The ABC of immunohistochemistry: Looking back to early developments

While the principles of what would ultimately be termed immunohistochemistry began to emerge during the 1930s, it was not until 1942 that the seminal work on immunofluorescence, undertaken and reported by Coons and colleagues, represented the first steps along the path to the now ubiquitous diagnostic methodology.

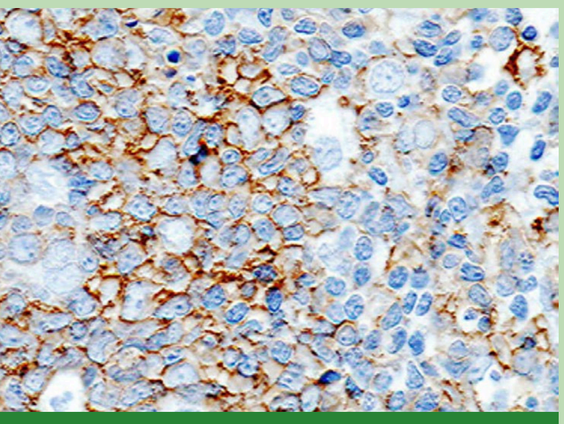
Birth of Immunofluorescence

In the early 1940s, American immunologist Dr Albert Coons and colleagues were the first to conceptualise and develop immunofluorescence (IMF) techniques for labelling antibodies, which could be detected by optical rather than analytic or radiographic methods.

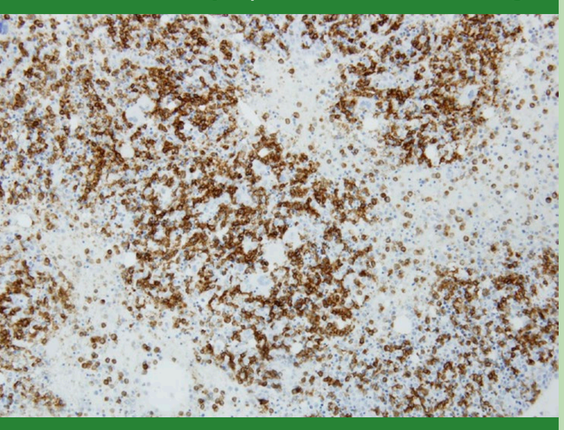
- Initial investigations determined the feasibility of using chemically labelled antibodies as reagents to detect and orientate antigenic material in mammalian tissue.
- This required the retention of specificity by the antibody-molecule during and after chemical manipulation, a stable chemical linkage between the antibody and its label, and a label that could be detected when present in minute quantities.
- Also important was a need to demonstrate effective separation of the labelled antibody solution from unconjugated tracer material.
- Such a method was deemed more useful if it were possible to determine not only those organs, but those cells that contained the antigen in question.
- Initially, Coons and colleagues used a direct technique in which fluorescein isothiocyanate (FITC)-labelled antibodies localised pneumococcal antigens in infected tissues.

Dr Albert Coons

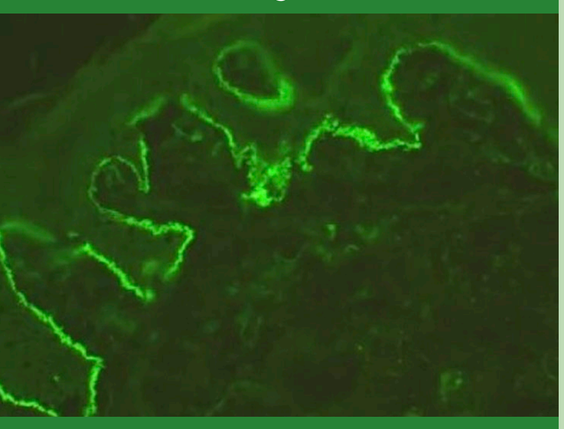
Early Applications in Haematopathology and Histopathology



