Rapid microbiological diagnostic test in the Primary Care Pediatric consultation

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Abstract

The general characteristics of rapid microbiological diagnostic tests and their potential usefulness and healthcare impact in pediatric primary care are described. Rapid determination of biological markers are also reported, since although they are not microbiological diagnostic tests, they are highly useful in the management of febrile infants and in the etiological diagnosis of community-acquired pneumonia.

Resumen

Se describen las características generales de los test de diagnóstico microbiológico rápido y las utilidades potenciales e impacto asistencial de su utilización en Atención Primaria pediátrica. Se describe también la determinación rápida de marcadores biológicos, ya que pese a que no son test de diagnóstico microbiológico, presentan gran utilidad en el manejo del lactante febril y en el diagnóstico etiológico de la neumonía adquirida en la comunidad.

Key words: *Rapid microbiological diagnostic test; C-reactive protein; Procalcitonin; Pediatric primary care.*

Palabras clave: Test de diagnóstico microbiológico rápido; Proteína C reactiva; Procalcitonina; Atención Primaria pediátrica.

OBJECTIVES

- To know the basic theoretical functioning of microbiological rapid diagnostic tests (RDTs) and their limitations, strengths and indications in the Primary Care (PC) Pediatric consultation.
- To be familiar with the main RDTs and their potential use in the Primary Care consultation.
- To make the PC pediatrician aware of the essential nature of adequately staffing the PC consultation to improve resolution.

Introduction

In daily clinical practice, there are many occasions in which we would like to have simple methods for rapid etiological diagnosis, which could modify the approach in a medical procedure, both from an epidemiological and fundamentally diagnostic and/or therapeutic point of view. It is in this space that certain rapid diagnostic tests (RDTs) play an important role, which, in recent years, have been gaining greater presence in emergency departments[1], but whose use in Pediatric consultations of Primary Care (PC), both in the public and private sectors, continues to be very marginal or practically non-existent, and which are the subject of this article.

Theoretical foundations for the use of rapid diagnostic tests

Characteristics of rapid diagnostic tests

They are defined as those that are designed to be performed in the consultation, during the medical procedure, by the own physician or his or her auxiliary staff, without help from the

laboratory. They must: offer simplicity in collecting and processing samples, be minimally invasive or bothersome, and offer a quick result, generally within minutes.

How do they work?

Basically, we could explain the operation of microbiological RDTs in the following way[2]: if in a clinical sample, in our case respiratory secretion, blood, urine or feces, the antigen (Ag) of the germ that we aim to detect is present, when specific antibodies (Ab) labeled against this microorganism are added, an Ag-Ab fixation reaction will occur and an objective effect or signal will appear that is clearly detectable by the clinician. A test strip or an immunodiffusion plate/cassette will be positive or a positive/negative indication will be given, if the test requires a reader. Currently, we have different methods adapted to the POC (point of care) format: immunochromatography, immunofluorescence and molecular diagnosis by isothermal amplification. Most of the tests that are going to be presented in this article are immunochromatographic tests. They are the most used today due to their convenience and simplicity. Their operation is as follows: they are made on a small strip of stratified nitrocellulose or on a horizontal optical immunodiffusion or lateral immunoflow plate (cassette). At the bottom of the strip or at the base of the plate, there are rabbit-specific Abs labeled with colloidal gold. In the middle part, unmarked Abs. At the top of the strip or end of the plate, Ab of another animal, usually goat, directed against rabbit Abs. If we add a liquid biological sample at the bottom or basal part, due to capillarity, the liquid migrates up the strip or diffuses into the plate. If the sample is positive, the Ag-Ab complexes are captured by the second zone, where we observe the colored band of the labeled Abs. The leftover Abs (alone or in complex with Ag) continue to migrate upward or towards the end, making a colored positive second line, regardless of the test result. This second line will be a control that the test has been performed with the correct technique (Fig. 1), although it does not presuppose adequate sample collection.

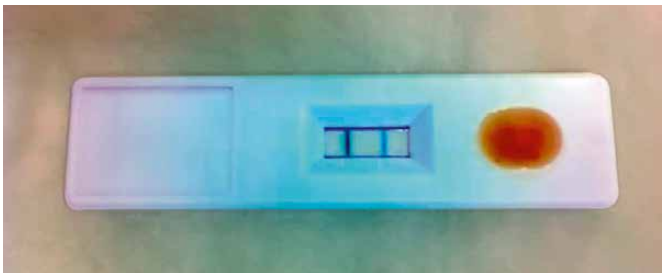

Figure 1. Lateral immunoflow plate/cassette with positive test line and control line. *Author´s personal observation.*

What do they inform us about?

In general, RDTs are qualitative tests, which give a positive or negative result, but do not allow the intensity of the bacterial inoculum or viral load to be quantified, nor to differentiate a carrier state from an active infection. Therefore, they are tests interpretable only within the clinical picture, which they cannot in any way replace. They do not give us a diagnosis, but rather inform us of the presence or absence of a certain germ in a biological sample. It is the professional who has to interpret this information and place it in its clinical and epidemiological context to use it appropriately.

