The Association for Clinical Biochemistry & Laboratory Medicine
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The Royal College of Pathologists
Pathology: the science behind the cure

Audit Template

Audit Title: A regional audit of cryoglobulin analysis in Scotland

Lead Auditor: Dr Karen Rankin and Dr Emma Dewar	**Audit date(s):** Nov 2021-Jan 2022
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Aims of the Audit:

To evaluate the current practice across Scotland for performing cryoglobulin analysis.

References:

1. Sargur, R., White, P., and Egner, W. Cryoglobulin evaluation: best practice? Annals of Clinical Biochemistry 2010; 47:8-16.
2. Vermeersch P, Gijbels K, Marie¨n G, et al. A critical appraisal of current practice in the detection, analysis, and reporting of cryoglobulins. Clin Chem 2008;54:39–43
3. Kolopp-Sarda M & Miossec P. Practical details for the detection and interpretation of cryoglobulins. Clin Chem 2022; 68: 282-290.
4. Sheldon, J. Cryoglobulin analysis & interpretation masterclass 2020.

Audit Method:
A 38 question survey was designed by ACB Scotland Clinical Audit Group and was sent to all 14 regional health boards in Scotland. The questionnaire remained open from 23rd November 2021 to the 31st January 2020. The survey sought relevant annual workload figures where possible.

Audit Outcomes:
Twelve responses to the questionnaire were received as well as one email response stating that cryoglobulin analysis was not offered within that health board. 11 labs who responded performed cryoglobulin analysis within their laboratory with one referring samples to an external laboratory.

1. **Which departments are involved in cryoglobulin analysis?**

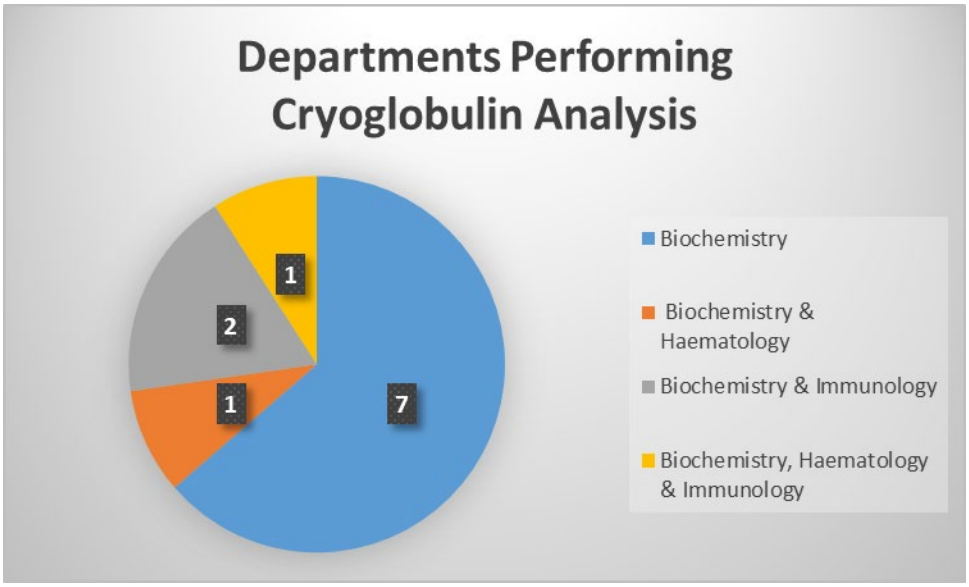


Figure 1. Departments performing cryoglobulin analysis.

Figure 1 demonstrates that in the majority of health boards the department of Biochemistry is solely responsible for cryoglobulin analysis.

