NEXT
MOLECULAR ANALYTICS

Laboratory Instruction Manual

Assay of Clinical Samples for L-Asparaginase Levels

11601 Ironbridge Rd
Chester, VA 23831
Email: clientservices@nextmolecular.com
www.nextmolecular.com

1. Contact Information

Client Services at NEXT Molecular Analytics can be reached at:

US and Canada Toll-Free: 844-812-7415
General Line: 804-977-6600
Fax: 804-977-6630
Email: clientservices@nextmolecular.com

For technical questions or questions regarding specimen collection or shipping, please contact:

Robert B. Harris, Ph.D., CSO	rharris@nextmolecular.com
Client Services	clientservices@nextmolecular.com

Client Services can be reached:

8:30 AM – 5:00 PM (Eastern Standard Time) Monday – Friday, except on Recognized Holidays.

Ship Specimens to:

NEXT Molecular Analytics
Sample Registration
Suite 101
11601 Ironbridge Rd
Chester, VA 23831 USA
Phone: 844-812-7415

2. Central Laboratory

2.1. Role of NEXT Molecular Analytics

NEXT Molecular Analytics has validated a laboratory assay which can be used to monitor asparaginase levels in patients treated with any of the current Pegylated or non-Pegylated L-asparaginase drug substances, including Oncaspar®, Asparlas™, Elspar ®, Kidrolase ®, or Erwinaze®.

NEXT Molecular Analytics is a CLIA accredited laboratory and maintains compliance with Good Clinical Practice (GCP), and the Health Insurance Portability and Accountability Act (HIPAA) of 1996 and 21 CFR Part 11.

3. Sample Handling Instructions

Please follow the instructions below for collection and shipment of **serum** or plasma samples for asparaginase testing.

3.1 Supplies Required at the Clinical Site

- Blood collection supplies (for standard venous collection)

3.2 Specimen Shipment Kits (available from NEXT Molecular Analytics; APPENDIX 1)

- Disposable pipettes
- Sample shipment tubes
- Sample Submission Form
- Bio hazard bag containing adsorbent paper
- Styrofoam insulated shipping container, foam cushion, gel pack
- Outer mailing sleeve

3.3 Sample Tube Labeling

- Label the sample shipment tube with a unique patient identifier, which may be a medical record number, a patient ID number, and Date of Birth.
- Information entered on the tube label must correspond to the information entered on the associated Sample Submission Form (**APPENDIX 2**).

3.4 Sample Collection

- **FOR PLASMA** collection, at each sample draw point, collect 2 mL whole blood into a lavender-capped (K2 EDTA) Vacutainer® Blood Collection tube (note: heparinized plasma is also acceptable)

    - Invert the tube gently 4 times to mix the blood with the EDTA (no longer than one minute after drawing the sample).
    - Place the tube on ice immediately after mixing (no longer than one minute after gently mixing the blood with the EDTA)
    - Centrifuge the tube immediately (within 15 minutes after blood collection), in a refrigerated centrifuge at a speed of approximately 3,000 rpm for 5 minutes.
    - Using the disposable pipette, transfer a portion of the plasma fraction (within 30 minutes of centrifugation) into a suitably pre-labeled specimen shipping tube.
        - **Make sure to transfer at least 0.3mL (300 µL) of plasma into specimen shipping tube.**
    - Record the sample date and time of collection on the sample submission form as well as the time and date of the last asparaginase administration.
    - Ship the samples by overnight express mail to NEXT Molecular Analytics (**Section 5,** below)

- **FOR SERUM** collection, draw about 2 mL blood in plain red top tubes or into a serum separator tubes (SST).

    - Leave the stopper intact throughout collection and centrifugation.
    - If using an SST tube, invert the tube gently five times as specified in the manufactures instructions
    - Allow blood to clot at room temperature for a minimum of 30, but not longer than 60 minutes.
    - Centrifuge the tube at approximately 3,000 rpm for 15 minutes.
    - Remove stopper avoid contaminating the serum with red cells
    - Using a disposable pipette, transfer a portion of the serum sample into a suitably pre- labeled specimen shipping tube.
        - **Make sure to transfer at least 0.3 mL (300 µL) of serum into specimen shipping tube.**
    - Record the sample date and time of collection on the sample submission form as well as the time and date of the last asparaginase administration.
    - Ship the samples by overnight express mail to NEXT Molecular Analytics (**Section 5**, below)

