Name of Policy:
Immune Cell Function Assay

Policy #: 381       Latest Review Date: November 2013
Category: Medicine      Policy Grade: C

Background/Definitions:
As a general rule, benefits are payable under Blue Cross and Blue Shield of Alabama health plans only in cases of medical necessity and only if services or supplies are not investigational, provided the customer group contracts have such coverage.

The following Association Technology Evaluation Criteria must be met for a service/supply to be considered for coverage:

1. The technology must have final approval from the appropriate government regulatory bodies;
2. The scientific evidence must permit conclusions concerning the effect of the technology on health outcomes;
3. The technology must improve the net health outcome;
4. The technology must be as beneficial as any established alternatives;
5. The improvement must be attainable outside the investigational setting.

Medical Necessity means that health care services (e.g., procedures, treatments, supplies, devices, equipment, facilities or drugs) that a physician, exercising prudent clinical judgment, would provide to a patient for the purpose of preventing, evaluating, diagnosing or treating an illness, injury or disease or its symptoms, and that are:

1. In accordance with generally accepted standards of medical practice; and
2. Clinically appropriate in terms of type, frequency, extent, site and duration and considered effective for the patient’s illness, injury or disease; and
3. Not primarily for the convenience of the patient, physician or other health care provider; and
4. Not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of that patient’s illness, injury or disease.
**Description of Procedure or Service:**
Careful monitoring of lifelong immunosuppression is required to ensure long-term viability of solid organ allografts without incurring an increased risk of infection. The monitoring of immunosuppression parameters attempts to balance the dual risks of rejection and infection. It is proposed that individual immune profiles, such as an immune cell function assay, will help assess the immune function of the transplant recipient and individualize the immunosuppressive therapy.

Currently, immunosuppression is determined by testing for clinical toxicity (e.g., leukopenia, renal failure) and by therapeutic drug monitoring (TDM) when available. However, drug levels are not a surrogate for overall drug distribution or efficacy because pharmacokinetics often differ among individuals due to clinical factors such as underlying diagnosis, age, gender, and race; circulating drug levels may not reflect the drug concentration in relevant tissues; and levels of an individual immunosuppressant drug may not reflect the cumulative effect of other concomitant immunosuppressants. The main value of TDM is the avoidance of toxic levels and monitoring patient compliance. Further, the appropriate level of immunosuppression may vary from person to person. Individual immune profiles, such as an immune cell function assay, could support clinical decision making and help to manage the risk of infection from excess immunosuppression and the risk of rejection from inadequate immunosuppression in immunosuppressed patients.

ImmuKnow® (Cylex) is an immune cell function assay cleared for marketing by the FDA in April, 2002 to detect cell-mediated immunity (CMI) in an immunosuppressed patient population. The assay measures the concentration of adenosine triphosphate (ATP) in whole blood following 15-18 hour incubation with the mitogenic stimulant, phytohemagglutinin (PHA). In cells that respond to stimulation, increased ATP synthesis occurs during incubation. Concurrently, whole blood is incubated in the absence of stimulant for the purpose of assessing basal ATP activity. CD4+ T-lymphocytes are immunoselected from both samples using anti-CD4 monoclonal antibody-coated magnetic particles. After washing the selected CD4+ cells on a magnet tray, a lysis reagent is added to release intracellular ATP. A luminescence reagent added to the released ATP produces light measured by a luminometer, which is proportional to the concentration of ATP. The characterization of the cellular immune response of a specimen is made by comparing the ATP concentration for that specimen to fixed ATP level ranges.

**Policy:**
Use of the immune cell function assay to monitor and predict immune function after solid organ transplantation does not meet Blue Cross and Blue Shield of Alabama’s medical criteria for coverage and is considered investigational.

Use of the immune cell function assay to monitor and predict immune function after hematopoietic stem cell transplantation does not meet Blue Cross and Blue Shield of Alabama’s medical criteria for coverage and is considered investigational.

Use of the immune cell function assay for all other indications does not meet Blue Cross and Blue Shield of Alabama’s medical criteria for coverage and is considered investigational.
Blue Cross and Blue Shield of Alabama does not approve or deny procedures, services, testing, or equipment for our members. Our decisions concern coverage only. The decision of whether or not to have a certain test, treatment or procedure is one made between the physician and his/her patient. Blue Cross and Blue Shield of Alabama administers benefits based on the members' contract and corporate medical policies. Physicians should always exercise their best medical judgment in providing the care they feel is most appropriate for their patients. Needed care should not be delayed or refused because of a coverage determination.

Key Points:
The ImmuKnow® (Cylex) assay has been examined in clinical trials for its potential use in monitoring immunosuppression medication regimens in solid organ transplant patients.

Assessment of a diagnostic technology typically focuses on three analyses: 1) analytic validity including comparison to a “gold-standard” test and test/re-test reliability; 2) clinical validity including sensitivity, specificity, and positive and negative predictive value in appropriate populations of patients; and 3) clinical utility, i.e., demonstration that the information from the diagnostic test results in improved health outcomes.

The sensitivity of a test is the ability to detect disease when the disease is present (true positive), while specificity indicates the ability to detect patients who do not have the disease (true negative). Evaluation of clinical validity, therefore, requires independent assessment by two methods in a population of patients who are suspected of having a disease but not all have the disease/disorder. In addition, demonstration of the clinical utility of the ImmuKnow® assay would require specifying abnormal levels prior to testing an immunosuppressed patient population, making treatment decisions based on the assay results, and documenting decreased morbidity and/or mortality (such as improved transplant organ survival and/or reduced infectious complications) following these treatment decisions.

