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

Diagnostic test: Plasma and urine protein electrophoresis and immunofixation
Test purpose: Differential diagnosis of primary antibody deficiency (rule out multiple myeloma)
ID number: PreSubmission_ID41_FullSubmission_ID15

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
   This is a rare collection of diseases causing deficiency in the immune system (~350 categories)
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
      Two individual studies referenced (not meta-analyses)
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: This test would typically be available at higher tiers than primary care. 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: There is no alternative to it currently in the EDL

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? There are two test formats proposed in this application: agarose gel electrophoresis and capillary zone electrophoresis. For quantitation, an additional total protein measurement must be made (already on EDL). Only 1 example of each type of instrument are presented. Also, the reference section includes documents from serum free light chain testing, but that test was not part of this submission.
   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

   However, they do describe the two primary alternative methodologies: gel electrophoresis and capillary zone electrophoresis.

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

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?

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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
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? Essentially no supporting studies have been provided on accuracy. However, gel electrophoresis and capillary zone electrophoresis (in conjunction with immunofixation using gel electrophoresis) are considered gold standards for identification and quantification of m-proteins (paraproteins).

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?

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


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  
These technologies (gel electrophoresis and capillary zone electrophoresis) are well-established for multiple myeloma, however studies were not included in the application. Also, no evidence was included in the application on the utility of excluding multiple myeloma in the process of diagnosing primary immunodeficiencies.

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

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

\(^3\) 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
How strong are these studies in terms of design and statistical power?
☐ weak
☐ strong

No studies were provided regarding the economic impact of electrophoresis in primary immunodeficiencies, and they likely do not exist. In the presubmission, there were a couple economic studies on the value of diagnosing primary immunodeficiencies in general. As electrophoresis likely plays a very minor role in diagnosing primary immunodeficiencies through myeloma rule-out, those studies are not very relevant.

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
      It is unclear if the cost of the instrument is included in the pricing.
   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.
      Given the pricing in the submission, these tests would be as accessible as many of the other tests in the EDL. However, the test is often not performed in-country in LMICs and this is perhaps because it can require manual expertise and/or interpretative expertise.

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: The cost and implementation of this test for the sole use of ruling out multiple myeloma in a patient suspected of primary immunodeficiency is not likely justified. As described in the submission, “It is considered that many PIDs can be diagnosed easily with two simple blood tests, a complete blood count including white cell differential and serum immunoglobulin levels”. The population of patients presenting with multiple myeloma has a median age of 66, while those presenting with primary immunodeficiencies are typically children or young adults. The most common primary immunodeficiency in adulthood is common variable immunodeficiency and most are diagnosed between the ages of 20 and 45 years of age, when patients with multiple myeloma not often presenting. Therefore, the utility of electrophoresis in the workup of primary immunodeficiencies is likely extremely limited. However, the utility of electrophoresis and immunofixation in the diagnosis and management of multiple myeloma and hemoglobinopathies/thalassemias is well-established and likely justified.

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

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

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)
      Paraproteins are typically stable but complements begin to deteriorate after a few days at room temperature, making interpretation more difficult.
      Typical laboratory practice is to keep samples refrigerated at 2-8C.

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

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? No, this testing requires typically 1 day as interpretation is required. If immunofixation is needed, then an additional day is typically required.

e. Where relevant to the IVD has ease of and effective use by trained lay providers been demonstrated?
   ☒ yes ☐ no
   This is performed in laboratories by medical technicians, with interpretation typically performed or approved by lab directors (e.g., PhDs, pathologists).

f. What equipment, if any, is required to perform this type of test?
   Agarose gel electrophoresis: these can be very simple and inexpensive plastic molded pieces designed to hold gel and apply an electric field, with a scanner that allows densitometry. This form would be largely manual, would be robust and low-cost, but suffers from low-throughput and quality control over time. More automated systems are available at higher prices. Antibody reagents and stains are required for immunofixation.

   Capillary zone electrophoresis: there are a limited number of manufacturers that produce these automated instruments. They are generally more expensive than automated gel electrophoresis instruments. A form of immunofixation can be performed by CZE (immunosubtraction) but agarose gel immunofixation is considered to have a lower limit of detection. So, sometimes both instrument types are employed.
g. Do instruments need to be calibrated, maintained, or serviced on a regular basis?
☒ yes ☐ no However, a simple, manual gel electrophoresis device would have only very minor maintenance performed by med techs.

h. How robust is the IVD?
Manual gel electrophoresis is very robust, while the automated instruments require preventive maintenance. In general, agarose gel electrophoresis is more robust than capillary electrophoresis.

i. What is the impact of an unreliable power supply, or can the IVD operate without a power supply? Reliable power is required.

