguably one of the more resource-intensive existing POC technologies. Other existing or in-development platforms, such as the AlereQ®, can run on battery power, are more compact, and hence more user friendly for more rural facilities. Nonetheless, the generalizability of our findings will not be limited to the GeneXpert® platform, as we are not validating this specific platform but rather testing a new approach to HIV management for children on ART which may include any POC VL testing platform. A key aspect of our proposal is leveraging existing POC technologies at these facilities, and, again, careful considerations will be made in selecting the most appropriate POC technologies for future studies. Third, crossovers or contamination may occur in our study if providers may want to utilize POC VL testing for children in the control

arm, since the POC VL testing is being implemented at the facility but only being offered to those children randomized to the intervention arm. Such crossovers will be minimized by limiting access to POC VL test ordering to study nurses to ensure only children randomized to the intervention arm undergo POC VL testing. Also, because all providers will receive additional training on management of children with virologic failure, it is possible that viral suppression will increase for both groups. Even if this occurs, we likely still have sufficient power to detect a significant difference between study arms. Fourth, prior research has shown that assessment of adherence—an integral element for achieving viral suppression—in this population is extremely difficult. We intend to use multiple methods to assess adherence including pill counts, pharmacy logs, and a standardized and validated pediatric assessment tool for children on ART in resource-limited settings.[75] However, none of these measures are 100% accurate. While measurement of drug concentrations, for example, adds accuracy to adherence assessments, the complexity of measuring timed blood collections, performing testing out of country, and inability to return results real-time to inform clinical management, makes its use less feasible and meaningful. Electronic pill monitoring devices may not reflect actual adherence or may be an intervention in of itself in promoting adherence. Ultimately, VL testing, in addition to targeted DRM testing, is arguably the most sensitive measure of adherence. Fifth, we have found some caregivers and children find blood draws difficult and may be, therefore, less willing to participate in this study. We plan to support demand creation for VL testing through health talks provided by research staff in the waiting areas to provide education on the importance of VL testing and address concerns for blood draws. Finally, there are few children newly initiating ART at study facilities, thus we will not have a sufficient sample to describe primarily transmitted DRMs nor will it be meaningful to block randomize the expected 24 ART-naïve children. However, our data on DRM in this group will certainly add to current knowledge and we will address any major imbalance in distribution of ART-naïve children between arms as described in our analysis.

## *F. Data Analysis Plan*

**Table 5: Effect sizes and power calculations**

| Viral suppression rates, control (SOC) vs. intervention (POC) arms | Difference (effect size) | Power |
|---|---|---|
| 65% vs. 70% | 5.0% | 0.22 |
| 65% vs. 72.5% | 7.5% | 0.45 |
| 65% vs. 75% | 10% | 0.71 |
| 65% vs. 76% | 11% | 0.80 |
| 65% vs. 77.5% | 12.5% | 0.89 |
| 65% vs. 80% | 15% | 0.98 |
| 70% vs. 80% | 10% | 0.76 |
| 70% vs. 82.5% | 12.5% | 0.93 |
| 70% vs. 85% | 15% | 0.99 |

**Sample size and power:** Power for the study is based on the primary outcome of viral suppression rates in children in the intervention vs. control arms at 12 months after enrollment for each child (Aim 1). With approximately 700 eligible participants at the study sites, 90% enrolling in our study, and 10% loss to follow-up over 12 months, we anticipate having a total of 567 children (or 284 per arm) with outcomes for analysis. With this number, we estimate power of ≥80% to detect a difference of 11% or higher between the intervention and control arms (**Table 5**). These calculations are based on Fisher's exact test, two-sided α=0.05, and initial viral suppression rates of approximately 65% (estimated from historical FACES facility data, stubbornly stable since 2014).

