Diagnostic molecular microbiology and its applications: Current and future perspectives

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Abstract

Infectious diseases are a major cause of morbidity and mortality throughout the world. Although the field of diagnostic microbiology has substantially evolved over the past few decades, it is still heavily reliant on cultures and serology, which while being cost effective is largely time consuming and sometimes less sensitive and specific. Molecular biology on the other hand has developed with rapid and confirmatory diagnosis. However, the merger of molecular diagnostics in routine diagnostic microbiology labs has been slow paced mostly due to higher costs and lack of infrastructure. Molecular methods have progressed beyond identification to detect antibiotic marker genes, fastidious bacteria, and uncultivable microbes. It has found a scope in mycology and parasitology, in which the basic conventional techniques may sometimes be unable to make a definitive diagnosis of the concerned pathogens. In the field of virology where culturing may be impractical in some clinical settings, it has developed various multiplexing procedures which can detect and quantitate the viral copies present in the crude specimen. Besides the field offers different types of permutations and combinations like direct sequencing for finding the variants, whole genome sequencing, epidemiological testing like plasmid profiling, RFLP etc. which can point out the infection and different types of PCR customisation such as nested, multiplex and Real Time (RT) PCR. In the present review, we have described the various molecular typing techniques and their application in microbial testing with a briefing of the tests that have been already standardised with relevant sample acceptance and rejection criteria for commonly encountered pathogens and pointed towards future directions converging automated closed DNA, RNA extraction/amplification platforms, Next Generation Sequencing (NSG), Microarrays and Digital PCR (dPCR) into its arsenal.

Introduction

Infectious disease is the main cause of mortality and morbidity worldwide. The different types of infection caused by various types of pathogens including bacteria, viruses, parasites and fungi are ever increasing [1]. Increasing age, diseases such as cancer, immunosuppressions etc are contributing factors. Although the era of antibiotic, antiviral, antimycotic, and antiparasitic has led to alleviation from these infectious agents, the rampant use of these agents has led to the emergence of multi drug resistant (MDR) pathogens which if not diagnosed and contained early, could spread to large geographical areas [2]. The role played by diagnostic systems is the ‘golden standard’ for enacting rapid treatment regimes. Routine clinical& microbiological procedures such as cultures, serology, and microscopy still remains the procedures of choice in terms of diagnosis and are also cost effective [3]. However the routine microbiological cultures are by themselves, not confirmatory tests for the accurate diagnosis of the pathogen. Even with cultivable bacteria, cultures fail to reveal an organism in many patients with sign and symptoms consistent with infectious disease. Due to the above reasons, there is a large scope for molecular biology procedures in the diagnostic clinical microbiology laboratory.

With the advent of PCR technology about 30 years ago came the era of molecular diagnostics. It is due molecular testing that the phenotypes exhibited by a pathogen can be genetically confirmed [4]. It ensures rapid diagnosis at a cost effective price, thus increasing the diagnostic arsenal for pathogen identification. This review enlists the various types of molecular typing methods which have been inculcated in molecular microbiology diagnostic labs. It also describes the need for more advanced molecular methods for increasing the sensitivity and specificity of diagnosis and also the routine protocols for various bacterial, viral, fungal, and parasites that have been already standardised at the genus and species levels with a briefing on sample acceptance and rejection criteria for the enlisted pathogens.

Molecular typing techniques

The advent of nucleic acid amplification techniques has led to the advancement of molecular typing techniques [5]. It offers the advantage of rapid confirmatory diagnosis. Since the start of the field of medical microbiology, the main techniques have relied on the identification of phenotypic characters like biochemical characterisation, morphological view, and cultures. The inert nature of the DNA molecule makes it the most suitable marker for confirmatory diagnosis [6]. The various types of molecular techniques have been reviewed in the following sections.

Restriction analysis

The microbial DNA consists of various sites containing sequences which are repetitive in nature. These repeats are tandemly repeated after constant intervals [6]. Also restriction enzymes or endonuclease (RE) a class of DNA–cleaving enzymes isolates from bacteria are used to cleave these DNA at a particular and specific sequence which results in the fragmentation of DNA molecule. This technique thus can detect the fragmentation pattern of the target and compare it with the in house pathogenic strains and handout results whether the isolated pathogens are similar or dissimilar in origins. Thus, in this technique...

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the DNA is cut with the restriction enzymes [7].

**Plasmid profiling**

In these techniques, plasmids which are extrachromosomal DNA elements are cut with restriction enzymes (RE). Transfer of plasmids is very common between members of the family Enterobacteriaceae. It is mainly useful in epidemiological outbreaks in which the plasmids are cut with the same RE which may result in same restriction patterns from plasmids isolated from various pathogens in the outbreak area thus showing the profiles in outbreak associated strains [8]. It has been carried out in opportunistic multidrug resistant *Pseudomonas aeruginosa* isolated from wound infections, which carried a pattern of resistance to various antibiotics [9]. A similar study was used for screening of *Neisseria gonorrhoeae* [10] and *Shigella spp.* [11] among others [12,13]. It has been also widely carried out to identify multidrug resistant isolates present in sewage associated with health care centres [14]. A study carried out used this technique for the study of the emergence of drug resistance in diarrheagenic *E. coli* in paediatric population in a developing country [15,16].

**Restriction fragment length polymorphism (RFLP)**

RFLP has been mainly used in multi drug resistant *Mycobacterium tuberculosis* outbreaks for simultaneous identification and differentiation in which polymorphisms present in DR locus which is characterised by repetitive sequence interspersed between non repetitive sequences has been hybridised by a probe known as spacer oligotyping or spoligotyping. It can differentiate *M. bovis* from *M. tuberculosis*, a distinction which is often difficult to make by traditional methods [17]. In parasitology PCR-RFLP has been used to diagnose and differentiate Old and New World *Leishmania* species [18] and also for differentiating *Fasciola hepatica* and *Fasciola gigantica* [19]. In a study carried out in outbreak in Japan characterization of enterohemorrhagic *Escherichia coli* O111 and O157 Strains were done by RFLP [20]. In case of the highly transmissible zoonotic infection Brucellosis, caused by the slow growing bacterium *Brucella spp.*, this technique has found importance [21].

