

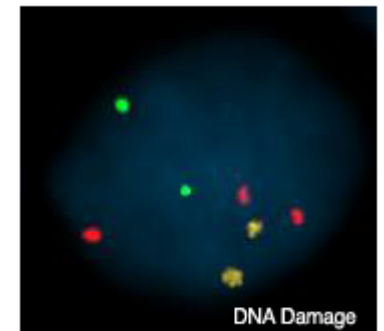
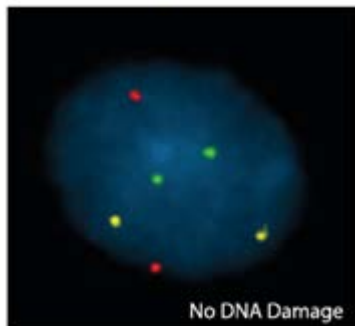
Fluorescence In Situ Hybridization (FISH) or Other In Situ Hybridization (ISH) Testing of Uterine Cervical Cells to Predict Precancer and Cancer

Katrin Uhlig, MD, MS

Tufts Evidence-Based Practice Center, Boston

MEDCAC meeting on Genetic Tests

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- **Disclaimer:** The authors of this report are responsible for its content. Statements in the report should not be construed as endorsement by the Agency for Healthcare Research and Quality or the U.S. Department of Health and Human Services.

Contributors

Tuft Evidence-based Practice Center

Katrin Uhlig, MD, MS
Amy Earley, BS
Jenny Lamont, MS
Issa J. Dahabreh, MD, MS
Esther E. Avendano, BA
Janet M. Cowan, PhD
(cytogeneticist)
Sarah Feldman, MD, MPH
(gynecological oncologist)

AHRQ Task Order Officers

Kim Wittenberg, MA
Elise Berliner, PhD

CMS

Jeff Roche, MD, MPH
Leslye K Fitterman, PhD

Background

- Cervical cancer has decreased in incidence secondary to widely adopted screening.
- Screening detects precancerous lesions and cancers in early stages which can be effectively treated.
- Almost all cervical cancers caused by infection with a high-risk human papillomavirus (HPV) genotype.
- HPV genotypes 16 and 18 alone are responsible for about 70 % of cancers

2012 Guidelines for Screening for Cervical Cancer

- U.S. Preventive Services Task Force
 - In women 21-65y, Papanicolaou test (Pap) every 3years
- American Cancer Society/American Society for Colposcopy and Cervical Pathology/American Society for Clinical Pathology (ACS/ASCCP/ASCP)
 - In women 21-65y Pap every 3 years
 - In women 30-65y Pap and HPV co-testing every 5 years preferred

Screening and Evaluation

- If abnormal screening test, follow up with colposcopy and tissue biopsy or with ablative treatment.
- Goal of screening is to detect most high-grade lesions on histology while minimizing unnecessary procedures
- Adverse effects of colposcopy, biopsy or treatment
 - pain and bleeding.
 - cervical incompetence with fetal loss and prematurity
 - cost

2001 Bethesda System for Interpretation of Epithelial Cell Abnormalities

- **NSIL** - Negative for squamous intraepithelial lesions
- **ASC** - Atypical squamous cells
 - ASC-US** Of undetermined significance
 - ASC-H** - Cannot exclude HSIL
- **LSIL** - Low-grade squamous intraepithelial lesion (encompassing: human papillomavirus/mild dysplasia/cervical intraepithelial neoplasia (CIN) 1
- **HSIL** - 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

Evaluation

- If NSIL, rescreen per guideline
- **If NSIL and HPV+, retest in 1 year**
- If ASCUS and HPV-, rescreen per guideline
- **If ASCUS and HPV+, do colposcopy**
- **If LSIL, do colposcopy**
- If HSIL, do colposcopy

Histology Grades of Cervical Intraepithelial Neoplasia (CIN)

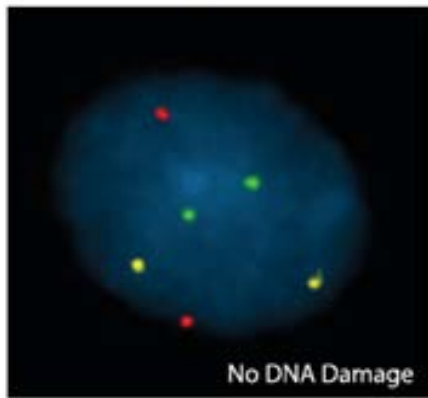
- **CIN1**
 - low-grade lesion. Mildly atypical cellular changes in the lower third of the epithelium
- **CIN2**
 - high-grade lesion. Moderately atypical cellular changes confined to the basal two-thirds of the epithelium
- **CIN3**
 - high-grade lesion. Severely atypical cellular changes encompassing $>2/3$ of epithelial thickness and includes full-thickness lesions
- **Invasive cancer**

CIN3+ as a Surrogate Outcome

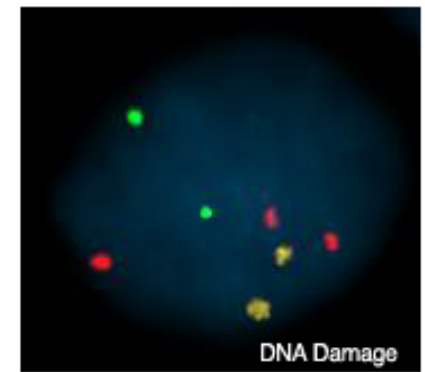
- Few studies have sufficient numbers of cancer cases to assess cancer risk directly.
- The absolute risk of CIN3, including the rare cases of cancer (CIN3+), is best measure of the risk of incident cervical cancer.
- In many studies, this is combined with CIN 2 as CIN2+.

