UK NEQAS
International Quality Expertise

Immunocytochemistry
& In-Situ Hybridisation

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

Suzanne Parry

22nd April September 2016

Outline Of Talk

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

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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'

UKAS PROFICIENCY TESTING

Proficiency Testing Provider
No. 7833

UK NEQAS Headquarters




Assessments are a team effort: Scientists and Pathologists

ASSESSORS

United Kingdom
Mr C Abbott, Bristol
Dr N Atkey, Sheffield
Dr M Arends, Cambridge
Dr M Ashton-Key, Southampton
Mrs J Bell, Nottingham
Mr N Bilbe, London
Mr D Blythe, Leeds
Mr J Brown, London
Dr L Carson, Aberdeen
Ms E Clark, Surrey
Mrs A Clayton, Preston
Mrs A Cramer, Manchester
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Mr A Dodson, Liverpool
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Mr R Fincham, Cambridge
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Mrs J Brincat, Victoria

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Prof. J Bartlett, Toronto

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Mr J Askaa, Copenhagen
Dr E Baslev, Herlev
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Hungary
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Prof E Kaye, Dublin
Mr K McAllister, Dublin
Dr T O'Grady, Dublin
Ms Yvonne Connolly, Dublin
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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

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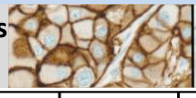
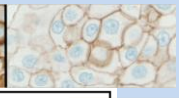
Breast HER2 IHC: Scoring system + Reports (From Run 106 – June 2014)





	Assessor1				Assessor2				Assessor3				Assesso			
Individual Assessor Scores	4				4				4				4			
Cell line / tissue	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	
ASSESSOR MEMBRANE SCORE	3+	2+	1+	0	3+	2+	1+	0	3+	2+	1+	0	3+	2+	1+	
Excellent standard of staining																
1+ stronger than expected																
Innapropriate/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

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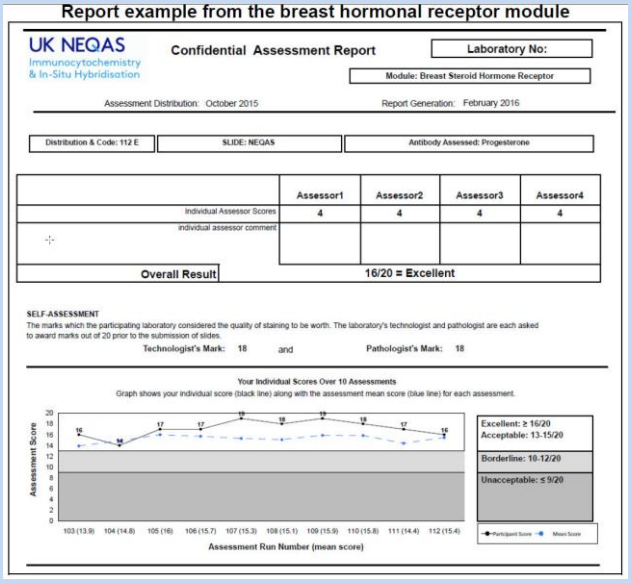
UK NEQAS ICC & ISH Reports

Report example from the breast hormonal receptor module



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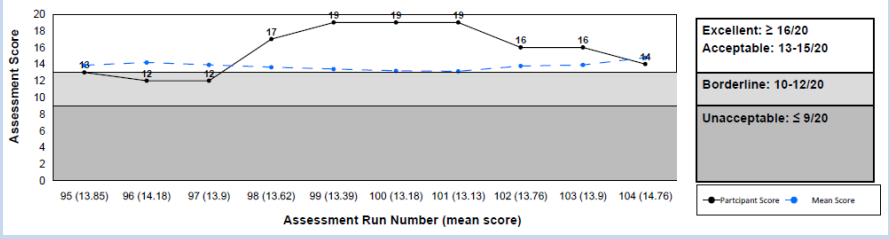
2.5 Year Rolling Performance Graphs

