Multiplex Urinary Antigen Detection for 13 Streptococcus pneumoniae Serotypes Improves Diagnosis of Pneumococcal Pneumonia in South African HIV-Infected Adults

Werner C. Albrich, Medical Research Council
Michael W. Pride, Pfizer Vaccine Research and Development
Shabir A. Madhi, Medical Research Council
Jan Callahan, Callahan Associates Inc
Peter V. Adrian, Medical Research Council
Roger French, Pfizer Vaccine Research and Development
Nadia van Niekerk, Medical Research Council
Shite Sebastian, Pfizer Vaccine Research and Development
Victor Souza, Pfizer Vaccine Research and Development
Jean-Noel Telles, Fondation Mérieux

Only first 10 authors above; see publication for full author list.

Journal Title: Journal of Clinical Microbiology
Volume: Volume 55, Number 1
Publisher: American Society for Microbiology | 2017-01-01, Pages 302-312
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1128/JCM.01573-16
Permanent URL: https://pid.emory.edu/ark:/25593/s3kb4

Final published version: http://dx.doi.org/10.1128/JCM.01573-16

Copyright information:
Copyright © 2016 American Society for Microbiology. All Rights Reserved.
Accessed October 18, 2023 10:00 AM EDT
Multiplex Urinary Antigen Detection for 13 Streptococcus pneumoniae Serotypes Improves Diagnosis of Pneumococcal Pneumonia in South African HIV-Infected Adults

Werner C. Albrich,a,f Michael W. Pride,c Shabir A. Madhi,a,b Jan Callahan,a Peter V. Adrian,a,b Roger French,a Nadia van Niekerk,a Shite Sebastian,c Victor Souza,c Jean-Noel Telles,h Glaucia Paranhos-Baccalà,h* Kathrin U. Jansen,c Keith P. Klugman,a,b,o*

Respiratory and Meningeal Pathogens Research Unit/Medical Research Council, Johannesburg, South Africa; National Institute for Communicable Diseases, Johannesburg, South Africa; Pfizer Vaccine Research and Development, Pfizer Inc., Pearl River, New York, USA; Biotechnology Clinical Development Statistics, Pfizer Inc., Pearl River, New York, USA; Callahan Associates Inc., La Jolla, California, USA; Division of Infectious Diseases and Hospital Epidemiology, Cantonal Hospital St. Gallen, St. Gallen, Switzerland; Hubert Department of Global Health and Division of Infectious Diseases, Emory University, Atlanta, Georgia, USA; Emerging Pathogens Laboratory, Fondation Mérieux, Lyon, France

ABSTRACT A serotype-specific urinary antigen detection (UAD) assay for 13 serotypes included in the pneumococcal conjugate vaccine (PCV13) was recently reported as a useful diagnostic tool for pneumococcal pneumonia. We aimed to assess the diagnostic accuracy of the UAD in HIV-infected South African adults. Urine specimens from a well-defined cohort of HIV-infected South African adults with pneumonia were evaluated retrospectively in the UAD assay. Pneumonia was considered pneumococcal if either sputum Gram stain, sputum culture, blood culture, or the immunochromatographic (ICT) BinaxNow S. pneumoniae test (composite diagnostic) was positive. Among 235 enrolled pneumonia patients, the UAD assay was more frequently positive (104 [44.3%]) than the composite diagnostic (71 [30.2%]; P < 0.001) and increased the pneumococcal etiology from 30.2% by an additional 22.6% to 52.8%. The UAD assay detected more pneumococcal etiologies (45.0%) than the serotype-independent ICT (23.4%, P < 0.001). UAD identified 6 out of 7 patients with PCV13 serotype bacteremia without misclassification of bacteremia episodes due to non-PCV13 serotypes. UAD was positive for 5.1% of asymptomatic HIV-infected persons, with higher rates among those with nasopharyngeal carriage. Concordance between serotypes identified by UAD and by Quellung reaction and PCR serotyping was 70/86 (81.4%). This study confirms the utility of the UAD assay for HIV-infected adults comparing favorably with other diagnostic tests. A highly valent UAD may become a new standard for detection of pneumococcal pneumonia in adults. Prior to PCV introduction, at least 53% of pneumonia cases were due to pneumococci in HIV-infected South African adults.

