# Novel Rejection Biomarkers in Kidney Transplantation

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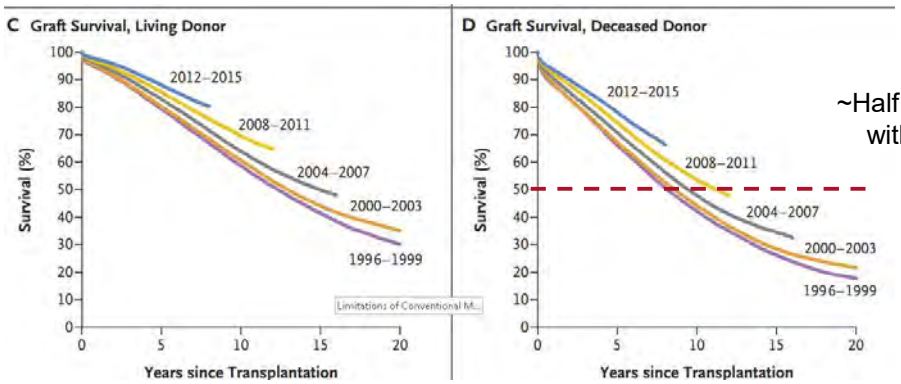
## The Challenge: Unacceptable Rates of Long-Term Kidney Graft Failure

*Kidney transplant is best treatment for ESKD

*Advances in pre-, peri-, and post-transplant management have improved short-term (1-year outcomes)




~Half of DDKTs fail within 10 year

Hariharan S, et al. *NEJM*, 2021.

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# Rejection is a Leading Cause of Late Graft Failure

## Conventional Approach to Allograft Monitoring

**BLOOD TESTS**

*Serial Measurements of serum creatinine (eGFR)

*HLA Donor Specific Antibodies

*Immunosuppression Drug Levels

**URINE TESTS**

*Urinalysis

*Urine Protein:Creatinine Ratio

**BIOPSY**

*For-Cause Biopsies

*Surveillance/Protocol Biopsies (e.g. 3 mo, 12mo)

**Conventional strategies are not sufficient --development and implementation of novel biomarkers is an unmet need

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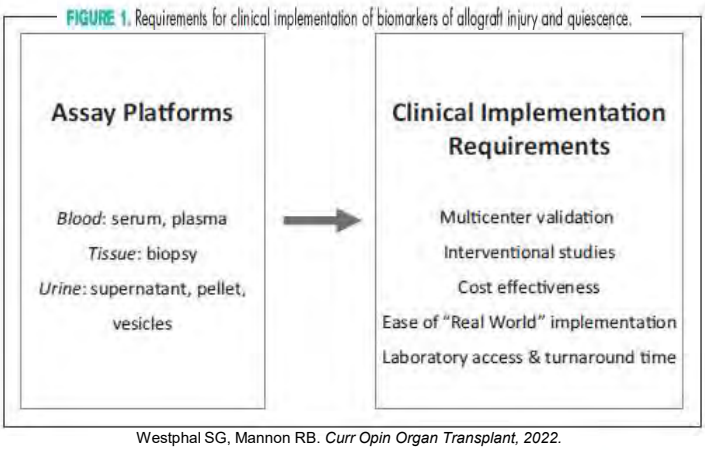
## NOVEL BIOMARKERS: FROM DISCOVERY to CLINICAL IMPLEMENTATION




Westphal SG, Mannon RB. *Curr Opin Organ Transplant, 2022.*

-Should be easily obtained, cost-effective, reliably indicate underlying biologic/pathologic process