When to use them?

RDTs should only be used in those cases in which, from the resulting information, potential changes in practical approach can be derived, not only in relation to the treatment, but also in terms of the epidemiological notion, information to parents, prediction of progress, isolation and evolutionary control.

Potential usefulness of the systematic use of rapid microbiological diagnostic tests in PC Pediatrics

Group A beta-hemolytic streptococcus

This RDT is now universal in all public sector PC consultations in Spain since 2018. When we evaluate a child with acute pharyngitis, we must carefully assess the clinical and epidemiological aspects before performing a RDT. If they go against the streptococcal etiology, the low probability of a positive result, which, in addition, would possibly reflect a carrier state (15% of school-age children), and the current low incidence of rheumatic fever and other serious secondary complications to *Streptococcus pyogenes* infection in our environment, would not justify the cost of indiscriminate use of the test in any process of pharyngodynia/pharyngeal hyperemia, mostly of viral cause. On the other hand, the pre-test assessment of a supposed high clinical and/or epidemiological probability of streptococcal pharyngitis has many false positives, even made by very experienced pediatricians, so the test would be indicated primarily in these cases, with the aim to use antibiotics (ATB) appropriately, significantly reducing their use. RDTs offer speed that reduces the spread of Group A beta-hemolytic streptococcus and promotes the child's rapid return to normal activity. Current immunochromatographic RDTs have high sensitivities, greater than 90%, similar to those obtained by culture, so the concomitant practice of culture can be avoided in negative cases[3]. The discrepancies in sensitivity studies probably depend on the methodology and the skill in collecting the sample: the use of a swab, with which you have to vigorously rub the two tonsillar surfaces, the posterior pharynx, the uvula, remove it without contacting the oral mucosa, the tongue or the teeth, and doing so as quickly as possible to reduce the inevitable discomfort of the child, is relatively simple, but requires a certain amount of practice and skill[4]. The decision to use or not use RDT in pharyngitis should be based on the pre-test intention to treat (do the test) or not treat (not do it), and not on clinical prediction scales (Centor-Mc Isaac) which have no consistency.

Cost-benefit studies with rapid tests demonstrate a reduction in costs derived from its systematic use[5,6].

The American Academy of Pediatrics concludes that, with the use of modern immunochromatographic tests, culture can be avoided in negative cases. This practice has not demonstrated an increase in suppurative and nonsuppurative complications of streptococcal infection.

The test is capable of detecting antigens up to 48 hours after starting antibiotic treatment, which makes it useful for suspending ATB treatments incorrectly instituted in presumably viral pharyngitis within this period, based on empirical diagnoses without etiological confirmation.

The tests have also been used with good results in the rapid diagnosis of potentially streptococcal skin pathology, mainly perianal cellulitis and, obviously, in scarlet fever.

Respiratory syncytial virus

Respiratory syncytial virus (RSV) causes annual epidemics of bronchiolitis and pneumonia in autumn-winter, and is the origin of a large number of outpatient visits and scheduled or spontaneous check-ups, spontaneous visits to the emergency room, and enormous pharmaceutical expenditure on medications of dubious value (bronchodilators and corticosteroids), but which some pediatricians continue to use, and of many hospital admissions, with a low, but not negligible, mortality, generally confined to high-risk infants. Due to its high transmissibility (5 to 12 days, sometimes up to 3 weeks), it is also the cause of school outbreaks and nosocomial infections, so infected children require management under adequate isolation conditions.

On a practical level, it seems that this need for isolation would be the fundamental indication of being able to have the test, obviously in a child who has to be admitted for bronchiolitis, but especially in the most frequent case of mild bronchiolitis with home monitoring, in which the patient should be isolated from daycare, a recommendation that is often forgotten if the child is afebrile and in good general condition. Documentation of the negativity of a previously positive test should be a criterion for re-entry into daycare. Some studies have suggested that RSV bronchiolitis has a course independent of the use of bronchodilator drugs, which would constitute a differential factor with bronchiolitis caused by other respiratory viruses and having a positive rapid test would be a definitive element in deciding therapeutic abstention. Other authors have not corroborated this observation, so therapeutic testing with bronchodilators continues to be common practice, regardless of the etiology, in those cases of moderate/severe symptoms with pulse oximetry <95%. It is a common observation that some RSV bronchiolitis objectively improve with the administration of beta 2 adrenergic receptor agonists.

From PC it seems that the rapid detection test could be of interest in the non-aggressive management of febrile infants with bronchiolitis. Various studies[7-9] have agreed in pointing out that the incidence of severe bacterial disease in children with positive RSV is initially very low, and that their documentation advises initially ignoring, in febrile children older than 1 month, any other complementary examination, with the possible exception of ruling out urinary tract infection, which is common in children with RSV infection. However, the immune depletion associated with RSV favors secondary bacterial superinfection, so the persistence of fever beyond 3-5 days should warn of this possible unfavorable progression.