**Data analysis and interpretation:**

Study Aim 1: For **Aim 1a**, we will collect individual-level data on viral suppression up to 24 months, time to viral suppression, and process outcomes, such as uptake of VL testing. We note that viral suppression is assessed on different schedules for the intervention arm (quarterly) versus control arm (6-monthly unless unsuppressed and then quarterly until suppression). Although testing frequency differs by arm, note that both arms include viral suppression testing at 12 months, which is our primary outcome. In addition, both arms test quarterly in those not currently suppressed, i.e., the group for analysis of our secondary outcome (VL suppression at 24 months and time to viral suppression). For both outcomes, the assessment plan is similar in spite of the overall difference in testing frequency by arm. We will provide descriptive statistics by randomization arm for study population baseline demographics and facility characteristics, viral suppression rates at baseline and quarterly afterwards, and process outcomes of interest, such as uptake of VL testing, turn-around time for the VL testing results, retention-in-care, and proportion of children switching ART. Analyses of the intervention will be intent-to-treat (ITT), meaning that all participants will be analyzed as randomly assigned, regardless of any issues in participant receipt of the intervention, crossovers, or any other post-randomization information.

Our primary analysis in this randomized study will compare the proportion of children with viral suppression at 12 months after enrollment for each child (primary outcome) in the intervention vs. control arms (primary predictor) using a logistic regression, adjusting for facility and age group strata (1-9 or 10-14 years). Because VL suppression in young

adolescents may differ from younger children due to unique adherence challenges, they may have a quite different response to our intervention. Therefore, a key secondary analysis will stratify by age group in the sense that we will estimate the effect of the intervention separately by age group, using an interaction term between age group and intervention group in the model. While the study is not powered for the intervention effect within age subgroups, particularly in the subgroup of 10-14 year old, this analysis will provide data for the intervention effect within each, as well as for the difference in effect between the younger and older children. Our randomized study design should protect us from bias between intervention and control groups, but should we find that strong predictors of viral suppression vary between these groups at baseline, we will also perform the analysis adjusting for any such differing characteristics. Additional analyses including multivariate logistic regression models will estimate associations between viral suppression and all potentially related individual-level factors, such as age, sex, duration on ART, prior viral suppression patterns, and family demographics, as well as facility, in order to explore predictors of viral suppression in this context. We will also do a secondary analysis separating the outlined outcomes for children on 1st vs. 2nd line therapy.

For our secondary analysis, we will compare the proportion with viral suppression at 24 months after enrollment and follow the analytical plan detailed above for the primary outcome. We will also compare time to viral suppression using a Cox regression model limited to individuals who were not already suppressed at 1st POC VL testing or those initiating ART. The primary predictor will be intervention vs. control arm, with stratified baseline hazards by site, adjusting for other predictors that could confound this relationship, such as the ones listed above. As noted above, although the VL testing schedule is generally different in intervention vs. control arms, the frequency of VL assessment is not different among those on ART but not suppressed; in both intervention and control arms, children without viral suppression at their first VL test while in the study are subsequently tested every 3 months until achieving suppression. The exception is for children newly initiating ART at study enrollment, the first VL test is at 6 months in the control arm and 3 months in the intervention arm; to align the outcome assessment in those newly initiating ART, we will exclude from the analysis the initial 3-month viral load in the intervention arm for those participants. In a sensitivity analysis, we will exclude those newly initiating ART altogether (estimated to be 24 individuals).

For **Aim 1b**, the quantified values will be transformed on a log scale. Then we will correlate log copies when detected, <40 copies/mL but detected, or not detected. We will use Cohen's kappa statistic, generating 95% confidence intervals, to determine the agreement in concordance and discordance of the POC VL testing against the gold-standard testing. We expect that as the quantity of viral copies/mL detected increases, so will the concordance rates between the two platforms. We will also assess the agreement between the two platforms using a Bland-Altman plot. For the discordant results, we will compare descriptively the results found in each platform.

Study Aim 2: To estimate **(Aim 2a)** the effect of providing timely DRM results on viral suppression for intervention participants undergoing DRM, separately from the effect of POC alone, a secondary analysis will be performed with outcome of time to viral suppression. As in Aim 1, we will use a Cox model with primary predictor of intervention vs. control arm. To distinguish the effect of DRM results from that of POC VL testing, we will add a time-varying covariate which indicates, for each visit with a VL test, whether the clinician was notified of a positive DRM result since the child's prior VL test. If implemented as expected, all children without viral suppression will be provided DRM testing and providers notified of the results. This model will allow us to divide the estimated effect of the intervention between the effect of POC VL testing alone (on those who do not have DRM), and the effect of POC VL testing plus DRM testing for those who do undergo DRM testing. We will perform these Cox models both unadjusted and adjusted, accounting for other factors likely to affect viral suppression, particularly those that may vary at baseline in the intervention vs. control arms.

