Technology Assessment

Technology Assessment Program

Fluorescence In Situ Hybridization (FISH) or Other In Situ Hybridization (ISH) Testing of Uterine Cervical Cells to Predict Precancer and Cancer

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Fluorescence In Situ Hybridization (FISH) or Other In Situ Hybridization (ISH) Testing of Uterine Cervical Cells to Predict Precancer and Cancer

Technology Assessment Report

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Tufts Evidence-based Practice Center

Katrin Uhlig, M.D., M.S.
Amy Earley, B.S.
Jenny Lamont, M.S.
Issa J. Dahabreh, M.D., M.S.
Esther E. Avendano, B.A.
Janet M. Cowan, Ph.D.
Sarah Feldman, M.D.

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Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-based Practice Centers (EPCs), sponsors the development of evidence reports and technology assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. The reports and assessments provide organizations with comprehensive, science-based information on common, costly medical conditions, and new health care technologies and strategies.

The EPCs systematically review the relevant scientific literature on topics assigned to them by AHRQ and conduct additional analyses when appropriate prior to developing their reports and assessments. To bring the broadest range of experts into the development of evidence reports and health technology assessments, AHRQ encourages the EPCs to form partnerships and enter into collaborations with other medical and research organizations. The EPCs work with these partner organizations to ensure that the evidence reports and technology assessments they produce will become building blocks for health care quality improvement projects throughout the Nation. The reports undergo peer review and public comment prior to their release as a final report.

AHRQ expects that the EPC evidence reports and technology assessments will inform individual health plans, providers, and purchasers as well as the health care system as a whole by providing important information to help improve health care quality.

We welcome comments on this evidence report. Comments may be sent by mail to the Task Order Officer named in this report to: Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850, or by e-mail to epc@ahrq.hhs.gov.

Carolyn M. Clancy, M.D.
Director
Agency for Healthcare Research and Quality

Jean Slutsky, P.A., M.S.P.H.
Director, Center for Outcomes and Evidence
Agency for Healthcare Research and Quality

Stephanie Chang, M.D., M.P.H.
Director
Evidence-based Practice Program
Center for Outcomes and Evidence
Agency for Healthcare Research and Quality

Kim Marie Wittenberg, M.S.
Task Order Officer
Center for Outcomes and Evidence
Agency for Healthcare Research and Quality

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The authors gratefully acknowledge the Peer Reviewers for their contributions to this project.

Peer Reviewers

Amy Clayton, M.D.
Assistant Professor of Pathology
Mayo Clinic
Rochester, Minnesota

Andrew H. Fischer M.D.
Professor of Pathology
University of Massachusetts
Worcester, Massachusetts

Anna-Barbara Moscicki, M.D.
Professor of Pediatrics
University of California San Francisco
San Francisco, California

Mark Schiffman, M.D., M.P.H.
Senior Investigator
National Cancer Institute
Bethesda, Maryland

Nicolas Wentzensen, M.D., Ph.D.
Investigator
National Cancer Institute
Bethesda, Maryland

David C. Wilbur, M.D.
Professor of Pathology
Harvard Medical School
Director of Pathology Imaging
Massachusetts General Hospital
Boston, Massachusetts

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Structured Abstract

Objectives: Screening for cervical cancer has the potential to detect precancerous lesions and cancers in early stages, which can be effectively treated. Screening tests currently used in the United States on cervical cell samples include the Papanicolaou (Pap) test to detect cellular changes, as well as tests for high-risk human papillomavirus (HPV) genotypes. A particular challenge is the management of women with test results of atypical squamous cells of unknown significance (ASCUS) or of low-grade squamous intraepithelial lesions (LSIL) on cytology or those with a normal Pap test but a positive test for high-risk HPV genotypes, since only a fraction of these women will have a finding on colposcopically directed tissue biopsy that warrants treatment (e.g., high-grade cervical intraepithelial neoplasia [CIN]). We aimed to examine the role of in situ hybridization (ISH) tests, including fluorescence ISH (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.

Data Sources: MEDLINE® (from inception to October 2011, week 2), the Cochrane Central Trials Registry (through the fourth quarter of 2011), and Scopus (including Embase) on November 7, 2011, with no language exclusion. The searches were updated on July 12, 2012.

Review Methods: We used established systematic review methods to identify articles on the basis of predetermined eligibility criteria: studies of ISH tests in cervical tissue from at least 10 women. We addressed four Key Questions (KQs).

For KQ1, a horizon scan of what ISH tests have been examined with what frequency, we included any study that tested ISH in cervical cytology or histology. While most studies evaluated an ISH assay examining multiple probes, this scan served to focus the subsequent detailed evidence review on the most commonly studied ISH tests, which included probes for the telomerase RNA component gene (TERC [3q26]), the myelocytomatosis oncogene (MYC [8q24]), HPV 16, or HPV 18, alone or in combination with other probes.

For KQ2, about the analytic validity of ISH testing, we included any study that used ISH with any of these four probes in cervical cytology or histology specimens and compared the ISH test with a non-ISH reference test.

For KQ3, about the clinical validity of ISH testing on cervical cytology for high-grade CIN on histology, cervical cancer or clinical outcomes related to morbidity and mortality from cervical cancer, we included any study using ISH with any of the four probes in cervical cytology specimens to detect high-grade CIN or cervical cancer (or clinical outcomes). Cervical cytology had to be classified according to the Bethesda classification as ASCUS or LSIL or as normal in combination with a positive HPV test. Histology outcomes had to be defined as CIN and had to be expressible as either CIN3+ (i.e., CIN3 or cervical cancer) or CIN2+ (i.e., CIN2, CIN3, or cervical cancer). Studies had to provide data that allowed for calculation of sensitivity and specificity.

For KQ4, about the clinical utility and possible harms of ISH testing, we reviewed studies that compared patient management strategies using different screening or testing algorithms, including ISH testing.

Results: The literature search yielded a total of 1462 abstracts, of which we screened 227 in full text. For KQ1, 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). (None compared a FISH test for TERC or MYC with a DNA-based reference test.) 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 CIN2+ or CIN3+. 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). HPV status was not known except in one study of women who were all HPV positive (type not specified). Meta-analysis was performed for studies of ISH for TERC in women with LSIL cytologic findings. For CIN2+, 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 CIN3+, 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.

Across all 10 KQ3 studies, there was a trade-off between sensitivity and specificity, which may be indicative of a threshold effect. There was also large clinical heterogeneity across populations and test probes. Assessment of risk of bias suggested low study quality and incomplete reporting. We rated the strength of evidence as low for KQ3, failing to show consistently better sensitivity or specificity with FISH testing for identification of CIN2+ or CIN3+ than would be expected by chance.

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.

Conclusions: 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.

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Background

Overview

Cervical cancer is a highly preventable disease. Most cases are related to infection with human papillomavirus (HPV).[1] Persistent cervical infection with high-risk, oncogenic HPV genotypes is necessary for the development of cervical cancer and its immediate precursor lesion, high-grade cervical intraepithelial neoplasia (CIN).[2] Progression is generally slow and screening for cervical cancer has the potential to detect precancerous lesions and cancers in early stages, which can be effectively treated. Thus, in the United States, regular screening is recommended for all women between the ages of 21 and 65 years.[3]

Incidence and mortality rates for cervical cancer vary globally, depending on the availability of cervical screening and prevention programs. In the United States, which has widespread screening practices, there were more than 12,000 new cases of cervical cancer and 4,220 related deaths in 2011.[1,4]

The screening tests currently used in the United States on cervical cell samples include the Papanicolaou (Pap) test to detect cellular changes, as well as tests for high-risk HPV genotypes. Although both tests identify a large proportion of women who harbor high-grade CIN, in a large number of women abnormalities detected on these tests will spontaneously resolve or will not be confirmed on histologic examination by colposcopy. A particular challenge is the management of women with test results of atypical squamous cells of unknown significance (ASCUS) or of low-grade squamous intraepithelial lesions (LSIL) on cytology or those with a normal Pap test but a positive test for high-risk HPV genotypes, since only a fraction of these women will have a finding on colposcopically directed tissue biopsy that warrants treatment. The median percentage of all Pap tests reported by various U.S. laboratories in 2009 for ASCUS ranged from 2.9 to 4.8 percent and for LSIL, from 1.2 to 2.8 percent, depending on the cytology preparation method (according to the College of American Pathologists Laboratory Accreditation Program).[5] Less than one-fifth of women with these findings will have a finding on colposcopically directed biopsy that warrants treatment.[6] Colposcopy incurs expense and may be associated with adverse events.[7,8] Another complication of an ablative procedure is that on subsequent cervical cancer screening, if a screening test comes back abnormal, it can be difficult to visualize the lesion and allow the lesion to be optimally treated. Thus, testing strategies that can more accurately triage patients to colposcopy are needed, to minimize overtreatment. Tests being marketed and offered by some laboratories include in situ hybridization (ISH) tests to detect chromosomal abnormalities or HPV DNA.

This Technology Assessment (TA) examines the role of in situ hybridization (ISH) tests, including fluorescence ISH (FISH), to detect chromosomal abnormalities or HPV DNA on cervical cytologic specimens and their clinical validity for identification of precancerous lesions or cervical cancer.

Terminology for Cervical Lesions

In the United States, most laboratories use the Bethesda System for reporting cervical cytology findings (**Table A**).[9]

Table A. The 2001 Bethesda System for Reporting Cervical Cytology[10]

Specimen Type

Indicate: conventional test (Pap test), liquid-based preparation, or other

Specimen Adequacy

- Satisfactory for evaluation (describe presence/absence or endocervical/transformation zone component and any other quality indicators, e.g., partially obscuring blood inflammation, etc.)
- Unsatisfactory for evaluation (specify reason)
    - Specimen rejected/not processed (specify reason)
    - Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason)

General Categorization (Optional)

- Negative for intraepithelial lesion or malignancy
- Other: see Interpretation/Result (e.g., endometrial cells in a woman aged 40 years or older)
- Epithelial cell abnormality: See interpretation/Result (specify "squamous" or "glandular" as appropriate)

Interpretation/Result

- Negative for intraepithelial lesion or malignancy (when there is no cellular evidence of neoplasia, state this in the General Categorization above, in the Interpretation/Result section of the report, or both-whether or not there are organisms or other nonneoplastic findings)
    - Organisms
        - Trichomonas vaginalis
        - Fungal organisms morphologically consistent with Candida species
        - Shift in flora suggestive of bacterial vaginosis
        - Bacteria morphologically consistent with Actinomyces species
        - Cellular changes consistent with herpes simplex virus
    - Other nonneoplastic findings (optional to report; list not inclusive)
        - Reactive cellular changes associated with
            - Inflammation (includes typical repair)
            - Radiation
            - Intrauterine device
        - Glandular cells status posthysterectomy
        - Atrophy
- Other (list not comprehensive)
    - Endometrial cells (in a woman aged 40 years or older) (specify if negative for squamous intraepithelial lesion)
- Epithelial cell abnormalities
    - Squamous cell
        - Atypical squamous cells (ASC)
            - Of undetermined significance (ASC-US)
            - Cannot exclude HSIL (ASC-H)
        - Low-grade squamous intraepithelial lesion (LSIL) (encompassing: human papillomavirus/mild dysplasia/cervical intraepithelial neoplasia (CIN) 1
        - High-grade squamous intraepithelial lesion (HSIL) (encompassing: moderate and severe dysplasia, carcinoma in situ; CIN2 and CIN3)
        - With features suspicious for invasion (if invasion is suspected)
        - Squamous cell carcinoma
    - Glandular cell
        - Atypical
            - Endocervical cells (not otherwise specified or specify in comments)
            - Endometrial cells (not otherwise specified or specify in comments)
            - Glandular cells (not otherwise specified or specify in comments)
        - Atypical
            - Endocervical cells, favor neoplastic
            - Glandular cells, favor neoplastic
        - Endocervical adenocarcinoma in situ (AIS)

▪ Adenocarcinoma • Endocervical • Endometrial • Extrauterine • Not otherwise specified • Other malignant neoplasms (specify)
Ancillary Testing Provide a brief description of the test method(s) and report the result so that it is easily understood by the clinician. Automated Review If case examined by automated device, specify device and result
Educational Notes and Suggestions (Optional) Suggestions should be concise and consistent with clinical followup guidelines published by professional organizations (references to relevant publications may be included)

Histologic changes (those detected on biopsy) are described as cervical intraepithelial neoplasia (CIN). CIN is categorized, according to the depth of involvement and the atypicality of the cell, into three degrees of severity. CIN1 is considered a low-grade lesion. It refers to mildly atypical cellular changes in the lower third of the epithelium (formerly called mild dysplasia). HPV-induced cytopathic effects (koilocytotic atypia) are often present. CIN2 is considered a high-grade lesion. It refers to moderately atypical cellular changes confined to the basal two-thirds of the epithelium (formerly called moderate dysplasia) with preservation of epithelial maturation. CIN3 is also considered a high-grade lesion, but it refers to severely atypical cellular changes encompassing greater than two-thirds of the epithelial thickness and includes full-thickness lesions (formerly called severe dysplasia or carcinoma in situ). Invasive cancer may also be diagnosed on histology.

Recent consensus recommendations by the Lower Anogenital Squamous Terminology (LAST) initiative aim to unify the terminology for HPV-associated lower anogenital lesions.[2] This new terminology classifies into a two-tiered system of low-grade squamous intraepithelial lesions (LSIL) and high-grade intraepithelial lesion (HSIL), similar to the Pap testing classification. The pathologist must still determine the CIN category on the basis of what proportion of the epithelium is involved (lower one-third, lower two-thirds, or full thickness) and then stratify to LSIL or HSIL. CIN1 under the old classification maps to LSIL, and CIN3 maps to HSIL. CIN2 is a biologically equivocal lesion and if this diagnosis is entertained by a pathologist, LAST recommends stratification to high-grade or low-grade disease on the basis of p16 immunohistochemistry. The LAST classification is not yet broadly used.

Generally, a higher grade of cytology indicates a greater risk for higher-grade histology, but cytology may also be associated with both more or less severe histologic findings.

Cervical Human Papillomavirus Infection in Cervical Cancer

HPV testing detects the presence of HPV DNA. Even though cervical abnormalities may not be present or may not develop in every person with an HPV infection, infection with specific high-risk genotypes of HPV is associated with risk for precancer or cancer. Of the approximately 30 to 40 HPV genotypes that infect the mucosa of the genital tract, 8 (types 16, 18, 31, 33, 35, 45, 52, and 58) are responsible for over 90 percent of cervical cancers and are therefore called "high-risk" types. Two of these types (types 16 and 18) are alone responsible for about 70 percent of cervical cancers.[11-13]

Cytologic Screening for Cervical Cancer

Screening tests are performed on a sample of cervical cells obtained from scraping the cervix during a speculum examination, called a Pap test. Conventional cervical samples are prepared by smearing the specimen on a slide. Liquid-based preparation involves placing the specimen into a liquid fixative solution (e.g., ThinPrep or SurePath).

The Pap test is widely performed to screen for precancerous or cancerous changes in cervical cells and is usually reported in the United States according to the Bethesda system (see Table A above). LSIL, especially in young women, is generally associated with a transient HPV infection, whereas a finding of high-grade squamous intraepithelial lesion (HSIL) is more likely to be associated with persistent HPV infection and a higher risk of progression to cervical cancer.[14]

If abnormal cells are detected on Pap testing, then further evaluation is conducted, with a colposcopy, a procedure in which the cervix is viewed at high magnification, and colposcopically directed cervical biopsy.[15] Increasingly, HPV testing is done either reflexively on abnormal Pap tests or concurrently with Pap testing to aid risk stratification before proceeding to colposcopy, as described in a section below.

Histology allows for triage to either a low or a high-grade lesion, with the latter requiring ablative therapy (loop electrosurgical excision procedure [LEEP], conization, or cryosurgery). Histologic examination is useful because it decreases the number of unnecessary ablative procedures, since some equivocal lesions on cytology will regress spontaneously or be so small that they cannot be identified on colposcopic sampling and presumably do not pose an immediate threat warranting definitive ablative therapies. On the other hand, high-grade lesions diagnosed by cytology have a low false positive rate. Therefore, a "see and treat" approach for patients with HSIL findings on cytology is acceptable. "See and treat" allows an ablation to take place upon the first colposcopic visit and avoids the cost of an additional colposcopic exam and histologic confirmation.

Current Guidelines for Cervical Cancer Screening and Treatment

Recent guidelines issued by the U.S. Preventive Services Task Force (USPSTF)[16] suggest screening with a Pap test every 3 years for all healthy women ages 21 through 65 years. The 2012 guidelines by American Cancer Society, the American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology (ASCP)[3] recommend cytology testing alone every 3 years, but in women aged 30-65 years, state a preference for HPV and cytology cotesting every 5-years. Given its improved performance over Pap testing alone in this age group, cotesting (Pap plus HPV testing) can be used for screening at less frequent intervals. Because few studies have sufficient numbers of cancer cases to assess cancer risk directly, the guidelines considered the absolute risk of CIN3, including the rare cases of cancer (CIN3+) prior to or at the visit after a given visit, as the best measure of the risk of incident cervical cancer. Screening by HPV testing alone (without concurrent Pap testing) is not currently recommended in the United States, but several clinical trials outside the United States in resource-poor settings have documented the value of "primary HPV screening"—that is, if the HPV test is negative, no other testing is needed, and if the test is positive, cytologic Pap testing is effective for triage because of the high specificity of Pap test findings.

Genotype-specific testing for HPV 16 or HPV 16/18 is only recommended as an option in one particular clinical setting: for women who have a normal Pap result and a positive HPV test.

In these women, both tests should be repeated at an interval, or alternatively these women can undergo genotyping to determine whether they have infection with HPV16 and/or HPV 18. If positive on HPV16/18 testing, then colposcopy is recommended. If negative, then retesting after 1 year is recommended.

Recent guidelines by the American College of Gynecology (ACOG) specify that women with ASCUS on Pap testing and a negative HPV test continue with routine screening as indicated for their age.[17]

All guidelines recommend against screening for cervical cancer in women over the age of 65 years who have had negative results on an adequate number of previous screening tests (with "adequate number" defined as three consecutive negative Pap results or two negative Pap and HPV tests in the prior 10 years, with the most recent within the previous 5 years). Finally, women who have been vaccinated against HPV should begin cervical cancer screening at the same age as unvaccinated women (i.e., at 21 years).

Principles of ISH

ISH is a technique that is used to detect and localize the presence or absence of a specific genetic sequence in cells using a probe with a complementary polynucleotide sequence. The probe is either directly tagged with a fluorescent compound such as Texas Red (in FISH) or indirectly tagged with biotin (historically) or digoxigenin or dinitrophenol (more recently). After binding to the complementary sequences in the cell, the probes are either visualized under ultraviolet (UV) light (in FISH) or located using antibodies to the chemical tag and visualized using direct light (in chromogenic in situ hybridization [CISH]) or UV light (in FISH). Gains of a region are seen as additional spots in the cell, while deletions are seen as a loss of spots. The majority of probes in use are "cocktails" that combine the region of interest with a control probe, and loss or gain is determined with respect to the number of control signals. The technique is used to detect recurrent diagnostic changes in hematological malignancies (such as a deletion in the long arm of chromosome 5 in myelodysplastic syndrome), to look for gene amplification (e.g., of the HER2/neu gene in breast cancer), and to detect specific gene rearrangements that can be treated with particular drugs (e.g., ALK gene rearrangement in non–small-cell lung cancer, translocation 9 and 22 in chronic myeloid leukemia).

The resolution of ISH is limited by the size of the probe available for a region. The majority of commercially available ISH probes range in size from ~100 Kb to ~900 KB and will detect a genetic sequence of this size. Smaller sequences will not be detected. Scoring of ISH tests can be automated, reducing the potential for operator errors such as the evaluation of the wrong cell population or the incorrect area of a tumor section. Any ISH final interpretation must be rendered by a pathologist. The technique is reliable but requires appropriate specimen preparation for consistent results.[18] Differentiating signals from experimental background can be problematic with CISH, but not with FISH.

Potential for ISH Tests for Cervical Cancer Screening

ISH has been proposed as an additional noninvasive test on cervical smears to detect chromosomal abnormalities (markers of chromosomal damage) or HPV DNA. ISH testing on cervical specimens is not yet widely established, but some laboratories have developed their own tests, and manufacturers are starting to promote the use of ISH testing to triage women to colposcopy on the basis of their cytology, HPV result, and ISH test finding (e.g.,

www.cervicaldnadtextest.com/casestudies.php). This raises the question about the role of ISH as an add-on test when prior screening tests have yielded abnormal results.

Key Questions

The four Key Questions in this TA were drafted by CMS and refined by the Evidence-based Practice Center through discussions with the Agency for Healthcare Research and Quality (AHRQ) Task Order Officer and CMS experts. Broadly, Key Question 1 asked for the results of a "horizon scan" to identify studies that have used any ISH tests on cervical cytologic or histologic samples and to identify the ISH probes most frequently studied; Key Question 2 asked to examine the analytic validity (technical performance) of the most frequently studied ISH tests for detection of markers of chromosomal damage or oncogenic HPV DNA; Key Question 3 asked to examine the clinical validity of ISH tests for detection of high-grade CIN or for prediction of cancer related clinical outcomes; and Key Question 4 asked to examine the clinical utility of ISH testing (i.e., how ISH testing impacts presumptive diagnosis, patient evaluation, management, and ultimately patients' clinical outcomes). The Centers for Medicare and Medicaid Services (CMS) requested this TA to inform its decisionmaking about the coverage of this technology.

Key Question 1. What ISH tests have been used in cervical cytology or histology specimens?

To refine the scope for the detailed evidence review, we conducted a horizon scan to better understand the extent of the use of ISH tests for cervical cancer. On the basis of the findings of the horizon scan, we focused the subsequent review on ISH tests including probes for TERC (the telomerase RNA component gene, on chromosome 3, band 3q26), MYC (the myelocytomatosis oncogene, on chromosome 8, band 8q24), HPV 16, or HPV 18.

Key Question 2. For ISH tests for TERC or MYC or HPV 16 or HPV 18 in cervical cytology or cervical histology:

a. What are the associations between ISH test results and reference test results? What thresholds were used for positive, indeterminate, and negative results of the ISH tests? What reference tests were used to assess the presence or absence of the genetic marker (TERC, MYC, or HPV 16 or 18)?
b. What is known about reliability and reproducibility of ISH tests? What genetic, environmental, or other factors are known to affect ISH test results (e.g., the presence of more than a certain proportion of necrotic tumor tissue in the sample or the presence of infection)?
c. Are there some conditions for which an ISH test is not able to give a clinically useable result?
d. What are the sample acceptance and rejection criteria for ISH tests?
e. What sample storage or preservation requirements are needed for a reliable ISH test result?
f. What variation occurs in results of the ISH test if performed in multiple laboratories?
g. What is the prevalence of the genetic marker(s) detected by the reference standards in Medicare beneficiaries by age or race/ethnicity?

Key Question 3. For ISH tests for TERC or MYC or HPV 16 or HPV 18:

a. What is the association between ISH tests on cytology for high-grade CIN or cervical cancer on histopathology or for clinical outcomes related to cervical cancer morbidity and mortality? What thresholds were used for positive, indeterminate, and negative results on the ISH tests?
b. How similar are the spectrum and prevalence of the histopathological abnormalities and cervical cancers found in the studies to the spectrum and prevalence in Medicare beneficiaries? How is diagnostic accuracy modulated by age, race, and ethnicity?

Key Question 4. For ISH tests for TERC or MYC or HPV 16 or HPV 18 in cervical cytology, what is the published evidence about the test's clinical utility and harms?

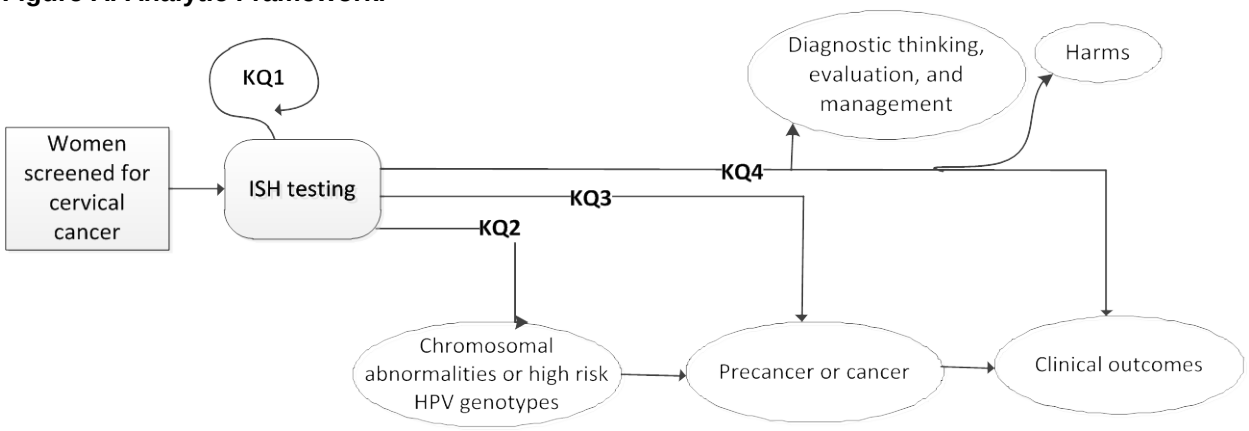
a. In comparative studies of ISH testing versus alternative testing (with similar or equivalent tests) or no testing, what is the effect on diagnostic thinking, evaluation, management, or clinical outcomes?
b. What are the clinical inclusion criteria in the studies? How similar are the populations to the core Medicare population (i.e., persons ≥65 years of age) overall as well as according to race/ethnicity?
c. How similar are the spectrum and prevalence of the cancers in these studies to the spectrum and prevalence in the core Medicare population?

Analytic Framework

The overarching analytic framework for the report is shown in **Figure A**. Key Question 1 relates to a horizon scan of the literature to identify the most relevant ISH tests for subsequent detailed evidence review. Key Question 2 pertains to analytic validity, Key Question 3 to clinical validity, and Key Question 4 to clinical utility.

Key Question 3 was further focused on specific clinical scenarios according to currently recommended screening options in 2012 clinical practice guidelines (described above).[3,16] Under current guidelines, a woman whose Pap results show HSIL or ASC-H would be referred to colposcopy; whereas a woman with normal Pap or a woman with a normal Pap and a normal HPV results would be retested after a certain period . This leaves women with ASCUS or LSIL on Pap testing and those with a positive test for high-risk HPV, for whom additional testing with ISH might have a role as an add-on test instead of directly proceeding to colposcopy and as an alternative to non–ISH-based HPV 16/18 testing.

Figure A. Analytic Framework.



The key questions (KQs) are shown within the context of the population, tests, and outcomes. KQ1 reviews the existing literature on what in situ hybridization (ISH) test has been used in women tested for cervical cancer; KQ2 addresses the analytic validity of ISH testing to detect chromosomal abnormalities or high-risk HPV genotypes; KQ3 addresses the clinical validity of ISH testing to detect precancer or cancer; and KQ4 addresses the clinical utility of ISH testing to predict clinical outcomes, to affect diagnostic thinking, evaluation, and management and to ascertain harms.

Methods

Data Sources, Study Selection, and Data Extraction

The search was conducted in MEDLINE®, SciVerse Scopus (including Embase) (Elsevier), and the Cochrane Central Register of Controlled Trials and had no language restrictions. Key words included terms related to the test of interest (in situ hybridization) and terms related to cervical cancer or abnormalities (cervical, precancerous, neoplasm, and cervical intraepithelial neoplasia). The first search was performed on November 7, 2011; the update search was conducted on July 12, 2012. We also searched the sections on gynecologic cancer for the past 2 years of proceedings of major gynecology and oncology conferences. Studies were eligible if they provided relevant data on cervical tissue samples from at least 10 women examined with ISH tests in a clinical or research setting.

For Key Question 1, we included studies that described any ISH testing and mentioned cervical cytologic grade (e.g., ASCUS, LSIL, or normal cytology in combination with a positive HPV test) or cervical histologic grade or cancer stage (e.g., CIN or squamous-cell carcinoma). We excluded studies of cervical cell lines and reviews without primary data.

For Key Question 2a, we included any study that examined an ISH test for TERC, MYC, HPV 16, or HPV 18 (with or without additional probes) in cervical cytology or histology specimens and compared these ISH tests with a non-ISH reference test. We included studies that applied both ISH and reference test in the same cervical specimen, either cytologic or histologic, regardless of classification. Studies had to provide data that allowed for the reconstruction of 2×2 tables for the results of index and reference tests. We described the agreement between tests as the percent of those with concordant results (either positive or both negative) divided by the number of all samples tested. For Key Questions 2b–f, we reviewed studies eligible for Key Questions 2a, 3, or 4 for pertinent narrative or quantitative information on reliability and reproducibility of ISH tests and possible factors interfering with analytic test performance. For Key Question 2g, we conducted a focused search for literature on the prevalence of the markers detected by ISH by age or race/ethnicity in the United States.

For Key Question 3a, we included any study that examined ISH testing for TERC, MYC, HPV 16, or HPV 18 (alone or in combination with other probes) in cervical cytology samples to detect high-grade CIN or cervical cancer (or related clinical outcomes). We extracted ISH findings for each of the following groups: ASCUS, LSIL along with HPV status, and normal cytology if HPV positive. While the Bethesda nomenclature has evolved to further divide ASCUS into ASCUS and ASC-H, we were limited to using ASCUS as the term was used in the studies. If the study differentiated ASC-US and ASC-H, we included results for ASC-US only. Histology outcomes had to be classified as CIN and had to be expressible as either CIN3+ (i.e., CIN3 or cervical cancer) or CIN2+ (i.e., CIN2, CIN3, or cervical cancer). Studies included in this review had not uniformly adopted the recently published LAST nomenclature to categorize histology into HSIL or LSIL. Studies had to provide data on clinical validity, including sensitivity and specificity. We also looked within each study for comparisons of different test combinations that included ISH tests. For Key Question 3b, we conducted a focused review for information on population-based prevalence of CIN2+ and CIN3+, stratified for LSIL or ASCUS in Medicare beneficiaries.

For Key Question 4, we searched for studies that compared patient management strategies using different screening or testing algorithms. We considered strategies that compare different test thresholds or different combinations of ISH and/or non-ISH tests. Outcomes of interest were impacts on diagnostic thinking, evaluation, and management and clinical outcomes.

Study data were extracted into customized forms and tables. Data elements were related to study design, population characteristics, cytologic classification, HPV status, sampling, test characteristics, outcomes, and study results.

Assessment of Risk of Bias and Completeness of Reporting for Individual Studies

For Key Question 2, we graded each study according to 11 items, based on an approach for assessing quality and reporting for studies on analytic validity recently proposed by Sun et al.[19] in an AHRQ Methods Report. For Key Question 3, study quality was assessed according to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) 2 instrument.[20]

Data Synthesis and Grading Strength of Evidence

Evidence tables summarize study and sample characteristics, detailed descriptions of index tests and reference tests, outcomes, study quality, and relevant study results. Results were graphed.

For our survey of the literature on the most commonly used ISH probes in Key Question 1, no grading was performed. Neither did we assess strength of evidence for Key Question 2. For Key Question 4, we planned to rate the body of evidence based on risk of bias, consistency, directness, and precision for comparative studies.[21,22] However, we found no comparative studies.

For Key Question 3, we followed the Methods Guide[21,22] to evaluate the strength of evidence with respect to four domains: risk of bias, consistency, directness, and precision. Risk of bias relied on the overall summary of the quality and reporting assessed with the QUADAS-2 tool. It was summarized as low, high, or unclear. We also rated the body of evidence on the basis of four strength of evidence levels (high, moderate, low, and insufficient[21,22]) to indicate our level of confidence that the evidence reflects the true effect for the major comparisons of interest.

Applicability

We did not assess applicability of studies reviewed for Key Question 2 for analytic validity because they addressed technical test performance, which is not pertinent to the issue of applicability to a patient population.

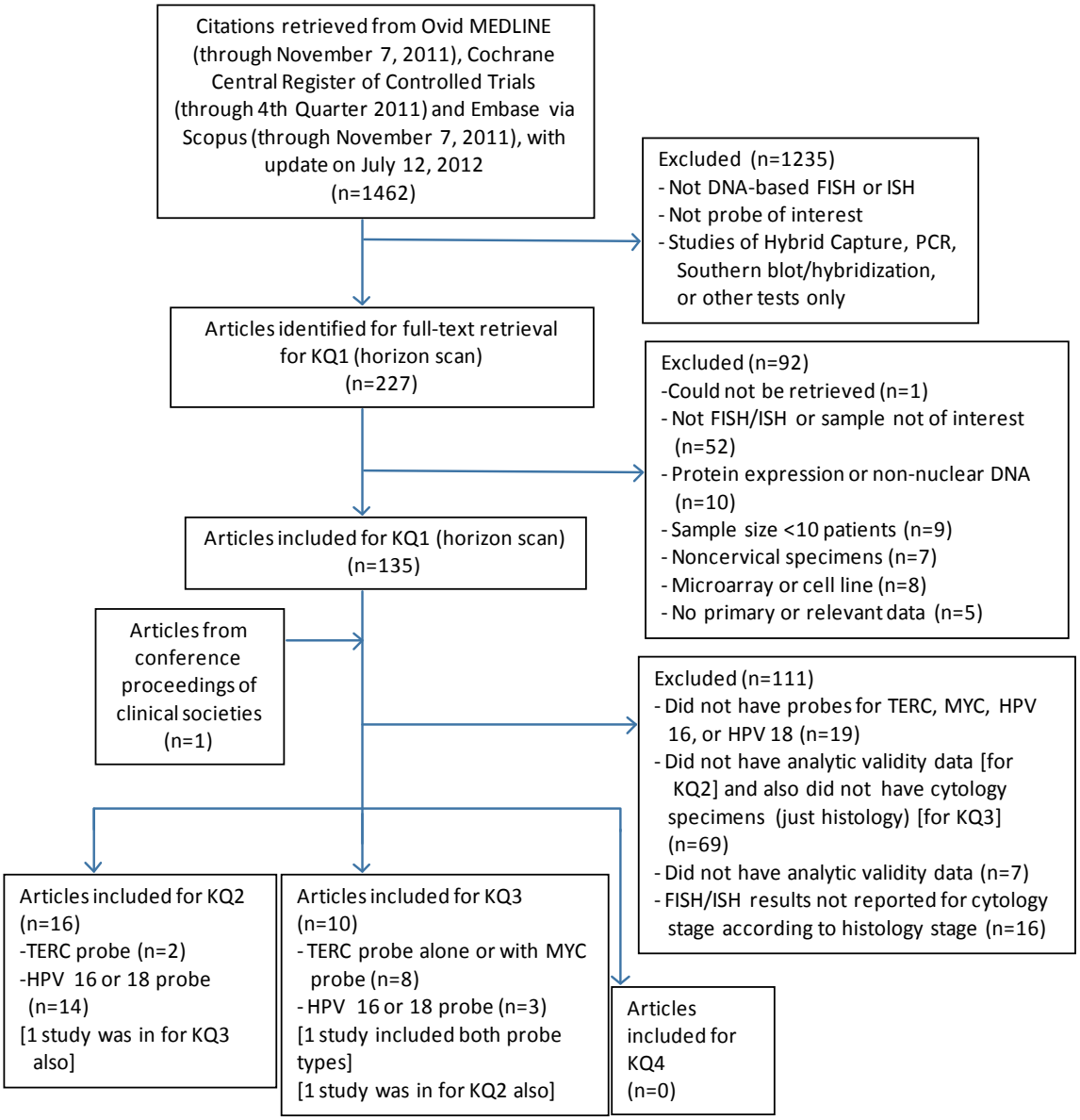
We did appraise the applicability of studies reviewed for Key Question 3 using the QUADAS-2 applicability items. We also considered how the study characteristics, for example study county, might impact applicability to the general U.S. population of screened women.

Results

Overall Literature Yield

Our searches identified a total of 1462 abstracts, of which we screened 227 in full text and included 135 in the horizon scan for Key Question 1 (**Figure B**). Twenty-five studies were included for Key Questions 2 and 3: 16 studies addressed Key Question 2, and 10 studies addressed Key Question 3, with 1 study providing data for both of these key questions. One study was in Chinese; the others were all published in English. No studies addressed Key Question 4.

