## Chapter 1. Purpose and Organization of the Procedures Manual

The Procedures Manual (PM) for the Medchal CATCH study documents in detail the plan for implementation of each component of the study protocol. The PM should be used throughout the study as a guide to maximize consistency in execution of each phase of the protocol, which will ensure that each participant receives an equally high standard of care, and that our ultimate scientific aims can be answered with quality data.

This research protocol provides the highest level of cervical cancer screening and treatment to the participants in the study. However, the women enrolled in the study are not only receiving quality care for themselves, their participation is critical to the development of a sustainable cervical cancer prevention effort in their communities – an achievement that will help to ensure the health of their daughters and granddaughters. Because we are indebted to these women for their critical help in this broad-reaching goal, it is our duty to ensure that the data we collect as part of this study protocol is of the highest quality and consistency. We must ensure that the women of Medchal Mandel receive the full benefit from their participation and be able to answer our research aim of identifying the most appropriate screening method for this community. We will do this by following the protocols laid out in the PM as closely and consistently as possible, from the very first enrollment to the last woman seen.

The PM is organized by numbered chapters that outline in detail specific components of the study protocol. Early chapters give overviews of the study objectives and the protocol by visit type. Subsequent chapters provide detailed protocols for implemented procedures that will be used during the participant visits. These protocols have been standardized through years of international research on cervical cancer, and should be followed in detail for each participant. Rare circumstances may necessitate a deviation from the documented protocol. This will inevitably happen in medical practice, and the study protocol should not be allowed to compromise patient care. When such deviations are required, a notation of protocol breach with explanation will be documented in the patient's study chart.

## Chapter 2. Scientific Background and Study Objective

### 2.1 General Background

Invasive cervical carcinoma is a major cancer of women in the developing world. Nearly one-fourth of the new cervical cancer cases worldwide are estimated to occur in India, where a large majority is identified in an advanced and inoperable stage of disease. This is a public health tragedy, since among all major human cancers; cervical cancer is potentially the most preventable. It can be easily diagnosed in its pre-invasive stage, because the cervix is readily accessible for inspection and sampling, and pre-invasive cervical abnormalities persist for many years and can be effectively treated. Proof of prevention by early detection is evident in that the cervical cancer burden in the USA in the 1940's was similar to that of India at present, but had decreased by over 70% by the 1980's as a result of effective Pap smear screening programs and treatment of identified pre-invasive lesions. Despite the success seen in the US and elsewhere, it has been difficult, for various reasons, to establish and maintain effective Pap smear programs in India and other areas of the developing world. However, in the past few years, there has been a marked progress in our understanding of the etiology and molecular pathogenesis of cervical cancer. It is now recognized that cervical cancer in all parts of the world is the end result of a process that is initiated by infection of the genital tract with high-risk human papillomaviruses (HPVs) and that only a small proportion of infected women develop invasive cancer many years after the initial infection. Many new strategies of cervical cancer screening monopolize on the understanding of HPV infection as a necessary cause of cervical cancer. These strategies may allow the application and development of more objective screening tools that can be successfully implemented in resource-poor settings. Furthermore, many viral and cellular factors mediate the development of cervical cancer, and it may be possible to incorporate some of these biomarkers in the new and promising strategies of primary cervical cancer screening.

Specific Aims:

**(1) To compare the test characteristics (sensitivity, specificity, positive and negative predictive values and referral to colposcopy rates) of each of four screening methods (Pap smears, visual inspection of the cervix (VIA), HPV DNA in clinician-collected specimens (HPV-C) and HPV DNA in self-collected specimens (HPV-V)), for the detection of prevalent high-grade squamous intra-epithelial lesions (HSIL) and/or invasive cervical carcinoma;**

(2) To evaluate the above methods for their ability to predict incident disease;

(3) To characterize viral factors (genotype, variants, viral load, viral persistence, and integration) for their roles in disease progression; and

(4) To characterize cellular events (p16 expression, 3q gain, loss of FHIT expression, and altered methylation patterns), for their roles in disease progression.

## 2.2 Cervical Cancer Screening in Rural India

In our targeted rural Indian population, Medchal Mandal, no effort has been made so far to screen for cervical cancer. Screening may be offered to women as they present for other medical care, but this occurrence is usually rare. The infrastructure for an organized program exists at MediCiti Hospital in Medchal, and in conducting this project we seek to not only find and treat all prevalent cervical cancer and precursors, but also to identify a sustainable screening program for this area.

## 2.3 Pap Smear

Pap smears represent the trademark of cervical cancer screening worldwide, and in regions with the financial support and infrastructure to sustain these programs, the use of Pap screening has contributed to a precipitous decline in the incidence of cervical cancer. Pap smear screening is based on the ability to collect exfoliated cervical cells using a brush or spatula, to spread the cells on a glass slide, and to detect morphologic abnormalities consistent with cancer or its precursor lesions with the aid of a microscope. The estimates of sensitivity and specificity of Pap smears, as measured against the reference gold standard of histology, vary widely. In a meta-analysis of Pap smear accuracy, the authors concluded that the Pap smear may be unable to achieve concurrently high sensitivity and specificity: specificity in the 90-95% range corresponded to sensitivity in the 20-35% range (Fahey et al 1995). Therefore, successful Pap smear screening is contingent on the ability to increase the cumulative sensitivity by screening at frequent intervals. Many regions have attempted to implement Pap smear-based screening strategies without a demonstrable reduction in the incidence of cervical cancer. Failures of Pap screening strategies stem from the difficulty in maintaining the solid infrastructure, training, and quality control required for successful early detection and intervention. A successful Pap smear program requires that (a) the smears contain adequate numbers of cells from the transformation zone and the endocervix, (b) the smear is fixed properly and promptly and is transported to the laboratory, (c) it is read by a trained cytopathologist and, if abnormal, confirmed by a pathologist, (d) the results are transmitted back to the clinician, (e) women who have abnormal cytology are informed and scheduled for a second clinic visit for a diagnostic work up, and (f) women requiring treatment are brought in for a third visit. Lack of any one or

more of these requirements, e.g., inadequacy of smears, lack of needed reagents and of trained cytopathology technicians and cytopathologists, poor follow-up of women with abnormal smears, and/or lack of treatment facilities, is the major cause for failure of the screening programs. Even in well-organized screening programs, reading of Pap smears is subjective; expert pathologists do not agree with one another in the interpretation of mild cytologic abnormalities (Sherman, et al. 1994).

## 2.4 HPV DNA Testing

The role of HPV infection as a necessary cause of invasive cervical cancer and cervical cancer precursor lesions has been firmly established. Because of this criterion (i.e., HPV as a necessary cause of cervical cancer), women who are HPV negative are considered to have no immediate risk for development of cervical cancer. Conversely, identification of HPV positive women should, in theory, select all women at risk of cervical cancer. HPV cannot be cultured, and serologic tests are relatively insensitive and lack the requisite genotype spectrum for diagnostic use. Therefore, HPV testing has relied on the identification of HPV DNA from exfoliated cervical and/or vaginal cells. A variety of assays are available for HPV diagnosis, the most common being the commercially available Hybrid Capture 2 (HC 2) assay (Digene Diagnostics, Gaithersburg, MD). HC 2 screens for the presence of one or more of 13 high risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). In an International Study of Invasive Cervical Cancer, over 95% of the HPV-positive cervical cancer specimens were positive for these types (Bosch, et al 1995). In a large number of investigations, the sensitivity of HPV DNA detection for the identification of HSIL and cervical cancer varied between 84% and 97% and was always higher than the corresponding estimate of Pap smear sensitivity in the same study. HC 2 results are uniformly scored relative to a known control standard, with an objective cut-off point to determine positive results.

### 2.4.1 Clinician-directed cervical cell sampling (HPV-C)

Most studies evaluating the role of HPV DNA testing in cervical cancer screening algorithms use samples that are collected directly from the cervix by the clinician administering the pelvic exam. This is considered the gold-standard sampling method for HPV DNA testing.

### 2.4.2 Self-directed vaginal cell sampling (HPV-V)

Self-directed vaginal cell sampling allows a woman to collect her own exfoliated cell sample for HPV DNA testing. This circumvents the need for a clinic visit, potentially increasing coverage of cervical cancer screening in remote areas, or in populations that are reluctant or unable to take the time for an annual screening visit. In addition, the privacy offered by

this technique may be more appealing in some cultures. The self-collected HPV test has been compared in many studies to Pap smear and clinician-collected HPV tests. Usually, the self-collected swabs perform comparably, but with some reduction in overall sensitivity. The test performance requires reconciliation with the increased coverage and potentially lower cost to make a final estimate of the feasibility of programmatic application.

## 2.5 Visual Inspection with Acetic Acid (VIA)

VIA offers the most inexpensive direct cost for a cervical cancer screening method. This technique involves the application of 3-5% acetic acid to the cervix to look for acetowhitening, which indicates areas of dysplasia. Field health care workers can be trained to perform this test, and it has compared favorably with HPV DNA testing and Pap smears in many studies. The overall test sensitivity of VIA is generally better than Pap, and comparable to the self-collected HPV DNA test. This method offers an advantage in non-compliant and transient populations in that results are immediate, and treatment can be performed without a further diagnosis. However, studies have shown that this 'screen and treat' approach results in over treatment by a 4:1 ratio.

## 2.6 Summary

The screening tests employed in the CATCH study have been validated and proven efficacious in multiple studies. The variation in the performance of each test usually varies by geography, and may be related to the intensity of training of medical personnel, the availability of necessary equipment, and the prevalence of HPV infection in the population screened. The CATCH study is not a randomized trial: we will perform all screening tests for each participant, and will be able to fully characterize the relative performance characteristics of each test such that the most suitable screening strategy for this community in rural India can be identified and established. This can prevent cervical cancer in the population for decades.

## Chapter 3. Overview of the Scientific Protocol

### 3.1 Eligibility and reasoning

Women residing in the defined 25 village area of rural Medchal Mandal (excluding Medchal township), will be targeted for recruitment into the CATCH Study. Eligibility will be based on the following criteria:

a. 25 years of age or older as of January 1, 2005
b. intact uterus
c. not currently pregnant (pregnant women to be deferred until 3 months post-partum).

The lack of an upper limit in targeted age group is based on the fact that there have been no substantial screening efforts in rural Medchal in the past, therefore prevalent disease is expected to be found in all of the older age groups. It is our intent to find and treat all prevalent disease. The lower limit of 25 years has been set based on two principles: (1) the vast majority of invasive cervical cancer will occur in women > 25 years, and (2) many women < 25 years are likely to have an abnormal screen by any of the testing methods (but particularly the HPV assay), most of which will represent transient infections that will not lead to invasive disease. Effective screening programs in this area cannot sustain the number of false positive screens that would result from identification of recent HPV infection in the young women that are, for the most part, likely to resolve. Even among the few of these women whose HPV infection will progress, it will not progress to invasion for at least a decade. Of course, there may be the rare circumstance of women who acquire HPV in their late teens/early twenties, and progress rapidly to cervical cancer. This is expected to happen in few to no women in our study. After the data from enrollment have been analyzed, and a preferred screening method identified for this population, we expect the health care system at MediCiti to begin implementation of a cervical cancer screening program, which should first target the younger women who were not participants in the formal CATCH study.

Hysterectomized women lack a cervix, and are therefore not at risk of the disease under investigation. Enrollment of pregnant women in the study will be deferred until they are 3 months post-partum to avoid any potential adverse effects on the pregnancy from the sample collection and pelvic exam procedures. Women in the active follow-up phase of the study who become pregnant during follow-up will be referred to the antenatal clinic and subsequent sample collection deferred till they are 3 months postpartum.

## 3.2 Triage plans based on screening results

All women with an abnormal screening result on any one or more of the three screening tests will be referred for immediate colposcopic examination **[AS OF 7/15/03, HPV-V SAMPLES WILL BE COLLECTED AND STORED FOR TESTING IN A NESTED CASE-CONTROL DESIGN AT A LATER DATE]**. A colposcopy-directed biopsy will be performed if indicated. Abnormal results for each screening assay will be defined as follows:

| Screening Assay | NORMAL | ABNORMAL |
|---|---|---|
| PAP SMEAR | Cytologic diagnosis < ASCUS | Cytologic diagnosis ≥ ASCUS |
| HPV-C | HR HPV DNA negative | HR HPV DNA positive |
| VIA | negative | positive |

Protocols for interpretation of results from each screening assay are included in this manual. The criteria above indicate simply the threshold results used for referral decisions.

## 3.3 Safety and monitoring

20% of women enrolled will be randomly selected for an immediate colposcopic examination, and will therefore serve as the screening control group since the majority will have completely normal screening results. If > 5% of the control women are found to have high grade disease (HSIL or cancer) after colposcopic examination, the follow-up protocol will be delayed, all women will be recalled for colposcopic examination, and the failure in the screening methods will be identified. This is expected to be an exceedingly unlikely event. All women with abnormal screens, but normal or low grade diagnoses, will be followed annually for 4 years to monitor progression. Any diagnosis of HSIL or cancer will be referred for immediate treatment, and censored from further study follow-up.

The Histopathology QC group at Johns Hopkins Medical Institutions (JHMI) will review 10% of the diagnostic histopathology slides. Participants with a diagnosis of HSIL or cancer by the Histopathology QC group that was less severely graded by the MediCiti histopathologist will be notified by the study staff and referred for treatment.

## 3.4 Management of positive screens and treatment

Participants who have a positive screening result by one or more of the three screening assays will be referred for colposcopic examination **[HPV-V TESTING DEFERED AS OF 07/15/03]**. The colposcopic exam should be scheduled within one month of notification of screening results. Colposcopic examination will be performed according to the procedures that are outlined in Chapter 11. Suspicious lesions will be biopsied, and final diagnosis will be based on the histopathologic report. **Confirmed diagnoses of CIN 2, CIN 3, CIS, or**

**invasive cancer will be referred for immediate treatment at no cost to the participant.** Treatment should be performed within one month of notification of histopathologic results. Treatment will be guided according to standard protocols, as outlined in Chapter 12.

## 3.5 Follow-up and exit plans

Women who were referred to colposcopy (positive screens plus controls), but who did not have a final diagnosis ≥ CIN 2 (i.e., did not require immediate treatment), will be asked to return for screening again in 1 year from the enrollment screen date. These women will constitute the active follow-up group for the study, and will be screened annually for 4 additional years (maximum of 5 screening visits in all) by all three screening assays. Referral to colposcopy following a follow-up screen will follow the rules as outlined in Section 3.4. Any participant diagnosed with CIN 2 or more severe disease at any point during follow-up will be treated at no cost to the participant, and censored from further study follow-up.

All women enrolled into the **CATCH** study who were not included in the active follow-up group will be asked to return for a final screening examination 5 years post-enrollment. Analysis of enrollment screening assay performance will be complete by this time, and the recommended screening protocol derived from this analysis will be used for exit screening. The exit visit should coincide with implementation of a routine cervical cancer screening program in Medchal Mandal.

## 3.6 Summary of analysis plans

The primary analyses for the **CATCH** study will concentrate on defining the performance characteristics of each of the four screening assays. The performance of each assay for detection of prevalent HSIL and cancer (CIN 2, CIN 3, CIS, and cancer) will be characterized by calculating assay sensitivity, specificity, positive and negative predictive values (PPV and NPV), and referral rates. The performance of each screening test will be compared to the histologic diagnosis as the gold standard. Sensitivity and specificity will be estimated by the Bayes formula to minimize the effect of referral bias (given that <100% of the women screened will be subject to diagnostic confirmation by colposcopy). PPV, NPV, and colposcopic referrals will be directly calculated by sample proportions.

The risk of cumulative incident HSIL and cancer (histologically confirmed) during the 4 year follow-up period according to enrollment screening result for each method will be estimated by calculating the odds ratio (OR) using logistic regression. Discrete proportional hazards

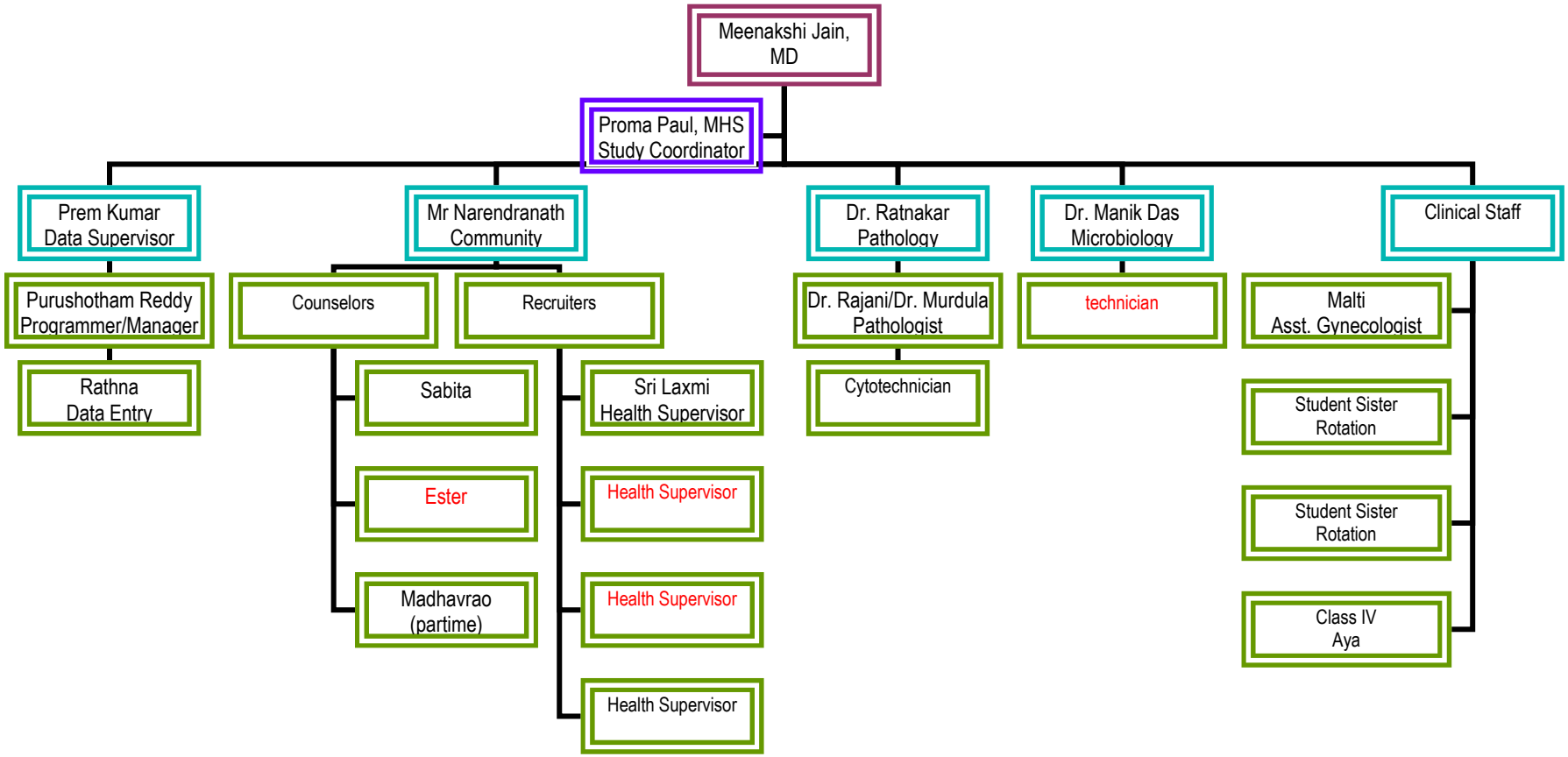
models will be used to estimate the risk of incident disease given a positive screening test at enrollment, both to account for censoring in the event of substantial losses to follow-up (>10%), and to incorporate time-to-diagnosis into the evaluation of test performance. This analysis will aid in recommending screening intervals in this population.

