

## Proficiency Testing Guidelines

### Purpose

Passed in 1988, the Clinical Laboratory Improvement Amendments (CLIA) establishes quality standards for all laboratory testing to ensure the accuracy, reliability and timeliness of patient test results regardless of where the test was performed. The CLIA regulations now include a requirement for verifying the performance specifications of unmodified, moderate complexity tests cleared or approved by the FDA. The following guidelines are proposed by Cylex for verification of the method performance characteristics for ImmuKnow.

### Background

Proficiency testing (PT) is a requirement of CLIA regulations. Because the ImmuKnow test is not included in any commercially available CLIA-approved PT program at this time, laboratories must prove their proficiency through an alternate process.

According to Sec. 493.1709, “If a laboratory performs tests that are not included under subpart I of this part [(e.g., ImmuKnow)], Proficiency Testing Programs, the laboratory must have a system for verifying the accuracy of its test results at least twice a year.”

### ImmuKnow Intended Use

ImmuKnow - the Cylex Immune Cell Function Assay detects cell-mediated immunity (CMI) by measuring the concentration of ATP from CD4 cells following stimulation. This measurement is made on heparin anti-coagulated whole blood using a luminometer and luciferin/luciferase. The assay is used for the detection of cell-mediated immunity in an immunosuppressed population.

### ImmuKnow Result

While ImmuKnow provides an ATP level that is numerical (1-1000ng/mL) in non-stimulated and stimulated samples, ImmuKnow is FDA cleared as a **qualitative** assay that reports results as Low, Moderate and Strong immune cell response.

### Proficiency Testing

The following table describes proficiency testing protocols and Cylex suggests a testing schedule that is compliant with CLIA requirements.

Proficiency Testing Protocol	Proposed Guidelines
Define number of samples.	Analyze 3-5 samples, or a quantity required by the institution, for proficiency testing.
Identify sample source.	<p>The samples should be representative of the range of ImmuKnow results (e.g., Low, Moderate and Strong immune cell response).</p> <p>Ideally, the samples should come from transplant patients receiving immuno-suppressive therapy. These samples must be set up within 30 hours of specimen collection.</p> <p>If samples must be collected from apparently healthy adult volunteers, the results might change after the first day of collection. Theoretically, however, testing the samples at multiple sites on the second day (but within 30 hours of collection) should yield equivalent results when compared.</p>
Perform appropriate sample collection and handling. Refer to reverse side of document for addendum concerning shipping guidelines.  <small>Note: Sites participating in proficiency testing must have the capability of maintaining proper sample handling at all times. Blood must be stored and shipped at room temperature only (18-28 C), in accordance with the ImmuKnow product insert.</small>	<p>For each sample, collect whole blood of equal volume into 1 tube containing sodium heparin anticoagulant for each participating site. Store 1 tube at room temperature (18-28 C) at the home site, and ship the other tube(s) at room temperature (18-28 C) to the participating parallel testing site(s). The parallel testing site(s) must receive and test all samples no later than 30 hours post-collection.</p> <p><small>Note: Ideally, the blood tubes that will be tested at the home site should be shipped out/returned to the home site. This will mimic shipping conditions of the tubes that are shipped to the parallel testing site(s).</small></p>
Perform ImmuKnow testing.	<p>The home site and the parallel testing site(s) must perform ImmuKnow on the same day, no later than 30 hours post-collection.</p> <p>The samples should be analyzed once, following the protocol described in the ImmuKnow product insert.</p>
Compare test results.	<p>ImmuKnow is a <b>qualitative</b> assay, and results should be reported as Low, Moderate and Strong immune cell response. Samples from all testing sites participating in the proficiency program should have the same qualitative result for proficiency testing to be acceptable.</p> <p>If qualitative results from the sites do not match, it is possible that the results are near Low/Moderate or Moderate/Strong ATP cutoff points. In this situation, compare the numerical ATP (ng/mL) levels at the sites by calculating the %CV between sites. For proficiency testing to be accepted, the %CV for these results should be <math>\leq 25\%</math> for Low/Moderate samples and <math>\leq 20\%</math> for Moderate/Strong samples.</p>