KEYWORDS diagnosis, human immunodeficiency virus, pneumococcal pneumonia, serotyping, urinary antigen

Streptococcus pneumoniae remains a leading cause of community-acquired pneumonia (CAP) (1–9), with a substantially increased burden in HIV-infected individuals in sub-Saharan Africa (10–12). The incidence of invasive pneumococcal disease (IPD) is 10


Editor Yi-Wei Tang, Memorial Sloan-Kettering Cancer Center
Copyright © 2016 American Society for Microbiology. All Rights Reserved. Address correspondence to Werner C. Albrich, Werner.albrich@kssg.ch.
* Present address: Glaucia Paranhos-Baccalà, bioMérieux, Sao Paulo, Brazil; Keith P. Klugman, Pneumonia Program, Bill and Melinda Gates Foundation, Seattle, Washington, USA.
to 35 times higher than in age-matched HIV-uninfected persons, even after the introduction of pneumococcal conjugate vaccines (PCVs) (13, 14). Nevertheless, pneumococcal diagnoses in patients with CAP seem to be declining, which may be related to a true decline due to secular changes, successful pediatric vaccination programs (14–16), and declining emphasis on microbiological diagnostics (4).

The diagnosis of pneumococcal pneumonia is hampered by the lack of a diagnostic gold standard (17). Blood culture is insufficiently sensitive (18–22). Obtaining sputum is sometimes difficult, and distinguishing between colonization and infection by Gram stain and culture can be challenging. The urinary immunochromatographic BinaxNow S. pneumoniae test (ICT) for the pneumococcal C-polysaccharide is affected by pneumococcal nasopharyngeal (NP) carriage and therefore not clinically useful for children, who have a high prevalence and density of NP carriage (23). In a meta-analysis in adults, the ICT had a high specificity, 94%, but its sensitivity was only 74% (24). Pneumococcal diagnosis is clinically important, as it allows antibiotic de-escalation (25, 26). In addition, serotype-specific surveillance after PCV introduction is needed to assess success of vaccine programs and detection of serotype replacement.

Recently, a novel serotype-specific urinary antigen detection (UAD) assay was developed to evaluate the efficacy of PCV13 in adults in the CAPiTA study (22, 27). This assay detects the PCV13 serotypes and was clinically validated using urine specimens from adult patients with CAP in the Netherlands, with an overall sensitivity and specificity of 97.1% and 100% (27). In this study, we aimed to assess the diagnostic accuracy of the UAD assay in a cohort of HIV-infected South African adults with CAP who were well characterized by traditional microbiological and molecular diagnostic tests. We determined the value of the UAD assay both as a diagnostic for pneumococcal pneumonia and as a serotyping tool.

(This work has been presented in part at the 9th International Symposium on Pneumococci and Pneumococcal Diseases [ISPPD] in Hyderabad, India, 9 to 13 March 2014.)

RESULTS

We enrolled all 235 HIV-infected patients with radiologically confirmed pneumonia and 297 HIV-infected outpatient controls who had a urine specimen available for UAD. No enrolled subject had previously been vaccinated with any pneumococcal vaccine. Demographic data of the enrolled cases and controls were previously reported (20).

Establishment of positivity cutoff limits. To maintain the high level of assay specificity of the UAD assay in the HIV-infected South African study cohort, the method of nonparametric tolerance was applied to the cohort of asymptomatic HIV-infected controls (n = 297) as previously described (27). As shown in Table 1, for this study population, the original cutoffs were maintained for five (3, 4, 5, 6A, and 7F) and revised for the remaining eight PCV13 serotypes (1, 6B, 9V, 14, 18C, 19A, 19F, and 23F). Of these

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Established cutoff (PnPS U/ml)</th>
<th>Cutoff used in this study (PnPS U/ml)</th>
<th>Upper limit of validated UAD assay range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.8</td>
<td>52.3</td>
<td>477.2</td>
</tr>
<tr>
<td>3</td>
<td>330.5</td>
<td>330.5</td>
<td>997.2</td>
</tr>
<tr>
<td>4</td>
<td>27.3</td>
<td>27.3</td>
<td>192.5</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>2.5</td>
<td>32.9</td>
</tr>
<tr>
<td>6A</td>
<td>106.7</td>
<td>106.7</td>
<td>472.1</td>
</tr>
<tr>
<td>6B</td>
<td>79.7</td>
<td>154.2</td>
<td>504.2</td>
</tr>
<tr>
<td>7F</td>
<td>6.6</td>
<td>6.6</td>
<td>165.4</td>
</tr>
<tr>
<td>9V</td>
<td>15.5</td>
<td>31.5</td>
<td>1,480.5</td>
</tr>
<tr>
<td>14</td>
<td>1.7</td>
<td>8.6</td>
<td>16.4</td>
</tr>
<tr>
<td>18C</td>
<td>4.3</td>
<td>9.3</td>
<td>15.2</td>
</tr>
<tr>
<td>19A</td>
<td>9.8</td>
<td>108.7</td>
<td>174.3</td>
</tr>
<tr>
<td>19F</td>
<td>149.9</td>
<td>164.7</td>
<td>428.6</td>
</tr>
<tr>
<td>23F</td>
<td>8.0</td>
<td>70.7</td>
<td>85.6</td>
</tr>
</tbody>
</table>

*Per reference 27. PnPS, serogroup-specific S. pneumoniae polysaccharide.
eight PCV13 serotypes, the cutoffs for serotypes 14, 18C, and 23F were adjusted to ensure that the revised cutoffs were within the validated assay limits, resulting in specificities of 95.5%, 96%, and 97%, respectively.

