Full Submission for inclusion of an IVD category to the EDL

Survey response 1

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Identification

Please indicate your response ID (unique identifier given to your screening application)
41.0000000000

Name of organization supporting this application:
IPOPI and IUIS

Please enter full applicant’s name
Johan Prevot and Stuart Tangye

4. Typical Characteristics of IVD’s in each format in which tests in the test category are available
Please provide detailed description of test components (reagents/instrumentation (where relevant), methodology and labelling for each test format available

<table>
<thead>
<tr>
<th>Test Components</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmatic and urine for protein electrophoresis - urine &amp; plasmatic dipstick and light microscopy</td>
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</table>

**Agarose gel electrophoresis**

This is an inexpensive and easy to perform screening procedure for the detection of a monoclonal protein, which can be performed on both serum and urine without sophisticated equipment. The technique involves the movement of charged molecules through a solvent by an electrical field. Thus, proteins migrate according to their net charge and are separated into six major fractions. Once the proteins have been separated, the gel is stained using a dye e.g. Coomassie Blue dye in order to be able to visualise the different protein bands. If a monoclonal band is detected, this can be quantified using a densitometer tracing of the gel, in conjunction with the total protein concentration.

**Capillary Zone Electrophoresis (CZE)**

This is an alternative and slightly more sensitive method of screening for protein abnormalities. In this technique, a capillary is filled with a conductive fluid at a certain pH which acts as the buffer solution, where the sample’s proteins are separated. After the sample is introduced, a high voltage is generated over the capillary and the sample’s proteins will migrate through the capillary at differing speeds due to the electrical field, thus allowing separation into six major fractions, with identification of monoclonal peaks. It also allows for quantification of these monoclonal peaks and immunofixation in order to identify the type of monoclonal. Abnormal bands in the electrophoretic pattern of serum proteins, primarily those in the beta globulin and gamma globulin zones are always suspect of being monoclonal proteins and therefore, an indication of gammapathy.

**Assay Principle**

Separation occurs in a liquid buffer pH~10 flowing through a fused silica capillary tube. A volume of sample is aspirated and introduced into the anodal end of the capillary. The buffer is alkaline relative to the pH of serum proteins. This allows the proteins to donate protons to the buffer and become negatively charged. When an electric field is applied the proteins remain at the anode.

However, the interior of the capillary has a strong negative charge relative to the buffer and the application of a high voltage creates a strong electroendosmotic flow of buffer ions to the cathode. The pull of this flow is much stronger than the attraction to the anode and so the proteins begin to move cathodally. Proteins with the lowest net negative charge at pH 10 have the weakest attraction to the anode. They travel rapidly in the electroendosmotic flow and reach the cathode first. Proteins with the highest net negative charge at pH 10 have the strongest attraction to the anode. This slows their movement and they reach the cathode later.

An ultraviolet detector at the cathodal end of the capillary detects the proteins as they pass via peptide bond UV absorbance at 214nm. From this a plot of absorption vs time (electropherogram) is constructed. A ‘normal’ serum protein electropherogram shows the same six major fractions as gel electrophoresis: five bands – Albumin, alpha-1, alpha-2, beta-1 and beta-2 – and a non-banded gamma region.

**Equipment required**

- V8 and Platinum software from Helena Biosciences (www.helena-biosciences.com)
- V-CE sample cups (supplied within V8 Serum Protein SPE kit – Catalogue No 800500)
- Filter for SPE buffer (supplied within V8 Serum Protein SPE kit – Catalogue No 800500)

**Reagents**

- SPE buffer (supplied within V8 Serum Protein SPE kit – Catalogue No 800500)
- SPE diluent ((supplied within V8 Serum Protein SPE kit – Catalogue No 800500)
- V8 storage buffer – Catalogue No 830100
- V8 maintenance buffer – Catalogue No 830200

**Sample requirements**

The test requires 500ul of serum which may be stored refrigerated (2 to 8°C). Haemolysed specimens may be used but it is essential that this information is recorded on the worksheet (extra band in b region due to haemoglobin). Avoid plasma samples where possible - fibrinogen gives a band close to the application point which may be mistaken for a monoclonal immunoglobulin.