Estimation of screening test performance characteristics will be performed for each test alone, and in relevant combinations to identify the best screening protocol for this community.

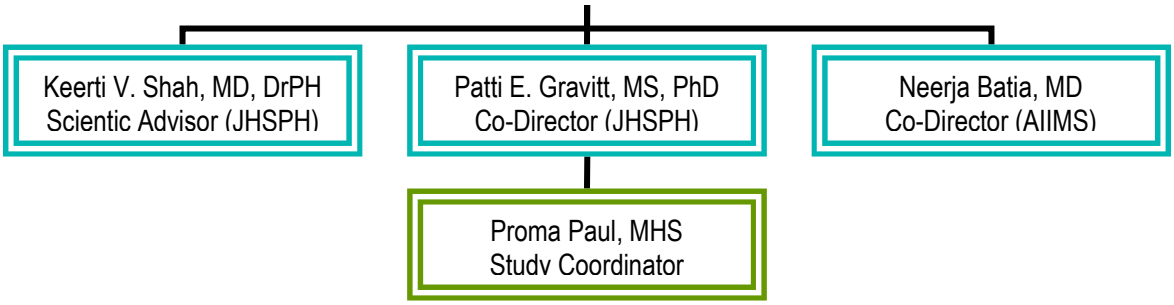
Additional analyses are planned to advance our understanding of the natural history of HPV-associated cervical cancer using the biomarker data that will be collected through the course of the study.

## Chapter 4. Organization and Management Structure

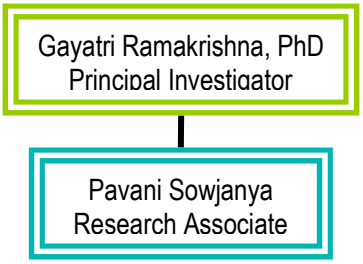
### 4.1 MediCiti Hospital, Medchal Mandal



## 4.2 Johns Hopkins Bloomberg School of Public Health (JHBSPH)




## 4.3 Center for DNA Fingerprinting and Diagnostics (CDFD)



## Chapter 5. Overview of specimens and related data flow

### 5.1 Introduction

A number of hard copy forms and biologic specimens will be collected throughout the course of the **CATCH** study. The biologic specimens and accompanying paperwork will require transport to and from multiple locations and must be keenly tracked. A complete history of the movement and disposition of each sample must be maintained and be available for ready access. A table detailing forms and specimens to be collected at each visit is presented below.

| | **Type of Visit** | | | |
|---|---|---|---|---|
| **Specimen/Form** | **Enrollment** | **Colposcopy** | **Treatment** | **Follow-up** |
| Refusal Questionnaire | ∇ | | | |
| Pre-enrollment contact form | pre | | | |
| Consent | √ | | | |
| Questionnaire | √ | | | √ |
| Pelvic exam form | √ | | | √ |
| VIA form | √ | | | √ |
| Cytology Report | √ | | | √ |
| Colposcopy exam form | | √ | | |
| Biopsy request form | | ∇ | | |
| Histology report form | | ∇ | ∇ | |
| Adverse events form | ∇ | ∇ | ∇ | ∇ |
| Blood | √ | | | |
| HPV-V | √ | | | √ |
| Pap smear – conventional | √ | | | √ |
| Pap smear – Liquid cytology | | √ | | |
| HPV-C (Digene STM) | √ | √ | | √ |
| HPV RNA (Digene STM) | | √ | | |

| | | |
|---|---|---|
| Paraffin-embedded biopsy | ∇ | |
| Endeocervical Curettage (ECC) | ∇ | ∇ |
| Paraffin-embedded Excised tissue | | ∇ |
| Snap-frozen Excised tissue | | ∇ |

∇ Where indicated

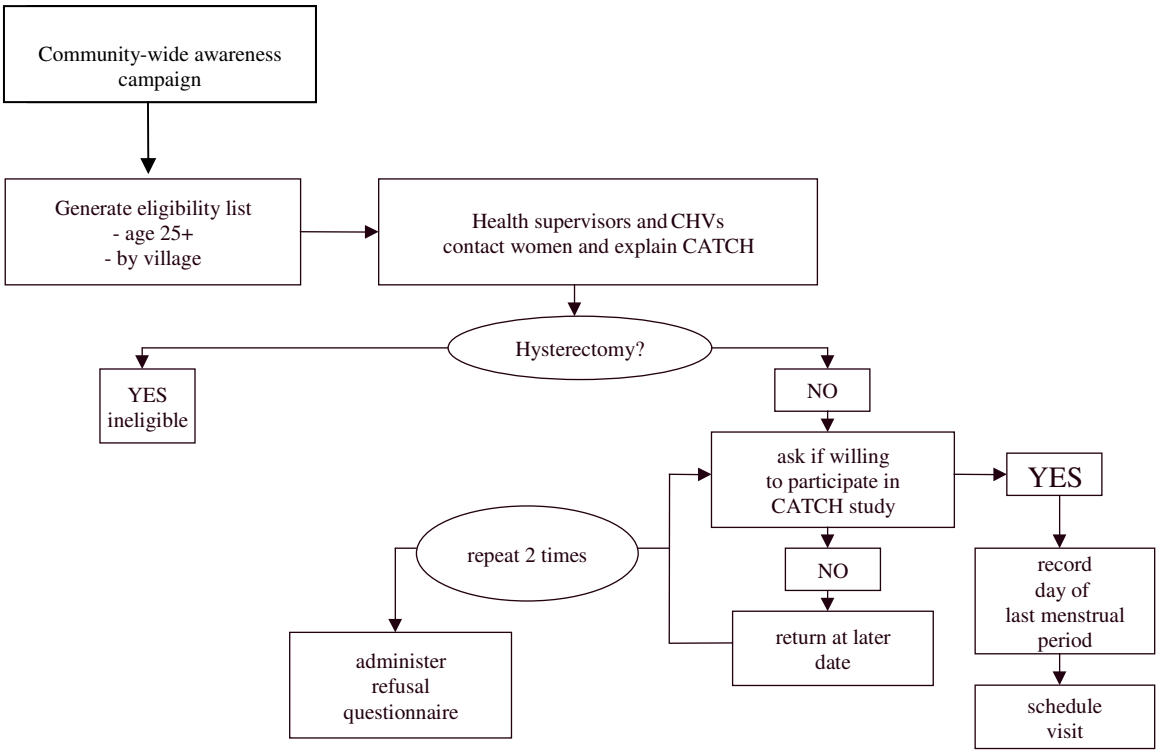
The primary and secondary uses of each biologic specimen are outlined in the following table.

| **Specimen Type** | **1° Purpose** | **2° Purpose** |
|---|---|---|
| Blood | Nutrition studies | Serology |
| HPV-V | HPV genotyping | |
| **Pap smear – conventional** | Patient triage based on evidence of ASCUS, LSIL, HSIL or cancer | |
| Pap smear – Liquid cytology | P16 staining, 3q gain | |
| **HPV-C (Digene STM)** | Patient triage based on HPV positive test | HPV genotyping, viral load, and variant analysis |
| HPV RNA | Assessment of HPV 16/18 integration status | mRNA quantitation |
| Paraffin-embedded biopsy | Histological diagnosis | P16 staining, FHIT loss |
| Endocervical Curettage (ECC) | Histological diagnosis | |
| Paraffin-embedded Excised tissue | Histological diagnosis | P16 staining, FHIT loss |
| Snap-frozen Excised tissue | Methylation status | |

The bolded specimens are those that will be used for patient triage **[HPV-V IMMEDIATE TESTING DEFERED AS OF 07/15/03]**. The specimen and data flow from each of these specimens and forms is outlined in detail in the following sections.

## 5.2 Pre-enrollment forms

The pre-enrollment forms will be used to identify and recruit eligible women. Health supervisors will be given a list of all eligible women, stratified by village, and will designate a community health volunteer (CHV) to help with recruitment of each woman. The recruiters will use a contact sheet to record initial and follow-up recruitment activities prior to enrollment. The contact sheets will aid in monitoring the recruitment activities and scheduling of enrollment visits. The flow of the contact sheet is outlined below.




In the event that an eligible woman declines to participate in the enrollment visit, a refusal questionnaire will be administered at the time of CHV contact. The refusal questionnaire will be administered by the CHV in the village, and will be returned to the Data Manager at MediCiti for Data Entry.

## 5.3 Enrollment forms

An enrollment log will be generated for each clinic day. This log will record the date, the on-site staff conducting the enrollment protocol that day, and the names and STUDY ID of all women successfully enrolled that day, including the number who registered per day and the number of women who consented per day. The enrollment log will also track the collection of the forms and biospecimens anticipated from the enrollment visit to allow rapid identification and resolution of missing data. This log will be reconciled with the number of forms that were collected at the end of the day.

Written informed consent will be obtained from each enrolled participant. Documentation that signed consent was obtained must be entered each day into the main participant tracking database. The original signed consent forms must be filed each day in a locked file drawer to be stored until the close of the study protocol.

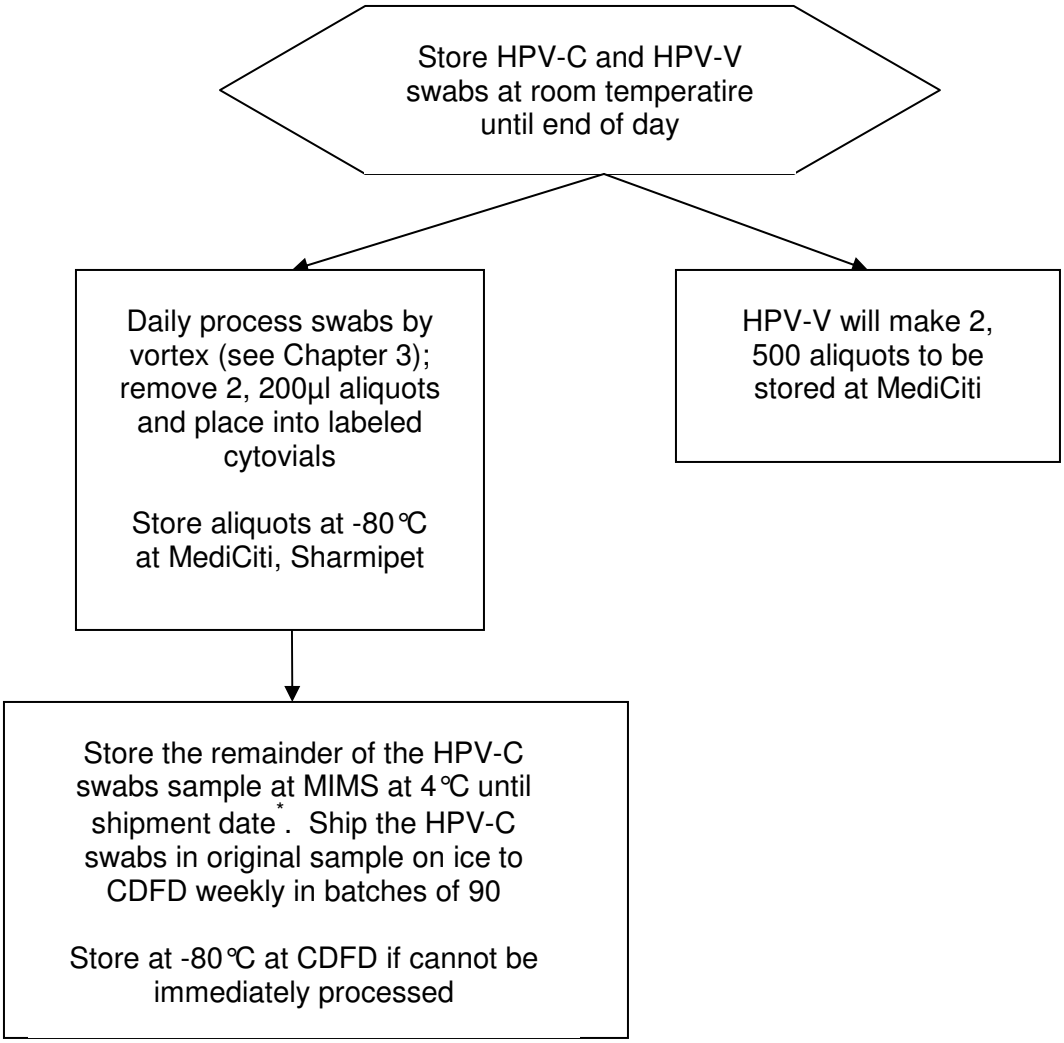
In the event that a woman who comes to the enrollment visit decides to refuse participation, a refusal questionnaire will be administered. Refusals will be entered into the enrollment log, and documented in the main database as "NOT CONSENTED". It is important to keep track that these women were successfully recruited but declined participation, so that they are not continually contacted regarding participation. Hard copies of the refusal questionnaire will be stored in a locked file drawer until the end of the study.

## 5.4 HPV-V **[SEE NOTE – PROTOCOL CHANGE EFFECTIVE 07/15/03]**

Each enrolled and consented participant will be asked to collect a self-swab for HPV DNA testing. The self-collected swab will be processed and aliquotted at MediCiti. Two aliquots of 500 μl each will be made at MediCiti permanently stored at MediCiti. The 500 μl aliquots are made prior to any additional sample preparation, and will be used for DNA extraction for PCR assays. The PCR aliquots will be selected for PCR-based viral load and variant. The specimen flow for the self-collected HPV-V biospecimens follows.

## 5.5 HPV-C

Cells collected by a physician-directed swab will be obtained from each participant during the pelvic exam to be used for HPV DNA testing. The physician-collected swab will be processed and aliquotted at MediCiti. Two aliquots of 200 μl each will be made at MediCiti before HC 2 testing and permanently stored at MediCiti. The remaining sample will be shipped to CDFD for HC 2 testing. The 200 μl aliquots are made prior to any additional sample preparation, and will be used for DNA extraction for PCR assays. DNA will be extracted and stored at CDFD for HPV genotyping from aliquots that are requested from Medchal. The remaining PCR aliquot will be selected for PCR-based viral load and variant testing. The specimen flow for the self-collected HPV-C biospecimens follows.




*Digene STM is stable at 4°C for 2 weeks

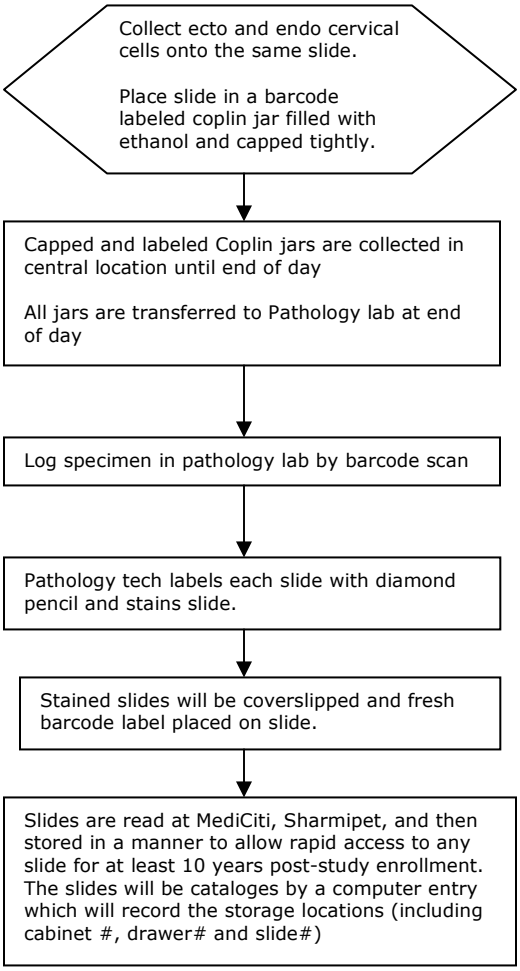
## 5.6 Blood

10 ml of blood will be collected in a red-top vacutainer from each consenting participant. The blood will be processed daily (see APPENDIX A) and stored at -80°C at MediCiti.

## 5.7 Pap smear

Conventional Pap smears will be used for patient cytology screening. The smears will be taken according to standardized protocols, as outlined in Chapter 20, Section 20.7. The clinician who administers the pelvic exam will ensure that a pre-labeled glass slide and proper fixatives are in place before beginning the exam. Fixed smears will be stored in a central location convenient to the exam room until the end of each clinic day. At the end of the day, a laboratory technician will log the collected Pap smears into the specimen tracking data base, and transport the smears to the cytopathology laboratory at Medchal. The cytopathology lab will stain and evaluate the slides, and generate a written report of the

cytologic results. One pathologist will read the slides and record their results on the cytology form with their ID code and signature; this result will be used for patient triage. This cytology report will be accessed by Data Management to generate the screening report card for each participant. The stained slides will be stored systematically in the cytopathology laboratory at Medchal, by case, drawer, and position number. At the end of the enrollment phase, a subset of slides will be selected for masked review at Johns Hopkins School of Medicine, Dept. of Pathology for assessment of the concordance of two independent readings of the same slide. Slides sent to JHMI will be read in the Dept. of Pathology, and stored there until the end of the study.



## 5.8 Colposcopy forms

Colposcopy forms will be available from the main data screen at the time of colposcopy and will be printed with patient ID and barcode. Completed forms will be collected from the colposcopy exam room at the end of each day, and data from the forms transferred into the database. Hard copies of the colposcopy exam forms will be filed in a locked file cabinet until the end of the study.

## 5.9 HPV RNA

A swab will be collected at the colposcopy visit and placed into an RNA-preserving medium (Digene sampler kit). This specimen will be split into two 500ul aliquots to be stored at -80°C.

## 5.10 Liquid cytology specimen

A brush will be used to obtain exfoliative cervical cells, which will be placed into a jar containing the liquid cytology medium (autocyte or cytyc). This sample will be stored at MediCiti Hospitals at 4°C.

## 5.11 Biopsies/endocervical curettage (ECC)

Biopsies and ECC samples will be placed in labeled bottles of 10% buffered formalin and sent to the pathology lab for processing.

## 5.12 Treatment visit forms

LEEP forms and treatment visit forms will be available from the main data screen and will be printed with the study ID and barcode. Forms should be collected and returned to the data center for electronic data entry.

## 5.13 Excised tissue

**TBD**

### 5.13.1 Snap Frozen

**TBD**

### 5.13.2 Paraffin-embedded

**TBD**

# Chapter 6. Overview of Data Management Systems

## 6.1 Overview

The database construction, coordination, and management for the study will be centralized in MediCiti, Medchal under the direction of Prem Kumar. The master database will house the identification number of each enrolled participant with the corresponding questionnaire and biologic data collected as part of the study design. This database will also coordinate participant tracking, ensuring maximum recruitment and retention of study participants during enrollment and follow-up. Tracking of biologic specimens from point of collection, through testing and storage, will be coordinated through the same data management system. The participant and specimen tracking systems will be linked to the master database, but will function as a separate component allowing broad access to relevant fields to allow patient scheduling, results tracking, and specimen location. The primary data will be maintained in the master database with limited access.

