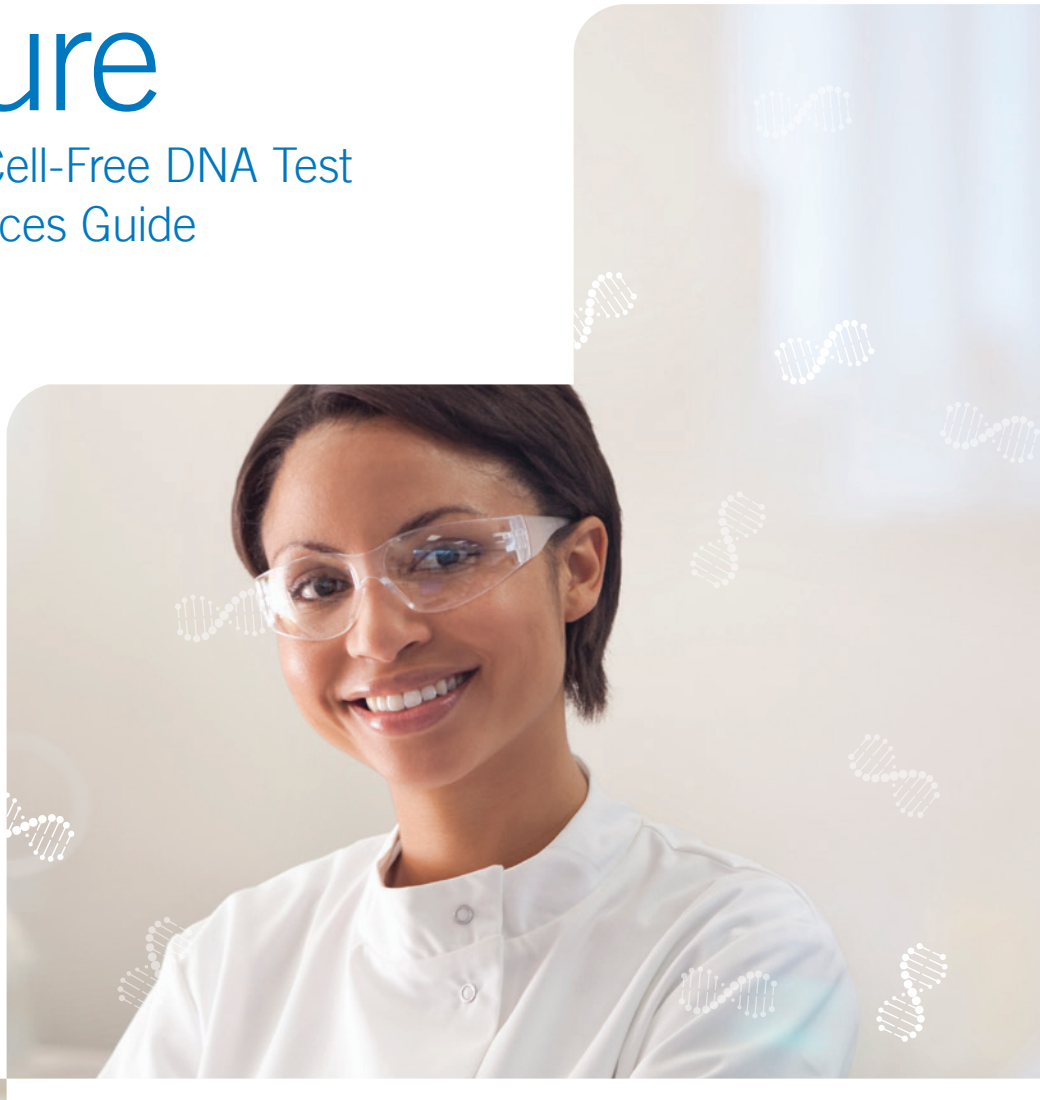
AlloSure

Donor-Derived Cell-Free DNA Test
Laboratory Services Guide



AlloSure®

Introduction

Performing Laboratory and Location:
CareDx
3260 Bayshore Blvd.
Brisbane, CA 94005
1-888-255-6627
CLIA No: 05D1029609
CAP ID: 7193868

Laboratory Directors:
Judith C. Wilber, PhD, D(ABMM)
Patrick Joseph, MD

TEST NAME

AlloSure® Donor-Derived Cell-Free DNA Test

CPT code: 81479

INTENDED USE

The AlloSure test is intended to assess the probability of allograft rejection in kidney transplant recipients with clinical suspicion of rejection and to inform clinical decision-making about the necessity of renal biopsy in such patients at least 2 weeks post-transplant in conjunction with standard clinical assessment.

