Streptococcus pneumoniae Antigen, Missing Opportunities of a Promising Point of Care Diagnostic Test

Magdalena Słomka[1], Ruby Gupta[2], Baltej Singh[3], Venkatesh Gondhi[4], Siddartha Bhandary[4], Ekezie Francis[2], and *Jose Bordon[4]

Abstract

Background: The etiologic diagnosis of community acquired pneumonia (CAP) and community-acquired meningitis (CAM) are still far from optimal accuracy and turnaround time. The most common bacterial pathogen identified in both CAP and CAM is *Streptococcus pneumoniae*.

Methods: We reviewed the literature on *S. pneumoniae* antigen to highlight opportunities to optimize its use as a point of care diagnostic test to maximize quality of patient care, antimicrobial stewardship outcomes and cost saving. A PubMed search was performed using key words "*S. pneumoniae* antigen; rapid diagnostic tests for *S. pneumoniae*". We selected articles in English and sorted into randomized controlled trials (RCT), case control, and retrospectives studies.

Results: The BinaxNOW *S. pneumoniae* antigen rapid immunochromatographic test (ICT) to detect C-polysaccharide cell wall antigen was approved by the Food and Drug Administration (FDA) in 2003. This test has a turn around time of 15 minutes, a sensitivity of 52-82% and specificity of 89.7-99.7% in urine and 95.4-100% and 99.3-100% respectively in cerebrospinal fluid (CSF) specimens. BinaxNOW detected the antigen in 100% of blood culture bottles that grew *S. pneumoniae*. *S. pneumoniae* urinary antigen has a sensitivity of 77-92% in bacteremic pneumococcal pneumonia and 52-78% non-bacteremic pneumococcal pneumonia. This test was reported to be inexpensive and useful in streamlining antibiotic treatment. In a physician survey, this test led to a narrowed antibiotic regimen, shorter duration of antibiotics treatment and fewer diagnostic tests.

Conclusion: *S. pneumoniae* ICT urinary antigen has an optimal yield to support the etiologic diagnosis of CAP and up to 15-minute turnaround time that could facilitate he selection of a one-antibiotic regimen upon the hospital admission of a patient with CAP. Similarly, *S. pneumoniae* CSF antigen test should be considered a standard test for the evaluation of patients with meningitis.

Introduction

Despite progress in diagnostic technology, the confirmation of etiology of community-acquired pneumonia (CAP) and community-acquired meningitis (CAM) is still far from optimal accuracy and turn-around time. Pneumonia has the highest hospitalization and mortality rate among all infections diagnosed in the adult population in the United States (US) [1]. Every year, 4,000 cases of bacterial meningitis are reported nationwide with 12% fatality rate and up to 34% of cases resulting in permanent disability among survivors [2, 3]. These serious complications prompt the initiation of antibiotic therapy in patients with presumptive bacterial CAP or CAM regardless of ability to identify the culprit pathogen.

The causative pathogen of CAP in hospitalized patients in the US remains unknown in up to 62% and on a lower scale in CAM [4]. The main diagnostic tools used for evaluation of CAP and CAM are microbiologic cultures; which have a relatively low yield. Hospitalized patients with CAP have positive blood cultures in less than 14% [5, 6]. The sensitivity of sputum culture for pneumococcal pneumonia is 60% [7]. The CSF cultures performed for CAM are positive in 70-85% cases however it takes an average of 48 hours to obtain the result. Although cerebrospinal fluid (CSF) Gram stain can identify the pathogen in 60-90%, this is only true for CSF with high bacteria load. The sensitivity drops to 25% for CSF with colony forming unit (CFU) $\leq 10^3$ [8]. Furthermore, if microbiology material is collected after initiation of antibiotic therapy the CSF culture sensitivity rate even further decreases.

