

# External Quality Control in Cellular Pathology: UK NEQAS ICC and ISH

**Suzanne Parry**

22<sup>nd</sup> April September 2016

## Outline Of Talk

- **Introduction to the scheme**
- **Module Specific Assessments:**
  - Alimentary Tract
  - Lymphoma
  - Breast Modules: HER2 IHC & ISH

## UK NEQAS

- Service now running for over 25 years
- Assess the quality of IHC & ISH & provide objective help & advice
- Commercially independent & 'not for profit'
- 4 Assessments per year – allows for continued improvement
- Accredited EQA scheme to International Standard ISO/IEO 17043:2010 'proficiency testing'



Proficiency Testing Provider  
No. 7833

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Immunocytochemistry & In-Situ Hybridisation

## UK NEQAS Headquarters



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# Assessments are a team effort: Scientists and Pathologists

## ASSESSORS

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Dr M Arends, Cambridge  
Dr M Ashton-Key, Southampton  
Mrs J Bell, Nottingham  
Mr N Bilbe, London  
Mr D Blythe, Leeds  
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Mr N Hand, Nottingham  
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Dr R Hunt, Stockport  
Dr M Ibrahim, London  
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Mrs H McBride, Belfast  
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Mr K Miller, London  
Ms J Moorhead, London  
Dr M Morgan, Cardiff  
Ms P Jones, London  
Ms A Newman, London  
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Dr G Orchard, London  
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Prof E Kaye, Dublin  
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Dr T O'Grady, Dublin  
Ms Yvonne Connolly, Dublin  
Dr Hilary Magee, Dublin

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Dr M Franco, Lisbon  
Dr F Schmitt, Porto  
Mr A Ferrero, Lisbon  
Mrs T Peniera, Lisbon  
Mr R Roque, Lisbon  
Mr J Matos, Lisbon

### Slovenia

Dr M Flezar, Ljubljana  
Mrs I Kirbis, Ljubljana

### South Africa

Mrs R Van Wijk, Cape town

### Sweden

Dr G Elmberger, Stockholm

### Switzerland

Prof. Pierre-Andre Diener, St Gallen

- >5000 slides assessed in 2–3 Weeks
- 1–4 days for each area of pathology
- 4 assessors & driver
- Pool of over 89 assessors

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## Quality controlling the analytic stage of IHC

Preanalytic  
e.g. Fixation

Analytic  
Technical  
IHC/ISH  
Staining

Postanalytic  
interpretation



1. Distribute unstained tissue/cell line samples

2. Stain samples as routine sections and return + provide methodology (antibody, dilution, retrieval methods etc)

3. Submit 'in-house control' stained at the same time as the QA slide

4. Assess slides for technical quality: 4 assessors provide interpretation & feedback to improve staining techniques

5. Individual reports provide feedback plus data on overall pass rate, antibodies, automation systems used, methods and photographic examples

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# EQA covers specialised areas of pathology

30% UK : 70% OS

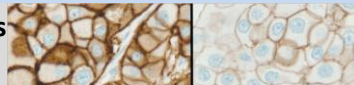
56 Countries taking part

MODULE	UK	OS	Total
1. General	191	172	363
2. Breast Hormonal	157	177	334
3. Neuropathology	32	41	73
4. Lymphoma	113	127	240
5. GIST	54	61	115
6. Cytology	35	42	77
7. Breast HER2 ICC	84	329	413
8. Breast HER2 ISH	55	186	241
9. HNPCC/Lynch	25	53	78
10. Gastric HER2 ICC	19	170	189
11. NSCLC EML4-ALK IHC	15	32	47
11. NSCLC PD-L1 IHC		New module for 2016	

### Difference between UK and Non-UK laboratories

- Accreditation Requirement that UK clinical laboratories participate in an EQA
- UK labs are monitored for under-performance

## Breast HER2 IHC: Scoring system + Reports (From Run 106 – June 2014)



Cell line / tissue	Assessor1				Assessor2				Assessor3				Assessor4			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Individual Assessor Scores	4				4				4				4			
ASSESSOR INTERPRETATION	3+	2+	1+	0	3+	2+	1+	0	3+	2+	1+	0	3+	2+	1+	0
Excellent standard of staining																
1+ stronger than expected																
Inappropriate/excessive membrane staining																
Weak membrane staining																
Excessive cytoplasmic staining																
Excessive background staining																
Recommend using a standardised kit/assay																
Morphology damaged																
Excessive antigen retrieval																
Insufficient antigen retrieval																
Excessive counterstain																
Weak counterstain																
Differentiate counterstain recommended																
Waterbath retrieval recommended																
Reassessment of slide																
Complete online methods. This is mandatory																
Individual assessor comment																
Overall Result	16/20 = Excellent															

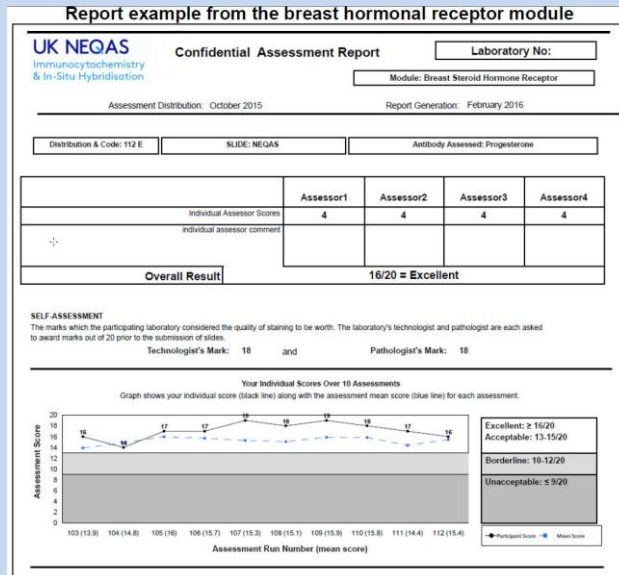
Membrane interpretation

Numeric score:  
• Four assessors mark out of 5  
• Scores summed to provide possible score out of 20

