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 testing
Test purpose: As stated by the applicant in the full application, ‘*Diagnosis of invasive aspergillosis.*’
ID number: PreSubmission_ID69_FullSubmission_ID04

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

Sections ‘7. Public health impact of the disease’, ‘8. Potential public health impact of the test’ and ‘20. Cost and cost-effectiveness’ of the applicant’s full application were reviewed. It is noted that information provided in the final paragraph of section 7 is unreadable. The applicant’s impact assessment is limited, particularly with regard to resources and budgets, and does not specifically consider the needs of low-middle income countries (LMIC) who may get the most utility out of the EDL.
3. Is any information provided showing the degree of access to diagnostic testing for the addressed disease in the primary care setting?

☐ yes
☒ no (not applicable)

Comment: The applicant states in their application to update the EDL that IVD products for Aspergillus antigen testing are for use by trained laboratory technicians/technologists only, and therefore are not intended for use in the 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: The testing of serum and/or bronchoalveolar lavage (BAL) fluids may offer improved access over the gold standard, as it would allow for testing of patients who are unable to tolerate resection and/or sampling procedures to collect specimens for histology and/or microscopy and culture.

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.**

Draft 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 that are available for the analyte of interest?
   - ☐ yes
   - ☒ no

The applicant has highlighted two (2) analytes of interest- GM and antigenic mannoproteins.

The applicant has provided a list of five (5) IVD products in Annex I accompanying the full application. The IVDs are named:

- Platelia™ *Aspergillus* Galactomannan Ag by Bio-Rad Laboratories*;
- Dynamiker *Aspergillus* Galactomannan Assay by Dynamiker Biotechnology (Tianjin) Co., Ltd*;
- Goldstream *Aspergillus* Galactomannan Assay by ERA Biology;
- sona *Aspergillus* Galactomannan Lateral Flow Assay by Immy; and
- AspLFD by OLM.

*Note that the full names of these companies have been identified through a Google search; see section 2.3 of this report for more information.

The IVDs listed by the applicant fall into three (3) categories-

- Three (3) enzyme-linked immunosorbency assays (ELISAs) for the qualitative detection of galactomannan (GM) in serum or BAL fluid;
- One (1) lateral flow assay (LFA) for the qualitative detection of GM in serum or BAL fluid; and
- One (1) LFA for the detective of antigenic mannoproteins in serum or BAL fluid.

It is noted that the applicant has not listed any latex agglutination tests, which are also commercially available for the qualitative detection of galactomannan to aid in the diagnosis of IA. It is also noted that this form of testing appears to have fallen out of favour in recent years, due to decreased sensitivity and specificity as compared to the immunochromatographic (ICT) techniques.

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

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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
No devices intended for use in the diagnosis of IA have been included on the WHO list of prequalified IVD products.

The applicant states in Annex I that all devices hold regulatory approval in the EU. An internet search was performed for all five (5) of the devices listed by the applicant in Annex I and confirms this to be the case.

To date, it appears that the Australian Therapeutic Goods Administration (TGA), Health Canada and United States Food & Drug Administration (FDA) have approved (of the devices listed by the applicant), only the Platelia™ Aspergillus Galactomannan Ag ELISA device from Bio-Rad Laboratories.

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?

Unable to locate any package inserts or instructions for use (IFU) in the applicant’s submission. An internet search was performed to identify any publicly-available IFUs for the devices listed. The search terms used were ‘[name of device as stated in Annex I] [manufacturer’s name as stated in Annex I] IFU’. The search identified the following:

- Product information for the sona Aspergillus Galactomannan Lateral Flow Assay by Immy [http://www.immy.com/aspergillus_galactomannan_lfa/]; and

This information has been used to inform other sections of this report. A limitation of this approach is that they may not represent the manufacturer’s current versions of IFU provided with these IVDs.
4. Have any independently published studies been provided, showing IVDs’ performances compared to a recognised gold standard? How strong are these studies?
   ☒ yes  ☐ no

For the purposes of this review, the gold standard for diagnosis of IA is as defined for the diagnosis of proven fungal disease as stated in the consensus statement from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) consensus group (de Pauw et al., 2008). This is the standard referenced most frequently in the submitted clinical studies and clinical practice guidelines.

