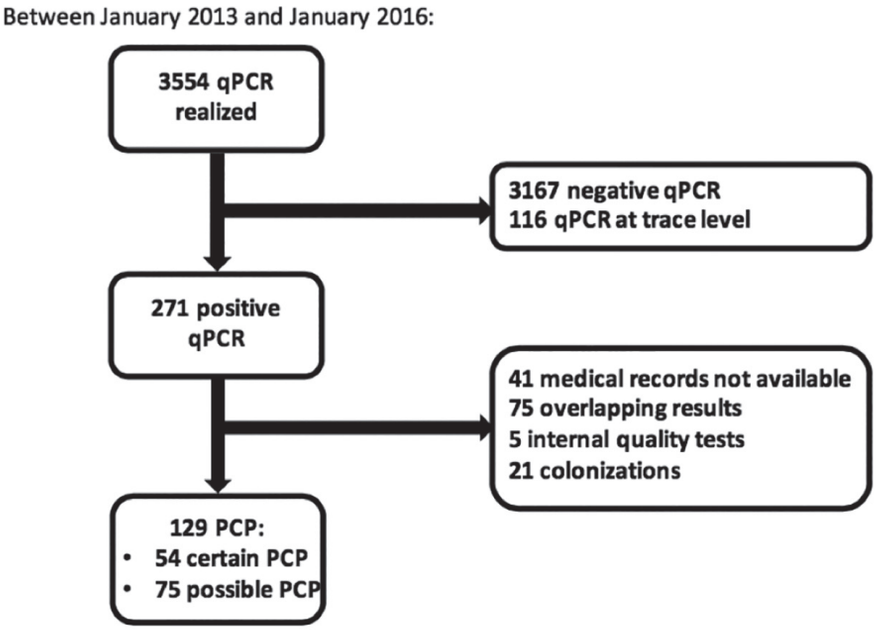

Suppl. Fig. 1. Flowchart of the study.

Supplementary method

Specimen processing and analysis:

The samples were treated in the Mycology Laboratory of Bordeaux University Hospital. All respiratory specimens, with the exception of BAL, were diluted 1:1 (v/v) in a 7.5% Sputasol solution (Oxoid), vortexed, and incubated for 30 minutes at 37°C. After centrifugation (2800g, 10 min), the pellet was re-suspended in 500 µL of the supernatant, and two drops were spotted on two slides for microscopy, and 200 µL was used for PCR. One slide was subjected to May-Grünwald-Giemsa staining, and the other to silver staining (1). Both were examined by experienced microscopists for the presence of asci and/or other trophic forms. DNA extraction of 200 µmol was performed on MagNa Pure 96 (Roche Diagnostics), PCR amplification on MagNa 480 (Roche Diagnostics) using a LightMix Pneumocystis jirovecii kit (TIB MOLBIOL) according to the manufacturer's instructions. The target gene was a multi-copy gene coding for Major Surface Glycoprotein (216-240 base pairs). The cut-off value to consider qPCR as significant was 1000cp/mL according to the commercial recommendations.

1. MUSTO L, FLANIGAN M, ELDABAWI A: Ten-minute silverstain for P. carinii and fungi in tissue sections. *Arch Pathol Lab Med* 1982; 106: 292-94.