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<tbody>
<tr>
<td>AGI</td>
<td>Alan Guttmacher Institute</td>
</tr>
<tr>
<td>AIDS</td>
<td>acquired immunodeficiency syndrome</td>
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<tr>
<td>BCG</td>
<td>Boston Consulting Group</td>
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<tr>
<td>BPV</td>
<td>bovine papillomavirus</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<tr>
<td>cDNA</td>
<td>complementary deoxyribonucleic acid (cDNA)</td>
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<tr>
<td>CFU</td>
<td>colony-forming unit</td>
</tr>
<tr>
<td>CIOMS</td>
<td>Council for International Organizations of Medical Sciences</td>
</tr>
<tr>
<td>CONRAD</td>
<td>Contraceptive Research and Development Program (Arlington, VA)</td>
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<tr>
<td>CSW</td>
<td>commercial sex worker</td>
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<tr>
<td>CT</td>
<td>Chlamydia trachomatis</td>
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<tr>
<td>DC</td>
<td>dendritic cell</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DSMB</td>
<td>Data and Safety Monitoring Board</td>
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<tr>
<td>FDA</td>
<td>United States Food and Drug Administration</td>
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<td>FHI</td>
<td>Family Health International</td>
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<td>FIV</td>
<td>feline lentivirus</td>
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<tr>
<td>GC</td>
<td>Neisseria gonorrhoeae</td>
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<tr>
<td>GMP</td>
<td>good manufacturing practice</td>
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<tr>
<td>HAART</td>
<td>highly active anti-retroviral therapy</td>
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<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
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<td>HPTN</td>
<td>HIV Prevention Trials Network (National Institutes of Health)</td>
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<td>herpes simplex virus</td>
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<tr>
<td>ICH</td>
<td>International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use</td>
</tr>
<tr>
<td>IND</td>
<td>investigational new drug</td>
</tr>
<tr>
<td>IWGM</td>
<td>International Working Group on Microbicides</td>
</tr>
<tr>
<td>MO-DC</td>
<td>monocyte-derived dendritic cells</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MSF</td>
<td>males having sex with females</td>
</tr>
<tr>
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<td>males having sex with males</td>
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<tr>
<td>MSM/MSF</td>
<td>bisexual men</td>
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<td>NDA</td>
<td>new drug application</td>
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<td>non-governmental organization</td>
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<td>Acronym</td>
<td>Definition</td>
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<td>NICHD</td>
<td>National Institute of Child Health and Human Development</td>
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<td>National Institutes of Health</td>
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<tr>
<td>NIAID</td>
<td>National Institute of Allergy and Infectious Diseases (Bethesda, MD)</td>
</tr>
<tr>
<td>OAR</td>
<td>Office of AIDS Research</td>
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<tr>
<td>OTC</td>
<td>over-the-counter</td>
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<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cell</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RTI</td>
<td>reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>SHIV</td>
<td>chimeric SIV containing HIV components</td>
</tr>
<tr>
<td>SIV</td>
<td>simian immunodeficiency virus</td>
</tr>
<tr>
<td>SPC</td>
<td>summary of product characteristics</td>
</tr>
<tr>
<td>STD</td>
<td>sexually transmitted disease</td>
</tr>
<tr>
<td>STI</td>
<td>sexually transmitted infection</td>
</tr>
<tr>
<td>TMWG</td>
<td>Topical Microbicide Working Group (FDA)</td>
</tr>
<tr>
<td>UN</td>
<td>United Nations</td>
</tr>
<tr>
<td>UNAIDS</td>
<td>Joint United Nations Programme on HIV/AIDS</td>
</tr>
<tr>
<td>USP</td>
<td>unique selling point</td>
</tr>
<tr>
<td>VLP</td>
<td>virus-like particle</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</table>
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**Section One - Target Identification and Lead Selection**

- **Chapter 1.** The Human Immunodeficiency Virus  
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- **Chapter 2.** Non-HIV Pathogens  
  Fulvia Veronese, Robert Eisinger, and Penny Hitchcock
- **Chapter 3.** Lead Selection  
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**Section Two - Pre-clinical Testing**

- **Chapter 4.** Lead Optimization  
  Alan Stone
- **Chapter 5.** Efficacy Testing  
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- **Chapter 6.** Toxicology and Safety Testing  
  Patricia Reichelderfer
- **Chapter 7.** Monitoring the Microbicides Pipeline  
  Polly Harrison

**Section Three - Clinical Trials**

- **Chapter 8.** Proof of Concept  
  Zeda Rosenberg
- **Chapter 9.** Non-HIV STIs and the Epidemiology of Success  
  Kelly Blanchard and Christopher Elias
- **Chapter 10.** Alternative Pathways for Development  
  Charlotte Ellertson, Dan Davis, and Debra Birnkrant
- **Chapter 11.** Clinical Trials Management  
  Peter Kilmarx
- **Chapter 12.** Regulatory Concerns and Global Options  
  Richard Bax

**Section Four - Manufacturing, Formulation, and End Use**

- **Chapter 13.** Contract Manufacturing  
  Anne-Marie Corner
- **Chapter 14.** Opportunities for Coordination  
  George Bene and Henry Gabelnick
- **Chapter 15.** Key Issues in Formulation  
  Bill Rencher
- **Chapter 16.** Market Segments and End Users  
  Florence Camus-Bablon
- **Chapter 17.** Social Science Research  
  Christa Coggins

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**Note:** The views expressed in this document do not necessarily reflect the opinions of individual members of the Working Group, nor the policies of their respective agencies or organizations.
A global priority
Although education and public health interventions have effectively slowed the AIDS pandemic in some parts of the world, the rate of HIV infection in many other areas has continued to accelerate. In some parts of sub-Saharan Africa, in particular, HIV seroprevalence has reached truly alarming levels, as high as 30 percent to 50 percent among certain populations. Beyond even the appalling toll in individual human misery, the pandemic in these areas has now reached levels at which the economic development of entire societies is threatened, with further devastating consequences.

As the pandemic continues to spread, its impact on women is becoming increasingly important. Due in part to deep-seated social inequalities, using condoms to prevent sexually transmitted infections is simply not a feasible option for many women—in many cases, they are both unable to decline sex and unable to negotiate condom use with their partners. Providing an additional means for women to protect themselves, by developing a woman-controlled method to interrupt the transmission of HIV (and potentially other sexually transmitted infections as well), could thus save many millions of lives and provide an additional critical avenue for helping to control the pandemic as a whole.

Furthermore, although the Microbicide Initiative has focused primarily on the development of vaginal microbicides, the Working Group recognizes that there is also considerable risk of HIV transmission via anal intercourse, both among men having sex with men and among heterosexual couples. Use of a safe and effective microbicide—a product used before intercourse to reduce infection—might offer an additional means of interrupting the spread of HIV via this route.

A microbicide would provide a critical adjunct to condoms, as well as complementing current efforts to develop a therapeutic or prophylactic HIV vaccine. A microbicide is likely to become available more widely and much more rapidly than a vaccine; will act at an earlier stage of the infection; and may also be able to interrupt the spread of other sexually transmitted infections (which an HIV vaccine would be unlikely to affect). The development of a safe, effective, and widely accessible microbicide offers a vital new approach to addressing the single most important public health challenge worldwide.

The Science Working Group
In response to this urgent need, in late 2000 the Rockefeller Foundation invited an international group of scientists, research organizations, pharmaceutical industry representatives, United Nations organizations, advocacy groups, and donors to come together to find ways to accelerate the development of a safe, effective, and accessible microbicide. Working Groups were established to examine five key elements in the process of creating a microbicide: (1) the potential market size and demand for such a product; (2) ways to ensure widespread access to a final product; (3) advocacy to encourage additional investment in the field; (4) the scientific and technical requirements for accelerating microbicide development; and (5) the public health impact.

The reports from other Working Groups are contained in companion volumes within this series. This document contains the consensus
The report of the Science Working Group of the Microbicides Initiative. The core Science Working Group was composed of twenty members with extensive experience in the microbicides field, drawn from private companies, governmental regulatory and research agencies, and non-governmental organizations. Their names have been individually listed at the front of this volume.

Over the course of six months of research and investigation, members of this core group completed extensive consultations with a wide variety of technical experts. They covered topics ranging from the basic science underlying a microbicide product, through the challenges of pre-clinical and clinical development, to key issues in manufacturing, formulation, acceptability, and end use. Participation in the group was voluntary, and selection was based solely on individual expertise and willingness to contribute. As a result, the conclusions and recommendations of the group, while reflecting a perhaps uniquely thorough understanding of the state of the art in microbicides development, in no way represent the official position of any particular sponsoring agency.

Strategies for product acceleration

Section I of this document outlines our current best understanding of the basic life cycles and processes of infection for HIV, as well as several other sexually transmitted pathogens. Effective microbicide development depends upon identifying and exploiting specific opportunities to interrupt these pathways, in order to halt the spread of a particular pathogen without damaging a human host. As more opportunities for interrupting these pathways have become apparent, and more potential products have become available, appropriate lead selection criteria to help guide sponsors’ investment decisions have also become increasingly important.

Section II of this document discusses a variety of current approaches to lead optimization and to the pre-clinical testing of safety and efficacy among candidate microbicides. In vitro and animal testing presents a particular challenge in the microbicides field, due to the lack of any product with demonstrated efficacy in humans against which to evaluate the results of pre-clinical models. As greater numbers of early-stage lead molecules move toward clinical testing, improved comparability among in vitro and animal tests as well as an increasingly cost-effective and clinically relevant pre-clinical development pathway will become increasingly valuable.

Section III of this document analyzes the many challenges facing microbicide clinical trials. Because the underlying event (transmission of HIV between sexual partners) happens relatively rarely, even among populations with the highest incidence of HIV infection, a clinical trial designed to prove a product’s efficacy must necessarily be quite large, involving thousands of women for extended periods of time. In addition, because AIDS is an incurable and fatal condition, condom promotion and safer-sex counseling are ethical imperatives during any microbicides trial, even though these interventions are likely to make it even more difficult to detect a beneficial effect attributable specifically to the microbicide product being tested. Finally, the appropriate regulatory pathway for a preventive treatment, such as a microbicide, may differ from the regulatory pathway for a therapeutic drug in several important ways.

Section IV sets forth a number of additional considerations in manufacturing and formulating a microbicide product. Since the organizations that are currently sponsoring the development of microbicides do not have dedicated manufacturing capabilities, contract manufacturing will be essential to producing sufficient product, even to complete clinical testing. Managing this process cost-effectively will be critical to a successful development program. In addition, the proper formulation of a microbicide product is critically important to both its effectiveness and its acceptability. Understanding
the relationship between end use, formulation, and manufacturing is critical to ensuring that successive generations of products are as widely used as possible.

**Conclusions and recommendations**

After more than a decade of research and development, the microbicides field is looking increasingly bright. An explosion of basic scientific research has identified many promising biological targets, encompassing a wide variety of mechanisms of action. Within each of these mechanisms of action, many new compounds are under active development. A wide variety of in vitro and animal models are now available to vet these products before clinical testing, and these models are continually being refined to improve their internal comparability and their specific relevance to human infection.

Significant progress has also been made in the process of bringing a potential lead compound to market. There have been important clarifications of the requirements for pre-clinical testing of safety and efficacy. The regulatory pathway for human studies is also becoming more transparent (at least up to the evaluation of a large phase 3 efficacy study, which has to date been completed only for existing spermicidal products containing nonoxynol-9). Finally, a substantial level of consensus has developed around the appropriate design of clinical trials and the ethical standards that apply to their conduct and management.

Despite this dramatic progress in the overall product development effort, the concept of a topical microbicide for preventing the transmission of HIV and other sexually transmitted infections has yet to be clinically proved. No major pharmaceutical firm has yet made a significant investment in developing a microbicide product, and public-sector support has fallen well short of providing the funding required for optimal progress. The recommendations of the Science Working Group have therefore focused on identifying specific areas for investment to accelerate the development of a first-generation microbicide. These key areas are:

1. **Improved funding for large-scale clinical effectiveness trials**
2. **Continued development of a robust and dynamic clinical trials infrastructure**
3. **Greater support and coordination of formulation, manufacturing, and delivery resources, on behalf of the entire field**
4. **Increased resources specifically devoted to the toxicological and pharmacodynamic testing of products currently in early development**
5. **Improved access by product sponsors to a coordinated program of animal and in vitro tests**
6. **Sustained resources for the development of microbicides through several product generations, in order to allow the field eventually to become commercially self-sustaining**

**Next steps**

Accelerating the development of microbicides is a realistic and important near-term opportunity. The challenges facing microbicide development are well understood and manageable. The first generation of microbicide products is now undergoing clinical testing, and, if effective, should be on the market well within this decade. Subsequent product generations will deliver improved effectiveness, a broader spectrum of activity, and enhanced acceptability for consumers. In addition, once clinical effectiveness for a lead product has been established, market mechanisms are likely to support most subsequent product development (although public support for access in resource-poor settings will probably be required in order to achieve the greatest possible public health benefit).

Proving clinical effectiveness in the first place, however, and demonstrating that microbicides
can offer a valuable weapon in the fight against AIDS and other sexually transmitted infections, is likely to require significantly greater resources than are currently being devoted to the field. As this document demonstrates, the science behind microbicides is developing rapidly, and the process for developing a marketable product is becoming increasingly clear. The only missing ingredient for developing a safe, effective, and accessible microbicide is sufficient investment and the sustained will to see these products created. As a global health priority, it is imperative that these ingredients do not remain missing for long.
Section I
Target Identification and Lead Selection

An important first step in developing an effective topical microbicide is to identify and understand the mechanisms of action by which the sexual transmission of particular pathogens may be blocked. As the relevant biology in this area becomes better understood, certain mechanisms of action are likely to prove either more effective or less prone to adverse effects than others.

In the microbicides field, this important background of scientific understanding has been most highly developed in the case of HIV, due to its overwhelming significance as a public health challenge. As a result, an earlier abundance of poorly understood leads, with some degree of efficacy in one model or another, have now been mostly sorted out according to a reasonably well-understood set of mechanisms for interrupting the transmission or replication of the virus. The field is now well on its way to understanding the relative in vitro safety and efficacy profiles for each of these various mechanisms of action, and for a wide portfolio of potential products.

In the case of non-HIV pathogens, however, many more questions about the basic physiology of transmission and infection remain unanswered. As a result, while microbicides directed against non-HIV pathogens may be targeted by virtue of some degree of overlap with the physiology of HIV transmission (for example, agents that disrupt the envelope surrounding HIV may also disrupt a similar envelope that surrounds the herpes simplex virus (HSV)), the most important mechanisms of action against many other pathogens remain unclear.

This section will therefore concentrate on the basic science behind microbicides targeted against HIV, with some additional mention of areas where there are overlapping mechanisms of action that may be effective against other sexually transmitted pathogens. It will also provide a reasonably comprehensive overview of the wide portfolio of lead molecules and other approaches that have been investigated or proposed to interrupt the transmission of HIV, as well as review areas of current research into the underlying biology. Finally, it will discuss the evolving process by which microbicide developers have been able to select the most appropriate leads for further investment and development. The process for optimizing these leads, and for ensuring their safety and efficacy prior to clinical trials, will be discussed in the next section.

Key conclusions and recommendations from this section include:

- As the biology of the field has become better understood, several interesting opportunities have arisen to interrupt the transmission of HIV and other sexually transmitted infections, without causing harm to a microbicide user.
- A large portfolio of early-stage products (or “leads”) has already been created.
- Lead selection is becoming increasingly rational, driven both by rapid testing and an improved understanding of the relevant characteristics for a successful microbicide product.
Section I Target Identification and Lead Selection

Chapter 1. The Human Immunodeficiency Virus (HIV)

Basic biology

The retroviral life cycle

There are several key steps in the life cycle of a retrovirus (outlined in figure 1):

1) Attachment of the virus to the cell surface, via both non-specific and specific receptors
2) Penetration of the virus core into the cell
3) Uncoating of the virus
4) Copying of the viral RNA into DNA by the viral enzyme reverse transcriptase
5) Importation of the viral DNA and some viral proteins into the nucleus
6) Integration of the viral DNA into the host cell DNA
7) Synthesis of viral RNA
8) Synthesis of viral proteins, including structural elements such as the envelope and core, as well as enzymes such as reverse transcriptase, integrase, and protease
9) Assembly, budding, and release of virus particles
10) Maturation of core proteins

Many of these steps represent potentially attractive drug targets. However, since the goal of a topical microbicide is to prevent viral infection as early as possible, this overview will concentrate on potential targets that arise during the earlier steps of the retroviral life cycle.

Attachment, binding, fusion, and entry

Retrovirus infection begins with a series of non-specific interactions between the viral envelope and certain molecules on the surface of human cells. Several mechanisms have been implicated in this initial process, including the association of regions on the HIV envelope with long, negatively-charged molecules known as heparan sulfate proteoglycans; the binding of the viral envelope to other cell-surface molecules, known as lectins; and a variety of other interactions between the virus particle and certain cell adhesion molecules.

After this initial association, the HIV gp120 protein binds to the human CD4 molecule on the surface of certain cells of the immune system.
This binding induces a structural change that allows a second stage of interaction with a family of molecules known as chemokine receptors, including CXCR4 and CCR5. Binding to these second co-receptors in turn triggers a process of envelope fusion, mediated by gp41, a trans-membrane protein in the HIV envelope. Several molecules have already been identified that block these early stages in viral association, binding, and fusion.

**Uncoating**

Once the virus particle has fused with the host cell, “uncoating” occurs, which results in the release of viral RNA and viral proteins into the substance of the cell. The process leading to uncoating is not yet well understood, and no specific inhibitors have yet been identified.

**Reverse transcription**

After uncoating, viral RNA is converted by the viral enzyme reverse transcriptase into complementary DNA (cDNA). Reverse transcription has long represented an attractive target for interfering with viral replication, since this process is critical for retroviruses but its inhibition does not usually disrupt cellular functions. A class of therapeutic anti-HIV drugs termed “reverse transcriptase inhibitors” (RTIs) targets this stage of the retrovirus life cycle.

**Nuclear importation**

In order to integrate into the host cell DNA, the viral cDNA needs to be brought into the cell nucleus. No inhibitors have yet been shown to block this stage of the viral life cycle.

**Integration**

The final step in the process of cell infection is the integration of viral cDNA into the host DNA, which is accomplished by the viral enzyme integrase. Integration is the process most unique to retrovirus replication. Other viruses use reverse transcription as part of their replication cycle; however, no other family of viruses integrates itself into the host DNA. While the first integrase inhibitors have been identified very recently, all of the molecules identified so far have had undesirable pharmacological properties.

**Mechanisms of transmission**

The precise sequence of events between exposure to HIV and the establishment of infection in the host has yet to be completely described. Even so, it is clear that either cell-free or cell-associated virus routinely comes into contact with a wide variety of host tissues, each with differing characteristics that are important to the eventual establishment of a host infection.

The mucous membranes of the female genital tract are largely composed of multiple layers of stratified epithelia. These structures generally offer a good barrier to viruses and pathogens that may be present in seminal fluids or associated with sperm. The cervix is considered the site most susceptible to HIV entry because (i) the endocervix mucosa is a monostratified epithelium; (ii) numerous potential target cells for HIV are present in the epithelia as well as in the submucosa, including Langerhans cells, monocytes, and macrophages; and (iii) the possibility of ectropion, particularly in young or pregnant women, increases the exposure of the endocervix mucosa to a genital inoculum of HIV.

The rectal mucosa is composed of a single layer of columnar epithelial cells, and offers a poor barrier to viruses and other pathogens. The rectum has little anatomical protection against trauma encountered during sex, and so can be easily damaged. When trauma does occur, bacteria and viruses are able to pass directly to the lamina propria, a layer of tissue located beneath the epithelium proper, populated with immune cells that may be particularly susceptible to HIV infection.

Several specific mechanisms have been proposed for the transfer of infectious virus particles across these mucous membranes. These mechanisms include the direct infection of epithelial cells, transmigration of infected cells across the epithelium, uptake of HIV by dendritic (or
antigen-processing) cells, and/or the direct breakdown of epithelial integrity (see figure 2).

Once viral particles have crossed the mucous membrane, however, there is general agreement that antigen-processing cells in the lamina propria actively transport the virus particles to regional lymph nodes. Viruses may also simultaneously infect nearby immune cells in the lamina propria, causing a local propagation of the infection. Although the relative balance of viral transport to distant sites versus local replication may be important for the rational design and use of microbicides, this topic is not yet well understood. In any event, once viruses associated with antigen-processing cells or infected T-cells reach the lymph nodes, they are then disseminated throughout the body’s lymphatic tree, and a systemic infection results.

**Physiologic and immunologic defenses against HIV**

**Vaginal pH**
The pH of the normal vagina is in the slightly acidic range (~ pH 4), and is detrimental to HIV and many other sexually transmitted pathogens. However, semen neutralizes the vaginal environment, increasing the pH to neutrality (pH 7.2). Although this pH is required to maintain sperm viability, it also allows cell-free and cell-associated viruses to persist in the ejaculate.

**Vaginal ecosystem**
The human vagina is a complex environment. A combination of microbiological, chemical, and physical barriers act to protect the normal vagina from infection. Lactobacilli (gram-positive bacteria that are normally found in the vagina) interfere with pathogens by releasing a variety of anti-microbial compounds such as lactic acid, hydrogen peroxide, bacteriocins, and biosurfactants. The loss of lactobacilli and/or overgrowth of abnormal vaginal flora have been associated with bacterial vaginosis and HIV infection.

**Natural immune defenses**
Epithelial cells are the first line of defense against pathogens and other inflammatory stimuli. Upon infection, epithelial cells synthesize anti-microbial peptides called defensins, cytokines, and chemokines, as well as other molecules that activate or recruit key immune cells. Defensins are small peptides that act rapidly against bacteria, fungi, and enveloped viruses. Epithelial cells also secrete cytokines, such as IL-7 and IL-5, which support the development and survival of resident intra-epithelial lymphocytes. Finally, pathogen-specific antibodies, such as IgA and IgG, are also widely present in cervicovaginal secretions.

---

**Figure 2. Potential mechanisms for HIV transmission**

[Diagram showing potential mechanisms for HIV transmission, including cell-free and cell-associated viruses, epithelial cells, defensins, cytokines, chemokines, and antibodies.]
Targets for interrupting transmission
There are a number of points at which a microbicide product might effectively and specifically interrupt HIV transmission or replication. Key areas of opportunity include physical or physio-chemical barrier methods; maintaining normal vaginal defenses (via buffered gels, supplementary vaginal micro-flora, antibodies, or antibiotic peptides); disrupting viral particles; inhibiting viral attachment or fusion; inhibiting reverse transcription; and inhibiting HIV uptake and active dissemination by antigen-presenting cells. In addition, preventing other sexually transmitted infections, which are major cofactors for HIV sexual transmission, also reduces the risk of transmitting HIV due to the reduction of associated inflammation or ulceration of the vaginal mucous membranes.

