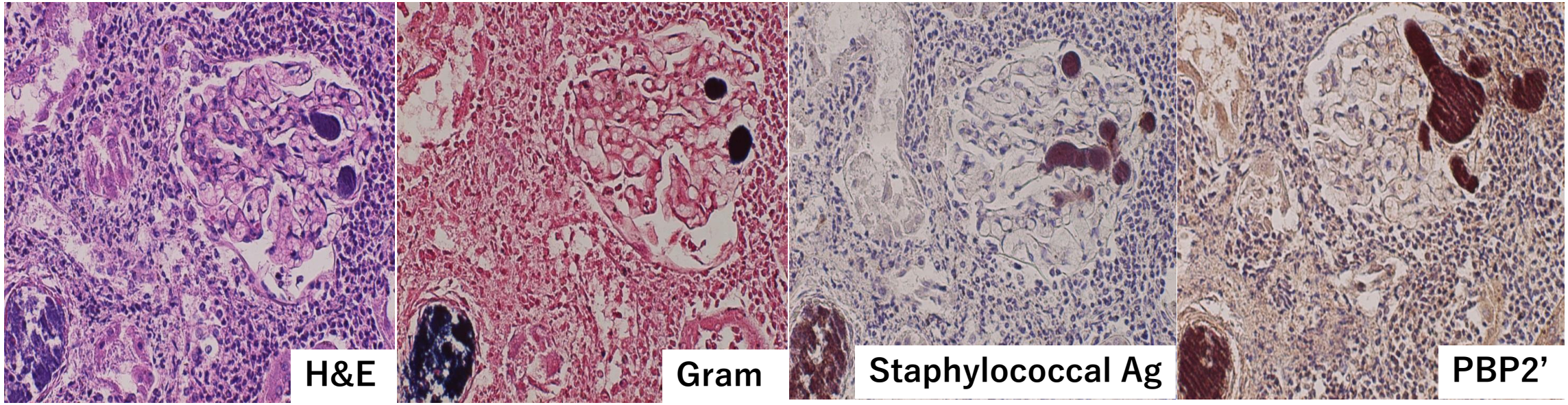
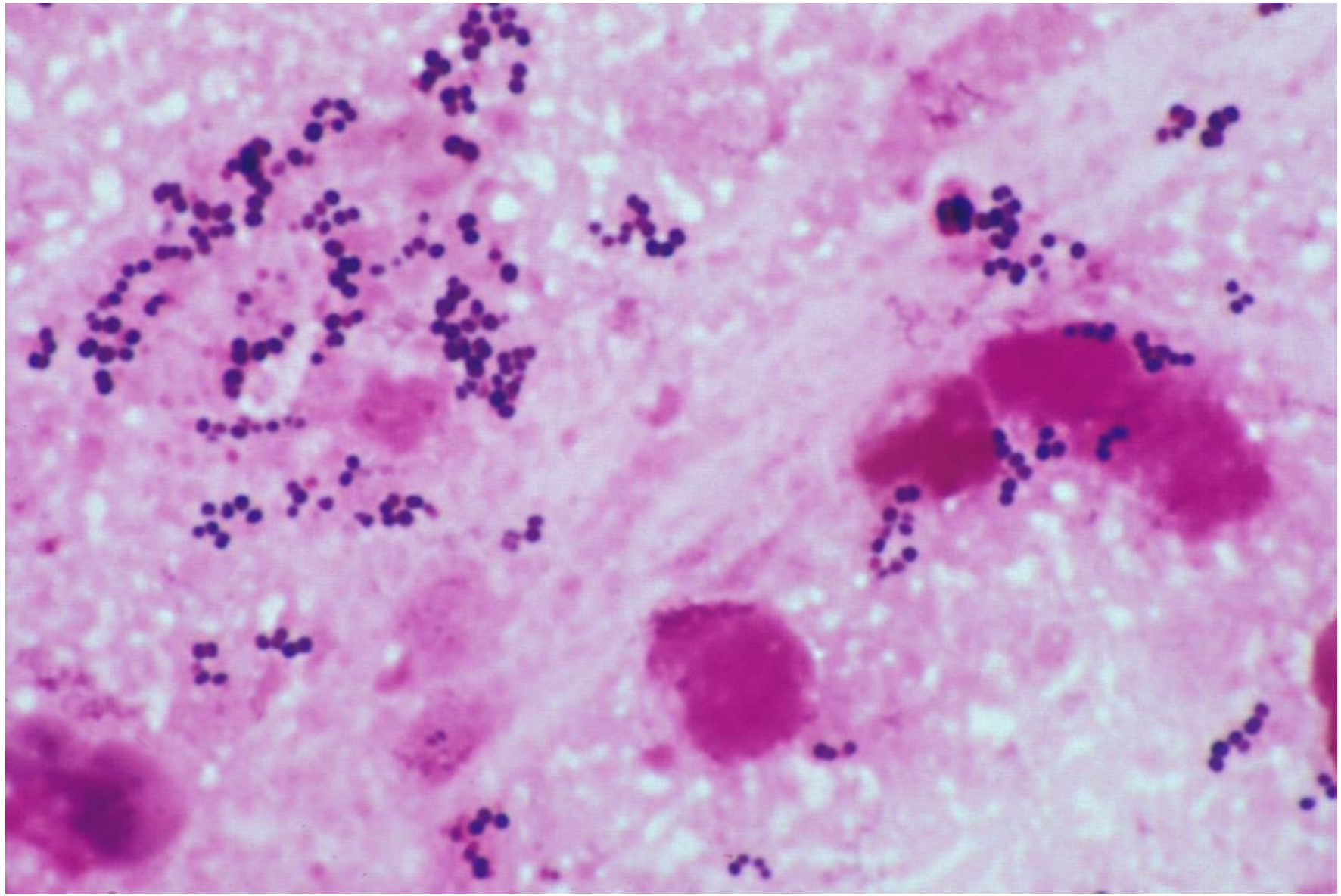
Methicilin-resistant *Staphylococcus aureus* (MRSA). Histochemical demonstration

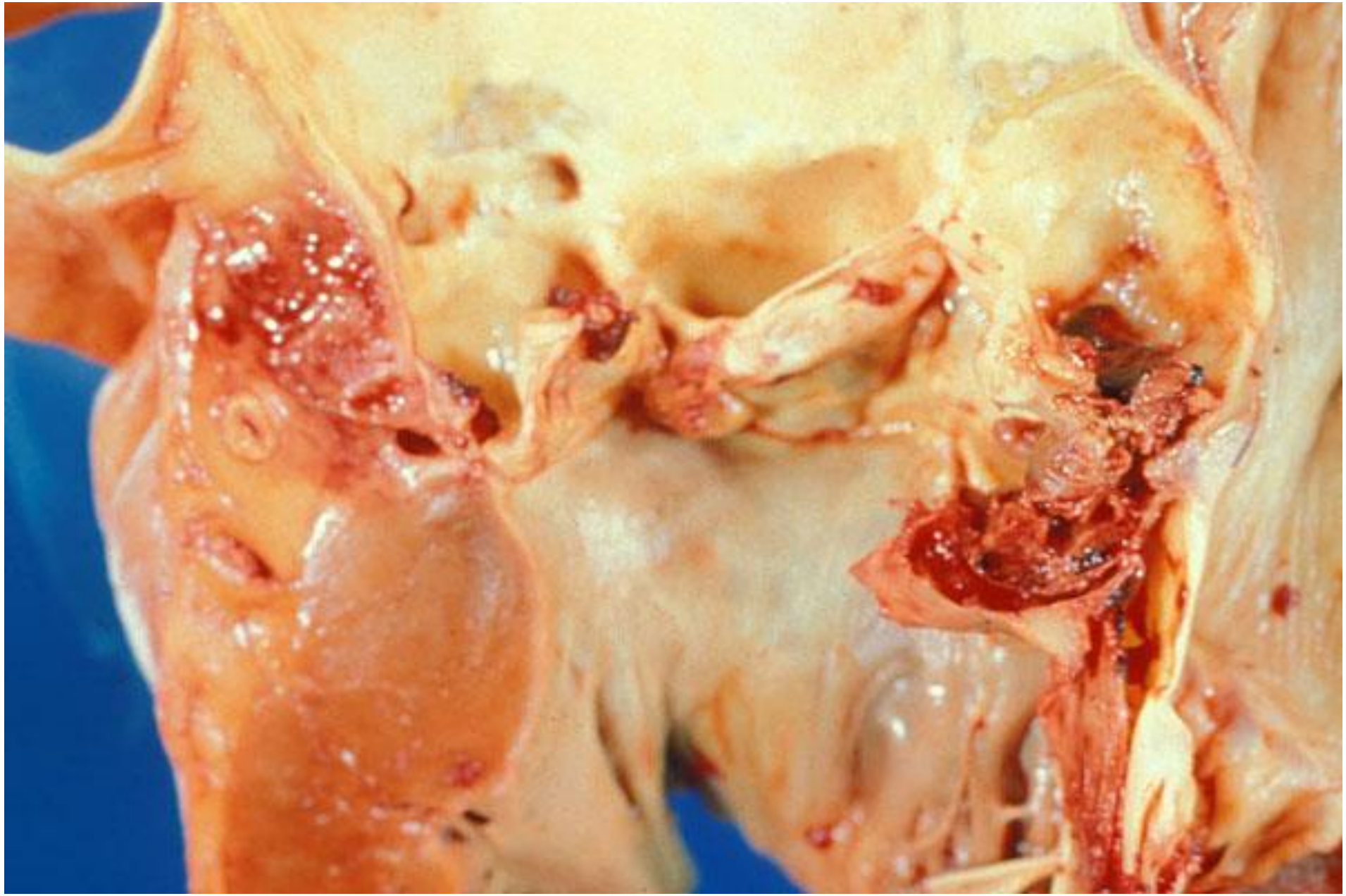
Methicillin-resistance of *Staphylococcus aureus* can be visualized with immunohistochemistry and *in situ* hybridization techniques. From the pathological point of view, it is important to identify MRSA in routinely prepared formalin-fixed and paraffin-embedded sections in order to analyze and prevent nosocomial infection by MRSA.



