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Review Article

A current overview of commercially available nucleic acid diagnostics approaches to detect and identify human gastroenteritis pathogens

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ABSTRACT

Purpose of review: Gastroenteritis is caused by a wide range of viral, bacterial and parasitic pathogens and causes millions of deaths worldwide each year, particularly in infant populations in developing countries. Traditional microbiological culture and immunological based tests are time consuming, laborious and often lack diagnostic specificity and sensitivity. As a result patients can receive suboptimal and/or inappropriate antimicrobial treatment. In recent years, rapid nucleic acid diagnostics (NAD) technologies have become available to complement or even bypass and replace these traditional microbiological culture and immunological based tests.

The main purpose of this review is to describe a number of recently available multiparametric commercial tests, to support the rapid and accurate clinical diagnosis of human gastroenteritis. These state of the art technologies have the ability to identify a wide range of microorganisms associated with enteric gastroenteritis. Following further technological innovation and more comprehensive clinical validation studies, these NAD tests have the potential to impact on the economic burden of health care systems. These rapid NAD tests can also be used to guide improved patient therapy in a timely manner which will reduce the extent of morbidity and mortality associated with these infections globally.

Contents

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1. Introduction

Gastroenteritis remains an important cause of morbidity and mortality and accounts for significant economic and societal loses [1]. Despite improved standards of living, advances in sanitation, water treatment and food safety awareness, an estimated 1.7 billion cases of diarrhoeal disease occur every year [2]. It is considered amongst the leading causes of death in children in developing countries, and with infants under five years of age it is estimated that diarrhoeal disease accounts for approximately 2 million deaths each year [3,4].

Infectious gastroenteritis is caused by a wide range of microorganisms which makes diagnosis of the causative agent of infection challenging using traditional microbiological methods. In developed countries, viral pathogens are considered the most common cause of gastroenteritis in humans [5]. Electron microscopy, and in more recent years antigen based tests have been widely used in virology diagnostic laboratories [6,7]. These methods are laborious and lack specificity and/or sensitivity [5]. Bacterial gastroenteritis also remains a significant cause of diarrhoeal disease worldwide and diagnosis is often limited to culturing on selective media, followed with a subsequent myriad of biochemical tests to identify the causative agent of infection. This can be time consuming (3–5 days), lack specificity and relies on the cultivation of viable organisms [8]. Finally, enteric protozoa are also considered the most important cause of parasitic infection [9]. Diagnosis of parasitic infection often relies on microscopy, which requires trained personnel and in some instances cannot differentiate between pathogenic and non-pathogenic species [10].

To address some of these difficulties in conventional gastroenteritis related diagnostics, a trend in recent years is the development of suites of nucleic acid based amplification techniques (NAAT's) to replace and/or complement traditional microbiological tests. Owing to the sensitivity, specificity and reproducibility of test results, highly multiplexed NAD technologies are becoming the method of choice in many clinical diagnostics laboratories [11]. In this review we aim to describe the current state of the art in molecular diagnosis of gastrointestinal infections. Particular emphasis is focused on multiparametric kits which offer highly multiplexed single test solutions for the identification of human associated gastrointestinal pathogens and also on algorithmic based tests, whereby a series of successive diagnostics tests may be performed to identify a causative agent of infection. Additional emphasis is also placed on fully integrated test platforms i.e. test platforms which have the capability to combine sample preparation, amplification, detection and reporting of the specific microorganism(s) present in a sample [12].

2. Polymerase Chain Reaction

Since its discovery, the Polymerase Chain Reaction (PCR) has become the molecular diagnostics cornerstone in clinical microbiology. In recent years it has been transformed by multiplex real-time PCR which allows for the rapid and accurate quantitative detection of multiple targets in a single closed tube system [13,14]. There are a number of commercially available real-time PCR platforms with single analyte detection kits available such as the Xpert *C. difficile* (Cepheid), BD MAX *C.diff* (Becton Dickenson). However, it is outside the scope of this review to describe all single gastroenteritis pathogen commercially available molecular based tests. Instead, this review focuses on platforms and technologies that have a capability of detecting at least four microorganisms and/or associated antimicrobial drug resistance markers. Below we discuss a number of advantages and disadvantages of a range of recent commercially available test platforms, the list of which may be non-exhaustive.

2.1. RIDA GENE-gastrointestinal kits

R-Biopharm (Darmstadt, Germany) offers a suite of Conformite Europeene – in vitro diagnostic (CE-IVD) marked RIDA GENE-Gastrointestinal kits which utilise multiplex real-time PCR and multiplex reverse transcriptase real-time PCR to detect a range of enteric pathogens (Table 1). Each individual kit has the ability to detect and identify 3–4 bacteria, viruses and or parasitic pathogens respectively [15]. An advantage of these rapid diagnostics tests is that they have been validated on most common real-time PCR platforms and hence can be readily adapted to many clinical diagnostics laboratories for routine use [16]. A disadvantage of these tests is that sample preparation is off line which means there is a requirement for external nucleic acid extraction and purification by the end user prior to use of the test.

2.2. EntericBio real-time Gastro Panel I

The EntericBio real-time Gastro Panel I (Serosep, Limerick, Ireland) is a real-time PCR based kit that allows for the detection of four bacterial enteric pathogens (Table 1). Briefly, this test procedure involves taking a swab from a stool sample and resuspending in a nucleic acid sample preparation solution. The resuspended sample is then heated to 97 °C for 30 min. Samples can then be automatically transferred to wells containing lyophilised diagnostics assay components and sealed. Subsequently, the diagnostics assay is performed using a predefined programme on a LightCycler 480 (Roche Diagnostics, Basel, Switzerland) which allows for the automated amplification, detection and analysis of the data generated [17]. A recent study has reported analytical specificities of 96–100% and sensitivities of 100% depending on the pathogen present in a sample [18]. The main advantage of this kit is that it can be used directly on faecal samples and the sample throughput is high. However, a disadvantage of this test is the relatively low multiplexing capacity.

2.3. Seeplex Diarrhea ACE detection

The Seeplex® Diarrhea ACE detection kit, by Seegene (Seoul, Korea) is a multiplex PCR based test that allows for the detection and identification of 14 viruses and bacteria (Table 1). The test procedure encompasses reverse transcription, 3 multiplex PCR assays utilising proprietary dual priming oligonucleotides (DPO) and subsequent separation and detection of various size PCR products using a capillary electrophoresis device [19]. Recent studies have reported analytical specificies of 96–100% and sensitivities of 40–100% depending on the pathogen present in a sample [19–21]. The main advantages of this test are the ability to detect both bacterial and viral enteric pathogens. A disadvantage of this kit is that no parasitic pathogens are detected by the assays. Also nucleic acid must be extracted and purified off line prior to use of this test [21].

2.4. Faecal pathogens A (16 plex)

AusDiagnostics (Beaconsfield NSW, Australia) offers a multiparametric kit utilising multiplexed tandem PCR for the detection of 16 faecal pathogens (Table 1) [22]. Briefly, multiplex tandem PCR consists of two amplification phases: Firstly a short (10–15 cycles) "primary amplification", which contains highly multiplexed reactions is performed. These products are then diluted and separated onto a 72 well base disc containing individual primer pairs for each of the target microorganisms and subsequently "secondary amplification" for highly specific and sensitive amplification of the targets of interest. This secondary amplification for each target occurs in "tandem" as opposed to traditional multiplexing, which allows for the use of one individual detection dye namely SYBR