Figure 3 presents a summary of the potential steps at which the transmission or replication of HIV might be blocked by a topical microbicide.

Compounds currently proposed or under investigation
Table 1 lists virtually every method currently proposed or under active investigation as an option to interrupt the transmission of HIV or other sexually transmitted pathogens. Most of these approaches have not been pursued any further than a limited demonstration of efficacy in an in vitro or animal model, although a limited number of these approaches have progressed as far as extensive clinical testing (see chapter 7).

Additional approaches
Many of the first approaches to develop microbicidal products for the prevention of sexually transmitted infections were based on the knowledge that had already accumulated about spermicidal agents. Unfortunately, these early microbicidal products, including surfactant agents such as nonoxynol-9, had a poor therapeutic index (a measure of the relationship between the potential therapeutic efficacy and adverse effects). Potential adverse effects from the application of these products became apparent during clinical trials. In fact, while having no protective effect, nonoxynol-9 has been demonstrated to potentially increase the risk of acquiring HIV infection. As research in the field has progressed, however, many new compounds have been identified that represent much more specific strategies to inactivate sexually transmitted...
viruses and other pathogens without damaging human cells in the process.

**Attachment, fusion, and entry**
A critical step in the HIV life cycle appears to be the secondary binding of the virus to chemokine receptors such as CCR5 and CXCR4 on the cell surface (after primary binding to CD-4). Several lines of evidence point to CCR5 as one of the most important biological determinants of HIV transmission. In addition, while loss of CCR5 on the cell surface has been correlated with natural resistance to HIV infection, there are no apparent adverse consequences due to the absence of this cell receptor. Thus, molecules that block CCR5 may represent effective vaginal and rectal microbicides. This is an extremely active area of research in both industry and academia.

**Table 1. Approaches proposed for interrupting HIV transmission, by primary mechanism of action**

<table>
<thead>
<tr>
<th>Physico-chemical barriers</th>
<th>Viral disruption</th>
<th>Inhibition of HIV uptake and dissemination by antigen-presenting cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Devices</td>
<td>Oxidants</td>
<td>No lead molecules are currently under investigation for this mechanism of action (but see further discussion of DC-SIGN in the following section).</td>
</tr>
<tr>
<td>Devices</td>
<td>Surfactants</td>
<td>Antiviral proteins</td>
</tr>
<tr>
<td>Devices</td>
<td>Attachment or fusion inhibitors</td>
<td>Other molecules</td>
</tr>
<tr>
<td>Devices</td>
<td>Sulfated and other charged polymers</td>
<td>Thermoreversible gel: a physico-chemical barrier that acts as a double barrier—(i) physical barrier, the gel by itself and (ii) chemical barrier, sodium lauryl sulphate, for viral inactivation.</td>
</tr>
<tr>
<td>Devices</td>
<td>Fusion inhibitors</td>
<td>Inhibitory agents</td>
</tr>
<tr>
<td>Devices</td>
<td>Inhibition of reverse transcription</td>
<td>Other uncharacterized or unknown mechanisms of action</td>
</tr>
<tr>
<td>Devices</td>
<td></td>
<td>anionic dendrimers</td>
</tr>
<tr>
<td>Devices</td>
<td></td>
<td>porphyrins and protoporphyrins</td>
</tr>
<tr>
<td>Devices</td>
<td></td>
<td>sodium dimandelic acid ether (SAMMA)</td>
</tr>
<tr>
<td>Devices</td>
<td></td>
<td>zinc gluconate gel (ZnG-Gel)</td>
</tr>
<tr>
<td>Devices</td>
<td></td>
<td>Doxovir-M® (cobalt-containing redox complex)</td>
</tr>
</tbody>
</table>

**Maintenance of normal vaginal defenses (including microflora)**

**Buffered acidic gels**
- Acidiform®
- BufferGel®

**Lactobacillus preparations**
- Lactobacillus suppositories
- CD4-expressing Lactobacillus (Viroshield®)

**Monoclonal antibodies**
- Plantibodies®

**Antibiotic peptides**
- defensins
- magainins
- protegrins
- Novispirin® G-10
- VenaGel®

**Other**
- hydrogen peroxide/peroxidases
- topical estrogens

**Viral disruption**
- povidone iodine
- oxidative chlorine
- haloperoxidases
- chlorhexidine

**Surfactants**
- C-31G (Savvy®)
- nonoxynol-9 (various doses and formulations)
- octoxynol-9 (Geda Plus®)
- benzalkonium chloride (BZK)
- octyl glycerol/milk lipids
- polybiquanides
- sodium dodecyl sulphate (SDS)
- Z-14 (acyl-carnitine analogue / hemocholinium and related lipids)

**Attachment or fusion inhibitors**
- carrageenan (PC-515/ Carraguard™)
- cellulose sulfate (CS/Ushercell®)
- naphthalene sulfonate polymer (PRO-2000/5)
- dextrin-2-sulfate (D2S/Emmelle®)
- heparan sulfate/cholic acid
- polyionic gp120 inhibitors
- polystyrene sulfonate (PSS)

**Inhibition of reverse transcription**
- loviride
- nevirapine
- tenofovir (PMPA)
- UC-781
- WHI-07 (an AZT derivative)
Several natural and synthetic CCR5 ligands with potent anti-HIV activity are already available, including:

- Small-molecule selective CCR5 and CXCR4 antagonists. This is the only approach that is targeting CXCR4 as well as CCR5, the other co-receptor thought to be important for in vivo infection. Complete protection might ultimately require a combination of agents that target CCR5, CXCR4, and possibly certain other co-receptors.

- Cyclodextrins. These are chains of sugar polymers that remove cholesterol from the cell membrane and inhibit both cell-free and cell-associated HIV infectivity in vitro. Removing cholesterol results in the reduction of chemokine receptors on the cell surface.

**Envelope interactions**

A recombinant chimeric protein has recently been developed that contains soluble CD4 (sCD4) attached via a flexible polypeptide to a monoclonal antibody against the 17b area of the gp120 protein. This is a highly conserved portion of the HIV gp120 protein that is involved in co-receptor binding, but which usually remains masked. The sCD4 in the chimeric protein binds to HIV gp120 and exposes the 17b-binding portion. The 17b antibody then binds to this previously hidden area, and blocks any subsequent interaction of the virus with co-receptors.

An alternative approach involves gp41, the trans-membrane component of the HIV envelope, which allows fusion with the cell membrane.

Blockage of this critical event impedes virus entry into susceptible cells. The multiple sequential steps involved in this fusion process make it an attractive target for the development of novel HIV therapeutics and preventatives. Several compounds or small molecules block fusion by interfering with important parts of the gp41 protein, including T20, T1249, C34, and C34-Q652L.

**Inhibition of viral uptake by antigen-presenting cells**

DC-SIGN is a binding protein that is highly expressed on the surface of human antigen-presenting cells within many types of mucous membranes. Blocking DC-SIGN should prevent the uptake of HIV and its transport to regional lymph nodes, and therefore its subsequent spread throughout the body. In theory, this effect could be achieved either by soluble forms of DC-SIGN or by DC-SIGN inhibitors, such as antibodies or small synthetic molecules. This is an additional area of active research.

**Topical estrogens**

Recent data have demonstrated that female macaques without ovaries were protected against infection after an intra-vaginal exposure to the simian immunodeficiency virus (SIV), if they had been pre-treated with estrogen. Estrogen did not protect against intravenous exposure to SIV, indicating that protection was due to a barrier effect. Thus, topical estrogens may be considered for future development as adjuncts to a microbicide product.
Other viral pathogens

Herpes simplex virus (HSV)

**Life cycle**

As opposed to HIV, which primarily infects cells of the immune system, the enveloped herpes simplex virus directly infects epithelial cells, as well as neurons and other cell types. Thus, a number of distinct attachment and entry pathways are likely to be involved in HSV entry into host cells. This attachment, while not yet fully understood, involves interactions between proteins in the viral envelope (glycoproteins gB, gD, gH, and gL) and a variety of cell surface molecules, including glycosaminoglycans, mannose-6-phosphate receptors, and C3b complement receptors. After this initial association and binding, direct fusion occurs between the viral envelope and the cell membrane.

Double-stranded DNA from the virus is then delivered to the cell nucleus, where the viral genes are expressed and assume control over the host’s protein manufacturing capability. Although the HSV genome is located in the nucleus of the host cell, it is not integrated into the host cell genome (unlike retroviruses). More than thirty viral proteins are imported from the cytoplasm into the cell nucleus to help accomplish viral gene transcription, viral genome replication, and the assembly of new virus particles.

**Mechanisms of transmission**

A break or tear in a mucous membrane or area of skin permits HSV to enter and replicate in the epithelial cells. This results in a localized primary infection, often accompanied by vesicles (blisters) and inflammation. During this primary stage of infection, some HSV particles enter peripheral sensory nerves and are transported to the central nervous system, thereby escaping immune surveillance. This latent infection of nerve cells is characteristic of HSV infection. When periodic reactivation of the virus occurs, HSV can migrate from the central nervous system back to the surface of the body, where the virus replicates and causes cell and tissue damage. Primary HSV infection may also be followed by spread via the bloodstream, resulting in a systemic infection.

Studies have demonstrated that HSV can be shed from both mucosal and skin surfaces. In heterosexual men, the penile skin has been shown to be the most common site for HSV shedding. In women, the vulvar and perianal areas represent the most common sites for HSV shedding. Genital HSV infection is then acquired by contact with infected secretions during sexual intercourse. Viral shedding may occur from various sites within the genital tract and rectum during both sub-clinical and symptomatic episodes of infection, although an increased risk of HSV acquisition has been demonstrated if intercourse occurs when an individual has clinical lesions present.

Asymptomatic shedding of HSV in cervical mucus continues after the primary stages of infection. This continued shedding, along with a failure to diagnose infection, are the main factors associated with the spread of HSV. While genital herpes remains a chronic infection, the frequency of symptomatic reactivation tends to decrease over time. Recent findings also suggest that an individual’s degree of infectivity declines over time as well, as both clinical and sub-clinical viral shedding gradually decrease.
Recent epidemiological studies have shown that infection with HSV-2 is highly prevalent in sub-Saharan Africa, and that HSV-2 seropositivity is a marker of high-risk sexual behaviour. HSV-2 infection is increasingly being recognized as one of the commonest causes of genital ulceration in many African countries. HSV-2 infection may act as a major cofactor for HIV transmission, a fact that has long been underestimated. Genital ulcers (caused by HSV-2) are by themselves cofactors of HIV transmission, either by increasing infectiousness of dually-infected individuals, or by increasing susceptibility of the seronegative partner. Second, HSV-2 infection may transactivate in vivo the genital replication of HIV, thereby increasing the infectiousness of dually-infected individuals.

**Human papillomavirus (HPV)**

**Life cycle**

Papillomaviruses are non-enveloped viruses that are highly species-specific and demonstrate specific affinity for either mucous membranes or skin. There are about twenty-five distinct types of HPV that can be transmitted sexually. HPV types that infect mucous membranes have been associated with cervical neoplasia and malignancy, while HPV types that infect skin usually cause benign lesions and genital warts.

HPV infection requires that the virus first reach a basal epithelial cell, perhaps by entering through a small wound. The initial steps of HPV replication, including virus uptake, uncoating, and nuclear entry of the viral DNA, all occur in the basal and supra-basal cells. While the specific steps of these processes are not fully known, several potential receptors have been identified that can bind the virus to the cell surface, including the alpha-6/beta-4 integrin complex, heparin, and cell surface glycosaminoglycans. These substances may permit a loose initial attachment, followed by specific receptor binding in a similar fashion to retroviruses.

**Mechanisms of transmission**

While the mechanisms of HPV transmission have not been clearly delineated, studies have demonstrated that genital HPV infection is associated primarily with sexual transmission, although non-sexual transmission also has been reported. HPV has been shown to be present in sperm cells from both HPV-infected and a few apparently healthy individuals. Potentially cancer-causing HPV DNA also has been obtained from exfoliated penile cells.

Risk factors associated with genital HPV infection in young women include both their own and their male partner’s sexual behaviors. Age, number of sexual partners, and previous chlamydia infection have been shown to be the main risk factors for cancer-causing HPV infection. Men tend to clear acute HPV infections more rapidly than women, which may explain the low prevalence of cancer-causing HPV DNA among men. Recent findings have indicated that hormonal factors (such as oral contraceptives) may also increase the risk of cervical neoplasia, by enhancing the persistence of HPV infection.

Additional research is needed to characterize how HPV infection is sexually transmitted. Studies also are needed to better define the HPV life cycle, its pathogenic mechanisms, and the immune responses that are produced by HPV infection. There is no clear evidence that condom use alone reduces the risk of HPV infection, but study results do suggest that condom use might cause some reduction in the risk of developing HPV-associated disease, including genital warts in men and cervical neoplasia in women.

**Non-viral infections**

Non-viral sexually transmitted infections are the cause of significant morbidity, and can also be associated with significant or even fatal sequelae. If not identified and treated, chlamydia and gonorrhea can lead to pelvic inflammatory disease and infertility. Untreated syphilis can lead to insanity, major organ damage, and death. Trichomoniasis is associated with early pregnancy loss, and bacterial vaginosis is a risk factor for premature labor and other neonatal complications.
Gonorrhea
Gonorrhea, caused by the bacteria Neisseria gonorrhoeae, is spread through sexual contact, with transmission occurring readily upon exposure to infectious fluids. The process of infection requires several distinct steps, including attachment, death of adjacent cells, internalization of the bacteria, intracellular replication, movement through adjacent cells, and exit from infected cells. Based on laboratory and human challenge studies, several bacterial surface components are thought to be involved in this process, including the Por protein, the Opa protein, iron-binding proteins, and bacterial lipopolysaccharide, all of which have sophisticated molecular mechanisms to avoid, ignore, or subvert specific host immune responses.

Complications of gonorrhea in women occur when the bacteria ascend into the upper reproductive tract and cause pelvic inflammatory disease, which can result in subsequent ectopic pregnancy and infertility, or in scarring of the peritoneal cavity, causing chronic pelvic pain. When born to an infected mother, a newborn infant can suffer from blindness, joint infections, or septicemia. In men, the gonococcus rarely moves into the upper genital tract, but painful inflammation of the epididymis or testicles can result when this occurs.

Chlamydia
While Neisseria gonorrhoeae bacteria cause infection by attaching to the microvilli of cells in the genital tract, Chlamydia trachomatis bacteria are apparently able to attach to the epithelial surface without specific ligand binding. This indicates that the mechanism of attachment to, and invasion of, cells by these two intra-cellular pathogens are quite different. In addition, once inside an epithelial cell, chlamydia bacteria are apparently able to actively modify their surroundings so as to persist for long periods of time. They may even be able to interrupt normal signals from the immune system that call for an infected cell to self-destruct (a process known as “apoptosis”).

According to recent data from the WHO, chlamydia may now be the most common sexually transmitted bacterial infection worldwide. Infections can often be asymptomatic, although even sub-clinical infections can result in scarring of the fallopian tubes and ectopic pregnancy or infertility. As a result, chlamydial infections are responsible for a significant and increasing proportion of the burden of pelvic inflammatory disease, chronic pelvic pain, and infertility, particularly in the developed world.

Syphilis
Untreated syphilis is a chronic disease with a waxing and waning course, the protean manifestations of which have been described by clinicians for centuries. It occurs worldwide, although incidence varies significantly with geographic location. Transmission is mainly by sexual contact. The disease has been arbitrarily divided into several stages. The primary stage is defined by a chancre at the site of inoculation, usually on the genitalia. The secondary stage is defined by a rash and other systemic manifestations. A variable period without symptoms usually follows, prior to the tertiary stage of the disease. This stage is the most destructive, and is marked by cardiovascular and neurological damage. Congenital infection is possible, and may result in both early and late symptoms.

Syphilis is caused by the spirochete Treponema pallidum, a corkscrew-shaped bacterium that resists culture in the clinical laboratory. Because this bacterium is one of the few prominent infectious agents that has not been cultured continuously in vitro, relatively little is known about its molecular virulence mechanisms. However, the complete genome sequence of T. pallidum has now been completed, and early analysis has identified a number of potential areas for further investigation. In addition, recent studies have shown that changes in the composition of the outer envelope of the bacterium can render it resistant to internalization by the immune cells usually responsible for bacterial clearance. This modification of the bacterial
envelope may explain the organism’s ability to evade host immune responses and to persist for many years after the spontaneous resolution of early lesions.

Another STI that is not discussed in detail in this document, but is important to consider because it produces ulcers, is *Haeophilus ducreyi*.

**Trichomonas**

Trichomoniasis is the single most common sexually transmitted infection, and can be associated with a variety of perinatal complications, male and female genitourinary tract symptoms, and an increased incidence of HIV transmission. *Trichomonas vaginalis*, a parasitic protozoan, is the etiologic agent.

The pathogenesis of *T. vaginalis* is very complex, involving adhesion, hemolysis, and the production of a number of soluble virulence factors. In addition, the parasite has been observed to internalize resident vaginal lactobacilli, as well as epithelial cells, leukocytes, and erythrocytes. This material is then digested by the parasite, possibly providing an efficient means for obtaining nutrients. Because the parasite lacks mitochondria, however, as well as adequate peroxide-reducing and radical-scavenging mechanisms, it is very sensitive to the presence of elevated oxygen levels. Since highly virulent strains of *T. vaginalis* have been noted to internalize the resident lactobacilli more rapidly than less virulent strains, the rapid ingestion of these peroxide-producing bacteria may also represent a significant factor in allowing persistent infection.

**Bacterial vaginosis**

Infectious vaginosis is the most common cause of abnormal vaginal discharge. The syndrome is associated with risk factors for STIs, such as multiple sexual partners and recent intercourse with a new partner, but no single sexually transmitted pathogen has been clearly implicated as the cause. One putative organism, *Gardnerella vaginalis*, has been isolated in low concentrations from the vagina of up to half of healthy women. Formerly considered a benign condition, bacterial vaginosis has now been implicated as a risk factor for inflammation of the fallopian tubes, premature labor, and related neonatal and perinatal complications.

Alteration of cervicovaginal microbial flora can lead to vaginosis, which is associated with an increased risk of HIV-1 transmission. Bacterial vaginosis is a common clinical condition, characterized by decreased hydrogen peroxide-producing lactobacilli and increased concentrations of anaerobic gram-negative rods, *Gardnerella* species, and genital mycoplasmas. It is accompanied by an increase in vaginal pH, a condition favorable to the vaginal establishment of many anaerobic organisms. Bacterial vaginosis has been shown to be associated with HIV infection. Subsequent to the determination that hydrogen-peroxide-producing lactobacilli deterred the growth of HIV-1, clinical data suggest that the presence of vaginal lactobacilli may protect against heterosexual transmission of HIV. Indeed, physiologic *Lactobacillus* flora is likely to be protective against the acquisition of HIV via sexual intercourse. In vitro studies have shown that lactobacilli can be cidal to HIV-1 via the peroxidase-halide system, and other substances may be produced by lactobacilli that have antimicrobial properties. For example, lactic acid is produced by all *Lactobacillus* species, regardless of hydrogen peroxide status, and HIV-1 is inactivated at acid pH ranges. Furthermore, microorganisms associated with bacterial vaginosis may increase a woman’s susceptibility to HIV-1 infection. For example, cell-associated or secreted products of microorganisms may affect the differentiation or proliferation state of HIV target cells in the genital tract, leading to increased susceptibility to HIV infection. Probiotics enriched in lactobacilli have been proposed as an alternative tool to antibiotics for the treatment of bacterial vaginosis.
Areas of overlap with anti-HIV mechanisms
The potential broad-spectrum protection provided by lubrication, epithelial coating, and acid buffering suggests that all three components should probably be included in any potential microbicide. Surfactant-based compounds have obvious broad-spectrum activity, but the relatively low therapeutic index of those tested to date may restrict their practical use. Some non-specific inhibitors of HIV attachment and fusion (such as the poly-anionic compounds) also appear to have effects on attachment and subsequent infection by other STI pathogens. However, agents designed to interfere specifically with the process of HIV attachment, fusion, and entry are unlikely to offer any appreciable target overlaps with other STIs.

For the bacterial infections, in particular, general and specific molecular strategies are possible, based on key differences between the various pathogens and host cells. The targets for these microbicides are likely to be bacterial surface proteins or the unique bacterial lipopolysaccharide surface molecule. Several putative products, including protegrins, acid buffer gels, Pro 2000/5, and certain lactobacilli, have shown potent bactericidal activity in laboratory-based assays, but whether these will prove to be safe and effective in human trials is not yet known.
Overview

In vitro product characteristics
The potential role of microbicides in preventing the mucosal transmission of HIV has been clearly established. However, rigorous pre-clinical evaluation of candidate microbicides is essential to the selection of the best compounds for continued development.

The International Working Group on Microbicides has already defined the biological and chemical requirements for an ideal vaginal microbicide (discussed more fully in chapter 4). This list has been more recently expanded to include potential requirements for a rectal microbicide, during the NIAID/OAR-sponsored Rectal Microbicide Workshop held in June 2001.

Additional anatomical and physiological considerations
The mechanisms of HIV transmission across genital and rectal mucous membranes have yet to be fully described. Even so, some basic anatomical and physiological considerations should be taken into account when selecting target compounds, to explain why one is unable merely to extrapolate in vitro efficacy to in vivo protection.

In the case of HIV transmission, both cell-free and cell-associated viruses are present in infectious semen, so any microbicide must be effective against both. Furthermore, since epithelial integrity provides a significant barrier to HIV transmission, microbicidal agents should not disrupt or damage epithelial surfaces. Candidate compounds with inflammatory potential should also be avoided, since they may increase the number of potential immune target cells in the rectum or genital tract. In addition, compounds targeted against susceptible sub-categories of cells (e.g., fusion inhibitors, chemokine antagonists, or cholesterol-depleting agents) must be able to reach their specific targets (e.g., antigen-presenting cells, macrophages, and T cells) at least as well as the infectious virus. Finally, any candidate compound must be effective under physiologically relevant conditions, including physiologic pH, and in the presence of semen, cervical mucus, and blood. As described below, many of these issues can be addressed by the rational design of an effective in vitro testing program specifically aimed at lead selection.

Opportunity analysis, by category
As the available mechanisms of action for candidate microbicides have become clearer, assessment of the strengths and weaknesses of each category can help to assign a relative value to specific lead opportunities. Availability and cost must often be balanced against effectiveness, while broad-spectrum agents can often be burdened by poor therapeutic ratios. As a result, an optimal microbicide may need to consist of a combination of agents, and may require several product generations before achieving an optimal balance of product characteristics.