**Pulse field gel electrophoresis (PFGE)**

With this technique in RFLP, there arose a need to perform high resolution separation on agarose gel to look out for similarity in banding patterns obtained after RE digestion. In 1982, Schwartz introduced the concept that DNA molecules larger than 50kb can be separated by using two alternating electric fields. This led to the technique of PFGE. It is based on the digestion of bacterial DNA with RE that recognises few sites along the chromosome, generating large DNA fragments (30-800Kb). The basis for PFGE separation is the size-dependent time-associated reorientation of DNA migration. When visualised electronically bacterial isolates with identical or very similar band patterns are more likely to be related genetically than with more divergent band patterns. PFGE has been used for the detection of *Listeria monocytogenes* and *Campylobacter spp.* which are food borne pathogen difficult to culture [22,23]. It is also widely applied to the diagnosis of eukaryotic DNA like parasites which are dense and complex in nature [24].

**Multiplex PCR**

Multiplexing is one of the most widely applied techniques in the field of diagnostic microbiology. It is a variant of PCR, which enables simultaneous amplification of many targets of interest in one reaction by using more than one pair of primers. Its main use is in the field of virology in which primers are designed to detect different types of viruses from a sample [25]. Different types of panels are available from brands such as Biomurix, Roche, life technologies, Qiagen etc., which are named syndromically e.g. Respiratory panel, Gastrointestinal panel, Meningitis panel etc. Multiplexing is also useful for differentiating at the species levels e.g. Multiplex PCR for identification of *Campyllobacter coli* and *Campyllobacter jejuni* from pure cultures and stools [26], rapid detection of Methicillin Resistant *Staphylococcus aureus* (MRSA) from blood culture bottles [27], acquired carbapenemase resistance [28]; and in parasite detection, e.g. for differential diagnosis of helminths like Taeniasis and Cysticercosis [29].

**Nested PCR**

Involves two consecutive PCR reactions of 25 cycles. The first PCR uses primers external to the sequence of interest. The second PCR uses the product of the first PCR in conjunction with one or more nested primers to amplify the sequence within the region flanked by the initial set of primers. It has been designed for various bacterial [30-33], fungal [34,35], and parasitic infections.

**Real time PCR (RT-PCR)**

It allows viewing the increase in the amount of DNA as it is amplified. All real-time PCR systems rely upon the detection and quantitation of the fluorescent reporter, the signal of which increases in direct proportion of the amount of PCR product in a reaction. The most economical reporter is the double strand DNA specific dye SYBR green, which upon excitation emits light. However SYBR green has its limitations as it will bind to any double stranded DNA in a reaction [36]. Two most popular alternatives to SYBR green are TaqMan and molecular beacons. Both technologies depend on hybridisation probes relying on fluorescence resonance energy transfer (FRET) and quantitation [37]. There are numerous laboratory-developed real time PCR tests, including assays for viruses like Dengue [38], Ebola [39] and bacteria like *Pseudomonas aeruginosa* [40].

**Direct sequencing**

Direct sequencing is the most reliable method for the detection of sequences at the molecular level. The first whole genome sequenced was that of bacteriophage φX174. Given below is a briefing of some of the methods applied in microbial sequencing. It is noteworthy that sequencing has helped to differentiate fungi and parasites at the species level which is difficult to achieve by current morphological and phenotypic techniques [41].

**Whole genome sequencing (WGS)**

WGS has changed the landscape of molecular biology with its advent in 2005 by Roche. It is the most cost effective approach for solving many epidemiologic outbreaks and confirmation of pathogens after cultures [42,43]. The near market platforms available are Roche 454, Pacific Biosciences, Ion Torrent, and Illumina MiSeq which can perform operations in 48hrs [44]. In terms of sample preparation for pathogens Oxford Nanopore is also very reliable [45].
Target gene sequencing

It is the most common method of sequencing used which relies on Sanger dideoxy chain terminator method. In this the primers complementary to the gene of interest are used to amplify the band containing the gene of interest and sequenced using an automated sequencer like the Applied Biosystems 8180xl genetic analyser. It is used for diagnosing multi drug resistance in microorganisms like presence of acquired carbapenemase resistance gene NDM-1 gene [28,46].

Universal gene target

These are specific sequences used in microbial diagnostics which are highly conserved and also impart certain phylogenetic traits at the genus and species levels.

Ribosomal RNA (rRNA)

The bacterial pathogens which are unidentifiable with routine testing, slow growers, uncultivable can be identified by amplification of DNA encoding ribosomal RNA genes followed by DNA sequencing. In bacteria there are three genes that make up rRNA functionality, i.e., 5S, 16S, and 23S rRNA. The 16S has been employed for identification purposes due to it being highly conserved and having a moderate copy number depending on the genus. Besides 16S rRNA genes are found in all bacteria and accumulate mutations at a slow, constant rate over time, hence serving as “molecular clocks” [47]. The use of this technique has led to the discovery of novel clinical isolates and culture negative infections [48]. It has been widely used to identify tuberculosis and non-tuberculosis infections [49] and biothreat agents like Brucella spp [50]. Recently, there have been several reports regarding the use of the large subunit (23S rRNA) which is specific for bacterial species recognition. It has been used frequently for the detection of Stenotrophomonas maltophilia from patients with cystic fibrosis [51].

Heat shock proteins

The use of 16S rRNA although applied routinely bears some limitations. Strains with less than 97.5% 16S rRNA gene sequence identity are unlikely to be related at the species level. However, there are a number of strains that share less than 50% DNA similarity by reassociation and therefore are classified as distinct species, but share 99% to 100% 16S rRNA gene sequence identity. For example, Mycobacterium chelonae and Mycobacterium abscessus have more than 99% 16S rRNA gene sequence identity, but their DNA similarity by reassociation is only 35% [52]. In such circumstance sequencing of essential genes such as the heat shock proteins (HSP; HSP60, HSP65, groEL, groER, etc.), have been shown to be useful [53,54]. The heat shock response is an important homeostatic response that enables the microbial cells survive the conditions of stress. This phenomenon correlates with phenotypic resistance has been designed for retroviruses in clinical labs. Genotypic mutation analysis of the virus which necessitated diagnosis by molecular techniques. The medically important viruses encountered in a diagnostic molecular microbiology laboratory at the genus and species levels for various clinically important pathogens including bacteria, fungi, parasites and viruses. We also include the sample acceptance and rejection criteria as cited in the literature.

Bacteriology

Most medically important classes of pathogens belong to the class of bacteria and their molecular detection is the need of the hour for rapid confirmatory diagnosis [61]. Molecular standardised protocols have been cited in Table 1. Whipple’s disease is a rare but fatal infection caused by Tropheryma whipplei the diagnosis of which until recently only relied on histopathology and electron microscopy, often from post mortem material. PCR now allows the diagnosis of neuro-Whipple’s disease and endocarditis by the detection of T. whipplei from non-invasive specimens [62]. Molecular diagnosis can also help to diagnose uncultivable pathogens like that of cat scratch disease Bartonella henselae, Q fever due to Coxiella burnetti, and male urethritis caused by Mycoplasma genitalium [63].

