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Evaluation and performance comparison between two commercial multiplex gastroenteritis diagnostic systems in a routine laboratory setting

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Master Degree Project in Infection Biology, 45 credits. 2021

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1. Abstract

Background: Gastroenteritis is a common infection and the leading cause of morbidity worldwide and is mostly caused by viruses. Outbreaks appear in both developed and developing countries and result in large economic costs. Rapid detection is important for appropriate treatment, control and to prevent the spread of infection.

Objective: Evaluation and performance comparison between the BioFire® FilmArray® Torch System gastrointestinal panel and the Molecular BD MAX™ enteric viral panel to indicate a multiplex method for viral gastroenteritis diagnostic in a routine laboratory setting.

Material and methods: In this study, 58 different samples were used which consisted of selected stool specimens from patients who were tested and treated for gastroenteritis infection at Uppsala Academic Hospital and Norrlands University Hospital in Umeå during 2018-2021, samples from Quality control for molecular diagnostics viral gastroenteritis EQA pilot study during 2018-2019 and cultivated strains of different adenovirus species from 2018. All samples were analyzed with both systems for comparison of detected pathogens.

Results: Sensitivity and specificity values were 95% and 100% respectively for the BioFire® FilmArray® Torch System and 100% and 93.3% for the BD MAX™ System.

Conclusions: Both systems are rapid and adequate diagnostic tools. The BioFire® FilmArray® Torch System with greater coverage has the ability of detecting more pathogens and is more promising particularly in the occasional infection circumstance. The BD MAX™ System demonstrated almost the same results and seems to be a better option in times of an outbreak when the numbers of patients are significantly higher.

2. Popular science summary

Gastroenteritis is a terrible experience, which everyone has had at least a few times during his or her lifetime. It is a common infection in the digestive system and a major reason of illness worldwide. Viruses mostly cause the infection and the sickness can be recognized from its typical symptoms such as abdominal pain, diarrhea and vomiting. It is extremely contagious and spread very easily and causes outbreaks in the society. Fortunately, in most cases the illness usually subsides after a couple of days without requiring medical care. Although, it is notable that it is harmful if it leads to dehydration complications, especially for young children, elderly and immunocompromised patients. The infection can be found everywhere in the world causing enormous economic costs. However, it has a larger impact in developing countries mostly due to poor hygiene and water sanitation problems.

Various viruses have the ability to cause gastrointestinal infections in humans and among those, noroviruses, sapoviruses, rotaviruses and enteric adenoviruses are the most commonly detected ones. There are different diagnostic methods developed for the detection of gastroenteritis agents. In this study, we tried to find a gastroenteritis diagnostic method for Uppsala Academic Hospital in a routine laboratory setting, which is both rapid and highly sensitive to be able to prevent the spread and control the infection in a short period of time. There were two interesting commercial instruments on the market that we compared.

The first instrument was the BD MAX™ System, which has the ability to detect five of the most important enteric viral pathogens. It provided an automated result for maximum 24 tests in one run, in approximately three hours.

The second instrument was the BioFire® FilmArray® Torch System. It has a broad coverage with the ability to detect 22 of the most common gastrointestinal pathogens in one single panel. In this instrument, each test was uploaded and analyzed independently and an automated result was provided in approximately one hour.

Both instruments had an easy and simple procedure, and offered rapid results, which is important for viral gastroenteritis diagnostic tests. We tested 58 different stool samples in both instruments. The BioFire® FilmArray® Torch System had 95% sensitivity and 100%

specificity and also detected more pathogens compared to the BD MAX™ System, which in the other hand had 100% sensitivity and 93.3% specificity.

The results demonstrated that both systems are reliable. The BioFire® FilmArray® was somewhat more specific and able to detect several more pathogens. Therefore, it has higher potential to increase correct and appropriate treatment as well as precise clinical management. These lead to quick control of the infection, particularly in the occasional infection circumstance and could therefore prevent an outbreak. The BD MAX™ System had almost the same results and it seems to be a better choice in times of an outbreak when the numbers of patients are higher. Therefor they could be used as each other's complement in different infection situations that are present in the society.

3. Key words

BD MAX™ System, BioFire® FilmArray® Torch System, Adenovirus F40/41, Human Astrovirus, Norovirus GI/GII, Rotavirus, Sapovirus.

4. Introduction

Gastroenteritis, generally known as stomach flu, is an inflammation of the gastrointestinal tract that is mostly caused by viruses, bacteria, parasites or fungus. The infection is extremely contagious and mainly characterized by diarrhea, abdominal pain, nausea, vomiting and in some cases even fever, headache, dehydration and muscle pain may appear in the infected populations. [1,2]

Gastroenteritis is a common and self-limiting infection that can be found throughout the world, however continuously persistent and untreated severe diarrhea or vomiting can lead to dehydration, which is a serious complication. Severe dehydration is dangerous and critical, especially for young children and elderly and requires rapid treatment. It can be noticed by different signs such as; abnormal breathing, rapid heartbeat, dark urine, skin´s elasticity and prolonged capillary refill time (CRT). [3,4]

Gastroenteritis is a leading cause of morbidity and hospitalization worldwide and with almost 1.3 million deaths every year it is a common cause of mortality globally with a great impact on infants, young children, elderly and immunocompromised populations. The infection is usually spread via the fecal oral route by eating food or drinking water, which is contaminated with infectious agents, close contact with infected persons or touching contaminated surfaces and objects. Infection and death occurs mainly in developing and low-income countries, especially in areas with malnutrition and mostly due to poor hygiene, water sanitation problems, improper disposal of human waste and incorrect management or lack of proper sewage systems besides lacking in efficient treatment. However, it is notable that it is still also a significant concern in developed and high-income countries and requires consideration. [5,6]

Among all agents, which can cause gastroenteritis, viruses are the most common source of infection and are solely responsible for more than 75% of the cases around the world. Many different viruses have the ability to cause gastrointestinal infection in humans and among those noroviruses, sapoviruses , rotaviruses, enteric adenoviruses 40/41 and human astroviruses are the most common and concerning viruses, which have been detected

worldwide. The mentioned viruses are also the ones that have been in the center of focus and observation in this study. [7]

