# Single-Cell Sequencing in Human Genital Infections

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**Abstract**

Human genital infections are one of the most concerning issues worldwide and can be categorized into sexually transmitted, urinary tract and vaginal infections. These infections, if left untreated, can disseminate to the other parts of the body and cause more complicated illnesses such as pelvic inflammatory disease, urethritis, and anogenital cancers. The effective treatment against these infections is further complicated by the emergence of antimicrobial resistance in the genital infection causing pathogens. Furthermore, the development and applications of single-cell sequencing technologies have open new possibilities to study the drug resistant clones, cell to cell variations, the discovery of acquired drug resistance mutations, transcriptional diversity of a pathogen across different infection stages, to identify rare cell types and investigate different cellular states of genital infection causing pathogens, and to develop novel therapeutical strategies. In this chapter, I will provide a complete review of the applications of single-cell sequencing in human genital infections before discussing their limitations and challenges.

**Keywords**

Single-cell sequencing · Bioinformatics · Genital Infections · Sexually transmitted infections · Urinary tract Infections

## 17.1 Introduction

Genital Infections (GIs) are one of the most common problems worldwide, which are commonly transmitted via sexual contacts (vaginal, anal, and oral), mother to fetus or mother to child during birth, and imbalance in the vaginal microbiota [1]. It would not be wrong to classify GIs into three different categories i.e., Sexually Transmitted Infections (STIs) [2], Urinary Tract Infections (UTIs), [3] and Vaginal Infections (VIs) [4]. STIs are gonorrhea, chlamydia, syphilis, genital herpes, genital warts, Acquired Immunodeficiency Syndrome (AIDS), and hepatitis B. Bacterial vaginosis, trichomoniasis, and candidiasis are the commonly known VIs and UTIs, respectively, collectively called as vulvovaginitis. UTIs are described as uncomplicated and complicated. Uncomplicated UTIs mostly affect the healthy individuals whereas complicated UTIs are linked with factors that compromise the host defense [5]. GIs are caused by different types of bacteria,

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viruses, and parasites, and associated with infertility, acute illnesses, and complications in the upper genital part. Most curable GIs are caused by bacteria belonging to the gram-negative strain category (*Neisseria gonorrhea*, *Chlamydia trachomatis*, *Escherichia coli*, *Treponema pallidum, Haemophilus ducreyi, and Klebsiella granulomatis* (also known as *Calymmatobacterium granulomatis*) [6]. While virus-mediated infections such as genital herpes, hepatitis B, AIDS, and genital warts are incurable. The complete information about each infection including symptoms and global estimates is given in Table 17.1.

Several difficulties are associated with genital infections. Firstly, life-threatening complications can happen when the untreated GIs disseminate into other parts of the body. For example, untreated gonorrhea, chlamydia, and bacterial vaginosis can cause pelvic inflammatory disease (PID), which can result in long-term reproductive disabilities such as ectopic pregnancy, infertility, and pelvic pain [21]. Furthermore, sexually acquired infections with hepatitis B and certain types of human papillomavirus are considered to be the leading cause of liver cirrhosis, hepatocellular carcinoma, and cervical cancer development, respectively [22, 23]. Some of the STIs induced diseases in the human host are shown in Fig. 17.1. Finally, coinfection with multiple pathogens is another obstacle. A growing body of evidence suggested that both bacterial and viral STIs, as well as bacterial vaginosis (BV), are known to be associated with the probability of HIV acquisition [24–26], and these infections appear to facilitate a vicious cycle of each other's transmission [27–31]. Furthermore, the chances of getting HPV-related cancers are higher in a person living with HIV and HPV coinfection [32].

The treatment therapy for GIs is based on the use of antibiotics such as penicillins, cephalosporins, quinolone, amoxicillin, erythromycin, azithromycin, doxycycline, macrolides, fluoroquinolones, and tetracycline [33]. However, the continuous rise of antibiotic resistance impeded the therapeutical strategies, especially due to the emergence of multidrug-resistant (MDR), extensive drug-resistant (XDR), and pan-drug-resistant (PDR) bacteria, which are resistant against a wide range of available classes of antimicrobial agents [34]. There are several well-defined genetics and mechanistic ways that these disease causing pathogens use to withstand the drug effects i.e., (1) resistance acquired by mutations and horizontal gene transfer and (2) biochemical resistance mechanisms e.g., target modification, antibiotic inactivation and changes in outer membrane permeability [35, 36]. Several studies have reported the increasing cases of antibiotic resistance in genital infection causing pathogens such as *Neisseria gonorrhea*, *Escherichia coli*, *Candida albicans*, Herpes simplex virus (HSV), HIV, etc. [37–42].

Recent advancements in next-generation sequencing technologies and analysis tools to study genomes and transcriptomes in bulk tissues/body fluids have provided new insights into the epidemiology and antimicrobial resistance/susceptibility pattern of genital infection causing pathogens. Reports are published on the applications of single-cell sequencing to investigate various human diseases such as cancer, infectious diseases, brain disorders, etc. [43, 44], however, in the case of GIs, limited information is available. Therefore, the focus of this chapter is to highlight the current applications of single-cell sequencing in human genital infection studies. As single-cell sequencing and analysis methods have already been reviewed in detail elsewhere [45, 46] so I will discuss them briefly in this chapter. Challenges and limitations are also highlighted along with the conclusions.

## 17.2 Single-Cell Sequencing: Different Types and Analysis

Single-cell sequencing has proven an important milestone in diverse fields of biology such as cancer research, metagenomics, developmental biology, reproductive health, drug discovery, immunology, microbiology, and neurobiology [43, 44]. Until the present, this platform has applied to study cell heterogenicity, host–pathogen interactions, clonal structure, clonal expansion and evolution, copy-number variants (CNVs), target

**Table 17.1** Types of genital infections in humans, causative pathogens, symptoms, and global estimates (adapted Passos [7])

| Types | Causative pathogens | Symptoms | Global estimates | References |
|---|---|---|---|---|
| Genital herpes | *Herpes simplex virus* | Genital ulcers | 19.2 million | Looker et al. [8] |
| Syphilis | *Treponema pallidum* | Genital ulcers | 19.9 million | Rowley et al. [9] |
| Gonorrhea | *Neisseria gonorrhoeae* | Urethral discharge, Endocervicitis/pelvic pain, testical pain/ swelling, proctitis, ophthalmia | 30.6 million | Rowley et al. [9] |
| Chlamydia | *Chlamydia trachomatis* | Urethral discharge, Endocervicitis/pelvic pain, testical pain/ swelling, proctitis, ophthalmia | 124.3 million | Rowley et al. [9] |
| Bacterial vaginosis | *Gardnerella vaginalis*, *Mobiluncus* sp., *Mycoplasma hominis*, *Bacteroides*, *Prevotella* sp., *Peptostreptococcus* sp., *Atopobium vaginae* | Vaginal discharge | Limited | Kenyon et al. [10] |
| Candidiasis | *Candida albicans* | Vaginal discharge | 138 million | Cai and Chen [11] |
| Trichomoniasis | *Trichomonas vaginitis* | Vaginal discharge, vulvar irritation | 110.4 million | Rowley et al. [9] |
| Chancroid | *Haemophilus ducreyi* | Papule | 7 million (cases are decreasing) | Steen [12], González-Beiras et al. [13] |
| Lymphogranuloma venereum | *Chlamydia trachomatis,* serovars L1,L2 and L3 | Painless papule | Limited | de Vries [14], Cole et al. [15] |
| Donovanosis | *Calymmatobacterium granulomatis* | Ulcerative lesions | Limited | O'Farrell [16] |
| Genital warts | *Human papillomavirus* | Papular or flat lesions | 160–289 cases per 100,000 person | Patel et al. [17] |
| Acquired immune deficiency syndrome (AIDS) | *Human immunodeficiency virus* | Genital sores | 36.9 million | UNAIDS 2017 global estimate |
| Hepatitis B | *Hepatitis B virus* | Dark urine, abdominal pain | 257 million | WHO 2015 global estimate |
| Scabies | *Sarcoptes scabiei* | Genital papular and itching | >200 million | Chandler and Fuller [18] |
| Urinary tract infection | *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii* | Pain/burning sensation on urination, | 92 million | Global Burden of Disease Study 2013 Collaborators [19] |
| Genital mycoplasmas | *Mycoplasma hominis, M. primatum, M. genitalium, M. spermatophilum, M. penetrans, Ureaplasma urealyticum* | Vaginal itching, pain during sex, burning sensation on urination | Limited | Baumann et al. [20] |