Virology

The field of virology has already adopted diagnosis by molecular methods and Table 2 sites the medically important viruses encountered in clinical labs. Genotypic mutation analysis of the virus which correlates with phenotypic resistance has been designed for retroviruses like HIV in treatment naïve and for viral rebound in patients already on treatment, to establish if resistance has developed which requires a change in treatment [64,65].

Mycology and parasitology

The molecular diagnosis of parasites and fungi has been a revolution as they are difficult to diagnose at the species level [24]. Although still in a developing phase in parasitology, the lack of culturing techniques has necessitated diagnosis by molecular techniques. The medically encountered parasites and fungi are elaborated in Table 3. In mycology the most common testing employed is for molecular detection of Pneumocystis jiroveci (Pneumocystis carinii) an opportunistic fungus causing severe pneumonia in HIV-infected patients, which has made the microscopic and silver staining of tissue specimens a thing of the past [66].

Future applications

The introduction of PCR technology by Nobel Prize winning scientist Dr Kary Mullis in 1983 has revolutionised the field of medical diagnostics including microbiology. A number of automated extraction/detection systems are available in the market which will become essential in molecular labs in the future. This system, such as QiASymphony (Qiagen) offers automated DNA extraction, PCR setup, including reagent preparation, dilution series and sample pipetting.
| Bacteria                        | Acceptable Specimen                                                                 | Unacceptable Specimen                                                                 | Limitations/Comments                                                                 | Primer Sequence/Probe and Method References |
|--------------------------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------|
| *Bordetella pertussis*         | Nasopharyngeal aspirates, nasal swabs or throat swabs. Sputum, endotracheal tube aspirates, and BAL fluids are also occasionally processed. | -                                                                                      | The potential for false *B. pertussis* detection due to *B. Holmsei* infection must also be kept in mind. | [72-75]                                    |
| *Bordetella parapertussis*     | Nasopharyngeal aspirates, nasal swabs or throat swabs. Sputum, endotracheal tube aspirates, and BAL fluids are also occasionally processed. | -                                                                                      | -                                                                                    | [72-75]                                    |
| *Brucellosis*                  | Bacterial isolates, Blood (EDTA), Serum, Buffy coat, fresh tissue (liver, spleen, thyroid, bone, synovial tissue and fluid) | -                                                                                      | Assays were validated by testing historical culture confirmed *B. suis* and *B. melitensis* patient isolates as well as cattle derived *B. abortus* isolates | [76,77]                                    |
| *Burkholderia cepacia*         | Designed for the identification of suspected *B. cepacia* complex and *B. gladioli* cultures and has not yet been validated for the detection of these organisms in clinical samples. | -                                                                                      | Species specific assays are not currently available for all of the *B. cepacia* complex species (e.g. *Burkholderia dolosa*, *Burkholderia pyrocinia*). | [78-81]                                    |
| *Chlamydia*                    | Acceptable specimens are urine, urethral swab, endocervical swab, dry swab (including throat, rectal and vaginal), tampon cells collected in liquid based, cytology medium PreservCyt™ (Hologic, Bedford, MA, USA) and SurePath™ (BD, Franklin Lakes, NJ, USA). | Not validated for other specimens                                                       | Most commercial assays, however, do not differentiate between LGV and other serovars of *C. trachomatis* | [82,83]                                    |
| *Chlamydophila*                | Respiratory material, including throat swabs, nose swabs, nasopharyngeal aspirates, bronchoalveolar lavage, whole blood (EDTA), urine and CSF, occasionally environmental/bird specimens. | Wound swabs, drainage fluid, faeces                                                   | No international controls are available for *C. psittaci* due to restrictions on transport of potential bioterrorism agents | [84-86]                                    |
| *Coxiellaburnetii*             | Blood, serum, bone marrow, biopsies (Including those imbedded in paraffin) and cerebrospinal fluid. | -                                                                                      | -                                                                                    | [63]                                       |
| Diarrheagenic Escherichia coli Pathotypes (DEP) Including *Enterohaemorrhagic (EHEC)/Shiga-toxin E. coli* (STEIC) | Infectious colonies isolated after processing. | -                                                                                      | -                                                                                    | [87-90]                                    |
| *Haemophilus ducreyi*          | Impression smears or press slides, swabs, and biopsies only. | -                                                                                      | -                                                                                    | [91-93]                                    |
| *Klebsiella granulomatis*      | -------Do--------- | -                                                                                      | Only *K. granulomatis* carries the restriction site specific to this organism. | [92]                                       |
| *Haemophilus influenzae* – Capsulated and Non-typeable Complex* | *H. influenza* cultures only | Not been validated for the detection of *H. influenza* in clinical samples | - | [94-96] |
| *Mycobacterium tuberculosis*  | Respiratory tract | Non-respiratory origin, the sensitivity and specificity of the assay has been estimated to be 69.8% and 95.6%, respectively. | Potential for environmental mycobacteria to contaminate samples | [94-96] |
| *Mycoplasma genitalium*        | Urine and genital swabs | - | M. genitalium DNA may be detected in asymptomatic individuals | [99,100] |
| *Mycoplasma pneumoniae*        | Sputum and Bronchoalveolar lavage (BAL) specimens, nasopharyngeal and throat swabs, nasopharyngeal aspirates, tracheal aspirates and pleural fluid specimens. | Not been validated for any other specimen than those listed. | M. pneumonia may be detected for some time after acute infection following the resolution of clinical symptoms | [101-103] |