The uncertainty of the etiology of both CAP and CAM leads to empiric treatment and this has become the standard practice. This standard practice results in overuse of broad spectrum antimicrobials, increasing antibiotic resistance and risk for

*Correspondence To: Jose Bordon, M.D., Ph.D.
Work: Address: Providence Health System, Center for Infectious Diseases 1140 Varnum Street, NE Washington, DC 20017
Work Email: jbordon@ascension.org

medication side effects including the deadly epidemic of C. difficile colitis [9]. The median length of stay (LOS) for CAP is 3 days [5]. In 2011 the cost of all CAP hospitalizations exceeded $10 billion [10]. Studies have already shown that de-escalation of antibiotics in hospitalized CAP cases is associated with decreased LOS without adversely affecting outcomes including mortality rate [11, 12]. The most common bacterial pathogen identified in both CAP and CAM is *S. pneumoniae* [3, 4]. Rapid *S. pneumoniae* c-polysaccharide antigen test (SPCAT) BinaxNOW has been approved by the Food and Drug Administration (FDA) to detect the antigen in the urine samples for diagnosis of pneumococcal pneumonia and in CSF for pneumococcal meningitis.

Methods

We reviewed the literature on *S. pneumoniae* antigen to highlight opportunities to optimize its use as a point of care diagnostic test to maximize the quality of patient care, antimicrobial stewardship outcomes and cost savings. A PubMed search was performed using key words "*S. pneumoniae* antigen; rapid diagnostic tests for *S. pneumoniae*". We selected articles in English and sorted them out in Randomized controlled trials (RCT), case control, and retrospectives studies.

Results

Molecular properties of *S. pneumoniae* antigen.
S. pneumoniae are lancet shaped Gram-positive bacteria that grow in pairs or short chains. Their pathogenicity is attributed to many structures, most of them located on the surface. Three major surface layers have been found; the capsule, the cell wall, and the plasma membrane [13, 14].

The capsule is made of a high molecular weight polymer units of repeating oligosaccharides. There may be additional components to this like acidic components or phosphorylcholine (PC) [15]. Based on differences in the capsular polysaccharide (PS), pneumococci are sorted out into 90 plus serotypes [16]. This has been recognized as a major virulence factor as it has been found that encapsulated strains have 105 times more virulence than non-encapsulated. Also, there is 50% difference in lethal dose between capsulated and non-encapsulated strains [13] [17].

The cell wall is mainly composed of peptidoglycan which is glycan chains cross-linked through peptide side chains. A complex teichoic acid containing PC residue known as cell wall polysaccharide (CWPS) is attached to peptidoglycan via N-acetyl muramic acid[18]. CWPS has various proposed mechanisms of virulence like inducing inflammation via activation of the alternative complement pathway, enhancement of vascular permeability, mast cell degranulation, polymorphonuclear leukocytes (PMN) activation and IL-1 production increase [19-21].

Capsular polysaccharide antigens of *S. pneumoniae* were first detected in urine of patients with pneumonia in 1917 [22]. The latex agglutination test was initially developed to detect urinary capsular polysaccharide of *S. pneumoniae* but the test was of limited usefulness because it was not easy to perform and could not detect all the different capsule serotypes [23]. Schaffner et al. used enzyme-linked immunosorbent assay (ELISA) for detection of this capsular polysaccharide in urine and found that the levels of this antigen varied depending on the severity of infection as well as the serotype of the bacteria. Notably, there is

more than 250 fold variation in serotype-specific clearance rates [24].

Later in 2003, the FDA approved BinaxNOW *S. pneumoniae* assay, a rapid immunochromatographic test (ICT) detecting CWPS antigen that has shown good utility for diagnosis of *S. pneumoniae* via urine with high sensitivity and specificity [25]. The high analytical sensitivity for CWPS antigen urine specimens make it the point of care test [26]. Some of the advantages that *S. pneumoniae* ICT antigen offers is that it is easy to perform in all settings with 15 minutes turnaround time, the test is not serotype dependent and prior antibiotic use has less influence on the diagnostic yield [25] [26]. In a study by Said et al, prior antibiotic use has shown reduction in the relative diagnostic yield for *S. pneumoniae* ICT antigen only by 26%, sputum cultures by 34%, blood cultures by 67% [27].

