**Objectives**: To assess the diagnostic value of serum β-D-glucan (BDG) and real-time PCR in detecting Pneumocystis jirovecii pneumonia (PCP), and to describe the respective contribution of each method to the diagnostic process. The aim is to explore how these tools can be used effectively, individually or in combination, in routine clinical practice to diagnose PCP in immunocompromised patients.

**Materials & Methods:** This retrospective study included 36 patients who underwent PCPPCR testing (ELITechGroup, Puteaux, France) on bronchoalveolar lavage (BAL) samples in the context of clinical suspicion of pneumocystosis, between October 2023 and April 2025. The study population consisted almost exclusively of non-HIV immunocompromised patients, with only one HIV-positive case included in the cohort. Serum BDG testing (Fujifilm Wako Chemicals, Richmond, VA, USA) was performed retrospectively on stored serum samples from these patients collected on the same day as the bronchoalveolar lavage (BAL). Patients were classified as probableornon-probable PCP based on the revised European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) criteria for PCP, taking into account host factors, clinical presentation, and radiological findings.. Receiver operating characteristic (ROC) curves were generated based on the classification of patients as probable or non-probable PCP. These curves were used to determine the optimal cut-off values for each assay, from which sensitivity and specificity were calculated. Inter-rater agreement between the two methods was also evaluated using the kappa coefficient, based on the diagnostic classifications obtained using the established ROC-derived cut-offs. Statistical analyses were performed using MedCalc® Statistical Software version 23.0.2.

**Results**: Of the 36 patients included, 14 were classified as probable PCPand 22 as non-probable PCP*.* ROC curve analysis identified the optimal diagnostic thresholds for each test: 6367 copies/mL (cycle threshold (ct) 30) for PCPPCR and 8 pg/mL for serum BDG. At these cut-offs, PCP PCR demonstrated 100% sensitivity and 100% specificity (AUC 1; P < 0.001), while BDG showed 100% sensitivity and 86% specificity (AUC 0.98; P < 0.001). No significant differences were observed in the ROC curves of the two methods for the diagnosis of probable PCP, indicating comparable overall diagnostic accuracy (differences between areas 0.016; P = 0,2673). Using these thresholds, the level of agreement (kappa) was 0.83 (standard error: 0.092; 95% confidence interval: 0.65 to 1.00), indicating strong agreement between the two assays. Three false-positive results were observed for BDG The median fungal load in probable PCP cases was 260300 copies/mL (Ct 25) for PCR and 116 pg/mL for BDG, compared to <97 copies/mL (Ct 45) and 7 pg/mL, respectively, in non-probable PCP cases.

**Conclusions**: Both serum BDG and BAL-based PCPPCR showed excellent diagnostic performance for PCP in this cohort. BDG, as a non-invasive biomarker, proved useful for early decision-making. BDG levels below 8 pg/mL may help defer bronchoalveolar lavage for PCP diagnosis in selected cases, provided that clinical and radiological findings are stable and close follow-up is ensured. These findings support the complementary role of BDG and PCR in guiding the diagnostic strategy for PCP.