Table 1. Standardised molecular protocols in diagnostic bacteriology.
| Virus                      | Acceptable Specimen | Unacceptable Specimen | Limitations/Comments                                                                 | Primer Sequence/Probe and Protocol References |
|---------------------------|--------------------|-----------------------|-------------------------------------------------------------------------------------|-----------------------------------------------|
| Neisseria gonorrhoeae     | Urine, urethral swab, endocervical swab, dry swab (including throat, rectal and vaginal) | Not been validated for any other specimen than those listed | PHILN guidelines for the use and interpretation of nucleic acid detection tests for Neisseria gonorrhoeae | [104-107] |
| Neisseria meningitidis    | EDTA whole blood, CSF and aspirates from joints. Heparinised or citrated samples can be tested | Plasma and serum samples show a decreased recovery of N. meningitides DNA | The cts/PCR assay is used as a diagnostic test for meningococcal disease | [108,109] |
| Pseudomonas aeruginosa    | P. aeruginosa isolates only | Used in sputum samples, but has not been validated | - | [110-113] |
| Rickettsia                | Blood, biopsies, and cerebrospinal fluid | Clotted blood, serum, swabs and urine | - | [114] |
| Streptococcus pneumoniae  | CSF and other sterile fluids. | Not appropriate to test respiratory specimens | The phy gene is not specific for pneumococci and been detected in Streptococcus mitis and Streptococcus oralis isolates | [115] |
| Treponema pallidum        | Swabs from skin lesions only | Not validated for other specimens | The gold standard for the detection of T. pallidum is probably rabbit inoculation | [116-118] |
| **Table 2. Standardised molecular protocols in diagnostic virology.** | | | | |
| **Adenovirus**            | Clinical specimens (throat or nasal swabs or faecal samples) | Not been validated for any other specimen than those listed | Most common technique is to passage Adenovirus in A549 cells before genetic analysis | [119-121] |
| **Cytomegalovirus (CMV)** | New born Screening card, fresh placenta, fresh umbilical cord, amniotic fluid, urine, serum, plasma, buffy coats, formalin-fixed, paraffin-embedded tissues | Not been validated for any other specimen than those listed | Generally two different CMV PCR are performed on each sample to minimise false positivity | [122-126] |
| Dengue Virus              | Serum | Not been validated for any other specimen than those listed | 'Not detected' should not be considered as absence of virus. | [127-131] |
| Enterovirus               | Faeces, CSF, nasopharyngeal aspirates, cell culture supernatant, throat, vesicle and rectal swabs | Blood and tissue samples | The specimen should be kept at 4 degree C | [132-135] |
| Epstein Barr Virus        | CSF, Blood, Biopsies | Not been validated for any other specimen than those listed | Stored at -20 degree C | [136,137] |
| Flavi virus               | CSF, CNS tissues and whole blood | Not been validated for any other specimen than those listed | West Nile virus assay also detects Kanjin virus. | [137] |
| Hepatitis A Virus         | Whole blood, serum, and EDTA tubes | Heparin tubes as it is a potent inhibitor of PCR | Haemolysed samples are causes PCR inhibition | [138] |
| Hepatitis B Virus         | - do - do - do - do | - do - do - do - do | - do - do - do - do | [138] |
| Hepatitis C Virus         | - do - do - do - do | - do - do - do - do | - do - do - do - do | [139] |
| Hepatitis D Virus         | - do - do - do - do | - do - do - do - do | - do - do - do - do | [140] |
| Hepatitis E Virus         | - do - do - do - do | - do - do - do - do | - do - do - do - do | [141] |
| Herpes Simplex Virus Type 1 and 2 | CSF, brain biopsy, FFPE | Not been validated for any other specimen than those listed | PCR detection has become a confirmatory test for HSV encephalitis | [142-147] |
| Human Bocavirus           | Respiratory secretions, nose/throat swabs, stool samples, whole blood | Not been validated for any other specimen than those listed | - | [148] |
| Human Coronavirus         | Respiratory specimens (Nasopharyngeal aspirates, nose and throat swabs and bronchial samples) | Not been validated for any other specimen than those listed | - | [149-151] |
| Human Herpes Viruses 6, 7 and 8 | EDTA blood, bone marrow and body fluids (amniotic fluid, CSF), biopsies, newborn screening cards | Not been validated for any other specimen than those listed | - | [122,124,125] |
| Human Papillomavirus      | Vaginal swab, tampon, FFPE, cells collected in liquid based cytology medium PreservCytTM (Hologic, Massachusetts, USA) and SurePathTM (BD, New Jersey, USA) | Not been validated for any other specimen than those listed | For keratinised tissues, specific methods should be followed | [152,153] |
| Human Polyomaviruses – JCV and BKV | EDTA blood, CSF, body fluids, urine, biopsies | Not been validated for any other specimen than those listed | JCV and BKV may occur at the same time in same patients | [93,154-158] |
| Human Polyomaviruses – KIV and WUV | Nasopharyngeal aspirates, bronchial washes and other respiratory secretions, nose/throat swabs, cerebrospinal fluid, urine, whole blood, and stool samples | Not been validated for any other specimen than those listed | - | [159-161] |
| Human Rhinoviruses        | Nasopharyngeal aspirates, nose and throat swabs, nasal or bronchial washings, broncho alveolar lavage or middle ear fluids | Not been validated for any other specimen than those listed | The assay may miss some viral strains | [162] |
| Influenza Virus A H3N1 (“Asian Influenza”) | Throat swab, nasal swab or nasal wash | Not been validated for any other specimen than those listed | Prior testing with current strains is mandatory | [130] |
In the future a diverse array of next generation sequencing (NGS) platforms like Roche 454 will be routinely used as it can sequence ~300 Gb of raw data per eight-lane flow cell in the form of a 100 bp reads and provide rapid sequencing [68]. The future is promising for bench top NGS analysers, like Ion PGM and the equipment which holds a huge scope is digital PCR (dPCR) which is still in its early stage of development. The sample is diluted and partitioned into hundreds or even millions of separate reaction chambers so that each contains one or no copies of the sequence of interest. By counting the number of 'positive' partitions (in which the sequence is detected) versus 'negative' partitions (in which it is not), scientists can determine exactly how many copies of a DNA molecule were in the original sample [71].

Table 3. Standardised molecular protocols in diagnostic mycology and parasitology.

| Fungi/Parasites | Acceptable Specimen | Unacceptable Specimen | Limitations/Comments | Primer Sequence/Probe and Method References |
|-----------------|---------------------|-----------------------|----------------------|-------------------------------------------|
| Aspergillus Species | BAL, EDTA whole blood, serum, fresh and paraffin embedded tissue, vitreous fluid, ascite fluid and CSF | Sputum and plasma | Penicillium spp. can also be amplified by the assay | [181-184] |
| Cryptosporidium | Fresh or frozen stool samples | - | PCR has a high sensitivity | [185-196] |
| Cyclospora | Fresh or frozen stool samples | - | The cited assay has a detection limit of 0.5 oocyst | [189] |
| Dientamoeba fragilis | Fresh or frozen stool samples | - | The cited assay amplifies 18s rDNA | [197-199] |
| Entamoeba histolytica | Fresh or frozen stool samples | - | The cited assay amplifies small subunit (18S) rDNA gene | [200-211] |
| Giardia | Fresh or frozen stool samples | - | - | [212-216] |
| Malaria (P. falciparum, P. vivax, P. malariae, P. ovale) | Whole blood samples | - | - | [155,217-219] |
| Microsporidia | Fresh or frozen stool samples | - | - | [220-227] |
| Pneumocystis jirovecii | Sputum, induced sputum, bronchial washings, bronchial lavages and lung biopsies | - | The cited assay can be used only as qualitative assay | [228,229] |
| Trichomonas vaginalis | Genital swabs and urine | - | The cited assay targets the β-tubulin and 18s rRNA genes | [230] |
| Toxoplasma gondii | CSF, CNS tissue, and FFPE | CSF supernatant, plasma, and serum | Negative results should be confirmed with serology | [231-233] |

Other automated systems such as the Abbott m1000 system, the ABI PRISM 6100 Nucleic Acid PrepStation, 6700 Automated Nucleic Acid Workstation, and the Corbett Robotics X-tractor Gene perform DNA extraction in a closed system.
one type of platform manufactured by Fluidigm and Life Technologies, reactions are created in within specially designed chips or plates. While in other platforms developed by BioRad and RainDance, reagents are sequestered into individual droplets i.e., Droplet Digital PCR (ddPCR).