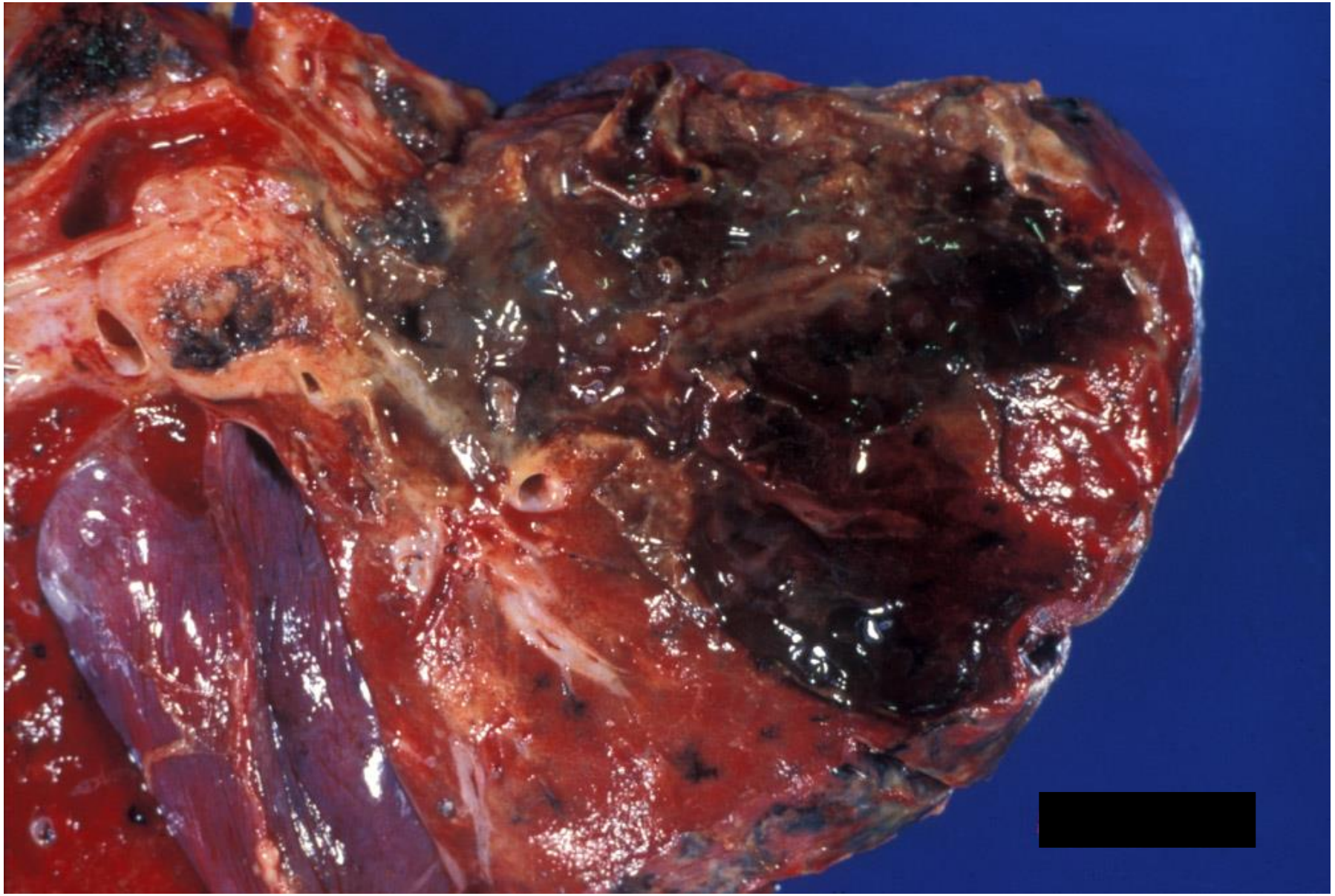
Representative example of immunohistochemical identification of MRSA in formalin-fixed and paraffin-embedded sections. Fulminant MRSA infection (kidney: H&E, Gram, immunostaining for staphylococcal antigen and PBP2’). Septic emboli in the glomerulus represent Gram-positive cocci with positivity for staphylococcal antigens and penicillin-binding protein 2’ (PBP2’). Immunohistochemistry confirmed MRSA septicemia. Marked adrenal hemorrhage with Waterhouse-Friderichsen’s syndrome was the direct cause of death in this patient.



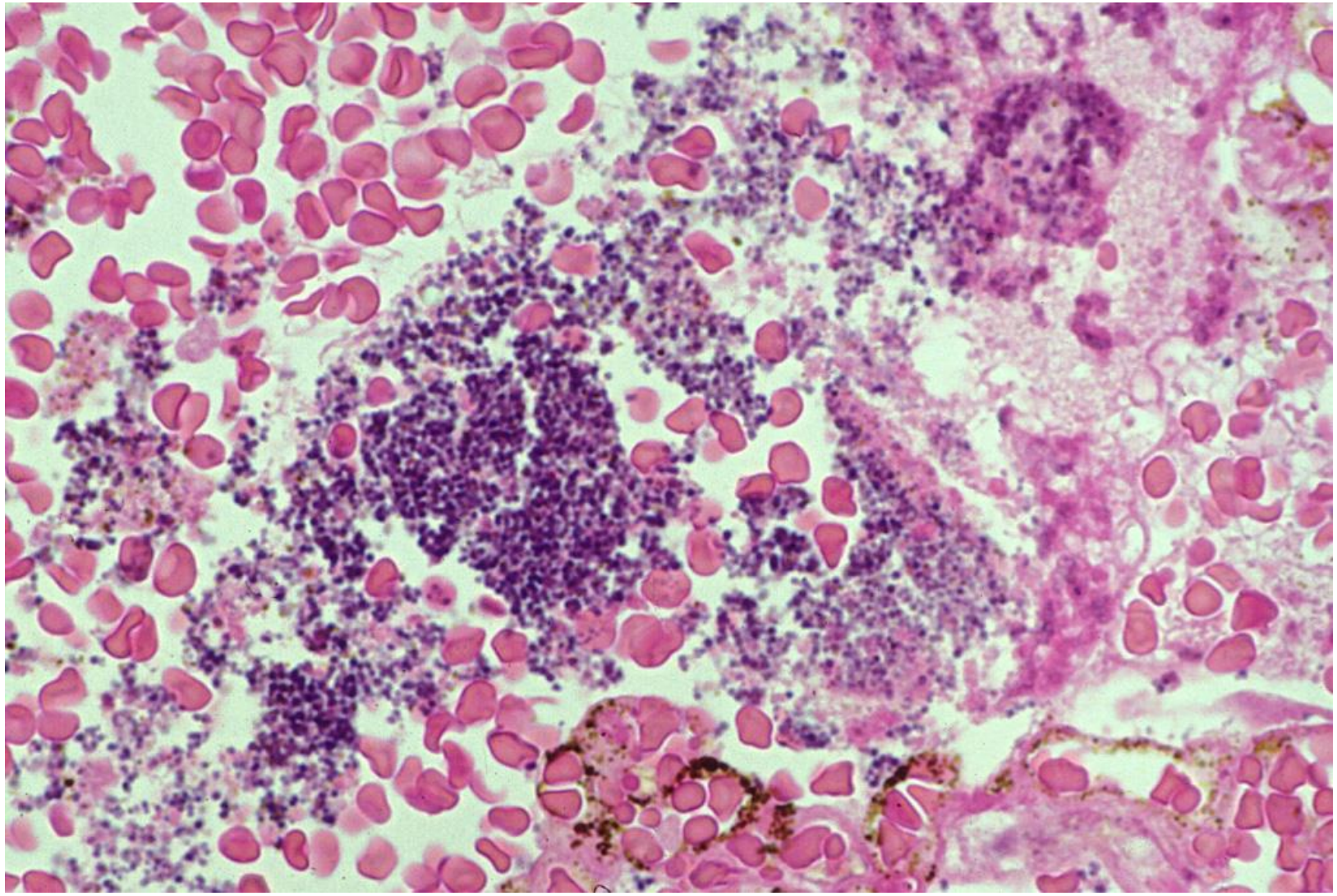
MRSA seen in the sputum (Gram). Grape-like aggregation of Gram-positive cocci are seen. Neutrophils phagocytize bacteria. MRSA pneumonia was proven.