4.1. Brief descriptions of the agents in this study

4.1.1. Adenovirus (AdV)

Adenoviruses are stable viruses belonging to the family *Adenoviridae* with the ability of infecting both humans and animals and have prolonged survival in the environment. Those, which infect humans, are called human adenoviruses (HAdV) and belong to the genus *Mastadenovirus* with ability of causing respiratory, ocular, urinary tract and gastrointestinal infection. They have double-stranded DNA, non-segmented, linear genome about 35 kilobases (kb) and a non-enveloped, icosahedral virion with a capsid that is approximately 90 nm in diameter. Until now, there are 88 assorted types of HAdV (1-88) discovered and they have been divided into seven characteristic species (A-G) where each species are associated with different conditions and special human diseases. Among them, adenoviruses F types 40/41 and G types 52 are called enteric adenoviruses and are associated with infection in gastrointestinal tract and ability of extremely prolonged viral shedding. [8,9]

4.1.2. Astrovirus (AstV)

Astroviruses belong to the *Astroviridae* family and are able to cause infection in both humans and animals, particularly avian and mammalian. They have a linear genome about 6.8 to 7 kb and a single stranded positive sense RNA. They are non-enveloped, non-segmented with icosahedral capsid about 35 nm in diameter. Astroviruses that infect humans are known as human astroviruses (HAstV) and cause gastroenteritis especially in children, elderly and immunocompromised populations. There are several different HAstV and currently, there are three groups and eight serotypes (1-8) of HAstV known worldwide. [10-12]

4.1.3. Norovirus (NoV)

Noroviruses are positive-sense RNA viruses with a linear, non-segmented genome and size of 7.5 kb. They have a capsid which is 38 to 40 nm in diameter, non-enveloped, with icosahedral structure and belong to the *Caliciviridae* family. Noroviruses are the most reported and major

cause of gastroenteritis single agent outbreaks and sporadic cases worldwide. They can infect humans of all ages and due to prolonged viral shedding, ability to survive in the environment and furthermore requiring extremely low amount of viral particles for transmission, have extremely high infectious potential. They are divided into seven different genogroups (GI-GVII), which among those, groups GI and GII are capable of developing infection in humans and particularly group GII is known as the most common strain of noroviruses in adult gastroenteritis outbreaks. [13-15]

4.1.4. Rotavirus (RoV)

One other virus type that was observed in this study is rotaviruses, which are the leading cause of severe diarrhea and gastroenteritis especially among infants and young children. According to Centers for Disease Control and Prevention (CDC), rotaviruses are accountable for being the leading cause of infant mortality worldwide, with approximately 352,000 – 592,000 deaths each year among young children under the age of five. Rotaviruses are double-stranded RNA viruses belonging to the family *Reoviridae* and can infect both humans and animals. They have an icosahedral, non-enveloped virion, which is about 80 nm in diameter and a segmented linear genome, which is about 18 kb. Currently, there are nine different rotavirus species known (A-J), which among those, rotavirus group A is responsible of more than 90% of all the rotavirus infections in humans and is consequently the most common species. According to the World Health Organization (WHO), there are four prequalified rotavirus vaccines accessible globally; Rotarix™, Rotateq™, Rotavac™ and RotaSiil™, and between those Rotarix™ and Rotateq™ vaccines are licensed. All four appointed vaccines are oral, live, attenuated, with high efficacy ability in preventing gastrointestinal disease. [16-19]

4.1.5. Sapovirus (SaV)

Sapoviruses are another group of viruses that also belong to the *Caliciviridae* family with a genome that is approximately 7.7 kb, linear, single stranded, positive-sense RNA. They have non-enveloped virion with icosahedral structure and a capsid that is about 27 to 40 nm in diameter. Sapoviruses are causative agent of gastroenteritis sporadic cases and outbreaks in humans and animals all over the world. Sapoviruses are classified into five different

genogroups (GI-GV) and all groups except group GIII, which is responsible for infection among procine species, are capable of infecting human especially young children. [20,21]

4.2. Laboratory diagnostic methods

There are many different diagnostic methods utilized for the detection of gastroenteritis viral agents in different countries. For many years, mainly the traditional diagnostic methods such as electron microscopy and culturing, based on direct visualization, have been used clinically. Currently, assortments of different molecular techniques have been developed and are used in routine setting in clinical laboratories, which offer more rapid diagnosis with higher sensitivity and specificity test results. [22]

Real-time PCR (RT-PCR) is one of the molecular diagnostic techniques that are used in routine laboratories for detecting viral gastrointestinal pathogens. The RT-PCR assays are easy to perform and has become a method of choice in most laboratories and frequently improved as an *in house* method since it has relatively high sensitivity and specificity and in addition is a low cost method. However, it is time consuming compared to the recently developed commercial methods and require various preparations before diagnosis, which can increase the number of erroneous diagnostic results. Furthermore, it can be difficult to develop a multiplex *in house* RT-PCR that can detect multiple targets in a single reaction. Also time, optimization and validation costs are crucial and must be considered for a successful implementation of multiplex PCR assays. [23,24]

In both developed and developing countries, in-house RT-PCR is used in many laboratories. However, in the developed and high-income countries, such as Sweden, it has become more common to use a commercial instrument as well for diagnosis whereas in developing countries, the higher price of these methods makes them unaffordable for use in routine laboratories. [25-28]

The BD MAX^{TM} System is one of the automated multiplex instruments on the market that performs molecular diagnosis and provides results by incorporating both nucleic acid extraction and RT-PCR. A large variety of BD MAX^{TM} assays are available on the market such as healthcare associated infections (HAI) assays, women's health and sexually transmitted infections (STIs) assays, respiratory and enteric syndrome assays. Enteric

syndrome assays contain four different panels; enteric bacterial, extended enteric bacterial, enteric viral and parasite panel. It can provide results for maximum 24 samples in one run, which can be a mix between the different panels. The BD MAX™ enteric viral panel has the ability to detect five of the most important enteric viral pathogens; norovirus GI/GII, rotavirus group A, adenovirus F types 40/41, sapovirus and human astrovirus, directly from unprocessed stool samples, in approximately three hours. It can achieve a rapid turnaround time with brief preparation, simple procedure and providing fast diagnostic results however, the diagnostic tests with BD MAX™ System is more expensive compared to the traditional and *in house* methods. [29, 30]