-Diagnostic, predictive, prognostic, surrogate

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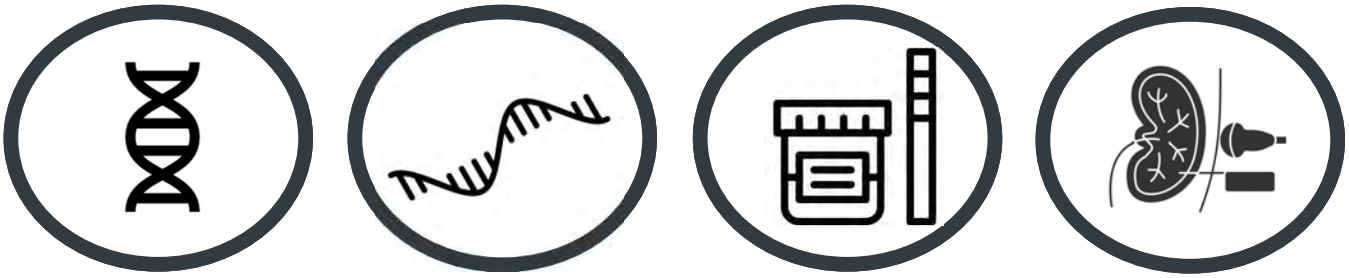
# Novel Biomarkers in Kidney Transplantation



**DONOR-DERIVED CELL-FREE DNA (dd-cfDNA)**

**mRNA GENE EXPRESSION PROFILE (GEP)**

**URINE BIOMARKERS**

**TISSUE-BASED GENE EXPRESSION**

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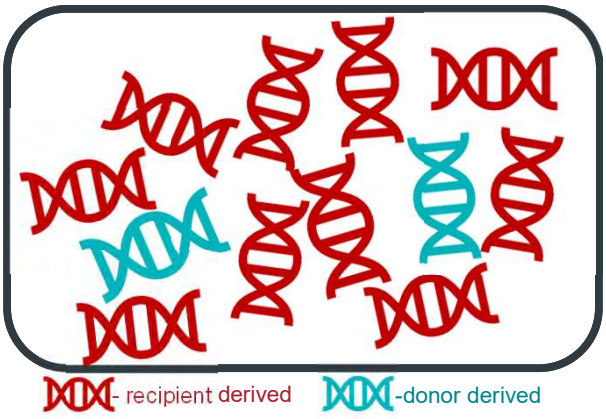
# Donor-Derived Cell-Free DNA




Commercially Available Assays with CMS approval

1. Allosure® (CareDx)
2. Prospera™ (Natera)
3. TRAC® (Eurofins Viracor)
4. VitaGraft™ (Oncocyte)

Non-encapsulated cell-free DNA is released by cells undergoing injury/apoptosis

Modern high throughput technology (PCR, NGS) can compare differences in SNPs to quantify donor-derived vs. recipient-derived without donor genotyping

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# DIAGNOSTIC PERFORMANCE OF dd-cfDNA ASSAYS

| Study | Design | Threshold | Sensitivity | Specificity | NPV | PPV | AUC |
|---|---|---|---|---|---|---|---|
| **Allosure® (CareDx)** | | | | | | | |
| *DART* [1] | *Prospective, 107 for-cause biopsies<br>*Rejection prevalence = 25% | 1.0% | 59% | 85% | 84% | 61% | 0.74 |
| *Admiral* [2] | *Prospective, Serial dd-cfDNA monitoring<br>*Surveillance and for-cause biopsies<br>*Rejection prevalence = 9.2% | 0.5% | 78% | 71% | 90% | 50% | 0.80 |
| *Gupta*[3] | *Prospective, n=208 biopsies (for cause and surveillance) (Rej Prevalence 38%) | 0.82% | 58-61% | 83-90% | | | 0.77-0.80 |
| **Prospera ™ (Natera)** | | | | | | | |
| *Sigdel* [4] | *Biorepository (For Cause/Surveillance)<br>*Rejection Prevalence = 14% | 1.0% | 89% | 73% | 95%* | 52%*- | 0.87 |
| *Trifecta*[5] | *Prospective, For-cause biopsies<br>*Rejection prevalence: 40% | 1.0% or 78 cp/ml | 74-83% | 81% | 83-91% | 68-71% | 0.82-0.88 |
| **TRAC® (Eurofins Viracor)** | | | | | | | |
| *Bixler* [6] | *Biorepository, For-Cause<br>*Rejection prevalence: 20% | 0.69% | 58% | 84.5% | 86% | 55% | 0.85 |
| **VitaGraft™ (Oncocyte)** | | | | | | | |
| Oellerich [7] | *Prospective, serial measurements<br>*Surveillance/For-cause biopsies<br>*Rejection prevalence: 8% | 52 cp/ml | 73% | 73% | 98% | 13% | 0.83 |

