



LLETZ (Large Loop Excision of the Transformation Zone) Fragmentation: Impact on Margin Assessment and Cervical Biopsy-LLETZ Correlation

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Cervical Cancer

- 306 new cases and 93 deaths annually in Ireland (NCRI 2015)
- Cervical screening effective in early detection (Arbyn *et al* 2010)
- Cervical cytology and Papanicolaou staining (Arbyn *et al* 2010)
- Originates in epithelial cells of transformation zone (Cervical Check 2013)

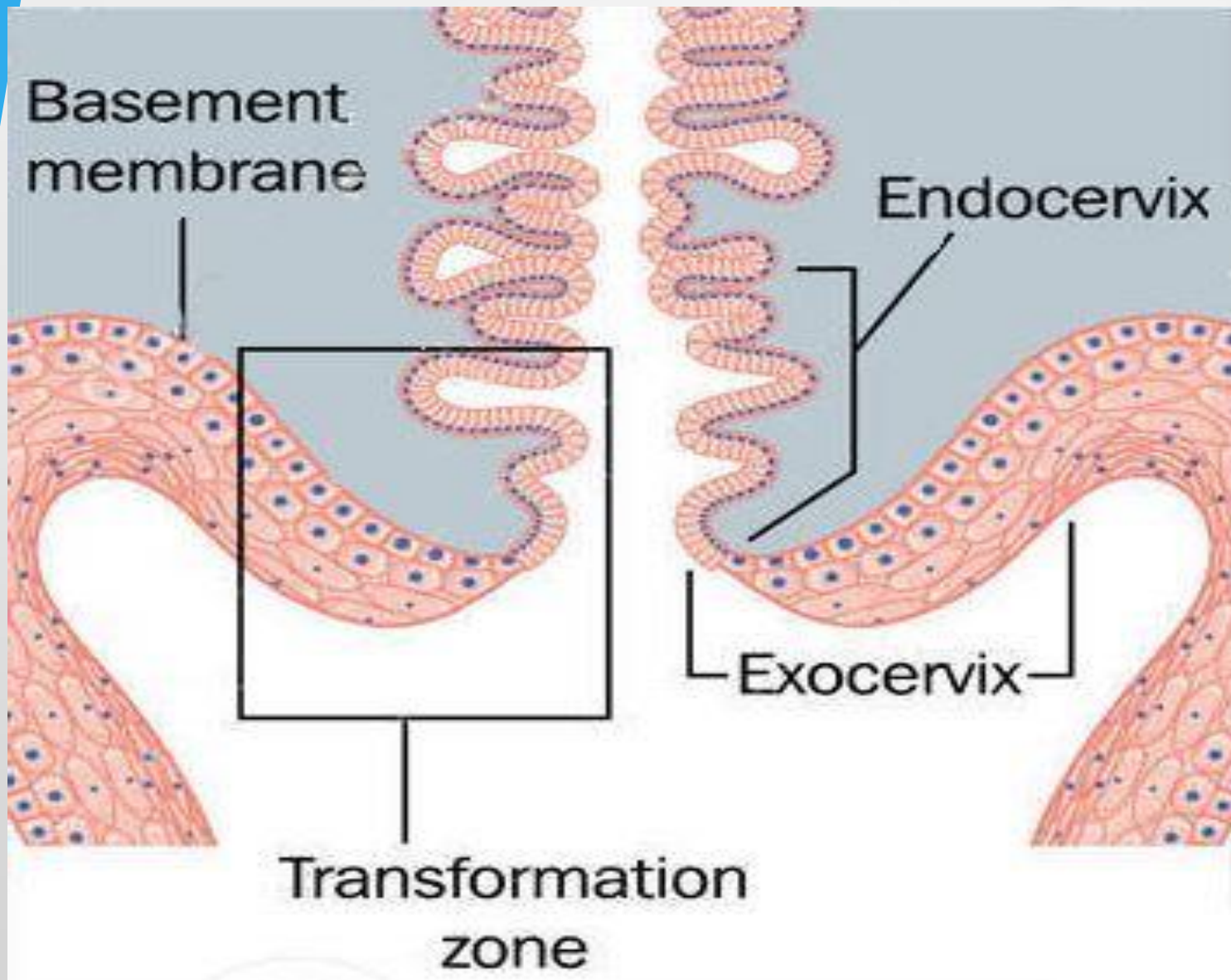
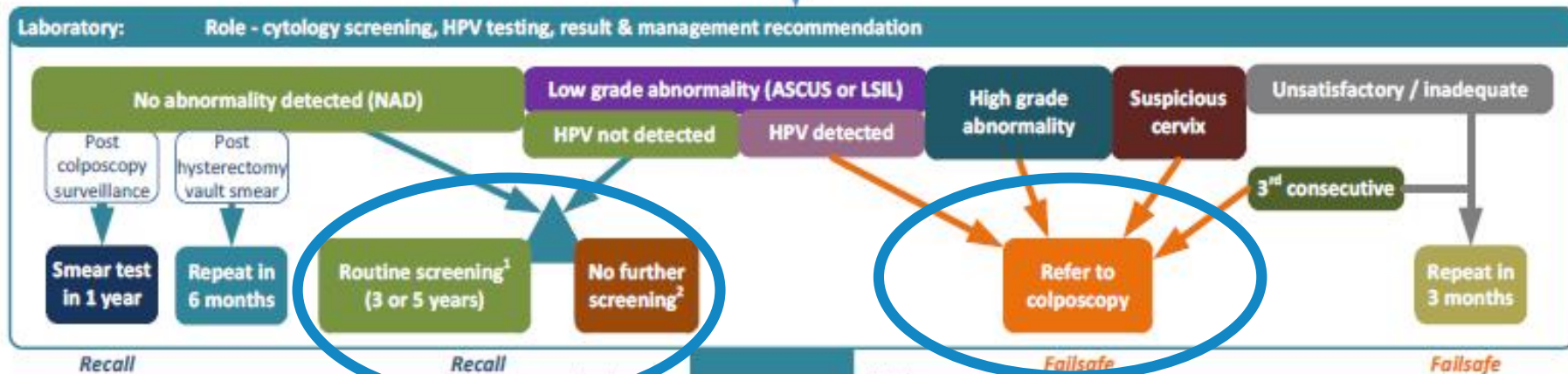
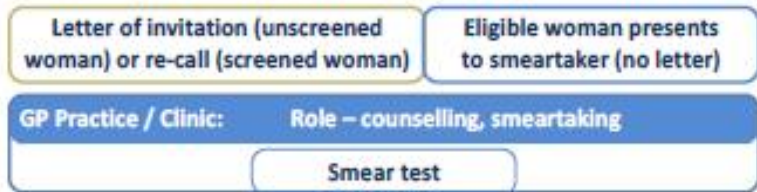


