Testing of Kappa and Lambda immunoglobulin light chain antibodies

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In this retrospective study, we have tested the new **kappa DB037-1 and lambda DB039-1 light chain antibodies, developed by DB Biotech** and compared to standard staining, performed with widely used mouse monoclonal antibodies from Producer A and Producer B..

Tested material:

For the study, we have retrieved trephine biopsy samples, from cases in which kappa and lambda immunohistochemical staining was performed in the diagnostic workup of suspected or already confirmed plasma cell myeloma (index and follow-up biopsies). Trephine biopsy samples were chosen, because they represent samples on which immunohistochemistry is difficult to perform due to artefacts caused by prior decalcification, crushing and large amounts of erythrocytes and blood serum. Altogether, forty three consecutive cases from years 2009 and 2008 were identified in the files of the Department of Pathology, to simulate routine practice.

Methods

4 micrometers thick sections were deparaffinized in xylene (3 x 10 min), rehydrated in benzylalcohol (3 x 10 min) and washed in distilled water (DW, 2 x 5 min). Endogenous peroxidase activity was blocked by immersion in 3% hydrogen peroxide solution, slides were than washed in DW water (2 x 5 min). Antigen retrieval was performed by microwave cooking in citrate buffer pH 6,0 (20 min, 600 W). After cooling (15-20 min) and washing slides in DW (2 x 5 min) and PBS buffer (2 x 2 min),primary antibody (**anti-Human Kappa Light Chain / DB 037-1, clone 1 /** ready to use; **anti-Human Lambda Light Chain / DB 039-1, clone 1** / ready to use) was applied for 1 hour (humid chamber, ambient temperature). After washing in PBS buffer (3 x 5 min), secondary antibody (EnVision+, Dual Link System-HRP) was applied for 30 min. Washing in PBS buffer followed (3 x 5 min) and diaminobenzidine (DAB) was used as chromogen. Standard finishing with hematoxylin counterstaining and mounting in aqueous medium followed.

Results of the staining were scored as negative, weakly positive or strongly positive. Interference with the background staining was also noted, as present or absent. Each case was subsequently classified as unequivocal or equivocal. Comparison of the results of „old“ (mouse monoclonal antibodies from Producer A and Producer B) and „new“ (DB Biotech antibodies) immunohistochemical stainings was then performed, case by case.

Results

From the forty three cases, results of the immunostaining with the new antibodies was evaluable in 39. In four cases, no diagnostic tissue was left on the slides prepared for the immunohistochemistry. These cases were excluded from further comparison.

Overall, results of 26,,old“ stainings were considered unequivocal. Remaining 13 cases, either showed weak positivity together with high background staining or were negative for both light chains. In this latter cases, diagnosis of myeloma was possible because of clear neoplastic infiltrate was present, positive for CD138 and/or CD56, together with monoclonal immunoglobulin in the serum and other clinical findings.

In contrast, 34 ,,new” cases were considered unequivocal, and only five cases remained undiagnostic. Four of the five latter cases were equivocal in both „old“ and „new“ staining (**Figures 1 to 5**). Diagnosis in the „new“ staining group was more straightforward despite the fact that significantly more „new“cases showed background staining (13 „new“ versus 7 „old“ for lambda and 5 „new“ versus 2 „old“ for lambda).

Conclusion

In a limited number of studied cases, the new DB Biotech kappa and lambda antibodies showed excellent sensitivity and specificity, compared to standard antibodies, used previously in our daily practice. With the use of these new antibodies, it would be possible to solve two thirds of cases previously considered as equivocal.

