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Corporate Medical Policy

Immune Cell Function Assay for Organ Transplant Rejection AHS-G2098

File Name: immune_cell_function_assay_for_organ_transplant_rejection
Origination: 1/1/2019
Last Review: 10/2023

Description of Procedure or Service

Immune cell function assays involve measurement of peripheral blood lymphocyte response (intracellular ATP levels, proliferation) following stimulation to assess the degree of functionality of the cell-mediated immune response (Buttgereit, et al., 2000).

Related Policies:
AHS-F2019 Flow Cytometry
AHS-M2091 Transplant Rejection Testing

******Note: This Medical Policy is complex and technical. For questions concerning the technical language and/or specific clinical indications for its use, please consult your physician.***

Policy

Reimbursement is not allowed for an immune cell function assay for organ transplant rejection for all applications.

Benefits Application

This medical policy relates only to the services or supplies described herein. Please refer to the Member's Benefit Booklet for availability of benefits. Member's benefits may vary according to benefit design; therefore member benefit language should be reviewed before applying the terms of this medical policy.

When Immune Cell Function Assay for Organ Transplant Rejection is covered

Not applicable

When Immune Cell Function Assay for Organ Transplant Rejection is not covered

Reimbursement is not allowed for an immune cell function assay (e.g., Pleximmune™, Pleximar) for all situations.

Policy Guidelines

Primary immunodeficiencies are a group of rare disorders in which part of the body's immune system is absent or functions incorrectly. These disorders occur in as many as 1:2000 live births and are most often categorized according to a combination of mechanistic and clinical descriptive characteristics (Bonilla et al., 2015). Specific cellular immunity is mediated by T cells, and defects affecting these T cells underlie

the most severe immunodeficiencies. As antibody production by B cells requires intact T cell function, most T cell defects lead to combined (cellular and humoral) immunodeficiency (Butte, 2023).

In vitro studies of T cell function measure peripheral blood T cell response to several different types of stimuli (Bonilla, 2008):

- Mitogens (such as the plant lectins phytohemagglutinin, concanavalin A, pokeweed mitogen, anti-CD3).
- Specific antigens (such as tetanus and diphtheria toxoids or Candida albicans antigens).
- Allogeneic lymphocytes (ie., mixed lymphocyte culture).

Exposure of T cells to stimulus leads to their metabolic activation and polyclonal expansion (Fernandez-Ruiz, et al., 2014). Response can be measured by indicators of proliferation, ATP synthesis and release, or expansion of specific subpopulations (Butte, 2023).

The evaluation of specific immune responses is essential for diagnosis of primary immune deficiencies. Screening tests used to evaluate patients with suspected primary immune deficiencies are relatively inexpensive, performed rapidly, and reasonably sensitive and specific (Notarangelo, 2010; Oliveira & Fleisher, 2010). Abnormal screening test results indicate the need for more sophisticated tests. This stepwise approach ensures efficient and thorough evaluation of mechanisms of immune dysfunction that underlie the clinical presentation, with narrowing of diagnostic options before using costly sophisticated tests that might be required to arrive at specific diagnoses (Bonilla et al., 2015). Abnormal T cell counts T cell mitogen responses that are absent or extremely low; this is a crucial element in the diagnosis of several primary immune deficiencies, most notably, severe combined immunodeficiency (SCID) (Picard et al., 2015). Additionally, T-cell recognition of alloantigens is the primary and central event that leads to the cascade of events that result in rejection of a transplanted organ (Vella, 2022). Several commercial assays have been developed based on the traditional assessment of T-cell stimulation to predict or assess transplant rejection.

Proprietary Testing

The Immunoknow assay measures the ability of CD4 T-cells to respond to mitogenic stimulation by phytohemagglutinin-L in vitro by quantifying the amount of adenosine triphosphate (ATP) produced and released from these cells following stimulation (Zhang et al., 2016). Since the CD4 lymphocytes orchestrate cell-mediated immunity responses through immunoregulatory signaling, measurement of intracellular ATP levels following CD4 activation is intended to estimate the net state of immune system in immunocompromised patients (Anglicheau et al., 2023) and one of the few well-established strategies for functional immune monitoring in solid organ transplant recipients (Sottong, et al., 2000).