Figure B. Literature Flow Diagram.



Studies could have had more than one reason for exclusion but only one reason for each is listed here.
FISH=fluorescence in situ hybridization; HPV=human papillomavirus; ISH=in situ hybridization; KQ=Key Question; MYC=myelocytomatosis oncogene; PCR=polymerase chain reaction; TERC=telomerase RNA component.

Key Question 1 (Horizon Scan)

Key Question 1: What ISH Tests Have Been Used in Cervical Cytology or Histology Specimens?

A horizon scan of the literature was performed, identifying 135 articles that described the use of an ISH probe on cervical cytology or histology samples. The probes most commonly used were TERC (31 studies), MYC (7 studies, all of which also used a TERC probe), HPV 16 (91 studies), and HPV 18 (used in 87 of the 91 studies with an HPV probe). We focused the subsequent detailed evidence review (i.e., Key Questions 2–4) on the 116 studies using one or more of these four most common ISH probes; the remaining 19 studies did not use these probes and were not reviewed further.

Focusing the detailed evidence review on ISH tests for TERC (with or without MYC) was supported by the frequency of its use in the literature and by our narrative review of microarray studies, which suggest that gain of TERC is linked to high-grade cervical cancer (see the Background section). Including ISH probes for HPV 16 (with or without HPV 18) was supported by the findings of a large amount of literature on these tests and because HPV 16 and HPV 18 are well characterized as the two high-risk genotypes most strongly associated with cancer development.

Key Question 2

Key Question 2a (Analytic Validity): For ISH tests for TERC or MYC or HPV 16 or HPV 18, what are the associations between ISH test results and reference test results? What thresholds were used? What reference standards were used?

Agreement Between ISH and Reference Tests

No studies provided data on the association between ISH for TERC or MYC and a DNA-based reference test with measurement on the same samples.

Fourteen studies compared ISH tests that included HPV 16 or HPV 18 with another HPV test in a total of 852 patients. The studies were heterogeneous with regard to the types of tissue, ISH test, and reference test; the HPV genotype; and the number of probes in either the ISH test or the reference test. The ISH tests used were specifically ISH in 10 studies, FISH in 1 study, catalyzed signal amplified colorimetric (CSAC) ISH in 1 study, catalyzed reporter deposition amplified (CARD) ISH in 1 study, and nonisotopic ISH in 1 study. The reference tests were PCR in 11 studies, in situ PCR in 2 studies, and Hybrid Capture 2 in 2 studies. The ISH test and the reference tests conspicuously varied in the HPV genotypes captured, both within and across studies.

The percent agreement between the ISH test and the reference test in each study (the sum of concordant results over the total number of test comparisons, expressed as percentage) ranged between 35 percent (95 percent CI, 15.4 to 59.2) to 100 percent (95 percent CI, 91.6 to 100). This variability in agreement was expected, given the true heterogeneity from comparison of tests with different principles of measurement and different target DNA. Also, the HPV probe sets used by ISH and by the reference test in each study often did not overlap. Given the substantial disagreement between tests across studies, is possible that index and reference tests

provide complementary information and that combining these tests could increase diagnostic or prognostic accuracy.

Assessment of Risk of Bias and Completeness of Reporting

In general, study reporting was variable across questions used to assess risk of bias. All studies described the performance of the index tests in sufficient detail to permit replication. About half the studies reported use of both positive and negative samples and use of the same type of tissue for those controls. Some criteria for scoring test results were established a priori in the majority of studies. No studies reported on the six remaining items (reproducibility on testing of the same specimen multiple times; reproducibility across operators, instruments, reagent lots, different days of the week, different laboratories; yield of useable results; and multisite collaborative, proficiency testing, or interlaboratory exchange programs). Information on cross-reactivity was provided in only two studies. Overall, the assessment shows deficiencies in reporting, likely because most of the studies were not designed to specifically address analytic validity.

Thresholds Used for Positive, Indeterminate, and Negative Results of the ISH Tests

We reviewed information from the 14 articles using ISH with HPV probes as well as 10 studies reviewed for Key Question 3 (8 reporting on FISH for TERC and 3 reporting on FISH for HPV). Most of the studies of FISH with a TERC or MYC probe defined test result positivity by the presence of additional signals in two or three or more cells, often in combination with a threshold for cellular positivity (typically a ratio of the TERC or MYC probe and the chromosomal control probe), but there were no standard thresholds for test positivity.

Two additional studies of FISH for TERC established the threshold for positivity for TERC gain by assaying cervical cytology samples from 20 women per study center who had normal Pap results and negative for HPV infection. The thresholds at four centers (one in one study and three in another) were 5.3 percent of cells with abnormal signals, 5.2 percent, 5.6 percent, and 6.4 percent. No statistical comparisons were performed.

For HPV, test positivity was usually defined simply by staining indicating the presence of HPV DNA in the nucleus of at least one cell, except in one study in which 30 or more cells had to have had staining for HPV for the sample to be deemed positive (for episomal infection).

Key Questions 2b–2f. b) What is known about reliability and reproducibility of FISH tests? What factors affect FISH test results? c) Are there some conditions for which a FISH test is not useable? d) What are the sample criteria? e) What are the sample storage or preservation requirements? f) What variation occurs across laboratories?

To address Key Questions 2b through 2f, we looked at the 14 articles describing ISH with HPV probes as well as the 10 studies reviewed in detail for Key Question 3.

None of the studies reported on the true reliability of FISH results within a study or the genetic, environmental, or other factors and their impact on FISH results or addressed whether there are some conditions for which a FISH test is not able to give a clinically useable result. None of the studies addressed variation in ISH results across multiple laboratories.

Regarding sample acceptance and rejection criteria for FISH tests, there was limited evidence from single reports on possible causes of unreliable results, such as the type of fixative used (reflecting the age of the samples). A few studies described the ascertainment of the quality of DNA, by means of beta-globin testing, before including samples. Another study reported that the cytologic sample preparation (ThinPrep vs. SurePath) did not significantly affect positivity or negativity of FISH using probes for HPV.

Overall, for questions related to preanalytic issues impacting analytic validity, the data were sparse and highlighted a lack of commonly agreed upon test and validation standards.

Key Question 2g. What is the prevalence of the genetic marker(s) detected by the reference standards in Medicare beneficiaries by age or race/ethnicity?

We conducted a focused search for literature on the prevalence of the markers detected by ISH (i.e., TERC, MYC, HPV 16, and HPV 18) by age or race/ethnicity in the United States. We did not find direct evidence.

Key Question 3

For Key Question 3, we reviewed studies that examined the sensitivity or specificity of ISH tests in cytology samples for the diagnosis of high-grade CIN. We examined the role of ISH for add-on testing in 1) women who have a Pap test showing LSIL or ASCUS without a HPV test, and 2) in women who have a Pap test showing normal cytology or ASCUS as well as a positive HPV test.

Key Question 3a (Clinical Validity): What is the association between FISH test results on cytology and CIN or cervical cancer on histopathology? What thresholds were used?

Ten studies provided information on the clinical validity of FISH tests for CIN2+ or CIN3+. Of these, eight provided results for FISH with a probe for TERC (as well as FISH for MYC, in three of these); three provided results of FISH for HPV 16 or 18, 1 study for both TERC or MYC and HPV separately. In one study, all women were HPV positive (type not specified). HPV status in the other studies was not known.

Clinical Validity in LSIL Cytology Samples

FISH for TERC or MYC

Seven studies compared the clinical validity of TERC in LSIL for CIN2+; two examined FISH for TERC or MYC. Only one study tested patients who were all positive for HPV. In these studies, the sensitivity ranged from 0.24 to 1.00 and specificity ranged from 0.38 to 1.00. Meta-analysis of 7 studies of TERC in LSIL for CIN2+ found summary sensitivity of 0.76 (95 percent CI 0.60, 0.86) and summary specificity of 0.79 (95 percent CI 0.50, 0.93).

Five studies compared the clinical validity of TERC in LSIL for CIN3+, with two testing FISH for TERC or MYC. Again, only one study tested patients who were positive for HPV. In these studies, the sensitivity ranged from 0.45 to 0.93and the specificity ranged from 0.42 to 1.00. Meta-analysis of five studies of TERC in LSIL for CIN3+ found summary sensitivity of 0.78 (95 percent CI 0.65, 0.87) and summary specificity of 0.79 (95 percent CI 0.51, 0.93).

FISH for TERC or MYC versus Other Tests

Two studies compared the performance of different tests or combinations of tests and their clinical validity in LSIL patients. One compared FISH testing for TERC or MYC, FISH for TERC or MYC or high-risk HPV, and Hybrid Capture 2 for high-risk HPV. For the diagnosis of CIN2+, testing with Hybrid Capture 2 for HPV was the most sensitive, whereas FISH for TERC or MYC was the most specific.

The other study compared FISH for TERC, Hybrid Capture 2 for HPV, and a combination of both. (We presumed that the combination was considered positive if either FISH or Hybrid Capture 2 was positive, although the study is unclear in this regard, given a consistent pattern of higher sensitivity and lower specificity from the combined test compared to either test alone.)

For the outcome of CIN2+, the combination of FISH and Hybrid Capture 2 appeared to be the most sensitive test, whereas FISH alone was the most specific. These results also held for the outcome of CIN3+.

FISH for HPV 16 or 18

Three studies examining FISH for HPV 16 or 18 (among other types) in women with LSIL provided data for the sensitivity and specificity in LSIL patients for the CIN2+ outcome. The sensitivities ranged from 0.75 to 0.81. The specificities ranged from 0.00 to 0.88. Two studies reported data for the outcome of CIN3+, showing similar sensitivities (0.83 and 0.80, respectively) with wide, overlapping CIs. The specificity was 0.42 in one study but only 0.17 in the other.

Clinical Validity in ASCUS Cytology Samples

FISH for TERC or MYC

Three studies assessed FISH for TERC (or TERC or MYC, in one study) and reported data by ASCUS cytology, one of which included patients positive for HPV (type not specified). Two of the studies provided data for the outcome of CIN2+: the sensitivity and specificity were 0.75 to 0.82 and 0.87 to 0.93, respectively. All three studies provided data for the CIN3+ outcome. Sensitivities ranged from 0.25 to 0.87. Specificities ranged from 0.67 to 0.89.

FISH for TERC versus Other Tests

One study compared FISH for TERC (not MYC), Hybrid Capture 2 for HPV, and a combination of FISH and Hybrid Capture 2 in women with ASCUS for CIN2+ and CIN3+. The results were similar to the corresponding findings in LSIL patients.

FISH for HPV 16 or 18

One study examined FISH for HPV 16 or 18 (among other types) in ASCUS patients for the CIN2+ outcome. The sensitivity was 1.00 and specificity was 0.57. Two studies examined FISH for HPV for the outcome of CIN3+, with sensitivities of 0.25 and 1.00 and specificities of 0.44 and 0.67.

Clinical Validity in HPV-Positive Normal Cytology Samples

We did not find relevant data in groups of women who were HPV test positive but had normal cytology.

Assessment of Risk of Bias and Completeness of Reporting for Individual Studies

Our assessment of study quality and reporting for the 10 studies of clinical validity (Key Question 3) was based on 18 questions related to assay performance and reporting thereof. Few studies reported information of recruitment and study design, although reporting of information on the index tests and references standards was generally adequate. Data on flow and timing was sparsely reported. All patients received the same reference standard, but inclusion of all patients was complete only in 50 percent of studies, resulting in variable clarity and bias resulting from patient flow. Overall, the reporting was frequently unclear, impeding the assessment of the risk of bias.

In contrast, concern regarding the applicability of studies to Key Question 3 was uniformly low, given the inclusion criteria for these studies.

Strength of Evidence

The strength of evidence for the studies on clinical validity reviewed for Key Question 3a was rated as low. The studies were generally small. The number of comparisons for each pair test–outcome pair was low. Reporting on items used for quality assessment was often unclear, yielding overall low methodological quality. Point estimates were heterogeneous. The CIs were often overlapping because of imprecise estimates. Across studies of FISH tests for HPV 16 or 18 (among other types), the panels of HPV probes used did not overlap, resulting in clinical heterogeneity.

Overall, the lower 95 percent confidence limit for sensitivity and specificity spanned 0.5 in a high proportion of studies, indicating that the test results may not distinguish between the presence or absence of FISH signals beyond chance. Thus, overall we have low confidence that the estimated clinical validity of the FISH test represents its true validity. The evidence was considered to be direct for clinical validity since the studies examined CIN2 or 3, which are meaningful outcomes for decisionmaking.

Key Question 3b: How similar are the spectrum and prevalence of the histopathological abnormalities and cervical cancers between the studies and Medicare beneficiaries?

We conducted a focused review for information on population-based prevalence of CIN2+ and CIN3+, stratified for LSIL or ASCUS, in Medicare beneficiaries. We did not find any evidence. Further, the primary studies did not provide any unbiased estimates.

Key Question 4: What are the clinical utility and harms for ISH tests in cervical cytology?

No studies compared patient care strategies resulting from different test thresholds or different combinations of ISH or non-ISH tests. This is not surprising, since ISH testing is not currently used in practice. Potential harms associated with colposcopy and biopsy include transient cervical bleeding and discharge or infection with fever and moderate-to-severe pain. Treatment with cervical conization can be complicated by cervical incompetence, resulting in fetal prematurity or infertility. An ablative procedure can also complicate subsequent testing for cervical cancer, as it makes it more difficult to visualize any lesions. Harms from false positive

findings are anxiety and unnecessary procedures. Harms from false negative findings are the missed opportunity for early and potentially curative treatment.

Discussion

Key Findings and Strength of Evidence

Cervical cancer screening remains an evolving field with ongoing reevaluation of Pap screening practices and the role of HPV testing, as well as development of new technologies including ISH testing for genetic abnormalities. The key findings of this review and the strength of evidence are summarized in **Table B**.

Table B: Key Findings and Strength of Evidence

Key Question	Population	Test/Assay	Outcome	Strength of Evidence Summary and Comments
1. Horizon scan	Women screened or tested for cervical cancer	Any ISH test	NA	SOE=NA 135 Articles described use of an ISH probe on cervical cytology or histology samples • 31 Studies used ISH for TERC; 7 of these examined both TERC and MYC • 91 Studies used ISH for HPV 16; 87 of these studies examined both HPV 16 and 18 • On the basis of these findings, we focused of the subsequent review on ISH for TERC, MYC, HPV 16, or HPV 18
2. Analytic validity	Women screened or tested for cervical cancer	Any ISH test for TERC, MYC, HPV 16, or HPV 18	Agreement with reference test	SOE=NA No studies compared ISH test for TERC or MYC with DNA-based reference test 14 Studies compared ISH tests for HPV 16 or 18 (among other types) with various reference tests (mostly PCR and Hybrid Capture 2). Agreement was variable, indicating differences in measurement techniques between ISH and reference tests, as well as nonoverlapping panels of HPV probes. Assessment of study quality shows deficiencies in reporting, which may indicate low study quality. Overall, evidence for analytic validity of various ISH assays was limited.
			Thresholds	SOE=NA 14 Studies included for KQ2 and 10 studies included for KQ3 were examined for information on thresholds of positivity on ISH testing. • Thresholds for ISH tests with TERC or MYC probes consisted of variable counts of signal-positive cells (three or more) and a range of different control probes for centromere or chromosome numbers. • Test positivity for HPV DNA was dichotomized as detection versus no detection in most studies (except for one, which used a cutoff of 30 cells as positivity for episomal infection). Two other studies provided information on threshold determination of FISH for TERC in samples from normal women across four laboratories (one in 1 study and three in the other); the value for a positive result ranged from 5.2–6.4 percent of cells with an abnormal signal (statistical comparison ND).
			Other preanalytic issues	SOE=NA For questions related to preanalytic issues impacting analytic validity, the data were sparse or not informative.
			Prevalence of genetic marker	SOE=NA No direct evidence for prevalence of the markers detected by ISH (i.e., TERC, MYC, HPV 16, and HPV 18) in US Medicare beneficiaries.
3a. Clinical validity	Women screened or tested for cervical cancer with finding of LSIL or ASCUS on cytology, with or without HPV infection	Any ISH test for TERC, MYC, HPV 16, or HPV 18	CIN2+ or CIN3+	SOE=low 10 Studies provided information on clinical validity of FISH tests for CIN2+ or CIN3+. Of these, 8 provided results for FISH on TERC (3 tested for TERC or MYC) and 3 studies provided results for FISH for HPV 16 or 18 (1 study tested both probe types, separately). In one study all women were HPV positive (type not reported); HPV status in the other studies was not known. Meta-analysis of 7 studies of TERC in LSIL for CIN2+ found summary sensitivity of 0.76 (95% CI 0.60, 0.86) and summary specificity of 0.79 (95% CI 0.50, 0.93). Meta-analysis of 5 studies of TERC in LSIL for CIN3+ found summary sensitivity of 0.78 (95% CI 0.65, 0.87) and summary specificity of 0.79 (95% CI 0.51, 0.93). 2 Studies compared different test combinations. • One compared results of FISH for TERC, Hybrid Capture 2 for high-risk HPV, and either test. FISH for TERC alone showed lower sensitivity but higher specificity than the combination of FISH or Hybrid Capture 2. • The other study compared three test strategies: FISH for TERC or MYC, Hybrid Capture 2 for high-risk HPV, and

Key Question	Population	Test/Assay	Outcome	Strength of Evidence Summary and Comments
				FISH for TERC, MYC, or HPV. FISH for TERC or MYC alone showed lower sensitivity but higher specificity than either other test strategy. For other cytology classifications and tests, the numbers of studies was limited. 3 Studies had data on FISH for TERC (without MYC) in women with ASCUS. One included only samples positive for HPV. There were also only 3 studies with data on FISH for HPV 16 or 18 in women with LSIL or ASCUS. Across all studies and tests, there was a trade-off between sensitivity and specificity, which may indicate a threshold effect. However, there was also great clinical heterogeneity across populations and test probes, Assessment of risk of bias showed low study quality or incomplete reporting. There was inconsistency in effect estimates and many were imprecise. The evidence was considered direct for clinical validity. Overall, the strength of evidence was graded as low, failing to show consistently better sensitivity or specificity with FISH testing for identification of CIN2+ or CIN3+ than would be expected by chance.
3a. Clinical validity	Women screened for cervical cancer with finding of LSIL or ASCUS, with or without HPV infection	Any ISH test for TERC, MYC, HPV 16, or HPV 18	Clinical outcomes	SOE=insufficient No studies examined the association of ISH test results with clinical outcomes.
3b. Prevalence of the outcome in comparison to the Medicare population		NA	Prevalence of disease (CIN2+ or 3+)	SOE=NA No direct evidence for prevalence of CIN2+ or 3+ in US Medicare beneficiaries.
4. Clinical utility	Women screened for cervical cancer	Any ISH test for TERC, MYC, HPV 16, or HPV 18	Clinical outcomes	SOE=insufficient No studies compared patient care strategies among various tests, thresholds, or combinations of ISH or non-ISH tests. Potential harms associated with colposcopy and biopsy are transient cervical bleeding and discharge or infection with fever and moderate-to-severe pain. Cervical conization can be complicated by cervical stenosis or incompetence resulting in fetal prematurity or infertility. An ablative procedure can also complicate subsequent testing for cervical cancer, as it makes it more difficult to visualize any lesions. Harms from false positive findings are anxiety and unnecessary procedures. Harms from false negative findings are the missed opportunity for early and potentially curative treatment.

ASCUS=atypical squamous cells of undetermined significance, CI=confidence interval; CIN=cervical intraepithelial neoplasia, FISH=fluorescence in situ hybridization, HPV=human papillomavirus, ISH=in situ hybridization, KQ=key question, MYC=myelocytomatosis oncogene, NA=not applicable; LSIL=low-grade squamous intraepithelial lesion, NHANES=National Health and Nutrition Examination Survey; PCR=polymerase chain reaction, SOE=strength of evidence, TERC=telomerase RNA component gene, US=United States.

The horizon scan conducted for Key Question 1 led to the subsequent focus on ISH tests for TERC, MYC, HPV 16, or HPV 18 as tests for cervical abnormalities or cancer.

Our review of data on analytic validity for Key Question 2 revealed a paucity of evidence. We found no studies examining the association between ISH for TERC or MYC and another genetic test in cytology or histology samples. For HPV, we identified some studies for which we could examine the correlation between ISH and reference tests, namely PCR and Hybrid Capture 2. However, these tests measure different biological parameters since, unlike ISH, the reference HPV tests are not restricted to detecting nuclear episomal or integrated HPV. (In situ ISH testing for HPV, which is the only ISH that can identify integration into the genome, may add information beyond the most common ISH testing for 13 or 14 types of HPV or ISH for HPV 16 and 18, which only indicate HPV infection, not integration.)

Further, the panels of HPV genotypes tested for by ISH and the reference tests varied and were not completely overlapping. This heterogeneity limits the conclusions that can be drawn about analytic validity. Not surprisingly, the agreement between ISH tests and reference tests was inconsistent across the studies.

Risk of bias assessment of analytic validity studies showed variable detail of reporting, which was particularly poor for the reference tests. Review of the evidence on thresholds for ISH tests also showed incomplete reporting as well as variable thresholds of positivity and chromosomal control probes used. Information on other preanalytic issues was sparse or not informative. The lack of data on reproducibility is a major deficiency in the evidence base. This suggests a need for research to explore thresholds and standardize test procedures.

For Key Question 3 on clinical validity, the strength of evidence for ISH testing was graded as low, failing to show that the addition of ISH tests resulted in better clinical validity. Clinical practice guidelines suggest that ISH is a potential add-on test after initial Pap testing, with subsequent HPV testing, or after initial Pap and HPV cotesting. In this context, it is more desirable for ISH to show high specificity than high sensitivity. In our review, FISH testing did not show consistently increased sensitivity for the identification of CIN2+ or CIN3+ on histology, although it was more specific than other tests or test combinations. However, we cannot conclude that ISH testing would increase clinical validity of an overall screening strategy. As compared with FISH or Hybrid Capture 2 testing for HPV, FISH for TERC or MYC alone was more specific and less sensitive than the test combinations.

Regarding Key Question 4, we found no studies examining the association of ISH test results with clinical outcomes. There were also no comparative studies of strategies that include ISH tests that examined clinical utility, which would be of particular interest for colposcopy rates and histology results.

Comparison with Current Knowledge

ISH tests are not used routinely used in screening for cervical cancer at this point. However, there is a need to improve the clinical validity of screening for cervical cancer. Thus there is a potential role for tests such as ISH. HPV tests for panels of high-risk genotypes have been shown to have a higher sensitivity but lower specificity that what we found for ISH tests.[23] Thus, when cotesting is used, add-on tests with greater specificity may be useful. However, HPV testing is evolving, and new reference tests for HPV testing will change the performance of add-on tests. ISH may need to be examined as an alternative to tests that can identify HPV 16 and 18 individually. Further, the recent launch of HPV vaccination in adolescents is expected to change the natural history of HPV-associated cervical carcinoma going forward.

Applicability

Formal appraisal of applicability of the Key Question 3 studies on clinical validity with the QUADAS-2 tool showed no major concern regarding applicability. However, studies included populations from around the world, with variable prevalence of HPV infections, CIN classes, and cervical cancer.

CMS has a particular interest in the Medicare population, whose core beneficiaries are 65 years of age or older. On the basis of the lower incidence of HPV infection and cervical cancer among older women who have undergone adequate screening than among younger women, the 2012 guidelines recommend cessation of screening after the age of 65 years (so long as screening tests were negative in the prior 10 years). Since a notable proportion of Medicare beneficiaries are younger than 65, the findings of the report are still relevant for CMS.

Implications for Clinical and Policy Decisionmaking

The current evidence base is insufficient to consider routine ISH testing in the clinical scenarios analyzed in the report. Specifically the evidence is insufficient to recommend routine ISH testing for TERC, MYC, HPV 16 or 18 in women screened or tested for cervical cancer with a finding of LSIL or ASCUS on cytology, with or without HPV infection.

Limitations

Our review is limited to published reports, which usually do not allow for detailed analysis of individual patient data for subgroups of interest. Studies evaluating more than one test approach did not include cross-tabulation of positive and negative test results across all tests. Our review addresses a limited scope based on what was determined to be the most meaningful clinical questions. Given our stringent inclusion criteria for articles, requiring the mention of cytologic or histologic sampling in the abstract, we may have missed studies that could have contributed additional data for the review of analytic validity.

Regarding Key Question 3 on clinical validity of ISH in particular, the identified evidence base was limited. Studies were generally small and those that we could meta-analyze yielded imprecise effect estimates. Study samples often were from sample banks or databanks, limiting the applicability to the screening population. With one exception, the included studies did not unequivocally report or stratify by HPV status. Studies conducted before the Bethesda terminology change that divided ASCUS into ASC-US and ASC-H may have included a mix of ASC-US and ASC-H in their ASCUS group. There was clinical heterogeneity among the results, given the variety of ISH probe panels used across studies and differences between ISH and the reference tests within studies. In addition, the reporting of study quality items was deficient. No studies examined risk prediction with ISH or the test's clinical utility or addressed screening for cervical adenocarcinoma in particular.

Research Gaps

Our review reveals four major research gaps. First, the assessment of the analytic validity of ISH (Key Question 2) highlights a need to establish common thresholds, probe sets, controls, and procedures. An expert conference may be helpful to agree on common measurement guidelines, a path that was successfully pursued to arrive at the consensus Bethesda classification for cytological abnormalities in cervical cancer. Scoring of ISH slides can be time-consuming.

Automated approaches are promising, but in order for ISH to become a routine test, the evaluation of test results needs to be standardized and accelerated.

Second, bigger studies are needed to yield more precise estimates and to have adequate numbers of individuals with CIN3+ on histology, which is considered a more valid marker for the risk to cancer progression than CIN2+. Third, future research should reflect changes in clinical practice. On the basis of the current guidelines, it can be expected that Pap with reflexive HPV testing or Pap–HPV cotesting will become more widely used. This will require study of the clinical validity of ISH as an add-on test in groups of women characterized as having a normal Pap or ASCUS or LSIL along with a positive or negative HPV test. It is also expected that HPV testing will eventually be able to routinely identify not only high-risk HPV genotypes broadly but also HPV 16 and 18 individually, with the use of either sequential or combined tests. This will require reevaluation of the role of ISH, which we considered in this review as a hypothetical alternative to testing for HPV 16 or HPV 18. Development of automated HPV testing may provide an incentive to explore the performance of up-front HPV testing rather than Pap testing, since testing of cervical cytologic specimens requires a trained human operator. This would generate another constellation in which to study the value added by ISH testing.

Fourth, changes in terminology should be followed in future studies, specifically the differentiation between ASC-US and ASC-H and the use of LAST terminology, including p16 staining in ambiguous cases for classifying histology as LSIL or HSIL. Fifth, further evaluation of clinical validity of ISH should be better designed to achieve this aim. Studies could examine ISH testing for not only a single probe (such as TERC) but also panels of probes, for example for both TERC and HPV. Ideally, large studies would allow for the comparison of multiple tests in order to make it possible to select tests with best analytic validity as well as clinical validity for CIN. However, to measure false negative rates, colposcopy would need to be performed in patients with negative screening tests. Such studies should therefore identify the tests, thresholds, and combinations that are most promising for further evaluation of clinical utility. Efficient exploration of the correct test use (i.e., the testing with the best performance) would again be conducted with several promising tests, thresholds, and test combinations studied simultaneously in a sufficiently large sample on the same specimens and follow patients with routine or test-directed care to assess impacts on diagnostic thinking, evaluation, management, and clinical outcomes. Projecting the clinical utility of different tests may entail modeling of data from different studies in decision analyses.

Lastly, the role ISH testing for detection of adenocarcinoma should be examined. The variability in chromosomal aberrations between squamous-cell cancer and adenocarcinoma suggests that a panel of ISH probes, rather than a single probe, would capture a greater variety of chromosomal changes.

Conclusions

This report shows an emerging body of literature on ISH testing for cervical cancer. Although ISH tests are marketed by some laboratories for triaging women with abnormal screening tests, there is a lack of standardization of probes and procedures that needs to be addressed. The role of add-on ISH testing has not been adequately examined in current screening contexts, that is, after HPV and Pap testing. Further, HPV testing is likely to evolve, for example with primary screening for 13 or 14 HPV genotypes or with wider availability of HPV16/18 testing. This will again require reexamination of the role of add-on tests such as ISH, and its impact not only on diagnostic utility but also on clinical utility (in particular colposcopy) and on

clinical outcomes. Thus, the evidence is currently too immature to suggest ISH testing for routine practice.

Acronyms

AHRQ	Agency for Healthcare Research and Quality
ASCP	American Society for Clinical Pathology
ASC-H	Atypical squamous cells, cannot exclude HSIL
ASCUS (cytologic classification)	Atypical squamous cells of undetermined significance
CIN	Cervical intraepithelial neoplasia, with mild dysplasia (CIN1), moderate dysplasia (CIN2), or severe dysplasia (CIN3)
CMS	Centers for Medicare and Medicaid Services
FISH	Fluorescence in situ hybridization
HPV	Human papillomavirus
HSIL	High-grade squamous intraepithelial lesion
ISH	In situ hybridization
LSIL	Low-grade squamous intraepithelial lesion
MYC	Myelocytomatosis oncogene (on chromosome 8, band q24)
Pap test	Papanicolaou test (of cervical cytology)
QUADAS-2	Quality Assessment of Diagnostic Accuracy Studies 2
SCC	Squamous-cell carcinoma
TERC	Telomerase RNA component gene (on chromosome 3, band q26)
USPSTF	U.S. Preventive Services Task Force

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Background

Overview

Cervical cancer is a highly preventable disease. Most cases are related to infection with human papillomavirus (HPV).[1] Persistent cervical infection with high-risk, oncogenic HPV genotypes is necessary for the development of cervical cancer and its immediate precursor lesion, high-grade cervical intraepithelial neoplasia (CIN).[2] Progression is generally slow and screening has the potential to detect precancerous lesions and cancers in early stages, which can be effectively treated. Thus, in the United States, regular screening is recommended for all women between the ages of 21 and 65 years.[3]

Incidence and mortality rates for cervical cancer vary globally, depending on the availability of cervical screening and prevention programs. In the United States, which has widespread screening practices, there were more than 12,000 new cases of cervical cancer and 4,220 related deaths in 2011.[1,4]

The screening tests currently used in the United States on cervical cell samples include the Papanicolaou (Pap) test to detect cellular changes, as well as tests for high-risk HPV genotypes. Although both tests identify a large proportion of women who harbor high-grade CIN, in a large number of women abnormalities detected on cytology will spontaneously resolve or will not be confirmed on histologic examination by colposcopy. A particular challenge is the management of women with test results of atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesions (LSIL) on cytology or those with a normal Pap test but a positive test for high-risk HPV genotypes, since only a fraction of these women will have a finding on colposcopically directed tissue biopsy that warrants treatment. The median percentage of all Pap tests reported by various U.S. laboratories in 2009 for ASCUS ranged from 2.9 to 4.8 percent and for LSIL, from 1.2 to 2.8 percent, depending on the cytology preparation method (according to the College of American Pathologists Laboratory Accreditation Program).[5] Less than one-fifth of women with these findings will have a finding on colposcopically directed biopsy that warrants treatment.[6] Colposcopy incurs expense and may be associated with adverse events.[7,8] Another complication of an ablative procedure is that on subsequent cervical cancer screening, if a screening test comes back abnormal, it can be difficult to visualize the lesion and allow the lesion to be optimally treated.

Thus, testing strategies that can more accurately triage patients to colposcopy are needed, to minimize overtreatment. Tests being marketed and offered by some laboratories include in situ hybridization (ISH) tests to detect chromosomal abnormalities or HPV DNA. This Technology Assessment (TA) examines the role of in situ hybridization (ISH) tests, including fluorescence ISH (FISH), to detect chromosomal abnormalities or HPV DNA on cervical cytologic specimens and their clinical validity for identification of precancerous lesions or cervical cancer.

Terminology for Cervical Lesions

In the United States, most laboratories use the Bethesda system for reporting cervical cytology (**Table 1**). The classification system was first developed in 1988 and has been revised several times since, most recently in 2001.[9] Squamous-cell abnormalities are the most common, although abnormal glandular cells can also be found.

Table 1. The 2001 Bethesda System for Reporting Cervical Cytology[10]

Specimen Type

Indicate: conventional test (Pap test), liquid-based preparation, or other

Specimen Adequacy

- Satisfactory for evaluation (describe presence/absence or endocervical/transformation zone component and any other quality indicators, e.g., partially obscuring blood inflammation, etc.)
- Unsatisfactory for evaluation (specify reason)
    - Specimen rejected/not processed (specify reason)
    - Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason)

General Categorization (Optional)

- Negative for intraepithelial lesion or malignancy
- Other: see Interpretation/Result (e.g., endometrial cells in a woman aged 40 years or older)
- Epithelial cell abnormality: See interpretation/Result (specify "squamous" or "glandular" as appropriate)

Interpretation/Result

- Negative for intraepithelial lesion or malignancy (when there is no cellular evidence of neoplasia, state this in the General Categorization above, in the Interpretation/Result section of the report, or both-whether or not there are organisms or other nonneoplastic findings)
    - Organisms
        - Trichomonas vaginalis
        - Fungal organisms morphologically consistent with Candida species
        - Shift in flora suggestive of bacterial vaginosis
        - Bacteria morphologically consistent with Actinomyces species
        - Cellular changes consistent with herpes simplex virus
    - Other nonneoplastic findings (optional to report; list not inclusive)
        - Reactive cellular changes associated with
            - Inflammation (includes typical repair)
            - Radiation
            - Intrauterine device
        - Glandular cells status posthysterectomy
        - Atrophy
- Other (list not comprehensive)
    - Endometrial cells (in a woman aged 40 years or older) (specify if negative for squamous intraepithelial lesion)
- Epithelial cell abnormalities
    - Squamous cell
        - Atypical squamous cells (ASC)
            - Of undetermined significance (ASC-US)
            - Cannot exclude HSIL (ASC-H)
        - Low-grade squamous intraepithelial lesion (LSIL) (encompassing: human papillomavirus/mild dysplasia/cervical intraepithelial neoplasia (CIN) 1
        - High-grade squamous intraepithelial lesion (HSIL) (encompassing: moderate and severe dysplasia, carcinoma in situ; CIN2 and CIN3)
        - With features suspicious for invasion (if invasion is suspected)
        - Squamous cell carcinoma
    - Glandular cell
        - Atypical
            - Endocervical cells (not otherwise specified or specify in comments)
            - Endometrial cells (not otherwise specified or specify in comments)
            - Glandular cells (not otherwise specified or specify in comments)
        - Atypical
            - Endocervical cells, favor neoplastic
            - Glandular cells, favor neoplastic

▪ Endocervical adenocarcinoma in situ (AIS) ▪ Adenocarcinoma • Endocervical • Endometrial • Extrauterine • Not otherwise specified • Other malignant neoplasms (specify)
Ancillary Testing Provide a brief description of the test method(s) and report the result so that it is easily understood by the clinician. Automated Review If case examined by automated device, specify device and result
Educational Notes and Suggestions (Optional) Suggestions should be concise and consistent with clinical followup guidelines published by professional organizations (references to relevant publications may be included)

Table 2. Recommendations for Lower Anogenital Squamous Terminology (LAST) for HPV-Associated Lesions).[2]

Squamous Intraepithelial Lesions
Recommendation:
1. A unified histopathologic nomenclature with a single set of diagnostic terms is recommended for all HPV-associated preinvasive squamous lesions of the lower anogenital tract (LAT)
2. A two-tiered nomenclature is recommended for noninvasive HPV-associated squamous proliferations of the LAT, which may be further qualified with the appropriate –IN terminology –IN refers to the generic intraepithelial neoplasia terminology without specifying the location. For a specific location, the appropriate complete term should be used. Thus, for an –IN 3 lesion: cervix = CIN3, vagina =VaIN 3, vulva =VIN 3, anus = AIN 3, perianus = PAIN 3, and penis =PeIN
3. The recommended terminology for HPV-associated squamous lesions of the LAT is LSIL and HSIL, which may be further classified by the applicable –IN subcategorization

Histologic changes (those detected on biopsy) are described as cervical intraepithelial neoplasia (CIN). CIN is categorized according to the depth of involvement and the atypicality of the cell into three degrees of severity. CIN1 is considered a low-grade lesion. It refers to mildly atypical cellular changes in the lower third of the epithelium (formerly called mild dysplasia). HPV-induced cytopathic effects (koilocytotic atypia) are often present. CIN2 is considered a high-grade lesion. It refers to moderately atypical cellular changes confined to the basal two-thirds of the epithelium (formerly called moderate dysplasia) with preservation of epithelial maturation. CIN3 is also considered a high-grade lesion, but it refers to severely atypical cellular changes encompassing greater than two-thirds of the epithelial thickness and includes full-thickness lesions (formerly called severe dysplasia or carcinoma in situ). Invasive cancer may also be diagnosed on histology.

