REVIEW ARTICLE

Point-of-Care Testing in Microbiology

The Advantages and Disadvantages of Immunochromatographic Test Strips

Enno Stürenburg, Ralf Junker

SUMMARY

Background: Point-of-care testing (POCT) for the demonstration of pathogens was introduced several years ago. The present study describes the current technical status of POCT, giving some examples, and summarizes the specific advantages and disadvantages of the POCT approach in microbiology.

Methods: Selective review of the literature found in medical databases under consideration of current German and international guidelines.

Results/conclusions: The test systems available today are technically mature and offer good to very good performance. For HIV, malaria, group A streptococci, and legionellae, POCT testing, when indicated, is on a par with conventional procedures. The information yielded by rapid tests for pneumococci and for influenza tends to be supplementary in nature. The rapid test for group B streptococci is unsuitable for routine use because its sensitivity is still too low compared with bacterial culture. POCT can be successful only if the tests are performed correctly by trained personnel, quality management procedures are followed, and the severity of illness and the epidemiological circumstances are taken into account when interpreting the results.

Key words: laboratory diagnosis, infection risk, immune diagnosis, rapid testing, diagnosis

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LADR GmbH, MVZ Geesthacht – Labor Dr. Kramer & Kollegen, Geesthacht: PD Dr. med. Stürenburg

Westfälische Wilhelms-Universität Münster: Prof. Dr. med. Junker

Point-of-care testing (POCT) has greatly increased in recent years *(box 1)*. Typical areas of use include blood gas analysis and blood sugar determination; use for cardiac markers is also increasing. This form of diagnosis has also been available for several years for problems related to infectious diseases—mostly as test strips or easy-to-operate cassette systems. Numerous products are commercially available in Germany for the point-of-care diagnosis of viral, bacterial and parasitic infections. A distinction must be made between POCT in the strict sense and so-called home testing, which includes blood sugar and PT/INR controls performed by the patient himself, and more recent developments, such as malaria and other rapid tests (including rapid tests to detect HIV antibodies), which are intended to be performed by people without medical training.

The use of these systems would have increased even more, were it not that their cost can hardly be covered in medical practices. There are few possibilities for reimbursement, either according to the GOÄ [Gebührenordnung für Ärzte; Directive on Medical Fees] or the EBM [einheitlicher Bewertungsmaßstab; Standard Evaluation Scale] (2, 3). Within hospitals, it must be decided in the individual case whether the extra costs are balanced by the benefits from more rapid diagnosis (4).

The basic principle in most systems is the immunochromatographic test of a specific microbial antigen (or more rarely, antibody) in the patient sample (urine, swab, whole blood), using the ELISA (enzyme linked immunosorbent assay) principle. There have also been some attempts to use molecular biological methods (mostly the polymerase chain reaction, PCR) for POCT, although these are technically demanding, so that they are not (yet) rapid tests in the strict sense *(box 1)*.

The most common argument for the use of these tests at the patient's bedside is the saving in time, as transport into the laboratory is now no longer necessary and tedious culture or nonculture analysis (depending on the problem) can be dispensed with. The diagnosis of a bacterial infection using culture requires at least 48 to 72 h. Diagnosis of viral or parasitic diseases—particularly in smaller hospitals—is either not available at all, is not performed rapidly or is performed within the laboratory with test strips (POCT).

BOX 1

Definition of POCT diagnosis

- Laboratory investigation performed near the patient
- With measurement systems that are easy to operate
- In the context of direct patient care
- With therapeutic relevance in patients at risk of death
- Within departments for in-patients, out-patient clinics or special functional areas (e.g. emergency admissions, operating theater, delivery room, endoscopy unit, interventional radiology)
- By personnel who have in general had no detailed training as medical technical assistants and no experience in laboratory medicine

Taken from Briedigkeit et al. 1998 (1)
POCT = point-of-care testing

A recent observational study on 2154 patients with septic shock has identified how important it is to start specific antibiotic therapy for the pathogen as early as possible. 83% of patients treated within the first 30 min (from initial onset of shock symptoms) survived; the survival rate was 6% poorer for patients given antibiotic therapy after 30 to 60 min (5). The mortality then increased by 7% for each additional delay of an hour (5).