Having this test available in PC could reduce the use of ATB in outpatient pneumonia. When there is a clinical suspicion of community-acquired pneumonia (CAP) in a child under 2 years of age, a positive rapid test for RSV during the epidemic should be a decisive factor in at least initial abstention from the use of ATB. Modern immunochromatographic tests have high sensitivity and specificity (89-94%), and provide results in 15 minutes. Samples can be obtained by nasopharyngeal swab or nasal wash-aspirate. Our group has published in this journal in September 2022, a decalogue for the use of RDTs for RSV in PC[10].

Flu

Pediatric flu is not as benign as it is believed, especially in young children, whose hospitalization rate for pneumonia is comparable to the classic risk group of those over 65 years of age and, above all, acts as a fundamental factor in the spread of the disease to high-risk groups, as children are the longest and most effective transmitters of the influenza virus, and have an attack rate of up to 50% in an epidemic.

The initial phase of influenza, before the appearance of respiratory symptoms, presents as a febrile syndrome without apparent focality (FSWF), and therefore considered in the differential diagnosis in infants and young children with the clinical risk of occult bacteremia (OB). The possibility of having a rapid test that allows children with FSWF to avoid the discomfort derived from the strict application of the OB protocol seems highly attractive.

The incidence of severe bacterial disease in a child with documented influenza is very low, and the presence of a positive result in a flu test allows the avoidance of other additional examinations and the use of ATB to be reduced[11-14].

Immunochromatographic influenza tests have lower sensitivities than other tests described in this article, between 45.5 and 80% depending on the series, higher for virus A, somewhat lower for virus B, but always lower than the viral culture, and a high specificity of 95%. There are large variations between different tests and sensitivity depends fundamentally on a correct technique for collecting the sample by nasopharyngeal swab (Fig. 2). Pneumonia is common in children with influenza and given the difficult clinical differentiation between viral pneumonia caused by the influenza virus itself or the much more frequent pneumococcal superinfection, a child with influenza who presents symptoms suggestive of pneumonia should undergo the same diagnostic procedure, regardless of the test result.

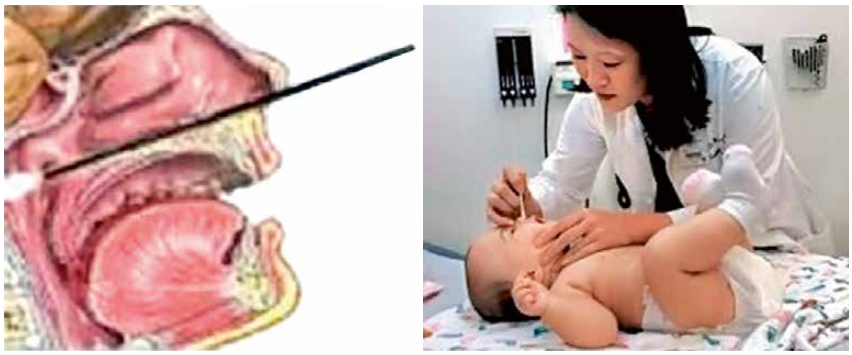

Figure 2. Respiratory sample collection technique by nasopharyngeal swab. *Author´s own elaboration and personal observation.*

The best period to perform the rapid test is between 12 and 48 hours from the onset of symptoms, and always within the first 5 days. There may be false negatives prior to 12 hours.

Modern immunochromatographic tests detect the influenza virus in less than 15 minutes, from a sample obtained by nasopharyngeal swab. Some kits can detect both viruses, A and B, and there are combinations for the detection of other respiratory viruses (RSV, adenovirus, SARS-CoV-2) and even for bocavirus, metapneumovirus and *Mycoplasma pneumoniae*. There are rapid immunofluorescence tests, which offer greater sensitivity than immunochromatography, although they require an automated reader. It is also available in a molecular diagnosis format by isothermal amplification, which increases sensitivity to values comparable to PCR, at a much lower cost and much more quickly (15 minutes), and the sample can be collected by nasal swab, which is more comfortable than the nasopharyngeal swab. Rapid POC molecular diagnosis has been recently extended to RSV.

In the first 2020-2021 pandemic season, RDTs have been incorporated into PC consultations in Catalonia and in some areas of Castilla-León and the Canary Islands. At the time of writing this article, its inclusion in the new common portfolio of services in PC of the Interterritorial Council of the Spanish National Health System (CISNS) is being evaluated. We consider that these tests are essential and have been shown to reduce return visits and the use of antibiotics[15].

SARS-CoV-2

Since the beginning of the pandemic caused by the new coronavirus, the etiological agent of COVID-19, sufficient consensus has been generated to affirm that, in addition to mobility restrictions, confinement and non-pharmacological interventions aimed at reducing infections (physical distance, face mask, hand hygiene, confinements and ventilation), the rapid identification of patients with symptoms compatible with COVID-19, close contacts of positive cases and asymptomatic individuals, quickly proceeding to their isolation or quarantine to cut off transmission chains, was a useful strategy to minimize the spread of the pandemic in its first waves. Currently, with growing population immunity due to vaccination and the circulation of less pathogenic variants, these interventions have been temporarily relaxed, pending the evolution of the virus, with the possible emergence of new, more pathogenic or more resistant to vaccination variants.

