Enferm Infecc Microbiol Clin. 2017;35(1):41–46

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Continuing medical education: Methods of rapid diagnosis in clinical microbiology

Methods of rapid diagnosis in clinical microbiology: Clinical needs☆

Jordi Vila[a,b,*], María Dolores Gómez[c], Miguel Salavert[d], Jordi Bosch[a,b]

[a] ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic – Universitat de Barcelona, Barcelona, Spain
[b] Servicio de Microbiología, Centro de Diagnóstico Biomédico, Hospital Clínic, Barcelona, Spain
[c] Servicio de Microbiología, Hospital Universitario y Politécnico La Fe, Valencia, Spain
[d] Unidad de Enfermedades Infecciosas, Hospital Universitario y Politécnico La Fe, Valencia, Spain

ARTICLE INFO

Article history:
Received 16 November 2016
Accepted 16 November 2016
Available online 22 January 2017

Keywords:
Methods
Microbiological diagnosis
Impact
Needs

ABSTRACT

The diagnostic methods of infectious diseases should be fast, accurate, simple and affordable. The speed of diagnosis can play a crucial role in healing the patient, allowing the administration of appropriate antibiotic treatment. One aspect that increasingly determines the need for rapid diagnostic techniques is the increased rates of serious infections caused by multidrug resistant bacteria, which cause a high probability of error in the empirical treatment. Some of the conventional methods such as Gram staining or antigen detection can generate results in less than 1 h but lack sensitivity.

Today we are witnessing a major change in clinical microbiology laboratories with the technological advances such as molecular diagnostics, digital microbiology and mass spectrometry. There are several studies showing that these changes in the microbiological diagnosis reduce the generation time of the test results, which has an obvious clinical impact.

However, if we look into the future, other new technologies which will cover the needs required for a rapid microbiological diagnosis are on the horizon. This review provides an in depth analysis of the clinical impact that the implementation of rapid diagnostic techniques will have on unmet clinical needs.

Métodos de diagnóstico rápido en microbiología clínica: necesidades clínicas

Palabras clave:
Métodos
Diagnóstico microbiológico
Necesidades
Impacto

RESUMEN

Los métodos para diagnosticar enfermedades infecciosas han de ser rápidos, precisos, sencillos y asequibles. La rapidez en el diagnóstico puede jugar un papel crucial en la curación del paciente, ya que permite la administración de un tratamiento adecuado. Un aspecto que condiciona cada vez más la necesidad de disponer de técnicas de diagnóstico rápido es el aumento de las tasas de infecciones graves causadas por bacterias resistentes a los antibióticos, lo que ocasiona una elevada probabilidad de error en el tratamiento antibiótico empírico. Algunos de los métodos convencionales, como la tinción de Gram o la detección de antígenos pueden generar resultados en menos de una hora pero adolecen de sensibilidad.

En la actualidad estamos asistiendo a un cambio importante en los laboratorios de microbiología clínica, en el que se incluyen avances tecnológicos tales como el diagnóstico molecular, la microbiología digital y las técnicas de espectrometría de masas. Existen diversos estudios que demuestran que dichos cambios en el diagnóstico microbiológico reducen el tiempo de generación de los resultados de las pruebas, lo cual posee un impacto clínico evidente.

Sin embargo, si miramos hacia el futuro, otras nuevas tecnologías están en el horizonte, las cuales permitirán cubrir las necesidades que se requieren en el diagnóstico microbiológico rápido. Esta revisión

☆ Please cite this article as: Vila J, Gómez MD, Salavert M, Bosch J. Métodos de diagnóstico rápido en microbiología clínica: necesidades clínicas. Enferm Infecc Microbiol Clin. 2017;35:41–46.

* Corresponding author.
E-mail address: jvila@ub.edu (J. Vila).

proporciona un análisis en profundidad del impacto clínico que la implementación de técnicas de diagnóstico rápido tendrá en las necesidades clínicas no satisfechas.

Introduction

The "trending topic" in the field of biomedicine right now is personalised medicine, also called precision medicine, stratified medicine, and P4 medicine (predictive, personalised, preventive and participatory). It is understood as using the right drug for the indicated person at the right time. Although this concept has been gaining more importance in the area of cancer, if we consider all the specialties, we could say that clinical microbiology, and specifically diagnostic microbiology, is the paradigm of personalised medicine. Several diagnostic methods can be used ranging from direct methods, by directly detecting the microorganism causing the infection, such as microscopy, cultures, specific gene detection and antigen detection, to indirect methods, such as serology, in which the levels of specific antibodies against certain microorganism antigens are detected. In general, diagnostic methods must be fast, precise, simple and affordable. Evidently, some of the above-mentioned methods, such as Gram-staining, antigen detection or gene detection, present several of these characteristics. However, the primary requirements for a diagnostic method are high specificity and sensitivity. Other interesting collateral properties, although not essential, would be the possibility of being automated and being cost-effective.