Recent consensus recommendations by the LAST initiative aim to unify the terminology for HPV-associated lower anogenital lesions (**Table 2**).[2]. This new terminology classifies into a two-tiered system of low-grade squamous intraepithelial lesions (LSIL) and high-grade intraepithelial lesion (HSIL), similar to the Pap testing classification. The pathologist must still determine the CIN category on the basis of what proportion of the epithelium is involved (lower one-third, lower wo-thirds, or full thickness) and then stratify to LSIL or HSIL. CIN1 under the old classification maps to LSIL, and CIN3 maps to HSIL. CIN2 is a biologically equivocal lesion and if this diagnosis is entertained by a pathologist, LAST recommends stratification to high-

grade or low-grade disease on the basis of p16 immunohistochemistry. The LAST terminology is not yet broadly used.

Generally, a higher grade of cytology indicates a greater risk for higher-grade histology but cytology may also be associated with both more or less severe histologic findings.

Natural History of Cervical Cancer

The cervix is the lower narrow portion of the uterus that consists of an ectocervix, lined by squamous epithelium and an endocervix, lined by glandular epithelium. The ectocervix transitions to the vagina inferiorly. The endocervix is superior to the ectocervix and transitions to the endometrial cavity of the uterus. The transition from ectocervical lining to endocervical lining is called the transformation zone.

Infection with sexually transmitted HPV can cause genital warts as well as cervical cancer. HPV infection can be acute or persistent. Acute infection can disappear within 1 to 2 years, most likely because of eradication by the woman's immune system. Persistent infection can occur in a small number of women who then are at increased risk of developing cancer.[11] The risk factors for persistent HPV infection include smoking, a compromised immune system, and possibly coinfection with other sexually transmitted viruses (e.g., herpesvirus).[12] Low socioeconomic status is also associated with an increased risk of cervical cancer.[13]

HPV infection and precancer do not cause symptoms but early genetic and morphologic changes can be seen in cervical cells with various tests. Early detection permits early treatment, which is more likely to be successful than treatment at later stages. Morphologic signs of precancer show up as abnormal-looking cells that can be seen under a microscope in a Pap smear or biopsy sample of cervical tissue. Genetic signs of precancer and HPV infection take the form of chromosomal changes (e.g., extra copies or too few copies of human genes) and the presence of HPV DNA, respectively, both of which can be detected by genetic tests such as ISH and PCR that involve probes (certain molecules) that bind to the DNA of interest. Other co-factors for cancer given infection are multiparity and long-term oral contraceptive use, but so far it is unclear in what stages in the carcinogenic process the co-factors act.

Epidemiology of Cervical Cancer

Incidence and Mortality

The incidence of and mortality from cervical cancer have decreased over time in the United States and in other developed countries, as screening has increased.[11] In 2012, there were more than 12,000 new cases and 4,220 deaths.[1,4] The age-adjusted incidence rate in the U.S. was approximately 15 cases per 100,000 women in 1975 and declined consistently over the next two decades to less than 7 cases per 100,000 women in 2009.[14] The corresponding rates of death have similarly decreased, with an estimated mortality of more than 5 women per 100,000 in 1975 to just over 2 women per 100,000 in 2009. The incidence remains high in developing countries, however, which carry more than 85 percent of the burden of disease[1,15]; and cervical cancer remains the second most common cause of cancer deaths among women worldwide.[3]

Squamous-cell carcinoma (SCC) accounts for about 80 to 90 percent of cases of cervical cancer, with adenocarcinoma accounting for the rest.[16] Although adenocarcinoma currently is diagnosed in fewer cases than SCC, its incidence is increasing and it can be harder to detect on

Pap testing because distinguishing features for glandular lesions are more subtle and challenging to interpret. Limited sampling may also play a role.

Incidence and mortality from cervical cancer are known to vary among racial/ethnic groups and among age groups in the United States. The incidence and mortality are lower among white women than among nonwhite women.[3,11] Hispanic women are most likely to get cervical cancer, followed by blacks, American Indians and Alaskan natives, whites, and Asians and Pacific Islanders.[14]

Cervical cancer tends to occur in midlife; the median age at diagnosis is 48 years.[14] Most cases are found in women younger than 50 years; few occur in women younger than 20.[11] Older women still have a risk of developing cervical cancer. More than 20 percent of cases of cervical cancer are found in women over 65 years of age. However, these cases are typically in women who had not been screened regularly for cervical cancer earlier in life.[17]

Precancer or localized cancer can be treated to prevent progression to cancer or metastasis. Although 91.5 percent of women will survive 5 years when the cancer is localized, only 12.6 percent will survive distant disease.[14]

Role of Human Papillomavirus Infection in Cervical Cancer

Pathogenesis

HPV testing detects the presence of HPV DNA. Infection with specific high-risk genotypes of HPV is associated with risk for precancer or cancer. Using modern HPV detection methods, 95 to 100 percent of squamous-cell cervical cancer and 75 to 95 percent of high-grade CIN lesions have detectable HPV DNA.[18] Many of the more than 150 genotypes of HPV can be divided into "high-risk" and "low-risk" categories on the basis of their association with cervical cancer (**Table 3**).

Table 3. High-Risk HPV Types.[19]

Risk Group	HPV Types
High	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 82

HPV=human papillomavirus.

Of the approximately 30 to 40 HPV genotypes that infect the mucosa of the genital tract, 8 (types 16, 18, 31, 33, 35, 45, 52, and 58) are responsible for over 90 percent of cervical cancers and are therefore called "high-risk" genotypes.[20-22] Two of these genotypes (16 and 18) are alone responsible for about 70 percent of cervical cancers.[22] In the United States, 20 to 44 percent of women are infected with one of the approximately 150 HPV genotypes[23] (high risk or other), which may either be cleared by the body or may persist and over time cause precancerous lesions and ultimately malignancy. Recent work (in 2012) has linked HPV-related cervical cancers to abnormalities in a small, discrete population of cells located at the transformation zone of the cervix.[24]

Epidemiology of HPV Infection

The Centers for Disease Control and Prevention estimate that nearly 5.5 million new genital HPV cases occur each year in the United States.[25] It is estimated that more than 20 million people in the U.S. are infected with HPV at any given time.[26]

Genital HPV is acquired through sexual and genital skin-to-skin contact. Prevalence generally is greatest within a few years after the median age of first sexual intercourse (which is at 17 years in the United States).[3] About 90 percent of HPV infections become undetectable within a year or two.[3] Persistence beyond this time period is predictive of CIN3 or more severe disease in the subsequent years. High-risk HPV 16 or 18 causes a greater proportion of adenocarcinomas (about 85 percent of cases) than SCCs (about 70 percent of cases).[27] (Note that virtually 100 percent of cases of cervical cancer are caused by infection with one of the high-risk HPV genotypes.)

Cytologic Screening for Cervical Cancer

Screening tests are performed on a sample of cervical cells obtained from scraping the cervix during a speculum examination, called a Pap test. Conventional cervical samples are prepared by smearing the specimen on a slide. Liquid-based preparation involves placing the specimen into a liquid fixative solution (e.g., ThinPrep or SurePath). Both of these preparations are a form of cytologic sample (i.e., the cells are separated from adjoining cells, either spread across the slide or suspended in solution). (This is in contrast with histologic specimens, which are pieces of tissue in which the cells remain intact; histologic tests are not done at the screening stage.)

Papanicolaou (Pap) Testing

The Pap test is widely performed to screen for precancerous or cancerous changes in cervical cells and is usually reported in the United States according to the Bethesda system (see Table 1 above). LSIL, especially in young women, is generally associated with a transient HPV infection, whereas a finding of high-grade squamous intraepithelial lesion (HSIL) is more likely to be associated with persistent HPV infection and a higher risk of progression to cervical cancer.[28]

HPV Testing

HPV testing detects the presence of (i.e., infection with) various types of HPV DNA, even though cervical abnormalities may not be present or may not develop. Testing for high-risk HPV has been proposed in combination with Pap testing or as an add-on test to follow up an abnormal Pap result. If liquid-based cytology sampling is performed, there is typically sufficient specimen left over after Pap testing to permit HPV testing as well. Specimens for HPV testing can also be collected from the endocervix and placed in an HPV test transport medium.[29]

In clinical practice, HPV test results are generally reported as positive or negative for the high-risk HPV types overall (rather than a specific type). A negative test means simply that no oncogenic HPV types are currently detected or that the patient may have been previously infected with a high-risk subtype and cleared the infection.

Currently there are four tests for high-risk HPV approved by the Food and Drug Administration (FDA) for primary screening as a cotest with Pap screening, all of which detect either 13 or 14 HPV types[30]:

- The Digene Hybrid Capture 2 High-Risk HPV test (the first to receive FDA approval, in 2003), which identifies the presence of any of 13 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) but cannot identify specific types.
- The Cervista™ HPV HR test (approved in 2009), which identifies the presence of any of 14 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).

- The Cobas HPV test (approved in April 2011), which detects the presence of HPV 16 or HPV 18 as well as a pooled result for an additional 12 high-risk types (HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).
- The APTIMA® HPV assay (approved in October 2011), which detects the presence of any of 14 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) by identifying its messenger RNA.

One other FDA-approved test is the Cervista™ HPV 16/18 test. It identifies the presence of the high-risk HPV 16 or HPV 18, the two types that cause most HPV-associated cancers and the most aggressive types of cancer. This test was approved in 2009 only for use in women 30 years of age and older, in whom it is intended as a follow-up test after a less-specific positive HPV screen for the 14 high-risk types and adjunctively with cytology. At the present this test is not widely adopted or available. However, as it is a more specific test than the HPV tests currently used to detect any of 13 or 14 HPV types, it could be a reflex test in some circumstances (see Figures 2–4) and therefore a theoretically credible alternative to non–ISH-based HPV 16/18 testing.

Generally data suggest that the prevalence of HPV infection in cytologic specimens increases with increasing severity of cytologic classification. In the United States in 2007, the prevalence of HPV infection was approximately 13 percent among women with normal cytology, 79 percent among women with low-grade lesions, 85 percent among women with high-grade lesions, and 87 percent among women with cervical cancer.[31] Another source claims that 85 to 95 percent of women with LSIL will be positive for HPV.[32] Therefore, it adds little to risk stratification in this group. However, HPV testing in individuals with ASCUS is helpful, as the risk of developing high-grade CIN would be considered to be the same in a patient with LSIL as in a patient with ASCUS and HPV positivity.

Management of Abnormal Screening Results

If abnormal cells are detected on Pap testing, then further evaluation is conducted with a colposcopy, a procedure in which the cervix is viewed at high magnification, and colposcopically directed cervical biopsy.[33]

Histology allows for triage to either a low or a high-grade lesion, with the latter requiring ablative therapy. Histologic examination is useful because it decreases the number of unnecessary ablative procedures, since some equivocal lesions on cytology will regress spontaneously or be so small that they cannot be identified on colposcopic sampling and presumably do not pose an immediate threat warranting definitive ablative therapies. On the other hand, high-grade lesions diagnosed by cytology have a low false positive rate. Therefore, a "see and treat" approach for patients with HSIL findings on cytology is acceptable. "See and treat" allows an ablation to take place upon the first colposcopic visit and avoids the cost of an additional colposcopic exam and histologic confirmation.

Ablative treatments use loop electrosurgical excision procedure (LEEP), laser therapy, or cryotherapy to remove or destroy the surface layers of the cervix and confirm that no invasive disease is present. More aggressive treatments for cancer include radical hysterectomy with pelvic lymph node dissection or radiation and chemotherapy.

Colposcopy and ablative treatments have adverse outcomes including discomfort, anxiety, risk of subsequent cervical incontinence with fetal loss or prematurity, and financial cost.[7,8] Thus, the goal of cervical-cancer screening is to enhance sensitivity and specificity for detecting

high-grade CIN (i.e., CIN2 or CIN3) on histopathology in order to maximize the true positive results and minimize the false positive ones.

Current Guidelines for Cervical Cancer Screening and Treatment

In March 2012, the U.S. Preventive Services Task Force (USPSTF) released new recommendations for screening women for cervical cancer.[18] At the same time, the American Cancer Society (ACS), the American Society for Colposcopy and Cervical Pathology (ASCCP), and the American Society for Clinical Pathology (ASCP) jointly published another set of screening recommendations.[3]

Guidelines issued by the USPSTF suggest screening with a Pap test every 3 years for all healthy women ages 21 through 65 years. The 2012 guidelines by ACS/ASCCP/ASCP[3] recommend cytology testing alone every 3 years, but in women aged 30-65 years, state a preference for HPV and cytology cotesting every 5 years. The guidelines by the ACS, ASCCP, and ASCP give preference to Pap and HPV cotesting, on the basis of evidence from randomized trials showing that cotesting results in earlier detection of high-grade CIN or invasive cancer, and women who have undergone cotesting have a lower risk of high-grade CIN and invasive cancer after the first screening round allowing for screening at less frequent intervals[34-36] Because few studies have sufficient numbers of cancer cases to assess cancer risk directly, the guidelines considered the absolute risk of CIN3, including the rare cases of cancer (CIN3+) prior to or at the visit after a given visit, as the best measure of the risk of incident cervical cancer. In addition, cotesting offers greater risk reduction for adenocarcinoma of the cervix and its precursors.

Screening by HPV testing alone (without concurrent or subsequent Pap testing) is not currently recommended in the United States , but several clinical trials outside the United States in resource poor settings have documented the value of "primary HPV screening"—that is, if the HPV test is negative, no other testing is needed, and if the test is positive, cytologic Pap testing is effective for triage because of the high specificity of Pap test findings. Genotype-specific testing for HPV 16 or HPV 16/18 is only recommended as an option in one particular clinical setting: for women who have a normal Pap result and a positive HPV test. In these women, both tests should be repeated at an interval, or alternatively they can undergo genotyping to determine whether they have infection with HPV16 and/or HPV 18. If positive on HPV16/18 testing, then colposcopy is recommended. If negative, then retesting after 1 year is recommended.

Recent guidelines by ACOG[19] specify that women with ASCUS on Pap testing and a negative HPV test continue with routine screening as indicated for their age.

All guidelines recommend against screening for cervical cancer in women over the age of 65 years who have had negative results on an adequate number of previous screening tests (with "adequate number" defined as three consecutive negative Pap results or two negative Pap and HPV tests in the prior 10 years, with the most recent within the previous 5 years). Finally, women who have been vaccinated against HPV should follow age specific recommendations as unvaccinated women (i.e., starting at 21 years).

Principles of ISH

ISH is a technique that is used to detect and localize the presence or absence of a specific genetic sequence in cells using a complementary polynucleotide sequence, the probe. The probe is either directly tagged with a fluorescent compound such as Texas Red (in FISH) or indirectly tagged

with biotin (historically) or digoxigenin or dinitrophenol (more recently). After binding to the complementary sequences in the cell, the probes are either visualized under ultraviolet (UV) light (in FISH) or located using antibodies to the chemical tag and visualized using direct light (in chromogenic in situ hybridization [CISH]). Gains of a region are seen as additional spots in the cell, while deletions are seen as a loss of spots. The majority of probes in use are "cocktails" that combine the region of interest with a control probe, and loss or gain is determined with respect to the number of control signals. The technique is used to detect recurrent diagnostic changes in hematological malignancies (such as a deletion in the long arm of chromosome 5 in myelodysplastic syndrome), to look for gene amplification (e.g., HER2/neu gene in breast cancer), and to detect specific gene rearrangements that can be treated with particular drugs (e.g., ALK gene rearrangement in non-small cell lung cancer, translocation 9 and 22 in chronic myeloid leukemia).

The resolution of ISH is limited by the size of the probe available for a region. The majority of commercially available ISH probes range in size from ~100 Kb to ~900 KB and will detect genetic sequence of this size. Smaller sequences will not be detected. Scoring of ISH tests can be automated, reducing the potential for operator errors such as the evaluation of the wrong cell population or the incorrect area of a tumor section. Any ISH final interpretation must be rendered by a pathologist. The technique is reliable but requires appropriate specimen preparation for consistent results.[37] Differentiating signals from experimental background can be problematic with CISH, but not with FISH.

The most common tags are nonisotopic (do not involve a radioactive isotope). Nonisotopic ISH (NISH) tests include FISH and chromogenic ISH (one test of which is colorimetric signal-amplified ISH [CSAC-ISH]). ISH tests can be made more sensitive by combination with other techniques such as catalyzed reporter deposition (CARD). Isotopic ISH is not commonly used because the chemical tag is radioactive and requires additional safety and handling procedures.

In the case of ISH involving probes for HPV, the HPV infection can be seen to be episomal, when a uniform HPV signal pattern is observed (contained within and covering the entire nucleus, suggesting the presence of HPV DNA in the nucleus but not yet integrated into the human chromosomes) or integrated, when a punctate signal pattern (consisting of discrete dots) is seen.

Potential for ISH Tests for Cervical Cancer Screening

ISH has been proposed as an additional noninvasive test on cervical smears to detect chromosomal abnormalities (markers of chromosomal damage) or HPV DNA. ISH testing on cervical specimens is not yet widely established, but some laboratories have developed their own tests, and manufacturers are starting to promote the use of ISH testing to triage women to colposcopy based on their cytology, HPV result, and ISH test finding (e.g., www.cervicaldnadtextest.com/casestudies.php). Quest Diagnostics, a commercial laboratory vendor, now advertises FISH testing for TERC, noting that "women with LSIL or ASC-H Pap results" are appropriate candidates (ASC-H is a category related to and generally less prevalent than ASCUS and stands for atypical squamous cells, cannot exclude HSIL) (http://www.questdiagnostics.com/testcenter/testguide.action?fn=HematOnc/Cervix/TS_CervicalCancer_TERC_FISH.htm).

Human chromosomal abnormalities have been observed in cervical cancers and premalignant stages on DNA-based microarrays. The microarray literature demonstrates that gain of 3q or loss of 3p are frequent changes in cervical cancer.[38-48] This is particularly the case for squamous-cell

carcinoma, whereas for adenocarcinoma the linkage to chromosomal abnormalities is less strong and if present it is more likely to be associated with gains of 1p, 1q and loss of 4q and 13q.

Chromosome 3 is the site of the telomerase RNA component (TERC) gene, located at band 3q26, which encodes the telomerase RNA component. Telomerase is activated relatively early in the progression to cervical cancer,[49] making it a logical target for ISH probes in cervical cancer screening. Another gene of interest implicated in cervical cancer is the myelocytomatosis oncogene (MYC), located on chromosome 8 (band 8q24). MYC has been shown to be a common site of HPV DNA integration,[50] specifically by a high-risk type of HPV (HPV 18).[51] Thus MYC also is of interest in using ISH testing for chromosomal changes associated with cervical cancer.

Given the prominent role of high-risk HPV infections for cervical cancer development, HPV DNA it is also a potentially informative target for ISH testing. HPV 16 and HPV 18 are the most studied types. This raises the question about the role of ISH as add-on tests when prior screening tests have yielded abnormal results.

Aim of the Technology Assessment

The objectives of this TA were to examine how ISH testing for either human chromosomal abnormalities or for HPV DNA in addition to Pap and HPV testing of cervical cells affects the detection of cervical cancer and related clinical outcomes. A finding of CIN3 carries a high risk of progression to cancer. Indeed, CIN3 on histopathology, combined with the rare cases of cancer as CIN3+, is considered the best surrogate measure of incident cervical cancer risk.

The specific aims were to: 1) conduct a "horizon scan" to identify studies that have used any ISH tests on cervical cytologic or histologic samples and to identify the ISH probes most frequently studied; 2) examine the analytic validity (technical performance) of the most frequently studied ISH tests for detection of markers of chromosomal damage or oncogenic HPV DNA; 3) examine the clinical validity of ISH tests for detection of high-grade CIN or for prediction of cancer related clinical outcomes; and 4) examine the clinical utility of ISH testing (i.e., how ISH testing impacts presumptive diagnosis, patient evaluation, management, and ultimately patients' clinical outcomes). The Centers for Medicare and Medicaid Services (CMS) has requested this TA to inform its decisionmaking about the coverage of this technology.

Key Questions

The four Key Questions in this TA, drafted by CMS and refined by the Evidence-based Practice Center (EPC) through discussions with Agency for Healthcare Research and Quality (AHRQ) Task Order Officer and CMS experts, broadly follow the first three domains of the ACCE framework for evaluating genetic tests—Analytic validity, Clinical validity, and Clinical utility—but did not directly address the associated Ethical, legal and social implications (www.cdc.gov/genomics/gtesting/ACCE/acce_proj.htm#T1).

Key Question 1. What ISH tests have been used in cervical cytology or histology specimens?

To refine the scope for the detailed evidence review, we conducted a horizon scan of the literature to better understand the extent of the use of ISH tests for cervical abnormalities or cancer. On the basis of the findings of the horizon scan, we focused the subsequent review on ISH tests including probes for TERC (the telomerase RNA component gene, on chromosome 3,

band 3q26), MYC (the myelocytomatosis oncogene, on chromosome 8, band 8q24), HPV 16, or HPV 18.

Key Question 2. For ISH tests for TERC or MYC or HPV 16 or HPV 18 in cervical cytology or cervical histology:

a. What are the associations between ISH test results and reference test results? What thresholds were used for positive, indeterminate, and negative results of the ISH tests? What reference tests were used to assess the presence or absence of the genetic marker (TERC, MYC, or HPV 16 or 18)?
b. What is known about reliability and reproducibility of ISH tests? What genetic, environmental, or other factors are known to affect ISH test results (e.g., the presence of more than a certain proportion of necrotic tumor tissue in the sample or the presence of infection)?
c. Are there some conditions for which an ISH test is not able to give a clinically useable result?
d. What are the sample acceptance and rejection criteria for ISH tests?
e. What sample storage or preservation requirements are needed for a reliable ISH test result?
f. What variation occurs in results of the ISH test if performed in multiple laboratories?
g. What is the prevalence of the genetic marker(s) detected by the reference standards in Medicare beneficiaries by age or race/ethnicity?

Key Question 3. For ISH tests for TERC or MYC or HPV 16 or HPV 18:

a. What is the association between ISH tests on cytology for high-grade CIN or cervical cancer on histopathology or for clinical outcomes related to cervical cancer morbidity and mortality? What thresholds were used for positive, indeterminate, and negative results on the ISH tests?
b. How similar are the spectrum and prevalence of the histopathological abnormalities and cervical cancers found in the studies to the spectrum and prevalence in Medicare beneficiaries? How is diagnostic accuracy modulated by age, race, and ethnicity?

Key Question 4: For ISH tests for TERC or MYC or HPV 16 or HPV 18 in cervical cytology, what is the published evidence about the test's clinical utility and harms?

a. In comparative studies of ISH testing versus alternative testing (with similar or equivalent tests) or no testing, what is the effect on diagnostic thinking, evaluation, management, or clinical outcomes?
b. What are the clinical inclusion criteria in the studies? How similar are the populations to the core Medicare population (i.e., persons ≥65 years of age) overall as well as according to race/ethnicity?
c. How similar are the spectrum and prevalence of the cancers in these studies to the spectrum and prevalence in the core Medicare population?

Analytic Framework

The overarching analytic framework for the report is shown in **Figure 1**. Key Question 1 relates to a horizon scan of the literature to identify the most relevant ISH tests for subsequent detailed evidence review. Key Question 2 pertains to analytic validity, Key Question 3 to clinical validity, and Key Question 4 to clinical utility.

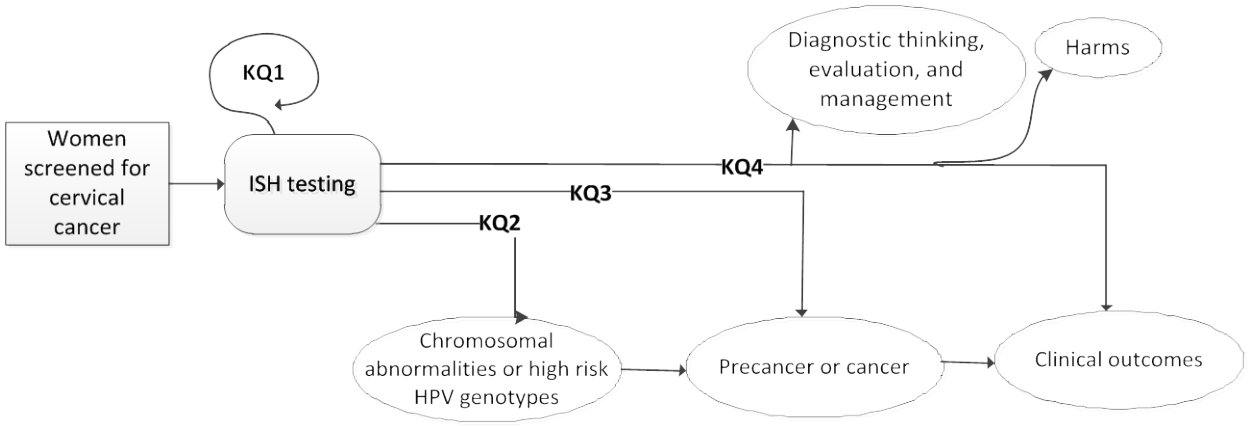
Key Question 3 was further focused on specific clinical scenarios according to currently recommended screening options in 2012 clinical practice guidelines (**Figures 2–4**).[3,18] In these clinical scenarios, the unshaded options show guideline-directed care, and the shaded options represent hypothetical choices for add-on testing with ISH or add-on testing for HPV 16 or HPV 18. Screening is recommended for women between 21 and 65 years of age.

Figure 2 starts with Pap testing which is followed by reflexive testing for HPV (13 or 14 types); this approach is currently recommended for women over 21 years of age. Figure 3 starts with cotesting for Pap and HPV (13 or 14 types), which is recommended as an alternative for women over 30 years of age. Finally, Figure 4 starts with HPV testing for 13 or 14 types. This is not currently recommended care.

Figures 2 through 4 show the test options in sequence; after the initial test, each test choice is contingent on the results of the prior test(s). All tests may be done on the same specimen, obviating repeated specimen acquisition and averting the need for repeated visits. An alternative to reflexive testing would be to do all testing simultaneously, but this would require increased resources.

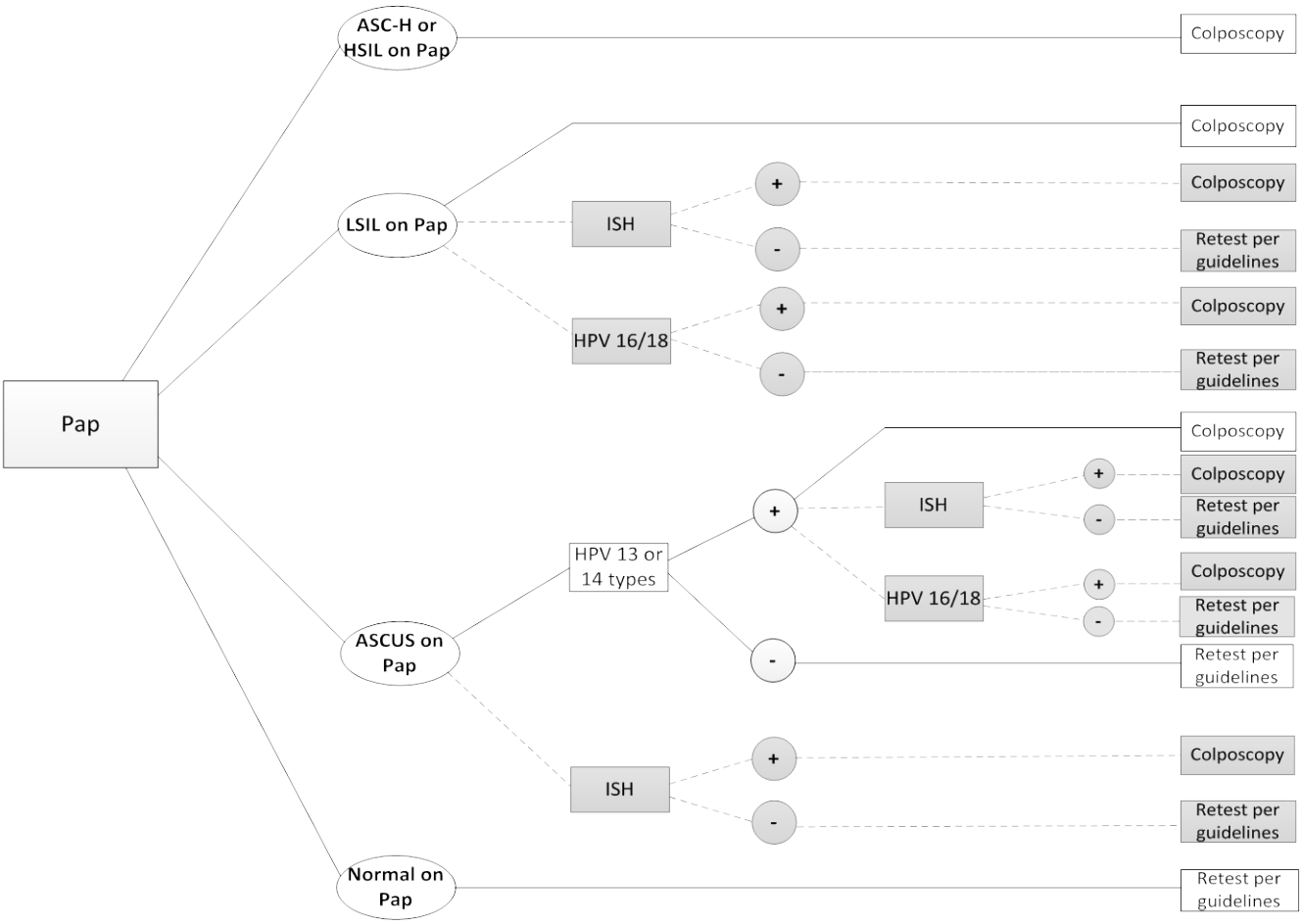
These figures show that a woman whose Pap results show HSIL or ASC-H would be referred to colposcopy; whereas a woman with normal Pap or with a normal Pap and a normal HPV results would be retested after a certain period. This leaves women with ASCUS or LSIL on Pap testing and those with a positive test for high-risk HPV, for whom additional testing with ISH might have a role as an add-on test instead of directly proceeding to colposcopy and as an alternative to non–ISH-based HPV 16/18 testing.

Figure 1. Analytic Framework.



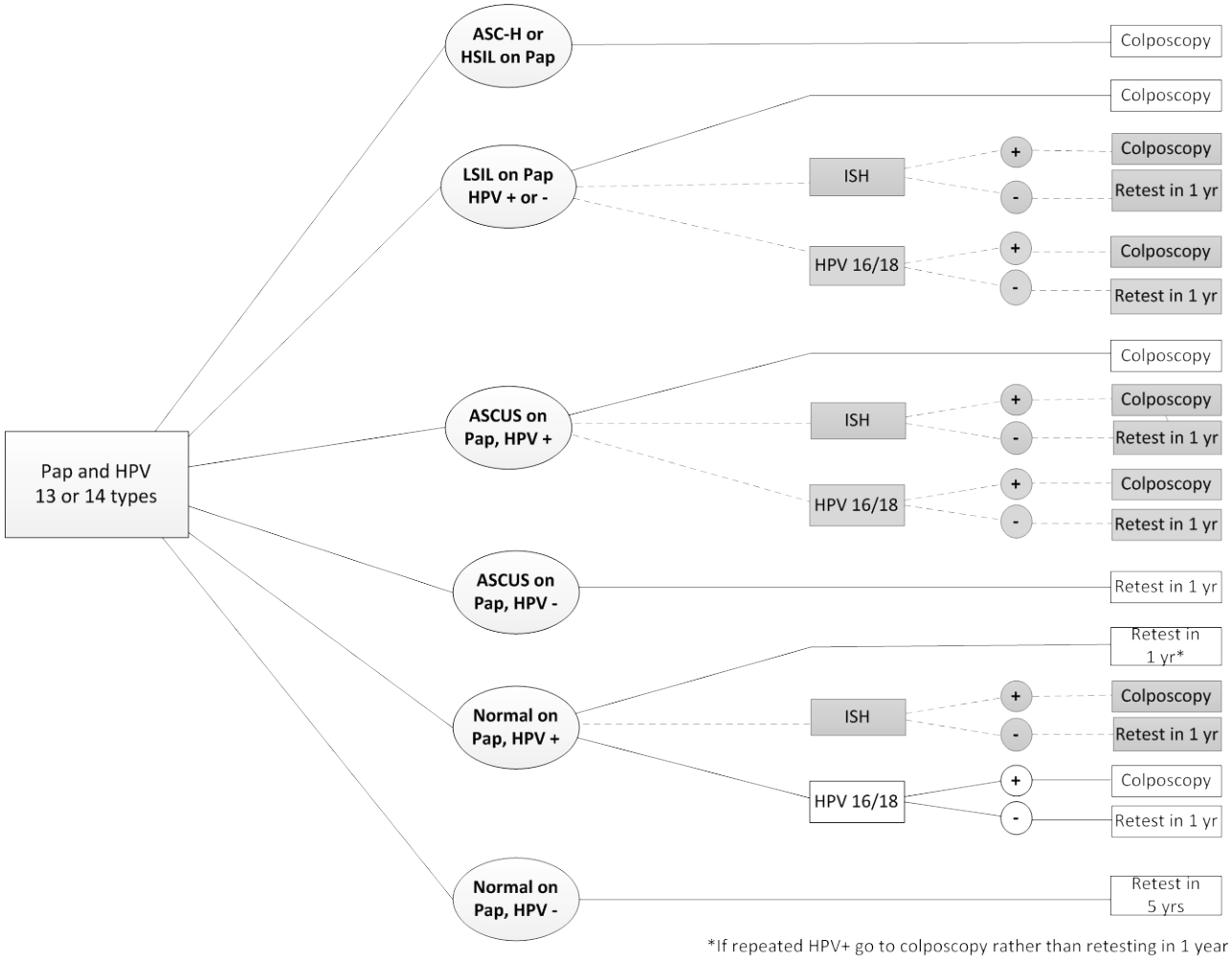
The key questions (KQs) are shown within the context of the population, tests, and outcomes. KQ1 reviews the existing literature on what in situ hybridization (ISH) test has been used in women tested for cervical cancer; KQ2 addresses the analytic validity of ISH testing to detect genetic abnormalities; KQ3 addresses the clinical validity of ISH testing to detect cervical dysplasia or malignancy; and KQ4 addresses the clinical utility of ISH testing to predict clinical outcomes, to affect diagnostic thinking, evaluation, and management, and to ascertain harms.

Figure 2. Testing Scenarios for Women Older Than 21 Years: Initial Papanicolaou (Pap) Testing Followed by Reflexive Testing for HPV (13 or 14 Types), as Recommended Under Current Guidelines.