Another of the latest commercial instruments in molecular diagnostics is the BioFire® FilmArray® Torch System, which is a multiplex PCR assay. It obtains four different comprehensive panels for detection of the most common pathogens that cause a particular condition and syndrome in humans; Meningitis and encephalitis, blood culture identification, respiratory and gastrointestinal panel. The gastrointestinal panel has a broad coverage and contains 22 of the most common gastrointestinal pathogens among bacteria, viruses and parasites in one single panel. The instrument can be composed of two to twelve modules, which are connected to a system base. Each sample uploads independently in a module and individual diagnostic tests are provided in different runs meaning you can run the same number of samples, as there are modules available. This makes it possible for analysis to be initiated at different desired times for different samples. BioFire® FilmArray® Torch is a rapid instrument which has a software that controls the process of analyzing and storing of the data. It generates an automated test result with the ability of data storage for each module. The results are provided and saved separately for each test and are available in approximately one hour after initialization of the run. With an easy and simple procedure, it offers a rapid turnaround time, which is important for viral gastroenteritis diagnostic tests. Nevertheless it is notable that despite all the benefits of this instrument, the diagnostic test cost is relatively high. [31]

Gastroenteritis is not just a leading cause of morbidity and hospitalization, but it is also the most common cause of outbreaks especially in healthcare facilities and hospitals and result in large economic expenses every year worldwide. Since viral gastroenteritis is a very contagious infection, non-diagnosed or misdiagnosed cases potentially affect clinical care and cause increase in spread of infection in the society. Consequently, it is essential to use an

effective diagnostic method to identify the agent of the infections and outbreaks more accurately and rapidly to be able to prevent the spread, provide appropriate treatment for patients, clinical management and control of the infection. [32]

5. Aim

The aim of this study was to evaluate and compare the performance of the BioFire® FilmArray® Torch System's gastrointestinal panel and the Molecular BD MAX™ System's enteric viral panel to indicate the most suitable multiplex method for gastroenteritis diagnosis in a routine laboratory setting.

6. Materials and methods

6.1. Ethics approval

All samples and data in this study were handled and analyzed anonymously and was approved through "Delivery of samples for another purpose," a decision of the section manager of Uppsala Academic Hospital.

6.2. Specimen collection and population

In total, 58 different samples were used in this study. The samples consisted of 23 selected stool specimens from patients who were tested and treated for gastroenteritis infection at Uppsala Academic Hospital and Norrlands University Hospital in Umeå during 2018 – 2021 (Table 1). These 23 samples had been selected after positive or negative results after analyzing once with either single- or duplex assays like: RIDA Quick Rotavirus/Adenovirus Combi test (r-biopharm) which is an antigen test; Xpert Norovirus (GenXpert system, Cepheid), duplex PCR for GG1 and GG2; Singleplex In house RT-PCR for Sapovirus and Singleplex In house RT-PCR for Adenovirus (Norrlands University Hospital in Umeå). Further, 20 synthetic samples from Quality control for molecular diagnostics (QCMD) viral gastroenteritis EQA pilot study during 2018-2019 (Table 2) and 15 cultivated strains of different adenovirus species from 2018 (Table 3) were included in this study.

Table 1. Pathogen content according to single- or duplex assays in selected patients' stool specimens from Uppsala and Umeå

Patients' samples nr.	*Analyzing methods used previously*	*Detected viral pathogens in patients' samples*
1	*RIDA Quick Rotavirus/Adenovirus Combi test (r-biopharm)*	*RoV*
2	*RIDA Quick Rotavirus/Adenovirus Combi test (r-biopharm)*	*RoV*
3	*RIDA Quick Rotavirus/Adenovirus Combi test (r-biopharm)*	*None*
4	*RIDA Quick Rotavirus/Adenovirus Combi test (r-biopharm)*	*None*
5	*RIDA Quick Rotavirus/Adenovirus Combi test (r-biopharm)*	*None*
6	*RIDA Quick Rotavirus/Adenovirus Combi test (r-biopharm)*	*RoV*
7	*RIDA Quick Rotavirus/Adenovirus Combi test (r-biopharm)*	*AdV 40/41*
8	*RIDA Quick Rotavirus/Adenovirus Combi test (r-biopharm)*	*RoV*
9	*Singleplex In house RT-PCR for Sapovirus*	*SaV*
10	*Singleplex In house RT-PCR for Sapovirus*	*SaV*
11	*Singleplex In house RT-PCR for Sapovirus*	*SaV*
12	*Singleplex In house RT-PCR for Sapovirus*	*SaV*
13	*Singleplex In house RT-PCR for Sapovirus*	*SaV*
14	*Singleplex In house RT-PCR for Sapovirus*	*SaV*
15	*Singleplex In house RT-PCR for Sapovirus*	*SaV*
16	*Singleplex In house RT-PCR for Sapovirus*	*SaV*
17	*Singleplex In house RT-PCR for Adenovirus*	*AdV 40*
18	*Singleplex In house RT-PCR for Adenovirus*	*AdV 41*
19	*Xpert Norovirus (GenXpert system, Cepheid)*	*NoV GII*
20	*Xpert Norovirus (GenXpert system, Cepheid)*	*NoV GII*
21	*Xpert Norovirus (GenXpert system, Cepheid)*	*NoV GII*
22	*Xpert Norovirus (GenXpert system, Cepheid)*	*NoV GII*
23	*Xpert Norovirus (GenXpert system, Cepheid)*	*NoV GII*

Table 2. Known content from QCMD samples

QCMD samples nr.	QCMD
1	NoV GII
2	AdV 41
3	RoV
4	NoV GI
5	NoV GII
6	RoV
7	None
8	NoV GII
9	HAstV
10	SaV
11	RoV
12	HAstV
13	RoV
14	None
15	SaV
16	NoV GII
17	NoV GII
18	AdV 41
19	NoV GII
20	Nov GI