In the four (4) out of five (5) primary studies submitted by the applicant, cases of proven IA were pooled with cases of probable IA. These will not be described further, as it is not clearly stated that diagnoses of probable IA were made independent of Aspergillus antigen testing results. Meersseman et al. (2008) conducted a cohort study in which they compared ante-mortem serum and BAL GM levels in patients found to have proven IA during post-mortem examination. From Meersseman et al., pg. 2-3/8-

Test Characteristics

**BAL GM levels.** On average, bronchoscopy with BAL was performed 6 days after admission to the ICU. A total of 156 procedures were performed. All 26 patients with proven IA had at least one BAL GM index ≥ 0.5 (range, 0.6–7.9). In 23 patients, the first BAL sample yielded a positive value, while in the remaining 3 patients, only the second BAL GM index was ≥ 0.5 (Table 2). The number of BAL GM-positive patients in the truly negative group (n = 46) was 6. As depicted in Figure 2A, the levels of GM in BAL were significantly higher in the

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Serum GM levels. A total of 397 serum samples were tested for GM (mean, 3.0 samples per patient). A total of 125 samples were analyzed from 26 patients with proven IA. 44% of these samples tested positive. The median value of GM in serum from proven cases was 0.3 (Figure 2B). Fourteen patients did not have a serum index ≥ 0.5 at the time of BAL positivity.

The study population in Meersseman et al. is small (n = 110) though this limitation is predominantly derived from the small number of patients meeting the inclusion criteria (~10% of those screened). Of those screened, only 26- or 2.3% - were found to have proven IA. Cohort studies are considered to represent level III evidence.

It is noted that there is variability in the design of the five (5) primary studies provided, the populations assessed and the reported outcomes (e.g. sensitivity and specificity of the assay).

Again, cases of proven IA were pooled with probable IA in all but two (2) of the meta-analyses. Comparison of GM results in proven IA is described in the meta-analyses by Zou et al. (2012) and Tong et al. (2018).

- **Zou et al. (2012)** looked at BAL GM in patients irrespective of patient factors. The reviewer notes the following from pg. 5/12-
• Tong et al. (2018) looked specifically at serum GM in paediatric patients. The reviewer notes the following from pg. 6/10:

3.3. Serum GM test for patients with proven IA vs. probable, possible or no IA

Of the studies included in this analysis, eleven studies [30,32–36,39–43] reported results of serum GM testing for proven IA. Among these studies, two [36,43] reported no proven IA patients and were excluded. The mean DOR was 7.69 (95% CI 2.56–23.09, I² = 91.5%, p = 0.000) (Figure S3). The pooled SEN and SPE were 0.76 (95% CI 0.54–0.89, I² = 24.21%, p = 0.26) and 0.71 (95% CI 0.59–0.81, I² = 92.15%, p = 0.000), respectively (Figure S3). The AUC was 0.80 (Figure S4). Pooled PLR and NLR were 2.63 (95% CI 1.67–4.15) and 0.34 (95% CI 0.16–0.72), respectively (data not shown).

The meta-analyses appear to have included sufficient volumes of literature (24). Both meta-analyses were performed using similar statistical methodologies. Both meta-analyses include discussion by the authors of the influence of study design on the variability in results observed. Neither of the meta-analyses included randomised studies.

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

5. Where relevant, have studies to demonstrate ease of use by trained lay providers been provided?
☐ yes ☒ no

a. What is your assessment of these studies? Not applicable, not for lay users

6. Where relevant, have studies been provided to show the IVD’s robustness in variable environmental conditions e.g. temperature and humidity?
☐ yes ☒ no


Draft questions:

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.
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

Recommend that the statistical power of the studies be considered by a suitable expert.