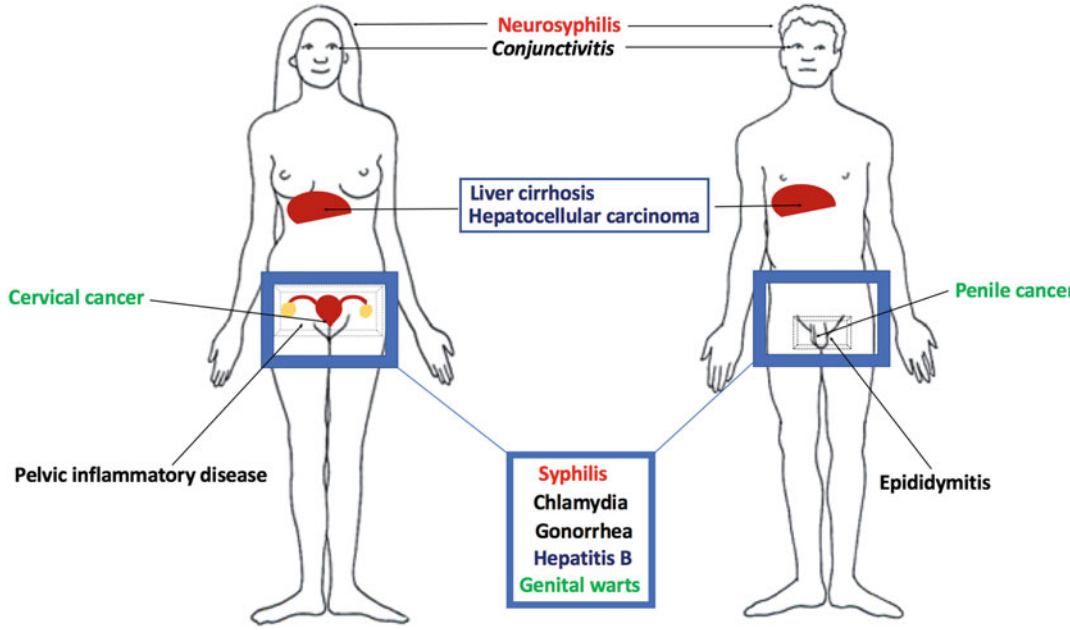



**Fig. 17.1** Different types of diseases that occur in the human host when the untreated STIs disseminate to the other parts of the body. Diseases (neurosyphilis, conjunctivitis, pelvic inflammatory diseases, epididymitis, liver cirrhosis, hepatocellular carcinoma, penile cancer, and cervical cancer) and genital infections (syphilis, chlamydia, gonorrhea, hepatitis B, and genital warts), caused by the same pathogens, are highlighted in similar colors. The basic human body drawings are copied from https://www.clipartqueen.com/human-body-diagram.html (these are free to use for academic purpose). Please note—chlamydia (caused by *Chlamydia trachomatis*) and gonorrhea (caused by *Neisseria gonorrhoeae*) are highlighted in the same color because they both can cause conjunctivitis, epididymitis, and pelvic inflammatory disease

therapy, expression profiling, and RNA splicing [47].

To understand biological processes and how they contribute to a particular disorder, it is important to study genetic regulation at all levels including genome, epigenome, protein, and metabolite. Single-cell sequencing provides the oppertunity to construct a multiomics profile that can give insights to link genotypes to phenotypes of the individual cells. The analysis using single-cell sequencing in any biological and/or clinical study starts with a research question, depending on which the most suitable technology is selected and subsequently sequencing is performed. Finally, the resulting raw sequencing data gets analyzed before interpreting the meaningful information from results (Fig. 17.2).

Single-cell sequencing approaches can be described into two categories i.e., low-throughput and high-throughput. Single-cell microscope, single-cell flow cytometry, single-cell imaging, and single-cell PCR (qPCR and RT-PCR) are some of the low-throughput traditional techniques, with a diverse spectrum of applications, which have been frequently used to detect certain markers of single cells until now [48–50]. However, these methods are only able to uncover the gene expression pattern of a very small set of genes. Thus, these limitations can be easily bypassed by using high-throughput modern global analytical technologies available for single-cell sequencing including single-cell genomics, single-cell epigenomics, single-cell transcriptomics, single-cell proteomics, single-cell metabolomics, and single-cell omics (Box

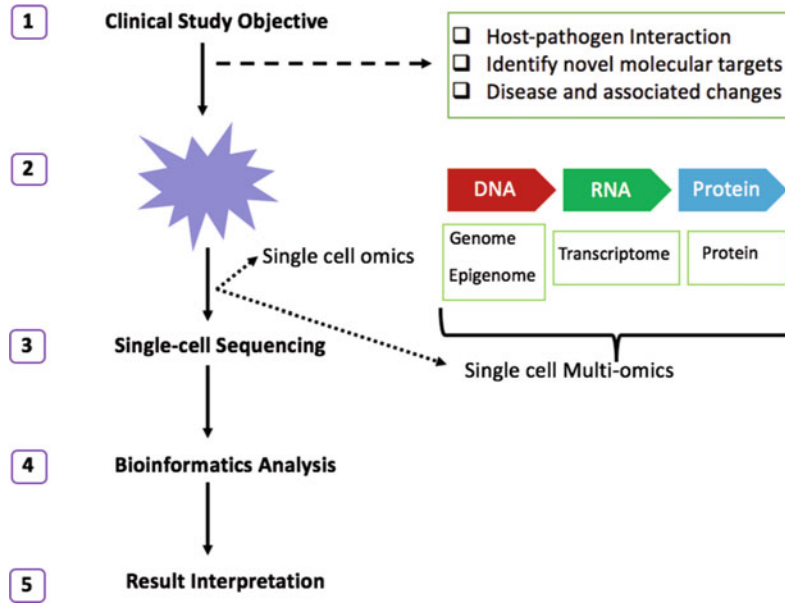



**Fig. 17.2** General flowchart showing the single-cell sequencing use in a clinical application. A typical analysis includes the following steps: (1) Selecting an objective, (2) Single-cell isolation, (3) Selection of "omics" technology and Sequencing, (4) Use of bioinformatics software to analyze the data, and (5) Inferring meaningful information

17.1) [48–50]. A variety of wet-lab and dry-lab analysis tools are chosen depending upon the nature of the research hypothesis [49, 51]. For example, if the objective of the study is to understand the cell heterogenicity and/or lineage tracing in early development then single-cell transcriptomics is the most popular choice (Box 17.1). Besides, the complete single-cell sequencing analysis procedure can be divided into three different steps, (1) selecting and isolating individual cells from the bulk population, (2) single-cell sequencing, and (3) downstream computational analysis. The complete single-cell sequencing workflow and several methods (traditional and modern) for cell isolation, sequencing as well as for bioinformatics analysis are explained somewhere else in detail [44, 52].

**Box 17.1 Summary of Available Single-Cell Sequencing Technologies and Their Applications**

| Technologies | Applications |
|---|---|
| Single-cell genomics | • To resolve variation between individual cells<br>• To study genetic alteration of rare cell types |
| Single-cell epigenomics | • To understand DNA modification as regulatory epigenetic mark<br>• To understand developmental process |
| Single-cell transcriptomics | • To identify novel cellular subtypes<br>• Cell heterogenicity analysis and lineage tracing<br>• Identification of diagnostic primers<br>• To detect emergence of resistance clone during chemotherapy<br>• Gene regulatory network construction |
| Single-cell proteomics | • To study protein–protein interactions<br>• To determine post-translational modifications |
| Single-cell metabolomics | • To understand the phenotypical variations between cells |

| Technologies | Applications |
|---|---|
| Single-cell omics | • To discover novel regulatory mechanisms<br>• To reveal relationship between different omics data types |

Multiple challenges exist for the bioinformatics and statistical analysis of single-cell sequencing data, which needs to fix using two important steps i.e., quality check and normalization, before applying any specialized algorithms. To decide whether the data generated from single-cell sequencing is valid for further analysis or not, several factors need to consider during quality check. For instance, removal of contaminated cells, genes that are always present in low quantity and cells with an elevated mitochondrial gene expression can tremendously increase the quality of the raw data [45, 53, 54]. Furthermore, the normalization step is very crucial to eliminate the batch effects generated due to technical variations.

