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**Title:** Evaluating a Pathogen-Specific IgG Binding Assay for Rapid Detection of Healthcare- Associated Infections

**Running Title:** IgG Binding in Diagnosing HAIs

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**ABSTRACT**

Rapid and accurate diagnosis of healthcare-associated infections (HAIs) is an unmet need for improving outcomes in intensive care units (ICUs). Traditional culture-based methods, while the gold standard, are time-consuming and can delay therapeutic interventions. In this study, we evaluated the diagnostic utility of pathogen-specific immunoglobulin G (IgG) binding levels using an enzyme-linked immunosorbent assay (ELISA). We measured IgG binding against pathogens including *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Staphylococcus aureus*. Diagnostic performance was evaluated through receiver operating characteristic (ROC) curve analysis, with culture results as the reference. The assay demonstrated a diagnostic accuracy of 83.2%, with a sensitivity of 85.4%, a specificity of 87.9%, and an area under the curve (AUC) of 0.910. Pathogen-specific cutoff values ranged from 0.918 to 1.534. Especially, *A. baumannii* showed the highest performance metrics, achieving a sensitivity of 94.7%, a specificity of 93.6%, and an AUC of 0.975. The pathogen-specific IgG binding levels can offer a novel and effective diagnostic tool for the initial assessment of HAIs, enhancing early detection and improving patient management across healthcare settings.

**Keywords:** Healthcare-associated infections, Pathogen-specific IgG, Intensive care unit, Rapid diagnostic test, Enzyme-linked immunosorbent assay, Antibiotic stewardship

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## INTRODUCTION

Healthcare-associated infections (HAIs) are a significant global challenge, with an average mortality rate of 10% among affected patients, especially in intensive care units (ICUs). In high-income countries, HAIs impact up to 30% of ICU patients and are frequently associated with comorbidities, immunosuppression, and invasive devices. Common causative agents include *Escherichia coli*, *Pseudomonas aeruginosa*, *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus*, and vancomycin-resistant *Enterococci* [1].

The conventional gold standard for diagnosing HAIs is the culture method, which requires 48 to 72 hours to provide results. This delay can lead to overuse of broad-spectrum antibiotics, extended hospital stays, increased morbidity, and increased healthcare costs. Consequently, there is a critical need for more rapid diagnostic methods to facilitate timely and precise therapeutic interventions to improve patient management and outcomes [2]. Rapid techniques such as matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), EUCAST Rapid Antimicrobial Susceptibility Testing (RAST), and multiplex polymerase chain reaction (PCR) panels accelerate identification but detect microbial components rather than host response and may not distinguish colonization from real infection or diagnose active infection under antibiotic suppression [3-6].

Recognizing these ongoing challenges, recent studies have focused on novel biomarkers for early detection of bacterial infections and have developed rapid, point-of-care tests for multidrug-resistant organisms [7,8]. While these technologies offer valuable insights, they still face limitations in sensitivity, specificity, and broader

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clinical applicability. Furthermore, the increasing prevalence of antibiotic resistance calls for enhanced diagnostic techniques that can not only quickly identify pathogens but also inform appropriate antibiotic therapy [9].

Immunoglobulin G (IgG) plays a pivotal role in host defense by binding specifically to bacterial antigens and promoting pathogen clearance. This key feature underlines the diagnostic potential of IgG-based assays for early bacterial infection detection [10,11]. Enzyme-linked immunosorbent assay (ELISA) techniques leverage this mechanism, providing a means to distinguish infected from non-infected patients. ELISA can be performed using standard laboratory equipment and delivers results within a few hours, thereby supporting early clinical decision-making [12].

In this study, we introduced a diagnostic approach utilizing ELISA to measure IgG binding levels against common pathogens such as *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Enterococcus faecium*. By comparing the efficacy of this method with traditional culture results, we aimed to establish a faster and more precise diagnostic tool, enhancing clinical outcomes through earlier and more targeted interventions in ICU settings.

## METHODS

### Study Settings

The study was conducted in the ICUs of Cerrahpaşa University Hospital and Istanbul Şişli Hamidiye Etfal Training and Research Hospital, two tertiary care hospitals in Istanbul. We included ICU patients who were admitted without infections and were not immunosuppressed or pregnant. Patients who developed infections with target pathogens during their stay were included. The target pathogens were *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Enterococcus faecium*. We selected these pathogens based on local epidemiology and clinical impact in the included ICUs. Non-target organisms were defined as microbial species not covered by the seven-pathogen IgG panel.

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**Sample Collection**

Serum samples for the IgG binding assay were collected within 24 hours of obtaining culture samples from different body sites, including blood, endotracheal aspirates (ETA), bronchoalveolar lavage (BAL), urine, sterile body fluids, and tissues. Sample collection procedures were blinded to culture results to mitigate bias**.** Samples were preserved at -80°C until processed. ELISA assays were performed weekly to ensure consistency (12).

We recorded demographic and clinical data for each patient, including age, sex, dates of admission and sample collection, and the duration of the hospital stay, using paper-based case report forms.

**Bacterial Strains and Sample Processing**

Routine culture samples were processed using standard microbiological methods in the Clinical Microbiology Laboratory. Pathogens were identified via Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS, Bruker Corporation). We defined specific criteria for significant bacterial growth, including the requirement for simultaneous growth in two or more sets of blood cultures from different veins; endotracheal aspirate (ETA) cultures with $\geq 10^5$ colony-forming units (CFU)/mL; bronchoalveolar lavage (BAL) cultures with $\geq 10^4$ CFU/mL; urine samples with $\geq 10^5$ CFU/mL; and pathogen growth of $\geq 10^3$ CFU/mL in sterile body fluids or tissues without normal flora [13]. Samples with over four pathogen types were excluded to prevent confounding.