Figure 1 Illustration of the opening to the cervix demonstrating the locations of the transformation zone, endocervix and ectocervix (adapted from dreamstime 2016).

Cervical Screening Process Chart



Programme office: Role - call / recall, result recommendation, failsafe

Recommendation to woman: Programme issues a letter to woman advising her that her result is available and what the recommendation is.

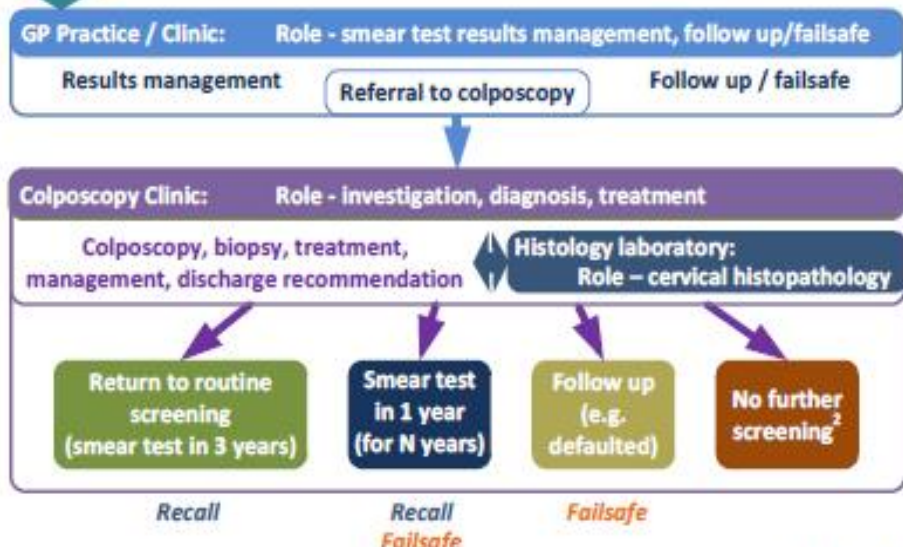
Recall: Programme will issue a letter to woman in advance of the next smear test due date.

Failsafe: Programme will issue letters to woman and to doctor after the due date of a recommended early repeat test or a referral to colposcopy, if these do not occur.

NOTES

1) **Routine screening:** Women aged 25-44 years -> interval = 3 years. Women aged 45+ years -> interval = 5 years after 2 normal results at 3-year interval.

2) **No further screening.** Woman is or will be over 61 years at next smear test due date and has had 2 successive normal – routine recall results OR woman is over age range and first test has normal result.



