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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

Table 1
Commercially available tests.

Test name (manufacturer)	Turnaround time to result	Technology	Regulatory status (FDA approved or CE marked)	Testing location	Number of multiparametric analytes detected	Microorganism panel portfolio
RIDA®GENE-kits (R-Biopharm)	1.5 h per test	Multiplex (rt) real-time PCR	CE marked	Centralised laboratory (high sample throughput)	3–4 bacterial, virus and parasites per test	**RIDA®GENE Bacterial Stool Panel:** *Campylobacter spp.*, *Salmonella spp.* and *Y. enterocolitica* **RIDA®GENE Hospital Stool Panel:** norovirus GI/GII, rotavirus and *Clostridium difficile toxin-genes A (tcdA)* and *B (tcdB)* **RIDA®GENE EHEC/EPEC:** *Enterohämorrhagic E. coli (EHEC)*, *Enteropathogeic E. coli (EPEC)* **RIDA®GENE ETEC/EIEC:** *Enterotoxic E. coli (ETEC)* and *Enteroinvasive E. coli (EIEC)/Shigella spp.* **RIDA®GENE Parasitic Stool Panel:** *Giardia lamblia*, *Cryptosporidium parvum*, *Entamoeba histolytica* and *Dientamoeba fragilis*
EntericBio real-time Gastro Panel I (Serosep)	~1.5 h	Multiplex real-time PCR	CE marked	Centralised laboratory (high sample throughput)	4 bacterial pathogens	*Campylobacter spp.* (*C. jejuni*, *C. coli*, and *C. lari*), *Shiga toxin-producing Escherichia coli (STEC) Shigella spp.*, and *Salmonella spp.*
Seeplex Diarrhea ACE detection (Seegene)	~10 h	Multiplex (rt) PCR	CE marked	Centralised laboratory (high sample throughput)	4–5 viruses and bacteria per test	**The Seeplex Diarrhea-V assay:** Astrovirus, Adenovirus, Rotavirus A, Noroviruses GI/GII **The Seeplex Diarrhea-B1 assay:** *Salmonella spp.*, *Shigella spp.*, *Vibrio spp.*, *Clostridium difficile (toxin B)*, *Campylobacter spp.* **The Seeplex Diarrhea-B2 assay:** *Clostridium perfringens*, *Yersinia enterocolitica Aeromonas spp.*, *E. coli O157:H7* and *Verocytotoxin-producing E. coli (VTEC)*
Faecal pathogens A (AusDiagnostics)	~3 h	Multiplex tandem PCR	No	Centralised laboratory (high sample throughput)	16 viruses, bacteria and parasites	Rotavirus A, norovirus G1and G2, Adenovirus group F and G, *Salmonella spp.*, *Shigella spp.*, *Campylobacter spp.*, *Clostridium difficile (toxin B)*, *Yersinia enterocolitica*, *Aeromonas hydrophila*, *Giardia lamblia*, *Cryptosporidium (parvum and hominis)*, *Dientamoeba fragilis*, *Entamoeba histolytica*, *Blastocystis hominis*
XTAG GPP (Luminex)	~5 h	Multiplex rtPCR, suspension array detection	FDA approved CE marked	Centralised laboratory (high sample throughput)	15 viruses, bacteria and parasites	Adenovirus 40/41, Norovirus GI/GII, Rotavirus A, *Campylobacter spp.*, *Clostridium difficile* (Toxin A/B), *Escherichia coli* O157, *Enterotoxigenic E. coli* (ETEC) LT/ST, *Shiga*-like Toxin producing *E. coli* (STEC) *stx1/stx2*, *Salmonella spp.*, *Shigella spp.*, *Vibrio cholera*, *Yersinia enterocolitica*, *Giardia spp.*, *Cryptosporidium spp.*, *Entamoeba histolytica*
CLART EnteroBac	~5 h	Multiplex PCR and array detection	CE marked	Near patient (high sample throughput)	8 bacterial pathogens	*Salmonella spp.*, *Aeromonas spp.*, *Shigella spp.*, *Escherichia coli [Enterohämorrhagic E. coli (EHEC)*, *Enteropathogeic E. coli (EPEC) Enterotoxic E. coli (ETEC)* and *Enteroinvasive E. coli (EIEC)]*, *Campylobacter spp.*, *Campylobacter jejuni*, *Campylobacter coli*, *Clostridium difficile (toxin B)*, *Yersinnia spp.*, *Yersinia enterocolitica*
Enteric Pathogens [EP] Test (Nanosphere)	~2 h	PCR, hybridisation to gold nanoparticle	No	Near patient (high sample throughput)	9 viruses, bacteria and toxins	Norovirus, Rotavirus *Campylobacter spp.*, *Salmonella spp.*, *Shigella spp. Shiga Toxin Gene (stx1and stx2)*, *Vibrio spp.*, *Yersinia enterocolitica*
Filmarray GI panel (Biofire)	~1 h	Nested PCR, Multiplex rtPCR, meltcurve analysis	FDA approved	Near patient (low sample throughput)	23 viruses, bacteria and parasites	Adenovirus F40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, Sapovirus (I, II, IV and V), *Campylobacter spp. (jejuni, coli and upsaliensis)*, *Clostridium difficile* (Toxin A/B), *Plesiomonas shigelloides*, *Salmonella spp.*, *Yersinia enterocolitica*, *Vibrio spp.* (*parahaemolyticus*, *vulnificus*, and *cholera*), *Vibrio cholera*, *Enteroaggregative E. coli* (EAEC), *Enteropathogenic E. coli* (EPEC), *Enterotoxigenic E. coli* (ETEC) *lt/st*, Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*, *E. coli* O157, *Shigella*/Enteroinvasive *E. coli* (EIEC), *Cryptosporidium spp.*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia lamblia*