**IgG Binding Assay**

The IgG binding assays were conducted independently of culture results to prevent bias. We used ATCC pathogen strains adjusted to 0.5 McFarland standards for assay preparations. IgG binding levels were quantified using ELISA in optical density (OD) units. A 100-mL bacterial suspension at 10 µg/mL was prepared from 1.25 mL of these cultures in 98.75 mL carbonate-bicarbonate buffer and added to 96-well plates (100 µL/well). The plates were incubated at 37°C for one hour, washed with phosphate buffered saline (PBS) containing 0.1% Tween 20, then coated with 1% bovine serum

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albumin (BSA) in PBS (200 µL/well), and incubated for another hour at 37°C. After subsequent washing, a 1:2 dilution of patient samples in 1% BSA (100 µL/well) was added and incubated for an additional hour at 37°C. Following a final wash, the substrate pNPP (100 µL/well, Sigma P7998) was added and incubated for 20 minutes at room temperature. The reaction was stopped with 1M $H_2SO_4$ (100 µL/well), and the absorbance was measured at 405 nm using a Thermo Scientific™ Multiskan GO spectrophotometer, with results calibrated using Thermo Scientific™ SkanIt™ Software version 3.2 (https://www.thermofisher.com/order/catalog/product/5187139). We tested this dilution against dilutions of patient samples (1:100, 1:50, 1:10, and 1:2), finding that 1:2 provided the most consistent results. Serum from patients with confirmed pathogen growth in blood cultures was used as a positive control, and bovine serum albumin was used as a negative control to establish the baseline and assess nonspecific reactions. Assays were performed in triplicate to ensure reproducibility [14]. IgG binding levels were quantified as the mean absorbance at 405 nm ($OD_{405}$) from triplicate wells. This $OD_{405}$ value was directly proportional to the amount of patient IgG bound to the pathogen antigens in the well and thus served as the quantitative measure of pathogen-specific IgG-binding titer.

**Data Analysis**

Data analysis was conducted utilizing GraphPad Prism™ version 10.5.0 (673) (GraphPad Software, San Diego, CA, USA; https://www.graphpad.com/scientific-software/prism/) [15]. Basic descriptive statistics were employed to outline patient demographics and clinical outcomes. We employed a chi-square test to assess the variance in pathogen distribution and the Kruskal-Wallis test with Dunn's post-hoc analysis to determine differences in IgG binding levels among pathogens. The Brown-Forsythe and Welch ANOVA tests were utilized to compare IgG binding levels across different infection sites. Changes in IgG binding levels over time were assessed using the Wilcoxon matched-pairs signed-rank test and Pearson's correlation coefficient. We employed Welch’s test to compare IgG binding efficacy between single and

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multiple pathogen infections and utilized a two-way ANOVA to analyze the effects of multiple simultaneous pathogens. We evaluated the diagnostic performance of pathogen-specific IgG levels using receiver operating characteristic (ROC) curve analysis, comparing IgG results to culture outcomes as the reference standard. ROC curves plot sensitivity against 1–specificity for various optical-density (OD) thresholds. The area under the ROC curve (AUC), calculated using Prism software, reflects diagnostic accuracy (values closer to 1.0 indicate better discrimination). Optimal cutoff values were determined using Youden's index (maximum sensitivity + specificity – 1). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated at these cutoffs. All results were presented with 95% confidence intervals, both overall and by pathogen type.

Finally, categorical analysis of test reliability involved calculating the Kappa statistic and odds ratio using Fisher's exact test to correlate IgG binding assay results with culture outcomes. All statistical tests were performed, considering a significance threshold set at $p < 0.05$ [16].

## RESULTS

### Demographic and Clinical Characteristics of ICU Patients

A total of 432 serum samples were collected from 225 ICU patients, of which 315 met the study criteria. The average age of the patients was 60.6 years, with an average hospital stay of 14.8 days. The gender distribution included 55% males and 45% females, with an overall mortality rate of 31%. Patients were predominantly from Cerrahpaşa University Hospital (68%), with the remaining 32% from Şişli Etfal Training and Research Hospital.

### Pathogen Distribution and Infection Characteristics

*Acinetobacter baumannii* was identified as the most prevalent pathogen, detected in 27.7% of culture-positive samples, followed by *E. coli* (17.5%), *P. aeruginosa* (16%), *K. pneumoniae* (11.6%), *E. faecalis* (11.6%), *E. faecium* (10.2%), and *S. aureus* (0.05%). The distribution of pathogens showed no significant variance within culture-positive samples (Chi-square, $p = 0.411$). Among non-target

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organisms, *Corynebacterium spp.* and *Proteus mirabilis* were most common in culture-positive patients, with *E. faecalis* significantly associated with concurrent pathogen growth ($p = 0.006$).

**IgG Binding Levels Across Different Pathogens and Infection Sites**

Significant differences in IgG binding levels among the target pathogens were observed ($p < 0.0001$, Kruskal-Wallis statistic = 52.61). Dunn's post-hoc tests indicated that *P. aeruginosa* exhibited the highest overall OD values, while both *E. coli* and *E. faecalis* demonstrated significantly higher values than *S. aureus* (Fig. 1).