On the other hand, critics have doubted whether the time gained with POCT can really be exploited for the patient's benefit. They argue that most dangerous infections can be equally well treated by immediately administering an empirically selected antibiotic against the pathogen or pathogens. This position is also evident in the current consensus criteria of many guidelines, so that the diagnosis is often explicitly no longer linked to the detection of the pathogen, but is primarily defined on the basis of clinical criteria (6, 7).

It may therefore be asked, what are the specific potential benefits and risks, advantages and disadvantages, of the diagnosis of infectious diseases with rapid tests. With the objective of analyzing the practical benefits of the strips in normal clinical work, the authors have evaluated the literature on some of the rapid tests which are of particular practical importance (pneumococci, legionellae, influenza, beta-hemolyzing streptococci of groups A and B, HIV, and malaria).

Methods

The selective literature evaluation on the theme of "POCT" and "microbiology" was performed in medical literature databases, in Medline/PubMed, in our own library, with Internet search engines, and by individual searches in relevant institutions (Centers for Disease Control and Prevention, CDC; World Health Organization, WHO) and medical societies (Association of Scientific Medical Societies in Germany, AWMF). The authors inspected the literature up to April 2008, selected relevant articles, and evaluated them. The following search terms were used in German and English: "antigen test," "rapid test device," "point-of-care-test," "POCT," "bedside test," "rapid test" and "immunochromatographic test." The titles of the identified publications were systematically examined for the following additional terms, to reduce the number of hits: "influenza," "Legionella pneumophila," "legionella urinary antigen," "Streptococcus pneumoniae," "pneumococcal urinary antigen," "human immunodeficiency virus," "HIV," "beta-hemolytic streptococci," "Streptococcus pyogenes," "group A streptococcus," "Streptococcus agalactiae," "group B streptococcus," "malaria," and "Plasmodium falciparum."

Results and discussion

Sensitivity and specificity

In accordance with the Act on Medical Devices, all in vitro diagnostic kits must have a CE marking [CE, Conformité Européenne, roughly "Agreement with European directives"]. This occasionally leads to the assumption that the tests have already been externally validated. This is however not the case. The CE certification, including sensitivity and specificity values, is performed on the responsibility of the manufacturer and only confirms that the product conforms to the basic requirements of the European directives for in vitro diagnostic kits (8). Only products for the diagnosis of "risk markers" (such as HIV, HCV, HBV, HTLV, and some blood groups) are also evaluated by notified bodies such as the TÜV (German Society for Technical Monitoring) and the Paul Ehrlich Institute, Germany, and only then awarded a CE marking (8). It follows that sensitivity and specificity as given by the manufacturer are somewhat unreliable parameters for the diagnostic quality of a test and are largely useless as objective criteria for evaluation.

It is more reliable to take the necessary data from review articles or from the guidelines of specialty societies *(box 2)*. Here too there are some reservations. In the first place, the values determined are usually based on comparisons between the rapid test and conventional methods, which are certainly not standardized. Thus it regularly happens that different "gold standards" are used for the same studies or to answer the same question, making an overall comparison more difficult. In the second place, the parameters "sensitivity", "specificity", "positive predictive value," and "negative predictive value" are only valid within the context of a given study. They may not be transferred unthinkingly to other situations (such as primary care), unless the prevalence and severity of the disease are the same. Finally, it should be borne in mind that negative test results—even in test systems with good sensitivity—do not reliably exclude the disease in question, as the parameters "sensitivity" and "specificity" do not take into account the distribution of healthy and ill individuals within the given group of patients (9, 10). This is particularly the case if the prevalence of the disease is low (see model calculation in *table 1*).

