

Assay Verification

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.

Regulations

“Each laboratory that introduces a new procedure for patient testing using a device (instrument, kit or test system) cleared by the FDA as meeting certain CLIA requirements for quality control, must demonstrate that, prior to reporting patient test results, it can obtain the performance specifications for accuracy, precision, and reportable range of patient test results, comparable to those established by the manufacturer. The laboratory must also verify that the manufacturer’s reference range is appropriate for the laboratory’s patient population.”¹

¹Reference: Code of Federal Regulations
Title 42, Volume 3, Part 493
493.1213 (b) (1)

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.

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The following table describes Cylex assay performance claims and suggests a testing schedule that is compliant with CLIA requirements.

Cylex ImmuKnow Claims	Verification Requirements	Proposed Testing Schedule								
The mean of the cell function results for Apparently Healthy Adults (non-immunosuppressed) and Transplant Recipients (immunosuppressed) are statistically different.	Accuracy	Analyze 10 samples from apparently healthy adults and 10 samples from transplant recipients and perform a two-tailed, unpaired t-test on the results (statistical analysis available in Microsoft® Excel®) to verify a statistical difference between the 2 populations. Note: Results from this assay run may also be used to verify the Reference Range.								
Inter-Assay Precision <225 ng/mL: %CV = approximately <25% >225 ng/mL: %CV = <20%	Precision between runs	Ideally, test a low, moderate and strong sample in 3 separate assay runs within 24 hours, preferably 1 person performing 3 separate runs. Calculate the ATP result for each stimulated sample according to the assay protocol. For each sample, calculate the %CV for each of the 3 ATP results to verify reproducibility across the 3 assay runs (statistical analysis available in Microsoft Excel).								
Intra-Assay Precision <225 ng/mL: %CV = approximately <25% >225 ng/mL: %CV = <20%	Precision within a run	Ideally test a low, moderate and strong sample 3 times within the same run. Treat each of the 3 replicate samples as independent samples and calculate the ATP result for each stimulated sample according to the assay protocol. Next, calculate the %CV for each of the 3 within-run ATP results to verify reproducibility for each sample testing with the assay run (statistical analysis available in Microsoft Excel).								
The reportable range for ImmuKnow is 1ng/mL ATP (lower limit of analytical sensitivity) to 1000ng/mL ATP (upper limit of reportable range). Linear regression analysis of the Calibration Curve shows linearity with an acceptable correlation coefficient (r^2) of ≥ 0.97 .	Reportable Range and Linearity	Dilute the 1000, 100 and 10 ATP Calibrators 1:2 in Lysis Reagent. Refer to steps 3 and 4 of Assay Part 3 in the ImmuKnow product insert and measure the ATP calibrators and their dilutions (e.g., 1000, 100, 10, 1 and 0ng/mL) in duplicate. After subtracting the 0ng/mL RLU as a reagent blank, plot the adjusted RLU value for each ATP Calibrator and dilution versus the corresponding concentration on a log-log scale; perform linear regression analysis. If the ATP signal is detectable across the 1-1000ng/mL ATP reportable range with acceptable linearity, the correlation coefficient (r^2) should ≥ 0.97 .								
The Reference Range includes 3 zones of immune response: Low, Moderate and Strong: <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>ATP ng/mL</th> <th>Immune Cell Response</th> </tr> </thead> <tbody> <tr> <td>≤ 225</td> <td>Low</td> </tr> <tr> <td>226-524</td> <td>Moderate</td> </tr> <tr> <td>≥ 525</td> <td>Strong</td> </tr> </tbody> </table>	ATP ng/mL	Immune Cell Response	≤ 225	Low	226-524	Moderate	≥ 525	Strong	Reference Range	Test 10 samples from apparently healthy adults and 10 samples from transplant recipients. Analyze results for population distribution. It is expected that the results from the 20 samples tested will fall within each of the 3 immune response zones: Low, Moderate, Strong. Note: Results from this assay run may also be used to verify Accuracy.
ATP ng/mL	Immune Cell Response									
≤ 225	Low									
226-524	Moderate									
≥ 525	Strong									