Table 3. Pathogen content in the cultivated strains of different adenovirus species

Cultivated samples nr.	Adenovirus species
1	AdV 1 (C)
2	AdV 3 (B1)
3	AdV 4a (D)
4	AdV 5 (C)
5	AdV 7 (B1)
6	AdV 8 (D)
7	AdV 11 (B2)
8	AdV 12 (A)
9	AdV 18 (A)
10	AdV 19a (D)
11	AdV 22 (D)
12	AdV 35 (B2)
13	AdV 39 (D)
14	AdV 40 (F)
15	AdV 41 (F)

6.3. Laboratory diagnostic methods used in this study

6.3.1 BD MAX™ System (BD)

A 5-µL loop was used to place the stool samples (either in medium or unpreserved) into the BD MAX enteric viral panel sample buffer tubes, which had been labeled with sample ID in advance. The tubes were first vortexed for 15 seconds and then the clear caps were replaced with blue septum caps. After creating a work list and choosing the enteric viral panel in the BD MAX instrument, the tubes barcodes and the samples ID were scanned and thereafter the tubes were placed in a BD MAX rack. The required number of enteric viral panel unitized reagent strips, depending on the number of samples, were taped onto the BD MAX rack. Extraction tube (D4) with white foil, Master Mix tube (D6) with green foil and master Mix tube (D5) with blue foil from the kit snapped respectively into the colored position in the unitized reagent strips. (Fig.1)

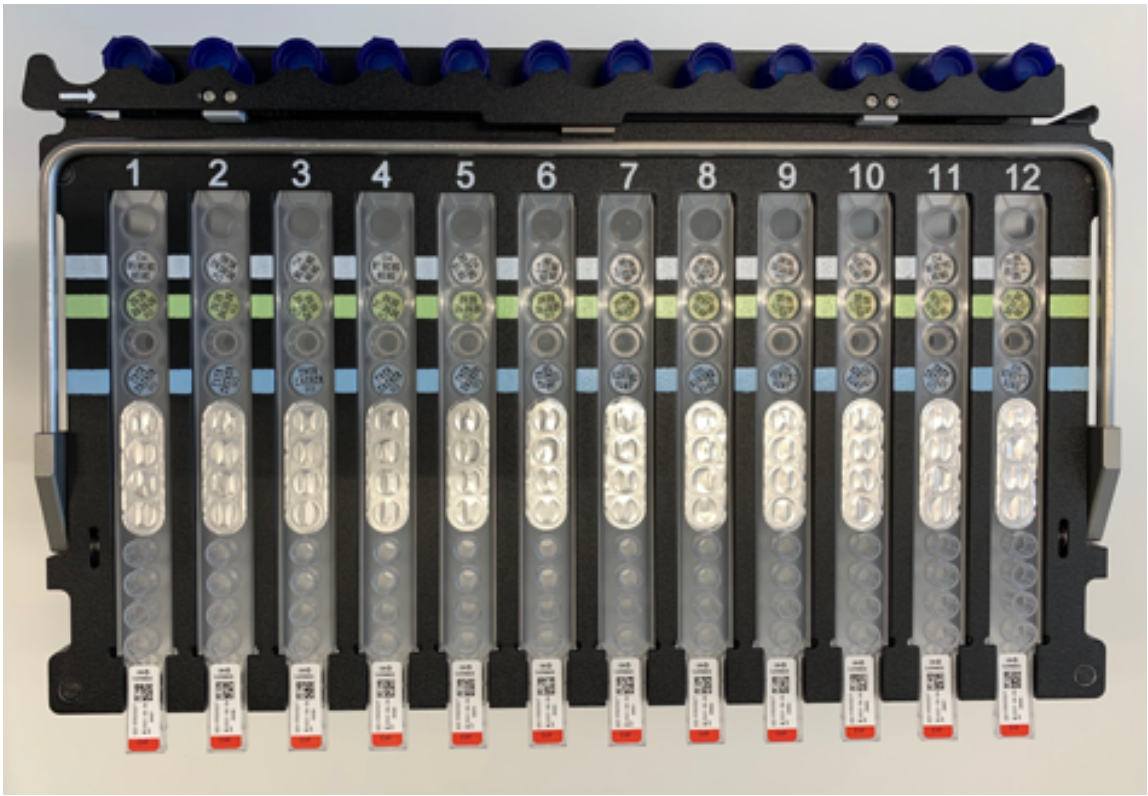

Figure 1. Placement of prepared sample buffer tubes with blue septum caps into the BD MAX rack and enteric viral panel's unitized reagent strips with placement of Master Mix tubes and extraction tubes (upside view). (Photograph by author)

The loaded racks and PCR cartridges were placed into the BD MAX instrument (Fig. 2). The lid was closed and the run was started. At the end of the run the automated results were checked and saved in the desired place.

Figure 2. BD MAX™ System with loaded PCR cartridge and racks (Photograph by author)

6.3.2 BioFire® FilmArray® Torch System (Biomerieux)

6.3.2.1. Preparing the sample

For diagnosis with BioFire® FilmArray® Torch System, 200 µL stool specimen (either in medium or unpreserved) was added into a 13 mL tube, which contained 2 mL TE-buffer (pH 7.5). The tube was vortexed and centrifuged for at least 10 min at 3000 rpm.

6.3.2.2. Preparing the Gastrointestinal (GI) Pouch

The FilmArray GI-pouch was removed from the vacuum-sealed package and placed in the FilmArray Pouch Loading Station, making sure the red and blue labels on the pouch were in line with the red and blue arrows on the Loading Station.