For some infections, early diagnosis and treatment may have a crucial role in curing the patient or in reducing their morbidity and mortality, since the right antibiotic treatment is administered at the right time when needed. One aspect that is increasingly conditioning the need for fast diagnostic techniques is the rise in the rate of severe infections caused by antibiotic-resistant bacteria, which causes a high probability of error in empirical treatment.

At present, we are witnessing a significant change in the clinical microbiology laboratories led by automation. This trend is supported by technological advances such as molecular diagnostics, digital microbiology and mass spectrometry techniques (MALDI-ToF and ESI-ToF). These advances open the door to greater standardisation in the processes and results, a new level of operational excellence and performance, as well as better laboratory efficiency. There are several studies demonstrating that such changes in diagnostic microbiology reduce the time for generating test results, which has a clear clinical impact.

Despite the fact that clinical microbiology laboratories are implementing the many advances taking place in our field, if we look towards the future, other new techniques are on the horizon, including next-generation sequencing. Although it is only used in a few laboratories at present, it is undoubtedly one method to keep an eye on, since the bioinformatics analysis time and cost are being optimised.

In this review, we intend to analyse in detail the clinical impact that implementing these rapid diagnostic techniques will have, as well as the unmet clinical needs.

Clinical impact and need for rapid diagnosis

Sepsis

The fact is clear that a delay in starting a suitable antibiotic treatment for sepsis increases the risk of mortality.[1] Until the advent of molecular diagnostic tests, blood cultures were and continue to be the standard method for routinely detecting pathogenic bacteria and fungi in blood. However, blood cultures have limitations inherent to the methodology, including the time delay in obtaining results. At present, implementing direct MALDI-ToF from the positive blood culture along with detecting certain resistance genes (essentially the *mecA* gene and genes coding for ESBLs and carbapenemases), as well as multiplex PCR-based techniques for detecting the pathogens that most often cause bacteraemia and their resistance determinants, have had a significant clinical and economic impact by reducing the time to establish the right treatment to 46 h.[2–5]

Sepsis is generally treated empirically, using broad-spectrum antibiotics. However, broad-spectrum antibiotics are not always sufficient for treatment since resistance to antimicrobials is increasing. Studies have demonstrated that every hour of delay in implementing an effective treatment in sepsis patients leads to a 7.6% increase in mortality.[1] Molecular diagnostic techniques that detect specific genes directly in blood produce results faster than blood cultures since they avoid the antimicrobial growth time. Nevertheless, these new diagnostic techniques also have their limitations. Interpreting the results is sometimes complicated given that these molecular tests detect the DNA of microorganisms rather than live pathogens, in addition to the risk of interference from contamination, the presence of "background" DNA in blood and the lack of an ideal "gold standard".[6] Another limitation is that the antimicrobial sensitivity results cannot always be provided simultaneously. For this reason, such techniques are usually seen more as a potentially useful tool that complement conventional blood cultures and not as a definitive method that would exempt the use of blood cultures altogether.[7] As a result, blood cultures continue to be the cornerstone for diagnosing sepsis, since it is a prerequisite for the antimicrobial sensitivity tests. The main future need for diagnosing sepsis is to identify the causative microorganism and, in addition, to ascertain the antibiotic sensitivity directly from blood. An ideal test would be capable of processing a small volume of blood and be fast, technically simple or automated, low cost, and not require batch processing. An additional advantage would be the possibility of being able to determine the bacterial load directly from the blood. The published data indicate that determining the bacterial load in clinical samples using quantitative PCR (qPCR) potentially represents a useful marker for assessing the efficacy of a treatment and the prognosis in patients with acute bacterial infections.[8]

qPCR-based diagnostic tests will continue to grow in the coming years, however new techniques will emerge, especially based on microfluidics and nanotechnology, which will enable antibiotic sensitivity to be determined directly from the microorganism present in the blood without having to pass through blood cultures.

Respiratory infections

Until recently, when the topic of rapid diagnostics in respiratory infections arose, many continued thinking about the various direct stains for respiratory tract samples. These classic techniques enabled us, and still enable us, to assess the sample cellularity, and thus approximate the clinical value of the isolate on the one hand, and to distinguish the presence of microorganisms typically considered respiratory pathogens on the other. Afterwards, direct immunofluorescence stains were added for diagnosing *Legionella pneumophila* and viral infections. The arrival of

immunochromatography techniques represented a new tool to be incorporated into the rapid diagnosis of respiratory infections: *Streptococcus pyogenes* (*S. pyogenes*), *Streptococcus pneumoniae* (*S. pneumoniae*) and *Legionella* antigens in urine, respiratory syncytial virus (RSV), adenovirus and influenza A and B virus.[9]

