Reviewer’s Questionnaire for Evaluation of Submissions for EDL v3
Based on the Criteria for Selection of Essential Diagnostics for the EDL

Diagnostic test: A panel of five antibodies for immunohistochemistry in FFPE tissues, as an aid to diagnosis in pediatric and adult solid tumors.

Test purpose: To enable more accurate classification of neoplasms for the purpose of prognosis and treatment, particularly in low and middle-income countries with limited resources for classification of tumors.

ID number: PreSubmission_ID63_FullSubmission_ID38

The selection process for essential diagnostics for the EDL will include consideration of a number of factors, including:

1. The public health and clinical need for the category of tests as determined for example, by disease burden and whether the proposed category of IVDs can help to bridge any existing gap in access to diagnostics that has been identified.

Questions:
1. Does the disease addressed by the test cause:
   ☒ a high burden of morbidity (human suffering)
   ☒ mortality
   ☒ cost on the populations and societies where it occurs

2. How strong is the evidence provided to support this?
   ☐ weak
   ☒ strong

Please complete the sub-questions below on evidence provided:
   a. Disease prevalence data?
      ☒ yes
      ☐ no
   b. Information on the disease impact on the quality of life of its sufferers?
      ☐ yes
      ☒ no
   c. Information on the disease impact on the quality of life of the families of sufferers and the communities in which they live? E.g. patients with high care needs, orphans, spread of infection
      ☐ yes
      ☒ no
d. Impact assessments on health care resources and budgets?
   ☒ yes
   ☐ no

2. Is any information provided showing the degree of access to diagnostic testing for the addressed disease in the primary care setting?
   ☐ yes
   ☒ no
   Comment: IHC assays are not for use in primary care setting.

   Does the submitted test category help to increase access in any way? E.g. reduced skill required, lower cost, improved performance vs alternative options
   ☒ yes
   ☐ no
   Comment: By providing guidance on the five most useful initial screening antibody immune-stains to perform when evaluating a solid tumor, it reduces cost overall of not performing unhelpful immunohistochemistry. The application also includes a reference for setting up a very cost-efficient immunohistochemistry lab.

Note: Answers to the questions above will have been assessed as part of the screening application and will have been deemed acceptable. Nevertheless, information provided on these matters in the full application may be commented upon in your assessment.

2. Availability of validated commercial diagnostic tests as indicated by sound and adequate data on quality, safety, performance, and regulatory status.

Questions:
1. How many commercially available IVDs are included in the application for this category?
   5
   a. Does the submission include a list?
      ☒ yes
      ☐ no
      Does the application consider IVDs of all technologies \(^1\) that are available for the analyte\(^2\) of interest?
      ☒ yes
      ☐ no

2. Which national regulatory bodies have approved these tests for market access e.g. CE IVD, US FDA, SFDA, WHO-PQ, others?

   Not applicable in this instance (the cancer biomarkers recommended are not FDA-approved)

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\(^1\) Technologies: It may be that, within the IVD category, there are tests that use different technologies to measure or detect the same analyte e.g. an RDT or and EIA for HIV antibody

\(^2\) Analyte: Marker that the IVDS in the category measures or detects
3. Have package inserts been provided showing studies demonstrating quality, safety, and performance of regulatory approved IVDs in this category?

Quality: ☒ yes  ☐ no
Safety:   ☒ yes  ☐ no
Performance: ☒ yes  ☐ no

   a. If so, what is your assessment of the strength of the study data described in the package inserts?

Comment: The example provided is for one of the antibodies (desmin). In the case of this application, the antibodies recommended in the panel are generic and very widely used in every laboratory evaluating tumor specimens by immunohistochemistry. Whilst specific clones and vendors have different sensitivity and performance, antibodies are typically similar in their safety.

4. Have any independently published studies been provided, showing IVDs’ performances compared to a recognised gold standard? How strong are these studies?

   ☒ yes  ☐ no

   a. If no gold standard exists, what is your assessment of the characterisation of the studies’ specimens?

