WG-ICQA Subgroup:

Harmonisation of reporting of protein electrophoresis and serum FLC, and quantification of small monoclonal proteins

- Improved patient safety and to promote alignment with clinical guideline practices
  - previous surveys in several countries indicate a lack of harmonisation in all related testing phases
- International baseline survey to inform protein laboratories globally of current practices
- 30 questions addressing specific aspects of pre-analytical, analytical and post-analytical phases of Total Testing Process
- To issue a survey report and to write a Position Paper on minimum recommended harmonised laboratory practices.
Survey questions

Sections A & B: Pre-analytical phase (6 Qs)
- Guidelines and test requesting for diagnosis of monoclonal gammopathies
- Guidelines and test requesting for monitoring disease response in monoclonal gammopathies

Section C: Analytical phase (11 Qs)
- Monoclonal IgD/E identification
- Analytical interference by therapeutic mAb immunotherapy
- Quantification

Section D: Post-analytical phase (8 Qs)
- Reporting
- Interpretive commenting

Section E: Demographics (5 Qs)
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245 labs from 31 countries. 347 labs participated.
Response rates to questions

Response Counts vs. Survey Progression

- Blue line: Answered
- Red line: Skipped
Q1: If you are asked to screen for a monoclonal gammopathy, which of the following describe best your laboratory procedure?

- Perform SPEP and/or UPEP (35%; 14%)
- Perform SPEP and reflex to/or request full IFE or IS (85%; 35%)
- Perform SPEP and reflex to/or request full IFE or IS, Igs, and SFLC (7; 3%)
- Perform screening IFE (i.e. 1 lane kappa/lambda or pentavalent antiserum) (33; 13%)
- Perform other tests
Q2: What tests are used in your institution to diagnose AL amyloidosis cases? Select all that apply.

<table>
<thead>
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<th>Option</th>
<th>Percent of Respondents</th>
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<tr>
<td>Q2 Opt.1: SPEP</td>
<td>74.3%</td>
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<td>Q2 Opt.2: Serum IFE or IS</td>
<td>67.3%</td>
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<td>Q2 Opt.3: SFIC</td>
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<td>Q2 Opt.4: UPEP</td>
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<td>Q2 Opt.5: Urine IFE</td>
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<td>Q2 Opt.6: Bence Jones Protein determination and quantification</td>
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<td>Q2 Opt.7: Ig quantitation</td>
<td>6.5%</td>
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<td>Q2 Opt.8: Serum heavy-light chain assay</td>
<td>10.2%</td>
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<tr>
<td>Q2 Free text (Other tests)</td>
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**Q3: How do you offer this laboratory diagnostic in your Institution?**

- **117; 48%**
  - All tests are orderable as standalone assays. Abnormalities are identified and laboratory will automatically add on additional tests if appropriate (reflex).

- **93; 38%**
  - All tests are orderable as standalone assays. Abnormalities are identified and laboratory will suggest additional tests if appropriate.

- **35; 14%**
  - Tests offered as a panel (i.e. screening for monoclonal gammopathy) and laboratory decides which tests to perform.
Q4: What tests are used in your institution to follow-up a treated myeloma case with the M-protein migrating in the gamma fraction? Select all that apply.

- Q4 Opt.1: SPEP and M-protein quantification
- Q4 Opt.2: IFE or IS if M-band visible on SPEP
- Q4 Opt.3: IFE or IS if M-band NOT visible on SPEP but previously detected on IFE/IS
- Q4 Opt.4: SFLC
- Q4 Opt.5: Serum heavy-light assay
- Q4 Opt.6: Ig quantitation
- Q4 Opt.7: Selective Ig quantitation e.g. IgA if IgA M-protein is <10 g/L (<1.0 g/dL)
- Q4 Opt.8: UPEP and IFE (and quantification of BJP if detected)
- Q4 Free text (Other tests)
Q5: What tests are used in your institution to follow-up a treated myeloma case with the M-protein migrating in the beta or alpha-2 fraction? Select all that apply.

- Q5 Opt.1: SPEP and M-protein quantification (80.4%)
- Q5 Opt.2: IFE or IS if M-band visible on SPEP (31.8%)
- Q5 Opt.3: IFE or IS if M-band NOT visible on SPEP but previously detected on IFE/IS (60.4%)
- Q5 Opt.4: SFLC (60.4%)
- Q5 Opt.5: Serum heavy-light assay (4.5%)
- Q5 Opt.6: Ig quantitation (54.7%)
- Q5 Opt.7: Selective Ig quantitation e.g. lgA if lgA M-protein in beta or alpha-2 fraction (32.2%)
- Q5 Opt.8: UPEP and IFE (and quantification of BJP if detected) (40.8%)
- Q5 Free text (Other tests) (13.5%)
Q6: What tests are used in your institution to follow-up a treated AL amyloidosis case? Select all that apply

- Q6 Opt.1: SPEP and M-protein quantification
- Q6 Opt.2: IFE or IS if M-band visible on SPEP
- Q6 Opt.3: IFE or IS if M-band NOT visible on SPEP but previously detected on IFE/IS
- Q6 Opt.4: SFLC
- Q6 Opt.5: Serum heavy-light assay
- Q6 Opt.6: Ig quantitation
- Q6 Opt.7: Selective Ig quantitation e.g., IgA if IgA M-protein in beta or alpha-2 fraction
- Q6 Opt.8: UPEP and IFE (and quantification of BJP if detected)
- Q6 Free text (Other tests)
Q7: Once a light chain (kappa or lambda) is identified on serum IFE or IS without a corresponding heavy chain for the first time, with no available history, what is the next step?