BOX 2

Evaluation and guideline recommendations on rapid microbiological tests

Pathogen/Test		Guidelines (literature reference)
Pneumococci:	The diagnostic standard is still sputum or blood culture and the Gram stain. A pneumococcal rapid test can be used to increase diagnostic yield. A negative test does not reliably exclude pneumococcal pneumonia.	(11–13)
Legionella:	Legionella testing is appropriate in all unclear cases of pneumonia. A test is recommended for each patient with pneumonia of unclear origin after admission to an intensive care ward, in epidemics, and when beta-lactam therapy fails. The diagnostic method of choice is antigen detection in the urine.	(11–13)
Influenza:	There should be no routine testing for influenza antigens. This may be helpful in outbreaks or before the decision to start antiviral therapy. A test should be used which can differentiate between influenza types A and B.	(11–13)
S. pyogenes:	The rapid test for group A streptococci is now established as a routine component of diagnosis. Specific use markedly reduces unnecessary antibiotic use.	(15, 19, 20)
S. agalactiae:	The rapid test for group B streptococci is currently not sensitive enough to replace detection in culture. Routine use is not recommended.	(21, 22, 24, 25)
HIV:	The rapid test for HIV has been fully developed in diagnosis and is just as reliable as conventional screening diagnosis with EIA. It can be used for patients who are difficult to reach, in regions with poor laboratory access, and in urgent decisions on possible prophylaxis after exposure or transmission.	(e2–e7)
Malaria (P. falciparum):	The rapid test is now a very good alternative to light microscopy, although it has not replaced this as "gold standard". It can be used when light microscopy is not available. The rapid test has failed in isolated cases in spite of high parasitemia.	(e10)

S. pyogenes, Streptococcus pyogenes; S. agalactiae, Streptococcus agalactiae; HIV, human immune deficiency virus; P. falciparum, Plasmodium falciparum.

Rapid tests for respiratory infections

Three established rapid tests of great practical importance are available for the diagnosis of respiratory infections *(table 2 and box 2)*. These are for the detection of the antigens of influenza, pneumococci, and legionellae. The greatest benefits of these systems are the improvement in diagnostic yield (pneumococci, legionellae) and in the time saved in diagnosis. For comparison, pneumococcal culture requires 24 to 48 h; influenza detection from short-term culture requires more than 3 days; legionella culture requires 3 to 7 days. For pneumococci, the pathogen can only be detected by sputum culture in 40% to 50% of patients with pneumococcal pneumonia, even in patients with bacteremia (11, 12). The main reasons for the failure of culture detection are nonoptimal sample isolation, excessive transport times, and prior antimicrobial therapy (11). In comparison, the pneumococcus rapid test is much less sensitive to interference, and detects pneumococcus pathogen in some patients with negative culture (sensitivity: 50% to 80%; specificity: 90%) (11, 13). On the other hand, the sensitivity of the urine antigen test is directly dependent on the severity of the disease. The sensitivity drops to 60% in patients with less severe disease *(figure)* (14). Together with the fact that pneumococci are almost always well covered by the most frequently selected antibiotics (beta-lactams), this leads to the conclusion that the pneumococci antigen test should currently only be regarded as a complement to routine tests (11, 13). There are also problems in the diagnosis of infections in children and infants, for as many of 20% of these may carry pneumococci as commensals (microbe carriers) and this can lead to false positive test results (13, 15).

Legionellae are important pathogens of both community-acquired and nosocomial pneumonia. They are particularly dangerous for patients with a weakened immune system, especially after an organ transplant. Legionella pneumophila of serotype 1 is responsible for about 60% to 70% of infections (11–13). Legionella can only be detected by culture in a few patients—in some hospitals, less than 10%—and usually requires 3 to 7 days (11, 13, 16). On the other hand, the infection may be peracute and rapidly fatal and requires special treatment (macrolide or fluoroquinolones). It follows that acute diagnosis by detecting the legionella antigen in urine is of great clinical value. The sensitivity of the tests is currently about 94% and the specificity 99% to 100%. This should be compared with the sensitivity of 10% to 80% in culture, with the specificity of 100% (11). Infections with serotypes other than 1 can be detected by cross-reactions, although the sensitivity is clearly lower (ca. 80%) (11).