**Methodology**

Samples are processed on COBAS first and then brought to the CZE bench. There are two in-house controls which are run daily. A sample with a monoclon of ~5g/L is run through all of the eight capillaries to control between-capillary variation and a sample with monoclon of ~2g/L is run through a single capillary to control detection of small proteins. Monoclonal peaks are quantified (%) for each result.

The CZE traces are read by suitably trained personnel. The traces should be read as soon as possible to allow identification of
significant bands relating to known and new monoclones. Reasons for performing immunofixation include a new band visible on serum protein electrophoresis (SPE), including the appearance of a second band, known bands not visible on SPE, e.g. in the beta region, suspicious looking beta region including Beta2 > Beta1 with increased IgA and “pointed” Beta1 or Beta2 with increased IgA, increased IgA (>5g/L) without evidence of either liver disease or beta-gamma bridging, known co-migrating bands, BMT patients excluding patients with previously identified and quantifiable bands, all samples referred by other laboratories for immunofixation, samples from clinical trials where immunofixation is part of the agreed profile, immune paresis and low immunoglobulins (first time only unless significant change): IgG

### Diagnostic accuracy: Please provide typical sensitivity, specificity, PPV, NPV) for each test format available. Note: Section 5 below request a summary of studies supporting performance criteria shown in this section.

### Capillary Zone Electrophoresis
This technique is generally slightly better than quantitation by densitometry and has a CV of under 25%.

### Identification of paraproteins (monoclonal gammopathies) in serum and urine by immunofixation
Generally CV is under 20%

### Specimen types: Please provide the range of specimen types that can be used with each format for which the tests are available.

<table>
<thead>
<tr>
<th>Format</th>
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<tr>
<td>2mls serum and 5mls urine for electrophoresis</td>
<td></td>
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<td>Identification of paraproteins (monoclonal gammopathies) in serum and urine by immunofixation</td>
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</table>

### Facility level: the kind of facility in which each test format is intended to be used. Include all that apply.

- [I: primary health care clinic with no laboratory] No
- [II: district/hospital laboratory] Yes
- [III: regional/provincial laboratory] Yes
- [IV: national reference laboratory] No

### User Skill level: minimum level of training the operator undergoes to effectively perform each of the test formats.

- Laboratory trained health care worker

### Throughput: number of specimens tested at one time for efficient use of each test format.

- High

### Time to result: length of time to report the result for each test format

- Identification of paraproteins (monoclonal gammopathies) in serum and urine by immunofixation: 2-4 hours
- Capillary Zone Electrophoresis: 1 hour

### Environmental stability (temperature, humidity) and shelf life for each test format.

- Operating: The above machines need to be maintained at room temperature.
- Storage: Reagents for the above techniques must be kept refrigerated, generally stored between 2-8°C.
- Transport: Specimens can be transported at room temperature.

### Disposal risks: risks posed by disposal of IVD components for each test format. Include all that apply.

- Biohazard: Yes
- Comment: Normal precautions required for handling all laboratory reagents should occur. Disposal of all waste material should be in accordance with local guidelines. All human material should be considered potentially infectious. Gloves and a laboratory coat should be worn and specimens handled with appropriate caution.
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<tr>
<td><strong>Capillary Zone Electrophoresis</strong> This uses the V8 maintenance buffer which contains Sodium Hydroxide, which is highly corrosive. Acid violet stain contains acetic acid and fume production needs to be minimised. Acidic violet stain can cause skin corrosion and irritation. Irritation to the respiratory tract and mucous membranes can also occur can be caused by destain, which contains citric acid. Agarose gels contain sodium azide</td>
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5. Evidence summary

Summary of laboratory evaluation studies covering reliability and reproducibility, analytical accuracy (sensitivity, specificity); and analyses of potentially interfering substances, cross reactivity, stability, sample type. All relevant studies should be reported, detailing the search strategy and eligibility criteria used, or providing a systematic review. Please indicate the organizations responsible for conducting each of the studies and provide links to full reports for each study.

