Ki67-QC international working group: whole section scoring protocol (global method)
2018-11-29

This document describes the scoring protocol for scoring Ki67 on whole section.

*Please note: a mobile application implementing this scoring protocol is freely available from the Google Play and Apple iTunes store (search term: “Ki67”). This app helps determine the number of fields to score and calculate the final Ki67 score.*

**Step 1 (of 4): specifying the percentages**

a) Examine the entire glass slide section using low-power magnification (with 4x, 10x objectives).

b) Estimate the percentages of the invasive tumour in the glass slide that exhibit the following Ki67 scores (be sure to exclude carcinoma *in-situ* and non-tumour tissue such as necrosis and fibrosis):

- Negative  i.e. contains invasive cells but a very low (including zero) percentage of positive invasive cells
- Low
- Medium
- High

If staining is homogeneous across all invasive tumour (i.e., equal Ki67 across the entire invasive tumour component), specify 100% in the appropriate Ki67 level, and 0% for the other Ki67 levels.

Areas with increased Ki67 positivity, including areas towards the edge of the tumour, as well as non-proliferating areas of invasive tumour cells, must be included in the assessment.

**IMPORTANT:** Heterogeneity of percentage of cells staining positive frequently occurs across a section.

Therefore, scorers should select regions for scoring that are high, medium, low or negative in relation to the overall percentage positivity.

Thus, “negative”, “low”, “medium” and “high” are meant to be relative determinations, based on each particular case, and do not reflect specific absolute values.
The following are three examples illustrating how to estimate the percentage of Ki67-stained invasive tumour nuclei:

A pictogram of an idealised whole section is shown at left-hand side. The percentages for each Ki67 staining category are as indicated.

In this whole section the invasive tumour represents 50% of the total nuclei present (the other 50% is non-invasive tumour or non-tumoural). Therefore, when estimating the percentages of invasive tumour nuclei exhibiting various categories of staining the calculation is as shown in the table:

<table>
<thead>
<tr>
<th>Category</th>
<th>Absolute % of total nuclei</th>
<th>Relative % of invasive tumour nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>10%</td>
<td>10/50 x 100 = 20%</td>
</tr>
<tr>
<td>Low</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Medium</td>
<td>10%</td>
<td>10/50 x 100 = 20%</td>
</tr>
<tr>
<td>High</td>
<td>10% + 20% = 30%</td>
<td>30/50 x 100 = 60%</td>
</tr>
</tbody>
</table>
ESTIMATING THE PERCENTAGE OF KI67 STAINED INVASIVE TUMOUR NUCLEI: EXAMPLE 2

A pictogram of an idealised whole section is shown at left-hand side. The percentages for each Ki67 staining category are as indicated.

Relative % of invasive tumour nuclei in a particular Ki67 staining category = \( \frac{\text{Total % of invasive tumour nuclei in that category}}{\text{Total % of all invasive tumour nuclei present}} \times 100 \)

In this whole section the invasive tumour represents 80% of the total nuclei present (the other 20% is non-invasive tumour or non-tumoural). Therefore, when estimating the percentages of invasive tumour nuclei exhibiting various categories of staining the calculation is as shown in the table:

<table>
<thead>
<tr>
<th>Category</th>
<th>Absolute % of total nuclei</th>
<th>Relative % of invasive tumour nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>30%</td>
<td>30/80 x 100 = 37.5%</td>
</tr>
<tr>
<td>Low</td>
<td>50%</td>
<td>50/80 x 100 = 62.5%</td>
</tr>
<tr>
<td>Medium</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>High</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

ESTIMATING THE PERCENTAGE OF KI67 STAINED INVASIVE TUMOUR NUCLEI: EXAMPLE 3

A pictogram of an idealised whole section is shown at left-hand side. The percentages for each Ki67 staining category are as indicated.