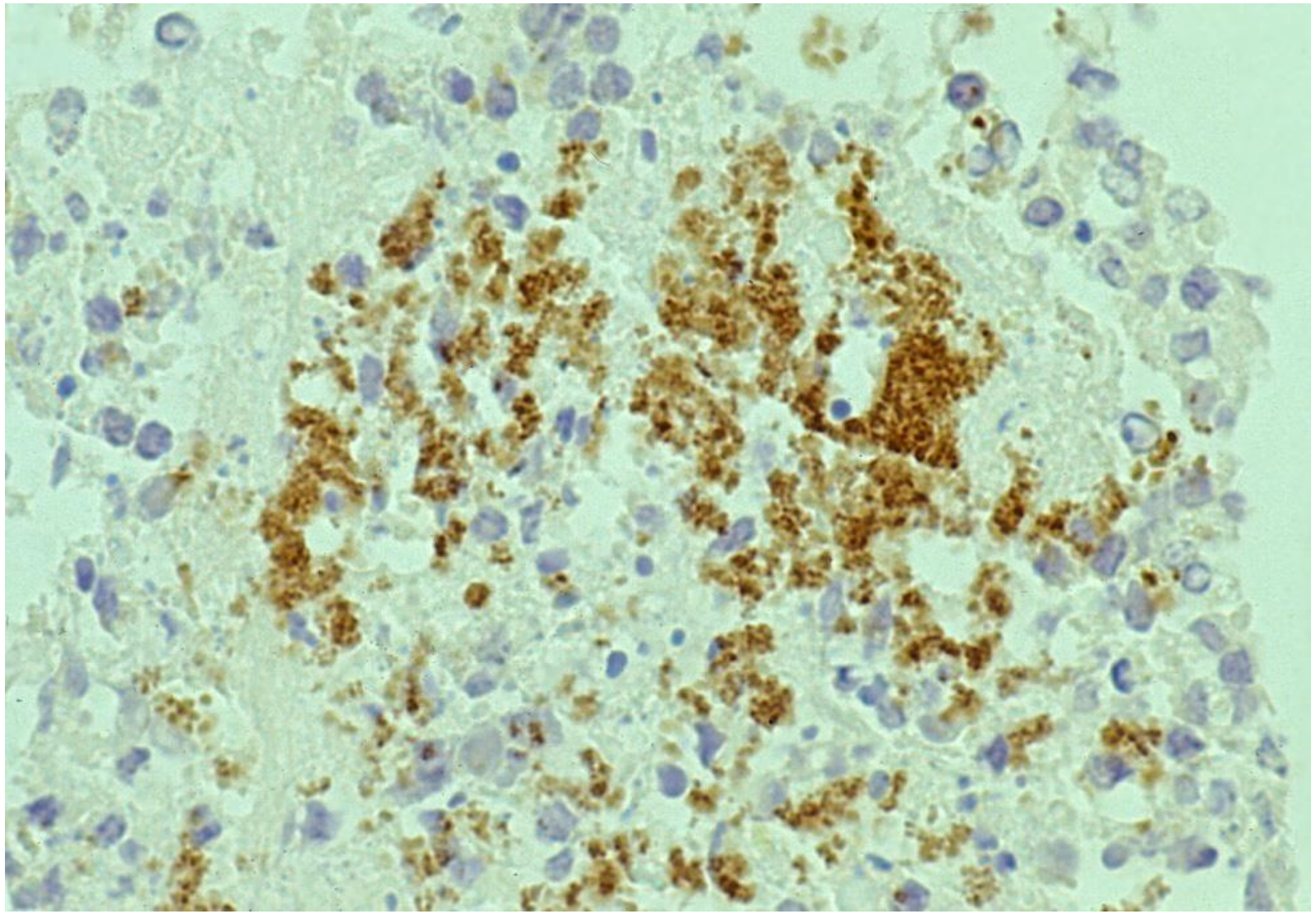
Gross features of MRSA endocarditis of the aortic valve. Valvular vegetations with ulcer formation are evident.



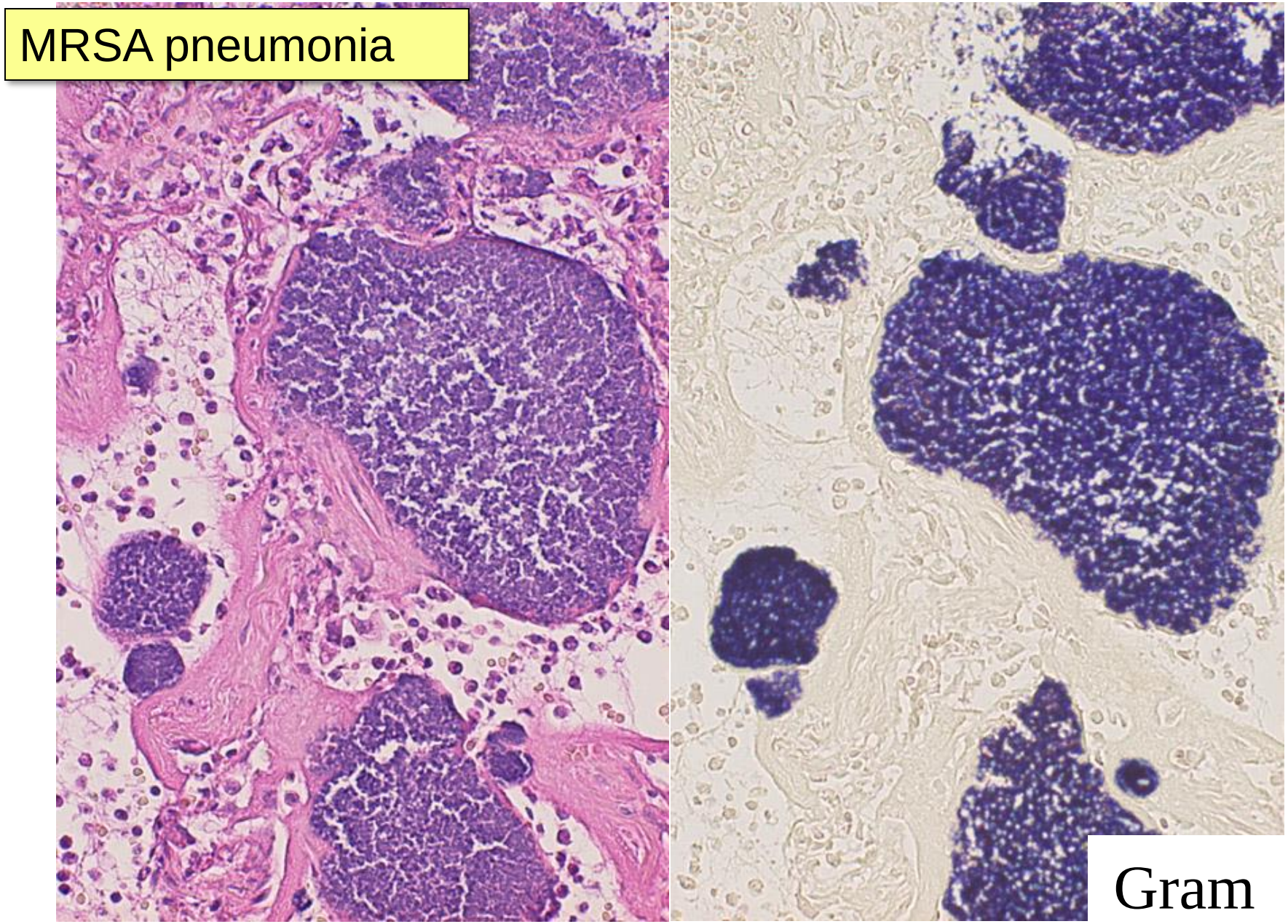
Macroscopic view of MRSA pneumonia with hemorrhagic lung abscess formation. Cavity-forming infection is often seen in MRSA pneumonia.