There are no published randomized controlled trials (RCTs) that compare immune cell function assays with current methods of assessing immune status. The majority of published studies of the ImmuKnow® assay are observational studies that correlate adenosine triphosphate (ATP) levels with clinical status. Some include additional analyses of the performance characteristics of the test. Systematic reviews of these observational studies have also been performed. No published trials have assessed the clinical utility of the immune cell function assays.

Ling et al. performed a systematic review and meta-analysis of studies published to July 2011 to assess the efficacy of ImmuKnow® assay in identifying risks of infection and rejection in adult transplant recipients. Nine studies published between 2008 and 2011 met the inclusion criteria. The meta-analysis of these nine studies incorporated 2,458 samples from transplant recipients, with 172 samples from patients with infection and 135 samples from patients with rejection. Three studies were among liver transplant recipients, three among kidney recipients, and one study each among heart, lung, and mixed organ recipients, respectively. The pooled estimates for the performance characteristics of the ImmuKnow® assay in identification of infection risk were a sensitivity of 0.58 (95% confidence interval [CI]: 0.52-0.64), a specificity of 0.69 (95% CI: 0.66-0.70), a positive likelihood ratio of 2.37 (95% CI: 1.90-2.94), a negative likelihood ratio of
0.39 (95% CI: 0.16-0.70), and a diagnostic odds ratio of 7.41 (95% CI: 3.36-16.34). The pooled estimates for ImmuKnow® assay in identifying risk of rejection were a sensitivity of 0.43 (95% CI: 0.34-0.52), a specificity of 0.75 (95% CI: 0.72-0.78), a positive likelihood ratio of 1.30 (95% CI: 0.74-2.28), a negative likelihood ratio of 0.96 (95% CI: 0.85-1.07), and a diagnostic odds ratio of 1.19 (95% CI: 0.65-2.20). Due to significant heterogeneity across studies, the review authors also conducted subgroup analyses in both liver and renal transplant patients. The subgroup analysis showed that the liver transplantation group had a relatively high pooled sensitivity of 0.85 and the renal transplantation group had a specificity of 0.80, indicating that the different types of organ transplants may be one source of this observed heterogeneity; however, the positive likelihood ratio of the liver group was low and the negative likelihood ratio of the renal group was high, suggesting that it may be inappropriate to use the assay result to identify the risk of infections in either liver or renal transplant recipients. Based on the overall findings, the current evidence suggests that ImmuKnow® assay does not have sufficient diagnostic accuracy to identify individuals at risk of infection or rejection. In particular, the sensitivity is low, and the likelihood ratios that are close to 1.0 indicate that this test does not alter the probability of the specified outcomes to a large degree. Additional studies are still needed to clarify the usefulness of this assay for identifying risks of infection and rejection in adult transplant recipients.

Rodrigo et al conducted a meta-analysis to identify studies (published to March 2012) documenting the use of ImmuKnow® assay to monitor immune function in adult liver transplant recipients. The authors identified five studies to analyze ImmuKnow® assay performance in infection and five studies in acute rejection. Two (of five) studies were also included in the above systematic review by Ling et al. The studies included a total of 651 cases in the infection meta-analysis and 543 cases in the acute rejection meta-analysis. Pooled sensitivity, specificity, positive likelihood ratio, diagnostic odds ratio and area under a summary receiver operating characteristic curve for infection were 0.84 (95% CI: 0.78-0.88), 0.75 (95% CI: 0.71-0.79), 3.3 (95% CI: 2.8-4.0), 14.6 (95% CI: 9.6-22.3), and 0.824 ± 0.034, respectively. The pooled estimates for acute rejection were 0.66 (95% CI: 0.55-0.75), 0.80 (95% CI: 0.76-0.84), 3.4 (95% CI: 2.4-4.7), 8.8 (95% CI: 3.1-24.8) and 0.835 ± 0.060, respectively. Heterogeneity was low for infection and high for acute rejection studies. Based on these findings, the ImmuKnow® assay could be considered a valid tool to know the risk of further infection in adult liver transplant recipients. However, there was significant heterogeneity across studies, which precluded concluding that ImmuKnow® assay identifies liver transplant patients at risk for rejection.