Even so, in the case of community-acquired pneumonia, nearly 50% of the cases lack a definitive aetiological diagnosis, and it is admitted that approximately the other 50% of the known microbial causes are due to *S. pneumoniae*. Thus, among other limitations, it is difficult to specify the true percentage and impact of "mixed" pneumonia caused by two or more pathogens, ranging between 3 and 12% of community-acquired pneumonia according to the potential microbiological diagnosis. The signs and symptoms of different pathogens are superimposed in respiratory infections; therefore, an aetiological diagnosis based only on the clinical symptoms is not reliable. The development of techniques based on nucleic acid amplification (NAAT) has led to the availability of molecular techniques that can be performed directly on the sample. These techniques include the possibility of determining more than one respiratory pathogen, like the available multiplex PCR techniques. This enables sidestepping by ruling out each microorganism according to an algorithm, which in turn involves repeatedly taking new samples and adding new diagnostic tests.

Incorporating rapid diagnostic microbiology techniques translates into a benefit for patients with infectious respiratory syndrome, since it enables an early, targeted treatment to be started and isolation and public health measures to be taken if necessary Nevertheless, when positioning the rapid tests, it should not be forgotten that molecular and antigen detection techniques do not distinguish between viable and non-viable microorganisms (antigenuria continues to be positive even months after the onset of the infection), and they do not differentiate between carrier and disease status, key aspects to take into account since most respiratory pathogens can be found in patients as simple respiratory tract colonisers. These limitations could be partially solved with quantitative molecular techniques and by choosing the gene to be amplified, e.g. *S. pneumoniae* and the *lytA* gene.[10] Moreover, by not using a culture, all the information provided by it is lost, such as antibiotic sensitivity or variations in the antigen composition for developing new vaccines.

We still do not know what types of diseases or what factors (seasonality, immune status, age, disease severity and stage) should coincide to take advantage of or benefit from all these new available rapid techniques. Studies on the evidence of their repercussion on clinical practice are scarce.[11] And until then, clinical-microbiological protocols should be applied to correctly interpret and assess the results.[12]

Gastroenteritis/enterocolitis

Most clinical microbiology laboratories continue using specific culture methods, antigen detection and microscopic exams to detect bacteria, viruses and parasites in stool samples from patients with diarrhoea. The percentage of samples in which the intestinal pathogen is not detected can be very high, for several reasons: (1) not all intestinal pathogens are routinely found; e.g. intestinal pathogens with a low prevalence, such as *Bacteroides fragilis*, *Edwardsiella tarda*, *Escherichia albertii*[13]; (2) the lability of some intestinal pathogens such as *Shigella*; (3) the low sensitivity of the method used to detect some intestinal pathogens; and (4) unknown intestinal pathogens.

Molecular techniques have been introduced into the routine diagnosis of diarrhoea in several microbiology laboratories around the world. In general, these techniques can be organised into two groups[14]: (1) using PCR to detect one or several genes from the same microorganism (e.g. detecting *Clostridium difficile* or norovirus); and (2) using multiplex PCR to detect concomitant gastroenteritis-causing bacteria, viruses and parasites. A significant advantage of the molecular methods is automating the laboratory work flow. The molecular panels available on the market are systems that include integrated sample extraction, target amplification and amplified product detection. These methods provide additional advantages such as speed (1–2 h), they do not need trained personnel, a minimum handling time and lower contamination risk. The main limitation of these integrated systems is their cost. Nevertheless, incorporating these panels could be profitable in certain patients with gastroenteritis or enterocolitis, as well as in immunosuppressed patients.[15,16] However, more studies are needed to assess the costs and benefits of the molecular panels in other types of patient with diarrhoea.

One of the main limitations related to these techniques is the inability to distinguish between infection and colonisation or detecting an insignificant pathogen load that may or may not be related with the patient's symptoms. Several studies have reported that quantifying the intestinal pathogen load can provide information about the role of the detected intestinal pathogen in causing the diarrhoea.[17,18] Therefore, a more precise method is needed to quantify the intestinal pathogens present in the stool samples.

Furthermore, all the microbiological results should be interpreted within a clinical context. In addition to detecting a low intestinal pathogen load, false positive results may be obtained due to detecting non-viable microorganisms, free DNA/RNA, or even due to non-specific amplification in the multiplex PCR.

Detecting antibiotic resistance using molecular methods is another difficult problem to solve in the case of stool samples, due to the impossibility of assigning the detected resistance marker to a particular pathogen. Therefore, we must still process the stool sample to isolate the bacteria to then be able to carry out the sensitivity testing. However, this culture could be performed only in those samples that give a positive result to a bacterial pathogen using multiplex PCR. There are still some questions to be answered, such as: Should diagnostic molecular tools be used in all diarrhoea patients or only in specific patient groups? Can these new tests be profitable in the case of community-acquired diarrhoea? Can we precisely differentiate infection from colonisation using only a molecular approximation?