We will also describe the proportion of samples within the intervention arm that have any targeted DRMs by HIV drug classes, e.g. NRTIs, NNRTIs, and PIs. We will report the proportion of samples with each type of mutation detected by drug class, and further group these mutations into major and minor ones. For example, we will examine major mutations in M184V/I and K65R for NRTIs, K103N, Y181C, G190A, and V106M for NNRTIs, and V82A, I76V, 184V, L47A, L90M, M46I, and D30N for PIs; one or more of the NRTI and NNRTIs mutations alone accounted for 99% of the mutations detected among adults on 1st line ART from resource-limited settings with similar findings in small studies conducted with children, though the rates of PI-based mutations are increasing with more children on PI-based regimens.[37] With 284 participants randomized to the intervention arm, we expect approx. 35% (100) to not achieve viral suppression at their 1st POC VL test and undergo DRM testing. With n=100, we will be able to estimate prevalence of DRMs to within +/- 5% to 10% of the 95% CIs. For example, for a class of DRMs with prevalence of 80%, we anticipate generating exact 95% CIs of 71.3%-87.0%; a less common DRM at 10% will generate 95% CIs of 5.1%-17.1%.

We will use logistic regression models to identify risk factors associated with major and any DRMs. Potential risk factors of interest include individual-level (age, sex, duration on ART, prior antiretroviral exposure, prior viral suppression patterns, WHO clinical stage), clinical parameters (such as weight- or height-for-age), immunological status, family-level, HIV status of caregivers, socioeconomic status, and facility-level factors, such as volume of patients and urban/rural location. Covariates with $p<0.20$ in univariate analyses will be included in the multivariate models. We will also consider including interaction terms for any suspected effect modifiers such as age group of the child. The above analytic approaches will be taken for analyzing data from the NGS genotyping and phenotyping; in addition to descriptively generating the

patterns and distribution of NGS-detected DRM, including for minority variants and for mutations relevant to DTG, we will also compare them to the results obtained via CS when concurrent results are available.

To evaluate **Aim 2b,** CS and OLA simple-based DRM testing (Aim 4): To evaluate the test performance of OLA Simple (Aim 4a), we will compare test results for each codon from the OLA Simple test against a composite "gold standard" constructed from CS and Illumina sequencing (the latter performed on samples that are negative for DRMs by CS), stratified by retro- or prospective testing. We will calculate sensitivity, specificity, and positive and negative predictive values for the detection of DRMs per codon, organized by drug classes, for the OLA Simple. The above are the fundamental metrics the WHO, for example, considers in its prequalification application for in-vitro diagnostic and laboratory technologies approvals;[40] achieving sensitivity and specificity >90% would demonstrate high test performance.

Study Aim 3: For **Aim 3a**, the interviewers themselves will transcribe the first few audio files from the interviews in the language that the interview occurred in. And if it is different from English, then translate them to English. A second study staff will verify the English translations against both the non-English transcripts and the audio files. Once we are confident of the accuracy of our transcription and translation, the interviewers will directly transcribe the non-English interviews into English. The English transcripts will be imported into Nvivo 10.1 for coding. Serial interviews for the same person will be coded together to maintain context for that person. We will develop a codebook documenting codes, definitions, guidelines on their use, and example quotes. 2-3 study staff will independently code the transcripts, including initial double coding. The initial, primary codes will be developed from the interview guides and expanded into more detailed, secondary codes during the coding process. After the initial round of coding, the researchers will meet to discuss their coding process, assess intercoder agreement, and resolve discrepancies through consensus. Once the coding is complete, for the first question guide, we will use framework analysis to organize the data for further analysis, prioritizing longitudinal over cross-sectional analysis.[76,77] Finally, analytic memos will be written to lift the primary and secondary codes into thematic analyses that represent a full range of perspectives, both convergent and divergent. Several measures will be taken to ensure high quality data and rigorous analysis, such as principles of reflexivity[78] and rigor.[79-81] For the second question guide, we will use thematic analysis using a deductive approach based on Nielsen's framework (Table 6). Special attention will be paid to identify domains of learnability, efficiency, memorability, error recovery, and satisfaction that could be improved in future iterations of the Excel-based model. For the FGD guide, thematic analysis using a deductive approach based on the concepts of acceptability, feasibility, and scalability of tools, as well as modifications, support, enhancement, or interference with process.