1. Bloom RD, et al. *JASN*, 2017
2. Bu L, et al. *KI*. 2022.
3. Gupta G, et al. *Transplantation*, 2022.
4. Sigdel TK, et al. *J Clin Med*, 2019.
5. Halloran PF, et al. *Transplantation, 2022.*
6. Bixler E, et al. *Online Publication*, 2020.
7. Oellerich M, et al. *AJT*, 2019.

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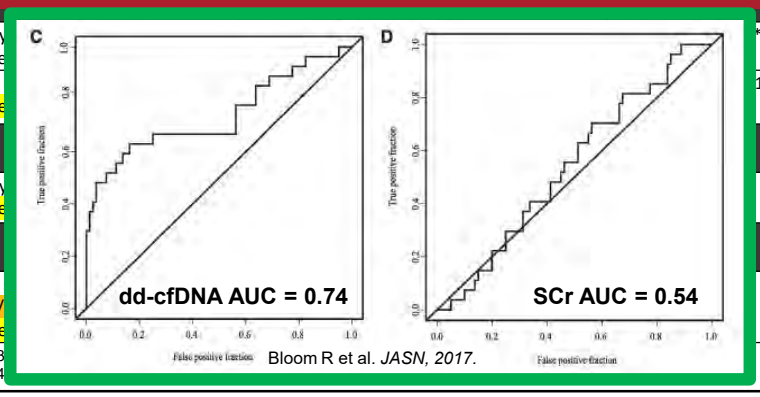
# DIAGNOSTIC PERFORMANCE OF dd-cfDNA ASSAYS

## Diagnostic Ranges Across Assays

*Sensitivity: 58-89%  *Specificity 71-90%

*NPV: 68-98%  *PPV: 13-71%

ROC-AUC: 0.74-0.88



Bloom R et al. *JASN, 2017.*

| Study | Design | Threshold | Sensitivity | Specificity | NPV | PPV | AUC |
|---|---|---|---|---|---|---|---|
| **Allosure® (CareDx)** | | | | | | | |
| *DART* [1] | | | | | | | |
| *Admiral* [2] | | | | | | | |
| *Gupta*[3] | | | | | | | |
| **Prospera ™ (Natera)** | | | | | | | |
| *Sigdel* [4] | *Biorepository<br>*Rejection Pre | | | | | *- | 0.87 |
| *Trifecta*[5] | *Prospective,<br>*Rejection pre | | | | | 1% | 0.82-0.88 |
| **TRAC® (Eurofins Viracor)** | | | | | | | |
| *Bixler*[6] | *Biorepository<br>*Rejection pre | | | | | | 0.85 |
| **VitaGraft™ (Oncocyte)** | | | | | | | |
| Oellerich[7] | *Prospective,<br>*Surveillance/<br>*Rejection pre | | | | | | 0.83 |

1. Bloom RD, et al. *JASN*, 2017
2. Bu L, et al. *KI*. 2022.
3
4
et al. *AJT*, 2019.

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# Uses and Pitfalls of dd-cfDNA in kidney transplant

**DIAGNOSTIC**

- Discrimination for ABMR >>TCMR (especially at higher thresholds and Banff 1a/Borderline)
- At low levels (e.g. <0.2%) good rule out test (strong NPV, especially in lower risk patient)
- Not specific for rejection (BKVN, pyelo, other injury can increase levels)

**PREDICTIVE/PROGNOSTIC**

- Elevated dd-cfDNA levels associated with eGFR decline, de novo DSA development, and future rejection
- Potential to monitor response to treatment

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# Blood Gene Expression Profiles (GEP)