S. pneumoniae antigen as the FDA-approved and non-approved diagnostic test
Though *S. pneumoniae* is a common pathogen in hospitalized patients with CAP, its microbiological diagnosis remains challenging. Conventional tests that have been used to identify the etiology of pneumonia include Gram stain of sputum, sputum cultures, and blood cultures. Other tests like latex agglutination, counter-immunoelectrophoresis that detect pneumococcal capsular antigens, have poor sensitivity and specificity [28]. They are not considered as useful diagnostic techniques in clinical practice. The ICT BinaxNOW has been cleared by the FDA to detect the pneumococcal antigen in both the urine and the CSF samples. It detects the pneumococcal C-polysaccharide present in the cell wall that is common to all serotypes [29].

Testing is not recommended in individuals vaccinated against pneumococcus in the last 5 days. *S. pneumoniae* ICT antigen was approved as an adjunct to culture for presumptive diagnosis of pneumococcal pneumonia. The most widely used indirect detection method for *S. pneumoniae* is the detection of pneumococcal antigen in urine.

Wellcogen bacterial antigen test is another simple rapid test. This test utilizes a solid phase coated with polyclonal antibody to detect antigen in the human urine sample. This is a latex agglutination test runs with antibody coated polystyrene beads that agglutinate in the presence of sufficient antigen. *S. pneumoniae* ICT antigen has also been detected in other body fluids like blood, pleural effusion or sputum samples. The FDA has not approved any of them yet as a standard of practice. Several studies showed the promising results of *S. pneumoniae* antigen ICT in blood (**Table 1**.) The test sensitivity and specificity in blood samples were 87.1-100% and 96.9-100% respectively revealing higher diagnostic yield than the urinary antigen. In pleural effusion, the *S. pneumoniae* ICT antigen resulted in a sensitivity of 79-88% and specificity of 71-93.6%; which should be considered as a valuable diagnostic tool in clinical practice.

Discussion

We strongly believe that physicians should consider the best utilization and the missing diagnostic opportunities of *S. pneumoniae* ICT antigen when serving patients with CAP and CAM. The determination of the etiologic agent for the pneumonia is essential to maximize the selection of an effective narrowed spectrum antibiotic treatment aiming to prevent the risks of adverse effects and antimicrobial resistance [30, 31].

Table 1. Diagnostic test performance of *S. pneumoniae* C-polysaccharide antigen

Body fluid sample	Sensitivity %	Specificity %	PPV %	NPPV %
URINE [42] [43] [32] [44]	52 – 82	89.7- 99.7	54.3-99.3	95.1-96.9
CSF [45] [46]	95.4 - 100	99.3 -100		
BLOOD [47] [41]	87.1 - 100	96.9 - 100		
SPUTUM [48] [49]	53.7- 90.9	61.1-94.8	74.1-82.9	81.3- 84.6
PLEURAL EFFUSION [50] [51]	79 - 88	71- 93.6	96	

CSF = Cerebrospinal fluid.

The SPCAT in urine and CSF support the etiological diagnosis in in patients with *S. pneumoniae* pneumonia and CAM. In adult patients, the SPCAT in urine was reported to have a sensitivity of 50-80% in cases without bacteremia and 75-85% in those with bacteremia; the specificity is approximately 95%; the use of concentrated urine by selective ultrafiltration slightly increases the sensitivity of the test [32].