A manufacturer-supported meta-analysis was published by Kowalski et al. The ImmuKnow® assay was completed on a total of 504 immunosuppressed transplant recipients (48% kidney, 30% liver, 17% heart, 5% small bowel) within 30 days after an episode of infection or rejection across ten centers throughout the United States. Because only 5% of patients with ATP levels between 130 ng/mL and 450 ng/mL demonstrated adverse events (either infection or rejection), the authors propose this as the target range for ATP level in immunosuppressed transplant recipients. Note that this analysis yielded different ATP threshold levels for infection risk and rejection risk than those developed in the earlier study and cited in the product insert. Further, a 2005 manufacturer-supported study of 37 stable pediatric kidney transplant recipients (mean age: 11.1 years) suggests that in children younger than 12 years of age, risk intervals are defined by ATP level greater than 395 ng/mL for rejection and less than 175 ng/mL for infection.
In its application for approval of ImmuKnow® by the U.S. Food and Drug Administration (FDA), Cylex submitted results from a multicenter study of 44 healthy adults and 78 transplant recipients. This study was expanded to include 115 apparently healthy adults and 127 solid organ transplant recipients (59% kidney, 34% liver, 2% pancreas, 5% simultaneous kidney and pancreas). Immunosuppressive therapies among the transplant recipients were not limited and included muromonab (lymphocyte-depleting antibody, OKT3), antithymocyte globulin, calcineurin inhibitors (e.g., cyclosporine, tacrolimus), steroids, and mycophenolate mofetil, a purine synthesis inhibitor. ImmuKnow® assays were performed less than one month to greater than four years after transplant. Additional details on testing were not specified. Ninety-two percent of the transplant patients had CD4+ adenosine triphosphate (ATP) levels less than 525 ng/mL, while 94% of apparently healthy controls had CD4+ ATP values greater than 225 ng/mL. The authors conclude that this defines three zones of patients’ immune response: ATP level at or less than 225 ng/mL indicates that the patient’s circulating immune cells are showing a low response to phytohemagglutinin (PHA) stimulation and suggests that the patient may be at increased risk of infection; ATP level at or greater than 525 ng/mL indicates that the patient’s circulating immune cells are showing a strong response to PHA stimulation and suggests that the patient may be at increased risk of transplant rejection; a moderate ATP level (i.e., between 225 and 525 ng/mL) represents a proposed ideal response to PHA stimulation. ATP level was not correlated with CD4+ T-cell count.

These transplant recipients were included in a follow-up manufacturer-supported study. The ImmuKnow® assay was completed on a total of 504 immunosuppressed transplant recipients (48% kidney, 30% liver, 17% heart, 5% small bowel) within 30 days after an episode of infection or rejection. Because only 5% of patients with ATP levels between 130 ng/mL and 450 ng/mL demonstrated adverse events (either infection or rejection), the authors propose this as the target range for ATP level in immunosuppressed transplant recipients. Note that this analysis yielded different ATP threshold levels for infection risk and rejection risk than those developed in the earlier study and cited in the product insert. Further, a 2005 manufacturer-supported study of 37 stable pediatric kidney transplant recipients (mean age: 11.1 years) suggests that in children younger than 12 years of age, risk intervals are defined by ATP level greater than 395 ng/mL for rejection and less than 175 ng/mL for infection.

A manufacturer-supported single-center study assessed 20 small bowel transplant recipients (70% isolated small bowel; 10% multivisceral; 10% modified multivisceral; 10% simultaneous liver, small bowel, and pancreas) undergoing tacrolimus tapering per protocol 60–190 days post-transplant. Among eight patients successfully tapered from tacrolimus, 70% of ATP levels clustered in the low range (less than 225 ng/mL), with 25% of ATP levels occurring in the moderate range and 5% occurring in the strong range. Incidence of infection was not reported. Twelve unstable transplant recipients (requiring addition of corticosteroid or OKT3) showed ATP levels with 30% in the low range, 43% in the moderate range, and 27% in the strong range. This study is often described as using ImmuKnow assay results to guide tacrolimus dosing. However, adjustments to the tapering protocol were determined by histologic examination of biopsy results and correlated with ATP levels, as described for the unstable group.
Two studies found no correlation between ATP levels as determined by the ImmuKnow® assay and outcomes in cardiac transplant recipients. Rossano et al. studied 83 pediatric patients (median age, 4.9 years) undergoing heart transplant. ImmuKnow® assays were performed at routine follow-up visits from three months to more than five years after transplant. There were 26 episodes of acute rejection, 20 (77%) of which were cell mediated, and the remainder were humoral rejection. There were 38 infections. No difference in ATP levels as measured by ImmuKnow® assay was detected between patients with or without acute rejection or with or without infection. Further, the manufacturer’s reported risk ranges for rejection (ATP level at or greater than 525 ng/mL) or infection (ATP level at or less than 225 ng/mL) were not predictive of rejection or infection, respectively. As noted, however, it may be that pediatric patients’ risks for post-transplant infection and rejection correspond to different ATP levels.

Gupta et al studied 125 adult heart transplant recipients, the majority of whom underwent ImmuKnow assay testing more than one year post-transplant. There was no apparent correlation between ATP level and rejection (n=3). For seven patients who developed infection, the median ATP level was 267 ng/mL and did not differ from the median ATP level in 104 patients who did not develop infection (282 ng/mL). There was a significant correlation between ATP level and white blood cell count but not between ATP level and absolute lymphocyte count, suggesting that non-lymphocytes also may influence the ATP response. This idea is supported by a 1994 study of CD4+ T-cell responsiveness to three stimulants (including phytohemagglutinin) in HIV-positive patients. The authors suggest that assays performed in clinical laboratories should profile immunoregulatory cytokines (e.g., interleukin-2), which modulate the complex interplay between cellular and humoral immune mechanisms.

Israeli et al correlated ImmuKnow® assay results with clinical status in 50 immunosuppressed heart transplant recipients (median age 58.5 years). The median ATP value of 280 blood samples collected from patients during clinical quiescence (i.e., good clinical status with normal heart function) was 351 ng/mL. ATP values were within the manufacturer’s “moderate” range of immune function (225-525 ng/mL) in 176 (63%) of these samples. The median ATP value of 22 blood samples collected during episodes of biopsy-proven acute rejection was 619 ng/mL, a statistically significant difference (p<0.05). The median ATP value of 19 blood samples collected during episodes of fungal or bacterial infection (i.e., requiring hospitalization for intravenous antimicrobial therapy) was 129 ng/mL, a statistically significant difference from the value during clinical quiescence (p<0.05). While these ATP values fall within the manufacturer’s defined ranges for increased risk of infection (<225 ng/mL) and increased risk of rejection (>525 ng/mL), the blood samples were drawn during the adverse event rather than before.