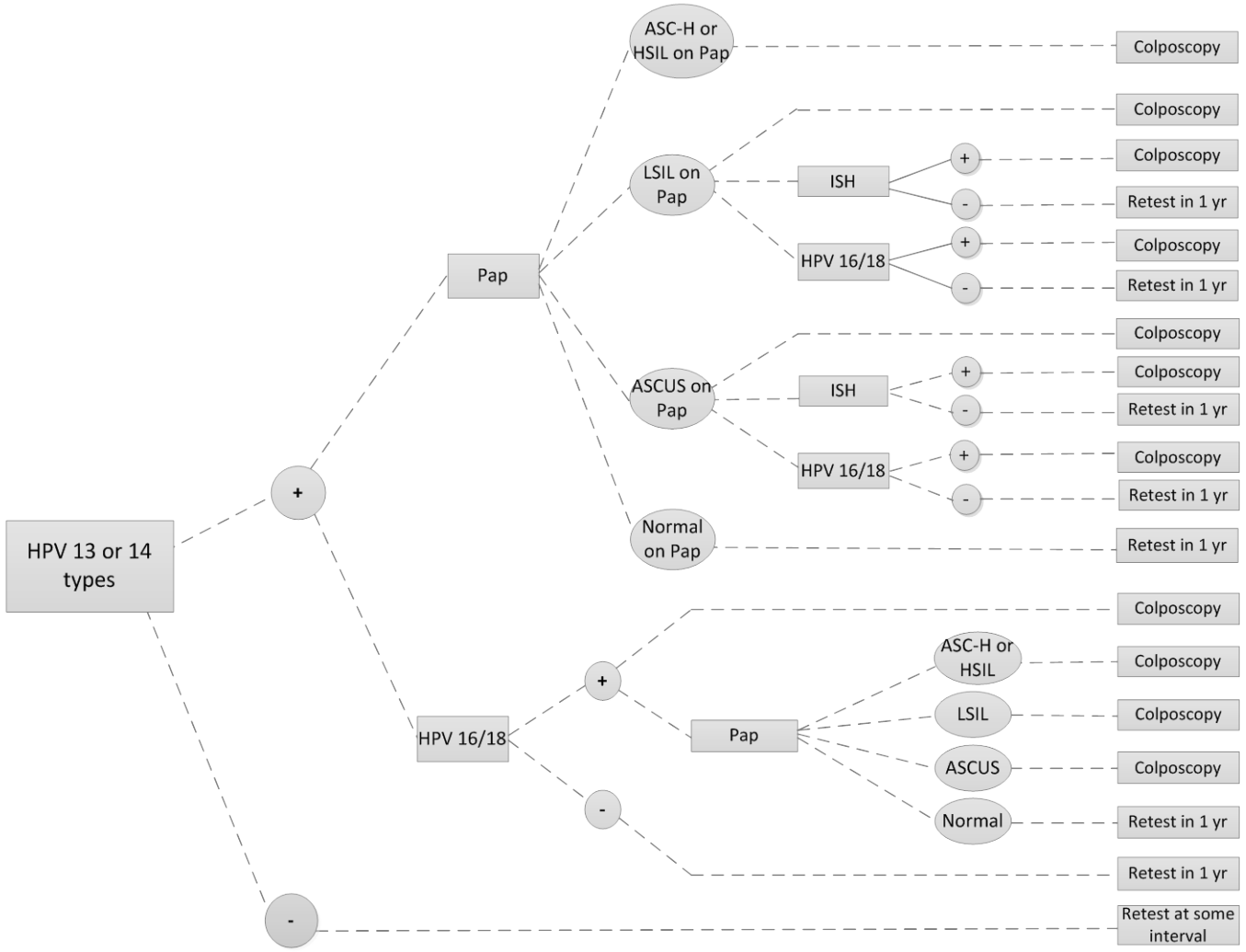
Test options are shown in sequence; after the initial test, each test choice is contingent on the results of the prior test(s). The dark gray–shaded test options with dashed lines are hypothetical alternatives to the currently recommended evaluation. Plus and minus signs indicate positive and negative results, respectively. "HPV 16/18" indicates HPV types 16 and/or 18 (both high-risk types), whereas "HPV 13 or 14 types" indicates testing for 13 or 14 types (depending on manufacturer of probe), a mix of low-, intermediate-, and high-risk types.
ASC-H= atypical squamous cells, cannot exclude HSIL; ASCUS=atypical squamous cells of undetermined significance; HSIL=high-grade squamous intraepithelial lesion; ISH=in situ hybridization; LSIL=low-grade squamous intraepithelial lesion; yr=years.

Figure 3. Testing Scenarios for Women Older Than 30 Years: Initial Cotesting with Papanicolaou (Pap) and HPV (13 or 14 Types).



Test options are shown in sequence; after the initial test, each test choice is contingent on the results of the prior test(s). The dark gray–shaded test options with dashed lines are hypothetical alternatives to the currently recommended evaluation. Plus and minus signs indicate positive and negative results, respectively. "HPV 16/18" indicates HPV types 16 and/or 18 (both high-risk types), whereas "HPV 13 or 14 types" indicates testing for 13 or 14 types (depending on manufacturer of probe), a mix of low-, intermediate-, and high-risk types.
ASC-H= atypical squamous cells, cannot exclude HSIL; ASCUS=atypical squamous cells of undetermined significance; HSIL=high-grade squamous intraepithelial lesion; ISH=in situ hybridization; LSIL=low-grade squamous intraepithelial lesion; yr=years.

Figure 4. Hypothetical Testing Scenarios with Primary Screening for HPV (13 or 14 Types) for Women 30 Years of Age or Older).



Test options are shown in sequence; after the initial test, each test choice is contingent on the results of the prior test(s). The dark gray–shaded test options with dashed lines are hypothetical alternatives to the currently recommended evaluation. Plus and minus signs indicate positive and negative results, respectively. "HPV 16/18" indicates HPV types 16 and/or 18 (both high-risk types), whereas "HPV 13 or 14 types" indicates testing for 13 or 14 types (depending on manufacturer of probe), a mix of low-, intermediate-, and high-risk types.
ASC-H= atypical squamous cells, cannot exclude HSIL; ASCUS=atypical squamous cells of undetermined significance; HSIL=high-grade squamous intraepithelial lesion; ISH=in situ hybridization; LSIL=low-grade squamous intraepithelial lesion; Pap=Papanicolaou test; yr=years.

Methods

The methods for this TA follow the methods suggested in the AHRQ *Methods Guide for Effectiveness and Comparative Effectiveness Reviews*[52](hereafter referred to as the Methods Guide; available at www.effectivehealthcare.ahrq.gov/methodsguide.cfm). We also referred to AHRQ's *Methods Guide for Medical Test Reviews*.[53]

Literature Search Strategy

Key Question 1 (Horizon Scan) and Scope Refinement

The four Key Questions and the guiding analytic frameworks are described in the Background section above. Briefly, Key Question 1 asked what ISH tests have been examined with what frequency in studies of cervical cytology or histology. This horizon scan served to focus the subsequent detailed evidence review on the most commonly studied ISH tests, namely ISH tests for TERC, MYC, or HPV 16 or 18. Key Question 2 examined analytic validity of these ISH tests, that is, the associations between ISH tests and reference tests for the corresponding chromosomal abnormality. Key Question 3 examined clinical validity of these ISH tests, specifically the clinical validity of these ISH tests on cervical cytology for high-grade CIN or cervical cancer. Key Question 4 examined the comparative effectiveness of ISH testing on clinical utility and possible harms.

Search Strategy

The search was conducted in MEDLINE®, SciVerse Scopus (including Embase) (Elsevier), and the Cochrane Central Register of Controlled Trials and had no language restrictions (**Appendix A**). Key words included terms related to the test of interest (in situ hybridization) and terms related to cervical cancer or abnormalities (cervical, precancerous, neoplasm, and cervical intraepithelial neoplasia). The first search was performed on November 7, 2011; the update search was conducted on July 12, 2012. We also searched the sections on gynecologic cancer for the past 2 years of proceedings of major gynecology and oncology conferences (e.g., ACOG, the American Society of Clinical Oncology, and the past year of the ASCCP) to identify recent but not yet published studies. We asked our technical experts to inform us of any potentially relevant articles. We did not contact authors for additional data.

Eligibility Criteria

The population of interest was women eligible for cervical cancer screening. The context was evaluation for cervical cancer after an abnormal screening test. Studies were eligible if they provided relevant data on cervical tissue samples from at least 10 women examined with ISH tests in a clinical or research setting

For Key Question 1, we included studies that described any ISH testing and mentioned cervical cytologic grade (e.g., ASCUS, LSIL, or normal cytology in combination with a positive HPV test) or cervical histologic grade or cancer stage (e.g., CIN or SCC). We excluded studies of cervical cell lines and reviews without primary data. We tabulated the frequency of studies for all ISH test probes examined in cervical cytology or histology specimens to identify those probes most frequently studied.

For Key Question 2a, we included any study that examined an ISH test for TERC, MYC, HPV 16 or HPV 18 (with or without additional probes) in cervical cytology or histology specimens and compared these ISH tests with a non-ISH reference test. We included studies that applied both ISH and reference test in the same cervical specimen, either cytologic or histologic, regardless of classification.

For TERC or MYC ISH tests, we looked for a DNA-based reference test for the same chromosomal abnormality. For HPV 16 or 18 ISH tests, we did not restrict the reference test to only those detecting nuclear DNA and instead accepted any "reference" test for HPV, including polymerase-chain-reaction (PCR) or Hybrid Capture 2 tests. Neither ISH tests nor reference tests for HPV were restricted to only HPV 16 or 18. Instead, they could test for panels of high-risk HPV genotypes. Further, there could be variability between the specific HPV genotypes targeted by ISH and reference tests. Studies had to provide data that allowed for the reconstruction of 2×2 tables for the results of index and reference tests.

Given the imperfection of HPV reference tests in terms of lack of specificity for intranuclear DNA and the variable overlap of HPV genotypes between ISH and reference tests, we described the agreement between tests as the percent of those with concordant results (both positive or both negative) divided by the number of all samples tested.

For Key Questions 2b–f, we reviewed studies eligible for Key Questions 2a, 3, or 4 for pertinent narrative or quantitative information on reliability and reproducibility of ISH tests and possible factors interfering with analytic test performance.

For Key Question 2g,we conducted a focused search for literature on the prevalence of the markers detected by ISH by age or race/ethnicity in the United States. PCR and Hybrid Capture 2 for HPV were the reference tests identified in studies reviewed for Key Question 2a. For Key Question 3a, we included any study that examined ISH testing for TERC, MYC, HPV 16, or HPV 18 (alone or in combination with other probes) in cervical cytology samples to detect high-grade CIN or cervical cancer (or related clinical outcomes). Cervical cytology had to be stratified by cytologic classification, and we extracted ISH findings for each of the following groups: ASCUS, LSIL, ASCUS and LSIL along with HPV status, and normal cytology if HPV positive. While the Bethesda nomenclature has evolved to divide ASCUS into ASC-US and ASC-H, we were limited to using ASCUS as the term was defined in the studies. If the study differentiated ASC-US and ASC-H, we included results for ASCUS only.

Histology outcomes had to be classified as CIN according to the Bethesda classification system and had to be expressible as either CIN3+ (i.e., CIN3 or cervical cancer) or CIN2+ (i.e., CIN2, CIN3, or cervical cancer). Studies included in this review had not uniformly adopted the recently published LAST nomenclature to categorize histology into HSIL or LSIL. Studies had to provide data that allowed for tabulation of 2×2 tables showing the relation of ISH test results and histologic results (CIN2+ or CIN3+) to calculate measures of clinical validity, including sensitivity and specificity. We also looked within each study for comparisons of clinical validity for different test combinations that included ISH tests.

For Key Question 3b, we conducted a focused review for information on the population-based prevalence of CIN2+ and CIN3+ stratified for LSIL or ASCUS in Medicare beneficiaries. We also looked for prevalence in subgroups by age, and race/ethnicity.

For Key Question 4, we searched for studies that compared patient management strategies using different screening or testing algorithms. We considered strategies that compare different test thresholds or different combinations of ISH and/or non-ISH tests. Outcomes of interest were impacts on diagnostic thinking, evaluation, and management and clinical outcomes.

Study Selection

For Key Question 1, each abstract was screened using *Abstrackr*,[54] singly by one of three reviewers, and queries were addressed at group meetings. For Key Questions 2 through 4, we further screened studies identified in the horizon scan based on their eligibility for these questions.

Full-text articles were retrieved for all potentially relevant abstracts. Studies excluded during full-text screening for Key Questions 2 through 4 and reasons for rejection are given in **Appendix B**. We ran an updated literature search using the same search strategy, on July 12, 2012, and added new eligible studies to the report.

Data Extraction

Data extracted by one reviewer were confirmed by at least one other reviewer, and queries or disagreements were resolved at meetings of the entire project team. Data were extracted into data tables in Excel or Microsoft Word that were customized for the question and piloted on several studies, with revision as necessary. For all studies we extracted the author, year of publication, journal, PMID, and country. For Key Question 1, we extracted data on study design. For each cohort or study group, we captured the number of women tested with ISH and the type of the specimen (cytologic or histologic). The sampling strategy was categorized as random, systematic (e.g., inclusion of every third patient), stratified (by any factor), convenience (i.e., using available specimens), or not described. Setting was described as screening, testing/diagnosis (i.e., followup for abnormal screening result), "mixed" (screening and/or testing/diagnosis), or not described. We captured the mean or median age and range. We categorized the probes as non-HPV or HPV and extracted the probe composition or kit name and manufacturer name and location. Finally, we described whether the study contained information on associations of probe results with cytologic grade, histologic grade, clinical outcomes, or reference tests for non-HPV or HPV (for analytic validity).

For Key Question 2, we further extracted information on the ISH assay methods, the reference standard, and the probe(s) used. We looked for information on thresholds or quantitation methods used for ascertaining positive, negative, and indeterminate results; blinding; and information on quality control, reproducibility, and factors affecting test performance, such as tissue sampling, sample handling, or variability due to operator or laboratory. Results from ISH testing compared with the reference test were captured in 2×2 tables or as sensitivity and specificity with 95 percent confidence intervals.

For Key Question 3, we extracted information on ASCUS and LSIL samples and recorded any information on HPV status in these cohorts. We also extracted data from samples of normal cytology with a positive HPV test. Results from ISH testing compared with high-grade CIN outcomes were captured in 2×2 tables or as sensitivity and specificity with 95 percent confidence intervals. For clinical outcomes we planned to record what clinical end points were examined, the mode of ascertainment, and measurements of association or risk. We recorded whether assessors were blinded to ISH result and grading scale used. We extracted information on study design, including power or estimated effect size, and time period between index test and reference test or duration of followup period.

For Key Question 4, we planned to extract information on populations, inclusion and exclusion criteria, description of testing and management strategies, study design, outcome definition, and results.

Assessment of Risk of Bias and Completeness of Reporting for Individual Studies

Each included study was assessed for study quality according to methods for evaluating study quality within the EPC Program.[52,55,56] For Key Question 2, we graded each study according to 11 items, based on an approach for assessing quality and reporting for studies on analytic validity recently proposed by Sun et al.[57] in an AHRQ Methods Report. We adapted the questions to those pertinent to the project. We showed the aggregate of responses across studies for each question (as low risk of bias, high risk of bias, or not reported).

For Key Question 3, study quality was assessed according to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) 2 instrument,[58] which builds on the validated QUADAS-2 list of quality items[59-61] for systematic reviews of medical tests. Briefly, the tool assesses four domains for risk of bias related to 1) patient selection, 2) index test, 3) reference standard test (outcome), and 4) flow and timing. After scoring each item, a summary risk-of-bias assessment is performed for each of the four domains. We show the aggregate of responses to each methodological quality item (as Yes, No, or Unclear) across studies. For Key Question 4, we planned to use the Cochrane risk of bias tool.[62,62] For all risk of bias assessment, we scored items as "unclear" or "not reported" if they were not clearly addressed in the article.

Data Synthesis

For Key Question 1, we summarized the included studies graphically and narratively. For Key Questions 2, 3, and 4, we presented summary tables that tabulate the important features of the study populations, design, index and reference tests or outcome, and results. We performed meta-analysis of sensitivity and specificity if the data were sufficiently clinically homogeneous and amenable to statistical pooling.

For Key Question 2, we calculated agreement between the tests used in each study. Percent agreement is the percentage of all concordant test pairs (both positive and negative) divided by all test pairs. For Key Question 3, we calculated sensitivity and specificity of ISH for CIN2+ or CIN3+. We adjudicated ISH results related to "polyploidy" found with a control probe. A result of polyploidy for the centromere of chromosome 3, which is sometimes used as a control for TERC, was considered a negative ISH test. Similarly, when the control was the centromere of chromosome 7, polyploidy was considered negative. In contrast, since studies in other malignancies have shown that one mechanism of MYC amplification is duplication of the whole chromosome rather than the MYC region alone,[63] we counted a ISH result of polyploidy of centromere 8 as positive for ISH tests for MYC.

We also reviewed all studies included for Key Question 3 for within-study comparisons of clinical validity with various combinations of ISH and non-ISH tests, to address Key Question 4.

Whenever possible, we present exact (binomial) 95 percent confidence intervals (CIs) for proportions (e.g., sensitivity, specificity, percent agreement). When at least five studies reported information on the clinical validity of a test for the same diagnostic outcome, we performed meta-analysis to quantitatively synthesize findings. We used a bivariate random effects model with the exact binomial likelihood to account for potential negative correlation of sensitivity and specificity across studies(e.g., due to threshold effects).[64,65] All analyses were performed using Stata IC, version 12.1 (Stata Corp., College Station, TX).

Grading the Strength of Evidence

For our survey of the literature on the most commonly used ISH probes in Key Question 1, no grading was performed. Neither did we assess strength of evidence for Key Question 2, because technical test performance does not directly inform medical decisions (it is, however, a prerequisite for the clinical use of tests).[55] Instead, we summarized our observations on the state of the literature, and in particular its limitations, in narrative form.

For Key Question 3, we followed the Methods Guide[52,66] to evaluate the strength of evidence with respect to four domains: risk of bias, consistency, directness, and precision. Risk of bias relied on the overall summary of the quality and reporting assessed with the QUADAS-2 tool. It was summarized as low, high, or unclear. We rated the consistency of the data as no inconsistency, inconsistency present, or not applicable (if there is only one study available). We did not use rigid counts of studies as standards of evaluation (e.g., four of five studies agree, therefore the data are consistent); instead, we assessed the direction, magnitude, and statistical significance of all studies and made a determination. We planned to describe our logic where studies were not unanimous. We assessed the directness of the evidence as direct (rather than indirect) for clinical validity given the choice of high-grade CIN or invasive cancer as the outcome of interest. This is an intermediate outcome with clinical significance in the evaluation for cervical cancer as the finding of high-grade CIN results in the recommendation for colposcopy, even though it is still only indirectly related to subsequent clinical outcomes, such as cancer related morbidity and mortality. Finally, we assessed the precision of the evidence as precise or imprecise on the basis of the degree of certainty surrounding each effect estimate. A precise estimate is one that allows for a clinically useful conclusion. An imprecise estimate is one for which the confidence interval is wide enough to include clinically distinct conclusions (e.g., both clinically important superiority and inferiority—a situation in which the direction of effect is unknown) and that therefore precludes a conclusion.

In addition, for Key Question 3, we also rated the body of evidence on the basis of four strength of evidence levels: high, moderate, low, and insufficient.[52,66]These indicate our level of confidence that the evidence reflects the true effect for the major comparisons of interest.

For Key Question 4, we planned to rate the body of evidence based on risk of bias, consistency, directness, and precision for comparative studies.[52,66] However, we found no comparative studies.

Applicability

We did not assess applicability of studies reviewed for Key Question 2 for analytic validity because they addressed technical test performance, which is not pertinent to the issue of applicability to a patient population.

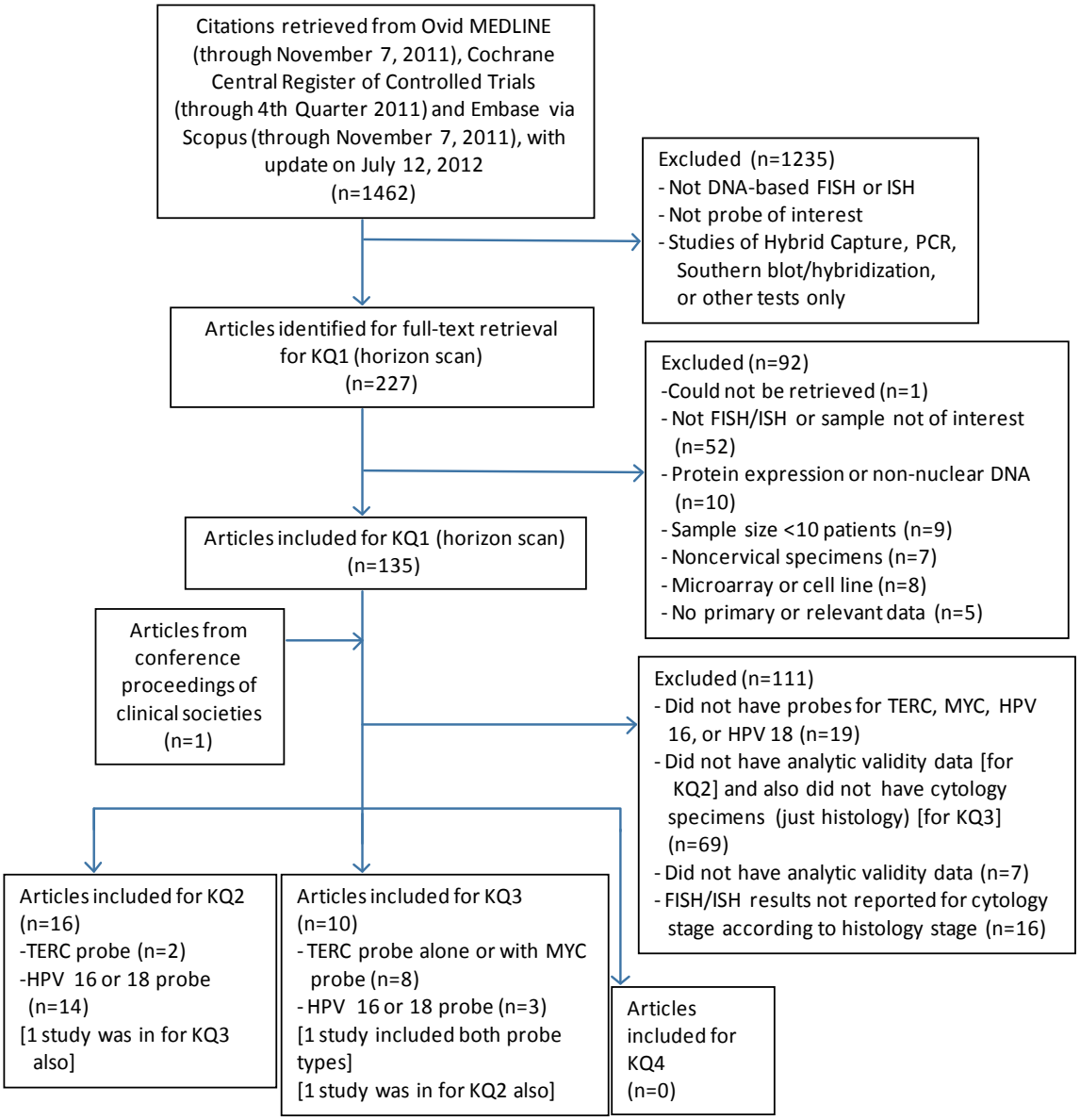
We did appraise the applicability of studies reviewed for Key Question 3 using the QUADAS-2 applicability items. We also considered how the study characteristics, for example study county, might impact applicability to the general U.S. population of screened women.

Results

Overall Literature Yield

Our searches identified a total of 1462 abstracts, of which we screened 227 in full text and included 135 in the horizon scan for Key Question 1 (**Figure 4**). Twenty-five studies were included for Key Questions 2 and 3: 16 studies addressed Key Question 2,[67-82] and 10 studies addressed Key Question 3,[49,75,83-90] with 1 study[75] providing data for both of these key questions. One study was in Chinese[84]; the others were all published in English. No studies addressed Key Question 4. The 110 studies from the horizon scan that were excluded for Key Questions 2 through 4 are listed, along with the reason for exclusion, in **Appendix B**.

Figure 5. Literature Flow Diagram.



Studies could have had more than one reason for exclusion but only one reason for each is listed here.
FISH=fluorescence in situ hybridization; HPV=human papillomavirus; ISH=in situ hybridization; KQ=Key Question; MYC=myelocytomatosis oncogene; PCR=polymerase chain reaction; TERC=telomerase RNA component.

Key Question 1 (Horizon Scan)

Key Question 1: What ISH Tests Have Been Used in Cervical Cytology or Histology Specimens?

Initial Review

Key Question 1 asked what ISH tests have been examined, with what frequency, in studies of cervical cytology or histology. A horizon scan of the literature was performed, identifying 135 articles that described the use of an ISH probe on cervical cytology or histology samples. The probes most commonly used were TERC (31 studies), MYC (7 studies, all of which also used a TERC probe), HPV 16 (91 studies), and HPV 18 (used in 87 of the 91 studies with an HPV probe) (see **Appendix Figures C1 and C2**). (Probes for chromosome 7 and chromosome 3 were not considered for narrowing our review. Chromosomal probes are used as controls for polyploidy in combination with other probes that target a more specific genetic region of interest.)

Focused Review

We focused the subsequent detailed evidence review (i.e., Key Questions 2–4) on the 116 studies using one or more of these four most common ISH probes: a probe for TERC, MYC, or HPV 16 or 18; the remaining 19 studies did not use these probes and were not reviewed further.

Appendix Tables C1 and C2 provide data on the 116 studies according to whether they used a TERC probe (31 studies [27 percent]) or either HPV probe (91 studies [78 percent]) (5 studies [4 percent] used probes of both types). Most studies were conducted in Europe (54 percent) and the United States (21 percent), but there were also studies from China, Japan, Brazil, Mexico, Egypt, India, Israel, and New Zealand (see **Appendix Figures C3 and C4**).

Fifty-four percent of the studies involved cytology specimens, and 73 percent involved histology specimens (studies could involve both). A total of 93 percent of the studies used convenience samples (the rest were not specified [4 percent] or used systematic [2 percent] or random sampling [1 percent]). Nine percent of studies included less than 30 patients.

None of the studies examined only patients 65 years of age or older. Although the mean or median age in most studies was less than 50 years, the age ranges were wide (range across all studies, 14 to 93 years) (data not shown). The majority of studies (75 percent) were cross-sectional (72 percent, with the rest being longitudinal [25 percent] or not specified [2 percent]).

Focusing the detailed evidence review on ISH tests for TERC (with or without MYC) was supported by the frequency of their use in the literature and by our narrative review of microarray studies, which suggest that gain of TERC is linked to high-grade cervical cancer (see the Background section). Including ISH probes for HPV 16 (with or without HPV 18) was supported by the findings of a large amount of literature on these tests and because HPV 16 and HPV 18 are well characterized as the two high-risk types most strongly associated with cancer development.

Key Question 2

Key Question 2a (Analytic Validity): For ISH tests for TERC or MYC or HPV 16 or HPV 18, what are the associations between ISH test results and reference test results? What thresholds were used? What reference standards were used?

Agreement Between ISH for TERC or MYC and Reference Tests

No studies provided data on the association between ISH for TERC or MYC and a DNA-based reference test with measurement on the same samples.

Agreement Between ISH for HPV 16 or 18 and Reference Tests

Fourteen studies compared ISH tests that included HPV 16 or HPV 18 with another HPV test in a total of 852 patients.[67-79,81] The studies were heterogeneous with regard to the types of tissue, ISH test, and reference test; the HPV genotype; and the number of probes in either the ISH test or the reference test (see **Table 4**). Study characteristics and results for agreement between FISH test and reference test are given in **Table 5**. Of the 14 studies, 4 used ISH on cytologic samples and 11 used ISH on histologic specimens (1 study[72] tested both types of sample). The studies varied in terms of sampling strategy and country; only 4 of the 14 were conducted in the United States.

The ISH tests used were specifically ISH in 10 studies, FISH in 1 study, catalyzed signal amplified colorimetric (CSAC) ISH in 1 study, catalyzed reporter deposition amplified (CARD) ISH in 1 study, and nonisotopic ISH in 1 study. For purposes of summary, we considered all these ISH variations as equivalent. ISH assays tested for variable combinations of HPV genotypes; some tested for a panel of high-risk HPV, some tested for specific genotypes. Studies did not clearly identify the specific probes of a positive test. The reference tests were PCR in 11 studies (1 of which used both PCR and real-time PCR[68]), Hybrid Capture 2 in 2 studies (1 of which also used PCR[72]), and in situ PCR in 2 studies. The ISH test and the reference tests conspicuously varied in the HPV genotypes captured, both within and across studies.

The percent agreement between the ISH test and the reference test in each study is shown in **Figure 6**. (The percent agreement is the sum of concordant results over the total number of test comparisons, expressed as percentage.) Three studies compared tests only in samples that were negative by ISH[69-71] but they were still included in the analysis of overall agreement. Overall, agreement was variable, as was the precision of the estimates. The agreement ranged between 35 percent (95 percent CI, 15.4 to 59.2) to 100 percent (95 percent CI, 91.6 to 100). Among the 11 studies using PCR as the reference tests, agreement ranged from 48.5 percent (95 percent CI, 36.2 to 61.0) to 100 percent (95 percent CI, 73.5 to 100.0). The numbers of studies using another shared reference test were too small for meaningful summary. Because of the across-study heterogeneity and clinical variability seen in the studies reviewed for Key Question 2, we refrained from meta-analysis of the results.

This variability in agreement was expected given the true heterogeneity from comparison of tests with different principles of measurement and different target DNA. ISH tests specifically for nuclear HPV DNA; other tests do not. In situ PCR, for example, quantifies messenger RNA and not DNA (thus looking at gene expression, not the actual number of copies of the gene). Other PCR-based tests cannot distinguish between HPV DNA in the nucleus and HPV DNA in the cytosol. Hybrid Capture 2 tests for HPV test for DNA of high-risk HPV types, but it cannot

determine the specific HPV types. The HPV probe sets used by ISH and by the reference test in each study often did not overlap. Given the substantial disagreement between tests across studies, is possible that index and reference tests provide complementary information and that combining these tests could increase diagnostic or prognostic accuracy.

Table 4. Patient and Study Characteristics in the 14 Studies Involving FISH Using HPV 16 or 18 Probes Included for Key Question 2.

Author Year Country PMID	Patient Population Age	Index Test and Probes [*Bold type indicates probes not included in reference probe set]	Index Test (ISH) Details	Index Test Definition of Positive Result	Reference Test and Probes [*Bold type indicates also included in FISH probe set]	Reference Test Details
Alameda 2011 Spain 21302019	80 Women with ASCUS or LSIL cytologic samples Age range, 19–62 yr	ISH Probes for high- risk HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 70 pooled	Automated detection system Ventana INFORM HPV (Atom, Ventana, Ventana Medical Systems, Tucson, AZ)	At least 1 positive cell (staining could be diffuse [episomal], multipunctate [integrated], or both)	PCR Probes for HPV 6,11, 16,18, 26, 31, 33, 35, 39,40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61,62, 66, 68, 70, 71, 72, 73, 81, 82, 83, 84, 85, and 89	Direct sequencing by BigDye v.3.1 kit (Applied Biosystems, Foster City, CA) and (for multiple genotypes in one sample) CLART HPV2 Kit (Genomica)
Andersson 2009 Sweden 19880826	78 Women with histologic specimens (7 and 16 women did not have data for comparison with PCR and real-time PCR, respectively) [Two nonindependent populations] Mean age, 35.3 yr (median, 33; range, 23–60)	FISH Probes for HPV 6, 18, 26, 30, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59. 66, 82 pooled	Probes from Abbott Molecular Inc.	All nuclei on slide evaluated using the Spectrum Green filter; positive results scored as episomal, episomal and integrated, or integrated pattern	PCR Probes for low-risk and high-risk HPV: 6, 11, 16, 18, 26,31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66–73, 81, 82, 83, 84, 89	LineBlot (Roche)
					Real-time PCR Probes for high-risk HPV: 16, 18, 31, 33, 35, 39, 45, 52, 58, 67	Quantovir
Ansari-Lari 2004 US 15043304	19 Women with endocervical adenocarcinoma in histologic specimens Age NR	ISH Probes for HPV 16 and 18 individually as well as 6, 11, 16, 18, 31, 33, 45, 51 pooled	Probe set from Dako Corp.	NR	PCR For “more than 35 HPV probes”	LineBlot (Roche)
Bernard 1994 France 7877628	20 Women with cervical lesions on biopsy that were ISH-negative Age NR	ISH Probes for HPV 6, 11, 16, 18, 31, 33, 51 pooled	NR	Staining in epithelial cells but not in any underlying connective tissue and repeat positivity on duplicate testing	In situ PCR Probes for HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52	NR
Bertelsen 1996 Norway 9048869	71 Women with CIN on biopsy specimens that were HPV-negative on ISH (some samples not accounted for) Age NR	ISH Probes for HPV 6, 11, 16, 18, 31, 33, 35, 42, 43, 44, 45, 51, 52, 56 pooled	Digene Tissue Hybridization Kit and HPV Omniprobe (Digene Diagnostics, Inc.)	Staining	PCR Probes for HPV 6, 11, 13, 16, 18, 30, 31, 32, 33, 35, 39, 40, 43, 45, 51, 52, 54–56, 58, 59, 66	NR

Author Year Country PMID	Patient Population Age	Index Test and Probes [*Bold type indicates probes not included in reference probe set]	Index Test (ISH) Details	Index Test Definition of Positive Result	Reference Test and Probes [*Bold type indicates also included in FISH probe set]	Reference Test Details
Birner 2001 Austria 11455003	86 Women with CIN3, HPV-positive cervical cancer biopsy specimens Age NR	CSAC-ISH Probes for HPV 16, 18, 31, and 33 individually as well as 6, 11, 16, 18, 30, 31, 33, 45, 51, 52 pooled	Probes from DAKO	Patchy signal unevenly distributed across nuclei (episomal HPV DNA) or a dot-like signal in nuclei (integrated HPV DNA)	PCR Probes for HPV 16, 18, 31, 33, 35, 45	NR
	21 Women with known high-risk HPV and cytologic and histologic specimens [independent of the 86 women above] Age NR				Hybrid Capture 2 Probes for low-risk HPV 6, 11, 42, 43, 44 and high-risk HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	Digene Corp.
Bulten 2002 Netherlands 12375262	56 Women with biopsy samples (5 normal, 11 CIN1, 13 CIN2, 18 CIN3, and 9 invasive carcinomas) Age NR	CARD-ISH Probes for HPV 16, 18 pooled	Probes from BRL	NR	PCR Probes for HPV 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74	Short-fragment PCR hybridization line probe assay for detection and genotyping
Cavalcanti 1996 Brazil 9070405	12 biopsy specimens pretested with Southern blotting (3 with "reactive changes" ["histologically normal tissues presenting only reactive/reparative changes"], 4 LSIL, 4 HSIL, and 1 SCC) Range of mean ages given by histologic grade, 31.5-46.9 yr	Nonisotopic ISH Probes for HPV 6, 11, 16, 18, 31, 33, 35 pooled	Probes home brewed or from Digene Corp.	Strong staining in upper epithelial cell nuclei	PCR Probes for HPV 6, 11, 16, 18, 31, 33, 35	Probes home brewed or from Digene Corp.
Hesselink 2004 Netherlands 14968413	76 Women with cytologic samples (normal or borderline, mild, moderate, or severe dysplasia) Mean age, 35 yr (range, 19–63)	ISH Probes for HPV 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, 68, 70 pooled	Ventana HPV lipopolysaccharide-binding protein test	Distinct nuclear staining in at least 1 cell	Hybrid Capture 2] For HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68	Digene Corp.
Kong 2007 US 17197917	25 Women with atypical squamous metaplasia on biopsy (n=28 but 3 did not have data for both tests) Mean age, 32.7 yr (median, 20; range, 20–63)	ISH Probes for HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66 pooled	Ventana HPVII test	Staining scored as strong or weak and also punctate, diffuse, or mixed	PCR Probes for HPV 6, 11, 16, 18, 30, 31, 33, 35, 39, 42–45, 51, 52, 53–56, 58, 59, 66, 68, 72, 83, 86, 87, 90, and 91	NR
Lie 1997 Norway 9113073	203 Women with CIN2 or CIN3 biopsy specimens Median age, 32 yr (range, 21–76)	ISH Probes for HPV 6, 11, 16, 18, 31, 33, 35 pooled	Vira-Type In Situ Kit (Digene Diagnostics Inc., Silver Spring MD)	NR	PCR Probes for "many types" of HPV (NR)	NR
Masumoto 2003 Japan 14506638	10 biopsy specimens for small-cell carcinoma of the cervix Mean age, 42.7 yr (range, 27–69)	ISH Probes for HPV 16, 18, 31, 33 individually as well as "wide spectrum" pooled	GenPoint kit (DakoCytomation, Kyoto, Japan)	Staining (also scored as diffuse, punctate, or both)	PCR Probes for "a broad range of genital HPVs"	Genotyping done through direct sequencing

Author Year Country PMID	Patient Population Age	Index Test and Probes [*Bold type indicates probes not included in reference probe set]	Index Test (ISH) Details	Index Test Definition of Positive Result	Reference Test and Probes [*Bold type indicates also included in FISH probe set]	Reference Test Details
Qureshi 2005 US 15839613	90 LSIL cytologic specimens by ThinPrep (n=47) or SurePath kit (n=43) Age NR	ISH Probes for HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 72 pooled	INFORM HPV High Risk kit (Ventana Medical Systems Inc., Tucson AZ)	NR except that pattern could be diffuse or punctate	PCR Probes for HPV 6, 11, 16, 18, 30, 31, 33, 35, 39, 42–45, 51, 52, 53–56, 58, 59, 66, 68, 72, 74, 83, 86, 87, 90, 91	HPV genotyped by Big Dye Terminator kit (Applied Biosystems)
Walker 1996 France 8727101	30 Women with biopsy samples Median age, 29 yr (range, 21–40)	ISH Probes for "a mix of HPV" types including 6, 11, 16, 18, 31, 33	Probes from Biohit, Finland, or Dakopatts, Denmark	NR	In situ PCR Probes for 40 HPV types including 6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 45, 52, 53, 54, and 59	NR

ASCUS=atypical squamous cells of undetermined significance; CARD=catalyzed reporter deposition amplified; CI=confidence interval; CIN=cervical intraepithelial neoplasia; CSAC=catalyzed signal amplified colorimetric DNA; FISH=fluorescence in situ hybridization; HPV=human papillomavirus; HSIL=high-grade squamous intraepithelial lesion; ISH=in situ hybridization; LSIL=low-grade squamous intraepithelial lesion; NR=not reported; PCR=polymerase chain reaction; SCC=squamous-cell carcinoma; yr=year(s).