The IDSA guidelines from 2007 recommend SPCAT combined with at least blood culture for all patients with CAP with suspected pneumococcal etiology and at least one of the following risk factors: failure of outpatient management, ICU admission, leukopenia, active alcohol abuse, chronic severe liver disease, asplenia or pleural effusion. A meta-analysis from papers published from 1996 to 2012 showed that higher pooled sensitivity and specificity of of the *S. pneumoniae* ICT urinary antigen testcompared to culture supporting its use as part of the workup for diagnosis of community acquired pneumonia [33]. In multiple studies, the urine *S. pneumoniae* assay has shown higher sensitivity for bacteremic pneumococcal pneumonia a (77-92%) than for non-bacteremic pneumococcal pneumonia (52-78%) [34, 35]. The indication of SPCAT in Healthcare Associated Pneumonias (HCAP) is controversial, and it is not recommended [36, 37]. With increasing concern regarding antibiotic stewardship, the ability to safely and quickly narrow antibiotic therapy becomes crucial. Physicians are encouraged to target antibiotic therapy towards *S. pneumoniae* even if the *S. pneumoniae* urinary antigen ICT is the only positive microbiologic test and there is no evidence of any other etiology. The underutilization of *S. pneumoniae* urinary ICT or CSF antigen ICT test may lead to a suboptimal outcome in terms of recovery. This leads to increased risk of antibiotic related complications like C. difficile diarrhea in those receiving broad spectrum antibiotics that could have been obviated by early diagnosis and treatment. According to a multi-hospital observational study from 2016, *S. pneumoniae* urinary antigen ICT is an inexpensive, noninvasive test that favorably influences antibiotic prescribing practices [38]. The costs were reduced significantly as the regimens of antibiotics were changed based on the *S. pneumoniae* urinary antigen ICT result [39]. In a survey in 2013 among Physician IDSA members, the result of *S. pneumoniae* urinary antigen ICT led to 84% narrowed antibiotic regimen, 28% shortened the course of antibiotics, and 67% ordered fewer diagnostic tests [40].

The growing emergence of antibiotic resistant organisms is a major public health and financial burden. The use of rapid, non-invasive *S. pneumoniae* ICT urinary antigen may favorably influence antibiotic prescribing practices. Thus, *S. pneumoniae* ICT urinary antigen can guide to safely de-escalate antibiotics once a positive result is obtained [30].

The BinaxNOW *S. pneumoniae* antigen test detected pneumococcal antigen in 100% of blood culture bottles that grew *S. pneumoniae*. False positive reactions were seen due to cross reactivity of polysaccharide antigen with *S. mitis* group [41].

Marcos et al. found that SPCAT testing in concentrated urine was positive in 70% of the patients with pneumococcal CAP proven by both culture and SPCAT 1 month after the diagnosis of CAP despite good clinical, laboratory and radiographic evolution. Therefore, further studies are needed to ascertain how long positive SPCAT results may persist after an episode of pneumococcal CAP to properly interpret the ICT test in clinical practice which will be of clinical significance especially in individuals who have recurrent pneumonia. They also found that five patients (11.1%) with bacteremic pneumococcal CAP tested SPCAT positive, only after concentrating the urine, which supports the necessity to concentrate the urine for this test [30]. One of the limitations of our review is that this is not a meta-analysis compiling the statistical data results. However, as part of the strengths of our paper is the systematic review of papers published in PubMed in the order of RTC, case-control studies and retrospective study.

Conclusion

The *S. pneumoniae* ICT urinary antigen is likely the best test to support the etiologic diagnosis of CAP with the optimal sensitivity and up to 15 minutes turn around result allowing to streamline the antibiotic de-escalation. The *S. pneumoniae* CSF antigen test, this test has optimal sensitivity and turnaround time; which should be considered a standard test for the evaluation of patients with meningitis.

References

1. Kochanek KD, Murphy SL, Xu J, Tejada-Vera B. Deaths: final Data for 2014. Natl Vital Stat Rep. 2016 Jun;65(4):1–122.

2. Thigpen MC, Whitney CG, Messonnier NE, Zell ER, Lynfield R, Hadler JL et al.; Emerging Infections Programs Network. Bacterial meningitis in the United States, 1998-2007. N Engl J Med. 2011 May;364(21):2016–25.

3. van de Beek D, de Gans J, Tunkel AR, Wijdicks EF. Community-acquired bacterial meningitis in adults. N Engl J Med. 2006 Jan;354(1):44–53.

4. Jain S, Self WH, Wunderink RG, Fakhran S, Balk R, Bramley AM et al.; CDC EPIC Study Team. Community-Acquired Pneumonia Requiring Hospitalization among U.S. Adults. N Engl J Med. 2015 Jul;373(5):415–27.

5. Campbell SG, Marrie TJ, Anstey R, Dickinson G, Ackroyd-Stolarz S. The contribution of blood cultures to the clinical management of adult patients admitted to the hospital with community-acquired pneumonia: a prospective observational study. Chest. 2003 Apr;123(4):1142–50.