Cabrera et al assessed the ability of the ImmuKnow® assay to differentiate between acute cellular rejection (ACR) and recurrent hepatitis C in 42 adult patients who had hepatitis C virus (HCV)-related endstage liver disease as the indication for liver transplant. All patients had liver enzyme abnormalities post-transplant and underwent liver biopsy to diagnose both ACR and recurrent HCV. The most sensitive indicator of HCV infection, HCV RNA detection by polymerase chain reaction (PCR), was not used to diagnose HCV. The ImmuKnow® assay was performed with blood collected prior to biopsy, and biopsy samples were interpreted by histopathologists blinded to the results of the ImmuKnow® assay. The median ATP value in 12 patients diagnosed with ACR was 283.3 (range: 241.1-423.0), and the median ATP value in 15 patients diagnosed with
recurrent HCV was 148.0 (range 33.7-186.0), a statistically significant difference (p<0.001). The median ATP value in 15 patients with mixed biopsy features of both ACR and recurrent HCV, but predominance of neither, was 234.0 (range: 155.3-325.0), a statistically significant difference from both the ACR group (p=0.02) and the recurrent HCV group (p<0.001). Of note, while 100% of patients with recurrent HCV had ATP values within the manufacturer’s range for increased risk of infection (<225 ng/mL), 100% of patients with ACR had ATP values outside of the manufacturer’s cutoff for increased risk of rejection (>525 ng/mL).

Torío et al grouped 227 samples from 116 kidney transplant recipients (mean age 51.2 years, range 19-77) by clinical course: stable (no infectious syndrome or acute rejection episode one month before and after immune cell assay; n=168), infection (fever plus at least one positive culture or positive PCR; n=24), or rejection (biopsy-proven acute rejection; n=35). Healthy blood donors served as controls (n=108). Immunosuppressive regimens included pre-transplant basiliximab (an interleukin-2 receptor inhibitor) or antithymocyte globulin and post-transplant tacrolimus, mycophenolate mofetil, and corticosteroid, or calcineurin inhibitors. Mean ATP levels in the stable group (375.3 ± 140.1 ng/mL) and in the control group (436.5 ± 112.0 ng/mL) were higher than in the infection group (180.5 ± 55.2 ng/mL; p<0.001 for both comparisons). No difference was observed between the rejection group (332.5 ± 131.7 ng/mL) and the stable group or the control group (p>0.05 for both comparisons).

**Role of ATP levels in monitoring immunosuppressive therapy**
Several single-center retrospective studies have been published to assess the use of ImmuKnow assay to guide immunosuppressive therapy in both adult and pediatric solid organ transplant patients. These studies demonstrate that this approach may help in more individualized immunosuppression across multiple patient groups. For example, in a Spanish series by Serrano and colleagues in 40 stable pediatric liver transplant patients, ATP values among patients with monotherapy (Cyclosporin A or tacrolimus) were significantly higher than in patients with double immunosuppressive therapy using either Cyclosporin A or tacrolimus and mycophenolate mofetil (p = 0.005). On the other hand, in a Chinese series by Zhou and colleagues in 259 adult kidney transplant patients, ATP values were reported to be significantly higher in the conventional group (receiving immunosuppressive triple-therapy) compared to the group receiving monotherapy (alemtuzumab depletion) at 180 days after transplantation (p < 0.001). Further prospective studies in larger patient populations using multiple time point measurements of ATP levels will be required to evaluate the utility of ImmuKnow assay in monitoring immunosuppression and improving net health outcomes.

**Test performance characteristics**
A smaller number of studies provide some evidence on the performance characteristics of the test, either by providing data on sensitivity, specificity, predictive value, or area under the curve on receiver operating characteristic (ROC) analysis. Other studies provide analogous information in the form of an odds ratio (OR) for the development of infection or rejection. These studies are discussed below.

The relationship between low post-transplant ATP levels (<225 ng/mL) and recent infection in 57 immunosuppressed adult lung transplant recipients was assessed by Bhorade et al. A total of 143 ImmuKnow® assays were performed at routine clinic visits when each patient was on a...
stable dose of tacrolimus. Fifteen patients developed infections (bacterial or fungal pneumonia, cytomegalovirus [CMV] infection); 14 of these (93%) had ATP levels less than 225 ng/mL at the time of their infections (sensitivity 93%). Among the 42 noninfected patients, 16 (38%) had ATP levels less than 225 ng/mL (specificity 62%). Without comparing postinfection ATP levels with preinfection ATP levels, it is not possible to draw conclusions about whether low ATP levels contributed to or resulted from the development of infection. In a US single-center study on 175 adult lung transplant recipients published in 2013, Shino and colleagues reported that the ImmuKnow assay had some predictive ability, but was unlikely to be sufficiently accurate for use in clinical care. ROC analysis showed that the area under the curve was relatively low at 0.61. At a cut-off of 525 ng/ml, there was a significant increase in the risk for acute cellular rejection (OR, 2.1; 95% CI, 1.1-3.8). However, at this cut-off the sensitivity was only 35%, with a specificity of 82%. When a cutoff of 425 ng/ml was used, the sensitivity was 53% and the specificity was 65%.