The bulk of the evidence provided is in the form of meta-analyses. Neither of the two meta-analyses comparing the test to the gold standard included any randomised controlled studies. There is variability in the design of the five (5) primary studies provided, the populations assessed and the reported outcomes (e.g. sensitivity and specificity of the assay).

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

The applicant’s cost effectiveness data described in their application is derived from two (2) sources that have not been provided as part of their submission. The sources are not sufficiently referenced to allow for them to be identified through a literature search performed by the reviewer. The sources are conflicting as to whether or not incorporating Aspergillus antigen testing into clinical work-up has any impact on the cost of treatment.

From pg. 8/9 of the full application-

3. 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

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Clinical utility: The likelihood of improved outcomes from use of diagnostic tests in the IVD category
b. 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.
   Unable to comment as no budgetary experience specific to LMIC settings. Suggest referral of this question back to the committee.
   It is noted that the tests are run serially; this is not a one-off diagnostic test and the associated costs would be cumulative. As an example, the IFU for the Platelia™ device states that at-risk patients should be screened twice weekly.

4. 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
   Unable to comment, insufficient information has been provided by the applicant in their submission. Refer this question back to the committee.

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 fluid.
   b. What skill level and training is required for specimen collection? E.g. Phlebotomist
      The procedure to collect blood for serum testing can be completed by a phlebotomist, however BAL fluid is collected by a respiratory physician during a bronchoscopy.
   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)
         The IFU for the Platelia™ device states that, ‘Unopened (serum) samples can be stored at 2-8°C for up to 5 days prior to testing’.
      ii. At what temperature is the processed specimen stored before testing (please specify if Celsius or Fahrenheit)
         The IFU for the Platelia™ device states -
         • For serum: ‘Unopened samples can be stored at 2-8°C for up to 5 days prior to testing. After initial opening, samples may be stored at 2-8°C for 48 hours prior to testing. For longer storage, store the serum at -70°C. Serum samples can be subjected to a maximum of 4 freezing/thawing cycles.’
         • For BAL: ‘BAL fluid samples must be collected in sterile saline and may be tested on neat samples (as is) or supernatants from
centrifuged samples (10,000 rpm for 10 min) before proceeding to treat the sample... Transport and store samples in sealed tubes, unexposed to air. After initial opening, samples may be stored at 2-8°C for up to 24 hours. For longer storage, store the BAL samples frozen (-20°C or less) up to 5 months. BAL samples can be subjected to a maximum of 4 freezing/thawing cycles.’

*Note that IFUs for the devices listed in the application were not provided. Therefore the responses above are provided based on the IFU for the Platelia™ device as it is the most established of the devices listed in Annex I for which an IFU could be identified; see section 2.3 of this report for further information.

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?
The applicant states in Annex I that the three (3) GM ELISAs take three (3) hours, while the LFAs are stated to have turnaround times of 25-30 minutes.

e. Where relevant to the IVD has ease of and effective use by trained lay providers been demonstrated?
☐ yes ☒ no (not applicable)

What equipment, if any, is required to perform this type of test?
The applicant states on pg. 7/9 of their full application that the following is needed-

17. Equipment required

<table>
<thead>
<tr>
<th>Considering the tests mentioned in 15 above, please describe in general terms what, if any, equipment is required other than that provided with the test:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifuge, pipettors and either nothing else or water bath and ELISA reader</td>
</tr>
</tbody>
</table>

Response below is based on a review of the IFUs and product information available for the devices. Aside from standard laboratory personal protective equipment (PPE), the following equipment is also required: a refrigerator for sample and reagent storage, heat-resistant microcentrifuge tubes, bench centrifuge, pipettes, plate reader, heat block or water bath, vortex agitator, microplate incubator, semi-automated or automated microplate washer and a microplate reader.

None of the equipment required is specific to this test alone, and it would be expected that the necessary equipment would be available in all tiers of laboratories from district up.

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

g. How robust is the IVD?
In relation to the storage properties of the IVD devices. The Platelia™* IFU states-
h. What is the impact of an unreliable power supply, or can the IVD operate without a power supply?
The IFUs and product information for all of the tests suggest that they cannot be performed without a reliable power supply; see section 2.3 of this report for more information.