ISH

- In situ hybridization uses a DNA probe to bind to a complementary DNA strand.
- Probes are visualized
 - under ultraviolet (UV) light in FISH
 - with another method in chromogenic in situ hybridization [CISH]



ISH



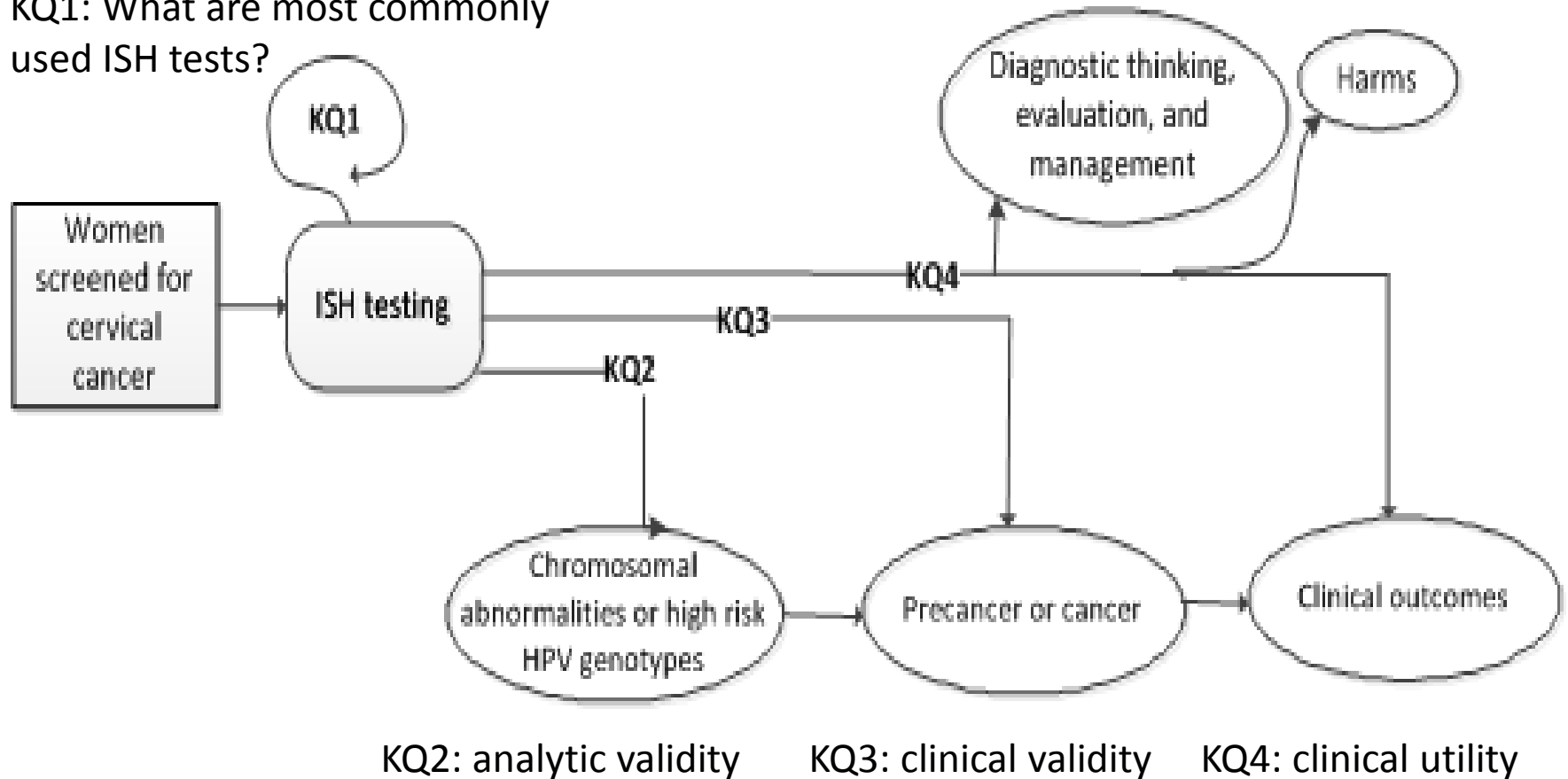
- Gains of a region are seen as additional spots in the cell, while deletions are seen as a loss of spots
- ISH test developed for cervical cancer detect
 - Gain of 3q26 which encodes **telomerase RNA component TERC**. This is activated early in the progression to cervical cancer
 - Gain of 8q24 which encodes **myelocytomatosis oncogene MYC**. This is a common site of HPV DNA integration, specifically HPV 18
 - DNA for high risk **HPV** genotypes, including HPV 16 and HPV 18

Marketing/Advertising of FISH

- Commercial laboratories offer FISH testing and advertise it for women with abnormal screening tests
 - NSIL and HPV+
 - ASCUS and HPV+
 - LSIL and HPV+, and
 - ASC-H

Analytic Framework: ISH for Cervical Cancer Testing

KQ1: What are most commonly
used ISH tests?



