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

Diagnostic test: Cerebrospinal (CSF) Profile (red blood cells, white blood cells, Glucose, Protein) by manual and automated analyzers

Test purpose: Diagnosis and Aid to Diagnosis
ID number: PreSubmission_ID 135 FullSubmission_ID63

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:
Does the submitted test category help to increase access in any way? E.g. reduced skill required, lower cost, improved performance vs. alternative options

The submitted test category may help to increase access to diagnosis, as the category is not listed in the current EDL as a General IVD for use in clinical laboratories except in the section on microscopy for bacteria. The cell counts in the CSF as well as estimation of CSF glucose and protein are not mentioned in the EDL 2nd Ed explicitly.

The submission refers to CSF analysis by both manual and automated analysers, but in the list of commercially available IVD products only automated analysers are mentioned, without any reference to commercially available hemocytometer which is the standard instrument used for cell counts in CSF.

The automated cell counters offer the option of performing CSF cell counts in settings which may not necessarily have skilled laboratory personnel. However the costs of cell counters are high in terms of procurement, running and maintenance, so they are not cost-effective in operations with low work volumes.

The reference standard for cell count on CSF is still manual counting on a hemocytometer, although this procedure itself may have high inter-observer variation. A problem of cell counting in CSF for automated cell counters are the low counts of WBCs in CSF. Some of the automated cell counters have not received FDA approval. Of the commercial IVDs mentioned in the application, Beckman Coulter LH 750 has been noted to have unacceptable rates of error at counts less than 200/μl, and the Sysmex – XT 4000i has limited precision below the cell count of 200/μl. (Hod EA, Brugnara C, Pilichowska M, et al. Automated cell counts on CSF samples: A multicenter performance evaluation of the GloCyte system. International journal of laboratory hematology 2018;40:56-65.)

It has been recommended that any laboratory should have documented linearity, background and correlation studies before using automated equipment for CSF cell counts because of concerns with low precision of automated equipment at low CSF counts. (See http://www.api-pt.com/Reference/Commentary/2005Bmicroscopy.pdf)
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

2. Which national regulatory bodies have approved these tests for market access e.g. CE IVD, US FDA, SFDA, WHO-PQ, others?
   The test equipment do have FDA clearance for use as cell counters for hematology purposes, but the FDA approval does not mention counting of cells in body fluids in the indications in many of the cell counters approved.

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? The package inserts do not provide any references on performance related data.

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1Technologies: 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
2Analyte: 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

The studies referring to the comparison with a gold standard (manual counts) have been conflicting with regard to the correlation between the automated counts and the manual ones. Concerns have been mainly on the limited precision or unacceptable rates of error at the lower cell counts in the CSF with some of these systems (LH 750, Sysmex XT-4000i). The current automated analysers are mostly those used for hematology which are also being used for estimation of cell counts in body fluids, but there are dedicated cell counters for estimation of cell counts in CSF which claim higher accuracy (e.g. Glocyte system: Automated cell counts on CSF samples: A multicenter performance evaluation of the GloCyte system. Hod EA, Brugnara C, Pilichowska M, Sandhaus LM, Luu HS, Forest SK, Netterwald JC, Reynafarje GM, Kratz A. Int J Lab Hematol. 2018 Feb;40(1):56-65. doi: 10.1111/ijlh.12728. Epub 2017 Sep 7)

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

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

The package inserts do refer to operating environment to be between 25-30% with humidity being to the level below 80%

3. **Clinical effectiveness\(^4\)** based on published peer reviewed data, safety and comparative cost-effectiveness.

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?

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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

\(^4\) Clinical effectiveness: The degree to which a particular healthcare 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
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
   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.
   The cost prices of the hematology analysers in markets like India are in the range of USD 5000 upwards, while the biochemistry analysers are in the range of USD 20,000 upwards which is quite substantial for health facilities in resource-constrained settings. Added to this are costs of providing a temperature controlled environment (these equipments have temperature related considerations which are relevant to countries with high ambient temperature in summer), consumables and maintenance costs. They would be cost-effective in laboratories e.g at district hospital level who have high workloads related to hematology.

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
This has to be contextualised to the clinical setting in which the test is done. In case the test is done in a teaching hospital/district hospital with a high workload, it may save on the salary cost of additional staff, while in a secondary care facility with lower workload, the costs may outweigh the benefits. The shorter turn around time of less than a minute by automated counters looks attractive than the turnaround time of 10-20 minutes for the manual count, quoted in a study, but abnormal cell counts by automated counters may also need a slide review.

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?

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

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

   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?