B-cell lymphoma: CD20 staining



Bone marrow trephine: T-cell CD3 staining



Bullous pemphigoid: direct IMF staining

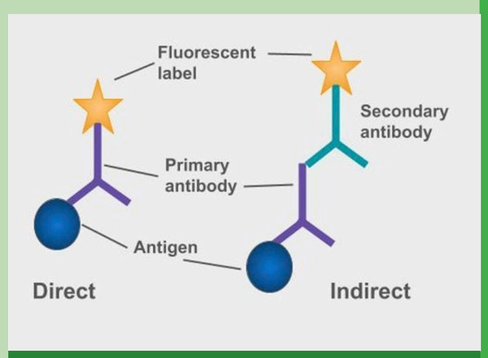
An early application of IHC proved helpful in the diagnosis and treatment of leukaemia and lymphoma. The sample type of choice was the trephine biopsy which produced a core of bone marrow, as opposed to the more fluid aspirate used to aid differential diagnosis by cytochemical staining using, for example, Romanowsky, periodic acid Schiff (PAS), Sudan Black (SB) and myeloperoxidase.

- Lymphoma
  - CD3, a pan T-cell marker expressed on most mature T/NK-cell lymphomas
  - CD20, a pan B-cell marker expressed on most mature B-cell lymphomas.
  - CD79a is an alternative to CD20, compensating for its loss due to the plasmocytic differentiation of tumour cells or history of rituximab administration.
  - CD56, a neuroendocrine marker is used to identify NK cells.
  - Overexpression of Bcl-2 and cyclin D-1 can be detected for follicular lymphoma (BCL2) and mantle cell lymphoma (CCND1), respectively.
  - With the development of immunotherapy, several antibodies against markers such as programmed death-ligand 1 (PD-L1), CD19 and CD30 have been used as biomarkers to identify therapeutic targets.
- Leukaemia
  - Diagnosis and classification of acute leukaemia relies on a combination of morphology, cytochemistry, immunophenotyping, cytogenetics, and molecular genetics.
  - Bone marrow examination is an integral part of the diagnostic work up and provides material for immunophenotyping by flow cytometry or IHC.
  - IHC can be used as an alternative method to primarily diagnose and classify acute leukaemia, especially in dry tap situations where aspirates are difficult to obtain.
  - Markers associated with specific leukaemic states include:
    + MPO and CD34 for acute myeloid leukaemia
    + PAX5 and TdT for acute B-cell lymphoblastic leukaemia
    + CD3 and TdT for acute T-cell lymhoblastic leukaemia
    + CD34 and TdT positive expression is generally associated with early cell lineage.
- Solid tumours
  - Epithelial membrane antigen (EMA) is positive in most carcinomas of glandular or luminal epithelial origin.
  - Cytokeratin 5/6 is a positive marker for malignant pleural mesothelioma in over three-quarters of cases. It is also present in certain lung and breast cancers.
- Dermatopathology
  - Direct and indirect IMF techniques used in the diagnosis of the skin lesions pemphigus vulgaris and bullous pemphigoid.

Introduction of Alternative Labels

In the late 1970s / early 1980s, an indirect technique saw the use of peroxidase anti-peroxidase and later avidin-biotin complex (ABC) technology.

- Soluble immune complexes of horseradish peroxidase (HRP) and antibody to peroxidase (PAP) became widely used in the so-called 'unlabelled antibody' method for the detection of cellular antigens.
- After initial use of a more complex technique, a simple and rapid method for preparation of PAP was facilitated by column chromatography of a mixture of the enzyme and the IgG fraction of antiperoxidase antiserum.
- In 1981, use of ABC technology was introduced, subsequently followed by labelled streptavidin–biotin (LSAB) methods.
- Use of labelled antibodies applied to tissue sections resulted in detection of an increasing range of cellular elements.