Score guide:  
16-20/20 = Excellent  
13-15/20 = Acceptable  
10-12/20 = Borderline  
≤9/20 = Unacceptable

More concise feedback on each sample

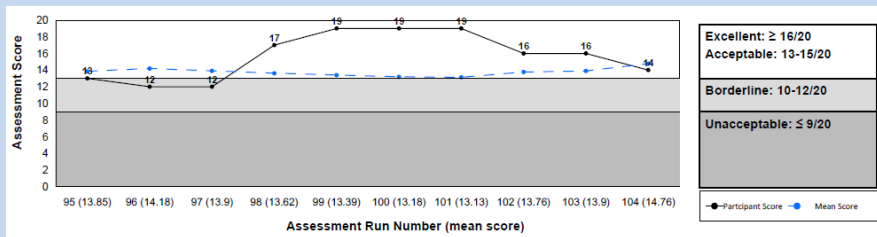
# UK NEQAS ICC & ISH Reports



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## 2.5 Year Rolling Performance Graphs

Benchmarking graphs on all individual assessments reports to better evaluate performance over time



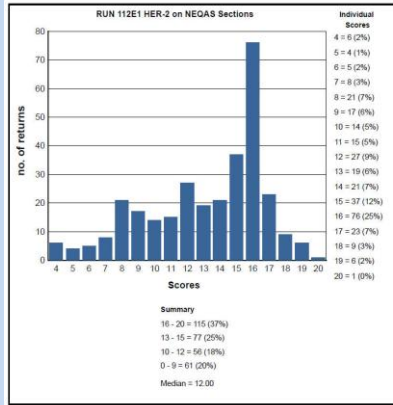
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# Breast ER IHC – Run 112 all participants

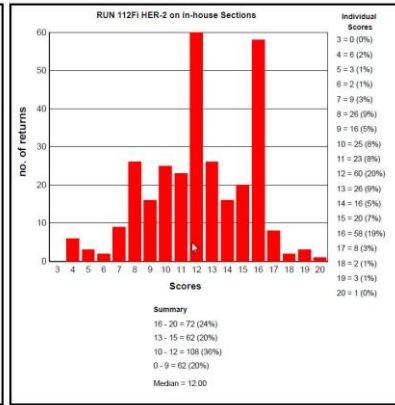
## NEQAS Samples

## In-house Samples

GRAPHICAL REPRESENTATION OF PASS RATES



20% of labs still show unacceptable staining



Many participants not using a composite control showing 3+, 2+ 1+ / 0 levels of expression

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## End of Year Participation Certificate



- Acknowledgement that participants are taking place in a recognised EQA service
- Provided to all participants who take part in at least 2/4 assessments in a single year

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# UK NEQAS ICC & ISH E-Journal

**Articles | Reports**



Participants Meeting 2015 2  
ICC 1519: Accreditation visit - ICC 5.0  
Cytology Module 2014 - 2015 report 5.0

**Immunocytochemistry Modules**

General Pathology: DAB & CK5E 3-12  
Breast Pathology: ER 10-21  
Breast Pathology: HER2/NEU 22-28  
Gastric: HER2/NEU 29-34  
Lymphoma Pathology: Cyclin D1 & BCL-6 37-42  
Neuropathology: Synaptophysin & GFAP 43-50  
Cytology: Mucicarmin & CD3 41-53  
Alternative Test: GMT: CD117 & CD34 45-47  
Alternative Test: Lymph Endothelium: CD31 & CD34 48-51  
In situ Hybridisation Modules

NECLC ALK IHC 57-64  
Breast: HER2/NEU (in situ) 65-68  
Breast: HER2/NEU (Tissue) 69-64

Click an sponsor logo below to go straight to the sponsor webpage

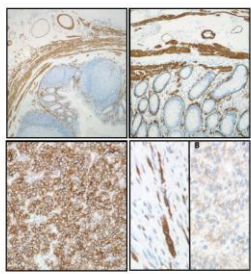



## Immunocytochemistry

Improving Immunocytochemistry for Over 25 Years

Results - Summary Graphs - Pass Rates  
Best Methods - Selected Images

Assessment Dates: 21st September – 8th October 2015



Cover Photo: Taken from the General Module

Top Left: Optimal SMA staining on the NEQAS appendix sample  
Top Right: Excellent field demonstration from an in-house appendix patient  
Bottom Left: Optimal CK5E staining on a representative positive NEQAS sample  
Bottom Right: Good appendix staining, weak neuroendocrine tumour (R) NEQAS CK5E

**Also in This Issue**

- UK NEQAS ICC & ISH Participants Meeting 2015
- UKAS ISO: 15189: accreditation visit - a positive IHC experience
- Cytology Module 2014 - 2015 EQA year report - sub-optimal staining

**UK NEQAS ICC & ISH Participants Meeting 2015**  
 Neil Gibb

**UK NEQAS ICC & ISH Participant Meeting November 16:** close to home, starting with two presentations from UK NEQAS ICC & ISH: support activities, Neil Gibb and Owen Wilkinson. Neil covered the new website which went live earlier this year. UK, EU and post-press, from over 60 individual laboratories. It is showing the old site and the need for a modern, 21st century one.

