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

Diagnostic test: Aspergillus antigen
Test purpose: Diagnosis of invasive aspergillosis (IA)
ID number: PreSubmission_ID69_FullSubmission_ID4

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.

Draft 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

3. Is any information provided showing the degree of access to diagnostic testing for the addressed disease in the primary care setting?
   ☐ yes
   ☒ no
Comment: No studies were provided to show the degree of access to diagnostic testing of *Aspergillus* antigen for the screening or diagnosis of IA in primary care settings. Information was provided for the use of *Aspergillus* antigen, galactomannan (GM), in the diagnosis of suspected IA in inpatients with immunocompromising conditions such as AIDS/HIV, neutropenia, cancer, intensive chemotherapy and leukaemia.

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:

Although quantitative detection of GM in serum or bronchoalveolar lavage (BAL) using enzyme-linked immunosorbent assay (ELISA) requires training and skilful laboratory personnel, qualitative lateral flow devices (LFD) [also called “lateral flow assays” (LFA)] for *Aspergillus* antigen can increase access to point-of-care screening for IA in primary health care settings with trained non-laboratory personnel. *Aspergillus* antigen test can improve the diagnostic performance in case of IA in immunocompromised patients to establish early diagnosis and avoid empirical treatment, particularly when used in adjunct with other traditional tests. Fungal cultures are neither sensitive nor specific and require several days, delaying the diagnosis and treatment that are crucial for saving lives of immunocompromised patients. Histopathologic sampling is invasive and challenging in many patients and its examination is time-consuming and usually insensitive, and molecular testing is not cost-effective in many LMICs and requires high technicality and expertise. Moreover, molecular assays for the detection of *Aspergillus* lack standardization.
2. **Availability of validated commercial diagnostic tests as indicated by sound and adequate data on quality, safety, performance, and regulatory status.**

Draft questions:
- How many commercially available IVDs are included in the application for this category?
  - [ ] Does the submission include a list?
    - ☒ yes
    - ☐ no
    
    The application includes five commercially available IVD brands for the detection of *Aspergillus GM*.
    - Three ELISA-based IVDs: Platelia Aspergillus antigen (Bio-Rad, France), *Aspergillus GM Assay* (Dynamiker) and Goldstream *Aspergillus GM Assay* (ERA Biology).
    - Two LFD-based IVDs: *sona Aspergillus GM Lateral Flow Assay* (Immy, Alpha Laboratories) and *AspLFD (OML)*

Does the application consider IVDs of all technologies that are available for the analyte of interest?
- ☒ yes
- ☐ no

- Which national regulatory bodies have approved these tests for market access e.g. CE IVD, US FDA, SFDA, WHO-PQ, others?
  - All brands are CE-marked
  - Platelia is US-FDA approved.

- 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

  - If so, what is your assessment of the strength of the study data described in the package inserts? No package inserts were provided with the application.

- Have any independently published studies been provided, showing IVDs’ performances compared to a recognised gold standard? How strong are these studies?
  - ☒ yes ☐ no
  - If no gold standard exists, what is your assessment of the characterisation of the studies’ specimens?

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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
The performance of serum and BAL GM ELISA in the diagnosis of IA among different groups of immunocompromised patients has been assessed by several systematic reviews and meta-analyses. Below are some examples:

- Cochrane systematic reviews and meta-analyses of the diagnostic performance of serum and BAL GM ELISA for the diagnosis of IA in immunocompromised using the European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC/MSG) criteria for IA as a reference method patients (Cochrane Reviews; Debets-Ossenkopp et al. 2015 and de Heer et al. 2019).

**Main findings:**
- The diagnostic accuracy of BAL GM ELISA is slightly better than serum GM ELISA.
- BAL GM ELISA has a higher sensitivity (88.0%) at cut-off value of 0.5 but a higher specificity at cut-off 1.0 (93.0%).

