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Technologies of the Future for the Clinical Microbiology Laboratory

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Introduction

The clinical microbiology laboratory still extensively relies on conventional techniques, such as culture, phenotypic, and biochemical assays, to identify microorganisms present in clinical specimens, despite technological advancements in laboratory diagnostics. This is caused in part by the clinical laboratory's specimens, which are complicated and diverse. The processing and culture media that are used for bacterial and fungal culture are determined by the specimen type and test order, and they also affect how the results of the culture are interpreted. Due to their higher sensitivity, specificity, and shorter turnaround times (TAT) compared to tests based on viral culture, testing based on molecular techniques have replaced viral culture-based tests in a large portion of clinical virology. Many molecular assays used in clinical laboratories are still created in-house or make use of reagents tailored to certain analytes (ASRs). Although these developments aim to enhance laboratory effectiveness and efficiency as well as the standard of patient care, they are not without problems. Through attrition and a loss of familiarity with fundamental knowledge and skills, such as the qualitative and quantitative streaking of culture media or appropriate work practises to minimise the risk of contamination when working with molecular assays, higher levels of automation of preanalytic and postanalytic processes could potentially reduce technologists' skill sets in those areas. Learning new abilities while retaining competence in traditional procedures is a difficulty in the education of technologists. The well-documented example of accepting new technologies is the change from viral culture to primarily molecular approaches.

Description

In the clinical laboratory, molecular techniques such as PCR, microarray, and nucleic acid sequencing have gained prominence. Through the amplification and detection of particular nucleic acid targets, these techniques allow sensitive and accurate identification of microbes or genetic variations. The a priori target selection restriction inherent to conventional PCR/probe-based assays has been eliminated by recent developments in high-density or massively parallel sequencing technology, which has expanded the diagnostic possibilities of these tests. Molecular diagnostics can produce more accurate diagnoses and shorter turnaround times regardless of methodology. Despite these obvious benefits, molecular diagnostic techniques have several disadvantages. The absence of an appropriate "gold standard" for comparison is a fundamental limitation of all nucleic acid amplification and non-culture-based approaches. When compared to culture methods, molecular and amplified nucleic acid approaches are frequently more sensitive. When specimens are NAAT positive but culture negative during validation of novel molecular tests, this can be troublesome. Utilizing clinical diagnosis as the gold standard is one approach, but when symptoms are vague, it can frequently be challenging to make a conclusive clinical diagnosis. A second verified molecular test that targets a genetic sequence different from the genetic sequence targeted by the test being validated or the use of well-characterized reference samples are two alternative ways for validating a new molecular test [1-4].

Molecular diagnostics in clinical microbiology laboratories today are based mostly on thermostable polymerase (PCR)-based nucleic acid amplification, which was first described. In order to detect and identify microorganisms in clinical specimens that are difficult to culture, such as fastidious bacterial or viral pathogens, it has been proposed to change the definition of the "gold standard" method. These properties include high sensitivity and specificity, an extremely low limit of detection, and rapid results. Although the fundamental idea behind nucleic acid amplification tests (NAATs) hasn't altered, technologies surrounding this idea, such as amplification strategy, amplicon detection, multiplexing of reactions, and automation of the entire process into sample-to-result platforms, have given molecular scientists a wide range of options.Studies of the mechanical properties of DNA polymers under sharp bending conditions are experimentally challenging due to the exceedingly low probability of spontaneous sharp bending caused by the high bending energy of DNA [1,5].

To accurately describe the DNA micromechanics under extreme bending conditions, one must properly enumerate the unusual, strongly bent conformations. The bulk of the methods described in the section before are no longer effective. For instance, single-molecule stretching studies are invalid since acute DNA bending is also prevented by tensile pressures. Despite the fact that AFM imaging measurements have been utilised to explore the likelihood of substantial bending angles across tiny contours, it is questionable if any of the sporadic acute bends found are DNA artefacts. These contentious findings highlight the intricate makeup of the bending stiffness of sharply bent DNA. The j-factor measurements that produced inconsistent results were performed at various temperatures, using various DNA lengths, or using various DNA sequences. As was already established, DNAsurface interactions and imaging analysis introduce uncertainty into AFM imaging investigations. Since DNA samples must be quickly (milliseconds) frozen for cryo-EM imaging research, it is possible that the DNA states before and after the cryo freezing procedure will vary [2,3].

Conclusion

The observed mechanical anomaly in 100 bp minicircles from these looping probability measurements is called into doubt because of the existence of pre-existing nicks and their capacity to promote flexible defect excitation at nicked locations. The abnormally high DNA flexibility shown in these DNA-looping tests may have resulted from a mix of defect excitation at both nicked sites and nick-free sections of the DNA, or it may have been generated solely by nick-dependent defect excitation. By examining the morphologies of the DNA minicircles in cryo-EM pictures, the elasticity of about 100 bp of looped DNA was also researched. Since no localised kinks were found in nick-free DNA minicircles, no flexible flaws were likely to be stimulated by this degree of bending.

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Conflict of Interest

The author reported no potential conflict of interest.

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