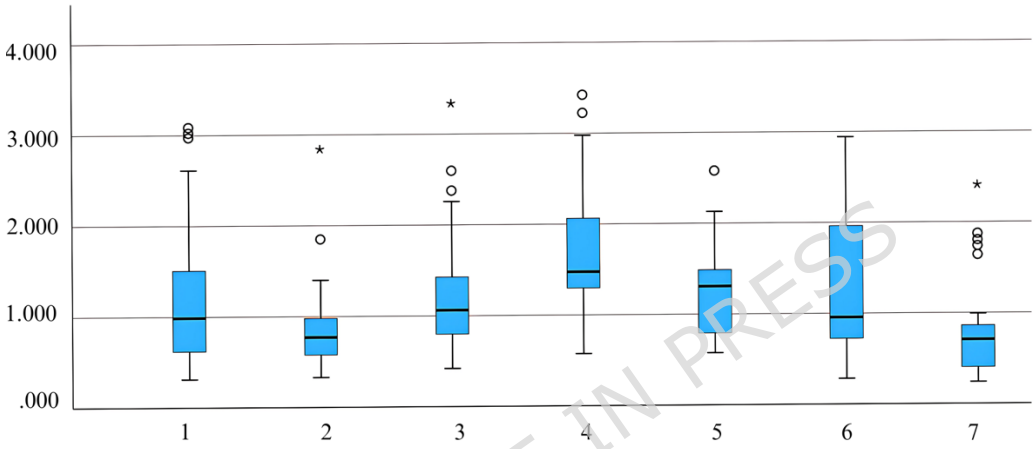



**Fig. 1.** IgG opsonization levels for different target pathogens. (1) *Acinetobacter baumannii*; (2) *Klebsiella pneumoniae*; (3) *Escherichia coli*; (4) *Pseudomonas aeruginosa*; (5) *Enterococcus faecalis*; (6) *Enterococcus faecium; (7) Staphylococcus aureus*

We stratified each pathogen by culture result, such as culture-positive and negative controls. There were significantly higher IgG binding levels in culture-positive samples for all pathogens ($p < 0.05$, unpaired two-tailed t-tests with Welch's correction). Table 1 detailed the differences in pathogen-specific IgG binding levels across each pathogen as well as grouped with culture results.

**Table 1:** IgG Binding Levels for Bacterial Isolates by Culture Results

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| Pathogen | Culture-positive (n) | *Mean* [a] *± SD OD* | Culture-negative controls (n) | *Mean ± SD OD* | *p* value | Total (n) |
|---|---|---|---|---|---|---|
| *A. baumannii* | 38 | 1.70 ± 0.58 | 47 | 0.66 ± 0.22 | <0.0001 | 85 |
| *K. pneumoniae* | 16 | 1.18 ± 0.54 | 24 | 0.65 ± 0.19 | 0.0015 | 40 |
| *E. coli* | 24 | 1.55 ± 0.65 | 26 | 0.86 ± 0.28 | <0.0001 | 50 |
| *P. aeruginosa* | 22 | 2.10 ± 0.72 | 21 | 1.27 ± 0.30 | <0.0001 | 43 |
| *E. faecalis* | 16 | 1.49 ± 0.46 | 12 | 0.88 ± 0.27 | 0.0005 | 28 |
| *E. faecium* | 14 | 2.11 ± 0.52 | 23 | 0.74 ± 0.23 | <0.0001 | 37 |
| *S. aureus* | 7 | 1.59 ± 0.56 | 25 | 0.59 ± 0.22 | 0.0029 | 32 |

[a] Pathogen-specific IgG binding level as *mean ± SD (*standard deviation) OD (optical density, measured at 405 nm); Controls: Cases with negative culture results for the target pathogen; p-values by unpaired two-tailed t-test with Welch's correction; $p < 0.05$ significant.

We compared the levels of pathogen-specific IgG binding based on the infection sites, which were classified into five groups: respiratory (38%), surgical site (27%), bacteremia (25%), urinary tract (8%), and other infections (2%). Only the non-infected sites had significantly lower binding levels than all other categories (Brown–Forsythe and Welch ANOVA, $p < 0.0001$; see Supplementary Fig. S1 online).

**IgG Binding in Recurrent Same-Pathogen Infections**

Among the 315 participants, a small post-hoc longitudinal subset (13/315 patients, 4.1%) had repeat positive cultures for the same pathogen at different time points. There was no overall change in the same pathogen-specific IgG binding levels between the two infection episodes (Wilcoxon matched pairs signed rank test, $p = 0.892$); however, a moderate positive correlation was observed (Pearson $r = 0.587$; $p = 0.034$). Only two patients in this subset reverted to seronegativity on day 3 and day 9 after the initial culture (see Supplementary Fig. S2 online).

**Impact of Pathogen Diversity on IgG Binding Efficacy**

We explored the impact of pathogen diversity by comparing IgG binding levels among patients with different target pathogens. Out of the cohort, 47% exhibited growth of a single target pathogen, whereas 53% presented with infections from multiple

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pathogens. Our analysis revealed no significant differences in pathogen-specific IgG binding levels between these groups (Welch's test, F = 0.037, p = 0.962, R-squared = 0.001).

We compared the pathogen-specific IgG binding levels in patients with simultaneous growths of two different targeted pathogens while excluding data from any third simultaneous pathogen to maintain data consistency. While the type of pathogen significantly impacted specific IgG binding levels (two-way ANOVA, p = 0.0379), the presence of multiple target pathogens did not alter the pathogen-specific IgG binding levels (p = 0.470).