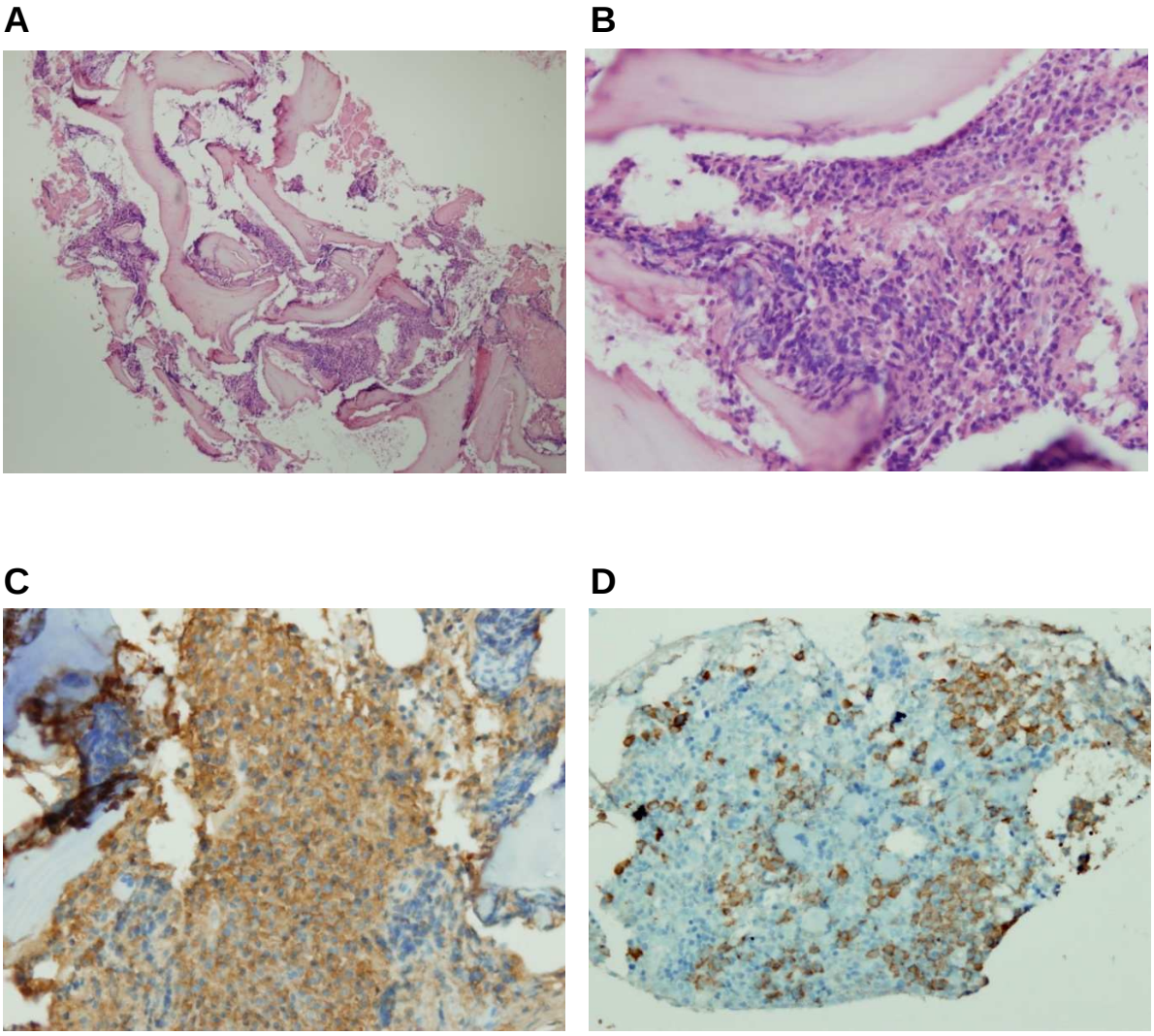

Figure 1
Crush artefacts in the trephine biopsy (A, B), leading to diffuse non-specific staining with *kappa from Producer A antibody* (C).
Specific staining of neoplastic cells with DB Biotech kappa antibody (D).