Barrier agents
Many vaginal and anal lubricants are already available over the counter. They are inexpensive and do not appear to affect resident microflora. They help reduce the risk of micro-trauma during intercourse, and provide a physical barrier by coating epithelial surfaces. However, they are unlikely to provide complete protection, since they rely on existing epithelial integrity, lack of micro-trauma or ulceration, and consistent distribution over all susceptible sites. Furthermore, since such compounds have no known direct effect on pathogen virulence, they cannot
eliminate the potential for infection. The development and use of thermo-reversible bio-adhesive gels may provide an opportunity for increased protection where condom use cannot be negotiated, although the issue of compound accumulation over time will need further assessment.

**Maintenance of normal vaginal defenses**
Maintaining an acidic vaginal pH, as well as supplementing normal microflora (particularly lactobacilli species) may prevent infection or colonization by a number of STI pathogens. No role for either pH or resident microflora has yet to be established in rectal transmission.

Strategies designed to maintain vaginal pH rely primarily upon the use of acid-buffering gels. These are inexpensive to manufacture, and are unlikely to have any effect on epithelial integrity or underlying inflammation. Additional approaches include active vaginal colonization with selected species of lactobacilli. Such agents will need to withstand the alkalinizing effects of semen; must be efficiently distributed over all potential target tissues; and must retain a sufficiently low pH to inactivate pathogens rapidly prior to potential infection.

**Virucidal or bactericidal agents**
Compounds designed specifically to disrupt viral and/or bacterial integrity would allow the rapid inactivation of STI pathogens. However, many of these compounds show little selectivity, being toxic also for host cells and resident microflora, and may have adverse effects on epithelial integrity and inflammation as well. Thus, rather than providing protection, such agents could actually increase transmission rates if the negative effects of irritation and inflammation outweighed the benefits of virucidal or bactericidal activity. This would be of special concern with inconsistent use, where irritation and inflammation might persist between coital events.

These concerns have been highlighted by the reported adverse events associated with nonoxynol-9 (N-9) in phase 3 trials (Col-1492), suggesting that the formulation of virucidal microbicides may not be as easy as previously hoped.

Recent studies have indicated that there may be significant differences between the phospholipid profile and fluidity of the HIV envelope membrane and the plasma membrane of host cells, providing an opportunity to design virucidal compounds with much-improved selectivity. Such compounds might include small molecules and peptides such as defensins, cathelicidins, and porphyrins, which demonstrate anti-viral and/or anti-bacterial activity. These agents are less likely to demonstrate significant cytotoxicity than surfactants or detergents, but have a more limited range of targets and are generally more costly.

**Fusion, binding, or attachment inhibitors**
An alternative strategy to block HIV infection has been the formulation of sulfated polysaccharides and other polyanionic compounds designed to interfere non-specifically with virus-cell attachment. These compounds include PRO-2000/5, dextrin-2-sulfate, cellulose sulfate, cellulose acetate phthalate (CAP), and the carrageenans. Many of these compounds also have broad-spectrum effects on the attachment of a number of other STI pathogens.

In addition to these non-specific inhibitors, HIV-specific proteins or peptides that target the attachment and fusion process may provide novel microbicide candidates. While these compounds may provide protection against HIV-1 transmission, they may not inactivate the virus itself, allowing any virus that escapes a localized concentration of such agents to retain virulence. In contrast, agents that bind directly to viral particles and render the virus non-infectious would be effective even if the virus subsequently
diffused or was transported away from a localized concentration of microbicide. Agents in this category include envelope-binding antibodies or proteins such as cyanovirin-N, which binds to gp120 and inhibits HIV-1 attachment and fusion.

**Replication inhibitors**
Inhibitors of HIV-1 reverse transcription have already been shown to be effective in post-exposure prophylaxis, providing a rationale for their inclusion in microbicide formulations. These small molecule inhibitors (including PM PA, UC 781, and WHI-05) may efficiently block HIV infection through localized diffusion to potential target cells. However, the transport of virus by migrating antigen-presenting cells may allow the virus to escape inhibitory concentrations of reverse transcriptase inhibitors, while rapid diffusion of agents into the bloodstream may lead to dilution and potential toxicity. The development of antiviral resistance should also be considered with regard to these agents. Recent evidence indicates that HIV continues to replicate in vaginal secretions, which would be exposed to a reverse transcriptase-containing microbicidal, although the effect of localized topical concentrations on viral evolution remains unclear.

**Combination microbicides**
Combinations expressing synergistic or additive effects may be particularly useful for a variety of reasons, including:

- maximizing activity, when direct synergy against a specific STI can be demonstrated;
- decreasing the potential for resistance;
- increasing the spectrum of STI activity; and
- reducing the required concentration of expensive or potentially toxic agents.

Potential synergy between compounds may be predicted based on the mechanisms of action of the individual agents. For example, agents that act at different points in the infection process (e.g., an HIV attachment inhibitor combined with an inhibitor of reverse transcriptase) are more likely to demonstrate synergy than those active against the same target (e.g., two attachment inhibitors). This can often be tested using existing in vitro assays.

Some estimate of the potential for resistance can also be predicted based on the mechanism of action of specific agents. For example, surfactants targeted at disrupting viral envelopes are less likely to produce resistance than agents that target reverse transcription. Where resistance to single agents can be induced in vitro, the effect of combinations on resistance can also be tested experimentally.

Full protection against all potential STIs is likely to be achieved only by the combination of several microbicidal agents. Indeed, full protection against HIV alone may also require a combination microbicide. The design of broad-spectrum combinations can be rationalized by understanding more fully the biological effects of individual agents, although such combinations will also need to be fully tested to rule out any potential for unanticipated antagonism between agents.

While there are many potential advantages to combination microbicides, increasing the number of agents in a product will also have to be balanced against the potential for unwanted side effects or toxicities; the added complexity of obtaining registration; and increased production costs. For example, combining a microbicidal agent targeted against HIV-1 with an acid-buffering gel would be likely to increase its spectrum of STI activity with little consequence, in terms of cost or potential toxicity. In contrast, the combination of two or more anti-viral proteins or peptides would have to be justified against increased production costs, while combinations that include surfactant products may increase the risk of adverse events or toxicity.

**Lead identification and selection**
Effective lead generation requires pre-clinical algorithms, either for internal use within an academic or commercial setting, or external use
by potential sponsors of microbicide development. The advantage of using such algorithms is that they may facilitate the acceleration of the best potential candidates into clinical trials and the rapid rejection of poor quality or potentially harmful agents. However, overly prescriptive algorithms may stifle inventiveness and lead to the rejection of potentially useful agents. Therefore, individual criteria must always be taken in context.

An example of a basic algorithm for the testing of an HIV candidate microbicide is given in figure 4. An incremental series of assays are performed, and at each stage agents are selected, rejected, or reformulated based on their performance. Screening of compounds for potential synergy is built into the algorithm. While an initial high-throughput screen should be performed prior to testing in animals, the latter part of the screening process may be performed in parallel with animal testing.

Figure 4. An algorithm for lead identification and selection among HIV microbicides
Using the illustrated algorithm, lead identification and pre-clinical development should progress through a series of phases.

**Exploratory phase**

Initial lead identification can be accomplished in several ways: extending the use of an existing product according to some scientific rationale; designing a new product to interrupt a specific pathway in the infection cycle (for example, fusion inhibitors); or large-scale screening of compound libraries for a desired activity. This phase includes:

1. defining a compound’s mechanism of action;
2. defining its activity under physiological conditions (i.e., in the presence of semen, blood, and cervical mucous, and across a range of pH values); and
3. screening for basic cellular toxicity.

The high-throughput nature of these assays means that individual agents selected by the screening process can also be screened for potential synergy.

**Feasibility analysis**

Initial screening leads to lead compound identification. At this stage, an analysis of potential manufacturing and regulatory issues needs to be undertaken for each identified lead. Selection for further testing is made based on a compound’s relative scientific, economic, and regulatory merits.

**Biocompatibility analysis**

The screening assays described above will lead to the rapid rejection of many potential agents. Only a limited number will progress to the biocompatibility phase. Here, more complex assays are performed to determine in vitro efficacy using primary cell cultures (PBMCs, macrophages, dendritic cells, and DC-T cell co-cultures) as well as tissue explants (cervical, vaginal, or rectal). The use of tissue explants may be important, as the cells within the explants contain the immune population first exposed to HIV-1 during sexual transmission. Furthermore, while certain agents may provide effective protection of cells in suspension, the use of explant tissues tests whether compounds can protect susceptible cells within natural tissue architectures.

**Other STIs**

Compounds predicted to have broad-spectrum activity should then be tested to find activity against a wider range of STI pathogens. This phase of in vitro testing would also consider the compound’s overall bio-compatibility, including analysis of its effects on epithelial integrity, immune function, co-receptor expression, cytokine and chemokine expression (as markers for inflammation), and associated micro-flora. Thus a complete activity and bio-compatibility profile can be constructed for any given agent, allowing objective strength and weakness analysis for any product candidate. Where synergy has been identified, or where there is scientific rationale for combining agents, testing can also be carried out on combination products.

**Formulation and sample production**

In the final phase of lead selection, fully formulated compounds must be evaluated. Assays need to be adapted to the technical issues presented by the analysis of creams, gels, foams, and sponges (discussed in chapter 15). The same selection criteria apply to formulated products, feeding back into the screening algorithm. The screening algorithm should allow side-by-side analysis of a number of formulations; those that show reduced efficacy or bio-compatibility can be either rejected or reformulated. Any final product for animal or clinical testing should thus be the result of a highly iterative process between formulation studies and continued in vitro testing.
Before a promising microbicide can be evaluated in human trials, it must first be tested for safety and potential efficacy against a wide variety of in vitro and animal models. The pre-clinical pathway can be optimized to select those compounds with the characteristics thought most likely to be safe and effective against the transmission of HIV. Since no microbicide product has demonstrated effectiveness in the clinic, however, the most appropriate set of pre-clinical tests cannot yet be fully evaluated.

Because the current portfolio of pre-clinical models has been developed across a wide variety of institutions, for a number of different purposes, significant variations remain in the way many testing procedures are conducted. This situation has resulted in data that cannot be compared directly across laboratories or development organizations; and, combined with the difficulty of predicting human efficacy in the absence of a proven product, it has greatly increased the difficulty of objectively identifying the most promising candidate molecules.

As a result, significant attention has recently focused on improving the comparability of animal and in vitro models across the microbicides field. Together with the development of criteria to assess optimal product characteristics, and the continued refinement of pre-clinical pathways for safety testing, further achievements in developing standard efficacy tests that are even more relevant to the process of human infection should allow the field to progress quite rapidly.

Finally, the microbicides field is particularly fortunate in being able to monitor this progress in real time, via a continually updated pipeline database maintained by the Alliance for Microbicide Development. By providing potential donors and investors with an up-to-date overview of the field, this integrated pipeline database has the potential to help generate additional resources and direct them to those candidate products that appear most likely to offer a substantial benefit.

Key conclusions and recommendations from this section include:

- **Product development for microbicides is likely to proceed through a series of generations, as lead compounds are optimized for effectiveness and acceptability, and as combination products and formulations are optimized.**

- **Tests for product efficacy should continue to be standardized for comparability, and improved in their relevance for human infection.**

- **Tests for product safety can be expensive and lengthy, and dedicated resources for toxicology and pharmaco-dynamic testing may be required.**

- **The microbicides pipeline is currently being monitored on a voluntary basis, but additional due diligence capabilities may help this effort to direct funding toward the most promising candidates for further development.**

- **Animal studies need to be further rationalized, and only those consistent with the compound’s mechanism of action should be priorities.**
Overview
As previously discussed, several dozen candidate microbicides have currently been proposed or identified. These fall into several different chemical categories, with diverse mechanisms of action. Some of these substances, after being incorporated into formulations suitable for vaginal application, are currently being evaluated in clinical trials for safety, acceptability, retention, effect on resident lactobacilli, and effectiveness against the transmission of HIV, gonorrhea, and chlamydia. Several other products are likely to enter clinical testing in the near future.

Most of the microbicides currently in clinical studies are substances that were already under development, or in use, to serve other needs—that is to say, they were not created specifically as microbicides, but rather, on investigation, were found to have useful microbicidal properties. For example, nonoxynol-9, the subject of several unsuccessful phase 3 trials, is a surfactant that has been on the market for several decades in various vaginal formulations for use as a spermicidal contraceptive. Carrageenan, currently undergoing phase 2 evaluation in South Africa, is a sulfated polysaccharide derived from seaweed that has long been used as an ingredient in foodstuffs and cosmetics. PRO-2000/5 (a polymer of naphthalene sulfonate) and dextrin-2-sulfate, both of which have reached phase 2 trials, were initially developed as potential systemic therapeutic agents for use in people infected with HIV. The active ingredient of BufferGel®, which is also undergoing clinical assessment, is a cross-linked polyacrylic acid widely used as a formulation agent in a variety of pharmaceuticals.

These products have progressed into clinical trials following pre-clinical demonstrations that they were active in vitro against HIV (and potentially against one or more other STI pathogens), and that they were likely to be safe for human use. The extent of efforts to optimize them further for a specific role as microbicides has varied. Some of the polymers have been selected from several alternatives in terms of chain length, number and location of charged groups, etc. But there have been few systematic attempts to engage in the sort of lead optimization process that is the usual practice in developing new drugs.

In conventional drug development, once a lead compound has been identified, optimization involves the application of procedures to select, from a broad list of molecular variations, a narrower group of molecules that interact more effectively or specifically with the drug target. One key objective is to enhance the therapeutic ratio—that is, the efficacy of the drug compared to any adverse side effects. Further chemical modification may then be appropriate in order to achieve even better outcomes. Finally, formulating the drug so that it is best suited to achieve its specific objective constitutes an additional stage in the optimization process.

Given the current state of microbicides research and funding, the absence of this optimization procedure has been both understandable and appropriate. First, optimization takes time, and microbicides—even products that are substantially less than 100 percent effective—are needed urgently to ameliorate an immense and growing public health problem. Second, the small biotech companies that own the intellectual property rights to many of these compounds survive to a great extent on venture capital and other forms of investment funding, and need to show a return on that investment in the short- to middle-term. Such returns can be best guaranteed
by a successful phase 3 trial using the company’s own particular microbicide.

A strong case can be made, however, that the next generation of clinical trials should evaluate products that have emerged from a comprehensive optimization process. Large-scale trials are costly and tie up human and other resources—including suitable trial populations—for considerable lengths of time, so the aim should be to test only products (that is, formulated active agents) that appear to be optimally constituted.

The International Working Group criteria
The International Working Group on Microbicides, whose membership includes representatives from twenty governmental and non-governmental organizations involved in microbicides development, recently proposed a set of guidelines for advancing candidate microbicides into human studies, with an emphasis on anti-HIV products. These guidelines offer assistance not only in the initial selection of lead compounds, but also for optimizing the leads selected. While there remains some debate about a few aspects of these guidelines, the overall approach of The International Working Group is as follows.

Obligatory criteria
If an agent fails to meet one of the following criteria, particularly during vaginal or penile testing, it most likely should not progress further.

- Highly active against free and cell-associated HIV in in vitro test systems, using a range of HIV strains and subtypes, as well as macrophages, lymphocytes, and dendritic cells, in the absence and presence of human semen. The mechanism of action may result in reversible or irreversible inactivation, e.g., via destruction of the virus, prevention of attachment or fusion, inhibition of replication, etc.
- Low cytotoxicity in vitro, e.g., against the cell types used in anti-HIV tests, and/or against cervical or vaginal mucosal cells or tissues.
- Non-mutagenic in standard in vitro tests.
- Capable of Good Manufacturing Practice (GMP) formulation suitable for vaginal and/or rectal use, without adversely affecting anti-viral activity.
- Stable in formulation for at least six months at the temperatures likely to be encountered in countries where it is to undergo clinical trials, and not dependent on the cold chain.
- Compatible in formulation with natural latex and other materials used in physical barrier devices.
- Not locally toxic in standard animal tests (e.g., rabbit vagina, penis, or eye; guinea pig skin). Evaluated in appropriate tests for toxicity when applied rectally.
- Not systemically toxic in animal tests, and without adverse effects on embryo or fetal development in rats.
- Evaluated in standard tests for spermicidal and contraceptive activity.

Additional preferable characteristics

- Little or no activity against vaginal lactobacilli in vitro.
- Without significant systemic absorption in animal studies.
- Acceptable color, odor, taste, and physical consistency in studies of potential users.
- Active against other sexually transmitted pathogens (e.g., Neisseria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum, Trichomonas vaginalis, herpes simplex virus-1, herpes simplex virus-2, and human papillomavirus), in vitro and/or in animal models.
Able to be industrially produced at an economic cost.

Able to be formulated as a gel, cream, foam, film, impregnated sponge, or suppository.

Methods for optimizing selected lead compounds
The above criteria can be used for both lead selection and lead optimization. During lead optimization the aim should be to enhance the performance of products over time against these criteria by chemically modifying selected lead compounds, by modifying the formulation to improve the expressed characteristics of an active ingredient, and/or by combining two or more microbicides in the same formulation.

Chemical modification
Chemical variations may include longer or shorter polymer chain lengths, amino acid substitutions, different side-chains, or overall charge and charge distribution, derivation of active fragments, etc. At present this may have to be carried out empirically on a trial-and-error basis, testing variants in appropriate systems and exploiting any observed trends. Eventually, however, it may become possible to design molecules with the desired characteristics.

A number of commercial contract organizations specialize in high-throughput screening and optimization, although not specifically for microbicides. Use of their services, if economically viable, could significantly reduce the time required for optimizing leads.

Modification of formulation
Modifications can include increasing or decreasing the concentration of the active ingredient in the product and altering the amounts and types of formulating ingredients so as to vary the product’s viscosity, pH, bio-adhesivity, etc. The aim would be to maximize the potency of the active ingredient within the formulation, both in terms of its molecular conformation, ionization, hydrogen bonding, etc., and also in terms of its bio-availability, while minimizing any potential adverse effects.

Microbicide combinations
As discussed above, synergy between two or more microbicides combined in the same product could result in greater potency, lower toxicity to the tissues and normal flora of the genital tract, and either greater pathogen specificity or a broader spectrum of activity. Appropriate combinations may result in products that are both anti-infective and contraceptive, or anti-infective only.

Throughout the optimization procedure it should be borne in mind that there is little point in increasing potency at the expense of safety. Also, a decision will need to be made at the outset as to whether a product is being developed for vaginal or for rectal use, or for both. Even if a product is intended for vaginal use only, some of it is bound to come into contact with the rectal epithelium, and some users will inevitably apply it for anal sex. Therefore it will still be important to optimize the end product for rectal safety. In addition, if it is intended to provide protection against rectal infection, it will also need to be optimized for rectal efficacy, and its pre-clinical development should include appropriate tests within appropriate tissue architectures.

Finally, while this discussion deals primarily with the pre-clinical aspects of lead optimization, products will also need to be optimized for user acceptability within a number of different user groups, as discussed in chapter 16. This implies the tuning not only of the product’s physical characteristics and volume but also the delivery mechanism by which it will be applied.
Chapter 5. Efficacy Testing

Background
We recognize that there is some doubt about the relevance of existing in vitro efficacy models vs. conditions encountered in humans, and that a microbicide proven safe and effective in the laboratory may not be safe and effective in human trials. Yet, as discussed above, high-throughput screening, followed by in vitro testing, are the first steps available toward identifying a viable candidate microbicide. Inhibition of HIV replication in a standard in vitro culture system may serve as a very preliminary indication of microbicidal potential—particularly in the case of agents that can block infection or significantly reduce viral replication when added prior to, but not following, virus exposure. A more compelling scientific rationale may be obtained by using mechanism-specific assays, in which early events in the infectious process or the viral replication cycle can be investigated. Examples of such mechanism-specific assays include viral binding, attachment, and fusion assays that target the steps required for HIV entry into the cell. Alternatively, a virucidal assay, in which a test agent is pre-mixed with a viral inoculum for a short duration prior to application of the virus to a cell culture system, may be used to determine the ability of the agent to inactivate virus particles directly. In general, it is desirable to determine microbicidal activity using both cell-free and cell-associated viral inoculums, given the presence of both viral forms in genital fluids and uncertainty regarding the exact nature of the infectious unit that permits the sexual transmission of HIV.

A successful candidate microbicide should be able to interrupt pathogen transmission without damaging host tissues. However, the majority of assay systems for evaluating microbicides use target cells, such as lymphocytes or isolated cell lines, that are not representative of the genital and rectal mucous membranes that will be exposed to an eventual product. Therefore, several new in vitro models are currently being developed, such as the measurement of inflammatory responses in immortalized human cervical and vaginal epithelial cells. In addition, ex vivo systems, or “explants,” which preserve tissue architecture and cellular background, have recently been developed to more closely simulate the relevant in vivo tissues. These systems include culture techniques for biopsies from the cervix, vagina, or rectum, as well as skin blisters (a potential model for vaginal squamous epithelium). While useful for evaluating the safety and efficacy of microbicide candidates that are more advanced in development, these culture systems are technically demanding, and are often not suitable for initial high-throughput screening.

In vitro tests of efficacy
HIV
Compounds demonstrating good efficacy against HIV in preliminary high-throughput screening (discussed in chapter 3) may then be selected for more detailed in vitro analysis. A stepwise series of assays, gradually increasing in technical complexity, usually represents the most time- and cost-effective approach. While some developers may be able to handle the majority of these assays in-house, other groups may have to subcontract work to laboratories with specific expertise.

The direct targets of primary HIV infection in the male and female genital tracts and in the rectum are not yet fully understood. However, the cells most likely to be infected include T lymphocytes, macrophages, and antigen-presenting cells. Thus, pre-clinical testing
requires the analysis of activity against the infection of these cells in culture. Even so, while primary cell cultures may provide important information for the evaluation of microbicides, anatomical, physiological, and immunological issues suggest they do not adequately model the events that occur in human mucous membranes. A comprehensive program of pre-clinical development will therefore also require that efficacy information be generated from several additional model systems, including a variety of human mucosal explant cultures.

Screening programs, based on cell lines, yield a large number of possible microbicides. A rational selection should be made for clinical trials. To this end, the use of cocultures of monocyte-derived dendritic cells (MO-DC) and autologous CD4 T cells has been proposed because these cells are representative of the first in vitro targets during sexual transmission.

**Non-HIV assays**

**Herpes simplex virus (HSV)**

Candidate compounds can be initially screened for anti-HSV activity using human cervical and colonic cell lines. Cultured cell lines are exposed to serial dilutions of a test compound prior to inoculation with HSV-1 or HSV-2. Anti-HSV activity is then assessed. Further pre-clinical evaluations should include analysis of anti-HSV activity using primary epithelial cultures, monocyte-derived macrophages, and cervical and other epithelial explants.

