Immune Cell Function Assay

Policy Description

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, Burmester, & Brand, 2000).

For guidance on procedures utilizing flow cytometry, please refer to AHS-F2019 Flow Cytometry.

Related Policies

<table>
<thead>
<tr>
<th>Policy Number</th>
<th>Policy Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>APEA-F2019</td>
<td>Flow Cytometry</td>
</tr>
</tbody>
</table>

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual’s benefit coverage at the time of the request

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient’s illness.

1. An immune cell function assay DOES NOT MEET COVERAGE CRITERIA for all indications including, but not limited to:
   a. Management of solid organ transplant rejection in an individual undergoing immunosuppressive therapy;
   b. Identification of risk for rejection prior to any solid organ transplantation;
   c. Management of autologous or allogeneic hematopoietic stem cell transplantation;
   d. Management of immunodeficiency disorders including human immunodeficiency virus (HIV) and severe combined immunodeficiency disease (SCID);
e. Management of or prediction of infection risk in immune mediated disorders including rheumatoid arthritis (RA), multiple sclerosis, and lupus nephritis;

f. Testing for urticaria;

g. Diagnosis and management of Lyme disease (for example, iSpot Lyme Test).

h. Management of inflammatory bowel diseases;

i. Monitoring immune response following surgery.

**Scientific Background**

Primary immunodeficiencies occur in as many as 1:2000 live births. They 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 (Stiehm, 2017).

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, Kumar, & Humar, 2014). Response can be measured by indicators of proliferation, ATP synthesis and release, or expansion of specific subpopulations (Stiehm, 2017).

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, are a crucial element in the diagnosis of several primary immune deficiencies, most notably, SCID (Picard et al., 2015). Additionally, T-cell recognition of alloantigen is the primary and central event that leads to the cascade of events that result in rejection of a transplanted organ (Vella, 2019). Several commercial assays have been developed based on the traditional assessment of T-cell stimulation to predict or assess transplant rejection.

The ImmuKnow assay measures the ability of CD4 T-cells to respond to mitogenic stimulation by phytohemagglutinin-L in vitro by quantifying the amount of adenosine triphosphatate (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 (Chon, 2018) and one of the few well-established strategies for functional immune monitoring in solid organ transplant recipients (Sottong, Rosebrock, Britz, & Kramer, 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).

**Clinical Validity and Utility**

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 ImmuKnow® (Viracor) 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® 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.” 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, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (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 (alloH SCT) patients. In patients with stable status, the 6-month post-alloHSCT ImmuKnow® levels were found to be 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”. 100 patients had “serial immune function” done (compared to 102 controls). 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).” 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.”

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. 135 infectious episodes were studied with 77 of them bacterial, 45 viral, and 13 fungal. The authors found “significantly lower” median ImmuKnow® intracellular ATP levels 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”.

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

Ashokkumar et al (2017) evaluated PlexImmune through the assessment of CD-154 T-cytotoxic memory cells. 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 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.” 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).”

**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. Measurement of serum immunoglobulin levels and lymphocyte responses to mitogens are useful indicators of global B- and T-cell development and function.”
It 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**

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® (AST, 2009; Costanzo et al., 2010).

**The American Society of Transplantation (AST, 2006)**

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).


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 are ongoing” (Kotton et al., 2018).

**State and Federal Regulations, as applicable**

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.

**Applicable CPT/HCPCS Procedure Codes**

<table>
<thead>
<tr>
<th>Code Number</th>
<th>Code Description</th>
</tr>
</thead>
</table>
Cellular function assay involving stimulation (eg, mitogen or antigen) & detection of biomarker (eg, ATP)

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

Evidence-based Scientific References


---

**Policy Implementation/Update Information**

7/1/20 New Policy
State and Federal mandates and health plan contract language, including specific provisions/exclusions, take precedence over Medical Policy and must be considered first in determining eligibility for coverage. The medical policies contained herein are for informational purposes. The medical policies do not constitute medical advice or medical care. Treating health care providers are independent contractors and are neither employees nor agents Blue KC and are solely responsible for diagnosis, treatment and medical advice. No part of this publication may be reproduced, stored in a retrieval system or transmitted, in any form or by any means, electronic, photocopying, or otherwise, without permission from Blue KC.