Moreover, a plethora of open-source computational tools have been developed, often free for academic users, and reviewed previously [46, 54–57]. To check the performance of different single-cell RNA-Seq analysis pipeline and complete workflow describing the best-practice recommendations, please see the recently published literature [58, 59]. Besides, a list of these tools is given in Table 17.2.

## 17.3 Applications of Single-Cell Sequencing to Study Human Genital Infections

Recently, single-cell sequencing technologies have exploited to investigate the bacteria and viruses that cause genital infections. Despite the dominance of bacteria in the STIs, scientists have mainly applied single-cell sequencing to study viral-mediated infections. The most direct applications of single-cell sequencing in genital infections are outlined in Fig. 17.3. In most of the studies reported in this chapter, high-throughput single-cell transcriptomic analysis has utilized. In the following sections, major single-cell sequencing applications are in.

### 17.3.1 Sexually Transmitted Infections

#### 17.3.1.1 Acquired Immunodeficiency Syndrome (AIDS)

HIV is one of the research areas that is greatly benefited from single-cell sequencing. Until the present, this technology has been widely applied to understand cell heterogenicity, clonal expansion, HIV-1 replication cycle, and expressed gene signature in HIV (Fig. 17.3A).

HIV is transmitted sexually and attacks the $CD4^+$ lymphocytes cells that fight against infections. The final stage of HIV infection (if left untreated) is called AIDS. The virus elects some strategies for its survival in the host. One of these strategies is latent reservoirs, using which the virus does not only persist in the infected person but also reactivate the infection if treatment is stopped. While antiretroviral therapy (ART) is the widely accepted treatment to inhibit the HIV replication in the host, ART has not been able to eradicate the virus. Thus, most single-cell sequencing studies of HIV have applied to research on HIV-infected individuals on ART.

Reactivation of latent reservoirs is one of the leading research focus to study viral persistence in host and to identify novel therapeutical strategies for HIV annihilation. To report the heterogeneous effect of latency reserving agents (LRA) on HIV-1 activation, Yucha et al., developed a microfluidic single-cell-in-droplet PCR assay [70]. The assay was then applied to measure the number of transcriptionally active $CD4^+$ T cells from HIV-1 infected individuals on ART [70]. Furthermore, in another study, working on the Interleukin-7 (IL-7) authors indicated that IL-7 stimulates the spread of latently infected cells by enhancing the residual level of viral production in HIV-infected subjects. Therefore, IL-7 is not a suitable candidate for future therapeutical strategies to eradicate HIV [71].

**Table 17.2** List of bioinformatics software and tools available for the single-cell sequencing data analysis

| Types | Software/tools | References |
|---|---|---|
| Pipelines | Scruff, Granatum, BALDR, ScPipe, SINCERA | Wang et al. [56], Zhu et al. [60], Upadhyay et al. [61], Tian et al. [62], Guo et al. [63] |
| R-packages | Seurat, Scran, SingleSplice, OncoNEM, batchelor, infercnv, scds, scMerge, scRecover, scater, monocle, MAST, SC3, AUCell, clusterExperiment, splatter, M3Drop,SCnorm, scmap, TSCAN, scDD, BASiCS, slingshot, cellTree, AneuFinder, scfind, cellity, switchde, MetaNeighbor, DEsingle, cicero, BEARsec, singleCellTK, MIMOSA, sincell, slalom, LineagePulse, cellscape, celaref, bayNorm, scFeatureFilter, scTensor, mbkmeans, Melissa, phemd, cellBench, scds, scRecover, scAlign, Oscope | https://www.bioconductor.org |
| Others | ASAP, SSCC, iS-CellR, ESAT,SC1, bigSCale | Gardeux et al. [64], Ren et al. [65], Patel [66], Derr et al. [67], Moussa and Măndoiu [68], Iacono et al. [69] |

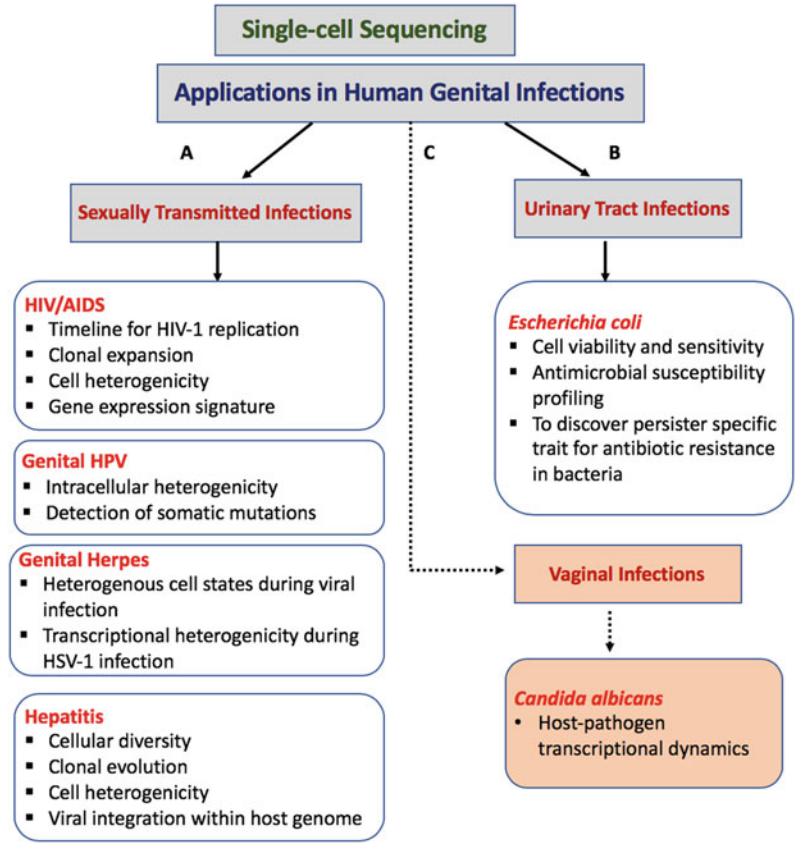



**Fig. 17.3** Applications of single-cell sequencing in human genital infections: Sexually transmitted infections (A), Urinary tract infections (B) and Vaginal infections (C). In the case of vaginal infections (dotted lines with orange box), single-cell sequencing technologies have not directly applied to the samples extracted from human vagina, instead, the experiment was performed on samples extracted from mouse bone marrow

Moreover, clonal expansion is one of the key mechanisms using which the HIV-1 reservoir persists in the latently infected cells [72]. An integral part of the latent reservoir, called provirus, reactivates the dormant infection by producing the unspliced RNA. Evidence of this clonal expansion using single-cell sequencing came from a previous study [73]. In this study, authors

developed a method, called CARD-SGS (cell-associated HIV RNA and DNA single-genome sequencing), to detect the proviral expression of HIV RNA in a single cell isolated from individuals with viremia or on long-term suppressive ART. Interestingly, this study identified different cells producing identical RNA molecules which indicates that these molecules could originate either from single infected cells undergoing clonal expansion or multiple cells infected by the same HIV variant before ART. Therefore, HIV-infected cells can perpetually express HIV RNA during suppressive ART through clonal expansion [73].

HIV-1 replication is another important field that has benefited from single-cell sequencing research. Using single-cell FACS-based and quantitative fluorescence microscopy-based analysis, Holmes et al., revealed the timeline of the key events in the HIV-1 replication cycle and measured the gene expression level in infected cells [74]. Also, significant heterogenicity in the HIV-1 replication cycle length and infected cells generate virions for only a few hours are some of the important observations of this study.

Another area in HIV research where single-cell sequencing has been a major asset is cell heterogenicity. Despite the presence of technical and computational challenges, high-throughput single-cell sequencing approaches opened up new perspectives in HIV latency research. Figure 17.4 highlights the types of different experiments that have conducted using high-throughput single-cell sequencing in the HIV research field.