- Abnormalities associated with increased risk of progression to invasive malignancy termed 'Cervical Intraepithelial Neoplasia' (Buckley *et al* 1982)
- CIN₁, CIN₂ and CIN₃
- Bethesda System
 - Atypical Squamous Cells (ASC)
 - Low Grade Squamous Intraepithelial Lesions (LSIL)
 - High Grade Squamous Intraepithelial Lesions (HSIL)

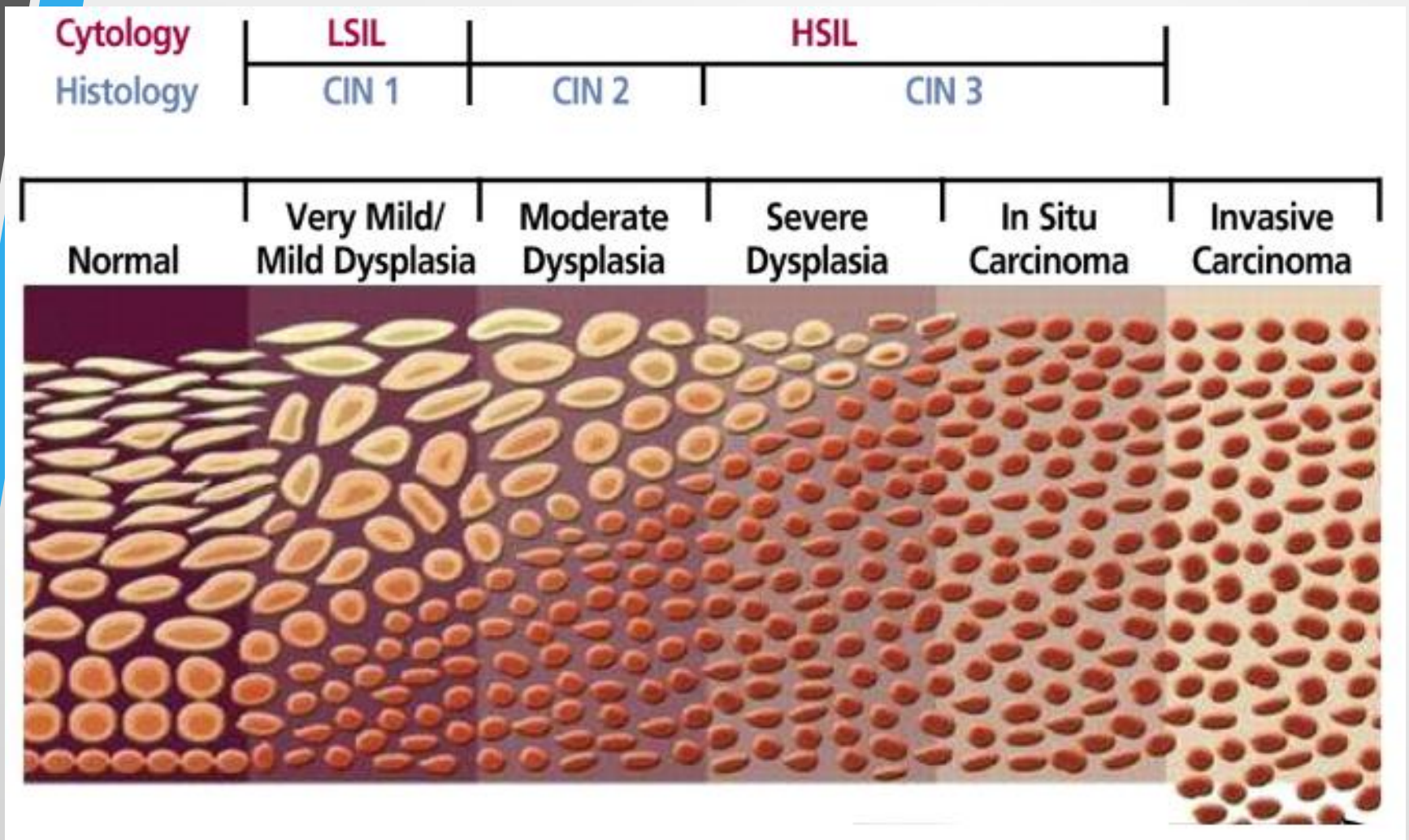


Figure 2 Progression of cervical cancer from 'normal' cervical epithelial cells to invasive carcinoma (Adapted from Ortoski, R. A. and Kell, C. S. 2011).

What Happens After Screening...?

- Colposcopy – A magnified visual examination aided by application on contrast solutions (Stoler *et al* 2011)
 - Acetowhite staining
- Histopathology – samples abnormal tissue in order to obtain diagnosis
 - Punch biopsy
 - Large Loop Excision of the Transformation Zone (LLETZ) → aims to excise abnormal lesion (European Commission 2008)






Figure 3 Image of intact LLETZ specimen in the Histopathology Department of Letterkenny University Hospital (longer demarcations represent centimetres).

Limitations of LLETZ

- Fragmentation
- Diathermy artefact (Bharathan *et al* 2013)
- Multiple guidelines developed to control quality of screening programme
 - National Health Service Cervical Screening Programme (NHSCSP) Guideline
 - Recommends removal of 80% of excisional specimens in a single fragment.