**Performance of UAD in relation to bacteremia.** Among 235 blood cultures drawn, 19 (8.1%) were positive by the BacTAlert FAN aerobic test, of which 7 were positive for PCV13 serotypes as determined by Quellung reaction from blood (serotypes 14 \(n=4\), 3 \(n=1\), 6A \(n=1\), and 6B \(n=1\)), 3 were positive for non-PCV13 serotypes (17B, 22, and 25) and 9 cultures indicated growth but failed to identify an organism. The UAD assay correctly identified the serotypes of 6 of 7 cases with a PCV13 serotype bacteremia (85.7%) and was negative for a single serotype 14 isolate; in this patient, the whole-blood (WB) \(\text{lytA}\) PCR was also negative. The UAD assay was also negative for urine samples from all 3 subjects with a corresponding blood culture positive for non-PCV13 serotypes. Thus, compared to blood culture results, the UAD assay had a sensitivity of 85.7% and a specificity of 100%. Among the 28 CAP patients with pneumococcal bacteremia (as detected by positive blood culture) and/or DNAemia (as detected by positive WB \(\text{lytA}\) real-time PCR [RT-PCR]) due to a PCV13 serotype, the UAD was positive for 25 (89.3%).

All of the 9 patients whose blood cultures indicated growth but failed to identify an organism were subsequently determined to be positive for pneumococcus via latex agglutination or nested PCR analysis. Urine samples from all of these subjects were positive in the UAD assay for a PCV13 serotype (serotypes 14 \(n=2\), 1 \(n=2\), 3 \(n=1\), 4 \(n=1\), 18C \(n=1\), and 19A \(n=2\)). For 4 of these 9 patients a serotyping PCR was available from WB, and classification of all 4 was in agreement with the UAD assay result \((n=1\) for each of serotypes 1, 3, 14, and 19A). For 8 of these 9 patients, a serotyping PCR (for the PCV13 serotypes) from a nasopharyngeal swab (NPS) was available: 5 (62.5%) of these 8 had an identical serotype in NPS and by the UAD assay (serotypes 1 \(n=2\), 3 \(n=1\), 4 \(n=1\), and 19A \(n=1\)); 1 patient had a discrepancy (serotype 19A in UAD assay and serotype 19F in PCR from NPS), and for 2 patients, the UAD assay showed PCV13 serotypes (14 and 18C), while the serotyping PCR from NPS did not detect a serotype.

**Performance of the UAD assay compared to other pneumococcal diagnostics.** For 222 CAP patients, both the UAD assay and urine ICT were performed on admission urine. The urine ICT was positive for 52 (23.4%) patients. Of these, 40 (76.9%) had a positive UAD (see Table S1 in the supplemental material). The UAD assay identified 60 additional cases when the urine ICT was negative and was more frequently positive than the urine ICT (45.0%; \(P < 0.001\)). Combining urine ICT and UAD assay resulted in attribution of CAP in 50.5% of patients to pneumococcus.

If restricted to the PCV13 serotypes (identified with serotyping PCR in WB or Quellung reaction from blood), the sensitivity of the UAD assay in relation to the urine ICT was 100% (17/17). The UAD assay was positive for 7 patients with negative ICT, and both assays were negative for 3 patients. The sensitivity of the UAD assay was higher (88.9% [24/27]) than that of the ICT (63.0% [17/27]) in relation to bacteremia and/or DNAemia due to a PCV13 serotype \((P = 0.03)\).

Among patients with PCV13 serotype bacteremia and/or DNAemia, the UAD assay was positive for 20 (90.9%) of 22 patients with a pneumococcal NP colonization density of \(\geq 8,000\) copies/ml (a surrogate for diagnosis of pneumococcal pneumonia [20]) and for all 3 patients with low NP pneumococcal colonization density (<8,000 copies/ml) (see Table S1). Among patients with identification of PCV13 serotype from any specimen (blood, sputum, nasopharyngeal specimen, or oropharyngeal specimen), the UAD was positive for 74.2% (62/83) of patients with an NP colonization density of \(\geq 8,000\) copies/ml and for 52.2% (12/23) of patients with an NP colonization density of <8,000 copies/ml \((P = 0.047)\).