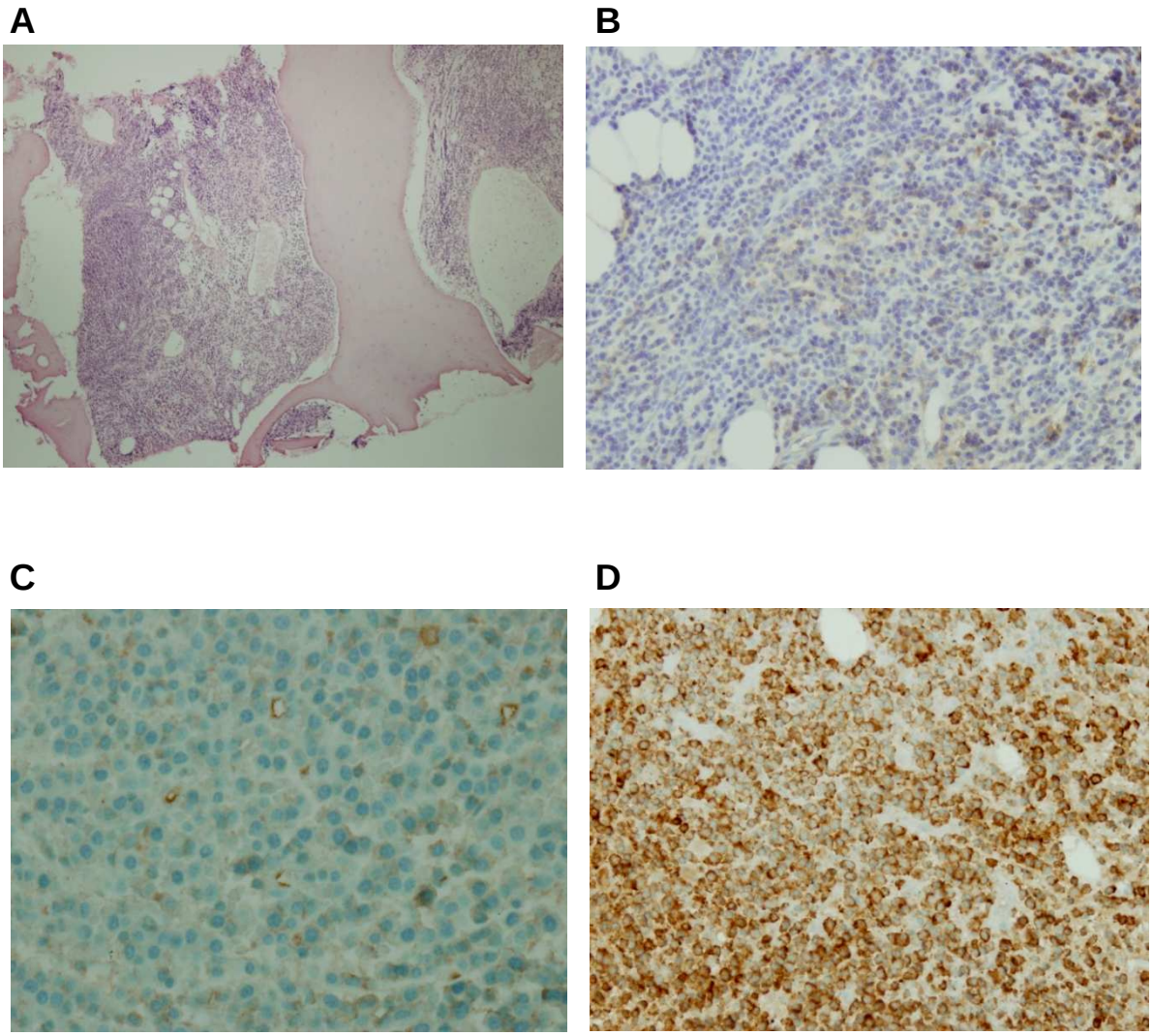

Figure 2
Frank neoplastic infiltrate of the bone marrow (A). Results with *kappa from Producer A* (B) and *lambda from Producer B* (C) are questionable, kappa is probably weakly positive.
In contrast, brisk positivity with DB Biotech lambda antibody (D).