**The Lymphoma Module** Run 111

David Blythe and Suzanne Parry

	Gold Standard	Second Antibody
Antigen(s) Assessed:	Cyclin D1	CD20
Tissue Sections circulated:	Reactive Tonsil and Mantle Cell	Reactive Tonsil and Follicular Cell

**The Cytology Module** Run 111

Number of F

Number of F

Samples sent to the participants, both originate from the same patient. Colloids are prepared and provided directly by the laboratory. In addition, a single Papanicolaou (Pap) smear for each antigen is sent to UK NEQAS ICC & ISH. Treat slides are then sub-divided into 2 or 3 smaller bottles before sectioning. Examples of the validation samples sent with the slides:

**Malacoplax (HM45, M1616, A, or S100)**

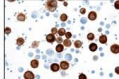


Fig 1: Mean A on cytospin prepared from 111R

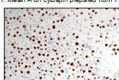


Fig 2: 100 on cell block prepared from 111R

**Features of:**

- Malacoplax is the tonsil.
- Malacoplax is characterized strongly by lymphocytic (Chan et al) implications to treatment!

**Features of:**

- Very weak in the tonsil.

**Assessment outcomes:**

There were 10 valid slides from 7 individual laboratories; three participants failed both their NEQAS and in-house (CS) assessment.

This was the eighth iteration of the two antigens, with only two failed NEQAS slides (R) and a single in-house control slide (R). The two failed slides both employed an S100 antibody, one on a CE, Dako poly 1:2000, with CC1 on the Benchmark Ultra (see fig 4); the second on a CE, Leica Bond RTU, no retrieval on the Bond Max. The assessors commented that the Dako CS slide had weak staining, with nonspecific demonstration of some components, allied with some background as well. The cells on the CS slide had been completely destroyed, although there was no mention of any associated preservation.

The single in-house failure (R) was a satisfactory piece of skin, weakly stained, using a Ventana pre-CD3, Marker A, S100, 1:1 antibody, with CC1 on the Benchmark Ultra.

\* S100 for Run 111 see [www.neqas.org.uk](http://www.neqas.org.uk)

**CD117 & U**

N.B. One participant stated that they rarely, if ever, used CD117, even though this was in their repertoire. They had requested a cytospin preparation and stained it with a Novocastra primary P53 clone diluted 1:200, without retrieval, formal on a cytospin sample on a Bond II. They had asked in their data entry for some antibody advice. This was failed both slides. Interestingly, two other labs also using this antibody, both achieved reasonable results (R20 spots), but both were on Papanicolaou smears, with CC1 retrieval, diluted 1:50, as per the supplier's guidelines. This was sent to the participant as requested. Without intending to delude in the future of introducing a new antibody, whether it is difficult to see how or if significant improvement can be made.

The other two participants who failed both (CS) slides, used:

- 1) Dako poly 1:50, with ER2 on a Bond II (CS) (see fig 9); and
- 2) Novocastra LM70, 1:200, without retrieval, again on a Bond II (CS). The staining on the CS sample (1) was off.

## 'Same Slide' NEQAS Material + Participant In-house control

Participants cut their control material alongside the NEQAS EQA samples



- Now Required for all NEQAS modules
- Reduce Laboratory EQA reagent costs
- Ensure same protocol is applied to all samples
- Recommend similar control setup for use with clinical cases

## In-house Control Preparation



- Simple Dermal Punch
- 3-4 mm cores

### Controls e.g

- **Breast HER2 IHC**
  - 3+, 2+, 1+/0
  - Invasive breast tumour BUT DCIS also acceptable
- **Breast hormonal receptors**
  - high (Allred: 7-8)
  - mid (Allred: 3-6)
  - negative

When clinically testing, ideally controls should also be placed alongside patient cases

## Alimentary Tract Module CD117 and DOG-1 Antibodies

### Sensitivity & Specificity



# Alimentary Tract CD117 (GIST) Module

## Appendix + GIST + Desmoid



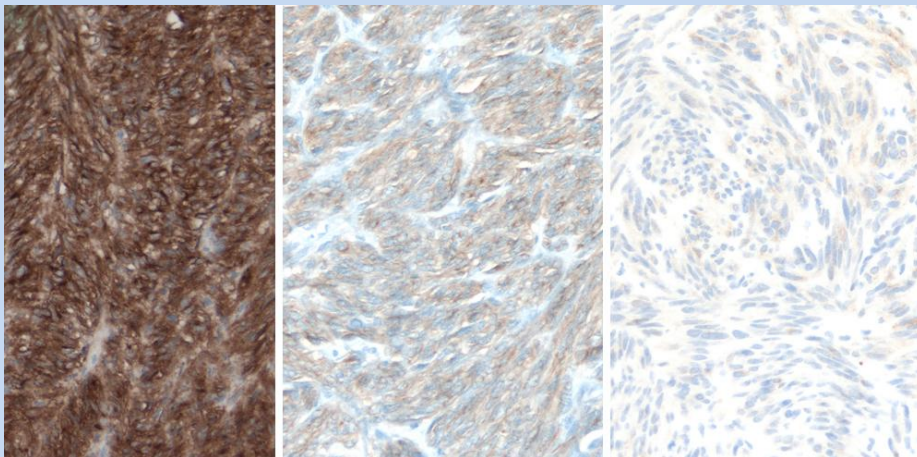
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## GIST: Main Reason for Poor NEQAS Scores Due to Lower Sensitivity in Staining