4. Sample Submission Form (APPENDIX 2)

- The Sample Submission Form is included in the Sample Collection kit, and can also be downloaded from our web site (www.nextmolecular.com), and is available upon request (**APPENDIX 2**).
- Complete a sample collection form for each patient at each blood draw.
    - ***IMPORTANT: IT IS IMPORTANT TO NOTE THE MATRIX (SERUM OR PLASMA) ON THE SAMPLE SUBMISSION FORM, AND MOREOVER, TO INDICATE THE TYPE OF PLASMA SAMPLE (EDTA•plasma; Heparin•plasma)***
- Enter the Patient and Billing information, as requested.
- **IF we are to bill the patient's insurance, you MUST Indicate the diagnosis code(s) (ICD-10) and the submission form MUST bear the signature of the health care provider.**
- Enter the sample collection date and time. Indicate pre- or post-treatment sample.
- If applicable enter the date and time of the last L-asparaginase administration.
    - It is preferable that the type of asparaginase (Oncaspar, Asparlas, Kidrolase, Erwinaze, Elspar) that was administered is indicated, but this information is not absolutely required.
- Note Special circumstances/errors – clearly indicate if any of the following occurred:
    - Specimen hemolyzed during processing
    - Any unusual appearance or occurrence during processing

5. Sample Shipment

- Place the sample shipment tubes in the long pouch of the bio hazard sample bag. Seal the pouch (see **Figure 1**).
- Place the sample collection form in the outer pocket of the bio hazard sample bag.
- Place the bio hazard bag into one half of the shipping container against the foam cushion.
- Place the frozen gel pack in the second half of the shipping container, against a foam cushion. (Note that the gel packs should be put in the freezer at least 24 hours ahead of use).
- Close the two halves of the shipping container, and place the shipping container in the outer mailing sleeve. Close the outer mailing sleeve.
- Samples should be sent Monday through Thursday to NEXT Molecular Analytics by overnight express mail. If necessary, samples may be stored refrigerated up to 5 days before shipment.
- Complete an airbill and affix to the outer mailing sleeve, or place the shipping container in an express mail container, or place the shipping container within a larger box.
- **Ship the Specimen(s) to:**

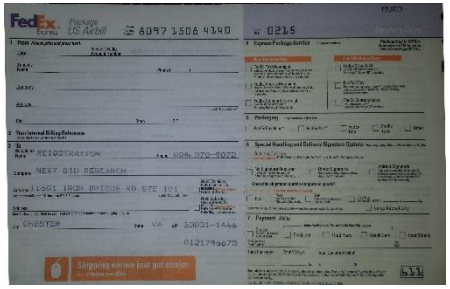
NEXT Molecular Analytics
Clinical Testing Services
Suite 101
11601 Ironbridge Rd.
Chester, VA 23831
Phone: 844-812-7415

FIGURE 1 Packaging the Samples for Shipment to NEXT Molecular Analytics

Place the shipping tube(s) in the large pocket of the specimen bag. DO NOT REMOVE the absorbent sheet

Place the Sample Submission form in the smaller pocket of the specimen bag

Place specimen bag in the shipping container against the cold pack



Affix the airbill to the mailing sleeve, or place the box inside a large container and send by express mail to NEXT Molecular Analytics

FREQUENTLY ASKED QUESTIONS

Q1: Why do this test?

There are two forms of hypersensitivity to asparaginase seen in the clinics. Clinical hypersensitivity ranges from a mild local injection site reaction to full blown anaphylaxis. Since asparaginase derived from *E. coli* is frequently used as first treatment, patients who develop such a reaction are switched to Erwinaze (derived from *Erwinia chrysanthemi*) to continue their treatment because of the lack of cross-reactivity of anti-E.coli antibodies to Erwinaze. The second form of hypersensitivity is when antibodies are formed, but the patients do not experience any clinical signs of hypersensitivity. Yet these antibodies can either inactivate the enzyme or enhance the metabolism of asparaginase such that the patient may not have adequate serum or plasma activity levels to achieve an anti-leukemic effect (often referred to as “silent inactivation” or “silent hypersensitivity”).

The asparaginase test will help clinicians identify these patients as well as to ensure that adequate asparaginase activity is present during asparaginase treatment. The assay is done on a plasma or serum sample obtained from the patient after treatment.

Q2: How is the assay done?

Asparaginase activity is determined by a coupled enzymatic assay. Briefly, aspartic acid formed from asparagine by the action of asparaginase reacts with α- ketoglutaric acid in the presence of glutamic-oxaloacetic transaminase, yielding oxaloacetic acid, which oxidizes reduced β-nicotinamide adenine dinucleotide in the presence of malic dehydrogenase, resulting in a decrease in absorbance at 340 nm. The rate of reaction at 37°C is a linear function of enzyme activity.

E. coli asparaginase purchased from Sigma-Aldrich (St. Louis, MO) is used as the analytical reference standard. A series of 11 calibration standards in normal human serum is used to prepare the assay calibration curve, and of these 11 points, 9 are within the quantitation range. Samples with activities exceeding the upper range of the calibration curve are re-assayed after diluting with blank human serum.

Q3: When should I take a sample and submit it for analysis?

An “algorithm” for sampling has been developed (see Bleyer et al, Pediatric Blood Cancer 2015: 1102-11095). An excerpted version of this algorithm is available on our web site (www.nextmolecular.com)

Q4: How much will it cost? Who will pay for the test?