## 6.2 Data Management at Coordinating Unit; MediCiti, Medchal

Personnel under the direction of Prem Kumar, and their responsibilities and duties follow:

- Data Entry (Pursotham)
    - Project development
    - Database management
    - Double key entry/Logic checks
- Data Entry (Rathna)
    - Daily data entry
    - Registration duties
    - Double key entry checking

The Medchal Data Coordinating Unit will be responsible for management of data collected at CDFD (HPV results) and from other collaborating sites. Data files including specimen ID and tracking information will be created and sent electronically to collaborating institutions. Data fields will be created as appropriate by CDFD and collaborating institutions and completed data files will be returned electronically to the Medchal Data Coordinating Unit who will merge new fields into the master database.

### 6.2.1 Computers and Software

- Database software: Microsoft access
- PC-based
- Computer stations required:
    - 1 – data management

- 1 – enrollment/registration
- 1 – pathology
- 1 – CDFD

### 6.2.2 Barcoding accessories

- Scanners needed:
  - 1 – enrollment/registration
  - 1 – pathology
  - 1 - CDFD
- Printers needed:
  - 1 – enrollment/registration

### 6.2.3 Research Data Component

The research data component is synonymous with the master database. This component will include all primary data collected during the course of the study, as well as derived summary data used for diagnosis and referral. This component will operate under restricted password access to ensure quality maintenance of the data**. Codes for primary data will be developed and summarized in an accompanying code book, and will be entered as numeric variables for ease of statistical summary.** In cases where a numeric variable is not possible (e.g., when exceptions or explanations must be documented), an indicator variable will be assigned that directs to explanatory field which allows string variables. All primary data will be entered into the master database (after manual editing where appropriate, see Chapter 15). Derived summary fields (e.g., final cytologic diagnosis as normal vs. abnormal) will be created in the master database. Read-only access to the derived summary fields will be available in the participant and specimen tracking data components. We defined in October what routine derived variables are required.

### 6.2.4 Study Tracking component

The study tracking component will allow access to specific fields appropriate to the management of participant visit scheduling, results tracking, and biospecimen tracking. These fields are outlined in the following table. The highlighted results fields should be accessible as read-only files if possible to ensure no inadvertent data editing.

| | | |
|---|---|---|
| A | Patient ID | |
| B | Date of enrollment | |
| C | VIA result | |
| D1 | Date HPV-C sent to CDFD | |
| D1a | Date HPV-C received at CDFD | |
| D2 | Late HPV-C result flag | D1a - B > 21 days |
| D3 | Date of CDFD contact for follow-up on missing result | |
| D4 | HPV-C result (positive vs. negative) | |
| D4a | Date of receipt: HPV-C Result | |
| E1 | Date HPV-V sent to CDFD | |
| E1a | Date HPV-V received at CDFD | |

| | | |
|---|---|---|
| E2 | Late HPV-V result flag | E1a – B > 21 days |
| E3 | Date of CDFD contact for follow-up on missing result | |
| E4 | HPV-V result (positive vs. negative) | |
| E4a | Date of receipt: HPV-V Result | |
| F1 | Date Pap smear sent to pathology lab | |
| F1a | Date Pap smear received in pathology lab | |
| F2 | Late Pap smear result flag | F1a – B > 21 days |
| F3 | Date of pathology lab contact for follow-up | |
| F4 | Cytopathology result (normal vs. abnormal) | |
| F4a | Date of receipt: Cytopathologic diagnosis | |
| G1 | Completed results due date | B + 35 days |
| G2 | Completed results (screen positive vs. negative) | |
| G2a | Completed results flag | Current date > G1 |
| G2b | Colposcopy indicated (yes, no, control) | |
| G3 | Date participant notified of results | |
| G3a | Scheduled date of colposcopy | |
| H | Date of colposcopic exam | |
| I | Biopsy(s) taken at colposcopy? | |
| I1 | Date biopsy(s) sent to cytopathology lab | |
| I1a | Date biopsy(s) received in cytopathology lab | |
| I2 | Late biopsy report flag | I1a – H > 21 days |
| I2a | Date contacted pathology lab for missing data FU | |
| I3 | Biopsy result (normal – follow in 1 year; treatment indicated) | |
| I3a | Treatment visit indicated? | |
| I4 | Scheduled date for treatment | |
| J | Date of treatment visit if I3a = YES | |
| J1 | Date of treatment follow-up | |
| K | Scheduled date for annual follow-up if I3a = NO | |

## 6.3 Data Management at CDFD

CDFD will obtain Specimen ID and tracking data fields from the Medchal Data Coordinating Center with the shipment HPV-C samples. Tracking and logging of specimens will be facilitated by linked barcoding, so that automatic notification with date of specimen receipt will be transmitted to the Data Coordinating Center. CDFD will be responsible for creating the necessary fields to track the processing and results of the Hybrid Capture 2 HPV testing, with a turnaround time of < 21 days. Completed results will be electronically transferred back to the Data Coordinating Center at Medchal in a timely manner. CDFD will be responsible for maintaining data for the following fields, in addition to any fields determined relevant by the CDFD PI.

- Date sample received
- Shipment batch number
- Adverse specimen events (e.g., inadvertent freeze-thaw)
- STM lot number

- Assay batch #
- HC2 Diluent lot #
- HC2 kit lot #
- Date of HC2 assay
- Adverse assay events
- Sample RLU/PC

CDFD will keep appended versions of the data files sent with each sample as a master list for the virology data collection. As HPV PCR genotyping data becomes available, this data will be added to the master virology list (which includes corresponding HC2 data). Completed genotyping data will be electronically transferred to the Medchal Data Coordinating Center where it will be merged with the master database. The PCR genotyping data transfer has no pre-specified timeline.

### 6.3.1 Computers and Software

CDFD will use PC-based Windows computing, and data will be managed using Microsoft Access. **Insert when known the freezerworks software for specimen inventory and barcoding software.**

### 6.3.2 Barcoding Accessories

**TBD**

## 6.4 Data Management at JHBSPH

Quality control for HPV testing, cytopathology, histopathology, and colposcopy will be conducted at the Johns Hopkins Medical Institutions. HPV sample aliquots and slides will be sent to the Shah/Gravitt lab at the School of Public Health for JHMI centralized processing and tracking. Tracking and logging of specimens will be facilitated by linked barcoding, so that automatic notification with date of specimen receipt will be transmitted to the Data Coordinating Center. A copy of colposcopic images will be transferred electronically to Dr. Connie Trimble and Dr. Neerja Bhatla. A database with specimen ID and tracking fields will accompany specimen shipment and will be used to create the QC data fields by JHMI investigators. The JHMI QC results will be entered into the database and when complete will be electronically transmitted back to the Medchal Data Coordinating Center. Return shipment of the slides and residual HPV sample will be tracked by bar code.

### 6.4.1 Computers and Software

PC-based windows operating systems using Microsoft Access and STATA software will be used for study data management at JHMI.

### 6.4.2 Barcoding Accessories

**TBD**

## 6.5 Transferring data between institutions

Data will be saved on the Johns Hopkins Bloomberg School of Public Health webdrive system to be transferred between MediCiti and Johns Hopkins.

## Chapter 7. Overview of MediCiti Clinic Responsibilities

### 7.1 Overview

The MediCiti Hospital will serve as the central location for patient consent and enrollment, all medical procedures, and overall study management. The Data Coordinating Center will be housed at MediCiti Hospital.

### 7.2 Physical location

During the enrollment phase of the study,

- Consent process/Waiting area
- Registration and enrollment area
- 1 Private room for self-sample collection (washroom necessary)
- 2-4 Private rooms/areas for questionnaire administration
- 1 Exam rooms for pelvic examination/colpo/tx with 3 partitioned exam stations
- 1 room for blood collection
- Data entry/management area
- Pathology lab
    - Pap smear staining area
- Microbiology lab for specimen processing
- Freezer storage (eventually need room for at least 4 freezers)
- Study office

### 7.3 Management tasks

- Study coordination (Proma Paul)
    - Participant scheduling and tracking
    - Results tracking
- Clinical management (Meenakshi Jain)
    - Clinical protocol implementation
    - Results notification/counseling
- Recruitment/retention (Proma Paul/Meenakshi Jain)
- Administration management (Proma Paul/TBA)
- Laboratory management (Manik Das)
    - Sample processing/storage
    - Sample shipment/tracking
    - Results reporting

## 7.4 Training and supervision of MediCiti Study Staff

All study investigators and staff will undergo a one-day formal introduction to the study protocol where the study objectives and organization structure will be clearly defined. The scope of the project will be presented so that each team member gains an appreciation for each aspect of the study protocol. In addition to the broad-scope training, specific staff training in the following areas will be provided.

### 7.4.1 Recruitment/retention

Counselors, health supervisors and community health volunteers will be trained by Sudha Sivaram and Meenakshi Jain on the study objectives, risks, and benefits to the participants. Mr. Naranderanath will supervise the education, recruitment, and retention efforts. Dr. Sudha Sivaram from JHBSPH will serve as consultant for this training effort.

### 7.4.2 Enrollment clinical exam

Staff gynecologists will receive training on VIA, Pap smear and HPV specimen collection at the Barshi study site. These gynecologists will train additional staff as necessary. Dr. Meenakshi Jain will supervise the clinical staff for the study protocol. Dr. Neerja Bhatla (AIIMS, Delhi) will serve as clinical consultant for the study.

### 7.4.3 Cytopathology/Histopathology

All staff pathologists will be formally trained and certified in cervical cytopathology/histopathology. A formal review of the modified Bethesda classification system will be performed before the onset of the study. Dr. Ratnakar will supervise the pathology staff. Dr. Dorothy Rosenthal (Johns Hopkins Medical Institution) will serve as pathology consultant for the study and will participate in the training review for the study pathologists.

### 7.4.4 Colposcopy/treatment

Dr. Meenakshi Jain, a trained gynecologist will perform the colposcopic exam and provide treatment as necessary. One additional gynecologist will be trained by Meenakshi Jain as a backup.

### 7.4.5 HPV testing

All technicians participating in the HC 2 testing at CDFD will undergo training from a Digene technical representative and obtain certification to perform the test. Dr. Gayatri Ramakrishna will supervise the virologic testing at CDFD. Dr. Ramakrishna has been trained to perform the HPV PCR and line blot genotyping assay by Dr. Patti Gravitt (Johns Hopkins Bloomberg School of Public Health), and will train additional CDFD staff to perform the HPV genotyping tests.

### 7.4.6 Laboratory staff

Dr. Patti Gravitt (JHBSPH) will train the laboratory staff in the proper processing, labeling, and storage of all biologic samples collected during the course of the study. This staff member will train future staff as necessary. Dr. Manik Das will supervise the laboratory technical staff throughout the course of the study. Dr. Gravitt will serve as consultant for laboratory aspects of the study.

## *7.5 Assignment of study ID*

*At the time of enrollment, all consenting participants will be issued a study folder, that will contain the barcoded participant study ID number on enrollment forms.*

## 7.6 Maintaining participant study folders

A full time administrative staff member will be responsible for coordinating between community, study, clinic, and laboratory staff to track collected hard copy data and keep it centralized in a location. Data sheets will be filed in binders for specific formm separated by village and time of enrollment. Forms should not be removed from binders, and binders must be checked out when removed – logging the name of the person in custody of the binder and its temporary location.

## 7.7 Recruitment and tracking of study participants

Proma Paul and Mr. Naranderanath will be responsible for all recruitment and tracking activities. The Health Supervisors will contact eligible participants and invite them to attend the enrollment visit. Proma Paul & Dr. Jain will arrange for community education reinforcement when recruitment is slow and will help to understand reasons for reluctance in participation.

## 7.8 Specimen collection supplies and kit assembly

All specimen collection kits and assembly when necessary will be performed by the laboratory staff and nurse attendants, under the supervision of Dr. Jain and Proma Paul.

## 7.9 Tracking and shipment of biological specimens

Laboratory staff will be responsible for shipment of the biological specimens to CDFD on a weekly basis. Courier service will be arranged through Dr. P.S. Reddy, and will operate on a predetermined schedule. Tracking of the biological specimens through the various laboratories will be facilitated through the barcoding scanner system.

## 7.10 Receipt of test results and patient management

Test results will be tracked by the Data Coordinating Center. Tracking will be maintained through the broad access data fields as described in Chapter 6.2.4. Fields that flag missing data after 21 days post-enrollment will help to maintain prompt recall for diagnostic exam. The Health Supervisors and CHVs are responsible for delivery of written results to the participants, with counseling provided by the Health Supervisors at the time of results delivery and by the medical staff at the time of the colposcopic visit.

## 7.11 Management of data collected

The Data Coordinating Center will be located at MediCiti, Medchal and will be responsible for all data management as outlined in Chapter 6.

## 7.12 Monitoring of study progress

Proma Paul and the Data Coordinating Center will monitor study progress by routine queries of the database to track recruitment, scheduling, timely reporting of the screening results and retention during follow-up.

## 7.13 Communicating with CDFD and JHBSPH

The Data Coordinating Center will be responsible for facilitating rapid communication between the participating centers. High speed internet connections (DSL) will allow quick communication between the sites.

## 7.14 Site visits

As necessary, the MediCiti staff will be required to undergo any site visits required by funding agencies. The JHBSPH and MediCiti staff will work together to coordinate preparation for the site visits.

## Chapter 8. Strategies for Outreach, Recruitment, and Retention

### *8.1 Community education and outreach*

A broad education campaign in the participating villages will be launched prior to formal recruitment of eligible women. Key community leaders and will be identified to help disseminate the information of the CATCH project and the importance of cervical screenings to prevent disease. Focus group discussions will be carried in the community to determine the knowledge and practices of cervical disease screening and cervical cancer in the targeted women's populations and in the male population of the community [See Appendix_]. Using this information we will begin to actively educate the women on the importance of getting screened. Gynecologists and/or counselors will attend mother's committee meetings to give a presentation regarding the importance of cervical cancer screening and afford an opportunity for women to ask questions about cervical cancer. This educational effort will culminate in a showing of an educational film [Appendix _]. After the viewing of the video in the community, recruitment for the project will begin immediately [Section 8.2]. Health supervisors will visit each eligible woman in her home to inform her about the aims of the study, and to invite her for participation.

### 8.2 Strategies for recruitment

The community education and outreach as described in Section 8.1 is the cornerstone of the recruitment efforts. Trained Health Supervisors familiar with the women in the village will help to recruit for participation in the prevention study. **The need to detect treatable lesions before they become symptomatic to prevent eventual cancer will be emphasized.** Eligible participants who refuse participation will be queried to assess reasons for reluctance. Patterns will be identified to assess primary reasons for non-participation (e.g., require husbands approval). Directed response by the study staff to help to alleviate any anxiety that is preventing participation will be made.

### 8.3 Enrollment goals

Our goal is to enroll 100% of the women eligible to participate in the cervical cancer prevention project.

### 8.4 Tracking and monitoring recruitment

Each of four Health Supervisors will be given a list of eligible women from geographically proximal villages. The Health Supervisors, under the direction of Dr. Meenakshi Jain, Proma Paul and Mr. Naranderanath, will be responsible for timely recruitment. Health Supervisors

will be given contact sheets to track their recruitment efforts. Each week, a meeting between the Health Supervisors, the CHVs and the study clinic staff will be held to review progress in meeting the recruitment goals. If reluctance to participate is encountered, reasons will be sought, and a targeted response including more education, village visits by a gynecologist, and other situation-specific methods will be implemented. On a monthly basis, a recruitment summary will be sent to the investigators at JHBSPH.

## 8.5 Overview of patient compliance and retention

On a weekly basis, summaries of patient compliance and retention will be prepared including the following parameters:

- % (n/N) ineligible due to current pregnancy
- % (n/N) ineligible due to hysterectomy
- % (n/N) enrolled (# enrolled & consented/# approached)
- Among unenrolled, % contacted 0 time
- Among unenrolled, % contacted 1 time
- Among unenrolled % contacted 2 times
- Reasons for non-participation (% stating reason)
- % agreed to self-collected sample (% giving self-swab/total enrolled)
- % agreed to blood draw (% giving blood/total enrolled)
- Average and range of time between enrollment and completed results.
- Average and range of time between completed results and results delivered to participant.
- % returned for colposcopy (# attending colpo/# asked to return)
- Lag time between enrollment and colposcopy (mean, full and interquartile range in days)
- % returned for treatment (# treated/# requiring treatment)
- Lag time between colposcopy and treatment (mean, full and interquartile range in days)

If enrollment response is low, remedial measures will be taken as outlined above based on observed reasons for non-participation. If response rate for the self-collected swab, or blood sample is below 50%, a meeting of the scientific advisory committee will be called to review reasons for non-compliance in these aspects of the study protocol. Based on this review, either steps to improve participation in these activities or discontinuation of specific sample collection protocols will be recommended.

# Chapter 9. The Pre-Enrollment Process

## 9.1 Overview

The pre-enrollment process includes all efforts made to reach eligible women to invite and schedule them to attend the enrollment visit.

## 9.2 Definition of targeted community

All women over the age of 25 years will be invited to participate in the project. Assessment of presence of an intact uterus (i.e., no hysterectomy) should be made during the first contact; hysterectomized women will not be invited to join as they are not at risk of cancer of the cervix. If an eligible woman is currently pregnant, enrollment into the study should be delayed until she is 3 months post-partum. Tracking of the pregnancy-deferred participants should be processed with the Data Coordinating center and logged onto the enrollment contact forms.

## 9.3 Documentation of pre-enrollment process

Each Health Supervisor will be given a list of eligible women in their assigned villages. This will serve as the pre-enrollment contact sheet. The HS should use this contact sheet to record the date of each contact and the response from the participant (e.g., eligible, scheduled enrollment, refused with reason for refusal). These contact sheets should be given on a weekly basis to the Data Coordinating Center so that updated lists can be generated and centralized tracking of the enrollment process facilitated.

## 9.4 Pre-enrollment process

### 9.4.1 Generation of eligibility list

The Data Coordinating Center will generate the eligibility list of each woman over 25 years, stratified by village. The lists will be provided as contact sheets with room to record each date of contact and outcome.

### 9.4.2 Training of CHVs and HSs on recruitment protocol

Sudha Sivarim and Meenakshi Jain will develop the appropriate message to use to recruit women into the study. Mr. Naranderanath will be responsible for ensuring appropriateness of the message and translation into Telugu.

### 9.4.3 Initial contact with eligible participants

Initial contact will occur after 1-2 months of intensive community education and awareness regarding cervical cancer and the CATCH study.