Dako A4502, 1:50  
Bond Max ER1

Dako A4502, 1:800  
Vent. XT CC1 Stand'd

Novocastra T595  
1:50 Bond III ER1



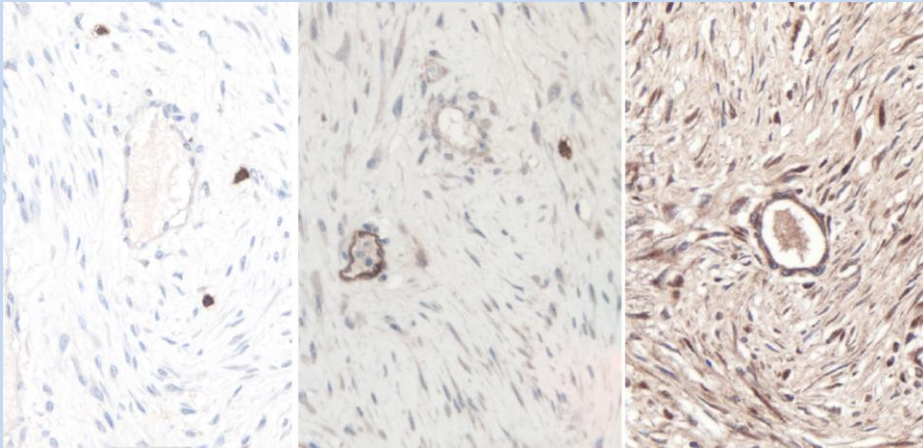
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Immunocytochemistry & In-Situ Hybridisation

## Desmoid as a Negative Control: Can Help to Gauge Non-specific Staining

**Dako A4502, 1:120**  
**Dako Autostainer - PC**

**Dako A4502, 1:500 Bond**  
**III- retrieval not stated**

**Dako A4502, 1:5**  
**NO RETRIEVAL**



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## Pass Rates Run 110 (Scores $\geq 13/20$ )

**90% of labs currently use: Dako A4502 rb poly Antibody**

Alimentary Tract Pathology Run: 110		
Primary Antibody : CD117		
Antibody Details	N	%
Cell Marque 117R/S-xx (YR145)	3	67
Dako A4502 (rb poly)	95	83
Epitomics AC-0029 (EP10)	1	100
Leica/Novocastra NCL-CD117 (T595)	1	100
Other	2	100
Ventana 790-2951 (9.7)	10	70

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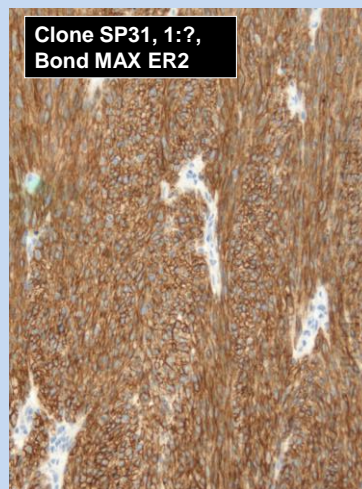
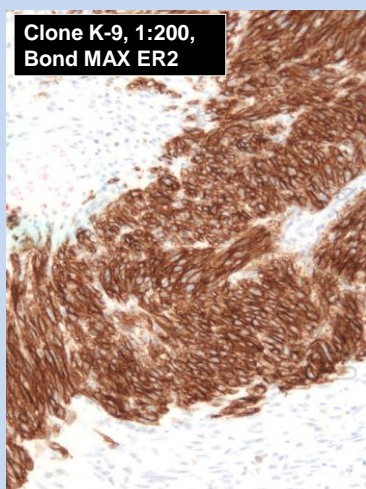
## DOG-1 Assessment

- **90% of Labs using CD117 Also Submit Slides for DOG-1**
- **Same Tissue Sent out as with CD117 (Appendix + GIST + Desmoid)**



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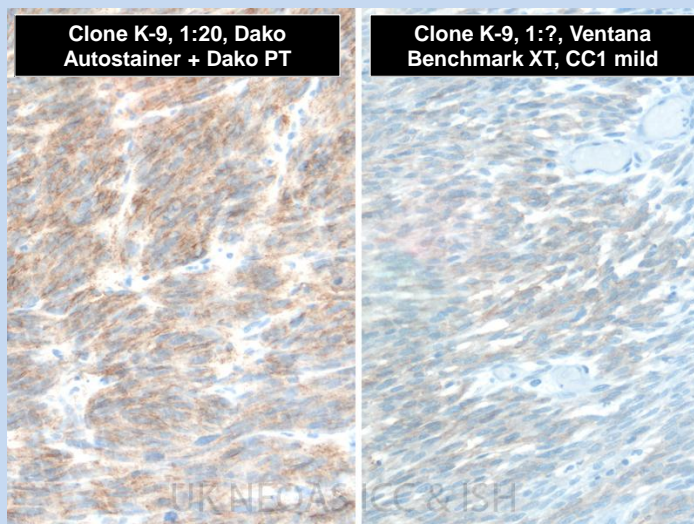
## Expected Level of Staining



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# Poor Level of Staining

Methods Still Need to Be Optimised



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## DOG-1 Pass Rates Run 110 (Scores $\geq 13/20$ )

71% of labs currently use: Leica/NCL (K9) Antibody

**Alimentary Tract Pathology Run: 110**

**Primary Antibody : DOG1**

Antibody Details	N	%
Abcam TMEM16A (ab53212)	1	0
Biocare CM 385 (1.1)	1	100
Cell Marque 244R-14/15/16 (SP31)	3	67
Cell Marque 244R-17/18 (SP31)	3	100
Diagnostic Biosystems Mob466 (DOG1.1)	1	0
Leica NCL-L-DOG-1 (K9)	53	94
Leica PA0219 (K9)	18	100
Menarini MP-385-CM01/1	1	0
Other	3	67
Spring Biosciences M3311 (SP31)	1	100
Thermo RM-9132-R7 (SP31)	1	100
Ventana (SP31) 760-4590	14	93

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# Lymphoma Module CD5 Antibody