Thus the future of diagnostic microbiology holds a tremendous scope offered by molecular diagnostics which has the potential to transform the precision, sensitivity and specificity of pathogen detection in a rapid and cost effective manner.

Competing interests
None declared.

References
None declared.

1. Leeuwen WB Van (2009) Molecular Diagnostics in Clinical Microbiology. 1: 5–20.
2. French GL (2010) The continuing crisis in antibiotic resistance. Int J Antimicrob Agents 36: S3–S7. [Crossref]
3. N Khodard (2014) Future of diagnostic microbiology. Indian J Med Microbiol 32: 371–377. [Crossref]
4. Fluit ADC, Visser MR, Schmitz F (2001) Molecular Detection of Antimicrobial Resistance. 14: 836–871. [Crossref]
5. Speers DJ (2006) Clinical Applications of Molecular Biology for Infectious Diseases. 27: 39–51. [Crossref]
6. Seeman NC(2003) DNA in a material world. Nature 421: 427–431. [Crossref]
7. Hammoudi D, Ayoub Moubareck C, Karam Sarkis D (2014) How to detect carbapenemase producers? A literature review of phenotypic and molecular methods. J Microbiol Methods 107: 106–118. [Crossref]
8. Mayer LW (1988) Use of Plasmid Profiles in Epidemiologic Surveillance of Disease Outbreaks and in Tracing the Transmission of Antibiotic Resistance. Clin Microbiol Rev 1: 228–243. [Crossref]
9. Road E, Harcourt P, Stunt RA, Amyes AK, Thomson CJ, Payne DJ, Amyes SG (1998) The production of a plasmid encoding the AmpC β-lactamase of Pseudomonas aeruginosa sequestered into individual droplets in other platforms developed by BioRad and RainDance, reagents are
10. Müller EM, Ashshi AM, Cooper RJ (2000) Multiplex PCR: Optimization and Application in Diagnostic Virology. Clin Microbiol Rev 13: 559–570. [Crossref]
11. Persson S, Olsen KEP (2016) Multiplex PCR for identification of Campylobacter coli and Campylobacter jejuni from pure cultures and directly on stool samples. J Med Microbiol 54: 1043–1047. [Crossref]
12. Louie DM, Goodfellow J, Mathieu P, Glatt A, Louie M, et al. (2002) Rapid Detection of Methicillin-Resistant Staphylococci from Blood Culture Bottles by Using a Multiplex PCR Assay. J Clin Microbiol 40: 2786–2790. [Crossref]
13. Poirel L, Walsh TR, Cuvillier V, Nordmann P (2011) Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 70: 119–123. [Crossref]
14. Yamashita H, Allan JC, Sato MO, Naka M, Sako Y, et al. (2004) DNA Differential Diagnosis of Taeniais and Cysticercosis by Multiplex PCR. J Clin Microbiol 42: 548–553. [Crossref]
15. Calsamiglia M, Pijoo C, Trigo A (2016) Application of a nested polymerase chain reaction assay to detect Mycoplasma hyopneumoniae from nasal swabs. J Vet Diagn Invest 25: 246–251. [Crossref]
16. Kim DM, Yoon NR, Yang TY, Lee JH, Yang JT, et al. (2006) Usefulness of nested pcr for the diagnosis of scrub typhus in clinical practice: a prospective study. Am J Trop Med Hyg 77: 542–545. [Crossref]
17. Carroll NM, Jaeger EE, Choudhury S, Dunlop AA, Matheson MM, et al. (2009) Detection of and Discrimination between Gram-Positive and Gram-Negative Bacteria in Intraocular Samples by Using Nested PCR. J Clin Microbiol 38: 1753–1757. [Crossref]
18. Song Q, Lange TA, Sahr AA, Adler G, Bode G (2016) Characteristic distribution pattern of Helicobacter pylori in dental plaque and saliva detected with nested PCR. J Med Microbiol 49: 49–53. [Crossref]
19. Rappelli P, Are R, Casu G, Fioi PL, Cappuccinelli P (1998) Development of a Nested PCR for Detection of Cryptococcus neoformans in Cerebrospinal Fluid. J Med Microbiol 36: 3438–3440. [Crossref]
20. Or HS, Hughes WT (2000) Search for Pneumocystis carinii DNA in upper and lower respiratory tract of humans. Diagn Microbiol Infect Dis 37: 161–164. [Crossref]
21. Klein D (2002) Quantification using real-time PCR technology: applications and limitations. Trends Mol Med 8: 257–260. [Crossref]
22. Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, et al. (2006) Real-Time PCR in Clinical Microbiology: Applications for Routine Laboratory Testing. Clin Microbiol Rev 19: 165–256. [Crossref]
23. Yeon Y, Heng C, Teng CH, Devi S (2006) Rapid detection, serotyping and quantitation of dengue viruses by TaqMan real-time one-step RT-PCR. J Virol Methods 138: 123–130. [Crossref]
24. Leroy EM, Baize S, Lu CY, Mccormick JB, Georges AJ, et al. (2000) Diagnosis of Ebolavirus/Hemorrhagic Fever by RT-PCR in an Epidemiological Setting. J Med Virol 467: 463–467. [Crossref]
25. Savli H, Karadenizli A, Kolayli F, Gundes S, Ozbek U, et al. (2016) Expression stability of six housekeeping genes: a proposal for resistance gene quantification studies of Pseudomonas aeruginosa by real-time quantitative RT-PCR. J Med Microbiol 2003: 403–408. [Crossref]
26. Balajee SA, Borman AM, Brandt ME, Cano J, Dannaoui E, et al. (2009) Species in the Clinical Mycology Laboratory: Where Are We and Where Should We Go from Here?. J Clin Microbiol 47: 877–884. [Crossref]
27. Didelot X, Bowden R, Wilson DJ, Peto TEA, Crook DW (2012) Transforming clinical
and serotyping of dengue virus in serum samples by multiplex reverse transcriptase PCR-ligase detection reaction assay. J Clin Microbiol 46: 3276–3284. [Crossref]

