Response to Reviews for EDL Submission

Basic panel with Immunohistochemical testing (IHC) for solid tumors

Pre-submission ID Number: 63
Full Submission ID Number: 38

Reviewer’s questions in bold font

#Reviewer 1

The antibodies put forward cover the major solid tumor types except for heme-malignancies (epithelial, mesenchymal, neuroectodermal, and neuroendocrine) which have different treatment algorithms in all but the most limited health-care settings. I generally recommend accepting this application, although I would like a little more explanation of the progressive use of the panel. For example, with the use of myogenin, my take is that this would only be performed if the desmin were positive to differentiate alveolar rhabdomyosarcoma from embryonal, and I would like to see LCA included as an alternative fifth antibody, to be used when keratin, S100 and desmin are negative. The breadth of IHC available in limited resources is not going to be limited to five IHC, labs will typically have a small number of heme-path markers to subclassify lymphoma and may have more than one keratin. The point of a limited panel is that to perform more five IHC to classify a tumor becomes very expensive, and essentially is prohibitive, so IHC can be performed in a step-wise manner to minimize waste of resources, and I would like to see a detailed explanation for the use of this panel put forward that includes whether to run four or five IHC in one go, or whether to go step-wise, applying the most likely positive result antibody first (keratin), followed by S100 and LCA if keratin negative (to rule out melanoma and nerve sheath tumors and almost all lymphomas), and then desmin, and then consider if the tumor has neuroendocrine features, to include synaptophysin etc.

1. Please put forward a step-wise algorithm for use of these antibodies and provide a sequence for use with explanation.

The essential panel is intended to acknowledge the fundamental role of IHC in the diagnosis and differential diagnosis of childhood cancer. However, the submission is not intended to work as guidelines or guidance for diagnosis of pediatric solid tumors, like a Path. As the panel was built in an evidence-based manner, we here provide an example of its step-wise utilization, to minimize waste of resources. Cellular morphology can orient the sequence of use of the IHC antibodies e.g. spindle cell or round cell
malignancy. An evidence based algorithmic immunohistochemical analysis for small round blue cell tumors is here reported (re-designed from: Bahrami A, Arch Pathol Lab Med 2008).

The tumors reported in the algorithm are to be intended as an example of the positive/ negative staining and not the sole diagnosis possible; multiple antibodies can be requested to confirm a diagnosis and a precise confirmation can require other antibodies, outside the essential panel. The intention of a minimalistic panel is to save resource and optimize the diagnostic algorithm.

2. **Please consider adding in LCA to this panel, as in reality, this would be a routinely performed IHC if the cytomorphology were compatible with a lymphoma in an unknown solid tumor diagnostic setting.**

Leukocyte common antigen (CD45/LCA) and keratin expression are generally mutually exclusive in diagnostic pathology. The panel here presented was not inclusive of LCA since a distinct panel for lymphoma has been submitted. However, as seen in the algorithm, the two panels are necessarily inter-linked in the diagnostic process of childhood cancer, particularly in the work-up of undifferentiated tumors. As the panel is intended for solid tumors and CD45 already part of the “hematological panel”, we voluntarily omitted here to avoid a duplicate. To respond to the reviewer, the LCA is absolutely part of the “essential” requirement of a laboratory IHC to diagnose childhood cancer, as suggested: the “solid tumors” and “hematological malignancies” panels are complementary.
Reviewer 2. No questions.