### 9.4.4 Eligibility/willingness screening

At the time of initial contact, the Health Supervisors will confirm participant eligibility (age, pregnancy status, and intact uterus), and invite them to come to MediCiti Hospital for an enrollment visit. The Health Supervisor will explain again that enrolling in the study will provide free screening for cervical cancer where some blood and cells from their cervix will be collected by a female doctor. They should emphasize that the participation of the women will help to establish cervical cancer screening in their village, so that their daughters and granddaughters will not have to worry about getting cervical cancer if they participate in the screening programs. Remind the women of the minimal risks involved in their participation. Be clear that transport and incentive will be provided free of charge for each visit they make to MediCiti. All treatment that is deemed necessary as a result of their screening will also be provided at no cost to them.

The Health Supervisors should be prepared to answer any questions that the women may have regarding the procedures or their participation. If they do not know the answers to any question, they should write it down, and follow-up promptly; preferably accompanied by a gynecologist from MediCiti.

### 9.4.5 Scheduling the enrollment visit

If the eligible participant agrees to come for enrollment, document her willingness on the contact sheet, and suggest a day that is convenient to the participant and outside of the first four days of their menstrual cycle for scheduling the visit. Remind the participant of the hours she will be away, but that transport will be provided free of charge. Document if there are extenuating circumstances that prevent the woman from attending a clinic visit (e.g., child/elder care). Arrangements to accommodate these situations should be made wherever possible. Give the participant a reminder card of the day agreed upon for the clinic visit and if possible, remind her of the visit 1-2 days before it is scheduled.

## 9.5 Entry of pre-enrollment data into DMS

Health supervisors should leave a copy of the contact sheet with the Data Coordinating Center each day (or minimally each week) for data entry.

## 9.6 Follow-up of patients not contacted or unwilling to participate

The Health Supervisors should continue to engage in pre-enrollment activities until all eligible women have been approached. After all willing participants have been enrolled, efforts to recruit reluctant participants should again be made by probing into the reasons for their refusal. However, if after 3 contacts a woman still refuses to participate, no further recruitment should be considered, and a refusal questionnaire administered.

## 9.7 Reporting back to MediCiti

The Health Supervisors should coordinate with the CHVs and the medical study staff at MediCiti to schedule enrollment for willing participants. The Health Supervisors should also report back to the study coordinators the general opinion of the community regarding the CATCH study and concerns that may have been voiced. This interaction will help to maintain a positive relationship with the members of the community and will work to help achieve the goal of developing.

## Chapter 10. The Enrollment Visit

### *10.1 Overview*

The following sections outline the activities, in chronologic order, that will occur as a part of the participant enrollment visit. Descriptions of each component may contain a reference to a subsequent chapter where a detailed protocol is provided.

### *10.2 Scheduling the enrollment visit*

The enrollment visit is scheduled on the preceding night's recruitment visit based on women's willingness and date of last menstrual period. Thirty or more women should be scheduled each day since many of them will not show up the next morning, ensuring the maximum actual per day enrollment.

### *10.3 Daily transportation procedures*

Bus will transport villagers in groups to the clinic 1-2 times per day.

### *10.4 Preparing for the enrollment visit*

Before the scheduled arrival of the daily participants, the following should be in order:

- List of scheduled appointments
- Consent audio
- Consent forms
- Study folder
- Questionnaires
- Clinician collected swab kits with labels
- Self-collected swab kits with labels
- Phlebotomy kits with labels
- Digene sampler kit with labels
- Liquid cytology sampler kit with labels
- Specula
- Ayre's spatulas
- Endocervical brushes
- Glass slides with labels (diamond pencil for labeling before EtOH)
- Coplin jars with 95% ethanol
- HPV swab kits with labels
- 5% acetic acid
- cotton swabs

- pelvic exam proformas
- VIA proformas
- 10% **buffered** formalin
- specimen vials for biopsy/ECC
- presence and working condition of equipment for pelvic exam (Chapter 18)
- presence and working condition of equipment for colposcopy (Chapter 19)

## *10.5 Registration*

At the time the participants arrive, they should be checked in at the registration desk. Checking in does not indicate consent to participate in the study. Do not refuse to enroll eligible women who present as unscheduled visits; record their names in the enrollment log and proceed as with the scheduled participants. If a woman is ineligible to participate, but wants a screen, please refer her to Meenakshi Jain and ensure that she is given a Pap smear and a VIA. Create a patient record for her so that results may be followed, but do not enter anything into the CATCH database.

## *10.6 Tracking enrollment visit activities*

The enrollment log will serve to record all women attending an enrollment clinic, the number of women consenting to enroll, reasons for refusal where applicable, and any notable events occurring within the course of the enrollment day. Each woman should be monitored over the course of the day to ensure that she has been approached to participate in all sample collection activities, whether or not she agreed to each activity, and that she has received lunch. **(CHECKLIST TO BE ATTACHED)**

## *10.7 Administering consent*

### 10.7.1 The consent process

The consent process is non-negotiable. All women who are enrolled in the study must provide this informed, written consent. Each woman must be clear about the aims of the study, the procedures involved, the risks and benefits incurred by their participation, and what consent means. Women will consent first to participate in the study protocol. Second, the women will be asked to provide consent so that we may store biological specimens collected during the course of the study for future research purposes. Refusal to consent to the biological specimen storage does not invalidate the consent to participate in the study. In addition, subjects may consent to the protocol, but decline providing one or more of the following samples; blood or self-collected vaginal swab.

Every effort must be made on the part of the study staff to ensure that the consent process is carried out as described in the PM, and ethical standards of human subjects research are maintained. **All study staff administering consent must have the JHBSPH Human Subjects Module certification on file at the beginning of the study, and this certification must be renewed as necessary.** A list of all of the individuals certified to take informed consent will be maintained by Proma Paul who will send updated lists to CHR at JHBSPH.

### 10.7.2 IRB approval of consent administration

Following this section are the signed approvals from the MediCiti Hospital, CDFD, and Johns Hopkins Bloomberg School of Public Health. Annual review and renewal of IRB approval from each institution should be made, and signed approval documents stored at the study site.

### 10.7.3 Administering the informed consent

Women arriving for the enrollment visit have not yet consented to participation in the study. When the group arrives, they will be given an audio presentation describing the aims of the study, the procedures involved, the risk and benefits to participation, and the consent process and the consent form read verbatim. Each woman should be given the opportunity to ask questions about the study or the consent process in a private location. One by one, women who agree to participation will sign (or make a thumbprint) on the consent form in front of a witness to indicate their willingness to join the study.

If a subject is reluctant to sign consent only because of apprehension regarding the self-collected samples or the blood sample, she may decline the provision of these samples. A checklist for consent to pelvic exam, self-collected swab or blood is available on the consent form. Participants should be encouraged to participate in all aspects of the study, but can opt out of any part.

## *10.8 Assigning study ID*

*After proper informed consent is obtained, the subject can be formally enrolled. A reception desk will be operational in the morning hours of the clinic day to assign a Study ID folder for each enrolled participant.*

## *10.9 Labeling forms*

All enrollment forms are pre-scanned into the Main Cervical Cancer Project database. All data collection forms and specimen barcode labels will be collated into a study folder the day before for each expected participant.

### *10.10 Procedures for administering the enrollment questionnaire*

Trained interviewers will administer a short questionnaire (APPENDIX B) to each enrolled participant to assess information regarding demographics, medical, contraceptive, and reproductive histories, as well as tobacco use. The interviews should take no longer than 15 minutes per participant. Start and stop times should be entered during questionnaire administration, and weekly review of interview length should be made to ensure comparability of exposure assessment. If any member of the interview staff leaves before the end of the study, a replacement interviewer must be trained prior to assuming the roles of the previous interviewer.

### *10.11 Procedures for self-collected samples (HPV-V)*

A nurse will be positioned outside of the private room designated for self-collection. When each participant arrives, she will be asked for her study folder. The labels for the self-collected vaginal and urine sample will be removed by the nurse and placed on the collection vials. The participant will then be instructed again briefly on the collection procedures (APPENDIX A), and asked if she has any questions. After the samples have been collected, the nurse will ensure that all vials are properly sealed, that all specimen residue on the outside of the vials has been cleaned off, and return the study folder to the participant and direct her to the pelvic exam room if one is available, or to the waiting area.

### *10.12 The enrollment pelvic examination*

The pelvic exam will be performed according to the detailed protocols outlined in Chapter 16. The order of specimen collection and procedures is outlined below, with reference to the Chapters contained detailed protocols. Each sample should be collected with care to avoid excessive bleeding. Collecting samples from each patient in a consistent manner in compliance with the PM will ensure reproducible and valid results. **Physician's code and signature of the staff completing the forms is required.**

#### 10.12.1 Pap collection

The Pap smear will be taken first, after general documentation of the appearance of the cervix and genital tract and gentle mucus removal. Chapter 17 outlines the procedures for collecting and fixing the Pap smear in detail.

### 10.12.2 HPV-C swab collection

The physician-directed exfoliated cell sample for HPV testing (collected into Digene STM) will be taken after the sample for the Pap smear. Chapter 17 outlines the procedures for collecting, processing and storage of the HPV sample.

### 10.12.3 VIA

The VIA will be performed last, to avoid acetic acid contamination of the Pap and HPV samples. VIA will be performed as described in Chapter 16, and results will be documented on the VIA proforma [Appendix __]. **Physician's code and signature of the staff completing the forms is required.**

### 10.12.4 General Pelvic exam results

In addition to the procedures involved in the cervical cancer screen, a medical exam proforma will be completed during each pelvic exam [Appendix D]. Please complete the proforma accurately, including any diagnosis and/or treatment prescribed. **Physician's code and signature of the staff completing the forms is required.**

## *10.13 Blood collection*

Each woman will be asked to donate ~10 mL of blood for micronutrient analysis. Any residual serum remaining after these analyses will be stored for future research, if the patient has signed the biological materials storage consent form. A private room with a table will be available for the blood draw. This will occur before the pelvic exam. Detailed protocols regarding the blood draw procedure, blood processing and storage are provided in Chapter 18.

## *10.14 Immediate Colposcopy randomization*

In order to ensure that a sample of women with completely normal screens receive the diagnostic (colposcopy), 20% of women enrolled each day will be randomly selected (random number generator) for immediate colposcopy. A number (1-30) will be assigned to each women scheduled for enrollment after they consent. Using the Stata 7.0 statistical package, a random 20% sample without replacement will be generated. The participants who correspond to the numbers selected will receive a standard colposcopy exam.

The women randomized to colposcopy at the enrollment visit will have a flag (colored clip) placed on patient folder to indicate their selection and have a colposcopy form inside the folder. They will follow all enrollment procedures, but will have pelvic exam performed on the colposcopy table. After completing all required enrollment samples, but before VIA, collect additional samples (second Digene sampler kit and liquid cytology specimen)

required for a colposcopy visit (See Chapter 11). Then, perform and record VIA (unaided) according to the enrollment procedure (See 10.12.3). Follow this with acetic acid application and standard colposcopy exam (See Chapter 19), recording results on colposcopy form. Biopsy should be taken only from suspicious lesions.

### *10.15 Ending the enrollment visit*

After the pelvic examination, the gynecologist will counsel the patient regarding any treatment/prescription advised as an immediate result of the exam. The gynecologist should ask the patient if they have any questions regarding the examination or participation in the study, reminding them that if they have such questions in the future they should contact the staff members at the number provided on the study ID card. The patients should be told that results of their screening tests will be made available to them in about 1 month. Remind them that an 'abnormal' result is common, and simply means that they should come back for further testing; this only very rarely will mean that they cancer. Tell them that it is likely that they will not have an abnormality after more testing, but in the few that might, it is important to detect it now when it is curable. Tell them that even if they have normal results, they may be selected to come back for further tests, and encourage them to do this. If patients complain of other medical problems, ensure proper referral.

### 10.16 *Disposition of specimens and forms from the enrollment visit*

Specimens will be collected by attendants in coordination with the laboratory technician and transported to a common area in the pathology lab. Forms collected during enrollment should be taken immediately to the data management group for data entry.

### *10.17 Reporting laboratory results to MediCiti*

The data management team will provide results reporting applications for the pathology lab and CDFD. These screens will be used to enter verified data, which will be linked to the results tracking module. All primary data will be stored in the main study database.

### *10.18 Lost specimens and inadequate test results*

Any specimen that is lost will require recalling the woman for a new collection. Inadequate test results (such as unsatisfactory Pap smear) will also result in recalling the participant for a new sample collection. Invalid Hybrid Capture HPV tests will simply require a repeat of the assay using the same sample; this will NOT result in recalling participants.

### *10.19 Triage decisions*

Women who have an abnormal VIA, an HPV-V positive, an HPV-C positive, OR ≥ ASCUS Pap result will be recalled for colposcopy. **[HPV-V TESTING FOR TRIAGE DEFERRED AS OF 07/15/03.** The following criteria will define VIA abnormal, HPV-V and HPV-C positive, and abnormal Pap:

- HPV-C: RLU/CO ≥ 1.0 from HC 2 test
- VIA: described in Chapter 19
- Pap: ASCUS or more severe disease. Benign reactive changes (including inflammation) will be considered a normal result.

If women with an abnormal screening result were randomized for colposcopy during enrollment, they will not be recalled for an additional colposcopy. The necessary follow up and treatment decisions will be determined on from the results of colposcopy test during enrollment and based on the criteria defined in the protocol (See 11.11).

### 10.20 *Reporting screening results to participant*

When all results are completed and registered in the database, a results card will be printed for each patient.

**TBD**

The health supervisors and counselors will report the screening results back to the participants. The counselors will counsel patients who require a colposcopy visit and schedule the colposcopy visit within 2 weeks of receiving screening results.

## Chapter 11. The Colposcopy Visit

### *11.1 Overview*

The following sections outline the activities, in chronologic order, that will occur as a part of the participant colposcopy visit. Descriptions of each component may contain a reference to a subsequent chapter where a detailed protocol is provided.

### *11.2 Recommended timeframe for Colposcopy visit*

Colposcopy should be performed within 2 weeks after notification of results.

### *11.3 Scheduling the Colposcopy visit*

**TBD by health supervisors and Dr. Meenakshi Jain.**

### *11.4 Transportation to the Colposcopy visit*

**TBD**

### *11.5 Preparing for the Colposcopy visit*

Before the scheduled arrival of the participants, the following should be in order:

- Study folder
- Colposcopy exam form
- Biopsy request form
- Specimen labels
- Digene sampler kit
- Liquid cytology sampler kit
- 10% **buffered** formalin
- specimen vials for biopsy/ECC
- presence and working condition of equipment for colposcopy (Chapter 19)

### *11.6 Registration*

When participants arrive at the hospital for the colposcopy visit, they should present their study ID card at the registration desk. Once the card has been scanned, and participant's identification verified, pre-printed forms and specimen labels should be generated, and given to an attendant to prepare for the exam. If the participant does not have a study ID card, identification must be verified by name, village, and husband's name. A new study ID card can be printed at this time if the participant has lost, rather than forgotten, her card.

### *11.7 Tracking Colposcopy visit activities*

The colposcopy log will serve to record all women attending a colposcopy visit and any notable events occurring within the course of the day. Each woman should be monitored over the course of the day to ensure that she has been approached to participate in all sample collection activities, and that she has received her incentive.

## *11.8 Collection of sample at the Colposcopy visit*

The following samples will be collected at the beginning of the colposcopy visit. These samples should be collected with care to avoid bleeding as much as possible to ensure a good colposcopic image.

### 11.8.1 Liquid cytology specimen collection

The cytobrush should be placed at the cervical os, and rotated clockwise gently 3 times. The brush should be placed directly into the specimen collection vial with appropriate label, and the lid firmly screwed on.

### 11.8.2 HPV RNA specimen collection

The Digene sampler kit will be used. Place the brush at the os, and rotate clockwise gently 3 times. Place the brush directly into the specimen collection tube and cap the tube securely.

### 11.8.3 Colposcopic exam

The colposcopic exam will be performed as described in Chapter 19.

### 11.8.4 Colposcopic image

Using the provided digital camera, a colposcopic image will be taken after acetic acid application. This image should be labeled and stored electronically for future QC access.

### 11.8.5 Ending the colposcopy visit

After the colposcopic examination, the gynecologist will counsel the patient regarding any treatment/prescription they received as an immediate result of the exam. The gynecologist should ask the patient if they have any questions regarding the examination or participation in the study, reminding them that if they have such questions in the future, they should contact the staff at the number provided on the study ID card. If the patient did NOT require a biopsy, she should be counseled that her diagnostic test did not detect any abnormalities, and that she will be called again for screening tests in one year. Patients who underwent a biopsy or ECC should be told that results of their screening tests will be available to them in about 2-3 weeks. Tell them that a health supervisor will inform them of the results of their biopsy, and schedule a treatment visit if necessary.

## *11.9 Disposition of forms and specimens obtained at the Colposcopy visit*

Specimens will be collected by attendants in coordination with the laboratory technician and transported to a common area in the pathology lab. Forms collected during colposcopy should be taken immediately to the data management group for data entry.

### *11.10 Obtaining histologic results*

Histologic results should be entered into the main database within 2 weeks of the date of biopsy. The consensus diagnosis from the 3 reviewing pathologists will be made and entered into the designated results field.

### *11.11 Treatment and follow-up decisions*

Women who had a normal colposcopic exam with no evidence of lesion will be referred for screening again in 1 year. Women with a biopsy result ≤ CIN 1 will be asked to return for screening in 1 year. Women with CIN 2 or 3 will be offered excisional treatment via LEEP or cone biopsy. Women with a diagnosis of invasive cancer will be referred for treatment as indicated. Some women with ≤ CIN2 refuse LEEP because of stated preference for full hysterectomy. If counseling cannot dissuade these women from over treatment, hysterectomy will be provided at some reduced cost to patient.

### *11.12 Reporting histology results to the participant*

Health supervisors will report the histology results to the participant. They will counsel patients who require treatment and schedule a treatment visit.

### 11.13 *Scheduling treatment or follow-up visit*

**TBD between Meenakshi and health supervisors.**

## Chapter 12. The Treatment Visit

### *12.1 Overview*

The following sections outline the activities, in chronologic order, that will occur as a part of the participant treatment visit. Description of each component may contain a reference to a subsequent chapter where a detailed protocol is provided.

### *12.2 Recommended timeframe for the treatment visit*

Treatment should be provided as soon as possible. No more than 3 weeks should elapse between reporting of histopathology results and treatment of the lesion. Be sure that the patient is scheduled at least 4 days after the start of her last menstrual period.

### *12.3 Scheduling the treatment visit*

**TBD by Meenakshi and health supervisors**

### *12.4 Transportation to the treatment visit*

**TBD**

### *12.5 Preparing for the treatment visit*

Before the scheduled visit, the following should be in order:

- Study folder
- Pre-printed forms and specimen labels
- Liquid nitrogen and cryovials
- 10% buffered neutral formalin and vials
- Electrosurgical generator
- Patient grounding (dispersive) pad
- Various sizes and shapes of loop electrodes
- Ball electrodes (3- and 5-mm sizes)
- Insulated electrode handle
- Nonconductive speculum with smoke-evacuator port
- Nonconductive vaginal sidewall retractor
- Smoke evacuator with filter system
- Colposcope
- 5% acetic acid, freshly prepared
- aqueous Lugol's iodine
- large cotton swabs

- local anesthetic with vasopressin (10 units vasopressin in 30 ml of 1% lidocaine)
- dental-type syringe with 27-gauge needles, 1.5" in length
- Monsel's paste or gel
- Specimen vials with 10% neutral-buffered formalin
- 12" needle-holder and 2-0 resorbable suture material
- roller gauze for packing

### *12.6 Registration*

When participants arrive at the hospital for the treatment visit, they should present their study ID card at the registration desk. Once the card has been scanned, and participant's identification verified, pre-printed forms and specimen labels should be generated, and given to an attendant to prepare for the exam. If the participant does not have a study ID card, identification must be verified by name, village, and husband's name. A new study ID card can be printed at this time if the participant has lost, rather than forgotten, her card.