- Evidence required to support guideline
- Associations between LLETZ fragmentation and;

-  Indeterminate margins
-  Margin involvement
-  Endocervical margin involvement

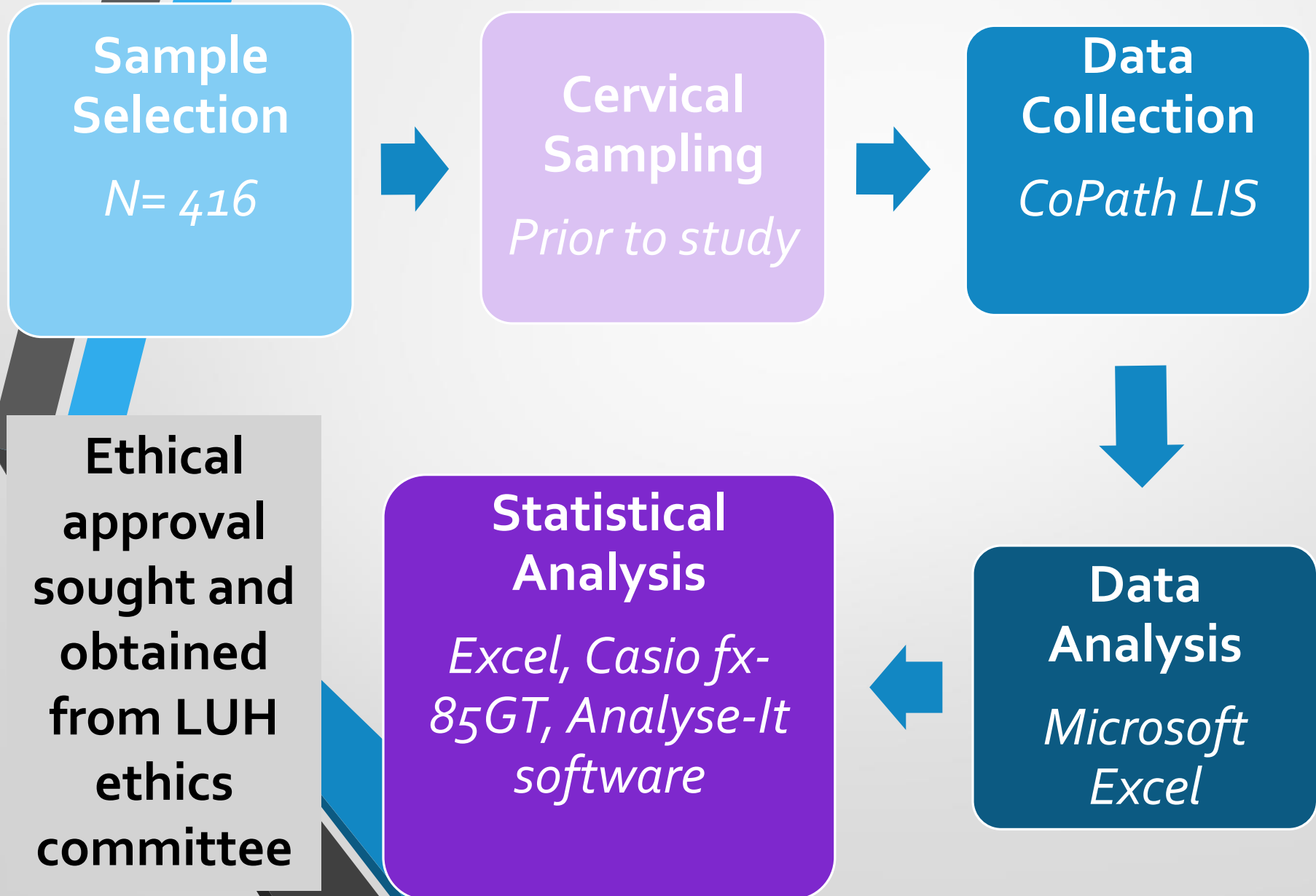
- (Bharathan *et al* 2013 and Papoutsis *et al* 2016)

- Cytology – Histopathology correlation used as a measure of quality (European Commission 2008)

Aims and Objectives

- To determine whether the NHSCSP recommendation for removal of LLETZ in a single piece is supported by scientific evidence.
- To investigate relationships between;
 - LLETZ fragmentation and margin assessment
 - LLETZ fragmentation and cervical biopsy-LLETZ agreement
 - Patient age and LLETZ fragmentation
 - Acetowhite grade and grade of subsequent cervical biopsy

Methodology



Results

- Age Range: 20-60 years
- Mean age: 32.3 years
- Final Diagnosis:
 - Negative in 15 (3.6%)
 - CIN1 in 54 (13%)
 - CIN2 in 187 (44.9%)
 - CIN3 in 160 (38.5%)

LLETZ Fragmentation and Margin Interpretation

- 43.7% removed intact and 56.3% removed in multiple pieces
- Single fragment → 97% margin interpretability
- Multiple Fragment → 73.4%