Relative % of invasive tumour nuclei in a particular Ki67 staining category = \( \frac{\text{Total % of invasive tumour nuclei in that category}}{\text{Total % of all invasive tumour nuclei present}} \times 100 \)

In this whole section the invasive tumour represents 10% of the total nuclei present (the other 90% is non-invasive tumour or non-tumoural). Therefore, when estimating the percentages of invasive tumour nuclei exhibiting various categories of staining the calculation is as shown in the table:

<table>
<thead>
<tr>
<th>Category</th>
<th>Absolute % of total nuclei</th>
<th>Relative % of invasive tumour nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Low</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Medium</td>
<td>10%</td>
<td>10/10 x 100 = 100%</td>
</tr>
<tr>
<td>High</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>
Step 2 (of 4): selecting the representative fields

a) Based on the percentage of Ki67 representation specified in the previous step, use the following algorithm to determine the number and staining category (high/medium/low/negative) of required fields (up to 4) to score.

1. For each staining category present on the full slide (non-zero representation) allocate 1 scoring field.
2. If any of the staining categories is not present on the slide (zero representation) less than 4 fields would have been allocated and the remaining field(s) will be allocated as follows:

   One field remaining?
   
   yes
   
   no

   Two fields remaining?
   
   yes
   
   no
   
   no (3 fields remaining)

   Allocate all three fields to the single non-zero percentage category.

   Do not allocate i.e. only 3 fields will be scored.

   Ties between the 3 non-zero percentages e.g. 20% low, 40% medium, 40% high.
   
   yes
   
   no

   Allocate this field to the category with the highest percentage.

   Absolute difference between the 2 non-zero percentages < 25%?
   
   yes
   
   no

   Allocate the remaining 2 fields to the category with the highest percentage e.g. 63% low, 37% medium → 3 fields low, 1 field medium.

   Allocate the remaining 2 fields to the 2 categories e.g. 62% low, 38% medium → 2 fields low, 2 fields medium.


b) Select the fields indicated by step a) and take note of the location of the selected fields.

Step 3 (of 4): scoring the representative fields

For each of the required fields selected in step 2, do the following

a) Position the high-powered (40x objective) microscope field to the area of the selected scoring field.

b) Count invasive tumor nuclei in a “typewriter” pattern (please refer to: Appendix A. Typewriter pattern) from the top of the selected scoring field, until either 100 invasive tumour nuclei in total has been counted or all invasive tumor nuclei in the entire scoring field has been counted, whichever comes first.

c) Note the number of nuclei counted that are stained positive or negative for Ki67. For the definition of positive/negative staining, please refer to: Appendix B. What counts as “positive”?
Step 4 (of 4): calculating the final Ki67 score

The final Ki67 score can be calculated as follows (there are two versions):

\[
\text{unweighted Ki67 score} = \frac{\text{total } \# \text{ of } +ve \text{ tumor nuclei counted in all fields}}{\text{total } \# \text{ of tumor nuclei counted in all fields}} \times 100
\]

\[
\text{weighted Ki67 score} = \sum_{i \ in \ \text{neg, low, med, high}} \frac{\% \text{ of slide with } i^{th} \text{ staining category}}{\text{total } \# \text{ of } +ve \text{ tumor nuclei counted in fields with } i^{th} \text{ staining category}} \times \frac{\text{total } \# \text{ of tumor nuclei in fields with } i^{th} \text{ staining category}}{\text{total } \# \text{ of tumor nuclei counted in all fields}} \times 100
\]
Appendix A. Typewriter pattern

The following image shows a typewriter nuclei counting pattern. The green circle indicates the selected scoring field.
Appendix B. What counts as “positive”? 

“Positive” = any definite brown staining in the nucleus of an invasive breast cancer cell, above the surrounding background in cytoplasm and extracellular matrix.

- Cells with any degree/intensity of brown nuclear staining are considered positive.
- Cells showing only blue haematoxylin counterstain (i.e. an absence of brown nuclear staining) are considered negative.

The following are example images of range of staining levels that should be considered positive (red squares) for Ki67 (unmarked & marked side by side).
The following are example images of range of staining levels that should be considered **negative (green circles)** for Ki67 (unmarked & marked side by side).

Negative nuclei are ordered from weakest to strongest blue stain.