Meningitis and encephalitis

Rapid clinical and microbiological diagnosis, along with early and effective treatment, are key aspects for minimising morbidity and mortality from central nervous system (CNS) infections, especially from meningitis and encephalitis of infectious origin, as the complications can be very severe and the sequelae devastating, despite survival.[19,20] Moreover, the costs associated with these infections are significant, both short-term, related to hospitalisation and treatment, and long-term, due to the loss of social and work contributions.[21] Their management differs from intracranial suppurative complications such as brain abscesses, subdural empyema and epidural abscesses. It is closer to a diagnostic approach similar to that of other purulent collections, except due to the caveat of the location, which is less accessible for sample collection. Because of this, for the clinical diagnosis, cytobiochemistry and microbiology of meningitis and encephalitis, the cerebrospinal fluid (CSF) comprises a sample of unquestionable value. The basic tests for studying CSF usually include rapid stains (Gram, Ziehl-Neelsen, India ink, etc.), detecting bacterial antigens, the different types of cultures (bacterial, mycobacterial and fungal, and rarely viral cultures) and increasingly include molecular methods. This is all paired with cellular analysis and biochemistry testing of a series of laboratory parameters.

The sample handling protocols usually require a significant volume of CSF to conduct extensive testing, which is not always easy to acquire and the sampling may have to be repeated to get it.

Suspected cases of meningitis with a negative CSF Gram stain are an important diagnostic and therapeutic challenge, since in most cases the causative microorganisms will be unknown. On occasion, a rapid antigen agglutination test can be used to diagnose it, and this option covers a wide range of bacterial microorganisms, but it is not very sensitive. The false positive results that have also been reported with this method have caused some institutions to abandon it.[22]

In the case of bacterial meningitis, the culture is useful, but it can require 2–5 days, and it may give a false negative if the microorganism is nutritionally demanding, there was prior antibiotic treatment or the sample was handled incorrectly. It might give a sterile result or the microorganism might not grow in conventional cultures. Therefore, it is possible that the advent of modern NAAT will solve, or compensate for, some of these limitations of traditional microbiology in processing, labour, performance and results from the samples. NAAT may be helpful for diagnosing, monitoring and resolving bacterial meningitis.[23] Two important questions about PCR are: (1) PCR does not require virus replication for detection, therefore a positive PCR should not necessarily lead to the presence of infection itself nor contribute to an aetiological diagnosis in the patients' post-infection recovery phase in which the pathological diagnosis remains unclear; (2) PCR should not eliminate, for now, the practice of microbiological cultures, which continue to be necessary for also determining the sensitivity of the isolates to antibiotics and for monitoring resistance in a specific epidemiological and geographic context.

Panels for multiplex detection of several pathogenic microorganisms involved in these CNS infections are already available. Being able to discriminate bacterial agents from viral agents helps lead to a more targeted treatment when using antibiotics and antivirals.

Sexually transmitted infections

The incidence of sexually transmitted infections (STIs) has increased considerably in recent years. The emergence of antiretroviral treatment against HIV infection caused a growing number of people to stop using condoms to prevent this and other infections.

At present there are three populations more susceptible and at risk of developing a STI: men who have sex with men, circles related to female and male prostitution, and lastly, heterosexuals who have sexual relations with multiple partners, especially younger people.

Arriving at a rapid microbiological diagnosis of these infections pursues a dual aim. First, to initiate an effective treatment that prevents the disease from advancing and its possible sequelae. And secondly, to establish targeted preventive measures to prevent the infection from spreading. The speed of the diagnosis is essential for achieving both aims due to the peculiarity of these infections, which often cause those who have it to conceal it and resist going to healthcare centres.

The existence of STI appointments[24] that offer patients, in addition to discretion, a fast clinical and microbiological diagnosis, undoubtedly contribute to slowing their spread. In this context, a rapid microbiological diagnosis should aim to establish whether or not the infection exists, as well as to discover its aetiological agent. Classically, microbiologists have had several rapid techniques based on microscopic examination available that continue to maintain their efficacy, such as Gram staining of urethral discharge (for gonococcal urethritis) or vaginal discharge (for bacterial vaginosis), vaginal discharge smears (for vaginitis due to *Trichomonas vaginalis* [*T. vaginalis*] or *Candida* spp.), or dark field microscopy for primary syphilis (although with its practical limitations).

Some serology tests, such as detecting reaginic antibodies using rapid agglutination techniques (RPR), may be negative in the initial phases of primary syphilis. Other rapid techniques, such as detecting *Chlamydia trachomatis* (*C. trachomatis*) or herpes simplex virus antigens, have fallen into disuse due to their low sensitivity. Others, such as nucleic acid hybridisation techniques (for detecting *T. vaginalis*, *Candida* spp. or *Gardnerella vaginalis* [*G. vaginalis*]) have been overtaken by NAAT.

And these NAATs, especially the real-time polymerase chain reaction (rt-PCR) techniques, including those multiplex PCR techniques that enable several microorganisms involved in a certain infection to be detected, are already enabling and will enable in the future the needs for a rapid microbiological diagnosis for several STIs to be met: (1) detecting *Neisseria gonorrhoeae*, *C. trachomatis*, *Mycoplasma genitalium* and *Ureaplasma* spp. in patients with urethritis, proctitis, cervicitis and pelvic inflammatory disease[25]; (2) detecting *T. vaginalis* and *Candida* spp. in patients with vaginitis or diagnosing bacterial vaginitis using techniques that quantify the presence or absence of *G. vaginalis* and *Lactobacillus* spp.,[26] or those aimed at detecting *Mycoplasma hominis*, *Atopobium vaginae* and *Mobiluncus* spp.; (3) detecting *Treponema pallidum*, the L1-L2-L3 strains of *C. trachomatis* which cause venereal lymphogranuloma, *Haemophilus ducreyi*, and the herpes simplex 1 and 2 virus, which causes genital, rectal and pharyngeal ulcers.[27,28]

Some rt-multiplex PCR platforms currently exist that enable some of these microorganisms to be detected in less than an hour.[25] Others enable seven or more genital pathogens to be detected,[29,30] although they require a longer time. The right combination of microorganisms capable of being detected for a certain STI, excellent sensitivity and specificity, increased technique speed and ease of performing it, in addition to its decreasing economic cost, will encourage the use of these multiplex NAATs as a rapid diagnostic method for STIs.

Rapid diagnostic microbiology in immunosuppressed patients

The right empirical antibiotic treatment administered early in the case of immunosuppressed patients, and especially in febrile neutropaenia patients,[31,32] has been repeatedly associated with improved survival in patients with sepsis and/or bacteraemia.[33] Just like many immunocompetent patients, up to 40% receive unsuitable antibiotic therapy until the first positive blood culture report comes back, generally with the Gram stain result.[34] At this point, 12–20% of patients may have not yet started antibiotic treatment and 30–45% of patients will require changes to the empirical antibiotic treatment as a result of the stain report. It has been demonstrated that the information from Gram staining has a much higher impact on the antimicrobial treatment than the definitive blood culture results, provided later by isolation in the cultures and the antimicrobial sensitivity tests.[35]

Blood cultures continue to be the "gold standard" in diagnosing bacteraemia, but they have a low sensitivity for so-called "fastidious" bacteria and fungi, a term that usually encompasses microorganisms with nutritional requirements, metabolism defects or that require special atmospheric and temperature conditions. Blood culture sensitivity also decreases when the blood sample is taken after the start of antimicrobial treatment. The specificity is challenged by contamination of the blood culture. Thus, isolating the usual contaminants in blood cultures (particularly taking into account that some of them, such as coagulase-negative staphylococci are also common causes of bacteraemia) make interpreting the results much more difficult.[36] Isolating a potentially

contaminating microorganism based on more than one blood sample extracted from independent peripheral vein punctures is typically considered a true bacteraemia diagnosis; nevertheless, molecular genetic testing of these strains of coagulase-negative staphylococci isolated from paired blood samples suggests that either contamination may occur under these circumstances or that the infections are often polyclonal.[37]

Given that the blood culture as the "gold standard" is slow and insufficiently sensitive, new techniques have been developed to detect bacteria and fungi in blood, as has been previously described. A new T2 magnetic resonance (T2MR)-based diagnostic platform was recently announced in the candidaemia field, capable of ultrasensitive and rapid detection of target fungi in whole blood, without the need for a prior blood culture.[38,39] This ability to quickly and precisely exclude the possibility of candidaemia, especially caused by non-*albicans* species, has important implications in clinical practice, since it makes it possible to decrease the number of patients received empirical antifungal therapy, thus reducing the incidence of resistant strains, the occurrence of adverse effects and the economic cost of the medical care. It remains to be proven whether this technological platform could be profitable and translatable to different types of hosts (immunocompetent and immunosuppressed), to both adult and paediatric patients, as well as to sample types other than blood.

Rapid diagnostic microbiology in children

Infectious diseases continue to be one of the main causes for seeking outpatient, inpatient and emergency medical care in the paediatric population. Starting even before birth until they reach maturity, the paediatric population is especially susceptible to many infections. Newborns and infants do not yet have a sufficiently developed immune system to fight off these diseases. In childhood, children continue developing this immunity, in part thanks to vaccines, although they are often subject to transmission, from both adults and other children. Respiratory tract and gastrointestinal infections, as well as more serious infections such as sepsis or meningitis, are common in this population that is particularly predisposed to them.

In this context, a fast clinical and microbiological diagnosis of these processes, and starting a suitable treatment, whether antibiotic or not, are essential not only for the child's life and subsequent development, but also to prevent other collateral effects, such as mass use of paediatric emergency departments and the inevitable anguish of the parents and family members.

Controlling paediatric infections starts when the child is still in their mother's womb. One of many examples of this may be the need to detect the carrier status for *Streptococcus agalactiae* in expectant mothers in whom a vaginal-rectal culture was not performed to detect this microorganism, either because the pregnancy has not been monitored, or because the birth is taking place prematurely. Or those women who present an intra-amniotic infection that can trigger severe symptoms of sepsis or neonatal meningitis. To date, microbiologists have had access to a limited number of fast techniques capable of being used in the paediatric population, from microscopic observation (CSF Gram staining in a child which suspected meningitis continues to be an essential technique for its aetiological diagnosis), to antigen detection techniques.

There are agglutination tests for detecting the antigens of some meningitis-causing bacteria in CSF, although their low sensitivity and the scant amount of CSF that is available limit their use to specific situations.

There are also immunochromatography techniques for detecting *S. pneumoniae* (in urine) and *S. pyogenes* (in the pharynx), or some respiratory viruses (such as respiratory syncytial virus [RSV] or influenza) or gastrointestinal viruses (such as rotavirus or certain adenoviruses) that have a certain usefulness because of their easy use, although they suffer from low sensitivity.

It was not until the emergence of NAAT, especially the rt-PCR and multiplex PCR techniques, when the rapid diagnosis of paediatric infections was developed, and will continue to be developed. Below are some examples of the clinical needs: (1) intrapartum detection of *S. agalactiae* on vaginal-rectal smears from the mother (there are already platforms enabling it to be detected in under an hour[40]) or detection of *Ureaplasma* spp., *S. agalactiae*, *Escherichia coli* and *Listeria monocytogenes* in samples of amniotic fluid; (2) detection of meningoencephalitis-causing agents: there are already platforms that enable bacteria (*S. agalactiae*, *E. coli K-1*, *L. monocytogenes*, *Neisseria meningitidis*, *Haemophilus influenzae* and *S. pneumoniae*), fungi (*Cryptococcus* spp.) or viruses (cytomegalovirus, enterovirus, herpes simplex 1 and 2 virus, varicella-zoster virus, human herpesvirus 6 and Parechovirus) to be detected in around an hour[41]; (3) detection of bacteraemia- and fungaemia-causing agents: there are several platforms that enable, using NAAT, microorganisms grown in the blood culture flasks to be identified or several resistance mechanisms to be detected,[42,43] although the true challenge would be to have methods available that are sufficiently sensitive as to be applicable directly to the patient's blood, taking into account the scarce volume of sample available from certain patients, such as premature newborns, as mentioned above; (4) detection of respiratory tract infection-causing microorganisms, both those caused by bacteria (including *S. pneumoniae*, *H. influenzae*, *Bordetella pertussis*, *Mycoplasma pneumoniae* or *Chlamydia pneumoniae*) and by viruses (influenza A and B, RSV, parainfluenza, adenovirus, etc.)[44]; (5) detection of pharyngotonsillitis-causing microorganisms, especially *S. pyogenes*[45] (but also other bacteria such as *Streptococcus dysgalactiae or equisimilis*, *Arcanobacterium haemolyticum* or *Fusobacterium necrophorum*) or the Epstein–Barr virus; (6) detection of gastroenteritis-causing microorganisms: from bacteria (including *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia enterocolitica* or *enterohaemorrhagic E. coli*), through parasites (*Giardia lamblia*) or viruses (rotavirus, adenovirus 40–41, norovirus, etc.), as well as detection of *Helicobacter pylori* in stools.[46]

The diversity of microorganisms liable to be detected, the need for a small sample volume, the sensitivity and specificity of the technique, the speed and ease of performing it and the decreasing economic cost will encourage the use of these multiplex NAATs as a rapid diagnostic method for paediatric infections.

Conflict of interests

The authors declare that they have no conflicts of interest.

References

1. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in humna septic shock. Crit Care Med. 2006;34:1589–96.
2. Perez KK, Olsen RJ, Musick WL, Cernoch PL, Davis JR, Peterson LE, et al. Integrating rapid pathogen identification and antimicrobial stewardship significantly decreases hospital costs. Arch Pathol Lab Med. 2013;137:1247–54.
3. Wolk DM, Struelens MJ, Pancholi P, Davis T, Della-Latta P, Fuller D, et al. Rapid detection of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in wound specimens and blood cultures: multicenter preclinical evaluation of the Cepheid Xpert MRSA/SA skin and soft tissue and blood culture assays. J Clin Microbiol. 2009;47:823–6.
4. Zboromyrska Y, Vergara A, Cosgaya C, Verger G, Mosqueda N, Almela M, et al. Rapid detection of β-lactamases directly from positive blood cultures using a loop-mediated isothermal amplification (LAMP)-based assay. Int J Antimicrob Agents. 2015;46:355–6.
5. Kothari A, Morgan M, Haake DA. Emerging technologies for rapid identification of bloodstream pathogens. Clin Infect Dis. 2014;59:272–8.
6. Nikkari S, McLaughlin IJ, Bi W, Dodge DE, Relman DA. Does blood of healthy subjects contain bacterial ribosomal DNA? J Clin Microbiol. 2001;39:1956–9.

7. Lehmann LE, Alvarez J, Hunfeld KP, Goglio A, Kost GJ, Louie RF, et al. Potential clinical utility of polymerase chain reaction in microbiological testing for sepsis. Crit Care Med. 2009;37:3085–90.
8. Rello J, Lisboa T, Lujan M, Gallego M, Kee C, Kay I, et al. Severity of pneumococcal pneumonia associated with genomic bacterial load. Chest. 2009;136:832–40.
9. Drancourt M, Michel-Lepage A, Boyer S, Raoult D. The point-of-care laboratory in clinical microbiology. Clin Microbiol Rev. 2016;29:429–47.
10. Sanchez JL, Cooper HJ, Myers CA, Cummings JF, Vest KG, Russell KL, et al. Respiratory infections in the US military: recent experience and control. Clin Microbiol Rev. 2015;28:743–800.
11. Doern GV. The value of outcomes data in the practice of clinical microbiology. J Clin Microbiol. 2014;52:1314–6.
12. Schreckenberger PC, McAdam AJ. Point-Counterpoint: large multiplex PCR panels should be first-line test for detection of respiratory and intestinal pathogens. J Clin Microbiol. 2015;53:3110–5.
13. Humphries RM, Linscott AJ. Laboratory diagnosis of bacterial gastroenteritis. Clin Microbiol Rev. 2015;28:3–31.
14. Zboromyrska Y, Vila J. Advanced PCR-based molecular diagnosis of gastrointestinal infections: challenges and opportunities. Expert Rev Mol Diagn. 2016;16:631–40.
15. Rand KH, Tremblay EE, Hoidal M, Fisher LB, Grau KR, Karst SM. Multiplex gastrointestinal pathogen panels: implications for infection control. Diagn Microbiol Infect Dis. 2015;82:154–7.
16. Goldenberg SD, Bacelar M, Brazier P, Bisnauthsing K, Edgeworth JD. A cost benefit analysis of the Luminex xTAG Gastrointestinal Pathogen Panel for detection of infectious gastroenteritis in hospitalised patients. J Infect. 2015;70:504–11.
17. Bennett A, Bar-Zeev N, Jere KC, Tate JE, Parashar UD, Nakagomi O, et al. Determination of a viral load threshold to distinguish symptomatic versus asymptomatic rotavirus infection in a high-disease-burden African population. J Clin Microbiol. 2015;53:1951–4.
18. Teunis PF, Sukhrie FH, Vennema H, Bogerman J, Beersma MF, Koopmans MP. Shedding of norovirus in symptomatic and asymptomatic infections. Epidemiol Infect. 2015;143:1710–7.
19. Brouwer MC, Tunkel AR, van de Beek D. Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. Clin Microbiol Rev. 2010;23:467–92.
20. Edmond K, Clark A, Korczak VS, Sanderson C, Griffiths UK, Rudan I. Global and regional risk of disabling sequelae from bacterial meningitis: a systematic review and meta-analysis. Lancet Infect Dis. 2010;10:317–28.
21. Portnoy A, Jit M, Lauer J, Blommaert A, Ozawa S, Stack M, et al. Estimating cost of care for meningitis infections in low- and middle-income countries. Vaccine. 2015;33 Suppl. 1:A240–7.
22. El Bashir H, Laundy M, Booy R. Diagnosis and treatment of bacterial meningitis. Arch Dis Child. 2003;88:615–20.
23. Tunkel AR. Meningitis. Available from: www.antimicrobe.org/e7.asp [accessed 28.02.16].
24. Gaydos C, Hardick J. Point of care diagnostics for sexually transmitted infections: perspectives and advances. Expert Rev Anti Infect Ther. 2014;12:657–72.
25. Gaydos CA, Cartwright CP, Colaninno P, Welsch J, Holden J, Ho SY, et al. Performance of the Abbott RealTime CT/NG for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. J Clin Microbiol. 2010;48:3236–43.
26. Cartwright CP, Lembke BD, Ramachandran K, Body BA, Nye MB, Rivers CA, et al. Development and validation of a semiquantitative, multitarget PCR assay for diagnosis of bacterial vaginosis. J Clin Microbiol. 2012;50:2321–9.
27. Glatz M, Juricevic N, Altwegg M, Bruisten S, Komericki P, Lautenschlager S, et al. A multicenter prospective trial to asses a new real-time polymerase chain reaction for detection of *Treponema pallidum*, herpes simplex-1/2 and *Haemophilus ducreyi* in genital, anal and oropharyngeal ulcers. Clin Microbiol Infect. 2014;20:O1020–7.
28. De Vrieze NH, van Rooijen M, Schim van der Loeff MF, de Vries HJ. Anorectal and inguinal lymphogranuloma venereum among men who have sex with men in Amsterdam, The Netherlands: trends over time, symptomatology and concurrent infections. Sex Transm Infect. 2013;89:548–52.