TABLE 1

Correlation between positive and negative predictive value and disease, using the example of a fictitious influenza rapid test

Prevalence (%)	False positives in 1000 persons examined	True positives in 1000 persons examined	Negative predictive value (%)*1	Positive predictive value (%)*1
0.1	50.0	0.8	100.0	1.6
1.0	49.5	8	99.8	13.9
2.5	48.8	20	99.5	29.1
5.0	47.5	40	98.9	45.7
10.0	45.0	80	97.7	64.0
25.0	37.5	200	93.4	84.2
50.0	25.0	400	82.6	94.1
75.0	12.5	600	61.3	98.0
90.0	5.0	720	34.5	99.3
95.0	2.5	760	20.0	99.7
97.5	1.3	780	10.9	99.8
99.0	0.5	792	4.6	99.9
99.9	0.0	799	0.5	100.0

Sensitivity of the test: 80%; specificity of the test: 95%

*1 Positive/Negative predictive value: probability (in %) that the test result accurately reflects the disease status.

Example: The proportion of correctly diagnosed patients is 1.6% when the prevalence of the disease is 0.1% in the patient group, but 84.2% when the prevalence is 25%

The development of the rapid test for influenza virus was greatly accelerated by the recognition that early therapy (within 48 h) with neuraminidase inhibitors is more likely to be successful (17). Currently available tests give a diagnosis with a sensitivity of 50% to 96% and specificity of 72% to 100%, depending on the selected "gold standard" (17). Additional factors include the type of test material—nasal swabs are better than throat swabs—and the patient's age (17). If there is an outbreak, with relatively high prevalence, current publications suggest that the positive predictive value of this test can be exploited in patient management—even though clinical evaluation by an experienced physician gives a similarly good positive predictive value (17).

In settings with low prevalence (for example, at the start of an outbreak or in an inter-epidemic phase), the reliability is greatly restricted by the low predictive value *(table 1)* (9, 10). In this phase, it is very probable that positive rapid test results are false positives. Therefore, they must be checked with a second independent test *(table 1)* (9, 10).

Rapid tests for detecting streptococci

For beta-hemolyzing streptococci, rapid tests are available for directly detecting the antigens of group A streptococci (GAS, S. pyogenes) and of group B streptococci (GBS, S. agalactiae) (15, 18). The tests are based on the extraction of the C-antigen from the cell wall, followed by detection with an immunological reaction. If GAS is directly detected during the examination of a tonsillitis patient, it is then possible to decide whether antimicrobial therapy is necessary. Studies have shown that this can reduce the unnecessary use of antibiotics in pharyngitis by at least a quarter (20). Moreover, the specificity of almost all modern systems is now good to very good (>85%), as is the specificity (>95%; cf. culture: sensitivity 80% to 97%, specificity 100%). Taken together, this has led many medical societies to include the GAS rapid test in their recommendations and guidelines for tonsillitis as a routine diagnostic component (15, 19). On the other hand, dispensing with culture excludes the possibility of testing for macrolide sensitivity, and macrolide resistance is a growing problem (15).

Group B streptococci (GBS) are a major cause of neonatal infections in industrial countries. Although there has been considerable progress in their diagnosis and treatment, GBS infections lead to high morbidity and mortality (21, 22). The most efficient strategy to reduce the frequency and severity of neonatal infection is currently thought to be culture detection of group B streptococci from rectovaginal screening swabs in weeks 35 to 37 of pregnancy and intrapartal chemoprophylaxis with ampicillin (22). If however culture screening is not possible because of a premature birth, there are a variety