2. How many samples does your laboratory analyse per year?

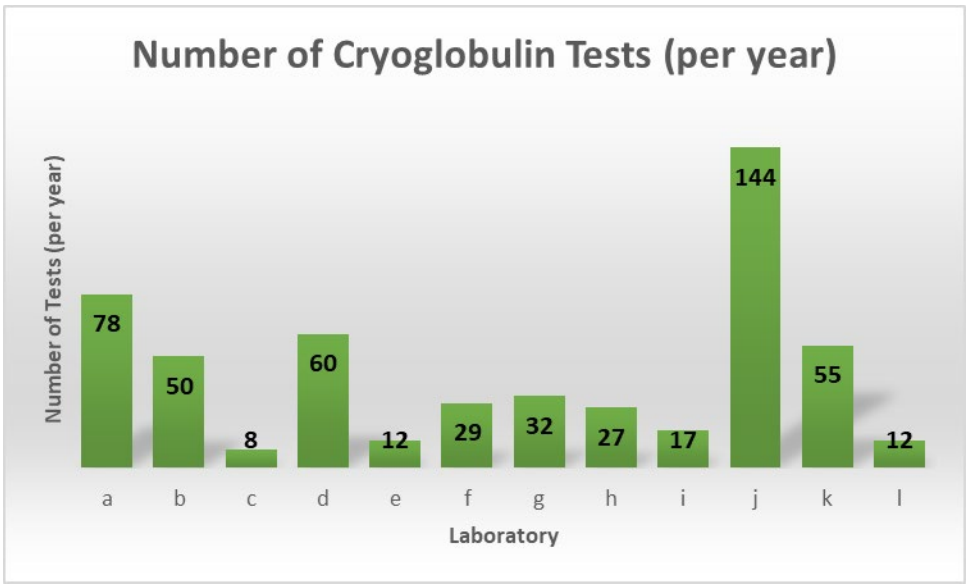


Figure 2. Anonymised work load data for each health board.

Work load for cryoglobulin analysis ranged from 8-144 samples per year (median = 30.5).

3. Which disciplines refer the most samples?

The majority of laboratories indicated that Rheumatology was the most frequent requester of cryoglobulin analysis. This was closely followed by Haematology, Renal, Dermatology, Critical Care, Neurology and General Medicine.

4. **Do you provide instructions for users to refer to when taking these samples?**

10 laboratories said Yes they do provide instructions. This was by various means including instruction cards, electronic pop up messages, laboratory handbooks and staff attending to collect or assist with the collection of samples. Of those that responded 'No' the reasons given were because requests are made by email and that sample requirements are discussed with requestors then a member of staff goes at a pre-arranged time with pre-warmed samples/flasks.

5. **Do you suggest measurement of C3, C4 and RH Factor as part of cryoglobulin work up?**

9/12 laboratories do not suggest measurement of C3, C4 and rheumatoid factor as part of a cryoglobulin work up. However, 1 of these laboratories intends to add this to their handbook. 3/12 do recommend measuring these analytes, with one laboratory only doing so if the cryoglobulin analysis is positive. Sargur et al., (2010), Kolopp-Sarda and Miossec (2022) as well as the Protein Reference Unit at St. Georges, recommend measurement of these analytes to assist with characterisation of the cryoprecipitate.

6. **Immunology tests easily accessible?**

When asked if immunology tests are easy to add on to cryoglobulin samples 6 laboratories stated 'Yes' while the remaining 6 laboratories stated "No'. Reasons provided for difficulties adding on immunology tests were largely due to logistical issues i.e. immunology being a separate department sometimes on another site; the sample would need renumbered and booked into LIMS separately making it difficult to track. Or that the collected sample type was not suitable for immunology tests.

7. **Are Immunology Tests run at 37°C?**

The majority of responders (8/12) were unsure if Immunology tests were analysed at 37°C and a further 3 laboratories stated that analysis was not performed at this temperature. One laboratory replied that rheumatoid factor is run at 37°C.

