organism using modifications of previously described mammalian cell co-culture. In vitro cultures of *T. pallidum* have now been maintained continuously for over 500 days, with full retention of multiplication rate, motility, structural integrity, and infectivity in a rabbit model. Genome sequencing of long-term in vitro cultured *T. pallidum* has revealed remarkable genetic stability, in that organisms from long-term in vitro culture had identical genome sequences and the same intrastrain heterogeneity observed in the original organisms used for inoculation. We have verified that replacement of Eagle’s MEM with CMRL 1066 as the basal medium was key to achieving long-term growth. Surprisingly, the reducing agent dithiothreitol (DTT) was not required for long-term multiplication in the tissue culture system. We have also examined the effects of the scale of culture, medium composition, and axenic vs. mammalian cell co-culture. Finally, we have utilized limiting dilution to generate clonal isolates of *T. pallidum*, an important first step in developing a system to genetically manipulate the bacterium. Further development of the *T. pallidum* in vitro culture system is likely to have far-reaching effects on many aspects of *T. pallidum* research, including studies of physiology, structure, genetics, gene regulation, antimicrobial susceptibility, pathogenesis, immune reactivity, and epidemiology.

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**S06.3** PIGTAILED MACAQUE MODEL OF STIs

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The Public Health Problem: Sexually transmitted infections (STIs) and their sequelae disproportionally affect young women, with cervical infections frequently ascending to the upper genital tract, leading to reproductive, pregnancy-related and newborn morbidity. Attributes of this Nonhuman Primate (NHP) as a Model: The pigtailed macaque (*Macaca nemestrina*) has several advantages over small animals for evaluating STIs, treatment and prevention. This nonhuman primate undergoes a regular menstrual cycle of 28–30 days and shows hormonal and genital tract changes similar to human females. Her microflora and reproductive tract tissues are similar in constituents and function to those of women. Use of the Model: The female pigtailed macaque model was initially developed in the early 1980’s to simulate human *Chlamydia trachomatis* infection (cervicitis, salpingitis, pelvic inflammatory disease), pathogenesis and disease outcome, which has been key to our understanding of human chlamydial pathogenesis and treatment. The immune responses and histopathological characteristics of infection in this model closely resemble those seen in humans. This NHP model has been expanded to include lower genital tract infections with *Trichomonas vaginalis*, *Mycoplasma genitalium* and simian/human immunodeficiency virus (SHIV). Consequently, this model lends itself to co-infection studies using multiple STIs. Summary and Future Direction: *M. nemestrina* is naturally susceptible to multiple human sexually transmitted infections including *C. trachomatis*, *T. vaginalis* and *M. genitalium*. Pretreatment with exogenous hormones are not required to initiate or sustain these infections. Current model refinement efforts focus on modeling *Neisseria gonorrhoeae* infection. These STI pathogens are unique in that the majority of infections in women are asymptomatic, vaccines are currently unavailable, and concerns about antimicrobial resistance are on the rise. Supported by NIH and CONRAD HHSN27017000151, N01 AI 95388, HHSN272201400016C, HHSN27220100006L, R21 AI 074898, P01 AI 39061, P51 OD010425 and MSA-02–315

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**S06.4** CHLAMYDIA, TRICHOMONAS AND SYPHILIS INFECTIONS IN MACAQUES: EFFECTS ON SIMIAN HIV ACQUISITION

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Epidemiologic studies have linked sexually transmitted infections (STIs) to an increased risk of HIV acquisition. Although the precise mechanism of this association is unclear, it is likely to be a combination of STI-induced local inflammation, disruption of mucosal surfaces, and recruitment of HIV target cells. Given that some experiments are logistically difficult or impossible to conduct in humans, nonhuman primates (NHP) as STI models of enhanced HIV susceptibility are invaluable in understanding mechanisms, magnitude of risk, and evaluating effectiveness of biomedical interventions. Advantages of using NHPs over other animal models include their relatedness to humans and availability of better immunological reagents. We have successfully developed NHP models of both vaginal and rectal STIs, and studied them in the context of simian HIV (SHIV) acquisition and coinfection, and pre-exposure prophylaxis (PrEP) efficacy. We demonstrated that vaginal *Chlamydia trachomatis* (CT) and *Trichomonas vaginalis* (TV) infections increase SHIV acquisition risk while rectal CT infections do not. Also, to study efficacy of Truvada® (the only anti-HIV medication FDA-approved for PrEP), we used a validated STI-NHP model of repeated SHIV exposures to mimic populations at high risk for HIV infection, and demonstrated that oral Truvada® maintained efficacy despite CT-TV infections, albeit with a modest loss of PrEP activity. We showed that another promising anti-HIV injectable, long-acting cabotegravir, maintained complete efficacy against vaginal SHIV acquisition in NHPs infected with CT and TV. However, these are non-ulcerative infections, which led us to develop the first NHP models for rectally and vaginally acquired syphilis, an ulcerative STI. More NHP studies are ongoing to assess risk of vaginal SHIV acquisition and PrEP efficacy in macaques coinfected with syphilis, CT, and TV. These STI-NHP models are also powerful tools to study interactions between STIs, concomitant alterations in clinical manifestations and host responses, and to evaluate specific STI-related interventions, including vaccines.

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