

An Introduction to Analysis of immunoglobulin heavy chain/light chain pairs (Hevylite™)

Summary: Immunoglobulin Hevylite (HLC) immunoassays:-

1. Are more sensitive than SPE for quantifying monoclonal immunoglobulins.
2. Provide quantitative information compared with IFE and can be more sensitive.
3. Are of clinical value when monitoring patients with monoclonal gammopathies.
4. Provide information on immunoglobulin light chain subsets in immunohistochemistry.

Introduction: limitations of immunoglobulin measurements

Typical analytical tests for monoclonal gammopathies are SPE with scanning densitometry and/or IFE together with sFLC immunoassays. While SPE is a simple, cheap test, it is not particularly sensitive so that quantification of proteins at low concentrations (1-3g/L) is inaccurate. This is particularly apparent for monoclonal IgA since its anodal electrophoretic migration positions it over other bands such as transferrin. Improved sensitivity is achieved with IFE but it is a non-quantitative assay. Nephelometry is also used for immunoglobulin measurements and is analytically accurate to low concentrations. However, patients' samples also contain non-tumour polyclonal immunoglobulins of both κ and λ types that are included in the analysis so that results are clinically inaccurate at normal serum concentrations. Furthermore, assessments of monoclonal IgG are unreliable because of variable catabolism as FcRn recycling receptors become saturated or reduced by chemotherapy.

In contrast, one of the great diagnostic benefits of serum FLC analysis is the κ/λ ratio. This is because:-1), it provides a quantitative assessment of FLC clonality, 2), it has typical high immunoassay sensitivity, 3), clinical ranges are wide due to immunosuppression of the non-tumour FLCs and 4), there is automatic compensation for variable renal metabolism and changes in blood volume.

Immunoglobulin heavy chain/light chain immunoassays -“Hevylite” (HLC), have similar analytical advantages.

Concept: immunoglobulin heavy chain/light chain assays

Intact immunoglobulin molecules contain unique junctional epitopes between the heavy chain (CH1) and light chain (CL) constant regions (Figure 1). These are the target of Hevylite (HLC) antibodies. Hence, they can separately identify the different light chain types of each immunoglobulin class, i.e. IgG κ , IgG λ , IgA κ , IgA λ , IgM κ and IgM λ (Figure 2). These molecules are then measured in pairs, e.g., IgG κ /IgG λ , to produce ratios of monoclonal immunoglobulin/background polyclonal immunoglobulin concentrations, in the same manner as sFLC κ/λ ratios.

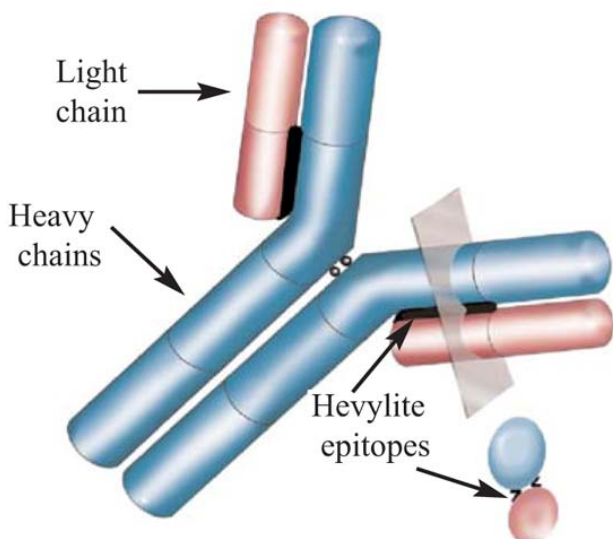


Figure 1: Target epitopes (in black) for Hevylite antibodies are on the constant regions (CH1 and CL) between the heavy and light chains of immunoglobulin molecules.

Antibody specificity

Inevitably, one of the most demanding aspects of HLC assay production is ensuring good specificity. As for FLC immunoassays, the reagents are polyclonal antibodies produced in sheep. Immunisation and adsorption techniques are designed to ensure no cross-reactivity. For example, IgG κ reagents do not react with free κ or IgG λ , or any other immunoglobulins.

