Freelite® and Hevylite® immunoassays:

- Are optimised for use on Binding Site Optilite® and SPAPLUS® analysers which include automatic redilution and prozone parameters.

37.1. Overview

Freelite immunoassays for the measurement of free light chains (FLCs) are optimised for Binding Site Optilite and SPAPLUS analysers. The assays utilise latex enhancement to allow detection of FLCs at low concentrations. The development of Freelite assays, normal ranges, and implementation and interpretation are covered in Chapters 5, 6 and 7, respectively.

Immunoglobulin heavy + light chain immunoassays (Hevylite; HLC) are also optimised for Binding Site instruments. Whilst IgGκ, IgGλ, IgAκ and IgAλ Hevylite assays are antisera-based assays, IgMκ and IgMλ Hevylite assays are latex-enhanced immunoassays. The development of Hevylite assays, normal ranges, and implementation and interpretation are covered in Chapters 9, 10 and 11, respectively.

37.2. The Binding Site Optilite

The Binding Site Optilite is an automated, bench-top turbidimeter allowing host interface capability with continuous sample loading, barcoded parameter entry, sample identification and reagent management systems (Figure 37.1). A full catalogue of assays is available for the Optilite, designed to meet the needs of the clinical laboratory.

On average, the Optilite requires 9.3 minutes to perform daily start-up and 20 minutes for daily quality control protocols (QC), giving a time to instrument availability of approximately 30 minutes. An independent assessment of Optilite performance in a clinical diagnostic laboratory setting by Stone et al.[1] reported a time to first result of 41.8 minutes. The authors commented that “The Optilite special protein analyser provides rapid start-up times, reduced QC times and improved result reporting which has a substantial impact on laboratory workflow, technician time and reporting speed”.

Thouless et al.[2] tested the analytical performance of the Optilite system under simulated routine conditions, with multiple analysers, assays and operators. In total, 12 assays were run across four different analysers by four operators over a two week period. This equated to a throughput of 280-350 tests/day from 80-110 samples/day. Under this variety of different permutations, the results demonstrated excellent agreement and the authors concluded that under the demanding conditions set in the place “…the Optilite systems proved to be robust and reliable.”
37.2.1. Overview of Freelite and Hevylite assays on the Optilite

Freelite and Hevylite kits supplied for the Optilite both contain a single vial calibrator, which is diluted automatically by the instrument to generate the calibration curves. The kits also contain two levels of kit-matched control fluid to validate the curves. Hevylite IgGκ and IgGλ kits also contain antigen excess control fluid (Section 37.2.3).

Performance characteristics of Freelite and Hevylite on the Optilite are summarised in Table 37.1. Starting sample dilutions are 1/10 for κ Freelite assays, 1/8 for λ Freelite assays, 1/20 for IgGκ and IgGλ, and 1/10 for all other Hevylite assays. Samples reporting outside of the standard assay measuring range are automatically re-diluted by the instrument to give a final result, for example the range of reportable results for Optilite Freelite κ and λ assays is approximately 0.6 – 127,000 mg/L and 1.3 – 139,000 mg/L, respectively (Section 7.3).

<table>
<thead>
<tr>
<th></th>
<th>Freelite</th>
<th>Hevylite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>κ</td>
<td>λ</td>
</tr>
<tr>
<td>Range</td>
<td>2.9 - 127 mg/L</td>
<td>5.2 - 139 mg/L</td>
</tr>
<tr>
<td>Standard dilution</td>
<td>1+9</td>
<td>1+7</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.6 mg/L</td>
<td>1.3 mg/L</td>
</tr>
<tr>
<td>Assay time</td>
<td>15 min</td>
<td>15 min</td>
</tr>
</tbody>
</table>

Table 37.1. Analytical performance of Freelite and Hevylite assays on the Binding Site Optilite.

37.2.2. Freelite and Hevylite Precision on the Optilite

Precision on the Optilite is very good due to the use of disposable cuvettes and stringent washing protocols for the sample probe, reagent probe and mixing paddle. During Freelite assay validation, assay precision was assessed using 3 control fluids that ranged in concentration from 4.7 mg/L to 71.8 mg/L. The maximum intra- and inter-assay imprecision for κ or λ Freelite assays was 3.3% and 3.2%, respectively. Similar studies of Hevylite reagents utilising control fluids that varied from 0.26 g/L IgAκ to 25.18 g/L IgGκ demonstrated a maximum intra- and inter-assay imprecision for 6 Hevylite assays of 3.2% and 4.7%, respectively.