Molecular diagnosis and specifically the polymerase chain reaction (RT-PCR, per its acronym in English: Reverse Transcription Polymerase Chain Reaction, or simply PCR) is the reference standard for the diagnosis of SARS-CoV-2 infection.

SARS-CoV-2 antigen detection tests in POC format offer the great advantage of providing the result in a few minutes. Furthermore, the simplicity of sample processing and the low cost compared to molecular diagnosis favor its use in PC. In general, the sensitivity of antigenic tests is lower than molecular techniques in any RDT, although with comparable specificity.

RDTs for SARS-CoV-2 are immunochromatographic tests. They exist in exclusive format or in combos with influenza, influenza and RSV, adenovirus and even with *Mycoplasma pneumoniae*. These combined formats are very useful for the differential diagnosis of clinically indistinguishable respiratory infections, and reduce patient discomfort by requiring a single sample. In the case of SARS-CoV-2, the viral load is very high in the nasopharynx in the early stages of the disease, allowing for high sensitivity. The RDT for SARS-CoV-2 detects the presence of the virus's nucleocapsid protein, which is the main antigenic determinant of the virus.

RDTs are a most useful instrument for the diagnosis of "possible COVID" cases during the first 5-7 days from the onset of symptoms, and sensitivities as high as 93%, with specificity of 99.58% have been published, and those infected with false negative results have low viral loads and low transmission capacity. In a recent review, the interested reader can delve deeper into the diagnosis of COVID-19[16].

Pneumococcus

An immunochromatographic technique for detecting pneumococcal PnC antigen in urinary samples has proven useful in adults and children for the diagnosis of pneumococcal pneumonia, bacteremic or not, offering convenience in sample collection and processing and results in less than 15 minutes. Although pneumococcus frequently colonizes the oropharynx of normal children (up to 25% of children under 7 years of age), the germ concentration in these cases is usually below the level of antigenic detection required by the test. However, the test offers no doubts regarding its sensitivity, but its specificity (85%) has been questioned at an early age, with a high prevalence of nasopharyngeal colonization by pneumococcus. It is speculated that the PnC antigen can reach the urinary tract, especially in case of concomitant respiratory infection that damages the nasopharyngeal mucosal barrier. In any case, it is a test with

a sensitivity-specificity profile higher than the leukocyte count, as a predictor of pneumonia or bacteremia due to pneumococcus, which gives it potential interest for its use in a PC consultation[17], especially in the differential diagnosis between pneumococcal and atypical pneumonia in children over 3 years of age, with suspicion of community-acquired pneumonia.

Rotavirus

Acute gastroenteritis (AGE) due to rotavirus (RV) is the most common in young children, so that practically all of them have had contact with the virus by the age of 5, also experiencing various reinfections of decreasing severity. In our environment it does not cause mortality or it is very low, but the disease has a great socioeconomic impact. Children with RV AGE present higher and longer-lasting fever, more vomiting, a greater number of more liquid stools, and a worse general condition and higher incidence of dehydration than in other viral AGE.

The practical relevance that rapid diagnosis may have in the management of the child may seem *a priori* scarce, since treatment based on the prevention and treatment of dehydration with oral rehydration solutions is common to all AGE and independent of the etiology. However, the test allows correct information to parents, in the sense of predicting a longer and potentially severe condition that probably requires scheduled clinical check-ups (at least after 24 hours) to reassess the state of hydration of the child.

The test is also of interest for a stricter indication of isolation. RV is eliminated in very abundant quantities through feces and survives for several hours in the hands of the child's caregivers, and for days to weeks on inanimate surfaces (telephones, school supplies, notebooks...), so viral transmission is very effective and the disease is very contagious, causing school and nosocomial outbreaks. Hygienic-sanitary measures are not useful in the control of this disease, which is transmitted mainly by the fecal-oral route and by aerosols of respiratory particles. Rotavirus is not only a digestive disease of infants, but a disease that affects all ages (especially severe in extreme ages), with possible respiratory and neurological effects (seizures) and viremia. Meticulous hand washing by staff caring for children reduces transmission, only if done with powerful antiseptics and not with soap and water, but it does not prevent it, although it is the only partially effective method to control the spread of the disease. Therefore, knowledge of the etiology is important to take extreme isolation measures. Taking into account that the virus can be excreted for prolonged periods of several weeks, even without diarrhea, documentation of the negativity of a previously positive test could be a criterion for re-entry into daycare, which has not yet been studied.

Finally, the etiological diagnosis allows us to objectively assess the impact of the disease and the need to recommend its prevention with vaccines. In our environment, anti-rotavirus vaccination coverage is low, which is fundamentally due to a low perception of the importance of the disease on the part of the health professional who must recommend the vaccine. The RTD for rotavirus is an immunochromatographic test with sensitivity and specificity of 99%.

Adenovirus

Acute adenovirus gastroenteritis mainly affects children under 2 years of age and occurs throughout the year. It can cause nosocomial outbreaks, although they are less frequent than those caused by rotavirus. The duration of diarrhea is longer (10 to 14 days), but the condition is much milder and has much less tendency to dehydration. It has occasionally been associated with intussusception. A positive rapid test for adenovirus allows a prediction of a prolonged but benign condition, and probably reduces successive unscheduled visits.