**Clinical Trials in Organ Transplantation 08 (CTOT-08)**

*Prospective, multicenter (discovery cohort and validation cohort)

*Paired microarray based molecular GEP assay with surveillance biopsies (stable patients) (42% and 28% of subjects in the 2 cohorts had subclinical rejection w/in 24 mo)

*Dichotomous result threshold: TX or Not-TX

| Data Set | Paired Samples, n | TX: subAR (% subAR prevalence) | Probability Threshold | % Negative (spared biopsy) | NPV | True Negative | False Negative | % Positive (pickup subAR) | PPV |
|---|---|---|---|---|---|---|---|---|---|
| Discovery Set | 530 | 400:130 (24.5) | 0.375 | 74.7 | 88 | 349 | 42 | 25.3 | 61 |
| Validation set 1 | 138 | 96:42 (30.4) | 0.375 | 71.7 | 78 | 77 | 22 | 28.3 | 51 |
| Validation set 2 | 129/138 | 93:36 (27.9) | 0.375 | 72.1 | 80 | 74 | 19 | 27.9 | 47 |

Friedewald JJ, et al. *AJT*, 2019.

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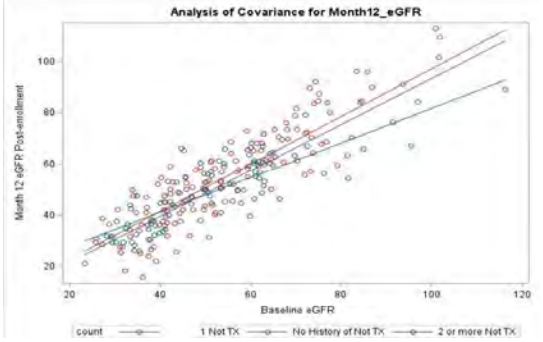
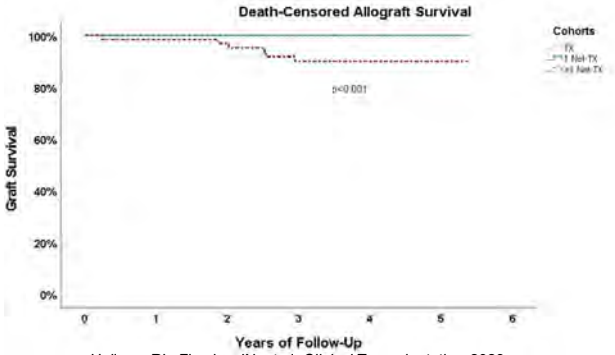
# TruGraf (Transplant Genomics) GEP Assay Correlates with Outcomes

**>1 Not-TX results -> greater loss of eGFR and dnDSA formation**




Leca N, Westphal S, Kew C, et al. *AJT*,S381,2023.

**>1 Not-TX results -> increased rates of rejection and lower death-censored graft survival**




Heilman RL, Fleming JN, et al. *Clinical Transplantation,* 2023.

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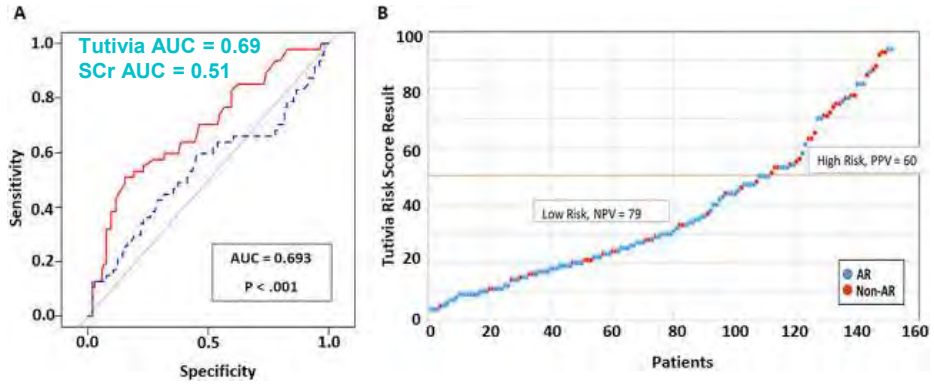
# Blood Gene Expression Profile: Tutivia™ (Verici Dx)

*17-gene mRNA signature developed from GoCAR study validated in recent prospective study with paired surveillance/for-cause biopsy samples (rejection rate 31%)




Bestard O, Augustin J, Wee A, Poggio E, Mannon R, et al. *AJT,* 2024.