Comment: There are a number of publications working through algorithms for immunohistochemistry use in diagnosing solid tumors. The issue in low and middle-income settings, is always cost of the immunohistochemistry, such that use of more than five immunostains to diagnose a tumor becomes cost-prohibitive. Thus, very careful decisions must be made regarding which antibodies will yield the most useful diagnostic information when applied to the tumor. The five-IHC panel outlined is practical and covers the most useful very basic differentiating antibodies in published panels, although myogenin would not be applied unless desmin were positive. Adding LCA to this antibody panel, for use when the cytomorphology is compatible with a lymphoma, and when keratin, S100, desmin and synaptophysin are all negative as an alternative “fifth” antibody would be in keeping with recommended antibody panels for “unknown tumor diagnosis”.

5. Where relevant, have studies to demonstrate ease of use by trained lay providers been provided?

   ☐ yes  ☒ no

   What is your assessment of these studies?

Comment: IHC assays cannot be performed by lay providers

6. Where relevant, have studies been provided to show the IVD’s robustness in variable environmental conditions e.g. temperature and humidity?

   ☒ yes  ☐ no

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3 Robustness: An IVD’s capacity to remain unaffected by small variations in method parameters, which provides an indication of its reliability during normal usage.
Comment: The product inserts of antibodies include instructions to store at 2 - 8°C, and not to use beyond the expiry date. When stored used according to manufacturer’s instructions, reagents for IHC are robust.

3. **Clinical effectiveness** based on published peer reviewed data, safety and comparative cost-effectiveness.

Questions:
1. Has the applicant provided strong peer reviewed clinical studies that demonstrate the clinical utility and effectiveness of IVDs in this category?
   - Clinical utility: ☒ yes ☐ no
   - Effectiveness: ☒ yes ☐ no

2. Are you satisfied that these studies are properly designed and sufficiently powered statistically to support their conclusions? ☒ yes ☐ no

3. Has the applicant provided cost effectiveness, health economics or budget impact studies demonstrating the value of IVDs in this category?
   - Cost effectiveness: ☒ yes ☐ no
   - Health economics: ☒ yes ☐ no
   - Budget impact studies: ☒ yes ☐ no
   
   How strong are these studies in terms of design and statistical power? (See Note above)
   - Weak ☐
   - Strong ☒

4. Has the applicant provided pricing information for commercially available IVDs in this category? ☒ yes ☐ no
   
   a. Is the pricing information given inclusive of instrument and service costs where relevant? ☒ yes ☐ no
   
   In your experience, based on the pricing information provided, how accessible are IVDs in this category to LMIC settings?
   - Accessible: ☒ yes ☐ no
   - Not accessible: ☐ yes ☐ no

   Please provide examples to support your conclusions.

Comment: The inclusion of a reference of how to set up a 10 antibody IHC panel in low resource settings in the most cost-efficient way, using manual staining, provides much of this information. In general, the issues of quality control for non-quantitative IHC testing (i.e. not ER, PR or HER2) are not as hard to control as those for ER, PR and HER2 testing. Therefore, this panel of antibodies can be expected to be very robust and cost-effective in classifying tumors, provided the pre-analytic, analytic and post-analytic factors are attended to. (cold-ischemic time, quality of tissue fixation and duration of fixation, laboratory quality

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4 Clinical effectiveness: The degree to which a particular health care intervention does more good than harm. It is measured by the number of lives saved, or by improvements of objective parameters of a morbid condition

5 Clinical utility: The likelihood of improved outcomes from use of diagnostic tests in the IVD category
controls, and skilled pathologists reviewing and reading the immunohistochemistry and pathology cytomorphology findings).

Whilst the cost of five IHC in this scenario is $50-100, the costs are typically higher in my experience, closer to $30 per antibody, so a panel of five IHC could be $150 to $300, depending on antibody clone and vendor. Plus, positive and negative controls must be run with every batch, and preferably on-slide control tissue used.