The blue-capped Hydration Injection Vial was placed in the blue wheel of the Pouch Loading Station and the Hydration solution was loaded into the pouch at the blue-end. The red-capped Sample Injection Vial was placed in the red wheel of the Pouch Loading Station. A sample buffer ampoule was added into the red-capped Sample Injection Vial and 200 µL prepared stool sample in the TE-buffer. After closing the lid of the Sample Injection Vial, it was turned upside down at least three times and thereafter inserted into the pouch at the red end of the Pouch Loading Station. (Fig. 3)

Figure 3. The BioFire® FilmArray® Torch System Loading Station and placement of GI-Pouch, the blue-capped Hydration Injection Vial and red-capped Sample Injection Vial (Photograph by author)

6.3.2.3. Loading the BioFire® FilmArray® Torch System

After selecting the available module in the BioFire® FilmArray® Torch System (Fig. 4), the GI-pouch barcode and sample ID was scanned. Thereafter the pouch was inserted into the selected module and the run was started. At the end of the run results was printed out automatically and saved in a desired place.

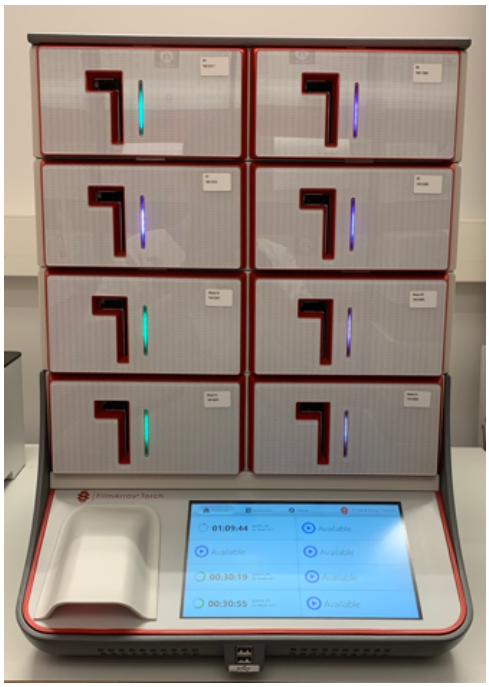

Figure 4. The BioFire® FilmArray® Torch System with 8 modules (Photograph by author)

7. Results

Collected samples were divided into three different groups: 23 stool specimens from patients, 20 samples from QCMD and 15 cultivated strains of different adenovirus species. All samples were analyzed with both the BD MAX™ System and the BioFire® FilmArray® Torch System.

7.1 Differing results of selected patients' stool specimens by the BD MAX™ System and the BioFire® FilmArray® Torch System

The subject of the investigation was to compare the results of the BD MAX™ System and the BioFire® FilmArray® Torch System, both between the two systems and with the results of previous testing of the patients' samples from Uppsala and Umeå. 23 patients' samples had been selected after positive or negative results after analyzing once with either single- or duplex assays. Therefore, the full content of these samples were not completely known.

After analyzing the samples we observed that in some samples the results agreed whereas in others they disagreed, both between the two systems and with previous results. Detected pathogens in patients' samples with both the BD MAX™ and the FilmArray® compared to

the previous testing results are shown in table 4. To clarify the differences between the BD MAX™ and the FilmArray®, the number of detected viral pathogens by the two systems in patients' samples were counted for each virus species (Table 5).

Since the real content of the patients' samples were not completely known, the varying results of the two systems obtained with these samples made it difficult to determine whether the results were true/false positives or true/false negatives.

Table 4. Detected pathogens in selected patients' stool specimens from Uppsala and Umeå by the BD MAX™ System and the BioFire® FilmArray® Torch System

Patients' sample nr.	*Content according to single- or duplex assays*	*BD MAX™ System*	*BD MAX™ System CT Value*	*BioFire® FilmArray® Torch System*
1	*RoV*	*RoV*	*18.4*	*RoV*
2	*RoV*	*RoV*	*20.6*	*RoV*
3	*None*	*RoV*	*24.5*	*RoV*
4	*None*	*RoV*	*25.9*	*RoV+Campylobacter*
5	*None*	*None*	*-*	*AdV 40/41+Campylobacter*
6	*RoV*	*RoV*	*21.4*	*RoV+EPEC*
7	*AdV 40/41*	*AdV 40/41+SapV*	*17.1+29.7*	*AdV 40/41+C. difficile*
8	*RoV*	*RoV*	*21.3*	*RoV+AdV 40/41+EPEC*
9	*SaV*	*SaV*	*20.4*	*SaV+AdV 40/41+C. difficile*
10	*SaV*	*SaV*	*33.1*	*AdV 40/41*
11	*SaV*	*SaV*	*23.0*	*SaV+AdV 40/41*
12	*SaV*	*SaV+HAtV*	*25.4+35.9*	*SaV+AdV 40/41+EPEC*
13	*SaV*	*SaV*	*29.6*	*SaV+AdV 40/41+RoV+EPEC*
14	*SaV*	*SaV*	*24.2*	*SaV*
15	*SaV*	*SaV*	*29.2*	*SaV+AdV 40/41+RoV*
16	*SaV*	*SaV*	*24.6*	*SaV+AdV 40/41*
17	*AdV 40*	*AdV 40/41*	*14.4*	*AdV 40/41*
18	*AdV 41*	*AdV 40/41*	*14.6*	*AdV 40/41*
19	*NoV GII*	*NoV GI/GII*	*22.2*	*NoV GI/GII+AdV 40/41*
20	*NoV GII*	*NoV GI/GII*	*27.2*	*NoV GI/GII*
21	*NoV GII*	*NoV GI/GII*	*24.9*	*NoV GI/GII*
22	*NoV GII*	*NoV GI/GII*	*25.5*	*NoV GI/GII*
23	*NoV GII*	*NoV GI/GII*	*25.4*	*NoV GI/GII*

Table 5. Comparison between the BD MAX™ System and the BioFire® FilmArray® Torch System for number of detected viral pathogens in the 23 patients' samples

Detected viral pathogens in patients' samples	*BD MAX™ System*		*BioFire® FilmArray® Torch System*	
	Positive	***Negative***	***Positive***	***Negative***
Enteric Adenovirus	*3*	*20*	*13*	*10*
Human Astrovirus	*1*	*22*	*0*	*23*
Norovirus	*5*	*18*	*5*	*18*
Rotavirus	*6*	*17*	*8*	*15*
Sapovirus	*9*	*14*	*7*	*16*

7.2 Consistent results of QCMD samples and cultivated strains of different adenovirus species samples by the BD MAX™ System and the BioFire® FilmArray® Torch System

To compare the BD MAX™ and the FilmArray® further, 20 synthetic samples from QCMD and 15 samples of cultivated strains of different adenovirus species, both with completely known content, were analyzed with the two systems. The detected viral pathogens in these samples by BD MAX™ and FilmArray® and their real content are shown in table 6 and 7.