**Diagnostic Accuracy of the IgG Binding Assay**

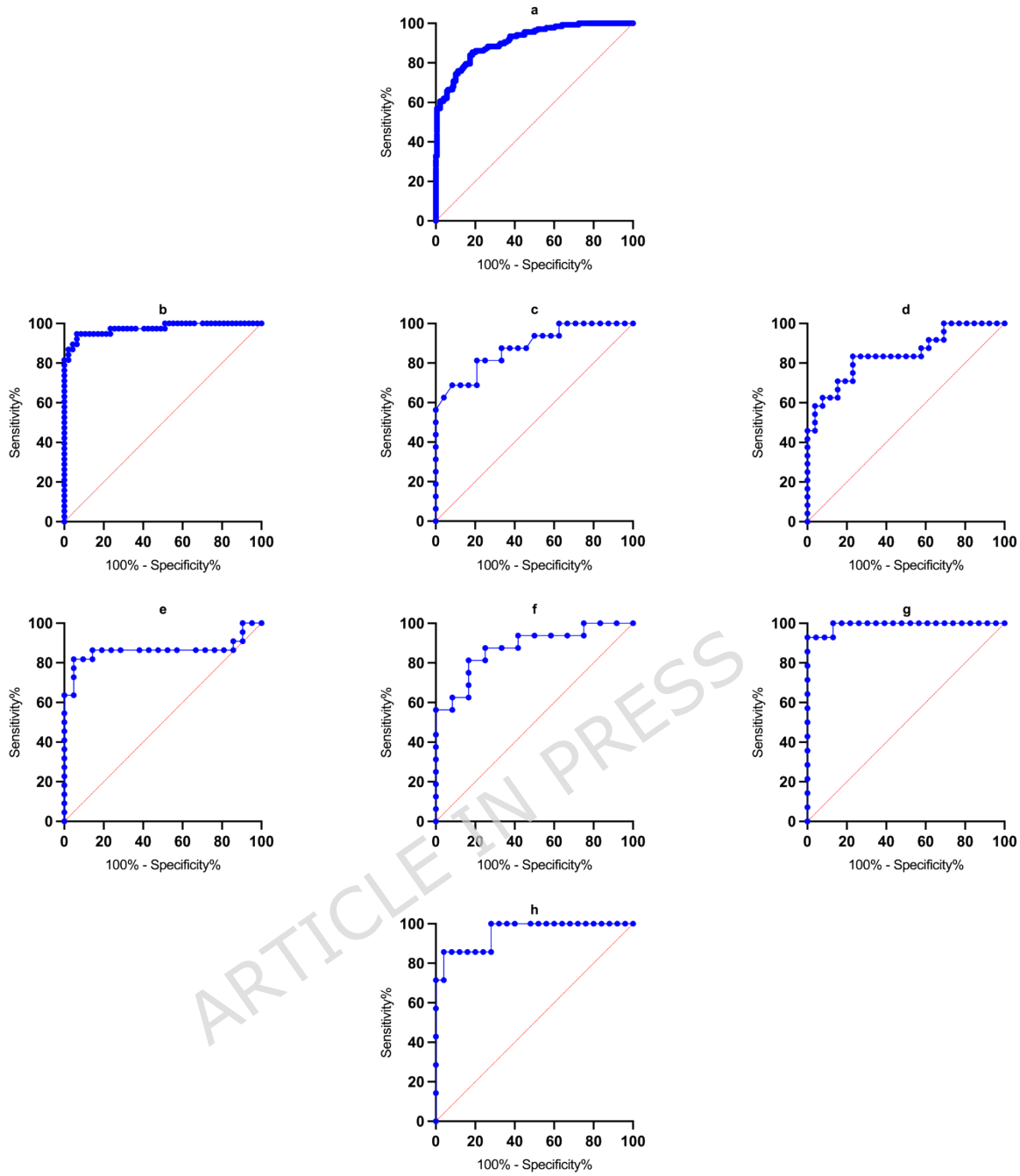
The optimized threshold value for the combined IgG binding assay was determined to be 1.009, based on ROC curve analysis using culture results as the reference standard. At this cutoff, the test was classified as an excellent A-category test with an ROC area of 0.910 (95% CI: 0.87-0.944, p < 0.0001). The analysis demonstrated high accuracy (83.2%), with a sensitivity of 85.4% and a specificity of 81.4%.

In addition to the global threshold, pathogen-specific OD cutoffs were calculated using Youden's index (sensitivity + specificity – 1) to optimize the performance of each individual assay. These cutoffs, determined via ROC analysis, represent the OD thresholds that best differentiate true positive from true negative results based on culture reference. ODs above a pathogen's cutoff were interpreted as positive IgG binding, indicating active or recent infection, whereas ODs below the cutoff indicated a negative result.

Among the pathogens tested, the *A. baumannii* IgG binding assay demonstrated the highest diagnostic accuracy (94.12%), with 94.7% sensitivity, 93.6% specificity, and an AUC of 0.975, followed by *E. faecium* and *S. aureus* (Table 2). Fig. 2 displayed the ROC curves of each target pathogen.

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**Fig. 2.** Receiver Operating Characteristic (ROC) curves for IgG binding levels across the targeted pathogens. (a) ROC curve for all pathogens combined; (b) *Acinetobacter baumannii*; (c) *Klebsiella pneumoniae*; (d) *Escherichia coli*; (e) *Pseudomonas aeruginosa*; (f) *Enterococcus faecalis*; (g) *Enterococcus faecium; (h) Staphylococcus aureus*

**Table 2:** Diagnostic Test Performance and Optimal Cutoffs of Binding Assay

| Pathogens | Sensitivity (%) | Specificity (%) | Accuracy (%) | PPV (%) | NPV (%) | AUC | Cutoff |
|---|---|---|---|---|---|---|---|

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *A. baumannii* | 94.7 | 93.6 | 94.1 | 92.3 | 95.6 | 0.975 | 1.041 |
| *K. pneumoniae* | 68.7 | 91.6 | 82.5 | 84.6 | 81.4 | 0.878 | 0.955 |
| *E. coli* | 83.3 | 76.9 | 80 | 76.9 | 83.3 | 0.842 | 1.050 |
| *P. aeruginosa* | 81.8 | 95.2 | 88.4 | 94.7 | 83.2 | 0.863 | 1.534 |
| *E. faecalis* | 81.3 | 83.3 | 82.1 | 86.7 | 76.9 | 0.875 | 1.266 |
| *E. faecium* | 71.4 | 87 | 81.1 | 76.9 | 83.3 | 0.990 | 1.327 |
| *S. aureus* | 85.7 | 93.7 | 93.7 | 85.7 | 96 | 0.954 | 0.918 |
| **Total** | **85.4** | **81.4** | **83.2** | **78** | **87.8** | **0.910** | **1.009** |

PPV: positive predictive value; NPV: negative predictive value; AUC: area under the Receiver Operating Characteristic (ROC) curve

**Categorical Analysis and Test Reliability**

We classified IgG binding OD values as positive or negative using pathogen-specific cutoffs derived from ROC curve analysis. Out of 315 samples, 132 (42.0%) were IgG-positive and 183 (58.0%) were IgG-negative for target infections. Of the 132 IgG-positive cases, 117 (88%, PPV) correlated with culture-positive results, whereas 15 did not. In contrast, 163 (89%, NPV) of the 183 IgG-negative results matched the control-negative samples, though 20 showed positive culture results (Table 3).