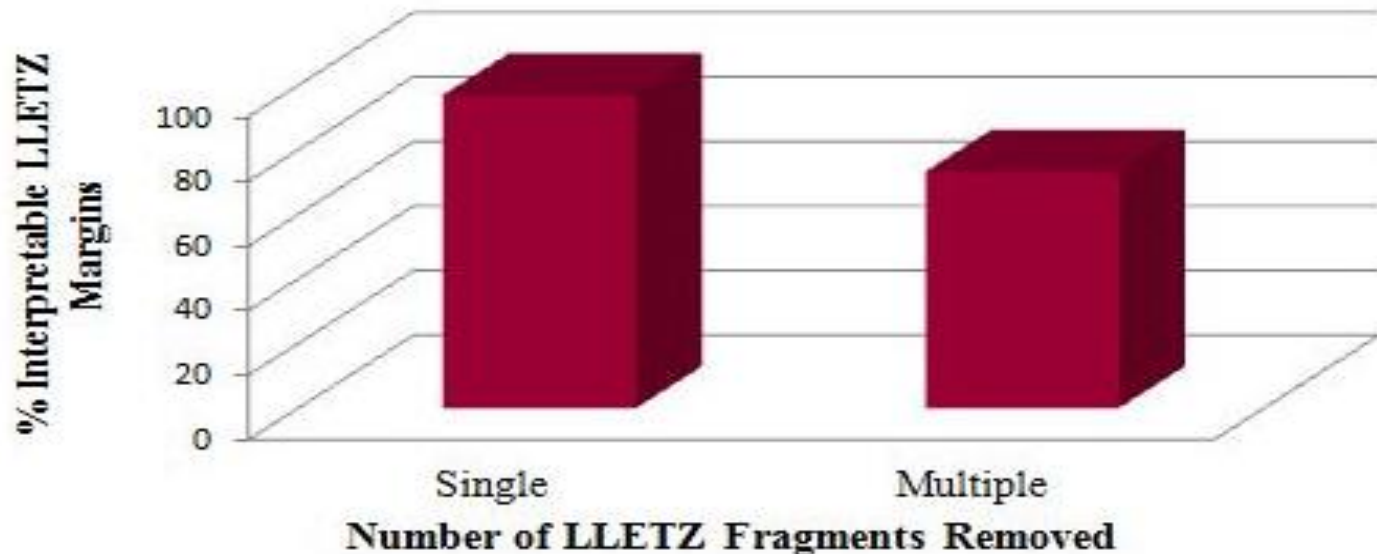


Figure 4 Column chart demonstrating the percentage of interpretable LLETZ specimen margins when the LLETZ biopsy is removed in a single or multiple fragment(s) (N=380).

- Chi-Square Test: $p\text{-value} < 0.0001$
- Rejection null hypothesis
- 7 outliers identified and excluded (N=373)
- Correlation co-efficient (r) = -0.95