In MRSA pneumonia, cocci are colonized in the lung. Hemorrhagic background without inflammatory cell infiltration is associated. H&E

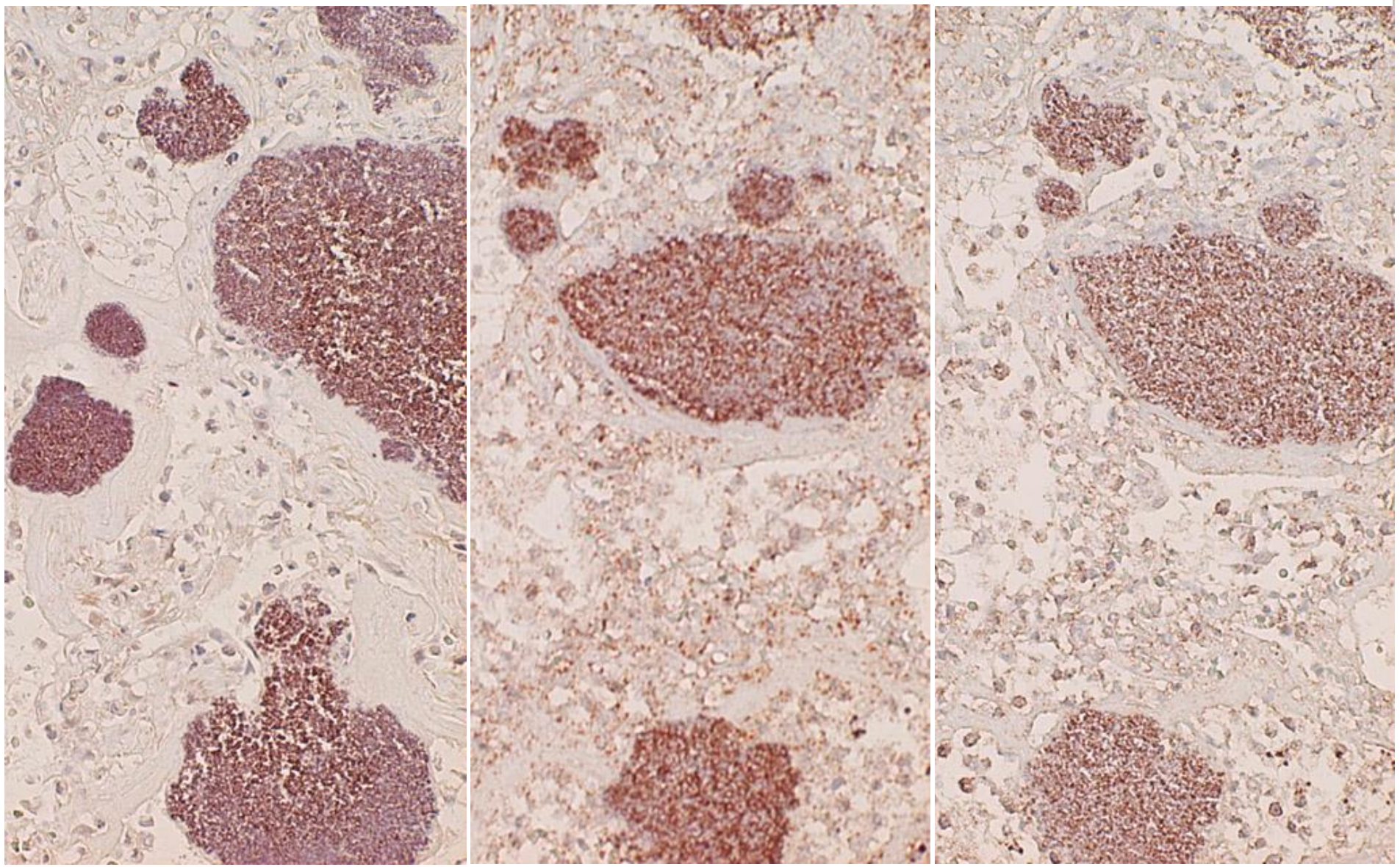


Immunostaining with a monoclonal Ab against PBP2’ (penicillin-binding protein 2’).
The infected Gram-positive cocci express PBP2’ antigen, a protein product of the mecA gene, indicating Methicillin resistance of the cocci.



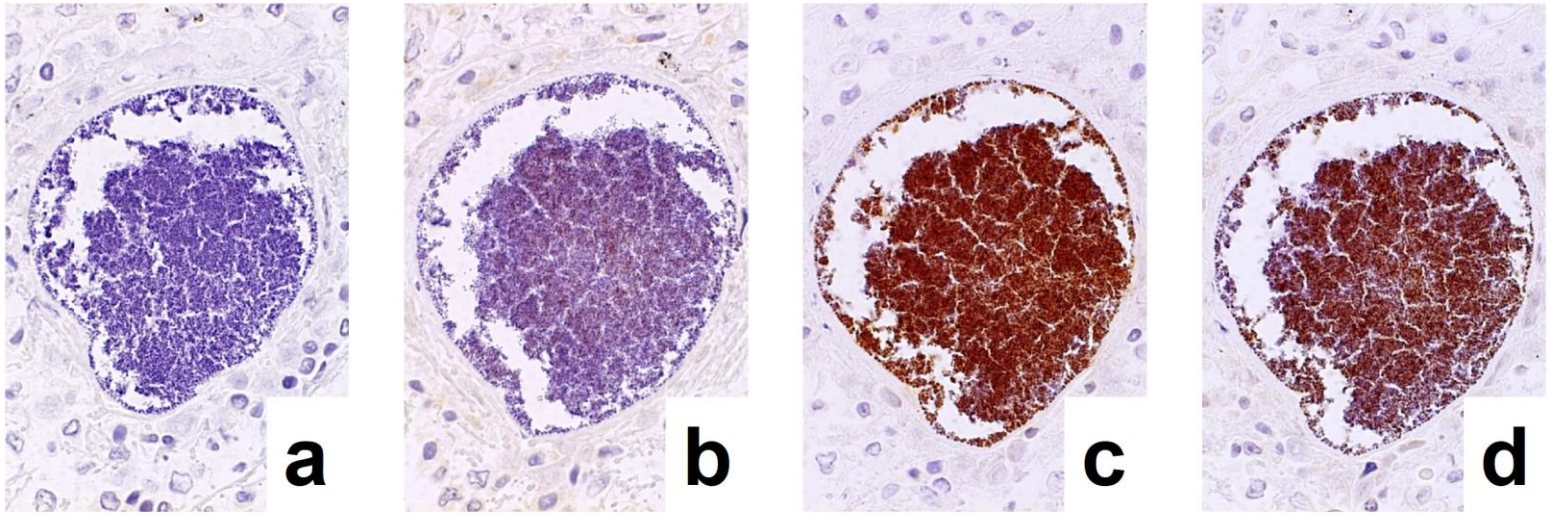
MRSA pneumonia with massive colonization of Gram-positive cocci.

IHC for Staphylococcal Ag | ISH for *S. aureus* 16S RNA | ISH for mec A



The colonies of Gram-positive cocci in the lung are proven as MRSA infection. They strongly express staphylococcal Ag (immunostaining) and 16S RNA of *S. aureus* and mec A gene (*in situ* hybridization).