6. Waterer GW, Wunderink RG. The influence of the severity of community-acquired pneumonia on the usefulness of blood cultures. Respir Med. 2001 Jan;95(1):78–82.

7. García-Vázquez E, Marcos MA, Mensa J, de Roux A, Puig

J, Font C et al. Assessment of the usefulness of sputum culture for diagnosis of community-acquired pneumonia using the PORT predictive scoring system. Arch Intern Med. 2004 Sep;164(16):1807–11.

8. Tunkel AR, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, Scheld WM et al. Practice guidelines for the management of bacterial meningitis. Clin Infect Dis. 2004 Nov;39(9):1267–84.

9. Brown KA, Khanafer N, Daneman N, Fisman DN. Meta-analysis of antibiotics and the risk of community-associated Clostridium difficile infection. Antimicrob Agents Chemother. 2013 May;57(5):2326–32.

10. Pfuntner A, Wier LM, Steiner C. Costs for hospital stays in the United States, 2011: Statistical Brief# 168.

11. Viasus D, Simonetti AF, Garcia-Vidal C, Niubó J, Dorca J, Carratalà J. Impact of antibiotic de-escalation on clinical outcomes in community-acquired pneumococcal pneumonia. J Antimicrob Chemother. 2017 Feb;72(2):547–53.

12. Rhedin S, Galanis I, Granath F, Ternhag A, Hedlund J, Spindler C et al. Narrow-spectrum ß-lactam monotherapy in hospital treatment of community-acquired pneumonia: a register-based cohort study. Clin Microbiol Infect. 2017 Apr;23(4):247–52.

13. AlonsoDeVelasco E, Verheul AF, Verhoef J, Snippe H. Streptococcus pneumoniae: virulence factors, pathogenesis, and vaccines. Microbiol Rev. 1995 Dec;59(4):591–603.

14. Skov Sørensen UB, Blom J, Birch-Andersen A, Henrichsen J. Ultrastructural localization of capsules, cell wall polysaccharide, cell wall proteins, and F antigen in pneumococci. Infect Immun. 1988 Aug;56(8):1890–6.

15. Sørensen UB, Agger R, Bennedsen J, Henrichsen J. Phosphorylcholine determinants in six pneumococcal capsular polysaccharides detected by monoclonal antibody. Infect Immun. 1984 Mar;43(3):876–8.

16. Habib M, Porter BD, Satzke C. Capsular serotyping of Streptococcus pneumoniae using the Quellung reaction. J Vis Exp. 2014 Feb;(84):e51208.

17. Watson DA, Musher DM. Interruption of capsule production in Streptococcus pneumonia serotype 3 by insertion of transposon Tn916. Infect Immun. 1990 Sep;58(9):3135–8.

18. Sørensen UB, Henrichsen J, Chen HC, Szu SC. Covalent linkage between the capsular polysaccharide and the cell wall peptidoglycan of Streptococcus pneumoniae revealed by immunochemical methods. Microb Pathog. 1990 May;8(5):325–34.

19. Geelen S, Bhattacharyya C, Tuomanen E. The cell wall mediates pneumococcal attachment to and cytopathology in human endothelial cells. Infect Immun. 1993 Apr;61(4):1538–43.

20. Johnston RB Jr. Pathogenesis of pneumococcal pneumonia. Rev Infect Dis. 1991 May-Jun;13 Suppl 6:S509–17.

21. Winkelstein JA, Tomasz A. Activation of the alternative pathway by pneumococcal cell walls. J Immunol. 1977 Feb;118(2):451–4.

22. Dochez AR, Avery OT. The Elaboration of Specific Soluble Substance by Pneumococcus during Growth. J Exp Med. 1917 Oct;26(4):477–93.

23. Werno AM, Murdoch DR. Medical microbiology: laboratory diagnosis of invasive pneumococcal disease.

Clin Infect Dis. 2008 Mar;46(6):926–32.

24. Schaffner A, Michel-Harder C, Yeginsoy S. Detection of capsular polysaccharide in serum for the diagnosis of pneumococcal pneumonia: clinical and experimental evaluation. J Infect Dis. 1991 May;163(5):1094–102.