### *12.7 Tracking treatment visit activities*

The colposcopy log will serve to record all women attending a colposcopy visit and any notable events occurring within the course of the day. Each woman should be monitored over the course of the day to ensure that she has been approached to participate in all sample collection activities, and that she has received lunch.

### *12.8 Excisional treatment procedures*

Treatment procedures are outline in Chapter 20.

### *12.9 Ending the treatment visit*

At the end of the treatment visit, the patient should be counseled about what to expect as a result of her treatment, and when she should contact the doctor because of a suspected complication. Some amount of bleeding or dirty discharge is to be expected. However if she passes clots, has foul-smelling discharge, significant pain, etc. she must immediately contact the doctor. She must definitely maintain abstinence for a period of one month till the wound at the mouth of her womb heals.

Tell the patient that someone will look at her tissue under a microscope, and she will be informed about the success of the procedure (perhaps stating that most of the time the procedure is successful). If the pathologist indicates some residual lesion, she may be required to come back for an additional treatment, but this does not happen often. Tell the

patient that with successful treatment, her risk of cancer cervix is very low. Thank her sincerely for her participation in the screening program. She will be exited from the study protocol at this time. However, she should be encouraged (particularly if young and still sexually active) to attend screening visits every 3 – 5 years.

### *12.10 Disposition of forms and specimens obtained during the treatment visit*

Specimens will be collected by attendants in coordination with the laboratory technician and transported to a common area in the pathology lab. Forms collected during treatment should be taken immediately to the data management group for data entry.

### *12.11 Obtaining and entering the histologic results*

Histologic results should be entered into the main database within 2 weeks of the date of biopsy. The consensus diagnosis from the 3 reviewing pathologists will be made and entered into the designated results field.

### *12.12 Reporting histologic results to the patient*

Health supervisors.

# Chapter 13. Participant Tracking

## *13.1 Overview*

Study coordinators and clinic staff will have access to a computerized tracking system that will coordinate recruitment, enrollment, results reporting, and follow-up of all participants with the aim of maintaining efficient patient care and tracking of all study data. Flags will be designed such that delinquent results or potential losses to follow-up can be identified on a weekly basis, and remedial action can be appropriately and promptly implemented as defined below.

## *13.2 Recruitment*

The recruitment tracking module will be designed using a database extracted from the REACH project which will identify all women over 25 years as the maximum eligibility list for the study. This module will ensure that all eligible women are asked to participate, while keeping repeated contacts of the same woman to a minimum.

### 13.2.1 Contact eligibiles

The initial contact of the eligible women is done by the CHV as part of a routine census updating check using some form. Once this list is updated each pair of HS will be given roster of eligible women to use to keep track of contacts for their records. The women they contact and agree for enrollment the day after the film will be recorded on that roster. In addition each pair of HS will be given a list of eligible women to contact. They will contact them the night before enrollment and will record their names. The next morning they will collect the participants from the list maintained the night before. Differences will be reported to Data Entry personnel during registration. The eligible women from the 40 villages (including hamlets) will be divided evenly between the Health Supervisors. The HS will keep track of contacts using the contact form [Appendix __]. After initial contact the HS will give the contact list to ITG and a new list will be created for those needing to be contact for a second and even third time. ***PREM- we should work with Mr. Naranderanath to see how best to divide the eligibles between the health supervisors. Minimally, we need to divide the eligible lists by village – if this could be done before I get there, that would be great. We will need to devise a contact form for this portion; let's do that during my visit.***

### 13.2.2 Enrollment visit scheduling

Scheduling for the study will be dependant on the village and the availability of staff. ***TBD with advice from Proma Paul, Prem, and Mr. Naranderanath.***

**13.2.3 Tracking attendance at scheduled enrollment**
***PREM _ how can we do this electronically?***

13.3 Visit tracking

*PREM – how can we best do this? We need to make sure that when each woman comes to the hospital on a study visit, that we will be able to tell how many times she has been seen, and for what purpose. We also must be able to summarize at any given time how many people have consented, enrolled, attended a colpo visit, etc.* **[create field that is coded for visits that is specifically defined, ie: approached for pre-enrollment (0-for not approached) P1(1), P2(2), P3(3); enrollment S1 (0-no/1-yes); colposcopy C1(0-no/1-yes) and a field how many times approached before agreeing to colposcopy; treatment (0-treatment refused), T1(1), T2(2), T3(3) and a field how many times approached before agreeing treatment; follow-up (0-loss to follow-up), F1(1), F2(2), F3(3), F4(4)]**

**13.3.1 Tracking enrollment visit**
*The hs contacted the village women and CHVs, in the evening they would talk door to door, those who "promise" list, HS go back in the morning, using the list and bring participants in. They handwrite 3 copies of the attendance list. One goes Rathna, ITG, and sister. Rathna: registered, printed out barcodes for forms, ITG: printed out barcodes for samples, Sister: got ready clinical supplies. Participants arrive by 9:30am (15women-left 2-2:30pm). Consent audio 10-15 minutes.*
*PREM – for this, we need to make sure that all women who responded positively to the recruitment and schedule an enrollment visit show up. There needs to be an algorithm set that will automatically identify women who are missing their visits.*

**13.3.2 Tracking the Colposcopy visit**
*Essentially the same concept as before*

13.3.2.1 ID participation to be scheduled for Colposcopy
Participants receiving a results card of "abnormal" should be scheduled for a colposcopy visit.

**13.3.3 Tracking the treatment visit**
*Same as above*

13.3.3.1 ID participants to be scheduled for treatment

Participants who have CIN 2 or more severe disease by histopathology should be scheduled for treatment.

### 13.3.4Tracking the follow-up visit

*Same as above, although can be deferred for a few months until enrollment is underway*

13.3.4.1 ID participant in routine follow-up

*We need to develop a unique code to be able to quickly identify participants that have been selected into active follow-up. This can be a separate field. Create an automatically generated field for women entering active followup.*

13.3.4.2 Scheduling annual screening visits

*We need to think of the best way to schedule the next annual exam for participants in active follow-up, with a weekly print-out of participants due back so that a timely reminder can be sent to them and travel arrangements to the clinic can be efficiently made.*

13.3.4.3 Monitoring regular follow-up intervals

*For this, we must ensure that all women under active follow-up are returning for their annual exams, and track down those that are missed as soon as possible.*

13.3.4.4 Documenting missed intervals for regular follow-up

*If participants are missing their scheduled follow-up appointments, and need to be tracked, we must devise a way to document the longer follow-up interval and reason.*

## *13.4 Tracking overall participation in the study*

On a weekly basis, summaries of patient compliance and retention will be prepared including the following parameters:

- % ineligible due to current pregnancy
- % ineligible due to hysterectomy
- % enrolled (# enrolled & consented/# approached)
- Among unenrolled, % contacted 1 time
- Among unenrolled % contacted 2 times
- Reasons for non-participation (% stating reason)
- % agreed to self-collected sample (% giving self-swab/total enrolled)
- % agreed to blood draw (% giving blood/total enrolled)
- Average and range of time between enrollment and completed results.
- Average and range of time between completed results and results delivered to participant.
- % returned for colposcopy (# attending colpo/# asked to return)
- Lag time between enrollment and colposcopy (mean, full and interquartile range in days)
- % returned for treatment (# treated/# requiring treatment)

- Lag time between colposcopy and treatment (mean, full and interquartile range in days)

### 13.4.1 Tracking participants through the recruitment process

On a weekly basis, summaries of patient compliance and retention will be prepared including the following parameters:

- % ineligible due to current pregnancy
- % ineligible due to hysterectomy
- % enrolled (# enrolled & consented/# approached)
- Among unenrolled, % contacted 1 time
- Among unenrolled, % contacted 2 times
- Reasons for non-participation (% stating reason)

# Chapter 14. Specimen Tracking

14.7 *Tracking the liquid cytology swab*

**14.7.1 Tracking the LC swab at MediCiti**

**14.7.2 Tracking the LC swab at JHMI**

**14.7.3 Tracking the monolayer slides at JHMI**

**14.7.4 Tracking the monolayer slides at NCI (Ried)**

**14.7.5 Tracking the monolayer slides at lab (TBD)**

14.8 *Tracking the Biopsy/ECC*

**14.8.1 Tracking the tissue at MediCiti**

**14.8.2 Tracking the block at MediCiti**

**14.8.3 Tracking the slides at MediCiti**

**14.8.4 Tracking the slides at JHMI**

**14.8.5 Tracking sections at MediCiti**

**14.8.6 Tracking sections at CDFD**

**14.8.7 Tracking sections at outside lab**

14.9 *Tracking the excised tissue*

**14.9.1 Tracking the snap frozen tissue at MediCiti**

**14.9.2 Tracking the snap frozen tissue at outside lab**

**14.9.3 Tracking the PE tissue at MediCiti**

**14.9.4 Tracking the PE tissue at outside lab**

## Chapter 15. Management of data preparation and data processing activities

### 15.1 *Overview*

### 15.2 *Manual editing of completed data forms*

If mistakes are made in the process of filling out a data form, a single slash should be made through the incorrect response, the correct response should be filled in, and both corrections should be initialed as shown below.

6. Visual Examination Findings:
   - 1 |__| Vesicles or ulcers on external genitalia
   - 2 |__| Excoriation marks on external genitalia, vagina
   - 3 |__| Cervical polyp
   - pg 4 |✓| Nabothian follicles
   - 5 | ✓ | Congenital transformation zone seen

### 15.3 *Manual editing of questionnaires*

Follow as per 15.2 above

### 15.4 *Training on use of DMS*

Training regarding use of the Data Management System will be provided by the MediCiti ITG, under the direction of Prem Kumar. All study staff should be trained on the DMS.

### 15.5 *Data entry*

Data will be entered on a daily basis (e.g., all enrollment forms and questionnaires will be entered the next day as a batch). A data entry screen for each form has been created to facilitate data entry. All data will be entered twice as double keyed entry, with notification of discrepancies and logic checks.

### 15.6 *Computer editing*

Data can be edited once entered only with the instruction of Meenakshi Jain or Patti Gravitt.

15.7 *Data updates*

15.8 *Management and tracking of missing procedure/forms*

15.9 *Data transmission*

## Chapter 16. The pelvic exam

### 16.1 *Overview*

This section outlines the standardized procedures to be followed while conducting the pelvic exam as part of the CATCH study.

### 16.2 *Examiner qualifications, training, and certification*

Medical Graduate (MBBS) from a recognized medical college with six months' house job in Obstetrics and Gynecology, OR Postgraduate (DGO/MD/DNBE) in Obstetrics & Gynecology.

### 16.3 *Preparing for the pelvic exam*

The room should be well illuminated and ventilated, and equipped as outlined below. The examination table should be placed behind a screen to allow for adequate privacy. Separate disposal bins should be available for different categories of biomedical waste.

The examiner washes his/her hands and wears sterile gloves. The patient is asked to empty her bladder and lie on the exam table in dorsal position.

### 16.4 *Equipment and supplies*

- Examination table with mattress, sheet and mackintosh
- Surgical table or stand for supplies
- A good halogen light for VIA/headlamp as used in ENT, e.g., Welch-Allen Physician's Head light code no. 49032
- Tray containing autoclaved vaginal speculums – Cusco's / Sims' & anterior vaginal wall retractors
- Sterile examination gloves of various sizes
- Cotton swabs
- Water soluble lubricant
- Supplies for taking Pap smear - Coplin jar, glass slides, 95% ethyl alcohol, Ayre's spatula/endocervical brush
- Supplies for taking HPV samples – brush, collection media, tubes, rack for standing tubes
- Pre-printed labels

- Cervical biopsy forceps
- Sponge-holding forceps
- Sterile pads
- Bucket with disinfectant bleach solution
- Disposal bins
- Light Microscope
- Specimen vials for collecting specimens for culture
- 10% neutral buffered formalin, 5% freshly prepared acetic acid
- Forms for documentation

## 16.5 *Step by step procedure for conduction the pelvic exam*

The patient is asked to empty her bladder. She is asked to lie in the dorsal position. The pelvic area is illuminated well. The examiner wears sterile gloves. The following order of the procedure is suggested for pelvic examination.

### 16.5.1 External genitalia

Inspect the mons pubis, labia majora, perineal body, and anal region for characteristics of the skin, distribution of the hair, contour and swelling. Separate the labia majora with the index and middle finger of the gloved hand and inspect the epidermal and mucosal characteristics and anatomical configuration of – the labia minora, clitoris, urethral orifice, introitus, hymen, perineal body, anus.

If disease of the Skene's glands is suspected, palpate the gland for abnormal secretions by milking the undersurface of the urethra through the anterior vaginal wall. If present, treat for gonorrhoea. If there is history of labial swelling, palpate for a diseased Bartholin gland with the thumb on the posterior part of labia majora and the index finger in the vaginal orifice.

### 16.5.2 Introitus

With the labia still separated by the middle and index finger, instruct the patient to bear down. Note the presence of the anterior wall of the vagina when a cystocele is present or bulging of the posterior wall when a rectocele is present. (Supporting structures of the pelvic outlet will be evaluated further when bimanual pelvic examination is done.)

### 16.5.3 Vagina and cervix

Inspection of the vagina and cervix using a speculum should precede palpation. The instrument should not be lubricated with an antiseptic if vaginal or cervical smears are to be obtained for the test.

Select the proper sized speculum: a smaller sized speculum helps the patient to relax. To introduce, separate the labia with fingers and introduce the instrument into vaginal orifice with blades horizontal and closed. Carry the speculum along the posterior vaginal wall, and after it is fully inserted, open the blades gently, maneuvering the speculum until the cervix is exposed between the blades and taking care not to traumatize it.

Inspect the vagina for the following

- The presence of blood
- Discharge. This should be treated syndromically for monilia, bacterial vaginosis, gonococci and chlamydia.
- Mucosal characteristics.
- Structural abnormality

Inspect the cervix for the following:

- Unusual bleeding from the cervical canal, except during menstruation, merits an evaluation for cervical or uterine neoplasia.
- Inflammatory lesions are characterized by mucopurulent discharge from the os and redness, swelling and superficial ulcerations of the surface.
- Polyps may arise either from the surface of the cervix projecting into the vagina or from the cervical canal, which may be inflammatory or neoplastic.
- Early carcinoma of the cervix may not change the appearance of the appearance of the cervix or may appear as a lesion similar to an inflammation.
- If a growth is seen, a punch biopsy should be taken and sent for histopathology. Pap smear and HPV sample is not to be taken. The patient is sent for her blood sample and asked to return with her histopathology report.

Collection of Pap smear: see Chapter 19
Collection of HPV sample: see Chapter 19

### 16.5.4 Bimanual palpation

The pelvic organs can be outlined by bimanual palpation; the examiner places one hand on the lower abdominal wall and the fingers of the other hand in the vagina. Either the right or left hand may be used for vaginal palpation, depending on the examiner's preference.

Introduce the well-lubricated index and middle finger into the vagina at its posterior aspect near the perineum. Advance the fingers along the posterior wall until the cervix is encountered. Note any abnormality of structure or tenderness in the vagina or cervix.

Press the abdominal hand, which is resting on the infraumbilical area, very gently downward, sweeping the pelvic structures towards the palpating vaginal fingers. Coordinate the activity of the two hands to evaluate the body of the uterus for the following: position, shape, size, symmetry, consistency, tenderness, mobility, tumor.

Continue the bimanual palpation and evaluate the cervix for position, consistency and tenderness, especially on mobility of the cervix.

The intravaginal fingers should then explore the anterior, posterior and lateral fornices. Place the vaginal fingers in the right lateral fornix and the abdominal hand on the right lower quadrant. Manipulate the abdominal hand gently downward towards vaginal fingers to outline the adnexa. Then palpate the left adnexal region, repeating the technique describe above on the left side.

Palpate the pouch of Douglas for any mass, tenderness, nodules, or fullness suggestive of fluid collection.

The bimanual examination may be followed with a rectovaginal-abdominal examination if the parametria or pouch of Douglas need assessment, e.g., if there are nodules palpable in the pouch of Douglas or in cases with a frank growth for staging of cervical cancer. Insert the index finger into the vagina and the middle finger into the rectum very gently. Place the other hand on the infraumbilical region.

### 16.5.5 Visual inspection with acetic acid (VIA)

5% freshly prepared acetic acid is applied on the cervix, wait for 1 minute and report the findings as 'negative', 'positive' or 'invasive cancer' using the criteria given below.

**Negative (-)**
VIA screening is reported negative when any of the following occur:

- No acetowhite lesions are observed in the cervix;
- Polyps protruding from the cervix with bluish white areas;
- Nabothian cysts appearing as button-like areas or as whitish acne or pimples;
- Faint line-like or ill-defined acetowhitening at the squamocolumnar junction;
- Dot like areas in the endocervix, which are due to grape-like columnar epithelium staining with acetic acid;
- When there are shiny or pinkish-white or cloudy-white or bluish white lesions or faint patchy lesions or doubtful lesions with ill-defined, indefinite margins, blending with the rest of the cervix;
- Angular, irregular, digitating acetowhite lesions, resembling geographical regions, far away from the transformation zone (satellite lesions);
- Streak like acetowhitening in the columnar epithelium;
- Ill-defined, patchy, pale acetowhite areas in an inflamed, unhealthy, ulcerated cervix are seen with bleeding and mucopurulent discharge;
- Red spots are observed in the cervix against pinkish white hue after the application of acetic acid.

**Positive (+)**

The VIA test outcome is scored positive when any of the following occur:

- There are sharp, distinct, well defined, dense (opaque or dull white or oyster white) acetowhite areas with or without raised margins, abutting the squamocolumnar junction in the transformation zone;
- Strikingly dense acetowhite areas in the columnar epithelium;
- Condyloma and leukoplakia occurring closer to the squamocolumnar junction turning intensely white after application of acetic acid.

**Invasive cancer**

The test outcome is scored as invasive cancer if there is clinically visible ulcero-proliferative growth on the cervix that bleeds on touch.