Reinsmoen et al studied 126 kidney transplant recipients to determine whether pretransplant immune parameters (ATP level, as well as human leukocyte antigen [HLA] mismatch, HLA-specific antibodies, and interferon-gamma precursor frequencies to donor or third-party cells) were associated with post-transplant early acute rejection, unstable creatinine course, and poor graft outcome. The mean pretransplant ATP level of recipients who had no clinical reason for a biopsy was significantly different from that of recipients who had biopsy-proven acute rejection at any post-transplant time point up to 36 months (285.3 +/- 143.2 vs. 414.3 +/- 138.5 ng/mL, respectively). Recipients who underwent biopsy but had no diagnosis of acute cellular or antibody-mediated rejection had an intermediate mean value of 333.7 +/- 156.3 ng/mL. Pretransplant ATP levels were also significantly higher for recipients with early (less than 90 days) unstable creatinine levels, a significant predictor of early acute rejection, than for recipients with stable creatinine values (362.8 +/- 141.2 vs. 283.4 +/- 146.4 ng/mL, respectively). Post hoc analysis using a cutoff ATP level of 375 ng/mL revealed that recipients with pretransplant ATP greater than 375 ng/mL were significantly more likely to experience acute rejection (OR: 3.67, 95% confidence interval [CI]: 1.195, 11.201). The immune parameters were not used to guide modifications of the immunosuppression protocol. Graft survival and incidence of infection were not reported in this study.

Serban et al assessed ImmunoKnow® assay results in 76 kidney transplant patients (mean age 50 years) receiving antithymocyte globulin induction and maintenance immunosuppression. ATP values were assigned to episodes of infection or rejection only if the ImmunoKnow® measurement was performed within the 30 days preceding the adverse event. Over a median of 10 months of follow-up, there was a statistically significant difference between ATP activity measured in 15 of 18 patients with infection requiring hospitalization (median approximately 110 ng/mL) and 44 stable patients (median approximately 220 ng/mL; p=0.002). The median ATP value of 9 of 11 patients with rejection (230 ng/mL) was not significantly different from that observed in stable patients (p value not reported). The results of three patients whose blood was sampled for ImmunoKnow® assay are unknown. ATP activity did not correlate with the number of CD4+ T-cells during the first five months post-transplant (r: 0.129; p=0.153) but did correlate with the number of neutrophils and total white blood cells within the first 3 months post-transplant (r>0.4; p<0.001). Because of substantial myeloid cell contamination of cells captured by the ImmunoKnow® assay in patients with low CD4+ T-cell counts, the authors conclude that cells of
the myeloid lineage substantially contributed to the ATP signal measured by ImmuKnow in these patients. Among 31 patients treated with darbepoetin, an erythropoiesis-stimulating agent often used in renal transplant recipients for the treatment of anemia, the median ATP value within the first two months post-transplant was approximately 260 ng/mL compared to 160 ng/mL in 38 patients who did not receive darbepoetin (p=0.017). There was no association between ATP values and development of rejection or infection at any time during the entire 10-month follow-up. The authors suggest that, in darbepoetin-treated patients, increased ATP activity is due to myeloid cell mobilization induced by darbepoetin rather than T-cell activation and does not justify increased immunosuppression. The relationship between ImmuKnow® results and infections was further analyzed using the ROC analysis. The area under the ROC curve (AUC) was 0.736, indicating a “fair” accuracy level of ImmuKnow® results for prediction of infection risk. The ATP cutoff value calculated based on the ROC curve was 165 ng/mL, and the corresponding positive and negative predictive values were 0.513 and 0.874, respectively. This cutoff value for increased risk of infection differs from the manufacturer’s cutoff value of 225 ng/mL. However, because of the specific effects of antithymocyte globulin induction, the results of this study cannot be extrapolated to transplant recipients receiving no induction therapy or receiving induction agents that do not cause vigorous lymphocyte depletion (e.g., alemtuzumab, an anti-CD25 monoclonal antibody).

Husain et al assessed the correlation of ImmuKnow assay results to different types of infections (bacterial, fungal, viral) in 175 adult lung transplant recipients receiving immunosuppression induction with alemtuzumab. Blood samples were collected prospectively as a part of routine surveillance in all patients during two to 48 months of follow-up. Periods of stability were defined as no infection occurring one month before or after the blood draw. For infectious episodes, only ATP values drawn within one month before the episode were analyzed. The median ATP value during stability was 174.8 ng/mL (25th–75th percentile, 97–306 ng/mL). Significantly lower median ATP values were seen in 13 cytomegalovirus (CMV) infections (49.3 ng/mL, p<0.001), five infections with other viruses (one Epstein-Barr virus, 2 rhinovirus, one influenza, and one parainfluenza; 70.3 ng/mL, p<0.05), and 14 bacterial pneumonias (92.4 ng/mL, p=0.002). The median ATP value in fungal disease (85 ng/mL) did not differ significantly from that in stability (p-value not reported). Four patients who developed invasive pulmonary aspergillosis all had ATP values less than 50 ng/mL. Generalized estimating equation (GEE) logistic regression analysis demonstrated an odds ratio (OR) of 2.81 (95% CI: 1.48, 4.98) for increased risk of infection with ATP values less than 100 ng/mL and an OR of 9 (95% CI not reported) with values less than 50 ng/mL. In comparison, a diagnosis of cystic fibrosis yielded an OR of 2.66 (95% CI: 1.26, 5.63) and CMV mismatch (donor positive, recipient negative) yielded an OR of 2.97 (95% CI: 1.52, 5.80). Note that all ImmuKnow® values, both during periods of stability and within the month before infectious episodes, fall below the manufacturer’s cutoff for increased risk of infection (225 ng/mL).