The Pleximmune™ blood test measures the inflammatory immune response of recipient T-cells to the donor in co-culture of lymphocytes from both sources (Ashokkumar et al., 2009; Ashokkumar et al., 2017; Sindhi et al., 2016). The Pleximmune test sensitivity and specificity for predicting acute cellular rejection was found to be 84% and 81%, respectively, in a training set–validation set testing of 214 children. Early clinical experience shows that test predictions are particularly useful in planning immunosuppression in the setting of indeterminate biopsy findings or in modifying protocol-mandated treatment when combined with all other available clinical information about an individual patient (Sindhi et al., 2016).

The iQue® Immune Cell Function Assay identifies immune cells based on cell surface markers or secreted soluble mediators. This assay quantifies cytokines, adhesion molecules, enzymes, and growth factors receptors and measures cell phenotypes, cell function markers, cell viability, cell count, proliferation and secreted effector cytokines in a single well. The iQue assay can be used to characterize T cells and measure various populations including memory T cells, cytotoxic T cells, and natural killer cells (Intellicyt, 2023).

Clinical Utility and Validity

A population-based study comparing the assay results in healthy controls and solid organ transplant recipients established three categories to define patient's cell-mediated immune response: strong

(⩾525 ng ml^{-1}), moderate (226–524 ng ml^{-1}) and low (⩽225 ng ml^{-1}) (Fernandez-Ruiz et al., 2014; Kowalski et al., 2006). Numerous authors have analyzed the predictive value of the Immunoknow assay for acute rejection, as recently summarized in a meta-analysis that found a relatively high specificity (0.75) but a low sensitivity (0.43), with significant heterogeneity across studies (Fernandez-Ruiz et al., 2014; Ling et al., 2012). The ImmuKnow® (Cylex) assay has been examined in clinical trials for its potential use in monitoring immunosuppression medication regimens in solid organ transplant patients.

Kowalski et al (2006) performed "a meta-analysis of 504 solid organ transplant recipients (heart, kidney, kidney-pancreas, liver and small bowel)" from 10 U.S. centers. The authors found that: "A recipient with an immune response value of 25 ng/ml adenosine triphosphate (ATP) was 12 times more likely to develop an infection than a recipient with a stronger immune response. Similarly, a recipient with an immune response of 700 ng/ml ATP was 30 times more likely to develop a cellular rejection than a recipient with a lower immune response value. Of note is the intersection of odds ratio curves for infection and rejection in the moderate immune response value (Kowalski et al., 2006). The authors also hypothesized an "immunological target of immune function," created by the intersection of odds ratio curves at 280 ng/ml ATP. The authors concluded "the Cylex ImmuKnow assay has a high negative predictive value and provides a target immunological response zone for minimizing risk and managing patients to stability (Kowalski et al., 2006)."

Wang et al. (2014) performed a meta-analysis of six studies which found "The pooled sensitivity, specificity, PLR, NLR, and DOR of ImmuKnow for predicting the risk of infection were 0.51, 0.75, 1.97, 0.67, and 3.56, respectively. A DOR of 13.81, with a sensitivity of 0.51, a specificity of 0.90, a PLR of 4.45, and an NLR of 0.35, was found in the analysis of the predictive value for acute rejection. The authors concluded, "Our analysis did not support the use of the ImmuKnow assay to predict or monitor the risks of infection and acute rejection in renal transplant recipients. Further studies are needed to confirm the relationships between the ImmuKnow assay and infection and acute rejection in kidney transplantation (Wang et al., 2014)."

Jo et al. (2015) "analyzed CD4 T-lymphocytes ATP levels along with lymphocyte subsets in 160 samples from 111 post-allogeneic hematopoietic stem cell transplantation (alloHSCT) patients. In patients with stable status, ImmuKnow levels changed over time and the 6-month post-alloHSCT levels were significantly higher than those tested within 6 months post-alloHSCT. ImmuKnow results 6 months post-alloHSCT showed low positive correlation with natural killer cell count (r = 0.328) and the values tested later than 6 months post-alloHSCT were positively correlated with CD4 T cell count (r = 0.425). However, ImmuKnow® levels for acute graft-versus-host disease (GVHD) or infection episodes were not significantly different compared to those for stable alloHSCT. The authors concluded that "the combined test of ImmuKnow levels and lymphocyte subsets may be helpful for immune monitoring following alloHSCT."

Ravaioli et al. (2015) aimed to "assess the clinical benefits of adjusting immunosuppressive therapy in liver recipients based on immune function assay results." A total of 100 patients received serial immune function testing via the ImmuKnow in vitro diagnostic assay (compared to 102 controls who received standard practice). The authors found that "based on immune function values, tacrolimus doses were reduced 25% when values were less than 130 ng/mL adenosine triphosphate (low immune cell response) and increased 25% when values were greater than 450 ng/mL adenosine triphosphate (strong immune cell response)" (Ravaioli et al., 2015). The authors also found that survival and infection rates were better in the treatment arm compared to the control arm. Overall, the investigators concluded "Immune function testing provided additional data which helped optimize immunosuppression and improve patient outcomes" (Ravaioli et al., 2015).