INDICATIONS FOR USE

AlloSure is indicated for use in renal transplant patients who are

- 18 years of age or older
- At least 2 weeks (14 days) post-transplant

Clinical validity of the AlloSure test was established in single-kidney transplant recipients who were 18 years of age or older and at least two weeks post-transplant.

INTRODUCTION TO ALLOSURE

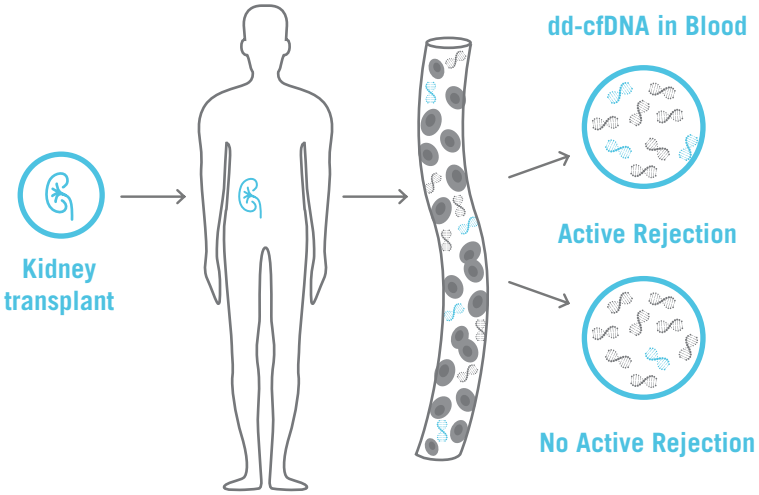
AlloSure is the first and only non-invasive blood test that assesses organ health by directly measuring allograft injury. AlloSure can accurately determine active rejection, enabling better management of kidney transplant patients.

The AlloSure test measures donor-derived cell-free DNA (dd-cfDNA) in renal transplant recipients. dd-cfDNA refers to fragments of DNA in the recipient's bloodstream that originate from cells undergoing cell injury and death.

Principle of the Test

The AlloSure test is a clinical-grade, targeted, next-generation sequencing (NGS) assay that measures single-nucleotide polymorphisms (SNPs) to accurately quantify dd-cfDNA in renal transplant recipients without separate genotyping of either the donor or the recipient. The assay quantifies the fraction of dd-cfDNA in both unrelated and related donor-recipient pairs.

Blood is collected from the patient, packaged, and shipped at ambient temperature to CareDx for testing. Donor-derived cell-free DNA is measured via targeted



amplification and sequencing of a set of carefully selected and validated SNPs. The AlloSure bioinformatics software calculates the percent dd-cfDNA in the sample tested and applies the QC criteria. Most AlloSure test results are reported to the ordering physician within 3 days from blood specimen collection.

Test Methodology and Validation

The test design, development, and validation are described in Grskovic et al., J Mol Diagn, 2016. Cell-free DNA is purified from plasma collected in Streck cell-free DNA BCT® tubes. Cell-free DNA extracted from plasma is used as the template in a pre-amplification PCR reaction with primer pairs that specifically amplify the genomic regions containing the AlloSure SNPs. The pre-amplification product is used as the template in parallel small-multiplex microfluidic PCR amplification library preparation and next generation sequencing. Based on a proprietary algorithm that uses the known population frequencies of the SNPs and expected distributions of alleles, the percentage of the cell-free DNA that is derived from the graft (dd-cfDNA) is computed.

Analytical performance of the assay was characterized and validated using 1,117 samples comprising the NIST-GIAB human reference genome, independently validated reference materials, and clinical samples. The assay can reliably measure dd-cfDNA with a limit of detection (LOD) of 0.19% and a limit of quantification (LOQ) of 0.37%.