- 159; 65%: Send-out to reference lab for confirmation and additional testing
- 27; 11%: Run IFE with anti-IgD and anti-IgE antisera
- 19; 8%: Run IFE with anti-IgD antiserum
- 16; 6%: Report a monoclonal light chain
- 24; 10%: Reflex IFE to serum FLC
Q8: Do you perform any routine testing to distinguish between an endogenous M-protein and a therapeutic mAb?

- No: 234 (96%)
- Yes: 11 (4%)
Q9: Which method do you use (or think will be able to use in the future) to detect this interference?

- DIRA, daratumumab-specific immunofixation electrophoresis reflex assay: 44 (18%)
- Mass spectrometry method able to identify the mAb molecular mass and compare it to a library: 25 (10%)
- Other IFE-based tests with anti-drug-antibodies immune-complex formation: 30 (12%)
- We are ready to send out these samples to more specialized labs: 146 (60%)
Q10: What methods do you use for SPEP? (select all that apply)

- Agarose gel electrophoresis: 43.7%
- Capillary zone electrophoresis: 63.7%
- One lane screening IFE followed by CE: 2.9%
Q11: How do you currently quantitate the M-protein migrating in the gamma fraction?

- Ig quantitation by nephelometry or turbidimetry: 27; 11%
- Perpendicular drop of M-spike only, including any polyclonal Ig background: 57; 23%
- Quantitation not performed - report qualitatively as small, medium or large: 6; 3%
- Tangent skimming of M-spike, not including the polyclonal Ig background: 155; 63%
Q12: What is your method’s limit of detection of an M-protein migrating in the gamma fraction on a low polyclonal Ig background of less than 5 g/L (0.5 g/dL). Select one concentration.

- 1 g/L (0.1 g/dL): 152; 62%
- 2 g/L (0.2 g/dL): 33; 13%
- 3 g/L (0.3 g/dL): 14; 6%
- Other (please state in free text): 46; 19%
Q13: What is your method’s limit of detection of an M-protein migrating in the gamma fraction on an elevated polyclonal Ig background of greater than 20 g/L (2.0 g/dL). Select one concentration

- 76; 31%
- 50; 20%
- 29; 12%
- 17; 7%
- 44; 18%
- 1 g/L (0.1 g/dL)
- 10 g/L (1.0 g/dL)
- 2 g/L (0.2 g/dL)
- 3 g/L (0.3 g/dL)
- 5 g/L (0.5 g/dL)
- Other (please state in free text)
Q14: If beta or alpha-2 fraction is increased or abnormal, but not enough for you to quantitate it, and a monoclonal gammopathy is suspected, what are the next tests that you would suggest to be performed? Select all that apply.

- Q14 Opt.1: IgA total quantitation
- Q14 Opt.2: Heavy-light chain pairs for IgA (IgAK/IgAL)
- Q14 Opt.3: SFLC
- Q14 Opt.4: Serum IFE
- Q14 Opt.5: Serum IS

Percent of Respondents: 21.2%, 3.7%, 22.0%, 89.8%, 15.9%
Q15: When do you quantitate the beta-fraction or beta-1 and beta-2 fractions? Select all that apply

- When there is a shoulder and the M-protein is visible and distinguishable from the normal protein background (58.5%)
- When the entire beta-fraction is greater than 20 g/L (2 g/dL) (35.5%)
- Don’t quantitate beta-migrating M-proteins using electrophoresis, only total beta-fraction concentration is reported. (31.3%)
- Don’t quantitate beta-1 and beta-2 fractions. (8.3%)
- Other (0.0%)
Q16: How do you quantify M-proteins overlapping normal proteins in the beta and alpha-2 fractions when the M-protein is not clearly separated?

- **Perpendicular drop of M-spike only, including any normal protein background**
  - 38; 16%
- **Quantitation not performed; rather the total beta or alpha-2 fraction containing the M-protein is reported**
  - 79; 32%
- **Recommend or reflex to Ig quantitation by nephelometry or turbidimetry**
  - 68; 28%
- **Recommend SFLC**
  - 28; 11%
- **Tangent skimming of M-spike, not including the normal protein background**
  - 9; 4%
- **Other (please write in free text)**
  - 6; 2%
Q17: Does your institution conduct a periodic ‘gating’ challenge among operators to assess variation in M-protein quantitation?