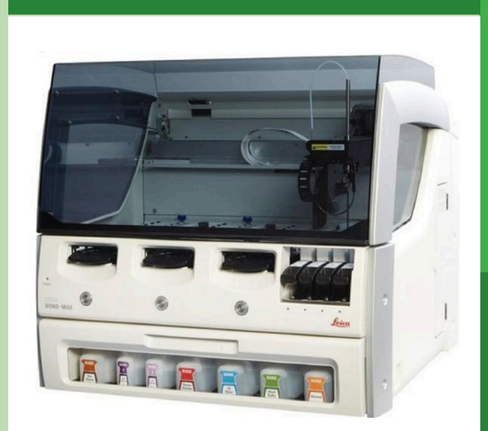


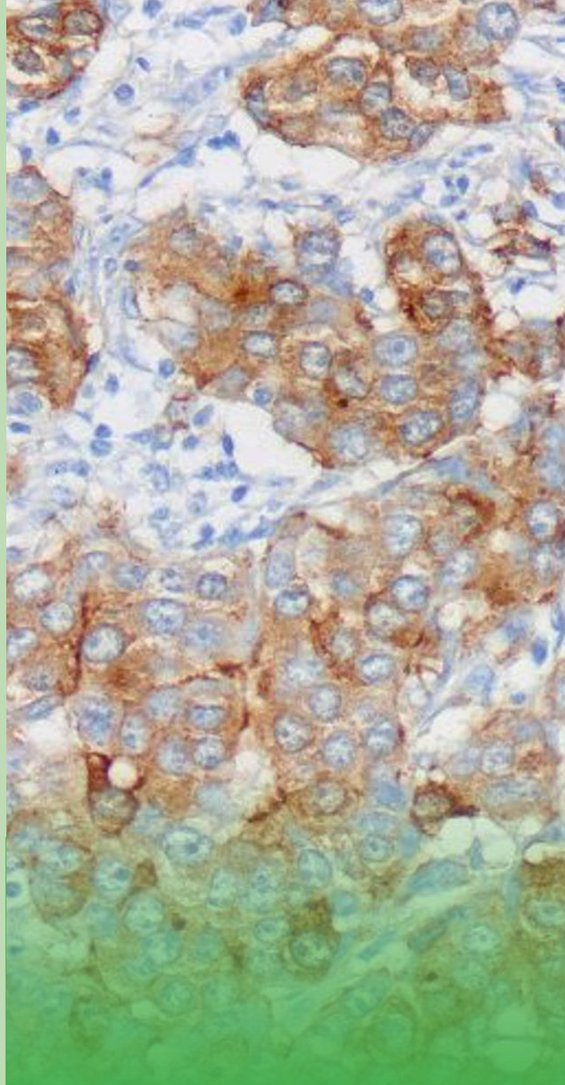
Immunofluorescence methods

Automation

Early manual IHC techniques were labour-intensive and required careful and accurate technique and attention to detail. One of the first companies that sought to automate these processes was Leica Biosystems, with the introduction of the automated BOND-MAX compact benchtop system, which was the first autostainer to deliver high-quality IHC results flexibly, efficiently and consistently fast.

Early automation…
Leica BOND-MAX





Secondary carcinoma deposit: epithelial membrane antigen staining

Antigen Retrieval: Proteases to Microwaves

In the development of immunohistochemistry, antigen retrieval proved to be one of the most important factors affecting the results of IHC staining, and as important as the sensitivity of the detection system. The two most important methods are enzymatic digestion and heat-induced antigen (epitope) retrieval (HIER), with the latter being responsible for the successful detection of an increasing number of specific protein biomarkers.

- Curran and Gregory in 1977/78 introduced the use of proteases (primarily trypsin) to expose antigens in cryostat sections and in sections of paraffin wax-embedded tissues.
- Shi and colleagues in 1991 described a new approach for retrieval of antigens from formalin-fixed, paraffin wax-embedded tissues and their subsequent staining by IHC techniques. This HIER performed in a microwave oven raised the temperature of tissue sections attached to microscope slides to temperatures up to 100^{0}C in the presence of buffered solutions.

Chromogens

The IHC localisation of antibodies has relied on use of two main enzyme labels, HRP and alkaline phosphatase (AP). These enzymes required chromogens in order to be detected by light microscopy.

Examples of chromogens to detect HRP have included:

- aminoethyl carbazole (AEC)
- 3,3′-diaminobenzidine (DAB)
- 3,3′5,5′-tetramethylbenzidine (TMB)

Examples of chromogens to detect AP have included:

- Fast Red
- Permanent Red

Bodies to Blocks:
A Brief History of Cell Science
Produced by the IBMS History Committee for Congress 2025