Pre-Analytics

8. **What sample types do you request?**

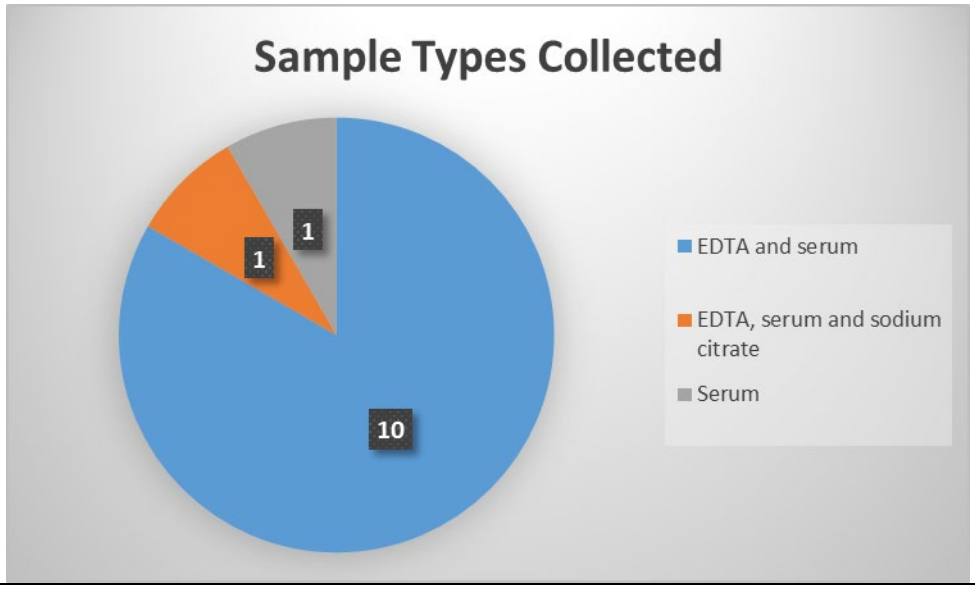


Figure 3. Sample types collected for cryoglobulin analysis

Sargur et al. (2010) recommend collecting EDTA as well as serum samples to exclude cryofibrogenaemia and the majority of laboratories do this. However, Kolopp-Sarda and Miossec (2022) state that tubes containing EDTA or citrate anticoagulants should not be used since they chelate calcium and many cryoglobulins require calcium to precipitate. They also indicate that heparin samples should not be used for cryoglobulin analysis due to spontaneous cryoprecipitation of complexes formed by heparin-fibrinogen-fibronectin when a sample is stored at 4°C. This may lead to false positives.

9. Are the sample tubes preheated prior to sampling?

Eleven out of 12 laboratories do preheat the sample tubes prior to sampling using various methods. One laboratory does not. Sargur et al., (2010), Kolopp-Sarda and Miossec (2022) as well as the Protein Reference Unit at St. Georges recommend using pre-warmed tubes for sampling.

10. At what temperature are the samples transported?

Samples should never be carried by hand to the laboratory. Instead, samples should be transported at 37°C to maintain sample integrity avoid false negative results (Sangur et al. (2010); Kolopp-Sarda and Miossec (2022) and the Protein Reference Unit at St. Georges). Where adequate temperature control cannot be ensured, it is recommended that cryoglobulin investigations should not be attempted. Encouragingly, 10 laboratories reported that sample tubes are heated to 37°C or above in order to maintain 37°C temp during collection and 1 laboratory heats water to 42°C. However, one laboratory keeps the sample at body temperature by transporting the sample in a staff member's armpit.

11. What type of container are samples transported to the laboratory in?

Eight laboratories follow the recommendation of using a flask containing either heated water or sand to transport cryoglobulin samples. The containers used by the remaining 4 laboratories included – flask with no additive, armpit, portable mini incubator and a flask with a heated gel pack inside. The temperature of the container used should remain between 38-40°C and not fall below 37°C (Sangur et al. (2010); Kolopp-Sarda and Miossec (2022) and the Protein Reference Unit at St. Georges).

12. What time interval is recommended between sampling and centrifugation?

A minimum of 1 hour clotting time (at 37°C) prior to centrifugation is recommended by Sargur et al. (2010). All laboratories follow this recommendation with timing between sampling and centrifugation ranging from 1-7 hours. The most common length of time to wait was 4 hours. However, Kolopp-Sarda and Miossec (2022) recommended allowing a minimum of 2 hours for samples to clot at 37°C.

13. Do you use a heated centrifuge and what temperature do you centrifuge samples at?

Again, to avoid false negatives, it is recommended that samples should be centrifuged at 37°C. Nine out of 12 laboratories do use heated centrifuges for cryoglobulin samples with the temperatures uses ranging from 37-40°C. The remaining 3 do not use heated centrifuges so centrifuge samples at room temperature. It is unclear whether this is due to lack of access to a temperature controlled

centrifuge. The guidance states that "When a warm centrifuge is not available, specimens should be allowed to separate at 37°C, and serum drawn off without centrifugation. Following separation, any haemolysis or lipaemia should be noted since this may interfere with visual interpretation of cryoprecipitation."

Analysis

15. Do you divide samples to be held at various temperatures? If yes, at what temperatures?

Eleven laboratories do divide samples to be held at various temperatures with 10 of these doing so at 4°C and 37°C which aligns with the recommendations by Sargur et al (2010). One laboratory divides samples to be stored at 4°C, 37°c and room temperature. One laboratory stated that this question was not applicable.

