AlloSure LUNG®

AlloSure Lung Interpretive Guide

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CareDx®

Test Description

The AlloSure® test is a clinical grade, targeted, next generation sequencing (NGS) assay that measures single-nucleotide polymorphisms (SNPs) to accurately quantify donor-derived cell-free DNA (dd-cfDNA) in organ transplant recipients without separate requisite genotyping of either the donor or the recipient.

Intended Use

The AlloSure Lung test is intended to assess the probability of allograft rejection in lung transplant recipients with clinical suspicion of rejection and to inform clinical decision making about the necessity of allograft biopsy in such patients in conjunction with standard of care clinical assessment.

Current Relative Contraindications For Use

- Recipients of transplanted multi organs
- Recipients of a transplant from a monozygotic (identical) twin
- Recipients of a bone marrow / hematopoietic transplant
- Recipients who are pregnant

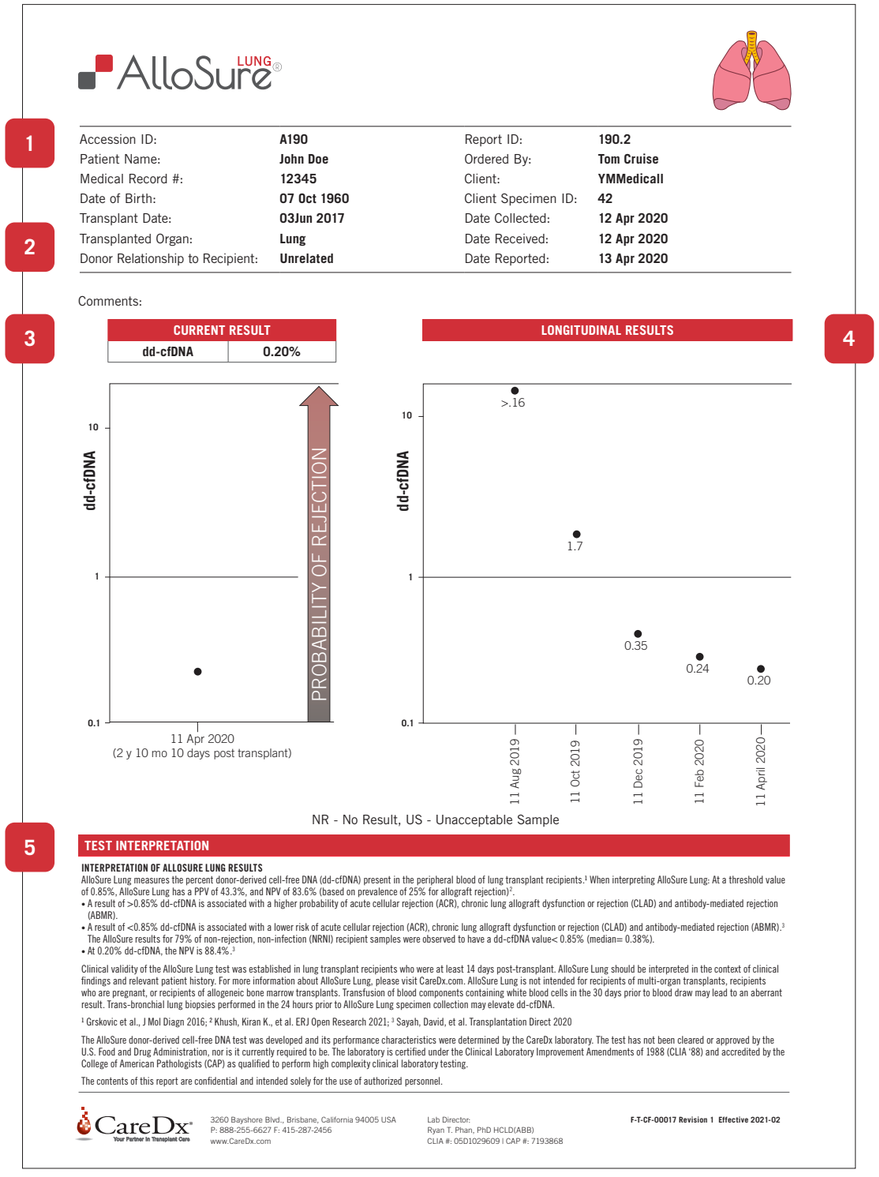
Limitations

AlloSure should NOT be used:

- Following blood transfusion that contains white blood cells for 30 days (washed or leukocyte-depleted RBCs are acceptable).
- AlloSure Lung does not provide information on specific allograft histomorphology. All AlloSure Lung results must be considered in the context of a patient's overall clinical presentation, including other diagnostic findings, history, and examination of the patient. There may be differences both within and between individuals in biological variability of baseline values of dd-cfDNA. Damage to the graft caused by invasive procedures such as trans-bronchial lung biopsy may cause a short-term elevation of dd-cfDNA. Until definitive studies are completed, AlloSure Lung should not be performed on patients within 24h following a trans-bronchial lung biopsy.