129. Lancioti RS, Calisher CH, Gubler DJ, Chang GJ, Vornad AM (1992) Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol 30: 545–551. [Crossref]

130. Smith G, Smith I, Harrower B, Warrillow D, Bletchly C (2006) A simple method for preparing synthetic controls for conventional and real-time PCR for the identification of endemic and exotic disease agents. J Virol Methods 135: 229–234. [Crossref]

131. Warrillow D, Northill JA, Pyke A, Smith GA (2002) Single rapid TaqMan fluorogenic probe based PRC assay that detects all four dengue serotypes. J Med Virol 66: 524–528. [Crossref]

132. Fowlkes AL, Honarmand S, Glaser C, Yagi S, Schnurr D, et al. (2008) Enterovirus-129. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV (1992) Rapid detection of enterovirus.

138. Nainan OV, Xia G, Vaughan G, Margolis HS (2006) Diagnosis of Hepatitis A Virus by reverse transcription-polymerase chain reaction using universal oligonucleotide primers. J Virol Methods 81: 109–113. [Crossref]

139. Simmonds P, Bukh J, Combet C, Deleage G, Enomoto N, et al. (2005) Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 42: 962–973. [Crossref]

140. Simpson LH, Battegay M, Hoofnagle JH, Waggoner JG, Di Bisceglie AM (1994) Hepatitis delta virus RNA in serum of patients with chronic hepatitis. Dig Dis Sci 39: 2650–2655. [Crossref]

141. Erker JC, Desai SM, Musahawar IK (1999) Rapid detection of Hepatitis E virus RNA by reverse transcription-polymerase chain reaction using universal oligonucleotide primers. J Virol Methods 81: 109–113. [Crossref]

142. Aberle SW, Puchhammer-Stöckl, E (2002) Diagnosis of herpesvirus infections of the central nervous system. J Clin Virol 25: 79–85. [Crossref]

143. Cinque P, Cleator GM, Weber T, Monteyne P, Sindic CJ, van Loon AM (1996) The General primer-mediated polymerase chain reaction for detection of enteroviruses: A tool for rapid species identification of uncharacterized enteroviruses. J Gen Virol 77: 1181–1187. [Crossref]

144. Mitchell PS, Espy MJ, Smith TF, Toal DR, Rys PN, et al. (1997) Laboratory diagnosis of congenital cytomegalovirus infection with cerebrospinal fluid specimens. J Clin Microbiol 35: 160–165. [Crossref]

145. Tebas P, Nease RF, Storch GA (1998) Use of the polymerase chain reaction in the diagnosis of herpes simplex virus encephalitis by using the polymerase chain reaction. J Clin Virol 10: 422–429. [Crossref]

146. Troendle-Atkins J, Demmler GJ, Buffone GJ (1993) Rapid diagnosis of herpes simplex virus infections by using the polymerase chain reaction. J Pediatr 123: 376–386. [Crossref]

147. Watzinger F, Suda M, Preuner S, Baumgartner R, Baskova L, et al. (2004) Real-Time Quantitative PCR Assays for Detection and Monitoring of Pathogenic Human Viruses in Immunosuppressed Pediatric Patients. J Clin Microbiol 42: 5189–5198. [Crossref]

148. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, et al. (2005) Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci USA 102: 12891–12896. [Crossref]

149. Gunson RN, Collins TC, Carman WF (2005) Real-time RT-PCR detection of 12 respiratory viral infections in four triplex reactions. J Clin Virol 33: 341–344. [Crossref]

150. Elden LJ, Loon AM, Alphen T, Hendriksen KA, Hoepelman AI, et al. (2004) Frequent detection of human coronaviruses in clinical specimens from patients with respiratory tract infection by use of a novel real-time reverse-transcriptase polymerase chain reaction. J Infect Dis 189: 652–657. [Crossref]

151. Tebas P, Nease RF, Storch GA (1998) Use of the polymerase chain reaction in the diagnosis of herpes simplex encephalitis: a decision analysis model. J Clin Virol 10: 422–429. [Crossref]

152. Whiley DM, Sloan TP (2007) Impact of competitive inhibition and sequence variation upon the sensitivity of malaria PCR. J Clin Microbiol 45: 1621–1623. [Crossref]

153. Chesters PM, Hedges J, Maceace DH, The S, Diseases I, et al. (1983) Persistence of DNA Sequences of BK Virus and JC Virus in Normal Human Tissues and in Tissues Treated with DNA Sequences of BK Virus and JC Virus in Normal Human Tissues. J Virol Methods 135: 32–42. [Crossref]

154. Pal A, Sirota L, Maudru T, Peden K, Lewis AM (2006) Real-time, quantitative PCR assays for the detection of virus-specific DNA in samples with mixed populations of polyomaviruses. J Virol Methods 135: 32–42. [Crossref]

155. Bilalsieiwicz S, Whitey DM, Nissen MD, Sloots TP (2007) Development and evaluation of real-time PCR assays for the detection of the newly identified KI and WU polyomaviruses. J Clin Virol 40: 9–14. [Crossref]

156. Gaynor AM, Nissen MD, Whiley DM, Mackay IM, Lambert SB, Wu G, et al. (2007) Identification of a novel polyomavirus from patients with acute respiratory tract infections. PLoS Pathog 3: 0595–0604. [Crossref]

157. Hansman GS, Katayama K, Maneekarn N, Peerakome S, Khamsin MH, et al. (2004) Improved Amplification of Genital Human Papillomaviruses Improved Amplification of Genital Human Papillomaviruses. J Clin Microbiol 38: 357–361. [Crossref]

158. Whiley DM, Bialasiewicz S, Whitey DM, Lambert SB, Gould A, Nissen MD, et al. (2007) Development and evaluation of real-time PCR assays for the detection of the newly identified KI and WU polyomaviruses. J Clin Microbiol 45: 203–204. [Crossref]

159. Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Hildesheim A, Schiffman MH, et al. (2000) Improved Amplification of Genital Human Papillomaviruses Improved Amplification of Genital Human Papillomaviruses. J Clin Microbiol 38: 357–361. [Crossref]

160. Holtz S, Llinas J, Ortega S, Paredes J, Bastida J, et al. (2004) Combined Analysis of Multiple Influenza A Viruses Present in Single Clinical Specimens Using Real-Time RT-PCR. J Virol Methods 123: 1321–1328. [Crossref]

