CD30: A DIFFERENTIATING BIOMARKER IMPACTING DIAGNOSTIC AND TREATMENT DECISIONS ACROSS VARIOUS LYMPHOMAS
The CD30 protein is expressed in several lymphoma subtypes

- Strong and homogeneous expression in:\n  - Classical Hodgkin lymphoma (cHL)
  - Systemic anaplastic large cell lymphoma (sALCL)
  - Primary cutaneous anaplastic large cell lymphoma (pcALCL)

- Expressed to varying degrees in other B- and T-cell lymphomas, including:
  - Subtypes of diffuse large B-cell lymphoma (DLBCL): anaplastic variant, primary mediastinal large B-cell lymphoma, primary effusion lymphoma, DLBCL not otherwise specified (NOS)\(^5,6\)
  - Epstein-Barr virus–positive B-cell lymphoproliferative disorders\(^5\)
  - Gray zone lymphoma*\(^7\)
  - Mycosis fungoides (MF) and transformed MF\(^5\)
  - Lymphomatoid papulosis\(^5\)
  - Subtypes of peripheral T-cell lymphoma\(^5\)

Select T-cell lymphomas expressing CD30 [WHO classification]\(^8\)

<table>
<thead>
<tr>
<th>SUBTYPE</th>
<th>PERCENTAGE OF CASES WITH CD30 EXPRESSION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral T-cell lymphoma, NOS (PTCL-NOS)</td>
<td>59%(^9)</td>
</tr>
<tr>
<td>Angioimmunoblastic T-cell lymphoma (AITL)</td>
<td>76%(^3)</td>
</tr>
<tr>
<td>Extranodal natural killer (NK)/T-cell lymphoma</td>
<td>52%-75%(^9,10)</td>
</tr>
<tr>
<td>Adult T-cell leukemia/lymphoma (ATLL)</td>
<td>0%-23%(^11,12)</td>
</tr>
<tr>
<td>Anaplastic large cell lymphoma (ALCL), ALK+</td>
<td>100%(^4)</td>
</tr>
<tr>
<td>ALCL, ALK−</td>
<td>100%(^3)</td>
</tr>
<tr>
<td>Enteropathy-associated T-cell lymphoma (EATL)</td>
<td>54%-100%(^9,13)</td>
</tr>
<tr>
<td>pcALCL</td>
<td>100%(^14)</td>
</tr>
<tr>
<td>MF/Sézary syndrome</td>
<td>11%-100%(^15,16)</td>
</tr>
<tr>
<td>Transformed MF</td>
<td>24%-100%(^15,17)</td>
</tr>
<tr>
<td>Lymphomatoid papulosis (LyP)</td>
<td>60%-100%(^18)</td>
</tr>
</tbody>
</table>

* B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL.
† Type I.
WHO = World Health Organization.
Since CD30 expression level is variable, it is important to evaluate and quantify expression level by immunohistochemistry (IHC)\textsuperscript{19}

\begin{itemize}
  \item 1. 100\% of Hodgkin Reed-Sternberg cells
  \item 2. 90\% of ALCL cells
  \item 3. 60\% of DLBCL cells
  \item 4. 40\% of cells in transformed MF
  \item 5. 5\% of immunoblasts in AITL
\end{itemize}

\textsuperscript{1}Images are examples and not intended for scoring purposes.

\textit{The CD30 transmembrane receptor...is expressed in a distinct, yet diverse set of lymphoproliferative disorders....Therefore, detection of CD30 expression when performed properly according to the standardized methods facilitates diagnosis of Hodgkin lymphoma, anaplastic large cell lymphoma, and other disorders expressing the receptor.}\textsuperscript{5}
**CD30 TESTING BY IHC IMPROVES DIAGNOSTIC ACCURACY AND SHOULD BE INCLUDED AS PART OF A TIERED APPROACH TO DIAGNOSING LYMPHOMA**¹,²⁰

A 2015 American Society for Clinical Pathology survey showed that²¹:

- 55% of pathologists (n = 100) and 42% of hematopathologists (n = 50) were unaware of the significance of CD30 IHC in T-cell lymphoma classification
- 48% of surveyed pathologists did not recognize the importance of T-cell lymphoma subtyping in determination of patient treatment