Clinical performance of AlloSure was validated using blood samples collected from nearly 400 renal transplant recipients participating in the DART study, including 102 patients for which blood was collected at the time of a clinically indicated biopsy. A cutoff of 1% discriminates active rejection from no active rejection with a positive predictive value (PPV) of 61% and a negative predictive value (NPV) of 84% (Bloom et al., J Am Soc Nephrol 2017). At the 1% cutoff, AlloSure was highly correlated with antibody-mediated rejection (PPV of 44% and NPV of 96%).

Patients in the DART multicenter study who did not experience rejection or other signs of allograft injury were considered stable transplant recipients. Monthly AlloSure tests in this stable population were used to define the baseline AlloSure (median = 0.21% dd-cfDNA) and month-month biological variation (increase ≤61% in consecutive dd-cfDNA results). (Bromberg et al., J Appl Lab Med 2017).

SUPPLIES REQUIRED AND SUPPLIED BY CAREDX

AlloSure Specimen Collection Kit – Description of Contents

- Streck Cell-Free DNA BCT® (Streck Tube)

  Streck Cell-Free DNA BCT is a 10 mL, direct draw, whole blood collection tube intended for collection, transport, and storage of blood samples. Cell-Free DNA BCT stabilizes cell-free plasma DNA. Cell-Free DNA BCT contains the anticoagulant K3EDTA and a cell preservative in a liquid medium. When stored at 2 °C to 30 °C, empty Cell-Free DNA BCT is stable through its expiration date. Cloudiness or precipitation visible in the tube reagent is an indication of deterioration. Do not use Cell-Free DNA BCT showing signs of deterioration and please call CareDx Customer Care to request new collection kits.
- Streck Cell-Free DNA BCT® Instructions for Use Reference Card
- AlloSure Test Requisition Form
- AlloSure Tube & Accession Labels card
- Absorbent 2-bay tube pouch
- 95 kPa bag
- Ambient gel pack
- Insulated foil envelope
- List of Contents Card
- FedEx shipping label and Clinical Pak

May be ordered individually:

- AlloSure Test Requisition Form, individual item
- AlloSure Tube & Accession Labels card, individual item

To order AlloSure Supplies

Phone 1-888-255-6627
Fax 1-415-287-2456
Email OrderSupplies@caredx.com

ORDERING ALLOSURE

WAYS TO ORDER ALLOSURE

AlloSure may be ordered in one of several ways:

- **Web Portal:** AlloSure may be ordered electronically, via the CareDx Web Portal. One-time tests or standing orders (up to 12 months) are available through the portal. Please contact Customer Care to sign up for the web portal.
- **AlloSure Test Requisition Form (TRF):** A paper test requisition form is available for ordering AlloSure. To obtain paper test requisition forms, contact us.
- **Other (order form):** Some customers prefer to use their own order forms to place laboratory orders. We will accept order forms assuming all Required Information for AlloSure Orders (see below) is included.

REQUIRED INFORMATION FOR ALLOSURE ORDERS

Regardless of the method used to place an AlloSure order, the following information must be collected. Without complete information, test results may be delayed.

Patient and Prescriber Information:

- Patient last name, first name
- Patient medical record number or other unique identifying number
- Patient date of birth
- Patient gender
- ICD-10 code
- Patient status
- Patient address (street, city, state, zip)
- Patient primary phone
- Prescriber name, NPI, and signature
- Transplant date
- Type of donor (living unrelated donor, living related donor, deceased donor)
  - If living related donor, relatedness (parent, child, grandparent, grandchild, sibling, half-sibling, fraternal twin, identical twin, aunt, uncle, niece, nephew, great-aunt, great-uncle, great-niece, great-nephew, cousin, other-specify

Phlebotomy Information:

- Draw date
- Phlebotomist initials

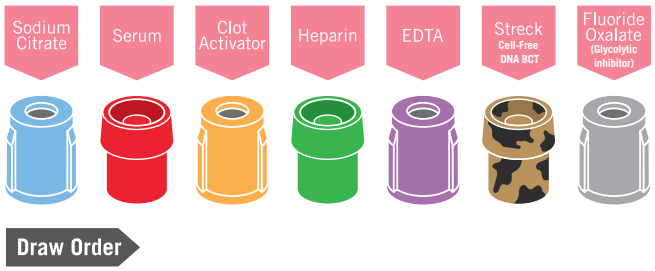
Insurance Information:

- Name of insured
- Insurance provider name
- Insurance Member ID number

SPECIMEN COLLECTION AND SHIPPING

AlloSure Specimen Collection Procedure

- Identify the patient according to your laboratory's patient identification policy, and prepare the patient for phlebotomy according to your standard procedure.
- Open the AlloSure Laboratory Kit and locate the following supplies:
  - Two Streck Cell-Free DNA BCT (check expiration date before use)
  - AlloSure Tube & Accession Labels card
  - AlloSure Test Requisition Form—use if the patient doesn't have an order form
- If collecting additional blood tests during the draw, follow this order of draw for Streck Tubes:



- Use a 22 gauge needle or larger bore for phlebotomy. Avoid drawing with a syringe and transferring through the tube's stopper; hemolysis may result.
- When using a winged (butterfly) collection set for venipuncture and the Streck Tube is the first tube drawn, draw a partially-filled non-additive or EDTA discard tube to eliminate air from the tubing.
- Since Streck Tubes contain chemical additives, it is important to avoid possible backflow from the tube. To guard against backflow, use the following precautions.

– Keep the patient's arm in the downward position during the collection procedure.

– Hold the tube with the stopper in the uppermost position so that the tube contents do not touch the stopper or the end of the needle during sample collection.

– Release the tourniquet once blood starts to flow in the tube, or within 2 minutes of application.

- Fill both Streck Tubes completely; one tube holds approximately 10 mL.
- Mix by gentle inversion at least 10 times.
- On the Tube & Accession Labels card, complete both labels with Patient Last Name, Patient First Name, Date of Birth, Draw Date, and Your Initials. Date format is MM/DD/YYYY. Do not write on the barcode label.
- Affix the completed labels to the Streck Tubes, vertically aligned. Do not cover the expiration date.

  – If using a lab-generated label, ensure that Patient Last Name, Patient First Name, Date of Birth, Draw Date, and Phlebotomist's Initials are included. Affix an extra barcode label to the tube as well.

  Note: The Tube & Accession Label card has extra barcode labels should you need them.

- You may have an order form from the web portal or an order form from the transplant center. If you do not have an order form, on the blank AlloSure Test Requisition Form, please complete:

  – Patient Last Name and Patient First Name

  – Patient Date of Birth

  – Prescriber Name and Location

  – Referring Lab Name

- Affix the first barcode label from the Tube & Accession Labels card to the upper right corner of the order form.

  Note: Specimen transport via pneumatic tube system is not advised as it may cause hemolysis. Hemolyzed specimens are not acceptable for testing.

- Ship the specimen as soon as possible following the Specimen Shipping Procedure. If you cannot ship the specimens immediately, hold specimens between 6 °C and 37 °C. Do not refrigerate or freeze. Specimens must arrive at CareDx with 7 days of drawing.

Specimen Shipping Procedure

To ship AlloSure specimen:

- Place the filled Streck tube in the pocket of the absorbent pouch.
- Place the pouch in the 95 kPa bag and seal.
- Wrap the 95 kPa bag in the gel pack and place inside the foil envelope, and seal.
- Place the sealed foil envelope in the shipper box.
- Fold the TRF or order form so that it fits in the shipper box and place it on top of the foil envelope.
- Place the Tube & Accession Labels card in the box.
- Place the List of Contents card on the very top; close and seal the box.
- Label the FedEx Clinical Pak with the shipping label and place the sealed box inside the Clinical Pak. Seal the Pak shut.
- Ship via FedEx the same day, or as soon as possible.