The rapid test for adenovirus in feces is an immunochromatography test that can be associated with the same diagnostic kit for rotavirus, astrovirus and norovirus. The technique is very comfortable, requires a small amount of stool and offers results in a maximum of 15 minutes. There is no previous experience of its use in PC. In hospital studies, the test has shown sensitivity of 90% and specificity of 99%, in relation to viral culture.

Astrovirus

AGE due to astrovirus lasts 5-6 days and generally has a favorable progress and, rarely, evolves towards dehydration, but it induces secondary lactose intolerance more frequently than other viruses. The immunochromatographic test can be associated in the same kit with rotavirus, adenovirus and norovirus. In hospital studies it has shown sensitivity of 94% and specificity of 99%.

Norovirus

AGE due to calicivirus (norovirus and sapovirus) is characterized by the predominance of vomiting, nausea, and abdominal pain over diarrhea. Accompanying systemic symptoms are common. Norovirus is the most common cause of viral AGE in adolescents and adults, although it can affect any age, and school, nosocomial and isolated outbreaks ("Cruise ship AGE") are common. Although it is more common in winter, it can occur throughout the year, and in countries where there are high rates of vaccination against rotavirus, it is already the leading cause of viral AGE. It is also observed with increasing frequency in our environment. There is an immunochromatographic test integrated in combos with rotavirus, adenovirus and astrovirus.

Enterovirus

Enterovirus is an RNA virus with more than 100 serotypes, very prevalent in pediatric infectious pathology, causing: fever without focality, upper respiratory tract infections, rash and aseptic meningitis. It is more common in spring and summer. It is easily recognized by the PC pediatrician in its presentations of herpangina and mouth-hand-foot disease. It is also a frequent cause of acute AGE, which can be diagnosed in an immunochromatographic RDT. The epidemic experienced in 2016 in our environment, of severe neurological disease with rhombencephalitis due to enterovirus A71, makes it advisable, when faced with a diagnosis of enterovirus

disease, to inform the family of the early signs of neurological complication, especially drowsiness, tremors or ataxia, encourage hand washing and emphasize the need for isolation.

Campylobacter

It is the most common bacterial AGE in children, and the second cause of traveler's diarrhea, only behind enterotoxigenic *Escherichia coli*. The typical picture of AGE is frequently accompanied by blood in the stool, but its absence does not eliminate the etiological possibility. In infants, bloody diarrhea without fever is a common presentation that guides the diagnostic suspicion. It has been related to the appearance of Guillain-Barré syndrome, probably due to an autoimmune cause. The infection is usually benign (more severe in poor countries) and self-limited (rarely with bacteremia), although treatment with macrolides drastically reduces the symptomatic period and prevents chronic infections and relapses, so the interest of RDT is that an early diagnosis allows useful treatment to be instituted. The qualitative immunochromatographic test has a sensitivity and specificity of 99%.

Salmonella

AGE due to *Salmonella* produces a typical AGE picture, but focal infections occur in 10% and, in children under 6 months of age, there is a risk of bacteremia. In uncomplicated AGE, antibiotic treatment is not indicated. The qualitative immunochromatographic test has a sensitivity of 99% and a specificity of 97%.

Shigella

Early treatment of moderate or severe forms of AGE due to *Shigella* with azithromycin or cotrimoxazole reduces the symptomatic and excretion period. The test is qualitative immunochromatographic and has a sensitivity and specificity of 99%.

Helicobacter pylori

Helicobacter pylori (HP) infection is very common in humans, especially in low socioeconomic environments, and prevalences of up to 50% have been reported in pediatric age, although many of these infections are transient and asymptomatic. All children persistently infected by this germ develop histological changes suggestive of chronic gastritis.

HP infection in Pediatrics can be asymptomatic, or manifested by abdominal pain, vomiting and, less frequently, iron deficiency anemia refractory to treatment due to occult blood loss in feces, and growth retardation. Chronic HP colonization increases the risk of developing peptic ulcer and stomach cancer. Classically, the diagnosis was made with the detection of IgG antibodies, and histological changes were confirmed with endoscopy and biopsy. Currently, serological tests are not recommended in children. Urea tests in exhaled air are considered the diagnostic gold standard. Currently, there are methods for antigen detection of HP in feces using immunochromatography techniques[18,19], which offer great convenience and sensitivity (94%) and specificity (99%), similar to exhaled air urea tests. There is strong discussion about in which cases the presence of HP should be determined, but there seems to be a consensus that asymptomatic cases should not be treated. In our group, the protocol is referral to gastroenterology for endoscopy of children with a positive RDT.

Giardia

Giardia lamblia infection has a wide spectrum of presentation, ranging from asymptomatic colonization to acute diarrhea, chronic diarrhea, malabsorption syndrome with growth retardation, and recurrent abdominal pain.