Methods

- Search key words
 - terms for test (in situ hybridization) and for disease (cervical cancer, precancer, neoplasm, CIN).
- Databases:
 - MEDLINE[®], Cochrane Central Register of Controlled Trials, Scopus (including Embase).
 - Last search date 7/2012.
 - No language restriction
- Population:
 - studies with cervical tissue from ≥ 10 women
 - clinical or research setting

KQ1: Horizon Scan

- What ISH tests have been examined most commonly?
- Horizon scan of studies of 135 studies using ISH on cervical specimens (cytologic or histologic)
- 116 used one or more of the four probes of interest
 - 31 TERC (7 also MYC)
 - 91 HPV 16 (87 also HPV 18)
- Subsequent review focused on ISH for TERC, MYC, HPV16 or 18

KQ2: Analytic Validity

- What are the associations between ISH test and reference test in cervical cytology or histology specimens?
 - ISH for TERC, MYC, HPV 16 or HPV 18
- Included studies that compared ISH test with a non-ISH reference test.
- Agreement between tests = % with concordant results
- Grading according to 11 items (ref Sun)

KQ2: Analytic Validity - Results

For FISH for TERC or MYC

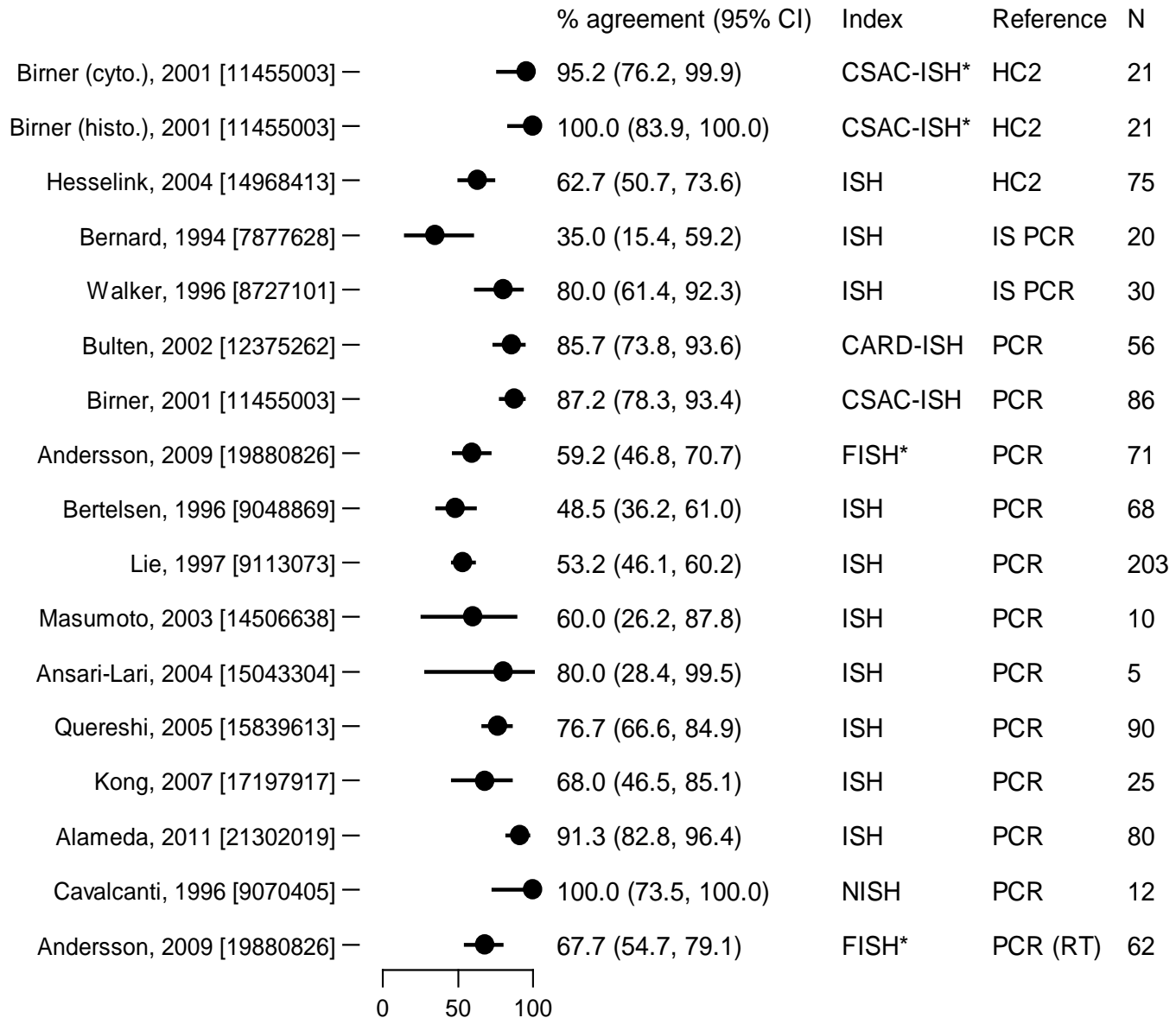
- No studies with a DNA-based reference test

For FISH for HPV

- 14 studies provided data on agreement
 - ISH for HPV 16 or 18 probe (among other HPV probes)
 - HPV reference tests (polymerase chain reaction [PCR] or Hybrid Capture 2).
- Agreement ranged from 35% to 100%
 - Differences in measurement techniques among ISH tests and reference tests
 - Use of non-overlapping panels of probes
- Quality assessment showed deficiencies in reporting.

KQ2: Analytic Validity - Results

% Agreement Between ISH Test for HPV and Reference Test (14 studies)



KQ2: Analytic Validity - Quality

- Deficiencies in reporting, likely because most of the studies were not designed to specifically address analytic validity.
- Studies did not explicitly describe laboratory procedures in detail because ISH tests and 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 reported in only 57%.

KQ3: Clinical Validity

- What is the association between FISH test results on cytology and CIN or cervical cancer on histology?

