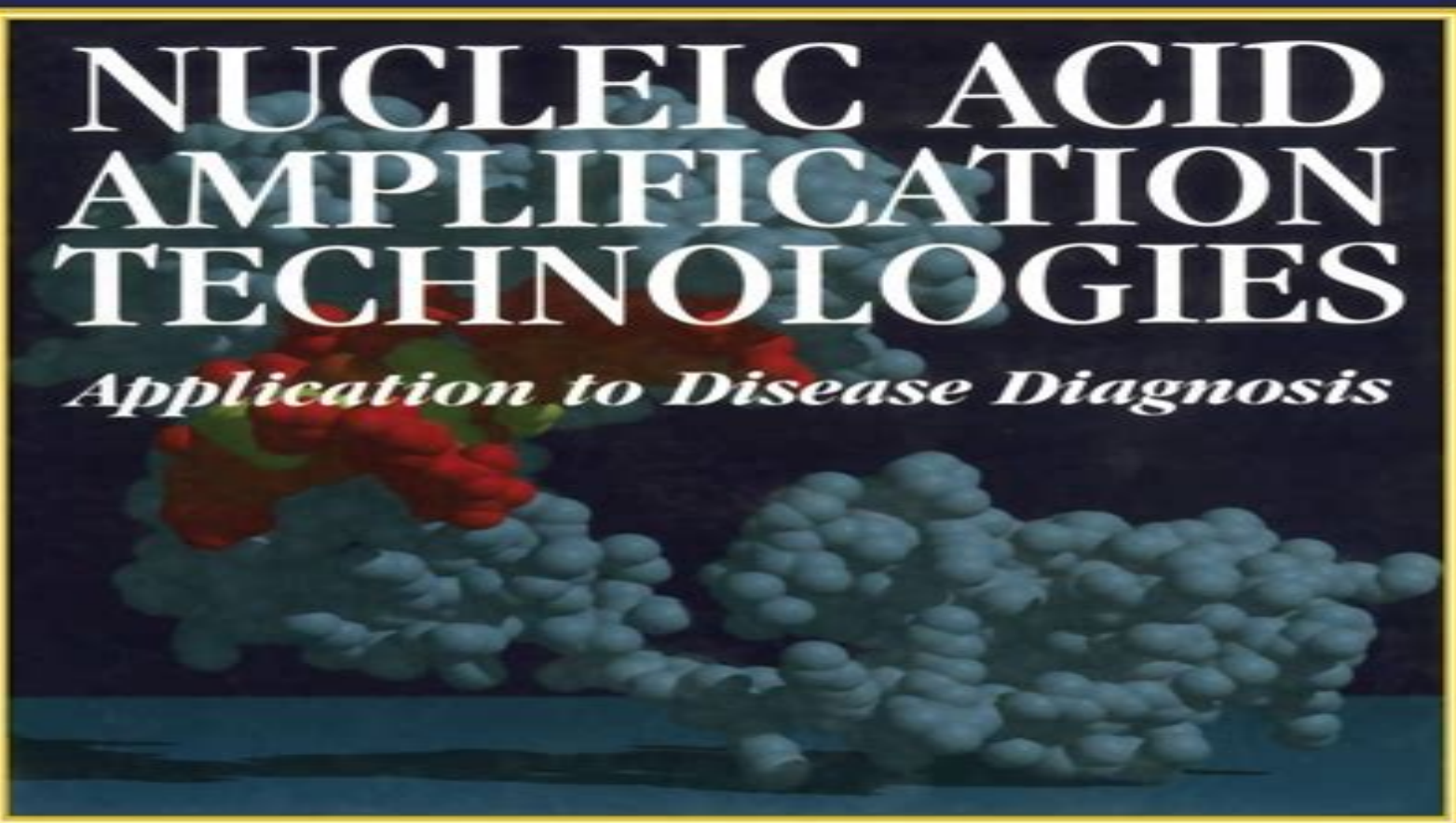


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Nucleic Acid Amplification Technologies:

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PCR amplification with isothermal amplification methods that do not require thermocycling equipment We present two devices that use isotachophoresis and isothermal amplification to extract and amplify nucleic acids from complex samples The first technology is named the NAIL device Nucleic acid Amplification using Isotachophoresis and Loop mediated isothermal amplification NAIL uses ITP and loop mediated isothermal amplification LAMP to extract and amplify nucleic acids from complex matrices in less than one hour inside of an integrated chip Fluid actuation is performed by passive capillary barriers and heated air chambers that act as valves and pumps respectively LAMP amplifies nucleic acids at constant temperature and produces a bright fluorescent signal that can be detected using a mobile phone camera equipped with fluorescent filters We show the limit of detection LoD of pathogenic E coli O157 H7 cells from whole milk samples to be 1000 CFU mL which is a two orders of magnitude improvement to standard tube LAMP reactions with diluted milk samples The second device is called ITP RPA which combines isotachophoresis with the isothermal amplification strategy recombinase polymerase amplification RPA This device offers improvements over NAIL because it simultaneously incorporates sample extraction with amplification removing the need for any pumps valves etc ITP RPA offers detection of nucleic acids in less than 15 20 minutes and uses low cost glass fiber substrates that make it amenable to POC applications We show the detection of nucleic acids spiked into serum and whole blood as we work towards creating a POC viral load monitoring diagnostic for HIV 1 antiretroviral therapy treatments We discuss the design of ITP chemistry that can incorporate the reagents required for RPA as well as how to adapt this chemistry for separating the reaction from sample inhibitors We show the current LOD of the device to be 10 000 copies mL in serum which is one order of magnitude higher than the clinical threshold Future work will aim to improve this value below the relevant threshold The introduction to these thesis discusses diagnostic devices with a focus on nucleic acid diagnostics It also presents the descriptions and relevant principles for ITP and isothermal amplification Chapter 2 reviews and summarizes relevant metrics for developing diagnostic devices in the laboratory as well as transitioning these devices to the clinic Appreciating and using these metrics will benefit test developers by providing consistent measures to evaluate analytical and clinical assay performance as well as guide the design of tests that will most benefit clinicians and patients Discussion topics include analytical laboratory statistics diagnostic sensitivity specificity and clinical thresholds clinical metrics and case studies from relevant commercially available tests

Review of Nucleic Acid Amplification Australian Health Technology Advisory Committee,1997 PCR Technology Tania Nolan,Stephen A. Bustin,2013-06-13 PCR s simplicity as a molecular technique is in some ways responsible for the huge amount of innovation that surrounds it as researchers continually think of new ways to tweak adapt and re formulate concepts and applications PCR Technology Current Innovations Third Edition is a collection of novel methods insights and points of view that provides a critical and timely reference point for anyone wishing to use this technology Topics in this forward thinking volume include The purification and handling of PCR templates The effect of the manufacture

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Rapid and Cost-effective Technologies to Detect the Pathogens in Food and Environment Xuejun Ma,Yi-Wei Tang,Xiyang Wu,Joseph Oliver Falkinham,2022-07-13 Dr Yi Wei Tang is currently an employee of Danaher Cepheid a biotech company produces in vitro diagnostic devices The other Topic Editors declare no competing interests with regard to the Research Topic project

Current and Emerging Technologies for the Diagnosis of Microbial Infections ,2015-11-23

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