16. What type of tubes do you aliquot serum and plasma into?

The Protein Reference Unit at St Georges and Kolopp-Sarda & Miossec (2022) suggest using conical bottom tubes. Only 2 laboratories currently use conical bottoms tubes. Eight laboratories uses Sarstedt tubes which have round bottoms and one of these laboratories is actively seeking to change to conical tubes. Of the remaining two laboratories – one uses Accuvettes and the other stated this question was not applicable.

17. Once divided how many days do you hold samples at the various temps?

Eleven laboratories meet the best practice criteria of keeping samples at the various temperatures for 3-7 days. One laboratory again stated that this was not applicable.

18. How often do you check for precipitate and how is this recorded?

Six laboratories check daily for precipitate and 2 laboratories check after 3 days. One laboratory checks at 4 hours, 24 hours, 48 hours and 7 days. One laboratory checks at 24 hours, 3 days and 7 days and one checks after 3 days and 7 days. One laboratory stated this is not applicable. Ideally samples should be stored for 3-7 days and inspected for precipitate every day. Only half of the laboratories who responded are following this recommendation. However, all laboratories record their findings on worksheets.

19. Do you keep a photo record?

The protocol from the Protein Reference Unit at St. Georges states that's photographs are taken of the specimen tubes, side by side, as a permanent record of what was observed. However, on 2 laboratories currently do this. Nine laboratories do not take photographs but two of these indicated that they would like to implement this. One laboratory said this was not applicable.

20. Do you confirm a positive by rewarming to 37°C?

If the precipitate does not become soluble once re-heated to 37°C for a few minutes then the result should be recorded as negative and no further investigation is required (Sargur et al 2010). Nine laboratories do reheat their samples and 2 laboratories do not. However one does as visual comparison with a sample kept in an oven at 37°C. One laboratory said this was not applicable.

21. Do you run electrophoresis on the 37°C samples?

One of the options to quantify a cryoglobulin is to perform capillary zone electrophoresis. Six laboratories perform electrophoresis at 37°C with most doing CZE and immunofixation. One laboratory doesn't do it routinely, only if electrophoresis is requested at the same time and 4 laboratories do not perform electrophoresis at 37°C. One laboratory said this was not applicable.

22. Do you measure TP, Albumin Igs on the 37 sample?

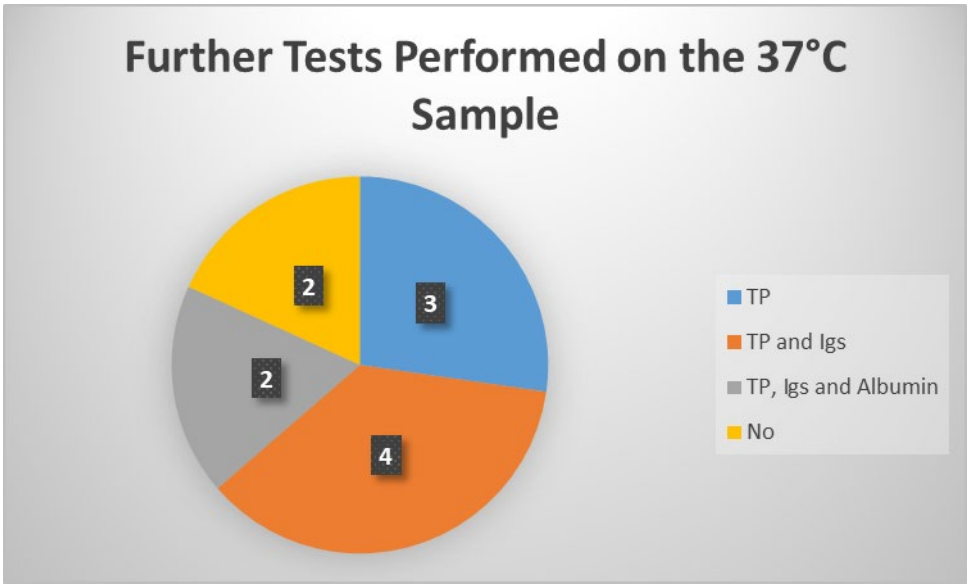


Figure 4. Further testing performed on the 37°C sample. TP = total protein, Igs = immunoglobulins.

One laboratory stated that total protein and immunoglobulins would only be performed if requested at the time, as part of an electrophoresis profile. One laboratory said this was not applicable.