**Inclusion of biomarkers such as CD30 can result in reclassification of a patient’s initial diagnosis**²⁰

A study assessed the usefulness of second-opinion pathology review as characterized by evaluating the rate of diagnostic concordance between referring center diagnoses and expert hematopathology review for 4 subtypes* of T-cell lymphomas at 7 tertiary National Comprehensive Cancer Network® (NCCN®) centers. 131 patient cases were reviewed.²⁰

- **24%** of patients had discordant diagnoses between referring centers and NCCN centers.
- **53%** of discordant samples were reclassified based on additional studies performed by NCCN centers.
  - Additional IHC, including standard T-cell marker or CD30 testing, was used to reclassify discordant samples
  - One of the most common reclassifications was a referral diagnosis of PTCL-NOS reclassified as AITL or ALCL, ALK−
- **44%** of patients with discordant diagnoses may have required changes in treatment if they had had expert reclassification.

*PTCL-NOS, AITL, ALK+ ALCL, ALK- ALCL.

Both College of American Pathologists (CAP) Guidelines and NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines™) recommend CD30 testing by IHC to assist in confirming a diagnosis in a variety of lymphomas²²-²⁷
CD30 TESTING BY IHC IMPROVES DIAGNOSTIC ACCURACY AND SHOULD BE INCLUDED AS PART OF A TIERED APPROACH TO DIAGNOSING LYMPHOMA

A tiered approach that included CD30 increased WHO diagnostic accuracy from 17% to 83% (n = 336)

A study involving 7 expert hematopathologists from 5 leading academic centers characterized the diagnostic accuracy and clinical relevance of a defined approach to the diagnosis and subclassification of peripheral T-cell and NK-cell lymphomas.

<table>
<thead>
<tr>
<th>OBJECTIVE</th>
<th>DIAGNOSTIC CONSENSUS (WHO DIAGNOSES)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIER 0</td>
<td>Assess morphology</td>
</tr>
<tr>
<td></td>
<td>H&amp;E review</td>
</tr>
<tr>
<td></td>
<td>Basic clinical and demographic data</td>
</tr>
<tr>
<td></td>
<td>17%</td>
</tr>
<tr>
<td>TIER 1</td>
<td>Distinguish B-cell lymphoma, HL, and reactive processes from suspected T-cell lymphoma</td>
</tr>
<tr>
<td></td>
<td>HL vs NHL vs reactive</td>
</tr>
<tr>
<td></td>
<td>CD3, CD5, CD10, CD20, CD21, <strong>CD30</strong>, CD45, PAX5</td>
</tr>
<tr>
<td></td>
<td>37%</td>
</tr>
<tr>
<td>TIER 2</td>
<td>Aid in confirmation of lymphoma and subclassification</td>
</tr>
<tr>
<td></td>
<td>T-cell lymphoma subtypes</td>
</tr>
<tr>
<td></td>
<td>CD2, CD4, CD7, CD8, CD23, PD-1, CD56, EBER, ALK, TIA1, TCRγ, TCRβF1</td>
</tr>
<tr>
<td></td>
<td>83%</td>
</tr>
<tr>
<td>TIER 2b</td>
<td>Secondary confirmation of lymphoma and subclassification</td>
</tr>
<tr>
<td></td>
<td>T-cell and/or B-cell receptor gene rearrangement</td>
</tr>
<tr>
<td></td>
<td>86%</td>
</tr>
</tbody>
</table>

*Percentage of 336 reviewed cases.
HE = hematoxylin and eosin; HL = Hodgkin lymphoma; NHL = non-Hodgkin lymphoma.