Retrieval of Fc-binding reactivity of protein A on *Staphylococcus aureus*

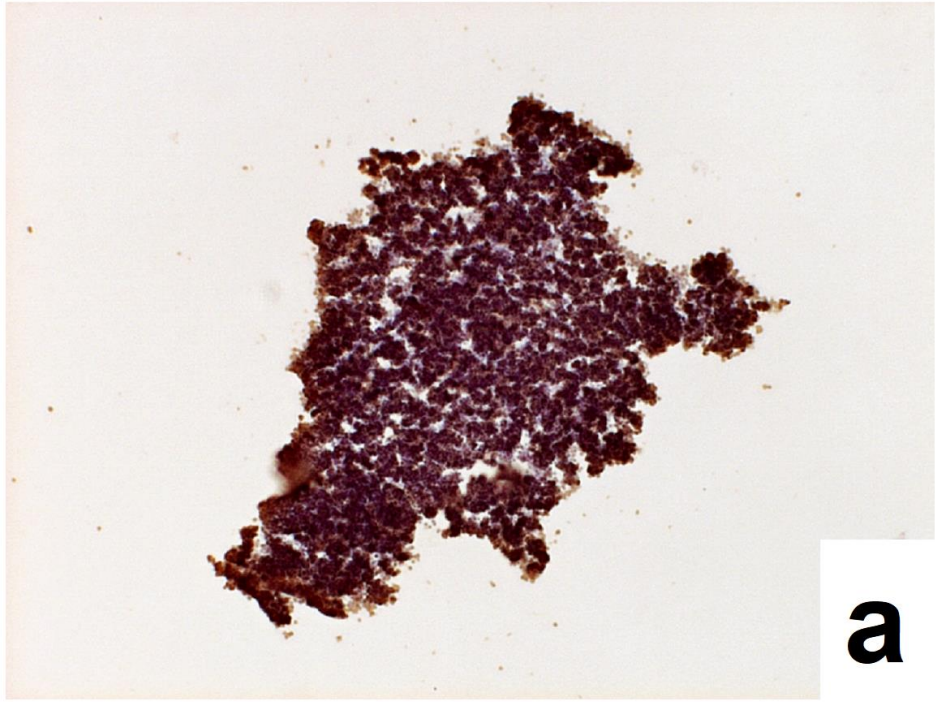


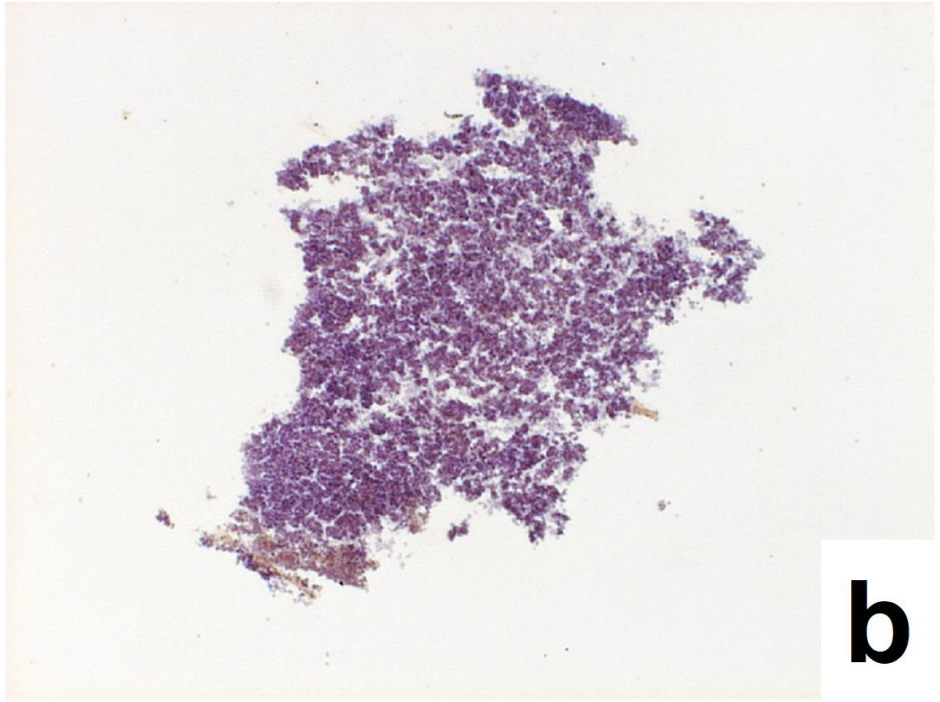
a) No primary Ab
b) F(ab)'$_2$ fragments of rabbit polyclonal Ab against mouse Igs
c) Whole Ig of rabbit polyclonal Ab against mouse Igs
d) Mouse monoclonal Ab against *Pseudomonas aeruginosa*
The Fc portion of Ig (c and d) bind to S. aureus expressing protein A.

It should be noted that the heat-induced epitope retrieval procedure retrieves IgG, Fc-binding reactivity of protein A on the *Staphylococcus aureus*. The MRSA colony in paraffin sections are positive, when whole IgG or indifferent monoclonal antibody was incubated.

Ref.: Shimomura R, Tsutsumi Y. Histochemical identification of Methicillin-resistant *Staphylococcus aureus*: contribution to preventing nosocomial infection. Semin Diagnost Pathol 2007; 24(4): 217-226. doi: 10.1053/j.semdp.2007.07.004

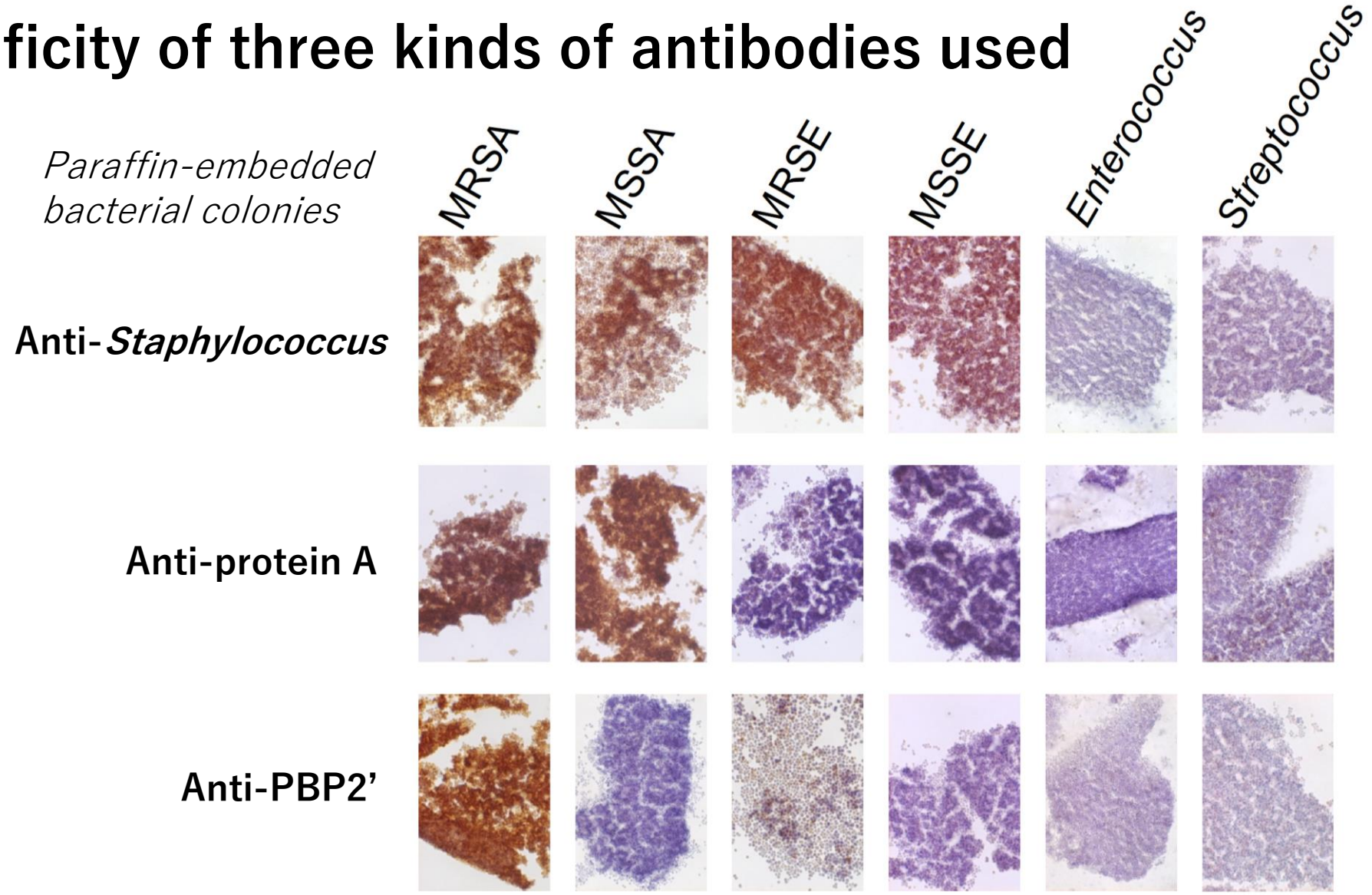
Blocking of heat-retrieved protein A-mediated Fc-binding reactivity by preincubation with swine serum