Zhou et al grouped 259 Chinese kidney transplant recipients (mean age 38.8 ± 12.3 years) by clinical course: stable (no adverse events seven days before and after immune cell assay; n=174), infection (clinical and imaging evidence of infection within seven days before or after assay; n=32), rejection (biopsy-proven acute rejection diagnosed within seven days before or after assay without antirejection therapy; n=16), or methylprednisolone (intravenous methylprednisolone given to treat biopsy-proven acute rejection within three days before or after assay; n=33). Post-
transplant immunosuppressive regimens included corticosteroids, calcineurin inhibitors, and mycophenolate mofetil. Median ATP levels in the infection group (116.4 ng/mL, range 66.3–169.2) and the methylprednisolone group (182.3 ng/mL, range 113.6–388.8) were lower than in the stable group (347.7 ng/mL, range 297.9–411.7, p<0.001 for both comparisons). Median ATP levels in the rejection group were higher than in the stable group (615.9 ng/mL, range 548.8–743.5, p<0.001). The ROC analysis was also evaluated to determine optimal ATP cutoff values for infection and rejection in this sample. With an ATP cutoff value for infection of 238 ng/mL, sensitivity and specificity were 92.9% and 100%, respectively (AUC=0.991). For rejection, a cutoff value of 497 ng/mL maximized sensitivity and specificity at 91.5% and 93.8%, respectively (AUC=0.988).

A retrospective study by Kobashigawa et al correlated ImmuKnow® assay results from 296 adult heart transplant recipients (mean age 54.6 ± 12.8 years) with infection or rejection episodes occurring within one month of assay. Assays were performed between two weeks and ten years post-transplant (N=864). Infection was diagnosed by the treating physician and resulted in antibiotic therapy. Rejection was defined as any treated episode of cellular or antibody-mediated rejection, with or without hemodynamic compromise. Heart transplant recipients without infection or rejection served as controls (n=818 assays). All patients received immunosuppression with tacrolimus, mycophenolate mofetil, and corticosteroids, without induction therapy. Oral prednisone bolus and taper was used for asymptomatic rejection, and antithymocyte globulin was used for rejection with hemodynamic compromise. Mean ATP level was lower in patients with infection (187 ± 126 ng/mL) than in controls (280 ± 126 ng/mL, p<0.001). Ten percent of ATP levels less than 200 ng/mL were associated with infection, and 2% of ATP levels greater than 200 ng/mL were associated with infection (p<0.001). Mean ATP levels did not differ between patients who developed rejection (327 ± 175 ng/mL) and controls (p=0.35). The 200 ng/mL cutoff was chosen based on ROC analysis to maximize sensitivity (71%) and specificity (73%; AUC=0.728).

Huskey et al conducted a single-center, retrospective analysis to assess the predictive ability of ImmuKnow® to identify kidney transplant recipients at risk for opportunistic infection or acute rejection when used in routine clinical management. ImmuKnow® assay results were categorized according to the manufacturer’s ATP cutoff values and correlated with subsequent infection or rejection occurring within 90 days after the assay. Patients matched for age, gender, and time of testing post-transplant who had neither infection nor rejection served as controls. Immunosuppressive regimens included prednisone, calcineurin inhibitors, and mycophenolate mofetil. Eighty percent of patients received pre-transplant antithymocyte globulin. Standard CMV and Pneumocystis carnii prophylaxis was administered. Ninety-four ImmuKnow® assays were performed in 85 patients with subsequent opportunistic infection and in matched controls. Mean ATP levels did not differ between cases (386 ng/mL) and controls (417 ng/mL; p=0.24). A low ATP level (≤225 ng/mL) was not associated with an increased risk of infection (OR: 1.34, 95% CI: 0.64, 2.82, p=0.43). Forty-seven ImmuKnow® assays were performed in 47 patients with subsequent acute rejection and in matched controls. Mean ATP levels did not differ between cases (390 ng/mL) and controls (432 ng/mL; p=0.25). A high ATP level (≥525 ng/mL) was not associated with an increased risk of rejection (OR 1.87, 95% CI: 0.47, 8.38, p=0.48).
To assess the ImmuKnow® assay’s ability to differentiate acute cellular rejection from recurrent HCV infection among patients transplanted for HCV-related liver disease, Hashimoto et al. (18) conducted a retrospective review of 54 allograft liver transplant recipients who had concomitant ImmuKnow® assay results available (mean age 52 years, range 40-63). Liver biopsies were performed every six months after liver transplantation and when clinically indicated due to elevated liver function tests. Biopsies were read by a pathologist who was blinded to ImmuKnow® assay results. PCR detection of HCV RNA was not used. Immunosuppressive regimens included basiliximab, calcineurin inhibitors, and mycophenolate mofetil. ImmuKnow® assays were collected before biopsy. Results were divided into four groups based on biopsy findings: acute cellular rejection (n=11), recurrent HCV (n=26), normal biopsy (n=12), and overlapping features of both acute cellular rejection and recurrent HCV. The mean ATP level in acute cellular rejection (365 ± 130 ng/mL, range 210-666) was higher than in normal biopsy (240 ± 71 ng/mL, range 142-387; p=0.006). The mean ATP level in recurrent HCV (152 ± 100 ng/mL, range 20-487) was lower than in both acute cellular rejection (p<0.001) and normal biopsy (p=0.019). The mean ATP level of patients with overlapping features of both acute cellular rejection and recurrent HCV (157 ± 130 ng/mL, range 25–355) did not differ from the other groups. Seventy-three percent of patients with acute cellular rejection had ATP levels in the manufacturer-defined moderate range. Eighty-eight percent of patients with recurrent HCV had ATP levels in the low range (p<0.001). ROC analysis yielded a cutoff level of 220 ng/mL with sensitivity of 88.5% and specificity of 90.9% (AUC=0.93, 95% CI: 0.85, 1.00).