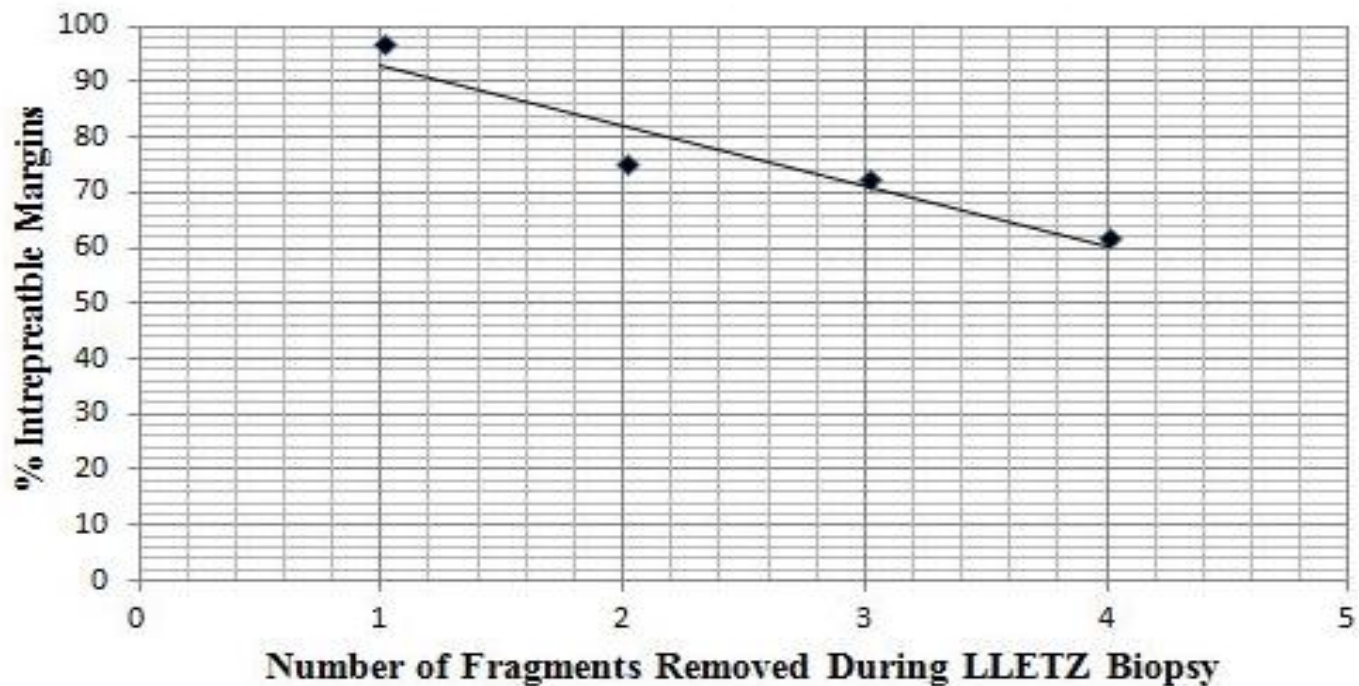


Figure 5 Scatter-plot demonstrating the strong negative relationship between the number of tissue fragments in which a LLETZ biopsy is removed and the percentage of subsequent LLETZ specimens in which the excisional margins are interpretable (N=373).

Causes of Indeterminate Margins

- 62 reports gave reason for indeterminate margins
 - 56 (90.3%) fragmentation
 - 5 (8.1%) artefact
 - 1 (1.6%) poor orientation

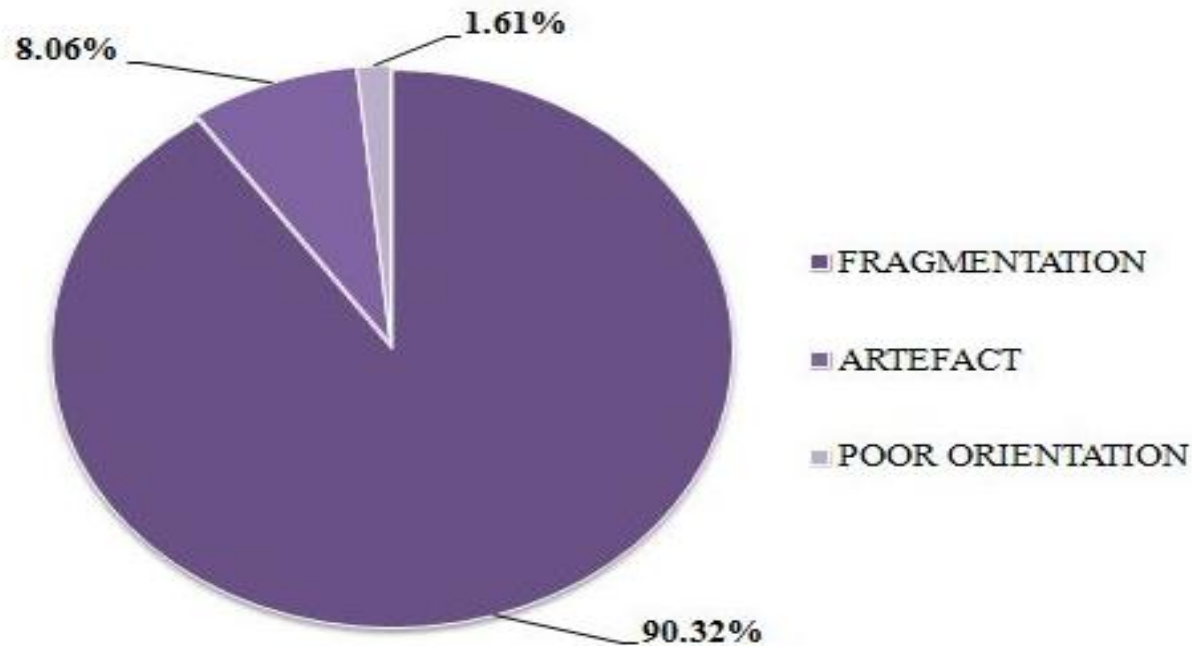


Figure 6 Pie chart demonstrating the three causes of indeterminate margins and their associated proportions (N=62).

Patient Age and LLETZ Fragmentation

- Correlation co-efficient (r) = 0.00
- No statistically significant relationship observed

