Incorrect specimen collection for investigation of cryoproteins can give mis-leading results

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Introduction

Cryoproteins are proteins that precipitate reversibly at temperatures below 37°C. Cryoproteins are classified as type I, II and III cryoglobulins, and cryofibrinogen. Type I cryoproteins are monoclonal proteins associated with B cell malignancies including Waldenstrom's macroglobulinaemia, myeloma and lymphoma. Type II and III cryoproteins are associated with chronic infection or inflammation e.g. hepatitis C, Sjogren's syndrome.

Cryoproteins can be associated with clinically significant symptoms, including hyperviscosity, purpura, neuropathy, renal impairment, Raynaud's phenomenon and even ulceration and necrosis. Cryoprotein symptoms can precede symptoms associated with any underlying malignancy.

Cryoproteins each have their own thermal profile; some precipitate within minutes of cooling below 37°C while others may only precipitate at room temperature or below, or precipitate slowly over days. It is vital that when investigating cryoproteins, samples are collected at 37°C and maintained at 37°C during clotting and sample separation, otherwise any precipitated cryoprotein may be centrifuged into the clot and discarded during sample processing.

Paraprotein concentration is important for assessing tumour burden at diagnosis, the need for urgent treatment e.g. plasmapheresis, and also as a measure of response to treatment. If the paraprotein is a cryoglobulin, the paraprotein concentration may be under-estimated if the sample is not collected appropriately, as presented here. This could have significant clinical consequences.

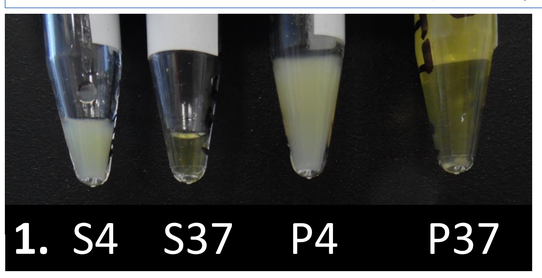
Type I cryoglobulins

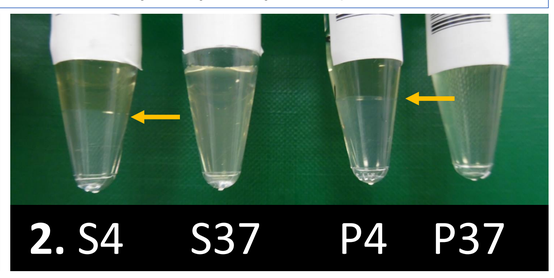
Type I cryoglobulins form precipitates that consist entirely of a monoclonal protein.

The precipitates may appear gel-like or 'fluffy', opaque or translucent, and may first be seen as an incidental observation during lab analysis.

Type I cryoglobulins can be associated with symptoms of hyperviscosity, due to high concentrations of the monoclonal protein, but can also be associated with symptoms of impaired perfusion due to cryoprecipitation in blood vessels in the extremities, depending on exactly what temperature the cryoprotein precipitates e.g. Raynaud's phenomenon, tissue necrosis affecting toes, ears, nose, or ears.

Example images of two Type I cryoglobulins. Case 1 had a milky, gel-like precipitate. Case 2 has a clear gel-like precipitate that is harder to see, but is obvious when the tube is tilted (arrow points to top of precipitate).





Case 1

Clinical information: A 67 Year old man diagnosed with Waldenstrom's macroglobulinaemia in August 2017.

Lab information: Initial samples showed an IgM kappa paraprotein. A precipitate was noted during analysis of a subsequent sample. The clinical team was alerted and a cryoprotein collection arranged. During subsequent monitoring, samples were collected at room temperature. These samples showed a decrease in paraprotein concentration when no treatment had been given. Analysis of samples collected for cryoprotein studies showed the true paraprotein concentration was 6 times higher.

Date	Temp of collection	Paraprotein conc. (g/L)
05.07.18	RT	34
15.04.19	RT	47
27.06.19	RT	7
03.07.19	37°C	42

Under-estimate = 90.5%

Case 2

Clinical information: A 87 Year old man diagnosed with myeloma in November 2018.

Lab information: Initial samples were noted to be viscous in the Blood Sciences laboratory and referred to Immunology for further investigation. An IgG kappa paraprotein was identified. During follow-up not all samples were collected correctly for cryoprotein analysis.

Date	Temp of collection	Paraprotein conc. (g/L)
15.01.19	37°C	32
28.02.19	RT	5
12.06.19	RT	8
17.06.19	37°C	51

Under-estimate = 84.3%

Case 3

Clinical information: A 76-year old man diagnosed with myeloma in August 2017. Patient presented with renal failure and hypercalcaemia.

Lab information: A large IgG lambda paraprotein was identified. During analysis of a second sample, an obvious precipitate was noted in the sample tube. Clinical team was contacted to discuss finding and cryoprotein collection arranged.

Date	Temp of collection	Paraprotein conc. (g/L)
24.08.17	RT	44
31.08.17	37°C	74

Under-estimate = 40.5%

Conclusions

1. It is vital that samples for cryoprotein investigations are collected directly into pre-warmed tubes and are maintained at 37°C during transport to the lab, clotting and sample separation. The steps should be validated to confirm that the temperature is maintained at 37°C.
2. It is vital that subsequent samples taken to monitor a patient with a cryoprecipitating monoclonal protein are ALWAYS collected according to the cryoprotein protocol, otherwise there is a risk of significant under-estimation of paraprotein concentration. This can be facilitated by maintaining good records and having clear communication by the laboratory that the patient is known to have a cryoprotein.
3. Paraprotein concentration results that do not make sense in the context of clinical treatment should be discussed immediately with the laboratory and the presence of a cryoprotein considered as a possible root cause.
4. Laboratory staff should be alert to the presence of precipitate in samples. Cryoprecipitates can be distinguished from a fibrin clot by incubating the sample overnight at 37°C – the cryoprecipitate should disappear back into solution. Clinicians should be alerted to the presence of a cryoprecipitate.

Methods

Serum samples were collected at room temperature or collected into samples tubes that had been stored in a 37°C incubator for >24h and transported to the lab in insulation socks in a thermos flask, to keep the temperature at 37°C, as previously published (5). The samples were allowed to clot for at least 30min before separating by centrifugation. The 37°C samples were separated using a temperature-regulated centrifuge which had been pre-warmed for 1h. An aliquot of the 37°C samples were incubated at 4°C for >72h then inspected to check for the presence of cryoproteins. Serum immunoglobulins (Immage rate nephelometer; Beckman Coulter, UK) and total protein (MIRA colorimeter; Cobas, UK) were quantified on the room temperature and 37°C samples. Serum protein electrophoresis was done using the Capillarys 2 (Sebia, UK). Monoclonal proteins were delimited (full drop) using Phoresis software (Sebia, UK).

References

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