Table 5. Results for Analytic Validity in the 14 Studies Involving ISH Using HPV 16 or 18 Probes Included for Key Question 2.

Author Year Country PMID	Sample Classification and Size	Tissue Preparation	Index Test Reference Test	Index Test ISH Result	No. + on Ref. Test	No. – on Ref. Test
Alameda 2011 Spain 21302019	ASCUS or LSIL (n=80)	Cytologic	ISH PCR	ISH +	32	0
				ISH -	7	41
Andersson 2009 Sweden 19880826	Any classification (n=71, with overlap with the 62 below)	Histologic	FISH PCR	ISH +	45	4
				ISH -	14	8
	Any classification (n=62, with overlap with the 71 above)	Histologic	FISH Real-time PCR	ISH +	30	14
				ISH -	6	12
Ansari-Lari 2004 US 15043304	Endocervical adenocarcinoma (n=5)	Histologic	ISH PCR	ISH +	NR	NR
				ISH -	1	4
Bernard 1994 France 7877628	Any classification (n=20)	Histologic	ISH In situ PCR	ISH +	NR	NR
				ISH -	13	7
Bertelsen 1996 Norway 9048869	Any class (n=68)	Histologic	ISH PCR	ISH +	NR	NR
				ISH -	35	33
Birner 2001 Austria 11455003	CIN3 (n=86, independent of the 21 below)	Histologic	CSAC-ISH PCR	ISH +	66	1
				ISH -	10	9
	CIN3, cytologic specimen (n=21, independent of the 86 above)	Cytologic	CSAC-ISH	ISH +	20	0

Author Year Country PMID	Sample Classification and Size	Tissue Preparation	Index Test Reference Test	Index Test ISH Result	No. + on Ref. Test	No. – on Ref. Test
			Hybrid Capture 2			
				ISH -	1	0
	CIN3, histologic specimen (n=21, same as the 21 above)	Histologic	CSAC-ISH Hybrid Capture 2	ISH +	21	0
				ISH -	0	0
Bulten 2002 Netherlands 12375262	Any classification (n=56)	Histologic	CARD-ISH PCR	ISH +	28	5
				ISH -	3	20
Cavalcanti 1996 Brazil 9070405	Any classification (n=12)	Histologic	Nonisotopic ISH PCR	ISH +	9	0
				ISH -	0	3
Hesselink 2004 Netherlands 14968413	Any classification (n=75)*	Cytologic	ISH Hybrid Capture 2	ISH +	46	0
				ISH -	28	1
Kong 2007 US 17197917	Atypical squamous metaplasia (n=25)	Histologic	ISH PCR	ISH+	4	0
				ISH -	8	13
Lie 1997† Norway 9113073	CIN2 or CIN3 (n=203)	Histologic	ISH PCR	ISH+	86	83
				ISH -	12	22
Masumoto 2003 Japan 14506638	Small-cell carcinoma of the cervix (n=10)	Histologic	ISH PCR	ISH+	6	0
				ISH -	4	0
Qureshi 2005‡ US 15839613	Any classification (n=90)	Cytology	ISH PCR	ISH+	52	13
				ISH -	8	17
Walker 1996 France 8727101	Any classification (n=30)	Histologic	ISH In situ PCR	ISH+	13	0
				ISH -	6	11

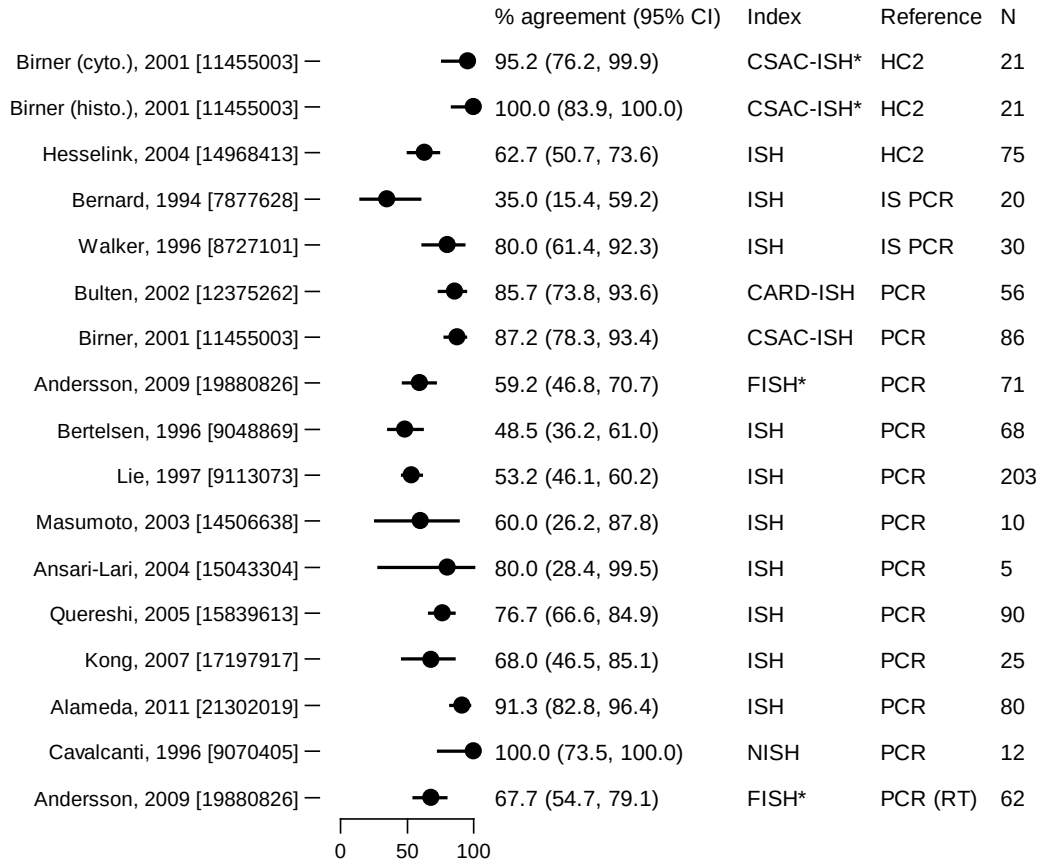
ASCUS=atypical squamous cells of undetermined significance; CARD=catalyzed reporter deposition amplified; CI=confidence interval; CSAC=catalyzed signal amplified colorimetric DNA; FISH=fluorescence in situ hybridization; HPV=human papillomavirus; ISH=in situ hybridization; LSIL=low-grade squamous intraepithelial lesion; NR=not reported; PCR=polymerase chain reaction.

*The study was of 76 samples but for 1 we could not ascertain both the index and reference test result.

† Counts were derived from sensitivity and specificity reported for ISH: sensitivity=0.51, specificity 0.65.

‡ For ISH: sensitivity (95% CI) 0.87 (0.75,0.94), specificity 0.57 (0.37,0.75).

Figure 6. Percent Agreement Between ISH (Index) Test Using HPV 16 or 18 Probes and Reference Test in the 14 Studies with Analytic Validity Data.*



This forest plot shows the percent agreement between the in situ hybridization (ISH) test (called the "index" test above) and the reference test for studies of ISH testing for human papillomavirus (HPV). Point estimates are shown as black circles and are listed to the right of the plot; horizontal lines denote 95% confidence intervals, which are listed in parentheses to the right of the plot after the point estimate. The PubMed identifier is listed in square brackets for each study. See Tables 4 and 5 for details for each study. The studies are ordered by the reference test (last column), then by index test (type of ISH), and finally by year of publication.

* Denotes overlapping patient populations between each of the two test pairs. Andersson 2009 reported data for two nonindependent samples for which FISH was compared with two separate reference tests (PCR and real-time PCR). Birner 2011 reported data for three comparisons, two of which were from the same population but one sample set was cytologic (cyto.) and the other was histologic (histo.).

CARD=catalyzed reporter deposition amplified; CSAC=catalyzed signal amplified colorimetric DNA; CI=confidence interval; HC2=Hybrid Capture 2; IS PCR=in situ PCR; NISH=nonisotopic in situ hybridization; PCR=polymerase chain reaction; PCR (RT)=PCR (real-time).

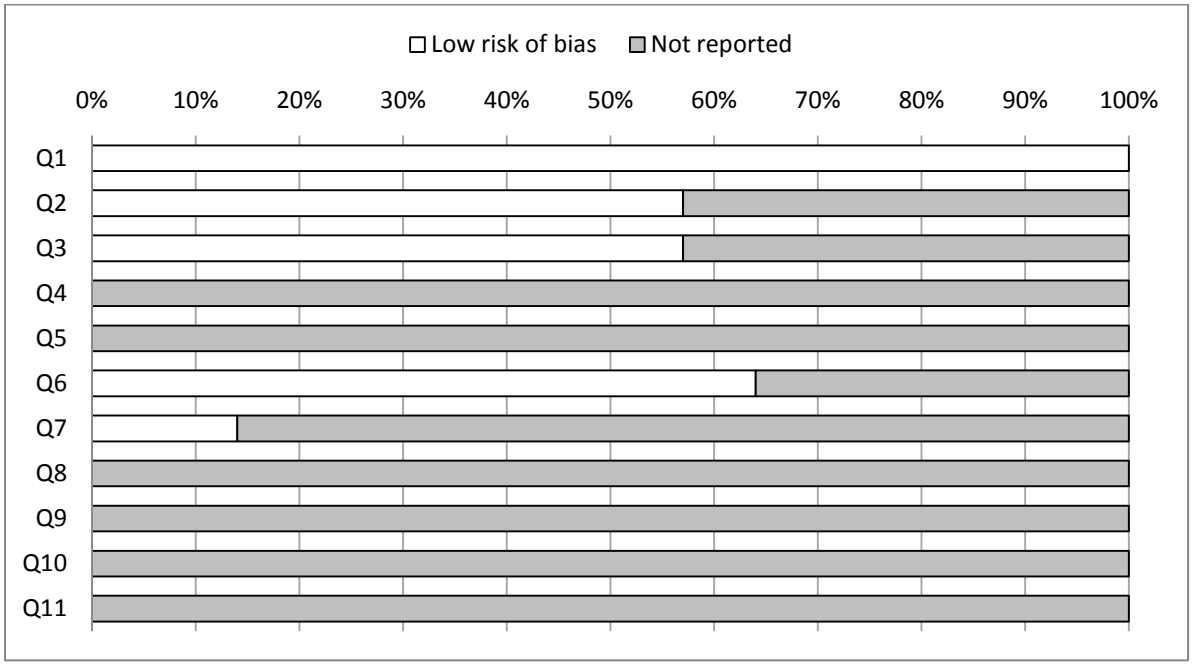
Assessment of Risk of Bias and Completeness of Reporting

Figure 7 summarizes our assessment of quality and reporting for the 14 studies reviewed for Key Question 2a. All studies used HPV probes. The assessment was based on 11 questions relevant to assay performance and reporting thereof (see **Appendix D** for scoring for each study). In general, study reporting was variable across questions. All studies described the performance of the index tests in sufficient detail to permit replication (Q1). Fifty-seven percent included the use of both positive and negative samples (Q2), and all 57 percent used the same

type of tissue for those controls (Q3). Blinding of testers and interpreters was not reported in any study (Q4, Q5). Some criteria for scoring test results were established a priori in the majority of studies (Q6). Only two studies provided information on cross-reactivity (Q7). Reproducibility on testing of the same specimen multiple times was not reported (Q8). Reproducibility across operators, instruments, reagent lots, different days of the week, and different laboratories was not described either (Q9), and no studies clearly described the numbers of samples with usable test results (Q10). There was no information on whether testing was performed with multisite collaborative, proficiency testing, or interlaboratory exchange programs (Q11).

Our assessment shows deficiencies in reporting, likely because most of the studies were not designed to specifically address analytic validity. Studies also did not explicitly describe laboratory procedures in detail because ISH testing and its reference standards (most often PCR assays) are well established in general (if not in particular for cervical specimens). Many of the reference tests were commercially available kits that probably included positive and negative controls, but we could not assume that this was the case and rarely was it reported.

Figure 7. Quality of Studies on Analytic Validity of In Situ Hybridization (ISH) with HPV 16 or 18 Probes.*



*Or other HPV types. The denominator for each question includes all 14 studies.

The 11 quality questions (Qs), adapted from Sun et al. 2011[57] were scored as yes (considered to reflect low risk of bias), no (high risk of bias), or not reported (including not applicable for a corollary questions that followed a question with an "not reported" score). No studies had a high risk of bias; thus this category is not represented in the key. The items are as follows:

Q1=Was the execution of the assay described in sufficient detail to permit replication?

Q2=Were both positive and negative control samples tested?

Q3=Were negative control materials from the same type of tissue, and collected, stored, and processed in the same way that sample materials used clinically for testing will be?

Q4=Were the tests performed with positive or negative control samples being blinded to the testers?

Q5=Were the testing results interpreted with positive or negative control samples being blinded to the interpreters?

Q6=Were criteria for determining a testing result as positive, negative, indeterminate, and uninterpretable set a priori?

Q7=Was any information on cross-reactivity of the test reported?

Q8=Was the reproducibility of the test when performed multiple times on a single specimen established?

Q9=Was the reproducibility of the test adequately established (across operators, instruments, reagent lots, different days of the week, different laboratories)?

Q10=Was the rate of yield [numbers] of usable (interpretable) results reported?

Q11=Were the study data from a multisite collaborative, proficiency testing, or interlaboratory exchange programs?

Thresholds Used for Positive, Indeterminate, and Negative Results of the ISH Tests

To describe the thresholds used for positive, indeterminate, and negative results of the FISH tests, we reviewed information from the 14 articles using ISH with HPV probes as well as 10 studies reviewed for Key Question 3 (8 reporting on FISH for TERC and 3 reporting on FISH for HPV) (Tables4 above and Tables 6, and 7 below).

Specific thresholds were expected to be given in the studies of FISH with probes for TERC or MYC, since the 3q and 8p chromosome arms are normally present; in this case FISH is used to detect an abnormality in the numbers of copies of the gene. An abnormal test is determined based on the number of ISH signals in relationship to the number of chromosomes or the average percentage of positive cells visualized, not merely the presence or absence of any signal. Most of the studies of FISH with a TERC or MYC probe defined test result positivity by the presence of additional signals in two or three or more cells, often in combination with a threshold for cellular positivity (typically a ratio of the TERC or MYC probe and the chromosomal control probe) (Table 6). However, there were no standard thresholds for test positivity.

Two studies of FISH for TERC not included for detailed review for Key Question 2 or 3 (data not shown) established the threshold for positivity for TERC gain by assaying cervical cytology samples from 20 women with normal Pap results and negative for HPV infection. One study used a single group of 20 women[82]; the other used three different groups of 20, one each at the three study centers.[80] Both studies established thresholds based on the mean plus 3 times the standard deviation for the percentage of cells with abnormal signals. The thresholds were 5.3 percent in the single-center study and 5.2 percent, 5.6 percent, and 6.4 percent in the three-center study. No statistical comparisons were performed.

For HPV 16 or 18 (and the other types tested for), test positivity was usually defined simply by staining indicating the presence of HPV DNA in the nucleus of at least one cell (Table 4 and Table 7), except in one study in which 30 or more cells had to have had staining for HPV for the sample to be deemed positive (for episomal infection).[90]

Reference Standards Used to Assess the Presence or Absence of the Genetic Marker

In the articles we reviewed, the reference tests for HPV 16 or 18 were PCR or Hybrid Capture 2 (see Tables 4 and 5 for details). Staining on ISH tests identifies episomal or nuclear HPV DNA. Most HPV PCR assays detect viral DNA, but PCR does not differentiate between episomal or nuclear DNA. Hybrid Capture 2 tests for DNA of high-risk HPV types, but it cannot determine the specific HPV types. Neither can be considered a true reference standard for FISH tests.

Key Questions 2b–2f. b) What is known about reliability and reproducibility of FISH tests? What factors affect FISH test results? c) Are there some conditions for which a FISH test is not useable? d) What are the sample criteria s? e) What are the sample storage or preservation requirements? f) What variation occurs across laboratories?

To address Key Questions 2b through 2f, we looked at the 14 articles describing FISH with HPV probes as well as the 10 studies reviewed in detail for Key Question 3. Overall, the studies varied widely in terms of the information reported about the technical aspects of performing a FISH test.

None of the studies reported on the true reliability of FISH results within a study or the genetic, environmental, or other factors and their impact on FISH results or addressed whether there are some conditions for which a FISH test is not able to give a clinically useable result. None of the studies addressed variation in ISH results across multiple laboratories.

Regarding sample acceptance and rejection criteria for FISH tests, we would expect that typical laboratory techniques of sample storage and preservation would be required for a reliable FISH result, since the test involves DNA detection. In one study of ISH for HPV detection,[69] the authors noted that the four true negative samples identified were suspect because they were the oldest samples in the study and used a different fixative than the rest; they believe the DNA was degraded. This was supported by a negative or weak result on beta-globin testing, indicating poor quality DNA; this test was also used by three other studies[72,74,75] to ascertain whether the samples were satisfactory for FISH testing. One of these studies explicitly stated that all samples had a positive signal for beta-globin[75]; the other two presumably excluded any poor samples but did not report this. One other study[77] reported that specimens with poor-quality DNA after testing by another means (amplification of a known genetic region) were excluded from index testing. Regarding the impact of sample storage or preservation requirements, we found a single study of 99 consecutive LSIL cytologic samples that were prepared at one of two centers (about half at each), one using ThinPrep and the other, SurePath. The authors reported that the method of preparation did not significantly affect positivity or negativity of FISH using probes for HPV.[79]

None of the studies included in our review addressed variations occurring in ISH results if performed in multiple laboratories. The two studies described above by Tu 2009[80] and Jin 2011[82] show that thresholds established for normal specimens varied only slightly (from 5.2 to 6.4 percent) across different labs (no statistical comparisons were performed). According to the College of American Pathologists and American College of Medical Genetics proficiency testing program, the FISH test generally has been found to be reliable across labs.[37] However, its proficiency was not tested for the chromosomal or HPV aberrations of interest in our review. One group suggested that incomplete sampling of the cervix by the cervical/endocervical brush can yield apparently negative FISH results owing to insufficient sampling of the lesion present[90]; this highlights the importance of operator care in obtaining cytologic samples.

Overall, for questions related to preanalytic issues impacting analytic validity, the data were sparse and highlighted a lack of commonly agreed upon test and validation standards.

Key Question 2g. What is the prevalence of the genetic marker(s) detected by the reference standards in Medicare beneficiaries by age or race/ethnicity?

We conducted a focused search for literature on the prevalence of the markers detected by ISH (i.e., TERC, MYC, HPV 16, and HPV 18) in Medicare beneficiaries by age or race/ethnicity. We did not find direct evidence.

Key Question 3

For Key Question 3, we reviewed studies that examined the sensitivity or specificity of ISH tests in cytology samples for the diagnosis of high-grade CIN. We examined the role of ISH for add-on testing in 1) women who have a Pap test showing LSIL or ASCUS without a HPV test, and 2) in women who have a Pap test showing normal cytology or ASCUS as well as a positive HPV test.

Eligible Studies

Ten studies were included into the systematic review for Key Question 3, all using FISH specifically (not another type of ISH test). Eight studies of 8,800 patients examined FISH testing for TERC gain.[1,3,4,6-9,11] One of these studies, with 235 women (Sokolova 2007), provided results for FISH for TERC and separately for FISH for HPV. Five of the eight TERC studies used only TERC probes, whereas three reported results for the combined use of FISH for TERC and MYC, with one of these also reporting results for FISH for TERC, MYC, and HPV.[90] All eight studies provided data for CIN2+ as an outcome; six had data for CIN3+ as well. There were 860 patients with ASCUS and 1033 patients with LSIL in these studies. In one study (Li 2010) all patients were HPV positive (type not specified) by Hybrid Capture 2; in the others the HPV status was not clear.

Three studies examined FISH for HPV detection in a total of 503 patients.[75,83,88] All had CIN2+ as an outcome, with CIN3+ also an outcome in two of these. Twenty-seven patients had ASCUS; 171 had LSIL. All patients were HPV positive, two by PCR and the third by FISH only.

Two of the eight TERC studies also compared the performance of FISH versus, or in combination with, Hybrid Capture 2for histologic outcomes.[49,90] They investigated several test combinations. Voss 2010 compared three test strategies: FISH for TERC or MYC, FISH for TERC or MYC or HPV, and Hybrid Capture 2 for high-risk HPV for the outcome of CIN2+ in 115 LSIL patients. Jiang 2010 compared FISH for TERC, Hybrid Capture 2 for high-risk HPV, and a combination of the two) in both 660 ASCUS patients and 601 LSIL patients, for the outcomes of CIN2+ and CIN3+.

Key Question 3a (Clinical Validity): What is the association between FISH test results on cytology and CIN or cervical cancer on histopathology? What thresholds were used?

The eight studies using FISH tests for TERC, MYC, or HPV 16 or 18 are described in **Table 6**.The three studies of FISH using a probe for HPV are described in **Table 7**.

Table 6. Patient and Study Characteristics in the Eight Studies Involving FISH Using TERC or MYC Probes Included for Key Question 3.

Author Year Country PMID	Patient Population	Age	FISH Probe(s) [Manufacturer, Location]	Thresholds	Cytology Description	HPV Test Description
Huang 2009 China NR	Women (20 controls, 100 with abnormal cytologic findings) who had cytologic smear and biopsy	NR	TERC or MYC [Beijing GP Medical]	NR	ThinPrep	NR

Author Year Country PMID	Patient Population	Age	FISH Probe(s) [Manufacturer, Location]	Thresholds	Cytology Description	HPV Test Description
Jalali 2010 US 20171606	Archival thin-layer cytologic slides of 31 women with LSIL	Range, 14-67 yr	TERC, CEP7 [Abbott Molecular, Des Plaines, IL]	Positive for TERC gain if ≥2 cells with ≥5 3q-FISH signals	NR	NR
Jiang 2010 China 20864639	7786 patients who underwent routine screening or were returning after abnormal cervical cytology result, HPV result, or symptoms of increased leukorrhea discharge or postcoital bleeding	Mean, 39.7 ± 9.7 yr (range, 18–93)	TERC, CSP3 [GP Medical Technologies Ltd., Beijing, China]	Abnormal signal if ratio of CSP3 to TERC was 2:3, 2:4, 2:5, 3:3, 4:4, etc.; positive if >2 TERC signals were observed	ThinPrep or Autocyte	"High risk" by Hybrid Capture 2
Kokalj-Vokac 2009 Slovenia 19837263	Prospective data for150 women	Mean, 37.3 ± 10.1 yr (range, 20–75)	TERC, CEP7 [Cancer Genetics, Rutherford, NJ]	For each sample, 30 abnormal nuclei were checked; 2 signals for CEP7 and >2 signals for TERC constituted an abnormal FISH pattern. Positive if ≥5 cells with abnormal FISH pattern	Cervical smears	NR
Li 2011 China 21035173	300 women with mild cytologic abnormality and positive HR-HPV DNA test.	Mean, 39.3 yr (range, 20–71)	TERC, CEP3 [GP Medical, Ltd. Beijing, China]	TERC signals in >6.5 nuclei.	ThinPrep	HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 by Hybrid Capture 2
Sokolova 2007 US 17975027	235 women with cytologic smear and concurrent biopsy	NR	TERC or MYC [Abbott Molecular, Des Plaines, IL]	≥3 TERC or MYC signals	ThinPrep	HPV 16, 18, 30, 45, 51, 58 by FISH
Sui 2010 China 20882876	63 women undergoing routine cytology screening	Mean, 42 yr (range 23–63)	TERC, CEP3 [China Medical Technologies Inc., Beijing China]	Positive for TERC if ratio >1.0 between the TERC and CEP3 copy number	SurePath	NR
Voss 2010 US 20701064	115 women with LSIL who underwent a same-day colposcopy-directed biopsy or had a follow-up biopsy within 1 yr after cytology specimen	Median, 24 yr; mean, 29 yr (range, 18–73)	TERC or MYC or HPV (16, 18, 30, 45, 51, or 58) [Abbott Molecular Inc., Des Plaines IL]	Positive gain of TERC or MYC if ≥3 positive cells. HPV positivity, ≥30 cells	ThinPrep	HPV 16, 18, 30, 45, 51, 58 by Hybrid Capture 2

CEP or CSP=centromere protein of the chromosome number specified; DNA=deoxyribonucleic acid; FISH=fluorescence in situ hybridization; HPV=human papillomavirus; HR-HPV=high-risk HPV; HSIL=high-grade squamous intraepithelial lesion;

LSIL=low-grade squamous intraepithelial lesion; MYC=myelocytomatosis oncogene (on chromosome 8q24); NR=not reported; TERC=telomerase RNA component; US=United States; yr=year(s)

Table 7. Results for Clinical Validity in the Three Studies Involving FISH Using HPV Probes Alone Included for Key Question 3.

Author Year Country PMID	Patient Population	Age	HPV Probe(s) [Manufacturer, Location]	Thresholds	Cytology Description
Fujii 2008 Japan 18936966	153 specimens from patients visiting Keio University Hospital, Tokyo, Japan. The population consisted of a mixture of asymptomatic women and those who were being followed up for previous atypical smears or were under treatment for previously diagnosed as CIN. All patients with HPV+ on PCR.	Median, 37 yr (range, 21–80)	HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 [NR]	Punctate pattern of at least 1 signal in the nucleus indicated HPV integration Diffuse nuclear pattern represented multiple copies of episomal HPV (viral replication)	ThinPrep
Hesselink 2004 Netherlands 14968413	115 women during a 3-month period in a routine gynecologic setting at the Department of Obstetrics and Gynecology, VU University Medical Center. Indications for visiting a gynecologist included having an abnormal cervical smear in the population-based screening program and monitoring after treatment for CIN3. All patients with HPV+ on PCR.	Mean, 35 yr (range, 19–63)	HPV 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, 68, 70 [Ventana Medical Systems, Tucson AZ]	NR	Cervical samples
Sokolova 2007 US 17975027	235 women with a concurrent biopsy. All patients with HPV+ by FISH.	NR	HPV 16, 18 [American Type Culture Collection, Manassas, VA] HPV 30, 45 [homebrew in lab in Heidelberg, Germany] HPV 51, 58 [Abbott Molecular, Inc., Des Plaines, IL]	Punctate staining, suggestive of integrated HPV state, was defined as at least 1 spot of staining Diffuse staining was suggestive of episomal HPV state	ThinPrep

CIN=cervical intraepithelial neoplasia; HPV=human papillomavirus; ISH=in situ hybridization; NR=not reported; PCR=polymerase chain reaction; US=United States; yr=year(s).

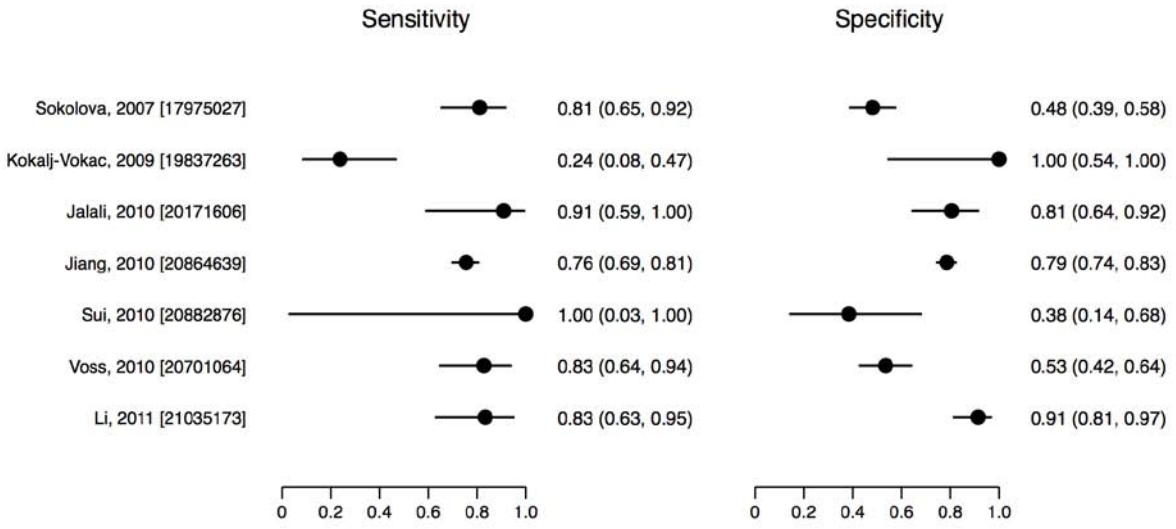
Summary of Findings on Clinical Validity

LSIL

FISH for TERC or MYC

Seven studies compared the clinical validity of TERC in LSIL for CIN2+.[3,4,6-9,11] Two of them, Sokolova 2007 and Voss 2010, examined FISH for TERC or MYC. Only one study tested patients who were all positive for HPV. [87] In these studies, the sensitivity ranged from 0.24 to 1.00, and specificity ranged from 0.38 to 1.00 (**Figure 8**). For the CIN2+ outcome, Sui 2010 had the highest sensitivity (1.00) and Kokalj-Vokac 2009 had the highest specificity (1.00).

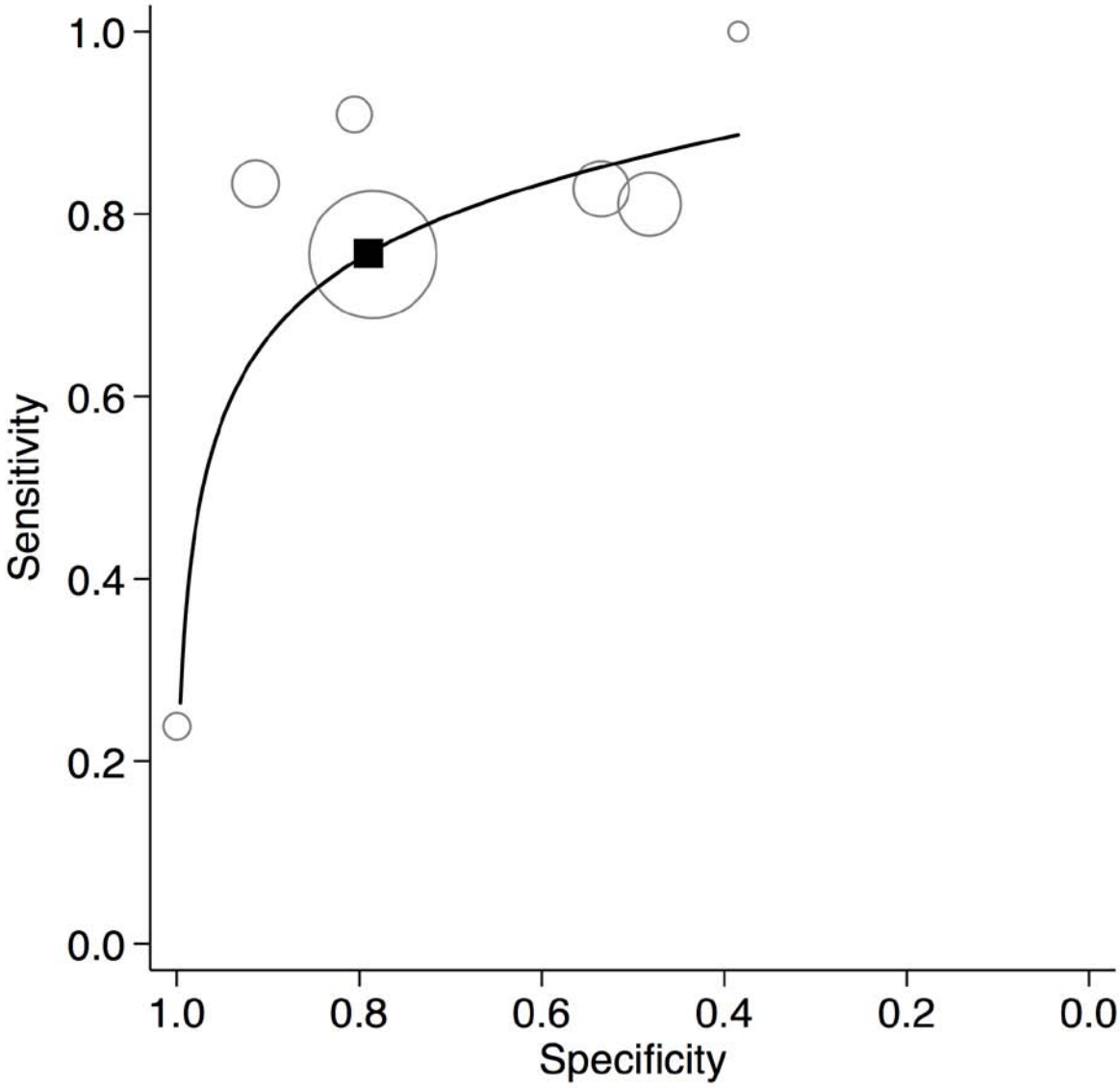
Figure 8. Sensitivity and Specificity of FISH Testing for TERC in LSIL Patients for an Outcome of CIN2+.



This forest plot shows the sensitivity (left plot) and specificity (right plot) of fluorescence in situ hybridization (FISH) testing for an outcome of cervical intraepithelial neoplasia (CIN) 2+ (i.e., CIN2 or CIN3 or cervical cancer) in low-grade squamous intraepithelial lesion (LSIL) specimens. Point estimates are shown as black circles and are listed to the right of the plot; horizontal lines denote 95% confidence intervals, which are listed in parentheses to the right of the plot after the point estimate. The PubMed identifier is listed in square brackets for each study. See Table 6 for details for each study.
TERC=telomerase RNA component gene.