Index test Cytology	Reference test Histology
NSIL, HPV+	CIN2+ , CIN3+
ASCUS	
LSIL	

- Extracted data on sensitivity, specificity
- Meta-analysis if 5 studies for test-outcome pair
- Grading with Quality Assessment of Diagnostic Accuracy Studies (QUADAS) 2 instrument

KQ3: Clinical Validity - Results

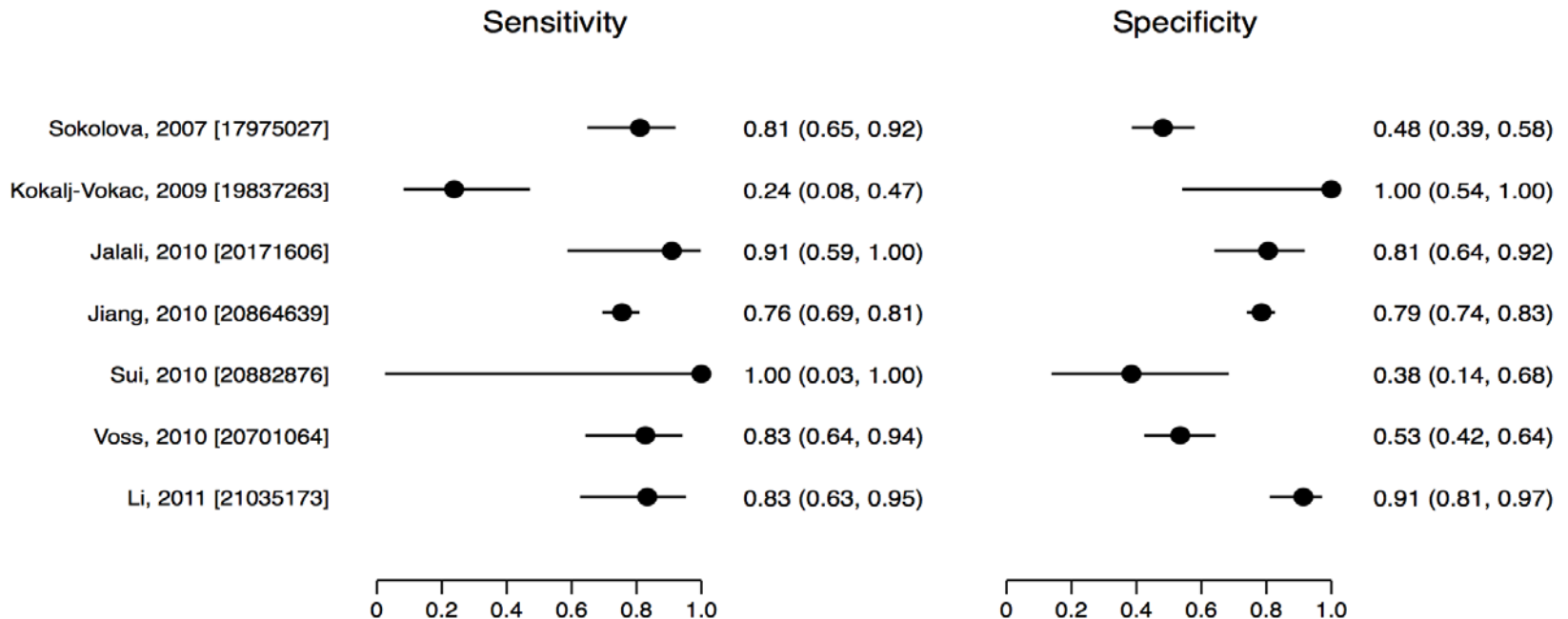
- 10 studies, all FISH (not another ISH test).
- 8 studies of **8,800** individuals examined FISH testing for TERC.
- One study from China with **7786** individuals.
- 5/8 TERC studies used only probes for TERC,
3/8 studies used probes for TERC and MYC
- In 3 studies all patients HPV positive by Hybrid Capture 2 or PCR; in others the HPV status was not clear.
- CIN3+ outcome results consistent with CIN2+

FISH Probe	Cytology	Outcome	# studies	N patients	Sensitivity	95% CI	Specificity	95% CI
TERC	LSIL	CIN2+	7	1033	0.76	0.60, 0.86	0.79	0.50, 0.93
		CIN3+	5	904	0.78	0.65, 0.87	0.79	0.51, 0.93
	ASCUS	CIN2+	2	789	0.75 to 0.82	0.60, 0.95^	0.87 to 0.93	0.83; 0.97^
		CIN3+	3	803	0.25 to 0.87	0.03, 0.98^	0.67 to 0.89	0.22, 0.96^
HPV	LSIL	CIN2+	3	38	0.75 to 0.81	0.19, 0.99^	0.00 to 0.88	0.00, 1.00^
		CIN3+	2	26	0.80 to 0.83	0.28, 1.00^	0.17 to 0.42	0.00, 0.64^
	ASCUS	CIN2+	1	12	1.00	0.48, 1.00	0.57	0.18, 0.90
		CIN3+	2	26	0.25 to 1.00	0.03, 1.00^	0.44 to 0.67	0.14, 0.96^
TERC or HPV	LSIL	CIN2+	1	115	0.90	0.73, 0.98	0.48	0.37, 0.59

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TERC or HPV	LSIL	CIN2+	1	115	0.90	0.73, 0.98	0.48	0.37, 0.59