5. In your experience, do you consider the cost of tests in this category (cost per test includes reagents, any amortised instrument capital expenditure and service contracts) to justify the clinical benefits. Please provide examples to support your conclusions.
☒ yes ☐ no

Examples

Whilst treatment options may be limited in low and middle-income settings, being able to differentiate between heme neoplasms, common carcinomas, melanomas and nerve-sheath tumors and sarcomas is very important for deciding treatment. Surgery is not the treatment of choice for heme neoplasms, but is the treatment of choice for many carcinomas, for melanomas and many sarcomas, with limited role for chemotherapy or radiation therapy. Being able to classify a tumor and direct a patient down a path of heme-chemotherapy, carcinoma-chemotherapy, sarcoma-chemotherapy, and/or surgery is essential. This depends on being able to make an accurate diagnosis.

4. Appropriateness of the IVD category for use at specified levels of the laboratory or health care system.
Answer questions 1 and 2 for each IVD technology in the category. A table may help with reaching your recommendation, the characteristics of each IVD represented by one row of the table

1. a. What specimen type is required?

Comment: Cancer tissue in the form of FFPE (formalin-fixed paraffin embedded blocks)

b. What skill level and training is required for specimen collection? E.g. Phlebotomist

Comment: Advanced skills – the tissue is procured by a doctor, typically a surgeon in low-resource settings and the specimen must be handled appropriately to avoid false-negative results with IHC testing (rapid placement into formalin and good quality grossing procedures followed).

c. Do specimens need to be processed in any way prior to analysis? E.g. centrifugation, microscope slide staining, etc. ☒ yes ☐ no
i. If so, for how long and at what temperature is the specimen stable before being processed (00:00:00 hours, min, seconds format)

ii. At what temperature is the processed specimen stored before testing (please specify if Celsius or Fahrenheit)

Comment: Pre-analytic issues: The tissue must be fixed in 10% formalin, processed and made into a paraffin block, with tissue sections cut and placed on a microscope slide requiring highly skilled technicians. Tissue fixation times must be monitored (6-72 hours), and cold-ischemic time should be <1hr to reduce the risk of false-negative IHC results.

d. How long does it take to get a result? E.g. can a result be obtained during a consultation i.e. < 10 minutes, or while the patient is at the facility i.e. 2 – 3 hours or specimens are tested in a batch using the IVD i.e. days?

Comment: An IHC run typically takes 2 - 3 hours, but the pre-analytic step takes a minimum of 12 hours, and result must also be read by a skilled pathologist. This is always too long for same-day results, but can be obtained within 24 hours.

e. Where relevant to the IVD has ease of and effective use by trained lay providers been demonstrated?

☐ yes ☒ no

f. What equipment, if any, is required to perform this type of test?

Comment: All IHC can be performed manually (with potential for quality assurance issues) or using an autostainer (automated process). Other requirements are standard lab equipment (e.g. heat bath) for handling anatomic pathology specimens and performing IHC on FFPE tissue.

g. Do instruments need to be calibrated, maintained, or serviced on a regular basis?

☒ yes ☐ no

h. How robust is the IVD?

Comment: Provided the test kit has been transported and stored according to instructions (at 2-8C), and all instructions for use are adhered to rigidly, the IVD is robust.
i. What is the impact of an unreliable power supply, or can the IVD operate without a power supply?
Comment: The test cannot be performed without a power supply.