<table>
<thead>
<tr>
<th>Cut-off value</th>
<th>Sensitivity % (95% CI)</th>
<th>Specificity % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum GM ELISA</td>
<td>BAL GM ELISA</td>
<td>Serum GM ELISA</td>
</tr>
<tr>
<td>0.5 ODI</td>
<td>78.0 (70.0-85.0)</td>
<td>88.0 (75.0-100.0)</td>
</tr>
<tr>
<td>1.0 ODI</td>
<td>71.0 (63.0-78.0)</td>
<td>78.0 (61.0-95.0)</td>
</tr>
<tr>
<td>1.5 ODI</td>
<td>63.0 (49.0-78.0)</td>
<td>---</td>
</tr>
</tbody>
</table>

- A Cochrane review (Crucciani et al. 2019) showed that a single polymerase chain reaction (PCR) has a sensitivity of 79.2% (71.0-85.5%) and specificity of 79.6% (69.9-86.6%) using the EORTC/MSG criteria for IA as a reference standard.
  - The sensitivity of a single blood PCR is comparable to and its specificity is lower than those of serum GM ELISA at a cut-off value of 0.5 ODI.
- A systematic review and meta-analysis to determine the diagnostic accuracy of serum and BAL GM in patients with suspected IA (Haydour et al. 2019; Ann Am Thorac Soc.) revealed the following:

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity % (95% CI)</th>
<th>Specificity % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum GM</td>
<td>71.0 (64.0-78.0)</td>
<td>89.0 (78.0-92.0)</td>
</tr>
<tr>
<td>BAL GM</td>
<td>84.0 (73.0-91.0)</td>
<td>88.0 (81.0-91.0)</td>
</tr>
</tbody>
</table>

- A meta-analysis to assess the diagnostic accuracy of serum GM in the diagnosis of paediatric IA using the EORTC/MSG criteria for IA as a reference standard showed that serum GM had a sensitivity of 85.0% (72.0-93.0), specificity of 88.0% (80.0-93.0), positive likelihood ratio (PLR) of 6.92 (4.40-10.88) and negative likelihood ratio (NLR) of 0.17 (0.09-0.32) (Tong et al. 2018; Microb Pathog).
- A systematic review and meta-analysis of the accuracy of BAL GM in the diagnosis of IA using the EORTC/MSG criteria for IA as a reference standard showed that BAL GM had a pooled sensitivity of 87.0% (79.0-92.0), pooled specificity of 89.0% (85.0-92.0), PLR of 8 (5.7-11.1) and NLR of 0.15 (0.10-0.23), where the optimal cut-off value was 1.0 ODI. On the other hand, BAL GM showed higher sensitivity than serum GM and PCR but lower specificity than serum GM (Zou et al. 2012; PLoS One).
- The performance of serum and BAL GM LFD in the diagnosis of IA among different groups of immunocompromised patients has been assessed by several systematic reviews and meta-
analyses, but the results of studies evaluating the diagnostic accuracy were conflicting. Below are some examples:

- A meta-analysis of 7 studies (2008-2015) on the accuracy of LFD in the diagnosis of proven/probable IA versus no IA according to EORTC/MSG showed that a good accuracy of Aspergillus LFD for the diagnosis of IA, with better performance of BAL LFD than serum LFD (Pan et al. 2015; J Med Microbiol). Performance values are shown below:

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity % (95% CI)</th>
<th>Specificity % (95% CI)</th>
<th>Diagnostic OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL LFD</td>
<td>86.0 (76.0-93.0)</td>
<td>93.0 (89.0-96.0)</td>
<td>65.9 (27.2-159.8)</td>
</tr>
<tr>
<td>Serum LFD</td>
<td>68.0 (52.0-81.0)</td>
<td>87.0 (80.0-92.0)</td>
<td>11.9 (3.5-40.0)</td>
</tr>
</tbody>
</table>

- A subsequent meta-analysis of BAL Aspergillus LFD for the diagnosis of probable/proven IA versus no evidence for IA in different patient cohorts showed that LFD had overall sensitivity of 73% and specificity of 90%, PPV of 61% and NPV of 94% (Heldt and Hoenigl 2017; Curr Fungal Infect Rep).
  - A major limitation of Aspergillus LFD is the reduced sensitivity in case of patients undergoing mould-active antifungals (86.0% versus 56%).

- A review of the diagnostic performance of Aspergillus-LFD in various cohorts of immunocompromised patients showed a sensitivity of 65-94%, specificity of 85-92%, PPV of 57-73% and NPV of 84-99% for BAL specimens, while serum Aspergillus-LDF showed sensitivity of 40.0-81.8% and specificity of 84.4-98.0% (Prattes et al. 2016: Curr Fungal Infect Rep).