To clarify the results, the number of true/false positive and true/false negative results obtained from QCMD and cultivated strains of different adenovirus species were counted and the result is shown in Table 8.

With these samples, both systems showed a much greater consistency with the known content as well as between the two systems. Only one false negative for FilmArray® and one false positive for BD MAX™ were observed.

Table 6. Detected pathogens in the 20 QCMD samples by the BD MAX™ System and the BioFire® FilmArray® Torch System

QCMD sample nr.	QCMD	BD MAX™ System	BD MAX™ System CT Value	BioFire® FilmArray® Torch System
1	NoV GII	NoV GI/GII	33.0	None
2	AdV 41	AdV 40/41	29.7	AdV 40/41
3	RoV	RoV	25.5	RoV
4	NoV GI	NoV GI/GII	32.8	NoV GI/GII
5	NoV GII	NoV GI/GII	32.4	NoV GI/GII
6	RoV	RoV	27.2	RoV
7	None	None	-	None
8	NoV GII	NoV GI/GII	28.5	NoV GI/GII
9	HAstV	HAstV	29.2	HAstV
10	SaV	SaV	31.5	SaV
11	RoV	RoV	25.9	RoV
12	HAstV	HAstV	26.9	HAstV
13	RoV	RoV	28.7	RoV
14	None	None	-	None
15	SaV	SaV	29.1	SaV
16	NoV GII	NoV GI/GII	29.2	NoV GI/GII
17	NoV GII	NoV GI/GII	30.5	NoV GI/GII
18	AdV 41	AdV 40/41	27.2	AdV 40/41
19	NoV GII	NoV GI/GII	29.8	NoV GI/GII
20	NoV GI	NoV GI/GII	34.3	NoV GI/GII

Table 7. Detected pathogens in the 15 samples with cultivated strains of different adenovirus species by the BD MAX™ System and the BioFire® FilmArray® Torch System

Adenovirus sample nr.	Adenovirus species	BD MAX™ System	BD MAX™ System CT Value	BioFire® FilmArray® Torch System
1	Adenovirus 1 (C)	None	-	None
2	Adenovirus 3 (B1)	None	-	None
3	Adenovirus 4a (D)	None	-	None
4	Adenovirus 5 (C)	Adenovirus 40/41	34.9	None
5	Adenovirus 7 (B1)	None	-	None
6	Adenovirus 8 (D)	None	-	None
7	Adenovirus 11 (B2)	None	-	None
8	Adenovirus 12 (A)	None	-	None
9	Adenovirus 18 (A)	None	-	None
10	Adenovirus 19a (D)	None	-	None
11	Adenovirus 22 (D)	None	-	None
12	Adenovirus 35 (B2)	None	-	None
13	Adenovirus 39 (D)	None	-	None
14	Adenovirus 40 (F)	Adenovirus 40/41	17.0	Adenovirus 40/41
15	Adenovirus 41 (F)	Adenovirus 40/41	16.7	Adenovirus 40/41

Table 8. Number of detected viral pathogens in QCMD and cultivated strains of different adenovirus species by the BD MAX™ System and the BioFire® FilmArray® Torch System

QCMD & Cultivated samples	*BD MAX™ System*				*BioFire® FilmArray® Torch System*			
	Positive		*Negative*		*Positive*		*Negative*	
	True	*False*	*True*	*False*	*True*	*False*	*True*	*False*
Enteric Adenovirus	*4*	*1*	*30*	*0*	*4*	*0*	*31*	*0*
Human Astrovirus	*2*	*0*	*33*	*0*	*2*	*0*	*33*	*0*
Norovirus	*8*	*0*	*27*	*0*	*7*	*0*	*27*	*1*
Rotavirus	*4*	*0*	*31*	*0*	*4*	*0*	*31*	*0*
Sapovirus	*2*	*0*	*33*	*0*	*2*	*0*	*33*	*0*

7.3 Sensitivity and specificity calculated from QCMD and cultivated strains of different adenovirus species

To be able to compute the sensitivity and specificity for the two systems, both true/false positive and true/false negative results were needed. Since these were not known in the case of the patients' samples, these results were excluded from the calculation and only results from the QCMD and cultivated strains were used.

$$Sensitivity = \frac{True\ positive}{True\ positive + False\ negative} \qquad Specificity = \frac{True\ negative}{True\ negative + False\ positive}$$

According to the results obtained from the QCMD and cultivated strains samples, the sensitivity and specificity of the FilmArray® and the BD MAX™ for each virus species were calculated (Table 9). The sensitivity and specificity in whole were also calculated for both systems and resulted in 95% and 100% respectively for the FilmArray® and 100% and 93.3% for the BD MAX™ (Table 10).

Table 9. Sensitivity, specificity and the lower 95% confidence interval (CI) of the BD MAX™ System compared the BioFire® FilmArray® Torch System for QCMD and cultivated strains of different adenovirus species for each virus species

QCMD & Cultivated samples	*BD MAX™ System*				*BioFire® FilmArray® Torch System*			
	Sensitivity	*Lower 95% CI*	*Specificity*	*Lower 95% CI*	*Sensitivity*	*Lower 95% CI*	*Specificity*	*Lower 95% CI*
Enteric Adenovirus	*100%*	*39.8%*	*96.8%*	*83.3%*	*100%*	*39.8%*	*100%*	*88.8%*
Human Astrovirus	*100%*	*15.8%*	*100%*	*89.4%*	*100%*	*15.8%*	*100%*	*89.4%*
Norovirus	*100%*	*63.1%*	*100%*	*87.2%*	*87.5%*	*47.4%*	*100%*	*87.2%*
Rotavirus	*100%*	*39.8%*	*100%*	*88.8%*	*100%*	*39.8%*	*100%*	*88.8%*
Sapovirus	*100%*	*15.8%*	*100%*	*89.4%*	*100%*	*15.8%*	*100%*	*89.4%*