## Choice of Antibody Clone and Ideal In-house Control Tissue

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### CD5: Choice of Antibody

Lymphoma Run: 88

Primary Antibody : CD5

Antibody Details	N	%
Biocare CM090B (4C7)	2	100
Biogenex MU430-UC (4C7)	2	50
Cell Marque 760-4280 (SP19)	1	0
Dako FLEX IR081 (SP19)	1	100
Dako IR081 (SP19)	1	100
Dako IS081 (SP19)	1	0
Dako M3633 (SP19)	5	60
<b>Dako M7194 (CD5/54/F8)</b>	<b>18</b>	<b>6</b>
Dako N1618 (CD5/54/F8)	1	0
Labvision MS-393-S (4C7)	1	0
Labvision RB 9008-p (SP19)	1	0
Labvision RM-9119 (SP19)	8	38
Leica Bond RTU PA0188 (4C7)	5	100
NeoMarkers MS-393-S (4C7)	1	100
Novocastra Bond PA0188 (4C7)	2	100
Novocastra NCL-CD5-4C7 (4C7)	66	71
Novocastra NCL-L- CD5-4C7 (4C7)	66	67
Novocastra RTU-CD5-4C7 (4C7)	5	60
Soytek (4C7)	1	0
Vector VP-C322 (4C7)	21	76
Ventana 760-4280 (SP19)	13	69
Ventana CD5 790-4451	2	100

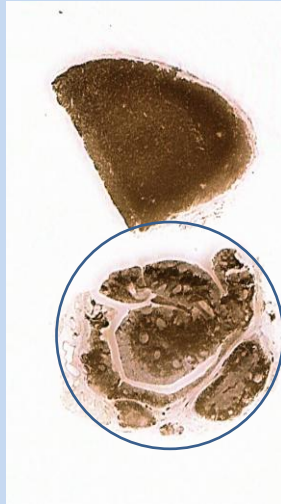
Lymphoma Run: 93

Primary Antibody : CD5

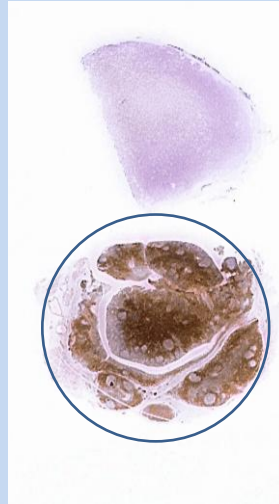
Antibody Details	N	%
Biocare CM090B (4C7)	1	0
Biogenex MU430-UC (4C7)	1	0
Cell Marque 205M/S-x (4C7)	2	50
Cell Marque 760-4280 (SP19)	3	33
Dako FLEX IR081 (SP19)	1	100
Dako IR081 (SP19)	5	40
Dako M3633 (SP19)	6	50
<b>Dako M7194 (CD5/54/F8)</b>	<b>5</b>	<b>20</b>
Labvision RB 9008-p (SP19)	1	100
Labvision RM-9119 (SP19)	3	67
Leica Bond RTU PA0188 (4C7)	8	88
NeoMarkers MS-393-S (4C7)	2	50
Novocastra Bond PA0188 (4C7)	5	100
Novocastra NCL-CD5-4C7 (4C7)	66	77
Novocastra NCL-L- CD5-4C7 (4C7)	75	77
Other	10	40
Spring Bio. M3190 (SP19)	1	100
Vector VP-C322 (4C7)	19	84
Ventana 760-4280 (SP19)	12	75
Ventana CD5 790-4451 (SP19)	11	91

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## Lymphoma Module: CD5 on Tonsil



Leica 4C7 Clone

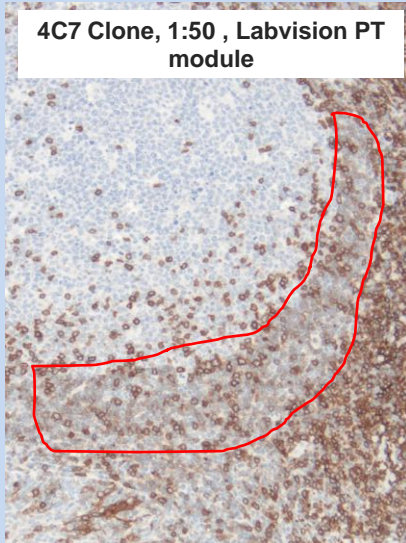


Dako CD5/54/F6  
Clone

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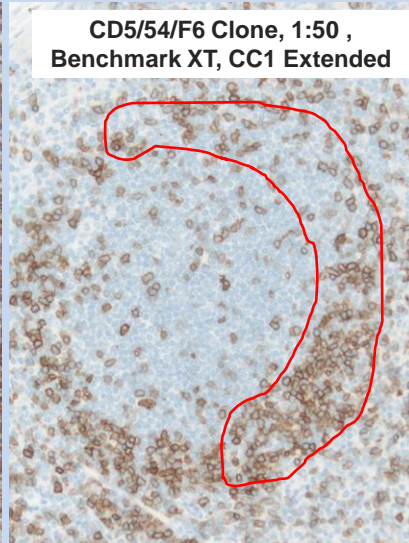
## Lymphoma Module: CD5 on Tonsil

4C7 Clone, 1:50 , Labvision PT  
module



Inter-follicular T cells and B-cells in  
Mantle Zone

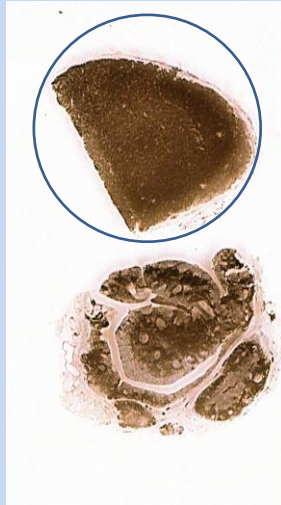
CD5/54/F6 Clone, 1:50 ,  
Benchmark XT, CC1 Extended



