Sample Collection: An Important First Step

Options for identifying epidermal growth factor receptor (EGFR) mutations in metastatic non–small cell lung cancer (NSCLC) may include tissue- or plasma-based testing.[1] While multiple test options are available, none are considered flawless. A negative result on one does not confirm that the patient is negative for EGFR mutations, either at initial diagnosis or at disease progression. Negative results should be followed up with a complementary test type (ie, a tissue biopsy) to give the patient the most appropriate treatment options at diagnosis or at progression.

Reminder: NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) recommend testing for all actionable biomarkers at diagnosis of NSCLC, including EGFR, BRAF, ROS1 mutations, ALK rearrangements, and PD-L1 expression.[2]

Reminder: A new sample is required to test for EGFR mutations at the time of progression.

Learn more about sample collection methods in the sections below.

Tissue-based samples

Tissue-based samples have long been considered the gold standard and can be used to test for EGFR mutations both at initial diagnosis (eg, exon 19 deletions, L858R), as well as at disease progression (eg, T790M mutations), but a new sample is needed at progression.[3]

The members of the multidisciplinary team responsible for obtaining tissue samples can include the interventional radiologist, pulmonologist, or thoracic surgeon.[4]

There are various methods used to collect tissue-based samples for diagnostic and EGFR mutations.[5,6] (See table below.)

Tissue-based Collection Modalities[5,6]

Diagnostic Modality	Specimen Types
Bronchoscopy ± EBUS	Endobronchial biopsy Transbronchial biopsy Brushing cytology Washing cytology FNA cytology
Surgical (eg, thoracoscopy, mediastinoscopy, resection)	Tissue biopsy
Image-guided (eg, TTNA, thoracentesis, bone biopsy)	CNB FNA cytology Fluid cytology Bone biopsy

CNB, core needle biopsy; EBUS, endobronchial ultrasound; FNA, fine needle aspiration; TTNA, transthoracic needle aspiration.

Tissue-based testing can be extremely useful in identifying EGFR mutations, but as with any testing protocol, there are also some drawbacks. The advantages and considerations are outlined below.[1,3,7]

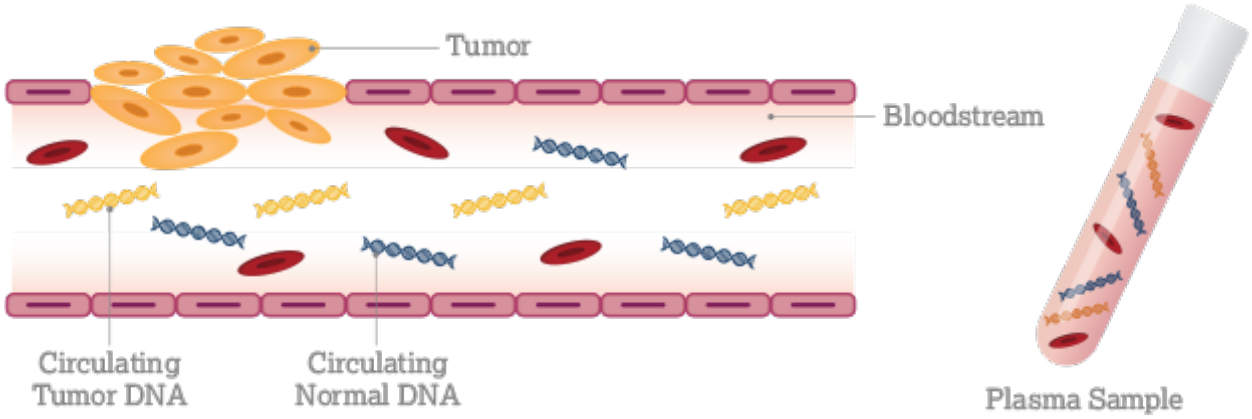
Advantages and Considerations of Tissue-based Testing for EGFR Mutations

Advantages	Considerations
Established testing method for identifying NSCLC mutations at diagnosis	Patients may not be positively identified due to heterogeneity of sample
Mutations can be identified with more certainty due to high sensitivity rates	Due to variable tumor quantity and quality in each biopsy or insufficient tumor material, a patient may not be eligible for tissue biopsy
	Complications may develop during the collection process

Plasma-based Testing: A Less Invasive Alternative

Plasma-based testing is effective because DNA from the tumor cells, known as circulating tumor DNA (ctDNA), often enters the patient's blood stream, a process known as shedding. When blood is collected from the patient, ctDNA from the tumor can be detected from plasma derived from whole blood.[3,8]

Tumors Shed ctDNA Into Blood Stream[3,8]



With plasma-based tests, a phlebotomist or nurse draws blood from a patient when the treating physician (eg, the oncologist) orders an EGFR mutation test.