The corresponding meta-analysis for FISH for TERC or MYC in LSIL patients for the outcome of CIN2+ is shown in **Figure 9**. We used a bivariate random effects model that allows for threshold effects (i.e., the trade-off between sensitivity and specificity across studies) and accounts for unexplained between-study heterogeneity. The summary receiver-operator-characteristic (ROC) curve derived from the model is shown, with each study plotted as a circle whose size is proportional to the number of study participants. The overall summary sensitivity was 0.76 (95 percent confidence interval [CI] 0.60, 0.86); the summary specificity was 0.79 (95 percent CI 0.50, 0.93). The largest study (Jiang 2010) is closest to the overall summary estimate, but the summary line fits to all the study estimates fairly well. The between-study correlation of sensitivity and specificity was negative, supporting an inverse relationship.

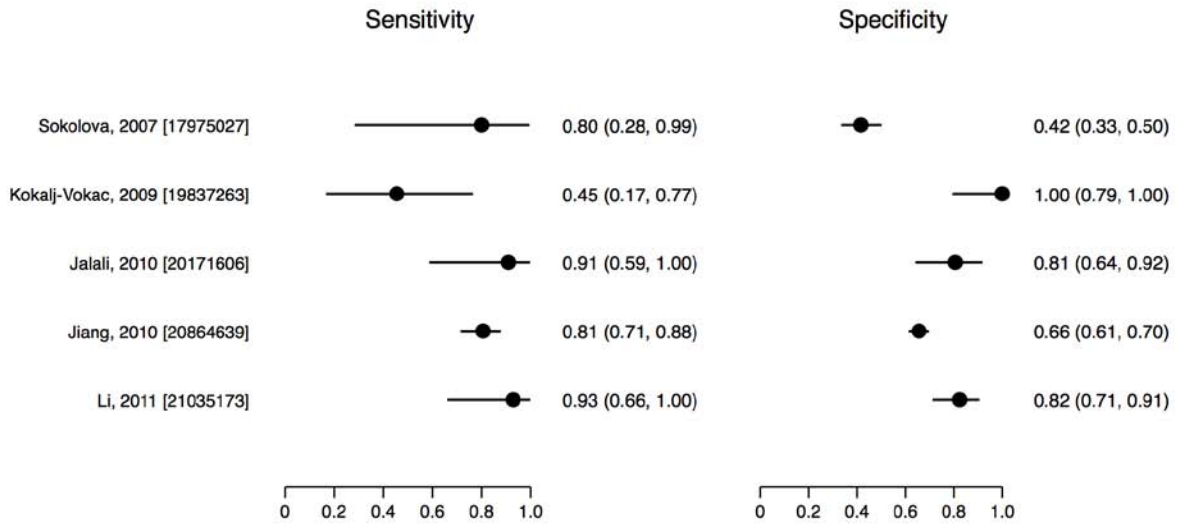
Figure 9. Summary ROC Curve for the Sensitivity and Specificity of FISH Testing for TERC or MYC in LSIL Patients for an Outcome of CIN2+.



This receiver-operating-characteristic (ROC) curve plots the sensitivity (y axis) and specificity (x axis) of fluorescence in situ hybridization (FISH) testing for an outcome of cervical intraepithelial neoplasia (CIN) 2+ (i.e., CIN2 or CIN3 or cervical cancer) in low-grade squamous intraepithelial lesion (LSIL) specimens. The summary estimate is represented by a black square, with each study plotted as a circle whose size is proportional to the number of study participants.
MYC=myelocytomatosis oncogene, TERC=telomerase RNA component gene.

Five studies compared the clinical validity of TERC in LSIL for CIN3+.2-6 Sokolova 2007 and Voss 2010 tested FISH for TERC or MYC. Again, only one study tested patients who were positive for HPV.[87] In these studies, the sensitivity ranged from 0.45 to 0.93, with Li 2011 showing the highest sensitivity. Specificities ranged from 0.42 to 1.00, with Kokalj-Vokac 2009 reporting the highest estimate (**Figure 10**).

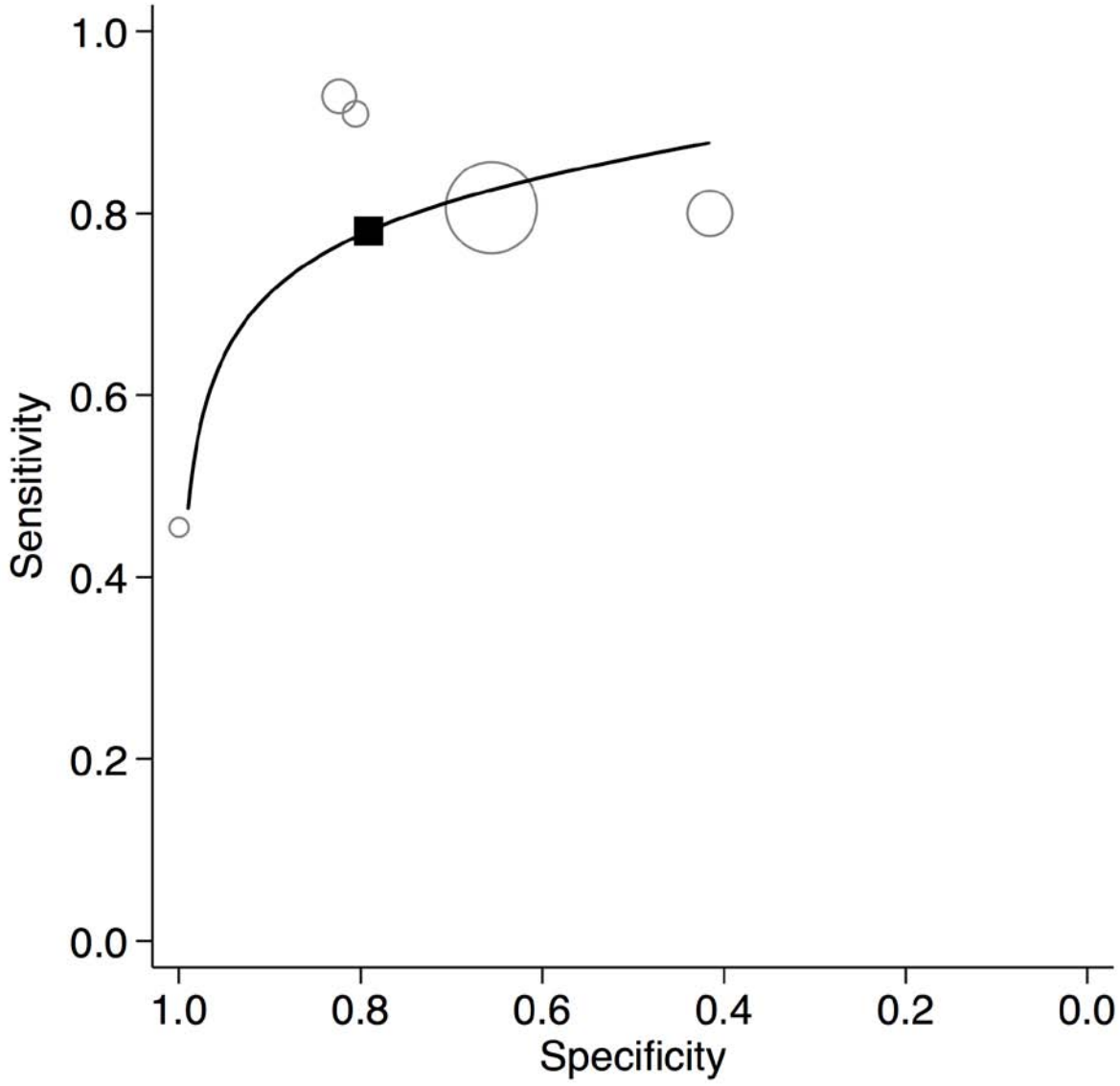
Figure 10. Sensitivity and Specificity of FISH Testing for TERC or MYC in LSIL Patients for an Outcome of CIN3+.



This forest plot shows the sensitivity (left plot) and specificity (right plot) of fluorescence in situ hybridization (FISH) testing for an outcome of cervical intraepithelial neoplasia (CIN) 2+ (i.e., CIN2 or CIN3 or cervical cancer) in low-grade squamous intraepithelial lesion (LSIL) specimens. Point estimates are shown as black circles and are listed to the right of the plot; horizontal lines denote 95% confidence intervals, which are listed in parentheses to the right of the plot after the point estimate. The PubMed identifier is listed in square brackets for each study. See Table 6 for details for each study.
MYC=myelocytomatosis oncogene, TERC=telomerase RNA component gene.

The corresponding meta-analysis for FISH for TERC or MYC in LSIL patients for the outcome of CIN3+ is shown in **Figure 11**. The overall 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). The between-study correlation of sensitivity and specificity was also negative. Visually, the curves in Figure 9 and Figure 11 appear to be similar, although the small number of studies in Figure 11 precludes conclusive findings.

Figure 11. Summary ROC Curve for the Sensitivity and Specificity of FISH Testing for TERC or MYC in LSIL Patients for an Outcome of CIN3+.



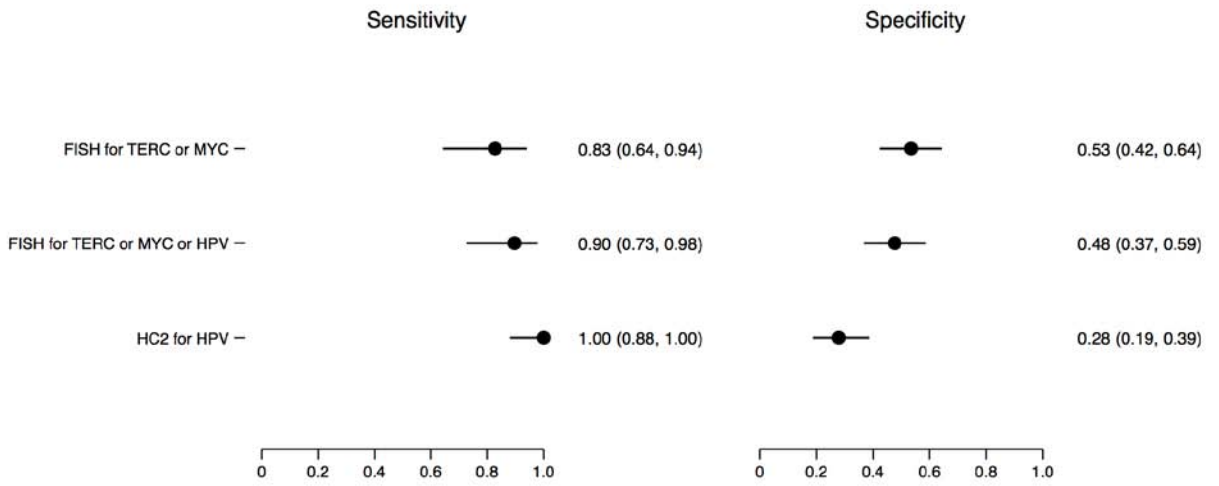
This receiver-operating-characteristic (ROC) curve plots the sensitivity (y axis) and specificity (x axis) of fluorescence in situ hybridization (FISH) testing for an outcome of cervical intraepithelial neoplasia (CIN) 3+ (i.e., CIN3 or cervical cancer) in low-grade squamous intraepithelial lesion (LSIL) specimens. The summary estimate is represented by a black square, with each study plotted as a circle whose size is proportional to the number of study participants.
MYC=myelocytomatosis oncogene, TERC=telomerase RNA component gene.

FISH for TERC or MYC versus Other Tests

Two studies compared the performance of different tests or combinations of tests and their clinical validity in LSIL patients. One study compared FISH testing for TERC or MYC, FISH for TERC or MYC or high-risk HPV, and Hybrid Capture 2 for high-risk HPV.[90] Using ROC curves to determine the optimum cutoff for producing the highest sensitivity without a great loss in specificity for the detection of CIN2+, Voss 2010 found the most favorable cutoff to be three or more cells with chromosomal gains or 30 or more cells with episomal HPV infection. These were cutoffs for positivity for testing FISH for TERC or MYC and FISH for TERC or MYC or

HPV. For the diagnosis of CIN2+, testing with Hybrid Capture 2 for HPV was the most sensitive, whereas FISH for TERC or MYC was the most specific (**Figure 12**).

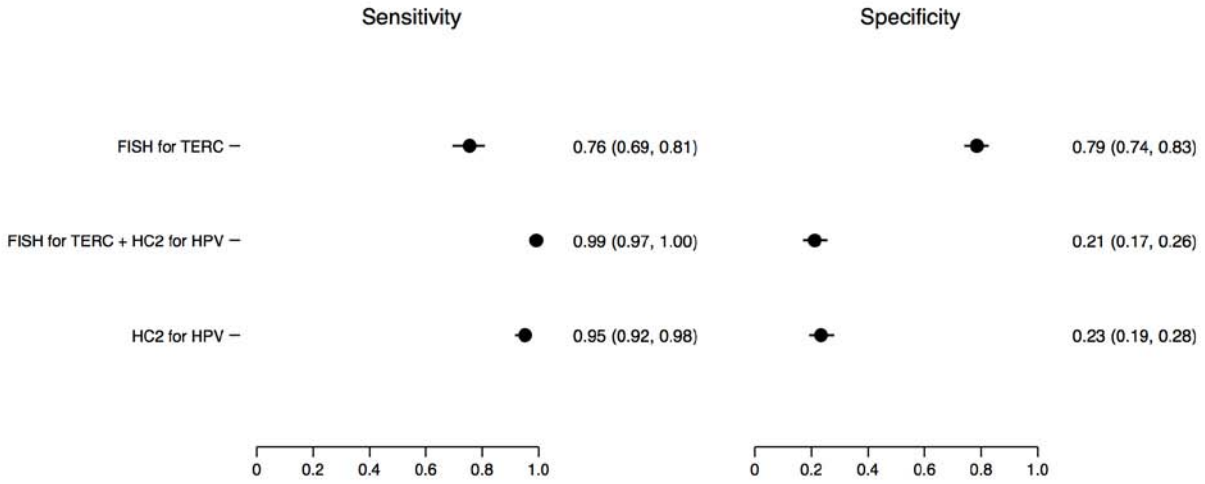
Figure 12. Sensitivity and Specificity of FISH Testing for TERC or MYC, FISH for TERC or MYC or High-Risk HPV, and Hybrid Capture 2 for High-Risk HPV for the Outcome of CIN2+ in LSIL Patients from Voss 2010.



This forest plot shows, for the Voss 2010 study (PMID 20701064), the sensitivity (left plot) and specificity (right plot) of fluorescence in situ hybridization (FISH) testing for TERC, Hybrid Capture 2 (HC2) for human papillomavirus (HPV), and the combination of both results for an outcome of cervical intraepithelial neoplasia (CIN) 2+ (i.e., CIN2 or CIN3 or cervical cancer) in low-grade squamous intraepithelial lesion (LSIL) specimens. Point estimates are shown as black circles and are listed to the right of the plot; horizontal lines denote 95% confidence intervals, which are listed in parentheses to the right of the plot after the point estimate. The See Table 6 for details of the study.
MYC=myelocytomatosis oncogene, TERC=telomerase RNA component gene.

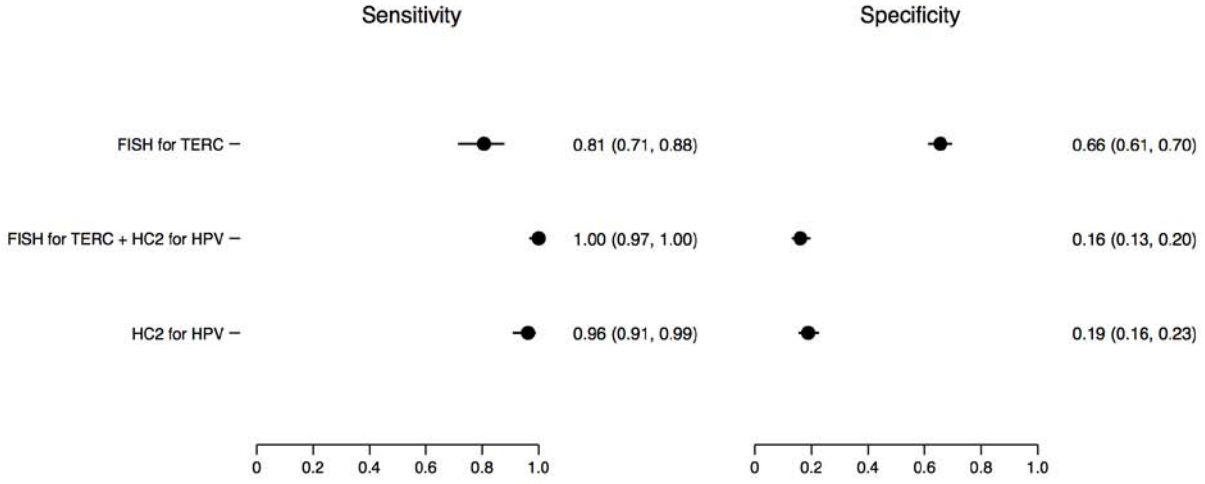
The other study compared FISH for TERC, Hybrid Capture 2 for HPV, and a combination of the two. Given the consistent pattern of higher sensitivity and lower specificity from the combined test compared to either test alone, we presumed that the combined test was considered positive if either FISH or Hybrid Capture 2 was positive; the study is unclear in this regard. For the outcome of CIN2+, the combination of FISH and Hybrid Capture 2 appears to be the most sensitive test while FISH alone was the most specific (**Figure 13**). These results also held for the outcome of CIN3+ (**Figure 14**). This combination provides a small gain in sensitivity as compared to FISH alone, but at the cost of much lower specificity.

Figure 13. Sensitivity and Specificity of FISH Testing for TERC, Hybrid Capture 2 for High-Risk HPV, and FISH for TERC or Hybrid Capture 2 for High-Risk HPV for the Outcome of CIN2+ in LSIL Patients from Jiang 2010.



This forest plot shows, for Jiang 2010 (PMID 20864639), the sensitivity (left plot) and specificity (right plot) of fluorescence in situ hybridization (FISH) testing for TERC, Hybrid Capture 2 (HC2) for human papillomavirus (HPV), and a combination of both results for an outcome of cervical intraepithelial neoplasia (CIN) 2+ (i.e., CIN2 or CIN3 or cervical cancer) in low-grade squamous intraepithelial lesion (LSIL) specimens. Point estimates are shown as black circles and are listed to the right of the plot; horizontal lines denote 95% confidence intervals, which are listed in parentheses to the right of the plot after the point estimate. See Table 6 for details of the study.
TERC=telomerase RNA component gene.

Figure 14. Sensitivity and Specificity of FISH Testing for TERC, Hybrid Capture 2 for High-Risk HPV, and FISH for TERC or Hybrid Capture 2 for High-Risk HPV for the Outcome of CIN3+ in LSIL Patients from Jiang 2010.



This forest plot shows, for Jiang 2010 (PMID 20864639), the sensitivity (left plot) and specificity (right plot) of fluorescence in situ hybridization (FISH) testing for TERC, Hybrid Capture 2 (HC2) for human papillomavirus (HPV), or a combination of both results for an outcome of cervical intraepithelial neoplasia (CIN) 3+ (i.e., CIN3 or cervical cancer) in low-grade squamous intraepithelial lesion (LSIL) specimens. Point estimates are shown as black circles and are listed to the right of the plot; horizontal lines denote 95% confidence intervals, which are listed in parentheses to the right of the plot after the point estimate. See Table 6 for details of the study.
MYC=myelocytomatosis oncogene, TERC=telomerase RNA component gene.

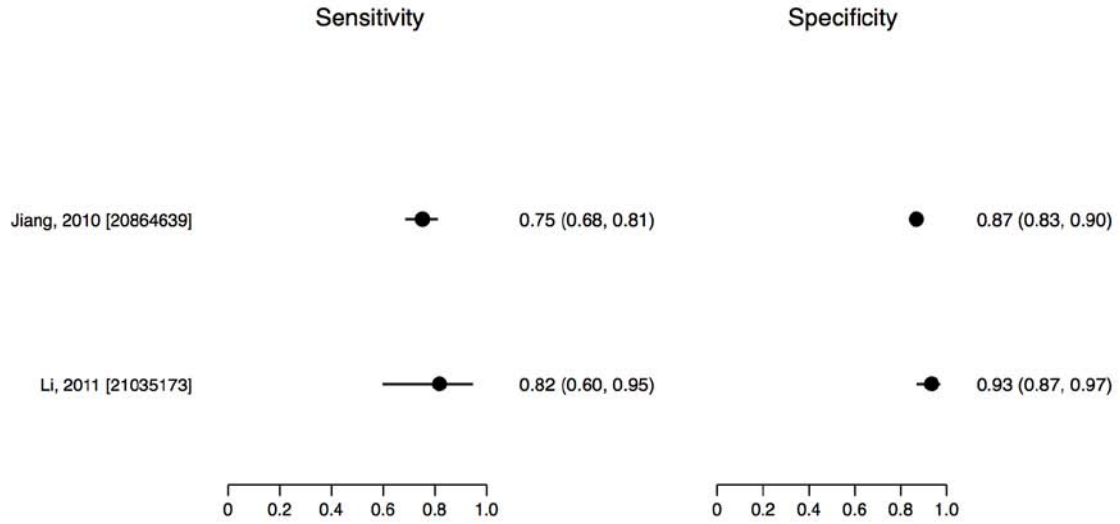
ASCUS

FISH for TERC or MYC

The data on FISH for TERC in women with ASCUS were too sparse to pool for purposes of a meta-analysis. There were only three studies, one of which included patients positive for HPV.[87]

Only two of these three studies, both testing for TERC only (not MYC), provided data for the outcome of CIN2+.[49,87] The sensitivity and specificity in these studies is plotted in **Figure 15**. Li 2011 demonstrated the higher sensitivity and specificity (0.82 and 0.93, respectively) with Jiang 2010 showing similar results with overlapping confidence intervals.

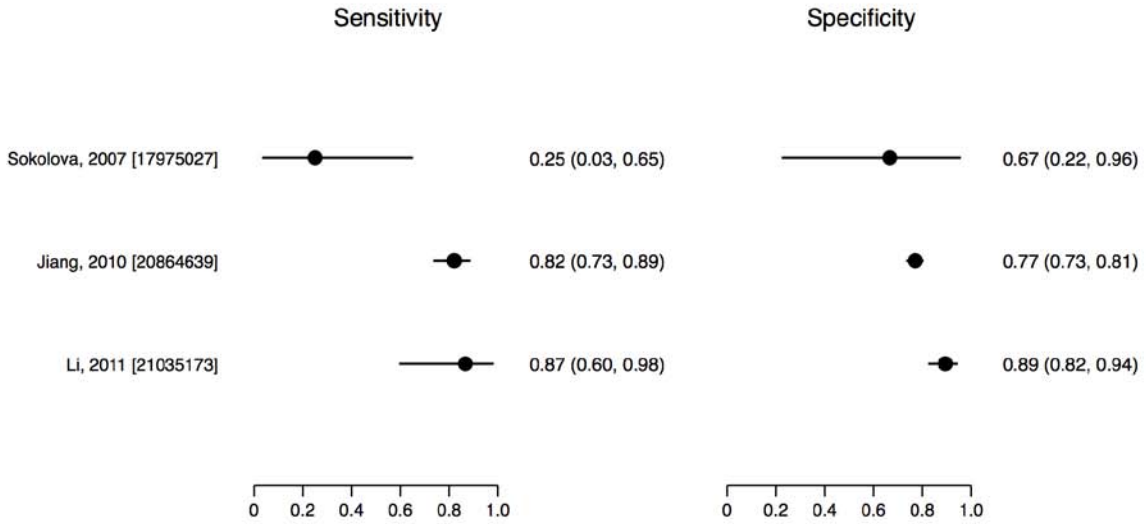
Figure 15. Sensitivity and Specificity of FISH Testing for TERC in ASCUS Patients for an Outcome of CIN2+.



This forest plot shows the sensitivity (left plot) and specificity (right plot) of fluorescence in situ hybridization (FISH) testing for an outcome of cervical intraepithelial neoplasia (CIN) 2+ (i.e., CIN2 or CIN3 or cervical cancer) in atypical squamous cells of undetermined significance (ASCUS) specimens. Point estimates are shown as black circles and are listed to the right of the plot; horizontal lines denote 95% confidence intervals, which are listed in parentheses to the right of the plot after the point estimate. The PubMed identifier is listed in square brackets for each study. See Table 6 for details for each study.
TERC=telomerase RNA component gene.

All three studies provided data for the CIN3+ outcome.[49,87,88] Sokolova 2007 examined TERC or MYC. Sensitivities ranged from 0.25 (in Sokolova 2007) to 0.87 (in Li 2011) (**Figure 16**). The estimates for Sokolova 2007 were imprecise, which may reflect the small sample size (N=14). Point estimates for specificities fell into a more narrow range from 0.67 to 0.89 percent, with Li 2011 reporting the highest estimate.

Figure 16. Sensitivity and Specificity of FISH Testing for TERC or MYC in ASCUS Patients for an Outcome of CIN3+.

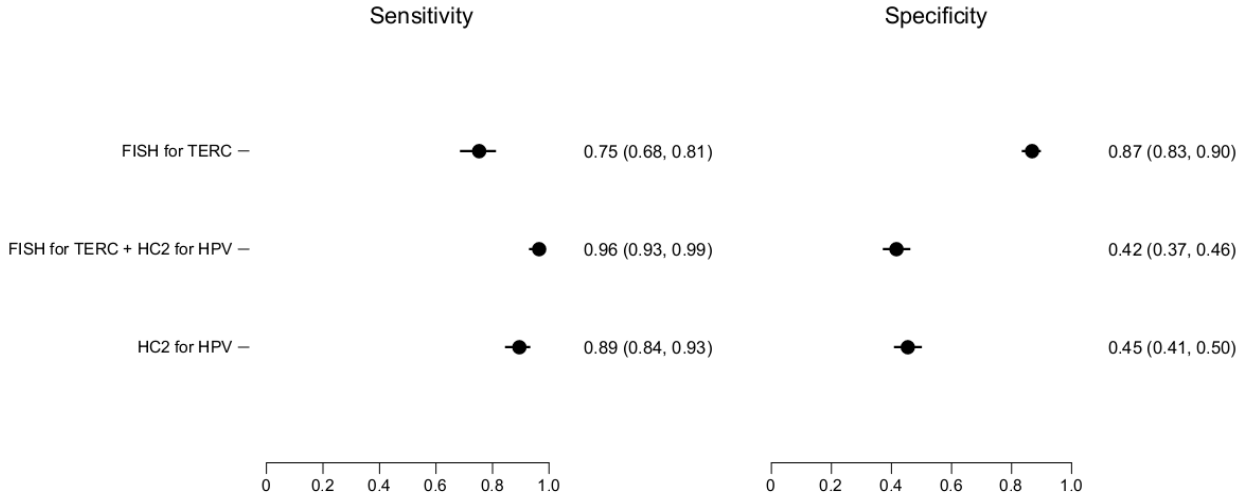


This forest plot shows the sensitivity (left plot) and specificity (right plot) of fluorescence in situ hybridization (FISH) testing for an outcome of cervical intraepithelial neoplasia (CIN) 3+ (i.e., CIN3 or cervical cancer) in atypical squamous cells of undetermined significance (ASCUS) specimens. Point estimates are shown as black circles and are listed to the right of the plot; horizontal lines denote 95% confidence intervals, which are listed in parentheses to the right of the plot after the point estimate. The PubMed identifier is listed in square brackets for each study. See Table 6 for details for each study.
MYC=myelocytomatosis oncogene, TERC=telomerase RNA component gene.

FISH for TERC versus Other Tests

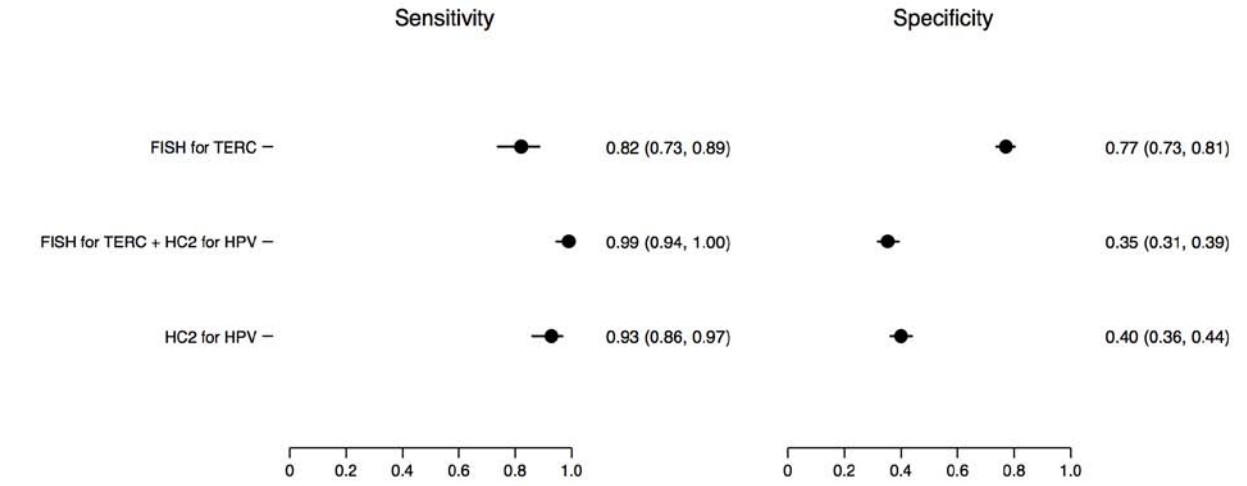
Jiang 2010 also provided comparative information on test performance in ASCUS patients by comparing sensitivity and specificity for FISH testing and Hybrid Capture 2 testing alone versus in combination (presumably with a positivity defined as a positive result on either FISH or Hybrid Capture 2) in ASCUS patients for CIN2+ and CIN3+.[49] The results (**Figure 17 and 18**) were similar to the corresponding findings in LSIL patients (Figures 13 and 14), in that the combination of tests was most sensitive for both CIN2+ and CIN3+ and FISH testing alone was the most specific. (Again we presumed that the combined test was considered positive if either FISH or Hybrid Capture 2 was positive, although the study is not entirely clear in this regard.) As expected, estimates of sensitivity were generally more precise (i.e., had narrower CIs) for the CIN2+ outcome because more patients are classified as being affected using this broader definition of disease as compared with the narrower CIN3+ definition.

Figure 17. Sensitivity and Specificity of FISH Testing for TERC, Hybrid Capture 2 for High-Risk HPV, and FISH for TERC or Hybrid Capture 2 for High-Risk HPV for the Outcome of CIN2+ in ASCUS Patients from Jiang 2010.



This forest plot shows, for Jiang 2010 (PMID 20864639),. the sensitivity (left plot) and specificity (right plot) of fluorescence in situ hybridization (FISH) testing for TERC, Hybrid Capture 2 (HC2) for human papillomavirus (HPV), or a combination of both results for an outcome of cervical intraepithelial neoplasia (CIN) 2+ (i.e., CIN2 or CIN3 or cervical cancer) in atypical squamous cells of undetermined significance (ASCUS) specimens. Point estimates are shown as black circles and are listed to the right of the plot; horizontal lines denote 95% confidence intervals, which are listed in parentheses to the right of the plot after the point estimate. See Table 6 for details of the study.
TERC=telomerase RNA component gene.

Figure 18. Sensitivity and Specificity of FISH Testing for TERC, Hybrid Capture 2 for High-Risk HPV, and FISH for TERC or Hybrid Capture 2 for High-Risk HPV for the Outcome of CIN3+ in ASCUS Patients from Jiang 2010.



This forest plot shows the sensitivity (left plot) and specificity (right plot) of fluorescence in situ hybridization (FISH) testing for TERC, Hybrid Capture 2 (HC2) for human papillomavirus (HPV), or a combination of both results for an outcome of cervical intraepithelial neoplasia (CIN) 3+ (i.e., CIN3 or cervical cancer) in atypical squamous cells of undetermined significance (ASCUS) specimens. Point estimates are shown as black circles and are listed to the right of the plot; horizontal lines denote 95% confidence intervals, which are listed in parentheses to the right of the plot after the point estimate. See Table 6 for details of the study.
TERC=telomerase RNA component gene.

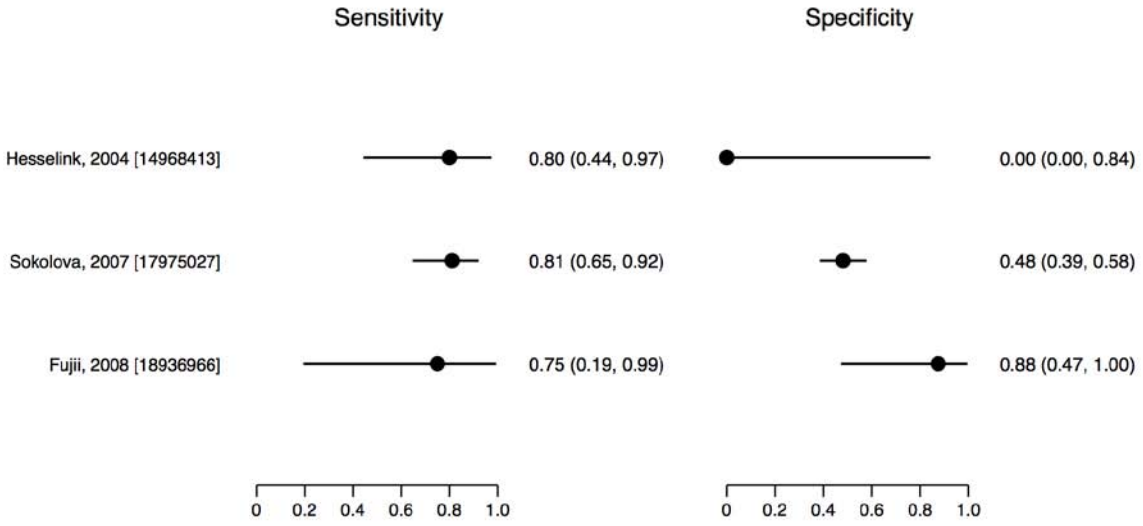
HPV 16 or 18

The data on FISH for HPV 16 or 18 in women with LSIL or ASCUS were too sparse to pool for purposes of meta-analysis; there were only three studies.[75,83,88]

LSIL

All three studies provided data for the sensitivity and specificity of FISH for HPV 16 or 18 in LSIL patients for the CIN2+ outcome (**Figure 19**). The sensitivities ranged from 0.75 to 0.81percent, with Sokolova 2007 reporting the highest estimate. The specificities ranged from 0.00, in Hesselink 2004, to 0.88, in Fujii 2008. The CIs were overlapping because of imprecise estimates and the point estimates of specificity were very heterogeneous, possibly reflecting nonoverlapping HPV probes being detected.

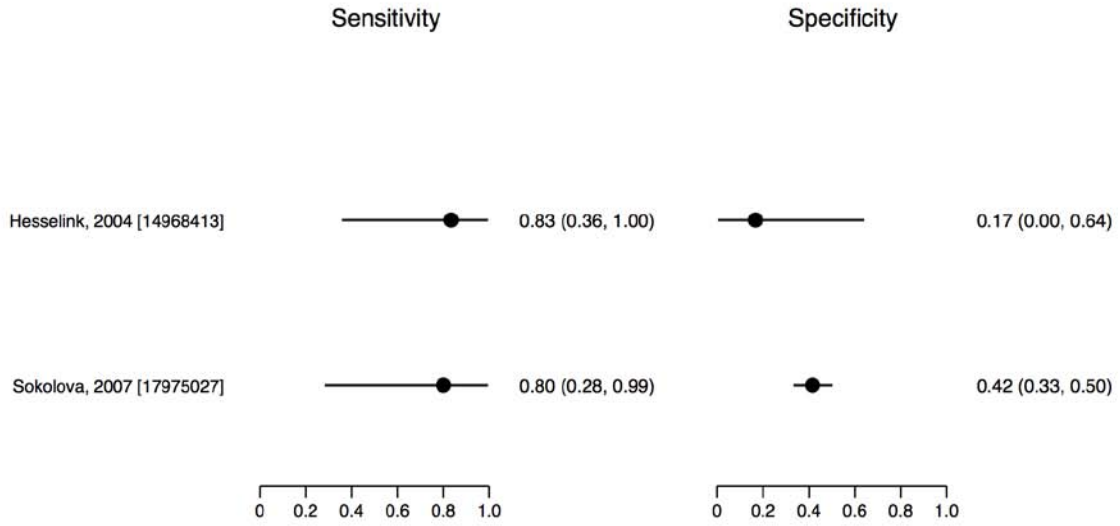
Figure 19. Sensitivity and Specificity of FISH Testing for HPV 16 or 18 in LSIL Patients for an Outcome of CIN2+.