## 16.6 *Treatment of Gynecologic Conditions*

### 16.6.1 Pelvic Inflammatory Disease

16.6.1.1 Criteria for clinical diagnosis of PID

- Minimum criteria for clinical diagnosis (all three must be present)

- Lower abdominal tenderness
- Bilateral adnexal tenderness
- Cervical motion tenderness
- Additional criteria useful in diagnosis (one or more necessary for diagnosis)
- Oral temperature > 101' F (>38.3 'c)
- Abnormal cervical or vaginal discharge
- Elevated ESR or CRP
- WBC >10,500/cu.mm
- Evidence of cervical infection with *Neisseria gonorrheae* or *Chlamydia trachomatis*
- Tubo-ovarian abscess on sonography
- Laparoscopic abnormalities consistence with PID
- Histopathologic evidence on endometrial biopsy
- CDC criteria for hospital admission
- Adolescent patient
- Concurrent HIV infection
- Diagnosis of PID uncertain
- Failure of outpatient treatment
- Inability of patient to follow or tolerate outpatient regimen
- Inability to exclude surgical emergency
- Pregnancy
- Severe illness or nausea and vomiting
- Suspected pelvic abscess
- Uncertainty about follow-up within 72 hours of starting antibiotics
- All nulliparous women

**<u>In-Patient Treatment Guidelines for PID</u> (TO BE MODIFIED ACCORDING TO AVAILABLE DRUGS – MEENAKSHI PLEASE)**

**Regimen A**

Cefoxitin sodium, 2g IV q. 6hr
Or
Cefotetan disodium, 2g IV q. 12hr
Plus
Doxycycline 100 mg IV (Vibramycin IV) q. 12 hr

- continue this regimen for atleast 48 hours after clinical improvement. After discharge the patient continues doxycycline 100mg p.o. b.i.d. for a total of 14 days

**Regimen B**

Clindamycin 900mg IV q. 8hr
Plus
Gentamycin in IV or IM (loading dose of 2 mg/kg of body weight followed by a maintenance dose of 1.5 mg/kg q. 8hr

- continue this regimen for atleast 48 hours after clinical improvement.
After discharge the patient continues doxycycline 100mg p.o.b.i.d. Or clindamycin 450 mg p.o.q.i.d. for a total of 14 days.

**Outpatient Treatment Guidelines for PID**

**Regimen A**

Cefoxitin 2g IM ; plus probenecid, 1g p.o. concurrently

Or

Ceftriaxone, 250 mg IM

Plus

Doxycycline 100 mg p.o.b.i.d. for 14 days

**Regimen B**

Ofloxacin 400 mg p.o.b.i.d. for 14 days

Plus

Clindamycin 450 mg p.o.q.i.d. for 14 days

Or

Metronidazole 500 mg p.o.b.i.d. for 14 days

### 16.6.2 Cancer cervix

Stages I through IIA can be treated equally effectively by surgery or radiotherapy, but the choice can be determined by the facilities available and the policy of each institute. In general, since radiotherapy facilities in India are limited, early stage disease is treated surgically wherever possible. Surgery is preferred over radiotherapy in the case of the young patient where ovarian function needs to be preserved, if there are associated adnexal masses or uterine tumours, e.g., leiomyomas, or if there is an associated pregnancy.

For advanced stages, i.e., $\geq$ IIB, treatment is by radiotherapy. Radiotherapy is also preferred over surgery for early stage disease if the patient is very obese, has a very bulky tumour or has associated medical disorders.

## 16.7 *Documenting the results of the pelvic exam*

Results from the pelvic exam will be recorded on the pelvic exam form, preprinted with participant's study ID and barcode.

## Chapter 17. Collection of cervical cancer specimens for cytology and HPV testing

### 17.1 *Overview*

During the enrollment pelvic exam, two exfoliated cervical cells specimens will be collected prior to VIA; a Pap smear and a swab for HPV DNA testing. Details of how to collect these samples are outlined below.

### 17.2 *Reasons to reschedule exam*

Women who are determined at the time of pelvic exam to be currently pregnant should be deferred and asked to reschedule their pelvic exam at 3 months postpartum. Samples should be taken from all other women. If the Pap smear is returned with a result of 'unsatisfactory for evaluation', the participant must be recalled for a repeat enrollment Pap smear. If any sample is lost before results have been recorded, the participant will be recalled for a repeat sample collection.

### 17.3 *Order of specimen collection*

The Pap smear should be taken first, followed by the HPV specimen. If there is visible discharge on the cervix, clean the cervix of the discharge gently with a saline-moistened cotton swab before collecting Pap.

### 17.4 *Examiner Qualifications, Training, and Certification*

Samples will be taken by trained gynecologists.

### 17.5 *Equipment and Supplies*

- Endocervical brush
- Ayer's spatual
- Coplin jar filled with 95% ethanol
- Red top vacutainers
- Other blood draw equip (needles, tubing, alcohol swabs)

- 2, Hybrid Capture collection kits per patient
- soap, water and towels for patient after self-collection
- racks for specimen tubes

## 17.6 *Handling of Specimen Collection Devices, Slides, and Vials*

Care should be taken when handling the collection devices not to mix up samples, or to cross contaminate the HC2 vials. Use gloves when handling specimen vials.

## 17.7 *Procedures for collecting the First Cervical Cell Sample (Pap)*

### 17.7.1 Reasons to defer collection of the Pap test

- Mentruation: Women will be advised by health supervisors not to attend an enrollment visit on days 1 – 4 of their menstrual cycle. In the event a woman is menstruating at the time of the pelvic exam, reschedule the visit for the following week.

- Severe atrophy: Postmenopausal women with atrophic changes in the cervix and vagina will be enrolled into the study and a Pap test taken at the time of the visit. However, women with severe atrophy will be advised to apply estrogen cream for 7-10 days in the event that they need a repeat Pap smear or are recalled for a colposcopic examination.

- Use of vaginal antiseptics: Patients should not use vaginal pessaries or vaginal douches immediately prior to the procedure. They should also be advised not to have sexual intercourse on the previous night.

### 17.7.2 Step-by-step instructions for Pap collection:

- The patient is positioned in dorsal position. There should be adequate exposure. Fans in the room should be switched off.

- No antiseptics are to be used. Cusco's speculum is introduced and the cervix visualised. Profuse discharge obscuring the cervix may be gently wiped off with help of a cotton swab soaked in saline.

- The Pap smear jar should be pre-labelled. A clean, dry slide is taken from the box. The Ayre's spatula is introduced through the speculum. The longer tip remains inside the os and the shorter tip overlies the exocervix and the TZ zone. With firm pressure the spatula is rotated by 360 degrees and quickly smeared on the dried slide, firmly pressing the flat slide of the spatula over the slide and moving in one direction. The same procedure is repeated with the reverse side of the spatula. The smear should be spread evenly. The slide should be immediately put into the jar containing 95% ethyl alcohol to prevent air-drying of the slide. It should remain in the fixative for at least 1-2 minutes.

- Next the endocervical brush is introduced inside the endocervical canal keeping the lowermost bristles in contact with the exocervix. The brush should not be rotated by more than 180 degrees, otherwise bleeding may occur which can contaminate the sample. The brush is taken out gently. It has now to be smeared on the same slide as

the exocervical smear. It is important to ensure that the exocervical smear should be allowed to fix for at least 1-2 minutes, after which the slide is taken out of the bottle partially and the brush smeared on the slide, rotating the bristles in the reverse direction. The slide is again dipped into the alcohol.

### 17.8 *Procedures for collecting the Second Cervical Cell Sample (HPV)*

- Remove the brush from the Digene Sampler kit.
- Introduce the brush into the cervical os with enough pressure to maintain contact with the epithelium but not induce bleeding. Twirl the handle 3 rotations in a clockwise direction.
- Remove the swab from the endocervical os and wipe the ectocervical portio with 3 circumferential strokes.
- Place the swab in the collection tube containing the STM (prelabeled with barcoded ID). Break off the end of the shaft protruding from the tube by bending it sharply against the rim of the tube. The shaft is prescored to facilitate breaking.

### 17.9 *Documenting the collection of cervical specimens*

Document the collection of the Pap and HPV specimens in the appropriate area of the pelvic exam form. If any problem with specimen collection was encountered, document the specifics of the problem in the area provided.

### 17.10 *Handling of the specimens collected and transfer to testing laboratory*

Pap smear specimens will be collected in barcode-labeled Coplin jars, capped and filled with 95% EtOH. At the end of each day of enrollment, a nurse attendant or lab technician will collect all the Coplin jars and transport them to the Pathology lab. The Pathology technician will register each smear as received by scanning the barcode on the jar.

HPV-C specimens will be capped and placed in a rack. At the end of each day of enrollment, a nurse attendant or lab technician will collect the HPV-C specimens and transport them to the Pathology lab for processing. The Pathology technician will register each HPV-C sample as received by scanning the barcode on the specimen tube. HPV-C specimens will be stored at 4°C until processed for PCR aliquots and the remainder of the specimen will be shipped to CDFD on ice.

# Chapter 18. Blood Collection (Microbiology)

## 18.1 *Overview*

## 18.2 *Order of the Blood Collection*

After women are consented, half of will have blood drawn while waiting to collect their HPV self sample swab. The other half will first collect their HPV self sample and then have their blood drawn.

## 18.3 *Equipment and Supplies*

- examination table
- vacutainer
- spirit/alcohol wipes
- cotton swabs
- needle
- needle destroyer
- study id labels
- vacutainer rack

## 18.4 *Phlebotomist Qualifications and Training*

## 18.5 *Preparing the Participant for the Blood Draw*

During counseling the participant is informed that 3 teaspoons of blood from the front of the elbow (cubital fossa) will be drawn.

## 18.6 *Step-by-step Procedures for the Blood Collection*

## 18.7 *Documenting the Blood Collection*

## 18.8 *Handling the Specimens after the Draw – see APPENDIX*

## 18.9 *Quality Control for Blood Specimens*

# Chapter 19. Colposcopy and Colposcopically-directed Biopsy

## 19.1 *Overview*

The following sections outline the standardized clinical procedures to be followed in the conduct of the colposcopic exam and collection of biopsy specimens.

## 19.2 *Collection of Study Specimens*

### 19.2.1 Liquid Cytology Swab – to be revised for AUTOCYTE vs. Cytyc collection

- Identify the transformation zone, if visible, and direct sampling efforts to encompass this area.
- Insert the long central bristles of the Papette broom into the os and gently push it inward until the lateral bristles bend against the ectocervix.
- Rotate the instrument 5 times (360°) in a clockwise direction.
    - NOTE: If it is difficult to insert the central bristles into a stenotic os, it may help to jiggle the brush so that the stiffer center bristle can 'guide' the others into the canal.
- Withdraw the Papette broom from the canal.
- Rinse the Papette broom in the liquid cytology vial by tapping the bristles on the bottom of the container 10 times with enough force to bend them, while simultaneously twisting the handle.
- Discard the Papette broom.
- Twist the cap onto the vial tightly so that the black torque lines cross.

### 19.2.2 HPV DNA

- Remove the brush from the Digene Sampler kit.
- Introduce the brush into the cervical os with enough pressure to maintain contact with the epithelium but not induce bleeding. Twirl the handle 3 rotations in a clockwise direction.
- Remove the swab from the endocervical os and wipe the ectocervical portio with 3 circumferential strokes.
- Place the swab in the collection tube containing the STM (prelabeled with barcoded ID). Break off the end of the shaft protruding from the tube by bending it sharply against the rim of the tube. The shaft is prescored to facilitate breaking.

### 19.2.3 HPV RNA

TBD

19.3 *Equipment and Supplies*

- Colposcope with extra light bulbs
- Optional attachments for colposcope (camera, TV monitor, computer for data storage)
- Examination table with heel cushion, provision for lithotomy position/stirrups
- Mattress, sheets, disposable plastic sheets, pillows
- A good light
- Stand or surgical table/trolley for supplies
- Sterile examination gloves of various sizes
- Cusco's vaginal speculum of various sizes
- Sponge-holding forceps
- Endocervical speculum (or long dissection forceps)
- Cervical biopsy forceps
- Endocervical biopsy forceps
- Endocervical curette
- Lateral vaginal wall retractor/plain spatulas
- Plastic jars for stocking cotton balls and other supplies
- Cotton-tipped fine swab sticks
- Larger cotton-tipped swab sticks
- Gauze pieces and cotton swabs, roller gauze, sterile pads, gauze pads 4"x4"
- Stainless steel containers for saline, acetic acid, Lugol's iodine
- Fresh supplies of saline, 5% acetic acid, Lugol's iodine
- 10% buffered neutral formalin
- Silver nitrate sticks for hemostasis (or Monsel's solution)
- Specimen collection containing vials with labels
- Bucket with disinfectant solution
- Bins for segregation and disposal of waste
- Facilities for autoclaving/Cidex tray
- Tissue holding forceps, toothpicks
- Needle holder, 1-0 chr. catgut, scissors
- Lens paper, acetone

## 19.4 *Examiner Qualifications, Training, and Certification*

- Medical Graduate (MBBS) from a recognized medical college with six months' house job in Obstetrics and Gynecology, OR
- Postgraduate (DGO/MD/DNBE) in Obstetrics & Gynecology with a training in colposcopy from one of the medical colleges or from an IARC Centre

## 19.5 *Colposcopy Protocol*

### 19.5.1 Preparing for Colposcopy

The patient is explained the nature of the procedure and reassured. She must understand that the colposcope is only a kind of binocular and that the procedure will not mean the insertion of any additional instruments or significant discomfort to her. She is asked to pass urine, remove her underclothes, and lie down in lithotomy position with the feet supported by stirrups or heel cushions. Ensure the placement of a fresh disposable plastic sheet under the patient's hips, a sheet over her knees and pillows under her head.

### 19.5.2 Visualization

The cervix is visualised by inserting the speculum as described in chapter 19. Ensure that the cervix is "looking at you". If necessary, use a cotton-tipped swab to gently manoevre it into position.

Look for the nature of the cervicovaginal secretions and any obvious findings, e.g., ectropion, polyp, nabothian follicles, leukoplakia, condyloma, ulcer, growth, atrophy, inflammation or infection.

Gently remove excess mucous from the cervix with saline-soaked cotton swabs. If any other swabs need to be taken because of suspicious signs or symptoms, it is done now, e.g., *Neisseria gonorrhoeae*, *Chlamydia trachomatis* or HPV.

### 19.5.3 Assessment

The cervix is examined at low-power magnification (5x to 10x), looking for surface abnormalities, e.g., leukoplakia, condylomas, the cervical capillaries and surface blood vessels. The green filter in the colposcope improves the evaluation of the blood vessels. The transformation zone is evaluated completely, using an endocervical speculum if required. If the transformation zone cannot be seen completely, the colposcopy is termed unsatisfactory.

Freshly prepared dilute glacial acetic acid, 5%, is applied liberally on the cervix using the cotton balls on a sponge-holder or swab stick. The effect develops over 60-120 seconds and then fades away, so the application may need to be repeated 2-3 times during the course of the examination. Look for acetowhite areas in the transformation zone, which reflect areas of probable dysplasia. If the mucous could not be removed completely it may need to be maneuvered with a fine-tipped cotton swab.

Lugol's iodine is applied if required. It stains the glycogen-containing squamous cells mahogany brown. Normal columnar epithelium does not stain, nor does immature squamous metaplastic epithelium, inflammatory or regenerating epithelium. Also, lesions like condylomata and abnormal transformation zones such as those with CIN or invasive cancer contain very little or no glycogen. The amount of staining is proportional to the degree of differentiation.

The findings of saline, acetic acid and iodine tests are integrated to make a colposcopic assessment:

_____ (00) NORMAL COLPOSCOPY
_____ (01) LEUKOPLAKIA
_____ (02) LOW GRADE CIN
_____ (03) HIGH GRADE CIN
_____ (04) UPPER LIMIT AW NOT VISIBLE
_____ (05) INVASIVE CARCINOMA
_____ (96) OTHERS, SPECIFY:_____
_____ (99) UNSATISFACTORY

The modified Reid score is useful in making a colposcopic assessment:

Modified Reid Colposcopic index

| Feature | **0 point** | **1 point** | **2 points** |
|---|---|---|---|
| Colour of acetowhite area (AW) | Low intensity acetowhitening; snow-white, shiny AW;<br>indistinct AW;<br>transparent AW;<br>AW beyond the transformation zone (TZ) | Gray-white AW with shiny surface | Dull, oyster-white;<br>Gray |
| AW lesion margin and surface configuration | Feathered margins;<br>angular, jagged lesions;<br>Flat lesions with indistinct margins;<br>microcondylomatous or micropapillary surface | Regular lesions with smooth, straight outlines | Rolled, peeling edges;<br>internal demarcations (a central area of high-grade change and peripheral area of low-grade change) |
| Vessels | Fine/uniform vessels;<br>poorly formed patterns of fine and/or fine mosaic;<br>vessels beyond the margin of TZ;<br>fine vessels within microcondylomatous or micropapillary lesions | Absent vessels | Well defined coarse punctation or coarse mosaic |
| Iodine staining | Positive iodine uptake giving mahogany brown colour;<br>Negative uptake of lesions scoring 3 points or less on above three categories | Partial iodine uptake-variegated, speckled appearance | Negative iodine uptake by a lesion scoring 4 or more points on the above three criteria |

**A score of 0-1 indicates subclinical HPV infection/atypia;**

**2-3 indicates CIN I;**

**4-6 indicates CIN II;**

**7-8 indicates CIN III.**

### 19.5.4 Endocervical Curettage

Endocervical curettage is required in the following situations:

- The squamocolumnar junction is not completely visualized, i.e., the transformation zone is extending into the endocervical canal, i.e., unsatisfactory colposcopy.
- The lesion extends into the endocervical canal.
- There is a report of atypical glandular cells on the Pap smear.

### 19.5.5 Cervical Biopsy

Cervical punch biopsy is taken with a Tischler forceps from all suspicious lesions (Reid score $\geq 1$) on the cervix. Asking the patient to cough while taking the biopsy helps to get a good

specimen as it gives a counter-pressure. Monsel's paste can be applied on the biopsy site. The patient is provided with a sanitary pad. Rarely, there may be significant bleeding from the biopsy site, which may require packing or suturing.
Endocervical biopsy can be taken with an endocervical biopsy forceps if required and similarly transported.

The specimens are immediately transferred to a labeled vial containing 10% formalin for transport to the laboratory. Delay in transferring it to formalin can result in autolysis of the specimen. The site(s) of the biopsy is(are) clearly marked on the form as well as on the label.

#### 19.5.6 Vaginal Exam

The vagina is examined as the speculum is being removed. Any area of abnormality is evaluated similarly with acetic acid with or without Lugol's iodine.

#### 19.5.7 Vulvar, Perineal, Per rectal Exam

See Chapter 16

## 19.6 *Exam Conclusion*

All excess of acetic acid and iodine should be mopped with a gauze piece. Patients who have undergone biopsy are usually prescribed a course of prophylactic antibiotics. They are counseled that they may expect some amount of bleeding. If the bleeding is excessive or if there is foul-smelling discharge they should contact the doctor at the no. given on the study ID card or return to the hospital. They will receive their result within 2 weeks.
All instruments are placed in a bucket containing bleach solution and then washed and autoclaved or sterilized in Cidex.
Patients who did not need a biopsy are reassured but told to come back after a year for the next screening visit when contacted by the study supervisor.

## 19.7 *Documenting Performance of Colposcopy and Colposcopically-directed Biopsy*

Results from the colposcopic exam will be recorded on the Colposcopy exam form, preprinted with participant's study ID and barcode. If biopsy was required, completion of a histology request form is also required.

## Chapter 20. Excision of High Grade Lesions

### 20.1 *Overview*

This chapter provides a standardized protocol for the clinical treatment of high grade lesions.