161. Hübschen JM, Kremer JR, De Landtsheer S, Muller CP (2006) Development and evaluation of real-time PCR assays for the detection of human papillomaviruses (HPV) DNA in PreservCyt samples by the Roche AMPLICOR and LINEAR ARRAY HPV tests. J Clin Microbiol 44: 2428–2433. [Crossref]

162. Arthur RR, Dagostin S, Shah K V (1989) Detection of BK virus and JC virus in urine and brain tissue by the polymerase chain reaction. J Clin Microbiol 27: 1174–1179. [Crossref]

163. El Mubarak HS, De Swart RL, Osterhaus ADME, Schutten M (2005) Development of real-time RT-PCR assays for comprehensive detection of human rhinoviruses. J Clin Microbiol 43: 533–539. [Crossref]

164. Whitey DM, Sloots TP (2005) A 5-nuclease real-time reverse transcription-polymerase chain reaction assay for the detection of a broad range of influenza A subtypes, including HSN1. Diagn Microbiol Infect Dis 55: 335–337. [Crossref]

165. Whiley DM, Bialasiewicz S, Whitey DM, Lambert SB, Gould A, Nissen MD, et al. (2007) Development and evaluation of real-time PCR assays for the detection of the newly identified KI and WU polyomaviruses. J Clin Virol 40: 9–14. [Crossref]

166. Gnirke A, Sirota L, Maudru T, Kedra E, Lewis AM (2006) Real-time, quantitative PCR assays for the detection of virus-specific DNA in samples with mixed populations of polyomaviruses. J Virol Methods 135: 32–42. [Crossref]

167. Whiley DM, Sloots TP, (2005) A 5-nuclease real-time reverse transcriptase-polymerase chain reaction assay for the detection of a broad range of influenza A subtypes, including HSN1. Diagn Microbiol Infect Dis 55: 335–337. [Crossref]

168. Gaynor AM, Nissen MD, Whitey DM, Mackay IM, Lambert SB, Wu G, et al. (2007) Identification of a novel polyomavirus from patients with acute respiratory tract infections. J Clin Microbiol 45: 603–607. [Crossref]

169. Hansman GS, Katayama K, Maneekarn N, Peerakome S, Khamsin MH, et al. (2004) Genetic Diversity of Norovirus and Sapovirus in Hospitalized Infants with Sporadic Vira H (2016) Diagnostic molecular microbiology and its applications: Current and future perspectives.
Vira H (2016) Diagnostic molecular microbiology and its applications: Current and future perspectives

Clin Microbiol Infect Dis, 2016
doi: 10.15761/CMID.1000105
Volume 1(1): 20-31

170. Jiang X, Huang PW, Zhong WM, Farkas T, Cubit DW, Matson DO (1999) Design and evaluation of a primer pair that detects both Norwalk- and Sapporo-like calicviruses by RT-PCR. J Virol Methods 83: 145–154. [Crossref]

171. Kojima S, Kagayama T, Fukushima S, Hoshino FB, Shinhara M, et al. (2002) Genogroup-specific PCR primers for detection of Norwalk-like viruses. J Virol Methods 100: 107–114. [Crossref]

172. Cubie HA, Inglis JM, Leslie EE, Edmunds AT, Totapally B (1992) Detection of respiratory syncytial virus in acute bronchiolitis in infants. J Med Virol 38: 283–287. [Crossref]

173. Elscher M, Prudlo J, Hotzel H, Otto P, Sachse K (2002) Nested reverse transcriptase-polymerase chain reaction for the detection of group A rotaviruses. J Vet Med B Infect Dis Vet Public Health 49: 77–81. [Crossref]

174. Gentsch JR, Glass RI, Woods P, Gouveia V, Gorzgilla M, et al. (1992) Identification of group A rotavirus gene 4 types by polymerase chain reaction. J Clin Microbiol 30: 1365–1373. [Crossref]

175. Gouveia V, Glass RI, Woods P, Taniguchi K, Clark HF, Forrester B, et al. (1999) Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool Polymerase Chain Reaction Amplification and Typing of Rotavirus Nucleic Acid from Stool Specimens. J Clin Microbiol 28: 276–282. [Crossref]

176. Simmonds MK, Armah G, Asmah R, Banerjee I, Damanka S, et al. (2008) New

177. Zeng SQ, Halkosalo A, Salminen M, Szakal ED, Puustinen L, Vesikari T (2008) One-Step Quantitative RT-PCR for the Detection of Rotavirus in Acute Gastroenteritis. J Virol Methods 153: 238–240. [Crossref]

178. Coupe S, Sarfai C, Hamane S, Derouin F, Coupe S, et al. (2005) Detection of Cryptosporidium parvum DNA in human feces by nested PCR. J Clin Microbiol 43: 1017–1023. [Crossref]

179. Hausfater P, Fillet AM, Rozenberg F, Arthaud M, Trystram D, et al. (2004) Prevalence of Cryptosporidium and Identification to the Species Level by Nested PCR and Restriction Fragment Length Polymorphism. J Clinical Microbiol 42: 1769–1772. [Crossref]

180. Ihekwaba UK, Kudesia G, McKendrick MW (2008) Clinical Features of Viral Respiratory Syncytial Virus Infection in Acute Bronchiolitis in Infants. J Vet Med B Infect Dis Vet Public Health 51: 251–259. [Crossref]

181. Coupee S, Sarfai C, Hamane S, Derouin F, Coupe S, et al. (2005) Detection of Cryptosporidium and Identification to the Species Level by Nested PCR and Restriction Fragment Length Polymorphism. J Clinical Microbiol 43: 1017–1023. [Crossref]

182. Halliday C, Wu QX, James G, Sorrell T (2005) Development of a Nested Qualitative Discriminatory Real-Time PCR Assay for the Detection of Cryptosporidium parvum. J Clin Microbiol 43: 783–789. [Crossref]

183.照亮 W, Halkosalo A, Salminen M, Szakal ED, Puustinen L, Vesikari T (2008) One-Step Quantitative RT-PCR for the Detection of Rotavirus in Acute Gastroenteritis. J Virol Methods 153: 238–240. [Crossref]

184. Williamson EC, Leeming JP, Palmer HM, Steward CG, Warnock D, Marks DI, et al. (1996) Detection of Cryptosporidium parvum. J Clin Microbiol 34: 1769–1772. [Crossref]

185. Cubie HA, Inglis JM, Leslie EE, Edmunds AT, Totapally B (1992) Detection of respiratory syncytial virus in acute bronchiolitis in infants. J Med Virol 38: 283–287. [Crossref]