For **Aim 3b**, the micro-costing data, time and motion studies, and clinical outcomes will be used to estimate the average cost per HIV-positive child achieving VL suppression and retained in care in the intervention compared to the control arm. The mathematical modeling will project clinical outcomes and estimate the cost-effectiveness of the intervention. These data are key to informing decision makers considering implementation of HIV adherence interventions for children. We expect to demonstrate that the intervention is cost-effective for improving VL suppression and retention in care. *Queuing model parameterization and analysis:* Finally, we will use our model to conduct analysis for a range of scenarios. The base case scenario of the model will be built to reflect 100 facilities in Kisumu, Kenya, using a fully SOC VL processing system (Figure 7). Several additional model scenarios will be run to represent: 1) a hub-and-spoke model, in which samples are sent from neighboring facilities (spokes) to a facility equipped with a stationary POC VL machine (hubs); and 2) a platform sharing model, in which a single POC machine is transported from one facility to the next on a regular basis, rather than transporting the samples in the hub-and-spoke model. Within the hub-and-spoke scenarios, we will model a range of combinations of SOC and already GeneXpert® POC-equipped sites (hubs), ranging from 2-30% of facilities having a dedicated POC machine. We will further modify the number of spoke sites. Within the platform sharing scenarios, we will model a range of combinations of the number of sites within a sharing network, duration of time that a machine stays stationary, and geographic distribution of networks. We will also include a combination model of both hub-and-spoke and platform sharing, pending our initial results. We will run the full queuing models with a range of constraints that reflect the public health priorities of Ministries of Health in resource-limited settings, including differing budgets available to purchase different POC machines and different turnaround times acceptable for VL sample return. *Use of data:* This model will yield direct, practical suggestions for POC machine placement at different budgetary levels in Kisumu, Kenya and may be easily adapted for other regions within Kenya. The model will also be modifiable for future use in other African regions and settings. The goal of this Excel-based model is to provide a user-friendly tool to Ministry of Health policymakers at the county or regional level to assist in resource allocation to optimize chronic care monitoring tests for populations.

## *G. Summarize Knowledge to be Gained*

The findings from this pilot study will inform programmatic scale-up of POC VL and DRM testing in Kenya and elsewhere. Additionally, our findings will provide crucial HIV drug resistance information for children on ART, which is currently lacking and very likely to undermine current management approaches for children with virologic failure. Specifically, data collected from this study will help optimize future POC VL and DRM testing strategies. This work will directly inform national policy approaches to ART regimen choices for children in Kenya. Children living with HIV have continually lagged adults regarding

HIV treatment access and health outcomes including viral suppression; our study strives to contribute to the evidence-base that will close this gap.

# HUMAN SUBJECTS PROTECTIONS

Ethical approval for this study will be obtained from all relevant institutional review boards before initiating any of the study data collection activities. In the US, UW and UCD's Institutional Review Boards will review this protocol. UCSF already has ethical approval from both UCSF and CDC for routine data collection and evaluation at FACES sites in Kenya that will be facilitated through UCSF. In Kenya, the protocols will be reviewed by AMREF IRB. We will ensure that all procedures conform to US, Kenyan, and international ethical standards regarding research involving human subjects. Future studies or analyses conducted with the data collected in this study will undergo separate ethical reviews.