TABLE 2

Comparison: rapid microbiological tests versus conventional diagnosis

Rapid test	Sample	Se% *1	Sp% *1	Conventional diagnosis	Se %*1	Sp %*1
Pneumococcal antigen	Urine (CSF)	50-80	90	Sputum culture	<40-50	100
Legionella antigen	Urine	94	99-100	Sputum culture	10-80	100
Influenza antigen	Nasal or throat swab	50-96	72-100	Rapid culture	ND	ND
S. pyogenes antigen	Throat swab	>85	>95	Culture throat	80-97	100
S. agalactiae antigen	Rectovaginal swab	11-79	91-100	Culture	91	89
HIV antibody	Blood	98-100	75-100	EIA	ND	ND
P. falciparum antigen	Blood	>90	>80	Microscopy	ND	ND

EIA, enzyme immunoassay; HIV, human immunodeficiency virus; ND, no available data; S. pyogenes, Streptococcus pyogenes; S. agalactiae, Streptococcus agalactiae; Se%, sensitivity %; Sp%, specificity %; P. facliparum, Plasmodium falciparum;

*1 In so far as they were available, the figures for sensitivity and specificity were taken from the review literature quoted in the text.

of rapid tests for intrapartal screening (18). The specificity of these tests is good (91% to 100%; cf. culture: 89%) (18, 23). However, the sensitivity of the test is much poorer (11% to 79%; cf. culture: 91%) (18, 23). This is not good enough in practice and is probably due to some pregnant women being colonized with a low bacterial inoculum. This is too low to be detected, but can nevertheless lead to infections (24). For this reason, routine use of the GBS rapid test is currently not recommended by specialty societies (22, 25).

Human immune deficiency virus (HIV)

Bedside rapid tests to detect HIV antibodies are now an equivalent alternative to the conventional antibody screening tests, as their sensitivity (98% to 100%) and specificity (86% to 100%, one outlier 75%) are comparable to the values found with the enzyme immunoassays (EIAs) performed in the laboratory (e1). They are particularly useful in areas with little access to laboratories (e.g. Africa) (e5), in people who are difficult to reach (e.g. drug addicts or the homeless), for the critical period in which a decision has to be made about prophylaxis after exposure, and after a birth where the HIV status of the mother is uncertain (e1–e7). Even though the specificity is 99% to 100% in some studies, a rapid test can always in principle give a false positive result. Although current experience suggests that the problem is less severe in practice than had been expected, it is essential that positive rapid test results should be confirmed by an alternate rapid test (if resources are limited) (e5) or by a conventional test (e.g. Western blot) (e1).

Plasmodium falciparum—falciparum malaria

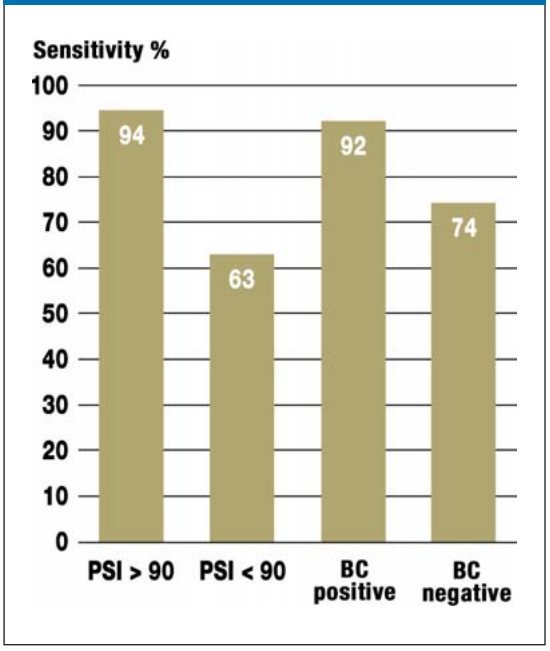
An infection with Plasmodium falciparum (falciparum malaria) can be detected with rapid tests to two specific antigens—histidine-rich protein 2 (HRP2) and parasite-specific lactate dehydrogenase (pLDH). These are an alternative to conventional diagnosis by light microscopy (thick drops and blood smear) (e8, e9). Current studies have found that the sensitivity of the tests is usually over 90% and the specificity over 80% (e8). False positives are possible, for example, because of rheumatoid factor. False negatives are also possible, usually if the parasitemia is very low (<100/µL) (e8, e10). It is also a problem that the rapid test sometimes fails in spite of high parasite density. If this is borne in mind, malaria rapid tests may be used for emergency diagnosis, in accordance with the recommendations of the German Society for Tropical Medicine, if light microscopy of a thick drop or smear examination is not available (e10).