The UAD assay was more frequently positive than the composite diagnostic (104/235 [44.3%] versus 71/235 [30.2%]; \(P < 0.001\) [Table 2]). Based on this composite diagnostic, the sensitivity of the UAD was 71.8% (51/71) and 84.9% (45/53) if restricted
to the PCV13 serotypes identified from any specimen (blood, sputum, nasopharyngeal specimen, or oropharyngeal specimen). Combining UAD assay and composite diagnostic resulted in a pneumococcal diagnosis in 124 (52.8%) patients, which was higher than the composite diagnostic alone (\( P < 0.001 \)) but not statistically different from the UAD assay alone (\( P = 0.07 \)). Adding the UAD assay to an extended composite diagnostic (composite diagnostic or WB lytA RT-PCR positive or lytA RT-PCR from NPS with \( \geq 8,000 \) copies/ml) resulted in 158 (67.2%) patients being shown to have pneumococcal etiology (Table 2).

**TABLE 2** Diagnostic performance of the UAD assay in relation to composite diagnostics

<table>
<thead>
<tr>
<th>UAD assay result</th>
<th>Composite diagnostic(^a)</th>
<th>Extended composite diagnostic(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>51</td>
<td>53</td>
</tr>
<tr>
<td>Negative</td>
<td>20</td>
<td>111</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>164</td>
</tr>
</tbody>
</table>

\(^a\)Composite diagnostic: positive if any of blood culture, good quality sputum Gram stain, culture or urine ICT were positive; negative otherwise. Pneumococcal detection (proportion of patients who had pneumococcus identified from UAD or the composite diagnostic) was 52.8% (124/235). Sensitivity of UAD was 71.8% (51/71). Sensitivity of UAD if restricted to PCV13 serotypes from any specimen was 84.9% (45/53).

\(^b\)Extended composite diagnostic: positive if any of blood culture, good-quality sputum Gram stain or culture, urine ICT, or WB lytA RT-PCR was positive or if lytA RT-PCR was positive from NPS with \( \geq 8,000 \) copies/ml; negative otherwise. Pneumococcal detection (proportion of patients who had pneumococcus identified from UAD assay or the extended composite diagnostic) was 67.2% (158/235). Sensitivity of UAD was 59.1% (78/132). Sensitivity of UAD if restricted to PCV13 serotypes from any specimen was 75.0% (69/92).

**Serotype distribution and concordance with other serotyping methods.** The UAD assay identified 2 PCV13 serotypes in 12 patients and 3 serotypes in 2 patients. The serotypes identified by the UAD assay were concordant with the serotypes detected by serotyping PCR and Quellung reaction in 70 patients and were different in 16 patients (Table 3).

In 11 of 15 patients with concordant serotypes and >1 serotype in blood or the nasopharynx, the UAD assay serotype correlated with the dominant serotype, i.e., the serotype with highest copy number. In 3 of 15 patients, the UAD assay identified not the dominant of two serotypes but the serotype which was consistently identified in other specimens and also by Quellung reaction. The remaining patient had serotypes...

**TABLE 3** Serotype discrepancies based on Quellung and PCR-based serotyping methodologies compared to UAD assay in pneumonia patients\(^a\)

<table>
<thead>
<tr>
<th>UAD assay serotype(s)</th>
<th>Serotype(s) identified from other specimens and methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19A (NPS PCR)</td>
</tr>
<tr>
<td>1, 4</td>
<td>23F (NW PCR, NPS/OPS Quellung)</td>
</tr>
<tr>
<td>1, 5</td>
<td>38 (NW PCR)</td>
</tr>
<tr>
<td>3</td>
<td>Non-PCV13 serotype by NPS PCR</td>
</tr>
<tr>
<td>6A</td>
<td>18C (NPS PCR), 34 (NPS/OPS Quellung)</td>
</tr>
<tr>
<td>9V</td>
<td>12F, 33F, 10A (NW PCR), 12F (NPS/OPS/sputum Quellung)</td>
</tr>
<tr>
<td>9V, 14</td>
<td>11A (NW PCR)</td>
</tr>
<tr>
<td>14</td>
<td>8 (NW PCR, NPS/OPS/sputum Quellung)</td>
</tr>
<tr>
<td>14</td>
<td>8 (NW PCR, NPS/OPS Quellung)</td>
</tr>
<tr>
<td>14</td>
<td>19A (NW PCR, NPS PCR, NP/OPS/sputum Quellung), 12F, 10A (NW PCR)</td>
</tr>
<tr>
<td>18C</td>
<td>15A, 35F (NW PCR)</td>
</tr>
<tr>
<td>18C</td>
<td>12F (NW PCR, sputum Quellung)</td>
</tr>
<tr>
<td>18C</td>
<td>11F (NPS/OPS/sputum Quellung)</td>
</tr>
<tr>
<td>19A</td>
<td>9V (NPS PCR, NPS/OPS Quellung), 19F (NW PCR)</td>
</tr>
<tr>
<td>19A</td>
<td>35F, 38 (NW PCR)</td>
</tr>
<tr>
<td>19A</td>
<td>19F, 5 (NPS PCR)</td>
</tr>
</tbody>
</table>