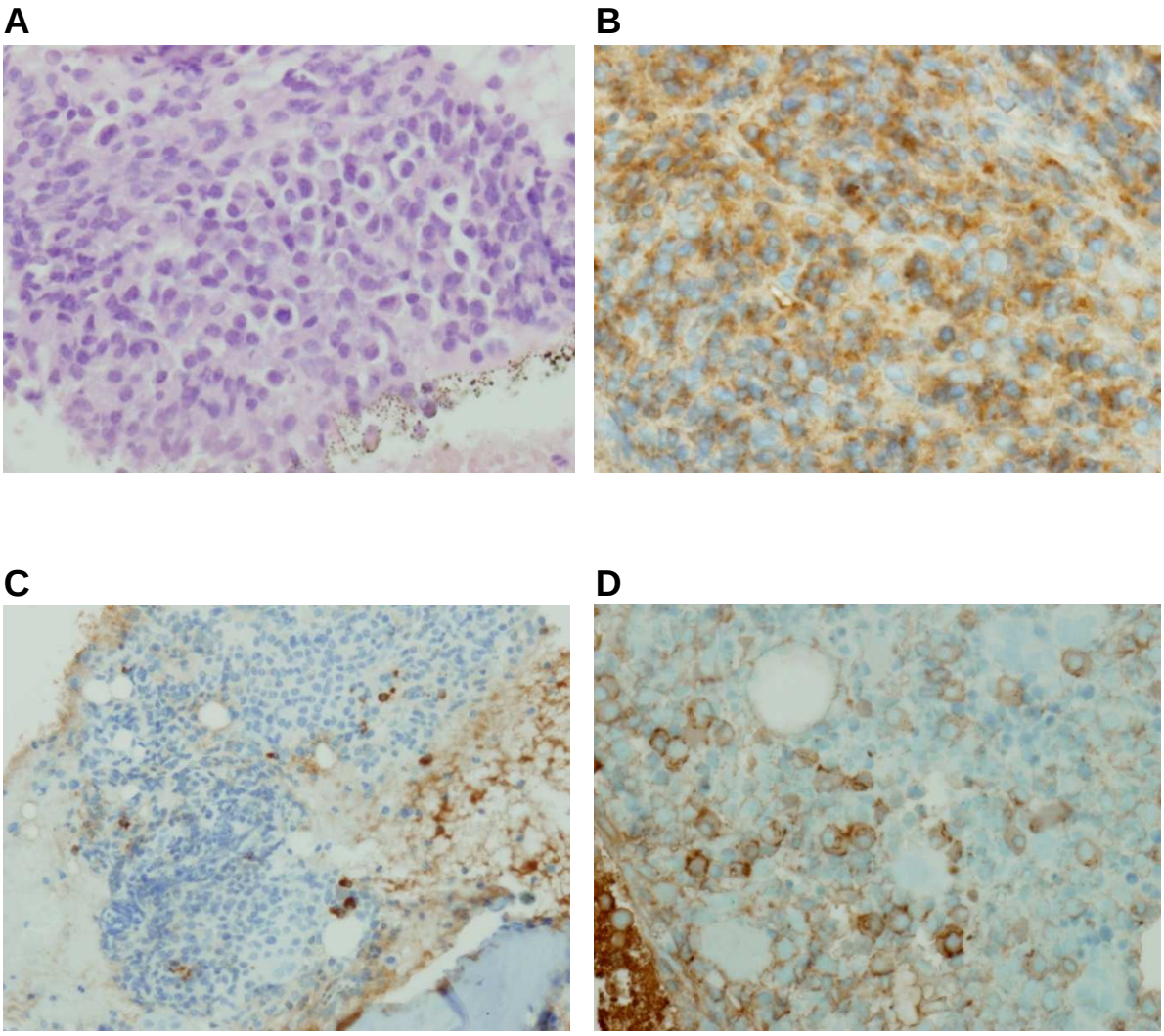

Figure 3
A case that was considered as unequivocal by both „old“ and „new“ staining. Neoplastic infiltrate (A) was positive for *kappa from Producer A* (B) and negative *lambda from Producer B*, showing only few positive cells (C).
However, staining with DB Biotech antibody for kappa light chain resulted in more specific pattern of staining (D).