Reasons for Specimen Rejection

- Unlabeled or improperly labeled specimens: Specimens must be labeled with 2 patient identifiers (i.e., First Name & Last Name, and DOB or MRN) and the date the specimen is drawn.
- Mishandled specimens: Specimens that are or have been frozen, or have been shipped on cold packs or ice, or have been shipped without an ambient temperature gel pack, or have evidence of mishandling or improper shipping.
- Hemolyzed specimens. Specimens showing hemolysis above a trace/slight amount by visual inspection.
- Specimens received >7 days after blood collection.
- Specimens collected in expired blood tubes.
- Specimens from patients for whom any of the following are true will not be tested:

  – Recipients of transplanted organs other than kidney

  – Recipients of a transplant from a monozygotic (identical) twin

  – Recipients of a bone marrow transplant

  – Recipients who are pregnant

– Recipients who are under the age of 18

– Recipients who are less than 14 days post-transplant

LIMITATIONS AND CONTRAINDICATIONS

Limitations

When more than two genomes may be present in the recipient plasma (more than recipient + donor), contribution of cfDNA from each genome is not differentiated by the test. This includes pregnancy, due to the presence of fetal genome DNA in the maternal plasma and multiple-organ transplants from different donors since the grafts each introduce a unique genome (e.g. kidney/pancreas) and contribute different basal levels of cfDNA confounding interpretation of the results.

Patients who received transfusions of whole blood or other blood transfusions that contain white blood cell components within one month prior to the AlloSure test may have an inaccurate result. Transfusions of washed red blood cells or leukocyte-depleted, packed red blood cell transfusions are allowed.

There are some indications that damage to the graft caused by invasive procedures such as renal biopsy may cause a short-term elevation of dd-cfDNA. Until definitive studies are completed, AlloSure should not be used on patients within 24h following a renal biopsy.

Contraindications

- Since the test evaluates differences in the genome between the donor and recipient, it is not possible to perform the test for a kidney transplant recipient that is a monozygotic twin to the donor.
- When more than two genomes may be present in the recipient plasma (more than recipient + donor), contribution of cfDNA from each genome is not differentiated by the test. This includes the presence of fetal genome DNA in maternal plasma during pregnancy, and multiple-organ transplants from different donors since each graft introduces a unique genome (e.g. kidney/pancreas) and contributes different basal levels of cfDNA, confounding interpretation of the results.
- A recipient of multiple transplanted organs that all originated from the same donor presents a situation where elevated levels of dd-cfDNA could have originated from one organ or another or both. If from both, they could be contributing at different baseline levels, confounding interpretation of the results. Therefore, AlloSure is not to be used for transplant recipients with multiple transplanted organs from the same donor.
- Recipients of allogeneic blood or bone marrow transplants who have received cells with a genome different from the recipient (e.g. non-monozygotic twin) should not receive AlloSure testing.

PERFORMANCE CHARACTERISTICS

Accuracy

Accuracy was assessed across seven "donor/recipient" DNA mixtures in three different panels (contrived specimens made from cell lines, range 0.2%-16%, sonicated to 160 bp fragments to mimic cfDNA). Each panel was run in 12 replicates for both 3ng and 8ng total cfDNA input mass. The slope, intercept, and correlation between digital PCR results and AlloSure results were determined for the set of 7 mixtures. Results:

3ng total cfDNA input mass

	Average	95% CI
Slope	**1.23**	**1.19 – 1.27**
Intercept	**-0.0009**	**-0.0016 – -0.0002**
R2	**0.997**	**0.996 – 0.999**

8ng total cfDNA input mass

	Average	95% CI
Slope	**1.28**	**1.25 – 1.30**
Intercept	**-0.0008**	**-0.0011 – -0.0006**
R2	**0.998**	**0.998 – 0.999**

Precision

Twelve replicate runs using contrived specimens (described above) were performed on 12 separate days by four operators using two Fluidigm Access Array systems, four Illumina MiSeq sequencing instruments, 2 manufacturing lots of Access Array chips, and 8 lots of sequencing kits. One lot of critical raw reagents was used. Mean CV across dd-cfDNA levels = 6.8% (Grskovic et al., J Mol Diagn 2016).

Sensitivity

At a cut-off level of 1.0%, the AlloSure test demonstrated 59% sensitivity (95% CI, 44-74%) to discriminate active rejection from no rejection. At a cut-off level of 1.0%, the AlloSure test demonstrated 81% sensitivity (95% CI, 67-100%) to discriminate antibody-mediated rejection (ABMR) from no ABMR.