**Human papillomavirus (HPV)**

There are no reliable methods for the in vitro propagation of HPV. However, cell-binding assays have been developed using virus-like particles (VLPs) generated by synthesizing viral proteins in various expression systems. These have the potential to be used in the screening of candidate compounds aimed at blocking viral attachment. Bovine papillomavirus-1 (BPV) has also been used for the initial investigation of microbicidal activity against HPV. More recently, a novel assay has been developed using COS-7 cells and “pseudo-virus” particles, in which capsid proteins surround a marker plasmid. Finally, HPV has been successfully propagated in a variety of explants, suggesting that these models may also be useful as in vitro systems for assessing efficacy against HPV.

**Non-viral pathogens**

Non-viral STI pathogens include *Neisseria gonorrhoea*, *Chlamydia trachomatis*, *Treponema pallidum*, *Trichomonas vaginalis*, and *Gardnerella vaginalis*. The size of these organisms, much larger than viral pathogens, means that they can easily be separated from test compounds by simple centrifugation. With the exception of *C. trachomatis*, these organisms can also be easily grown on either specialized agar plates or in broth culture. Minimum inhibitory concentrations for candidate microbicides can easily be established by incubating a known concentration of the pathogen for a fixed time period with serial dilutions of a test compound. Organisms are then washed to remove the test compound, and serial dilutions are plated onto relevant culture media. After several hours of culture growth, plates are scored for the number of colony-forming units (CFUs) to indicate in vitro efficacy. Using such methods, compounds can be rapidly tested for activity against a range of non-viral STI pathogens.

**Lactobacilli sensitivity testing**

The ideal microbicide should have little effect on commensal organisms of the vagina, such as *Lactobacillus* species. In addition to the above in vitro procedures, all potential test compounds should be tested in parallel to determine inhibitory values against lactobacilli.

**Animal testing**

After demonstrating in vitro efficacy and safety, evaluating a microbicide in vivo is a key step in proving biologic plausibility. Many different animal models exist, each with particular strengths and weaknesses. The specific models used for testing a given compound should be considered carefully, depending on the
compound’s mechanism of action and degree of specificity. For instance, a SIV/macaque vaginal challenge would not be appropriate for evaluating a candidate reverse transcriptase inhibitor, since, despite demonstrated success as an HIV therapeutic, this class of agents lacks efficacy against SIV reverse transcriptase.

**Murine models**

Murine models can be particularly valuable, since large numbers of animals can be used to evaluate the dosages of microbicides and pathogens, as well as to vary the timing of microbicide application. New murine models using immuno-compromised mice are also being developed to more closely model STI transmission and infection in humans. Finally, human vaginal epithelial xenografts in immuno-compromised mice have been successfully infected with HPV and HSV-2, paralleling the characteristics of patient lesions. Such xenografts in mice, reconstituted using human immune cells, may also allow infection by HIV; however, they are technically intensive and require significant expertise, escalating the cost of using such murine models. A murine model in which SCID OK mice have been seeded with human peripheral blood-derived lymphocytes (hu-PBL-SCID) has been recently developed. The more relevant models are much more expensive and limited in number, and to the extent to which donor tissue can be distributed among the mice, sufficiently large numbers of animals could be made available to allow experiments of a size that allows statistical analysis of the data even in the absence of 100 percent infection of the control animals.

**Feline models**

A model for vaginal feline lentivirus (FIV) infection in cats has recently been established using cell-associated viral challenges. The feline model represents an improvement over murine models, in that FIV is a naturally occurring cat pathogen, while no similar pathogen has been identified for mice. Although cats are significantly more expensive than mice, they are much more cost-effective than non-human primates, the primary alternative system in which a natural pathogen similar to HIV has been identified. However, the FIV model may be of limited relevance to human sexual transmission due to significant differences in genital tract anatomy and physiology, including differences in viral co-receptor specificity between cats and humans.

**Primate models**

Non-human primates inoculated intravaginally with SIV or SHIV (a chimeric SIV containing HIV components) are the most frequently used animal models for evaluating HIV microbicide efficacy. These non-human primate models are particularly attractive because the macaque species closely parallels humans with respect to their vaginal anatomy, physiology, pH, and micro-flora. Reproducible infection can be achieved using a cell-free virus challenge, although progestin pre-treatment of the animal is required to enhance susceptibility to most viral stocks. The most widely used current procedure may not accurately reflect the dynamics of human sexual transmission, since it is based on a single, high-dose, intra-vaginal inoculation of virus shortly after a single application of microbicide. Nevertheless, biologic proof-of-concept has been established for a number of microbicide candidates, several of which are poised for phase 2/3 clinical trials.

Meanwhile, the development of animal models that are more directly comparable to the circumstances of human infection (i.e., multiple low-dose exposures to HIV, prolonged microbicide use, and no progestin pretreatment) should be vigorously pursued.

In addition, the feasibility of exploiting a single, high-dose, intra-rectal challenge for evaluating rectal microbicide candidates in the macaque has recently been reported. The inoculating dose required in this model is significantly lower than that used in the intra-vaginal models. The relative ease of rectal, compared to vaginal, infection may be due, at least partly, to the
inadequate physical barrier provided by the single layer of columnar epithelia in rectal tissue. Few microbicide candidates have been evaluated in this model.

A macaque model to assess the efficacy of microbicides targeted against *Chlamydia trachomatis* has also been established. Multiple microbicide candidates have been evaluated in this model for the ability to prevent vaginal infection with a human variant of chlamydia.

In view of the expense and limited availability of female non-human primates, it is often not economically feasible to use these efficacy models in sufficient numbers to routinely achieve statistical significance. Because of the slow breeding rates for macaque populations in captivity, even immediate short-term investment is not likely to be able to significantly expand the number of animals available for testing programs. Nevertheless, the non-human primate models provide valuable information regarding the biologic plausibility for microbicide candidates, albeit under conditions that augment animal susceptibility to infection and discount the potential effects of chronic microbicide application.
Background
A complete program of toxicology testing can cost between $2.5 million and $5 million per product, representing a significant financial strain for a small product sponsor early in the development process (although this sum is much less than what will eventually be required for clinical testing). Because governmental agencies and academic institutions generally support basic and translational research, rather than specific product development efforts such as toxicology and safety testing, public funding for further development of a specific compound is often difficult to obtain. Therefore, donors specifically interested in supporting microbicides development may wish to target a significant proportion of their support to encourage the timely completion of these critical studies.

While in some instances a vaginal microbicide might be amenable to development as an over-the-counter (OTC), non-prescription medication, the sponsors should consider the same safety and pharmaco-dynamic issues for an OTC product as for a prescription medication. In the case of vaginal OTC products that have already been marketed, investigating the safety profile in animals is unnecessary. For already-marketed pharmaceuticals that are being reformulated for vaginal application, however, some bridging non-clinical studies may be needed to address any potential toxicities relevant to a new route of administration. In any event, it is highly desirable that all studies be conducted using active ingredients and formulations produced in compliance with GMP guidelines. All pivotal pre-clinical toxicology studies should also use the desired clinical formulation whenever possible.

One of the major considerations in the safety assessment of a product to be used as a topical microbicide is whether or not the product is absorbed from the vagina. A second major consideration is whether the product is metabolized. For products that are absorbed, blood levels need to be measured. These measurements should include maximum plasma concentrations, time to maximum plasma concentration, the area under the time/concentration curve and the volume of distribution of the product, its presence in other organs, and the rate of clearance. If the product is metabolized, all of the above measurements need to be considered for each metabolite as well. Performing these analyses may require the synthesis of radio-labeled products. In any event, microbicide development is likely to be greatly facilitated if the product is not appreciably absorbed or metabolized.

Non-clinical safety tests
The following inventory of tests is intended for those products being developed through the FDA’s standard pathway, although the process for other regulatory authorities is quite similar.

Inventory
For a new product the FDA and most other regulatory authorities require safety testing across five general categories:

- general toxicology
- irritation and inflammation
- genetic toxicity
- carcinogenicity
- reproductive toxicology

The FDA requires completion of the first two items above, and at least two of the three types of tests used to examine genetic toxicity (see next page) prior to beginning phase 1 clinical trials. In
general, the length of the toxicity studies submitted should be equal to or greater than that of the proposed clinical trials. An initial series of reproductive toxicology studies (“segment I”) should be submitted prior to the initiation of phase 1 trials, but certainly prior to phase 2/3 trials. Although the second series of reproductive toxicology studies (“segment II”) are not usually required before beginning phase 3 trials, it may be prudent to conduct them even prior to beginning phase 1 trials because of the close proximity of the vaginal products to any developing embryo. The final series of reproductive toxicology studies (“segment III”) are expected during phase 2/3 trials. Carcinogenicity studies are due prior to submission of a new drug application (NDA). In addition to these required studies, there are often a large number of “recommended” studies, which depend on the precise compounds and mechanisms of action under investigation.

While not intended to be an in-depth exploration of the wide variety of potential safety tests that exist, the following should serve as examples from each of the above areas, to offer an indication of the complexity and length of time required for these assays. Many guidance documents are available from the FDA’s web site; these explain each test in greater detail.

**General toxicology**

Subacute or subchronic studies of toxicity are performed for thirty to ninety days, during which animals receive the product via both oral and vaginal routes of administration. For absorbed compounds, models will be required for both oral and vaginal absorption and excretion of the compound and any metabolites, following intravenous, oral, and vaginal administration.

**Irritation and inflammation**

Irritation studies include a ten-day vaginal challenge in rabbits, during which the product is generally compared to nonoxynol-9. Hypersensitivity studies can also be done to assess irritation to rabbit skin and eyes. Similar hypersensitivity studies have been carried out with multiple applications to the arms of human volunteers. A delayed hypersensitivity test in guinea pigs is also available.

**Genetic toxicity**

Genetic toxicity studies must be completed prior to phase 1 clinical trials. These tests assess mutagenic and other properties of the product. Products that are mutagenic may be associated with an increased risk of cancer. The following standard test battery is recommended: (1) a test for gene mutation in bacteria; (2) an in vitro test with cytological evaluation of chromosomal damage to mammalian cells, or an in vitro mouse lymphoma tk assay; and (3) an in vivo test for chromosomal damage using rodent hematopoietic cells.

**Carcinogenicity**

Carcinogenicity tests must be completed before the final product can be marketed. These tests take at least two years, and are generally done using rats and mice. The route of administration should be the same as that intended for the marketed product, although systemic routes may be acceptable. Such tests are designed to assess the carcinogenic potential of the product.

**Reproductive toxicity**

Repeat-dose toxicity studies in rodents and non-rodents are used to define maximum and minimum doses of active ingredient and to measure the effect of the product on fertility and reproductive health (segment I), as well as embryo-fetal development (segment II). These tests are usually short in duration, taking place over a few weeks. There may be several routes of administration, but the route by which the eventual product will be administered must certainly be included.

Chronic reproductive toxicology testing (segment III) must be complete before beginning phase 2/3 clinical trials. These tests require six months in a rodent species (rats) and nine months in a non-rodent species (dogs and monkeys) to assess the toxicological effects of long-term exposure to the
product. These doses must be given via the route that the product will be administered, e.g., intra-vaginally, although systemic routes may be acceptable.

**Key issues in safety testing**
The current approach to toxicology testing allows for the process to be individualized for each product under development. However, this approach requires constant interaction between the developer and the appropriate regulatory authority, such as the FDA. Since FDA guidelines may vary according to individual assessments, there can be inconsistency between recommendations among similar products. Also, the current process favors products that are not absorbed from the vaginal tract and that are not subsequently metabolized. For example, the number of toxicology studies for a product with little or no absorption could be in the range of ten to fifteen, while the studies needed for an absorbed compound might be twenty to thirty.

The FDA and many other regulatory bodies have taken a conservative approach towards safety testing for new products intended to be used by healthy women of childbearing age. After a new product class has been approved and shown to be effective and safe among its intended recipients, this position may relax somewhat. As a result, products that enter safety testing early in the development of a new product category are likely to be treated differently than those entering later.

A particular hurdle for many sponsors has been carcinogenicity testing. These tests require daily administration for up to two years in animals, with a substantial additional period required for data analysis. Although not required before beginning phase 3 testing, these data will be required for eventual approval. Thus, unless the tests have been undertaken in parallel with phase 3 trials, there may be significant delays before registration can be granted. On the other hand, if the product proves to be unsuccessful during phase 3 testing, money will have been spent to very little use.
Within the microbicides field, the challenge of monitoring the development of a diverse group of pipeline candidates across many separate development entities has been taken up by the Alliance for Microbicide Development. Established in 1998, this database has been regularly updated, and attempts to monitor the progress of the entire microbicides pipeline. It is available online at www.microbicide.org.

Beyond its use as a general resource, the Alliance also fulfills two critical surveillance purposes. One is a “watchdog” function. A variety of motivations—difficulties along the development pathway, frustration with slow progress against a background of public health urgency, simple naiveté, and/or pressures for a return on investment—might precipitate inappropriate testing or even product introduction where safety and efficacy are unknown. Such actions could potentially jeopardize legitimate microbicides research. Proactive pipeline surveillance is also essential to strategic decision making. Beyond its simple communication function, the current surveillance process could be substantially reinforced with a due diligence process to permit evidence-based determinations about the most appropriate areas for continued donor investment.

At present, there is no large pharmaceutical company engaged in microbicide research and development. While there has been a real increase in donations, out-licensing, and information-sharing, such examples remain painfully few. The profile of the field continues to be dominated by small biotechnology or biopharmaceutical firms, and a growing number of nonprofit research entities. Of these small companies, only a handful are publicly traded. Table 2 lists many of the current institutions directly involved in microbicides research.
Table 2. Current microbicide research and developers

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<tr>
<th>Product Sponsors</th>
<th>Nonprofit Research Entities</th>
<th>Public Sector Entities</th>
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<tr>
<td>Biofilm, Inc.</td>
<td>Cincinnati Children's Hospital</td>
<td>Centers for Disease Control and Prevention (CDC)</td>
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<td>Biosyn, Inc.</td>
<td>Columbia University Department</td>
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<td>- National Cancer Institute (NCI)</td>
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<td>- National Institute of Allergy and Infectious Diseases (NIAID)</td>
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<td>CONRAD Program</td>
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Note: The above listing does not include many nonprofit entities where researchers are performing supporting activities such as screening and animal model development. For-profit entities such as SRI that perform contracted support activities have likewise not been included.
Section III
Clinical Trials

Microbicides have shown remarkable effectiveness in interrupting the transmission of HIV in animal models and in vitro, and several products have already been demonstrated as safe for human use. Even so, the only way to demonstrate effectiveness at preventing HIV transmission among people is to conclude a successful large-scale phase 3 clinical trial. This is an unusually difficult proposition, both because of the low incidence of seroconversion (even among populations with very high rates of HIV prevalence) and because the disease being prevented is both lethal and incurable. The ethical conduct of a clinical trial requires the provision of condoms and safer-sex counseling, as well as interventions to cure existing sexually transmitted infections—requirements that are likely to further reduce already low rates of seroconversion.

Proving the concept of a clinically effective microbicide to any reasonable degree of certainty will require many thousands of woman-years of observation, often in areas of the world currently lacking the infrastructure to conduct large clinical trials. However, there have been several large-scale randomized clinical trials conducted in sub-Saharan Africa, and a number of research groups have considerable experience in this area. Some developers have begun investigating the possibility of proving the concept of a topical microbicide against the transmission of a pathogen other than HIV. But while other sexually transmitted infections are a serious public health concern, the incurable and lethal nature of HIV infection sets it apart. The prevention of HIV transmission should be a critical barometer of success, although a product that prevents the transmission of other STIs would certainly be desirable.

The regulatory pathways developed by the FDA and other regulatory agencies in the developed world to allow the marketing of therapeutic products may be poorly adapted to considerations that are important in developing a preventative product, such as a microbicide. The cost/benefit calculations that apply to a product marketed in an area with relatively little exposure to HIV may be different from those that apply to areas in which the HIV epidemic is spreading much faster. As a result, continued dialogue with the FDA and other developed-world authorities, as recently initiated under the auspices of the WHO, is particularly important. There should also be continued exploration of opportunities to launch a carefully developed product directly in those markets where there is the greatest need.

Finally, the availability of life-saving, highly active anti-retroviral therapy (HAART) in the developed world has raised new ethical concerns about the conduct of appropriate, sustainable, and economically viable trials in the developing world.

Key conclusions and recommendations from this section include the following:

- The principal aim is to conduct a large-scale phase 3 efficacy randomized clinical trial. While many of the issues are common with the development of new drugs for therapy or prevention, a number of features are unique to the prevention of HIV and complicate the design and conduct of such a trial.
- The steps required to accomplish this goal are increasingly well understood, and appear to be eminently achievable,
given sufficient investment of time, effort, and resources.

- The prevention of HIV transmission is the primary objective. Secondary endpoints for non-HIV pathogens should be considered for inclusion in HIV trials wherever possible.

- The regulatory pathway for registration of a microbicide should be carefully considered, and coordinated between developed and developing-world authorities.

- Community involvement and decision making, along with some degree of medical care, are critical components of an ethical trial—particularly in the developing world.
Topical antiseptics and antibiotics have long been used to prevent infections in traumatic injuries, during surgical procedures, and in catheter insertions. Within the last twenty years, topical anti-virals have been found to be effective in the treatment of a variety of conditions, such as herpes lesions and genital warts. Topical vaginal antibiotics and anti-fungals are commonly used for the treatment of candidiasis as well as bacterial vaginosis. Vaginal spermicides have proven the concept of a safe, vaginally administered product with some degree of effectiveness in preventing unwanted pregnancy. However, no topical product currently exists with proven effectiveness in preventing the transmission of HIV or other STIs.

Prevention versus treatment

Proving the concept for a prevention technology, such as a microbicide, can be significantly more complex than for a treatment technology. In the latter case, a treatment is administered to an ill patient, and the patient’s response can then be directly monitored. In the former case, a preventative is given to a healthy patient, who may then stay healthy either because of the preventative or with no relationship to the preventative whatsoever. Only by noting a statistically significant difference in the incidence of a given condition between otherwise comparable groups—one group receiving, and one not receiving, the preventative—can one have confidence that a prophylactic treatment is effective. Such a difference is particularly difficult to demonstrate when the background incidence of the condition to be prevented is low.

This distinction is critical for the process of product development. In drug development for treatment indications, preliminary indicators of effectiveness can be observed early in the clinical trials program. Early drug safety studies in participants with specific illnesses can often contribute effectiveness data either directly (through the resolution of symptoms) or indirectly (through effects on well-characterized surrogate markers of improvement). However, studies of primary prevention modalities must be conducted in healthy populations, and generally require long-term follow-up of large numbers of participants who are at high risk for developing the condition of interest.

Proof of concept for a microbicide will require the demonstration of a statistically significant reduction in the number of new HIV infections in at least one well-conducted, randomized, controlled clinical trial. Such a trial could involve use of the microbicide by women not infected with HIV to prevent acquisition of new HIV infection, or by HIV-infected women to prevent transmission to an uninfected sexual partner, or both.

As detailed in table 6, the number of participants in a clinical trial will almost certainly number in the thousands, since HIV seroincidence is typically under 5 percent per year—even in heavily affected populations (although in certain areas, such as South Africa’s KwaZulu-Natal region, annual incidence rates in some communities may be as high as 12 percent). As a result, these studies are likely to require enrollment at multiple sites in order to ensure an adequate number of study participants in a reasonable time frame.

Approaches for achieving proof of concept

Several approaches to streamline this process have been considered. One approach might be to use other, more common STIs as a means to prove the concept that interruption of HIV transmission
might be possible. With the higher incidence of common STIs such as chlamydia and trichomoniasis, the study populations needed would be smaller and the trials easier to conduct. Because the infections resulting from failure of the microbicide are easily treatable, the ethical issues surrounding such trials might be significantly less complex. However, if the mechanism of action of the candidate microbicide is HIV-specific, this approach will not be possible.

Even in the case of broad-spectrum microbicides, there are several disadvantages to using other STIs as surrogates for HIV. While a positive result would certainly provide biological plausibility, a definitive effectiveness trial with an HIV endpoint would be needed anyway. On the other hand, if a microbicide were not shown to be effective against a given STI, it might still be effective in preventing HIV, since the per-contact infectivity of HIV is usually much lower. In that case, an otherwise useful compound might be inappropriately eliminated from further development. In any event, a trial demonstrating a reduction in new HIV must be performed.

Although a single successful trial is needed for the microbicides field as soon as possible, it will undoubtedly take several more iterations before a product can be shown to be broadly effective. Resources commensurate with this requirement will continue to be urgently required (see chapter 11). Even after the concept has been proven, a relentless effort to achieve maximum protection through superiority and equivalency studies with subsequent product generations—using different active agents and formulations, both alone and in combination—must be undertaken.

The higher incidence of HIV infection among certain populations of female sex workers reduces the sample size requirements for studies among these vulnerable women. However, given their potentially high rates of product application and other factors that might enhance the rate of vaginal irritation (e.g., frequent coitus, regular douching, etc.), results among sex workers may not be fully representative of product safety or efficacy among other groups of women—particularly those at lower risk or using product in lower frequencies. Careful consideration must be given to the issue of external validity in choosing optimal study populations and designing trial protocols.

Figure 5 (see next page) from the Alliance for Microbicide Development’s pipeline database, lists the current status of completed, planned, or ongoing clinical trials for ten products currently undergoing human testing. Note that, for the purposes of microbicides trials, the content of the various phases has been defined in tables 3, 4, and 5, in chapter 10.
### Figure 5. Current status of microbicide-related clinical trials

<table>
<thead>
<tr>
<th>Product</th>
<th>Phase 1 Vaginal HIV uninfected</th>
<th>Phase 1 Vaginal HIV infected</th>
<th>Phase 1 Penile HIV uninfected</th>
<th>Phase 1 Penile HIV infected</th>
<th>Phase 1 Recanalization</th>
<th>Phase 1 Post-canal activity</th>
<th>Phase 1 Expanded safety</th>
<th>Phase 2</th>
<th>Phase 2/3</th>
<th>Contraceptive efficacy</th>
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Because AIDS is a lethal and incurable disease, microbicide development has been focused on the prevention of HIV transmission. But other sexually transmitted infections are also significant causes of morbidity and mortality. Their spread has been facilitated by many of the same social dynamics that have led to the staggering growth of the HIV epidemic, such as the lack of woman-controlled preventive methods and a general failure to achieve the widespread use of condoms.

Three key reasons make it important to consider other STIs. First, other STIs, particularly ulcerative STIs, are important co-factors for HIV transmission. Second, although the majority of people at risk for HIV infection live in developing countries, where a preventive technology will need to be inexpensive in order to be accessible, many people in developed countries are at much greater risk for other STIs. The purchasing power of consumers in industrialized countries could provide a significant market for a microbicide product, and could encourage the pharmaceutical industry to become more involved in microbicide development. Finally, many of the products currently being evaluated as HIV microbicides have overlapping mechanisms of action, particularly against other viral pathogens like the herpes simplex virus.