### 20.2 *Equipment and Supplies*

- Electrosurgical generator, with remote electrode monitoring (REM) facility, blend ratios of cutting and coagulation that should perform equally well UP TO 70W and should t be able to maintain a voltage of over 200 V throughout the procedure.
- Patient grounding (dispersive) pad
- Various sizes and shapes of loop electrodes. Loops used for LLETZ should be made of 0.20 mm hard stainless steel or platinum wire.
- Ball electrodes (3- and 5-mm sizes)
- Insulated electrode handle
- Nonconductive speculum with smoke-evacuator port
- Nonconductive vaginal sidewall retractor
- Smoke evacuator with filter system
- Colposcope and all requirements of colposcopy as detailed above
- 5% acetic acid, freshly prepared
- aqueous Lugol's iodine
- large cotton swabs
- local anesthetic with vasopressin (10 units vasopressin in 30 ml of 1% lidocaine)
- dental-type syringe with 27-gauge needles, 1.5" in length
- Monsel's paste or gel
- Specimen vials with 10% neutral-buffered formalin
- 12" needle-holder and 2-0 reabsorbable suture material
- roller gauze for packing, sterile pads
- Pre-printed forms and specimen labels
- Liquid nitrogen and cryovials
- 10% buffered neutral formalin and vials

## 20.3 *Registration*

Once patient registers at the treatment visit, treatment visit forms will be generated with preprinted Study ID and barcode.

## 20.4 *Clinician Qualifications and Training*

Postgraduate (DGO/MD/DNB) in Obstetrics & Gynecology

## 20.5 *Protocols for Excisional Procedures*

### 20.5.1 Indications and Contraindications

*Indications:*

- Cytological or colposcopic suspicion of CIN 2 or worse (including micro-invasion)
- Likelihood of a glandular intraepithelial abnormality
- Unsatisfactory colposcopic examination in the presence of convincing cytological abnormality
- Persistent CIN 1 (of more than 12 months duration)
- CIN 1 where the likelihood of follow-up is low or when a patient requests treatment.

*Contraindications:*

- Pregnancy (exclude by pregnancy test if necessary)

### 20.5.2 Procedure for Electrosurgical Excision of High Grade Lesions (LEEP)

Preparing for LEEP

1. LEEP is preferentially performed in the follicular phase of the cycle but can, if required, be done at any time in the menstrual cycle, provided pregnancy has been excluded.
2. Premedication is generally not required if the patient is counseled well. In extreme cases a short-acting anxiolytic may be administered.
3. The patient is explained that the reason for the procedure, that local anaesthetic will be administered and a small piece of tissue removed which will cure her of the problem. Written informed consent is thus obtained and a review of potential complications and post-LEEP management recommendations is completed.
4. The smoke evacuator is switched on prior to the procedure and the patient explained that it will generate a lot of noise which should not worry her.

Procedure for LEEP

1. The patient is positioned as described in the section on colposcopy.
2. The ground pad is applied on the patient's leg.
3. A plastic or insulated speculum that is comfortable for the patient is placed in the vagina, fully exposing the cervix. Use of as small a speculum as possible keeps the patient more relaxed and facilitates the procedure.
4. Colposcopy is performed as detailed above. This ensures that a more severe (i.e., invasive) lesion has not been missed and also thoroughly assesses the anatomy of the cervix to tailor the LEEP.
5. The cervix and upper vagina are bathed in Lugol's solution to facilitate resection of the entire lesion and the squamocolumnar junction and also to provide antisepsis.

6. Using 1% lidocaine with 1:100,000 units of epinephrine, a circumferential intracervical block is performed using a **25**-gauge dental/spinal needle. The cervix is injected in each of the four quadrants (2,4,8 and 19 o'clock positions) with the needle being inserted to a depth of 2 cm and withdrawn as pressure is placed on the plunger, injecting 0.5 ml at each site. Adequate time is given for the local anaesthetic to act.
7. Select a loop that will encompass the entire lesion and the squamocolumnar junction. Depending on the loop size, a power setting from 40 to 55 watts of pure cutting current is used for excision.
8. Relax your whole body and especially your shoulders. Put the electrocoagulation on before you enter the tissue. Perform an excision of the ectocervix, taking care to control the movement of the loop in the tissue. Every attempt is made to remove the entire lesion in a single specimen and with a single pass. The specimens must be labeled and inked in a manner to assure that orientation can be clearly determined by the processing pathologist and preserved in formalin.
9. Should endocervical excision be needed, a smaller loop (usually 0.8 cm in width) is used for the second pass using the same rules of tissue collection and submission. A cotton-tipped applicator may be required to identify the endocervical canal. The power setting may be set lower when using the smaller loop (e.g., 40 watts, pure cutting, or keep the same setting as before). The second specimen should be placed in a separate jar.
10. Collect an ECC.
11. Once all specimens have been collected, cautery of the entire crater is done using the ball electrode and a pure coagulation current of 60 watts is performed. This ensures hemostasis from any evident bleeding vessels and also takes care of residual disease in the margins to a large extent. Coat the surgical defect with Monsel's paste to assure hemostasis once the vasoconstrictive effect of the injected epinephrine has worn off.
12. Inspect the vagina for any inadvertent thermal injury while the speculum is being removed. Remove the ground pad from the patient's leg and inspect the site to ensure that there is no trauma.
13. Provide the patient with a sanitary towel and help her to get up from the table and dress.

### 20.5.3 Procedure for Cone Excision

The procedure should ideally be done in the immediate post menstrual period.

The transformation zone and abnormal areas should be identified pre-operatively by colposcopy/ visual inspection with Lugol's iodine.

The procedure can be done under general or regional analgesia/anaesthesia.

The patient is placed in a lithotomy position. There should be adequate exposure and illumination. The patient is cleaned and draped. Special care is taken to clean the vagina gently so as to avoid abrading the cervical epithelium.

Haemostatic measures to reduce blood loss : Deep cervical sutures applied at 3 o'clock and 9 o'clock positions to occlude the descending cervical vessels with absorbable suture materials are usually adequate. Additional stitches can be placed at 12 and 6 o'clock positions if required. These sutures also help to provide traction.
An alternative method of reducing blood loss includes injecting saline or dilute adrenaline into the substance of the cervix but this is not usually needed.

A marker stitch is placed at 12 o'clock position using a non-absorbable suture to facilitate orientation of the specimen and reporting of site of abnormality.

Depending on the extent of transformation zone visible on cervix or prior ECC reports, the type of cone to be excised is planned, i.e. long/short or truncated cone. In case of a long cone, a length of 1.5 to 2 cm. will usually suffice and it is not necessary to extend up to the internal os except in rare cases. Introduction of a cervical dilator is not necessary if traction stitches are placed prior hand as dilators may injure the endocervical epithelium or the transformation zone. A circumferential incision is given with a no. 11 scalpel blade 5 mm beyond the transformation zone or abnormal area. The incision is deepened up to the desired depth by tangential cuts and a cone taken out. Handling the surface epithelium must be avoided. An ECC should be performed at the end. This is especially useful if there are doubts regarding the adequacy of the length of the cone.

Haemostasis: The cone bed and margins can be electro- coagulated with a roller ball electrode. This has the advantage of also getting rid of residual disease. Monsel's paste (ferric subsulphate) can be applied on the residual crater. Alternatively, packing with a ribbon gauze is also effective, but it will need removal the following day. Large bleeders will require the placement of hemostatic sutures. The Sturmdorf suture to cover the raw cone bed is not to be practiced as it distorts the cervix and poses difficulty for further cervical biopsies if required in future.

*Follow up*
Patients should be observed for bleeding and the vital signs checked regularly.
Routine catherisation of the urinary bladder is not required. Patients can be discharged the following day.

### 20.5.4 Patient Instructions following Excisional Therapy

- A course of prophylactic antibiotics, analgesics and haematinics are usually prescribed.
- Abstinence is advised for 4 weeks to allow for healing.
- Appropriate contraceptive advice and supplies should be given for subsequent use.

- Patient should follow up with the histopathology report and after 6 weeks. They should be encouraged to report if there is any complication, e.g. excessive bleeding, pain or foul-smelling discharge.

## 20.6 *Documenting excision of High Grade Lesions*

A LEEP form is provided with specimen ID and barcode to document the procedures for excision and submission to the pathologist.

## 20.7 *Submitting Tissue to the Pathologist*

Immediately upon removal, the specimen is cut open and with the endocervical surface facing upwards, pinned to a surface board and fixed in 10% formalin. The marker stitch at 12 o'clock helps in orientation. The pathologist's report is expected to mention the following information the degree of intraepithelial neoplasia, whether crypts are involved or not, whether there is any invasion or not if invasion is present, its depth and extent along with information regarding lymphatic channel involvement. Whether the lesion has been completely excised, at both endo- and exocervical margins should be commented upon.
A point should be made if no abnormality is found but epithelial loss is present. It is important to distinguish between a negative biopsy and one that is inadequate because of epithelial loss. Other incidental findings should be recorded too. All this information helps to plan the future management of the patient.

## Chapter 21. Treatment of Other Gynecologic Conditions

### 21.1 *Overview*

This section is intended to provide a guide to some conditions that may be encountered during the screening of patients for cervical cancer and precancer.

### 21.2 *Vaginitis*

Already written, Meenakshi/Asima please check, maybe this is also in AIIMS protocol

### 21.3 *Cervicitis*

It is characterized by a severe inflammation of the mucosa and submucosa of the cervix. The primary pathogens of mucopurulent cervicitis are *C.trachomatis* and *N.gonorrhoeae*, both of which are sexually transmitted. They can be diagnosed by gross inspection and gram's stain can confirm the diagnosis.

*CDC Treatment Recommendations*

*N.gonorrhoeae* endocervicitis

Ceftriaxone 125mg i.m. (Single dose) or

Ofloxacin 400mg orally (single) or

Cefixime 400 mg orally (single dose) or

Ciprofloxacin 500mg orally (single dose)

Treatment should be given to both partners

*C.trachomatis* endocervicitis

Doxycycline 100 mg orally, b.i.d for 7 days or

Azithromycin 1 g. orally (single dose) or

Ofloxacin 300mg orally b.i.d

Erythromycin base 500 mg q.i.d for 7 days

## 21.4 *HIV/Other Immune Compromise (?)*

Just being sent for post test counseling.

## 21.5 *Pregnancy*

Exclusion criteria. If woman is pregnant at time of enrollment, they will be invited to participate 3 months after post-partum.

## 21.6 *Condylomata acuminata*

This is an infection of the vulva, vagina, or cervix with HPV of the low-risk types. It is sexually transmitted. Gross inspection and colposcopic examination clinches the diagnosis and can be confirmed with biopsy and HPV detection techniques.

Treatment

Symptomatic lesions can be excised. There is no therapy for complete eradication of the virus.

- Podophyllin - paint the lesion each week for 4-6 weeks. Podophyllin should be washed off in 6 hrs.This treatment is contraindicated in pregnancy.
- Trichloroacetic acid –apply every 1-2 weeks until the lesion sloughs off.
- Topical 5-Fluorouracil - daily application for 7-10 days
- Imiquimod cream 5% -apply 3 times per week up to 16 weeks.
- Cryotherapy, Electrocautery or Laser treatment may be used for larger lesions.

## 21.7 *Gross Lesions of the Cervix*

## 21.8 *Atrophy*

## 21.9 *Documenting the Occurrence and Treatment of Other Gynecologic Conditions*

# Chapter 22. Reporting Adverse Events and Medical Complications

## 22.1 *Overview*

It is the responsibility of the study staff to monitor and document adverse events and to ensure that safety of the patients is not compromised. This chapter outlines potential adverse events that could occur as a result of study procedures, and methods to document and monitor these events.

## 22.2 *Adverse Events or Complications from Study Procedure*

Trauma from the speculum, uterine cramping, and/or bleeding may occur during the pelvic examination, collection of cervical cells, and colposcopic examination. Burning after application of acetic acid may occur during the VIA and colposcopic examination. Biopsies, ECC, and LEEP procedures may induce bleeding, severe cramping and pain, and may rarely lead to infection. Some reaction to anesthesia, excessive tissue removal with stenosis or cervical incompetence, and/or thermal injury to vagina or other unintended site may occur during the LEEP procedure. Dizziness, fainting, pain, and/or hematoma may result from the blood draw.

## 22.3 *Document Adverse Events*

### 22.3.1 AE occurring DURING a procedure

Adverse events occurring during the course of an examination or study visit will be recorded on the visit exam form.

### 22.3.2 AE reported AFTER a procedure

Adverse events requiring an unscheduled clinic visit will be documented on an adverse event study form and all symptoms treated according to local protocol.

## 22.4 *Quality Control Procedures*

The study coordinator will be responsible for summarizing adverse events and report these monthly to the Hopkins PIs. Any unforeseen adverse events that is deemed by the scientific advisory committee to be compromising the care of the participants will warrant a suspension of study activities until remedial procedures can be adopted.

## Chapter 23. Cytopathology

23.1 *Overview*

*This chapter contains standardized protocols for the cytopathology processing and interpretation of the Pap smears collected during the CATCH enrollment protocol.*

23.2 *Labeling Requirements*

23.3 *Forms*

23.4 *Coverslips*

23.5 *Papanicolaou Staining*

23.6 *Standard Dotting Recommendations*

## Chapter 24. HPV Testing at CDFD

### 24.1 *Overview*

HPV testing at CDFD will be performed using the Digene Hybrid Capture 2 HPV test using the HPV Probe B cocktail **only** for the detection of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Testing will be performed strictly according to the manufacturer's instructions. Details about documentation and study flow are outlined below.

### 24.2 *Technician training and validation of the CDFD Lab*

All CDFD staff performing the HC2 testing will be required to obtain certification from a Digene representative. Dr. Gayatri Ramakrishna will arrange and ensure appropriate training for all CDFD study staff. Validation panels will be provided by Digene, and 'passing' grade on the validation runs must be obtained.

#### 24.2.1 Initial training at CDFD by Digene staff

The instrument was installed by Dr. Gupta on 12.12.03. The system installed is Hybrid Capture-II. The following instruments were obtained from Digene corp: DML 2000 luminometer, Dell computer system, EPSON printer, Rotary shaker, Microplate heater, Multichanell pipettes (4 channels) and its stand.

The following consumables were also obtained: 1000 sample collection kits (already sent to MediCiti through Meenkashi and Patti), two assay kits, extra long pipettes (400 tips), plate sealers, Kim-wipe towels.

The first test performed on 12.12.03 was done using the test panel and it passed the test. The second test was done on 12.16.03 using the positive samples sent by Dr. Gupta but that failed. Repeated tests were then conducted using the kits and the samples provided by Dr. Gupta some of which had passed and others failed, the reports are in the system itself.

On 04.25.2003 13 samples from MediCiti were obtained, which were analyzed using only the high risk probes. Demonstration for correct handling was done by Patti. In the same test run 5 samples sent by Dr. Gupta were also run. The same MediCiti samples have now been analyzed using the low risk probes as the amount left for high risk probe was not sufficient.

### 24.2.2 Validation runs

The validation run was done using the Test panel provided with the kit which included the low risk, high risk and the negative controls. Initially we had run both the high risk and low risk probes and the assays had passed. The reports are stored in the system also repeat test runs were done using the high risk probes for some of positive cervical-samples sent by Dr. Gupta.

1st July 2003, Pavani had joined in as a project associate in the HPV-Group. A separate bench area has been designated and a new set of pipettes are ordered which will be dedicated only for HPV sample assay. Pavani has since repeated the test with both the high and the low risk types using both the MediCiti samples and Dr. Gupta's samples. One of the MediCiti samples turned positive for low risk HPV. Check reports and data book of Pavani. Of the five tests done by her one had failed.

Till date (14th July, 2003)
A) 11 tests have passed and these included:
1) testing of High risk Test panel with Negative control
2) Testing of low risk test panel with negative control
3) Testing with medicity samples
4) Testing with samples of Dr. Gupta

B) 7 testes have failed:
Reasons are: 1) calibrator out of range
2) The NC % CV too high
3) The NC mean is out of specific range
4) LRC %CV is too high

July 5th orders placed with Dr. Gupta for the 8 sampler kits

Mr. Prem Kumar's staff at MediCiti made two visits one in April followed by another in July where they are trying to help transfer the data-report generated to MediCiti. Scanning for specimens has still not been installed.

### 24.2.3 QC testing of validation panels

Q: Should we run the test panel with each test run or the calibrator is enough?

### 24.2.4 Validation acceptability parameters

Q: At times the test is validated but it appears in the report as " test failed" !

### 24.2.5 Training for new technicians

New technicians hired for HC2 testing in CATCH over the course of the study will be required to receive HC 2 certification from a Digene representative.

A: Ms. Pavani Sowjanya has been hired and she will be running all the tests. How to get the certificate?

## 24.3 *Receiving the HPV-V and HPV-C Vials*

If for the same patient two samples are collected then how will the ID be given. The system takes only one Id #, can we put ID#V and ID#C for both the samples from same patient, e.g., if patient # is 001 then 001V and 001C for both the samples collected from same women

### 24.3.1 Labeling requirements

Samples received from MediCiti will be labeled with a barcode. This barcode will identify the participant ID, the date of sample collection, the visit number, the specimen type, and the aliquot number. CDFD will be equipped with a barcode scanner. When the samples are scanned at CDFD, the date will be registered in the main database under 'date of receipt: HPV-V (or –C) at CDFD', and a log of study ID numbers will be generated for automatic entry into the HC 2 software.

## 24.4 *Digene Hybrid Capture 2 HPV DNA Assay*

### 24.4.1 Principle of the Assay (from the manufacturer)

The Digene HPV Test using Hybrid Capture 2 technology is a signal amplified hybridization antibody capture microplate assay that utilizes chemiluminescent detection. Specimens containing the target DNA hybridize with a specific HPV RNA probe cocktail. The resultant RNA:DNA hybrids are captured onto the surface of a microplate well coated with antibodies specific for RNA:DNA hybrids. Immobilized hybrids are then reacted with alkaline phosphotase conjugated antibodies specific for the RNA:DNA hybrids, and detected with a chemiluminescent substrate. Several alkaline phosphotase molecules are conjugated to each antibody. Multiple conjugated antibodies bind to each captured hybrid resulting in substantial signal amplification. As the substrate is cleaved by the bound alkaline phosphotase, light is emitted which is measured as relative light units (RLU) on a luminometer. The intensity of the light emitted denotes the presence or absence of target DNA in the specimen. An RLU measurement equal to or greater than the Cutoff Value indicates the presence of HPV DNA sequences in the specimen. An RLU measurement less than the Cutoff Value indicates the absence of the specific HPV DNA sequences tested or HPV DNA levels below the detection limit of the assay.