What is the minimal skill level and training required for personnel to perform this test?
☐ Unskilled
☒ Skilled
☐ Highly trained
However, interpretation of results requires highly trained staff and is typically performed or at least approved by the director of the lab or pathologists.

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
<table>
<thead>
<tr>
<th>Proposed answer table:</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><strong>Infrastructure requirements</strong></td>
<td>Power, controlled laboratory temperature, refrigeration, water, benchtop for instrument, biohazard disposal</td>
<td>Power, controlled laboratory temperature, refrigeration, water, benchtop for instrument, biohazard disposal</td>
<td>Power, controlled laboratory temperature, refrigeration, water, benchtop for instrument, biohazard disposal</td>
<td></td>
</tr>
<tr>
<td><strong>Specimen types</strong></td>
<td>Serum</td>
<td>Serum</td>
<td>Serum</td>
<td></td>
</tr>
<tr>
<td><strong>Testing volumes expected</strong></td>
<td>Very low for ruling out myeloma in patients suspected of primary immunodeficiency. Much higher for diagnosing patients with multiple myeloma or haemoglobin variants.</td>
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<td></td>
</tr>
<tr>
<td><strong>Complexity of specimen handling</strong></td>
<td>Low complexity, only centrifugation. Some patients with cryoglobulinaemia require specimen transport and storage at 37C.</td>
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<td></td>
</tr>
<tr>
<td><strong>Infrastructure for transporting specimens</strong></td>
<td>Samples ideally transported at 2-8C, but likely stable at room temperature for a couple days.</td>
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<td></td>
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<tr>
<td>Result turn-around times required</td>
<td>1-2 days</td>
<td>1-2 days</td>
<td>1-2 days</td>
<td></td>
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<tr>
<td>Reagent shipping, storage and operating conditions required</td>
<td>Reagents must be kept at 2-8°C.</td>
<td>Reagents must be kept at 2-8°C.</td>
<td>Reagents must be kept at 2-8°C.</td>
<td></td>
</tr>
<tr>
<td>Instrument operating conditions required</td>
<td>Operation of automated instruments in temperature controlled laboratories, with electricity, water, refrigeration.</td>
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<td></td>
</tr>
<tr>
<td>Required qualifications, training and skill levels</td>
<td>Phlebotomy for blood draws. Skilled techs to operate instrument, highly skilled staff to interpret results.</td>
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<td></td>
</tr>
<tr>
<td>Quality management requirements</td>
<td>Depending on the country, sufficient skilled labor may not be staffed at district hospitals.</td>
<td>Quality resources likely present to operate instruments.</td>
<td>Quality resources likely present to operate instruments.</td>
<td></td>
</tr>
</tbody>
</table>

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

My recommendation to SAGE IVD is that this testing (gel electrophoresis and capillary zone electrophoresis) does not provide sufficient utility for the sole purpose of excluding multiple myeloma in patients suspected of primary immunodeficiencies. As described in the submission, “It is considered that many PIDs can be diagnosed easily with two simple blood tests, a complete blood count including white cell differential and serum immunoglobulin levels”. The population of patients presenting with multiple myeloma has a median age of 66 and only 10 and 2 percent of patients are younger than 50 and 40 years, respectively (UpToDate), while those presenting with primary immunodeficiencies are typically children or young adults. The most common primary immunodeficiency in adulthood is common variable immunodeficiency and most are diagnosed between the ages of 20 and 45 years of
age. Therefore, the utility of electrophoresis in the workup of primary immunodeficiencies is likely limited.

Also, the authors included references for serum free light chain analysis, however did not include that test formally in the application. This is a very useful test for multiple myeloma, particularly when the patient has light chain myeloma. However, urine electrophoresis with densitometry and immunofixation can be used as an alternative and serum free light chain testing reagents are typically expensive.

That being said, the utility of protein electrophoresis and immunofixation in the diagnosis and management of multiple myeloma is well-established. Furthermore, gel electrophoresis and capillary zone electrophoresis are well-established for hemoglobinopathies and thalassemias (for thalassemias, capillary zone electrophoresis is preferred, or high-performance liquid chromatography). Protein electrophoresis is essential for proper diagnosis and management of these conditions, and the condition burdens are high. Furthermore, there are WHO EML medicines for both multiple myeloma and hemoglobinopathies/thalassemias. Finally, capillary zone electrophoresis can also be used to measure haemoglobin A1c. For these reasons, protein electrophoresis should be considered as an addition to the EDL.

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

If the authors believe a significant number of patients with multiple myeloma will benefit from protein electrophoresis being performed in patients suspected of primary immunodeficiencies, please submit estimates of the number of patients along with references.