Using a combination of experiments, Cohn et al., (Fig. 17.4a) purified and characterized single reactivated latent cells from HIV-1 infected individuals on suppressive antiviral therapy [75]. Furthermore, by performing single-cell RNA sequencing (scRNA-Seq) on 227 cells (Control/uninfected; 109, LURE purified $Gag^{+}Env^{+}$; 85, YU2 infected; 33) from three subjects, authors demonstrated several conclusions. Firstly, read sequences from LURE purified $Gag^{+}Env^{+}$ mapped both to the human genome and HIV, and recovery of full-length HIV-1 from the reactivated latent cells is possible by using scRNA-Seq. Secondly, hierarchical clustering segregated the gene expression data into three distinct clusters, indicating the heterogeneous nature of the cells. Moreover, genes associated with HIV-1 latency are highly expressed in LURE purified $Gag^{+}Env^{+}$ cells as compared to control cells. Finally, gene set enrichment analysis of eight (among 240) overlapping genes between clustering and differential gene expression analysis are related to significantly enriched biological processes (immune system function), indicated that in response to the pathogen LURE purified $Gag^{+}Env^{+}$ and control cells are different in their gene expression. However, to reveal more differentially expressed genes and pathways contributing to maintaining HIV latency in the host cells, additional confirmation by performing this analysis on more donors are needed.

Working with the $CD4^{+}$ T cells, Golumbeanu et al., (Fig. 17.4b) reported transcription heterogenicity during the HIV reinfection and latency stage. They also found similar in-vivo expression pattern of 134 gene-specific signature identified among untreated, SAHA (vorinostat), and TCR (T-cell receptor)-treated $CD4^{+}$ T cells isolated from HIV+ individuals, a finding that could benefit LRA (latency-reversing agents) identification [76]. Moreover, a second study in which exploring cellular heterogenicity has been a primary focus includes Rato et al., (Fig. 17.4c), suggesting that the main factor of transcriptional heterogenicity at the single-cell level is cellular activation state, which results into diverse levels of HIV permissiveness. Besides, HIV-permissive cell-specific signature is identified, most of which are involved in innate immunity [77]. Furthermore, using single-cell transcriptomics, Bradley et al., (Fig. 17.4d) revealed a specific set of cellular genes that are associated with the HIV expression during latency. They further identified the latency-associated signature and concluded that viral transcript expression during latency is influenced by the host cell transcriptional program [78].

Another study including virologically suppressed HIV individuals by Farhadian et al., (Fig. 17.4e), identified potentially novel myeloid

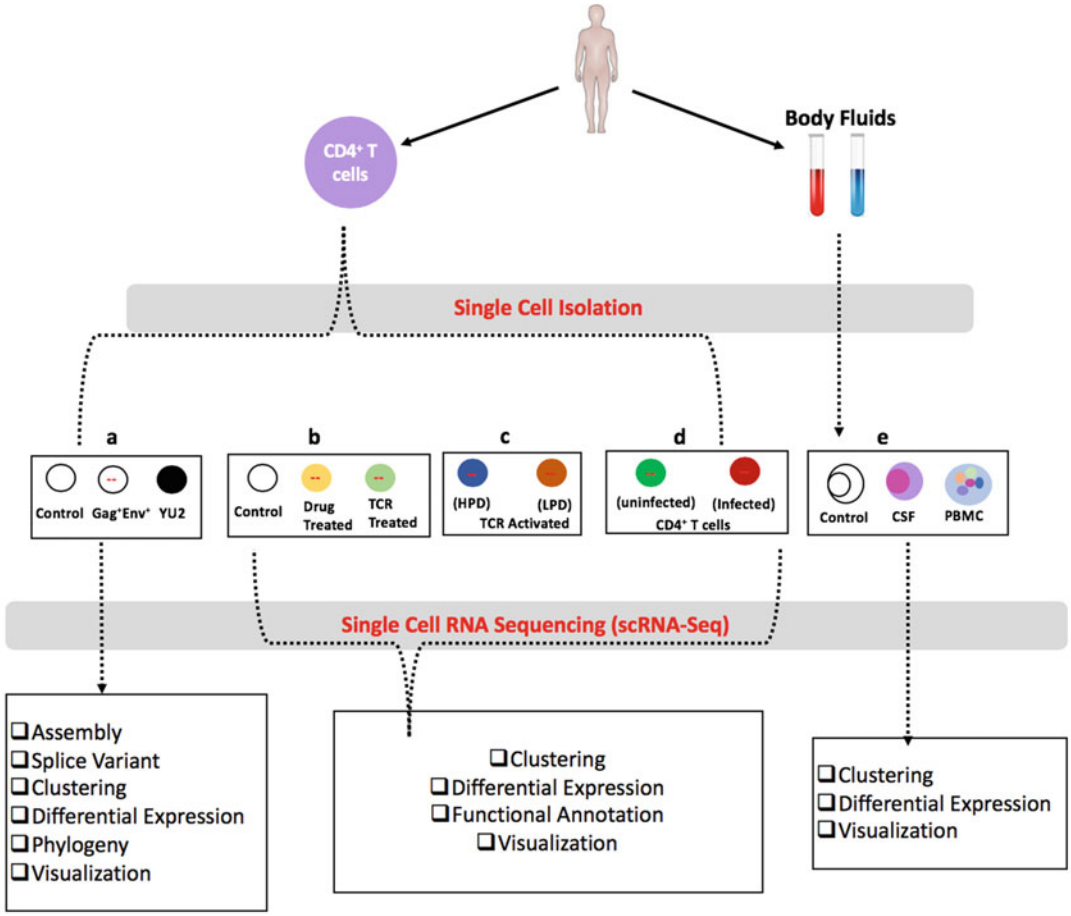



**Fig. 17.4** Types of experiments conducted using high-throughput single-cell sequencing in HIV studies: (a) Cohn et al. [75]; (b) Golumbeanu et al. [76]; (c) Rato et al. [77]; (d) Bradley et al. [78]; (e) Farhadian et al. [79] (only single-cell sequencing transcriptomics experiments are reported from all these studies). $gag^{+}Env^{+}$ cells expressing both *env* and *gag*, *YU2* HIV-$1_{YU2}$, *HPD* high permissive TCR-activated $CD4^{+}$ T cells, LPD low permissive TCR-activated $CD4^{+}$ T cells, *CSF* cerebrospinal fluid, *PBMC* peripheral blood mononuclear cell, *TCR* T-cell receptor

cell populations that are associated with central nervous system (CNS) immune activation by performing single-cell RNA sequencing of blood cells and cerebrospinal fluid (CSF) samples collected at different time points. Furthermore, the gene expression signature present in these myeloid cells intersects with neurodegenerative disease-associated microglia in an animal model. Therefore, indicating a common mechanistic link between neuron injury in HIV and other neurodegenerative disease pathways. The small number of participants is the biggest limiting factor of this study and thus requires a large cohort study to confirm the association between CSF immune cells and markers of neurodegenerative disease [79].

HIV infection impaired the host immune system by attacking the cells such as $CD4^{+}$ T and memory B cells (MBC) that fight against infections. As an example of this, de Armas et al., applied single-cell RT-PCR methods to interrogate the cellular state of MBC from HIV-infected individuals. As a result, gene expression data revealed the overexpression of

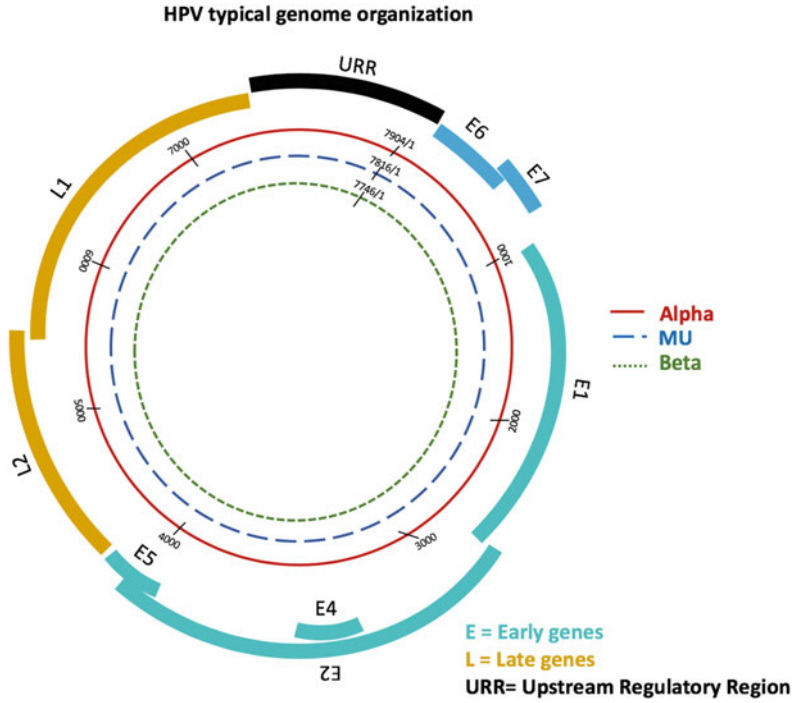



**Fig. 17.5** Typical HPV genome organization for an Alpha, Mu, and Beta. (Adapted Doorbar [81]; Harari [82])

*PTEN* in H1N1-specific MBC from HIV-infected as compared to healthy individuals. Moreover, the authors concluded that as compared to age-matched healthy controls, H1N1-specific MBC demonstrates diverse gene expression signature in HIV, ART suppressed individuals [80].