### 24.4.2 Laboratory Requirements, Equipment, and Supplies

24.4.2.1 Materials provided in each HC 2 kit

| **Reagents and materials provided** | |
|---|---|
| Name | Description |
| Indicator Dye | |
| Denaturation Reagent | Dilute NaOH solution |
| Probe diluent | Buffered solution with Na azide |
| HPV Probe B | HPV 16/18/31/33/35/39/45/51/52/56/58/59/68 RNA probe cocktail |
| Negative calibrator | Carrier DNA in STM with Na azide |
| Positive Calibrator B | 1.0 pg/ml constructed HPV 16 DNA and carrier DNA in STM with Na azide |
| Capture Microplate | Coated with anti-RNA:DNA antibodies |
| Detection reagent 1 | Alkaline phosphotase-conjugated antibodies to RNA:DNA hybrids in buffered solution with Na azide |
| Detection Reagent 2 | CDP-Star with Emerald II (chemiluminescent substrate) |
| Wash Buffer Concentrate | Contains Na Azide |

24.4.2.2 Other equipment required and source

| Equipment required | Source (e.g., Vendor) |
|---|---|
| DML 2000 Luminometer | On loan from JHBSPH |
| Rotary Shaker with adjustable speed | **On loan from JHBSPH** |
| Wash apparatus | **XXX** |
| Specimen collection tube rack | Digene |
| EXPAND-4 pipettor | On loan from JHBSPH |
| EXPAND-4 pipettor stand | On loan from JHBSPH |
| PC System | Digene |
| Water bath | **Julabo, already available** |
| Vortex mixer with cup attachment | **Bangalore Genei** |
| Micropipettor; 20 – 200 μl volumes | Gilson |
| Repeating + displacement pipettor | Not present |
| 4-channel pipettor | On loan from JHBSPH |
| Timer | **Sigma** |

24.4.2.3 Other materials required and source

| Accessories required | Source (Vendor, Make, or Manufacturer) |
|---|---|
| Microtubes | **Axygen** |
| Microtube racks | Tarson |
| Plate sealers | Need to find |
| Extra long pipette tips | Need to find, At present getting from Digene |
| Disposable reagent reservoirs | **XXX** |
| Disposable bench cover | **XXX** |
| Disposable powder-free gloves | Local agent |
| Sodium hypochlorite solution (bleach) | Local agent |
| Parafilm or equivalent | Sigma |

| | |
|---|---|
| Disposable aerosol-barrier tips | **Axygen** |
| Disposable repeat pipettor tips | |
| Kimtowels Wipers | Not found |
| 5 ml or 15 ml snap cap round bottom tubes | Tarson |

### 24.4.3 Safety and Special Handling Precautions

***Safety precautions*:**

- HANDLE ALL ASSAY SPECIMENS AND DISPOSED MATERIALS AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS.
- Do not pipette by mouth.
- Do not smoke, eat, or drink in areas where reagents or specimens are being handled.
- Wear disposable power-free gloves while handling reagents or specimens. Wash hands thoroughly after performing the test.
- All materials used in this assay, including reagents and specimens, should be disposed of in a manner that will inactivate infectious agents.
    - SOLID WASTES: autoclave
    - LIQUID WASTES: Add bleach to a final concentration of 1.0% (1:5 dilution of household bleach). Allow 30 minutes for decontamination.
- SPILLS: Non-base-containing spills should be wiped thoroughly with a 5% sodium hypochlorite solution (full strength household bleach). Base-containing spills should be neutralized, wiped dry, and then the spill areas should be wiped with a 5% sodium hypochlorite solution. The wiped area should be covered with absorbent material, saturated with a 5% sodium hypochlorite solution and allowed to stand for at least 10 minutes. A glass or plastic cover or tray can be used to reduce exposure to fumes. **All wiping materials should be treated as hazardous waste.**
- **CORROSIVE REAGENT:** Denaturation Reagent contains sodium hydroxide which is poisonous and can cause severe burns. Do not ingest, breathe vapor or make contact with eyes, skin, or clothing. Wash with copious amounts of water in the event of contact. Always wash after handling. **Take care and wear powder-free gloves when removing tape from around the bottle cap. Bottle cap may contain corrosive solids or liquids.**

### 24.4.4 Notes on Specimen Handling and Reagent Preparation and Storage

- Do not use the reagents beyond the expiration date on the outer box label.
- **DO NOT** interchange components from other sources or from different lots. These have been validated only as a kit.
- Nucleic acids are very sensitive to environmental nuclease degredation. Nucleases are present on human skin and on surfaces or materials handled by humans.
    - **Clean and cover work surfaces with disposal pads and change pads between each assay. Drain detection reagent 1 in a designated area separated from the area used to perform the rest of the assay.**
    - **Wear powder free gloves when performing all steps.**
- **Covering the capture microplate after the wash step and during detection reagent 2 incubation is absolutely imperative!! Exogenous alkaline phosphatase may react with Detection Reagent 2 producing false positive results.** Substances which may contain alkaline phosphatase include Detection Reagent 1, bacteria, saliva, hair and oils from the skin.

- Protect Detection Reagent 2 from prolonged exposure to direct light. Use reagent immediately after aliquoting and avoid direct sunlight.
- The correct volumes of reagents MUST be delivered to the reaction tubes and microplates at ALL steps and COMPLETE mixing should be done after each reagent addition. Prime the repeating pipette in advance of reagent delivery and checked for large air bubbles periodically. Excessive amounts of large air bubbles in the repeating pipettor tip may cause inaccurate delivery. The repeating pipettor MUST be filled, all liquid dispensed, and the refilled before aliquots are made.
- Pipetting to dispense Detection Reagents 1 and 2 must be performed using reverse pipetting technique using either the single or multichannel pipettor. To reverse pipette, depress pipette plunger below the first stop and then draw up liquid fully; a greater volume than the setting on the pipettor will be aspirated. To dispense the correct volume of liquid, depress the pipette plunger only to the first stop; the remaining liquid creates a reservoir that will allow precise delivery of the required volume without creating air bubbles and aerosolization that typically occur when total pipette tip volume is dispensed.
- Each microwell MUST BE washed thoroughly during EVERY WASH! Inadequate washing will result in increased background and will cause false positive results. Residual wash buffer in wells will result in reduced signal or poor reproducibility.

### 24.4.5 Frequency of HC 2 Assay Runs

Approximately 300 samples per week will be collected during enrollment at MediCiti. These samples will be batched into groups of 90, which will include quality control samples. Therefore, CDFD should plan for 3 assays per week.

### 24.4.6 Equipment calibration

- All pipettors should be sent for calibration on an annual basis.
- A blank plate should be read on the luminometer to ensure proper positioning once monthly. The result of this calibration should be recorded in the data log.
- The HPV DNA Panel should be run, alone, at 3 month intervals to ensure quality performance.

## 24.5 *Preparation of Samples and Running the HC 2 Assay: Step-by-Step*

**Remove the specimens and ALL required reagents from the refrigerator PRIOR TO BEGINNING THE ASSAY.**

### 24.5.1 Sample Preparation

Samples will arrive at CDFD in the original collection tube with a screw cap. Samples will be shipped at room temperature (once collected, the un-denatured samples are stable for two weeks at room temperature). **If the samples are to be tested in the same week, they may be stored at 2 - 8°C. If the samples will be tested later than one week, store them at -20°C.** The cervical brush will remain in the collection tube, and does NOT need to be removed. The sample will contain 500 µl total volume, as aliquots will have been removed at MediCiti for PCR.

- Prepare denaturation reagent: Add 5 drops of Indicator Dye to the bottle of Denaturation Reagent and mix thoroughly. The Denaturation Reagent should turn to a uniform, dark purple color.
  - Once prepared, the Denaturation Reagent is stable for three months when stored at 2-8°C. If the color fades, add 5 drops of Indicator Dye and mix thoroughly before using.
- Prepare calibrators:
  - Allow calibrators to reach 20 - 25°C for at least 15 to 30 minutes.
  - Place calibrators in the rack so that NC correspond to wells A1, B1, C1, and PC correspond to wells D1, E1, F1.
  - Remove screw cap on calibrators and carefully place the cap on a clean tissue with the open end facing up. Take care not to touch inside of cap.
  - Pipette 1000 µl of Denaturation Reagent into the Negative Calibrator using an adjustable pipettor. **DO NOT touch the sides of the tube with the pipettor or cross-contamination of the samples will occur.** Replace the cap and tighten.
  - Pipette 500 µl of Denaturation Reagent into the Positive Calibrator using an adjustable pipettor. **DO NOT touch the sides of the tube with the pipettor or cross-contamination of the samples will occur.** Replace the cap and tighten.
- Prepare samples:
  - All specimens to reach 20 – 25°C for at least 15 to 30 minutes.
  - Remove screw cap on samples and carefully place the cap on a clean tissue with the open end facing up. Do **NOT** remove the specimen collection device prior to denaturation. Take care not to touch inside of cap.
  - Pipette 500 µl of Denaturation Reagent into the sample using an adjustable or repeating pipettor. **DO NOT touch the sides of the tube with the pipettor or cross-contamination of the samples will occur.** Replace the cap and tighten.
  - Vortex each tube individually at high speed for 5 seconds. **There must be a visible vortex in each tube such that the purple liquid washes the entire inner surface of the tube.**
    - The calibrators and specimens should turn purple. **Some cervical specimens may contain blood or other biological material which can mask the color changes after addition of the Denaturation Reagent. Failure to exhibit the proper color change will NOT affect the results of the assay.**
    - *Calibrators and specimens in denaturation reagent are stable if stored at 2-8°C overnight, or at -20°C for up to 3 months. Mix well before using.* ***If specimens have been stored under conditions beyond these recommendation ranges, document the sample number, batch number, and storage conditions in the MediCiti datafile.***
  - Incubate denatured samples and calibrators in a 65 ± 2°C water bath for 45 ± 5 minutes. Prepare HPV Probe B cocktails during this incubation.

### 24.5.2 Hybridization

- Preparing the probe: NOTE – extreme care should be taken at this step to prevent RNase contamination of probe and probe mix. Use aerosol-barrier pipette tips for pipetting probe.
  - Centrifuge the vial of Probe B briefly to bring the liquid to the bottom of the vial. Tap gently to mix.

- Transfer 3.5 ml of probe diluent to a new 5ml capacity disposable container. *Probe diluent is viscous – wait 5 seconds before removing the pipette tip from the liquid to ensure complete aspiration.*
- Pipette Probe B into the probe diluent by placing the pipette tip against the inner wall of the tube just above the meniscus and expelling the contents. **Do NOT immerse the tip into probe diluent.**
- Vortex for at least 5 seconds at maximum speed. A visible vortex must be produced. Unused probe mix should be discarded. *If fewer than 72 samples are to run in a single assay, refer to manufacturers instructions for recommended partial assay probe volumes.*
- Pipette 25 µl of probe mix into each microtube using a repeating pipettor (for an Eppendorf pipettor, use 1.25 ml tips at a setting of 1). **Follow pipetting instructions carefully!**
  - Holding the repeating pipettor vertically, insert the tip of the pipettor approximately ¼ inch into the center of the tube. Dispense the volume of the probe into all tubes such that the probe strikes the microtube near the bottom.
  - Gently tap the rack to assure that all of the probe mixture falls to the bottom of the microtubes. Inspect the rack from underneath to verify that all wells have received the appropriate amount of probe mix and that it is at the bottom of each tube.

- Aliquoting samples and calibrators:
  - Remove calibrators and specimens from the water bath after incubation.
  - Vortex each tube individually for at least 5 seconds.
  - Using an extra-long tip, pipette 75 µl of each calibrator or specimen to the bottom of the appropriate hybridization microtube – **take care to follow template so that samples are maintained in the correct order in the microtube array. False positive results will occur if sample aliquots are not carefully transferred. Do not touch pipette tip to inside of specimen/calibrator tube. Do not immerse tip in probe mix when dispensing the sample into the microtube.**
  - Cover the microtubes with a plate sealer. Place rack cover on top of rack.
  - Shake the microtube rack on rotary shaker set at 1100 ± 100 rpm for 3 ± 2 minutes. *The calibrators and specimens should turn yellow after shaking except for bloody samples that may not turn yellow; lack of color change in these samples will not affect the assay.*
  - If the non-bloody samples do not turn yellow after shaking, they may not have received the proper amount of probe. Add an additional 25 µl of probe mix to these samples and shake again. If tubes remain purple after this, the test must be repeated.
  - Incubate in a 65 ± 2°C water bath or the Digene microplate heater for 60 ± 5 minutes. If using the water bath, the water level must be sufficient to cover the entire volume of hybridization solution. The microtube rack may float in the water bath. Program the luminometer during this incubation.

### 24.5.3 Preparing the HC 2 Assay

- With a marker, number each column in the capture microwell plate 1 – 12. Add the samples according to the pre-specified layout, eg.

| Row | Column | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| A | NC | SPEC 3 | SPEC 11 |
| B | NC | SPEC 4 | SPEC 12 |
| C | NC | SPEC 5 | SPEC 13 |
| D | PC | SPEC 6 | SPEC 14 |
| E | PC | SPEC 7 | SPEC 15 |
| F | PC | SPEC 8 | SPEC 16 |
| G | SPEC 1 | SPEC 9 | SPEC 17 |
| H | SPEC 2 | SPEC 10 | SPEC 18 |

etc.

### 24.5.4 Hybrid Capture

- Carefully remove microtube rack containing calibrators and specimens from water bath. Immediately remove the rack lid and slowly pull plate sealer up and across the rack.
- Using a multichannel pipettor, transfer the entire 100 µl contents of the microtubes to the bottom of the corresponding capture microwell. Use new pipette tips for each column and allow each pipette tip to drain well. The pipettor may be steadied by resting the **middle** of the pipette tips on the top edge of the microwells. *Do not pipette vertically or allow tip to touch the side of the well.*
- Cover microplate with a new plate sealer and shake on a rotary shaker at 1100 ± 100 rpm at 20-25°C for 60 ± 5 minutes.
- Prepare wash buffer during this incubation
  - Dilute 100 ml wash buffer concentrate with 2.9 L of distilled or deionized water and mix well for a final volume of 3.0 L.
  - Seal container to prevent contamination or evaporation and record date of preparation on the container. Once prepared, the wash buffer is stable for 3 months at 2 - 25°C. If kept refrigerated, equilibrate to 20 - 25°C before using.
- Remove the capture microplate from the shaker and carefully remove the plate sealer.
- Fully invert the plate over sink and shake hard with a downward motion. **Do NOT reinvert plate.**
- Blot by tapping firmly 2-3 times on clean kimtowels absorbent paper. Ensure that all liquid is removed from the wells and the top of the plate is dry. If liquid remains, tap firmly again on clean kimtowels until dry.

### 24.5.5 Hybrid detection

***NOTES: Make additions in left-to-right direction using an 8-channel pipettor. Use reverse pipetting technique described in section 27.4.4. Do not touch pipette tips to the sides of the microwells.***

- Carefully pipette 75 µl of detection reagent 1 into each well of the capture microplate using an 8-channel pipettor. *Verify that all wells have been filled accurately by observing that all wells have the same intensity of pink color.*
- Cover plates with clean Parafilm and incubate at 20 - 25°C for 30 ± 3 minutes.

### 24.5.6 Washing Method

- Remove detection reagent 1 from the wells by placing clean Kimtowels absorbent paper on top of the plate and carefully inverting. Before inverting, ensure that the paper is in contact with the entire surface area of the plate.
- Allow plate to drain for 1 – 2 minutes. Blot well on clean Kimtowels. Carefully discard use Kimtowels to avoid alkaline phosphatase contamination of later steps.
- Hand wash the plate 6 times. Each well must be washed to overflowing to remove conjugate from the top of the wells. Washing begins at A1 and continues in a serpentine fashion to the right and downward. After all the wells have been filled, decant liquid into sink with a strong downward motion. The second wash is started at well H12 moving in a serpentine fashion to the left and upward. This sequence of 2 washes is repeated 2 more times.
- After washing, blot the plate by inverting on clean Kimtowels and tapping firmly 3 – 4 times. Replace the toweling and blot again.
- Leave the plate inverted and allow to drain for 5 minutes. Blot the plate one more time.

### 24.5.7 Signal Amplification

***NOTE: Use a clean pair of gloves for handling Detection Reagent 2. Detection Reagent 2 addition should be made without interruption. The incubation times of all wells must be consistent. Take care not to touch the sides of the microwell or splash reagent back onto the tips, as cross-contamination of specimens can occur.***

- Using an 8-channel pipettor, carefully pipette 75 µl of Detection Reagent 2 into each well of the capture microplate. *All microwells should turn a yellow color.* Verify that all wells have been filled accurately by observing the same intensity of yellow color.
- Cover microplates with clean parafilm and incubate at 20 – 25°C for 15 minutes. **Time this step precisely.** Avoid incubating in direct sunlight.
- Read the microplate on the DML 2000 Luminometer immediately after 15 minutes of incubation (and no later than 30 minutes of incubation).
- Assay specific software will allow the entry of pertinent run information directly into the spreadsheet.

### 24.5.8 Quality Control for HC 2

- The negative calibrator and positive calibrator must be run in triplicate with each test run. The negative calibrator and positive calibrator results should show a coefficient of variation (%CV) of ≤ 25%. If the %CV is > 25%, discard the calibrator value with a RLU value furthest from the mean as an outlier, and recalculate the mean using the remaining two calibrator values. If the difference between the mean and each of the two values is ≤ 25%, proceed to the next step; otherwise, **the assay is invalid and must be repeated.**
- The mean of the negative calibrator results should be ≤ 250 RLUs. If the mean of the negative calibrator is > 250 RLUs, **the assay is invalid and must be repeated.**
- The positive calibrator mean (PCmean) and negative calibrator mean (NCmean) results are used to calculate the PCmean/NCmean ratio.
- Calculate the PCmean/NCmean ratio. If the ratio is ≥ 2.0, proceed to the next step. If any of the ratios fail, **the assay if invalid and must be repeated.**
- **Interpretation of specimen results:**
    - Specimens with RLU/Cutoff ratios ≥ 1.0 are considered 'positive' for one or more of HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 68.

- Specimens with RLU/Cutoff ration < 1.0 are considered 'negative' or 'none detected' for the 13 types tested. HPV DNA sequences are either absent or the HPV DNA levels are below the detection limit of the assay.

### 24.5.9 Transmitting Data to MediCiti

## Chapter 25. Histopathology at MediCiti

# Chapter 26. Pathology Quality Control

# Chapter 27. Colposcopy Quality Control

## 27.1 *Overview*

## 27.2 *Colposcopy Safety Monitoring* -

## 27.3 *Colposcopy QC Plan*

All women having a colposcopic exam will have a still digital image of the cervix after acetic acid application taken and stored electronically. A 10% random sample of these images will be selected and sent electronically to Dr. Neerja Bhatla at AIIMS, and Dr. Connie Trimble at JHMI for masked review. We will perform this masked QC on a monthly basis for the first 3 months of enrollment into the study to evaluate performance of the local colposcopy. If **x%** disagreement is found, Dr. Bhatla will visit Medchal and conduct an intensive colposcopy workshop highlighting areas which were identified as having been deficient, or the colposcopists will be retrained at the cervical cancer screening site in Barshi.

If agreement is satisfactory after the first 3 months of enrollment, QC will be performed every 3 months until the end of the study.

## 27.4 *QC Data Management*

## 27.5 *QC Intervention*

### 27.5.1 Equipment Deficiencies

If equipment is found to be deficient, colposcopic examination will be postponed until equipment can be brought up to specification or new equipment purchased and delivered.

### 27.5.2 Colposcopist Deficiencies

If deficiencies in subjective interpretation of colposcopic impression are found, colposcopic examination will be postponed until an intensive retraining workshop has been completed by all study colposcopists. These workshops will particularly highlight documented areas of deficiency.