23. If there is precipitate do you characterise and type the samples?

Sargur et al. (2010) insist that characterisation and typing of cryopreciptate should always be performed to assist clinicians in determining the cause and prognosis. All laboratories offer this service either in-house (6 laboratories) or by referring to another laboratory (5). One laboratory said this was not applicable.

24. If you characterise and type in house do you remove the supernatant and wash the precipitate?

All six laboratories that this question is applicable to, answered 'Yes'.

25. How many times is the precipitate washed?

Five laboratories wash the precipitate three times and one laboratory washes the precipitate twice. This question was not applicable to the remaining six laboratories. A previous audit by UKNEQAS (Vermeersch et al., 2008) highlighted significant variation in the number of washes performed since each wash step risks a loss of protein due to dilution. However, Sargur et al. (2010) and Kolopp-Sarda and Miossec (2022) recommend 3 successive washes with cold saline.

26. Do you immunofix on serum and plasma?

Of the six laboratories that perform immunofixation of cryoglobulins in-house, 3 laboratories

perform immunofixation on both. However, 3 laboratories only perform immunofixation on serum.

27. Do you immunofix both the supernatant and precipitate?

All of the six laboratories that perform immunofixation in-house do this on both the supernatant and the precipitate as per the protocol describe by the Protein Reference Unit at St George's.

28. Do you quantify the precipitate if isolated?

Only three laboratories quantify isolated precipitate. One laboratory does this using total protein analysis of the precipitate re-dissolved at 37°C while another uses a more elaborate method of Cryoprecipitate (g/L) = TP of warmed room temperature sample – TP of warmed 4°C supernatant. The third laboratory uses CZE. A further 4 laboratories stated they do not quantify the precipitate and the remaining 5 laboratories indicated this question was not applicable.

29. What do you report if precipitate is identified as fibrinogen?

Six laboratories report "cryofibrinogen detected" or "cryofibrinogen positive" some with additional comments including:

- Results must be interpreted in view of the clinical picture
- 'It should be noted that the prevalence of CF in healthy subjects has been estimated to be from 2% to 9% (3% of blood donors). A false-positive result may occur if heparin has been given therapeutically to the patient'. This particular lab also reports the concentration of cryoprecipitate with a reference range.

Six laboratories stated this was not applicable.

30. Do you type for fibrinogen? If yes, how?

The majority of laboratories stated no (6) or not applicable (4). Of the two laboratories that do type for fibrinogen, one acts as a referral centre for the other. Typing is performed using total protein analysis and immunofixation using anti-fibrinogen anti-sera.

Post-Analysis

31. Which department is responsible for reporting cryoglobulin results?

Biochemistry is solely responsible for the reporting of cryoglobulin results in the majority of health boards (7 laboratories). Three laboratories stated that Biochemistry and Immunology share responsibility for cryoglobulin reporting and at another the Biochemistry and Haematology are responsible. One laboratory responded that this question was not applicable.

32. Which members of staff report cryoglobulins?

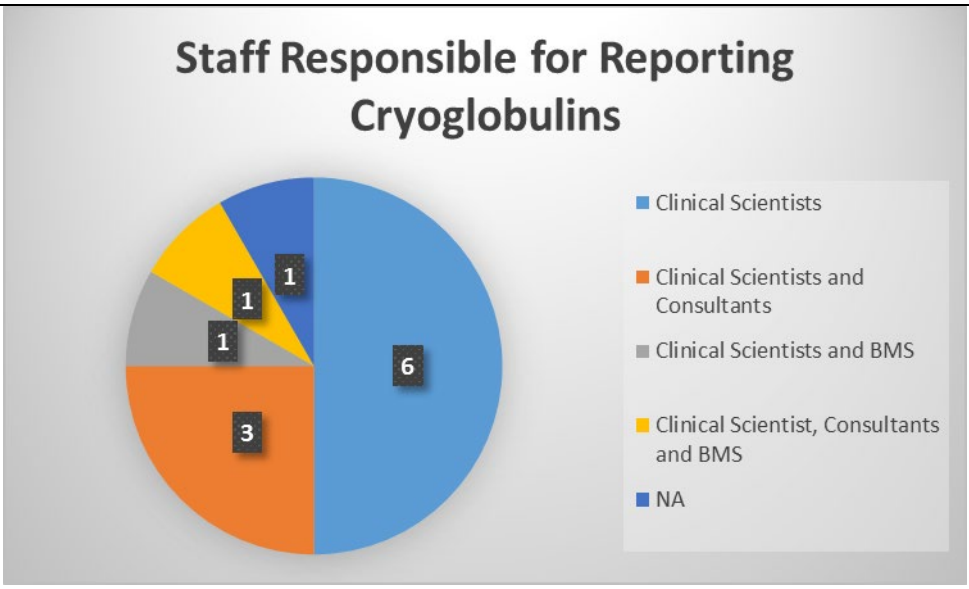


Figure 5. Staff responsible for cryoglobulin reporting.

