

EXAMPLES OF IMMUNOFLUORESCENCE AND NEGATIVE CONTROLS

There is autofluorescence in formalin-fixed paraffin-embedded tissue that is not completely abolished by borohydride treatment. This allows the background anatomy to be discerned superimposed on which is the brilliant positive immunofluorescent signal. However, red blood cells (RBCs) are also brilliantly autofluorescent and it is not possible to eliminate the capture this without eliminating the positive staining. In the illustrations of the test sections the digital camera exposure is identical to the exposure that eliminates capture of the autofluorescence to the point where only RBCs are evident in the negative control. At this exposure Biondi bodies, small linear, curvilinear or spherical inclusions in choroid plexus epithelial cells that express beta amyloid and accumulate with aging, also autofluoresce. Thus, the resulting fluorescent image in the test section represents positive staining as well as non-specific autofluorescence of RBCs and Biondi bodies, referenced to the negative control. In the illustrations to help orient the reader, a low-exposure bright-field image is superimposed on the fluorescence image to outline the underlying choroid plexus anatomy.

Examples of Immunofluorescence and Negative Controls for C1q, C3d

C1q (A-F): Bright-field in A and D, fluorescence in B and E, merge in C and F. The negative control (A-C) shows autofluorescence of some RBCs (asterisks). Immunofluorescence for C1q shows the autofluorescence of RBCs (asterisks), as well as positive staining of a concretion (c), the epithelial basement membrane overlying the concretion (BMc), the stroma (S) and a single epithelial cell (EP).

C3d (G-L): Bright-field in G and J, fluorescence in H and K, bright-field and fluorescence in I and L. The negative control (G-I) shows autofluorescence of RBCs (asterisks), which is also evident in the test slide stained for C3d (J-K). There are C3d-positive deposits in the superficial and deep regions of a concretion (c) and focally in the stroma (S).

C4d (not shown) demonstrated specific immunofluorescence relatively infrequently.

Examples of Immunofluorescence and Negative Controls for C9, C9neo

C9 (A-F): Bright-field in A and D, fluorescence in B and E, merge in C and F. In the negative control (A-C) autofluorescence of RBCs (asterisks) and Biondi bodies (examples indicated by arrows) is evident (asterisks). Autofluorescent Biondi bodies, in this field assuming linear or curvilinear shapes, are also evident in the immunofluorescence slide for C9 (D-F) where specific staining of two concretions (c) is also shown.

C9neo (G-L): Bright field in G and J, fluorescence in H and K, merge in I and L. In the negative control (G-I) fluorescence of RBCs (asterisks) is evident. Immunofluorescence for C9neo shows autofluorescence of some Biondi bodies (examples indicated by arrows) and positive staining of a concretion (c).

