EuMyoNet

Serology Collection and Standardisation

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Importance

- Homogeneity
- Reliability and significance of the results

Non specific general consideration

- Local ethics committee approval should be obtained before sample collection
- Patient anonymization should be secured

Patients to be included

- All patients with idiopathic inflammatory myositis (IIM), with antisynthetase syndrome or with antisynthetase autoantibodies without a specific diagnosis
- Patients with uncertain diagnosis could be included with clear words of caution in the diagnosis or with use of diagnosis of unspecific myositis

Types of analysis

Usually performed in the individual centres

- Muscle enzymes
- ESR, CRP
- Some (classical) autoantibodies
- Some viral analyses (serology)

Possibly performed in the individual centres

- Cytokines
- Coagulations assays

Performed centrally

- Multiple autoantibody screening by **line blot test** (LB): Peter Charles at the Kennedy Institute, London
- **Immunoprecipitation** (IP) for rarer antibodies not detectable with LB) : Zoe Betteridge at Bath Institute of Rheumatic Diseases, Bath UK
- Possibly cytokines in defined projects
- Possibly some viral analyses (serology) in defined projects
- Possibly other (not defined yet) tests in defined projects

Type of samples to collect

EDTA blood or serum; ideally at least 1 EDTA blood sample (see below for amounts and storage)

EDTA Plasma: adequate for
- Autoantibodies
- Cytokines
- Viral analysis (serology)

Serum: adequate for
- Autoantibodies
- Cytokines
- Viral analysis (serology)

NB: preparation process of plasma is easier, and allows parallel collection of DNA. **Heparin** must be **avoided** because it interferes with serology analyses.

Frequency and time of blood sample collection

At the time of inclusion in the cohort
- Ideally at the time of diagnosis, before initiation of immunosuppressive agents (can quickly modify the levels of autoantibodies and cytokines, see below)

Additionally At the time of significant changes in therapy
- Ideally: 3-6 months later

NB: repeat measurements are potentially important for cytokine analysis and potentially interesting for autoantibodies.

Effects of treatments on samples

Autoantibody analyses
- Glucocorticoids (GC): probably no significant change for 2 months
- Conventional immunosuppressives (IS): probably no significant change for 2 months
- Biologics (anti CD-20 (Rituximab-Mabthera®): possible significant changes after 1 month
- Plasmapheresis: probable immediate profound changes
- IvIg: possible immediate changes (modest)

Cytokine analyses
- GC: possible changes within days
- Conventional IS: possible changes within weeks
- Biologics: possible immediate changes

Therefore, plasma and serum samples should definitely be **collected before treatment initiation**, w**henever possible.**
Treatments should definitely be **recorded** and remain **traceable**!

Processing

Pre-analytic procedures are probably not critical for detection of autoantibodies or cytokines, but:

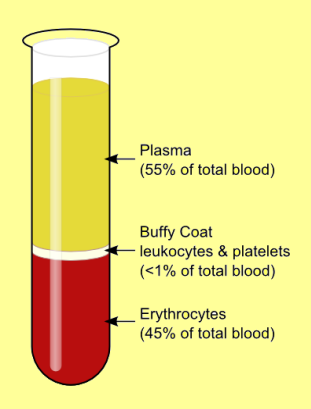
- Hemolytic, lipemic, and icteric sera should be recorded, as they may influence the test system.
- Processing should take place within 4 hours of blood drawing (room temperature).
- Standardized procedure might be preferable! Numerous protocols are equivalent and possible. Detailed below is just one example.

Example of standard procedure for plasma processing (with possible DNA preparation in passing):

1. Draw the blood from the patient using a straight needle or butterfly, depending on the needs of the vein.
2. Attach a lavender-topped Vacutainer tube to the needle so that its vacuum pressure sucks the blood into the tube. A lavender-topped tube contains EDTA.
3. Place the tube in a centrifuge (3,000 RPM) for 10 minutes. When it is done spinning, the blood will be separated into red blood cells (the bottom, red in color), white blood cells (upper part of the RBC plug ("buffy coat")) and plasma (the top, which is colorless).
4. Make properly labelled aliquots and freeze them, if possible at -70°C (see below).

Example of standard procedure for DNA preparation during plasma processing

1. Collect the upper parts of the RBC plugs ("buffy coat") that contains the white blood cells and rapidly freeze them, if possible at -70°C (see below).



Example of standard procedure for serum processing:

2. Obtain fresh blood in sterile glass vacutainer tubes.
3. Put the blood to clot at RT for 1 hour.
4. Pull the rubber stopper off the top, and use a long Pasteur pipette to "ring" the clot, i.e. separate it from the sides of the glass tube. Alternatively, just invert the tube; if the clot pulls free from the glass walls of the tube, then you don't have to ring the clot.
5. Decant the serum away from the clot into a new centrifuge tube. Some red blood cells and bits of clot will come along with the serum. Spin the serum at 3,000 RPM for 10 min to remove the remaining clots/red blood cells and other insoluble material.
6. Make properly labelled aliquots and freeze the serum if possible at -70°C (see below).

Amount of samples

At entry

Ideally a minimum of **5 aliquots, each 250 µl** of plasma or serum.
An absolute **minimum** of **one 100µl** of plasma or serum is required for entry in the EuroMyositis biobank.

At follow-ups

If possible, a minimum of 1-2 aliquots of 250 µl serum at follow-up visits

Labeling

EuroMyositis Database coding-labelling, with **date**, is preferable

Storage - temperature

- **Principal storage** should take place **in the individual centres** but some **samples,** from all patients at entry in the EuroMyositis biobank, if possible, should be **shipped promptly** to the **reference centre** for serology analyses (Peter Charles, Kennedy Institute, London, United Kingdom, see below). If preferred, in case of limitation of storage capacity for instance, all samples can be shipped at once to the Kennedy Institute.
- **Ideally**, serum, EDTA-plasma samples and white blood cells for DNA should be stored at **-70°C** in cryovials. Temperature of **-20°C** is **acceptable** if the freezer is adequately controlled, and any problems properly recorded. For storage exceeding 4 months, samples should be transported to centres where conservation at -70°C can be carried out.

Shipping to the serology analysis centre

- Samples must be placed in **dry ice**.
- Shipping should take place early in the week, preferably on **Mondays or Tuesdays**.
- A notice should be e-mailed to Peter Charles beforehand (peter.charles@kennedy.ox.ac.uk)
- Address:
    - P. J. Charles
    - Kennedy Institute
    - 1 Lurgan Avenue
    - London W6
    - United Kingdom