**Other sexually transmitted infections**

**Curable STIs**

There are more than 340 million new cases of curable STIs each year, including:

- 170 million cases of trichomoniasis;
- 89 million cases of chlamydia;
- 62 million cases of gonorrhea;
- 12 million cases of syphilis; and
- 7 million cases of chancroid.

Excluding chancroid, this translates to:

- 73 million new infections in North America and Western Europe (incidence 91 per 1000 and 77 per 1000, respectively);
- 65 million new infections in sub-Saharan Africa (254 per 1000);
- 160 million new infections in South and Southeast Asia (160 per 1000); and
- 35 million new infections in Latin America and the Caribbean (145 per 1000).

In developed countries, particularly in the United States, there is an epidemic of chlamydia among young people. In 1996, the prevalence of chlamydia among patients under the age of twenty-four was estimated at 2 million, and annual incidence at 3 million. Gonorrhea incidence in this sub-population was estimated at 650,000 and syphilis at 70,000. According to this data, one in three Americans is likely to have contracted at least one STI by age twenty-four.

Data from developing countries are harder to locate, due to absent or poorly functioning surveillance systems. Recent data from the screening activities during a microbicides trial in South Africa showed that the prevalence of chlamydia was 10%; gonorrhea, 17%; syphilis, 12%; and HIV, 22%.

**Non-curable STIs**

Non-curable STIs also pose significant health problems worldwide. Herpes virus causes ulcers that can facilitate HIV transmission. Treatment
to manage symptom outbreaks is expensive and generally not available in developing countries. According to recent estimates, the prevalence of HSV-2 in nine cities was close to 30% (Concordia, Argentina, 37.4%; Guanacaste, Costa Rica, 41.8%; Cuernavaca, Mexico, 33.1%; Barcelona, Spain, 10.0%; Busan, Korea, 41.2%; Songkla, Thailand, 28.3%; Lampang, Thailand, 30.8%; Ho Chi Minh City, Vietnam, 35.2%; Hanoi, Vietnam, 11.3%). Data from one South African study indicate that in some communities, up to 80% of women of reproductive age have antibodies to HSV-2. In the United States, it is estimated that 45 million people are infected with herpes virus, and 1 million new cases occur each year.

Human papillomavirus (HPV), another important non-curable STI, can cause cervical cancer. Our knowledge of the natural history and other factors that lead to the actual development of cervical cancer is incomplete. In the U.S., however, it is estimated that the prevalence of HPV is 20 million cases per year, and the incidence is 5.5 million cases per year.

By way of comparison, UNAIDS/WHO recently estimated that 5.3 million people were newly infected with HIV in 2000. There are more than 36 million people living with HIV or AIDS worldwide, and 3 million people died of AIDS in 2000 alone.

Considerations for microbicides testing

Adding STI endpoints to HIV trials

One way to address the issue of other STIs is to include their measurement as secondary endpoints in trials focused on the prevention of HIV infection. In most, if not all, populations where HIV incidence is high enough to field HIV effectiveness trials, there are significant rates of other STIs as well. If the incidence of other STIs is at least as high as HIV in the target population, trials powered to detect a specified reduction in HIV incidence should also be adequately powered to detect similar, if not greater, reductions in the incidence of other STIs. Adding additional STI endpoints, though, makes trials more expensive and more complex, since the epidemiology of

Figure 6. Prevalence and incidence rates for assorted STIs, worldwide

- **US 1996**
  - CT prev 2 million
  - CT incid 3 million
  - GC incid 650,000
  - Syphilis incid 70,000
  - HSV-2 prev 45 million
  - HSV-2 incid 1 million

- **Cuernavaca, Mexico**
  - HSV-2 33.1%
  - HSV prev 20 million
  - HPV incid 5.5 million

- **Guanacaste, Costa Rica**
  - HSV-2 41.8%

- **Baltimore**
  - 24% trich
  - 6% CT
  - 8% GC

- **UK**
  - 10.1% GUM clinic

- **Barcelona, Spain**
  - HSV-2 10%

- **Abidjan, Ivory Coast**
  - 18% trich
  - CSW

- **Songkla, Thailand**
  - HSV-2 28.3%

- **Ho Chi Minh City, Vietnam**
  - HSV-2 35.2%

- **Amsterdam**
  - 1.4% Syphilis
  - MSM @ STI clinic

- **RSA**
  - CT 10%
  - GC 17%
  - Syphilis 12%
  - HIV 22%
  - Microbicide trial
  - Screening pop
each pathogen may be different and may require different sample collection logistics as well as different laboratory resources.

In many cases, these additional burdens should require only a marginal increase in effort. For example, if pelvic exams are already being done, adding the collection of a sample for HPV should not require a significant increase in labor. Even so, the increase in cost and technical expertise to store, transport, and test these additional samples may be significant. In many cases, there will be local lab capacity to test for gonorrhea or chlamydia, but there is less likely to be expertise in testing for HSV or HPV; and indeed there may not even be agreement on the appropriate tests or methodologies to use.

Adding tests for additional pathogens may also lead to additional ethical issues. Treatment for HSV, for example, is generally not available where most microbicide efficacy tests are planned, and may not be indicated except where patients are symptomatic. This raises important issues of whether or not to provide such treatment in the context of the trial. Deviating from the local standard of care could constitute an undue inducement to join a trial, and sustainable services may not be possible for trial participants.

This issue is even more complex for pathogens like HPV, where we have a very rudimentary understanding of the course of disease. As a result, it is difficult to power trials for measuring incidence reductions, particularly with respect to potential sequelae such as cervical cancer. These questions can confuse the correlation between a reduction in incidence and any reduction in relevant morbidity or mortality. It seems reasonable, though, on balance, to make every effort to measure other STI endpoints in effectiveness trials wherever possible.

**Concurrent trials**

The high incidence of other STIs in populations not ideal for HIV effectiveness testing raises the possibility of concurrent studies, with one study looking at impact on HIV, and others—perhaps at some distance from the HIV study—being conducted with other pathogen endpoints. This is the approach taken by the U.S. National Institutes of Allergy and Infectious Disease in its Sexually Transmitted Diseases Clinical Trials Unit. There are a number of benefits to this approach, including potentially smaller sample sizes and shorter studies where the incidence of other STIs is significantly higher than HIV, as well as the ability to conduct trials in developed countries.

These trials might help the microbicides field to satisfy the desire of developed-country regulatory authorities for data from their own populations, as well as addressing the commonly expressed concern that women in developing countries are bearing the entire burden of research related to the clinical testing of new products. If the incidence of the endpoint infection is sufficiently high, it may be possible for these trials to be conducted more quickly than HIV trials, moving the field along at an accelerated pace. Finally, showing effectiveness in a developed-country population might help demonstrate a potential market for microbicide products, bringing the pharmaceutical industry (and other private investors) to the table much more quickly.

The main obstacle to concurrent trials is cost. Trials are generally more expensive to conduct in developed countries, and it may be difficult to identify appropriate study populations. We may learn a great deal in this regard from recent efforts to develop vaccines for sexually transmitted pathogens like HSV and HPV. Given limited funding for microbicides research, supporting concurrent trials also raises the issue of whether resources might be diverted from testing against HIV. This concern might be reduced if there were a realistic expectation that demonstrated success against other pathogens might result in increased investment or additional resources across the entire field.

**Consecutive trials**

Consecutive trials are also an option. An initial trial could focus on HIV or another STI pathogen, and subsequent trials would add information
about effectiveness against additional pathogens so that labeling claims and public health promotion efforts could be expanded.

Addressing a non-HIV endpoint first, in a population where the incidence of the pathogen allowed the study to be completed more quickly, could speed approval of a product, potentially at a reduced overall cost.

A key drawback to this approach might be confusion among consumers; for example, if a product proved successful against chlamydia but not gonorrhea (which, based on potential mechanisms of action for some candidate compounds, is one possible outcome). Individuals are unlikely to have a clear understanding of which precise pathogens they are at risk of acquiring, and integrating a pathogen-specific product into their safer-sex practices might be confusing and difficult. On the other hand, a product proven effective against any pathogen would allow the field to begin work on the acceptability and distribution challenges that will confront the widespread introduction of a microbicide effective against HIV.

In addition, proving that a product is effective against any sexually transmitted pathogen might limit the possibility of testing further compounds against an inactive placebo. If a product were to show demonstrated activity against a non-HIV pathogen, while also effective against HIV (whether or not this had been shown), further trials to demonstrate an HIV endpoint would likely become significantly more difficult. This is because they would be expected to show activity above and beyond a comparison compound with some unknown degree of anti-HIV activity, rather than being required to show activity only versus an inactive placebo.

Deciding when and how to address other STIs is a complex issue. Considerations of cost, diverting scarce funds from HIV research, and identifying appropriate populations need to be balanced against the health benefits of preventing other STIs, the potential to bring new researchers and investors to the field, and the opportunity to prove the underlying concept of microbicides more quickly. Addressing other STIs might also help to meet regulatory requirements in the developed world, by including populations from developed countries. Finally, it might also provide an important hedge against diminishing confidence in the idea of a microbicide. For example, even if the next clinical trial of a microbicide against HIV does not prove the product’s effectiveness, having proved a compound’s ability to prevent the transmission of another STI is likely to encourage continued investment and interest in the field. This should support the development of subsequent product generations that do have effectiveness against HIV.

While recognizing these potential advantages, most members of the Science Working Group felt that, in light of limited resources for clinical trials and the urgency of the HIV pandemic, a first trial of a microbical product should address HIV as the primary endpoint. The prevention of other pathogens could be included concurrently as secondary endpoints, or studied in subsequent consecutive trials. As stated above, many trials geared to show protection against HIV would be likely to show protection against other pathogens incorporated as secondary endpoints. If resources were unavailable, or other technical issues preclude this, any product shown effective against HIV might later be tested for activity against other pathogens.

The final calculation on this matter will largely depend upon the resources available, researcher interest, and the perceived clinical, financial, and public health potential of a given product to prevent STIs other than HIV. The Science Working Group strongly recommends including
all practical STI endpoints within the currently planned series of HIV-focused pivotal trials. This is likely to require additional resources for STI testing, particularly for HSV and HPV, since local expertise in conducting these tests is unlikely to exist in areas where pivotal trials for HIV prevention are currently feasible.
Section III  Clinical Trials

Chapter 10. Alternative Pathways for Development

Overview
This chapter is intended to stimulate creative thinking among the microbicides community to promote faster, stronger, and more reliable clinical trials of candidate microbicides. In particular, this chapter will highlight areas where trial designs for potential vaginal microbicides might differ from the designs used for other new drug approvals and propose ideas for handling a variety of situations that arise as a result.

To begin, a word about what this chapter is not. It is not a review of the standard pathway that a typical drug, even a typical contraceptive, follows on its journey from lab bench to medicine cabinet. Interested readers should look to a report

<table>
<thead>
<tr>
<th>Table 3. Phase 1 studies - Summary of IWGM recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Objectives:</strong> Local and systemic safety</td>
</tr>
<tr>
<td>Pharmacokinetics</td>
</tr>
<tr>
<td>Acceptability</td>
</tr>
<tr>
<td>Dose and formulation selection</td>
</tr>
<tr>
<td><strong>Study designs:</strong> A) Open-label</td>
</tr>
<tr>
<td>No control group</td>
</tr>
<tr>
<td>Once-, then twice-daily, application for 7 days, escalating doses</td>
</tr>
<tr>
<td>Parallel or crossover design</td>
</tr>
<tr>
<td>B) Inactive or active control group</td>
</tr>
<tr>
<td>Parallel or crossover design</td>
</tr>
<tr>
<td><strong>Population:</strong> 10-20 per treatment group</td>
</tr>
<tr>
<td>HIV-uninfected, in good health</td>
</tr>
<tr>
<td>Not at risk for pregnancy or STDs</td>
</tr>
<tr>
<td>Sexually abstinent; then sexually active with condom; then sexually active without condoms</td>
</tr>
<tr>
<td>Parallel or crossover design</td>
</tr>
<tr>
<td>HIV-infected women may be studied in later Phase I trials</td>
</tr>
<tr>
<td><strong>Site:</strong> First trial or cohort: country of product origin</td>
</tr>
<tr>
<td><strong>Endpoints:</strong> Local safety: genital symptoms, gross exam, colposcopic exam, vaginal microflora</td>
</tr>
<tr>
<td>Systemic absorption: blood levels of the product and its metabolites</td>
</tr>
<tr>
<td>Systemic safety: appropriate lab tests</td>
</tr>
<tr>
<td>Acceptability: interview, focus groups, questionnaires</td>
</tr>
<tr>
<td><strong>Ancillary studies:</strong> Vaginal lavage for viral load</td>
</tr>
<tr>
<td>Spreading and bioadhesiveness</td>
</tr>
</tbody>
</table>


Neither is much of this chapter devoted to reviewing the standard pathway for developing a microbicide. Very recently, the International Working Group on Microbicides (IWGM) published its recommendations on the suggested requirements for phase 1, 2, and 3 clinical trials (Mauck, C., Z. Rosenberg, and L. Van Damme. 2001. Recommendations for the clinical development of topical microbicides: An update. AIDS 15:857-868), summarized in tables 3, 4, and 5, respectively. The IWGM recommended that the clinical phases of development might be streamlined by:

1) running different studies on a given product in parallel whenever possible;
2) testing multiple products (including products from different sponsors) in a single trial, thereby reducing the number of women required for control arms; and
3) using a phase 2/3 “run-in” study design whenever possible.

Although there are some statistical questions about the second of these recommendations, the Science Working Group generally agrees with all three.

Table 4. Phase 2 studies - Summary of IWGM recommendations

<table>
<thead>
<tr>
<th>Objectives:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local safety</td>
</tr>
<tr>
<td>Acceptability</td>
</tr>
<tr>
<td>If conducted as run-in to phase 3, will have eventual objective of effectiveness against HIV/STIs</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study designs:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Stand-alone study</td>
</tr>
<tr>
<td>Randomized, double-blind comparison with inactive control</td>
</tr>
<tr>
<td>Parallel or crossover design</td>
</tr>
<tr>
<td>Monthly visits for 2 to 6 months</td>
</tr>
<tr>
<td>B) Run-in to phase 3 (“phase 2/3”)</td>
</tr>
<tr>
<td>Preferred over stand-alone design due to urgency of need for microbicide</td>
</tr>
<tr>
<td>Interim analysis to determine whether study can be expanded to phase 3</td>
</tr>
<tr>
<td>Should include condom promotion run-in phase whenever possible</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Population:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Several hundred participants</td>
</tr>
<tr>
<td>HIV-uninfected</td>
</tr>
<tr>
<td>Representative of target population for product</td>
</tr>
<tr>
<td>Sexually active; condoms encouraged (condom promotion run-in phase to be utilized whenever possible)</td>
</tr>
<tr>
<td>Parallel design</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Endpoints:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local safety: genital symptoms, gross exam, colposcopic exam on subset</td>
</tr>
<tr>
<td>Acceptability: questionnaires</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ancillary studies:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additional pharmacokinetic studies</td>
</tr>
<tr>
<td>Use in anal intercourse</td>
</tr>
</tbody>
</table>

Key issues in the clinical testing of microbicides

Topical microbicides represent a new category of medication with many unique features. Microbicides are just part of a complex HIV prevention package that currently counts on behavioral strategies (abstinence or mutual monogamy), devices (male or female condoms), and public health interventions (treating other sexually transmitted infections) as the standard of care. Beyond this prevention package (of unknown efficacy), candidate microbicides must be tested against supposed placebos (also of unknown efficacy). All of this is further complicated by the lack of any accepted surrogate endpoint that links events more common (and therefore easier to study) than HIV seroconversion to eventual negative outcomes.

Motivations for clinical trials

Both the public health sector and the relevant regulatory agencies, including the FDA, clearly want a safe and effective microbicide to become available as soon as these product claims can be reasonably substantiated. Differences in approach result primarily from the different mandates of these groups. The primary job of a regulatory agency is to ensure that approved drugs are safe.

<table>
<thead>
<tr>
<th>Table 5. Phase 3 studies – Summary of IWGM recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Objectives:</strong> Evaluate effectiveness in preventing HIV/STDs</td>
</tr>
<tr>
<td>Local safety</td>
</tr>
<tr>
<td>Acceptability and compliance</td>
</tr>
<tr>
<td><strong>Study designs:</strong></td>
</tr>
<tr>
<td>A) Stand-alone study</td>
</tr>
<tr>
<td>B) Follow-on to phase 2 (“phase 2/3”)</td>
</tr>
<tr>
<td>Preferred over stand-alone design, due to urgency of need for microbicide</td>
</tr>
<tr>
<td>Interim analysis used to determine whether phase 2 study can be expanded to phase 3</td>
</tr>
<tr>
<td>In either case:</td>
</tr>
<tr>
<td>Should include condom promotion run-in phase whenever possible</td>
</tr>
<tr>
<td>Randomized, double-blind, inactive control(s)</td>
</tr>
<tr>
<td>Inactive controls include placebo and no-treatment arm—latter to be used if placebo may reduce HIV/STI incidence, although behavioral differences in non-treatment group should be evaluated</td>
</tr>
<tr>
<td>May test several products against one inactive control</td>
</tr>
<tr>
<td>Multi-center whenever possible</td>
</tr>
<tr>
<td>Follow-up about every 3 months for 12 months</td>
</tr>
<tr>
<td><strong>Population:</strong></td>
</tr>
<tr>
<td>Several hundred to several thousand participants</td>
</tr>
<tr>
<td>HIV-uninfected (although confidentiality issues may preclude exclusion of HIV-positive women)</td>
</tr>
<tr>
<td>Representative of target population for product</td>
</tr>
<tr>
<td>Sexually active; condoms encouraged (condom promotion run-in phase to be utilized whenever possible)</td>
</tr>
<tr>
<td>Parallel design</td>
</tr>
<tr>
<td><strong>Endpoints:</strong></td>
</tr>
<tr>
<td>HIV/STD incidence</td>
</tr>
<tr>
<td>Local safety: genital symptoms, gross exam, possibly colposcopic exam on subset</td>
</tr>
<tr>
<td>Acceptability: questionnaires</td>
</tr>
<tr>
<td>Compliance: product use for coital acts closest in time to study visits</td>
</tr>
</tbody>
</table>

and effective. In the case of microbicides, the FDA has taken extra steps through its topical microbicide working group (TMWG) to expedite its specific pre-IND, IND, and NDA processes to help address the urgent public health problems posed by the HIV pandemic.

In this chapter, we discuss some areas in which microbicides trials motivated by a desire to curb the spread of HIV in the populations most at risk might diverge from microbicides trials motivated by a desire to obtain marketing approval from the FDA or other regulatory agency. We then make some recommendations for resolving these differences.

Areas of potential divergence

Three particular areas might benefit from further compromise or clarification. These include (1) potential deviations from the standard clinical testing pathway; (2) the ability to generalize data from specific microbicide trials to a variety of other populations; and (3) labeling issues for the eventual marketing of a candidate microbicide.

Deviations from the classical pathway

- Efficacy evaluations for HIV endpoints cannot realistically take place in phase 2 trials
One of the standard purposes of a phase 2 trial is to show preliminary “activity” against a given endpoint. In the case of microbicides, however, an adequate number of seroconversions will not occur in a study of only “several hundred” participants over the short period of time generally considered standard for such trials. For this reason, smaller enrollment in phase 2 studies could be permitted, depending on the prior safety profile of the candidate microbicide. An alternative solution, already endorsed by the IWGM, is to consider the phase 2 trial as a run-in for phase 3, where infections and woman-years of exposure collected in phase 2 would also count toward the numerators and denominators of safety and effectiveness for phase 3. Because participants in phase 2 trials may receive more intensive early follow-up and testing than do those in phase 3, cost considerations will be important in deciding to pursue this option.

- Dose finding cannot meaningfully take place in most microbicides development pathways
Another classical aspect of phase 2 testing is to establish the lowest effective dose of a candidate drug. In the case of many microbicides, however, a dose response curve is unlikely to emerge, especially as early as a phase 2 trial. Ideally, the FDA would like to see some justification behind the selection of a given dose (volume, concentration, and formulation), but exactly how best to select the lowest effective dose in the case of many microbicides is still unclear, particularly when testing against an HIV endpoint. However, provided the selected dose is shown to be safe when used over a prolonged period of time, this should not be a critical issue.

- Placebo and other necessary trial features are of unknown efficacy
Consensus in the field holds that ethical and scientifically rigorous trials need to offer condoms as well as locally available STI treatment to participants. Trials also need to randomize women to receive either test microbicide or placebo. One problem, however, is that the field is too new to have established estimates of the efficacy of these various trial interventions. Without such estimates, it may be impossible to predict (ahead of time) or correct for (after the fact) the magnitude of any such effects on the apparent effectiveness of a microbicide.

In the case of a placebo, the field is again too new to have a well-documented portfolio of placebo substances known to be inert and ineffective. The placebo should preferably be similar in volume and formulation to the study drug, to permit double blinding throughout the trial. The IWGM recommends “vehicle” placebos, especially in phase 1 and maybe phase 2, but these are not always possible. The “vehicle” of a given
candidate microbicide might be a compound that looks or feels obviously different from the microbicide itself. Even more potentially confounding, the vehicle itself might also have microbicidal properties. (For example, see the brief mention of putative anti-microbial properties among sexual lubricants in chapter 1.)

One possible recommendation is to have a third study group (aside from test microbicide and placebos) that receives only the standard package of condoms, counseling, and STI treatment (a “condom-only” arm). The statistical and clinical effects of these interventions themselves would also have to be evaluated, however, in order to justify claims about microbicide effectiveness relative to this alternative “baseline.” It is not yet clear how this could be accomplished. Another potential pitfall raised by some critics of this approach is that women assigned to receive a study drug might well share with women who had been assigned to receive no study drug. It is unclear whether such sharing could be documented or corrected for in the analysis of trial results. In addition, a condom-only arm cannot be blinded due to its very nature, and sexual risk-taking behavior might differ as compared with the condom-plus-gel arms, making data interpretation extremely difficult. Finally, a condom-only arm would also raise the required number of study participants, as well as the cost of a trial, by about 50 percent, unless there are more than three arms in the trial, i.e., more than one active product arm.

Application of data across populations
In combating a global epidemic, such as HIV, it is inevitable that regulatory bodies other than the USFDA will play a role in the development and approval of microbicides. The USFDA already participates in cross-national agreements with selected other regulators (as mentioned in chapter 12), but it will be important to work with agencies from countries outside the current group (limited mainly to those from developed countries) to ensure an accelerated path. One important acknowledgement is that the FDA is already willing to travel to manufacturing sites and clinical trials for good practice audits, no matter where in the world these sites may be.

In general, the FDA and the scientific community share the problem of extrapolating from clinical trial populations to the broader populations that will eventually use a given product. In the FDA’s case, this broader population is U.S. women and men. By contrast, in the case of the public health community, it is women and men worldwide at risk for infection with HIV. The majority of these people live in developing countries, where the calculus of risk and benefit differs greatly from that which confronts an average American.