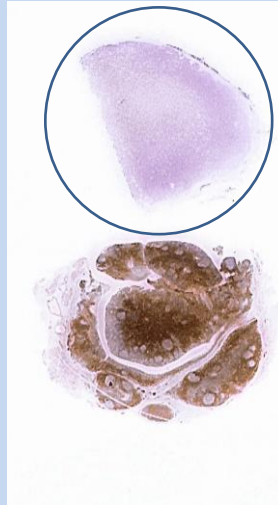
Inter-follicular T cells BUT **Absence**  
**of** B-cells in Mantle Zone

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## Lymphoma Module: CD5 in Mantle Cell Lymphoma



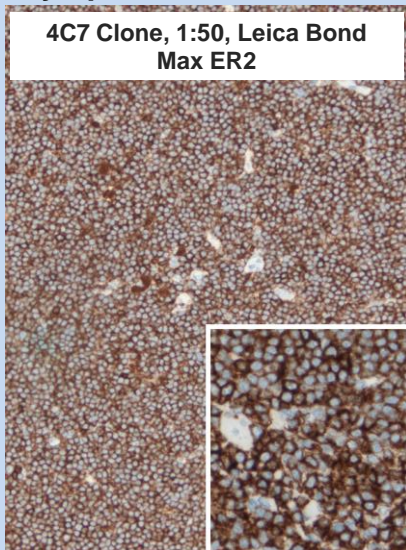
Leica 4C7 Clone



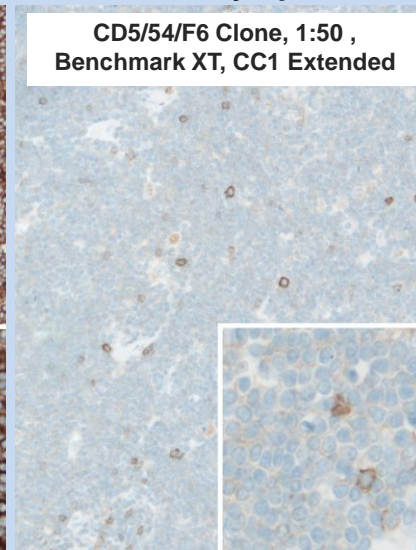
Dako CD5/54/F6 Clone

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## Lymphoma Module: CD5 in Mantle Cell Lymphoma



Expected Level of Staining of Tumour with Strong B-cell staining



Absence of Tumour Staining, with Only a Few T-cells Stained

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## CD5 Summary

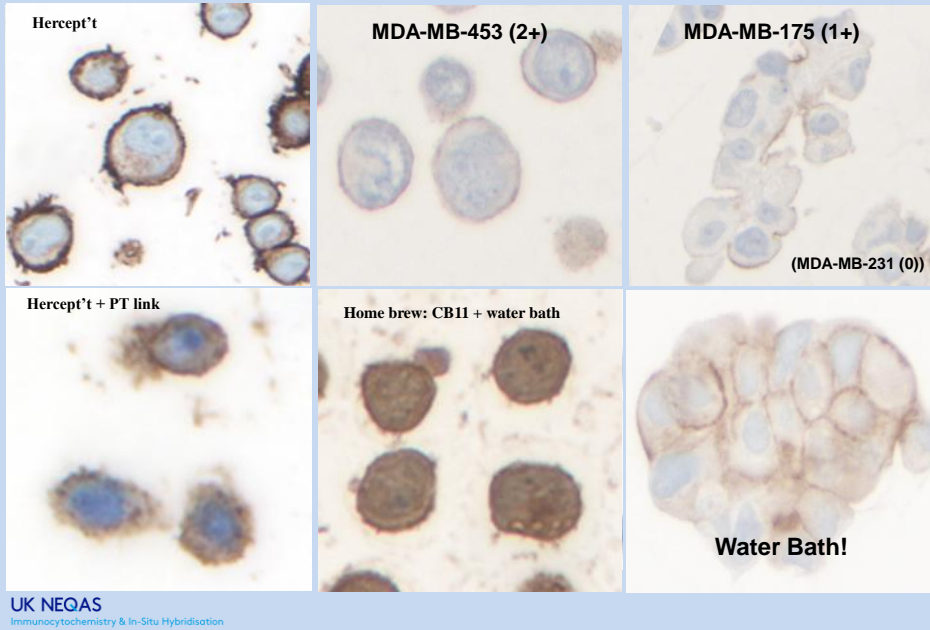
- Labs need to Validate the Antibody and carefully choose their Control Tissue
- Ideal control would be a multi block of both tonsil and MCL
- More recent assessments of CD5 have shown that (**most**) laboratories have changed their working practice and switched to another clone