25. Song JY, Eun BW, Nahm MH. Diagnosis of pneumococcal pneumonia: current pitfalls and the way forward. Infect Chemother. 2013 Dec;45(4):351–66.

26. Clerc O, Greub G. Routine use of point-of-care tests: usefulness and application in clinical microbiology. Clin Microbiol Infect. 2010 Aug;16(8):1054–61.

27. Said MA, Johnson HL, Nonyane BA, Deloria-Knoll M, O'Brien KL, Andreo F et al.; AGEDD Adult Pneumococcal Burden Study Team. Estimating the burden of pneumococcal pneumonia among adults: a systematic review and meta-analysis of diagnostic techniques. PLoS One. 2013;8(4):e60273.

28. Van der Auwera P, André A, Bulliard G, Legrand JC, Gordts B, Van Landuyt H et al. Comparison of latex agglutination and counterimmunoelectrophoresis in the diagnosis of acute Streptococcus pneumoniae infections. Eur J Clin Microbiol. 1983 Dec;2(6):534–40.

29. Jennings HJ, Lugowski C, Young NM. Structure of the complex polysaccharide C-substance from Streptococcus pneumoniae type 1. Biochemistry. 1980 Sep;19(20):4712–9.

30. Marcos MA, Jiménez de Anta MT, de la Bellacasa JP, González J, Martínez E, García E et al. Rapid urinary antigen test for diagnosis of pneumococcal community-acquired pneumonia in adults. Eur Respir J. 2003 Feb;21(2):209–14.

31. van der Eerden MM, Vlaspolder F, de Graaff CS, Groot T, Bronsveld W, Jansen HM et al. Comparison between pathogen directed antibiotic treatment and empirical broad spectrum antibiotic treatment in patients with community acquired pneumonia: a prospective randomised study. Thorax. 2005 Aug;60(8):672–8.

32. Murdoch DR, Laing RT, Mills GD, Karalus NC, Town GI, Mirrett S et al. Evaluation of a rapid immunochromatographic test for detection of Streptococcus pneumoniae antigen in urine samples from adults with community-acquired pneumonia. J Clin Microbiol. 2001 Oct;39(10):3495–8.

33. Sinclair A, Xie X, Teltscher M, Dendukuri N. Systematic review and meta-analysis of a urine-based pneumococcal antigen test for diagnosis of community-acquired pneumonia caused by Streptococcus pneumoniae. J Clin Microbiol. 2013 Jul;51(7):2303–10.

34. Smith MD, Derrington P, Evans R, Creek M, Morris R, Dance DA et al. Rapid diagnosis of bacteremic pneumococcal infections in adults by using the Binax NOW Streptococcus pneumoniae urinary antigen test: a prospective, controlled clinical evaluation. J Clin Microbiol. 2003 Jul;41(7):2810–3.

35. Rosón B, Fernández-Sabé N, Carratalà J, Verdaguer R, Dorca J, Manresa F et al. Contribution of a urinary antigen assay (Binax NOW) to the early diagnosis of pneumococcal pneumonia. Clin Infect Dis. 2004 Jan;38(2):222–6.

36. Helbig JH, Uldum SA, Bernander S, Lück PC, Wewalka G, Abraham B et al. Clinical utility of urinary antigen detection for diagnosis of community-acquired, travel-associated, and nosocomial legionnaires' disease. J Clin Microbiol. 2003 Feb;41(2):838–40.

37. Micek ST, Kollef KE, Reichley RM, Roubinian N, Kollef MH. Health care-associated pneumonia and community-acquired pneumonia: a single-center experience. Antimicrob Agents Chemother. 2007 Oct;51(10):3568–73.

38. West DM, McCauley LM, Sorensen JS, Jephson AR, Dean NC. Pneumococcal urinary antigen test use in diagnosis and treatment of pneumonia in seven Utah hospitals. ERJ open research. 2016 Oct 1;2(4):00011-2016.

39. Oosterheert JJ, Bonten MJ, Buskens E, Schneider MM, Hoepelman IM. Algorithm to determine cost savings of targeting antimicrobial therapy based on results of rapid diagnostic testing. J Clin Microbiol. 2003 Oct;41(10):4708–13.