The proposed research will be conducted in collaboration with Family AIDS Care and Education Services (FACES), a CDC/PEPFAR-funded HIV prevention, care, and treatment program operating in western Kenya since 2005, which already has long-standing positive relationships with the local Ministry of Health (MOH) and the study communities. Our study design takes advantage of the extensive existing infrastructure of FACES and current laboratory infrastructure. Additionally, the feasibility, acceptability and potential for scale up of the proposed interventions were carefully considered with MOH and FACES investigators. The proposed research builds on our findings and our experience protecting human subjects in the proposed study regions gained during the implementation and operational research by FACES such as the Rapid Results Initiative for PMTCT as well as the MOTIVATE Study (Mother and Infant Visit Adherence and Treatment Engagement Study) for which Dr. Abuogi is a multiple-PI (NICHD 1R01HD080477-01).

**Risks to the Subjects**

**Human subjects involvement, characteristics, and design**

Our proposed study design is an open-label randomized, controlled study piloting the use of POC VL testing and targeted DRM testing among HIV-positive children on ART age 1-14 years over a 12-month period in high-volume HIV treatment facilities in Kisumu, Kenya. At each facility, eligible children will be randomized 1:1 to either receive the intervention testing, consisting of quarterly POC VL testing and targeted DRM testing, or standard-of-care (SOC) testing based on the existing Kenyan national guidelines. We will follow the viral outcomes up to 24 months after enrollment for each child and compare VL suppression rates, defined as VL <1000 copies/mL by the Kenyan national guidelines, among intervention vs. control arms.

**Aim 1** of this study aims to determine the impact of point of care (POC) VL on the proportion of children achieving viral suppression and time to viral suppression for those who are either initiating ART or not suppressed.

The target population for aim 1 of the study includes approximately 700 HIV-infected children and young adolescents aged 1-14 years on ART at one of the intervention sites supported by FACES. Children who are outside of this age range will be excluded. These children will be randomized 1:1 to intervention vs. control arms. Therefore, approximately 350 children will receive the intervention (POC VL and DRM testing) and another 350 will receive the current standard of care as recommended by the Kenyan Ministry of Health, including routine viral load monitoring through centralized laboratory testing and DRM testing only for those failing 2$^{nd}$ line ART.

We conservatively estimate that around 90% of HIV infected children and adolescents and their families will agree to participate in the intervention portion of the study. Our sample size takes into account the potential for as much as 10% attrition due to loss to follow up, transfers out, or death based on current retention rates within the FACES program for children. Therefore, even if we experience as much as 10% attrition, we will still have a sufficient number of participants to evaluate the impact of the interventions.

**Aim 2** will explore the impact of targeted HIV drug resistance mutation (DRM) testing and patterns of DRM among HIV-infected children on ART without viral suppression.

In this aim we will determine the impact of DRM testing of children with viral load $\geq$ 1000 copies/ml among the 350 children 1-14 years on ART randomized to the intervention arm only. Based on current viral suppression rates in children at FACES sites, we anticipate at least 35% of children will have one or more POC VL results of $\geq$ 1000 copies/ml during the first 12 months of study follow up. This translates to around 100 DRM tests which allows us to generate point estimates with reasonable confidence intervals to assess our outcomes for this pilot study.

**Aim 3** of this study aims to better understand how our POC VL intervention functions using qualitative research methods and estimate the costs and time and feasibility of implementing pediatric POC VL testing and DRM monitoring at scale.

**Sources of materials**

Data for Aims 1&2 will be collected in the following manner:

As part of standard care at participating health facilities, a standardized Ministry of Health routine HIV encounter form is filled for each child at every clinic visit. This form will provide baseline demographic and clinical data on participants at intervention and non-intervention sites. Additionally, clinic registers which document laboratory testing and results, patient visits, and pharmacy refills will also be utilized. A standardized, electronic data collection tool will be used to capture supplemental study information for both intervention and control participants. Data will be abstracted from by trained research data clerks using direct data entry via tablets into REDCap. REDCap meets all required standards for protection of personal health information. Data collected from study activities, namely process and outcome data on POC VL and DRM testing, will also be captured into the REDCap-supported data collection forms.

Finally, because currently VL results from routine VL testing are erratically recorded on the routine HIV encounter form, and therefore not always available in patient charts or electronic medical records, we will supplement VL outcome information from the National AIDS and STI Control Programme (NASCOP) online VL database and facility laboratory VL log books.