### Serum free light chain quantification

1. Optilite User Training Manual APDOC39 v3
7. Laboratory standard operating procedure for Freelite assays, Gambro Dialyser study protocol, The Binding Site Limited

### Capillary Zone Electrophoresis

Summary of studies of clinical accuracy evaluating the test in patient pathways in clinical settings covering sensitivity, specificity, and predictive values. Full evidence of all relevant studies should be reported, detailing the search strategy and eligibility criteria used, or providing a systematic review. Please indicate the organizations responsible for conducting each of the studies and provide links to full reports for each study.

Providing robust predictive value and performance criteria in diagnosis for rare and heterogeneous patient populations, such as primary immunodeficiencies is not possible. The reference ranges by which subnormal immunoglobulin production are established are long established on normal populations.

The performance of paraprotein detection methods in the context of general lymphoid haematological malignancy are well established for Immunoglobulins, electrophoresis, immunofixation and free light chain examination.

Summary of evidence of the impact of using tests in clinical practice on diagnoses, treatment, and patient outcomes. Please summarise model based evaluations and empirical studies where available and provide links to full reports. The use of these tests in the diagnosis of immunodeficiency is well established in international practice and incorporated into recommendations of the American Academy of Allergy, Asthma & Immunology (AAAAI), the Jeffrey Modell Foundation (JMF) and the European Society for Immunodeficiencies (ESID) best practice recommendations and diagnostic criteria.

Summary of available non-clinical data (appraisal of evidence of quality manufacturing, ease of use and test disposal) and links to any relevant references.

There are no relevant studies.

Please attach relevant documents. Documents should be published in the last 5 years, except when not available.

Filecount - Please attach relevant documents. Documents should be published in the last 5 years, except when not available. 0

### 6. Societal impact information

**Ethical issues: please detail any important ethical consideration by the type of test and consequences**

**Capillary Zone Electrophoresis**

None anticipated

Identification of paraproteins (monoclonal gammopathies) in serum and urine by immunofixation

None, as long as the patient is aware that the investigation of immunodeficiency involves excluding a malignancy, and informed consent has been provided.
Equity and human rights issues: please indicate if it reduces inequities or increase equity and accessibility

Primary immunodeficiencies represent more than 350 different disorders and taken as a whole, they represent a significant proportion of the world’s population. Recent studies have shown that primary immunodeficiencies may be more common than previously estimated and that as many as 1% of the population may be affected with a primary immunodeficiency when all types and varieties are considered. Antibody deficiency disorders are chronic, rare and often diagnosed late or not diagnosed at all, even in developed countries with effective and widely available healthcare access. Once patients with primary immunodeficiencies are diagnosed, they can be prescribed the right treatment and, following their specialist doctor’s advice, they can lead a “normal” life and lower the risk of infections.

Receiving a prompt diagnosis is a key priority for patients with primary immunodeficiencies and can be life-saving. A prompt diagnosis will increase their chances of accessing appropriate treatment, as well as achieving successful management and care. A majority of patients with primary immunodeficiencies (around 60%) will need life-long treatment with immunoglobulin replacement therapies, which are considered to be essential medicines by the World Health Organisation both for adults (WHO Model List of Essential Medicines, 20th edition: http://apps.who.int/iris/bitstream/handle/10665/273826/EML-20-eng.pdf?ua=1) and children (WHO Model List of Essential Medicines for Children: http://apps.who.int/iris/bitstream/handle/10665/273825/EMLc-6-eng.pdf?ua=1).

By accepting the suggested diagnostic in vitro tests, the WHO would help close the gap between access to diagnosis and access to treatment. Inclusion of these tests will provide much needed support to ensure patients are diagnosed as swiftly as possible and increase their chances to access their essential medicines in a timely and potentially lifesaving manner. This will lead to better care, lower rate of acute and chronic complications (such as respiratory infections) as well as lower rate of sequelae (and death), along with a better quality of life. All of this is most likely to be cost-effective.

Additionally, by including the above-mentioned tests in the WHO list of Essential In Vitro Diagnostics, the WHO would help tackling a worrying international figure that considers that between 70 and 90% of people living with a primary immunodeficiency still remain undiagnosed worldwide (Primary Immunodeficiencies (PID) – Driving Diagnosis for Optimal Care in Europe, European Reference Paper. Available from: http://worldpiweek.org/sites/default/files/basic_page_documents/PI_European_Reference_Paper.pdf).