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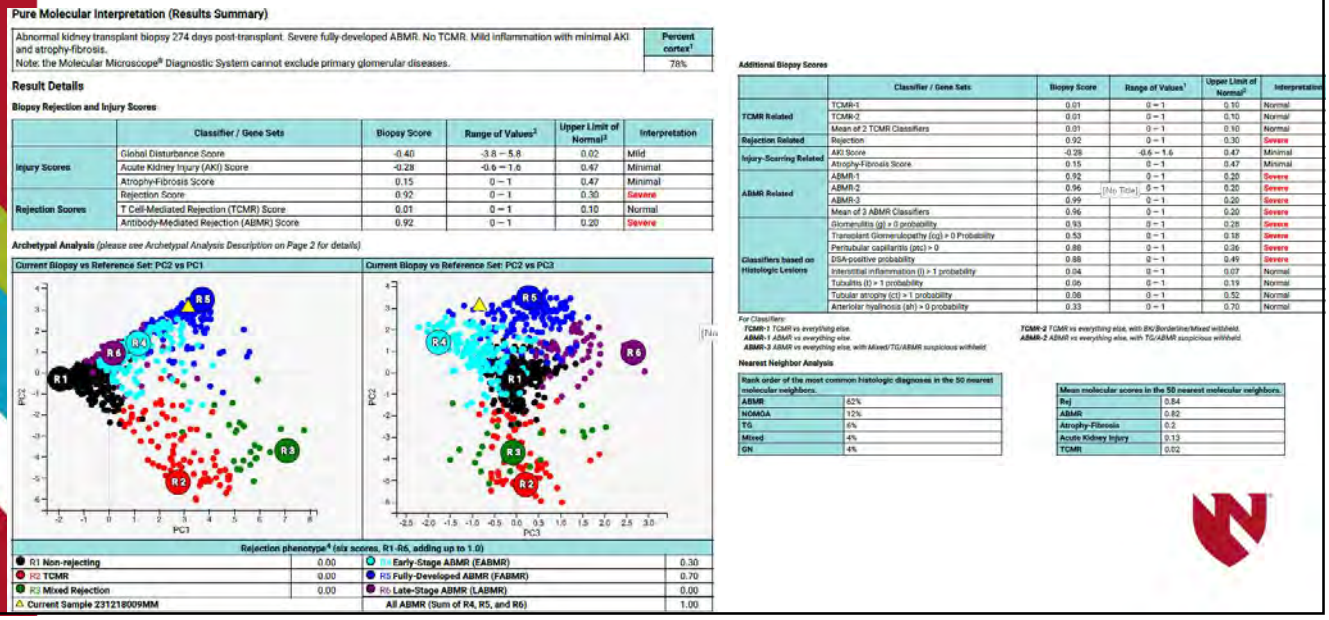
## Tissue Based Gene Expression: Results from the Molecular Microscope

Case: 39 yo 9 mo post DDKT. SCr = 0.8 mg/dl (stable). dd-cfDNA 3.6%. DR52 DSA (MFI 1365).
Histology – peritubular capillaritis (very rare/scant C4d + staining)



**Pure Molecular Interpretation (Results Summary)**

| | Percent cortex[1] |
|---|---|
| Abnormal kidney transplant biopsy 274 days post-transplant. Severe fully-developed ABMR. No TCMR. Mild inflammation with minimal AKI and atrophy-fibrosis.<br>Note: the Molecular Microscope® Diagnostic System cannot exclude primary glomerular diseases. | 78% |