■ Requiring data from two trials versus one very large one may not be justified
In considering drug approval and subsequent labeling claims, the FDA would like to ensure that data from phase 3 trials can be widely generalized. One way to accomplish this is to require data from more than one trial and from different patient populations. This is especially true when there is reason to believe that the drug at issue might not actually work with the same effectiveness in different populations, due to differences in sexual practices, personal hygiene, use of other vaginal products, incidence of various STIs, etc. In the case of microbicides trials, however, effectiveness endpoints—even in the populations with the highest incidence of HIV—will be uncommon. For this reason, it is particularly important not to under-power the trial, and phase 3 microbicide trials will almost invariably need to be so large as to require several study sites. (See table 6 for some illustrative sample sizes for possible phase 3 trials.)

In the opinion of the Working Group, although two or more trials would provide more convincing evidence than a single larger trial, most phase 3 trials could not feasibly be powered to break them into two or more single-site studies. Most single-site studies could not provide statistically significant information, even if the sites were to follow a common protocol. The FDA is, in fact, willing to consider data from a single multicenter trial, despite its declared preference for
two trials in some cases. In general, these are cases in which a single, multi-center study of excellent design has provided highly reliable and statistically strong evidence of an important clinical benefit, such as an effect on survival; and in which a confirmatory study would be difficult to conduct on ethical grounds. Even here, though, the FDA also generally requires additional supporting data. For microbicides, activity data from phase 2 or other sources would also be needed, but may be difficult to obtain.

Current microbicides trials should be designed to meet the announced standards required for a single trial. Aside from the familiar theme of financial constraints, there are logistical and ethical obstacles to a “second trial” for a candidate microbicide. In the first place, potential testing sites may be scarce enough that they should be carefully apportioned, and not used merely to reinforce a finding that has already met a scientifically rigorous burden of proof. In addition, an ethical review board would likely be reluctant to approve a second placebo-controlled trial if a large, rigorous study had already demonstrated a candidate microbicide to be more effective than placebo over an extended duration of use.

Some members of the Working Group feel this latter problem could be solved by fielding the two pivotal phase 3 trials simultaneously, and not sequentially. They urge further that the development of future clinical trial sites throughout the world be strongly encouraged and supported financially. Other members of the panel maintain that the millions of dollars needed to field a second phase 3 trial would not be well spent before some effectiveness data could emerge from a first phase 3 trial to help prioritize among additional candidate microbicides still awaiting testing.

Table 6. Illustrative sample sizes for phase 3 microbicides effectiveness trials

<table>
<thead>
<tr>
<th>Incidence rates</th>
<th>25% reduction</th>
<th>33% reduction</th>
<th>50% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>80% power</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.0%</td>
<td>7133</td>
<td>3825</td>
<td>1534</td>
</tr>
<tr>
<td>4.0%</td>
<td>5301</td>
<td>2844</td>
<td>1141</td>
</tr>
<tr>
<td>5.0%</td>
<td>4202</td>
<td>2256</td>
<td>906</td>
</tr>
<tr>
<td>6.0%</td>
<td>3490</td>
<td>1863</td>
<td>749</td>
</tr>
<tr>
<td><strong>90% power</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.0%</td>
<td>9548</td>
<td>5121</td>
<td>2053</td>
</tr>
<tr>
<td>4.0%</td>
<td>7096</td>
<td>3809</td>
<td>1527</td>
</tr>
<tr>
<td>5.0%</td>
<td>5625</td>
<td>3019</td>
<td>1202</td>
</tr>
<tr>
<td>6.0%</td>
<td>4644</td>
<td>2493</td>
<td>1002</td>
</tr>
</tbody>
</table>

This table assumes 95% two-sided test, 80% or 90% power, incidence rates in the placebo-plus-condom (“control”) arm ranging from 3.0% to 6.0%, reductions of 25%, 33%, and 50% in the “active”-plus-condom (“test”) arm. The calculations are the customary ones based on the normal approximation of the binomial distribution.

Note that the grid reflects woman-years, not women. If we assume that each woman will contribute, on average, six months rather than one year, we need to double the numbers in each cell to see how many women to enroll. As a practical matter, we will also need to increase the figures significantly for loss to follow-up, protocol violations, and early withdrawals, as these will realistically occur.

To read this grid, look, for instance, at the first line of the first panel. We would say that a two-group 95% two-sided significance level will have 80% power to detect the difference between a proportion, \( \hat{A}_1 \), of 0.03 (i.e., 2.5% incidence in the placebo-plus-condom arm) and a proportion, \( \hat{A}_2 \), of 0.0225 when the sample size in each group is 7,133.
As a practical matter, nearly all phase 3 microbicicides trials already entail the likelihood of variation in use and in populations across several study sites, and sometimes in several countries. Most protocols on the drawing boards now for phase 3 studies have converged on very similar designs, as the result of extensive consultation, and of logistical and other pressures.

**Data from phase 3 trials may not be best gathered from sites in the United States**

Given that the USFDA is charged with regulating product approval and labeling for only the U.S. market, it is understandable that the FDA would like to see at least some “bridging” data characterizing the use of candidate microbicides in American populations. From a global public health perspective, however, fielding multiple sites of a large phase 3 microbicides effectiveness trial in the United States may not be cost-effective or feasible.

As one recommendation, the FDA could accept bridging data from a smaller clinical study, or possibly from a study with a slightly different endpoint, such as prevention of HIV transmission via the rectal route. Such a study could provide limited data on potential differences in the use of condoms and other vaginal or rectal products, and study drug use and effectiveness in the U.S. compared to elsewhere, but would not entail the expense of supporting several U.S. sites in a phase 3 effectiveness trial. Alternatively, bridging data from a smaller number of U.S. sites in a phase 3 trial may be acceptable.

Phase 1 designs could also be expanded to collect “bridging” data, considering that phase 1 studies are indeed typically feasible in the United States. The fact remains, however, that public health funds devoted to discovering the effectiveness of candidate microbicides are best spent in sites with a high incidence of HIV and other STIs, particularly given the enormous cost differences between fielding microbicides effectiveness trials in other countries as compared to the U.S.

**Labeling issues for marketing claims**

**Labeling for trial sub-populations will probably be required**

As widely recognized in the microbicides field, it would be unethical to field a clinical trial that did not offer women the option of latex condoms. Yet widespread use of condoms within a trial considerably reduces the trial’s ability (at a fixed sample size) to detect a protective effect due to a candidate microbicide.

Some groups, including the IWGM, have proposed to solve the condom dilemma by the “condom run-in phase,” in which women are excluded from phase 3 trial participation if they are able to use condoms with any degree of consistency. After the “condom run-in phase,” only those participants who are not likely to exercise the advice they must (for ethical reasons) receive would be enrolled in the phase 3 effectiveness trial(s). This concept may present ethical and logistical challenges, but those can likely be overcome. More important, it also leaves open a data-interpretation and labeling challenge, discussed below.

Assuming that any study is likely to result in a mix of participants according to both test microbicide use and condom use, the FDA could reasonably permit conditional language to let consumers know the level of benefit they might expect to receive according to their use of the product. Contraceptive labels, for instance, sometimes provide two failure rates: the perfect-use failure rate and the typical-use failure rate. Because of the lethal and incurable nature of HIV infection, however, microbicide labels should always contain language recommending the use of condoms along with the use of the microbicide, in order to ensure the maximum possible protection against infection.

Because of the probable efficacy of condoms in preventing the transmission of HIV, women who comply perfectly with the protocol by using condoms and gel at every act of intercourse
should show lower absolute numbers of infections (as compared to those using condoms and placebo) than typical (or imperfect) users who do not comply with the advice to use condoms. Moreover, the number of infections prevented among perfect users will be higher, under almost any reasonable set of assumptions, than the same number among the subset of these latter women who use the study microbicide consistently and correctly but never use condoms. For this reason, presentations of “perfect-use” and “typical-use” effectiveness rates adopted by analogy to the contraceptive labels could be confusing. The protective effects of variable condom use must first be filtered out in order to produce a fair assessment of the independent efficacy of the microbicide. The same is true with regard to the hypothesized protective effects of concurrent STI treatment.

For example, consider the sub-population of women who do not use condoms, but who are inconsistent in their use of the test microbicide or placebo. These women are arguably the exact population most likely to need microbicides, using them in the precise way they are most likely to be used once marketed. The measured protection of a vaginal microbicide that is actually 80% effective will appear to vary between 13% and 53% in this population. Measured protection in the scenario where the majority of the woman-years in the trial comply with the protocol will be only 40%. (In fact, the measured protection will be a direct function of the ratio between product-only users and those who use nothing.)

It is clear that documenting actual use patterns as accurately as possible should be an important part of any phase 3 trial, given the importance of these patterns on the eventual trial result. But beyond that, all regulatory agencies will need to consider claims founded on sub-populations of phase 3 participants in order to produce labels meaningful to the users most likely to need microbicides. A full discussion of this issue is beyond the scope of this chapter, but will be essential before methodologically sound guidance can be issued on this topic.

Candidate HIV microbicides could be tested and labeled for prevention of other STIs, or even as sexual lubricants, as such claims may be faster and easier to substantiate

Although the primary endpoint for most phase 3 microbicide trials will be HIV prevention, trial designs currently on the drawing boards also test for other common sexually transmitted infections. Vaginal microbicides might reduce or increase transmission of these pathogens, and they might change the nature of any co-factor relationships that these pathogens have with HIV infection. A possibility is that one or more candidate microbicides could show efficacy against one or more STI pathogens, even if effectiveness against HIV is unclear.

Current microbicide protocols will oblige the investigators to treat the STI infections where possible and allow the women to continue in the study. Because some infections (such as bacterial vaginosis) have a less accurate or readily available test of cure, it may be more difficult to calculate precise incidence rates or precise microbicide protection figures to substantiate labeling claims regarding these particular STI endpoints.

There are certain conditions under which the FDA would permit labeling claims for endpoints beyond HIV in a trial primarily targeted at HIV prevention. We recommend additional dialogue and comments that could lead to broader guidance regarding the selection of appropriate endpoints for the STI prevention field as a whole.
Site requirements
Clinical trials are complex undertakings, which require extensive resources and experience. Effectiveness trials of vaginal microbicides must be able to enroll large numbers of women at risk for HIV infection via sexual transmission. For the most part, this limits these trials to locations in developing countries with uncontrolled HIV epidemics. In these areas, however, the infrastructure to perform research is typically weak, and sites that have both high rates of infection and an adequate infrastructure to perform microbicide effectiveness trials are severely limited.

Clinical trial sites must have the following:

- National and local political support to perform microbicides research. This is critical in order to obtain approval from ethical review committees, obtain product import permits, get collaboration with local institutions, and enroll and retain study volunteers.

- Willing and able local scientific collaborators.

- Large populations of women at substantial risk for HIV infection. For populations with an incidence of new HIV infection of less than 1 percent, efficacy studies would require a sample size of tens of thousands of women, and would probably not be feasible.

- An effective national and/or local human research ethical review committee, to protect the rights of potential study subjects. In many cases international accreditation of this body will be required.

- Medical care facilities for evaluation and treatment of conditions that may be related to the trial, such as adverse effects of study products, as well as conditions identified through (but not caused by) study participation. These include abnormal Pap smears, and HIV infection or sexually transmitted infections detected at the first screening visit or during follow-up.

- Medical clinics or other physical space for study participant visits. Hundreds of women may participate at each site, requiring ample room for simultaneous interviews and examinations.

- Behavioral science capacity to ensure that the research is acceptable to study participants and the community, and that potential participants can understand the study enough to give truly informed consent and adhere to instructions on study gel use.

- A trained staff, including counselors, interviewers, clinicians, behavioral scientists, laboratory technicians, and data managers.

- Administrative capacity to manage millions of dollars and dozens of employees.

- Laboratory capacity to perform tens of thousands of HIV and STD tests.

- Data management capacity to handle tens of thousands of case record forms.

Site creation and management
As resources for HIV prevention research increase, the small number of sites and limited human resources and infrastructure at these sites are creating a critical bottleneck. Several candidate microbicides are now ready for advanced human trials (see figure 5, in chapter 8) and it seems likely that there will be competition
among developers for access to limited trial sites. New HIV vaccines are also entering advanced clinical trials, and, while current vaccine trials are focused primarily on populations at risk for transmission via anal sex or injection drug use, there is likely to be competition between microbicide and vaccine sponsors for trial sites. Innovative ways of stimulating healthy competition and collaboration are urgently needed.

The following preparatory steps should be taken prior to beginning microbicides trials:

1. Reviewing the available literature from scientific journals, meeting abstracts, books, and government reports on HIV and other STIs, as well as reproductive health epidemiology and knowledge, perceptions, and behaviors in potential study populations.

2. Engaging local collaborators and advocates to support microbicides development.

3. Convening national and local consultations on microbicides research.

4. Assessing local capacity for human trials ethical review, and strengthening it (if necessary) through additional consultation and training.

5. Performing formative research on potential clinical trial populations and procedures. Key informant interviews and focus groups should be used to consider potential populations for recruitment, study venues, recruitment methods, and potential barriers to participation.

6. Performing behavioral research among potential trial participants on knowledge, attitudes, and practices regarding HIV and other STIs, sexual behavior, and vaginal product use.

7. Developing participant information tools, and assessing the comprehension and willingness to participate among potential volunteers.

8. Performing an initial cohort study to assess enrollment and follow-up rates; HIV and other STI prevalence, incidence, and risk factors; and response to prevention activities. If rates of enrollment, follow-up, or HIV incidence are too low, microbicide efficacy studies will not be feasible.

9. Performing pilot studies of vaginal product use as a stand-alone study or as a run-in to an effectiveness trial.

To date, large phase 2 and phase 3 microbicide trials have been performed at a total of only about a dozen international sites. In most cases, these sites had previous international support for HIV prevention or reproductive health research. The microbicides work has generally built on existing capacity and infrastructure, established over at least several years.

There are several models for ongoing site management with varying levels of continuity and central coordination. At one extreme, international research sites may be developed de novo for each new clinical trial, as was done for a study of nonoxynol-9 film in Cameroon. In this model, researchers need to go through some or all of the steps outlined above before initiating a clinical trial, with all of the attendant time and monetary costs. An advantage of this approach is that a strong combination of geographic sites, institutions, personnel, and methods can be brought together. Outdated, inefficient, or ineffective legacy elements from prior activities need not be incorporated.

In a second model, independent sites are able to maintain continuity by performing multiple concurrent studies, including microbicide trials, over time—potentially on behalf of many different sponsors or funding agencies. An advantage of this model is that a site can leverage the considerable infrastructure required over many individual studies. There still may be funding gaps, however, and substantial time
and energy must be continuously directed to identifying new resources.

There is a third model for research sites that have relatively stable, long-term support, usually from donor governments, such as Projet RETRO-CI in Côte d'Ivoire. Investigators at these sites are able to participate in microbicide trials with guaranteed support to maintain staffing and perform other research during interim periods. These sites may be able to establish strong infrastructures, but they have little incentive to eliminate inefficiencies.

A final model is the competitive funding and support of networks of sites such as the U.S. National Institutes of Health HIV Prevention Trials Network (HPTN). This model provides continuity and strong scientific guidance to sites, but may limit local investigator flexibility.

Any of these models may be best suited to a variety of different conditions. Since there is an urgent need to develop microbicides, and multiple products waiting to be tested, the absence of any clear “best model” for site creation and management implies that resources should be directed to support a variety of different models for establishing and maintaining a clinical trials infrastructure. If possible, it is also advantageous to engage investigators and sites with a proven track record of successful, completed studies, ideally including other clinical trials.

Ethical issues in microbicide research

The women who are potential study volunteers are likely to be already marginalized by the very characteristics that put them at risk for HIV infection, including female sex, engaging in commercial sex work, or living in a developing country with high HIV infection rates. Such women may also be unsophisticated, less educated, have poor baseline access to health care, and have substantial language and cultural barriers between them and the scientific investigators. Special attention must be given to ensuring protection for the thousands of women who will be enrolled in microbicide trials in the near future.

Sources of guidance

Guidance on the protection of human research subjects can be obtained from several sources. The Nuremberg Code, established after the Second World War, focuses on the voluntary consent and safety of study participants. The first Helsinki Declaration established ethical principles for medical research involving human subjects, and was adopted by the World Medical Association in 1964 with amendment in October 2000. Internationally recognized guidelines have also been developed by the Council for International Organizations of Medical Sciences (CIOMS), in the International Guidelines for Ethical Review of Epidemiological Studies in 1991, and the International Guidelines for Biomedical Research Involving Human Subjects in 1993. Two documents guiding HIV vaccine research, which are also relevant to microbicide research, were published by UNAIDS in 2000. A report from a 1997 international symposium on ethical issues, sponsored by the Women’s Health Advocates on Microbicides and the Population Council, is available, as is a recent review from the IWGM. Guidance for the ethical conduct of U.S. government-sponsored research is provided by The Belmont Report, published in 1979. Finally, the U.S. National Bioethics Advisory Commission has issued recommendations on research involving human participants in general, and on research in developing countries, in particular. Many other guidelines have been issued, including national guidelines in countries where HIV prevention research is performed.

Informed consent

The Nuremberg Code established as its first principle that “the voluntary consent of the human subject is absolutely essential.” However, most potential participants in HIV prevention trials are unfamiliar with clinical trial methods. Many languages have no direct translation for
words describing key study concepts such as “placebo” or “randomization,” which can lead to considerable confusion.

The following activities can help ensure that consent is truly “informed,” allowing potential participants to understand the study well enough to decide whether or not to join:

1. Mock study introduction sessions can be useful to ensure good comprehension, allowing potential problem areas in participant understanding to be identified and addressed.

2. Participant education should be monitored and used to guide additional efforts as needed. A comprehension examination is useful to demonstrate adequate baseline knowledge, allowing potential participants to decide whether or not to join the study.

3. Staff with participant contact should be well trained in communication and counseling, and should not have substantial language or cultural barriers separating them from participants.

4. Consent forms should be readable and easily understood by potential participants.

5. Special media such as videos, flip charts, booklets, or role-plays should be prepared to help educate study participants. These materials, along with the consent forms, should be reviewed and translated (if necessary) by staff experienced in HIV research with the participant population, as well as by community representatives.

6. Informed consent does not end at enrollment. Study participants should be aware that they are free to leave the study at any time, and education of participants should continue as the study progresses.

7. Participants’ understanding should continue to be re-evaluated as the study progresses. Repeated formal comprehension examinations, e.g., every three to six months, may be useful to assess individual participants’ understanding, and to suggest areas for broader communication with the study group. Questions to reassess comprehension and consent can be included in the regularly administered study data-collection questionnaires.

**Risk reduction**

Ethical guidelines also emphasize that any risks from research should not outweigh the potential benefits. The Declaration of Helsinki states that “considerations related to the well-being of the human subject should take precedence over the interests of science and society.” A particular challenge is the tension and potential conflict of interest between studies to determine whether or not a microbicide effectively prevents infection with HIV versus the prevention needs of study participants and their communities. Existing guidelines suggest that “high-quality” HIV prevention counseling and condoms must be given to study participants. However, if these activities are very successful in preventing HIV infection, the primary purpose for the trial may well be undermined, and any societal benefit negated.

Most advocates and researchers recommend that the following steps be taken to reduce and monitor risk behavior:

1. Study participants should receive counseling along with condoms, demonstrations of condom use, and screening and treatment for other STIs that may increase susceptibility to HIV infection. Partner counseling and testing should also be made available, if appropriate.

2. The results of preparatory studies performed in the study population should be used to improve HIV prevention services.

3. Innovative methods, such as the involvement of relevant community leaders in prevention programs, should be used when appropriate.

4. To avoid potential conflict of interest, a group independent of the researchers may provide prevention services. However, in many settings strong independent programs are not
available and the researchers themselves are best suited to offer the highest quality services.

5. Evaluation of prevention services should be data driven, using behavioral data collected during routine study follow-up visits and other markers, such as rates of other STIs.

6. At a minimum, indicators of risk should be no worse during a study than they were at enrollment. If risk appears to increase over the baseline, a false sense of security may have developed, indicating a failure in participant education and prevention counseling.

7. A data and safety monitoring board (DSMB), including members from the host country, should review risk indicator data. Education and prevention efforts should be strengthened considerably, or a study terminated, if there is a significant increase in risk over baseline.

Prevention activities may be broad-based programs that lower infection rates in the entire potential study population, or may be available only to study participants. In some cases, HIV prevention activities have been so successful that HIV seroincidence rates have become too low to support microbicides research. In Thailand, for example, efforts including the “100% condom program” to promote condom use during commercial sex have brought HIV seroincidence—both in sex workers and other women of reproductive age—to rates so low that microbicide efficacy studies are no longer feasible. There were very few seroconvertors in the Col-1492 microbicide study site in Thailand. Although a generalized HIV epidemic continues in Côte d’Ivoire, there were also very few seroconvertors at the Col-1492 study site in Abidjan. This may have been the result of specific prevention activities for the female sex workers at that site, developed over years of prevention research. Nevertheless, phase 1 and phase 2 safety and acceptability studies may still be carried out at both of these sites.

Community involvement
The inclusion of community representatives on ethical review boards is recommended by many ethical guidelines, and is required for studies involving the U.S. Department of Health and Human Services. The UNAIDS Guidance Document, Ethical Considerations in HIV Preventive Vaccine Research, states that “to ensure ethical and scientific quality of the proposed research, its relevance to the affected community, and its acceptance by the affected community, community representatives should be involved in an early and sustained manner in the design and development, implementation, and distribution of results of HIV vaccine research.”

Microbicide researchers have worked constructively with community representatives in the following ways:

- Community consultation—with, for example, a community advisory group representing the community from which study participants will be recruited—can help with the conduct of studies and protect the rights of study subjects.

- Such a group should review the study protocol and, while major changes in study design may not be possible, their suggestions for making the study more acceptable to participants should be incorporated.

- The group should also review the study consent forms and educational materials. Their suggestions for making those materials more comprehensible to study participants should be incorporated.

- Community advisory group members can help directly with study introduction and the recruitment of participants in their communities.

- Community advisory group members should be informed about study progress and problems, and help interact with the communities of study participants. For example, they can help clarify misconceptions in the community about study procedures and risks.
Where appropriate, community advisory group members may meet with study participants. In some cases—e.g., if the study participants engage in stigmatized or illegal behaviors such as sex work—such meetings may not be appropriate. In any case, study participants should be aware of who will be present, and thus be free to choose whether or not to attend.

Follow-up meetings with community representatives to report study results back to them are also important, and can help refine messages for communication to study participants and the broader local community.

