



Molecular Biology Techniques, Inclusive of PCR and Complete Genome Sequencing in HIV and SARS-CoV-2 Pandemics

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Introduction

Molecular biology techniques, inclusive of PCR and complete genome sequencing (WGS), have grown to be effective gear for epidemiological research. PCR techniques shape the premise for lots excessive-sensitivity diagnostic exams and might complement conventional touch tracing and surveillance techniques to outline chance networks and transmission patterns. WGS techniques can in addition outline the causative dealers of sickness, hint the origins of the pathogen, and make clear routes of transmission [1]. When coupled with scientific datasets, along with digital scientific file data, those techniques can inspect co-correlates of sickness and pathogenesis. In the continued HIV epidemic, those techniques had been correctly deployed to discover remedy gaps, transmission clusters and chance elements, aliven though enormous obstacles to speedy or real-time implementation stay crucial to overcome. Likewise, those techniques had been a success in addressing a few questions of SARS-CoV-2 transmission and pathogenesis, however the nature and speedy unfold of the virus have posed extra demanding situations. Overall, molecular epidemiology techniques provide specific benefits and demanding situations that supplement conventional epidemiological gear for the progressed know how and control of epidemics [2].

Description

Fundamental purpose of epidemiology

The fundamental purpose of epidemiology is to discover the causative and correlative elements that power a sickness to permit a rational foundation for contamination prevention and sickness manipulate. This consists of addressing the fundamental questions of what's the causative agent, how is it unfold, who's at chance, in which is it prevalent, whilst is it a threat, and why does it purpose sickness? At the start of the HIV pandemic, those questions have been addressed *via* using touch tracing, case finding, and nicely completed case manipulate studies, the fundamental gear of infectious sickness epidemiologic research. With advances in molecular biology, maximum substantially PCR and gene sequencing, new molecular primarily based totally techniques to carry out epidemiological investigations have been

evolved, frequently at once in reaction to the HIV epidemic itself [3]. Today, molecular epidemiology is crucial to the research of a brand new sickness or sickness outbreak. Indeed, those techniques have been deployed very early with inside the SARS-like coronavirus 2 (SARS-CoV-2) epidemics to tell pressing extraordinary questions of purpose, origin, transmission, and chance [4]. Despite those successes, numerous obstacles to those techniques had been uncovered *via* way of means of those unique epidemics, especially with reference to implementation. Here, we talk a number of the gear of molecular epidemiology, their use and obstacles as implemented to the HIV epidemic, and classes we've discovered to this point in making use of those techniques to SARS-CoV-2 [5]. One of the primary demanding situations confronted *via* way of means of clinicians, public fitness experts, and epidemiologists in the course of a brand new epidemic is the want for correct and touchy diagnostic gear [6]. Traditionally, diagnostic strategies have been closely depending on scientific symptom tracking, records of publicity, and trendy microbiology practices to isolate infectious dealers. Although regularly enough for extracting populace degree trends, those gear by myself are regularly complex *via* way of means of variability in scientific presentation, incomplete scientific histories, inadequate assets or insufficient protocols for microbial isolation, and excessive costs of normal uncertainty. The improvement of molecular diagnostic gear for the detection of unique pathogens, or an immune reaction to unique pathogens, revolutionized our ability to diagnose infectious illnesses appropriately inside huge populations. Although the sensitivity of those exams remains in large part depending on the best and timing of pattern series relative to the contamination time course, their specificity is typically excessive whilst nicely controlled [7].

The first molecular diagnostic device for HIV become an IgG antibody take a look at evolved in 1985, simply 2 years after the isolation and discovery of the virus because the causative agent of AIDS. This first technology take a look at become an ELISA that used HIV-1 inflamed mobileular lysates because the constant antigen over which affected person serum might be implemented. Anti-HIV antibodies might stick with the HIV antigens for detection with IgG-unique secondary antibodies that might be quantified *via* way of means of chemiluminescence readout. In order to rule out fake-advantageous exams (because of pregnancy, autoimmune sickness, and different undetermined reasons) and in addition differentiate HIV-1 from HIV-2, next validations of those outcomes become required *via* way of means of immunoblotting or immunofluorescence [8]. This component trying out set of rules (serology with secondary confirmation) might be delicate with inside the 2D and 1/3 generations to enhance the breadth of HIV subtypes that might be detected and to standardize the antigens used as bait for mass production. Although those algorithms had excessive sensitivity and specificity, their reliance on antibody detection dictated an enormous lag time among publicity and diagnosis. In different words, because of the time it takes for the frame to mount a particular antibody reaction detectable with inside the blood (*i.e.* the time to seroconversion), those exams have been now no longer be capable of come across contamination for 3-12 weeks following publicity. To slim this poor window, fourth-technology exams that included direct antigen detection have been evolved, first turning into to be had in 1997. These exams further depended on ELISA methodology, however for detection of each HIV p24 antigen in addition to anti-HIV antibodies. Fifth-technology exams that protected separate readouts for antigens and antibodies have been evolved in 2015. These exams are typically powerful at

detecting HIV contamination inside 18–45 days following publicity. These later exams additionally allowed for greater speedy and progressed differentiation among HIV-1 and HIV-2 contamination.

Serological exams for diagnostic functions are pretty cheap, have a low barrier to access and may be easily tailored to speedy, at domestic or point of care trying out platforms [9]. These ‘speedy’ exams typically depend upon immunochromatography in which variations in antibody motion withinside the presence or absence of its antigen may be detected *via* way of means of laminar flow. Rapid exams typically have decrease specificity than the conventional ELISA primarily based totally exams, and so require end result confirmation, however permit outreach and trying out to a miles broader populace than in any other case might be accessible. There is presently one FDA accredited speedy self-take a look at for HIV within side the United States (OraQuick), which detects anti-HIV antibodies from an oral swab. Rapid, point-of-care exams also are to be had that use an unmarried drop of blood from a fingertip (*i.e.* Alere Determine, amongst others) [10].

Serological assays

Although serological assays are the encouraged diagnostic exams for HIV, PCR primarily based totally trying out is likewise a useful adjunct in positive diagnostic situations. Rather than detecting the virus-unique antibodies or viral proteins, those exams depend upon detection of viral nucleic acids. In those exams, viral RNA is extracted from blood samples, transformed into complementary DNA (cDNA), and amplified with HIV-unique oligonucleotide primers. If achieved in a quantifiable way, those assays also can be used to decide viral load, which may be a crucial determinant of transmission and pathogenesis. Although those exams are typically greater expensive, greater susceptible to fake positivity than the modern antibody trying out algorithms, and may be situation to longer turnaround times, they may be used to come across HIV contamination inside 3-14 days following publicity, regularly previous to the advent of quantifiable p24 antigen or anti-HIV antibodies with inside the blood. PCR primarily based totally assays are on the whole used as a diagnostic adjunct in instances in which acute contamination is suspected very quickly after an excessive chance publicity (with follow-up antibody set of rules confirmation), for the detection of mother-to-infant transmission, and for surveillance of blood donations. Following acute contamination, a few people can also additionally have viral set factors beneath the restrict of detection for PCR primarily based totally assays, so

serological exams are regularly favored at time factors past the instantaneously post-publicity period. As such, PCR assays for plasma HIV RNA are on the whole used for scientific control and now no longer typically encouraged as a stand by myself diagnostic.

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