The rapid test for giardia in feces[20] is an immunochromatography test that offers results in 10 minutes. There is no previous experience published in PC. Hospital studies show excellent agreement with: microscopic examination of stool, sensitivity, specificity and positive and negative predictive values greater than 99%.

Cryptosporidium

Initially, infection by the protozoan *Cryptosporidium parvum* was considered pathogenic only in immunosuppressed patients. It is now recognized as a common cause of acute diarrhea in healthy children around the world and of outbreaks in daycare centers. It produces abundant watery diarrhea, without blood, accompanied by intermittent abdominal pain, nausea, vomiting and anorexia. 80% of cases present with vomiting and may also be accompanied by headache, myalgia and weakness. Clinically, it is indistinguishable from other causes of AGE. 30-50% manifest fever. Diarrhea can last for weeks, and the infection is self-limiting in immunocompetent individuals, requiring treatment only in immunosuppressed or persistent cases.

The rapid test for *Cryptosporidium* in feces, which can be part of the same kit for the rapid diagnosis of giardia, is an immunochromatographic test that uses specific monoclonal antibodies, which detect all forms of the parasite's life cycle. There is no previous experience published in PC. Hospital studies show excellent agreement with: microscopic examination of stool, sensitivity, specificity as well as positive and negative predictive values greater than 99%.

Entamoeba histolytica

Amoebic dysentery is a common cause of acute watery or bloody diarrhea and chronic diarrhea, with possible liver involvement. The stool immunochromatographic test can be associated, in a *combo* format, with the determination of giardia and *Cryptosporidium*.

Infectious mononucleosis

Infectious mononucleosis (IM) is a self-limiting disease caused by the Epstein-Barr (EB) herpesvirus. The most common symptoms are: fatigue, pharyngitis, fever, lymphadenopathy, splenomegaly and liver disease. In rare cases, complications may occur such as: lymphoproliferative syndrome, severe thrombocytopenia, hemolytic anemia, pericarditis, myocarditis, pneumonia, pancreatitis, Reye's syndrome, encephalitis and other neurological syndromes. In industrialized countries, the peak incidence of IM occurs between 14 and 18 years of age. In developing countries or in areas with high population density, most children are infected before the age of 3, and

symptoms may be mild or clinically inapparent. EB pharyngitis (exudative and, sometimes, with petechiae) can pose diagnostic problems in PC and is easily confused with that caused by Group A beta-hemolytic streptococcus, and it is not uncommon for both to coexist. Its appearance can also be confused with that caused by adenovirus. It should always be considered in case of a suspected or confirmed streptococcal pharyngitis that does not improve within 3 days of correct treatment with ATB.

During the acute phase of the disease, heterophilic antibodies appear in 90% of IM, the presence of which can be demonstrated from the week of illness, reaching its maximum concentration at 2-4 weeks and decreasing at 12 weeks, being detectable even up to a year later; however, they are often undetectable in children younger than 5 years with IM.

The RDT for Epstein-Barr is based on the detection by immunochromatography of heterophile IgM antibodies in plasma, serum or whole blood. The sample can be easily obtained by capillary puncture. There is no previous experience of its use in PC. In comparative hospital studies with EIA and hemagglutination techniques, sensitivity and specificity have been greater than 99%.

C-reactive protein

Although it is not a microbiological diagnostic test, we include it here because of its potential usefulness in the evaluation of febrile children or those with pneumonia.

CRP is synthesized in the liver in response to high levels of cytokines, starting 4-6 hours after the onset of inflammation or tissue aggression, and its values double every 8 hours until reaching a peak at 36 hours. It acts as an immune modulator, promoting complement synthesis through the classical pathway and favoring phagocytosis. Currently, CRP is once again being claimed as a useful instrument in the evaluation of febrile children. In a reference meta-analysis and with a proposed cut-off level of 30 mg/L (reduced to 20 in the latest Cochrane review), it has been shown to have similar diagnostic accuracy as procalcitonin (PCT), generally considered more sensitive and specific; however this conception arises from studies in which there is a severity bias, with a high rate of more severe BD such as sepsis or meningitis. It is considered, based on these data, that PCT would be more a marker of the severity of the bacterial infection than a basic differentiator of viral versus bacterial disease. PCT has the advantage that it rises earlier than CRP, which can have a silent window of about 8 hours. However, the cost of CRP determination is significantly lower. Recently, CRP has even been proposed again as a useful complementary instrument in the etiological assessment of community-acquired pneumonia[21,22].

The determination of CRP in the PC consultation is very useful for a more precise assessment of FSWF in the age group at risk for OB, with the consequent reduction in unnecessary hospital referral. The new techniques for rapid determination of CRP in capillary blood are very comfortable for the child, have a low cost and allow the result to be available in a few minutes, so practical decisions can be made in the same medical procedure. Recently[23], its adequate correlation with conventional laboratory techniques and its usefulness in an emergency department for the management of febrile infants without focus have been demonstrated.

Procalcitonin

The emergence of a RDT for PCT, with potential use in PC, means the availability of a potentially very useful and decisive technique for the practical pediatrician, by overcoming the limitations of CRP, which has a silent window of about 8 hours, which is the time it take for the liver to start synthesizing it. PCT, sharing similar properties to CRP, appears earlier and is more specific than CRP and its levels are related to the severity of the infection[24,25].