Piloni et al (Piloni et al., 2016) evaluated 61 lung recipients who underwent follow-up for lung transplantation between 2010 and 2014 in order to correlate ImmuKnow® values with functional immunity in lung transplant recipients. The authors found that 71 out of 127 samples (56%) showed an over-immunosuppression with an ImmuKnow® assay mean level of 112.92 ng/ml (SD ± 58.2) vs. 406.14 ng/ml (SD ± 167.7) of the rest of our cohort. In the over-immunosuppression group, the authors found 51 episodes of infection (71%). The mean absolute ATP level was significantly different between patients with or without infection (202.38 ± 139.06 ng/ml vs. 315.51 ± 221.60 ng/ml). The

authors concluded that "the ImmuKnow assay levels were significantly lower in infected lung transplant recipients compared with non-infected recipients and in RAS patients" (Piloni et al., 2016).

Chiereghin et al (2017) evaluated symptomatic infectious episodes that occurred during the first year after an organ transplant. A total of 135 infectious episodes were studied with 77 of the infections bacterial, 45 viral, and 13 fungal. Significantly lower median ImmuKnow® intracellular ATP levels were identified in patients with bacterial or fungal infections compared to infection-free patients, whereas patients with viral infection did not have a significantly different median ATP level compared to non-infected patients. The authors concluded that bacteria were responsible for most symptomatic infections post-transplant and that ImmuKnow measurements may be useful for "identifying patients at high risk of developing infection, particularly of fungal and bacterial etiology" (Chiereghin et al., 2017).

Liu et al. (2019) studied the potential of the ImmuKnow assay to diagnose infection in pediatric patients who have received a living-donor liver transplant. A total of 66 patients participated in this study and were divided into infection (n=28) and non-infection (n=38) groups. The researchers report that the "CD4+ T lymphocyte ATP value of the infection group was significantly lower compared with that of the non-infection group" (Liu et al., 2019). This suggests that for pediatric patients who have received a living-donor liver transplant, low CD4+ T lymphocyte ATP levels may be related to infection rates. The ImmuKnow assay may be a helpful tool in this scenario to predict infection.

Weston et al., (2020) used the ImmuKnow assay to adjust immunosuppression in heart transplant recipients with severe systemic infections. In particular if a patient developed an infection, the ImmuKnow assay was used to recommend adjustments in immunosuppression. This assay was used on 80 patients; thirteen of these patients developed a more serious infection. The researchers conclude that "Heart transplant recipients with severe systemic infections presented with a decreased ImmuKnow®, suggesting over immunosuppression. ImmuKnow® can be used as an objective measurement in withdrawing immunosuppression in heart transplant recipients with severe systemic infections (Weston et al., 2020)."

Ashokkumar et al. (2017) evaluated PlexImmune through the assessment of CD-154 T-cytotoxic memory cells. A total of 280 samples (158 training set, 122 validation) from 214 children were examined. Recipient CD-154 cells induced by stimulation with donor cells were expressed as a fraction of those induced by human leukocyte antigen (HLA) nonidentical cells, and a resulting immunoreactivity index (IR) ≥1 implied increased rejection-risk. The authors found that "an IR of 1.1 or greater in posttransplant training samples and IR of 1.23 or greater in pretransplant training samples predicted liver transplant (LTx) or intestine transplant (ITx) rejection with sensitivity, specificity, positive, and negative predictive values of 84%, 80%, 64%, and 92%, respectively, and 57%, 89%, 78%, and 74%, respectively (Ashokkumar et al., 2017)." The authors concluded that "Allospecific CD154+T-cytotoxic memory cells predict acute cellular rejection after LTx or ITx in children. Adjunctive use can enhance clinical outcomes (Ashokkumar et al., 2017)."

However, at the present time, there is no consensus on the utility of these tests, despite the amount of literature devoted to determine real value for predicting post-transplant complications (Clark & Cotler, 2022; Fernandez-Ruiz et al., 2014; Kowalski et al., 2006; Ling et al., 2012; Rodrigo et al., 2012).