This forest plot shows the sensitivity (left plot) and specificity (right plot) of fluorescence in situ hybridization (FISH) testing for human papillomavirus (HPV) 16 or 18 for an outcome of cervical intraepithelial neoplasia (CIN) 2+ (i.e., CIN2 or CIN3 or cervical cancer) in low-grade squamous intraepithelial lesion (LSIL) specimens. Point estimates are shown as black circles and are listed to the right of the plot; horizontal lines denote 95% confidence intervals, which are listed in parentheses to the right of the plot after the point estimate. The PubMed identifier is listed in square brackets for each study. See Table 6 for details for each study.

For the outcome of CIN3+, reported in two studies, Hesselink 2004 and Sokolova 2007 reported similar sensitivities (0.83 and 0.80, respectively) with wide, overlapping CIs (**Figure 20**). The specificity data were less congruent, with 0.42 specificity in Sokolova 2007 but only 0.17 specificity in Hesselink 2004. However, the 0.17 estimate had a wide CI that included the 0.42 estimate.

Figure 20. Sensitivity and Specificity of FISH Testing for HPV 16 or 18 in LSIL Patients for the Outcome of CIN3+.



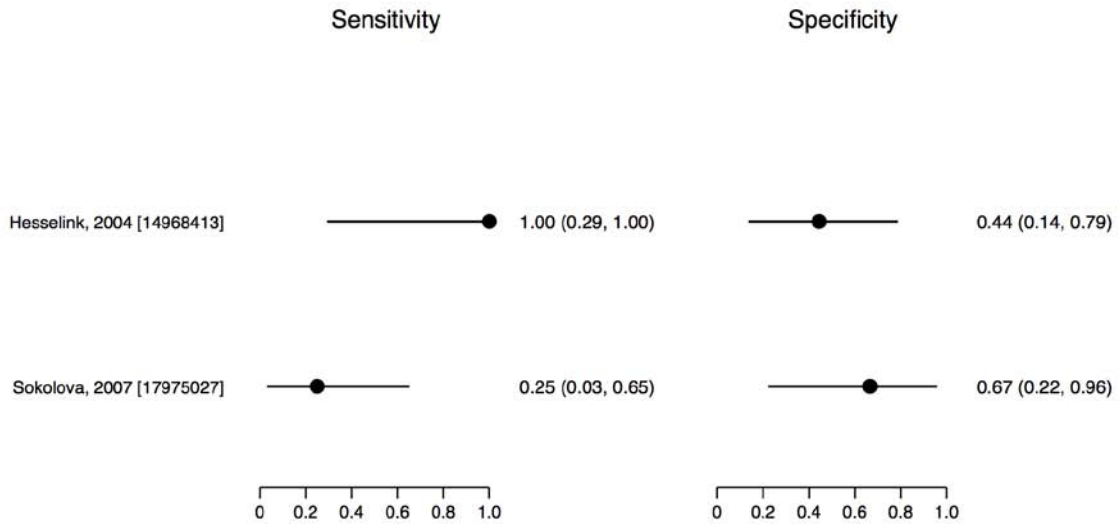
This forest plot shows the sensitivity (left plot) and specificity (right plot) of fluorescence in situ hybridization (FISH) testing for human papillomavirus (HPV) 16 or 18 for an outcome of cervical intraepithelial neoplasia (CIN) 2+ (i.e., CIN2 or CIN3 or cervical cancer) in low-grade squamous intraepithelial lesion (LSIL) specimens. Point estimates are shown as black circles and are listed to the right of the plot; horizontal lines denote 95% confidence intervals, which are listed in parentheses to the right of the plot after the point estimate. The PubMed identifier is listed in square brackets for each study. See Table 6 for details for each study.

ASCUS

One study examined FISH for HPV 16 or 18 in ASCUS patients for the CIN2+ outcome.[75,88] Hesselink 2004 demonstrated a sensitivity of 1.00 (95% CI 0.48, 1.00), and reported 0.50 specificity (95% CI 0.18, 0.90).

Two studies examined FISH for HPV 16 or 18 for the outcome of CIN3+ (**Figure 21**). Again Hesselink 2004 showed the higher sensitivity with CIs overlapping those of the Sokolova 2007 estimate. For specificity, however, the Sokolova 2007 estimate was higher. The CIs were overlapping and wide.

Figure 21. Sensitivity and Specificity of FISH Testing for HPV 16 or 18 in ASCUS Patients for an Outcome of CIN3+.



This forest plot shows the sensitivity (left plot) and specificity (right plot) of fluorescence in situ hybridization (FISH) testing for human papillomavirus (HPV) 16 or 18 for an outcome of cervical intraepithelial neoplasia (CIN) 3+ (i.e., CIN2 or CIN3 or cervical cancer) in atypical squamous cells of undetermined significance (ASCUS) specimens. Point estimates are shown as black circles and are listed to the right of the plot; horizontal lines denote 95% confidence intervals, which are listed in parentheses to the right of the plot after the point estimate. The PubMed identifier is listed in square brackets for each study. See Table 6 for details for each study.

Clinical Validity in HPV-Positive Normal Cytology Samples

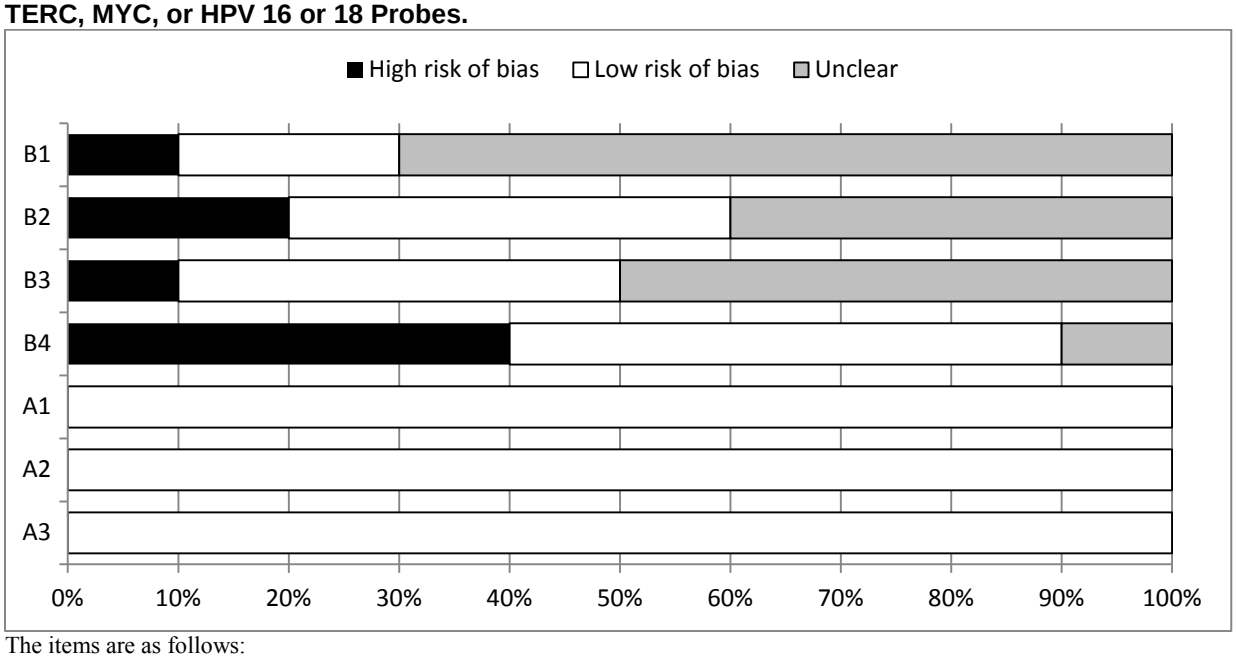
We found no relevant data in groups of women who were HPV test positive but had normal cytology.

Assessment of Risk of Bias and Completeness of Reporting for Individual Studies

Our assessment of study quality and reporting for Key Question 3 studies of clinical validity was based on 18 questions related to assay performance and reporting thereof. **Appendix E** shows scoring of each of the 10 studies for each item, and **Figure 22** shows the aggregated results. In general, study reporting was fair at best. Few studies reported information of recruitment and study design (questions S1–S3 in Appendix E, question B1 in Figure 22), although reporting of information on the index tests and references standards was generally adequate (questions S4–S5, and S6–S7 in Appendix E, questions B2 and B3 in Figure 22). Data on flow and timing was sparsely reported (questions S8 and S9 in Appendix E). All patient received the same reference standard (question S10 in Appendix E), but inclusion of all patients was complete only in 50 percent of studies (question S11 in Appendix E), resulting in variable clarity and bias resulting from patient flow (question B4, Figure 22).

Overall, the reporting was frequently unclear, impeding the assessment of the risk of bias (Figure 22). In contrast, concern regarding the applicability of studies to Key Question 3 was uniformly low, given the inclusion criteria for these studies.

Figure 22. Risk of Bias and Applicability of All 10 Studies of Clinical Validity of FISH Testing with TERC, MYC, or HPV 16 or 18 Probes.



The items are as follows:
B1=Risk of bias: Could the selection of patients have introduced bias?
B2=Risk of bias: Could the conduct or interpretation of the index test have introduced bias?
B3=Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?
B4=Risk of bias: Could the patient flow have introduced bias?
A1=Concerns about applicability: Concerns that the included patients do not match the review question?
A2=Concerns about applicability: Concerns that the index test, its conduct, or its interpretation differ from the review question?
A3=Concerns about applicability: Are there concerns that the target condition as defined by the reference standard does not match the review question?
FISH=fluorescence in situ hybridization, HPV=human papillomavirus, MYC=myelocytomatosis oncogene, TERC=telomerase RNA component gene.

Strength of Evidence

The strength of evidence for the studies on clinical validity reviewed for Key Question 3a was rated as low. The studies were generally small. The number of comparisons for each pair test–outcome pair was low. Reporting on items used for quality assessment was often unclear, yielding overall low methodological quality. Point estimates were heterogeneous. The CIs were often overlapping because of imprecise estimates. Across studies of FISH tests for HPV 16 or 18 (among other types), the panels of HPV probes used did not overlap, resulting in clinical heterogeneity.

Overall, the lower 95 percent confidence limit for sensitivity and specificity spanned 0.5 in a high proportion of studies, indicating that the test results may not distinguish between the presence or absence of FISH signals beyond chance. Thus, overall we have low confidence that the estimated clinical validity of the FISH test represents its true validity. The evidence was considered to be direct for clinical validity since the studies examined CIN2 or 3, which are meaningful outcomes for decisionmaking.

Key Question 3b: How similar are the spectrum and prevalence of the histopathological abnormalities and cervical cancers between the studies and Medicare beneficiaries?

To address this question, we conducted a focused review for information on population-based prevalence of CIN2+ and CIN3+, stratified for LSIL or ASCUS in Medicare beneficiaries. We did not find any evidence. Further, the primary studies did not provide any unbiased estimates.

Key Question 4: What are the clinical utility and harms for ISH tests in cervical cytology?

No studies compared patient care strategies resulting from different test thresholds or different combinations of ISH or non-ISH tests. This is not surprising, since ISH testing is not currently used in practice. Potential harms associated with colposcopy and biopsy include transient cervical bleeding and discharge or infection with fever and moderate-to-severe pain. Treatment with cervical conization can be complicated by cervical incompetence, resulting in fetal prematurity or infertility. An ablative procedure can also complicate subsequent testing for cervical cancer, as it makes it more difficult to visualize any lesions.

Harms from false positive findings are anxiety and unnecessary procedures. Harms from false negative findings are the missed opportunity for early and potentially curative treatment. These potential harms highlight the need for enhancing diagnostic accuracy.

Discussion

Key Findings and Strength of Evidence

Cervical cancer screening remains an evolving field with ongoing reevaluation of Pap screening practices and the role of HPV testing, as well as development of new technologies, including ISH testing for genetic abnormalities. The key findings of this review and the strength of evidence are summarized in **Table 8**.

Table 8: Key Findings and Strength of Evidence

Key Question	Population	Test/Assay	Outcome	Strength of Evidence Summary and Comments
1. Horizon scan	Women screened or tested for cervical cancer	Any ISH test	NA	SOE=NA 135 Articles described use of an ISH probe on cervical cytology or histology samples • 31 Studies used ISH for TERC; 7 of these examined both TERC and MYC • 91 Studies used ISH for HPV 16; 87 of these studies examined both HPV 16 and 18 • On the basis of these findings, we focused of the subsequent review on ISH for TERC, MYC, HPV 16, or HPV 18
2. Analytic validity	Women screened or tested for cervical cancer	Any ISH test for TERC, MYC, HPV 16, or HPV 18	Agreement with reference test	SOE=NA No studies compared ISH test for TERC or MYC with DNA-based reference test 14 Studies compared ISH tests for HPV 16 or 18 (among other types) with various reference tests (mostly PCR and Hybrid Capture 2). Agreement was variable, indicating differences in measurement techniques between ISH and reference tests, as well as nonoverlapping panels of HPV probes. Assessment of study quality shows deficiencies in reporting, which may indicate low study quality. Overall, evidence for analytic validity of various ISH assays was limited.
			Thresholds	SOE=NA 14 Studies included for KQ2 and 10 studies included for KQ3 were examined for information on thresholds of positivity on ISH testing. • Thresholds for ISH tests with TERC or MYC probes consisted of variable counts of signal-positive cells (three or more) and a range of different control probes for centromere or chromosome numbers. • Test positivity for HPV DNA was dichotomized as detection versus no detection in most studies (except for one, which used a cutoff of 30 cells as positivity for episomal infection). Two other studies provided information on threshold determination of FISH for TERC in samples from normal women across four laboratories (one in 1 study and three in the other); the value for a positive result ranged from 5.2–6.4 percent of cells with an abnormal signal (statistical comparison ND).
			Other preanalytic issues	SOE=NA For questions related to preanalytic issues impacting analytic validity, the data were sparse or not informative.
			Prevalence of genetic marker	SOE=NA No direct evidence for prevalence of the markers detected by ISH (i.e., TERC, MYC, HPV 16, and HPV 18) in US Medicare beneficiaries.
3a. Clinical validity	Women screened or tested for cervical cancer with finding of LSIL or ASCUS on cytology, with or without HPV infection	Any ISH test for TERC, MYC, HPV 16, or HPV 18	CIN2+ or CIN3+	SOE=low 10 Studies provided information on clinical validity of FISH tests for CIN2+ or CIN3+. Of these, 8 provided results for FISH on TERC (3 tested for TERC or MYC) and 3 studies provided results for FISH for HPV 16 or 18 (1 study tested both probe types, separately). In one study all women were HPV positive (type not reported); HPV status in the other studies was not known. Meta-analysis of 7 studies of TERC or MYC in LSIL for CIN2+ found summary sensitivity of 0.76 (95% CI 0.60, 0.86) and summary specificity of 0.79 (95% CI 0.50, 0.93). Meta-analysis of 5 studies of TERC or MYC in LSIL for CIN3+ found summary sensitivity of 0.78 (95% CI 0.65, 0.87) and summary specificity of 0.79 (95% CI 0.51, 0.93). 2 Studies compared different test combinations. • One compared results of FISH for TERC, Hybrid Capture 2 for high-risk HPV, and either test. FISH for TERC alone showed lower sensitivity but higher specificity than the combination of FISH or Hybrid Capture 2. • The other study compared three test strategies: FISH for TERC or MYC, Hybrid Capture 2 for high-risk HPV, and

Key Question	Population	Test/Assay	Outcome	Strength of Evidence Summary and Comments
				FISH for TERC, MYC, or HPV. FISH for TERC or MYC alone showed lower sensitivity but higher specificity than either other test strategy. For other cytology classifications and tests, the numbers of studies was limited. 3 Studies had data on FISH for TERC (without MYC) in women with ASCUS. One included only samples positive for HPV. There were also only 3 studies with data on FISH for HPV in women with LSIL or ASCUS. Across all studies and tests, there was a trade-off between sensitivity and specificity, which may indicate a threshold effect. However, there was also great clinical heterogeneity across populations and test probes, Assessment of risk of bias showed low study quality or incomplete reporting. There was inconsistency in effect estimates and many were imprecise. The evidence was considered direct for clinical validity. Overall, the strength of evidence was graded as low, failing to show consistently better sensitivity or specificity with FISH testing for identification of CIN2+ or CIN3+ than would be expected by chance.
3a. Clinical validity	Women screened for cervical cancer with finding of LSIL or ASCUS, with or without HPV infection	Any ISH test for TERC, MYC, HPV 16, or HPV 18	Clinical outcome	SOE=insufficient No studies examined the association of ISH test results with clinical outcomes.
3b. Prevalence of the outcome in comparison to the Medicare population		NA	Prevalence of disease (CIN2+ or 3+)	SOE=NA No direct evidence for prevalence of CIN2+ or 3+ in US Medicare beneficiaries
4. Clinical utility	Women screened for cervical cancer	Any ISH test for TERC, MYC, HPV 16, or HPV 18	All clinical outcomes	SOE=insufficient No studies compared patient care strategies among various tests, thresholds, or combinations of ISH or non-ISH tests. Potential harms associated with colposcopy and biopsy are transient cervical bleeding and discharge or infection with fever and moderate-to-severe pain. Cervical conization can be complicated by cervical stenosis or incompetence resulting in fetal prematurity or infertility. An ablative procedure can also complicate subsequent testing for cervical cancer, as it makes it more difficult to visualize any lesions. Harms from false positive findings are anxiety and unnecessary procedures. Harms from false negative findings are the missed opportunity for early and potentially curative treatment.

ASCUS=atypical squamous cells of undetermined significance, CI=confidence interval; CIN=cervical intraepithelial neoplasia, FISH=fluorescence in situ hybridization, HPV=human papillomavirus, ISH=in situ hybridization, KQ=key question, MYC=myelocytomatosis oncogene, NA=not applicable; LSIL=low-grade squamous intraepithelial lesion, NHANES=National Health and Nutrition Examination Survey; PCR=polymerase chain reaction, SOE=strength of evidence, TERC=telomerase RNA component gene, US=United States.

The horizon scan conducted for Key Question 1 led to the subsequent focus on ISH tests for TERC, MYC, HPV 16, or HPV 18 as tests for cervical abnormalities or cancer.

Our review of data on analytic validity for Key Question 2 revealed a paucity of evidence. We found no studies examining the association between ISH for TERC or MYC and another genetic test in cytology or histology samples. For HPV, we identified some studies for which we could examine the correlation between ISH and reference tests, namely PCR and Hybrid Capture 2. However, these tests measure different biological parameters since, unlike ISH, the reference HPV tests are not restricted to detecting nuclear episomal or integrated HPV. (In situ ISH testing for HPV, which is the only ISH that can identify integration into the genome, may add information beyond the most common ISH testing for 13 or 14 types of HPV or ISH for HPV 16 and 18, which only indicate HPV infection, not integration.)

Further, the panels of HPV genotypes tested for by ISH and the reference tests varied and were not completely overlapping. This heterogeneity limits the conclusions that can be drawn about analytic validity. Not surprisingly, the agreement between ISH tests and reference tests was inconsistent across the studies.

Risk of bias assessment of analytic validity studies showed variable detail of reporting, which was particularly poor for the reference tests. Review of the evidence on thresholds for ISH tests also showed incomplete reporting as well as variable thresholds of positivity and chromosomal control probes used. Information on other preanalytic issues was sparse or not informative. The lack of data on reproducibility is a major deficiency in the evidence base. This suggests a need for research to explore thresholds and standardize test procedures.

For Key Question 3 on clinical validity, the strength of evidence for ISH testing was graded as low, failing to show that the addition of ISH tests resulted in better clinical validity. Clinical practice guidelines suggest that ISH is a potential add-on test after initial Pap testing, with subsequent HPV testing, or after initial Pap and HPV cotesting. In this context, it is more desirable for ISH to show high specificity than high sensitivity. In our review, FISH testing did not show consistently increased sensitivity for the identification of CIN2+ or CIN3+ on histology, although it was more specific than other tests or test combinations. However, we cannot conclude that ISH testing would increase clinical validity of an overall screening strategy. As compared with FISH or Hybrid Capture 2 testing for HPV, FISH for TERC or MYC alone was more specific and less sensitive than the test combinations.

Regarding Key Question 4, we found no studies examining the association of ISH test results with clinical outcomes. There were also no comparative studies of strategies that include ISH tests that examined clinical utility, which would be of particular interest for colposcopy rates and histology results.

Comparison with Current Knowledge

ISH tests are not used routinely used in screening for cervical cancer at this point. However, there is a need to improve the clinical validity of screening for cervical cancer. Thus there is a potential role for tests such as ISH. HPV tests for panels of high-risk genotypes have been shown to have a higher sensitivit, but lower specificity that what we found for ISH tests.[91] Thus when cotesting is used, add-on tests with greater specificity may be useful. However, HPV testing is evolving, and new reference tests for HPV testing will change the performance of add-on tests. ISH may need to be examined as an alternative to tests that can identify HPV 16 and 18 individually. Further, the recent launch of HPV vaccination in adolescents is expected to change the natural history of HPV associated cervical carcinoma going forward.

Applicability

Formal appraisal of applicability of the Key Question 3 studies on clinical validity with the QUADAS-2 tool showed no major concern regarding applicability. However, studies included populations from around the world, with variable prevalence of HPV infections, CIN classes, and cervical cancer.

CMS has a particular interest in the Medicare population, whose core beneficiaries are 65 years of age or older. On the basis of the lower incidence of HPV infection and cervical cancer among older women who have undergone adequate screening than among younger women, the 2012 guidelines recommend cessation of screening after the age of 65 years (so long as screening tests were negative in the prior 10 years). Since a notable proportion of Medicare beneficiaries are younger than 65, the findings of the report are still relevant for CMS.

Implications for Clinical and Policy Decisionmaking

The current evidence base is insufficient to consider routine ISH testing in the clinical scenarios analyzed in the report. Specifically the evidence is insufficient to recommend routine ISH testing for TERC, MYC, HPV 16 or 18 in women screened or tested for cervical cancer with a finding of LSIL or ASCUS on cytology, with or without HPV infection.

Limitations

Our review is limited to published reports, which usually do not allow for detailed analysis of individual patient data for subgroups of interest. Studies evaluating more than one test approach did not include cross-tabulation of positive and negative test results across all tests. Our review addresses a limited scope based on what was determined to be the most meaningful clinical questions. Given our stringent inclusion criteria for articles, requiring the mention of cytologic or histologic sampling in the abstract, we may have missed studies that could have contributed additional data for the review of analytic validity.

Regarding Key Question 3 on clinical validity of ISH in particular, the identified evidence base was limited. Studies were generally small and those that we could meta-analyze yielded imprecise effect estimates. Study samples often were from sample banks or databanks, limiting the applicability to the screening population. With one exception, the included studies did not unequivocally report or stratify by HPV status. Studies conducted before the Bethesda terminology change that divided ASCUS into ASC-US and ASC-H may have included a mix of ASC-US and ASC-H in their ASCUS group. There was clinical heterogeneity among the results, given the variety of ISH probe panels used across studies and differences between ISH and the DNA-based reference tests. In addition, the reporting of study quality items was deficient. No studies examined risk prediction with ISH or the test's clinical utility or addressed screening for cervical adenocarcinoma in particular.

Research Gaps

Our review reveals four major research gaps. First, the assessment of the analytic validity of ISH (Key Question 2) highlights a need to establish common thresholds, probe sets, controls, and procedures. An expert conference may be helpful to agree on common measurement guidelines, a path that was successfully pursued to arrive at the consensus Bethesda classification for cytological abnormalities in cervical cancer. Scoring of ISH slides can be time-consuming.

Automated approaches are promising, but in order for ISH to become a routine test, the evaluation of test results needs to be standardized and accelerated.

Second, bigger studies with larger numbers of patients with HSIL are needed to yield more precise estimates.

Third, future research should reflect changes in clinical practice. On the basis of the current guidelines, it can be expected that Pap with reflexive HPV testing or Pap–HPV cotesting will become more widely used. This will require study of the clinical validity of ISH as an add-on test in groups of women characterized as having a normal Pap or ASCUS or LSIL along with a positive or negative HPV test. It is also expected that HPV testing will eventually be able to routinely identify not only high-risk HPV genotypes broadly but also HPV 16 and 18 individually, with the use of either sequential or combined tests. This will require reevaluation of the role of ISH, which we in this review considered as a hypothetical alternative to testing for HPV 16 or HPV 18. Development of automated HPV testing may provide an incentive to explore the performance of up-front HPV testing rather than Pap testing, since testing of cervical cytologic specimens requires a trained human operator. This would generate another constellation in which to study the value of ISH testing added to HPV genotyping.

Fourth, changes in terminology should be followed in future studies, specifically the differentiation between ASC-US and ASC-H and the use of LAST terminology, including p16 staining in ambiguous cases for classifying histology as LSIL or HSIL.

Fifth, further evaluation of clinical validity of ISH should be better designed to achieve this aim. Studies could examine ISH testing for not only a single probe (such as TERC) but also panels of probes, for example for both TERC and HPV. Ideally, large studies would allow for the comparison of multiple tests in order to make it possible to select tests with best analytic validity as well as clinical validity for CIN. However, to measure false negative rates, colposcopy would need to be performed in patients with negative screening tests. Such studies should therefore identify the tests, thresholds, and combinations that are most promising for further evaluation of clinical utility. Efficient exploration of the correct test use (i.e., the testing with the best performance) would again be conducted with several promising tests, thresholds, and test combinations studied simultaneously in a sufficiently large sample on the same specimens and follow patients with routine or test-directed care to assess impacts on diagnostic thinking, evaluation, management, and clinical outcomes. Projecting the clinical utility of different tests may entail modeling of data from different studies in decision analyses.

Lastly, the role ISH testing for detection of adenocarcinoma should be examined. The variability in chromosomal aberrations between squamous-cell cancer and adenocarcinoma suggests that a panel of ISH probes, rather than a single probe, would capture a greater variety of chromosomal changes.

Conclusions

Our report shows an emerging body of literature on ISH testing for cervical cancer. Although ISH tests are marketed by some laboratories for triaging women with abnormal screening tests, there is a lack of standardization of probes and procedures that needs to be addressed. The role of add-on ISH testing has not been adequately examined in current screening contexts, that is, after HPV and Pap testing. Further, HPV testing is likely to evolve, for example with primary screening for 13 or 14 HPV genotypes or with wider availability of HPV16/18 testing. This will again require reexamination of the role of add-on tests such as ISH and its impact not only on

diagnostic utility but also on clinical utility (in particular colposcopy) and on clinical outcomes. Thus, the evidence is currently too immature to suggest the ISH testing for routine practice.

Acronyms

ACOG	American Congress of Obstetricians and Gynecologists
ACS	American Cancer Society
AHRQ	Agency for Healthcare Research and Quality
ASCCP	American Society for Colposcopy and Cervical Pathology
ASCP	American Society for Clinical Pathology
ASC-H	Atypical squamous cells, cannot exclude HSIL
ASCUS	Atypical squamous cells of undetermined significance
CIN	Cervical intraepithelial neoplasia, with mild dysplasia (CIN1), moderate dysplasia (CIN2), or severe dysplasia (CIN3)
CMS	Centers for Medicare and Medicaid Services
EPC	Evidence-based Practice Center
FDA	Food and Drug Administration
FISH	Fluorescence in situ hybridization
HPV	Human papillomavirus
HSIL	High-grade squamous intraepithelial lesion
ISH	In situ hybridization
LSIL	Low-grade squamous intraepithelial lesion
MYC	Myelocytomatosis oncogene (on chromosome 8, band q24)
Pap test	Papanicolaou test (of cervical cytology)
PCR	Polymerase chain reaction
QUADAS-2	Quality Assessment of Diagnostic Accuracy Studies 2
SCC	Squamous-cell carcinoma
TA	Technology Assessment
TERC	Telomerase RNA component gene (on chromosome 3, band q26)
USPSTF	U.S. Preventive Services Task Force

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Appendix A. Search Strategy

We performed two automated searches on November 7, 2011: one in MEDLINE and the Cochrane Central Register of Controlled Trials; the other was in the Scopus database to capture citations in Embase. After reduplication of the two search yields, we identified a total of 1441 unique abstracts to screen for inclusion. We reran the same two searches on July 12, 2012, to update our yield; this search identified 21 additional abstracts.

SEARCH 1
Databases:

1) **Ovid MEDLINE ® without Revisions** 1996 to October Week 2 2011
2) **Ovid MEDLINE ®** 1948 to October Week 2 2011
3) **Ovid MEDLINE ® In-Process & Other Non-Indexed Citations** October 24, 2011
4) **EBM Reviews-Cochrane Central Register of Controlled Trials** 4th Quarter 2011

Table A1. Search Terms

#	Searches	Brief description of terms	Number of abstracts
1	in situ hybridization.af	Terms related to test of interest	106611
2	in situ hybridization.sh		45501
3	(situ and hybridization).af		108832
4	in situ hybridization, fluorescence.sh		31122
5	(situ and hybridization and fluorescence).af		37588
6	fluorescence in situ hybridization.af		17824
7	in situ hybridization, fluorescence.af		31126
8	or/1-7		108832
9	uterine cervical neoplasms.sh	Terms related to the disease of interest	55036
10	(uterine and cervical and neoplasms).af		56825
11	uterine cervical neoplasms.af		55047
12	(cervical and neoplasm).af		18198
13	cervical neoplasm.af		92
14	cervical intraepithelial neoplasia.sh		6478
15	uterine cervical dysplasia.af		3420
16	uterine cervical dysplasia.sh		3416
17	or/9-16		63287
18	((precancerous conditions and cervic$) or cervix uteri).af		25551
19	((precancerous conditions and cervic$) or cervix uteri).sh		21360
20	18 or 19		25551
21	17 or 20		79043
22	8 and 21		**1198**

The above search strategy was tested against the studies referenced in the bibliography of the test manufacturer's website. The search identified all relevant studies.

SEARCH 2
Database: Scopus
Search terms: ALL(in situ hybridization OR fluorescence in situ hybridization) AND ALL(cervical neoplasms OR cervical intraepithelial neoplasia OR cervical dysplasia)
Number of abstracts: 1441 from all years (included all 1198 captured in the above search)

Appendix B. Excluded Studies

The 111 studies excluded after full-text review for Key Questions 2, 3, and 4 are listed in alphabetical order by first author under each main reason for exclusion (bold headings): No probes for TERC, MYC, or HPV 16 or 18 (n=19), no analytic validity data and no cytology specimens (just histology) (n=69), no analytic validity data (n=7), and FISH results not reported for cytology classification according to histology classification (n=16). PMIDs are given at the end of each reference, when available.

No Probes for TERC or MYC or HPV 16 or 18 (n=19)

Cortes-Gutierrez EI, vila-Rodriguez MI, Fernandez JL, et al. DNA damage in women with cervical neoplasia evaluated by DNA breakage detection-fluorescence in situ hybridization. Anal Quant Cytol Histol 2011 Jun;33(3):175-81. *PMID:21980621.*

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Olaharski AJ, Sotelo R, Solorza-Luna G, et al. Tetraploidy and chromosomal instability are early events during cervical carcinogenesis. Carcinogenesis 2006 Feb;27(2):337-43. *PMID:16123119.*

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No Analytic Validity Data and No Cytology Specimens (n=69)

Yuan Yl, He CN, Xu Mt, et al. [Detection of TERC gene amplification by fluorescence in-situ hybridization in cervical intraepithelial lesions]. Zhonghua Bing Li Xue Za Zhi 2011 Mar;40(3):182-86. *PMID:21575390.*

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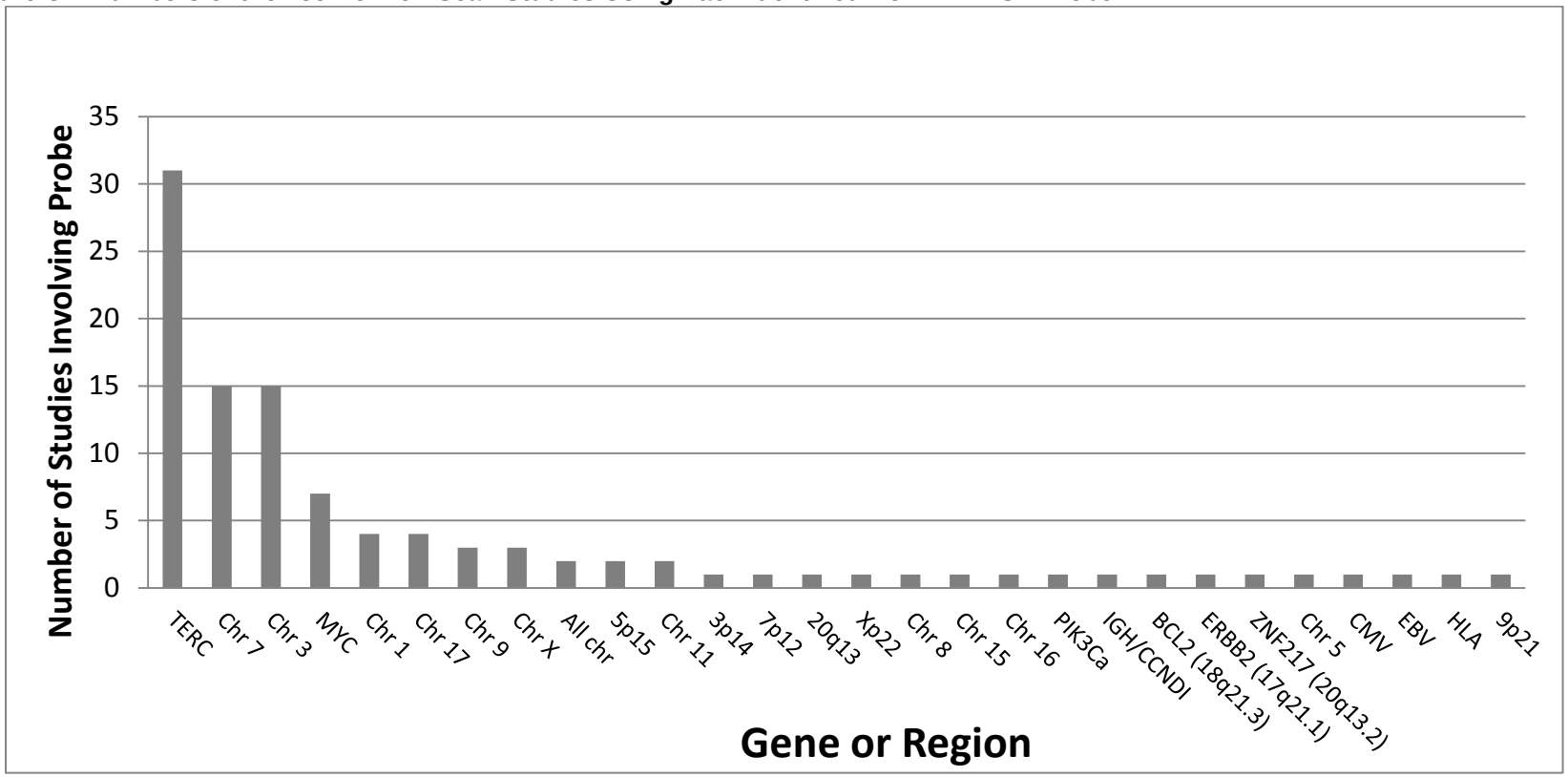
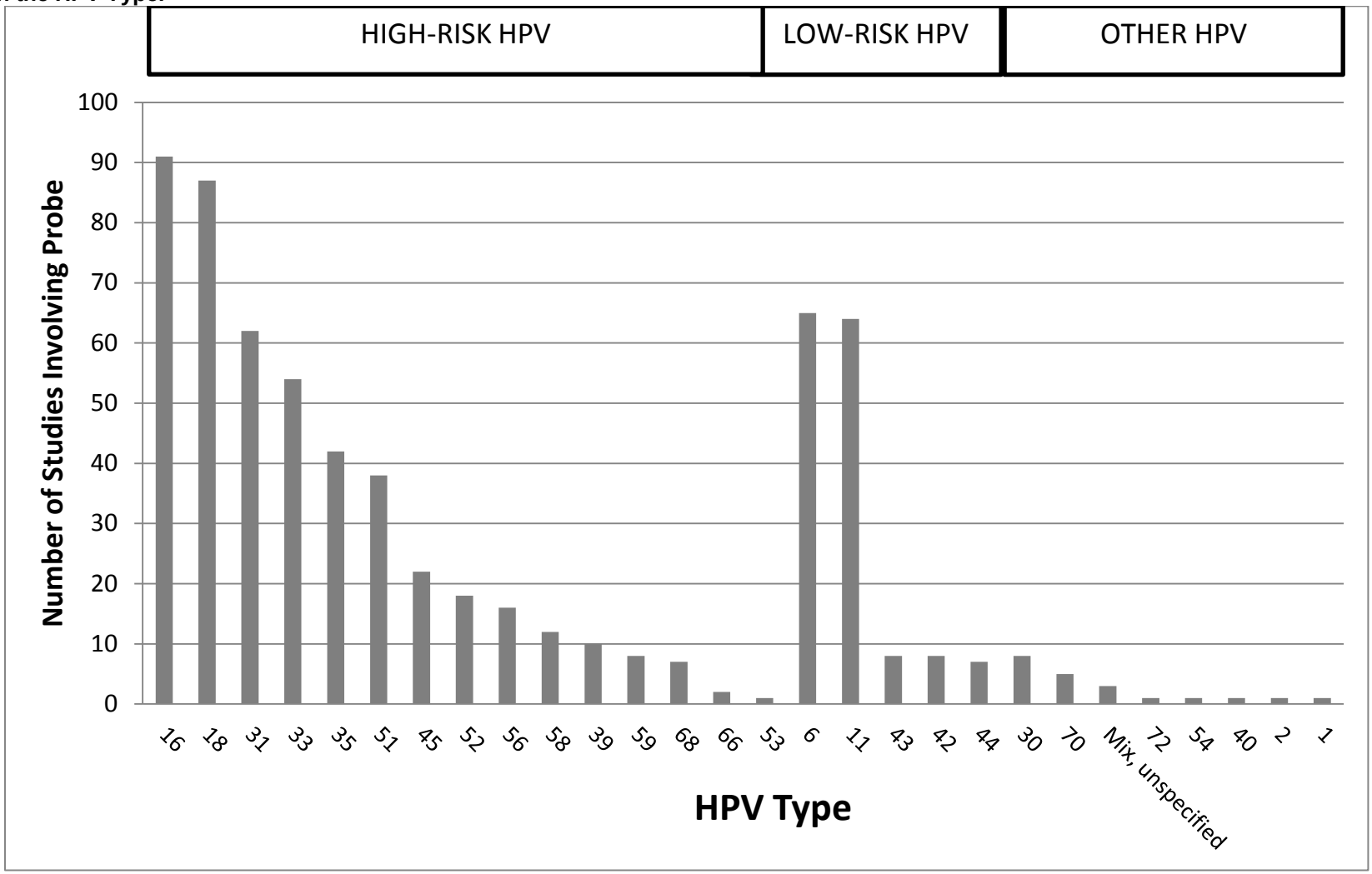
Appendix C. Key Question 1 (Horizon Scan) Figures and Tables

Figure C1. Numbers of the 135 Horizon Scan Studies Using Each Identified Non–HPV ISH Probe.