#### 17.3.1.2 Genital Human Papillomavirus Infection (Genital Warts)

Human papillomavirus (HPV) is transmitted mainly via sexual contacts, but the infected individual's immune system rapidly clear the infection in the majority of cases. There are more than 200 HPV types that have been identified and characterized. Based on their evolutionary analysis, HPV types are categorized into five diverse genera, the majority of which reside in two largest groups (Alpha and Beta) and one smallest group (Mu) [81] (Fig. 17.5). Moreover, depending on their carcinogenicity, HPV types are classified as low-risk and high-risk subtype [83]. Low-risk HPV subtypes are associated with low-grade cervical abnormalities and genital warts, while high-risk HPV subtypes are associated with six anogenital cancers (cervical, penile, anal, vaginal, vulvar, and oropharyngeal) [84]. Few studies have employed single-cell sequencing strategies (Fig. 17.3A) to characterize cell heterogenicity and mutations in human cervical cancer, however, none of them examine genital warts and low-grade cervical abnormalities.

Using HeLa cells, heterogenicity of viral alternative transcripts is revealed by Wu et al. In brief, using a novel platform, called MIRALCS (microwell full-length mRNA amplification and library construction system), single-cell RNA sequencing were performed on 40 HeLa S3 cells to understand the heterogenicity of HPV+ cancer cell lines in gene expression, alternative splicing, and fusion events. Also, at the single-cell level, they classified cells based on cell cycle states and identified that E6 and E7 viral oncogenes were residing in a cluster of the co-expressed gene [85]. Another study in which heterogenicity of HPV status has exposed at a single-cell level include Shen et al., validated the likelihood of single-cell analysis of HPV infection. Moreover,

to verify the coexistence of multiple HPV types, the authors measured the viral load in single cells using qPCR and confirmed the results by performing PCR-based DNA sequencing [86].

Furthermore, Yang et al., investigated the intracellular heterogenicity in tumour cells earlier and after radiotherapy. They performed single-cell whole-genome sequencing on 25 cervical tumour cells including the detection of somatic mutations, virus integration site mapping and clustering. Consequently, they concluded that the HPV integration site in *POU5FIB* might be responsible for radiotherapy resistance [87].

#### 17.3.1.3 Genital Herpes or Genital Ulcer

Herpes Simplex Virus (HSV) is the main cause of genital herpes, a chronic life-long infection in the sexually active population. It belongs to the herpesviridae family of the virus with a linear double-stranded genome ranging from 152 to 155 kb in size. There are two serotypes of this virus i.e., HSV-1 (the main cause of oral lesions) and HSV-2 (the main cause of genital lesions). A growing number of pieces of evidence concluded that genital ulcer cases due to HSV-1 have risen in high-income countries [88, 89].

In the case of HSV, single-cell sequencing has used to highlight the heterogeneous cell states in viral infections (Fig. 17.3A). For instance, in a recent study, Wyler et al. used single-cell transcriptomic analysis to quantify transcripts of human fibroblast at various time points during early HSV-1 infection. Consequently, authors identified cell cycle phases S/G2/M carried more viral transcripts and as compared to cells in the G1 phase provide a more favourable environment to establish the infection [90]. Furthermore, in a separate study, transcriptional heterogenicity has also characterized during HSV-1 infection [91]. By observing differences at the level of viral infection dynamics, gene expression and cellular host response in infected cells, authors concluded that highly infected cells activate multiple developmental pathways due to transcriptional reprogramming [91].

#### 17.3.1.4 Hepatitis

Hepatitis B virus (HBV) causes an acute and chronic infection which transmitted very efficiently through sexual contacts (heterosexual and/or male homosexual contacts). Coinfection with other viral forms i.e., Hepatitis C virus (HCV) and Hepatitis D virus (HDV) have become chronic as well [92]. Like HBC, HCV can be sexually transmitted [93], and both are associated with liver cirrhosis and hepatocellular carcinoma [23].

In the case of hepatitis, single-cell sequencing has proven a powerful tool to study viral integration within the host genome, cellular diversity, clonal evolution, and cell heterogenicity (Fig. 17.3A). In an early study, single-cell RNA viral sequencing was performed to study viral–host interactions during HCV infection to reveal quasispecies diversity in individual cells [94]. Also, the existence of independent evolution at the cellular level indicated by the diverse (in bulk cells population) and unique (in single cells) quasispecies in infected cells.

Furthermore, Eltahla et al., proposed a novel computational pipeline, called VDJPuzzle, to analyze the single-cell transcriptome of flow-sorted Ag-specific $CD8^+$ T cells, and to reconstruct the native TCRαβ [95]. Moreover, to compare the differences in $CD8^+$ T cell exhaustion, Wang et al., performed the reanalysis of the publicly available single-cell sequencing data from chronic hepatitis B (CHB) and Hepatocellular carcinoma (HCC). The authors further confirmed that cell exhaustion exists in both CHB and HCC along with genetic and phenotypic differences [96].

Another study performed single-cell whole-genome sequencing of 96 tumour cells and 15 normal liver cells from patients with HBV-associated hepatocellular carcinoma to gain insight into the intratumor heterogenicity [97]. Also, Chen et al., used single-cell viral capture sequencing to identify tumour heterogenicity from a patient with multifocal HCC without active replication and successfully identified viral integration within the host genome [98].

### 17.3.2 Urinary Tract Infections

Urinary tract infections are caused by both grams negative and positive bacteria. Uropathogenic *Escherichia coli* (UPEC) is the most frequent cause of UTI in humans, affecting mostly women. Treating UPEC has become challenging due to emerging resistance to the commonly used antibiotics.

Although there are other pathogens such as *Pseudomonas aeruginosa* [99], *Klebsiella pneumonia* [100] that cause UTI, *Escherichia coli* is the only bacteria that have benefited with the single-cell sequencing technologies. In the case of UTIs (caused by *Escherichia coli*), single-cell sequencing has applied to study cell viability and sensitivity, antimicrobial susceptibility profiling, and bacterial persister specific trait for antibiotic resistance (Fig. 17.3B).

A previous study [101] used an infected mouse model to understand the host and bacterial characteristics leading to UTI and recurrent UTI. Duraiswamy et al., developed a simple method to isolate pure IBCs (Intracellular Bacterial Communities) from mouse bladder during acute experimental UTI. Using micro pipetting they isolated the individual IBCs and further verified the sensitivity and purity based on microscopy, gene expression, and culture-based methods. Besides, they found $10^3$ viable bacteria in an early IBC (6 h post-infection) [101]. Furthermore, Yang et al., reported the complete procedure to extract IBCs from a mouse infected experimentally in the urinary tract [102].

While antibiotics are the most effective treatment for UTI, recurrent infections (due to antibiotic-resistant bacteria) complicate the situations. Incorrect prescription is one of the key factors involved in the development and spread of antimicrobial-resistant bacterial strains. Certainly, identifying the antibiotic resistance/susceptibility profile of the infecting bacteria could facilitate the use of effective antibiotics. The previous study showed that the susceptibility of an antibiotic can be detected in less than 30 min using single-cell technology (such as imaging, microscope, etc.) [103]. Thereby, these findings further support the development of a point-of-care test to direct the correct UTI treatment.

Bacterial persistence plays a very important role in the evolution of antibiotic resistance and the reoccurrence of infections. Goormaghtigh and Melderen devised a single-cell approach and applied it to analyze the cell persistence in wild-type *E.coli* to ofloxacin in steady-state growth conditions using microfluidics together with fluorescence microscopy. Consequently, after antibiotic treatment, the analysis discovered persister specific traits during recovery [104]. Therefore, this finding could provide valuable insights to tackle the AMR issue in UPEC.