green [23,24]. This approach has been successfully applied for the detection of fungal pathogens from blood, enteric protozoa from clinical stool samples and other diarrhoeal pathogens from stool samples [24–26]. An advantage of this test is the ability to rapidly detect a large number of viruses, bacteria and parasites. A disadvantage of this test is that nucleic acid must be extracted and purified off line prior to use of this test.

3. End point PCR – array technologies

Traditional PCR and real-time PCR based assays are limited by their ability to only detect 5–6 analytes per test. While new adaptations, such as tandem PCR above, may help to increase the number of analytes detected, alternative detection platforms are more commonly used to achieve multiparametric detection of pathogens. For example, there are a number of commercially available tests for the identification of faecal pathogens, which utilise multiplex PCR followed by hybridisation to microarray/macroarray. Examples of some of these technologies are outlined below.

3.1. Luminex Gastrointestinal Pathogen Panel

The Food and Drug Association (FDA) approved and CE-IVD marked Luminex Gastrointestinal Pathogen Panel (xTAG GPP) is a qualitative assay which allows for the detection of a broad range of virus, bacteria and parasites in a single test (Table 1). This test procedure includes nucleic acid extraction, multiplex PCR and reverse transcriptase PCR, subsequent hybridisation to a bead array, and detection on the Luminex 100/200 or Magpix instrument. Recent studies have demonstrated the test sensitivity range to be between 90 and 100%, depending on the pathogen present, and specificity range between 91 and 99% [27,28]. The main advantages of this test are the high sample throughput that can be achieved; the number of different analytes detected by the test; and also the ability to determine co-infection in patients [29–31]. However, a disadvantage of the test is it is not an integrated platform. There is a requirement for an off line nucleic acid extraction and also post PCR amplification handling of samples which increases the potential likelihood of cross contamination giving rise to false positives [30].

3.2. CLART EnteroBac

CLART EnteroBac (Genomica, Madrid, Spain) is a PCR array based technology that allows for the detection and identification of 8 bacteria to the genus and species level (Table 1) [32]. The test is proposed for use directly on stool without the requirement for any sample enrichment. The test procedure utilises DNA extraction, multiplex PCR, hybridisation to low density microarrays and subsequent automated reading and interpretation of results [32]. An advantage of this technology is that it is the high throughput and initial manufacture performance data is promising [33]. However, a disadvantage of the CLART Enterobac test is that it is limited to the detection of bacterial pathogens.