Chr=chromosome(s); CMV=cytomegalovirus; EBV=Epstein–Barr virus; HLA=human leukocyte antigen; MYC= myelocytomatosis oncogene (on chromosome 8q24); TERC=telomerase RNA component (on chromosome 3q26).
Studies could have used more than one type of probe, in which case they are counted once for each probe, such that the total number of studies across the plot is greater than 135.
Probes for whole chromosomes are typically control probes for overall amplification (vs. amplification of a specific gene or region).

Figure C2. Numbers of the 135 Horizon Scan Studies Using Each Identified HPV ISH Probe, According to the Risk of Cancer Associated with the HPV Type.



HPV =human papillomavirus.
Studies could have used more than one type of probe, in which case they are counted once for each probe, such that the total number of studies across the plot is greater than 135.
High-risk probes were of greatest interest since they are most associated with a risk of progression to cervical cancer. Low-risk HPV types 6 and 11 are often used as controls.

Figure C3. Worldwide Distribution of the 116 Horizon Scan Studies with Probes of Interest. For detail on the 63 European studies, see Fig. C4.

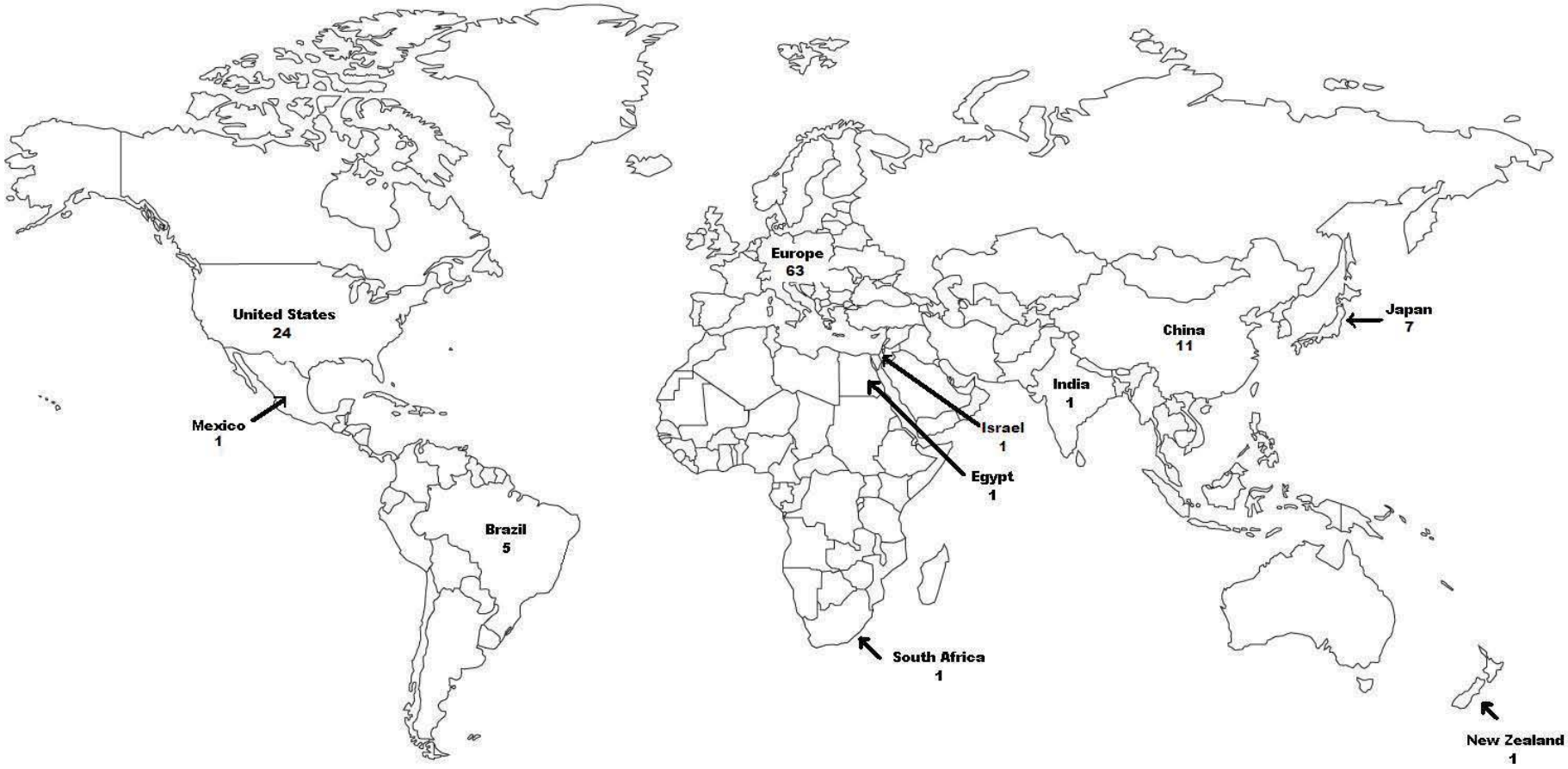


Figure C4. Distribution of the 63 European Studies in the Horizon Scan with Probes of Interest.

Table C1. Description of Probes Used, Outcomes Assessed, and Study Characteristics of the 31 Studies of ISH Using a TERC or MYC (Non-HPV) Probe (and Therefore Eligible for Key Questions 2–4).

A "1" indicates that the study used the probe indicated or had data on the outcome listed for that column. (Note that, by definition, all studies in the table must have had a "1" in either the TERC column or the MYC column.) Studies could have used more than one probe. There is then one column for each of four possible outcomes; our review focused on analytic validity for Key Question (KQ) 2 and cytology grade versus histology grade for KQ3.

Chr=chromosome, cyto=cytology, histo=histology, HPV=human papillomavirus, ISH=in situ hybridization, MYC= myelocytomatosis oncogene, TERC=human telomerase gene, UK= United Kingdom, US=United States.

Author Year PMID	TERC	MYC	Chr 3	3p14	5p15	20q13	Xp22	Chr 7	Chr 8	Chr 15	PIK3Ca	IGH/ CCNDI	BCL2 (18q21.3)	ERBB2 (17q21.1)	ZNF217 (20q13.2)	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome	Histo specimen & outcome	Country	Setting	Sampling method	N
Alameda 2009 19540557	1							1									1			Spain	Testing/Diagnosis	Convenience	55
Andersson 2006 16847471	1		1					1											1	Sweden	Testing/Diagnosis	Convenience	12
Andersson 2009 19880826	1	1						1								1	1	1	1	Sweden	Mixed	Convenience	78
Caraway 2008 18433848	1							1									1	1		US	Testing/Diagnosis	Convenience	66
Costa 2009 19475528	1	1			1							1	1	1	1				1	Spain	Testing/Diagnosis	Convenience	63
He 2010 nd	1																	1		China	Testing/Diagnosis	Convenience	90
Heselmeyer-Haddad 2003 14507648	1		1					1									1			US	Testing/Diagnosis	Convenience	57
Heselmeyer-Haddad 2005 15793301	1		1					1									1			Germany	Testing/Diagnosis	Convenience	59
Hopman 2006 17054308	1		1					1											1	Netherlands	Testing/Diagnosis	Convenience	37
Huang 2009 nd	1																1			China	Testing/Diagnosis	Convenience	100
Jalali 2010 20171606	1							1									1	1		US	Testing/Diagnosis	Convenience	47
Jin 2011 21875260	1		1					1												China	Testing/Diagnosis	Convenience	130
Jiang 2010 20864639	1															1	1	1	1	China	Mixed	Convenience	7787
Kokalj-Vokac 2009 19837263	1							1									1	1		Slovenia	Mixed	Convenience	102

Author Year PMID	TERC	MYC	Chr 3	3p14	5p15	20q13	Xp22	Chr 7	Chr 8	Chr 15	PIK3Ca	IGH/ CCNDI	BCL2 (18q21.3)	ERBB2 (17q21.1)	ZNF217 (20q13.2)	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome	Histo specimen & outcome	Country	Setting	Sampling method	N
Li 2011 21035173	1		1														1	1		China	Testing/Diagnosis	Systematic	300
Policht 2010 20712890	1	1	1	1		1	1			1							1		1	US	Testing/Diagnosis	Convenience	118
Ramsaroop 2009 19191295	1							1									1			New Zealand	Screening	ND	36
Seppo 2009 19394683	1							1			1						1			Greece and US	Screening	Convenience	257
Sokolova 2007 17975027	1	1															1	1	1	US	Screening	Convenience	455
Song 2010 19626623	1	1							1								1			US	ND	Convenience	15
Sui 2009 20009881	1		1																1	China	Testing/Diagnosis	Convenience	110
Sui 2010 20882876	1		1														1	1		China	Testing/Diagnosis	Convenience	63
Takac 2009 19930867	1							1										1		Slovenia	Testing/Diagnosis	Convenience	101
Theelen 2010 20813962	1		1					1								1	1	1	1	Netherlands	Testing/Diagnosis	Convenience	158
Tu 2009 19389503	1																1	1		China	Screening	ND	1033
Voss 2010 20701064	1	1																1		US	Testing/Diagnosis	Convenience	115
Wilting 2006 16538612	1				1														1	Netherlands	ND	Convenience	26
Yuan 2011 21575390	1																		1	China	Testing/Diagnosis	Convenience	150
Zhang 2009 19513624	1																1			China	ND	Convenience	70
Zheng 2010 20683395	1																1	1	1	China	Testing/Diagnosis	Convenience	120
Total	31	6	9	1	2	1	1	14	1	1	1	1	1	1	1	3		14					

Table C.2. Description of Probes Used, Outcomes Assessed, and Study Characteristics of the 91 Studies of ISH Using an HPV 16 or HPV 18 Probe (and Therefore Eligible for Key Questions 2–4).

A "1" indicates that the study used the probe indicated or had data on the outcome listed for that column. (Note that, by definition, all studies in the table must have had a "1" in either the HPV 16 column or the HPV 18 column.) Studies could have used more than one probe. There is then one column for each of four possible outcomes; our review focused on analytic validity for Key Question (KQ) 2 and cytology grade versus histology grade for KQ3. The final columns describe basic aspects of the study.

HPV=human papillomavirus, ISH=in situ hybridization, MYCUK= United Kingdom, US=United States.

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
Akasofu 19957697216	1	1																													1	Japan	ND	Convenience	39
Alameda 20112130 2019	1	1						1	1	1	1					1	1	1			1	1	1		1	1		1	1			Spain	Testing/Diagnosis	Convenience	107
Alejo 1996 nd	1	1			1	1		1		1							1											1			1	Spain	Testing/Diagnosis	Convenience	70
Alonso 19921281009	1	1			1	1		1		1							1											1			1	Spain	Testing/Diagnosis	Convenience	52
al-Saleh 19979155714	1	1			1	1		1	1	1			1	1	1	1	1	1			1									1		Belgium	Testing/Diagnosis	Convenience	87
Amortegui 19902174026	1	1			1	1		1																							1	US	Testing/Diagnosis	Convenience	615

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
Andersson 2009 19880826	1	1					1									1	1					1						1	1	1	1	Sweden	Mixed	Convenience	78
Ansari-Lari 2004 15043304	1	1			1	1		1	1							1	1											1			1	US	Testing/Diagnosis	Convenience	19
Arafa 2008 18542030	1				1																										1	Belgium	Testing/Diagnosis	Convenience	71
Badr 2008 18425044	1	1			1	1		1	1	1	1					1	1	1			1			1				1			1	US	Testing/Diagnosis	Convenience	56
Balbi 1996 8927276	1	1			1	1																									1	Italy	Testing/Diagnosis	Convenience	70
Bar 2001 11396132	1	1																													1	Poland	Mixed	Convenience	70
Bejui-Thivolet 1992 1317560	1	1			1	1																									1	France	Testing/Diagnosis	Convenience	36

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
Bernard 1994 7877628	1	1			1	1		1	1								1											1			1	France	Testing/Diagnosis	Convenience	20
Bertelsen 1996 9048869	1	1			1	1		1	1	1			1	1	1	1	1	1			1							1			1	Norway	Testing/Diagnosis	Convenience	111
Bertelsen 1999 9926893	1	1			1	1		1	1	1																					1	Norway	Testing/Diagnosis	Systematic	132
Berthier 1999 1022 7090	1	1			1	1		1	1								1												1			France	Screening	Convenience	45
Bettinger 1999 1056 3251	1	1			1	1		1		1							1														1	France	Testing/Diagnosis	Convenience	30
Birner 2001 1145 5003	1	1			1	1	1	1	1							1	1	1										1	1	1	1	Austria	Testing/Diagnosis	Convenience	86
Bulten 2002 1237 5262	1	1																										1			1	Netherland	Testing/Diagnosis	Convenience	56

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
																																s			
Cadorin 1992 1283127	1	1			1	1		1		1							1														1	Italy	Testing/Diagnosis	Convenience	54
Calore 1998 9836004	1	1			1	1																									1	Brazil	Testing/Diagnosis	Systematic	37
Cavalcanti 2000 1076 2117	1	1			1	1		1	1	1																				1		Brazil	Testing/Diagnosis	Convenience	514
Cavalcanti 2000 9070405	1	1			1	1		1	1	1																		1			1	Brazil	Testing/Diagnosis	Convenience	230
Choi 1991 1849699	1	1			1	1																							1			US	Testing/Diagnosis	Convenience	676
Cooper 1991 1646237	1	1			1	1		1	1	1																					1	SouthAfric	Testing/Diagnosis	Convenience	145

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
																																a			
D'Amico 1999 nd	1	1			1	1		1	1	1			1	1	1	1	1	1			1										1	Italy	Testing/Diagnosis	Convenience	39
Daste 1994 8157887	1	1			1	1		1	1	1																			1	1	1	France	Testing/Diagnosis	Convenience	156
Davidson 1997 9421072	1	1			1	1		1	1																						1	Israel	Testing/Diagnosis	Convenience	50
de Marcedo 1904 7903	1	1			1	1	1	1	1	1			1	1	1	1	1	1			1	1			1	1					1	US	Testing/Diagnosis	Systematic	87
De Marchi 2009 1900 7972	1	1						1	1	1	1					1	1	1			1	1	1		1						1	Brazil	Testing/Diagnosis	Convenience	74
Dellas 1996 8682581	1	1			1	1		1		1							1														1	Switzerland	Mixed	Convenience	112

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
Dellas 19969042198	1	1			1	1		1	1								1														1	Switzerland	Mixed	Convenience	93
el-All 20071761 0742	1	1			1	1	1	1	1	1						1	1	1													1	Egypt	Testing/Diagnosis	Convenience	217
Evans 20021248 1016	1	1			1	1		1	1	1				1							1									1	US	ND	Convenience	50
Fujii 20081893 6966	1	1						1	1	1	1					1	1	1			1	1	1		1			1	1	1	1	Japan	Mixed	Convenience	153
Gitsch 19911665 685	1	1			1	1		1	1																				1	1	1	Austria	Testing/Diagnosis	Convenience	148
Hara 19902167 347	1	1			1	1																									1	Japan	Testing/Diagnosis	Convenience	40
Hennig 19991060 6184	1																														1	Norway	Testing/Diagnosis	Convenience	15

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
Herrington 1992 1319766	1	1			1	1			1																				1			England	Screening	Convenience	262
Herrington 1996 8763265	1	1						1	1																			1	1	1	1	England	Testing/Diagnosis	Convenience	309
Hesselink 2004 14968413	1	1						1	1	1						1	1	1			1	1	1		1	1		1	1	1	1	Netherlands	Screening	Convenience	76
Hopman 2004 14694518	1	1																												1	1	Netherlands	ND	Convenience	47
Hopman 2006 17054308	1	1																													1	Netherlands	Testing/Diagnosis	Convenience	37

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
Hording 1991 1649773	1	1				1																							1	1	1	Denmark	Testing/Diagnosis	Convenience	216
Ji 1991 1684763	1	1			1	1		1	1																						1	Finland	Testing/Diagnosis	Convenience	426
Kalantari 2001 1127 7395	1																														1	Sweden	Testing/Diagnosis	Convenience	55
Kong 2007 1719 7917	1	1						1	1	1	1					1	1	1			1	1		1				1	1			US	Mixed	Convenience	81
Kotrsova 1995 8599 698	1	1							1																						1	Czechoslovakia	Testing/Diagnosis	Convenience	45
Laksmi 2009 1946 8254	1	1			1	1		1	1	1																				1		India	Testing/Diagnosis	Convenience	177

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
Lie 1997 9113 073	1	1			1	1		1	1	1																		1			1	Norway	Testing/Diagnosis	Convenience	203
Lizard 1998 9725 458	1	1			1	1		1	1								1														1	France	Testing/Diagnosis	ND	414
Masumoto 2003 1450 6638	1	1						1	1																			1			1	Japan	Testing/Diagnosis	Convenience	10
Menezes 2001 1172 6118	1	1			1	1	1	1	1	1	1		1	1	1	1	1	1			1				1	1			1			US	Mixed	Convenience	239
Meyer 1991 1653 262	1	1			1	1		1	1	1																					1	US	Testing/Diagnosis	ND	806
Mittal 1998 9475 188	1	1			1	1		1	1	1																					1	US	Testing/Diagnosis	Convenience	35
Monsonego 1997 9197 877	1	1			1	1			1																						1	France	Testing/Diagnosis	Convenience	292

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
Mougin 1991 1663402	1	1			1	1		1		1							1														1	France	Testing/Diagnosis	Convenience	131
Nagai 1994 7896562	1	1			1	1																							1	1	1	Japan	Testing/Diagnosis	Convenience	43
Neumann 1990 2157319	1	1																													1	Germany	Testing/Diagnosis	Convenience	18
Nuovo 1991 1654025	1	1			1	1		1	1	1																			1			US	Testing/Diagnosis	Convenience	132
Nuovo 1998 9836071	1	1			1	1	1	1	1	1	1		1	1	1	1	1	1			1	1	1		1	1			1			US	Testing/Diagnosis	Convenience	82
O'Leary 1998 9828814	1	1			1	1		1	1																						1	Ireland	Testing/Diagnosis	Convenience	40
Omori 2007 17638654	1	1						1	1	1	1					1	1	1			1	1	1						1			Japan	Testing/Diagnosis	Convenience	77

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
Pich 1992 1329 676	1	1			1	1																									1	Italy	Testing/Diagnosis	Convenience	57
Pollanen 1993 8227 412	1	1			1	1		1	1																						1	Finland	Testing/Diagnosis	ND	43
Pollanen 1993 8314 222	1	1			1	1		1	1	1																					1	Finland	Testing/Diagnosis	Convenience	81
Quereshi 2005 1583 9613	1	1						1	1	1	1					1	1	1			1	1	1				1	1	1			US	Testing/Diagnosis	Convenience	99
Rihet 1996 8944 607	1	1							1																						1	France	Testing/Diagnosis	Convenience	115
Samama 2008 1838 7664	1	1			1	1		1	1								1												1			France	s	ND	241
Sassi 1993 8390 639	1	1																													1	Italy	Testing/Diagnosis	Convenience	14

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
Schon 1991 1646654	1	1			1	1		1		1							1												1	1	1	Austria	Screening	Random	179
Shepherd 1992 1325072	1	1			1																										1	England	Testing/Diagnosis	Convenience	54
Soini 1996 8611192	1	1			1	1		1	1																						1	Finland	Testing/Diagnosis	Convenience	80
Sokolova 2007 17975027	1	1					1									1	1					1							1	1	1	US	Screening	Convenience	455
Sopracordevole 1993 8393793	1	1			1	1		1		1							1													1		Italy	Testing/Diagnosis	Convenience	39
Spinillo 1993 8381376	1	1			1	1		1		1							1														1	Italy	Mixed	Convenience	60
Spinillo 1996 8886703	1	1			1	1		1		1							1														1	Italy	Testing/Diagnosis	Convenience	16

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
Symmans 1992 1328078	1	1			1	1		1	1	1			1	1	1	1	1	1			1							1			1	US	Testing/Diagnosis	Convenience	88
Tabbara 1992 1738511	1	1			1	1		1	1	1																			1	1	1	US	Testing/Diagnosis	Convenience	119
Tase 1989 2542853	1	1																													1	US	Testing/Diagnosis	Convenience	69
Theelen 2010 20813962	1	1																										1		1	1	Netherlands	Testing/Diagnosis	ND	158
Tichy 1998 9929943	1				1	1																									1	Czechoslovakia	Testing/Diagnosis	Convenience	260

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
Twedel 19948314133	1	1			1	1		1	1	1																					1	US	Testing/Diagnosis	Convenience	23
Vassallo 20001104541	1	1			1	1		1		1							1														1	Brazil	Testing/Diagnosis	Convenience	78
Vocaturo 20021214585	1	1			1	1		1	1								1											1	1			Italy	Screening	Convenience	126
Voss 20102070064	1	1					1									1	1					1								1		US	Testing/Diagnosis	Convenience	115
Walker 19968727101	1	1			1	1		1	1	1	1	1	1			1		1	1	1			1					1			1	France	ND	Systematic	30
Weaver 19902175897	1	1			1	1		1	1																						1	US	Testing/Diagnosis	Convenience	53
Xiao 20011153292	1	1	1	1	1	1		1	1																			1	1	1	1	Japan	Testing/Diagnosis	Convenience	54

Author Year PMID	HPV 16	HPV 18	HPV 1	HPV 2	HPV 6	HPV 11	HPV 30	HPV 31	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54	HPV 56	HPV 58	HPV 59	HPV 66	HPV 68	HPV 70	HPV 72	Analytic validity (KQ2)	Cyto specimen & outcome	Cyto specimen; histo outcome (KQ3)	Histo specimen, histo outcome	Country	Setting	Sampling method	N
Ziol 1998 9781643	1	1			1	1		1	1																						1	France	Mixed	Convenience	68
Total	91	87	1	1	65	64	8	62	54	42	10	1	8	8	7	22	38	18	1	1	16	12	8	2	7	5	1	23		20					

Appendix D. Assessment of Risk of Bias and Completeness of Reporting of Studies for Key Question 2.

Table D1. Risk of Bias and Completeness of Reporting of Studies for Analytic Validity of ISH using TERC or MYC or HPV 16 or 18 Probes.*

Author Year Country PMID	ISH Probe	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11
Tu 2009 China 19389503	TERC	YES	NR	NR	NR	NR	YES	NR	NR	NR	NR	NR
Jin 2011 China 21875260	TERC	YES	NR	NR	NR	NR	YES	NR	NR	NR	NR	NR
Alameda 2011 Spain 21302019	HPV	YES	NR	NR	NR	NR	YES	NR	NR	NR	NR	NR
Andersson 2009 Sweden 19880826	HPV	YES	NR	NR	NR	NR	YES	YES	NR	NR	NR	NR
Ansari-Lari 2004 US 15043304	HPV	YES	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Bernard 1994 France 7877628	HPV	YES	YES	YES	NR	NR	YES	YES	NR	NR	NR	NR
Bertelsen 1996 Norway 9048869	HPV	YES	YES	YES	NR	NR	YES	NR	NR	NR	NR	NR
Birner 2001 Austria 11455003	HPV	YES	YES	YES	NR	NR	YES	NR	NR	NR	NR	NR
Bulten 2002 Netherlands 12375262	HPV	YES	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Cavalcanti 1996 Brazil 9070405	HPV	YES	YES	YES	NR	NR	YES	NR	NR	NR	NR	NR
Hesselink 2004 Netherlands 14968413	HPV	YES	YES	YES	NR	NR	YES	NR	NR	NR	NR	NR
Kong 2007 US 17197917	HPV	YES	YES	YES	NR	NR	YES	NR	NR	NR	NR	NR
Lie 1997 Norway 9113073	HPV	YES	YES	YES	NR	NR	NR	NR	NR	NR	NR	NR
Masumoto 2003 Japan 14506638	HPV	YES	NR	NR	NR	NR	YES	NR	NR	NR	NR	NR
Quereshi 2005 US 15839613	HPV	YES	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Walker 1996 France 8727101	HPV	YES	YES	YES	NR	NR	NR	YES	NR	NR	NR	NR

HPV=human papillomavirus, ISH=in situ hybridization, NR=not reported, TERC=telomerase RNA component, US=United States.
The 11 quality questions (Qs), adapted from Sun et al. 2011, were scored as yes, no, or not reported (NR) (including for a corollary that follows a question with an NR score). The items are as follows:
Q1 = Was the execution of the assay described in sufficient detail to permit replication?
Q2 = Were both positive and negative control samples tested?
Q3 = Were negative control materials from the same type of tissue, and collected, stored, and processed in the same way that sample materials used clinically for testing will be?
Q4 = Were the tests performed with positive or negative control samples being blinded to the testers?
Q5 = Were the testing results interpreted with positive or negative control samples being blinded to the interpreters?
Q6 = Were criteria for determining a testing result as positive, negative, indeterminate, and uninterpretable set a priori?
Q7 = Was any information on cross-reactivity of the test reported?
Q8 = Was the reproducibility of the test when performed multiple times on a single specimen established?
Q9 = Was the reproducibility of the test adequately established (across operators, instruments, reagent lots, different days of the week, different laboratories)?
Q10 = Was the rate of yield [numbers] of usable (interpretable) results reported?
Q11 = Were the study data from a multisite collaborative, proficiency testing, or interlaboratory exchange programs?

Reference: Sun F, Bruening W, Erinoff E, et al. Addressing challenges in genetic test evaluation. Rockville, MD: Agency for Healthcare Research and Quality; 2011. Available at: http://www.effectivehealthcare.ahrq.gov/ehc/products/105/704/Genetic-Test-Evaluation_Final-Report_20110615.pdf. Accessed on November 4, 2011.

Appendix E: Assessment of Risk of Bias and Completeness of Reporting of Studies for Key Question 3

Table E1. Risk of Bias and Completeness of Reporting of Studies for Clinical Validity of ISH Using TERC or MYC or HPV 16 or 18 Probes, According to QUADAS-2 Domain.*

		Patient Selection				Index Test			Reference Standard			Flow and Timing					Patient Selection	Index Test	Reference Standard
Author Year Country PMID	ISH Probe	S1	S2	S3	B1	S4	S5	B2	S6	S7	B3	S8	S9	S10	S11	B4	A1	A2	A3
Huang 2009 China NR	TERC	NO	NO	YES	High	NR	NR	Unclear	YES	NR	Unclear	NR	YES	YES	YES	Low	Low	Low	Low
Jalali 2010 US 20171606	TERC	NR	NO	YES	Unclear	YES	YES	Low	YES	YES	Low	NR	NO	YES	NO	High	Low	Low	Low
Jiang 2010 China 20864639	TERC	NR	NO	NR	Unclear	YES	YES	Low	YES	NR	Unclear	NR	NO	YES	NO	High	Low	Low	Low
Kokalj-Vokac 2009 Slovenia 9837263	TERC	NR	NO	YES	Unclear	NR	YES	Unclear	YES	NR	Unclear	NR	YES	YES	NO	Unclear	Low	Low	Low
Li 2011 China 21035173	TERC	YES	NO	YES	Low	YES	YES	Low	YES	YES	Low	NR	YES	YES	YES	Low	Low	Low	Low
Sui 2010 China 20882876	TERC	NR	NO	NR	Unclear	YES	YES	Low	YES	YES	Low	NR	YES	YES	YES	Low	Low	Low	Low
Fujii 2008 Japan 18936966	HPV	NR	NO	NR	Unclear	NR	YES	Unclear	YES	NO	High	YES	NO	YES	NO	High	Low	Low	Low
Hesselink 2004 Netherlands 14968413	HPV	NO	NR	NR	Unclear	NR	YES	Unclear	YES	NR	Unclear	NR	NO	YES	NO	High	Low	Low	Low
Sokolova 2007 US 17975027	TERC HPV	YES	NO	YES	Low	YES	NO	High	YES	YES	Low	NR	YES	YES	YES	Low	Low	Low	Low
Voss 2010 US 20701064	TERC HPV	YES	NO	NR	Unclear	NR	NO	High	YES	NR	Unclear	NR	YES	YES	YES	Low	Low	Low	Low

HPV=human papillomavirus, ISH=in situ hybridization, NR=not reported, QUADAS=Quality Assessment of Diagnostic Accuracy Studies, TERC=human telomerase gene, UK= United Kingdom, US=United States.

*The questions are those of the QUADAS-2 tool. The 11 signaling questions (S) were scored as yes, no, or not reported (NR). The 5 questions about bias (B) were scored as high, low, or unclear risk of bias. The three questions about applicability (A) were scored as high, low, unclear concerns about applicability. The items are as follows:

S1 = Consecutive or random sample of patients enrolled?
S2 = Case-control design avoided?
S3 = Study avoided inappropriate exclusions?
B1 = Risk of bias: Could the selection of patients have introduced bias?
S4 = Index test results interpreted without knowledge of results of reference standard?
S5 = If threshold used, was it prespecified?
B2 = Risk of bias: Could the conduct or interpretation of the index test have introduced bias?
S6 = Reference standard likely to correctly classify the target condition?
S7 = Reference standard results interpreted without knowledge of index test results?
B3 = Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?
S8 = Appropriate interval between index test and reference standard?
S9 = All patients received a reference standard?
S10 = All patients received the same reference standard?

S11 = Were all patients included in the analysis?
B4 = Risk of bias: Could the patient flow have introduced bias?
A1 = Concerns about applicability: Concerns that the included patients do not match the review question?
A2 = Concerns about applicability: Concerns that the index test, its conduct, or its interpretation differ from the review question?
A3 = Concerns about applicability: Are there concerns that the target condition as defined by the reference standard does not match the review question?

Appendix F. Prevalence of Histopathologic Abnormalities by CIN Grade and Cytologic Classification.

Table F1. Prevalence of CIN2+ from FISH Studies*

Author Year Country PMID	Prevalence of CIN2+ in all patients	Prevalence of CIN2+ in LSIL patients	Prevalence of CIN2+ in ASCUS, HPV status unknown	Prevalence of CIN2+ in ASCUS, HPV positive
Jalali 2010 US 20171606	23.4% (11/47)	NR	NR	NR
Jiang 2010 China 20864639	30.2% (2028/6726)	NR	NR	NR
Kokalj-Vokac 2009 Slovenia 19837263	67.6% (69/102)	10.8% (11/102)	NR	NR
Li 2011 China 21035173	36.1% (108/299) [HPV positive patients]	29.3% (24/82) [HPV positive patients]	NR	17.2% (22/128)
Sokolova 2007 US 17875027	46.9% (97/207)	25.9% (38/147)	100% (14/14)	NR
Sui 2010 China 20882876	36.5% (23/63)	7.1% (1/14)	0% (0/18)	NR
Voss 2010 US 20701064	2.6% (3/115)	NR	NR	NR

* Prevalence given as percentage (no. of CIN2+ cases/total no. of patients).

ASCUS=atypical cells of undetermined significance, CIN=cervical intraepithelial neoplasia, FISH=fluorescence in situ hybridization, HPV=human papillomavirus, LSIL low-grade squamous intraepithelial lesion, TERC=human telomerase gene, US=United States.

Table F2. Prevalence of CIN3+ from FISH Studies.*

Author Year Country PMID	Prevalence of CIN3+ in all patients	Prevalence of CIN3+ in LSIL patients	Prevalence of CIN2+ in ASCUS, HPV status unknown	Prevalence of CIN3+ in ASCUS, HPV positive
Jiang 2010 China 20864639	30.2% (2028/6726)	NR	NR	NR
Kokalj-Vokac 2009 Slovenia 19837263	90.2% (92/102)	21.6% (21/102)	NR	NR
Li 2011 China 21035173	32.3% (74/229) [HPV positive patients]	17.1% (14/82) [HPV positive patients]	NR	11.7% (15/128) [HPV positive patients]
Sokolova 2007 US 17875027	19.8% (41/207)	3.4% (5/147)	57.1% (8/14)	NR
Sui 2010 China 20882876	30.2% (19/63)	0%	0%	NR
Voss 2010 US 20701064	2.6% (3/115)	NR	NR	NR

* Prevalence given as percentage (no. of CIN2+ cases/total no. of patients).

ASCUS=atypical cells of undetermined significance, CIN=cervical intraepithelial neoplasia, FISH=fluorescence in situ hybridization, HPV=human papillomavirus, LSIL low-grade squamous intraepithelial lesion, TERC=human telomerase gene, US=United States.