Examples of Immunofluorescence and Negative Controls for IgA, IgG and IgM

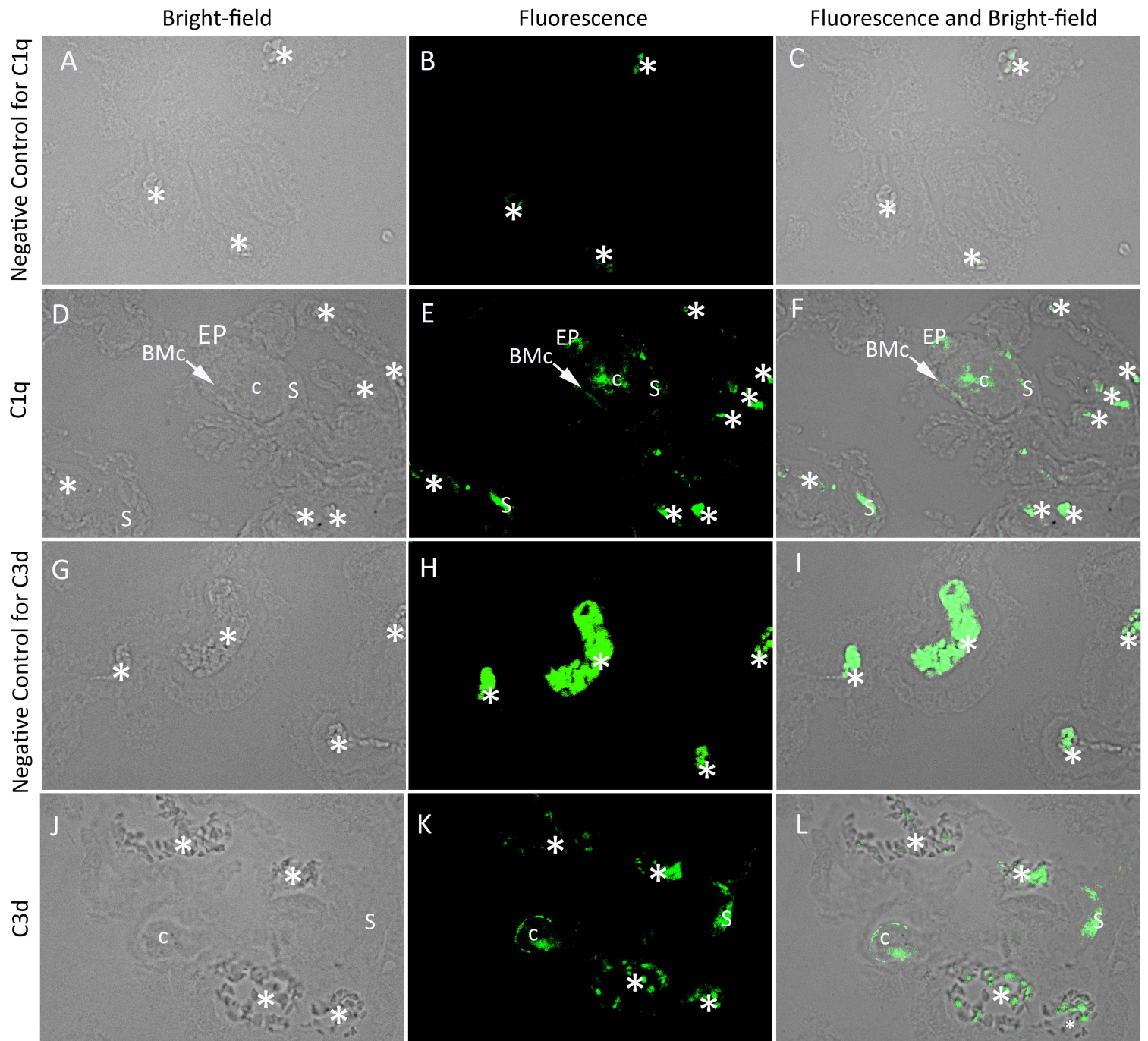
IgA (A-F): Bright-field in A and D, fluorescence in B and E, merge in C and F. The negative control (A-C) shows brilliant autofluorescence of many RBCs (asterisks). IgA (D,E,F) immunofluorescence referenced to the negative control shows positive staining of the choroid plexus (CP) stroma (S); note the brilliant autofluorescence of the RBCs (asterisks).

IgG (G-L): Bright-field in G and J, fluorescence in H and K, merge in I and L. The negative control (G-I) shows autofluorescence in some RBCs (asterisks). This is also evident (asterisks) in the immunofluorescence for IgG (J-L), which also shows positivity in the CP stroma (S) and epithelial cells (EP), particularly in their apical regions.