After it is drawn, the blood is processed and shipped to a testing laboratory where it can be tested for EGFR mutations.

As with tissue-based samples, plasma-based samples have advantages and drawbacks, which are outlined in the table below.[1,3,8]

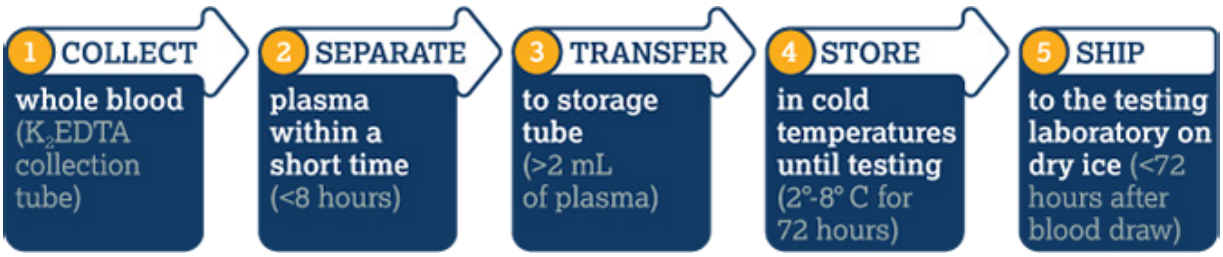
Advantages and Considerations of Plasma-based Testing for EGFR Mutations

Advantages	Considerations
Collection is less invasive to patients and has fewer limitations than other options	Results can be inconclusive due to differences in tumor biology • Variables such as tumor burden and number of tumor cells shed can influence results • DNA may be insufficient for positive identification
Potential for faster turnaround time	
May save procedure costs	
A greater sample of the patient population is able to be tested	

Plasma-based testing may reduce turnaround time and can be used to quickly determine mutation status while awaiting tissue-based test results.

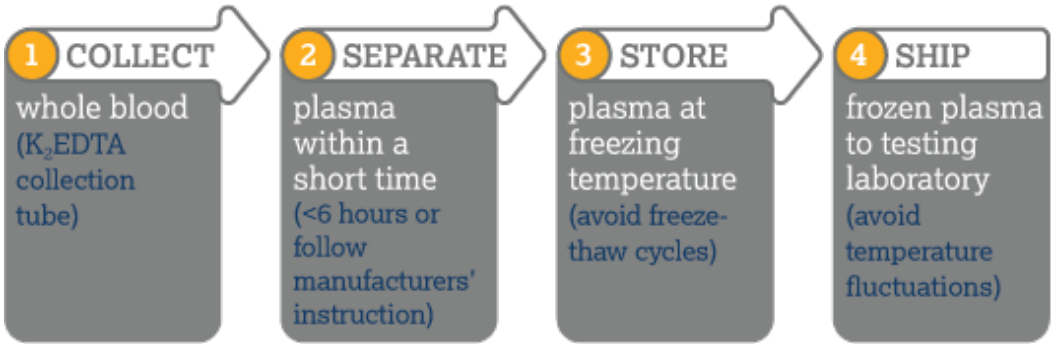
The plasma testing process may vary. Two example workflows for plasma-based testing are shown below:

Workflow for the Use of a Plasma-based Sample With the cobas® EGFR Mutation Test v2[9]



K_2EDTA, dipotassium ethylenediaminetetraacetic acid.