40. Harris AM, Beekmann SE, Polgreen PM, Moore MR. Rapid urine antigen testing for Streptococcus pneumoniae in adults with community-acquired pneumonia: clinical use and barriers. Diagn Microbiol Infect Dis. 2014 Aug;79(4):454–7.

41. Petti CA, Woods CW, Reller LB. Streptococcus pneumoniae antigen test using positive blood culture bottles as an alternative method to diagnose pneumococcal bacteremia. J Clin Microbiol. 2005 May;43(5):2510–2.

42. Domínguez J, Galí N, Blanco S, Pedroso P, Prat C, Matas L et al. Detection of Streptococcus pneumoniae antigen by a rapid immunochromatographic assay in urine samples. Chest. 2001 Jan;119(1):243–9.

43. Gutiérrez F, Masiá M, Rodríguez JC, Ayelo A, Soldán B, Cebrián L et al. Evaluation of the immunochromatographic Binax NOW assay for detection of Streptococcus pneumoniae urinary antigen in a prospective study of community-acquired pneumonia in Spain. Clin Infect Dis. 2003 Feb;36(3):286–92.

44. Molinos L, Zalacain R, Menéndez R, Reyes S, Capelastegui A, Cillóniz C et al. Sensitivity, Specificity, and Positivity Predictors of the Pneumococcal Urinary Antigen Test in Community-Acquired Pneumonia. Ann Am Thorac Soc. 2015 Oct;12(10):1482–9.

45. Samra Z, Shmuely H, Nahum E, Paghis D, Ben-Ari J. Use of the NOW Streptococcus pneumoniae urinary antigen test in cerebrospinal fluid for rapid diagnosis of pneumococcal meningitis. Diagn Microbiol Infect Dis. 2003 Apr;45(4):237–40.

46. 46. Moïsi JC, Saha SK, Falade AG, Njanpop-Lafourcade BM, Oundo J, Zaidi AK, Afroj S, Bakare RA, Buss JK, Lasi R, Mueller J. Enhanced diagnosis of pneumococcal meningitis with use of the Binax NOW immunochromatographic test of Streptococcus pneumoniae antigen: a multisite study. Clinical infectious diseases. 2009 Mar 1;48(Supplement_2):S49-56.

47. 47. Moïsi JC, Moore M, Carvalho MG, Sow SO, Siludjai D, Knoll MD et al.; Laboratory Evaluation of Assays for Pneumococcus (LEAP) Study Investigators. Enhanced Diagnosis of Pneumococcal Bacteremia Using Antigen- and Molecular-Based Tools on Blood Specimens in Mali and Thailand: A Prospective Surveillance Study. Am J Trop Med Hyg. 2016 Feb;94(2):267–75.

48. Mukae H, Yatera K, Noguchi S, Kawanami T, Yamasaki K, Tokuyama S et al. Evaluation of a rapid immunochromatographic ODK0501 assay for detecting Streptococcus pneumoniae antigens in the sputum of pneumonia patients with positive *S. pneumoniae* urinary antigens. J Infect Chemother. 2015 Mar;21(3):176–81.

49. Fukushima K, Nakamura S, Inoue Y, Higashiyama Y, Ohmichi M, Ishida T et al. Utility of a Sputum Antigen Detection Test in Pneumococcal Pneumonia and Lower Respiratory Infectious Disease in Adults. Intern Med. 2015;54(22):2843–50.

50. Andreo F, Domínguez J, Ruiz-Manzano J, Prat C, Blanco S, Lores L et al. Usefulness of pneumococcal antigen detection in pleural fluid samples by immunochromatographic assay for diagnosis of pneumococcal pneumonia. Clin Microbiol Infect. 2006 Jul;12(7):682–4.

51. Casado Flores J, Nieto Moro M, Berrón S, Jiménez R, Casal J. Usefulness of pneumococcal antigen detection in pleural effusion for the rapid diagnosis of infection by Streptococcus pneumoniae. Eur J Pediatr. 2010 May;169(5):581–4.