Quality assurance

To obtain valid measurement results and also to protect the user, care should be taken that the test is used properly. This includes correct sampling and compliance with the manufacturer's instructions for performing the test. As the systems are so simple, extensive training is generally unnecessary. The responsible physician evaluates the results of the laboratory diagnostic tests and makes the diagnosis. This applies to POCT, just as with "classical" laboratory diagnosis.

The current guideline of the German Medical Association on quality assurance of laboratory medical investigations (RiLiBÄK) should also be considered when using the POCT devices. There are special simplified regulations for POCT when these are the so-called unit-use reagents and the corresponding measurement systems. This means that reagents for single determinations should be split into portions and used up during a single investigation. The RiLiBÄK currently only applies to quantitative tests. However, most of the test strips used for the diagnosis of infectious diseases are intended for the qualitative detection of an antigen or antibody. It can be expected that the RiLiBÄK will be extended to cover this (e11).

FIGURE 1



Sensitivity of the pneumococcal antigen test (Binax NOW) in dependence on the severity of the pneumonia, as measured with the pneumonia severity index (PSI) or the status of the blood culture (BC). The sensitivity of the test was 94% with PSI>90 and 63% with PSI<90 ($p<0.001$). The sensitivity of the test was 92% with positive blood culture and 74% with negative blood culture (p-value, not significant). The specificity of the test was 100% in the patient group examined (95% confidence interval 99.7% to 100%). Taken from Roson et al., 2004 (14).

Conclusions

In general, it may be concluded that immunochromatography test strips to detect infectious pathogens are technically fully developed and that they exhibit a series of specific advantages, but also disadvantages *(box 3)*. With modern immunochromatography tests, investigations can be performed rapidly and simply, without requiring special instruments or expertise in the method. As the sensitivity and specificity of many

BOX 3

Advantages and disadvantages of rapid microbiological tests

Advantages	Disadvantages
● Immediate initiation of specific antibiotic therapy is possible.	● Older POCT systems perform more poorly (before the introduction of immunochromatographic techniques)
● Reduction in unnecessary antibiotic consumption	● Lack of data on pathogen sensitivity
● Reduction in selection pressure	● Increased risk of operator becoming infected
● Immediate recognition of infection chains	● Operator's qualifications may be inadequate.
● Reduction in pre-analytical interference	● Double or multiple infections are more likely to be overlooked than in culture.
● Extension of diagnostic instrumentarium; independent of culture	● Necessity of performing measures for quality control
● Better compliance with patients who are difficult to reach	

From Reinert, 2007 (15)

test procedures are now really high, rapid tests can be extraordinarily useful in answering specific questions and thus in helping to orientate diagnosis, uncovering chains of infection, and in deciding to start early specific antimicrobial therapy or drug prophylaxis. The precondition of the proper use of these tests is that they should be properly handled by medical personnel, that quality assurance measures should be in place and that the interpretation of the results should consider the severity of the clinical presentation and the epidemiological situation.

Conflict of interest statement
The authors declare that no conflict of interest exists according to the guidelines of the International Committee of Medical Journal Editors.

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Corresponding author
Priv.-Doz. Dr. med. Enno Stürenburg
LADR GmbH
MVZ Geesthacht—Labor Dr. Kramer & Kollegen
Lauenburger Str. 67
21502 Geesthacht, Germany
stuerenburg@ladr.de

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