Your choice to include CD30 may improve diagnostic accuracy, impact treatment choice, and ultimately affect patient outcomes.
Factors commonly impacting IHC results

IHC challenges exist but can be addressed with best-practice methods. Factors that can impact IHC include:

• Variable consistency
• Poor reproducibility
• Quality assurance disparities
• Lack of standardization resulting in poor concordance, validation, and/or verification

The NordiQC study highlights a need for an increase in sufficient CD30 IHC staining

NordiQC, an independent scientific organization that promotes the quality of IHC, assessed CD30 staining over a 13-year period. Tonsil tissue was used as a positive control.
The NordiQC 2017 assessment evaluating CD30 IHC staining results among 282 laboratories concluded²⁹:

96% (n = 46/48) of laboratories failing quality control did so due to insufficient staining results, often caused by weak or false-negative staining reactions

- Insufficient performance may compromise staining and thus the interpretation of test results

Frequent causes of insufficient IHC staining results are:

- Suboptimal tissue fixation or tissue processing
- Insufficient heat-induced epitope retrieval (HIER), with heating time being too short or the temperature too low
- Low concentration of the primary antibody
- Use of low-sensitivity detection systems
- Technical issues

Additionally, nonspecific and/or false-positive staining can be caused by:

- Crushed or damaged cell samples
- Necrotic and apoptotic cells (due to release of oxidative enzymes)

“Virtually all laboratories were able to determine CD30 in high-level antigen expressing cells…. However, demonstration of CD30 in low-level antigen expressing cells…was more challenging and required optimally calibrated protocols.”²⁹

—NordiQC
# ADDRESSING IHC CHALLENGES: BEST PRACTICES IDENTIFIED BY AND FOR PATHOLOGISTS

## Preanalytic considerations to address sample consistency issues

<table>
<thead>
<tr>
<th>Preanalytic Consideration</th>
<th>Recommendations</th>
</tr>
</thead>
</table>
| Inadequate tissue sample | Obtain an excisional biopsy to ensure adequate tissue for morphologic and molecular analysis.\(^{30}\)  
  - A core biopsy may be acceptable for a difficult-to-access site\(^ {30}\)  
  - Fine needle aspiration is not useful in this case\(^ {31}\)  
  - Inform the clinician as to which specimens are inadequate or suboptimal and why\(^{22}\) |
| Suboptimal fixation protocol | Consider formalin fixation, as zinc formalin and B5 may impair CD30 immunostaining.\(^{22}\)  
  - Tissue fixation should be in a 10% neutral-pH phosphate-buffered formalin solution for a minimum of 8 hours\(^{28}\)  
  - Avoid over-fixation of >24 hours in formalin\(^{22}\) |

## Analytic considerations to increase accuracy of results

<table>
<thead>
<tr>
<th>Analytic Consideration</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficient HIER</td>
<td>Consider antigen retrieval in an alkaline buffer or a modified low pH buffer (ie, Target Retrieval Solutions pH 6.1, Dako or Diva Decloaker pH 6.2, BioCare).(^{29})</td>
</tr>
<tr>
<td>Low concentration of the primary antibody</td>
<td>Perform primary antibody calibrations using best-performing anti-CD30 antibodies (ie, Ber-H2, CON6D/5, and JCM182, all of which have obtained optimal staining results).(^{29})</td>
</tr>
</tbody>
</table>

---

**VISIT NORDIQC.ORG:**

- For details on assay validation with CD30 proficiency testing through the Institute of Pathology, Aalborg University Hospital (click **Info** tab and **Subscription** link on homepage)
- For recommended protocols for CD30 and other markers
### Validation and verification considerations

<table>
<thead>
<tr>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unclear validation</strong></td>
</tr>
</tbody>
</table>
| Test a minimum of 25 separate tissue specimens by an alternative validated method in the same laboratory or by a validated method performed in another laboratory.  
  - ≥10 samples should have high levels of the target antigen  
  - ≥10 samples should have intermediate to low levels of the target antigen  
  - ≥5 samples should have no IHC evidence of the target antigen  
  - Keep in mind that as the complexity of the IHC assay increases, verification requires a significantly larger number of samples |

| **Uncertain verification** |
| Calibrate protocols to address CD30 detection in low-level antigen-expressing cells. |