29. Samra Z, Rosenberg S, Madar-Shapiro L. Direct simultaneous detection of 6 sexually transmitted pathogens from clinical specimens by multiplex polymerase chain reaction and auto-capillary electrophoresis. Diagn Microbiol Infect Dis. 2011;70:17–21.
30. Kriesel JD, Bhatia AS, Barrus C, Vaughn M, Gardner J, Crisp RJ. Multiplex PCR testing for nine different sexually transmitted diseases. Int J STD AIDS. 2015, pii:0956462415615775.
31. Rosa RG, Goldani LZ. Cohort study of the impact of time to antibiotic administration on mortality in patients with febrile neutropenia. Antimicrob Agents Chemother. 2014;58:3799–803.
32. Virizuela JA, Carratalà J, Aguado JM, Vicente D, Salavert M, Ruiz M, et al. Management of infection and febrile neutropenia in patients with solid cancer. Clin Transl Oncol. 2016;18:557–70.
33. Averbuch D, Orasch C, Cordonnier C, Livermore DM, Mikulska M, Viscoli C, et al. ECIL4, a joint venture of EBMT, EORTC, ICHS, ESGICH/ESCMID and ELN. European guidelines for empirical antibacterial therapy for febrile neutropenic patients in the era of growing resistance: summary of the 2011 4th European Conference on Infections in Leukemia. Haematologica. 2013;98:1826–35.
34. Uehara Y, Yagoshi M, Tanimichi Y, Yamada H, Shimoguchi K, Yamamoto S, et al. Impact of reporting Gram stain results from blood culture bottles on the selection of antimicrobial agents. Am J Clin Pathol. 2009;132:18–25.
35. Bouza E, Sousa D, Muñoz P, Rodriguez-Creixems M, Fron C, Lechuz JG. Bloodstream infections: a trial of the impact of different methods of reporting positive blood culture results. Clin Infect Dis. 2004;39:1161–9.
36. Weinstein MP. Blood culture contamination: persisting problems and partial progress. J Clin Microbiol. 2003;41:2275–8.
37. Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, Garey KW, Alangaden GJ, Vazquez JA, et al. T2 magnetic resonance assay for the rapid diagnosis of candidemia in whole blood: a clinical trial. Clin Infect Dis. 2015;60:892–9.
38. Neely LA, Audeh M, Phung NA, Min M, Suchocki A, Plourde D, et al. T2 magnetic resonance enables nanoparticle-mediated rapid detection of candidemia in whole blood. Sci Transl Med. 2013;5, 182ra54.
39. Seo SK, Venkataraman L, DeGirolami PC, Samore MH. Molecular typing of coagulase-negative staphylococci from blood cultures does not correlate with clinical criteria for true bacteremia. Am J Med. 2000;109:697–704.
40. El Helali N, Nguyen JC, Ly A, Giovangrandi Y, Trinquart L. Diagnostic accuracy of a rapid real-time polymerase chain reaction assay for universal intrapartum group B streptococcus screening. Clin Infect Dis. 2009;49:417–23.
41. Messacar K, Breazeale G, Robinson CC, Dominguez SR. Potential clinical impact of the film array meningitis encephalitis panel in children with suspected central nervous system infections. Diagn Microbiol Infect Dis. 2016;86:118–20.
42. Quiles MG, Menezes LC, Bauab Kde C, Gumpl EK, Rocchetti TT, Palomo FS, et al. Diagnosis of bacteremia in pediatric oncologic patients by in-house real-time PCR. BMC Infect Dis. 2015;15:283.
43. Tschiedel E, Steinmann J, Buer J, Onnebrink JG, Felderhoff-Müser U, Rath PM, et al. Results and relevance of molecular detection of pathogens by SeptiFast – a retrospective analysis in 75 critically ill children. Klin Padiatr. 2012;224:12–6.
44. Mengelle C, Mansuy JM, Pierre A, Claudet I, Grouteau E, Micheau P, et al. The use of a multiplex real-time PCR assay for diagnosing of acute respiratory viral infections in children attending an emergency unit. J Clin Virol. 2014;61:411–7.
45. Dunne EM, Marshall JL, Baker CA, Manning J, Gonis G, Danchin MH, et al. Detection of group A streptococcal pharyngitis by quantitative PCR. BMC Infect Dis. 2013;13:312.
46. Onori M, Coltella L, Mancinelli L, Argentieri M, Menichella D, Villani A, et al. Evaluation of a multiplex PCR assay for simultaneous detection of bacterial and viral enteropathogens in stool samples of paediatric patients. Diagn Microbiol Infect Dis. 2014;79:149–54.