\(^a\)NPS, nasopharyngeal swab; OPS, oropharyngeal swab; NW, nasal wash. Note that with serotyping PCR from NW, serotypes 6A and 6B and serotypes within serogroup 18 could not be distinguished.
1 and 18C identified by UAD, while serotyping PCR detected serotypes 7F (10⁶ copies/ml), 1 (400 copies/ml), and 18C (170 copies/ml) from NPS and serotype 1 from NW.

Lack of correlation of colonization density and UAD in CAP. Serotype-specific quantitative NP colonization densities, as measured with serotyping RT-PCR, were correlated with quantitative UAD values separately for each serotype in pneumonia patients. When restricted to only those patients whose UAD values were within the linear assay range for each serotype, there was no significant correlation for any serotype (Table 4).

Performance of UAD assay for asymptomatic HIV-infected South African controls. PCR serotyping from NPS and UAD results were collected from 297 asymptomatic HIV-infected South African controls. UAD was positive for 15 controls (5.1% [Table 5]), 9 without pneumococcal NP carriage and 6 with the corresponding PCV13 serotype identified by serotyping PCR from NPS. There were 33 controls with PCV13 serotype carriage, of whom 6 had a corresponding serotype in the UAD assay (18.2%). Of 238 controls without pneumococcal carriage, UAD was less frequently positive (n = 9) than in controls with identified carriage (P = 0.005). In addition, the only control person who had a positive urine ICT also had a negative UAD result, and there was no evidence of NP carriage by culture or lytA RT-PCR.

DISCUSSION

In this study, the UAD assay was successfully adapted to be used in a cohort of HIV-infected South African adults with pneumonia and proved to be a diagnostic test of high accuracy. There was good correlation with serotypes identified by Quellung reaction from bacteremic patients. UAD demonstrated high specificity and sensitivity in bacteremic patients and detected 15 times more PCV13 serotype infections than blood cultures, 4 times more than blood cultures and WB lytA PCR combined, and twice as many as the commercial urine ICT. The UAD assay detected an additional 23% of cases beyond currently available standard pneumococcal tests, resulting in an overall pneumococcal etiologic fraction of 53%. UAD was more frequently positive for asymptom-

### Table 4: Correlation between colonization density (serotyping RT-PCR from NP swab) and quantitative UAD result in pneumonia patients

<table>
<thead>
<tr>
<th>Serotype</th>
<th>n</th>
<th>Spearman correlation coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>-0.02</td>
<td>0.95</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-0.5</td>
<td>0.67</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6A</td>
<td>2</td>
<td>-0.8</td>
<td>NA</td>
</tr>
<tr>
<td>6B</td>
<td>4</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>7F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9V</td>
<td>2</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>0.5</td>
<td>0.67</td>
</tr>
<tr>
<td>18C</td>
<td>3</td>
<td>0.5</td>
<td>0.67</td>
</tr>
<tr>
<td>19A</td>
<td>4</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>19F</td>
<td>7</td>
<td>0.64</td>
<td>0.12</td>
</tr>
<tr>
<td>23F</td>
<td>2</td>
<td></td>
<td>NA</td>
</tr>
</tbody>
</table>

*a* Analysis was restricted to pneumonia patients with UAD values within the linear range. NA, not applicable.

### Table 5: UAD in asymptomatic controls

<table>
<thead>
<tr>
<th>UAD result</th>
<th>Carriage with PCV13 serotype</th>
<th>Carriage with non-PCV13 serotype</th>
<th>No carriage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>6*</td>
<td>0</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Negative</td>
<td>27</td>
<td>26</td>
<td>229</td>
<td>282</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>26</td>
<td>238</td>
<td>297</td>
</tr>
</tbody>
</table>

*All 6 had identical serotype identified by UAD and PCR/Quellung reaction from nasopharyngeal swabs.*
atic controls with pneumococcal carriage, but UAD values did not correlate with colonization density.