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



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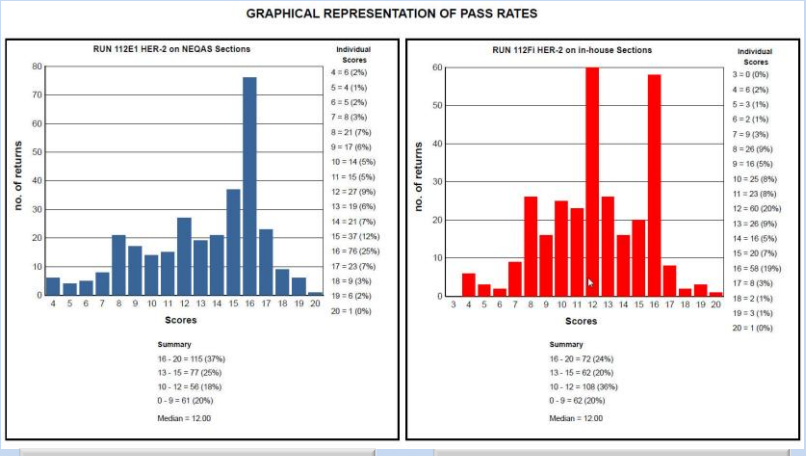
Breast ER IHC – Run 112 all participants

NEQAS Samples

In-house Samples



20% of labs still show unacceptable staining

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

End of Year Participation Certificate


UK NEQAS ICC & ISH
For Immunocytochemistry and In Situ Hybridisation
CPA
Accredited EQA Scheme No: 044

Certificate of Participation

This is to certify that

has participated in UK NEQAS ICC & ISH during the period April 2013 to March 2014

This certificate is not an indication of the overall performance of the participating laboratory, and as such is not an endorsement of the quality of staining produced

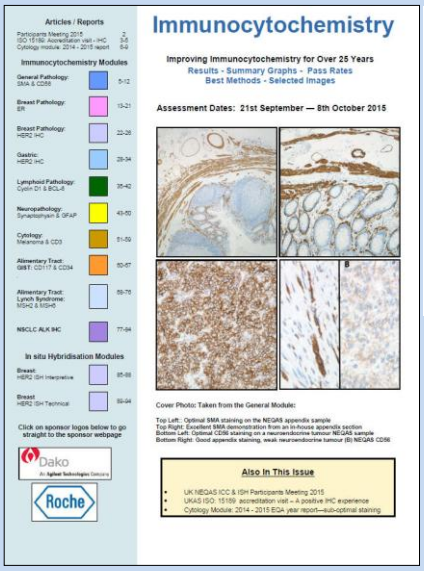
Mr Keith Miller (Scheme Director)

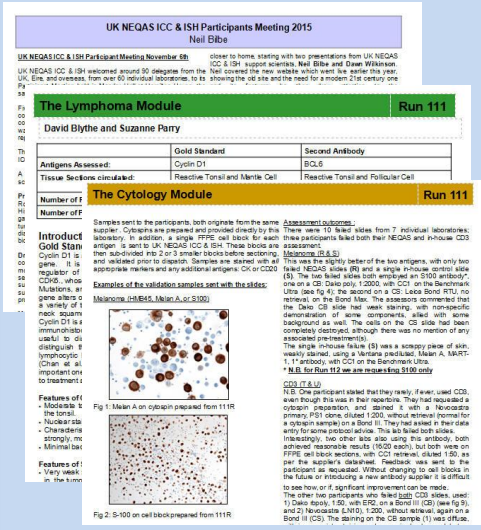
Dr Merdol Ibrahim (Scheme Manager)

UK NEQAS ICC & ISH 2013 - 2014

- 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

UK NEQAS ICC & ISH E-Journal





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'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

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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

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Alimentary Tract Module
CD117 and DOG-1 Antibodies

Sensitivity & Specificity

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Alimentary Tract CD117 (GIST) Module

Appendix + GIST + Desmoid

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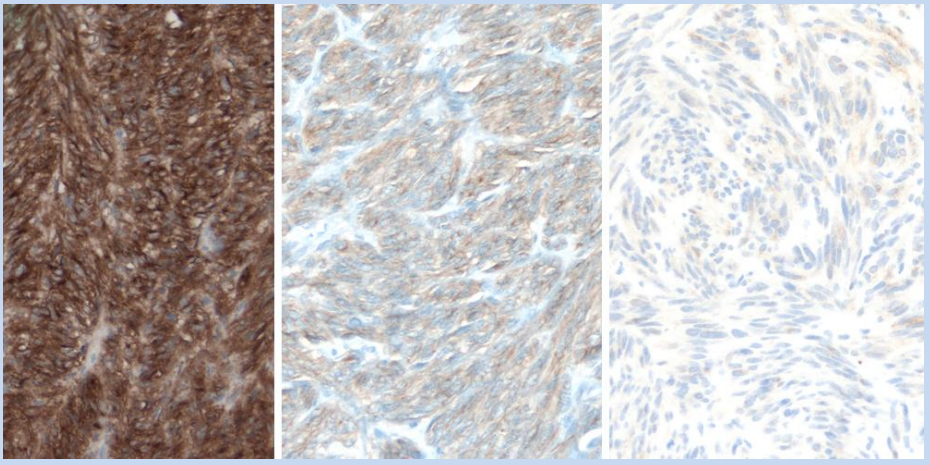
GIST: Main Reason for Poor NEQAS Scores Due to Lower Sensitivity in Staining