   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?

   g. Do instruments need to be calibrated, maintained, or serviced on a regular basis? □ yes □ no

   h. How robust is the IVD?
i. What is the impact of an unreliable power supply, or can the IVD operate 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 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</th>
<th>District hospitals/lab</th>
<th>Regional hospitals/lab</th>
<th>National hospitals/Reference lab</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Specimen types</th>
<th>CSF</th>
<th>CSF</th>
<th>CSF</th>
<th>CSF</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Testing volumes expected</th>
<th>1-2/month</th>
<th>4-5/month</th>
<th>20-30/month</th>
<th>50-100/month</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Complexity of specimen handling</th>
<th>Lumbar puncture required for collection of CSF may not be performed at this level, because of lack of expertise</th>
<th>Specimen collection and processing possible</th>
<th>Specimen collection and processing possible</th>
<th>Specimen collection and processing possible</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Infrastructure for transporting specimens</th>
<th>Collection may be difficult as mentioned above</th>
<th>Possible</th>
<th>Possible</th>
<th>Possible</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Result turn-around times required</th>
<th>60 minutes for manual count, 2 hours for biochemical analysis</th>
<th>60 minutes for manual count, 15 minutes for an automated count 2 hours for biochemical analysis</th>
<th>60 minutes for manual count, 15 minutes for an automated count 2 hours for biochemical analysis</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Reagent shipping, storage and operating conditions required</th>
<th>No</th>
<th>Yes</th>
<th>Yes</th>
<th>Yes</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Instrument operating conditions required</th>
<th>Yes</th>
<th>Yes</th>
<th>Yes</th>
<th>Yes</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Required qualifications, training and skill levels</th>
<th>Unlikely to be available</th>
<th>Likely to be available</th>
<th>Likely to be available</th>
<th>Likely to be available</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Quality management requirements</th>
<th>Unlikely to be available</th>
<th>May be available</th>
<th>Likely to be available</th>
<th>Likely to be available</th>
</tr>
</thead>
</table>
What is your recommendation to SAGE IVD? Please summarise the key points you considered in reaching your conclusion.

I agree with the applicants and would strongly recommend that this test category of the basic CSF profile be added to the WHO EDL as it involves disease conditions of public health importance and of a life-threatening nature which need rapid and accurate diagnosis and early institution of effective treatment. Timely access to a basic CSF profile will help in the diagnosis and management of CNS infections including bacterial meningitis, tubercular meningitis and fungal meningitis, which are still widely prevalent in low-middle income countries, and have unacceptable levels of morbidity and mortality.

The tests involved are those which are within the capacity of a hospital with a laboratory facility to perform. The procedure for cell counts are similar to those performed on peripheral blood, while the biochemical tests are those which are also routinely performed on peripheral blood.

I would recommend both the manual counter (still the gold standard) as well as the automated systems (which are now improving in precision and accuracy, and lower limits of quantification). Laboratory personnel in resource-constrained settings are trained and expected to diagnose infections like malaria, perform total and differential cell counts and these skills are still relevant even in the age of automated systems, in the context of the realities of the health systems.

The automated cell counters have multiple manufacturers employing a range of technologies (impedance technology, cytochemical reactivity, differential cell lysis, and flow cytometric analysis of light scatter and nuclear fluorescence staining intensity, etc), and not all of them may have received regulatory approval for performance of cell counts on CSF. Some of them have limited precision or unacceptable rates of error at low cell counts (see Hod et al International Journal of Laboratory Hematology 2018;40:56-65).

It would be important for the EDL to consider that automated hematology analyzers should have a body fluid mode, validated by the manufacturer and approved by a regulatory agency before they are used for CSF analysis. It would be therefore be important to review the information provided by the manufacturer on which types of body fluids (including CSF) have been validated on the analyzer and the analytical measurement range for CSF, and these claims must be verified. (Sandhaus LM. Clinics in laboratory medicine, 2015 Mar; 35(1):93-103). All cell counts on CSF should be made on automated analyzers only after changing the mode of the analyzer to the appropriate Body Fluid Mode.

If the manufacturer’s intended use statement does not include measurements on CSF then these counters would require validation, which may be beyond the capacity of laboratories in developing countries. So the recommendation to use automated cell counters may be
qualified by the need for further validation studies as well as a requirement for regulatory approval for use of the automated counter for CSF cell counts.

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

The applicants have suggested using automated hematology analysers as well as biochemical analysers for performing a basic CSF profile, and this assumes that these analysers are already present in the laboratory for performance of cell counts and biochemical counts on blood. In many low-middle income countries like India in primary and even at first level referral facilities, this is not true.

There are a multiplicity of devices for performing automated cell counts in the market, which is reflected in the literature provided and it would be useful to have a compendium on which of these have a body fluid mode, what kind of validation has been done and which of these have regulatory approval for estimation of cell counts in CSF.

The initial capital costs for cell counters as well as of fully automated biochemistry analysers (mentioned in the application) are also beyond the usual budgets of districts hospitals. Therefore it is usual for laboratories to continue performing manual cell counts as well as biochemical tests on semi-automated analysers. It would be good to review data on the performance characteristics of such techniques and technologies.

The application could provide some idea of the running and maintenance costs and cost-effectiveness of automated cell counters across levels of care which have different workloads. Finally the application could address briefly the quality assurance mechanisms for cell counters and how this can be implemented in laboratories in health systems with constrained budgets.