The UAD assay demonstrated very good sensitivity and specificity, 89% and 100%, respectively, and outperformed the urine ICT, which was positive for only 4 of 7 patients with PCV13 serotype bacteremia. The UAD assay was developed to detect pneumococcal pneumonia due to a PCV13 serotype irrespective of the presence of bacteremia (27, 28). As a limit assay with either a positive or a negative result, positivity cutoff values were originally derived from urine samples obtained from an adult population with no clinical suspicion of CAP. These cutoff values for serotype-specific pneumococcal polysaccharides ranged between 1.7 and 330.5 U/ml (27). The UAD assay was recently suspected to yield false-positive results for several adults with pneumonia from whom the urine was collected shortly after receipt of the 23-valent pneumococcal polysaccharide vaccine (29). As detailed by Pride et al. (27), to avoid false positives, it is important that the UAD assay results take into account clinical and radiological findings at the time of urine specimen collection. The suitability of the UAD assay should be first evaluated in the intended study population (27). Consistent with these recommendations and to maintain the high specificity of the UAD assay in our study population, we first evaluated the appropriateness of the established UAD cutoffs by examining asymptomatic HIV-infected adults without history of a respiratory tract infection within the last 30 days.

A relatively high positivity rate (~13% [data not shown]) was observed with the established CAPiTA cutoffs in the HIV-infected controls, resulting in an overall UAD assay specificity of 89% for this population. The main reason for the necessity to change some of the cutoff values was likely the higher carriage rate in our asymptomatic cohort, which was similar to previously reported carriage rates in Soweto, South Africa (30), compared to the likely considerably lower carriage prevalences in European and North American adults (31, 32) that were used to establish the CAPiTA cutoffs. To account for the higher carriage rate in this cohort and to improve the specificity of the UAD assay, cutoffs were adapted based on previously used methodology (27), using urine specimens from our controls. The overall UAD assay specificity increased from 89% to at least 95% with the adapted cutoffs. The 100% specificity of the UAD assay in relation to bacteremia allows the extrapolation of these findings to patients with nonbacteremic pneumococcal pneumonia. While the urine ICT is serotype independent and should therefore identify all pneumococcal episodes, UAD was twice as frequently positive as the ICT (45% versus 23%) in patients with bacteremic and nonbacteremic CAP. The observed sensitivity of the urine ICT in this study falls in the lower range of reported pooled sensitivities of 68 to 74% (with ranges from 29% to 87%) in recent meta-analyses in HIV-uninfected persons (24, 33). Sensitivities of the urine ICT are similar for HIV-infected and HIV-uninfected adults (24) and higher for bacteremic patients (34, 35). The UAD assay was 100% sensitive in relation to the ICT if restricted to patients with PCV13 serotype bacteremia. Irrespective of any serotype, UAD was significantly more frequently positive than a predefined pneumococcal composite diagnostic of blood culture, sputum culture and Gram stain, and urine ICT. Adding the UAD to the composite diagnostic increased the presumed pneumococcal etiology up to 52.8% (75% relative increase in etiology). Since the UAD assay was positive in 89% (25/28) of PCV13 serotype bacteremias and/or DNAemia and only 63% (38/60) of episodes of bacteremia and/or DNAemia were due to a PCV13 serotype, we extrapolate that 78% of pneumonia episodes in this South African cohort of HIV-infected adults might be pneumococcal. This is close to proportions which were reported in the 1930s (4, 36, 37) and considerably higher than contemporary pneumococcal proportions (18), which vary from 5% (38) to 57% (7). Few recent comprehensive etiology studies with African adults are available. In Kenya, 46% of adult pneumonias were pneumococcal (1, 39). Importantly, our cohort was recruited prior to the introduction of PCV7/13 into the South African pediatric vaccine schedule. South African, UK, and U.S. data reproducibly showed that pediatric PCV reduced IPD in adults (14–16), and U.S. surveillance revealed a decrease of all-cause pneumonia in adults (40). Thus, our data likely represent...
maximum pneumococcal proportions in adults prior to the introduction of childhood PCVs.

In some cases, the serotype declared by the UAD assay was different from the carriage serotype. Since, similar to our results, the serotype identified by the UAD assay had been shown in a previous study to correlate 100% with the serotypes from blood cultures in patients with bacteremic CAP (27), alternative explanations need to be considered for the discrepancy from carriage serotypes, not taking into account the possibility of technical error or mislabeling (41). This finding might reflect a previous infection rather than an incorrect detection of the carriage serotype. If pneumonia is due to an invasive serotype such as 1, 4, and 5, which are rarely carried or carried at low densities in the NP, these serotypes might be missed with NP sampling (42, 43). In contrast, in a longitudinal study of pneumococcal carriage and infection in U.S. infants, the infecting serotype was detected in the nasopharynx in all 31 infants at the time of infection (44). The commercially available urine ICT can remain positive for months after infection (45), but this period is still unknown for the UAD. With molecular serotyping assays, the proportion of multiple serotype carriage has increased in comparison to conventional techniques (46, 47). Quantitative PCR furthermore allows identification of a predominant serotype with the highest colonization density (47, 48). In patients with multiple serotype carriage and bacteremic and/or DNAemic pneumococcal pneumonia, the dominant carriage serotype usually correlated with the serotype identified in WB, as recently reported for this cohort (48). Accordingly, the UAD assay usually correlated with either the dominant carriage serotype or the serotype most consistently identified from other specimens or from blood.