**Result Details**

**Biopsy Rejection and Injury Scores**

| | Classifier / Gene Sets | Biopsy Score | Range of Values[2] | Upper Limit of Normal[3] | Interpretation |
|---|---|---|---|---|---|
| Injury Scores | Global Disturbance Score | -0.40 | -3.8 – 5.8 | 0.02 | Mild |
| | Acute Kidney Injury (AKI) Score | -0.28 | -0.6 – 1.6 | 0.47 | Minimal |
| | Atrophy-Fibrosis Score | 0.15 | 0 – 1 | 0.47 | Minimal |
| Rejection Scores | Rejection Score | 0.92 | 0 – 1 | 0.30 | Severe |
| | T Cell-Mediated Rejection (TCMR) Score | 0.01 | 0 – 1 | 0.10 | Normal |
| | Antibody-Mediated Rejection (ABMR) Score | 0.92 | 0 – 1 | 0.20 | Severe |

**Archetypal Analysis** *(please see Archetypal Analysis Description on Page 2 for details)*

Current Biopsy vs Reference Set: PC2 vs PC1 — Current Biopsy vs Reference Set: PC2 vs PC3

| Rejection phenotype[4] (six scores, R1-R6, adding up to 1.0) | | | |
|---|---|---|---|
| R1 Non-rejecting | 0.00 | R4 Early-Stage ABMR (EABMR) | 0.30 |
| R2 TCMR | 0.00 | R5 Fully-Developed ABMR (FABMR) | 0.70 |
| R3 Mixed Rejection | 0.00 | R6 Late-Stage ABMR (LABMR) | 0.00 |
| Current Sample 231218009MM | | All ABMR (Sum of R4, R5, and R6) | 1.00 |

**Additional Biopsy Scores**

| | Classifier / Gene Sets | Biopsy Score | Range of Values[1] | Upper Limit of Normal[2] | Interpretation |
|---|---|---|---|---|---|
| TCMR Related | TCMR-1 | 0.01 | 0 – 1 | 0.10 | Normal |
| | TCMR-2 | 0.01 | 0 – 1 | 0.10 | Normal |
| | Mean of 2 TCMR Classifiers | 0.01 | 0 – 1 | 0.10 | Normal |
| Rejection Related | Rejection | 0.92 | 0 – 1 | 0.30 | Severe |
| Injury-Scarring Related | AKI Score | -0.28 | -0.6 – 1.6 | 0.47 | Minimal |
| | Atrophy-Fibrosis Score | 0.15 | 0 – 1 | 0.47 | Minimal |
| ABMR Related | ABMR-1 | 0.92 | 0 – 1 | 0.20 | Severe |
| | ABMR-2 | 0.96 | 0 – 1 | 0.20 | Severe |
| | ABMR-3 | 0.99 | 0 – 1 | 0.20 | Severe |
| | Mean of 3 ABMR Classifiers | 0.96 | 0 – 1 | 0.20 | Severe |
| Classifiers based on Histologic Lesions | Glomerulitis (g) > 0 probability | 0.93 | 0 – 1 | 0.28 | Severe |
| | Transplant Glomerulopathy (cg) > 0 Probability | 0.53 | 0 – 1 | 0.18 | Severe |
| | Peritubular capillaritis (ptc) > 0 | 0.88 | 0 – 1 | 0.36 | Severe |
| | DSA-positive probability | 0.88 | 0 – 1 | 0.49 | Severe |
| | Interstitial inflammation (i) > 1 probability | 0.04 | 0 – 1 | 0.07 | Normal |
| | Tubulitis (t) > 1 probability | 0.06 | 0 – 1 | 0.19 | Normal |
| | Tubular atrophy (ct) > 1 probability | 0.08 | 0 – 1 | 0.52 | Normal |
| | Arteriolar hyalinosis (ah) > 0 probability | 0.33 | 0 – 1 | 0.70 | Normal |

For Classifiers:
*TCMR-1* TCMR vs everything else.
*ABMR-1* ABMR vs everything else.
*ABMR-3* ABMR vs everything else, with Mixed/TG/ABMR suspicious withheld.

