Immunophenotyping cells of the immune system of birds
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Introduction
Immunohistochemistry is an histopathological process based in the use of special designed antibodies for antigen specifically binding. This technique is widely used as a diagnostic tool in birds in general to bind some specific antigens, specially virical. However, the immune system cell of birds are not so easy to stain with this technique; in fact, only the CD3 antibody in front CD3 T cells is accepted as the only useful antibody in this task.

Objectives
To test new antibodies towards immune system cells of birds (chicken specially), whose combined with specific immunohistochemistry techniques, would become an useful tool for study and diagnosis.

Materials and methods
• Formalin-fixed paraffin-embedded (FFPE) tissue sections from young chicken including: bursa of Fabricius, thymus, liver, kidney, spleen and heart.
• Antibodies:
  o Anti-CD4 Mouse antichicken CD4 Isotype Mouse (BALB/C) IgG1k, 0.5 mg/mL.
  o Anti-CD8α Mouse antichicken CD8α Isotype Mouse (BALB/C) IgG1k, 0.5 mg/mL.
  o Anti-CD79α [HM47/A9] Mouse monoclonal [HM47/A9] Isotype IGG1, 0.2 mg/mL.
  o Lysozyme EC.3.2.1.17 Polyclonal rabbit anti-human.

Results
The multiple results are presented in the table below (see Table 1), according the used antibody and its dilution and incubation temperature along the antigen retrieval method in every single organ. The positive tests provided by lysozyme are visible in the images next to.

Table 1. Results are reported by each organ in a coloured way: absence of staining, non specific staining or specific staining

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Antibody</th>
<th>Dilution</th>
<th>Antigen retrieval method</th>
<th>Bursa of Fabricius</th>
<th>Thymus</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CD4</td>
<td>1:50°C PBS</td>
<td>Formalin</td>
<td>EDTA</td>
<td>Brown</td>
<td>Red</td>
<td>Blue</td>
<td>Yellow</td>
<td>Green</td>
<td>Brown</td>
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<tr>
<td>Anti-CD8α</td>
<td>1:50°C PBS</td>
<td>Formalin</td>
<td>EDTA</td>
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<tr>
<td>Lysozyme</td>
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<td>Brown</td>
<td>Red</td>
<td>Blue</td>
<td>Yellow</td>
<td>Green</td>
<td>Brown</td>
</tr>
</tbody>
</table>

Discussion & Conclusions
The results obtained from the different tests are very poor. Neither anti-CD4, anti-C84 or anti-CD79 provided any positive result in any of the tested tissues. Only the lysozyme offered positive staining in some cell of the bursa of Fabricius and thymus, despite non specific staining was observed in the other organs. In other studies, some of these antibodies have shown their efficiency in chicken (but in frozen tissues), and some in other species too. So there is no reason to give up on them as useful antibodies for this technique. Further research is needed.

Bibliography