Among controls, pneumococcal NP carriage was associated with a 4-fold risk of a positive UAD. This association was previously shown for the commercial urine ICT for children, but not adults, which therefore lacks utility for children (23). No data on the performance of the UAD assay for children has been published yet. Alternatively, positive UAD in controls might result from undocumented pneumococcal infection more than 30 days prior to specimen collection. More data are required on the duration of positivity of the UAD assay and the role of NP carriage. However, even if a 5% false-positivity rate related to carriage in asymptomatic persons will be confirmed in future studies, given a 50% proportion of pneumococcus among pneumonia episodes, with 85% sensitivity and 95% specificity the ratio of true-positive to false-positive UAD would be 17:1, rendering the UAD assay highly useful for HIV-infected patients with pneumonia to diagnose pneumococcal pneumonia while at the same time providing information on the presence of the PCV13 serotypes. It is therefore useful in the clinical setting as well as for epidemiological and research studies. In contrast, for asymptomatic controls—who may have only a 0.1% risk of developing pneumococcal pneumonia annually—the UAD would lead to 50 times more false-positive than true-positive results, rendering this assay a poor diagnostic test for asymptomatic persons; however, the UAD assay was not designed for this purpose.

The main strength of this study is that it was performed in a well-characterized patient cohort with extensive pneumococcal testing from a large number of specimens. Thus, the utility of the UAD assay can be extended to South African HIV-infected adults with some adaptations of the cutoff values. It also can be considered a proof of concept study of how to adjust cutoff values. The high sensitivity and specificity and the resulting proportion of pneumococcal etiology approaching historic figures support our findings.

The main limitations are that this was a retrospective single-center study with relatively few patients with bacteremic pneumococcal pneumonia and the limited number of cohort-matched controls. As discussed above, due to the unknown duration of positivity, our data are not able to conclusively distinguish carriage from recent infection. Since adjustments of the positivity cutoffs were necessary in order to maintain the high specificity, reevaluation of cutoffs is advisable when this assay is introduced for use in evaluating other populations which have not been studied yet. Addition of nonvaccine serotypes would be useful to study serotype replacement after
vaccine implementation and thus provide information which serotypes should be included in novel vaccine formulations.

In conclusion, our data confirm the high diagnostic accuracy of the UAD assay as a research tool to detect pneumococcal pneumonia with simultaneous determination of PCV13 serotypes in HIV-infected South African adults after slight adaptation of cutoff values. Therefore, the UAD also allows determining direct and indirect effects of vaccination programs on adult pneumococcal infections. Our data support further evaluation of expanded-serotype UAD assays, which may become a new standard for detection of pneumococcal pneumonia in adults. Information on duration of positivity, effects of carriage, and utility in children is urgently needed.

MATERIALS AND METHODS

Patients. Adult patients hospitalized with radiologically confirmed CAP at Chris Hani Baragwanath Hospital, Soweto, South Africa, were enrolled between 2005 and 2007, prior to introduction of PCV7 (2009) and PCV13 (2011) into the national pediatric vaccination schedule (20). This analysis was restricted to HIV-infected persons. HIV-infected adult outpatients without signs of respiratory infection were enrolled as controls.

Specimen collection and microbiological methods. A single aerobic FAN blood culture (BacT Alert FAN aerobic using 10 ml of blood), Gram stain, and cultures of induced sputum, urine, oropharyngeal and NP swabs (OPS and NPS) were obtained on admission as previously reported (20). Nasal washes (NW) were performed by reaspiration after applying 4 ml of 0.9% NaCl. Quantitative lytA real-time PCR (RT-PCR) was performed on NP swabs (20) and Fast-track Diagnostic respiratory 21-plus test (Fast-track Diagnostics) for 19 respiratory viruses and 5 bacteria on NW. Whole blood (WB) was tested with an in-house triplex RT-PCR for pneumococcus (lytA), Haemophilus influenzae type b, and Staphylococcus aureus (49). Urine was tested with the ICT within 12 h (BinaxNow S. pneumoniae test). Remaining urine was stored at −20°C prior to further processing.