Table 10. Total number of detected viral pathogens, sensitivity, specificity and the lower 95% CI for QCMD and cultivated strains of different adenovirus species by the BD MAX™ System and the BioFire® FilmArray® Torch System

QCMD & Cultivated samples	*BD MAX™ System*	*BioFire® FilmArray® Torch System*
True positive	*20*	*19*
False positive	*1*	*0*
True negative	*14*	*15*
False negative	*0*	*1*
Sensitivity	*100%*	*95%*
Lower 95% CI (Sensitivity)	*83.2%*	*75.1%*
Specificity	*93.3%*	*100%*
Lower 95% CI (Specificity)	*68.1%*	*78.2%*

7.4 General comparison between the BD MAX™ System and the BioFire® FilmArray® Torch System

To be able to have an overview comparison between the BD MAX™ and the FilmArray®, the pathogen coverage, turn around time and estimated hands on time were also documented (Table 11). Both systems had similar, short hands on time however turn around time was shorter and pathogen coverage greater with FilmArray®.

Table 11. General comparison between the BD MAX™ System and the BioFire® FilmArray® Torch System

	BD MAX™ System	*BioFire® FilmArray® Torch System*
Pathogen coverage	*5 viruses*	*5 viruses, 11 bacteria, 2 bacterial toxin, 4 parasites*
Hands on time	*2 min/sample*	*2 min/sample*
Turn around time	*3 h/(1-24) sample(s)*	*1h /sample*

8. Discussion

Viral gastroenteritis is one of the common causes of morbidity and mortality especially among young children, elderly and immunocompromised populations and is an infection that is present all around the world with greater impact in developing countries. [33,34]

There are several different methods for detection of viral gastroenteritis. Conventional routine PCR is a common molecular diagnostic technique due to the low cost, however it is time-consuming and therefore development of a more efficient multiplex PCR assay for detecting several viral targets at the same time is necessary. This is difficult; however it seems to be possible according to previous studies in this area. It requires time and accurate optimization beside validation costs, which must come into consideration for a successful implementation of multiplex PCR assays. Since the Microbiology department at Uppsala Academic Hospital was overwhelmed with Covid-19 patients' samples during the pandemic, implementation of a

multiplex PCR assay for viral gastroenteritis was not a possible option during this study and could therefore be considered in the future. [28,35]

According to current studies there is also evidence that due to the viruses' high prevalence, co-infection can potentially occur and exposure to multiple agents at the same time is common. For detection of enteric viral co-infections, multiplex methods are preferable in order to find several different pathogens with the advantage of saving time and cost compared to singleplex assays. [36,37]

The Microbiology department at Uppsala Academic Hospital has recently been equipped with two commercial multiplex diagnostic systems to provide a reliable and rapid diagnostic test; the BioFire® FilmArray® Torch System and the BD MAX™ System. The FilmArray® was already evaluated for the respiratory panel and was an operative system for emergency Covid-19 infection diagnosis. Part of this project was to also evaluate its gastrointestinal panel. In this study these two commercial methods were compared for pathogen coverage, hands on time, turn around time, workflow, cost, sensitivity and specificity to be able to indicate a multiplex method for viral gastroenteritis diagnosis in the routine laboratory setting.

The FilmArray® GI Panel is a multiplex PCR assay which has a broad coverage and is able to identify co-infections. The instrument incorporates software that examines the result from a high-resolution melt analysis of the PCR-products performed by the instrument, before assessing a final result. This feature makes Ct-values redundant for the qualitative pathogen detection. Beside viral pathogens, it detects some important bacteria, bacterial toxin and parasitic pathogens at the same time. It identifies 22 targets including 11 bacteria: *Campylobacter (jejuni, coli & upsaliensis), Plesiomonas shigelloides, Salmonella* spp*, vibrio (parahaemolyticus, vulnificus & cholera), Yersinia enterocolitica,* Enteroaggregative *E. coli* (EAEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC) *lt/st, E. coli* O157, and *Shigella*/Enteroinvasive *E. coli* (EIEC), two bacterial toxins: *Clostridium difficile toxin A/B* and Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2, four parasites: *Cryptosporidium, Cyclospora cayetanensis, Entamoeba histolytica and Giardia lamblia* and five viruses: adenovirus F 40/41, human astrovirus, norovirus GI/GII, rotavirus A and sapovirus. In comparison, the BD MAX™ System enteric viral panel detects just five of the most important enteric viral pathogens: Adenovirus F 40/41, human astrovirus, norovirus GI/GII, rotavirus A and sapovirus. Diagnosis with FilmArray® is a good option especially in

the beginning of an outbreak when the infection source is not identified and therefore the search target is unknown. In this situation a broad coverage is desirable which leads to finding the cause more rapidly and better patient management. [30,31]

Hands on times are short, a few minutes, and are about the same for both systems. However, in this study the stool samples were not in Cary-Blair enteric transport medium but had to be treated with TE-buffer and centrifuged for at least 10 min, which increased the hands on time in this case. Due to this, FilmArray® required slightly longer time to load because of the preparations needed for every sample. However, analyzing the stool samples with the BD MAX™ System required the samples to be taken to the lab location with biosafety level 3 (BSL-3), since in Uppsala Academic Hospital the instrument was located there. On the other hand, the FilmArray® instrument was located close to the samples arrival and labeling station.