Monforte et al. (2021) studied the prognostic value of ImmuKnow® for predicting non-cytomegalovirus (CMV) infections in lung transplant patients. After their lung transplants, 92 patients were followed for six to twelve months and the assay was carried out at 6, 8, 10, and 12 months. Twenty-five percent of the patients developed non-CMV infections between 6-12 months after the transplant. At six ~~6~~ months, 15.2% of patients had a moderate immune response and 84.8% of patients had a low immune response to the infection. In the following 6 months, only one of the patients with a moderate immune response developed a non-CMV infection compared to the 28.2% of low immune response patients who developed a non-CMV infection. The ImmuKnow® assay had a sensitivity of 95.7%, specificity of 18.8%, positive predictive value (PPV) of 28.2%, and negative predictive value (NPV) of 92.9% in detecting a non-CMV infection. The authors conclude that "although ImmuKnow® does not seem useful to predict non-CMV

infection, it could identify patients with a very low risk and help us define a target for an optimal immunosuppression" (Monforte et al., 2021).

In an open-label prospective cohort study, Xue et al. (2021) studied the use of the Cylex immune cell function assay for diagnosis of infection after liver transplant in pediatric patients. A total of 216 infants with liver transplants were followed and Cylex ATP values were measured before and after the liver transplant at weeks 1, 2, 3, 4, 8, 12 and 24. Post-surgery, 74.1% of the transplant patients had a diagnosed infection, 20.4% were clinically stable, and 5.6% experienced acute rejection. The median Cylex ATP value in infant PLTs post-surgery reduced significantly in the infection group compared to stable group. ROC curve analysis determined that the cut-off value of Cylex ATP was 152 ng/mL for diagnosis of infection. The authors conclude "In this study, we demonstrated that low Cylex ATP represented partly over-immunosuppression and had diagnostic value in infant PLTs with infections, which might assist individualized immunosuppression in PLT patients" (Xue et al., 2021).

Maidman et al. (2022) performed a retrospective observational study on patients from 2018 to 2020 who underwent orthotopic cardiac transplantation in a single center to investigate the predictive value of pre-transplant ImmuKnow results on rejection. When separating the patients into cohorts of low activity and moderate-high activity with the test results, they found that in the no patients experienced early organ rejection in the low pre-transplant ImmuKnow group, but 24.2% of patients experienced early rejection in the high pre-transplant ImmuKnow group with statistical significance. The researchers ultimately concluded a potential utility of utilizing pre-transplant ImmuKnow results to predict possible risk of early heart transplant rejection, and thus promote earlier intervention and immunosuppression when appropriate (Maidman et al., 2022).

State and Federal Regulations, as applicable

Food and Drug Administration (FDA)

ImmuKnow® (Viracor, previously, Cylex) is an immune cell function assay cleared for marketing by the U.S. Food and Drug Administration (FDA) in April 2002 to detect cell-mediated immunity (CMI) in an immunosuppressed patient population. Cylex obtained 510(k) clearances from the FDA to market the Immune Cell Function Assay based on substantial equivalence to two flow cytometry reagents. 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.

In August 2014, Pleximmune™ (Plexision, Pittsburgh, PA) was approved by FDA through the humanitarian device exemption process. The test is intended for use in the pre-transplantation and early and late post-transplantation period in pediatric liver and small bowel transplant patients for the purpose of predicting the risk of transplant rejection within 60 days after transplantation or 60 days after sampling.

Guidelines and Recommendations

The American Academy of Allergy, Asthma & Immunology (AAAAI) and the American College of Allergy, Asthma & Immunology (ACAAI)

The American Academy of Allergy, Asthma & Immunology (AAAAI) and the American College of Allergy, Asthma & Immunology (ACAAI) published practice parameters for the diagnosis and management of primary immunodeficiency (Bonilla et al., 2015) which stated that:

“Evaluation of specific immune responses is essential for diagnosis of PIDDs (primary immunodeficiency diseases). Measurement of serum immunoglobulin levels and lymphocyte responses to mitogens are useful indicators of global B- and T-cell development and function.”

The guidelines also lists “In vitro proliferative response to mitogens and antigens” as an advanced test used when “Abnormal screening test results indicate the need for more sophisticated tests”. The screening test indicated is: Flow cytometry to enumerate CD4 and CD8 T cells and NK cells.

Normal or abnormal T cell response to mitogen stimulation is listed in the diagnostic algorithm for the diagnosis of combined or syndromic immunodeficiencies. Specifically, it states that "Infants with low TREC counts should have secondary screening by using flow cytometry to enumerate T-cell numbers and the proportion of naive cells. T-cell counts of less than 1500/mm3 or a proportion of naive cells of less than 50% should be followed up measuring the in vitro response to a mitogen, such as PHA. It is also listed as a characteristic laboratory finding for WAS, AT related disorders, Good syndrome, XLP1, MSMD, MyD88, WHIM, EV and in the management of DGS, and immuno-osseous dysplasias.