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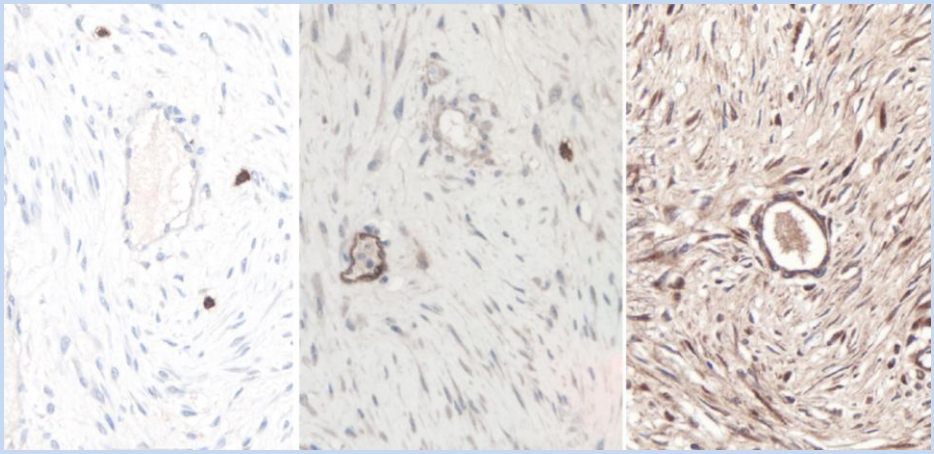
Desmoid as a Negative Control: Can Help to Gauge Non-specific Staining

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 ≥ 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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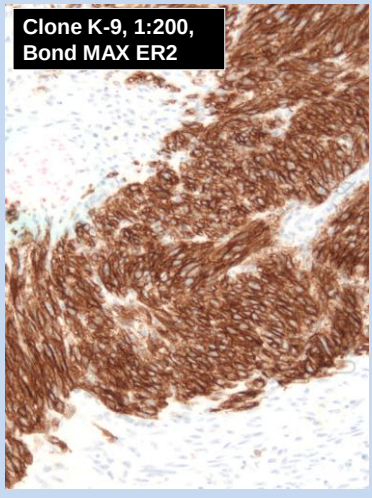
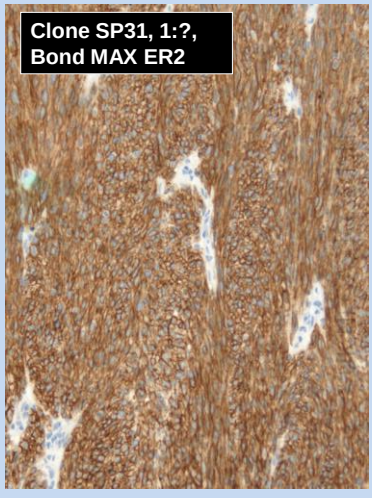
DOG-1 Assessment