In the majority of health boards Clinical Scientists report cryoglobulin results.

33. How do you report negative results?

Eleven laboratories use a standard comment. Examples include:

- No cryoglobulin or cryofibrinogen detected
- No cryoprecipitation detected
- A negative cryoglobulin assay may not exclude the diagnosis if the clinical suspicion remains high. Poor collection technique may lead to false-negative results.

One laboratory stated that this question was not applicable.

34. How do you report positive results?

This question generated extensive responses as follows:

- Standard comment: sometimes expanded to describe characteristics of the sample which may allow typing
- Standard comment: Comment on cryoglobulin screen/fix includes: 1.Cryoglobulinaemia type detected 2. Results of immunofixation 3. Examples of conditions associated with cryoglobulinaemia type. eg. “This is a type 1 cryoglobulin consisting of monoclonal IgG lambda. This may be associated with underlying lymphoproliferative disease e.g. Waldenstrom’s. Comment on EP states that EP was analysed at 37oC as part of cryoglobulin screen. If type 1 cryo is detected, report includes comment that future EP samples must be collected at 37oC. Note also added to patient's Clinical Portal record to this effect.
- Concentration and type of cryoprecipitate is reported as free text
- Standard comment: Cryoglobulin detected. See laboratory comment.
- Standard comment: positive (sample would be sent to Immunology at referral lab to confirm type)

- Standard comment
- Standard comment:No precipitate in 4C plasma tube but precipitate in serum 4C tube = Cryoglobulins detected, cryofibrinogen excluded OR Precipitates in serum and plasma 4C tubes = Cryoglobulins detected, cannot exclude cryofibrinogen.
- Detected. Then once cryoglobulin identification has been performed there is a standard comment for type 1, type 2 and type 3:
    - TYPE1 comment: "This cryoglobulin consists of monoclonal (enter immunofixation result here). This may be associated with underlying lymphoproliferative disease eg Waldenstrom's Macroglobulinaemia or myeloma and may be associated with hyperviscosity.'
    - TYPE2 comment: "This cryoglobulin consists of mononclonal (enter immunofixation result here) and polyclonal IgG with rheumatoid factor activity. Type 2 Cryoglobulins may be idiopathic or associated an underlying problem eg lymphoproliferative or connective tissue disease or chronic infection eg Hepatitis B or C".
    - TYPE3 3 comment: "This cryoglobulin consists of polyclonal immunoglobulin with rheumatoid factor activity. Type 3 cryoglobulins may be associated with connective tissue disease, other chronic inflammatory or infective disorders eg viral hepatitis".
- Positive for cryoglobulin (on day x) - sample will be referred for additional characterisation
- We append all referral lab comments
- Other: State that the sample shows a cryoprecipitating paraprotein (include type), state it has been confirmed by precipitation, washing and immunofixation of precipitate. If C3/4 and RF available mention the results and any clinical condition it could be associated with.

35. Do you include C3, C4 and Rheumatoid Factor?

The majority of laboratories do not include these analytes (8 laboratories) or stated this was not applicable (2). One laboratory includes rheumatoid factor and the other includes them in the interpretation if the results are available.

36. Do you suggest cryoglobulin type?

Eight laboratories suggest a cryoglobulin type or append the comments provided by a referral laboratory. However, 1 laboratory only does this if the type is obviously clear based on all sample, patient history, presentation, other immunological, serological and biochemical tests. Three laboratories do not suggest a cryoglobulin type and one laboratory stated this question was not applicable.

37. Are you registered with and EQA scheme?

Ten out of 12 laboratories are registered with and EQA scheme and only 1 is not. The remaining laboratory said this question was not applicable.