The Asia-Pacific Economic Cooperation, in its Recommendations for Enhancing Access to Safe Therapy for Persons with Immunodeficiency and Bleeding Disorders, includes in its first recommendation the need to “improve laboratory diagnosis and assist healthcare personnel to standardise the early identification of patients with plasma protein deficiency. Accurate diagnosis is the first step in treatment”. In its fifth recommendation, it also calls for including “an educational curriculum about bleeding disorders and immune deficiencies in medical and nursing schools to strengthen the recognition of symptoms and improve access to laboratory testing, including training on quality/GMP standards for blood products to hospitals, doctors, medical and nursing schools” (Available at: http://bloed.apec.org/wp-content/uploads/2018/01/17_isil2_agr05.7_Access-to-Safe-Therapy-recommendations-FINAL.-docx.pdf).

Lastly inclusion of these tests would enable the WHO to come full circle in its approach, by on one hand supporting the essential nature of immunoglobulin replacement therapies in the treatment of primary immunodeficiencies through the WHO EMLs and on the other hand enabling faster diagnosis of primary immunodeficiencies without which access to immunoglobulin replacement therapies would not be feasible. Availability of these diagnostic tools will therefore significantly reduce inequities and increase equity and accessibility to healthcare for this patient population.

Acceptability: please indicate the acceptability by patient or by health care worker, benefits and harms

Where informed consent has been provided, there are few harms associated with standard blood sampling and urine collection procedures. No acceptability issues are anticipated.

7. Budget and resources impact
Summary of data on comparative cost and cost-effectiveness, if available

Little comparative data on cost effectiveness is available in this context for these rare diseases.

Capillary Zone Electrophoresis

Typical cost: £5.00 (around 6.4 USD) in a country like the United Kingdom.
High initial costs for analysers and staffing. May only be suitable for hub laboratories in resource poor areas.
In India, the typical cost would be of 120INR (around 1.66 USD) per test for the laboratory and around 150 INR (around 2.08 USD) would be charged to the patient.

Identification of paraproteins (monoclonal gammopathies) in serum and urine by immunofixation

Moderate initial costs for analysers and staffing.
May only be suitable for hub laboratories in resource poor areas.
Typical cost: £10.00-20.00 (12.9 – 25.85 USD) in a country like the United Kingdom.
In India, the typical cost would be of 750INR (around 10.40 USD) per test for the laboratory and around 1000 INR (around 13.8 USD) would be charged to the patient.

Resources and budget impact on health care systems, including specialized human resources, training, maintenance issues as available to support implementation.

All of these techniques require significant resource in manpower, training and maintenance.
Some such as Capillary Zone Electrophoresis require dedicated machines.

Automated tests performed on random access platforms. Minimal human resources required to perform analyses.
Semi-automated tests performed on batch analyser; medium human resources required to perform analyses. Expertise and significant training required for interpretation.
Manually performed test; significant human resources required to perform analyses.
Expertise and significant training required for interpretation.

8. Environmental impact

Please enter any relevant information
Disposal of chemicals, plastics and containers from routine operations.

9. Proposed (new/adapted) text for the EDL.

Please enter any relevant information

Additional information and signature

Please provide additional information that you would like to be considered.

We would like to emphasise that the inclusion of these tests would enable the WHO to come full circle in its approach, by on one hand supporting the essential nature of immunoglobulin replacement therapies in the treatment of primary immunodeficiencies through the WHO EMLs and on the other hand enabling faster diagnosis of primary immunodeficiencies without which access to immunoglobulin replacement therapies would not be feasible. Availability of these diagnostic tools will therefore significantly reduce inequities and increase equity and accessibility to healthcare for this patient population.

Through the electronic signature below, I acknowledge that I have provided appropriate information to support this submission. I acknowledge that WHO reserves the right to format and select the information provided as necessary and agree that the information is publicly disclosed by WHO. [Electronic Signature (type your full name to sign):]

Johan Prevot

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