- The utility of serum/BAL GM has been recommended in well-established guidelines, including:
  - The American Thoracic Society (ATS) strongly recommends with high-quality evidence the use of serum GM testing in severely immunocompromised patients suspected of invasive pulmonary aspergillosis and the use of BAL GM testing in patients suspected of IA with a negative serum GM or with possibly false-positive serum GM results (Hage et al. 2019; Am J Respir Crit Care Med).
  - According to the 2016 guidelines of the Infectious Diseases Society of America (IDSA) for the diagnosis of IA, serum and BAL GM are strongly recommended with high-quality evidence as accurate markers for the diagnosis of IA in adult and paediatric patients when used in certain patient sub-groups (hematologic malignancy and haematopoietic stem cell transplantation). BAL GM (but not serum GM) is strongly recommended for screening patients receiving anti-mould therapy or prophylaxis (Patterson et al. 2016; Clin Infect Dis).
  - According to the 2017 guidelines of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), the European Confederation of Medical Mycology (ECMM) and the European Respiratory Society (ERS), serum and BAL GM measures are recommended as markers for the diagnosis of IA (Ullmann et al. 2018; Clin Microbiol Infect).
  - According to the 2015 guidelines of the International Society for Heart and Lung Transplantation (ISHLT), BAL GM (but not serum GM) can be used for the diagnosis of IA and to distinguish between colonization and IA (Husain et al. 2016; J Heart Lung Transplant).
  - According to the clinical practice guidelines for the diagnosis of IA infections in the Middle East (2012), serum and BAL GM measures are recommended as markers for the diagnosis of IA and that these should be available in typical tertiary care centres with high number of patients at risk of IA (Al-Abdely et al. 2014; J Infect Pub Health).
• Where relevant, have studies to demonstrate ease of use by trained lay providers been provided?
  □ yes ☒ no

What is your assessment of these studies? Not applicable.

• Where relevant, have studies been provided to show the IVD’s robustness\(^3\) in variable environmental conditions e.g. temperature and humidity?
  □ yes ☒ no


Draft questions:
1. Has the applicant provided strong peer reviewed clinical studies that demonstrate the clinical utility \(^5\) 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
   Not applicable
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
   b. In your experience, based on the pricing information provided, how accessible are IVDs in this category to LMIC settings?
      accessible: □ yes ☒ no

\(^3\) Robustness: An IVD’s capacity to remain unaffected by small variations in method parameters, which provides an indication of its reliability during normal usage

\(^4\) 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
The cost per test (12 and 50 USD for ELISA and 15-18 USD for LFD) may not be affordable in many low- and middle-income countries (LMICs). Although the cost of the ELISA equipment is high (5000 USD), this can be purchased once by tertiary care hospitals.

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

In LMICs, the cost per test poses high financial burden because the immunocompromised patients may need serial testing for IA.

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

a. What specimen type is required?
   Serum or BAL

b. What skill level and training is required for specimen collection? E.g. Phlebotomist
   A phlebotomist is required to collect venous blood for serum GM assays.
   A Clinician or well-trained nurse is required to collect BAL.

c. Do specimens need to be processed in any way prior to analysis? E.g. centrifugation, microscope slide staining, etc.
   ☒ yes  ☐ no  **Centrifugation and incubation**
   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)
      Serum: 2-8°C (up to 5 days for unopened specimens and 2 days for open samples). At -70°C for longer storage.
      BAL: 2-8°C for up to 24 hours for opened samples. At -20°C for longer storage of up to 5 months.

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?
   3 hours for ELISA and 25-30 min. for LFD.

e. Where relevant to the IVD has ease of and effective use by trained lay providers been demonstrated?
   ☒ yes  ☐ no  **In case of LFD only.**

f. What equipment, if any, is required to perform this type of test?
   **Centrifuge (to separate the serum)**
   Pipettes and micropipettes
   **Refrigerator (for reagent and sample storage)/freezer for long storage**
Microplate incubator and washer
Water bath

g. Do instruments need to be calibrated, maintained, or serviced on a regular basis?
☒ yes ☐ no For ELISA reader

h. How robust is the IVD?
The application does not include information about the robustness of *Aspergillus* GM assays (ELISA or LFD).

i. What is the impact of an unreliable power supply, or can the IVD operate without a power supply?
Unreliable power supply can negatively impact the performance of ELISA readers.
What is the minimal skill level and training required for personnel to perform this test?
☐ Unskilled
☒ Skilled
☒ Highly trained

*A skilled laboratory technician is required to perform ELISA.*
*Highly trained non-laboratory personnel to perform LFD.*

2. Considering a 4-tier laboratory system, with the following levels:
   i. Primary care
   ii. District hospitals/laboratories
   iii. Regional hospitals/laboratories and
   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
Proposed answer table:

<table>
<thead>
<tr>
<th>Infrastructure requirements</th>
<th>Primary care level</th>
<th>District hospitals/lab</th>
<th>Regional hospitals/lab</th>
<th>National hospitals/Reference lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refrigerator, centrifuge and water-bath or heat block (for qualitative detection using LFD)</td>
<td>Primary care level</td>
<td>District hospitals/lab</td>
<td>Regional hospitals/lab</td>
<td>National hospitals/Reference lab</td>
</tr>
<tr>
<td>ELISA reader, refrigerator, water-bath or heat block</td>
<td>District hospitals/lab</td>
<td>Regional hospitals/lab</td>
<td>National hospitals/Reference lab</td>
<td>Reference lab</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specimen types</th>
<th>Serum</th>
<th>Serum or BAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testing volumes expected</td>
<td>300 μl for initial processing</td>
<td></td>
</tr>
<tr>
<td>Complexity of specimen handling</td>
<td>Both serum and BAL specimens should be uncontaminated and need prior treatment</td>
<td></td>
</tr>
<tr>
<td>Infrastructure for transporting specimens</td>
<td>Cold chain</td>
<td></td>
</tr>
<tr>
<td>Result turn-around times required</td>
<td>25-30 min. (using LFD)</td>
<td>3 hours</td>
</tr>
<tr>
<td>Reagent shipping, storage and operating conditions required</td>
<td>Not required (kits can be stored at ambient temperature)</td>
<td>The kit components require storage at 2-8°C.</td>
</tr>
<tr>
<td>Instrument operating conditions required</td>
<td>Not required (for LFD)</td>
<td>Calibration and standardization of the ELISA reader are required</td>
</tr>
<tr>
<td>Required qualifications, training and skill levels</td>
<td>Highly trained non-laboratory personnel</td>
<td>Skilled laboratory technician</td>
</tr>
<tr>
<td>Quality management requirements</td>
<td>Positive controls are provided with the kit</td>
<td>Positive, negative and cut-off control sera are provided with the kits.</td>
</tr>
</tbody>
</table>

5. **What is your recommendation to SAGE IVD? Please summarise the key points you considered in reaching your conclusion.**

I recommend the inclusion of *Aspergillus* antigen test in the third EDL for the screening (using LFDs) and aid in diagnosis (using ELISA) of suspected IA as an adjunct to other tests in immunocompromised patients not undergoing antifungal prophylaxis or treatment. LFD can be used in primary care settings after training personnel on its performance, while serum or BAL GM ELISA (at generally accepted cut-off values of 0.5 for serum and 1-1.5 for BAL for positivity) can aid in the diagnosis of IA at the district or higher levels of health care with the required facilities and personnel. Published systematic reviews and meta-analyses revealed that GM ELISA had sensitivity levels of 71.0-78% using serum and 84.0-88.0% using BAL, and specificity levels of 85.0-89.0% using serum and 81.0-88.0% using BAL. On the other hand, *Aspergillus*-LFD showed sensitivity levels of 40.0-82.0% using serum and 65.0-94.0% using BAL, and specificity levels of 84.0-98.05 using serum and 85.0-93.05 using BAL.
The inclusion of *Aspergillus* antigen test in the third EDL is justified by the fact that fungal cultures are neither sensitive nor specific besides requiring several days. Although histopathologic evidence is crucial to confirm IA through determining whether *Aspergillus* growth in culture was significant, its sampling is invasive and challenging in many patients and its examination is time-consuming and usually insensitive. On the other hand, PCR is not cost-effective in many LMICs and requires high technicality and expertise. Moreover, molecular assays for the detection of *Aspergillus* lack standardization (Powers-Fletcher and Hanson, 2016; *J Clin Microbiol*). Moreover, the use of serum/BAL GM in the diagnosis of IA in immunocompromised patients is strongly recommended with high-quality evidence as per the guidelines of several societies concerned with invasive fungal infections, including the ATS, IDSA, ESCMID, ECMM, ERS, and ISHLT.

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

- Negative and positive predictive values of ELISA and LDF for detecting *Aspergillus* antigen.
- Optimization of the cut-off values of ELISA for the detection of *Aspergillus* antigen using different samples and among different categories of immunocompromised patients because there is a lack of consensus with this respect.
- Degree of diagnostic access to and cost-effectiveness of *Aspergillus* antigen LFDs for the screening of IA in primary care settings, particularly in LMICs.
- Stability of serum or BAL specimens collected from suspected patients before and after processing for the examination of *Aspergillus* antigen at different temperatures that cover the range in endemic countries.
- Robustness of *Aspergillus* antigen tests (LFD and ELISA) for the screening and diagnosis of IA in different situations and conditions.