American
Clinical Laboratory
Association

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Dear Dr. Duvall, Dr. Hambrick, and Ms. Smith,

Thank you very much for taking the time to meet with representatives of the American Clinical Laboratory Association ("ACLA") on March 5, 2014 to discuss recent National Correct Coding Initiative policy changes regarding fluorescent in-situ hybridization ("FISH") codes. We feel that it was a very productive discussion, and we appreciate your interest in this topic. Per your request, this letter summarizes our discussion and our answers to the questions you asked, and it includes a copy of the Power Point presentation we shared with you. We hope that our discussion, along with this letter, demonstrates why the recently-implemented coding policy does not reflect the way that FISH tests are performed and that it will result in wholly inadequate compensation for laboratories performing the tests. We urge you to rescind the policy.

In this letter, we explain the purpose of a FISH test and how they are performed, along with some examples of FISH tests used for specific diseases. We then review the CPT codes for FISH tests, the recent NCCI policy change, and why the policy change is not appropriate for how FISH tests are used and performed.

I. Background on FISH tests

A. Purpose of FISH tests

FISH tests are diagnostic tests used to detect, identify, and localize certain chromosomal abnormalities, and they are used for hematological or solid tumor cancers. With fluorescent tagging, a pathologist who specializes in genetics can visualize specific genes or portions of genes. Identification, interpretation, and analysis of genetic abnormalities can help determine eligibility for a course of treatment. For example, FISH testing can help a pathologist or geneticist determine whether a woman with invasive breast cancer may be a good candidate for a targeted therapy like

Herceptin (which is expensive), or whether Herceptin would be ineffective in treating her breast cancer. FISH testing also can help a pathologist or geneticist to stratify risks and determine a patient's likely prognosis, such as when certain chromosomal abnormalities are present in a patient with multiple myeloma and other abnormalities are not. Another use for FISH testing is to see whether a current course of therapy is having its intended effect, by looking for a particular chromosomal abnormality that may or may not still be present after therapy. Currently, there are very few FDA-approved FISH in vitro diagnostic kits on the market; almost all FISH tests are laboratory-developed tests ("LDTs").

B. Process of performing FISH tests

Using FISH, a pathologist or geneticist can identify where a particular gene is located on an individual patient's chromosome. The technique can reveal whether there has been translocation (part of one chromosome has broken off and relocated to another chromosome), deletion (part of a chromosome is missing), duplication (an extra copy or part of a chromosome), amplification (a cell contains many copies of a gene or part of a chromosome), or inversion (part of a chromosome is in reverse order). Translocations, duplications, deletions, amplifications, and inversions of particular chromosomes are associated with particular disease states.

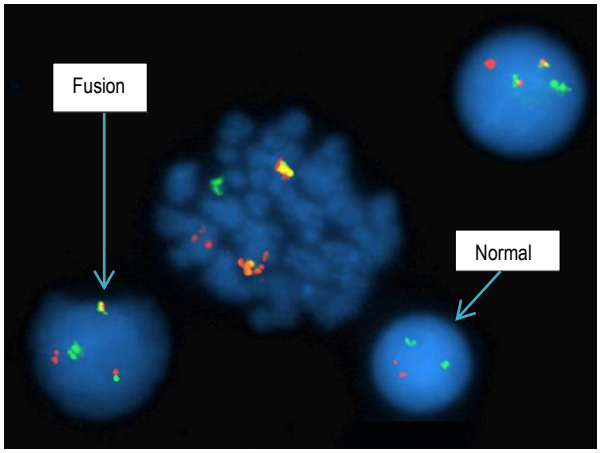
FISH testing is based on the principle that a specific DNA sequence will hybridize – or bind – to its complementary DNA sequence. The first step in FISH testing is preparation of a short sequence of single-stranded DNA that matches a portion of the target gene or genes. These short sequences are called "probes." The probes are labeled using one of a few different colored fluorphores (a fluorescent chemical compound). Typically there are only four colors used in FISH testing: red, green, yellow, and aqua. These four colors have different wavelengths on the color spectrum, which ensures that each color is distinguishable from others and that their wavelengths do not overlap with others. Since the probes are single-stranded, they can bind to the complementary strand of DNA on a patient's chromosome, and the fluorescent probe provides a way to determine the location of the target DNA sequence.

Cells are fixed onto the surface of a slide, and then the slide is treated so that the chromosomal DNA is denatured into single strands. The fluorescently labeled DNA probes are applied to the slide and are allowed to hybridize with the denatured chromosomal DNA. After hybridization, any excess probe is washed away. The slide is then examined using a microscope outfitted with filters that detect the colors emitted by the fluorescent probes. The examiner looks to see if the normal number of probe signals is present. This is usually two red signals and two green signals, but it could but two red signals, two green signals, and two aqua signals, depending on what probe set is used. If several cells have a probe signal missing, if there is an extra probe signal, or if two signals are fused together, the specimen is abnormal. Although the cell nuclei appear flat on the slide, they actually are three-dimensional. Thus, the examiner must view different "slices" of each nucleus in order to ensure that none of the probes are hidden.

Example 1 – BCR/ABL: In the picture below, a pathologist or geneticist is looking for the fusion of the BCR gene from the 22nd chromosome with the ABL gene from the 9th chromosome to diagnose the BCR/ABL gene fusions that is diagnostic of chronic myelogenous leukemia ("CML") (it is also seen in some cases of acute lymphoblastic leukemia). The cell in the lower

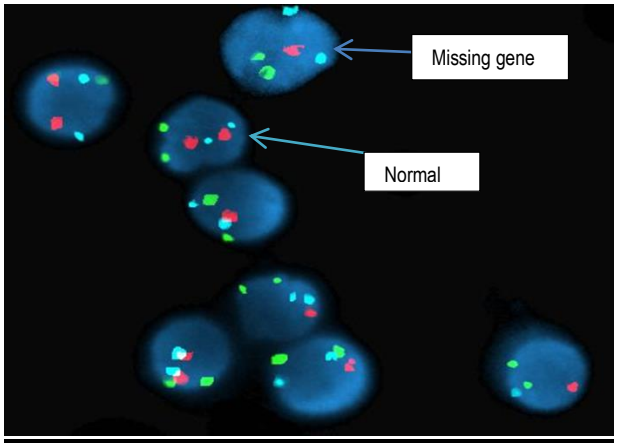
right-hand corner of the picture is normal: it has two separate copies of the BCR gene (green) and two separate copies of the ABL gene (red). The other cells in the picture have one copy of the BCR gene, one copy of the ABL gene, and two fusions of BCR and ABL (yellow). This is one example of the necessity of having more than one probe for a FISH test. One color probe could detect copies of BCR or copies of ABL but not fusions of the two.

BCR/ABL: Dual color, dual fusion



Example 2 – CLL: The next example shows a FISH test for chronic lymphocytic leukemia ("CLL"). Instead of looking for fusion, this test looks for deletions. Deletions of different genes are associated with different prognoses for the progression of CLL. In our example below, the reviewer uses three probes – one for a gene on chromosome 12 (aqua), and two for separate genes on chromosome 13 (red and green). A normal cell has two copies of each gene, showing as two copies of red, two copies of green, and two copies of aqua. The picture below shows normal cells and five cells with one "red" gene having been deleted. Again, without multiple colored probes, it would not be possible to determine which genes have been deleted or to determine a prognosis.

Chronic Lymphocytic Leukemia



These examples show FISH tests that use two and three probes. A FISH test also may use four separate colored probes to detect chromosomal abnormalities associated with four separate genes. Other times, it is necessary to look for abnormalities associated with more than four chromosomes, such as with testing for multiple myeloma, but the color spectrum limits the number of fluorescent probes to essentially four colors. In that case, a pathologist or geneticist may use a number of different "probe sets" or pairs on different slides, tagging one probe with red and another with green on one slide, and tagging another gene with red and another gene with green on a separate slide, and so on.

Occasionally, it is necessary to use only one probe on a slide. A pathologist or geneticist may use only one probe to determine whether an abnormality that previously was detected with a FISH test still is present. For example, in a patient with myelodysplastic syndrome ("MDS"), trisomy 8 would have been detected with the MDS FISH panel on the diagnostic bone marrow specimen. The MDS FISH panel, which looks for certain deletions, uses a total of six probes in three different hybridizations (three different slides from one specimen). When the patient has a follow-up bone marrow study after therapy, only the probe for trisomy 8 needs to be run to look for residual disease.

Some portions of FISH tests can be automated, but these steps always require human review and confirmation. For example, it is possible in some cases for an automated instrument to "count" gene signals in a specimen, but a pathologist still must review the work and perform the analysis and interpretation.

While both methodologies can be used to detect and/or diagnose cancers, FISH tests differ from assays utilizing stains, such as immunohistochemistry ("IHC"), in important ways. The term "stain" should not be used with FISH testing. IHC stains detect the presence of critical marker proteins in tissue samples, while FISH tests detect target DNA sequences. IHC stains use primary antibodies that bind to target proteins and detection systems that link to the primary antibody to provide a visual indication of the presence and location of a particular protein using bright field microscopy. As we have described above, FISH tests uses probes that bind to a target DNA sequence. For IHC stains and FISH tests, different detection systems are used to visualize the presence of the target sequence: FISH uses fluorophores and fluorescent microscopy, while ISH tests use chromogen stains and bright field microscopy. Also, certain IHC stains may be produced in combination as "cocktails" or "multi-stains", but generally, the fluorophores used in creating FISH probes are not created or marketed in such combinations.

II. Recent NCCI policy

Following are the CPT codes and descriptors for FISH tests:

> 88365 – In situ hybridization (*e.g.*, FISH), each probe (do not report 88365 in conjunction with 88367, 88368 for the same probe).
>
> 88367 – Morphometric analysis, in situ hybridization (quantitative or semi-quantitative) each probe; using computer-assisted technology.

> 88368 – Morphometric analysis, in situ hybridization (quantitative or semi-quantitative) each probe; manual.

Effective January 1, 2014, the following appears in the NCCI Policy Manual, Ch. X, Laboratory Services:

> The unit of service for in situ hybridization reported as CPT codes 88365, 88367, or 88368 is each probe staining procedure per specimen. If a single probe staining procedure for one or more probes is performed on multiple blocks from a surgical specimen, multiple slides from a cytological specimen, or multiple slides from a hematological specimen, only one unit of service may be performed for each separate specimen. Physicians should not report more than one unit of service for CPT codes 88365, 88367, or 88368 per specimen for a probe staining procedure even if it contains multiple separately interpretable probes.

III. NCCI policy is premised on misunderstanding of FISH tests and contradicts CPT code descriptions

As we discussed above, for each of the chromosomal abnormalities a pathologist is reviewing, short strands of DNA – probes – are prepared with a fluorophore of one color or another. One or more slides are prepared with a specimen and the relevant probes that are to bind to the targets. A pathologist views each probe separately through a special microscope filter that can detect the probe's fluorescent color and repeats this step for each probe color. The pathologist then views all probe colors together and analyzes and interprets the resulting patterns.

In light of the process used for FISH tests, it is clear that the NCCI policy does not reflect the procedure. The NCCI policy refers to a unit of service as a "probe staining procedure per specimen." As we discussed above, FISH tests differ fundamentally from staining procedures such as IHC tests, and in the context of FISH tests, the word "staining" is misleading and technically incorrect. The policy also refers to a "single probe staining procedure for one or more probes;" probes are not stained, and they always are tagged separately, not as part of the same procedure.

The policy refers to "one unit of service" being performed, regardless of the number of slides prepared from a specimen. Because of the limited number of colors of fluorophore available to label FISH probes, it sometimes is necessary to prepare several slides from the same specimen in order to look for multiple genetic abnormalities that may help diagnose and risk-stratify hematological malignancies and solid tumors. Our earlier example of FISH testing for multiple myeloma is such a case, and many laboratories offer FISH testing for this disease as a panel for just this reason. Such specimens may require several separate probes to complete the procedure properly. In any event, there are very few FISH tests performed where only one probe is used, so it does not make sense to issue a policy whereby "physicians should not report more than one unit of service...per specimen for a probe staining procedure, even if it contains multiple separately interpretable probes." Virtually all FISH tests contain multiple separately interpretable probes, by the very nature of the test.

The CPT code descriptors themselves weigh heavily against the NCCI policy. All of the codes' descriptors refer to "each probe." It is not possible to use any of the CPT codes properly and to comply with the NCCI policy at the same time.

IV. Reimbursement under the NCCI policy would be inadequate to cover the resources involved in performing a FISH test

Reimbursing a laboratory for one procedure, regardless of how many probes must be used for a test, results in inadequate compensation compared to the costs and fails to account for the resources associated with preparing and analyzing each probe in a properly-conducted FISH test. As is reflected in the CPT code descriptors for FISH tests, there is a cost associated with preparation of each individual probe and with the preparation of each slide, both in terms of the technologist's time and the required materials. Purchasing two probes, even if they come together in one vial, typically costs double what it costs to purchase a single probe. A pathologist or geneticist must analyze each fluorescent probe using single pass filters to determine the number of gene "signals" in each cell; since each probe must be viewed individually, there are no efficiencies to be gained by analyzing all probes at once when the procedure is performed properly. A pathologist or geneticist then must view all of the probes in concert through a filter that can detect all of the relevant colors in order to look for chromosomal abnormalities, interpret the findings, and issue a report.

V. Conclusion

In sum, we request that CMS rescind the NCCI policy. When conducting FISH tests, two or more probes must be used for virtually all patient specimens. Multiple probes are necessary to see almost all chromosomal translocations, duplications, deletions, amplifications, and inversions. Additionally, for some disease tests, multiple probes must be performed on a series of slides due to limitations in the number of fluorophores available. Further there are very few efficiencies, if any, gained when using multiple probes on a single specimen, since each probe itself has an added cost, and each must be analyzed and interpreted. We understand CMS's concerns about appropriate utilization of molecular diagnostic tests, and we would not oppose implementation of a reasonable edit that identifies claims submissions for FISH tests that clearly are wrong. However, the NCCI policy is too blunt an instrument for this kind of test, especially because multiple probes are essential to practically all FISH testing.

We appreciate your attention to this matter, and we remain available to you to discuss any further questions you have. We hope you will give strong consideration to removing the NCCI policy on FISH probes from the policy manual. Thank you for your time.

Sincerely,

JoAnne Glisson, Senior Vice President
American Clinical Laboratory Association