Informing participants of HIV test results
In many settings HIV infection is highly stigmatized, and needing to learn one’s HIV infection status may be a major barrier to study participation. In some cases, investigators have allowed women to participate in microbicide efficacy studies without learning their infection status. If they indicate that they do not want to learn their test results, HIV-infected women are told that they are not eligible, without being told the reason why. Alternatively, HIV-infected women may be allowed to participate without knowing their status, even though their participation will not help determine whether the microbicide under study prevents HIV infection.

In these cases it may be difficult for study staff to interact repeatedly with women whom they know to be infected without being able to inform them. Furthermore, institutional review boards responsible for reviewing and approving research may not allow HIV testing of participants without informing them of their results. The objection to nondisclosure is that participants may benefit from knowing their infection status, especially HIV-infected women who can receive counseling, including partner notification counseling and referral for medical care. Accordingly, most researchers currently require women to learn their HIV status in order to participate.

Medical care for trial participants
The extent of responsibility for the medical care of HIV trial participants is an evolving and contentious issue. Some advocates contend that donors and product sponsors are responsible for providing medical care equal to that available in the sponsor country for study participants, including long-term, highly active anti-retroviral therapy (HAART) for participants who acquire HIV infection during a trial. Others counter that researchers should not have any special responsibility to provide medical care not otherwise available in the community, because new HIV infection is not caused by trial participation. If anything, the HIV infection rate should be lower for trial participants than it would be if they did not take part.

HAART requires rigorous attention to adherence, response to treatment, emergence of resistance, and monitoring for side effects, each of which requires substantial human or financial resources. The cost of HAART can be thousands of dollars a year, although it is lower in some developing countries that produce the medications domestically or receive discounts from manufacturers. Providing medical care not otherwise available in the community could be considered an undue incentive for study enrollment. In addition, requiring the provision of lifelong medical care for a subset of trial participants is likely to discourage many potential sponsors from entering the microbicide field, thus potentially depriving society of this method for the prevention of HIV transmission.

The 1998 Women’s Health Advocates on Microbicides symposium concluded that “researchers have an obligation to provide some support to participants who seroconvert during the trial,” but that “there was no consensus that this should extend to anti-retroviral treatment.” The issue was also addressed in a series of UNAIDS-sponsored workshops (with regard to HIV vaccine research), again with no overall
consensus about the level of care that should be provided.

Some microbicide researchers have provided basic medical care, such as prophylaxis of opportunistic infections for HIV seroconvertors, but not anti-retroviral medication. Other researchers have provided financial support to non-governmental organizations that provide medical care and other services to people with HIV infection or AIDS, and have then referred seroconvertors to those organizations. Still others have referred HIV-infected study participants to medical care available in the community, and have subsidized that care directly for a limited period of time, or until the end of the study.

However, HIV infection has several years of latency before anti-retroviral treatment is generally considered necessary (although many experts would also consider therapy for patients in whom seroconversion has been documented to have occurred within the previous six months). In any event, long-term treatment is likely to be needed, but phase 3 trials last only a few years, at most. Therefore, study sponsors may not be available in the community when study participants eventually need treatment. Some researchers are exploring the option of purchasing medical insurance for study participants, which would include the treatment of HIV infection and would be available after the study is completed.

While the availability of anti-retroviral medications, the appropriate standard of medical care, and the ethical scrutiny of clinical trials in developing countries are all increasing, there remains no clear guideline for the provision of medical care by researchers. Most would agree, however, that researchers have at least some responsibility for ensuring medical care for trial participants. The specific package of care should be established in a local consultative process, and seek to be at least at the level of the best locally available and sustainable standard. Moreover, it is important to recognize that what pertains now may be superseded in the not-so-distant future.
A clinical development strategy must take into account a wide variety of elements, including the level of regulatory risk that the sponsor of the study is prepared to accept. The contents of a clinical development program should be based on what regulatory authorities are likely to accept as evidence, and should provide all of the critical information required for a future user to be able to employ the product safely and effectively.

**Draft product characteristics**
A draft label and summary of product characteristics (SPC) should be developed early in the process in order to detail the minimal features required to complete phase 3 studies. It is important that microbicide developers create strong and effective working relationships with regulators, and that they consider all questions likely to be raised by the regulatory authorities. Such questions are likely to include an outline of the relevant risk/benefit ratios, and a well-thought-out OTC switching strategy. Phase 3 clinical trials should also be designed to serve the needs of a wide variety of regulatory authorities, in order to allow the widest possible appropriate registration for an eventual product.

**Current U.S. and international practice**
The details of clinical data analysis, as well as key issues in document preparation, are often discussed between sponsors and the USFDA before the start of key studies, especially during phase 3. In the case of microbicides, however, no phase 3 regulatory precedents or guidelines exist because there are no marketed or registered products.

The International Working Group on Microbicides has recently published recommendations for the clinical development of topical microbicides (see chapter 4). This comprehensive effort reviewed development objectives, study designs, key populations, potential sites, and endpoints for phase 1, 2, and 3 studies. The guidelines are intended to provide a strategic framework for the efficient clinical evaluation of candidate microbicide products.

Since most of the heterosexual transmission of HIV occurs in the developing countries of Africa and Asia, vaginal microbicides with the potential to reduce the heterosexual spread of HIV will therefore produce the greatest benefit in these regions. It makes good sense to prioritize study conduct, registration, and availability within these territories. This should not preclude parallel U.S. activities, however, since the FDA is likely to require U.S. studies before allowing domestic registration.

**One path forward**
In many of these developing regions, regulatory activities are not well developed. The urgent clinical and humanitarian need for an effective microbicide argues for a new regulatory paradigm. Organizations such as the WHO, along with selected NGOs, should meet together with key regulators from the developed and developing worlds to decide on the appropriate regulatory pathways. As of this writing, plans for a microbicides regulatory meeting are currently underway, probably to be held in early 2002.
of Technical Requirements for Registration of Pharmaceuticals for Human Use (the “ICH”—see www.ifpma.org/ich1.html). This effort is a unique project that brings together the regulatory authorities of Europe, Japan, and the U.S. with experts from the pharmaceutical industry to discuss the scientific and technical aspects of product registration. The objective of such harmonization is to use human, animal, and material resources more effectively, while maintaining a high standard of patient protection.

No single product area has been considered to date in such a setting. However, the ICH process, or a modification thereof, might be considered as an opportunity to support the widest possible registration of an HIV microbicide, perhaps also including the related area of HIV vaccines.
Section IV
Manufacturing, Formulation, and End Use

In addition to the pre-clinical and clinical development challenges outlined in the previous two sections, microbicides face a number of parallel challenges in manufacturing, formulation, acceptability, and end use.

The small size of most development organizations in the microbicides field means that there is little, if any, internal manufacturing capacity. As a result, contract manufacturers must be engaged to produce products to allow clinical testing and to prepare for eventual product launch. While contract manufacturers can allow small organizations to participate in microbicides development, their efficient use requires careful and proactive management. In addition, because many microbicides development efforts are likely to be sub-scale, at least until phase 3, coordination of the manufacturing process among a number of separate entities may allow each participating developer to benefit from lower costs and quicker turnaround times.

The proper formulation of a vaginal microbicide is critical, both for efficacy and for consumer acceptability. Understanding the key issues that drive both effective intra-vaginal coverage and improved usability can help support productive investment decisions and make sure that, once developed and made available, a microbicide product will actually be used.

Key conclusions and recommendations from this section include:

- **Opportunities exist in both manufacturing and formulation to coordinate investment in the field as a whole.**

- **Formulation, in particular, requires particular attention to ensure that an eventual product is as widely used as possible.**

- **Research into the drivers of access and acceptability must be incorporated into the development of microbicide products as early as possible, and mechanisms to ensure that this occurs should be actively investigated.**
Chapter 13. Contract Manufacturing

Identifying a potential manufacturer
There are three key criteria for identifying and selecting potential contract manufacturers for a microbicide product: semi-solid manufacturing experience, accessible location, and multiple manufacturing sites.

To be an effective maker of microbicides, a contract manufacturing organization must have a proven track record of manufacturing large-scale quantities of semi-solid products. Manufacturing semi-solids in large quantities is more complex than simply mixing ingredients together in a vessel, so the potential manufacturer must have experience with the proper equipment and be familiar with a number of unique manufacturing processes.

In addition, the manufacturer should be accessible to the sponsor’s primary facility. Maintaining a well-informed person from the sponsor in the manufacturer’s facility during the actual manufacturing process can often be advantageous. As a result, it is often preferable to be within a reasonable travel distance, to avoid the need for repeated overnight stays.

Finally, identifying at least two potential manufacturing sites can allow a sponsor to compare the benefits and services being offered by a contract manufacturer on a head-to-head basis. Multiple manufacturing sites within the same contract manufacturing organization can also help to avoid or ameliorate supply risks.

Selecting a potential manufacturer
The key elements to consider in selecting a potential contract manufacturer include price, timing, capacity, and compliance. While specific selection criteria may vary depending on the project, these elements are nearly always important. In addition, some selection issues may be influenced by the existence of a coordinated manufacturing agency, as described in the next chapter.

In the event that individual sponsors are contracting on their own behalf, however, price is likely to be the most critical issue. Manufacturing semi-solids according to Good Manufacturing Practice (GMP) guidelines can cost anywhere from US$10,000 to US$100,000 per batch, depending on batch size and the complexity of the process. Most manufacturing firms will need at least ninety days to prepare for the manufacturing run.

The capacity of the manufacturer should also be evaluated. Some extremely large manufacturing firms will only make batches of 100 liters or more. This large size may be inappropriate for a small microbicides development firm.

Finally, if FDA compliance is important for the product being manufactured, the plant chosen must be GMP-compliant and must have been inspected within the last two years. Additionally, for the production of material destined for a phase 3 trial to be submitted to the FDA, the site must have the capacity for commercial-scale production and must have been audited and approved by the sponsor as part of the NDA filing process.

Contracting
Key elements in the contracting process include developing a contract that contains all relevant work scope plans and timelines, completing review by a reputable and trusted attorney, and concluding a signed document before work starts.
Once a manufacturing site has been selected, work should not begin before a contract has been negotiated and signed by both parties. The manufacturer usually draws up the contract in the first instance, spelling out the work detail and payment schedule, so that all activities to take place have been clearly identified and each partner’s responsibility is defined.

**Managing the process**

Once the contract has been signed, the manufacturing process can proceed. In these cases, managing the process means communicating frequently with the manufacturer to ensure that all timelines are met and that the process is progressing according to the sponsor’s requirements. Contract manufacturing firms are important assets to the microbicides field, and to pharmaceuticals in general. Without them, small firms with experimental medications might never get a chance to enter clinical trials. Even so, tight management of the contract manufacturing relationship is critical to ensuring a successful outcome for both parties.
Chapter 14. Opportunities for Coordination

Introduction and background
Manufacturing a microbicide across the several phases of drug development is a complicated process, potentially requiring a great deal of time and coordination. Numerous contractors and subcontractors may be involved, and inexperienced developers undertaking the process alone face many difficulties. The ultimate chance of successfully bringing a product to market may be reduced by working alone, and each step in the process will almost certainly take longer.

Manufacturing overview
Different stages of the drug development process require a wide variety of manufacturing support, in terms of quantities of drug substance produced, necessary testing, and FDA compliance documentation. Manufacturing can generally be split into two broad areas:

- Drug synthesis, or the manufacture of the active pharmaceutical ingredient
- Product production, which includes formulation, packaging, analysis, and testing of a completed microbicide

Few manufacturers offer both synthesis and production services, and it may be necessary to use separate contractors for several functions. Examples include the manufacturing of applicators, the development of analytical methods, the monitoring of clinical supplies, quality-control testing, packaging, applicator filling, and labeling. In general, it is best to choose a single manufacturer who can make the product, perform quality control tests (on raw materials, bulk dosage form, and packaged product), and package the product. If these steps are subcontracted separately, then each step will likely take longer, cost more, and be more difficult to coordinate.

Requirements by phase
Manufacturing requirements for each development phase can vary considerably. For example, in the late discovery phase, drug synthesis is focused on producing small, lab-scale quantities of active substance (about 1 kg per batch, depending on the potency of the active ingredient). Further work at this stage is directed toward developing a commercially viable synthesis process that has a good yield and reasonable costs. As the product moves toward IND submission, the sponsor must begin to coordinate the production of up to thousands of delivery units for early clinical studies, and must select and evaluate possible applicators and package configurations, while considering performance, acceptability, and stability.

During phase 1 and 2 of clinical testing, drug synthesis is focused upon producing larger quantities of active ingredient (up to 10 kg, depending on active ingredient potency). This often requires the transfer of production to a small synthetic contractor with GMP certification, like a university lab. If possible, synthesis pathways in phase 1 should scale easily to facilitate FDA compliance; by characterizing the raw material impurity profile in phase 1 and using the same lots for pre-phase 3 toxicology and phase 3 clinical studies, significant re-work can be avoided. Drug production during this phase is directed toward the production of 1000 to 4000 filled and packaged applicators for each product, although this production may be wasted if the drug trial does not progress from phase 1 to phase 2.

During phase 3 of clinical testing, drug synthesis focuses upon changing to a commercially feasible process, patented if at all possible, and
potentially switching to a larger-scale manufacturer. Drug production during this stage is concerned with producing as many as one million applicators per product per clinical trial arm, improving analytical methods, and changing the formulation process (if necessary) for commercial quantities. Formulation changes may be necessary, depending on the production equipment available, but should be avoided if at all possible, since changes may require repeat toxicology and other safety testing. Formulators and process development scientists from the manufacturer must meet with the developer and the production staff at this stage to ensure a smooth transition to larger-scale manufacturing. Finally, packaging may be changed, if necessary, or adjusted to meet commercial specifications. The manufacturer must be experienced in packaging, since ordering, installing equipment, and training employees can be a long and complicated process.

Finally, during the transition to commercial production, synthesis focuses upon validating processes and producing sufficient quantities of active ingredient for commercial launch, while production must also ramp up to accommodate significantly increased quantities of the final product. A commercial developer is likely to begin absorbing many of these costs once phase 3 testing shows substantial promise.

Cooperation may save money and time
Due to the complexity of drug development, and the number of potential contractors, project managers usually require industry experience and access to consultants and technical experts to efficiently manage the manufacturing process. Since many microbicide developers lack resources and manufacturing experience, cooperation may save the field considerable time and money.

The costs associated with manufacturing a microbicide are generally split into two categories:

1. **Direct costs** are driven by the volume of production. They include materials, such as chemicals; the labor used to make and test the product; and shipping and packaging costs.

2. **Indirect costs** are not driven directly by production volumes. These costs include administrative labor, consulting fees, and travel expenses.

In general, direct costs may be reduced by purchasing raw materials in large quantities to obtain volume discounts, while indirect costs may be minimized by increasing efficiency and reducing the amount of time that organizations spend performing required tasks. Cooperation among microbicide developers is most likely to save money in two ways:

1. Bulk applicator purchasing and agreement on a standard applicator design sourced from a single contractor could save hundreds of thousands of dollars per developer.

2. Indirect cost sharing could potentially save the field up to US$30 million net over ten years if an organization were established to coordinate manufacturing more efficiently than is possible with individual developers.

Figure 7 (see next page) summarizes these savings against the current pipeline of microbicides, assuming no active portfolio management.

**Applicator savings**
Applicators represent a relatively minor area for potential savings. There are two primary ways to save money with applicators: bulk purchasing and avoidance of cost sharing.

**Direct cost savings through bulk purchasing**
Applicator unit prices fall with increasing order volumes (see figure 8), and one way to save money might be to purchase applicators in large quantities. Savings via this method are somewhat limited, though, since most manufacturing-scale savings are realized by the time a single developer purchases a million or more units for a phase 3 trial. Savings beyond the one-million-unit mark are small, ranging between US$0.01 and US$0.03 per unit for volumes above ten million. Consequently, the only realistic savings arise from purchasing phase 1 and phase 2 supplies at the phase 3 unit price.
Applicator unit costs would fall from approximately $0.25 each (for quantities of around 10,000) to $0.08—$0.10 each (for quantities over 1 million).

Purchasing in lots of 1 million or greater could significantly reduce lead times for applicator availability.

Two or more products could be tested in the same clinical study, since they would be available in identical packaging.

Volume guarantees might persuade manufacturers to invest in multiple injection-mold machines to increase production capacity and reduce unit costs even further.

**Avoidance of cost sharing**

When a developer contracts with an applicator manufacturer for a new applicator design, the developer is usually expected to share the cost of new equipment. This investment can be substantial, since new applicator molds cost between US$100,000 and US$200,000, and additional parts and tooling costs can add another US$20,000 to US$50,000 for product package fillers. Using a standard applicator design, sourced from a single manufacturer, could help prevent each developer from incurring these costs separately. Another benefit of using a well designed, high-quality standard applicator would be that additional device testing might be avoided. Each time a different applicator is used with a given microbicide, a number of tests must be conducted to assess potential reactivity between the drug and the applicator materials, toxicity, and stability, among others. These tests may be avoided if the developer limits the number of applicator design changes by developing a model that can be as broadly accepted as possible.

In order to make these savings possible, developers must agree to use a common applicator design, and they will have to reach consensus on a number of specifications and options. Single-use applicators are presumed best for phase 3 trials because they are easier to employ, and reduce the chance that a product will appear ineffective merely due to improper use. Single-use models are also preferred for commercial

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**Figure 7. Net potential savings via coordination of direct and indirect costs**

*GIVEN EXISTING PIPELINE ASSUMPTIONS, THERE MAY BE UP TO $30M IN POTENTIAL SAVINGS OVER TEN YEARS*

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<tr>
<td>4.5</td>
<td>Applicator savings</td>
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<td>6.6</td>
<td>Indirect cost savings</td>
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<tr>
<td>7.0</td>
<td>Total</td>
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<td>33.5</td>
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(1) Figures presented are after subtraction of coordinating cost, estimated at $1.2 million per year, with all developers cooperating

Assumptions: All products developed on schedule, probabilities from clinical trials pipeline model, no portfolio management

(Source: BCG interviews with developers)
production because they are easier, faster, and more convenient to use, albeit more expensive, than other designs. On the other hand, single-use applicators are often bulky, difficult to dispose of, and less ecologically sound. Single-use applicator components and specifications that would require agreement among developers include the following:

- Overall design to include size, shape, cap, and plunger (if any), with particular attention to minimizing any risk of physical trauma to the vaginal mucous membranes
- Barrel material
- Piston materials (e.g., rubber or plastic)
- Design, if any, to improve product distribution in the vagina
- Lubricants, if any, to facilitate piston movement
- Preset pistons to reduce packaging complexity and costs
- Versatility to accommodate different dose sizes for different drugs
- Overwraps for safety, cleanliness, and to extend product shelf life
- Cosmetic characteristics for acceptability

A minimum of two applicator designs will probably be necessary to accommodate different microbicide dose forms and to avoid wasting product due to improper applicator size. Wastefulness becomes an even more important consideration in later development phases and for commercial production, when product unit costs must be minimized.

**Potential indirect cost savings**

Indirect costs arise from activities that developers must perform to manage contract manufacturing, but that are not affected by product volume. Indirect costs amenable to reduction through a coordinated manufacturing approach can be divided into five major categories:

- **Salaries and benefits** paid to full-time developer employees who coordinate, manage, and provide administrative support for contracted manufacturing. The proportions of salaries and benefits allocated as manufacturing indirect costs depend on the estimated time that the employees spend working on manufacturing coordination. Developers generally employ one primary project manager with administrative support for each drug.

- **Travel** expenses are incurred by developers when they visit contractors for evaluation, contracting, process transfer, and production, and also when they employ consultants to perform various functions in conjunction with the manufacturer.

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**Figure 8. Scale effects in applicator purchasing**

Applicators offer scale advantages but most scale realized within single developer phase 3 trial.

However, coordination may still offer incremental advantages.
- **Overhead** costs include expenses like rent, equipment, supplies, and utilities that developers must pay to operate. A share of overhead is allocated as a manufacturing indirect cost based on the time that employees spend on manufacturing-related activities.

- **Cost-sharing** expenses are paid by developers to manufacturers when new equipment must be purchased to accomplish product-specific tasks like processing or analysis. These costs may be saved if frequent switching between contractors can be avoided.

- **Consulting** fees are incurred when developers hire technical experts to perform tasks that their organizations cannot accomplish alone. These functions range from providing legal advice to analytical and process development expertise. Many consulting functions are so highly specialized that a coordinating organization would also be forced to outsource them, making savings in this area quite difficult to achieve.

Considering the current microbicide pipeline without portfolio management, and all clinical phase developers cooperating, a reasonable set of background assumptions indicates that potential savings from these sources could add up to more than US$30 million over the next ten years, net of the estimated cost of the coordinating organization. On the other hand, if the entities were unable to agree on a coordinated approach, an unsuccessful attempt to create a coordination entity might waste significant resources.
Interactions among active ingredients, formulations, and background physiology have been studied extensively for transdermal, oral, ophthalmic, and intramuscular or injectable products, but these interactions for vaginally and rectally administered products are much less defined. However, once the formulation for a product has been developed, subsequent pharmaceutical development, manufacturing, and packaging steps are often more or less routine. This chapter will highlight many of the assessment tools necessary to develop and evaluate the best formulation for a given drug; identify the team members essential to coordinate this work; and estimate the potential cost savings associated with coordinated formulation development and manufacture.

Developing the best formulation
The ideal microbicide product formulation should meet three objectives:

- Rapid release, distribution, and retention of sufficient quantities of active ingredient to permit protection during immediate coitus.

- Long-lasting residence in the vagina or rectum, if applied several hours prior to coitus, so as to permit discreet use. Sufficient quantities of the drug should be present to “coat” the vulvo-vaginal surface without producing adverse effects, disturbing beneficial micro-flora or reducing consumer acceptance due to adverse aesthetics.

- Drug residence time needs to outlast the life of the pathogen following coitus.

Currently, most microbicides are formulated into gels. Water-soluble and stable drugs are easy to formulate as gel products blinded for clinical studies by packaging into pre-filled vaginal applicators. For those drugs in early development that are not stable in water or are not highly water soluble, non-aqueous gels or alternative dosage forms will be necessary. Alternative dosage forms include solids (tablets, suppositories, or dissolving sponges/donuts), emulsions (creams), drug-in-device systems (sponges), films, aerosols, and foams.

Evaluation techniques
Present evaluation techniques for prototype vaginal formulations follow traditional physical and chemical stability laboratory tests, as well as the standard battery of toxicology studies used for transdermal or orally administered products. Additional assessment tools are necessary for vaginally administered drug products. To develop the most effective formulation for a given drug requires knowledge of the likely interactions between active ingredient, formulation, and vaginal physiology.