Specificity

At a cut-off level of 1.0%, the AlloSure test demonstrated 85% specificity (95% CI, 79-91%) to discriminate active rejection from no rejection. At a cut-off level of 1.0%, the AlloSure test demonstrated 83% specificity (95% CI, 78-89%) to discriminate ABMR from no ABMR. 96% of stable kidney transplant recipient samples are below 1.0% dd-cfDNA.

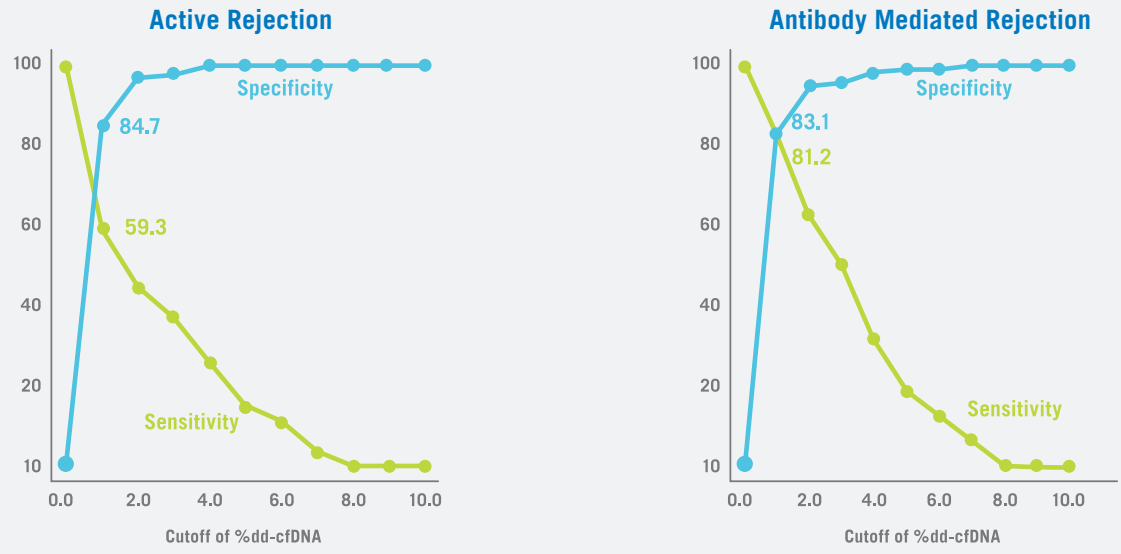
Reproducibility

Reproducibility was assessed on a set of 37 patient specimens: 26 “no rejection” (dd-cfDNA range 0%-0.94%); 11 “active rejection” (dd-cfDNA range 1.32-13.0%). Two replicate runs were performed for each paired tubes of specimens from the same venipuncture. In total, these were run by 5 operators across 21 separate days using two Fluidigm Access Array systems, four Illumina MiSeq sequencing instruments, 3 manufacturing lots of Access Array chips, and 7 lots of sequencing kits. 1-2 lots of critical raw reagents were used. There was 100% concordance of the results (95% CI: 90.5-100%) between the replicate specimens (>1% or ≤1%). For those in the quantitative range of the assay (22 specimens), the average CV for samples <2% dd-cfDNA was 7.7%; and for samples >2% dd-cfDNA, 4.5%.

Coefficient of Variation

Twelve replicate runs were performed on 12 separate days by four operators using two sets of amplification instruments and four sequencing instruments. Mean CV across dd-cfDNA levels = 6.8%.

SENSITIVITY (%) AND SPECIFICITY (%)



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Blood specimen collection

Streck Cell-Free DNA BCT® Instructions for Use (visit www.Streck.com for most up-to-date version)

AlloSure publications

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Additional relevant publications

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CAREDX CLINICAL LABORATORY QUALITY MANAGEMENT

Information regarding the CareDx clinical laboratory Quality Management program and current laboratory licensure status will be provided upon request.

Additional laboratory licenses and permits held by the CareDx Laboratory are listed below.

California: Lab ID: CLF 00332008
Florida: License #800020200
Maryland: Permit #1254
New York: Permit #8193
Pennsylvania: Lab ID #29355A
Rhode Island: LCO01129

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Hours (PST): Monday–Friday, 6:00 am – 5:00 pm, Saturday, 8:00 am – 3:00 pm, closed Sunday
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Better Surveillance for Better Outcomes
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