Cheng et al evaluated the ability of the ImmuKnow® assay to predict recurrence of hepatocellular carcinoma (HCC) in Chinese patients undergoing liver transplantation for HCC. A threshold ATP level of 175 ng/mL was initially determined from 176 assays of 60 patients with HCC (mean age 49.8 ± 8.7 years), 60 (34%) from patients with recurrent HCC post-transplant, and 116 (66%) from stable patients without HCC recurrence, infection, or biopsy-proven rejection. Mean ATP levels in patients with recurrent HCC (137.8 ± 66.4 ng/mL) were lower than in those without recurrence (289.2 ± 133.9 ng/mL, p<0.01). Sensitivity and specificity for the 175 ng/mL threshold value were 83.3% and 83.6% respectively (AUC=0.869). ImmuKnow® was then administered to a second cohort of 92 patients with HCC undergoing liver transplantation (mean age 50.1 ± 10.3 years). Patients were stratified by high immune response (mean ATP level >175 ng/mL) and low immune response (mean ATP level ≤175 ng/mL). Seventeen of 73 patients (23.3%) in the high response group and 16 of 19 patients (84.2%) in the low response group developed HCC recurrence (p<0.001). Mean ATP levels were 295.3 ± 85.4 ng/mL and 126.6 ± 37.9 ng/mL in the high and low immune response groups, respectively (p<0.001). High immune response was associated with recurrence-free survival (OR 7.28, 95% CI: 3.23, 16.13) but not overall survival (OR 2.20, 95% CI: 0.56, 8.65). This study also correlated ImmuKnow® assay results with clinical status (infection or rejection) among a cohort of the original 60 patients with HCC plus 45 additional patients with non-malignant liver diseases. ImmuKnow® assays were collected during infection (diagnosed by clinical features, positive microbiologic tests, and imaging), biopsy-proven acute or chronic rejection, and stability (defined as good liver function and good general health at least two weeks after transplantation, without evidence of infection, rejection, or tumor recurrence). Immunosuppressive regimens were not defined. Rejection episodes were treated with bolus steroids or antithymocyte globulin. Mean ATP levels during infection (145.2 ± 87.0 ng/mL) and rejection (418.9 ± 169.5 ng/mL) differed from the mean level during stability (286.6 ± 143.9 ng/mL, p<0.01 for both comparisons). ROC analysis showed that
the optimum cutoff ATP value for infection was 200 ng/mL with sensitivity of 79.2% and specificity of 75.0% (AUC=0.842). The cutoff value for rejection was 304 ng/mL with sensitivity of 79.6% and specificity of 76.4% (AUC=0.806). Another retrospective study of 87 liver transplant recipients utilized a cutoff level for rejection of 407 ng/mL based on ROC analysis with sensitivity and specificity of 85.7% and 80.9%, respectively (AUC=0.869).

Two studies examined the role of ImmuKnow® in hematopoietic stem cell transplantation (HSCT), one in autologous transplants and one in allogeneic transplants. Manga et al assessed ATP levels in 16 adult patients (mean age 52 years) with hematologic malignancies (multiple myeloma, B- or T-cell lymphoma, and acute myeloid leukemia) undergoing mobilization with granulocyte-colony stimulating factor (G-CSF) with or without granulocyte-macrophage-colony stimulating factor (GM-CSF) for autologous HSCT. Mean ATP level on day five of G-CSF therapy in ten patients who survived more than two years after mobilization (673 ± 274 ng/mL) was higher than in five patients who died within two years (282 ± 194; p=0.014). ROC analysis identified an ATP cutoff value of 522 ng/mL for predicting patient survival with sensitivity and specificity of 0.8 and 1.0, respectively (AUC=0.880). Gesundheit et al examined 170 ATP levels collected from 40 patients (median age 34 years, range 3-64) following engraftment of allogeneic HSCT for various malignant (acute and chronic myeloid leukemia, acute and chronic lymphocytic leukemia, non-Hodgkin lymphoma, multiple myeloma, myelodysplastic syndrome, and ovarian, breast, and testicular cancer) and non-malignant (severe aplastic anemia, thalassemia major, and adrenoleukodystrophy) diseases. ImmuKnow® assay results were categorized “low” or “normal” according to the manufacturer’s ATP cutoff values and correlated with post-engraftment clinical course. Overall survival for the immunocompetent (“normal”) group was 83% (10 out of 12 patients) at 13 months of follow-up. Overall survival for the immunocompromised (“low”) group was 12% (3 out of 25 patients) at 12 months of follow-up.