Thouless et al[2] reported excellent inter-assay precision for Freelite Optilite assays under routine simulated conditions, with a maximum imprecision of 7.0%. This was supported by Leung et al.[6] who reported CVs of <8% at every level for multiple serum assays (including Freelite) run on the Optilite in routine practice. A number of studies have reported very good Hevylite Optilite assay precision[3][4][5][7][8] with typical CVs of 3-8%.

Jamil et al[9] assessed the performance of the Freelite Mx kits for CSF analysis (Chapter 36) and reported that the assays were linear, with acceptable CVs, and produced results that compared well to those generated by the predicate method.

37.2.3. Freelite and Hevylite antigen excess detection on the Optilite

Selected assays on the Optilite include automated antigen excess checks (prozone detection). The Optilite has three methods of identifying samples that may be in antigen excess: control addition, sample addition and reaction kinetics monitoring.
Freelite and IgM Hevylite assays use reaction kinetics monitoring to detect antigen excess (Sections 7.5 and 11.4). The Optilite measures the rate of the reaction at 3 different time windows in order to identify samples that have a relatively high initial rate of reaction, and therefore may be at risk of being in antigen excess. The ratio between the change in absorbance for time windows 2 and 3 is compared with that of the other time windows, which is then compared to prozone limits defined within the assay parameters. If one or both of the calculated values is lower than defined limits, the sample may be in antigen excess and so the software applies a “High Activity” error and automatically retests the sample at a higher dilution.

Hevylite IgG assays use a control addition method, and the kits are supplied with a vial of IgG antigen excess control fluid, which must remain on the analyser during testing. In this method, a set amount of the antigen excess control fluid is added at the end of the reaction. Samples that are not in antigen excess demonstrate an increase in absorbance following the addition of extra antigen.

The efficacy of the antigen excess checks employed by Freelite Optilite assays was analysed by Coley et al.[10], who reported that antigen excess was correctly flagged for Freelite κ and λ in 98% and 99% cases, respectively, and that there were no examples of undetected antigen excess. This indicates that the antigen excess detection protocols employed by the Optilite are robust and the occurrence of undetected antigen excess experienced on this platform is rare (Sections 7.5.3 and 11.4).

37.3. The Binding Site SPA PLUS

The Binding Site SPA PLUS is a compact, automated, bench-top, batch-mode turbidimeter with host interface capability, barcoded sample identification and reagent management systems (Figure 37.2). A full catalogue of assays is available for the SPA PLUS, designed to meet the need of the routine clinical diagnostic laboratory.

The instrument has a good overall performance compared with other analysers, and provides good throughput for patient samples in spite of its relatively small size. Marionneaux et al.[11] reported that the time-to-first-result on the SPA PLUS was 15 minutes, comparable to that achieved on the Beckman Coulter IMMAGE®. The SPA PLUS was shown to be capable of approximately 105 tests/hour.

37.3.1. Overview of Freelite and Hevylite assays on the SPA PLUS

Freelite and Hevylite kits supplied for the Binding Site SPA PLUS each contain sets of calibrator fluids that are used to generate calibration curves. These are then validated using two levels of kit-matched control fluids. Samples are initially measured at the standard programmed sample dilution, and if out of range, the instrument automatically re-measures the sample at the appropriate higher (1/100) or lower (1/1) dilution (Section 7.3). Very high level samples may require further manual offline dilutions.

For example, the range of reportable results for SPA PLUS Freelite κ and λ assays is 0.4 – 180,000 mg/L and 0.45 – 165,000 mg/L, respectively. Good analytical sensitivity of Freelite SPA PLUS assays was demonstrated by Matters et al.[12]. Performance characteristics of Freelite and Hevylite on the SPA PLUS are summarised in Table 37.2.

A particular advantage of the SPA PLUS Hevylite assays is their wide measuring range. In comparison with the Siemens BN™II, SPA PLUS assays require fewer dilutions to reach the upper limit of the reportable range. Measuring ranges are discussed further in Section 9.4.3.
### Table 37.2. Performance characteristics of Freelite and Hevylite assays on the Binding Site SPA PLUS.