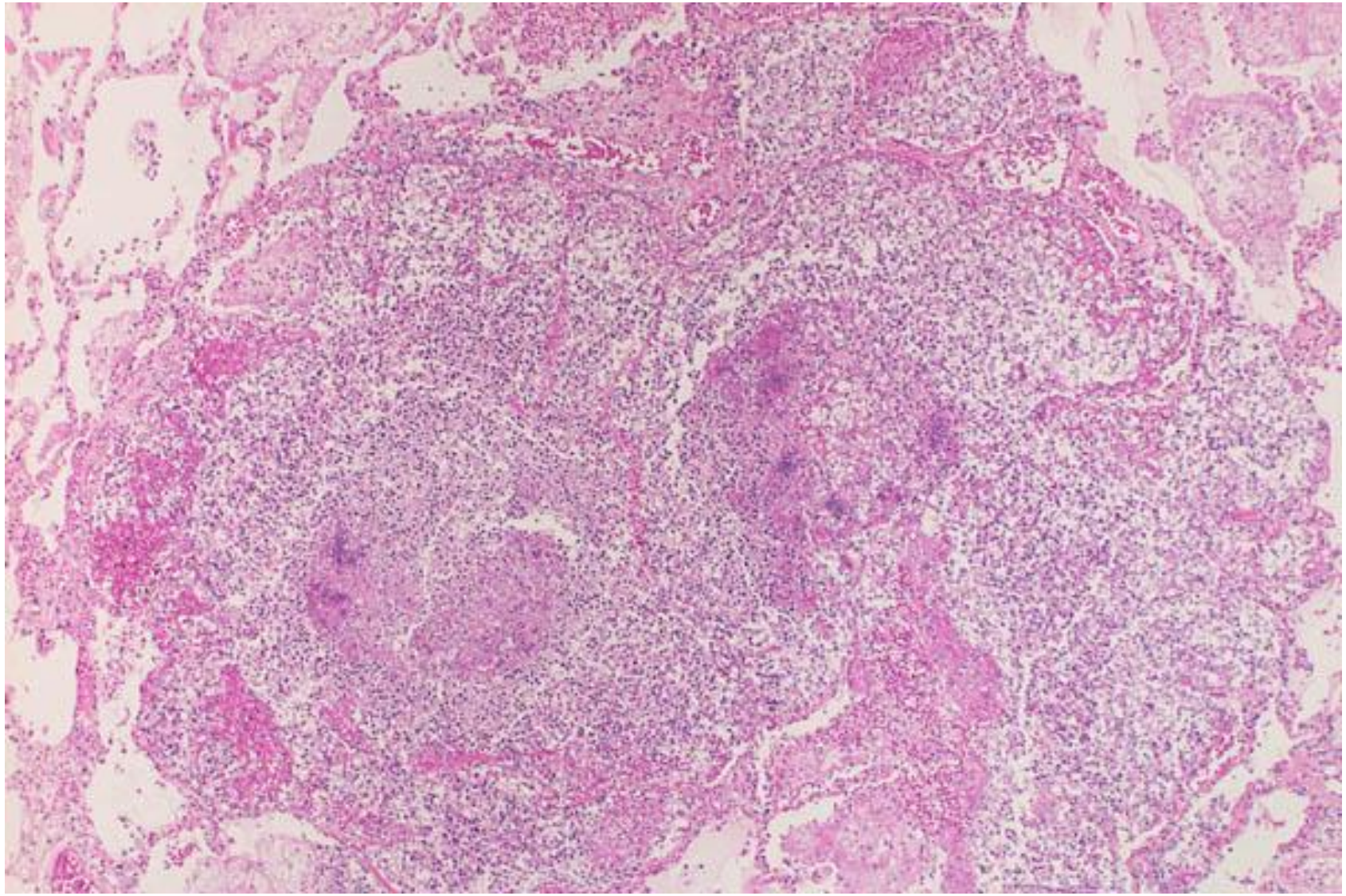


Formalin-fixed, paraffin-embedded sections of MSSA colonies
Primary Ab: anti-PBP2’ mouse monoclonal Ab
Epitope retrieval: heating in 1 mM EDTA, pH 8.0, in pressure pan at 121C for 10 min
a) without blocking, resulting in false positive reactivity
b) after blocking by preincubation with swine serum, resulting in little staining

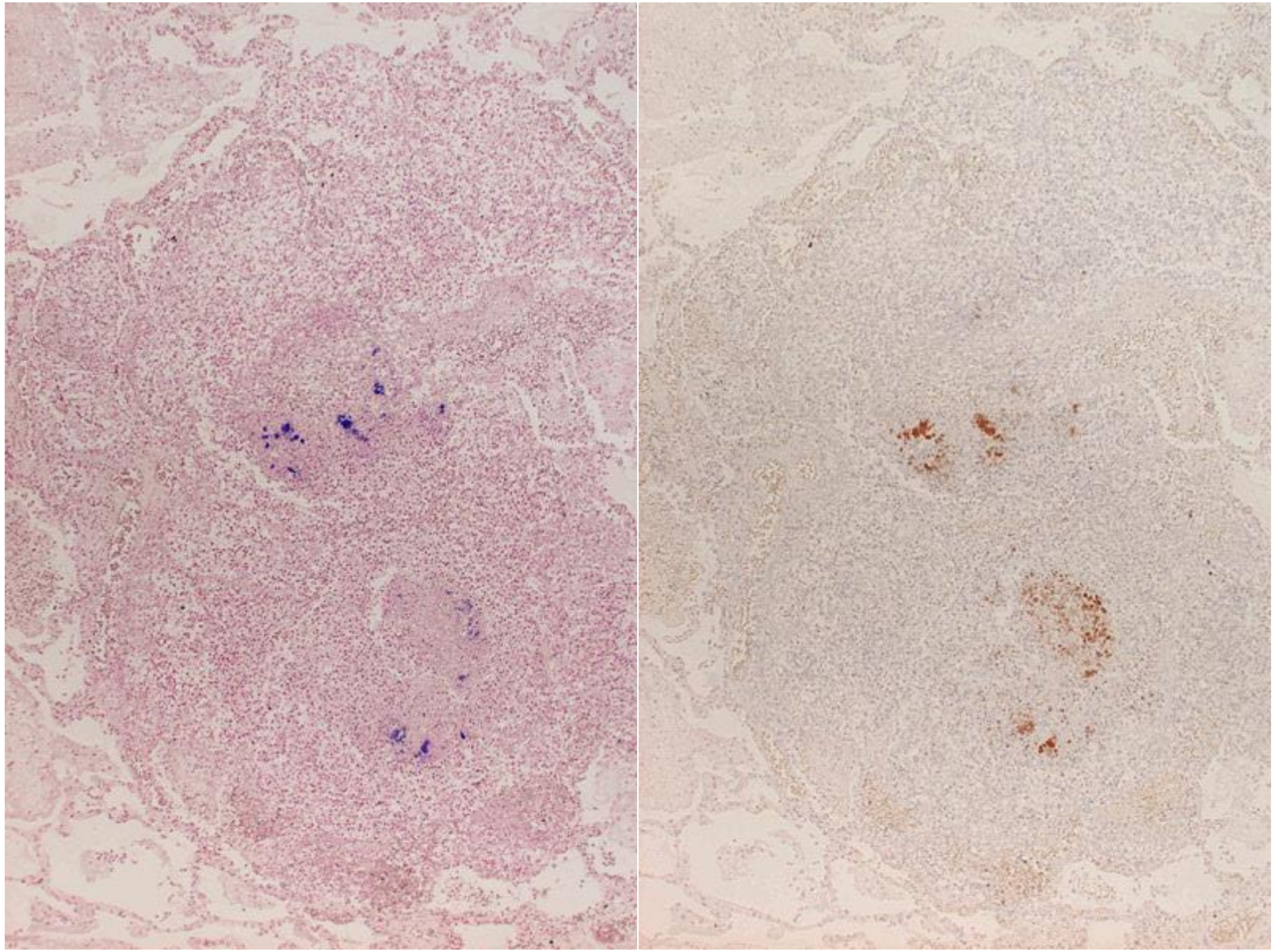
Specificity of three kinds of antibodies used



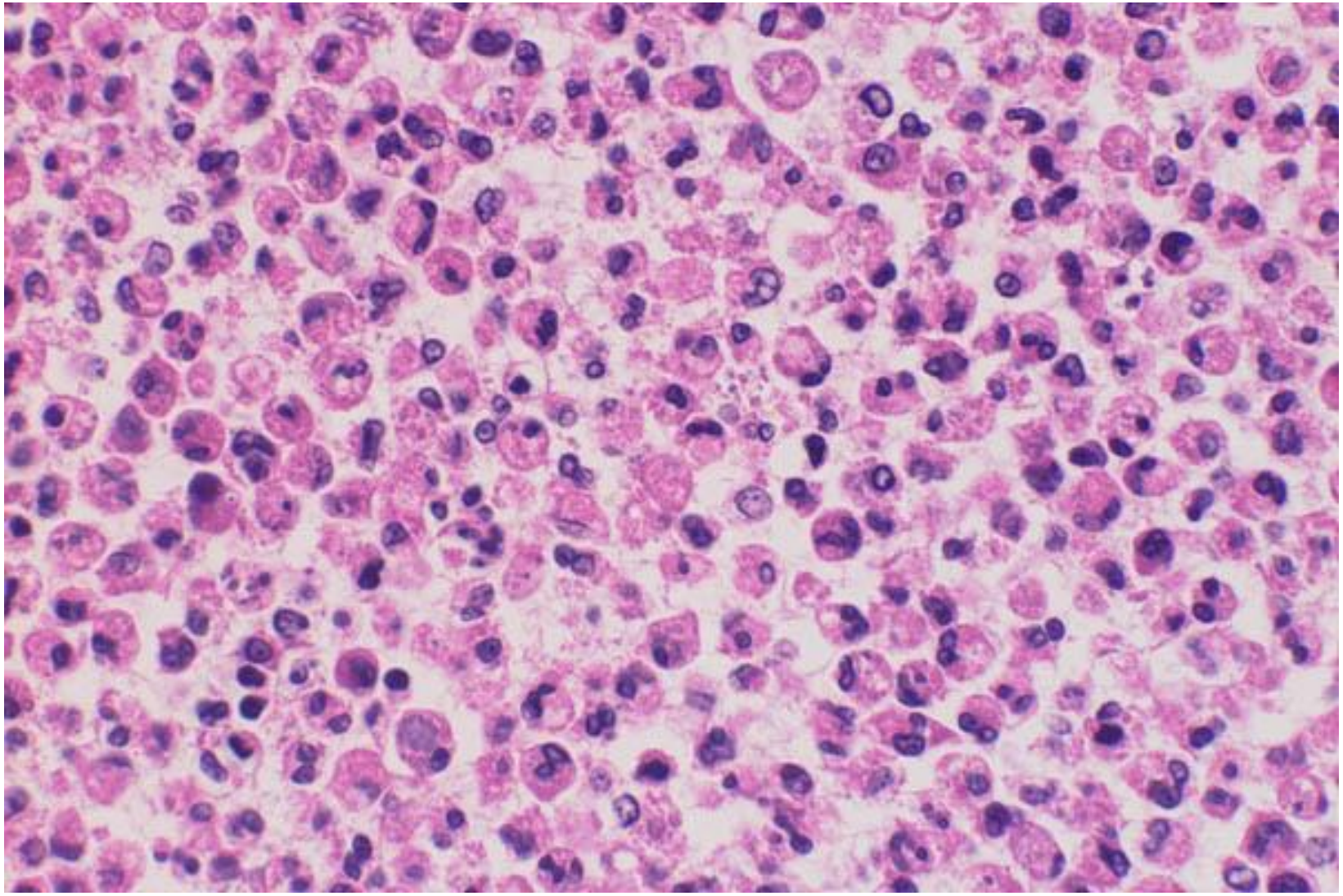
Staphylococcus Ag is expressed on MRSA, MSSA, MRSE and MSSE.
Protein A is expressed on MRSA and MSSA.
PBP2' is expressed only on MRSA.



