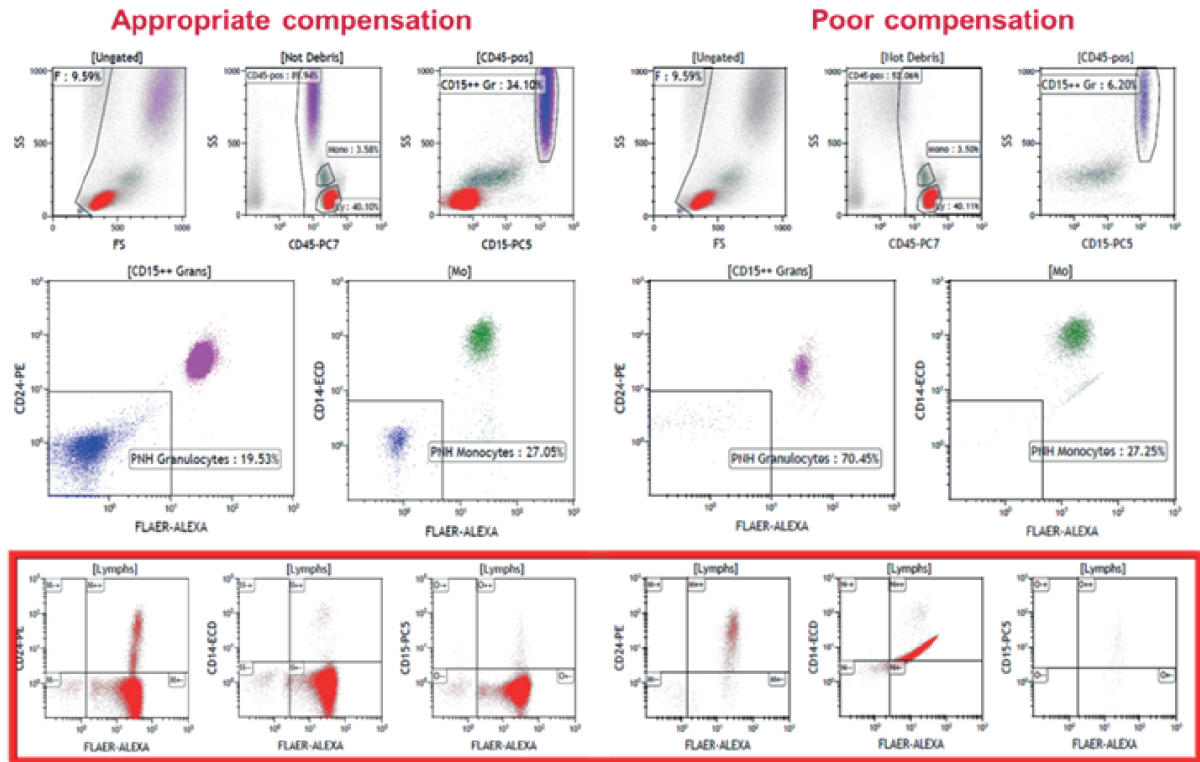


Quality control on lymphocytes*

In addition to optimising antibody concentrations and validating the sensitivity of the assay, quality controls should be put in place to confirm antibody and instrument performance.

Lymphocytes, although not recommended for the detection of paroxysmal nocturnal haemoglobinuria (PNH) clones, can serve as excellent quality-control material.

Compensation check on lymphocytes



FLAER, fluorescent aerolysin; FS, forward scatter; PE, phycoerythrin; SS, side scatter

The top row shows gating dot plots for PNH testing, the middle row shows diagnostic dot plots for PNH testing, and the bottom row shows some additional 'control' dot plots using PNH lymphocytes (red box).

Starting from left to right, the first dot plot (fluorescent aerolysin [FLAER]/CD24) shows good signal-to-noise ratio for the FLAER-positive normal lymphocytes versus the FLAER-negative PNH lymphocytes; both populations are visibly 'on scale'. The purpose of the PNH lymphocytes is not to assess the PNH clone (which is always much smaller than in the granulocytes), but rather to ensure that the negative cells are visible and not 'crushed'. This dot plot also shows the FLAER+/CD24+ B-cells and includes confirmation that CD24 was added. This may be important for cases with no, or rare, PNH granulocytes, which could also be seen if the antibody were not added.

The second (FLAER/CD14-ECD) and third (FLAER/CD15-PC5) dot plots also serve as confirmation for antibody performance and instrument settings.

*These protocols were developed in close collaboration with Mrs Andrea Illingworth of Dahl-Chase Diagnostic Services in Bangor, ME, USA, Drs Thomas Matthes and Mathieu Hauwel of the Swiss Flow Cytometry School at the University Hospital of Geneva, Switzerland, and Dr Iuri Marinov of Hematology and Blood Transfusion in Prague, Czech Republic. Images were provided with permission from the netflow Steering Committee and Swiss Flow Cytometry School.