KQ3: Clinical Validity - Results

FISH for TERC in LSIL for CIN2+

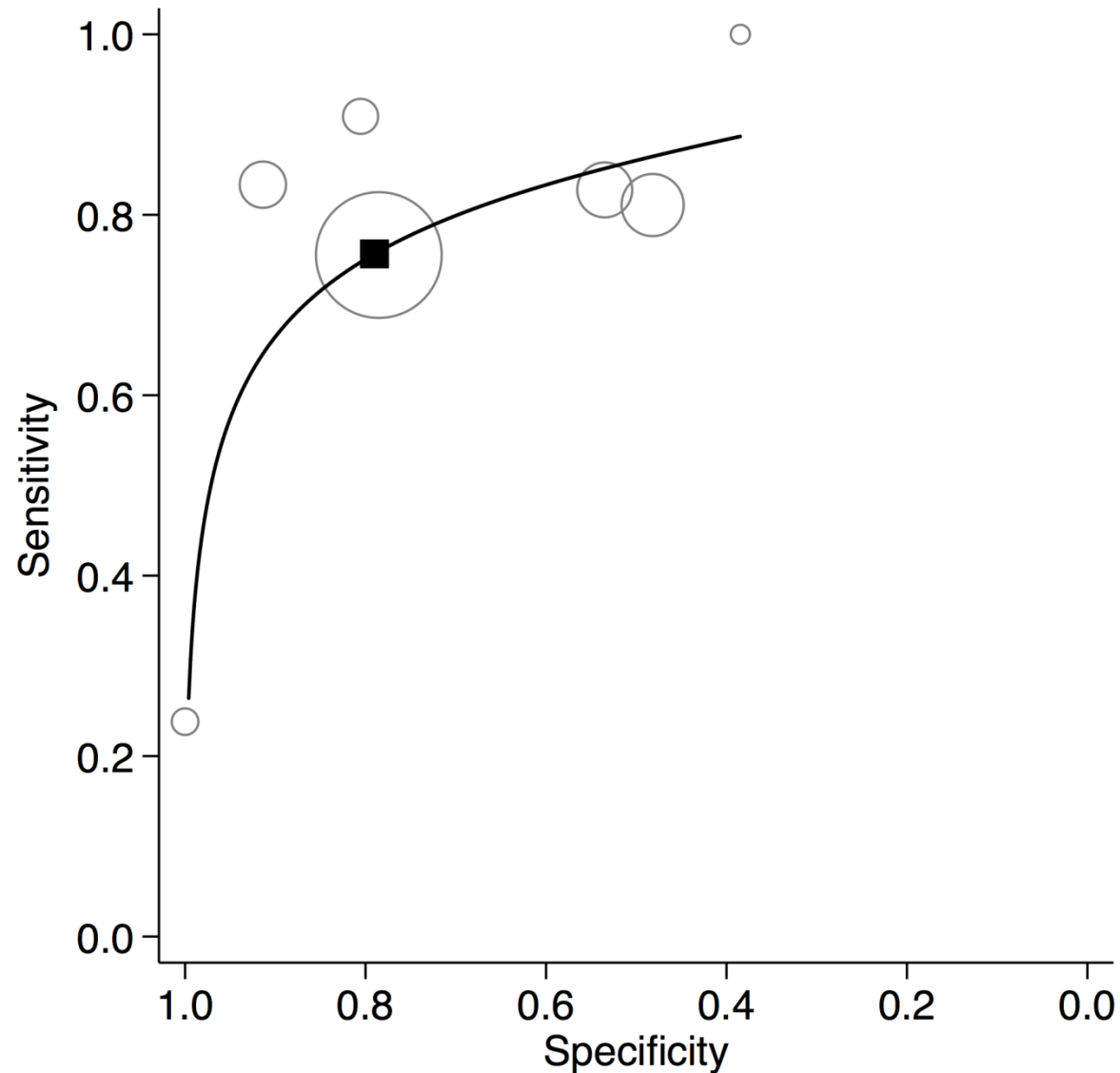


CIN2+ [7 studies, 1033 cases] sensitivity 0.76 (95% CI 0.60, 0.86); specificity 0.79 (95% CI 0.50, 0.93)

Not Shown:

CIN3+ [5 studies, 904 cases] sensitivity 0.78 (95% CI 0.65, 0.87); specificity 0.79 (95% CI 0.51, 0.93)