Data for Aim 3: For **Aim 3a**, the interviewers will audio record the KIIs or FGDs with a digital recorder and take field notes. Later the audio files will be transcribed from the interviews in the language that the interviewer occurred in, and if different from English, then translate them to English. Quantitative surveys assessing learnability, efficiency, memorability, error recovery, and satisfaction will be captured either on paper or electronically and stored on REDCap. For **Aim 3b**, we will use standardized cost and time menus to collect site costs and times, including start-up costs, clinic space, human resources, supplies, VL test costs, and timing of client arrival at a facility and VL sample collection, processing, and return. When data are not available from our cohort, we will utilize data from population-based studies from Kenya and sub-Saharan Africa. Additional cost data may be obtained from health facilities, published government information on labor costs, and health economics literature.

Of note, prior to shipment of any samples outside of Kenya, permission from appropriate authorities and agencies will be pursued as is standard in such cases.

**Potential Risks**

Potential risks to participants in this study include social risks involved if information about participants or their family members, specifically HIV status, or other personal details were to be disclosed outside of the research setting. In particular, children and caregivers might face serious social risks (disruption of family, discrimination, and/or physical harm) if their HIV status were to be disclosed without their consent. These risks could be posed if a) clinic staff do not maintain confidentiality, or b) laboratory specimens are not handled in a manner that maintains confidentiality. The proposed study approach considers these potential risks. Clinic staff including service providers, laboratory technicians and phlebotomists, are already trained on maintaining privacy and confidentiality for all patients and this will be reinforced during clinic-wide trainings pre-study initiation.

Neither POC viral load testing nor DRM testing are anticipated to pose significant additional risks to study participants outside of the risks they are exposed to regularly as part of routine HIV care. Phlebotomy required for POC VL testing and DRM testing is also required for routine lab and viral load monitoring already a part of HIV care in Kenya. However, this will be more frequent than routine lab monitoring. Participants may experience some additional pain and anxiety due to this. This risk will be minimized by utilizing trained study nurses with experience collecting blood in children. Children undergoing to POC VL and DRM testing may switch to 2$^{nd}$ or 3$^{rd}$ line more frequently than those at non-intervention sites. This may present a risk if they are not adherent to 2$^{nd}$ or 3$^{rd}$ line treatment as 3$^{rd}$ line and salvage regimen options are limited in Kenya. However, more frequent VL testing may help to identify these children sooner and intervene such that they are able to re-suppress on their 2$^{nd}$ line regimen. DRM will also inform if 3$^{rd}$ line treatment options are needed which will facilitate more rapid transition to 3$^{rd}$ line when needed.

The use of ART in children within this study will follow Kenya National ART guidelines. ART risks include potential medication side effects, induced viral resistance from poor adherence or long-term exposure to ART limiting future drug options, and the burden of initiating lifelong treatment at an early stage of disease. However, Kenyan ART guidelines have clear recommendations on side effect monitoring and management and our study is intended to reduce the potential for drug resistance. It is important to note that this study is not intended to evaluate the safety and efficacy of ART for children in Kenya but rather to explore interventions that will maximize virologic suppression and therefore health outcomes for children with HIV.

**Adequacy of protection against risks**

**Recruitment and informed consent**

**Aims 1&2:** Participants will be recruited at the study facilities during routine clinic visits. Caregivers accompanying HIV infected children 1-14 years of age receiving or initiating ART will be approached and invited to participate. Recruitment may occur as part of pre-clinic information sessions provided by trained study staff (e.g. study nurses), by clinic staff during care, or by study staff before or after routine visits. We will offer study site providers a small incentive for referring patients who are eligible and enroll in the study. There is currently precedence among other local studies to offer a small incentive for successful study referrals. Site providers have been educated on enrollment eligibility and will be directed to not coerce or otherwise influence study enrollment. If the caregiver expresses interest in participating in the study, they will be invited into a private room to conduct the informed consent process where study staff will confirm if the caregiver is a parent or legal guardian. Informed consent will be obtained from at least one parent, or from the primary caregiver if both parents are deceased. Informed consent will be obtained by a study staff, namely study nurses, in the local language preferred by the caregiver. Caregivers and children over 13 years of age will be invited to participate and will be given clear explanations that this is something separate from their child's regular care and that they have the option of refusing to participate in any part of the research without any effect on their or their child's routine care received at the facility.