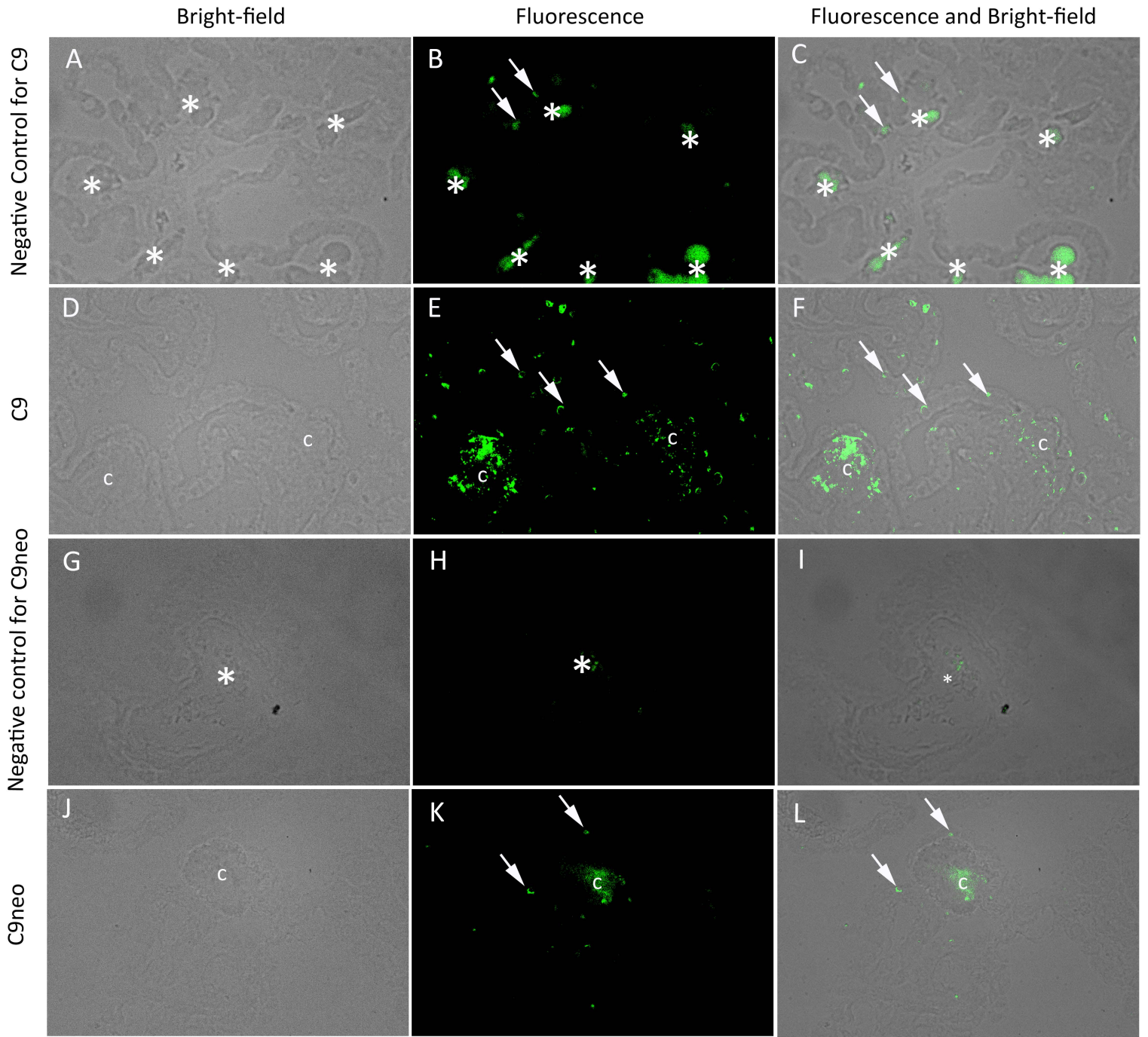
IgM (M-R): Bright-field in M and P, fluorescence in N and Q, merge in O and R. The negative control (M-O) shows autofluorescence of many RBCs (asterisks) and some Biondi bodies (arrows). As anticipated, both the autofluorescence of RBCs (asterisks) and Biondi bodies (arrows) is also seen in immunofluorescence stain for IgM, which also shows multifocal IgM-positive deposits in the stroma (S) immediately beneath the epithelium.

IgD (not shown) did not demonstrate specific immunofluorescence and IgE (not shown) only occasional showed showed specific immunofluorescence.

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