The IgG assay demonstrated a strong correlation with culture results, achieving a categorical agreement of 88.9% and a Kappa statistic of 0.773, indicating substantial agreement. The odds ratio of 63.57 underscores the assay's strong predictive value for detecting true infections (Fisher's exact, 95% CI: 31.48–122.4, p <0.0001, two-sided).

**Table 3.** Contingency table of IgG binding assay vs. culture results

| **Culture result** | **IgG binding assay positive (n, %)** | **IgG binding assay negative (n, %)** | **Total** |
|---|---|---|---|
| Positive | 117 (85.4 %) | 20 (14.6 %) | 137 |
| Negative | 15 (8.4 %) | 163 (91.6 %) | 178 |
| **Total** | 132 (42.0 %) | 183 (58.0 %) | 315 |

Values are n (%) within each culture-positive or culture-negative group.

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All 20 IgG-negative/culture-positive discordant cases had at least one target pathogen growth, but only 4 of these were monomicrobial infections *with Enterococcus faecalis, Pseudomonas aeruginosa, Escherichia coli*, and *Acinetobacter baumannii.* The other 16 discordant cases involved polymicrobial growth: seven (Cases 5–11) involved polymicrobial infections with two or more target pathogens; in these instances, serology was positive for some targets while co-isolates remained IgG-negative, and four also had non-target organisms. The remaining nine discordant cases (Cases 12–20) were likewise polymicrobial but featured only one target pathogen alongside non-target organisms, most commonly *Proteus mirabilis, Candida spp., Serratia marcescens,* and *Corynebacterium striatum* (see Supplementary Table S1 online).

The second discordant case, the 15 IgG-positive/culture-negative group, was clinically milder. Only five truly single-pathogen cases (2.8% (5/178)) were missed among culture-positives. Ten were drawn during empirical antibiotic treatment, and three of those had the same pathogen upon repeat culture within 72 h. The 20 IgG-negative / culture-positive group had increased ICU mortality (55 % vs 20 %; $p = 0.0461$), higher inflammation (CRP median 165 mg $L^{-1}$ vs 86 mg $L^{-1}$; $p = 0.0323$), and needed more frequent mechanical ventilation (90 % vs 40 %; $p = 0.0028$) compared to the 15 IgG-positive / culture-negative group (see Supplementary Table S2 online).

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## DISCUSSION

In this prospective observational cohort study conducted in two ICUs, we included patients who developed infections with one of seven target pathogens and analyzed their serum samples to compare the performance of an ELISA-based IgG binding assay with the standard culture method for diagnosing HAIs. Although culture remains the reference standard, its utility in ICU settings is limited by false negatives in antibiotic-treated patients and false positives arising from colonization [17,18].

IgG binding accurately identifies infections, aligning with established literature [19, 20], and ELISA demonstrates high specificity and sensitivity in identifying pathogens in polymicrobial environments [21, 22]. Despite the high incidence of secondary pathogens in our cohort, no significant impact on target pathogen-specific IgG

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binding levels was observed, demonstrating the assay's accuracy without cross-reactivity. IgG effectively targets pathogens in both single and polymicrobial infections and plays a fundamental and stable role in the body's defense mechanisms, regardless of the infection's complexity. This highlights the dependable and wide-ranging effectiveness of IgG in adaptive immunity [23, 24]. In our study, we found no significant differences in IgG binding levels between single and multiple target pathogen infections; this suggests that pathogen-specific IgG binding levels were consistent, regardless of the target pathogen complexity. There was a small subset of patients (4.1% of the cohort) with positive cultures of the same pathogen, and we observed no overall change in IgG-binding levels between the infection episodes.

Only two individuals reverted to seronegativity on day 3 and day 9, respectively. The rapid decline observed in these two cases may reflect the immediate but short-lived nature of IgG–FcγR interactions, which prioritize rapid response over long-term persistence typical of adaptive immunity [25,26].

Studies broadly emphasize the role of IgG in immune responses without specific references to the infection locations [27, 28], but they are more influenced by the severity of the disease, supporting the utility of IgG assays across different types of infections [29, 30]. In our study, there were also no significant differences in IgG binding levels between sample types or infection sites, indicating that the IgG binding assay can be used for any type of infection without losing sensitivity or specificity.

Our previous study [31], which included immunosuppressed individuals, demonstrated minimal impact on the effectiveness of the IgG binding assay, addressing concerns about the variability in immune response, especially in immunocompromised patients. Out of the 79 patients in that study, only two exhibited a low phagocytic index. This indicates that the assay's diagnostic accuracy remains reliable across diverse patient populations, including those with compromised immune responses.

Previous research supports our findings on the utility of IgG binding in infection management. Goh et al. observed a 48% increase in *Salmonella*

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*typhimurium* phagocytosis linked to IgG3 isotypes [32]. Similarly, Dunnen et al. reported that IgG binding boosts Th17 cell production in response to *S. aureus* and *K. pneumoniae* [23], and another study highlighted opsonins' role in enhancing *S. epidermidis* phagocytosis [33], thus improving pathogen recognition and ingestion by phagocytes. Bardardottir et al. demonstrated increased binding post-vaccination, indicating that immune activation enhances IgG levels and pathogen targeting [34]. Our results extend these observations by demonstrating high sensitivity (85.4%) and specificity (91.57%) in distinguishing culture-positive from control-negative cases through IgG binding. These findings align with the performance metrics of established diagnostic tests [35, 36].

