SECTION EIGHT

DAHL-CHASE DIAGNOSTIC SERVICES

MOLECULAR TESTING

INDEX

Section 8 Page

DAHL-CHASE PATHOLOGY ASSOCIATES

MOLECULAR REQUISITION M-

417 State Street, Suite 540
Bangor, ME 04401
(207) 941-8200 / 1-800-660-1626

PLEASE PRINT – COMPLETE ALL AREAS **OR** ATTACH A DEMOGRAPHIC SHEET **AND** FILL IN THE SHADED SECTIONS

Patient Name: (Last, First)			**Medicare #**
Date of Birth:	**Sex:**	**Telephone:**	**Medicaid #**
Social Security:	**MR#**		**Commercial Insurance Name:**
Address: (Mailing)			**Commercial Insurance Policy:** / **Group #**
(City, State, Zip)			**Commercial Insurance Address (Medical Claims)**
Requesting Clinician Name (Last, First):			**(City, State, Zip)**
Physician Office/Clinic/Hospital Name:			**Copy to Clinician Name (Last, First, and office location):**
Collection Date and Time: ____/____/____ ____:____			**Requisition Completed By:**

LOCATION:

☐ **Physician Office Patient** ☐ **Hospital Lab**

CLINICAL HISTORY:

Specimens accepted Monday through Friday

- ❑ **Urine UroVysion FISH for Bladder Cancer Recurrence –**
  Follow urine collection instructions in the Urocyte Kit.
  Transport at refrigerated temperature.

- ❑ **HER2-FISH PathVysion Gene Amplification status For Breast, Gastric, or Esophageal Cancers**
  Send selected paraffin block containing invasive CA.
  Please include a copy of the pathology report for this case.

- ❑ **K-RAS Mutation Assay For Colorectal Cancers**
  Send formalin fixed tissue containing a sufficient amount of tumor (generally at least several mm of tumor tissue submitted in the tissue block

- ❑ **BRAF Mutation Assay For Colorectal Cancers**
  Send formalin fixed tissue containing a sufficient amount of tumor (generally at least several mm of tumor tissue submitted in the tissue block)

- ❑ **Microsatellite Instability (MSI) For Colorectal**
  Send formalin fixed tissue containing a sufficient amount of tumor and a normal tissue block (generally at least several mm of tumor tissue submitted in the tissue block)

Lung Carcinoma Molecular Analysis

- ❑ **Epidermal Growth Factor Receptor (EGFR) Mutation Analysis**
- ❑ **FISH for ALK Rearrangements.**
  Send formalin fixed tissue containing a sufficient amount of tumor

SECTION EIGHT

DAHL-CHASE DIAGNOSTIC SERVICES

MOLECULAR TESTING

URINE UROVYSION BLADDER CANCER FISH COLLECTION

Methodology

Vysis UroVysion Bladder Cancer FISH Assay (FDA approved)

Principle

The UroVysion Bladder Cancer FISH assay is designed to detect aneuploidy for chromosomes 3, 7, 17, and loss of 9p21 locus via fluorescence in situ hybridization (FISH) in urine specimens from persons with hematuria suspected of having bladder cancer. Results from the UroVysion FISH assay are intended for use, in conjunction with and not in lieu of current standard diagnostic procedures, as an aid for initial diagnosis of bladder carcinoma in patients with hematuria and subsequent monitoring for tumor recurrence in patients previously diagnosed with bladder cancer.

Specimen Requirements

Urine specimens for Vysis UroVysion FISH testing are collected using the ThinPrep Urocyte Urine Collection Kit. Kits are available through Dahl Chase Diagnostic Services. A minimum of 33mls of urine (maximum 60ml) must be collected.

Specimens are transported at refrigerated temperature and must be processed within 48hours of collection. Specimen collection should be Monday through Friday.

Ordering UroVysion FISH testing

UroVysion FISH testing is ordered on a Molecular requisition available through Dahl Chase Diagnostic Services and is included in the collection kit.

Materials and Reagents

Completed Molecular DCDS requisition
ThinPrep Urocyte Urine Collection Kit (available through DCDS-call 941-8202)
Ice packs (or refrigeration)

Specimen Collection Procedure:

1. Record patient demographic and insurance information (Name, Address, SSN, DOB,date, physician) on the Molecular requisition.
2. On requisition, check box for FISH-UroVysion testing.
3. Open the Urocyte Urine Collection Kit.
4. The specimen collection cup has the blue cap. Write the patient's full name and date of birth on the specimen label where indicated.
5. Collect urine directly into the collection cup. If urine volume exceeds 60cc (see side ofcontainer), pour off excess. A minimum of 33cc of urine must be collected in order toperform the test.
6. The urine preservative (PreservCyt) is in the vial with the white cap.

7. Add entire contents of PreservCyt into specimen collection cup IMMEDIATELY AFTER COLLECTION! Mix well.
8. Tightly seal cap of specimen container (Keep turning ¼ inch after audible click is heard).
9. Place urine specimen in provided bag containing biohazard markings and seal. Place the sealed specimen back into the urine collection kit box.
10. Fold the molecular requisition and place in the box. Close the box.
11. Refrigerate the entire collection kit.
12. Specimens must be transported at refrigerated temperature and be processed within 48 hours of collection. Uniship courier pick up is available. If an additional courier pickup is needed, please call Uniship at 848-7546. If possible, please call the molecular department at 941- 8228 to notify them that a specimen is coming.

DAHL-CHASE Focus

Technical Bulletin: FISH for Recurrent Bladder Cancer

Technical Brief

FISH for Recurrent Bladder Cancer: Detection of Genetic Alterations in Bladder Cancer Cells

Dahl-Chase Diagnostic Services is now offering analysis of chromosomal abnormalities in urine specimens using multicolor, multi-target UroVysion FISH probes for the detection of bladder cancer recurrence.

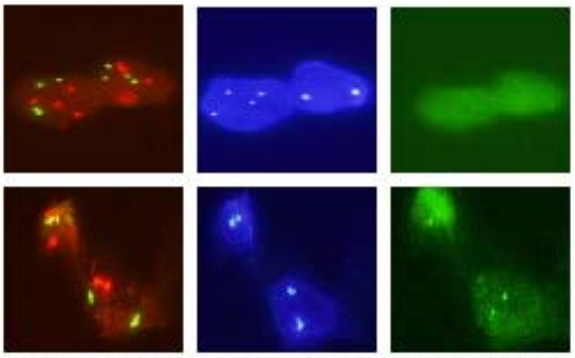
Background Information

Bladder Cancer is the fifth most common type of urothelial carcinoma in the United States. Ninety percent of all bladder cancer cases are urothelial carcinomas (UC). At presentation, about 75% of tumors are superficial, of which 50% to 80% will have one or multiple recurrences, and 15% to 25% will progress to muscle-invasive tumors. Follow-up cystoscopy and urine cytology have been used to detect recurrence and tumor progression in patients with superficial UC. However, low-grade tumors tend to have false negative cytology results.

Several genetic alterations have been identified to occur at high frequency in bladder cancer. These include the loss of a portion of chromosome 9 (presumably carrying a tumor suppressor gene), as well as aneuploidy of chromosome 3, 7, and 17 (1). Several studies have demonstrated that detection of chromosomal abnormalities by fluorescence *in situ* hybridization (FISH) has higher sensitivity in detection of UC recurrence than cytology, while maintaining high specificity (2-4). The presence of chromosomal abnormalities also has been shown to predict bladder cancer recurrence and is detectable by UroVysion (5,6). The UroVysion Bladder Cancer Kit (UroVysion, Abbott Molecular, Vysis, Des Plaines, IL) is an FDA approved in-vitro diagnostic assay used to detect aneusomy for chromosomes 3, 7, and 17 and loss of 9p21 locus.

Clinical Indications

UroVysion FISH testing is performed on persons with hematuria suspected of having bladder cancer and for subsequent monitoring of tumor recurrence in patients with previously diagnosed bladder cancer. FISH analysis of urine specimen can be utilized as an ancillary test thereby increasing the sensitivity of urine cytology for the detection of UC.



An illustrative image of UroVysion FISH from a bladder cancer patient (top row from left to right) demonstrates gains of chromosomes 3 (red), 7 (green), and 17 (aqua), and loss of 9p21 locus (gold). Nuclei of benign urothelial cells (bottom row) demonstrate two copies of each probe.

Methodology

The UroVysion Bladder Cancer Kit is an FDA approved In-Vitro Diagnostic assay designed to detect aneusomy of chromosomes 3, 7, and 17 and loss of 9p21 locus via FISH in urine specimen using a 4-color, four-probe mix of fluorescently labeled DNA probes. Urine specimen are collected and resuspended with PreservCyt (Thin Prep UroCyte Urine Collection Kit). Urine specimens are to be refrigerated and shipped on ice within 24 hrs of collection. Thin Prep UroCyte Urine Collection kits

may be obtained from Dahl-Chase Diagnostic Services (207) 941-8202.

Cells recovered from urine specimen are fixed on slides. The slides are protease digested, fixed in formaldehyde, washed, and dehydrated. The DNA is denatured to single stranded form and allowed to hybridize with the UroVysion DNA probes. Probe hybridization is performed using a DNA probe mixture containing fluorophore-labeled DNA probes to the peri-centromeric regions of chromosomes 3 (CEP3-red), 7 (CEP7-green,), and 17 (CEP17-aqua), and to the locus 9p21 (LSI 9p21-gold). Unbound and non-specifically bound probe is removed by a series of washes, and nuclei are counter stained. Morphologically abnormal nuclei are enumerated using fluorescence microscopy.

Interpretation

Morphologically atypical nuclei containing fluorescent signals for CEP 3 (red), 7 (green), and 17 (aqua) and the 9p21 locus (gold) are enumerated for minimum of 25 nuclei.

A positive result:
4 or more nuclei with a signal gain of 2 or more chromosomes (3, 7, and 17) in the same nucleus and/or homozygous loss of the 9p21 locus in 12 or more nuclei.

Assay Limitations

Urine specimen submitted for evaluation that are of low cellularity, low volume or any other situation that may result in a specimen containing less than 25 urothelial cells.

Test Overview

Test Name	FISH for bladder cancer
Patient Preparation	None
Reference Range	Fluorescent signal quantification of morphologically abnormal nuclei (see positive result definition above)
Specimen Requirements	A minimum of 33-60cc of fresh voided urine poured immediately into urine preservative (PreservCyt). Mix and refrigerate. Transport refrigerated. Submit with molecular requisition.
CPT Codes	88365 X 4

References

1. Sandberg AA, Berger CS. Review of Chromosome studies in urological tumors: Cytogenetics and molecular genetics of bladder cancer. J Urol 1994;151;545-560.
2. Halling K, King W, Sokolova I et al: A comparison of cytology and fluorescence in situ hybridization for the detection of bladder carcinoma. J Urol 2000;165:1768-1175.
3. Bubendorf L, Grilli B, Sauter G et al. Multiprobe FISH for enhanced detection of bladder cancer in voided urine specimens and bladder washings. AM J Clin Pathol 2001;116:79-85.
4. Halling K, King W, Sokolova I et al. A comparison of BTA stat, hemoglobin dipstick, telomerast and Vysis UroVysion assays for detection of urothelial carcinoma in urine. J Urol. 2002;167(5):2001-6.
5. Yoder BJ, Skacel M, Hedgepeth R et al. Reflex UroVysion testing of bladder cancer surveillance patients with equivocal or negative urine cytology: A prospective study with focus on the natural history of anticipatory positive findings. AM J Clin Pathol. 2007 Feb;127 (2):1-7.
6. Sarosdy MF, Schellhammer P, Bokinsky G et al. Clinical evaluation of a multi-target fluorescent in situ hybridization assay for detection of bladder cancer. J Urol. 2002 Nov;168 (5):1950-4.

Technical Information Contact:
Michael Babcock
(207) 941-8228

Scientific Information Contact:
Marek Skacel, M.D
(207) 941-8200

Collection/Submission Contact:
Lisa Grant
(207) 561-2415

DAHL-CHASE Focus

January, 2009

TECHNICAL BULLETIN: FISH for *HER2* Gene Amplification

Technical Brief

FISH for *HER2* Gene Amplification

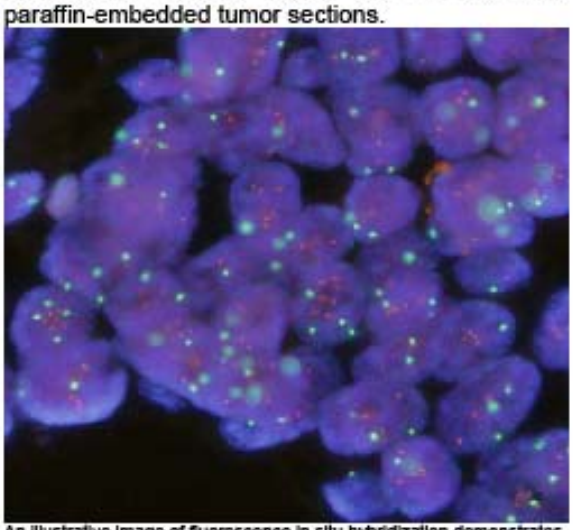
Background Information

Breast cancer in the United States is a devastating illness, affecting more than 200,000 newly diagnosed patients and resulting in more than 40,000 deaths annually (1). Modern diagnostic procedures allow earlier detection, and excision of small tumors at an early clinical stage offers the best hope for cure.

Breast tumors differ in their biological behavior. One important challenge is the identification of tumors of low clinical and/or pathologic stage that display phenotypic or genotypic characteristics that indicate potential for aggressive malignant behavior (metastasis, recurrence, and early death).

Proteins expressed on tumor cells have become recognized as markers of biological behavior. Amplification and over expression of the oncogene *HER2* (Her-2-neu, c-erbB2) occurs in 20-30% of human invasive breast carcinomas (2,3). *HER2* amplification in patients with invasive breast carcinoma is associated with a significant reduction in metastasis-free survival, and similarly staged patients without *HER2* gene amplification have a high likelihood of remaining cancer-free years after removal of the primary tumor (2,5). *HER2* positive patients are more likely to respond to regimens containing adriamycin and less likely to respond to tamoxifen (6,7). Furthermore, patients with metastatic breast carcinoma whose tumors have amplification of the HER2 gene and overexpress the protein it encodes, may benefit from therapy with the humanized monoclonal antibody trastuzumab (Herceptin) either as a single agent or in combination with other chemotherapy (8,9).

Dahl-Chase Diagnostic Services offers *HER2* gene amplification analysis of breast carcinoma utilizing an FDA-approved interphase fluorescence *in situ* hybridization (FISH) assay on formalin-fixed paraffin-embedded tumor sections.



An illustrative image of fluorescence in situ hybridization demonstrates HER2 gene amplification (increased numbers of orange signals) in invasive breast carcinoma cell nuclei (DAPI stain). Green signals identify centromeric regions of chromosome 17.

Clinical Indications

HER2 gene amplification analysis is offered specifically as a prognostic molecular marker for invasive breast carcinoma and as an adjunctive test in the management of patients with advanced breast carcinoma for whom therapy with humanized monoclonal antibody is contemplated. FISH also may be especially useful as a confirmatory assay for breast carcinoma cases for which a 2+ *HER2* score has been identified previously by immunohistochemistry (IHC).

Interpretation

HER2 gene amplification is reported as present, equivocal, or absent. The *HER2* gene copy

number is indicated on the report, and the *HER2*/CEP17 ratio is specified. A *HER2*/CEP17 ratio >2.2 is positive for *HER2* gene amplification. An equivocal result is reported for a *HER2*/CEP17 ratio between 1.8 and 2.2. Equivocal *HER2* FISH results are subjected to HER2 IHC. *HER2*/CEP17 results < 1.8 are report as *HER2* gene amplification absent (10).

Limitations of the Assay

The FISH assay performs well using formalin-fixed paraffin-embedded tissue sections. Inconsistent FISH results are obtained for specimen received in fixatives and preservatives that do not contain 10% formalin. Bone marrow core biopsies produce inconsistent results, likely due to decalcification effect, therefore, *HER2* FISH analysis of bone marrow specimens should be performed on the clot sections whenever possible.

Methodology

The assay consists of an interphase FISH probe assay (PathVysion, Abbott Molecular, Vysis, Des Plaines, IL) that quantifies *HER2* gene amplification in formalin-fixed paraffin-embedded human breast carcinoma tissue.

Test Overview

Test Name	FISH for *HER2* gene amplification analysis
Reference Range	*HER2*/CEP17 ratio <1.8 = *HER2* gene amplification ABSENT 1.8 – 2.2 = Equivocal >2.2 = *HER2* gene amplification PRESENT
Specimen Requirements	Paraffin block containing breast carcinoma (formalin fixation required) transported at room temperature
CPT Codes	88365 X 2 and 88313

References

1. www.cancer.org/docroot/STT/stt_0.asp
2. Ro J, El-Naggar A, Ro J et al. C-erb B-2 amplification in node negative human breast cancer. Cancer Research 1989;49:6941-44.
3. Seshadri R, Firgaira FA, Horsfall DJ et al. Clinical significance of HER2/neu oncogene amplification in primary breast cancer. Jour of Clin Ocol 1993:11(10):1936-42.
4. Slamon DJ, Godolphin W, Jones L et al. Studies of the HER2/neu proto-oncogene in human breast and ovarian cancer. Science 1989;244(4905):707-712.
5. Tsuda H, Hirohashi S. Shimosato Y et al. Correlation between long-term survival in breast cancer patients and amplification of two putative oncogene coamplification units: hst-1/int- 2 and c-erb B-2/er-1. Cancer Research 1989;49(11):3104-08.
6. Paik S, Bryant J, Park C et al. erbB-2 and response to doxorubicin in patients with axillary lymph node-positive, hormone receptor-negative breast cancer. J Natl Cancer Inst 1998;90(18):1361-1370.
7. Thor AD, Berry DA, Budman DR et al. erbB2, p53, and efficacy of adjuvant therapy in lymph node-positive breast cancer. J Natl Cancer Inst 1998;90(18):1346-1360.
8. Seidman AD, Fornier MN, Esteva FJ et al. Weekly Trastuzumab and Paclitaxel therapy for metastatic breast cancer with analysis of efficacy by Her-2 immunophenotype and gene amplification. J Clin Oncol 2001;19:2587-2595.
9. Slamon DJ, Leyland-Jones B, Shak S et al. Use of chemotherapy plus a monoclonal antibody against Her-2 for metastatic breast cancer that overexpresses Her-2. N Engl J Med 2001;344:783-92.
10. Wolf AC, Hammond EH, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane, LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, and Hayes DF. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. J Clin Oncol 2007:25(1):1-28.

Technical Information Contact:
Michael Babcock
(207) 941-8228

Scientific Information Contact:
Marek Skacel, M.D
(207) 941-8200

Collection/Submission Contact:
Lisa Grant
(207) 561-2415

DAHL-CHASE Focus

September 1, 2009

NEW TEST ANNOUNCEMENT: K-RAS Mutation Assay

Technical Brief
K-RAS Mutation Assay For Colorectal Cancers

Dahl-Chase Diagnostic Services is now offering *KRAS* mutation detection by direct sequencing on formalin-fixed paraffin-embedded tissue specimens.

Background Information

An estimated 30 to 40 percent of colorectal carcinomas carry mutant forms of the gene *KRAS* (*KRAS* mutations). Patients with advanced colorectal carcinoma who have such mutations in their tumors should not receive epidermal growth factor (EGFR) antibody therapy. This therapy is designed to block the activity of the epidermal growth factor receptor (EGFR) protein, which is often overactive in colorectal cancer. Since this therapy is unlikely to be effective in patients with *KRAS* mutations, testing for the presence of these mutations has become an essential step in determining which patients will benefit from treatment by EGFR antibodies and which patients should be spared of their side-effects and cost.

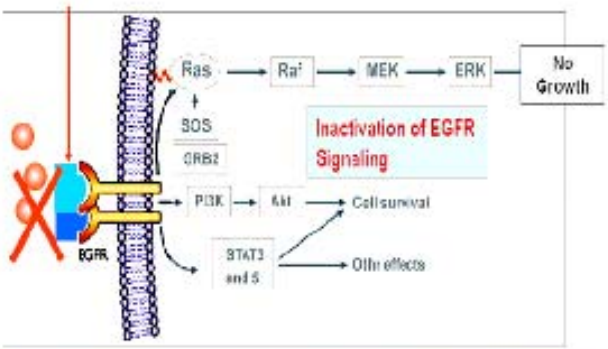


Fig 1. Schematic diagram of the EGFR pathway illustrating the site of action of the EGFR antibody therapy. Only patients with wild-type (normally functioning) *KRAS* gene benefit from this therapy as *KRAS* mutation causes constitutive activation of this pathway and the antibodies are ineffective.

Clinical Indications

The American Society of Clinical Oncologists (ASCO) recommends routine testing for *KRAS* mutations for all colorectal carcinomas considered for therapy with cetuximab.

Methodology

Of all known *KRAS* mutations, vast majority have been identified in human cancers involving codons 12 and 13 of *KRAS*. The assay performed at the Dahl-Chase laboratory performs optimally in formalin-fixed tissue to detect *KRAS* mutations in these two codons by direct sequencing when mutated DNA comprises greater than 25% of the sample DNA (the industry standard for this assay). To enrich for tumor content in the analyzed sample, microdissection from tissue sections of tumor is routinely performed prior to sequencing. The *KRAS* direct sequencing mutational analysis has been rigorously validated by our laboratory, and sequencing is performed bidirectionally in each case to confirm the results.

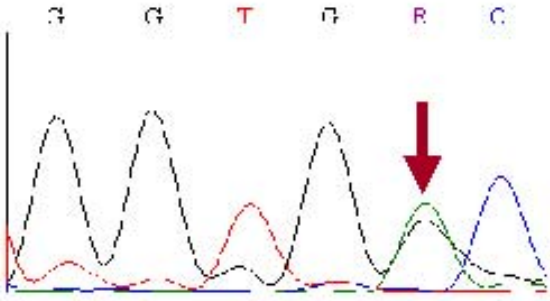


Fig 2. A positive *KRAS* mutation result as depicted in a gene sequencing electropherogram. A green peak superimposed on a black peak (arrow) indicates presence of an abnormal nucleotide (adenine) at this position, indicating *KRAS* gene mutation (GGC->GAC at codon 13 in this tumor). The smaller black peak at this position is the normal guanine nucleotide derived from background normal DNA in the sample.

Test Overview

Test Name	K-RAS Mutation Assay
Individuals Suitable for Testing	Patients with colorectal cancer prior to determining the use of EGFR-targeted therapy.
Limitations	A pathologist's evaluation of the tissue section used for DNA extraction is required to ensure that tumor cells are present in adequate quantity/concentration. The *KRAS* test utilized by our laboratory will detect mutations as long as they constitute at least 25% of the DNA sample mix.
Reference Range	*KRAS* mutation not detected (negative result). *KRAS* mutation detected (positive result).
Specimen Requirements	Formalin fixed tissue containing a sufficient amount of tumor (generally at least several mm of tumor tissue submitted in the tissue block)
Turn-around time	Assay is started every Monday and typically takes 4 days to complete, with results reported on Thursday
CPT Codes	83890, 83892 X 2, 83898, 83904 X 2, 83907, 83909 X 2, and 83912

References

1. Lievre A, Bachet JB, Le Corre D, et al: *KRAS* Mutation Status Is Predictive of Response to Cetuximab Therapy in Colorectal Cancer. Cancer Res 66: 3992-3995, 2006.

2. De Roock W, et al. *KRAS* wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with Cetuximab. Ann Oncol 19:508-515, 2008.

3. Plesec TP, Hunt JL. *KRAS* mutation testing in colorectal cancer. Adv Anat Pathol. 2009 Jul;16(4):196-203.

Contact information

Technical Information Contact:	Scientific Information Contact:	Collection/Submission Contact:
Michael Babcock	Marek Skacel, M.D	Lisa Grant
(207) 941-8228	(207) 941-8200	(207) 561-2415

Our current molecular diagnostics test menu also includes:

- UroVysion bladder cancer FISH assay
- *HER2* gene amplification FISH assay
- Cystic Fibrosis carrier detection

Dahl-Chase Diagnostic Services' state-of-the-art laboratory is located in Bangor, Maine, but serves physicians, hospitals, laboratories, and clinics throughout northern New England, with specialty testing that draws from across the United States. Dahl-Chase Diagnostic Services is fully accredited by the College of American Pathologists (CAP), with CLIA certification. We offer cytopathology, including human papilloma virus (HPV) screening, surgical pathology, hematopathology, molecular diagnostics, and extensive flow cytometry testing. Dahl-Chase is a national leader in flow cytometric testing for paroxysmal nocturnal hemoglobinuria (PNH), a rare but serious blood disorder. Dahl-Chase Diagnostic Services is a subsidiary of **Dahl-Chase Pathology Associates, P.A.**, northern New England's leading provider of pathology services. Our 13 board-certified pathologists cover the breadth of subspecialty pathology.

DAHL-CHASE Focus

October 18, 2010

TECHNICAL BULLETIN: BRAF V600E MUTATION ANALYSIS

Technical Brief
BRAF V600E Mutation Analysis

Background Information

BRAF is a kinase-encoding gene in the RAS/RAF/MAPK pathway. Oncogenic mutations have been identified in codon 600, including the V600E mutation. This activating mutation has been shown to be associated with resistance to EGFR antibody therapies for metastatic colorectal carcinoma.[1] BRAF V600E mutation has only rarely been seen in Lynch syndrome [2] but occurs in up to 68% of microsatellite unstable (MSI-H) colorectal carcinomas with absent MLH1/PMS2 immunostaining but no evidence of MLH1 mutation (sporadic MSI-H tumors).[3] When present, BRAF V600E mutation in MSI-H colorectal carcinoma indicates that the tumor is most likely sporadic and not associated with Lynch syndrome. Therefore, incorporating BRAF mutation testing in Lynch syndrome screening algorithm can reduce the number of patients needing mismatch repair gene sequencing analysis.

BRAF V600E mutation is highly specific for papillary thyroid carcinoma and only rarely occurs in other well-differentiated thyroid neoplasms or colloid nodules.

BRAF V600E mutation is commonly found in malignant melanoma and the role of BRAF mutation testing in this clinical setting is evolving, in concert with the development of BRAF inhibitor therapies.

Clinical Indications

BRAF V600E mutation analysis is useful in determining patient eligibility for targeted EGFR inhibitor therapy for metastatic colorectal carcinoma. While BRAF mutation testing is currently not viewed as mandatory by the National Comprehensive Cancer Network (NCCN), the most recent guidelines (Version I. 2011; March 2010) state: "Patients with a BRAF V600E mutation appear unlikely to benefit from anti-EGFR monoclonal antibodies", and suggest performing BRAF mutation testing in all patients with wild-type KRAS tumors.[4] Our laboratory will typically reflex KRAS wild-type carcinomas to BRAF mutation testing. There is no need to test patients with a KRAS mutation for BRAF mutation because evidence suggests that these two mutations are mutually exclusive.

A separate indication for BRAF V600E mutation testing is in the screening algorithm for Lynch syndrome. Our laboratory will typically reflex carcinomas with MSI-H or absent MLH1 expression to BRAF V600E mutation testing.

Methodology

The assay performs optimally in formalin-fixed tissue to detect mutations in codon 600 of exon 15 of the BRAF gene, when mutated DNA comprises greater than 10% of the sample DNA. To enrich for tumor content in the analyzed sample, microdissection from tissue sections of tumor is routinely performed prior to DNA analysis. The BRAF V600E mutational assay is performed by fragment length polymorphism analysis of primer extension products as validated in our laboratory.

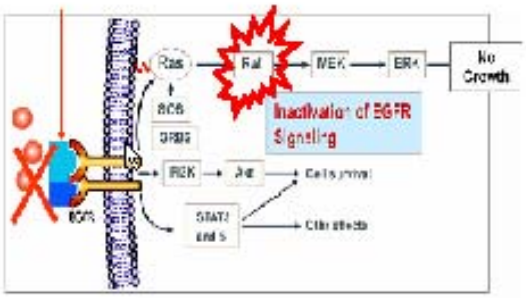


Fig. 1. Schematic diagram of the EGFR pathway illustrating the site of action of the EGFR antibody therapy. Only patients with wild-type (normally functioning) KRAS and BRAF genes will benefit from this therapy. BRAF V600E mutation causes constitutive activation of this pathway and the therapy appears ineffective.

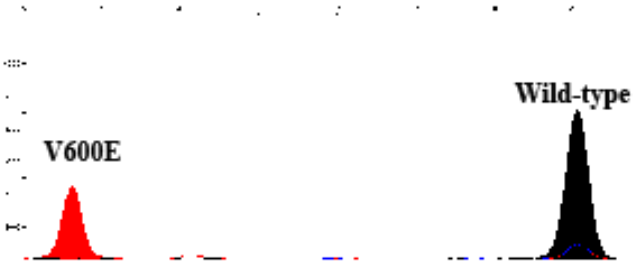


Fig. 2. A positive BRAF V600E mutation result is depicted in a capillary electropherogram. A red peak at 27.5 base pairs (bp) indicates the presence of a BRAF V600E mutation (GTG>GAG at codon 600 of exon 15). The black peak at 41 bp indicates the presence of the wildtype BRAF nucleotide sequence (GTG) in the sample.

Test Overview

Test Name	BRAF V600E Mutation Analysis
Individuals Suitable for Testing	Determining eligibility of patients with colorectal carcinoma for EGFR antibody-based therapy. Part of Lynch syndrome testing algorithm for MSI-H carcinomas.
Limitations	Pathologist's evaluation of the tissue section used for DNA extraction is required to ensure that tumor cells are present in adequate quantity/concentration. The BRAF V600E assay will detect mutations as long as they constitute at least 10% of the DNA sample mix. Only V600E (GTG>GAG), V600A (GTG>GCG), and V600G (GTG>GGG) are detected by this assay. Other codon 600 mutations or mutations in other locations within the BRAF gene will not be detected.
Reference Range	BRAF V600E mutation not detected (negative result) BRAF V600E mutation present (positive result)
Specimen Requirements	Formalin fixed tissue containing sufficient amount of tumor (generally at least several mm of tumor tissue submitted in the tissue block)
Turn-around time	Assay is started on Thursday and takes 2 days to complete, with results reported on Friday
CPT Codes	**88381, 83890, 83891, 83892, 83894, 83898, 83900, 83901 X 3, 83907, and 83912**

References

1. Loupakis F et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinothecan in KRAS codon 12 and 13 wild-type metastatic colon cancer. Br J Cancer 2009;101:715-21.
2. Senter L et al. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. Gastroenterol 2008;135:419-29.
3. Palomaki GE et al. EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. Genet Med 2009;11:42-65.
4. Colon cancer NCCN Guidelines Version I. 2011: http://www.nccn.org/professionals/physician_gls/PDF/colon.pdf

Contact information

Technical Information Contact:
Michael Babcock
(207) 941-8228

Scientific Information Contact:
Marek Skacel, M.D
(207) 941-8200

Collection/Submission Contact:
Lisa Grant
(207) 561-2415

Our current molecular diagnostics test menu also includes:

- UroVysion bladder cancer FISH assay
- *HER2* gene amplification FISH assay
- KRAS codon 12 & 13 Mutational Analysis
- Lynch syndrome screening by MSI testing

Dahl-Chase Diagnostic Services' state-of-the-art laboratory is located in Bangor, Maine, but serves physicians, hospitals, laboratories, and clinics throughout northern New England, with specialty testing that draws from across the United States. Dahl-Chase Diagnostic Services is fully accredited by the College of American Pathologists (CAP), with CLIA certification. We offer cytopathology, including human papilloma virus (HPV) screening, surgical pathology, hematopathology, molecular diagnostics, and extensive flow cytometry testing. Dahl-Chase is a national leader in flow cytometric testing for paroxysmal nocturnal hemoglobinuria (PNH), a rare but serious blood disorder. Dahl-Chase Diagnostic Services is a subsidiary of **Dahl-Chase Pathology Associates, P.A.**, northern New England's leading provider of pathology services. Our 14 board-certified pathologists cover the breadth of subspecialty pathology.

DAHL-CHASE Focus

October 18, 2010

TECHNICAL BULLETIN: MICROSATELLITE INSTABILITY ANALYSIS

Technical Brief

Screening for Lynch Syndrome by Microsatellite Instability

Background Information

Microsatellite instability (MSI) results from defective function of mismatch repair (MMR) proteins (MLH1, MSH2, MHS6, or PMS2). MSI is a key feature of Lynch syndrome, the most common inherited cause of colorectal cancer, due to mutation of one of the MMR genes. Close to 90% of Lynch syndrome-associated colorectal carcinomas display high degree of MSI (MSI-H) and show loss of expression of one (or more) of the MMR genes.[1] Identification of Lynch Syndrome patients is critical, given their high risk for additional carcinomas, as well as the risk of cancer affecting their family members. Screening to detect Lynch syndrome in individuals with newly diagnosed colorectal carcinoma was proposed in 2009 as a strategy to reduce morbidity and mortality in their relatives by the EGAPP (Evaluation of Genomic Applications in Practice and Prevention).[2]

MSI status has also become increasingly important in sporadic colorectal carcinomas, approximately 15% of which also show MSI-H and loss of MMR protein expression (typically MLH1 and PMS2).[3] MSI-H in sporadic carcinomas is most commonly due to methylation of the MLH1 gene promoter. While it does not indicate the presence of Lynch syndrome in these cases, there is evidence that sporadic carcinomas with MSI-H have a better prognosis than those without.[4] In addition, patients with MSI-H carcinomas do not appear to benefit from adjuvant fluorouracil-based chemotherapy.[5,6]

There is high concordance between PCR-based MSI status testing and loss of MMR protein expression as detected by immunohistochemistry (IHC).[7] MSI testing can occasionally detect tumors with defective MMR that show no expression loss by IHC, as some mutations may not affect protein detection by IHC. Conversely, IHC testing can occasionally detect cases that show only low levels (or even an absence) of MSI (e.g. tumors with MSH6 mutations). Based on the EGAPP review, overall sensitivities of MSI PCR and IHC are 89% (in cases with MSH6 defect 77%) and 83%, respectively, with specificity of both techniques being is comparable at about 90%.[2] IHC helps to focus further analysis on the MMR gene likely causing MSI. This can increase cost efficiency of the subsequent testing by eliminating the need of expensive and laborious DNA sequencing of all 4 major MMR genes. Therefore, MSI PCR testing and MMR IHC can be viewed as complementary.

Clinical Indications and testing algorithm

Our laboratory performs PCR to assess the MSI status on most newly diagnosed colorectal carcinomas. MSI-H tumors are reflexed to MMR IHC testing and BRAF V600E mutation. Since this mutation only rarely occurs in Lynch syndrome, its presence helps to identify sporadic (non-germline) MSI-H (see separate technical brief for more information about BRAF mutation testing).

Methodology

The MSI assay performs optimally in formalin-fixed tissue. Testing is performed by PCR analysis on genomic DNA extracted from patient tumor and normal tissue, using PCR amplification of seven loci (five mononucleotide repeats BAT-25, BAT-26, NR-21, NR-24 and MONO-27 and two pentanucleotide repeats (PentaC and PentaD). Fluorescently labeled PCR products are subjected to capillary electrophoresis. Allele sizing data are compared for normal and tumor samples and the presence of MSI is determined by the appearance of new alleles in the tumor sample. The pentanucleotide repeats serve only to verify specimen identity and are not used to determine MSI status.

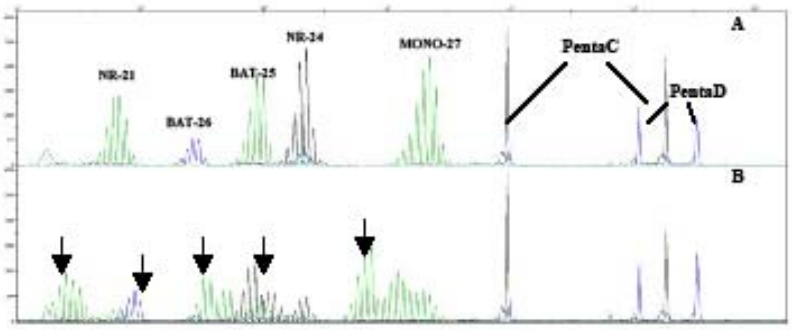


Fig. 1. – Capillary electropherogram showing a downward shift of 5 of the 5 analyzed microsatellite loci (arrows) in the cancer (B) compared to normal DNA (A), indicating high microsatellite instability (MSI-H). The identity markers PentaC and PentaD match between normal and tumor specimens.

Test Overview

Test Name	Microsatellite Instability Analysis
Individuals Suitable for Testing	Patients with newly diagnosed colorectal carcinoma, Lynch syndrome screening
Limitations	Pathologist's evaluation of the tissue section used for DNA extraction is required to ensure that tumor cells are present in adequate quantity/concentration. The MSI assay will detect mononucleotide repeat shifts as long as they constitute at least 10% of the DNA sample mix.
Reference Range	Microsatellite Stable (MSS): none of the 5 microsatellite markers unstable High microsatellite instability (MSI-H): ≥2 of 5 microsatellite markers unstable Low microsatellite instability (MSI-L): 1 of 5 microsatellite markers unstable
Specimen Requirements	Formalin-fixed tissue containing a sufficient amount of tumor (generally at least several mm of tumor tissue in the tissue block). Normal tissue sample for allelic comparison
Turn-around time	Assay is started every Monday and typically takes 3 days to complete, with results reported on Thursday
CPT Codes	**88381 X2, 83891 X2, 83900 X2, 83901 X10, 83907 X2, 83909 X2, and 83912**

References

1. Lynch HT, Lynch PM, Lanspa SJ, Snyder CL, Lynch JF, Boland CR. Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. *Clin Genet*. 2009;76:1-18.
2. Recommendations from the EGAPP Working Group: Genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet Med* 2009;11:35-41.
3. 3. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science*. 1993;260:81 6-819.
4. Jass JR. Pathology of hereditary nonpolyposis colorectal cancer. *Ann N Y Acad Sci*. 2000;910:62-73.
5. Ribic CM, Sargent DJ, Moore MJ et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med*. 2003;349:247-257.
6. Sargent DJ, Marsoni S, Monges G et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol*. 2010;28:3219-3226.
7. Boland CR, Koi M, Chang DK, Carethers JM. The biochemical basis of microsatellite instability and abnormal immunohistochemistry and clinical behavior in Lynch syndrome: from bench to bedside. *Fam Cancer*. 2008;7:41-52.

Contact information

Technical Information Contact:	Scientific Information Contact:	Collection/Submission Contact:
Michael Babcock	Marek Skacel, M.D	Lisa Grant
(207) 941-8228	(207) 941-8200	(207) 561-2415

Our current molecular diagnostics test menu also includes:

- UroVysion bladder cancer FISH assay
- *HER2* gene amplification FISH assay
- KRAS codon 12 & 13 Mutational Analysis
- BRAF V600E Mutation Analysis

Dahl-Chase Diagnostic Services' state-of-the-art laboratory is located in Bangor, Maine, but serves physicians, hospitals, laboratories, and clinics throughout northern New England, with specialty testing that draws from across the United States. Dahl-Chase Diagnostic Services is fully accredited by the College of American Pathologists (CAP), with CLIA certification. We offer cytopathology, including human papilloma virus (HPV) screening, surgical pathology, hematopathology, molecular diagnostics, and extensive flow cytometry testing. Dahl-Chase is a national leader in flow cytometric testing for paroxysmal nocturnal hemoglobinuria (PNH), a rare but serious blood disorder. Dahl-Chase Diagnostic Services is a subsidiary of **Dahl-Chase Pathology Associates, P.A.**, northern New England's leading provider of pathology services. Our 14 board-certified pathologists cover the breadth of subspecialty pathology.

DAHL-CHASE Focus

October 17, 2011

LUNG CARCINOMA MOLECULAR ANALYSIS

EGFR Mutation and ALK Rearrangement analysis for Lung Carcinoma

Dahl-Chase Diagnostic Services is now offering a lung carcinoma molecular analysis that includes: Epidermal Growth Factor Receptor (EGFR) Mutation Analysis and FISH for ALK Rearrangements.

Background Information

Non small-cell lung carcinoma (NSCLC) accounts for approximately 85% of lung cancers. Targeted therapies based on genetic alterations in the tumor are appropriate for selected cases, currently principally in non-squamous NSCLC. Identifying of mutations in oncogenes associated with NSCLC, physicians can distinguish patients who are more likely to benefit from certain therapies, such as tyrosine kinase inhibitors (TKI) erlotinib and gefitinib in case of EGFR mutation or critozinib in case of ALK rearrangement.

EGFR Mutation Analysis

This test is used to detect specific mutations of the EGFR gene (exons 18-19, 20 and 21), including point mutations, deletions and insertions reported to occur in characteristic locations throughout the above exons. This testing allows identifying patients who are most likely to respond to targeted lung cancer therapy, including tyrosine kinase inhibitors erlotinib and gefitinib. These mutations occur in between 10% up to 50% of patients (lower in Caucasians, higher in Asian patients).

ALK Rearrangement

ALK rearrangements can be found in 2-7% of NSCLC, more commonly in adenocarcinomas of never or light smokers, whose tumors lack EGFR and KRAS mutations. Patients with ALK rearrangements, characteristically due to ALK-EML4 fusion, rendering tumors sensitive to ALK inhibitors such as critozinib (while resistant to EGFR tyrosine kinase inhibitor therapies).

Interpretive Information

Activating EGFR mutations predict response or resistance to erlotinib or gefitinib therapy (with the exception of the T790M mutation, which is associated with resistance to this therapy). ALK rearrangement predicts response to critozinib therapy.

Clinical Indications

The American Society of Clinical Oncologists (ASCO) and National Comprehensive Cancer Network (NCCN) guidelines recommend lung cancer molecular analysis for patients with NSCLC who are being considered for EGFR TKI or critozinib therapies.

Methodology

EGFR: DNA was extracted by the MagneSil DNA extraction method. PCR amplification, PCR product clean-up, and primer extension were conducted according to the recommended protocol for the TrimGen Mutector™ II Assay for EGFR mutation detection using STA Core Reagents A and C and the EGFR Primer Sets GP07-01 and GP07-02. Capillary electrophoresis was performed using an ABI 3130 and fragment analysis was performed using the GeneMapper v4.0 software. The EGFR mutational status was determined by either the presence of a point mutation and/or insertion or deletion by an additional peak when compared to a wildtype and mutational controls.

ALK: The Vysis ALK Break Apart FISH Probe Kit (Vysis Inc., Des Plaines, IL) is an FDA approved In Vitro Diagnostic (IVD) assay designed to detect rearrangements involving the ALK gene via fluorescence *in situ* hybridization (FISH) in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissue specimen to aid in identifying patients eligible for treatment with crizotinib. Detection of the ALK probe hybridization to the 2p23 ALK region is performed using a fluorescence microscope. The pattern of orange and green signals are analyzed in a minimum of 50 nuclei and interpreted for the presence or absence of ALK gene rearrangement. A test is considered present if one orange and one green signal are present in the same nucleus and separated by at least two signal diameters in ≥15% of analyzed nuclei. Alternatively, a single orange signal (deletion of green signal) in addition to a fused or broken apart signal may be seen.

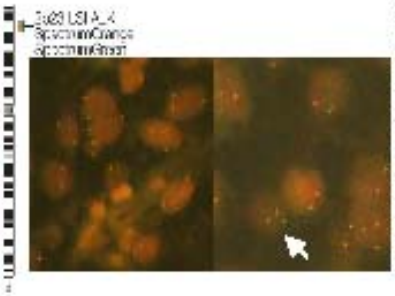


Figure A. An illustrative image of fluorescence in situ hybridization (FISH) demonstrating an ALK gene rearrangement (arrow). Adjacent orange and green signals are indicative of a fused signal (normal).

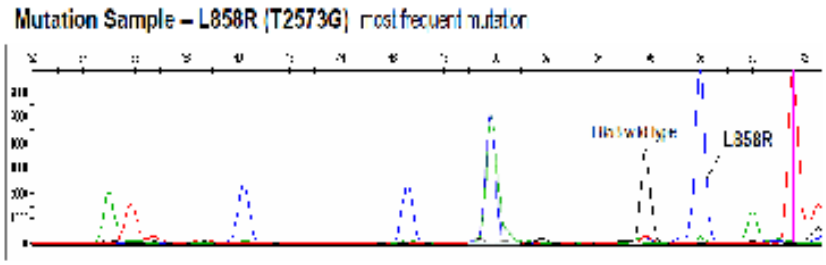


Figure B. A positive EGFR mutation result as depicted in a primer extension electropherogram. The presence of a blue peak adjacent to the wildtype L858 at position 56 base pairs confirms the presence of an L858R (T2573G) mutation.

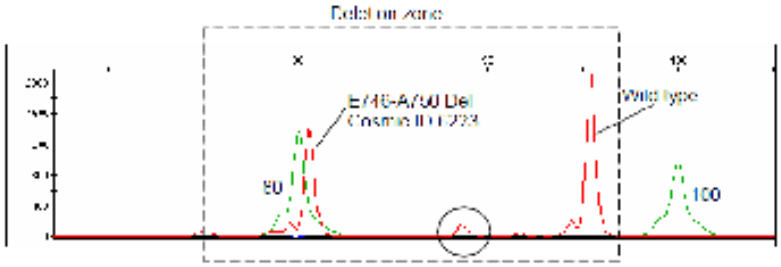


Figure C. A positive EGFR mutation result as depicted in a primer extension electropherogram. The presence of a red peak at 81 base pairs downstream of the exon 19 wildtype peak at 96 base pairs confirms the presence of an EGFR exon 19 deletion mutation.

Test Overview

Test Name	EGFR Mutation Analysis	ALK p23 Rearrangements
Individuals Suitable for Testing	Patients with NSCLC who are being considered for treatment with EGFR TKI	Patients with NSCLC who are being considered for treatment with critozinib
Limitations	Mutations of unknown clinical significance will not be detected by this assay. Mutations in low tumor cell populations may not be detected.	The probe set for this assay is only designed to detect breaks in the ALK gene region of chromosome 2 located at 2p23 and not to detect rearrangement partners, such as EML4.
Reference Range	EGFR mutation present (positive result) EGFR mutation not detected (negative result)	ALK gene rearrangement present (positive result) ALK gene rearrangement not detected (negative result)
Specimen Requirements	Formalin fixed tissue containing a sufficient amount of tumor (generally at least several mm of tumor tissue submitted in the tissue block)	Formalin fixed tissue containing a sufficient amount of tumor (generally at least several mm of tumor tissue submitted in the tissue block)
Turn-around time	7 - 10 Working days	7 - 10 Working days
CPT Codes	83891, 83892X2, 83900X2, 83901X9, 83907, 83909X2, 83912, 83912-TC, 83912-PC, 83917X8, 88380	88368X2

References

1. NCCN Clinical Practice Guidelines in Oncology. Non-small cell lung cancer v.3.2011.

Contact information

Technical Information Contact:
Michael Babcock
(207) 941-8228

Scientific Information Contact:
Marek Skacel, M.D
(207) 941-8200

Collection/Submission Contact:
Lisa Grant
(207) 561-2415

Our current molecular diagnostics test menu also includes:

- UroVysion bladder cancer FISH assay
- *HER2* gene amplification FISH assay
- KRAS Mutation Analysis of Colon Cancer
- BRAF V600E Mutation Analysis
- MSI Screening for Lynch Syndrome

Dahl-Chase Diagnostic Services' state-of-the-art laboratory is located in Bangor, Maine, but serves physicians, hospitals, laboratories, and clinics throughout northern New England, with specialty testing that draws from across the United States. Dahl-Chase Diagnostic Services is fully accredited by the College of American Pathologists (CAP), with CLIA certification. We offer cytopathology, including human papilloma virus (HPV) screening, surgical pathology, hematopathology, molecular diagnostics, and extensive flow cytometry testing. Dahl-Chase is a national leader in flow cytometric testing for paroxysmal nocturnal hemoglobinuria (PNH), a rare but serious blood disorder. Dahl-Chase Diagnostic Services is a subsidiary of **Dahl-Chase Pathology Associates, P.A.**, northern New England's leading provider of pathology services. Our 13 board-certified pathologists cover the breadth of subspecialty pathology.

SECTION EIGHT
DAHL-CHASE DIAGNOSTIC SERVICES
MOLECULAR TESTING
HPV TESTING

Methodology
Dygene Hybrid Capture II

Principle
HPV hybrid testing is conducted to assess if a patient with previous abnormal cytology or surgical pathology testing has the human papilloma virus. The Digene HPV test is a nucleic acid hybridization microplate assay with signal amplification that uses chemiluminescent detection. This is used as additional information by the clinician in deciding clinical follow up.

Specimen Requirements
HPV testing may be done off the original SurePath vial as long as the test is ordered within 2 weeks of the collection date. If a SurePath pap is not needed and only an HPV test is needed, the Digene cervical sampler vial should be used. These are available through Dahl-Chase purchasing department. Once collected, the cervical sampler vials may be stored at room temperature for 2 weeks, refrigerated for 3 weeks, or frozen for up to 3 months. Cervical biopsy specimens less than 5 mm are to be placed in Digene specimen transport media and frozen at -20 degrees immediately.

Ordering HPV Testing
For Recommended guidelines for ordering HPV, please visit the ASCCP website at www.asccp.org

Reflexive testing based on age recommendations:
HPV testing may be ordered up front on the pap requisition by checking the box for the situation in which testing should be performed. For example, if the provider would like HPV testing done only if the patient's pap result is ASCUS, then simply check the box for SurePath pap with HPV DNA test for ASCUS. If the provider would like HPV testing done on a patient age 30 or older for a negative or ASCUS pap result, check the box for SurePath pap with HPV DNA test for negative or ASCUS pap result.

Adding on HPV testing:
If the provider wishes to add on HPV testing after the pap result has been finalized, the provider may do this by faxing an order to add HPV testing to the SurePath vial. The provider must provide the patient name, date of the SurePath pap, and, if available, the "G" number on the report. See the fax form following this procedure. We will fax this form back to you as a confirmation of receipt of the order.

Ordering an "HPV only":
If the provider wants only HPV testing, the provider will need to specify on the requisition that this test is for "HPV ONLY". This way, the patient will not be charged for a SurePath pap in addition to the HPV. This should be collected in a Digene cervical sampler tube and ordered on the requisitions as such.

DAHL-CHASE
DIAGNOSTIC SERVICES

Last revised November 1, 2012

Request for HPV Testing on SurePath PAP Specimen

Fax to 941-8287

****HPV testing must be ordered within 14 days of date of collection****

- ❑ **Reflex HPV DNA Test per ASCCP consensus guidelines for HPV Testing**
  - ➢ Age 21-29 with ASCUS pap result
  - ➢ Age 30 and older with ASCUS or negative Pap result

  **References:* *Am J Obstetrics and Gynecology 2007;197(4); 346-355*
  Am J Clin Pathol 2009;131:708-709, Am J Clin Pathol 2009;131:770-773
  http://www.asccp.org/consensus.shtml

- ❑ **HPV DNA test outside of ASCCP consensus guidelines for HPV Testing.** Please note that some insurance companies may not cover HPV testing; therefore please submit Advanced Beneficiary Notice (ABN) form along with this request.
  - ➢ Please complete the following:
    - Age _____
    - Pap diagnosis _____
    - Have the ordering provider sign this order_____ (Signature)
    - Patient needs to sign ABN form www.dahlchase.com/ABN form

- ❑ **HPV DNA Genotyping for types 16/18**
  - ❑ Known HPV positive specimen
  - ❑ Reflex if add-on HPV is positive

G# on PAP Report	Patient Name	Date of Collection

Clinician Requesting HPV: _____

Person sending fax HPV request: _____

Your fax number: _____

A Dahl-Chase Diagnostics customer representative will fax a confirmation that your HPV request has been received and processed. Call us at 941-8282 if you do not receive confirmation within 1 day.

You will receive your HPV report within 2-3 days, however the genotyping report may take up to 7 days

DCDS Internal use only – Order confirmation

DCDS customer representative processing HPV "Fax-Back Order:

_____ Name _____ Date

X:\HPV Information\HPV Fax Back Sheet, 11-12.doc

SECTION EIGHT

DAHL-CHASE DIAGNOSTIC SERVICES

MOLECULAR TESTING

ANAL HPV SCREENING PROTOCOL

Principle

The “Anal Pap” is a screening tool used in at-risk populations to identify individuals who have premalignant cytologic changes in their anal epithelium.

Procedure

1. Moisten the Dacron swab with water, not lubricant.
2. Insert Dacron swab* approximately 1.5 to 2 inches into the anal canal.* It is important to use Dacron and not a cotton swab, as cells tend to cling to cotton and do not release easily into cytology collection fluid.
3. Once inserted deep enough into the anus (necessary in order to collect both rectal columnar and anal squamous cells), the swab should be pulled out, applying some pressure to the wall of the anus, rotating the swab in a spiral motion along the way.
4. The collection device should be thoroughly rinsed and swirled in the SurePath vial. After rinsing, the collection device may be discarded.
5. Place the cap on the vial and tighten.
6. Place the SurePath vial in a plastic biohazard bag and use zip lock to seal. Insert completed Non-Gyn Cytology Request Form in the outside pocket.

Human Papilloma Virus (HPV) Testing may be ordered, in addition to routine cytology screening. However, HPV Testing on anal samples has not been validated or FDA approved. Therefore, the significance of these results is uncertain. A negative result does not exclude the possibility of an infection. If you wish to have the sample tested for HPV, please indicate on the requisition.

Materials and Reagents

SurePath Vial
Dacron Swab - Moistened with Water
Non-Gyn Cytology Request Form
Biohazard Bag

Reference: ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108

SECTION EIGHT

DAHL-CHASE DIAGNOSTIC SERVICES

MOLECULAR TESTING

NEISSERIA GONORRHOEAE (GC) AND CHLAMYDIA TRACHOMATIS (CT) TESTING

Methodology
Digene Hybrid Capture II

Principle
The Digene Hybrid Capture test for Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC) test is a nucleic acid hybridization microplate assay with signal amplification that uses chemiluminescent detection. These tests are done together to confirm either Chlamydia Trachomatis or Neisseria Gonorrhoeae infections.

Specimen Requirements
GC/Chlamydia testing must be done on a Digene cervical sampler vial. These are available through Dahl-Chase purchasing department. Once collected, the cervical sampler specimen may be stored at room temperature for 2 weeks, refrigerated for 3 weeks, or frozen for up to 3 months.

Ordering GC/Chlamydia Testing
GC/Chlamydia testing is ordered on a Gyn Cytology form available through Dahl-Chase Diagnostic Services.