AlloSure Lung Report

AlloSure test results are provided via electronic medical record integration alongside other lab results or via fax directly to the transplant clinic. If results are required in alternative formats, please contact us for further discussion.



1 **Recipient Information**

Recipient information includes: date of birth, demographic data, date of blood draw, and type of lung transplant (unilateral versus bilateral). Lung transplants are assumed as unrelated, deceased type donor unless otherwise stipulated. The accuracy of this information should be verified when interpreting the AlloSure results and any discrepancies reported to CareDx immediately.

2 **Specimen collection date for the current result**

The specimen collection date for the current result (day, month, and year), are shown.

3 **Current AlloSure**

The current AlloSure result is shown as the percent of dd-cfDNA of the total cell free DNA (cfDNA) in the "Results Box".

4 **Longitudinal graph with AlloSure results from the last 12 months**

Serial AlloSure results within the last 12 months are plotted on the longitudinal graph to assist with evaluating patient results over time. Results less than 0.12% are plotted near the bottom of the graph. Results greater than 16% are plotted near the top of the graph.

5 **Information to assist with interpretation of the results**

Important information to assist with interpretation of the AlloSure result is provided directly on the report for convenience. (Detailed information can be found below).

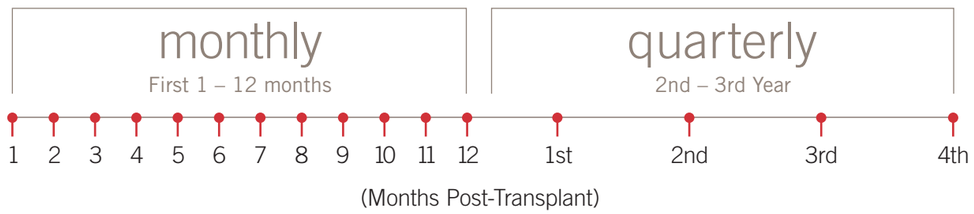
Allosure Results

The AlloSure result is the percent of dd-cfDNA of the total cf-DNA present in organ transplant recipients. When dd-cfDNA levels are so low that a value different from zero cannot be quantified, results are reported as "<0.12%". Results are reported as ">16%" when they are greater than the established quantifiable range.

AlloSure Lung may be used in the context of serial testing, which is thought to allow physicians to observe trends and reference baseline values when evaluating patient graft health.

The AlloSure Lung Routine Schedule (ALRTS) is based on the protocol used for the ALARM-1 Study. All testing should be performed when medically necessary, in accordance with a physician's guidance. The ALAMO study will continue to assess the clinical utility of this testing interval.

ALRTS Timeline



All specimens submitted for testing and billed to third party payers must support the medical necessity of testing when a test is ordered, consistent with clinical judgment and applicable payer policies.

Clinical Validity

De Vlaminck, et al, assessed 51 lung transplant recipients with 398 serial plasma samples Genome Transplant Dynamics (GTD) Study [NCT01985412]. The investigators reported elevated dd-cfDNA during episodes of acute cellular rejection (ACR) versus no rejection or infection (NRNI).

Using a "threshold" dd-cfDNA ≥1%, the reported sensitivity was 100%, specificity 73% and AUC 0.9 for detection of moderate-severe (ISHLT ≥A3) episodes of ACR.[1]

These results were validated during several studies. Khush et al, reported that AlloSure levels were a median of 0.38% as the reference baseline for normal healthy recipients and 1.06% in an aggregated cohort with allograft rejection, (p = 0.02). At an 0.85% threshold, AlloSure dd-cfDNA had 55.6% sensitivity to detect rejection and 83.6% negative predictive value (Fig. 1).[2]