38. Do you have any other comments to add?

Eight laboratories had no comments to add. The remaining 4 provided additional comments as

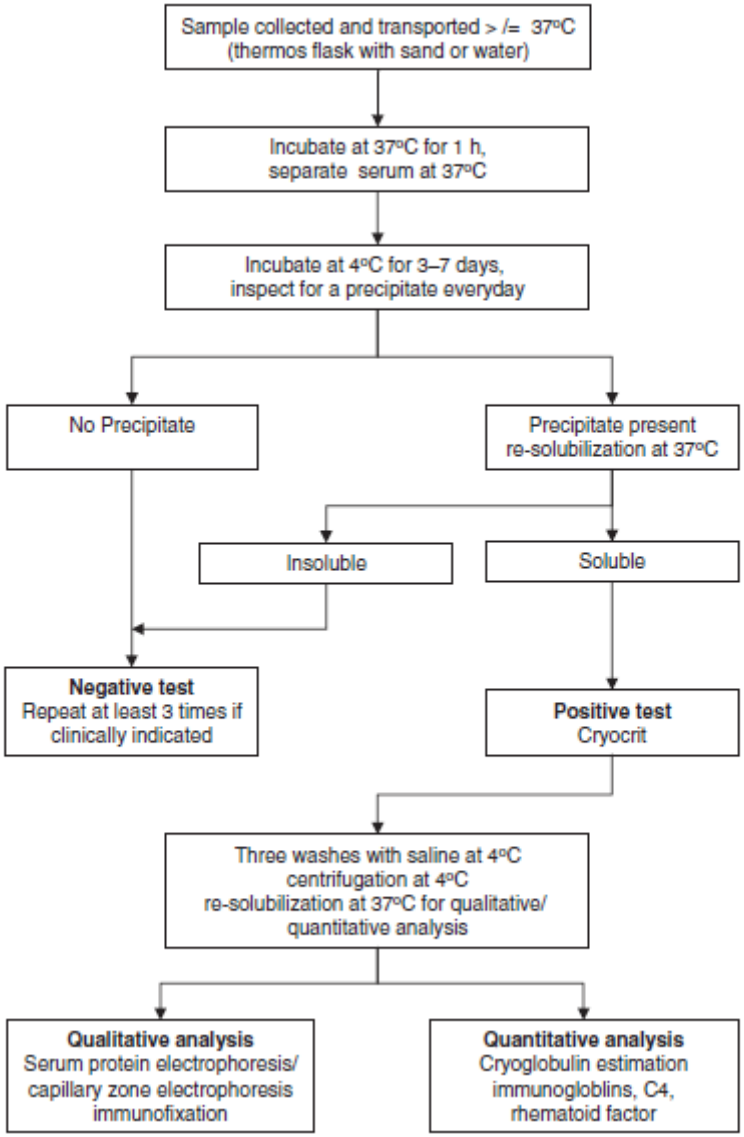
follows:

- Despite measures above which have improved our success rate, it is difficult to ensure ward/clinic staff adhere to guidance on sample collection (e.g. They have been observed using vacutainers other than those supplied in flask)
- We have verified our sample collection system to verify we can maintain the sample temp >37°C during collection and separation for UKAS
- In process of reviewing processes and revising and formalising new protocol to cover preanalytical, analytical and post-analytical aspects
- Our guidance has been recently updated (from 2010 Sargur paper), but still a lack of detail available on best practice / how to improve/standardise. For example preferred method of collection, type of thermometer, type of flask, how useful is the characterisation etc.

Audit Recommendations / Standards:

This audit demonstrates that there is variation in practice across Scottish NHS health boards and areas for improvement. As per the guidelines referenced within this audit we recommend the following:

- A fresh specimen of blood should be taken directly into a warmed container at 37°C
- Samples should be delivered to the laboratory ≥ 37°C
- Samples should be allowed to clot at 37°C – minimum 1 h.
- Serum should be separated by centrifugation at 37°C
- Aliquots of separated serum should be kept at 4°C and 37°C for at least three days
- Labs should record observation of any cryoprecipitate formation at 48C, which should become soluble again when placed at 37°C
- Labs should provide a reasonably robust quantitation estimate
- Labs should analyse the proteins to classify the type of cryoglobulin
- Labs should advise that C3, C4 and RF analysis should be performed
- Labs must be prepared to repeat the sample on multiple occasions to ensure that false-negative results do not occur when clinical suspicion remains high
- Labs must be part of EQA scheme for measurement of other proteins in the protocol



Please indicate to whom and when audit presented &/or circulated &/or published:

Scottish ACB Audit Group – 03/03/23

Audit recommendations / standards ratified by … and when: Scottish ACB Audit Group – 16/06/23

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