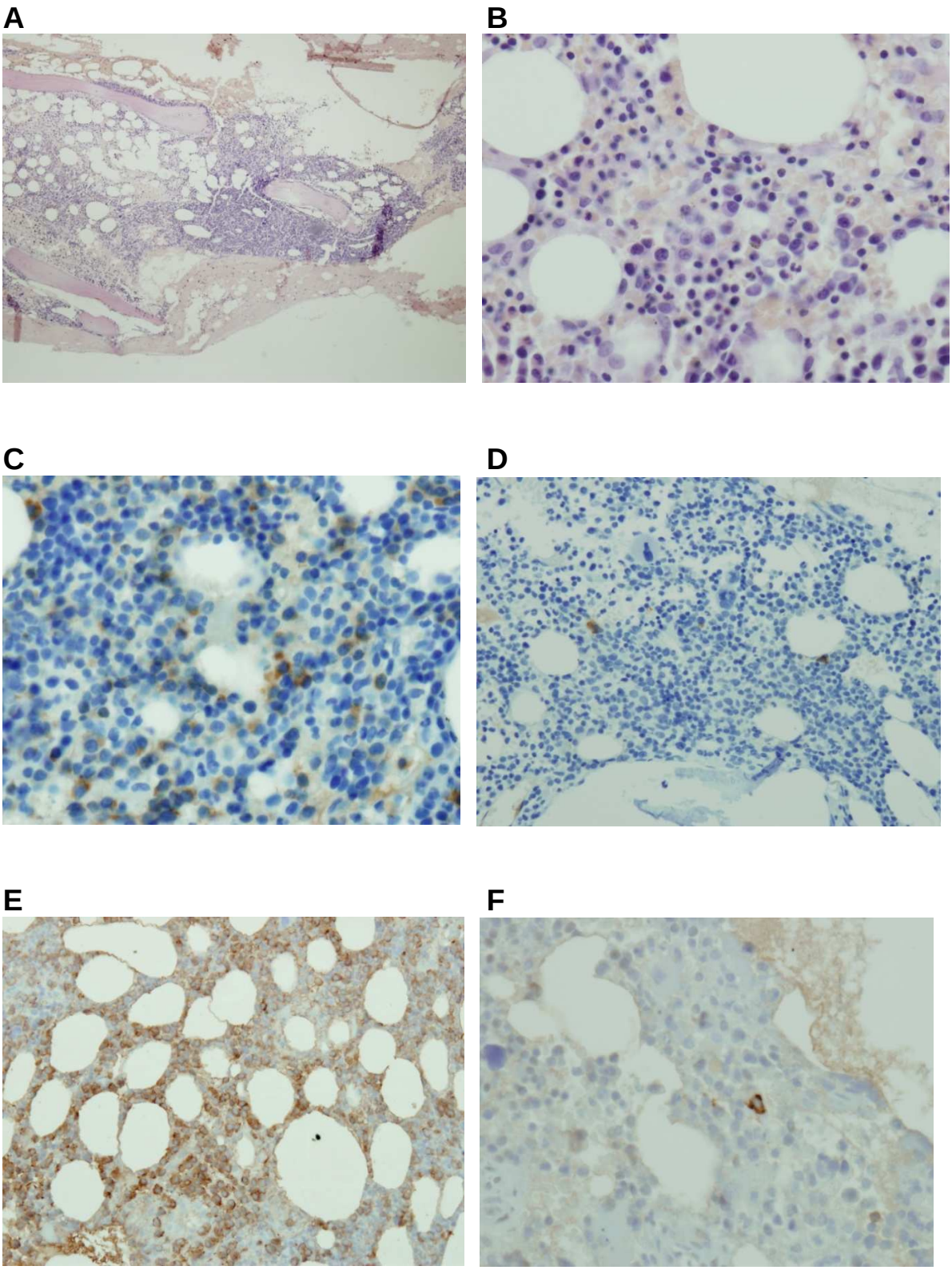

Figure 4
Diffuse pattern of infiltration by neoplastic plasma cells (A, B), confirmed by staining with CD138 antibody (not shown). However, stains of kappa and lambda antibodies from *Producer A and B* gave an impression that the plasma cells are not clonal (C, D).
In contrast, DB Biotech antibodies confirmed, that there is immunoglobulin light chain restriction (kappa,E; lambda F).