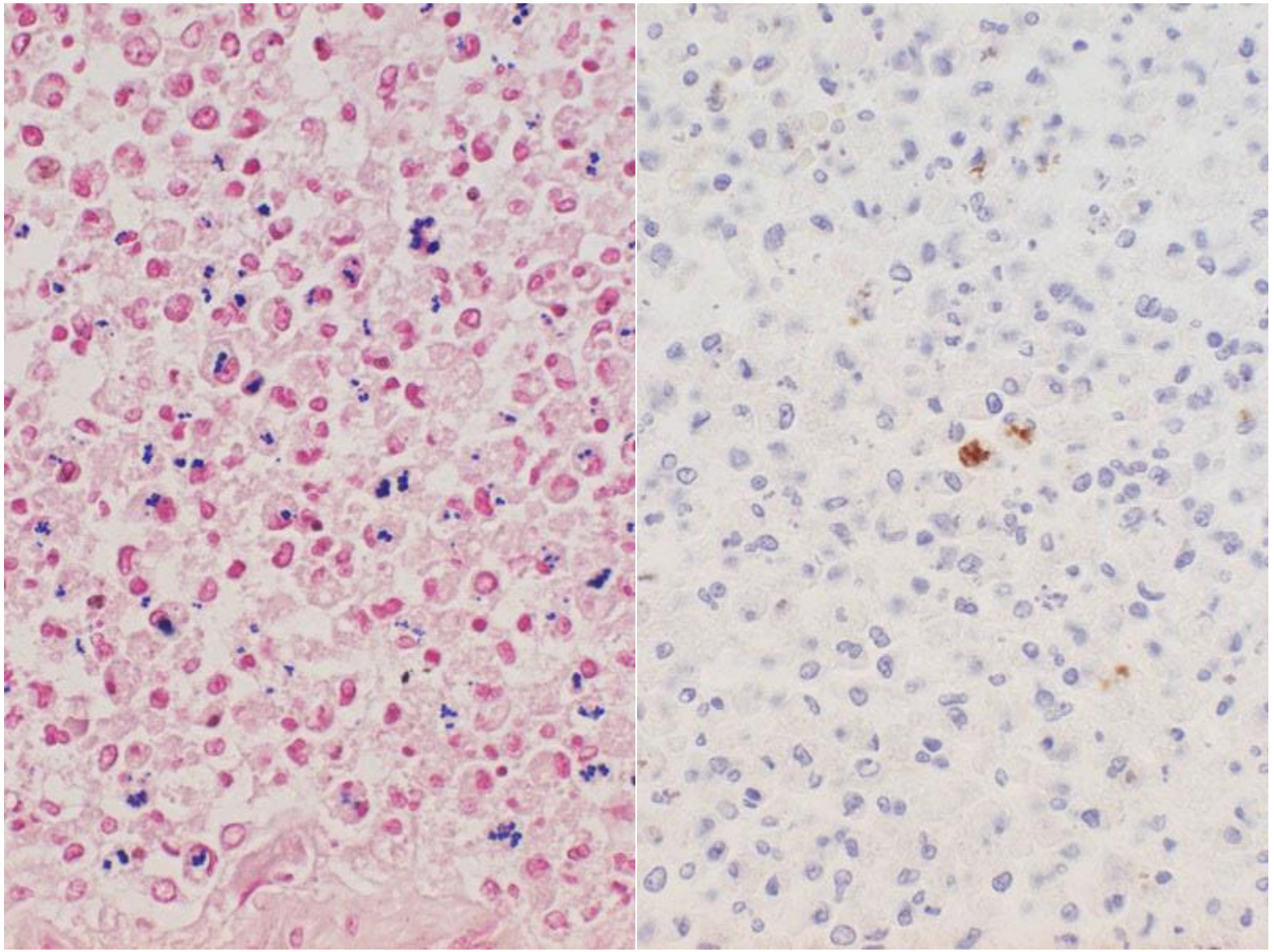
A 68 y-o male patient after surgery for esophageal cancer. Hospital-acquired MRSA bronchopneumonia was seen at autopsy. No microbial culture was performed. Bronchopneumonia with coccal colonization is observed. H&E



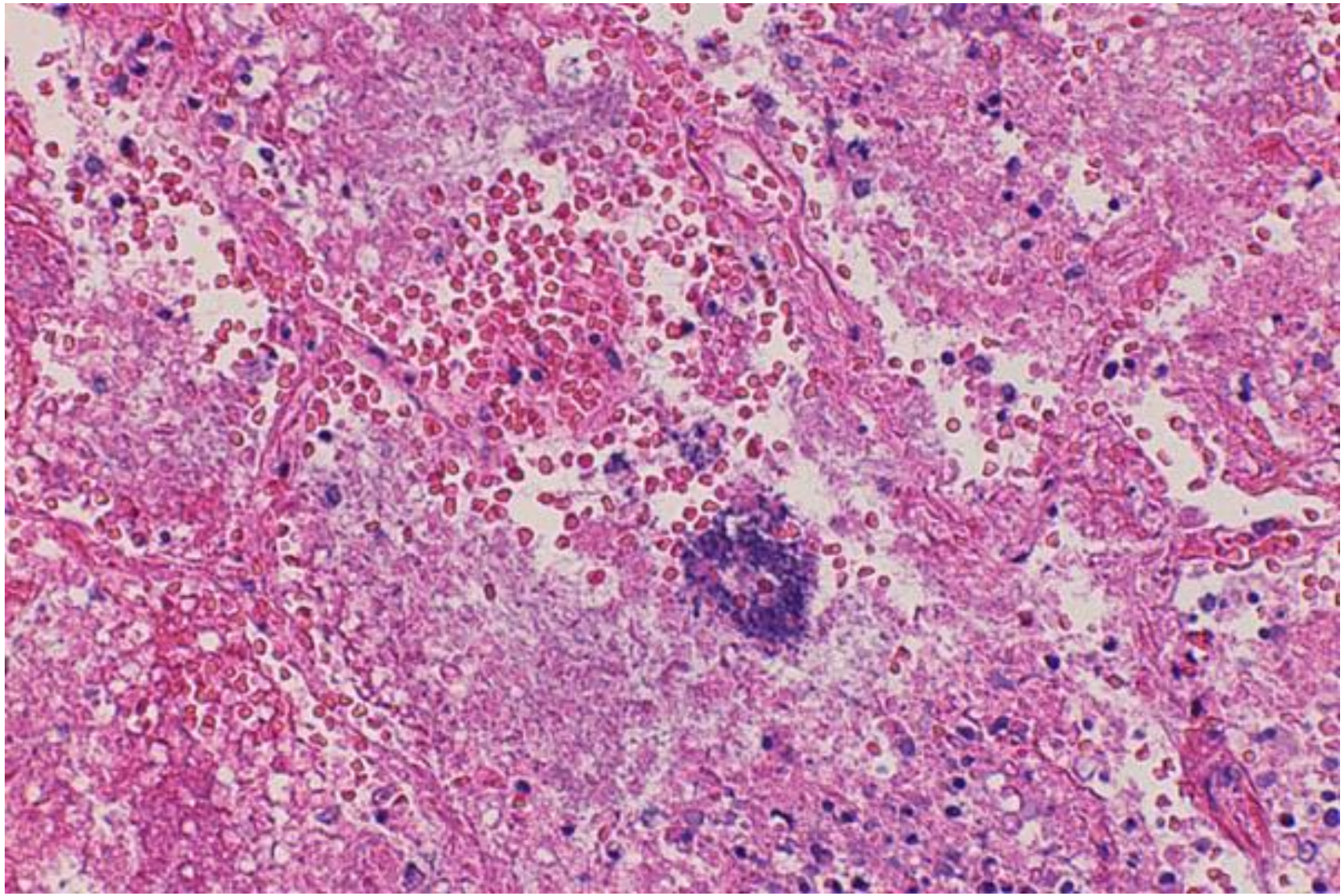
The cocci in the pneumonia lesion are Gram-positive (left) and express PBP2' (right). MRSA infection was proven.