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 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></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>N/A</td>
<td>Probably</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Specimen types</td>
<td>N/A</td>
<td>Yes- serum</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Probably- BAL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testing volumes expected</td>
<td>N/A</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Complexity of specimen handling</td>
<td>N/A</td>
<td>Probably</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Infrastructure for transporting specimens</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Result turn-around times required</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Reagent shipping, storage and operating conditions required</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Instrument operating conditions required</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Required qualifications, training and skill levels</td>
<td>N/A</td>
<td>Probably</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Quality management requirements</td>
<td>N/A</td>
<td>Probably</td>
<td>Yes</td>
<td>Yes</td>
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</table>

It is considered that laboratories falling into the categories ‘District’ (with appropriately skilled staff) and above would be suited to handle the complexity of IVD products in this category.

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

Although the submission provided by the applicant was limited, it is considered there is sufficient evidence to warrant inclusion of *Aspergillus* antigen testing on the EDL- in the formats outlined in Annex 1- as a test that can be used in conjunction with other tests to aid in the diagnosis of IA.

It is suggested that if the test is included, that it is listed using an alternate intended purpose from that stated by the applicant.

The applicant states the test purpose should be, ‘*Diagnosis of invasive aspergillosis.*’
It is suggested instead that the test purpose be listed as, ‘The qualitative detection of Aspergillus antigen in serum and/or bronchoalveolar lavage (BAL) fluid to aid in the diagnosis of invasive aspergillosis.’

In making their recommendation to include the test, the following has been considered:

- IA has a reported mortality rate as high as 90% in certain patient populations;
- The mortality rate in IA is stated to be predominantly affected by the timing and initiation of antifungal therapy;
- Use of the test may increase early diagnosis rates owing to the improved specificity over culture;
- The negative predictive value (NPV) of the test—while variable between studies—generally supports the use of the test to screen certain patient populations for pre-emptive antifungal therapy over universal prophylactic therapy;
- Use of the test to screen patients for pre-emptive antifungal therapy in place of universal prophylactic therapy may represent good microbial stewardship, which is important in resource-limited settings such as may be encountered in LMIC; and
- Use of the test is demonstrated to have been incorporated into clinical practice guidelines in several different fields and from bodies around the world.

In making a suggestion to re-word the intended purpose from what it stated by the applicant, the following has been considered:

- The wording more closely aligns with the intended purpose stated in the IFU for the leading global IVD product for this purpose, the Platelia™ Aspergillus Ag device from Bio-Rad Laboratories.
- Clinical practice standards and the EORTC/MSG guidelines require that the results of the test not be used as stand-alone diagnostics, and that the results are evaluated against other clinical signs and information (De Pauw et al., 2008).

The following limitations in the applicant’s submission were noted and may need consideration by the committee:

- The applicant’s full application document is limited in detail, and much of the information necessary to complete this review has needed to be inferred from the supporting material submitted;
- The applicant has not provided all of the references listed in their full application for review;
- Limited, albeit promising, evidence has been presented for the role of non-GM Aspergillus antigen testing, such as is utilised by the AspLFD device named in Annex I;
- The role of the assay in patients without relevant host factors (e.g. immunocompromised) is unclear as the applicant has not provided a source endorsing a clinical algorithm to diagnose IA in patients without host factors listed in the EORTC/MSG guidelines (De Pauw et al., 2008);
- The applicant has provided conflicting evidence as to whether there is any clear financial benefit to use of the device for screening for pre-emptive treatment with antifungal agents vs. empirical or prophylactic therapeutic approaches;
- The applicant has not provided any information regarding the burden of IA on LMIC;
• The applicant has not provided any information regarding whether or not there are differences in the analytical and/or clinical performance of the test depending on the invading Aspergillus spp; and

• The studies and meta-analyses included suggest that test performance, especially sensitivity, is significantly improved when it is combined with other indirect mycological tests not currently included on the EDL, such as those for 1,3-beta-D-glucan and PCR for Aspergillus spp.

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