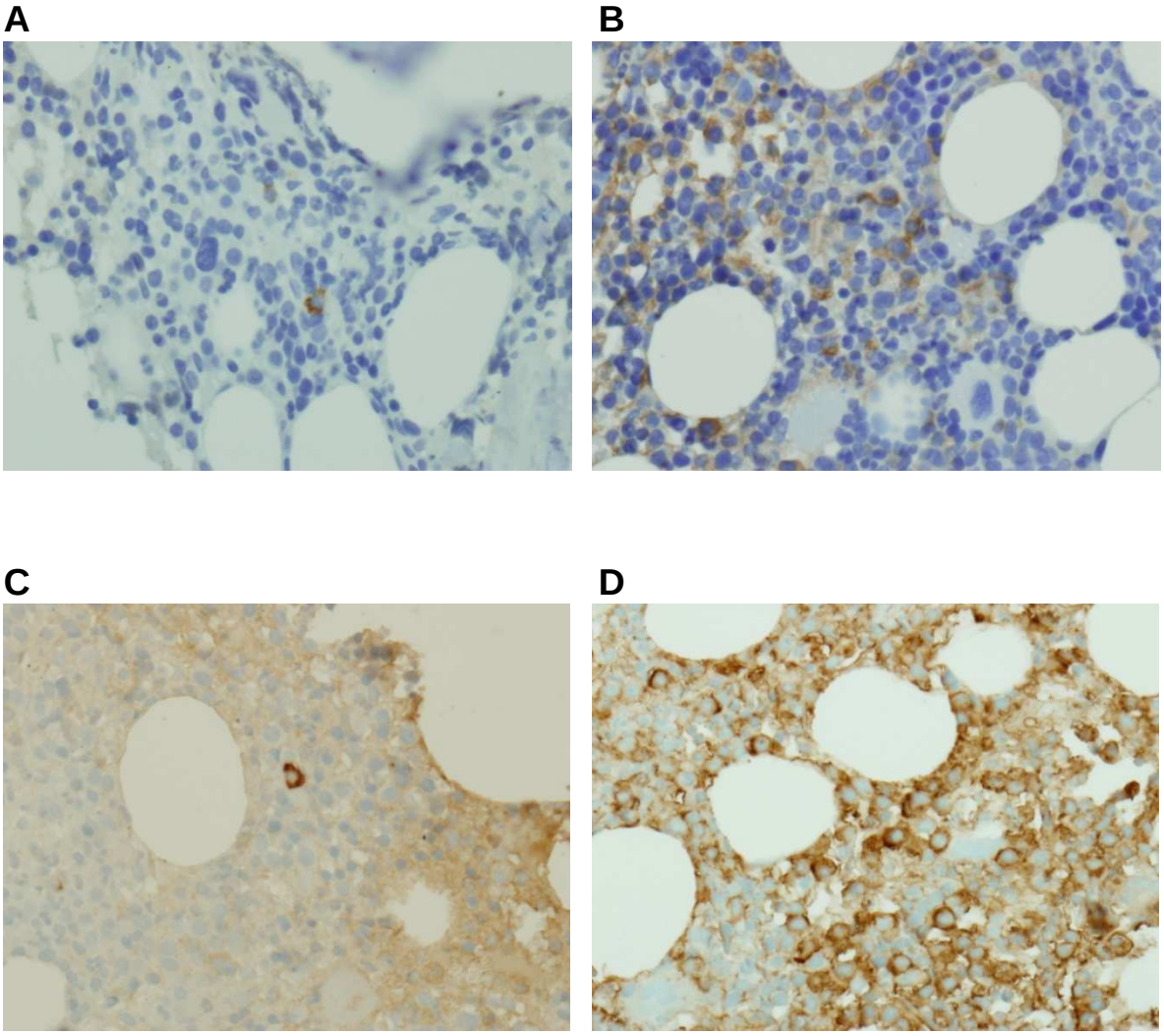

Figure 5
Another case of diffuse pattern of infiltration, that showed only weak lambda staining, but considered diagnostic (A , *kappa from Producer A,* B, *lambda from Producer B*).
However, DB Biotech lambda gave clear and intensive staining result, making the interpretation much easier (C , kappa; D, lambda).