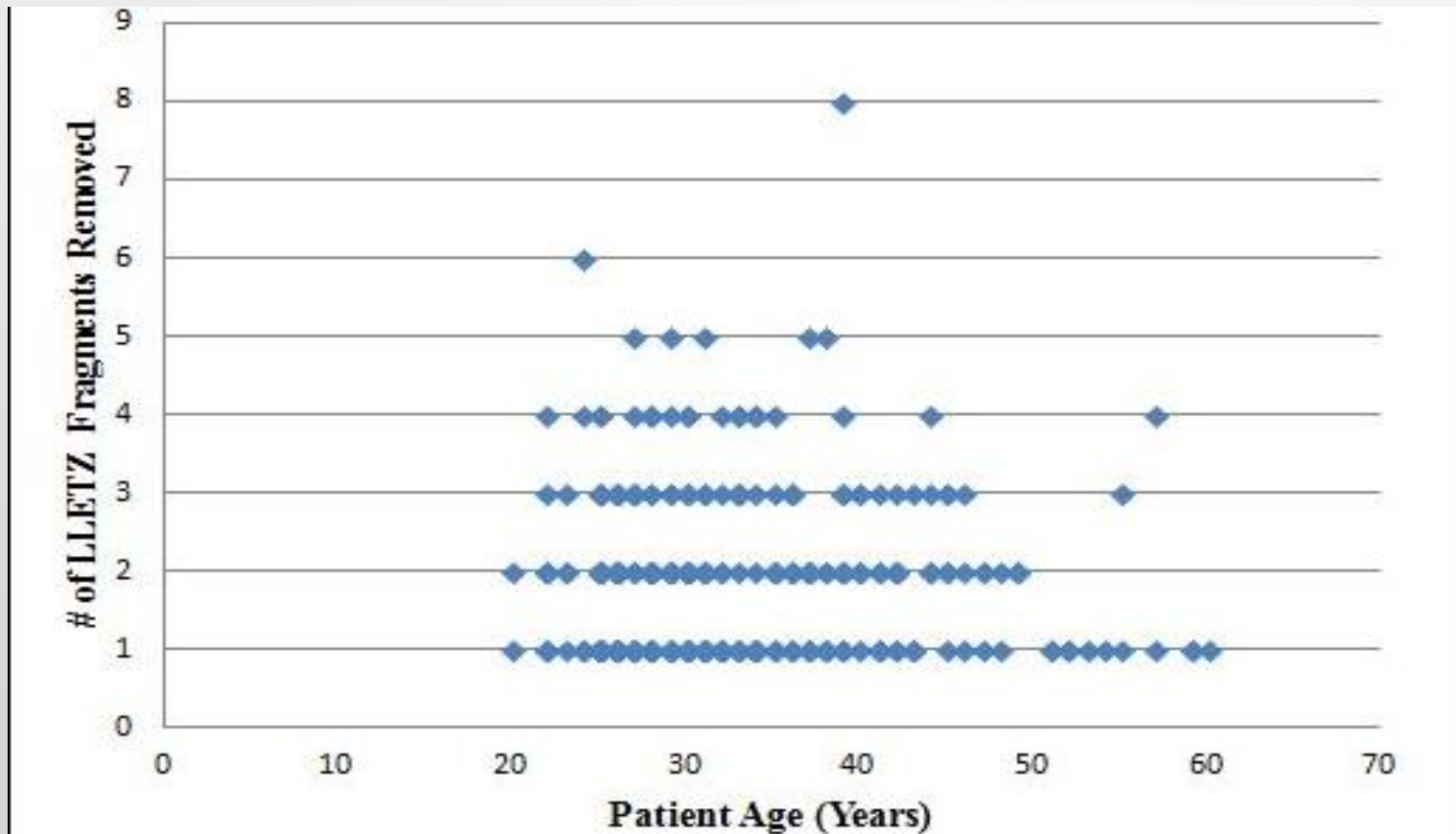


Figure 7 Scatter-plot demonstrating the absence of a relationship between the patient age (years) and the number of tissue fragments removed during the LLETZ biopsy (N=416).

LLETZ Fragmentation and Margin Involvement

• ↑ fragments → ↑ margin involvement

• ↑ fragments → ↑ endocervical involvement

Table 1 The Percentage of LLETZ specimens with margin involvement when the LLETZ is removed in 1, 2 or ≥ 3 fragments and the proportion of involved margins which are endocervical, ectocervical, both endocervical and ectocervical or unnamed (but true) margins (N=318)

# LLETZ Fragments	% Margin Involvement	% Endocervical	% Ectocervical	% Both	% Unnamed
1	40.4	40	46.2	9.2	4.6
2	44.1	53.3	28.9	17.8	0
≥ 3	52.7	55.2	37.9	6.9	0

Agreement between Punch Biopsy and LLETZ





- 44.5% single piece and 55.5% multiple piece
-  fragments →  kappa score
- 3 or more fragments →  perfect agreement
- 3 or more fragments → slightly  practical agreement

Table 2 Summary of the perfect agreement, the practical agreement and the calculated kappa score for each group of punch biopsy and LLETZ biopsy grades when the LLETZ biopsies were removed in 1, 2 or ≥ 3 fragments (N=416)

	Group		
	1 Fragment	2 Fragment	≥ 3 Fragments
Perfect Agreement	61.1%	63.7%	51.8%
Practical Agreement	95.1%	95.2%	92.9%
Kappa Score	0.38	0.33	0.23

Acetowhite and Punch Biopsy Grade Agreement

- Perfect agreement = 43.5% (163/375)
- Low kappa value of 0.13
- Positive predictive value (PPV) = 93%