As part of this informed consent process, caregivers will be informed of the purpose and methods of the study, interventions, procedures to protect the confidentiality of the information, their rights to withdraw from the study at any time, randomization procedures, the fact that their participation or non-participation will not affect the medical care/performance evaluation that they or their child receives at the study health facilities, and persons to contact if they have any questions about the study. They will be asked to sign an informed consent form, or if illiterate, to provide a thumbprint in the presence of a non-study staff witness (the standard practice used in our other Kenyan studies, as approved by the Kenyan IRB). Caregivers will receive a small transport and time reimbursement at enrollment of ~$5 USD. This is similar to enrollment incentives being offered locally by other research studies. We do not feel this enrollment reimbursement will be coercive or unduly influence caregivers to enroll as it is in line with other research being conducted in the same setting. Transport reimbursement of ~$5 USD will also be offered at the 12-month, and possibly 24-month, visits to incentivize return for collection of endpoint VL samples. The same transport reimbursement will also be offered during the observational phase of the project after a 12-month study visit for every 6-month visit up to 5 years, since blood collection may be required out of routine care visit or phlebotomy for our study monitoring of DRM.

Children age 13 and older will be given the opportunity to provide assent for participation in the study. They will be provided information at an age appropriate level and given the opportunity to assent or decline separate from their caregiver.
For those children who are not aware of their HIV positive status, which will be ascertained by asking their caregivers first, we will provide a modified assent form which removes any mention of HIV or HIV-related terms. All study participants will be informed that they will be followed up for a minimum of 12 months and up to 24 months.

Families who do not wish to participate, will continue to receive standard HIV services within the facility.

**Aims 3:** We will use purposeful sampling to identify key informants for each subgroup of key informants (**Table 3**). We will purposefully sample Groups 1 and 2 from both the control and intervention arms, ensuring we are capturing a diversity of key informant characteristics such as caretakers of younger vs. older children, children with and without viral suppression, male vs. female informants, etc. Study staff will recruit these participants from our enrolled study sample. For Group 3, we will again use purposeful sampling to interview providers and other facility staff, such as pharmacists, medical supervisors, nurses in charge, etc., with the goal of ensuring we capture a breath of professionals within the health facilities. Study staff, including our study coordinator, will recruit these participants from the facilities where we are conducting our study. For Group 4, we will use a combination of snowball and purposeful sampling, relying on our community partners and those attending our community stakeholder meetings to help us generate our initial pool of key informants. Once a person agrees to interview, we will also ask that person to help us identify another 2-3 persons to consider for interviews. For the focus

groups, we will use purposeful sampling to identify participants from national and county levels. We will ensure we capture a diversity in perspectives including local county and national policy makers, NGO staff, and academics working in this field and country. Study staff, including the lead investigators, will recruit these participants from known community partners and stakeholders.

**Protection against risk**

In order to minimize social risks to participants in this study, all study and participating facility staff will be thoroughly trained on procedures for maintaining confidentiality and will be asked to sign a pledge of confidentiality. All potential study participants will benefit from the facility- based training for staff on pediatric treatment failure identification and management.

All consent and will be kept in locked cabinets, initially at the health facility, and later be transferred for secure storage at the central study office in Kisumu. All personal identifiers from study data will be in electronic format only in password protected devices and files. When these procedures are followed, it is highly unlikely that any participant information will be disclosed to anyone outside the research team.

Given that some caregivers/young adolescents may not be literate, all explanations will be made orally in a language well understood by the participant. The caregivers/adolescents will also be given a copy of the study informed consent/assent form written in the local language to keep if he/she wishes. This page will include names and phone numbers of persons to contact with any questions regarding the study. The informed consent forms will be available in Dholuo, Swahili, and English.