There are 4 HLC epitope regions per molecule -one on each side of the heavy chain/light chain contact regions and the same on the other arm of the molecule. Because there are 4 per molecule, immune complexes readily form to produce good homogeneous immunoassays that are suitable for nephelometry and turbidimetry. Latex enhancement is not necessary for IgG, A and M HLC assays because of their high concentrations, but may be useful for IgD κ/λ assays and CSF samples.

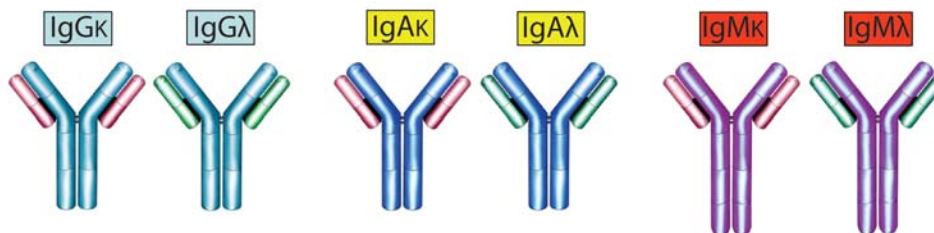


Figure 2. Heavy chain/light chain pairs of IgG, IgA and IgM molecules showing the target epitopes for Hevylite immunoassays in black.

Normal ranges of hevylite assays

Intact immunoglobulin concentrations are normally controlled within narrow limits, as are their HLC κ and λ subsets. The results are from testing blood donor panels. Pearson rank correlations for summation of IgG $\kappa+\lambda$ Hevylite samples to total IgG was ~ 0.9 ($p<0.01$), IgA $\kappa+\lambda$ to total IgA was 0.9 ($p<0.01$) and IgM $\kappa+\lambda$ to total IgM was 0.9 ($p<0.001$).

Ranges that include older individuals, hospital populations and patients with chronic infections and autoimmune diseases are required. Initial studies have indicated that HLC κ/λ ratios in diseases with raised polyclonal immunoglobulins are maintained within the narrow normal limits observed for blood donors (as with sFLC κ/λ ratios).

Publications on Hevylite assays

There are already a number of publications on Hevylite assays and we will continue to update you with developments in future editions of InSite.

- 1. Bradwell AR, Harding S, Drayson M, Mead G.** Novel nephelometric assays give a sensitive measure of residual disease in multiple myeloma (MM). Br J Haem 2008; **141**(s1): p39: Abstr 107.
- 2. Bradwell AR, Harding S, Fourrier N, Harris J, Sharp K, Hobbs J, Drayson M, Mead G.** Separate nephelometric immunoassays for IgA kappa and IgA lambda for the assessment of patients with multiple myeloma (MM). Clin Chem 2008; **54**: A136: Abstr C-116.
- 3. Harding S, Drayson M, Hobbs J, Mead G, Bradwell A.** Analysis of the involved IgG kappa/IgG lambda ratios may give a more sensitive measure of response to treatment in multiple myeloma. Haematologica 2008; 93(s1): Abstr 662.
- 4. Harding S, Drayson M, Lachmann H, Hawkins P, Hobbs J, Mead G, Bradwell AR.** Novel nephelometric immunoassays for the sensitive detection of IgA monoclonal gammopathies in multiple myeloma and AL amyloidosis. Haematologica 2008; 93(s1): Abstr 668.
- 5. Harding S, Mead G, Drayson M, Bradwell AR.** Monitoring of Residual Disease in Multiple Myeloma (MM) Patients Using Novel Immunoglobulin Assays. Ann of Oncology 2008; Abstr in Press.
- 6. Mead G, Harding S, Pratt G, Basu S, Jacob A, Beardsmore C, Bradwell AR.** Serum immunoglobulin and free light chain abnormalities in non Hodgkin Lymphoma. Ann of Oncology 2008; Abstr in Press.
- 7. Wallis GLF, Walsh P, White E, Fourrier N, Harding S, Mead GP, Bradwell AR.** Preparation of polyclonal immunoglobulin G and a reference material for calibration of nephelometric HevyLite™ assays. Clin Chem 2008; **54**: A135: Abstr C-111.