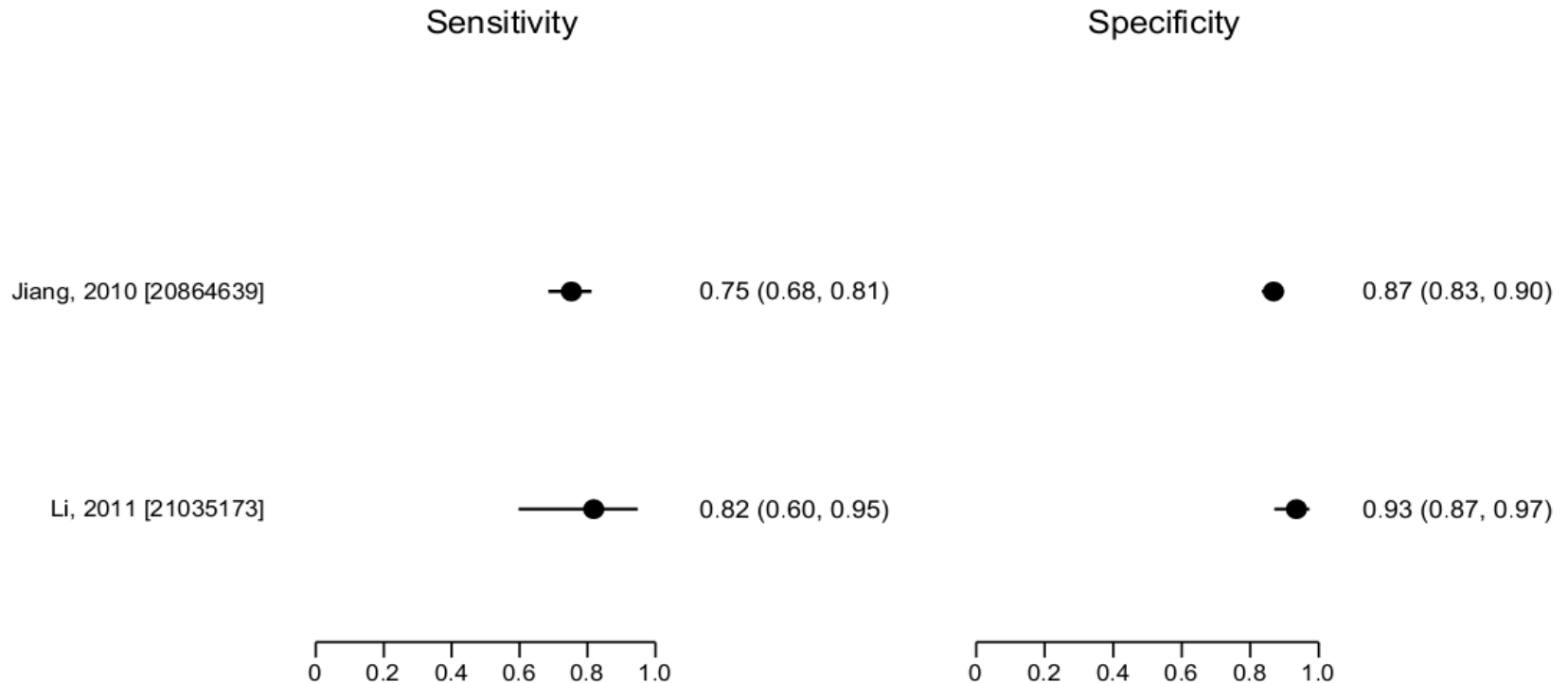
KQ3 Results-Summary ROC Curve: FISH for TERC or MYC in LSIL for CIN2+



FISH Probe	Cytology	Outcome	# studies	N patients	Sensitivity	95% CI	Specificity	95% CI
TERC	LSIL	CIN2+	7	1033	0.76	0.60, 0.86	0.79	0.50, 0.93
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		CIN3+	2	26	0.25 to 1.00	0.03, 1.00^	0.44 to 0.67	0.14, 0.96^
TERC or HPV	LSIL	CIN2+	1	115	0.90	0.73, 0.98	0.48	0.37, 0.59

KQ3: Clinical Validity - Results

FISH for TERC in ASCUS for CIN2+



CIN 2+ [2 studies, 789 cases] sensitivity range 0.75 to 0.82; specificity range 0.87 to 0.93

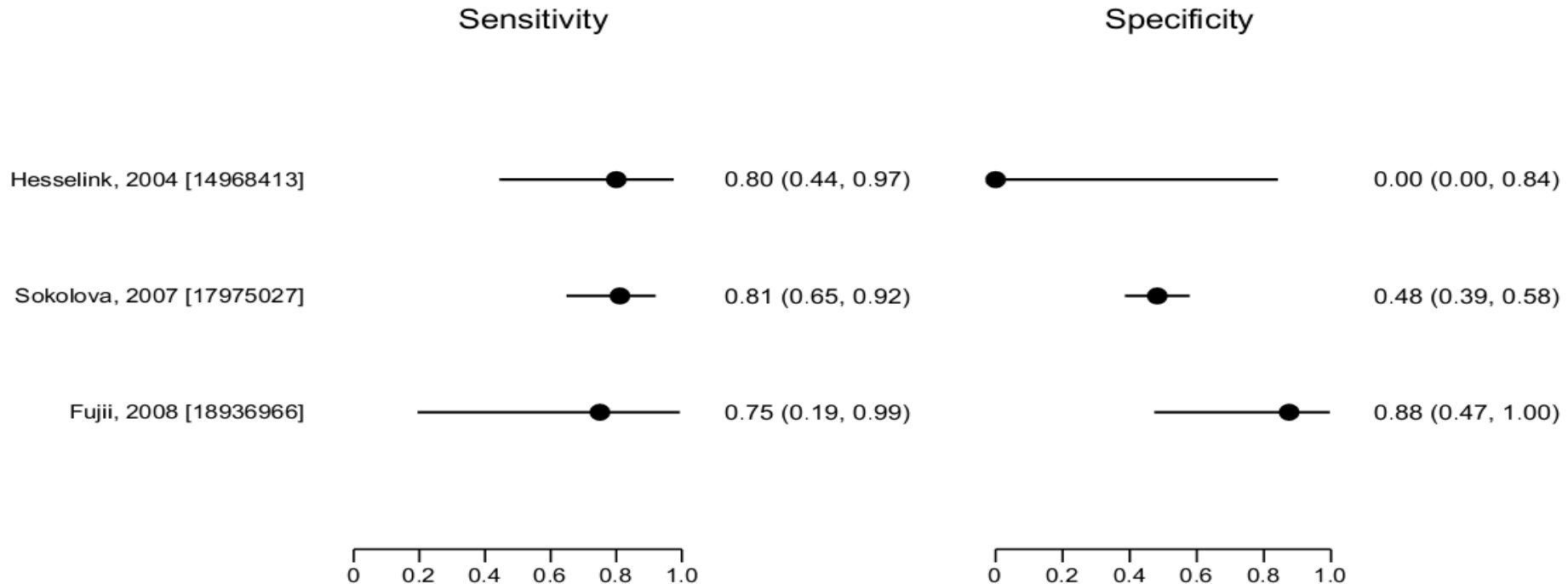
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CIN3+ [3 studies, 803 cases] sensitivity range 0.25 to 0.82; specificity range 0.67 to 0.89