Workflow for the Use of a Plasma-based Sample According to ASCO/CAP Guidelines[10]

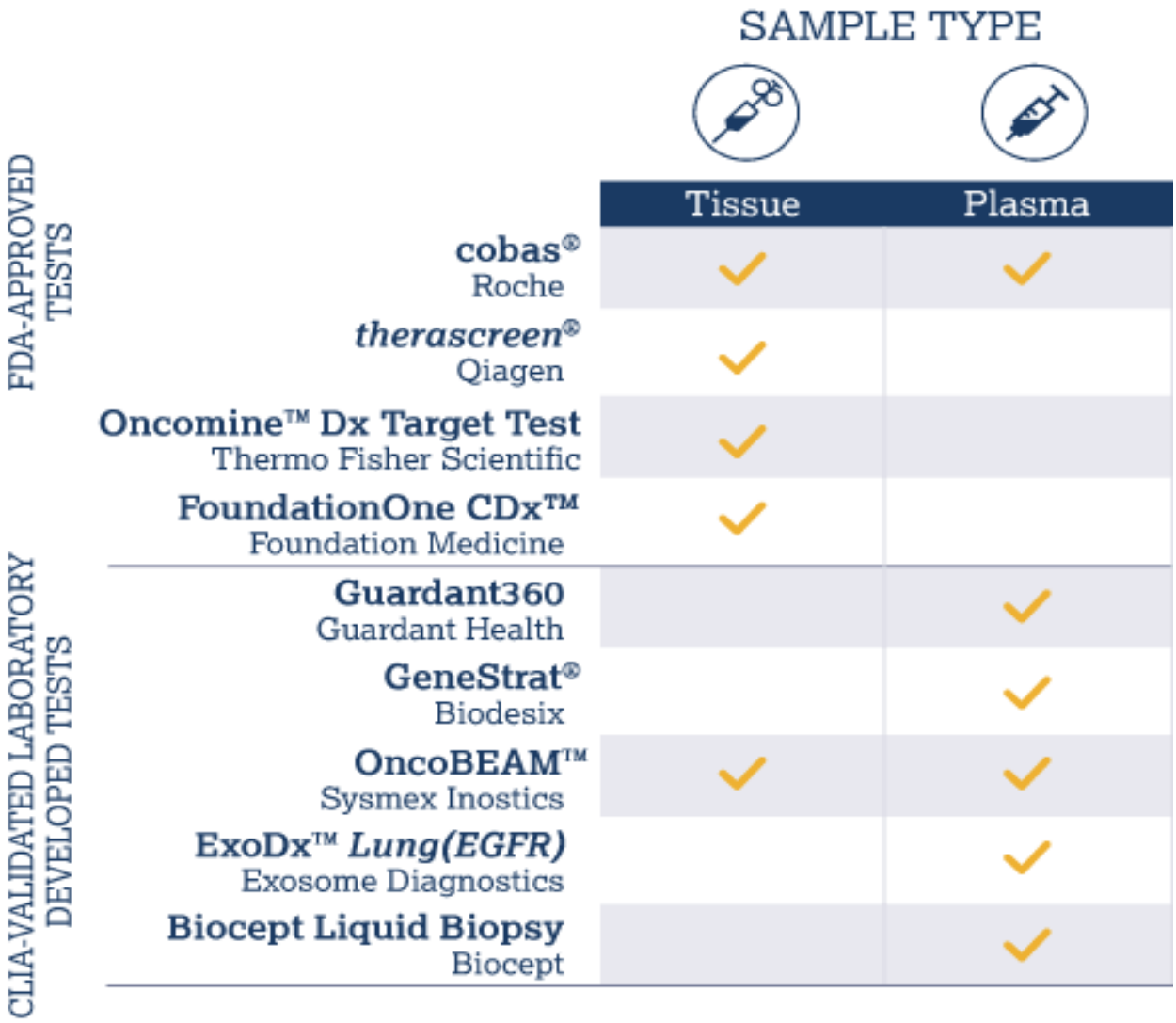


You will notice that the cobas® EGFR Mutation Test v2 requires, and the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines recommend, that you separate plasma after the blood draw before shipping the sample to your preferred laboratory.[9,10]

Please check with your pathologist or laboratory to verify the preferred method of sample preparation.

Sample types acceptable for use with various EGFR mutation tests

The table below outlines the sample types that are appropriate for use with various available EGFR mutation tests.[11-18]



ALK, anaplastic lymphoma kinase; BRAF, v-Raf murine sarcoma viral oncogene homolog B; PD-L1, programmed death-ligand 1; ROS1, ROS proto-oncogene 1, receptor tyrosine kinase.

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References: 1. Reckamp KL, Melnikova VO, Karlovich C, et al. A highly sensitive and quantitative test platform for detection of NSCLC EGFR mutations in urine and plasma. *J Thorac Oncol*. 2016;11(10):1690-1700. doi:10.1016/j.jtho.2016.05.035. **2.** Adapted with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V2.2018. © 2017 National Comprehensive Cancer Network, Inc. 2018. All rights reserved. Accessed January 26, 2018. To view the most recent and complete version of the guideline, go online to NCCN.org. **3.** Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J Clin Oncol*. 2014;32(6):579-586. doi:10.1200/JCO.2012.45.2011. **4.** Levy BP, Chioda MD, Herndon D, et al. Molecular testing for treatment of metastatic non-small cell lung cancer: how to implement evidence-based recommendations. *Oncologist*. 2015;20(10):1175-1181. doi:10.1634/theoncologist.2015-0114. **5.** Ofiara LM, Navasakulpong A, Beaudoin S, Gonzalez AV. Optimizing tissue sampling for the diagnosis, subtyping, and molecular analysis of lung cancer. *Front Oncol*. 2014;4:253. doi:10.3389/fonc.2014.00253. **6.** Wu JS, Goldsmith JD, Horwich PJ, Shetty SK, Hochman MG. Bone and soft-tissue lesions: what factors affect diagnostic yield of image-guided core-needle biopsy? *Radiology*. 2008;248(3):962-970. doi:10.1148/radiol.2483071742. **7.** Chen S, Zhao J, Cui L, Liu Y. Urinary circulating DNA detection for dynamic tracking of EGFR mutations for NSCLC patients treated with EGFR-TKIs [published online ahead of print July 28, 2016]. *Clin Transl Oncol*. doi:10.1007/s12094-016-1534-9. **8.** Bordi P, Del Re M, Danesi R, Tiseo M. Circulating DNA in diagnosis and monitoring EGFR gene mutations in advanced non-small cell lung cancer. *Transl Lung Cancer Res*. 2015;4(5):584-597.doi:10.3978/j.issn.2218-6751.2015.08.09. **9.** Roche Diagnostics. The cobas® EGFR Mutation Test v.2: sample collection for tissue and plasma. http://www.cobasegfrtest.com/sample-collection.html. Accessed February 21, 2018. **10.** Merker JD, Oxnard GR, Compton C, et al. Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists joint review [published online March 5, 2018]. *J Clin Oncol*. 2018. doi:10.1200/JCO.2017.76.8671. **11.** *therascreen*® EGFR RGQ PCR Kit [instructions for use (handbook)]. Manchester, UK: QIAGEN; 2017. **12.** Oncomine™ Dx Target Test Part I: Sample preparation and quantification [user guide]. Waltham, MA: Thermo Fisher Scientific Inc.; 2017. **13.** Foundation Medicine. FoundationOne CDx™ specimen instructions. https://www.foundationmedicine.com/genomic-testing/foundation-one-cdx. Accessed February 21, 2018. **14.** Guardant Health. How Guardant360™ works. http://www.guardanthealth.com/guardant360/#how-it-works. Accessed February 21, 2018. **15.** Biodesix. GeneStrat® genomic test. http://www.biodesix.com/genestrat. Accessed February 21, 2018. **16.** Sysmex Inostics. Sysmex OncoBEAM™ EGFR. http://www.sysmex-inostics.com/our-services/product-single-view/oncobeamTM-egfr-3805.html. Accessed February 21, 2018. **17.** Exosome Diagnostics. Lung cancer. http://www.exosomedx.com/lung-cancer-0. Accessed February 21, 2018. **18.** Biocept Inc. You need a complete answer to provide the best treatment. http://biocept.com/technology/lung-cancer-offering. Accessed February 21, 2018.