Training on VL interpretation and treatment failure recognition and management in children will be conducted at all study facilities prior to the launch of the intervention. This training will also include additional training on interpretation of DRM results and when to switch to second line. Expert pediatric HIV consultation will be available for complex cases provided by the multiple-PIs and Scientific Advisory Committee members, including Dr. Betsy McFarland, a pediatric HIV expert at the University of Colorado, in collaboration with local experts. Ongoing support for health care providers will be available through the study staff, FACES technical support teams and the Ministry of Health.

Importantly, this research will be conducted in collaboration with health facilities where the FACES program already provides comprehensive HIV education, counseling, and services. Thus, it will be possible for the study staff to provide immediate referrals to FACES staff for research participants who are in need of help with medical or social issues regarding HIV, including issues related to disclosure of HIV status and non-HIV related health problems that may be identified during the course of the study.

Children will be receiving the standard of care at all sites based on current Kenyan national ART guidelines. All study sites are already providing ART to children living with HIV. All sites have the necessary clinical, laboratory, and psychosocial support to provide ART to children. FACES technical support is available to all sites and includes consultation on HIV care and treatment, treatment failure, laboratory networking, psychosocial support, and routine support supervision visits.

**COVID-19 precautionary measures**

We are adapting study procedures in compliance with COVID-19 precautionary measures recommended by the Country and County governments. We have developed standard operating procedures in-line with current guidance to minimize risk of COVID-19 infection and transmission among participants and research staff. In order to minimize the risk of COVID-19, we will administer most of the questionnaires and conduct interviews through virtual means (by phone, WhatsApp etc.). In case the participants access the health facility for routine follow up or specific study procedures, facility screening procedures for COVID-19 will be repeated by phone by research staff to identify participants with symptoms or recent exposure to COVID-19. If no symptoms or recent COVID-19 exposure, then we shall ensure that both the interviewer and participant have their masks on, observed hand hygiene, have their temperature assessed and the requirement 1 meter – physical distance to carry out study activities. Of note, collection of POC VL samples does not allow for physical distancing

but close contact will be minimized, and above precautions taken. Study participants may benefit from POC VL testing at all study time points to achieve viral suppression.

**Potential benefits of the proposed research to the subjects and others**

Through their participation in the interventions to be tested (Aims 1&2), intervention participants will potentially benefit from intervention activities designed to achieve viral load suppression. If the interventions are found to be effective, there is the potential to scale up this intervention to include all FACES-supported health facilities in Kisumu, Kenya and for further scale up through our collaboration with CDC and the Kenyan Ministry of Health. Participants in the control are likely to benefit from the increased training and additional awareness of facility providers on VL testing and management of treatment failure in children. In addition, the research findings have the potential to benefit other Kenyan and sub-Saharan African HIV infected children in the future.

**Adverse event reporting**

All serious adverse events associated with the procedures of this study will be reported in the specified time-frame to the appropriate Institutional Review Boards. The primary risk to participants in this study is social harm associated with HIV status disclosure. Field staff will be trained to complete descriptions of adverse events that will then be communicated to the onsite Study Coordinator immediately, and then sent electronically to both the U.S. co-Principal Investigators and the Kenyan co- Investigators within 24 hours.

**Data safety monitoring plan**

In addition to the above plans which outline thorough assessment and mitigation of participant risk, the multiple-PIs, Drs. Patel and Abuogi, will ensure ongoing routine data safety monitoring to ensure participant safety. We will work with our local collaborators, study staff, facility staff, and other key stakeholders to ensure feedback to the PIs regarding potential increased risks or adverse events. If any increased risks are identified, the PIs will review and determine the adequacy of human subject protections, making recommendations for enhancing these protections if deemed necessary. The study team will create detailed procedures to be followed in cases of adverse events involving study participants. The PIs will immediately review each adverse or serious adverse event report and will establish criteria for stopping the study if any arm of the study is associated with increased severe adverse events. As this is a very low risk study, attempting to optimize clinical care delivery through enhancing the laboratory monitoring components of HIV care, a formal Data Safety Monitoring Board is not required. We believe the above protections and procedures will minimize any potential risk to participants.

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