### 17.3.3 Vaginal Infections

Despite the involvement of several pathogens in vaginal infections, researchers have only applied single-cell sequencing to study *Candida albicans* (a commensal residence of healthy human gastrointestinal and urogenital tract, mainly casing candidiasis). Until the present, single-cell sequencing has applied to study host–pathogen transcriptional dynamics (Fig. 17.3C), however, this technology has not directly applied to study *Candida albicans* extracted from human vaginal samples.

Macrophages play an essential role in detecting, engulfing and destroying a pathogen. In a recent study, single-cell transcriptomics is employed to access gene expression variability between host and *C.albicans* [105]. Using four different infected murine macrophages i.e., infected macrophages with dead, phagocytosed *C.albicans*, infected macrophages with live *C. albicans*, macrophages exposed to *C.albicans* that remained uninfected, and *C.albicans* exposed to macrophages that remained unengulfed, authors examined pathogen interactions with host cells. They further observed a tightly coordinated shift in transcriptional dynamics and revealed bimodality in expression and changes in splicing patterns that may direct infection outcomes. Although this study was performed using bone-marrow-derived macrophages from mice model, this data provides useful insight

that could be applied to study host–pathogen interactions during vaginal infections.

## 17.4 Limitations and Challenges

While pure bacterial culture is one of the essential requirements to study pathogenic virulence and antibiotic resistance/susceptibility pattern, inability to grow all of the microorganisms in a manner that enough genetic material can be preserved is one of the main challenges in applying single-cell sequencing technologies to study human genital infections [106]. Low amount of nucleic acid content in a single cell and contamination hinders their sequencing [49], especially in the case of clinical samples where microbial culturing is more difficult and requires specialized training. Sexually transmitted infection causing pathogens such as *Chlamydia trachomatis* and *Treponema pallidum* require special culture conditions. For example, *Treponema pallidum* grows slowly and it proliferates only in laboratory animals (rabbits) and *Chlamydia trachomatis* requires specialized level 3 laboratories [106]. Furthermore, other factors such as single-cell lysis, cDNA synthesis and amplification in the case of microorganisms are particularly challenging due to the presence of a rigid cell wall that inhibits lysis [107]. Thus, appropriate cell isolation strategies need to be selected carefully.

Another significant challenge in single-cell sequencing is to correct technical and biological variations during expression analysis which raises substantial questions throughout the computational analysis. Differences in tissue sampling and handling, and during sequencing when the cells from one condition are cultured and sequenced separately than another are the potential causes of technical variations. Furthermore, the key reasons for the biological variations are the random biochemical reactions, transcriptional bursting causing stochastic gene expression, and uneven genome coverage due to stochastic primer binding and chimeric fragments [108].

Artifacts introduced during library preparation and PCR amplification are responsible for artificial mutations and sequencing bias. These challenges are further exacerbated by the presence of missing gene expression values in the case of some cells which could lead to spurious results, including incorrect data integration and interpretation. Without proper study designs, results can be significantly affected by batch effects. Thus, correcting technical and biological variations is crucial for accurately analyzing the single-cell sequencing data. Finally, it also quite challenging to make sure that only data from single and live cells are included in the downstream analysis so that data from compromised cells do not negatively affect the result interpretation [53].

Moreover, another major limitation is the lack of scRNA-Seq computational analysis pipelines with a graphical user interface that could be easily accessible or useful for users with limited bioinformatics skills.

Finally, the key challenge is related to resource and infrastructure requirements. The emergence of high-throughput single-cell sequencing technologies is responsible for an exponential increase in the amount of data. Efficient approaches are required to store, distribute and analyze this data. Besides, along with storage, data confidentiality, security, and integrity are equally important and needs to be handled properly

## 17.5 Conclusions

This chapter introduced single-cell sequencing technologies and its applications in human genital infections i.e., sexually transmitted, urinary tract, and vaginal infections. Although in the field of viral causing sexually transmitted infections single-cell sequencing has paved the way to a broad range of applications, its use in bacterial causing genital infections is still in infancy. These technologies that are used to analyze genital infections from a single-cell perspective can deliver valuable insights into the discovery of acquired drug resistance mutations, cell to cell variations, deeper interrogation of cellular states and identify rare cell types, where the field of bulk analysis present difficulties. Furthermore, several

limitations such as complications in microbial culturing, biological and technical variations, unavailability of user-friendly computational tools, and proper storage slow down the research.

## Glossary

**Antimicrobial Resistance** The ability of a microorganism to withstand the effect of an antibiotic.

**Antiretroviral Therapy** A treatment to control AIDS with the use of a combination of antiretroviral agents.

**Chronic hepatitis B** When the host body's immune system is not able to clear the infection and the HBV remains in the blood and liver.

**Clonal expansion** The process of unpredictable increase in the number of cells.

**Extensive Drug Resistance** The ability of a microorganism to withstand the effect of at least one agent in all but two or fewer antimicrobial categories.

**Genital Infection** Infection related to human reproductive organs such as vulva, vagina, cervix, urethra, penis, fallopian tube, pelvic, testicles, scrotum, epididymis.

**Hepatocellular carcinoma** Type of liver malignancy that occurs in people with chronic liver diseases caused by hepatitis B and C infection.

**HIV permissiveness** The feature of a cell that supports the growth of HIV.

**Horizontal gene transfer** The process of transfer genetic material between unrelated organisms.

**Latent reservoir** These are the collections of infected immune cells that go into a dormant state and not actively produce new HIV.

**Multidrug Resistance** The ability of a microorganism to withstand the effect of at least one agent in three or more antimicrobial categories.

**Pan-drug Resistance** The ability of a microorganism to withstand the effect of all agents in all antimicrobial categories.

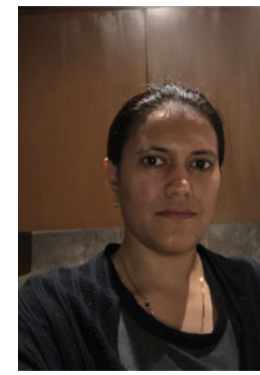
## References


1. Mendling W (2016) Vaginal microbiota. Adv Exp Med Biol 902:83–93
2. Smith L, Angarone MP (2015) Sexually transmitted infections. Urol Clin North Am 42(4):507–518
3. Karam MRA, Habibi M, Bouzari S (2019) Urinary tract infection: pathogenicity, antibiotic resistance and development of effective vaccines against uropathogenic Escherichia coli. Mol Immunol 18:56–57
4. Mashburn J (2012) Vaginal infections update. J Midwifery Womens Health 57(6):629–634
5. Flores-Mireles AL, Walker JN, Caparon M et al (2015) Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 13(5):269–284
6. Chen MY, Tabrizi SN (2015) Challenges to the management of curable sexually transmitted infections. BMC Infect Dis 15:337
7. Passos MR (ed) (2017) Atlas of sexually transmitted diseases: clinical aspects and differential diagnosis. Springer, Cham
8. Looker KJ, Magaret AS, Turner KME et al (2015) Global estimates of prevalent and incident herpes simplex virus type 2 infections in 2012. PLoS One 10(1):e114989
9. Rowley J, Vander Hoorn S, Korenromp E et al (2019) Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. Bull World Health Organ 97(8):548
10. Kenyon C, Colebunders R, Crucitti T (2013) The global epidemiology of bacterial vaginosis: a systematic review. Am J Obstet Gynecol 209(6):505–523
11. Cai YM, Chen XS (2018) Concern regarding bias in estimates of recurrent vulvovaginal candidiasis. Lancet Infect Dis 18(11):1180
12. Steen R (2001) Eradicating chancroid. Bull World Health Organ 79:818–826
13. González-Beiras C, Marks M, Chen CY et al (2016) Epidemiology of Haemophilus ducreyi infections. Emerg Infect Dis 22(1):1
14. de Vries HJ (2019) Lymphoganuloma venereum in the Western world, 15 years after its re-emergence: new perspectives and research priorities. Curr Opin Infect Dis 32(1):43–50
15. Cole MJ, Field N, Pitt R, Amato-Gauci AJ, Begovac J, French PD, Keše D, Klavs I, Lepej SZ, Pöcher K, Stary A (2019) Substantial underdiagnosis of lymphogranuloma venereum in men who have sex with men in Europe: preliminary findings from a multicentre surveillance pilot. Sex Transm Infect 96 (2): sextrans-2019
16. O'Farrell N (2002) Donovanosis. Sex Transm Infect 78:452–457
17. Patel H, Wagner M, Singhal P et al (2013) Systematic review of the incidence and prevalence of genital warts. BMC Infect Dis 13:39