The turn around time for FilmArray® is about one hour while it is approximately three hours for the BD MAX™ System. The turn around time is obviously shorter for FilmArray®, since the diagnosis was for just one sample at a time. However in times of an outbreak when there are higher numbers of the population infected, the BD MAX™ System offer shorter turn around time since it could analyze 24 samples in one run.
By offering more rapid and comprehensive results, both FilmArray® and the BD MAX™ System have the ability to streamline workflow, reduce total cost and improve care. The streamlined workflows in both systems are also documented in other studies. [38-40]

Analyzing with FilmArray® is approximately four to six times more expensive per sample than with the BD MAX™ System. However, since it can detect more pathogens in one run the total diagnostic cost would be almost the same in the situation where the cause of infection is unknown and a broad coverage is desirable. In this case the BD MAX™ System reagents price need to be taken into account since more runs are required by this system in order to reach the same coverage. On the other hand, if we are looking for a specific pathogen and if the number of samples is high, the BD MAX™ System is more economical due to its higher capacity per run.

The samples from QCMD and the cultivated strains of different adenovirus species had completely known content. On the other hand, the real content of the selected patients' stool

specimens from Uppsala and Umeå were unknown since they were analyzed only once and with different single- or duplex assays, which detected a specified pathogen in each sample. For example, all the norovirus positive samples were analyzed with GeneXpert, which was used to detect only norovirus. In some samples our result differed from the original results. For instance, in patients' samples number 3 and 4, both systems detected rotavirus. Prior to being used in this study these samples had been analyzed with the antigen test RIDA Quick Rotavirus/Adenovirus Combi test (r-biopharm) for rotavirus/adenovirus with negative results. However, antigen tests are known to have poor sensitivity, which could explain why the rotavirus had not been detected in these samples. There were also differences between FilmArray® and the BD MAX™ System in the patients' samples results. For example, the FilmArray® detected more positive samples of adenovirus compared to the BD MAX™ System. This could question the specificity of FilmArray®. However, this was not the case neither when analyzing the 20 QCMD samples (Table 6), nor in the 15 samples of cultivated strains of different adenovirus species (Table 7). Therefore a more probable cause is that the patients' samples contained a sufficiently low level of adenovirus 40/41 for the BD MAX™ System not to detect them. This could be confirmed in a future study with a third method, such as a RT-PCR Adenovirus to draw a more certain conclusion. The FilmArray® also detected rotaviruses in patients' samples 13, 15 and 16 which prior to this study had been tested only with singleplex in house RT-PCR Sapovirus. Since gastroenteritis is commonly caused by co-infections, this shows the advantage of a multiplex method compared to single- or duplex assays. This is also in line with our findings of several pathogens in some of the patients' samples. FilmArray® also detected bacteria in eight of the patients' samples. This study was about viral gastroenteritis pathogens and does not address these results, however these findings indicate that the use of a broad coverage diagnostic system would probably be beneficial for the treatment of the individual patient as well as the clinical management of the spread of the infection. [41-43]

Since the differing results were not compared with a third method it was not possible to know if the results of FilmArray® and the BD MAX™ System were reliable concerning the patients' samples. Therefore the results of the patients' samples were not used in order to calculate the sensitivity and specificity for each system in this study.

According to the results obtained from QCMD samples and cultivated strains of different adenovirus species, the FilmArray® had a sensitivity of 95% and a specificity of 100%. The

values for the BD MAX™ System would be 100% sensitivity and 93.3% specificity, respectively. There are also other studies that show high sensitivity and specificity for the FilmArray® GI panel in comparison to conventional diagnostic methods and other PCR assays, which correlate with the findings in this study. There are also some studies of the BD MAX system's evaluation and performance, in which it demonstrates as a reliable method for detection of viral enteric pathogens. [44-49]

There were some limitations in this study, which are important to point out and discuss since it could affect the data and furthermore the results provided. First, the analyzed stool specimens were not in Cary-Blair enteric transport medium as is recommended by BioFire®. Therefore, the stool samples used in this study, either in medium or unpreserved, were prepared with TE-buffer before diagnosis. Cary-Blair enteric transport medium has very low nutritive content and is used for collection and preservation of samples and facilitates survival of the organism without allowing multiplication. The samples used in this study were stored in freezer or refrigerator without this medium. It is unlikely that it had any effect of the results but still it is not in line with the recommendations of BioFire®, therefore it cannot be entirely ruled out. Secondly, access to stool samples from patients with viral gastroenteritis was limited. This limitation was probably due to the Covid-19 pandemic, which caused travel restrictions, more hand washing, isolation and social distancing overall resulting in fewer infections. Finally, there were low numbers of positive human astroviruses and sapoviruses among the analyzed samples with known content in this study, which affected the reliability of the sensitivity and specificity values for these specific pathogens. To be able to draw a more accurate conclusion a 95% confidence interval (CI) is preferable and at least 40% is required. In the case of enteric adenovirus and rotavirus, the lower 95% CI for sensitivity was 39.8% and for human astrovirus and sapovirus the lower CI was even less (15.8%). In order to achieve more reliable data for individual species, more samples need to be analyzed and also more samples with positive results. However, as shown in Table 10, the general lower 95% CI for sensitivity and specificity was 83.2% and 68.1% for BD MAX™ and 75.1% and 78.2% for FilmArray® respectively.

Achieved results in this study have showed that both systems have high sensitivity and specificity. This demonstrates that FilmArray® with its higher specificity as well as greater coverage has the ability of detecting several gastrointestinal pathogens in one run. With more accurate and rapid identification, it increases the likelihood of correct and appropriate

treatment for patients and precise clinical management. This also leads to quick control of the infection, particularly in the occasional infection circumstance and therefore has the potential to reduce the spread of infection in society and preventing outbreaks. The BD MAX™ System enteric viral panel showed almost the same results and seems to be a better option in times of an outbreak when the numbers of patients are significantly higher.

In conclusion, both the BioFire® FilmArray® Torch System GI panel and the BD MAX™ System enteric viral panel can be reliable for detection of viral enteric pathogens. Both systems are able to provide a rapid and accurate gastroenteritis diagnosis and can complement each other depending on different infection situations that are present in the society. In order to be able to draw a more definite conclusion, further research is needed in this area with a higher number of stool samples and particularly samples in Cary-Blair medium to diagnose with FilmArray® in the recommended condition.

9. Acknowledgements

I would like to thank and dedicate this work to the Microbiology Department of Uppsala Academic Hospital that supported this study. My special thanks to my supervisor Christina Larsson, without whom this work would not have been possible.

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