## Breast HER2 IHC & ISH Module

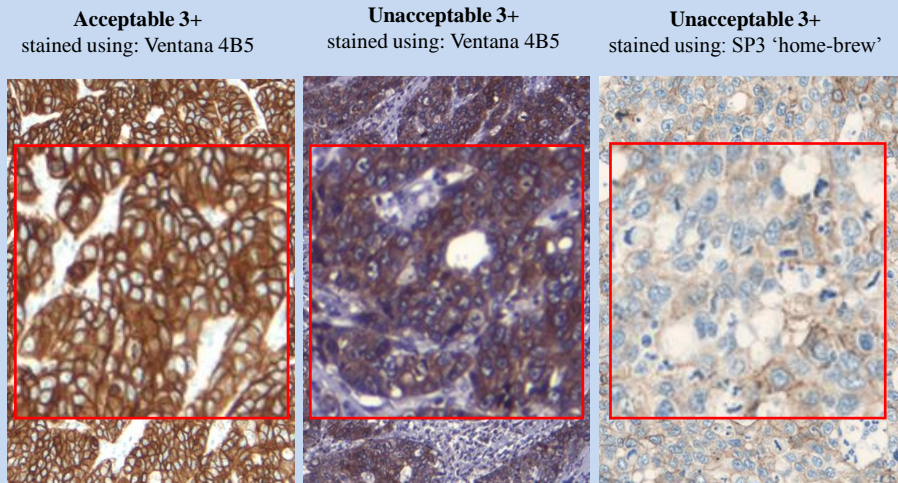
### Ongoing Improvements



## The 'Gold Standard' and The 'Unacceptable'



## Acceptable and Unacceptable Results



## Breast HER2 IHC : Main Methods Run 112- 2016

Breast HER2 ICC Run: 112		Pass Rates	
Primary Antibody	N	%	
Biocare CME 342 A,B (EP1045Y)	4	25	
BioGenex (EP1045Y) rb mono	4	50	
Biogenex AM134-5M (CB11)	3	0	
Cell Marque 237R (SP3)	5	40	
Cell Marque CMA 601 (CB11)	2	0	
Dako A0485 C-erbB-2 (poly)	30	40	
Dako HercepTest K5204 (poly)	4	25	
Dako HercepTest K5205 (poly)	1	0	
Dako HercepTest K5207 (poly)	4	100	
Dako Link HercepTest SK001 (poly)	14	79	
Labvision / Neomarkers RM-9103 (SP3)	7	0	
Leica Oracle HER2 Bond IHC (CB11)	19	79	
Novocastra NCL-L-CB11 (CB11)	10	20	
Novocastra NCL-L-CBE356 (10A7)	1	100	
Novocastra RTU-CB11 (CB11)	1	0	
Other	10	20	
Ventana Confirm 790-4493 (4B5)	39	67	
Ventana Pathway 790-100 (4B5)	11	82	
Ventana Pathway 790-2991 (4B5)	125	76	

Laboratory-derived methods (home-brew) have the lowest EQA pass rates

## EQA of HER2 ISH

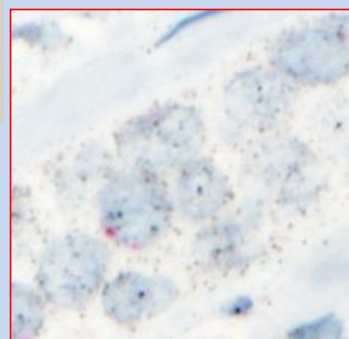


- EQA initially involved interpretation i.e send back 'scores' but no slides

**Lab interpretation is very Good**

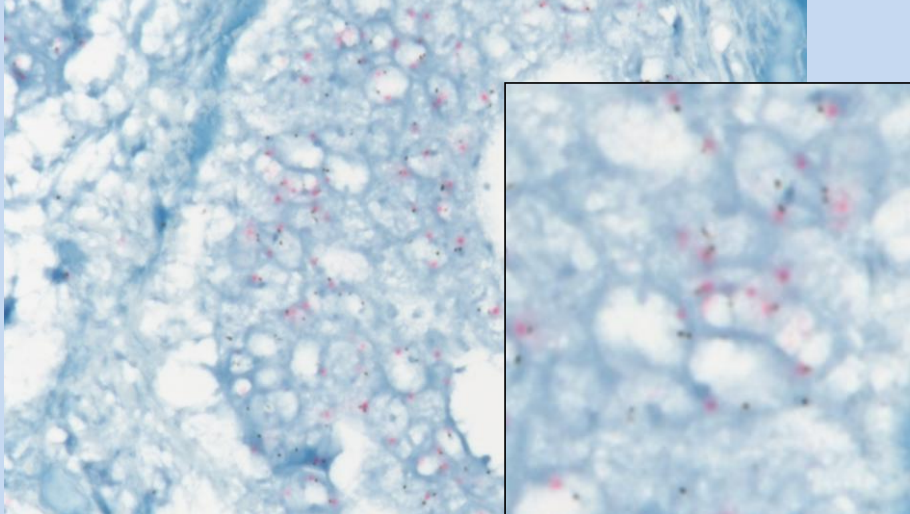
Number of cells counted	Average	Average	Average	Amplified/ Non-Amplified/ Borderline	Score
	HER2 Copy Number	CEP 17 Number	RATIO		
Result 1					
C: 20	1.65	1.4	1.18	Not Amplified	3

Quality of submitted slide



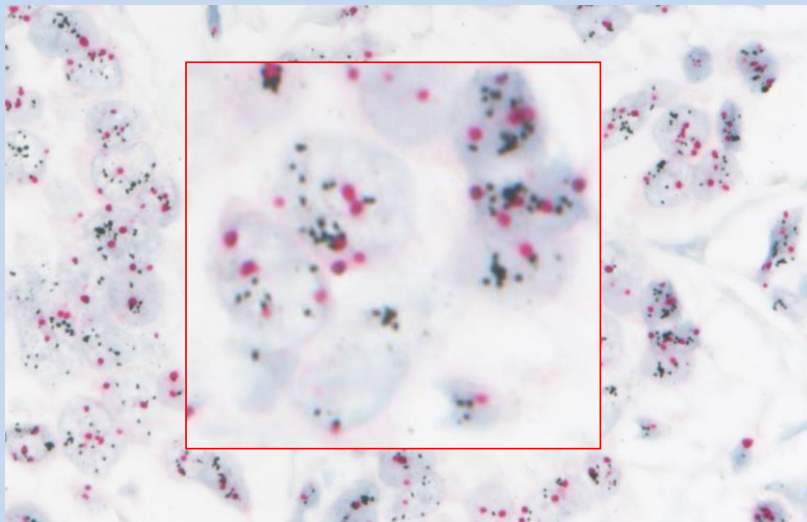
**Technically Not Good... BUT somehow lab has correct 'not amplified' status BUT lots of background HER2 and CEP 17 very low**