18. Chandler DJ, Fuller LC (2019) A review of scabies: an infestation more than skin deep. Dermatology 235:79–90
19. Global Burden of Disease Study 2013 Collaborators (2015) Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 386 (9995):743–800. https://doi.org/10.1016/S0140-6736(15)60692-4
20. Baumann L, Cina M, Egli-Gany D et al (2018) Prevalence of mycoplasma genitalium in different population groups: systematic review and meta-analysis. Sex Transm Infect 94(4):255–262
21. Brunham RC, Gottlieb SM, Paavonen J (2015) Pelvic inflammatory disease. N Engl J Med 372:2039–2048
22. Ciotti M, Paba P, Bonifacio D et al (2006) Single or multiple HPV types in cervical cancer and associated metastases. Oncol Rep 15:143–148
23. Ringehan M, McKeating JA, Protzer U (2017) Viral hepatitis and liver cancer. Phil Trans R Soc B 372:20160274
24. Juzlova K, Rob F, Zakoucka H et al (2018) The first case of lymphogranuloma venereum in a woman in East-Central Europe and its multiple co-infections. Folia Microbiol 63:93–95
25. Mutagoma M, Nyirazinyoye L, Sebuhoro D et al (2017) Syphilis and HIV prevalence and associated factors to their co-infection, hepatitis B and hepatitis C viruses prevalence among female sex workers in Rwanda. BMC Infect Dis 17:525
26. Peebles K, Velloza J, Balkus JE et al (2019) High global burden and costs of bacterial vaginosis: a systematic review and meta-analysis. Sex Transm Dis 46(5):304–311
27. Dreyer G (2018) Clinical implications of the interaction between HPV and HIV infections. Best Pract Res Clin Obstet Gynaecol 47:95–106
28. Mungati M, Machiha A, Mugurungi O et al (2018) The etiology of genital ulcer disease and coinfections with chlamydia trachomatis and Neisseria gonorrhoeae in Zimbabwe: results from the Zimbabwe STI etiology study. Sex Transm Dis 45 (1):61–68
29. Orlando G, Bianchi S, Fasolo MM et al (2017) Cervical human papillomavirus genotypes in HIV-infected women: a cross-sectional analysis of the VALHIDATE study. J Prev Med Hyg 58:E259–E265
30. Ortiz AP, Tamayo V, Scorsone A et al (2017) Prevalence and correlates of cervical HPV infection in a clinic-based sample of HIV-positive Hispanic women. Papillomavirus Res 4:39–44
31. Reda S, Gonçalves FA, Mazepa MM et al (2018) Women infected with HIV and the impact of associated sexually transmitted infections. Int J Gynecol Obstet 142:143–147
32. Badial RM, Dias MC, Stuqui B et al (2018) Detection and genotyping of human papillomavirus (HPV) in HIV-infected women and its relationship with HPV/HIV co-infection. Medicine 97:14
33. Wagenlehner FME, Brockmeyer NH, Discher T et al (2016) The presentation, diagnosis and treatment of sexually transmitted infections. Dtsch Arztebl Int 113:11–22. https://doi.org/10.3238/arztebl.2016.0011
34. Theuretzbacher U (2016) Global antimicrobial resistance in gram-negative pathogens and clinical need. Curr Opin Microbiol 39:106–112
35. Kapoor G, Saigal S, Elongavan A (2017) Action and resistance mechanisms of antibiotics: a guide for clinicians. J Anaesthesiol Clin Pharmacol 33:300–305
36. Munita JM, Arias CA (2016) Mechanisms of antibiotic resistance. Microbiol Spectr 4(2). https://doi.org/10.1128/microbiolspec.VMBF-0016-2015
37. Bodie M, Gale-Rowe M, Alexandre S et al (2019) Addressing the rising rates of gonorrhea and drug resistant gonorrhea: there's no time like the present. Can Commun Dis Rep 45(2/3):54–62
38. Hamers LR, Rinke de Wit TF, Holmes BC (2018) HIV drug resistance in low-income and middle-income countries. Lancet HIV 5:e588–e596
39. Jiang YC, Feng H, Lin YC et al (2016) New strategies against drug resistance to herpes simplex virus. Int J Oral Sci 8:1–6
40. Mazzariol A, Bazaj A, Cornaglia G (2017) Multi-drug-resistance gram-negative bacteria causing urinary tract infections: a review. J Chemother 29:S1
41. Sheikh AF, Veisi H, Shahin M et al (2019) Frequency of quinolone resistance gene among extended-spectrum ß-lactamase (ESBL)-producing *Escherichia coli* strains isolated from urinary tract infections. Trop Med Health 47:19
42. Zhang JY, Liu JH, Liu FD et al (2014) Vulvovaginal candidiasis: species distribution, fluconazole resistance and drug efflux pump gene overexpression. Mycoses 57:584–591
43. Strzelecka PM, Ranzoli AM, Cvejic A (2018) Dissecting human disease with single-cell omics: application in model systems and in the clinic. Dis Models Mech 11:dmm036525
44. Wang Y, Navin NE (2015) Advances and applications of single-cell sequencing technologies. Mol Cell 58:598–609
45. AlJanahi AA, Danielsen M, Dunbar CE (2018) An introduction to the analysis of single-cell RNA-sequencing data. Mol Ther Methods Clin Dev 10:189–196
46. Hwang B, Lee JH, Bang D (2018) Single-cell RNA sequencing technologies and bioinformatics pipelines. Exp Mol Med 50:96
47. Tang X, Huang Y, Lei J et al (2019) The single-cell sequencing: new developments and medical applications. Cell Biosci 9:53
48. Brehm-Stecher BF, Johnson EA (2004) Single-cell microbiology: tools, technologies, and applications. Microbiol Mol Biol Rev 68(3):538–559