Table 3 The acetowhite grade, available for 375 patients, and the grade of the corresponding and subsequent cervical punch biopsy (N=375)

Biopsy	Acetowhite Grade				Total
	Negative	Grade 1	Grade 2	Grade 3	
Negative	8 (14%)	2 (1.9%)	2 (1.2%)	1 (2.2%)	13
CIN1	12 (21.1%)	7 (6.7%)	10 (5.9%)	2 (4.4%)	31
CIN2	30 (52.6%)	82 (78.9%)	119 (70.4%)	13 (28.9%)	244
CIN3	7 (12.3%)	13 (12.5%)	38 (22.5%)	29 (64.5%)	87
Total	57 (100%)	104 (100%)	169 (100%)	45 (100%)	375

To summarise study findings...

- Correct sample chosen for investigation
- Selection criteria introduced a bias
- Rate of intact LLETZ (43.7%) lower than recommended 80%
- Increased LLETZ fragments → Decreased interpretable margins
 - Agreement with other studies
- Fragmentation is the primary cause of indeterminate margins

- No relationship between age and LLETZ fragmentation
- Relationship between LLETZ fragmentation and increased endocervical margin involvement
 - Clinically significant
- Decreased punch biopsy – LLETZ agreement with increased fragments
 - Not statistically significant
- Acetowhite is suitable for confirming presence of CIN and guiding biopsy
 - Not suitable as predictor of histopathological grade

Limitations and Further Study

- Absence of data regarding colposcopist
- Size of sample subgroups
 - Should be larger and relatively equal in size
- 1st prize in Allied Health, in the 6th Annual Multidisciplinary Research Symposium in Letterkenny University Hospital

Conclusion

- NHSCSP guideline supported
- Increased LLETZ fragments;
 - negatively impacts margin interpretation
 - Increases likelihood of endocervical involvement
- Patient age does not induce LLETZ fragmentation
- Specific acetowhite grades are not clinically useful

Sources of Reference

- Arbyn, M., Anttila, A., Jordon, J., Ronco, G., Schenck, U., Segnan, N., Wiener, H., Herbert, A., and von Karsa, L. (2010) 'European Guidelines for Quality Assurance in Cervical Cancer Screening. Second Edition – Summary Document.' *Annals of Oncology*, 21 (3), pp.448-458.
- Bharathan, R., Sagoo, B., Subramaniam, A., Larsen-Disney, P. And Fish, A. (2013) 'LLETZ Specimen Fragmentation: Impact on Diagnosis, Outcome, and Implications for Training', *The Journal of Obstetrics and Gynaecology of India*, 63 (5), pp.332-336.
- Buckley, C. H., Butler, E. B. and Fox, H. (1982) 'Cervical Intraepithelial Neoplasia', *Journal of Clinical Pathology*, Volume 35, pp.1-13.
- Cervical Check (2013) *Guidelines for Quality Assurance in Cervical Screening Second Edition*, NCSS/PUB/Q-1 Rev 2, Limerick: National Screening Service.
- Cervical Check (2015) *Programme Report 2013-2014*, CS/PR/PM-19 Rev 1, Limerick: National Cancer Screening Service.
- Dreamstime (2016) *Transformation zone of cervix* [online], available: <http://www.dreamstime.com/stock-illustration-transformation-zone-cervix-overview-female-reproductive-organs-detail-image41147460> [accessed 8 May 2016].
- European Commission (2008) *European Guidelines for Quality Assurance in Cervical Cancer Screening Second Edition*, Luxembourg: Office for Official Publications of the European Communities.
- National Cancer Registry Ireland (2016) *Incidence Statistics* [online] available: <http://ncri.ie/data/incidence-statistics> [accessed 10 March 2016].
- Ortoski, R. A. and Kell, C. S. (2011) 'Anal Cancer and Screening Guidelines for Human Papillomavirus in Men', *The Journal of the American Osteopathic Association*, 11, pp. S35-S43.
- Papoutsis, D., Panikkar, J., Gornall, A. and Blundell, S. (2016) 'Does the number of tissue fragments removed from the cervix with excisional treatment for CIN pathology affect the completeness of excision and cytology recurrence at follow-up? An observational cohort study', *Journal of Obstetrics and Gynaecology*, 36, pp.251-256.
- Public Health England (2016) *NHS Cervical Screening Programme Colposcopy and Programme Management*, NHSCSP publication number 20, London: Public Health England.

Stoler, M. H., Vichin, M. D., Ferenczy, A., Ferris, D. G., Gonzalo, P., Paavonen, J., Jouna, E. A., Djursing, H., Sigurdsson, K., Jefferson, L., Alvarez, F., Sings, H. L., Lu, S., James, M. K., Saah, A. and Haupt, R. M. (2011) 'The accuracy of colposcopic biopsy: analyses from the placebo arm of the Gardasil clinical trials', *International Journal of Cancer*, 128, pp.1354-1362.



Thank you
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