PCT is a polypeptide precursor of calcitonin, and due to the effect of the convertase of the C cells of the thyroid gland, it is fractionated into several components, one of which is calcitonin, with a role in calcium homeostasis. Normally, PCT is undetectable in blood, since it is fractionated before being secreted.

The circulating PCT concentration in healthy individuals remains below 0.1 ng/ml and is not affected by viral infections or bacterial colonization, but the level increases rapidly with bacterial infections. In these, due to the effect of toxins, both Gram positive and negative, the production of PCT is stimulated in various tissues, also inactivating the convertase enzyme, which inhibits the proteolysis of PCT and it is immediately released into the bloodstream (on the contrary, this process is blocked in viriasis). All of this allows PCT to be detected in the blood in about 3 hours, with a maximum peak at 12 hours, a level that persists for several days. It has a plasma half-life of about 24-30 hours. PCT is considered the most specific and earliest marker for the detection of sepsis. Serum levels correspond to the severity of the condition and the response to treatment, which gives it great diagnostic and prognostic value in bacterial infections and sepsis. High levels indicate the existence of a systemic, severe and/or bacterial infection, rather than a viral or inflammatory one. It also serves as an assistant in the monitoring of the evolution and treatment of children with bacterial infections and as a diagnostic aid in cases with fever without apparent focality, even in immunosuppressed, neutropenic, oncological and transplant patients and, likewise, in the monitoring of non-infectious inflammatory states. Early confirmation of the viral *vs.* bacterial etiology implies better management in the use of antibiotics, avoiding them in febrile symptoms without focus, and in a more rational use of referral to hospital emergency rooms and the request for complementary tests.

In localized infections, it can reach 0.5 ng/ml. In a state of bacterial sepsis with systemic repercussions, PCT begins to increase 3-6 hours after the stimulus occurs, reaches its maximum concentration between 12 and 36 hours later, with values even higher than 10 ng/ml, and then, when this stimulus disappears, it begins to decline. Its half-life is 25-30 hours. This increase

of several times its normal value makes it an ideal marker for bacterial sepsis. When sepsis is not of bacterial origin, levels remain in the lower range (<0.1 ng/ml), which is very useful in a differential diagnosis of viral infections and allergic states.

When levels are between 0.5 and 2 ng/ml, bacterial infection cannot be excluded and another determination is recommended within 6-24 hours, observing clinical signs and symptoms. It is advisable to repeat the test every 24 hours in patients at risk of developing sepsis for monitoring.

Persistently elevated PCT concentrations or continued plasma increases generally indicate that the infection is not resolved, is not under control and/or therapeutic measures are not effective. On the contrary, the return of PCT to basal levels indicates that the infectious process is resolving and that the treatment is effective. An advantage of PCT-guided treatment is to avoid unnecessary prescription of antibiotics or excessive duration of antibiotic therapy (successive controls), with beneficial effects on antimicrobial resistance.

The working range is 0.1 to 100 ng/ml:

- <0.1 ng/ml: normal values.
- 0.1 to 0.25 ng/ml (cutoff point): viriasis and unlikely bacterial infection.
- 0.25 to 0.5 ng/ml: possible bacterial infection.
- 0.5 to <2 ng/ml: probable bacterial infection.
- >2 ng/ml to >10 ng/ml: bacterial infection, sepsis, very likely.
- >10 ng/ml: compatible with sepsis.

Joint determination of *Myxovirus* resistance protein A with CRP

Both CRP and PCT share the limitation of the assessment of intermediate quantitative ranges: CRP values between 20/30-70 mg/L, and PCT values between 0.25-0.50 ng/ml may correspond, both to a viral or bacterial infection. It is common to observe these values in infections caused by adenovirus and, to a lesser extent, by the influenza virus and SARS-CoV-2, as well as in cytomegalovirus infections and measles[20]. The objective of obtaining maximum sensitivity in PC to minimize false negatives means that these ranges are usually handled as if they were bacterial infections, even reducing specificity, thereby increasing false positives.

Recently, a new biological marker has been introduced, *Myxovirus* resistance protein A (MxA), which increases specifically in viral infections[21-25], in which a cellular response is produced that includes the secretion of type I interferons. These interferons are part of innate immunity and have immunomodulatory, antiproliferative and antiviral effects. Although type I interferon has been proposed as a marker of viral infection, it does not perform well as a diagnostic test, given its reduced half-life in blood. The antiviral activity of type I interferons is mediated by the induction of various proteins, among which is MxA[26].

RDTs have been marketed that jointly analyze semiquantitative (immunochromatographic test) and quantitative (immunofluorescence) values of CRP and MxA in a capillary blood sample. In comparative studies it has been shown that the joint analysis of the two markers increases the sensitivity and specificity of either of the two separately. Although most of the experiences have been developed in adult respiratory pathology, very promising data have also been published in children.