4. Integrated platform tests

In the context of molecular diagnostics, platforms which are fully integrated to generate “sample in to result out” offer significant advantages in a clinical setting. For example, they typically require less hands on time; are often more user friendly to perform; reduce the requirement for highly trained personnel; and with automated recording and reporting of results they are often less subjective. In recent years, many commercial entities have strived to provide such platforms, and in the context of faecal pathogen detection, a number of such tests are described below.

4.1. Enteric Pathogens (EP) Test Nanosphere’s Verigene

The Verigene Enteric Pathogens (EP) Test (Nanosphere) has been developed for use on the FDA approved Verigene platform. Briefly, this approved platform consists of the Verigene Processor and a Verigene reader. The Verigene processor is used for extraction and purification of nucleic acid, amplification if required and hybridisation of sample to gold nanoparticle conjugated capture probes immobilised on a glass slide. Upon completion of hybridisation, the slide is transferred to the Verigene Reader for result interpretation [34]. The EP test, designed for use on stool, has the ability to identify a number of viruses and bacteria (and associated toxins) and is reported to provide a result in approximately 2 h (Table 1). An advantage of this kit is the little hands on time required to perform the test and the fact it is integrated from sample to result. While each Verigene reader can only process one sample at a time, sample throughput can be increased if multiple processors are linked to each individual reader.

4.2. Filmarray GI panel

The Filmarray platform (Biofire Diagnostics, Salt Lake City, UT, USA) is a fully integrated system including sample preparation, nested RT-PCR followed by multiplex PCR and detection by melt curve analysis. The Filmarray GI panel, which was recently FDA approved, is intended for use of the Filmarray platform and allows for the detection of 23 viruses, bacterial and parasitic pathogens (Table 1) [35]. An advantage of this kit is the little hands on time required to perform the test and the fact it is integrated from sample to result [31]. Furthermore, the filmarray GI panel detects and identifies the greatest number of viruses, bacteria and parasites on the market at present. However, a disadvantage of the Filmarray platform is that sample throughput is low [36].

5. Future prospectives

This review focuses on recent developments in commercially available nucleic acid based tests for pathogens associated with human gastroenteritis. Alternative technologies such as PCR mass spectrometry and next generation sequencing also have the potential for use in this disease setting. For example, Abbott Molecular has developed the CE-IVD marked Plex ID system which combines PCR with electrospray ionisation mass spectrometry for the detection of a wide range of microorganisms [37]. While there are currently no commercially available kits for the specific detection of microorganisms associated with human gastroenteritis based on these platforms, such approaches will be developed further in the future.

6. Concluding remarks

In recent years, there has been a growing trend to adapt NAAT’s to complement traditional microbiological testing methods, or where possible bypass and replace such methods in the clinical setting. This is partly due to the increased specificity, sensitivity and turnaround time to result. However, the requirement for highly trained personnel, cost and infrastructure requirements remain limiting factors for routine use of NAAT’s in a clinical setting [38]. In an effort to combat some of these limitations, multiplex assays, and more recently integrated multiparametric platforms, have been described for the identification and management of syndromic disease [31,39]. Owing to the wide spectrum of microorganisms associated with gastroenteritis, rapid detection and identification of the causative agent of infection remains challenging. In this disease setting, multiparametric tests which have the ability to

identify viruses, bacteria and parasites are particularly useful. They can aid in rapid high throughput routine screening directly from stool; monitor epidemiological outbreaks; and ultimately guide patient therapy through improved antimicrobial stewardship and management in a timely manner.

In this review, we have described a range of state of the art commercial gastroenteritis diagnostics tests which vary greatly in both the number and type of pathogens which can be detected and identified. The true clinical utility and validation of many of these tests remains unproven until more extensive peer reviewed studies defining performance characteristics, including diagnostic specificity and sensitivity are described. Further development of automated multiparametric integrated NAAT systems which can identify viruses, bacteria, parasites and also some drug resistance markers, will result in the routine use of molecular tests for gastroenteritis in a clinical setting in the near future.

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