What is the minimal skill level and training required for personnel to perform this test?
☐ Unskilled
☐ Skilled
☒ Highly trained

2. Considering a 4-tier laboratory system, with the following levels:
   i. Primary care
   ii. District hospitals/laboratories
   iii. Regional hospitals/laboratories
   iv. National hospitals/Reference laboratories

in your judgement, which level would be best suited to handle the required complexity of the relevant IVD?? Please include your answer in the table based on the likely availability of the following at district, regional and national laboratory level:
   a. Infrastructure requirements e.g. instrument size and complexity, biosafety requirements
   b. Specimen types
   c. Testing volumes expected (sample throughput required)
   d. Complexity of specimen handling e.g. biosafety level required, centrifugation or complex protocols requiring highly skilled laboratory technicians
   e. Availability of infrastructure for transporting specimens
   f. Result turn-around times required
   g. Reagent shipping, storage and operating conditions required
   h. Where relevant, instrument operating conditions required
   i. Required qualifications, training and skill levels needed for test performance and result interpretation e.g. non-laboratory personnel for a simple rapid test, trained laboratory technician to perform routine testing, medically trained personnel for result interpretation, Ph.D. level scientist required for highly complex and variable methodologies
   j. Quality management requirements based on complexity of facilities & support required to perform the test

Comment: I would recommend this assay is for use in national hospitals/reference laboratories that are accredited and can adhere to internal and external quality control procedures. It can also be used by regional hospitals/laboratories, provided those hospitals can demonstrate their quality control procedures and what they are doing to ensure accuracy of the results they report out. Breast cancer biomarker testing in laboratory settings with low volume IHC should be discouraged, given the
semi-quantitative skilled nature of the scoring algorithm, and large variety of pre-analytic and post-analytic factors that can influence the test result.

Proposed answer table:

<table>
<thead>
<tr>
<th></th>
<th>Primary care</th>
<th>District hospitals/lab</th>
<th>Regional hospitals/lab</th>
<th>National hospitals/Reference lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infrastructure requirements</td>
<td>No</td>
<td>No</td>
<td>Possibly – if have access to tissue processing facilities</td>
<td>yes</td>
</tr>
<tr>
<td>Specimen types</td>
<td>No</td>
<td>Yes</td>
<td>Yes: at least 5 tests/week</td>
<td>Yes: Much greater than 5 tests/week</td>
</tr>
<tr>
<td>Testing volumes expected</td>
<td>None</td>
<td>None</td>
<td>Yes: Much greater than 5 tests/week</td>
<td>Yes: Much greater than 5 tests/week</td>
</tr>
<tr>
<td>Complexity of specimen handling</td>
<td>Not available</td>
<td>Not available</td>
<td>Yes. If technical staff are trained to perform IHC</td>
<td>Yes. Technical staff should be trained to perform IHC</td>
</tr>
<tr>
<td>Infrastructure for transporting specimens</td>
<td>Rarely available in quality and timely manner</td>
<td>Rarely available in quality and timely manner</td>
<td>Likely available Poor tissue handling is always an issue in LMICs</td>
<td>Likely available Poor tissue handling is always an issue in LMICs</td>
</tr>
<tr>
<td>Result turn-around times required</td>
<td>-</td>
<td>-</td>
<td>24 hours minimum from tissue procurement</td>
<td>24 hours minimum from tissue receipt</td>
</tr>
<tr>
<td>Reagent shipping, storage and operating conditions required</td>
<td>-</td>
<td>-</td>
<td>Yes, a lab should be able to order and store reagents appropriately. May not have technical expertise to run assay</td>
<td>Yes, a lab should be able to order and store reagents appropriately, and have technical expertise to run assay</td>
</tr>
<tr>
<td>Instrument operating conditions required</td>
<td>Not available</td>
<td>Not available</td>
<td>Equipment maintenance is essential, and contracts may not be available</td>
<td>Equipment maintenance is essential, and contracts should be in place</td>
</tr>
<tr>
<td>Required qualifications, training and skill levels</td>
<td>Not available</td>
<td>Not available</td>
<td>Possibly available</td>
<td>Available and required</td>
</tr>
<tr>
<td>Quality management requirements</td>
<td>Not available</td>
<td>Not available</td>
<td>Required for accuracy of assay</td>
<td>Required for accuracy of assay</td>
</tr>
</tbody>
</table>
5. What is your recommendation to SAGE IVD? Please summarise the key points you considered in reaching your conclusion.

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.

6. Please list the items that require further clarification from the originator of this submission.

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

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.