The cost for assay of EACH sample is $165.00. If the patient is enrolled in a clinical trial, in many instances, the clinical trial sponsor will pay for the additional testing (please inquire of the clinical trial sponsor). Otherwise, NEXT Molecular Analytics will submit payment

claims to the submitting institution or will accept pre-payment from the patient by personal check or a credit card.

NEXT Molecular Analytics can also accept payment from Medicare, third party insurance companies, and Medicaid (in certain states; please inquire). If insurance payment is indicated, NEXT will bill the insurance company for the cost of the asparaginase assay ($165.00), but we are obligated to bill the patient for any co-pays or deductibles.

Please complete payment information portion of the sample submission form.

Q5: What is the expected turn-around time?

Assay results are routinely returned on the same day as the sample is received.

Q6: Where will the results be sent?

To the requesting clinician, and to those persons specified by the submitting physician as authorize to receive the sample results report. The results report will be sent by fax, or by encrypted email, as specified on the sample submission form.

Q7: How are the results reported?

The results will be reported in terms of IU/mL of L- asparaginase in the sample.

Q8: How do I interpret the test?

Only experienced physicians treating patients with asparaginase should interpret whether the asparaginase activity level is adequate. There are no established treatment guidelines, and the minimal threshold therapeutic level, once considered to be 0.1 IU/mL, may now be 0.05 IU/mL, and may even be lower. The decision to modify asparaginase treatment to achieve a target level must be made by the physician.

Q9: How can I order sample collection kits?

Fax the Request for Additional Supplies (**APPENDIX 1**) to 804-977-6630, or call Clinical Testing Services at 844 812 7415 **or e-mail:** clientservices@nextmolecular.com

APPENDIX 1
REQUEST FOR ADDITIONAL SUPPLIES

NEXT MOLECULAR ANALYTICS

LDT ASPARAGINASE ASSAY
REQUEST FOR SUPPLIES

Submit Completed Supply form to Next Molecular Analytics at:
FAX: 804-977-6630 or via EMAIL: clientservices@nextmolecular.com

Name of Requesting Entity _____

Shipping Address (no P.O. Boxes) _____

_____

City, State, Zip Code _____

Phone _____

Fax _____

Contact _____

Email _____

Quantity Needed	Item	Item Number
	Complete Sample Collection Kit	SK-LASP-001

*Collection kit includes all supplies needed for testing and shipping.

**Turnaround time for delivery of supplies is 2-3 business days.

11601 Iron Bridge Road, Ste. 101, Chester VA 23831
Phone 844-812-7415

LASPSO-001-1015 <SID>

APPENDIX 2
SAMPLE SUBMISSION FORM

ASPARAGINASE ASSAY SAMPLE SUBMISSION FORM

NEXT MOLECULAR ANALYTICS

11601 Iron Bridge Rd, STE 101, Chester, VA 23831

Phone (toll free) 844-812-7415
Phone 804-977-6600
Fax 804-977-6630
Email clientservices@nextmolecular.com

ASN21 _____ (NEXT USE ONLY)

PHYSICIAN INFORMATION

SEND REPORT TO

Organization: _____

Name (print) _____

Address _____

_____

Phone _____

Fax _____

Email _____

Signature _____

SAMPLE INFORMATION

Heparinized plasma ____ EDTA Plasma ____

Serum ____ Other ____

Date of Collection _____

Time of Collection _____

Today's Date _____

Did the patient receive a previous dose of Asnase (Y/N) ____

If Yes: Date and Time of last dose: _____

Drug Administered:

____ ____ ____ ____

Oncaspar Erwinaze Asparlas Other

Person Completing this form: _____

DIAGNOSIS (ICD-10) CODE(S)

Required if billing insurance

_____

Comments: _____

PATIENT INFORMATION

_____

Last Name First Name MI

Address: _____

_____

SEX Male ☐ Female ☐

Patient ID Number _____

DOB _____

BILLING INFORMATION

Institutional Payment Information PO No _____

Bill to address _____

_____

_____

Phone _____

Email _____

Charge card Payment (or enclose personal check, payable to NEXT Bio-Research Services, LLC)

Card Number _____

Name on Card _____

Expiration Date _____

Security Code _____

Amt to be charged (up to $ 165 per sample)

By signing this form, you authorize NEXT Bio-Research Services to charge your card for the amount listed above.

Cardholder Signature _____

Insurance Billing

(Medicaid approved in AZ,CO,DC,KY,MD,MS,NC,MO,NE,NJ,NM,OH,OK,VA)

Attach copies of insurance card(s), front and back.

Provide Charge Card Information.

Policy/ID# _____ Group # _____

Insured's Name _____

SSN _____ DOB _____

Insurance Carrier _____

Claim Address _____

_____

Phone _____

Samples should be shipped cold by overnight express mail Monday through Friday