**Another Poor Example: Roche/Ventana DDISH  
On 'Non Amplified' NEQAS Tissue Section**



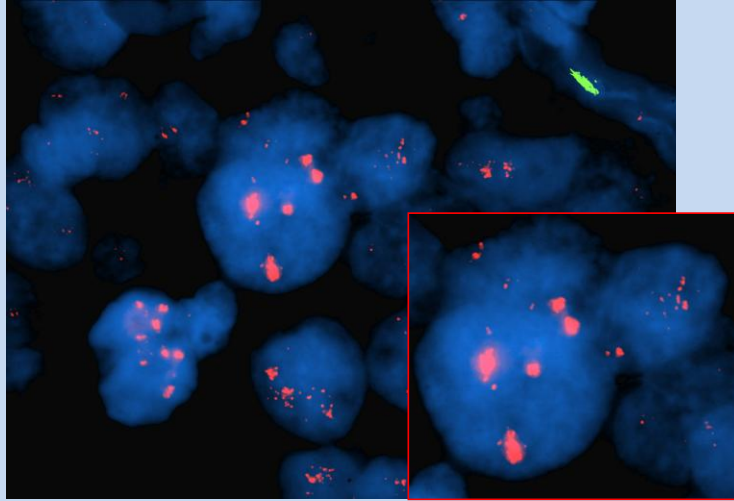
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**Good Example: Roche DDISH  
On 'Amplified' In-House Tissue Control**



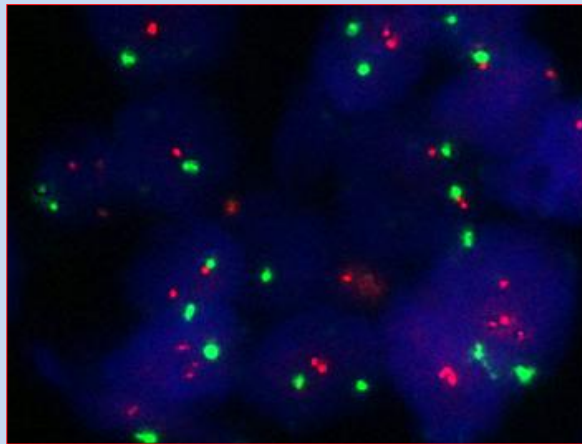
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**FISH on 'Amplified'  
(IHC 2+) NEQAS Cell line**



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**What We Should See!**



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# UK NEQAS ICC & ISH Website

**BREAKING NEWS: We are pleased to announce that UK NEQAS ICC & ISH is now ISO:17043 Accredited**  
See the [Downloads](#) and [News](#) pages!

UK NEQAS International Quality Expertise  
Immunocytochemistry & In-Situ Hybridisation

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## THE UK NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME FOR IMMUNOCYTOCHEMISTRY AND IN SITU HYBRIDISATION (ICC & ISH)

30 YEARS OF EXPERTISE

The External Quality Assessment Scheme for Immunocytochemistry was founded in 1985 by Mr Gerry Reynolds at Mount Vernon Hospital.

- Four assessment runs per year
- Specific modules catering for the specialised

### ASSESSMENT DATES

MEETINGS & WORKSHOPS

ASK A QUICK QUESTION

Your Name:   
Email:

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# UK NEQAS ICC & ISH Website

**РАЗРУШАЮЩАЯ НОВОСТИ: Мы рады сообщить, что Великобритания NEQAS ICC & ISH теперь ISO: 17043 Аккредитованный**  
Смотрите [Загрузки](#) и [Новостные](#) страницы!

UK NEQAS International Quality Expertise  
Immunocytochemistry & In-Situ Hybridisation

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Главная | Около | Модули | Журналы | Руководство участника | Календарь событий | Специализация Даты | Спонсоры | Загрузки | Вопросы и ответы

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## UK NATIONAL ВНЕШНЕЕ ОЦЕНКА КАЧЕСТВА СХЕМА ИММУНОЦИТОХИМИЮ И IN SITU ГИБРИДИЗАЦИЯ (ICC & ISH)

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### ASSESSMENT СПОКИ

ВСТРЕЧИ & МАСТЕРСКИЕ

ASK A QUICK QUESTION

Your Name:   
Email:

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Immunocytochemistry & In-Situ Hybridisation



**Searchable  
'Best  
Methods'  
Database**

**СДЕЛАЙТЕ СВОЙ ВЫБОР НИЖЕ** каждой формы поле / области ниже будут отображаться соответствующие параметры на основе первого выбора

1 \* Область Патологии: грудь

2 \* Первичное антигено: ER

3 \* Антигено Постажи: Dako (EP1) M3643

4 \* Автоматизация Методика: Dako Autostainer Ссылка 48

5 \* Обнаружение Kit или 2-го цвета: Dako EnVision FLEX + (K8002 / 12)

**ВАШИ ЛУЧШИЕ МЕТОДЫ РЕЗУЛЬТАТЫ**

Способ 1

Первичное антигено: Dako (EP1) M3643, 1:50, 20 мин Автоматизация: Dako Autostainer Ссылка 48 Heat Антиген Retrieval: Dako FTLink 20 минут Фермент Антиген Retrieval: Не применяемо Обнаружение: Dako EnVision FLEX + (K8002 / 12), 1:50, 20 минут хромогена: Dako FLEX DAB, 20 минут

ЭЛ. АДРЕС    РАСПЕЧАТАТЬ    ДОЛЯ    f    t    G+    in

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## UK NEQAS Workshop Meetings

### Bangkok



# Thank You