Our ROC-derived cutoffs revealed wide interspecies differences in assay accuracy. *P. aeruginosa* antigens (largely lipid A variants) demonstrated the highest median OD but a broader distribution (AUC 0.863), consistent with extensive lipopolysaccharide heterogeneity among clinical isolates [37, 38]. By contrast, *A. baumannii*'s conserved outer-membrane proteins may be clustered tightly (AUC 0.975), driving superior sensitivity (94.7%) and specificity (93.6%) [39]. Species with known immune-evasion features, *K. pneumoniae* (polysaccharide capsule) [40,41] and *S. aureus* (protein A), showed lower sensitivities (68.8% and 85.7%, respectively) despite high specificity, reflecting masked or variable epitope exposure [42,43]. *E. coli* and *E. faecalis*, with readily accessible surface antigens, delivered intermediate performance (AUC 0.842 and 0.875) [44,45].

In our cohort, despite excellent overall performance (AUC 0.91), we observed 20 IgG-negative/culture-positive and 15 IgG-positive/culture-negative discordant cases. Only 4/20 cases (2.9% of culture-positives) were IgG-negative with single target-pathogen growths, and 5/15 cases (2.8% of culture-negatives) were IgG-positive without antibiotic pressure; these likely represent the true upper limit for false negatives and false positives, respectively. Sixteen of the IgG-negative/culture-positive discordances involved polymicrobial infections. In seven mixed-target cases (5–11), serology was positive for some targets while co-isolates remained IgG-negative. Four of these seven

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379 also had non-target organisms (see Supplementary Table S1 online).
380 Such pathogen-specific immunodominance of one target over another [46, 47, 48], or
381 sepsis-associated immunoparalysis as a profound systemic inflammation delaying IgG
382 class switching, has been well documented in other settings [49,50]. In the remaining
383 13/20 (65%) cases, we recovered non-target organisms (e.g., *Proteus*
384 *mirabilis*, *Serratia marcescens*, and *Corynebacterium striatum*) alongside one target
385 pathogen. These patterns suggest that traditional culture may recover
386 colonizers under heavy antibiotic pressure rather than the principal immunogenic
387 pathogen [51,52].
388 The 15 IgG-positive/culture-negative cases further illustrated this host-pathogen
389 disconnect: 10/15 (67%) were drawn during empirical antibiotic therapy; antibiotics
390 likely suppressed culture growth, even though early IgG seroconversion had
391 occurred, as confirmed by repeat cultures in 3/10 within 72 h. Empirical antibiotic
392 administration prior to sample collection is well known to suppress culture positivity
393 [53, 54].
394 The IgG-negative/culture-positive subgroup had markedly worse clinical parameters
395 (median ICU stay 26.8 vs. 6.1 days; CRP 165 vs. 86 mg/L; 90% vs. 40% on ventilators;
396 55% vs. 20% mortality; all $p < 0.05$), compared with the IgG-positive/culture-negative
397 cases. This suggests that their extreme inflammatory state and immune exhaustion
398 may prevent a detectable IgG, explaining why they deviate from the overall mono- vs.
399 polymicrobial patterns [55]. Together, these data underscore that culture and
400 serology are complementary: culture confirms viability, whereas IgG reveals host
401 engagement, and integrating both, with clinical context, can optimize HAI diagnosis
402 in the ICU.
403 Implementing the IgG binding assay in ICUs can cut pathogen identification from days
404 to roughly 4 hours, enabling earlier, more informed antibiotic choices [56]. Although
405 our IgG assay does not provide antimicrobial-susceptibility results, studies of other
406 rapid diagnostics in bloodstream infections have demonstrated that quick turnaround
407 can shorten time to targeted therapy and reduce broad-spectrum empiric use [57].

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Multiplex PCR panels identify pathogens and resistance markers in 2 h, and MALDI-TOF MS provides species ID in <1 h post-isolation, but both require specialized instruments and do not assess host response [3,5]. EUCAST RAST uses standard blood-culture incubators and disk readers to provide resistance profiles in 4 h [4]. In contrast, the ELISA-based pathogen-specific IgG binding assay runs on common 96-well readers and delivers pathogen-specific host-response data in 4 h [14]. Crucially, by measuring pathogen-specific IgG binding levels, it reports on the host's immune engagement, enabling detection of true infections even under antibiotic therapy, early in disease progression, or when culture and molecular tests give false positives due to colonization or residual non-viable DNA [12, 58].

This study successfully optimized an IgG-binding ELISA targeting a specific panel of seven critical ICU pathogens, achieving a swift turnaround while maintaining high sensitivity and specificity. The high performance metrics reflect both the controlled nature of our study cohort and the targeted optimization of the assay. The collection and analysis of blind samples effectively reduced bias, while advanced statistical methods reinforced the strength of our findings. However, our study was conducted in a single geographic setting with a predefined panel of seven pathogens, so findings may not generalize to other institutions or non-target organisms. The use of standard culture as the sole reference point limited accurate determination of true-positive and true-negative rates, since culture often fails to detect infections in patients receiving antibiotic therapy or to distinguish colonization from invasive disease. In our small paired subset, the clinically defined follow-up times precluded mapping of seroreversion kinetics. Finally, while our assay rapidly confirms pathogen presence, it does not directly provide data on antimicrobial susceptibility. Therefore, future work should focus on (1) longitudinal sampling to map seroconversion kinetics; (2) expanding antigen libraries to cover strain heterogeneity; and (3) integrating rapid IgG profiling with molecular diagnostics and fast AST platforms to deliver a comprehensive, one-stop workflow for pathogen identification, host-response assessment, and susceptibility testing.

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In conclusion, our pathogen-specific IgG binding assay offers a rapid (≈ 4 h) and accurate complement to culture for diagnosing HAIs in ICU patients. By enabling earlier, targeted therapy, it has the potential to improve clinical outcomes, shorten lengths of stay, reduce unnecessary antibiotic usage, and ultimately lower healthcare costs.