49. Chen Z, Chen L, Zhang W (2017) Tools for genomic and transcriptomic analysis of microbes at single-cell level. Front Microbiol 8:1831
50. Liang J, Cai W, Sun Z (2014) Single-cell sequencing technologies: current and future. J Genet Genomics 14:513–528
51. Chen G, Ning B, Shi T (2019) Single-cell RNA-seq technologies and related computational data analysis. Front Genet 10:317
52. Valihrach L, Androvic P, Kubista M (2018) Platforms for single-cell collection and analysis. Int J Mol Sci 19(3):807
53. Ilicic T, Kim JK, Kolodziejczyk AA et al (2016) Classification of low quality cells from single-cell RNA-seq data. Genome Biol 17:29
54. Yalcin D, Hakgunder ZM, Otu HH (2016) Bioinformatics approaches to single-cell analysis in developmental biology. Mol Hum Reprod 22:182–192
55. Ji F, Sadreyev RI (2019) Single-cell RNA-seq: introduction to bioinformatics analysis. Curr Protoc Mol Biol 127:e92. https://doi.org/10.1002/cpmb.92
56. Wang Z, Hu J, Evan W (2019) scruff: an R/Bioconductor package for preprocessing single-cell RNA-sequencing data. BMC Bioinformatics 20:222
57. Wang T, Li B, Nelson CE et al (2019) Comparative analysis of differential gene expression analysis tools for single-cell RNA sequencing data. BMC Bioinformatics 20:40
58. Luecken MD, Theis FJ (2019) Current best practices in single-cell RNA-seq analysis: a tutorial. Mol Syst Biol 15:e8746
59. Vieth B, Parekh S, Ziegenhain C et al (2019) A systematic evaluation of single cell RNA-seq analysis pipelines. Nat commun 10(1):1–1
60. Zhu X, Wolfgruber TK, Tasato A et al (2017) Granatum: a graphical single-cell RNA-seq analysis pipeline for genomics scientists. Genome Med 9 (1):108
61. Upadhyay AA, Kauffman RC, Wolabaugh AN et al (2018) BALDR: a computational pipeline for paired heavy and light chain immunoglobulin reconstruction in single-cell RNA-seq data. Genome Med 10:20
62. Tian L, Su S, Dong X et al (2018) scPipe: A flexible R/Bioconductor preprocessing pipeline for single-cell RNA-sequencing data. PLoS Comput Biol 14 (8):e1006361
63. Guo M, Wang H, Potter SS et al (2015) SINCERA: a pipeline for single-cell RNA-seq profiling analysis. PLoS Comput Biol 11(11):e1004575
64. Gardeux V, David FPA, Shajkofci A et al (2017) ASAP: a web-based platform for the analysis and interactive visualization of single-cell RNA-seq data. Bioinformatics 33(19):3123–3125
65. Ren X, Zheng L, Zhang Z (2019) SSCC: a novel computational framework for rapid and accurate clustering large-scale single-cell RNA-seq data. Genomics Proteomics Bioinformatics 17:201–210
66. Patel MV (2018) iS-CellR: a user-friendly tool for analyzing and visualizing single-cell RNA sequencing data. Bioinformatics 34(24):4305–4306
67. Derr A, Yang C, Zilionis R et al (2016) End sequence analysis toolkit (ESAT) expands the extractable information from single-cell RNA-seq data. Genome Res 26(10):1397–1410
68. Moussa M, Măndoiu II (2018) SC1: a web-based single cell RNA-seq analysis pipeline. In: 2018 IEEE 8th international conference on computational advances in bio and medical sciences (ICCABS) 2018 Oct 18, IEEE, p 1
69. Iacono G, Mereu E et al (2018) bigSCale: an analytical framework for big-scale single-cell data. Genome Res 28(6):878–890
70. Yucha RW, Hobbs KS, Hanhauser E et al (2017) High-throughput characterization of HIV-1 reservoir reactivation using a single-cell-in-droplet PCR assay. EBioMedicine 20:217–229
71. Vandergeeten C, Fromentin R, DaFonseca S et al (2013) Interleukin-7 promotes HIV persistence during antiretroviral therapy. Blood 121(21):4321–4329
72. Kwon KJ, Siliciano RF (2017) HIV persistence: clonal expansion of cells in the latent reservoir. J Clin Invest 127(7):2536–2538
73. Wiegand A, Spindler J, Hong FF et al (2017) Single-cell analysis of HIV-1 transcriptional activity reveals expression of proviruses in expanded clones during ART. Proc Natl Acad Sci U S A 114(18):E3659–E3668
74. Holmes M, Zhang F, Bieniasz PD (2015) Single-cell and single-cycle analysis of HIV-1 replication. PLoS Pathog 11(6):e1004961
75. Cohn LB, da Silva IT, Valieris R, Huang AS, Lorenzi JC, Cohen YZ, Pai JA, Butler AL, Caskey M, Jankovic M, Nussenzweig MC (2018) Clonal CD4+ T cells in the HIV-1 latent reservoir display a distinct gene profile upon reactivation. Nat Med 24(5):604
76. Golumbeanu M, Cristinelli S, Rato S et al (2018) Single-cell RNA-seq reveals transcriptional heterogeneity in latent and reactivated HIV-infected cells. Cell Rep 23:942–950
77. Rato S, Rausell A, Muñoz M et al (2017) Single-cell analysis identifies cellular markers of the HIV permissive cell. PLoS Pathog 13(10):e1006678
78. Bradley T, Ferrari G, Haynes BF et al (2018) Single-cell analysis of quiescent HIV infection reveals host transcription profiles that regulate proviral latency. Cell Rep 25:107–117
79. Farhadian SF, Mehta SS, Zografou C et al (2018) Single-cell RNA sequencing reveals microglia-like cells in cerebrospinal fluid during virologically suppressed HIV. JCI Insight 3(18):e121718
80. de Armas LR, Pallikkuth S, Pan L et al (2019) Single cell profiling reveals PTEN overexpression in influenza-specific B cells in aging HIV-infected individuals on antiretroviral therapy. Sci Rep 9:2482
81. Doorbar J (2018) Host control of human papillomavirus infection and disease. Best Pract Res Clin Obstet Gynaecol 47:27–41
82. Harari A, Chen Z, Burk RD (2014) Human papillomavirus genomics: past, present and future. In: Human papillomavirus, vol 45. Karger Publishers, Basel, pp 1–18

83. Harden ME, Munger K (2017) Human papillomavirus molecular biology. Mutat Res 772:3–12
84. Forman D, Martel CD, Lacey CJ et al (2012) Global burden of human papillomavirus and related diseases. Vaccine 305:F12–F23
85. Wu L, Zhang X, Zhao Z et al (2015) Full-length single-cell RNA-seq applied to a viral human cancer: applications to HPV expression and splicing analysis in HeLa S3 cells. GigaScience 4:51
86. Shen Z, Liu X, Morihara J et al (2015) Detection of human papillomavirus infections at the single-cell level. Intervirology 58:324–331
87. Yang D, Zhang W, Liu Y et al (2018) Single-cell whole-genome sequencing identifies human papillomavirus integration in cervical tumour cells prior to and following radiotherapy. Oncol Lett 15:9633–9640
88. Lafferty WE, Downey L, Celum C et al (2000) Herpes simplex virus type 1 as a cause of genital herpes: impact on surveillance and prevention. J Infect Dis 181:1454–1457
89. Parra-Sánchez M (2019) Genital ulcers caused by herpes simplex virus. Enferm Infecc Microbiol Clin 37(4):260–264
90. Wyler E, Franke V, Menegatti J et al (2019). Single-cell RNA-sequencing of herpes simplex virus 1-infected cells connects NRF2 activation to an antiviral program. Nat Commun 10(1):1–4
91. Drayman N, Patel P, Vistain L et al (2019) HSV-1 single-cell analysis reveals the activation of anti-viral and developmental programs in distinct sub-populations. elife 8:e46339
92. Brook MG (2002) Sexually acquired hepatitis. Sex Transm Infect 78:235–240
93. Chan PCD, Sun HY, Wong THH et al (2016) Sexually acquired hepatitis C virus infection: a review. Int J Infect Dis 49:47–58
94. Leitch ECM, McLauchlan J (2013) Determining the cellular diversity of hepatitis C virus quasispecies by single-cell viral sequencing. J Virol 87 (23):12648–12655
95. Eltahla AA, Rizzetto S, Pirozyan MR et al (2016) Linking the T cell receptor to the single cell transcriptome in antigen-specific human T cells. Immunol Cell Biol 94:604–611
96. Wang X, He Q, Shen H et al (2019) Genetic and phenotypic difference in CD8+ T cell exhaustion between chronic hepatitis B infection and hepatocellular carcinoma. J Med Genet 56:18–21
97. Duan M, Hao J, Cui S et al (2018) Diverse modes of clonal evolution in HBV-related hepatocellular carcinoma revealed by single-cell genome sequencing. Cell Res 28:359–373
98. Chen XP, Long X, Jia W et al (2019) Viral integration drives multifocal HCC during the occult HBV infection. J Exp Clin Cancer Res 38:261
99. Paz-Zarza VM, Mordani SM, Maldonado AM et al (2019) Pseudomonas aeruginosa: pathogenicity and antimicrobial resistance in urinary tract infection. Rev Chil Infectol 36(2):180–189
100. Behzadi P, Behzadi E, Yazdanbod H et al (2010) A survey on urinary tract infections associated with the three most common uropathogenic bacteria. Maedica 5(2):111
101. Duraiswamy S, Chee LYJ, Chen S et al (2018) Purification of intracellular bacterial communities during experimental urinary tract infection reveals an abundant and viable bacterial reservoir. Infect Immun 86 (4):e00740–e00717
102. Yang E, Chee JL, Duraiswamy S et al (2019) Isolation of single intracellular bacterial communities generated from a murine model of urinary tract infection for downstream single-cell analysis. J Vis Exp 146:e58829
103. Baltekin Ö, Boucharin A, Tano E et al (2017) Antibiotic susceptibility testing in less than 30 min using direct single-cell imaging. PNAS 114 (34):9170–9175
104. Goormaghtigh F, Melderen LV (2019) Single-cell imaging and characterization of Escherichia coli persistence cells to ofloxacin in experimental cultures. Sci Adv 5:eaav9462
105. Muñoz JF, Delorey T, Ford CB et al (2019) Coordinated host-pathogen transcriptional dynamics revealed using sorted subpopulations and single macrophages infected with Candida albicans. Nat Commun 10:1607
106. Lagier J-C, Edouard S, Pagnier I et al (2015) Current and past strategies for bacterial culture in clinical microbiology. Clin Microbiol Rev 28:208–236
107. Zhang Y, Gao J, Huang Y et al (2018) Recent developments in single-cell RNA-seq of microorganisms. Biophys J 115(2):173–180
108. Yuan GC (ed) (2019) Computational methods for single-cell data analysis. Humana Press, Totowa



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