Serotype-specific UAD assay. The urinary antigen detection (UAD) assay is a noncommercial Luminex technology-based multiplex serotype-specific assay that utilizes spectrally unique microspheres coated individually with serotype-specific antipneumococcal polysaccharide monoclonal antibodies (capture antibodies) capable of detecting the following serotypes: 1, 3, 4, 5, 6A/C, 6B, 7F/A, 9V/A, 14, 18 (C, A, 8, and F), 19A, 19F, and 23F (22, 27). For the UAD assay, positivity cutoff limits, based on antigen concentrations read off a standard curve, were established for each serotype using 400 control urine specimens obtained from subjects ≥50 years of age in the Netherlands and United States without clinical suspicion of CAP in the 2 months prior to obtaining the urine sample. Nonparametric tolerance intervals were computed from these concentrations, giving a range predicted to contain 98% of negative urine samples with 99% confidence, thus achieving at least 97% assay specificity for each serotype. For each serotype the cutoff was based on the second highest control sample concentration (except for serotypes 1 and 5, for which the highest UAD value was used). These positivity cutoff values were clinically validated in a pilot study in the Netherlands prior to use in the CAPiTA study (27, 28).

The suitability of the established cutoff values were evaluated for use in this study cohort (HIV-infected South African adults with CAP) and adjusted, if needed, using similar nonparametric methods on the 297 asymptomatic HIV-infected South African control specimens to achieve ≥95.5% assay specificity for each serotype. To be conservative, it was agreed that the existing cutoffs would not be lowered. If a positivity cutoff needed to be increased, it was set to the second highest urine value from
among the asymptomatic HIV-infected South African controls. If a proposed cutoff based on the second highest value was above the upper limit of the assay’s validated range, the cutoff was based on the highest control urine value that was still within the validated assay range.

**Diagnostic criteria for pneumonia.** Pneumonia was considered pneumococcal if any of the composite diagnostic criteria were positive: blood culture, good-quality (>25 neutrophils and <10 epithelial cells per low-power field) sputum Gram stain or culture (20), or urine ICT.

**Serotyping.** Serotyping of all isolated pneumococci was performed using the Quellung method. No attempt was made to identify multiple serotypes; i.e., a single Quellung reaction for serotyping was performed per visually appreciated colony morphology. We also performed 13 serotype-specific PCRs for the PCV13 serotypes from NPS and from sputa if one-step duplex lytA PCR (20) was highly positive (lytA threshold cycle \( C_{\text{lytA}} \geq 35 \)). Serotyping PCR from NW and WB was performed by a quantitative multiplex RT-PCR for 40 pneumococcal serotypes, including all PCV13 serotypes (Fig. 1) (48).

The serotypes identified by UAD and by serotyping PCR and Quellung reaction were compared. If there was ≥1 serotype which was identified both by UAD and by any other method from any specimen (NPS with PCR and Quellung; NW with PCR, OPS with Quellung; sputum with PCR and Quellung; blood with PCR and Quellung), the result was judged concordant. Results were judged discordant if one or more PCV13 serotypes identified by UAD were different from all results identified by PCR serotyping or Quellung reaction. UAD specimens positive for >2 serotypes were considered indeterminate in previous studies, but for the purpose of this analysis all serotypes above the serotype-specific cutoff values were reported. While UAD results are usually provided as positive, negative, or indeterminate, the calculated UAD value (serogroup-specific \( S. pneumoniae \) polysaccharide [PnPS] units per milliliter) for a sample was used to test whether there was a correlation with NP colonization density from the same subject.

**Statistical methods.** Proportions were compared with Pearson’s \( \chi^2 \) test or Fisher’s exact test, as appropriate. Correlations between continuous variables were assessed by Spearman’s correlation coefficient (r). \( P \) values were considered statistically significant if they were \( \leq 0.05 \).

This study was approved by the ethics committees of the University of the Witwatersrand and Emory University. Informed consent was provided by all patients and controls.

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.01573-16.

**TEXT S1**

PDF file, 0.09 MB.

**ACKNOWLEDGMENTS**

W.C.A. received an honorarium from GlaxoSmithKline (GSK) and support from BRAHMS Thermo Fisher and bioMérieux to attend meetings and fulfilled speaking engagements. S.A.M. received research funding and honoraria from Pfizer vaccines and GSK and institutional grant support from Wyeth. K.P.K. received consulting and research funding from Pfizer Vaccines and consulting funding from GSK. M.W.P., R.F., S.S., V.S., and K.U.J. are employees of Pfizer Inc.

We thank Pfizer for performing the lytA RT-PCR and Binax for supplying ICT, BinaxNow Streptococcus pneumoniae, free of charge.

This work was supported by Centers for AIDS Research (CFAR) National Institutes of Health (NIH) grant P30 A1050409 to K.P.K.

The funding source had no influence on data analysis, the draft of the manuscript, or the decision to submit the manuscript for publication.

**REFERENCES**