Fig. 1

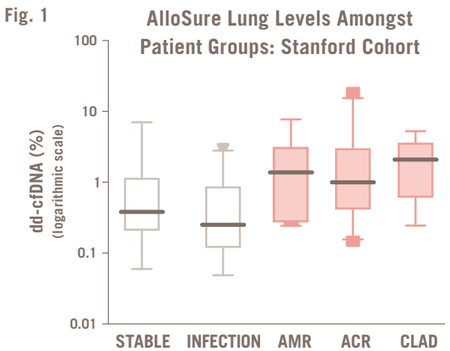
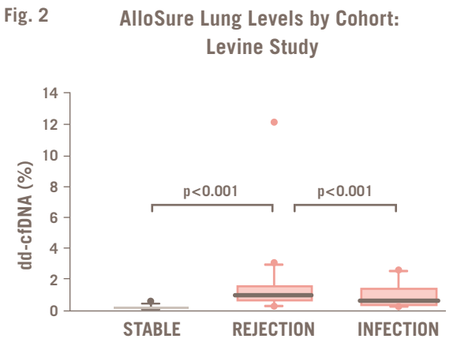


Fig. 2



STABLE = Normal allograft function **ACR = Acute cellular rejection**
AMR = Antibody mediated rejection **CLAD = Chronic lung allograft dysfunction**

Levine et al also confirmed stable lung transplant patients had very low levels of AlloSure dd-cfDNA [0.150% (IQR: 0.15-0.195)] while allograft rejection was associated with 6-fold increase [0.95% (IQR: 0.59-1.53)] (p<0.001) and antibody-mediated rejection (AMR) levels were of higher level [1.20% (IQR: 0.82- 1.73)] (Fig 2).[3]

Agbor-Enoh, et al. reported that elevation in the median dd-cfDNA during the initial 3 months post- transplantation correlated with subsequent risk for development of allograft failure[4]. Categorizing patients as High, Medium, and Low terciles, the median dd-cfDNA of 3.6%, 1.6%, and 0.7%, was respectively observed. High tercile patients demonstrated a 6.6-fold increased hazard ratio (HR) for subsequent development of chronic lung allograft dysfunction (CLAD), the primary etiology for allograft failure.[4]

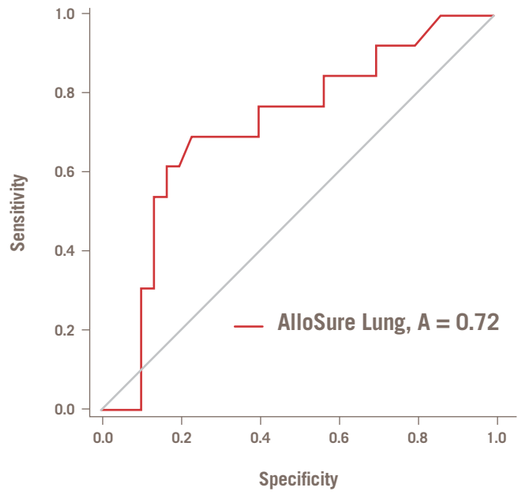
In a separate report, Agbor-Enoh, et al. evaluated "adjudicated episodes" of ACR and AMR where higher degrees of "allograft injury" were assessed. The increase in dd-cfDNA levels (1.1% versus 5.4%), were noted to precede the clinical detection of AMR by a median of 2.8 months.[5]

Sayah et al, published results from the Lung Allograft Rejection Gene expression Observation (LARGO) Study and assessed 69 unique lung transplant recipients less than 1-year post-transplant, across nine international centers. AlloSure dd-cfDNA levels in ACR episodes were significantly elevated [1.52% (IQR: 0.520-2.2550)] compared to the normal stable patients [0.485% (IQR: 0.220-0.790) (p=0.026)]. The AUC-ROC analysis for acute allograft rejection in this series was 0.717 (95% CI: 0.547-0.887; p=0.025). At a threshold dd-cfDNA of 0.87%, sensitivity= 73.1%, specificity= 52.9%, positive predictive value = 34.1% and negative predictive value = 85.5%.[6]

Performance in Infection

Importantly AlloSure is a molecular marker of injury, not diagnostic only for allograft rejection. Other causes of injury such as allograft infection may cause elevation in dd-cfDNA levels. This is important, suggesting that all results should be reviewed in the context of the complete clinical picture. The studies by Khush, et al. and Sayah, et al. identified that for patients with airway infection, the AlloSure median was 0.39% (IQR: 0.18-0.67), similar to patients without allograft rejection or infection. Airway microbiologic colonization may occur without significant allograft injury and therefore requires clinical and radiologic correlation.