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**Figure Legends:**

**Fig. 3.** IgG opsonization levels for different target pathogens. (1) *Acinetobacter baumannii*; (2) *Klebsiella pneumoniae*; (3) *Escherichia coli*; (4) *Pseudomonas aeruginosa*; (5) *Enterococcus faecalis*; (6) *Enterococcus faecium; (7) Staphylococcus aureus*

**Fig. 4.** Receiver Operating Characteristic (ROC) curves for IgG opsonization levels across the targeted pathogens. (a) ROC curve for all pathogens combined; (b) *Acinetobacter baumannii*; (c) *Klebsiella pneumoniae*; (d) *Escherichia coli*; (e) *Pseudomonas aeruginosa*; (f) *Enterococcus faecalis*; (g) *Enterococcus faecium; (h) Staphylococcus aureus*

**Declarations**

**Ethics Approval and Consent to Participate:** This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Clinical Research Ethics Committee of the Cerrahpaşa Faculty of Medicine (Date: 10.05.2018/No: 171778). Written informed consent was obtained from all individual participants included in the study.

**Availability of data and material:** The data that support the findings of this study are not openly available due to sensitivity reasons and are available from the

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corresponding author upon reasonable request. The data are stored in controlled - access storage systems by the corresponding author.

**Consent for publication:** Not applicable

**Conflicts of Interest:** All other authors have no competing interests to declare.

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**Author Contributions:** AK has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the study's conception and design. Material preparation and analysis were performed by AK, data collection was performed by MK, and visualization was performed by MAK. The first draft of the manuscript was written by AK. YD and GA provided critical revisions and supervision. All authors revised it critically for important intellectual content. All authors read and approved the final manuscript. All authors gave final approval for the version to be published.

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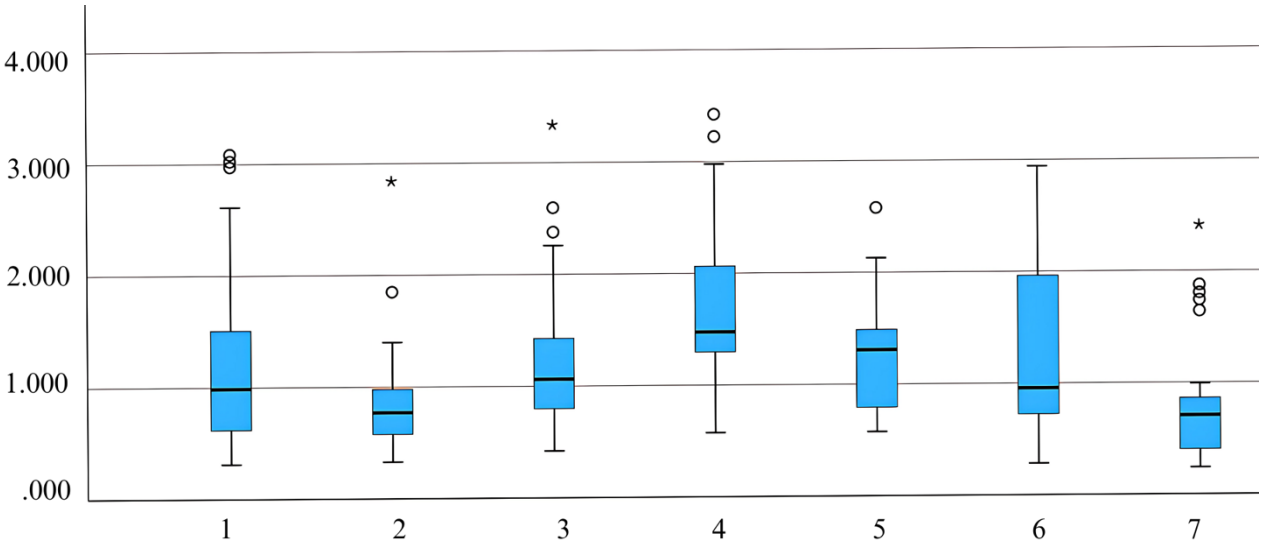
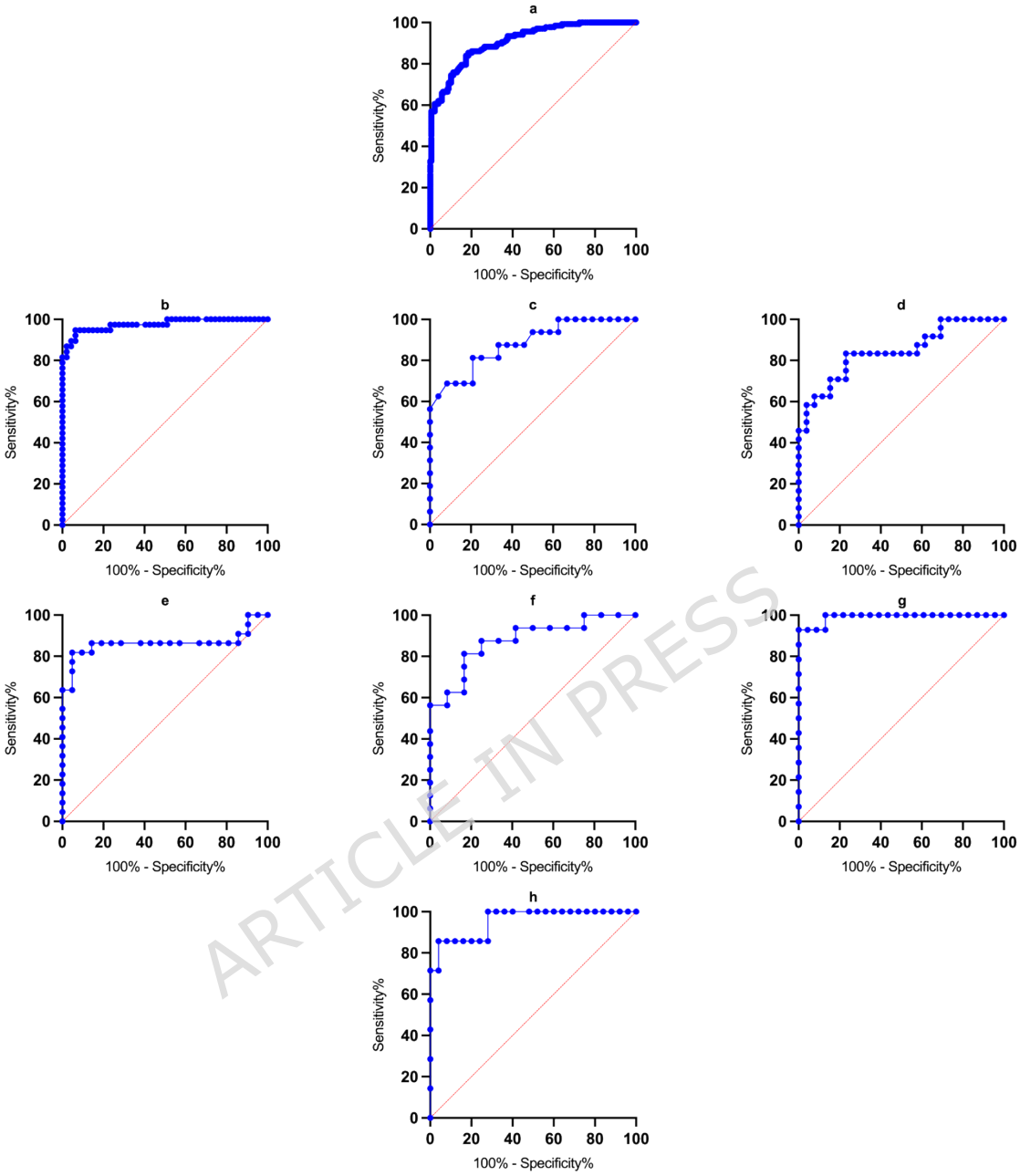