186. Balatbat AB, Jordan GW, Tang YJ, Silva JJ (1996) Detection of Cryptosporidium parvum. J Clin Microbiol 34: 1769–1772. [Crossref]

187. Coupe S, Sarfai C, Hamane S, Derouin F, Coupe S, et al. (2005) Detection of Cryptosporidium and Identification to the Species Level by Nested PCR and Restriction Fragment Length Polymorphism. J Clinical Microbiol 42: 1769–1772. [Crossref]

188. Fotedar R, Stark D, Beebe N, Marriott D, Ellis JT, et al. (2007) PCR detection of Cryptosporidium parvum. J Clin Microbiol 43: 1017–1023. [Crossref]

189. Garcia LS. (2007) Diagnostic Medical Parasitology FAQ ‘ s. ASM Press, Washington, DC.
Current status in Australia. Med J Aust 186: 412–416. [Crossref]

212. Ghosh S, Debnath A, Sil A, De S, Chattopadhyay DJ (2000) PCR detection of Giardia lamblia in stool: targeting intergenic spacer region of multicityp rRNA gene. Mol Cell Probes 14: 181–189. [Crossref]

213. Oster N, Gehrig-Feistel H, Jung H, Kammer J, McLean JE (2006) Evaluation of the immunochromatographic CORIS Giardia-Strip test for rapid diagnosis of Giardia lamblia. Eur J Clin Microbiol Infect Dis 25: 112–115. [Crossref]

214. Savioli L, Smith H, Thompson A (2006) Giardia and Cryptosporidium join the “Neglected Diseases Initiative.” Trends Parasitol 22: 203–208. [Crossref]

215. Verweij JJ, Schinkel J, Loeijendecker D, Van Rooyen MAA, Van Lieshout L, Polderman AM (2003) Real-time PCR for the detection of Giardia lamblia. Mol Cell Probes 17: 223–225. [Crossref]

216. Weitzel T, Dittrich S, Möhl I, Adusu E, Jelink T (2006) Evaluation of seven commercial antigen detection tests for Giardia and Cryptosporidium in stool samples. Clin Microbiol Infect 12: 656–659. [Crossref]

217. Charles DM, Hart J, Davis WA, Sullivan E, Dowse GK (2005) Notifications of imported malaria in Western Australia, 1990-2001: Incidence, associated factors and chemoprophylaxis. Med J Aust 182: 164–167. [Crossref]

218. Perandin F, Manca N (2004) Development of a real-time PCR assay for detection of Plasmodium falciparum, Plasmodium vivax, and Plasmodium ovale for routine clinical diagnosis. Journal of Clinical Virology 24: 1214–1219.

219. Whiley DM, LeComte GM, Baddeley A, Savill J, Symms MW, et al. (2004) Detection and differentiation of Plasmodium species by polymerase chain reaction and colorimetric detection in blood samples of patients with suspected malaria. Diagn Microbiol Infect Dis 49: 25–29. [Crossref]

220. Brasil P, de Lima DB, de Paiva DD, Lobo MS, Sodré FC, et al. (2000) Clinical and diagnostic aspects of intestinal microsporidiosis in HIV-infected patients with chronic diarrhea in Rio de Janeiro, Brazil. Rev Inst Med Trop Sao Paulo 42: 299–304. [Crossref]

221. Breton J, Bart-Delabesse E, Biligu S, Carbone A, Seiller X, et al. (2007) New highly divergent rRNA sequence among biodiverse genotypes of Enterocytozoon bieneusi strains isolated from humans in Gabon and Cameroon. J Clin Microbiol 45: 2580–2583. [Crossref]

222. Dowd SE, Gerba CP, Javier Enríquez F, Pepper IL (1998) PCR amplification and species determination of microsporidia in formalin-fixed, fixated tissues after immunomagnetic separation. Appl Environ Microbiol 64: 335–336. [Crossref]

223. Fedorok DP, Nelson N a, Cartwright CP (1995) Identification of microsporidia in stool specimens by using PCR and restriction endonucleases. J Clin Microbiol 33: 1739–1741. [Crossref]

224. Franzen C, Miller A, Hartmann P, Hegenauer P, Schrappe M, et al. (1998) Polymerase chain reaction for diagnosis and species differentiation of microsporidia. Folia Parasitologica (Prague) 45: 140–148. [Crossref]

225. Rinder H, Janitschke K, Aspöck H, Da Silva AJ, Deplazes P, et al. (1998) Blinded, externally controlled multicenter evaluation of light microscopy and PCR for detection of microsporidia in stool specimens. The Diagnostic Multicenter Study Group on Microsporidia. J Clin Microbiol 36: 1814–1818. [Crossref]

226. Stark D, Van Hal S, Barratt J, Ellis J, Marriott D, Harkness J (2009) Limited genetic diversity among genotypes of Enterocytozoon bieneusi strains isolated from HIV-infected patients from Sydney, Australia. J Med Microbiol 58: 355–357. [Crossref]

227. Verweij JJ, ten Hove R, Brien E AT, van Lieshout L (2007) Multiplex detection of Enterocytozoon bieneusi and Encephalitozoon spp. in fecal samples using real-time PCR. Diagn Microbiol Infect Dis 57: 163–167. [Crossref]

228. Morris A, Wei K, Adhikari K, Huang L (2008) Epidemiology and clinical significance of pneumocystis colonization. J Infect Dis 197: 10–17. [Crossref]

229. Palladino S, Kay I, Fonte R, Flexman J (2001) Use of real-time PCR and the LightCycler system for the rapid detection of Pneumocystis carinii in respiratory specimens. Diagn Microbiol Infect Dis 39: 233–236. [Crossref]

230. Hardick J, Yang S, Lin S, Duncan D, Gaydos C (2003) Use of the Roche LightCycler Instrument in a Real-Time PCR for Trichomonas vaginalis in Urine Samples from Females and Males. J Clin Microbiol 41: 5619–5622. [Crossref]

231. Filisetti D, Goicci M, Pernot-marino E, Villard O, Candolfi E (2003) Diagnosis of Congenital Toxoplasmosis : Comparison of Targets for Detection of Toxoplasma gondii by PCR. J Clin Microbiol 41: 4826–4828. [Crossref]

232. Homan WL, Vercammen M, De Braekeleer J, Verschueren H (2000) Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in Toxoplasma gondii, and its use for diagnostic and quantitative PCR. Int J Parasitol 30: 69–75. [Crossref]

233. James GS, Simpchenko VG, Dickeson DJ, Gilbert GL (1996) Comparison of cell culture , mouse inoculation, and PCR for detection of Toxoplasma gondii : effects of storage conditions on sensitivity. J Clin Microbiol 34: 1572–1575. [Crossref]