FISH Probe	Cytology	Outcome	# studies	N patients	Sensitivity	95% CI	Specificity	95% CI
TERC	LSIL	CIN2+	7	1033	0.76	0.60, 0.86	0.79	0.50, 0.93
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		CIN3+	2	26	0.25 to 1.00	0.03, 1.00^	0.44 to 0.67	0.14, 0.96^
TERC or HPV	LSIL	CIN2+	1	115	0.90	0.73, 0.98	0.48	0.37, 0.59

KQ3 Results

FISH for HPV 16 or 18 in LSIL for CIN2+



CIN2+ [3 studies; 38 cases] sensitivity range (0.75 to 0.81); specificity range (0.00 to 0.88)

Not Shown:

CIN3+ [2 studies, 26 cases] sensitivity range (0.80 to 0.83); specificity range (0.17 to 0.42)

FISH Probe	Cytology	Outcome	# studies	N patients	Sensitivity	95% CI	Specificity	95% CI
TERC	LSIL	CIN2+	7	1033	0.76	0.60, 0.86	0.79	0.50, 0.93
		CIN3+	5	904	0.78	0.65, 0.87	0.79	0.51, 0.93
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HPV	LSIL	CIN2+	3	38	0.75 to 0.81	0.19, 0.99^	0.00 to 0.88	0.00, 1.00^
		CIN3+	2	26	0.80 to 0.83	0.28, 1.00^	0.17 to 0.42	0.00, 0.64^
	ASCUS	CIN2+	1	12	1.00	0.48, 1.00	0.57	0.18, 0.90
		CIN3+	2	26	0.25 to 1.00	0.03, 1.00^	0.44 to 0.67	0.14, 0.96^
TERC or HPV	LSIL	CIN2+	1	115	0.90	0.73, 0.98	0.48	0.37, 0.59

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TERC or HPV	LSIL	CIN2+	1	115	0.90	0.73, 0.98	0.48	0.37, 0.59

KQ3: Clinical Validity - Results

Two studies compared three test strategies.

Voss 2010

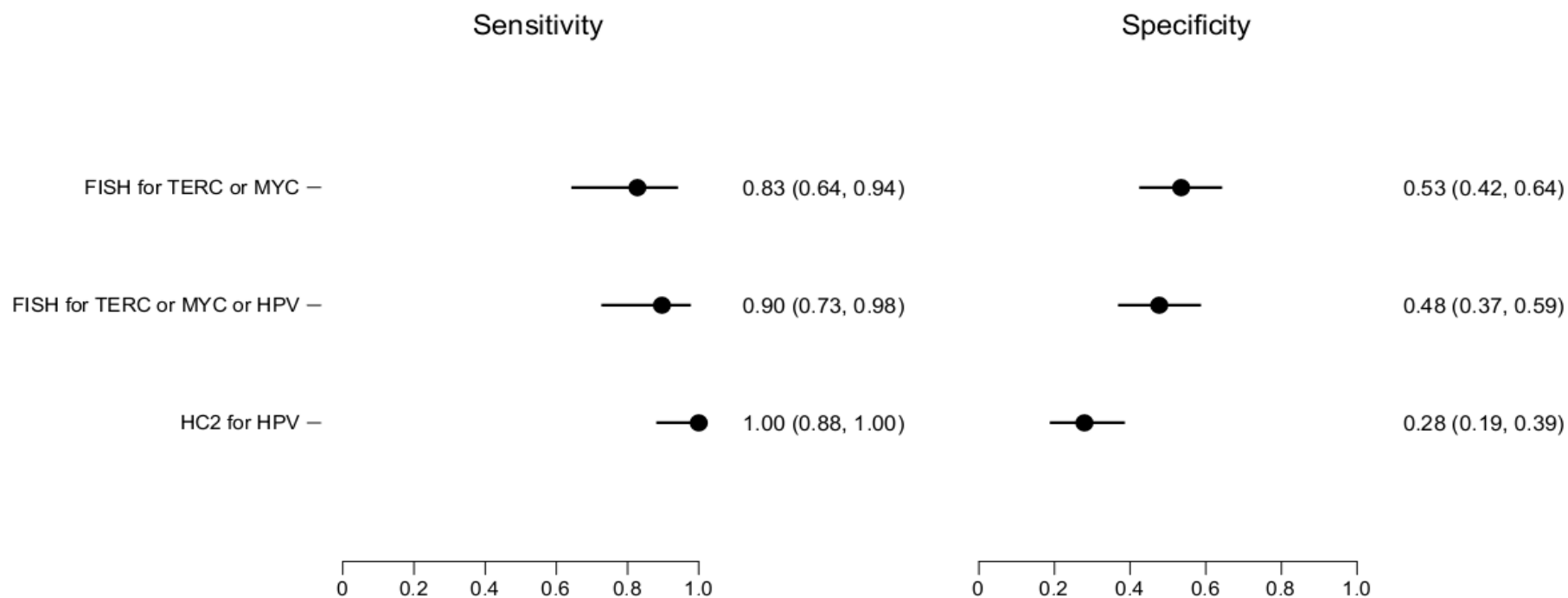
- FISH for TERC or MYC
- FISH for TERC or MYC or HPV
- Hybrid Capture 2