### Assay sensitivity considerations

<table>
<thead>
<tr>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inappropriate control sample</strong></td>
</tr>
</tbody>
</table>
| Select appropriate control samples.  
  - Tonsil tissue is the recommended control tissue for CD30  
  - Some lymphomas have a moderate or low level of CD30 expression  
  - An HL sample will not provide information on the limit of detection and should not be used as a control sample  
  - Using a Hodgkin lymphoma sample may impair the ability to evaluate CD30 in neoplasms with low-level expression |

| **Inconclusive staining** |
| Follow a staining protocol that provides a weak-to-moderate, but distinct, membranous signal of interfollicular activated B- and T-cells and activated B-cells mainly located in the rim of the germinal centers. Virtually all other cells must be negative. |

| **False-positive staining** |
| Be aware that plasma cells, macrophages, and endothelial cells may test positive depending on primary antibody clones.  
  - Plasma cells can be positive for Ber-H2  
  - Endothelial cells and macrophages can be positive for JCM182 |

---

No single IHC assay produces consistent, high-quality results across all antigens, antibodies, and tissue types. Consider changing antibody clones if an assay cannot be optimized.
Seattle Genetics recommends using the following descriptors to aid in characterizing CD30 expression in pathology reports.

<table>
<thead>
<tr>
<th>Report descriptors</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD30 detected (≥1% expression)</strong></td>
<td>Presence of ≥1% membranous and/or Golgi staining in tumor (neoplastic) cells. Staining at any intensity should be counted. In cases where it is difficult to differentiate tumor cells from normal lymphocytes, use total lymphocytes as the denominator for determination of percentage.</td>
</tr>
<tr>
<td><strong>CD30 not detected (&lt;1% expression)</strong></td>
<td>Presence of &lt;1% staining in tumor cells.</td>
</tr>
<tr>
<td><strong>Staining intensity</strong></td>
<td>1+, 2+, 3+</td>
</tr>
</tbody>
</table>

**Examples of samples with and without CD30 detection**

1. PTCL-NOS <1%; negative CD30
2. AITL 5%; scattered immunoblasts (G and M)
3. Transformed MF 40%; strong expression (M and G)
4. DLBCL 60%; intermediate, mainly G and some cytoplasmic
5. Unclassifiable lymphoma with features between HL and DLBCL 80%; strong expression (M and G)
6. ALCCL 90%; strong expression (M and G)
7. HL 100%; strong expression (M and G)

Percentage of CD30 expression

1. PTCL-NOS <1%; negative CD30
2. AITL 5%; scattered immunoblasts (G and M)
3. Transformed MF 40%; strong expression (M and G)
4. DLBCL 60%; intermediate, mainly G and some cytoplasmic
5. Unclassifiable lymphoma with features between HL and DLBCL 80%; strong expression (M and G)
6. ALCCL 90%; strong expression (M and G)
7. HL 100%; strong expression (M and G)

Pattern of staining: M (membranous) and G (Golgi).
Sample report useful for both pathologist and clinician

Interpretation: Final diagnosis: Hodgkin lymphoma

<table>
<thead>
<tr>
<th>Antibody/probe</th>
<th>Detected/not detected</th>
<th>Percentage of expression detected in tumor cells</th>
<th>Staining intensity 1+, 2+, 3+</th>
<th>Sensitivity of the assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD30</strong></td>
<td>Detected</td>
<td>100%</td>
<td>3+</td>
<td>1%</td>
</tr>
<tr>
<td>PAX5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBER</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUM1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CD30 should be consistently reported as a percentage
CD30: IMPROVING DIAGNOSTIC ACCURACY TO INFORM
TREATMENT DECISIONS

- CD30 is an important marker that aids in the differential diagnosis and classification of certain lymphomas and should be considered in all initial diagnostic HC panels.1,22-27
- CD30 testing has been shown to directly impact diagnosis and treatment decisions.1,20
- Accuracy is important for sample interpretation; IHC challenges exist but can be addressed with best-practice methods.22,28-30
- Standardized reporting helps improve the communication to clinicians.
  - Report the differential diagnosis.
  - List each marker run by IHC; report CD30 as “detected” or “not detected” AND include the percentage of CD30 expression in tumor cells.
    - “Detected” defined as ≥1% membranous and/or Golgi staining in tumor cells; “not detected” defined as <1% staining.

VISIT NORDIQC.ORG:
- For details on assay validation with CD30 proficiency testing through the Institute of Pathology, Aalborg University Hospital (click Info tab and Subscription link on homepage)
- For recommended protocols for CD30 and other markers

References: