Clinical Policy Title: Fluorescence in situ hybridization (FISH) for cervical cancer screening

Clinical Policy Number: 01.01.02

Effective Date: April 1, 2015
Initial Review Date: January 21, 2015
Most Recent Review Date: March 16, 2016
Next Review Date: March 2017

Related policies: None

ABOUT THIS POLICY: Arbor Health Plan has developed clinical policies to assist with making coverage determinations. Arbor Health Plan’s clinical policies are based on guidelines from established industry sources, such as the Centers for Medicare & Medicaid Services (CMS), state regulatory agencies, the American Medical Association (AMA), medical specialty professional societies, and peer-reviewed professional literature. These clinical policies, along with other sources, such as plan benefits and state and federal laws and regulatory requirements, including any state- or plan-specific definition of “medically necessary,” and the specific facts of the particular situation are considered by Arbor Health Plan when making coverage determinations. In the event of conflict between this clinical policy and plan benefits and/or state or federal laws and/or regulatory requirements, the plan benefits and/or state and federal laws and/or regulatory requirements shall control. Arbor Health Plan’s clinical policies are for informational purposes only and not intended as medical advice or to direct treatment. Physicians and other health care providers are solely responsible for the treatment decisions for their patients. Arbor Health Plan’s clinical policies are reflective of evidence-based medicine at the time of review. As medical science evolves, Arbor Health Plan will update its clinical policies as necessary. Arbor Health Plan’s clinical policies are not guarantees of payment.

Coverage policy

Arbor Health Plan considers the use of fluorescence in situ hybridization (FISH) for cervical cancer screening to be clinically unproven and, therefore, not medically necessary.

Limitations:

- All other uses of FISH for cervical cancer screening are considered clinically unproven and, therefore, not medically necessary.

Note: The following CPT/HCPCS codes are not listed in the Nebraska Medicaid fee schedule:

88364 - Each additional probe stain procedure

88366 - Each multiplex probe stain procedure
**Alternative covered services:**

- Pap smear.
- Cervical tissue biopsy.

**Background**

Cancer of the uterine cervix is often preventable and effectively treatable in its early stages. Most cases are related to persistent human papilloma virus (HPV) infection, for which the natural history makes the disease amenable to screening programs, such as the Papanicolaou (Pap) test or high-risk HPV genotypes, both of which detect large proportions of women with high-grade cervical intraepithelial neoplasia (CIN), the precursor to cervical cancer.

In high-resource settings such as North America, screening strategies to identify precursor lesions have reduced the incidence and mortality of cervical cancer by greater than 50 percent. However, screening technologies are relatively inefficient and require longitudinal testing over a lifetime.

Significant numbers of women with positive results on these tests will experience spontaneous resolution or lack of confirmation on histology. Particularly problematic are cytologic diagnoses of atypical squamous cells of unknown significance (ASCUS) or low-grade squamous intraepithelial lesions (LSIL), for either of which subsequent colposcopy and histology will fail to confirm a need for treatment in 20 percent of women. Colposcopy is associated with individual and system costs, as well as adverse events or complications, encouraging alternate approaches to identify and triage those women for whom colposcopy is warranted.

A screening is a specialized use of diagnostic tests, discussed at length in epidemiology texts, most of them citing a standard definition. Gray (1997) provides a particularly cogent statement:

“In a screening program, a test, or a series of tests, is performed on a population that has neither the signs nor symptoms of the disease being sought, but whose members have some characteristic that identifies them as being at risk from that disease, the outcome of which can be improved by early detection and treatment… “

“Thus, the effectiveness of any screening program is determined by:

- The sensitivity of the series of tests applied to the population.
- The effectiveness of the therapy offered to those individuals discovered to have the condition.”

“... Screening programs, like any other intervention, have the potential to do both good and harm. However, the balance between good and harm will change with the frequency of testing and the quality of the program. The ratio of benefits to harms changes as the number of individuals screened increases. If the quality of the screening program is low, the benefits are reduced and adverse effects increase; furthermore, if an adequate level of quality is not achieved, there may be a point at which the harm done by screening is greater than the good. Thus, the decision to introduce screening must be taken with the greatest of care.”
Mausner (1985) elaborates the same theme:

“...it is perhaps superfluous to note that since screening is designed to be applied to large groups of people, screening tests should be innocuous, rapid, and inexpensive; they should also be able to be carried out largely by technicians.”

Fletcher (1988) further refines the discussion:

“The very nature of screening for disease in asymptomatic people means the prevalence of disease is usually very low, even among high-risk groups selected for age, sex, and other characteristics. A good screening test must therefore have high sensitivity, in order not to miss the few cases of disease that are present, and a high specificity, to reduce the number of people with false positive results who require further workup.”

“Because of the low prevalence of most diseases, the positive predictive value of screening tests is likely to be low, regardless of how specific a given test is. Clinicians who want to practice preventive health care by performing periodic health examinations on their patients, therefore, must accept the fact that they will have to work up many patients who will not have disease. However, they can minimize the problem by concentrating their screening efforts on people with a higher prevalence for disease.”

Sackett (1991) discusses the interactions of early diagnosis with the natural history of disease:

“It is this orderly progression from biologic onset, to the point where early diagnosis is possible, to the time of usual diagnosis, and ultimately to its outcome that renders a disease vulnerable to assault through screening, case finding, and the periodic health examination. This orderly progression is not enough, however, because another assumption underlies attempts at early diagnosis. This element was described by Hutchinson in 1960 and consists of a ‘critical point’ in the natural history of disease, before which therapy is either more effective or easier to apply than afterward. Now, a disease may have several critical points (arguably pulmonary tuberculosis) or may have none (arguably several cancers), and the location of these critical points along its natural history is critical to the value of early diagnosis....”

“It is only when a disease possesses a critical point between the time when early diagnosis becomes possible and the time of usual clinical diagnosis that screening and case finding hold any promise of improving the outcomes of those with the target disorder.”

Finally, Sackett (1991) details “admissible evidence” for screening strategies:

“How do we tell whether a disease has a critical point at position 2 (defined above) and its detection is worth our critical effort? Unfortunately, the only way to tell for sure is to track down a properly executed a randomized controlled trial (RCT) in which individuals were randomly allocated to receive or not receive the screening or case-finding maneuver. The best standard therapy would have been provided to the experimental patients detected early and to any other patients (experimental or control) detected at the usual time of diagnosis. All patients would then have been followed up to see whether they succumbed to the target disease.”
Searches

Arbor Health Plan searched PubMed and the databases of:

- UK National Health Services Centre for Reviews and Dissemination.
- Agency for Healthcare Research and Quality’s National Guideline Clearinghouse and other evidence-based practice centers.
- The Centers for Medicare & Medicaid Services (CMS).

We conducted searches on March 1, 2016. Search terms were “fluorescence in situ hybridization” and “cervical cancer screening.”

We included:

- **Systematic reviews**, which pool results from multiple studies to achieve larger sample sizes and greater precision of effect estimation than in smaller primary studies. Systematic reviews use predetermined transparent methods to minimize bias, effectively treating the review as a scientific endeavor, and are thus rated highest in evidence-grading hierarchies.
- **Guidelines based on systematic reviews.**
- **Economic analyses**, such as cost-effectiveness, and benefit or utility studies (but not simple cost studies), reporting both costs and outcomes — sometimes referred to as efficiency studies — which also rank near the top of evidence hierarchies.

Findings

Currently, there is insufficient evidence regarding the clinical value of the onco-FISH cervical test. Furthermore, there are no guidelines from leading medical professional organizations or public health agencies that recommend FISH measurement of 3q26 in cervical cancer screening.

Rodolakis (2012) examined if 3q26 gain can predict which LSILs and ASCUSs will progress to high-grade squamous intraepithelial lesions (HSIL). Liquid cytology specimens of LSIL and ASCUS from 73 women were examined using FISH for the detection of 3q26 gain. All women underwent colposcopy and biopsy at the initial visit and 40 of them with histology showing CIN 1 or HPV infection (koilocytosis) were included in the study. They were re-evaluated with liquid cytology, colposcopy, and biopsy after a median follow-up of 17.5 months. A total of 40 cases were analyzed (31 LSIL and 9 ASCUS). Of these cases, 8 (20 %; 6 LSIL and 2 ASCUS) were positive and 32 (80 %) were negative for 3q26 gain according to FISH. Three of the 8 positive women (38 %) progressed to HSIL/CIN or worse, whereas none of the 32 negative women did so. 3q26 gain could predict progression with a negative-predictive value of 100 % (95 % confidence interval: 89.1 % to 100 %). In addition, women positive for 3q26 gain had a significantly lower regression rate compared with negative women (p = 0.009). The authors concluded that in this first prospective study, 3q26 gain in LSIL/ASCUS cytology exhibited an impressive negative predictive value for progression to HSIL/CIN or worse. The authors stated that 3q26 gain may be useful in stratifying patients’ risk for progression and possibly alter management and reduce cost of follow-up.

Verri (2011) stated that studies have demonstrated a correlation between the gain in 3q26 copy number and the severity and stage of cervical disease progression. A recent study has examined the potential of using a measure of 3q26 gain as a predictor of regression, persistence, or progression of LSIL of the cervix. The case described in this report documented a marked progression in both the severity and
extent of this patient's lesion from atypical squamous cells on cytology to biopsy established high-grade CIN over the span of 1 year. The initial gain of at least 5 copies of 3q26 in only 3 nuclei in this patient's first cervical smear may be an indication of the significance and sensitivity of this degree of gain, even in a small number of cells at a low level of disease, and may suggest the potential of predicting the progression of the lesion. The subsequent gain of at least 5 copies of 3q26 1 year later in the very large number of 264 nuclei may reflect both the severity and extent of disease progression. Nevertheless, since it is not possible to exclude the possibility that a high-grade CIN already existed at the time of the initial cytology, the presence of the 3q26 gain, even in a small number of cells, may also serve as an indicator of the possible presence of a high-grade lesion in those cervical specimens in which a definitive cytological diagnosis is not or cannot be made. The authors concluded that this case report supported the findings of other investigators on the potential utility of using 3q26 gain in predicting, at an early stage, the progression or non-progression of low-grade pre-neoplastic lesions of the cervix.

Policht (2010) evaluated 35 genomic regions associated with cervical disease and selected those which were found to have the highest frequency of aberration for use as probes in FISH. The frequency of gains and losses using FISH were assessed in these 35 regions on 30 paraffin-embedded cervical biopsy specimens. Based on this assessment, 6 candidate fluorescently labeled probes (8q24, Xp22, 20q13, 3p14, 3q26, CEP15) were selected for additional testing on a set of 106 cervical biopsy specimens diagnosed as normal, CIN, and SCC. The data were analyzed on the basis of signal mean, % change of signal mean between histological categories, and % positivity. The study revealed that the chromosomal regions with the highest frequency of copy number gains and highest combined sensitivity and specificity in high-grade cervical disease were 8q24 and 3q26. The cytological application of these 2 probes was then evaluated on 118 samples diagnosed as normal, ASCUS, LSIL, HSIL and cancer to determine utility as a tool for less invasive screening. Using gains of either 8q24 or 3q26 as a positivity criterion yielded specificity (normal + LSIL + ASCUS) of 81.0 % and sensitivity (HSIL + cancer) of 92.3 % based on a threshold of 4 positive cells. The authors concluded that the application of a FISH assay comprised of chromosomal probes 8q24 and 3q26 to cervical cytology specimens confirmed the positive correlation between increasing dysplasia and copy gains and showed promise as a marker in cervical disease progression.

Seppo (2009) evaluated an automated FISH assay for detection of 3q gain in liquid cytology samples as a potential tool for risk stratification and triaging. Slides prepared from 257 liquid cytology specimens (97 negative, 135 LSIL and 25 HSIL) were hybridized with a single-copy probe for the chromosome 3q26 region and a probe for the centromeric alpha-repeat sequence of chromosome 7, using standard FISH methods. Using automated analysis, the total number of nuclei and the number of nuclei with greater than 2 signals for 3q26 were determined, using a 20x objective. The nuclei were rank ordered based on number of 3q26 FISH signals. The 800 nuclei with the highest number of signals were scored using both FISH probes and nuclei with increased numbers of 3q signals were enumerated. Analysis of 257 specimens demonstrated that a fully automated FISH scoring system can detect 3q gain in liquid cytology samples. The authors concluded that a fully automated method for determination of 3q gain in liquid cytology may be the assay necessary to implement routine testing; and they stated that additional studies to validate the utility of this technology are needed.

Caraway (2008) noted that chromosomal aberrations have been documented in cervical carcinomas, especially chromosome 3q. The human telomerase ribonucleic acid (RNA) gene (hTERC) is located in the chromosome 3q26 region, and its product, telomerase, is involved in the maintenance of chromosome length and stability. Up-regulation of telomerase is in general associated with tumorigenesis. In this study, cervico-vaginal specimens were analyzed by FISH for gain of chromosome 3q26 containing hTERC,
and FISH findings were compared with the cytologic and histologic diagnoses. Slides prepared from 66 liquid-based preparations from cervical specimens with cytologic diagnoses of negative for SIL or malignancy (NILM, n = 4), atypical squamous cells of undetermined significance (ASCUS, n = 15), LSIL (n = 20), HSIL (n = 24), or cervical squamous cell carcinoma (SCCA, n = 3) were analyzed for aberrations of 3q26 using a commercially available 2-color FISH probe. The results of the cytologic analysis and those of concurrent or subsequent biopsies, when available, were compared with the FISH-detected 3q26 abnormalities. The Wilcoxon rank-sum test was used to assess associations between 3q26 gains and diagnoses. Gain of 3q26 was significantly associated with the cytologic diagnosis (p < 0.0001). Patients with HSIL or SCCA cytology diagnoses had significantly higher percentages of cells with 3q26 gain than did patients with NILM or ASCUS cytologic diagnoses. The authors concluded that FISH can be performed on cervico-vaginal liquid-based preparations to detect gain of 3q26; gain of 3q26 is associated with HSIL and SCCA. They stated that this test may be an adjunct to cytology screening, especially high-risk patients. This study did not have enough correlative data to be useful.

Policy updates:


Earley (2014) found a total of 11 studies which examined FISH tests for telomerase RNA component gene (TERC), myelocytomatosis oncogene (MYC), or HPV type 16 or 18 in samples exhibiting ASCUS or LSIL. None examined HPV-positive, cytologically normal samples. These investigators extracted data on the sensitivity and specificity for high-grade CIN 2. Only 1 study testing for TERC specified HPV status. In meta-analysis, FISH for TERC in LSIL (9 studies, 1,082 cases) had a summary sensitivity of 0.76 (95 % CI: 0.63 to 0.85) and a summary specificity of 0.78 (95 % CI: 0.57 to 0.91) for CIN 2+. FISH for TERC in ASCUS (3 studies, 839 cases) showed sensitivities ranging from 0.75 to 1.00 and specificities from 0.87 to 0.93 for CIN 2+. For high grade CIN sensitivity and specificity appeared similar, although the small number of studies precluded firm conclusions. The authors concluded that the evidence on FISH testing in screening for cervical cancer is limited given the small number of studies for each cytology subgroup and the lack of studies in well-defined screening contexts stratifying participants by HPV status.

An Agency for Healthcare Research and Quality (AHRQ) assessment on FISH or “other in situ hybridization of uterine cervical cells to predict precancer and cancer” (Uhlig 2013) concluded that, “Overall, the evidence of the analytic and clinical validity of in situ hybridization (ISH) tests in screening for cervical cancer was limited. Further research is needed to standardize techniques, compare clinical validity, thresholds, and combinations across different ISH tests, and compare the clinical utility of combinations of probes as add-on tests to HPV and cytology tests.”

Summary of clinical evidence:

<table>
<thead>
<tr>
<th>Citation</th>
<th>Content, Methods, Recommendations</th>
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<tbody>
<tr>
<td>NCCN 2015</td>
<td><strong>Key points:</strong> NCCN’s clinical practice guideline on cervical cancer does not mention FISH as a diagnostic tool.</td>
</tr>
<tr>
<td>Earley 2014</td>
<td><strong>Key points:</strong> Eleven studies examined FISH tests for telomerase RNA component gene (TERC), myelocytomatosis oncogene (MYC), or human papillomavirus (HPV) type 16 or 18 in samples exhibiting atypical squamous cells of unknown significance (ASCUS) or low-</td>
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| Uhlig 2013    | grade squamous intraepithelial lesions (LSIL).  
- Data on the sensitivity and specificity for high-grade CIN was collected.  
- FISH test probes and thresholds varied across studies.  
- Only 1 study testing for TERC specified HPV status.  
- In meta-analysis, FISH for TERC in LSIL (9 studies, 1,082 cases) had a summary sensitivity of 0.76 (95% confidence interval = 0.63-0.85) and a summary specificity of 0.78 (95% confidence interval = 0.57-0.91) for CIN.  
- FISH for TERC in ASCUS (3 studies, 839 cases) showed sensitivities ranging from 0.75 to 1.00 and specificities from 0.87 to 0.93 for CIN.  
- For high-grade CIN sensitivity and specificity appeared similar, although a small number of studies preclude firm conclusions.  
- For FISH tests for HPV, we found only few studies with small sample sizes.  
- The evidence on FISH testing is limited given the small number of studies for each cytology subgroup and the lack of studies in well-defined screening contexts stratifying participants by HPV status.  
- AHRQ examined the role of ISH tests, including FISH, to detect chromosomal abnormalities or DNA from high-risk oncogenic HPV genotypes on cervical cytologic specimens to increase the clinical validity of identification of precancerous lesions or cervical cancer.  
- A literature search yielded a total of 1462 abstracts, of which 135 articles described use of ISH on cervical specimens (cytologic or histologic), and 116 involved ISH using one of the four probes of interest:  
  31 used an ISH probe for TERC, with 7 of these also using probes for MYC; and 91 studies used an ISH probe for HPV 16, with 87 of these also using a probe for HPV 18.  
  Five studies used both a TERC probe and an HPV 16 or 18 probe.  
- For KQ2, 14 studies provided data on agreement between ISH tests with an HPV 16 or 18 probe (among other HPV probes) and HPV reference tests (polymerase chain reaction [PCR] or Hybrid Capture® 2).  
- The agreement between each ISH–non-ISH test pair was variable, reflecting differences in measurement techniques between the ISH tests and reference tests as well as the use of nonoverlapping panels of probes. Assessment of study quality showed deficiencies in reporting.  
- For KQ3, 10 studies provided information on the clinical validity of FISH tests for high-grade CIN. Of these, eight provided results for FISH using a TERC probe (with three using probes for both TERC and MYC); three studies provided results for ISH using a probe for HPV 16 or 18 (one study was of FISH with all four probes).  
- For intermediate grade CIN, with data from seven studies, the summary sensitivity was 0.76 (95 percent confidence interval [CI] 0.60, 0.86) and the summary specificity was 0.79 (95 percent CI 0.50, 0.93). For high-grade CIN with data from five studies, the summary sensitivity was 0.78 (95 percent CI 0.65, 0.87) and the summary specificity was 0.79 (95 percent CI 0.51, 0.93).  
- Also for KQ3, two studies compared combinations of FISH tests with reference tests, with both defining positivity on combination testing as positivity of either FISH or the reference test. In one, FISH testing alone, for TERC, showed lower sensitivity but higher specificity than did combined testing with FISH and Hybrid Capture® 2.  
- The other study showed that FISH testing for TERC or MYC had a lower sensitivity but higher specificity than did FISH for TERC, MYC, or HPV and Hybrid Capture 2® for high-risk HPV. For other KQ3 comparisons, the number of studies was limited.  
- Only three studies had data on FISH for TERC in ASCUS specimens, and only three had data on ISH for HPV in LSIL or ASCUS samples.  
- Assessment of risk of bias suggested low study quality and incomplete reporting. |
There were no standard thresholds for test positivity across KQ2 or KQ3 studies of ISH for TERC or MYC. For other questions related to preanalytic issues impacting analytic validity, the data were sparse or not informative.

For KQ3, no study in the specified contexts examined the association of FISH test results with clinical outcomes.

For KQ4, no study compared patient care strategies resulting from different tests, thresholds, or combinations of ISH and/or non-ISH tests.

Overall, the evidence of the analytic and clinical validity of ISH tests in screening for cervical cancer was limited.

Further research is needed to standardize techniques; compare clinical validity, thresholds, and combinations across different ISH tests; and compare the clinical utility of combinations of probes as add-on tests to HPV and cytology tests.

**ACOG 2013**

**Key points:**
- FISH not addressed.

**Canadian Task Force on Preventive Care 2013**

**Key points:**
- FISH not addressed.

**Patenwala 2013**

**Key points:**
- Sensitivity to detect CIN: 0.8 – 2.1.
- More sensitive than cytology but higher false positives than colposcopy rates.

**Pierson 2013**

**Key points:**
- RCTs or cohort, evaluating convention or liquid cytology or HPV vs. no screening, – Nov. 2012.
- 21 studies (97,627 subjects).
- Screening reduced mortality from advanced disease but not overall incidence.

**ACOG 2012**

**Key points:**
- FISH not addressed.

**Kaiser Permanente 2010**

**Key points:**
- FISH not addressed.

**Cantor 1998**

**Key points:**
- Effectiveness data from studies published 1973 – 1996 (accuracy of colposcopy in patients with abnormal Pap smear vs. biopsy); costs in USD from 1995.
- Lowest incremental cost-effectiveness ratio (ICER): 8,596/case of CIN detected.

**Glossary**

**Colposcopy** — An invasive diagnostic procedure to illuminate and visually examine the uterine and vaginal interiors, usually for the purpose of selecting sites for biopsy.

**Cytology** — Diagnosis using disaggregated cells (obtained by scraping surfaces as in the Pap test or from body fluids such as urine) in contrast to histologic diagnosis using intact tissues obtained by surgical excision or biopsy. Cytology, lacking normal tissue architecture, is inherently more difficult and often interpreted by specialist pathologists.

**Fluorescence in situ hybridization (FISH)** — FISH is a laboratory technique capitalizing on the ability of specific DNA or RNA sequences to fluoresce when combined with “probes” matched for and attached to those sequences (some of which identify cancer or pre-cancer), which are then examined by fluorescence microscopy.
Sensitivity — A measure of diagnostic test accuracy indicating the proportion of people with the disease of interest who have positive test results. Perfectly accurate tests will have sensitivity and specificity approaching 100 percent but the accuracy of tests is inherently imperfect and dependent on the prevalence of disease in the population being tested.

Specificity — A measure of diagnostic test accuracy indicating the proportion of people without the disease of interest who have negative results. Perfectly accurate tests will have sensitivity and specificity approaching 100 percent but the accuracy of tests is inherently imperfect and dependent on the prevalence of disease in the population being tested.

References

Professional associations/other:


Peer-reviewed references:


**Clinical Trials:**

Searched clinicaltrials.gov on March 6, 2016 using terms “fluorescence in situ hybridization (FISH) for cervical cancer” | No studies found.

**CMS National Coverage Determinations (NCDs):**

No NCDs identified as of the writing of this policy.

**Local coverage determination (LCDs):**

No LCDs identified as of the writing of this policy.

**Commonly submitted codes**

Below are the most commonly submitted codes for the service(s)/item(s) subject to this policy. This is not an exhaustive list of codes. Providers are expected to consult the appropriate coding manuals and bill accordingly.

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<th>CPT Code</th>
<th>Description</th>
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<td>88364</td>
<td>Each additional probe stain procedure</td>
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<tr>
<td>88365</td>
<td>In situ hybridization (e.g., FISH), per specimen; initial single probe stain procedure</td>
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<tr>
<td>88366</td>
<td>Each multiplex probe stain procedure</td>
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