The International Society of Heart and Lung Transplantation (ISHLT)

In their recommendations for non-invasive monitoring of acute heart transplant rejection, the ISHLT made a new Class III recommendation that "use of the immune cell function assay (ImmuKnow) cannot be recommended in adult and pediatric heart transplant recipients for rejection monitoring" with a B Level of Evidence (Velleca et al., 2022).

An ISHLT consensus document for the management of antibodies in a heart transplantation was published in 2018. This document does not mention the ImmuKnow or Pleximmune assays, but does state that "Solid-phase assays, such as the Luminex SAB assay, are recommended to detect circulating antibodies" (Kobashigawa et al., 2018).

An ISHLT consensus document for the antibody-mediated rejection of the lung was published in 2016. This consensus document does not mention the ImmuKnow or Pleximmune assays (Levine et al., 2016).

The American Society of Transplantation (AST)

The American Society of Transplantation does not include the use of the ImmuKnow assay in its publication: "Recommendations for Screening, Monitoring and Reporting of Infectious Complications in Immunosuppression Trials in Recipients of Organ Transplantation" (Humar & Michaels, 2006).

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) also do not include ImmuKnow® (AST, 2009).

Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-organ Transplantation

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 2018. In it, they note that "Clinical utility studies demonstrate that alteration of patient management based on the results of an immune-based assay is feasible, safe, and cost-effective" (Kotton et al., 2018).

Billing/Coding/Physician Documentation Information

This policy may apply to the following codes. Inclusion of a code in this section does not guarantee that it will be reimbursed. For further information on reimbursement guidelines, please see Administrative Policies on the Blue Cross Blue Shield of North Carolina web site at www.bcbsnc.com. They are listed in the Category Search on the Medical Policy search page.

Applicable service codes: 0018M, 81560, 86352

BCBSNC may request medical records for determination of medical necessity. When medical records are requested, letters of support and/or explanation are often useful, but are not sufficient documentation unless all specific information needed to make a medical necessity determination is included.

Scientific Background and Reference Sources

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Specialty Matched Consultant Advisory Panel 3/2020

Medical Director review 3/2020

Medical Director review 10/2020

Specialty Matched Consultant Advisory Panel 3/2021

Medical Director review 3/2021

Medical Director review 11/2022

Medical Director review 10/2023

Policy Implementation/Update Information

1/1/2019 New policy developed. Immune cell function assay for organ transplant rejection is considered investigational for all applications. Medical Director review 1/1/2019. Policy noticed 1/1/2019 for effective date 4/1/2019. (lpr)

10/1/2019 Policy Statement revised to read: Reimbursement is not allowed for an immune cell function assay for organ transplant rejection for all applications. Wording revised in the Not Covered section. "Investigational" changed to read "Reimbursement is not allowed…" Deleted coding grid. Notification given 10/1/2019 for effective date 12/2/2019. (an)

12/10/19 Reviewed by Avalon 3rd Quarter 2019 CAB. No change to policy statement. Medical Director review 11/2019. (lpr)

3/31/20 Specialty Matched Consultant Advisory Panel review 3/18/2020. No change to policy statement. (lpr)

11/10/20 Reviewed by Avalon 3rd Quarter 2020 CAB. Literature review only. Updated references and added Related Policy section. Medical Director review 10/2020. (lpr)

4/6/21 Specialty Matched Consultant Advisory Panel review 3/17/2021. No change to policy statement. (lpr)

11/16/21 Reviewed by Avalon 3rd Quarter 2021 CAB. Updated policy guidelines and references. Medical Director review 10/2021. (lpr)

12/30/21 Added CPT code 81560 to Billing/Coding section for effective date 1/1/22. (lpr)

5/17/22 Added CPT code 0018M to Billing/Coding section. (lpr)

12/13/22 Reviewed by Avalon 3rd Quarter 2022 CAB. References updated. No change to policy statement. Medical Director review 11/2022. (lpr)

12/5/23 Reviewed by Avalon 3rd Quarter 2023 CAB. Medical Director review 10/2023. Updated policy guidelines; added references. Edited "when not covered" section for clarity; removed all bullets under this section. (lpr)

and diagnosis of disease. Medical practices and knowledge are constantly changing and BCBSNC reserves the right to review and revise its medical policies periodically.