<table>
<thead>
<tr>
<th></th>
<th>Freelite</th>
<th>Hevylite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \kappa )</td>
<td>( \lambda )</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>4.0 - 180.0 mg/L</td>
<td>4.5 - 165.0 mg/L</td>
</tr>
<tr>
<td><strong>Standard dilution</strong></td>
<td>1+9</td>
<td>1+9</td>
</tr>
<tr>
<td><strong>Sensitivity (neat assay dilution)</strong></td>
<td>0.4 mg/L</td>
<td>0.5 mg/L</td>
</tr>
<tr>
<td><strong>Assay time</strong></td>
<td>15 min</td>
<td>15 min</td>
</tr>
</tbody>
</table>

#### 37.3.2. Freelite and Hevylite Precision on the SPA PLUS

The SPA PLUS maintains precision through a combination of acid/alkali cuvette washing and an innovative reaction cuvette mixing system. Air pressure is used in place of stirrers to mix the reaction mixture in a U-shaped cuvette. No physical contact is made with the reaction mixture, thereby removing any possibility of carry-over on a stirrer. Assessment of the performance of SPA PLUS CSF assays by Benard et al.\(^{173}\) and Laurent et al.\(^{14}\) indicated that carry over was not observed during routine use, and that serum and CSF analysis can be effectively integrated on a single SPA PLUS instrument to optimise laboratory workflow.

During Freelite assay validation, assay precision was assessed using 3 control fluids that ranged in concentration from 7.2 mg/L to 142.1 mg/L. The maximum intra- and inter-assay imprecision for \( \kappa \) or \( \lambda \) Freelite assays was 3.4% and 4.2%, respectively. An independent study by Ludwig et al.\(^{15}\) reported within run precision of 2.5-7% and 2.2-2.3% for Freelite \( \kappa \) and \( \lambda \) sFLC assays, respectively. The authors also assessed the day-to-day performance and identified precision of 3.3-3.6% and 5.3-6.1% for Freelite \( \kappa \) and \( \lambda \), respectively. These findings have been replicated by a number of other laboratories\(^{18}\)\(^{14}\)\(^{16}\)\(^{17}\).

A precision study of the 6 Hevylite reagents, by Mirbahai et al.\(^{18}\) reported maximum intra- and inter-assay imprecision of 3.2% and 4.7%, respectively. Jacobs et al.\(^{19}\) assessed the routine performance of Hevylite assays using routine clinical samples and a number of EQA samples. All 6 Hevylite assays demonstrated excellent precision, with CV results that varied between 2.2-7.4%.

These findings were supported by Arkir et al.\(^{20}\) who described intra- and inter-assay precision of 0.7-2.9% and 1.5-8.3%, respectively.

#### 37.3.3. Freelite and Hevylite antigen excess detection on the SPA PLUS

Freelite and Hevylite IgM\( \kappa \) and IgM\( \lambda \) SPA PLUS assays include antigen excess parameters (Sections 7.5 and 11.4), and are based on reaction kinetics monitoring, using the same method as the Optilite (Section 37.2.3). Samples detected as being in antigen excess are automatically flagged by the instrument with a prozone (P) flag and the sample is automatically re-assayed at the higher re-dilution. Cha et al.\(^{21}\) observed that the SPA PLUS algorithm for antigen excess detection correctly identified samples in antigen excess.

#### 37.4. Other analytical platforms

In addition to the Binding Site instruments listed above, Freelite immunoassays are also available for a number of nephelometric and turbidimetric laboratory instruments including: 1) Beckman Coulter IMMAGE\( ^{\text{R}} \) and IMMAGE 800; 2) Roche Cobas\( ^{\text{R}} \) c501, c502 and Integra (400,400 plus and 800); and 3) Siemens ADVIA\( ^{\text{R}} \) (1650, 1800 and 2400), BN\( ^{\text{TM}} \)II and BN ProSpec\( ^{\text{R}} \).
Figures

Figure 37.1. The Binding Site Optilite.

Figure 37.2. The Binding Site SPAPLUS.

View source:
- 37.2. The Binding Site Optilite
- 37.3. The Binding Site SPAPLUS
References