**Fig. 1.** IgG opsonization levels for different target pathogens. (1) *Acinetobacter baumannii*; (2) *Klebsiella pneumoniae*; (3) *Escherichia coli*; (4) *Pseudomonas aeruginosa*; (5) *Enterococcus faecalis*; (6) *Enterococcus faecium; (7) Staphylococcus aureus*

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**Table 1:** IgG Binding Levels for Bacterial Isolates by Culture Results

| Pathogen | Culture-positive (n) | *Mean [a] ± SD OD* | Culture-negative controls (n) | *Mean ± SD OD* | *p* value | Total (n) |
|---|---|---|---|---|---|---|
| *A. baumannii* | 38 | 1.70 ± 0.58 | 47 | 0.66 ± 0.22 | <0.0001 | 85 |
| *K. pneumoniae* | 16 | 1.18 ± 0.54 | 24 | 0.65 ± 0.19 | 0.0015 | 40 |
| *E. coli* | 24 | 1.55 ± 0.65 | 26 | 0.86 ± 0.28 | <0.0001 | 50 |
| *P. aeruginosa* | 22 | 2.10 ± 0.72 | 21 | 1.27 ± 0.30 | <0.0001 | 43 |
| *E. faecalis* | 16 | 1.49 ± 0.46 | 12 | 0.88 ± 0.27 | 0.0005 | 28 |
| *E. faecium* | 14 | 2.11 ± 0.52 | 23 | 0.74 ± 0.23 | <0.0001 | 37 |
| *S. aureus* | 7 | 1.59 ± 0.56 | 25 | 0.59 ± 0.22 | 0.0029 | 32 |

[a] Pathogen-specific IgG binding level as *mean ± SD (*standard deviation) OD (optical density, measured at 405 nm); Controls: Cases with negative culture results for the target pathogen; p-values by unpaired two-tailed t-test with Welch's correction; $p < 0.05$ significant.

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**Table 1:** Diagnostic Test Performance and Optimal Cutoffs of Binding Assay

| Pathogens | Sensitivity (%) | Specificity (%) | Accuracy (%) | PPV (%) | NPV (%) | AUC | Cutoff |
|---|---|---|---|---|---|---|---|
| *A. baumannii* | 94.7 | 93.6 | 94.1 | 92.3 | 95.6 | 0.975 | 1.041 |
| *K. pneumoniae* | 68.7 | 91.6 | 82.5 | 84.6 | 81.4 | 0.878 | 0.955 |
| *E. coli* | 83.3 | 76.9 | 80 | 76.9 | 83.3 | 0.842 | 1.050 |
| *P. aeruginosa* | 81.8 | 95.2 | 88.4 | 94.7 | 83.2 | 0.863 | 1.534 |
| *E. faecalis* | 81.3 | 83.3 | 82.1 | 86.7 | 76.9 | 0.875 | 1.266 |
| *E. faecium* | 71.4 | 87 | 81.1 | 76.9 | 83.3 | 0.990 | 1.327 |
| *S. aureus* | 85.7 | 93.7 | 93.7 | 85.7 | 96 | 0.954 | 0.918 |
| **Total** | **85.4** | **81.4** | **83.2** | **78** | **87.8** | **0.910** | **1.009** |

PPV: positive predictive value; NPV: negative predictive value; AUC: area under the Receiver Operating Characteristic (ROC) curve

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**Table 3:** Contingency table of IgG binding assay vs. culture results

| **Culture result** | **IgG binding assay positive (n, %)** | **IgG binding assay negative (n, %)** | **Total** |
|---|---|---|---|
| Positive | 117 (85.4 %) | 20 (14.6 %) | 137 |
| Negative | 15 (8.4 %) | 163 (91.6 %) | 178 |
| **Total** | 132 (42.0 %) | 183 (58.0 %) | 315 |

Values are n (%) within each culture-positive or culture-negative group.

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