The positivity of the test for CRP is set at the level of 20 mg/l (lower than that described in the CRP section, which increases sensitivity at the expense of reducing specificity), and that of MxA at the level of 40 ng/ml for immunochromatography, and 10 mg/l and 15 ng/ml for immunofluorescence. A test that is + CRP and negative MxA is suggestive of bacterial infection. A test + to MxA is suggestive of viral infection, regardless of the high or low value of the CRP, in such a way that it provides extra specificity to cases of CRP or PCT in the intermediate range, which actually correspond to viral infections. In our experience, when we have used it as a rescue test in cases of PCT in the intermediate range, the results have almost always shown a viral pattern (MxA +), decisively modifying the diagnostic-therapeutic approach.

The sample is conveniently obtained in capillary blood, with an integrated lancet-cassette device with the nitrocellulose strip, where immunochromatography is performed, and the results are available in 10 minutes.

To date, no comparative studies have been carried out with PCT.

Conflict of interests

There is no conflict of interest in the preparation of the manuscript. Declaration of interests: none.

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The asterisks show the interest of the article in the authors' opinion.

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Updated review of the TECDIAP on rapid tests and diagnostic tools in the pediatric Primary Care consultation.

Accreditation quiz

The Accreditation Questionnaires for FC topics can be done at "On line" through the web: www.sepeap.org and www.pediatriaintegral.es. To obtain the single continuous training accreditation from the accreditation system for health professionals for the entire national health system, 85% of the questions must be answered correctly. The accreditation questionnaires on the different issues in the journal may be carried out during the period stated in the online questionnaire.

Accreditation quiz

Subsequently, the following accreditation quiz of Pediatría Integral collects questions on this topic, which must be answered online through the website: www.sepeap.org.
In order to obtain certification by the Spanish "formación continuada" national health system for health professionals, 85% of the questions must be answered correctly. The accreditation quizzes of the different numbers of the journal may be submitted during the period indicated in the "on-line" quiz.

Rapid microbiological diagnostic test in the Primary Care Pediatric consultation

33. With regards to rapid microbiological diagnostic tests, one of the following statements is INCORRECT:

a. They are tests designed to be performed in the office, without laboratory help.
b. Agglutination tests are not the most suitable for the diagnosis of viral infections.
c. They detect specific antibodies.
d. They are qualitative tests.
e. Immunochromatography tests are performed on a reagent strip.

34. Immunochromatography tests for the detection of streptococcus are CHARACTERIZED by:

a. Offering results in a few hours.
b. Being very sensitive, but not very specific.
c. Being very specific, but not very sensitive.
d. Being indicated in situations of low clinical-epidemiological probability of streptococcal infection.
e. Being sufficient for diagnosis in areas of low incidence of severe complications associated with streptococcal infection.

35. A positive RSV rapid detection test in a PC consultation would allow the pediatrician to adopt all of the following behaviors, EXCEPT ONE:

a. To provide more specific and convincing instructions to parents in case of not administering medication.
b. To avoid additional examinations to assess possible occult bacteremia in a child with bronchiolitis, high fever and good general condition.
c. Avoidance in the use of antibiotics in case of clinical suspicion of pneumonia.
d. To take utmost isolation measures and remove the child from daycare.
e. To avoid a therapeutic trial with bronchodilators in bronchiolitis.

36. With regards to the rapid diagnostic test of flu, one of the following statements is INCORRECT:

a. There are tests that detect viruses A and B in the same kit.
b. They are very specific, but less sensitive than the viral culture.
c. They are performed by collecting the sample by nasopharyngeal swab.
d. A positive test allows the avoidance of antibiotics in pneumonia.
e. A positive test allows the avoidance of carrying out other additional tests to assess possible risk of hidden bacteria.

37. One of the following characteristics of the determination of CRP in capillary blood is INCORRECT:

a. It has comparable sensitivity/specificity to procalcitonin.
b. The test presents earlier changes than procalcitonin.
c. A value less than 20/30 mg/l is suggestive of viral infection.
d. It is more economical than the determination of procalcitonin.
e. It presents a sensitivity/specificity profile higher than leukocyte counts.

38. Rapid microbiological diagnostic tests must meet the following requirements, EXCEPT for:

a. Results must be available within 30-60 minutes.
b. Tests should be minimally uncomfortable and invasive to the patient.
c. Results should be available at the same time as the consultation or after a brief period in the waiting room.
d. Sensitivity should be acceptable, with few false negatives.
e. Specificity should be excellent, with almost no false positives.

39. With regards to immunofluorescence techniques adapted to Primary Care (point of care), one of the following statements is INCORRECT:

a. They require an automated reader for interpretation.
b. They increase the sensitivity of immunochromatographic techniques.
c. They reduce false negatives caused by very weak positives not detectable by the human eye.
d. Sample collection is more uncomfortable for the patient.
e. They have a higher cost than immunochromatography.

40. The sensitivity of a rapid immunochromatographic microbiological diagnostic test depends on many factors. Please point out which is the MOST IMPORTANT:

a. The commercial brand used.
b. The sample collection technique.
c. The sample processing method.
d. The prevalence of the disease.
e. The pretest clinical diagnosis.