Assessment tools
- Active ingredient, formulation, and formulated product physical and chemical stability
- In vivo semi-quantitative monitoring, using magnetic resonance imaging (MRI), gamma scintigraphy, visual/fluorescence monitoring, colposcopy and gravimetric measuring
- In vivo assessment of micro-flora, including vaginal cultures from animals and humans
- In vitro evaluation of drug and dosage from:
  - tissue and cell affinity
  - drug/host and drug/pathogen cell binding assays
- tissue adhesive forces and muco-adhesive forces
- sperm function and antimicrobial activity
- Animal testing, including contraception, antimicrobial activity, micro-flora cultures, and toxicity testing
- Trans-epithelial transport, including Franz diffusion cell models using vaginal tissue
- Epithelial cell uptake, including the extent of drug absorption, adsorption, pinocytosis or phagocytosis, and any corresponding physiological or toxicological effects

The formulation team
A cohesive team of scientists and clinicians is needed to establish a comprehensive formulation development plan. A well-organized project manager can ensure team communication and help activities to be run in parallel whenever possible, in order to save time and project resources and momentum. Essential team members for product development include:

- an experienced topical formulation scientist, with support staff;
- analytical chemists, for physical and chemical evaluation of the drug in the prototype formulation. Methods must be developed to fully characterize the drug and possible formulation/active ingredient interactions, including solubility and degradation (the so-called pre-formulation studies);
- analytic and pharmaceutical scientists with Franz cell diffusion expertise, to assess potential local and systemic absorption;
- a toxicologist for safety, bioavailability, retention, and metabolism evaluation, capable of performing the cell uptake studies necessary to characterize local absorption potential and likely toxicities;
- biologists, for biological activity evaluations;
- clinicians for safety, vaginal distribution, and efficacy evaluations.

Potential benefits from coordination
Potential benefits from a coordinating organization might include both improved capabilities and reduced costs.

Improved capabilities

- Performing gap analysis on existing project teams, and suggesting experts to strengthen areas where there are deficiencies.
- Assisting in the assembly of a productive team of scientists to develop and evaluate a series of prototypes for more than one drug.
- Helping to fund investigators or contract labs that will perform vaginal prototype evaluation tests and the ability to build on repeated experience.
- To a limited extent, coordinating specific activities for project teams.
- Establishing collaborations with GMP manufacturers to accelerate the routine process of clinical supply production. This includes establishing long-term rapport with GMP manufacturers, purchasing a dedicated packaging line for microbicides, using a standard vaginal applicator, and establishing a robust clinical supply distribution system.

Cost savings
Cost savings from coordination might be available through:

- reducing learning curve effects as new generations of drugs move through the pipeline, from prototype formula evaluation, through clinical supplies delivery, to clinical sites;
- creating a common FDA manufacturing interface for the microbicide field. As the prototype evaluation process matures, and as in vitro and in vivo correlations are established, fewer phase 1 clinical studies may be necessary. (Only by building trust and familiarity with regulators might this be possible, however.)
increasing donor confidence in the field by demonstrating the ability to assess safety and potential efficacy for new drugs earlier in development, thus saving clinical resources for the best candidates; and

reducing the cost of manufacturing clinical supplies.
Overview
A topical microbicide not only needs to fulfill a minimum set of safety and efficacy requirements, it should also have additional characteristics that will increase its acceptability to end users. Key drivers of acceptability include protection against HIV and the enhancement of sexual pleasure. For many women, the notion of vaginal health and vaginal cleansing is also very important. To date, the most often preferred formulation for microbicides appears to be a gel applied with an applicator. Men’s attitudes toward microbicides can also be important to acceptance by women, particularly in developing countries. Furthermore, in some developing countries, an applicator is necessary, but its disposal may be problematic, while shelf life can be an issue at high ambient temperatures.

The largest potential market segment, or user group, for microbicides is married women at risk because either they or their partners have other partners (a group estimated at 11.7 million women in the U.S., or about 17 percent of those women 15 to 49 years old). In addition, several additional market segments need to be specifically considered because of the impact of their special needs and preferences on product development. These categories include minorities in industrialized countries, adolescents, people engaging in anal sex, sex workers, and women in “dry sex” areas (see figure 9). Menopausal and pre-menopausal women constitute another group with special needs, but for whom the public health impact of an effective microbicide may be less dramatic. This chapter will discuss the unique selling points (USPs), or key drivers, needed to ensure market acceptance for each of these important groups. In addition, for many of these groups, market acceptance is likely to be driven by two or more USPs, both or all of which must be emphasized during product development and eventual market introduction.

Three important market segments are likely to require specific safety studies: sex workers, adolescents, and people who engage in anal sex. In addition, rectal use is also likely to require specific effectiveness studies, which may also be required among sex workers. Social marketing is likely to be necessary, not only to facilitate microbicide acceptance, but also to influence the habit of douching among some market segments.

Characteristics to increase product acceptance
A successful topical microbicide not only needs to meet a set of minimum safety and efficacy requirements (see the IWGM criteria, presented in chapter 4), but should also have additional characteristics to increase its overall acceptance. Based on previous acceptability studies for microbicides, clinical trials conducted to date, and studies on barrier contraceptives, the following characteristics are important for increasing product acceptability and use. Microbicides should:

- not only not disrupt sex, but also have a positive effect on sexual pleasure;
- be distributed over the counter;
- have pharmacokinetics which allow:
  - application prior to intercourse—from several hours to immediately before;
  - efficacy for up to eight hours (one-third of interviewees in the U.S. AGI study would not use a microbicide if it had to be reapplied before each act of intercourse); and
**Industrialized Countries**
- Women in partnership: she or partner has several partners
- Efficacy, safety, latex compatibility
- Positive effect on sexual pleasure
- Anti-HIV
- Contraceptive / non-contraceptive
- Long duration of action
- Extra lubrication
- Easy to use, applicator
- Long shelf life
- OTC
- Low price
- Tasteless
- Stable
- Non-colored or white

**USP Vaginal health + protection**

**Minorities**
Intense use of douches
- Efficacy?
- Acceptability?
- Vaginal health/cleansing

**USP Vaginal health + protection**

**Developing Countries**
- Men’s attitude key to acceptance
- Touching genitalia may be problematic
- Disposal of applicator may be difficult
- Perception of an hygienic effect may increase acceptance
- Film and gel favored formulations
- Stability at high temperatures

**USP Vaginal health + protection**

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**Anal Sex**
- MSM and heterosexual couples
  - High risk
  - Rectal use
  - More lubrication
  - Easier acceptance/use lubricants

**USP Extra lubrication + protection Safe for rectal use**

**Adolescents < 17 years of age**
- Safety?
  - Less vaginal fluid
  - Formulation?
  - Efficacy?
  - Acceptability?

**Acceptability?**
- Not used to vaginal products
- Less vaginal fluid
- Reluctance to touch genitalia
- Ease of use and packaging
- Size of applicator
- Contraceptive

**USP Contraception + protection**

**Dry Sex Regions**
- Lack of vaginal moisture
  - Formulation
  - No extra lubrication
  - Efficacy?
  - Vaginal cleansing

**USP Vaginal health + protection**

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**Sex Workers**
Intensive use → safety
  - absence of mess
  - Douches → efficacy?

**USP Vaginal health + protection**

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**(Pre)menopausal Women**
Vaginal atrophy → affect profile?
  - More lubrication

**USP Extra lubrication + protection**
provide extra lubrication in appropriate sub-populations. The degree of wetness is critical. It should not lead to discharge or messiness, especially if used twice a day or more. Too much lubrication is not acceptable because of messiness, and because it may trigger a negative reaction or interpretation by the partner.

Suppositories are not acceptable for most women: they are too messy, too wet, and tend to melt in hot regions.

Foam is also regularly rejected because of its excessive lubrication and messiness.

The microbicide should also be easy to apply.

Ease of insertion is critical.

The clotrimazole applicator used in previous acceptability studies was appropriate in length (11.4 cm) and color (opaque) for the Brazilian population involved; but, at 1.5 cm, it was too wide for 40 percent of subjects.

In order to offer maximal protection against STD pathogens, the applicator should allow a uniform distribution of the product to both the vagina and the cervix.

In addition, the partner’s support, or at least acquiescence, is often critical for acceptance among women. Male acceptability criteria include:

- safety, particularly long-term safety, and the maintenance of fertility;
- no interference with sexual pleasure, including oral sex;
- no smell; and
- no excessive lubrication.

Microbicide use is recommended to complement condoms, not in lieu of condoms. Therefore, any degree of efficacy will be beneficial for condom users as well as for non-users, as long as the marginal benefit of the microbicide is perceived to be appropriate in relation to its price.

The vital role of formulation

For a microbicide to be safe and effective, the delivery vehicle is critical. There is likely to be no universal formulation acceptable to all users, although a gel appears to be the formulation most likely to be accepted by the largest number of women. To maximize product acceptance, several gel formulations will need to be available, depending upon the environment (e.g., ambient temperature), the active ingredient, and the user’s wetness preferences—unless a “smart gel,” with a wide range of wetness and flow characteristics, can be developed.

An optimal gel should:

- distribute itself over the entire surface of the vagina or rectum, blocking the cervical os;
- be functional as soon as it is applied, despite any lack of ambient fluid;
- mix rapidly and efficiently with the ejaculate;
- retain some degree of coating during and after sex, ideally for up to eight to twelve hours;
- and then be entirely eliminated.

In some acceptability studies, the film formulation was selected by a number of users. However, it is not acceptable for women who prefer not to touch their genitalia, nor is it usable by those who lack vaginal fluid at the initiation of coitus.

Product characteristics by market segment

Women in developing countries

The following characteristics appear to be important for a microbicide to be accepted in developing countries:

- The microbicide must be accepted by men. Men’s attitudes toward microbicides is generally important for women’s acceptance, and particularly so in many developing countries.
- Touching genitalia may not be acceptable in some regions. In these settings, additional
counseling may be necessary or an applicator will be mandatory.

- The disposal of the applicator can be a problem and must be taken into consideration.
- The notion of cleaning the vagina is important, hence the perception of an hygienic product effect may drive its use; however, in some rural areas, women are generally not used to using anything but local natural products for this purpose.
- A range of product formulations will ultimately be needed, given that women’s formulation preferences vary considerably, both within specific populations and between population groups.
- Stability at high ambient temperature and a long shelf life are critical, and may limit the choice of formulations. Tablets or capsules might be best in certain more challenging settings.

Other preferences are less specific—the microbicidal should generally be available over the counter, and should be available either with or without a contraceptive effect. USPs in this population are likely to relate to vaginal health as well as to protection against HIV.

### Minorities in industrialized countries

A key market segment for microbicides will be minorities in industrialized countries. This segment is important because minority women are often at an increased risk for HIV, and are also likely early adopters of microbicides—in part because many minority women are already accustomed to using other vaginal products.

Up to 40 percent of black American women reportedly use douches for vaginal hygiene. This practice has three potential consequences relevant for microbicides:

1. **The immediate use of douches after intercourse might decrease the efficacy of the microbicidal product.**

2. **Women may wish to be able to douche immediately after intercourse, and the ability to do so with a particular product may improve its acceptability.**

3. **Familiarity with vaginal products is likely to facilitate the acceptance of topical microbicides.**

Other than the above points, there do not appear to be specific formulation preferences in this market segment.

The implications of the douching habit are most likely a matter for social marketing. USPs for minority women focus on highlighting the potential cleansing and vaginal health properties of a microbicidal, in addition to a protective effect.

### Young women, particularly adolescents

Young women, particularly adolescents, are also an important group to consider for the development of a microbicide. Adolescent girls represent a very high-risk population for contracting and spreading STIs. They often have multiple partners, and generally do not use condoms (condom use increases markedly with age at first intercourse). Due to cervical ectopy, the cervix of a young woman is biologically more susceptible to infection by gonorrhea, chlamydia, and HIV. In addition, adolescents may be more likely to engage in oral or anal sex, believing that these behaviors carry less risk of becoming infected.

In some parts of sub-Saharan Africa, up to 24% of girls aged fifteen to nineteen are already infected with HIV. In the U.S., 40% of chlamydia cases are reported among teens aged fifteen to nineteen years old; over one girl in ten is infected. Similarly, gonorrhea most dramatically affects teens.

Developing an appropriate microbicide will likely require specific safety and effectiveness studies in adolescents seventeen and younger.

- A safe product in adults is not necessarily safe in adolescents, given anatomical differences: the columnar epithelium extends to the outer
surface of the cervix until it matures, at sixteen to eighteen years of age. This cervical ectopy leads to a greater risk of injury. Once safety has been proven in adults, it will be critical also to assess it in the fifteen to eighteen age group.

- Young adolescents often have less vaginal fluid to dissolve some microbicide formulations. However, the quantity of available vaginal fluid appears to be more related to the lack of sexual experience than to age itself. Limited amounts of vaginal fluid may prevent the use of certain formulations, e.g., films, and might also impact microbicide efficacy as well as acceptability.

Acceptance in this group may also be particularly difficult.

- Adolescents may be particularly reluctant to touch their genitalia: additional counseling or an applicator may be necessary. The clotrimazole applicator mentioned above (11.4 x 1.5 cm), used in a Brazilian acceptability study, was too long and too wide for 40% and 39% of adolescent subjects, respectively.

- Ease of use and packaging will be critical, since adolescents may be more prone to having sex outside the home.

- A contraceptive microbicide would be best; many adolescent girls are more concerned about pregnancy than about STIs.

The USPs for this population should emphasize dual use: both infection prevention and contraception.

**Anal sex**

The risk of HIV infection is greater with anal sex than with vaginal intercourse, and there are very few studies that have actually evaluated condom effectiveness during this activity. Anal sex is frequent among males having sex with males (M SM), bisexual men (M SF/M SM), and among heterosexual couples. Indeed, it has been shown that more than 10% of American women and their partners engage in anal sex with some degree of regularity. In some studies, up to 32% of sexually active heterosexual American women and over 50% of Latin American women have engaged in anal sex, and approximately seven times more women than homosexual men engage in unprotected receptive anal intercourse. About half of sexually active M SM s do not use condoms consistently for anal sex, and the prevalence of HIV infection reaches 30% among African-American M SM s and 15% among Latino M SM s.

The development of an anal microbicide requires specific safety and effectiveness studies in both women and M SM s. Little is known about the necessary rectal mucous membrane coating required to prevent HIV. In addition, the product needs to be effective in an environment in which feces and gas may also be present.

Over 80% of M SM s engaging in anal sex use a lubricant, generally in quite high volumes. In one recent study, 65% of the subjects used two teaspoons or more. Therefore, the volumes at issue—and hence, the required microbicide concentrations—differ substantially from that of vaginal microbicides. Safety issues in the rectum are primarily related to the higher volume of microbicidal product used for anal sex, and to the increased risk of injury to the relatively more sensitive rectal epithelium. For instance, rectal use of nonoxynol-9 containing products has been shown to cause a rapid exfoliation of extensive areas of epithelium, which may in turn increase the risk of infection by HIV or other sexually transmitted pathogens. Favored formulations for rectal lubricants might be gels packaged in tubes with nozzles, like hemorrhoid creams; films that dissolve after insertion, such as Vaginal Contraceptive Film; or formulations that may be applied directly to the penis.

Microbicide acceptance should be quite rapid in the population of M SM s and M SF/M SM s, since an overwhelming majority already use lubricants. However, those who engage in anal sex and do not use lubricants will need to be educated to overcome any concern about the high volumes
of microbicide likely to be required. In several studies, a majority of M SM s and M SF/M SM s have expressed high interest in participating in microbicide studies. The characteristics of the optimal microbicide among this population do not differ significantly from those for a majority of potential users of vaginal microbicides. Effectiveness in preventing HIV and other STIs is the most important feature; physical and secondary effects and the logistics of use come second; and the physical attributes of the microbicide are next. However, cost was shown to be less of a concern in a U.S. study with Latino M SM s. The USPs for this market segment should emphasize protection and lubrication as well as the enhancement of sexual pleasure, in addition to safe and effective rectal use.

Sex workers and victims of violence or coercion
Acceptance and use of a microbicide by sex workers is critical, given their importance in the spread of the epidemic. The HIV infection rate is consistently highest among sex workers, in part because they often do not use condoms with their regular partners, and because they use them inconsistently with clients. Indeed, in many cases, sex workers can charge more for sex without condoms than for sex with condoms.

Specific safety and efficacy concerns are important for this group:

- Safety needs to be specifically evaluated, since sex workers are likely to be exposed to high quantities of the microbicide. Frequent sex increases their risk of epithelial injury, and also the risk of toxicity.

- Several factors may influence efficacy within this group:
  - Many sex workers douche immediately after intercourse. For example, nearly 100% of sex workers in Cameroon report doing so. Douching immediately might decrease either the efficacy of a microbicide or its acceptability (if the user is discouraged from douching). There are no data on the impact of douching on microbicide efficacy.

  - In addition, sex workers in “dry sex” areas may also douche or wipe out the vagina before sex as well, and they may regularly use vaginal drying and tightening agents. These behaviors may well compromise microbicide use and increase condom breakage.

These concerns warrant specific safety and effectiveness studies. Safety and efficacy often cannot be evaluated in smaller sub-groups because the studies are unable to attain sufficient power.

Key acceptance drivers in this group include:

- Modifying the douching habit with social marketing.

- As with most potential users, film and gel applications are the preferred formulations.

- The possibility of covert use is important in the context of coercion and violence. For sex workers, covert use may not be as important as long as the product does not interfere with the client’s pleasure.

USPs in this market segment are likely to relate both to protection and vaginal health.

Women in “dry sex” regions

The implications for a microbicide among this population include:

- A lack of vaginal moisture may prevent the use of certain formulations, particularly films, capsules, and tablets. A gel or a ring that dissolves immediately with semen might be used instead.
- A lack of vaginal moisture as well as vaginal practices to dry or tighten the vagina may affect product efficacy.

- Despite the desire for a dry vagina, a gel formulation was relatively well accepted in one South African trial.

- The notion of vaginal cleaning would be perceived as an important hygienic effect of the product, and increase its acceptability.

USPs here—as for developing countries more generally, minorities in industrialized countries, and sex workers—are likely to involve both vaginal health and protection against HIV.

**Pre-menopausal and menopausal women**

Pre-menopausal and menopausal women also have a set of specific needs and preferences:

- Age-related hormonal changes affect vaginal lubrication, which influences the choice of products. These women are likely to prefer wetter products, which may be perceived as too wet by other sub-populations. More women in this population would be likely to accept a suppository.

- Vaginal atrophy might also alter the safety and efficacy profile of the product.

The USPs within this market segment are likely to relate to the availability of extra lubrication, in addition to the protection conferred by a microbicide.
Chapter 17. Social Science Research: A Critical Element of Product Development

Throughout microbicides development, there are many relevant and critical opportunities for social science research to be incorporated into the “scientific” research process. Social science research is essential to provide product developers with critical information concerning how and whether a given product will actually be used, once approved. Before the end product is available, various social science methods can inform researchers about the conduct and viability of the clinical trial—and provide opportunities for ongoing improvement during the clinical trial process itself. Social science research can be conducted over the course of the development process—prior to clinical trials, parallel to clinical trials, in conjunction with clinical trials, and after clinical trials have been completed.

Prior to, or parallel with, actual clinical trials of a given product, studies can be designed to explore aspects of user preferences, anticipate possible use concerns, and evaluate possible introduction strategies. These kinds of studies can be associated with a specific product under development, or can explore broader issues of acceptability, use, and introduction facing microbicides as a concept. Another aspect of microbicide use that can be explored prior to actual product testing are questions of (male) partner knowledge and participation: what are men’s attitudes toward the concept of a microbicide? To what degree is it important for a woman to be able to use the product without her partner’s knowledge? Or is that even a possibility for most women? Because most microbical formulations will probably not be able to be used completely without a partner’s knowledge, social science research could explore how a woman can introduce and manage use of microbicides with her partner. Finally, social science research prior to actual product development can include studies to increase researchers’ understanding of what characteristics are most likely to support or undermine women’s willingness and ability to use a microbicide, and the cultural and individual preferences that influence them.

Social science research to be carried out in conjunction with the clinical trial structure includes studies that would enroll actual trial participants. These kinds of studies can examine the informed consent process—how well participants truly understand the clinical trial process and concepts such as placebos and randomization, and whether counseling around the informed consent process has any impact on levels of participant understanding. Studies can evaluate women’s concerns that relate to the clinical trial process itself—for example, about having to undergo a colposcopic exam, or being tested for HIV—and explore ways to address concerns and make such procedures more acceptable to participants. Also, during the clinical trial process, social science research can take advantage of the fact that the study product is actually being used to learn about use dynamics in quasi-real life situations. In such ways, social science studies carried out in conjunction with clinical trials can be essential to increasing participant retention rates, thereby offering the potential for improving clinical trial design on an ongoing basis. Such studies can provide researchers with greater understanding of participant motivation and, consequently, improve the quality of data collected and the interpretation of findings.

Social science studies that can be carried out prior to, parallel to, and following approval of a given new product may explore preferences for packaging and applicators, and they may examine strategies around how best to introduce and
position a new product in the market. Introduction strategies to be evaluated should include effectiveness of hierarchical messages, and the impact of method choice on coverage. Studies examining product use can be used to inform product developers about actual microbicide use patterns in a variety of scenarios.

Apart from any specific product, social science research studies can be used to explore how social and cultural norms affect women’s ability to access and use such products in a given regional context. For example, studies should be conducted to examine the development of skills, expertise, and leadership within countries and communities for incorporating microbicides into HIV prevention programs. Social science research conducted outside the structure of clinical trials can be used to define the special needs of populations who are not involved in clinical trials, but who may represent potential users of topical microbicides. This research addresses the individual, interpersonal, and contextual factors that may influence consistent and correct use of the product.

Findings from research examining how best to communicate key concepts like partial effectiveness and hierarchy of protection to users can be used to ensure that women are well informed and able to decide if microbicides are appropriate for them. Research exploring the development and testing of educational and training materials for users, health care providers, and distribution outlets will be critical to ensuring that microbicides are actually used correctly and consistently.

We can also learn how to address the concerns of policymakers and providers and what kinds of social marketing schemes might best address the needs of a given community for education regarding microbicide use.
As demonstrated repeatedly in this report, the microbicides field is currently at the brink of several exciting breakthroughs. Key phase 3 effectiveness trials are nearly up and running, a rapidly maturing set of pre-clinical pathways is being created, and an explosion of basic research and scientific understanding has created deep new insights into ways to interrupt the transmission of HIV and other sexually transmitted infections. The key requirements for managing clinical trials, expanding the available trials infrastructure, and handling ethical concerns in an effective and humanitarian way are both widely agreed upon and manageable.

Investment in this global priority is now needed as never before. Since large-scale pharmaceutical industry involvement is unlikely until a phase 3 trial has demonstrated human efficacy, this investment will be required from public agencies and from private philanthropies. Prioritizing an investment in the microbicides field offers a realistic, manageable, and near-term chance to seize a powerful opportunity to promote the public good.
Those interested in additional information may benefit from consulting the following articles or publications.