*TCMR-2* TCMR vs everything else, with BK/Borderline/Mixed withheld.
*ABMR-2* ABMR vs everything else, with TG/ABMR suspicious withheld.

**Nearest Neighbor Analysis**

| Rank order of the most common histologic diagnoses in the 50 nearest molecular neighbors. | |
|---|---|
| ABMR | 62% |
| NOMOA | 12% |
| TG | 6% |
| Mixed | 4% |
| GN | 4% |

| Mean molecular scores in the 50 nearest molecular neighbors. | |
|---|---|
| Rej | 0.84 |
| ABMR | 0.82 |
| Atrophy-Fibrosis | 0.2 |
| Acute Kidney Injury | 0.13 |
| TCMR | 0.02 |

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## Urine Biomarkers

**mRNA GENE EXPRESSION**

**CHEMOKINES (CXCL9 & CXCL10)**

**Exosomes and Vesicle RNA**

**Others: miRNA, dd-cfDNA, peptides**

***Non-invasive and easy to collect**
***May best approximate allograft microenvironment**

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LCD Reference Article | Billing and Coding Article

## Billing and Coding: MolDX: Molecular Testing for Solid Organ Allograft Rejection

https://www.cms.gov/medicare-coverage-database/view/article.aspx?articleid=58061&ver=18&=

### Intended Use Requirements for Coverage:

For-Cause:

1. "Concern for rejection and would otherwise obtain for-cause biopsy"

2. "Assess adequacy of immunosuppression and would otherwise obtain biopsy (or considering doing so"

3. "Assess the probability of rejection with concerning clinical information (clinical pre-tests that inform whether a subsequent biopsy would likely be avoided"

4. "Results of an inconclusive biopsy (wherein the test may subsequently preclude another biopsy"

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LCD Reference Article | Billing and Coding Article

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https://www.cms.gov/medicare-coverage-database/view/article.aspx?articleid=58061&ver=18&=

### Intended Use Requirements for Coverage:

Surveillance:

"If a patient population exists such that they are at particularly high risk for rejection, and the pre-test clinical information would otherwise indicate a biopsy to evaluate the allograft health based on the risk of that population, the use of these services may fit within the intent of the policy coverage language"…

"As such, the use of a molecular test for surveillance (protocol) testing can only be reasonably seen as compliant with the policy if the patient would otherwise receive a surveillance (protocol) biopsy. Providers must demonstrate that such a practice (for protocol biopsies) would otherwise be performed to meet policy requirements. Other uses of surveillance testing are not compliant with the policy language"

Other:

*Test/biopsy cannot be simultaneous or within "short window"

*Only one molecular test can be used for a given encounter

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# 2024 Nebraska Medicine Surveillance/Biomarker Protocol

**applies to <u>stable</u> patients (e.g. stable renal function w/out new proteinuria, DSA, etc.)

Conventional Surveillance

Biomarker/Biopsy Surveillance

**Group 1: Conventional Monitoring Only**

<u>Low Immunologic Risk</u>

*Age >65 + unsensitized

*Age < 65 + unsensitized + zero DR/DQ match

*Zero antigen mismatch

*Physician discretion

**Group 2: Biomarker Surveillance**

<u>Most Patients</u>

*Gene Expression Profile at month 3, 6, 12, 18, 24

*Response to abnl GEP
A. Biopsy
B. Repeat GEP 1-3 in mo.
C. dd-cfDNA
D. Other eval (DSA, BK, etc)

**Group 3: Biopsy Surveillance**

<u>Default</u>

*Surveillance biopsy at:

3-6 months
6-12 months
12-24 month

No biopsy if risk prohibitive

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# 2024 Nebraska Medicine: Utilization of For-Cause Novel Biomarkers

**For-Cause**

If there is clinical, laboratory or physiologic suspicion of rejection, a biopsy will be performed

OR

dd-cfDNA (any of the assays) obtained to decide if a biopsy should be performed (results used in conjunction with clinical picture to decide need for biopsy

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