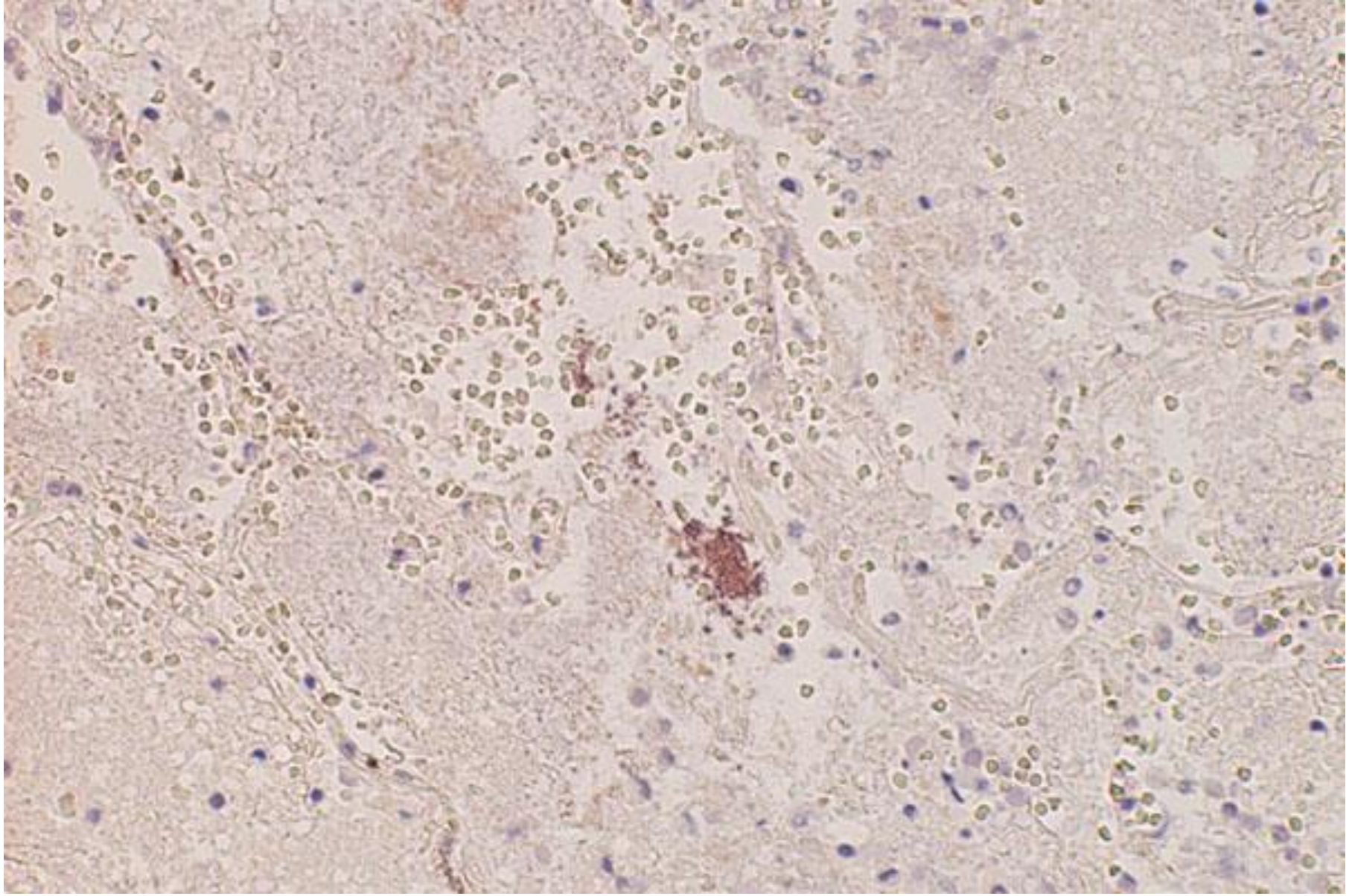
A 76 y-o male patient with metastatic gastric cancer.　At autopsy, active bronchopneumonia was seen but without colony formation. MRSA was cultured from the lung lesion.



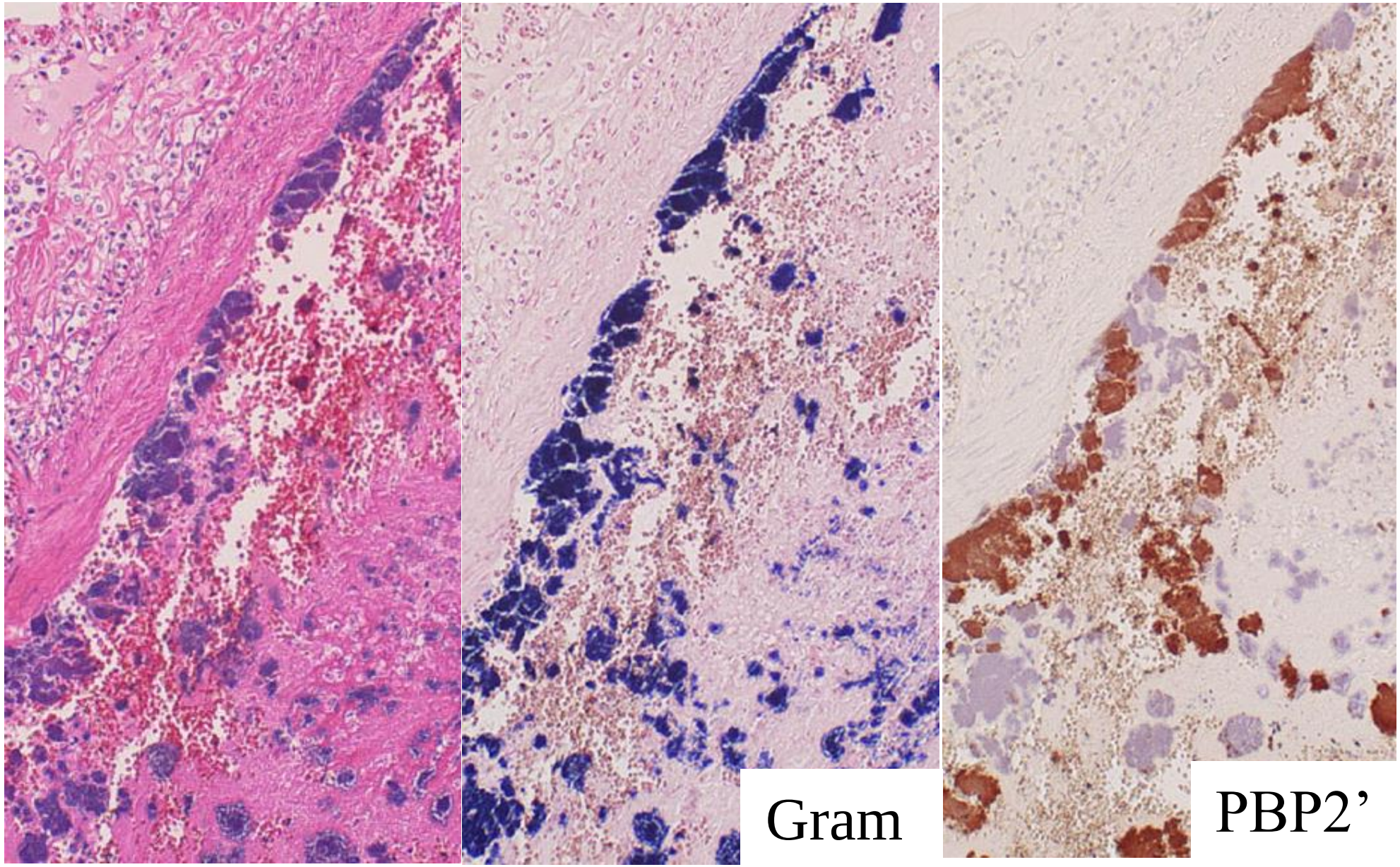
Gram-positive cocci were phagocytized by neutrophils (left), and they were immunoreactive for PBP2’ (right), confirming infection of MRSA.



A 67 y-o female patient with neutropenia. At autopsy, mixed infection of Pseudomonas aeruginosa and MRSA was seen. Coccal colonies were distributed in the hemorrhagic lung lesion but with little inflammatory reactions. H&E



The colonies in the poorly inflamed lung are immunoreactive for PBP2’.



A 68-year-old male patient. Mycotic embolus of MRSA in pulmonary artery.
The Gram-positive cocci express PBP2’.