Jiang 2010

- FISH for TERC
- Hybrid Capture 2
- FISH for TERC or Hybrid Capture 2

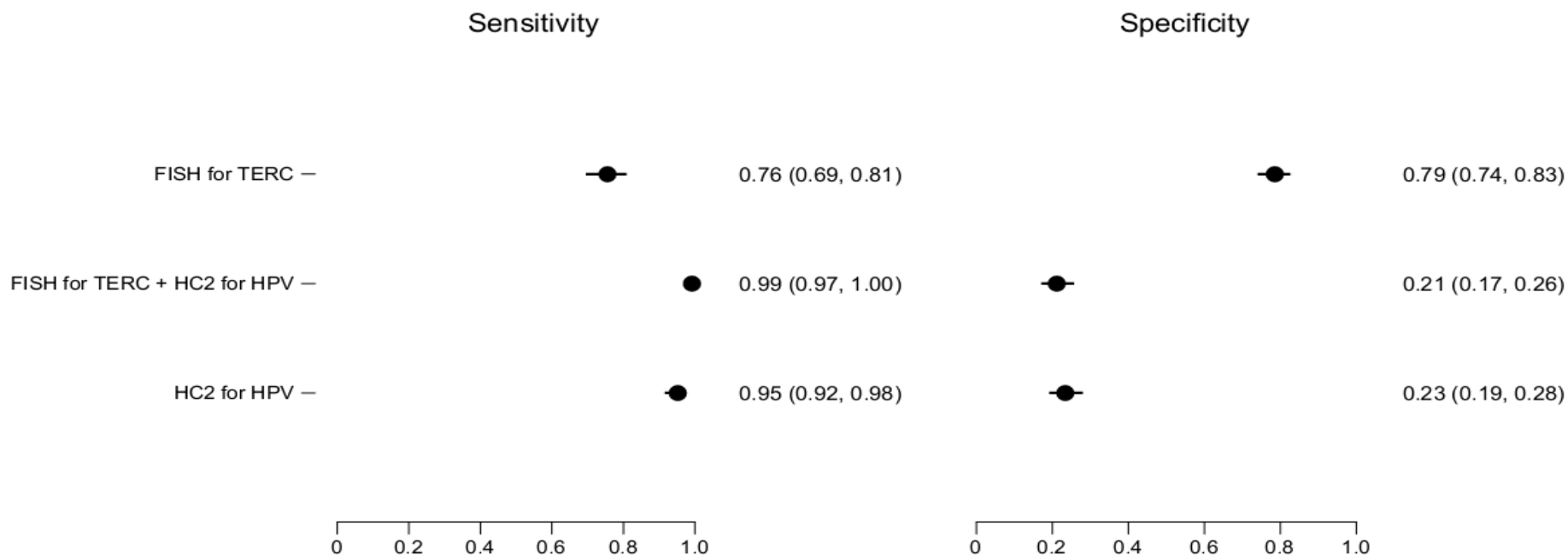
KQ3: Clinical Validity – Results Voss 2010

LSIL for CIN2+



KQ3: Clinical Validity – Results Jiang 2010

LSIL for CIN2+



KQ3: Clinical Validity – Results Jiang 2010

ASCUS for CIN2+

Sensitivity

Specificity

FISH for TERC –



0.75 (0.68, 0.81)



0.87 (0.83, 0.90)

FISH for TERC + HC2 for HPV –



0.96 (0.93, 0.99)



0.42 (0.37, 0.46)

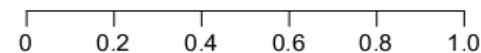
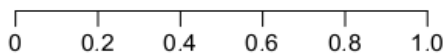
HC2 for HPV –



0.89 (0.84, 0.93)



0.45 (0.41, 0.50)



Results KQ3: Clinical Validity - Summary

- Sensitivity and specificity estimates often had wide CIs (even when meta-analysis was possible), indicating considerable uncertainty about the tests' ability to identify women with CIN2+ or CIN3+.
 - TERC in LSIL for CIN2+
sensitivity 0.76 (0.60, 0.86), specificity 0.79 (0.50, 0.93)
- Strength of evidence low
- Majority of studies of did not stratify women based on HPV results.

KQ3: Clinical Validity - Limitations of the Evidence

- Studies used convenience samples and were not conducted in a well-defined screening context.
- Sample sizes were generally small leading to imprecision
- Few studies for each test–outcome pair of interest.
- Reporting on items used for risk of bias assessment was often incomplete.
- Thresholds for test positivity varied across studies and point estimates were heterogeneous.
- Panels of HPV probes for HPV 16 or 18 among other types had variable overlap resulting in irreconcilable clinical heterogeneity.
- Confidence in the test performance of FISH was low.

KQ3: Clinical Validity – Evidence Gaps

- No data for NSIL and HPV+
- No study examined the association of FISH test results with clinical outcomes.

KQ4: Clinical Utility

- What are the clinical utility and harms for ISH tests in cervical cytology?
- No study compared patient care strategies resulting from different tests, thresholds, or combinations of ISH and/or non-ISH tests or examined testing strategies including ISH testing

Conclusion

The current evidence is insufficient to support routine FISH testing for TERC, MYC, HPV 16 or 18 in women with LSIL, ASCUS or NSIL on cytology, with or without HPV infection

Evidence Gaps

- Lack of standardization of pre-analytic issues
 - thresholds, probe sets, controls, and procedures
- Nomenclature updates
 - Bethesda system divides ASCUS into ASCUS and ASC-H
 - New system for histology (LAST) now suggests triaging CIN2 to HSIL or LSIL
- New testing recommendations
 - Co-testing of Pap with HPV
 - HPV tests in evolution
- No clinical outcome studies

Future Research Needs

- Standardize ISH techniques and thresholds
- Study ISH as add-on test after Pap and HPV co-testing
- Study larger samples
- Compare clinical validity for different test combinations
- Consider impact of newer HPV tests
- Examine the role of ISH testing to detect adenocarcinoma

End