- Yes: 162; 66%
- No: 83; 34%
Q18: Do you report quantitative electrophoretic result changes comparing to previous measurements on the same patient?

- Yes: 117 (48%)
- No: 128 (52%)
Q19: If yes, when do you consider an M-protein change significant?

- Change by absolute amount of 10 g/L (1 g/dL): 72; 29%
- Change by absolute amount of 5 g/L (0.5 g/dL): 41; 17%
- Change is greater than 25% (increase or decrease) from previous sample: 21; 9%
- Haematologist calculates change in M-protein using LIS cumulative results: 5; 2%
- Subjective interpretation by MD or PhD reading the gel: 106; 43%
Q20: How do you report a normal serum protein electrophoresis pattern? Select all that apply

- Q20 Opt.1: Normal pattern (39.6%)
- Q20 Opt.2: Normal pattern. M-protein not detected (35.5%)
- Q20 Free text (Other) (30.2%)
Q21: How do you interpret a normal serum protein electrophoresis pattern (if ordered as a standalone test) but the clinical context suggests suspicion of plasma cell dyscrasia? Select all that apply.

- Normal pattern. M-protein not detected (36.3%)
- Suggest UPEP and IFE, and/or SFLC if clinically indicated (if not already done/ordered) (58.8%)
- Other (22.9%)
Q22: How do you report an oligoclonal banding pattern with 2 or more small bands on a polyclonal Ig background on serum protein electrophoresis? Select all that apply.

- Q22 Opt.1: Oligoclonal bands are present
- Q22 Opt.2: Oligoclonal bands are present. This can occur in a number of infectious or autoimmune conditions.
- Q22 Opt.3: Suggest review in 3–6 months if clinically indicated
- Q22 Free text (Other)

Percent of Respondents:
- 51.0%
- 29.0%
- 48.6%
- 15.5%
Q23: How do you report the first presentation of a small abnormal band on serum protein electrophoresis/immunofixation in a patient with no known M-protein? Select all that apply.

- Q23 Opt.1: There is a small band (type: e.g. IgG kappa) band approximately (amount: e.g. 1 g/L [0.1 g/dL]).
- Q23 Opt.2: Its clinical significance is uncertain.
- Q23 Opt.3: Suggest UPEP and IFE, or SFLC.
- Q23 Opt.4: Repeat SPEP in 3–6 months if clinically indicated.
- Q23 Free text (Other)
Q24: How do you report a new, small abnormal band with different electrophoretic mobility from the original M-protein in a patient with a known M-protein? Select all that apply.

- 35.1% Q24 Opt.1: There is a small (type: e.g. IgG kappa) band approximately (amount: e.g. 1 g/L) on a background of a polyclonal and/or oligoclonal pattern.
- 49.0% Q24 Opt.2: The new band is different from the original M-protein.
- 9.0% Q24 Opt.3: The clinical significance of the new band is uncertain.
- 30.6% Q24 Opt.4: In the case of the band being identified as IgG kappa: "A new small monoclonal IgG kappa band has been found in the gamma fraction on immunofixation. This could...
- 21.6% Q24 Free text (Other)
Q25: How do you report a positive kappa or lambda Bence Jones protein in random and/or 24 hour urine collections? Select all that apply.

- 66.5%: BJP detected
- 13.9%: BJP cannot be quantitated but is present in small or trace amounts on IFE
- 9.8%: BJP concentration in mg/L
- 3.3%: BJP concentration in g/L
- 0.0%: BJP concentration in mg/dL
- 0.0%: BJP concentration in g/dL
- 14.3%: BJP output in mg/24 h
- 20.4%: BJP output in g/24 h
- 18.4%: BJP/creatinine ratio in g BJP/mol creatinine (or mg/mmol)
- 1.2%: BJP/creatinine ratio in g BJP/g creatinine (or mg/mg)
- 18.8%: Free text (Other)
Q26: Please indicate your laboratory affiliations: Select all that apply.
Q28: Please indicate your approximate annual volume of SPEP testing

- Less than 1,000: 2; 1%
- 1,000 to 10,000: 109; 45%
- 10,000 to 50,000: 89; 37%
- 50,000 to 500,000: 13; 5%
- 500,000 to 1 million: 29; 12%
Q29: Please indicate your approximate annual volume of UPEP testing

- Less than 100: 14 (6%)
- Less than 1,000: 54 (23%)
- 1,000 to 10,000: 87 (37%)
- 10,000 to 50,000: 82 (34%)

Legend:
- Blue: Less than 100
- Red: Less than 1,000
- Green: 1,000 to 10,000
- Purple: 10,000 to 50,000
Q30: Please indicate your approximate annual volume of SFLC (by immunoassay) testing

- Less than 1,000
  - 97; 41%
- 1,000 to 10,000
  - 111; 47%
- 10,000 to 50,000
  - 25; 11%
- 50,000 to 500,000
  - 3; 1%