Summary
The published studies to date have primarily been small single-center retrospective studies. The studies described above present evidence that adenosine triphosphate levels vary among transplant patients who have evidence of acute infection or transplant rejection, compared to clinically stable patients. The sensitivity and specificity of immune cell function assay have varied in studies reporting these parameters. Based on the results from two 2012 systematic reviews of observational studies of the ImmuKnow® assay in adult transplant recipients, estimates of sensitivity range from 52-88% for infection and 34-75% for rejection. Estimates of specificity, on the other hand, range from 66-79% for infection and 72-84% for rejection. Given the significant heterogeneity observed across studies, the performance characteristics of the ImmuKnow® assay have not been conclusively demonstrated. Further, it remains unclear whether different types of organ transplants or different immunosuppressive regimens affect CD4+ T cells’ response to phytohemagglutinin stimulation variably or whether cutoff values require adjustment for various clinical scenarios. Prospective trials are needed to better define the predictive ability of the ImmuKnow® assay compared to current methods of assessing immune status.

The clinical utility of the ImmuKnow® assay to impact net health outcome in comparison to current methods of care for solid organ transplant recipients has not been evaluated using prospective trials with multiple time point measurements of ATP levels. Thus, it is not known
how current methods of assessing immune status in solid organ transplant recipients, e.g., immunosuppressant drug-level monitoring or empiric use of anti-infective agents, might be changed by use of the ImmuKnow® assay. Therefore, the ImmuKnow® cell function assay is considered investigational.

**Practice Guidelines and Position Statements**

The International Cytomegalovirus (CMV) Consensus Group of the Transplantation Society published an international consensus statement on the management of CMV in solid organ transplant in 2010. The authors state that “there are no clinical studies demonstrating that management decisions based on immunologic monitoring affect patient outcomes.” Routine immunologic monitoring is not recommended.

Guidelines for the care of heart transplant recipients published in 2010 by The International Society of Heart and Lung Transplantation do not include ImmuKnow®. Educational guidelines for the management of kidney transplant recipients in the community setting and for infectious diseases in transplant recipients published in 2009 by the American Society of Transplantation (AST) do not include ImmuKnow®.

In 2006, the AST published recommendations for the screening, monitoring, and reporting of infectious complications in immunosuppression trials of organ transplant recipients. These recommendations define relevant infectious complications to be included in the reporting of immunosuppression trials and recommend specific laboratory monitoring and surveillance methods. The immune cell function assay is not included in these recommendations.

**Key Words:**
Cylex, ImmuKnow®, ImmuKnow® assay, Immune Cell Function Assay, Transplantation Immune Cell Function Assay

**Approved by Governing Bodies:**
In April 2002, Cylex obtained 510(k) clearance from the FDA to market the Immune Cell Function Assay based on substantial equivalence to two flow cytometry reagents (“predicate devices”) manufactured by Becton Dickinson, the TriTest™ CD4 FITC/CD8 PE/CD3 PerCP Reagent and the MultiTest™ CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC Reagent. These reagents are used to determine CD4+ T-lymphocyte counts in immunocompromised patients. The FDA-indicated use of the Cylex Immune Cell Function Assay is for the detection of cell-mediated immunity in an immunosuppressed population. A subsequent 510(k) marketing clearance for a device modification was issued by the FDA for this assay in 2010. There were no changes to the indications or intended use.

**Benefit Application:**
Coverage is subject to member’s specific benefits. Group specific policy will supersede this policy when applicable.
ITS: Home Policy provisions apply
FEP does not consider investigational if FDA approved. Will be reviewed for medical necessity.
Pre-certification requirements: Not applicable

**Current Coding:**
CPT Codes: **86352** Cellular function assay involving stimulation (e.g., mitogen or antigen) and detection of biomarker (e.g., ATP)

**References:**

Policy History:
Medical Policy Group, August 2009 (2)
Medical Policy Administration Committee, September 2009
Available for comment September 4-October 19, 2009
Medical Policy Group, August 2010 (1) Key Points updated, no policy statement coverage change
Medical Policy Group, September 2010 (3)
Medical Policy Panel, November, 2011
Medical Policy Group, February, 2012 (4): Policy updated with literature search; additional investigational indication added for HSCT and all other indications; title changed to “Immune Cell Function Assay.” Key Points and References updated to support policy changes.
Available for comments March 15 – April 30, 2012
Medical Policy Panel, November 2012
Medical Policy Group, March 2013 (2): Policy updated with literature review through September 2012; no change in policy statement; Key Points and Reference updated.
Medical Policy Panel, November 2013
Medical Policy Group, November 2013 (3): Updates to Key Points and References; no change in policy statement

This medical policy is not an authorization, certification, explanation of benefits, or a contract. Eligibility and benefits are determined on a case-by-case basis according to the terms of the member’s plan in effect as of the date services are rendered. All medical policies are based on (i) research of current medical literature and (ii) review of common medical practices in the treatment and diagnosis of disease as of the date hereof. Physicians and other providers are solely responsible for all aspects of medical care and treatment, including the type, quality, and levels of care and treatment.

This policy is intended to be used for adjudication of claims (including pre-admission certification, pre-determinations, and pre-procedure review) in Blue Cross and Blue Shield’s administration of plan contracts.