AlloSure Lung Area Under the Curve[6]

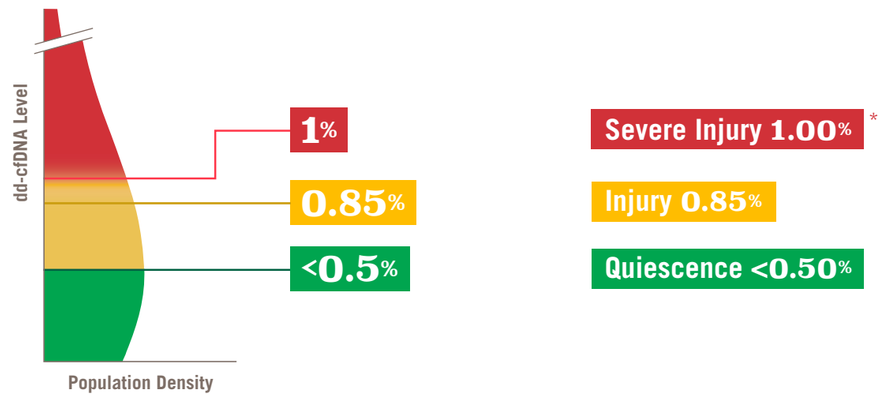


AlloSure Lung Performance for Aggregated Rejection Events

AlloSure dd-cfDNA	Aggregated Rejection Events	
>0.85%	Sensitivity 55.6% [44.6-66.2] Specificity 75.8% [69.3-82.3] PPV 43.3% [35.6-51.8] NPV 83.6% [80.4-87.2]	Threshold for highest overall accuracy
<0.20%	Sensitivity 90.3% [84.4-96.9] Specificity 24.8% [18.1-31.5] PPV 28.6% [26.3-31.0] NPV 88.4% [81.0-96.1]	Data point at which NPV is maximized

TABLE 1: AlloSure Lung performance based on data described in this document.[2, 3, 6]

Interpretation of AlloSure in Lung Transplant[1,7]



*The severe injury category indicates a higher risk of allograft rejection, while DSA formation and other allograft injury can occur at lower levels

Violin Plot showing the distribution of the AlloSure results from De Vlaminck et al. (2015) and Keller et al. (2021). Minimum = 0.05, 25th Percentile = 0.22, Median = 0.44, 75th Percentile = 1.04, Maximum = 17.80, Mean = 1.072, SD = 1.96, SEM = 0.14. Note: four AlloSure values >7% were included in the analysis, but excluded from the graph, (7.58%, 8.26%, 8.75%, and 17.80%).

Financial Assistance

For patients who are uninsured, under-insured, or have insurance that does not cover the cost of AlloSure Lung testing, and who meet financial eligibility criteria, CareDx offers a financial assistance program.

Questions, please email the CareDx Reimbursement Team at financialassistance@caredx.com.

References

1. De Vlaminck, I., et al., *Noninvasive monitoring of infection and rejection after lung transplantation*. Proc Natl Acad Sci U S A, 2015. 112(43): p. 13336-41.

2. Khush, K. K., De Vlaminck, I., Luikart, H., Ross, D. J., & Nicolls, M. R. (2021). *Donor-derived, cell-free DNA levels by next-generation targeted sequencing are elevated in allograft rejection after lung transplantation*. ERJ Open Research, 7(1).

3. Levine et al. (2020). *Single Center "Snapshot" Experience With Donor-Derived Cell-Free DNA After Lung Transplantation*. Biomarker Insights, 15, 1177271920958704.

4. Agbor-Enoh, S., et al., *Donor-derived cell-free DNA predicts allograft failure and mortality after lung transplantation*. EBioMedicine, 2019. 40: p. 541-553.

5. Agbor-Enoh, S., et al., *Late manifestation of alloantibody-associated injury and clinical pulmonary antibody-mediated rejection: Evidence from cell-free DNA analysis*. J Heart Lung Transplant, 2018. 37(7): p. 925-932.

6. Sayah, D., Weigt, S. S., Ramsey, A., Ardehali, A., Golden, J., & Ross, D. J. (2020). *Plasma Donor-derived Cell-free DNA Levels Are Increased During Acute Cellular Rejection After Lung Transplant: Pilot Data*. Transplantation Direct, 6(10).

7. Keller, M. B., Mutebi, C., Shah, P., Levine, D., Aryal, S., Timofte, I., ... & Agbor-Enoh, S. (2021). *Performance of Donor Derived Cell-Free DNA in Routine Clinical Care of Lung Transplant Recipients, a Multi-Center Study*. The Journal of Heart and Lung Transplantation, 40(4), S148.

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