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

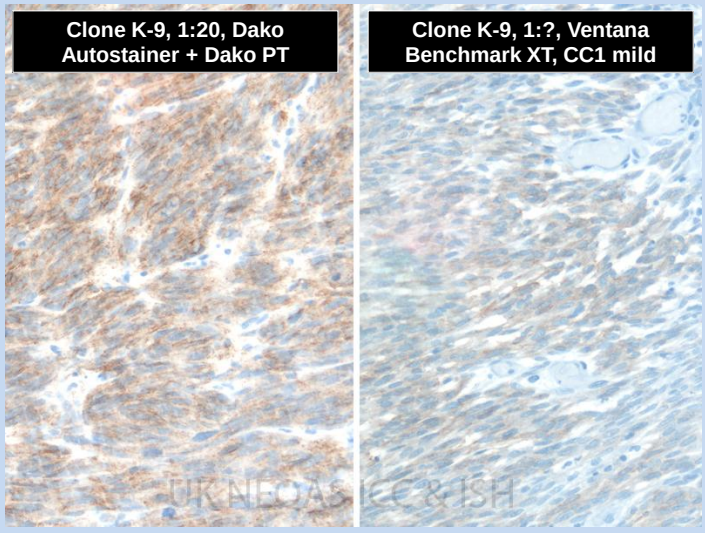
Expected Level of Staining





Poor Level of Staining

Methods Still Need to Be Optimised



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DOG-1 Pass Rates Run 110 (Scores ≥ 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 CM099B (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
Dako M7194 (CD5/54/F6)	18	6
Dako N1618 (CD5/54/F6)	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 PA0168 (4C7)	5	100
NeoMarkers MS-393-S (4C7)	1	100
Novocastra Bond PA0168 (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
Scytek (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 CM099B (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
Dako M7194 (CD5/54/F6)	5	20
Labvision RB 9008-p (SP19)	1	100
Labvision RM-9119 (SP19)	3	67
Leica Bond RTU PA0168 (4C7)	8	88
NeoMarkers MS-393-S (4C7)	2	50
Novocastra Bond PA0168 (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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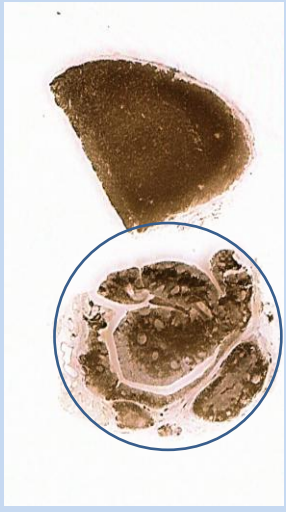
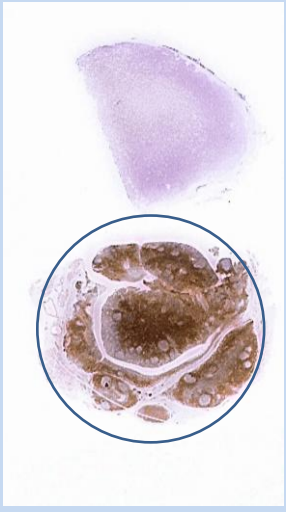
Lymphoma Module: CD5 on Tonsil



Leica 4C7 Clone



Dako CD5/54/F6 Clone

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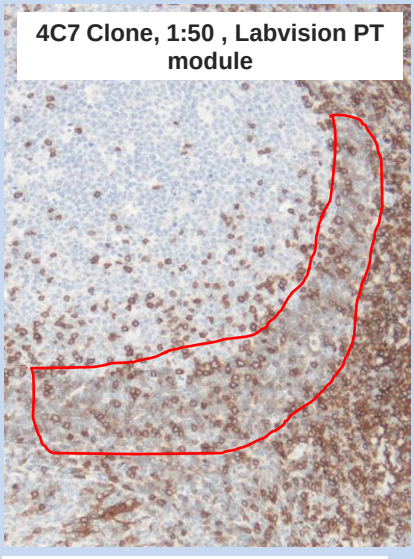
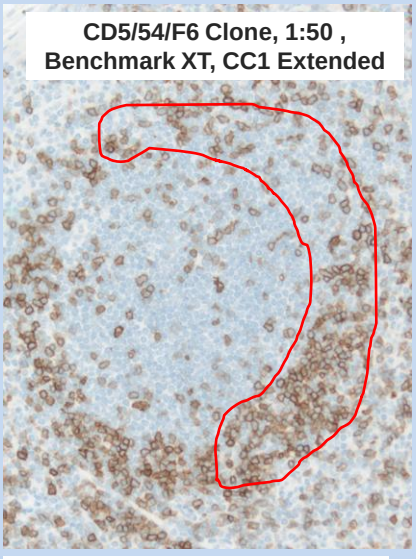
Lymphoma Module: CD5 on Tonsil

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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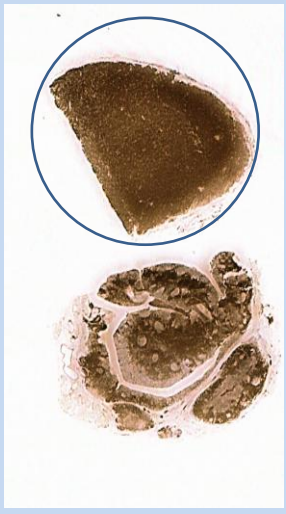
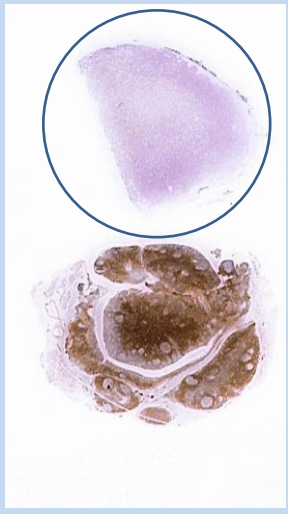
Lymphoma Module: CD5 in Mantle Cell Lymphoma



Leica 4C7 Clone



Dako CD5/54/F6 Clone

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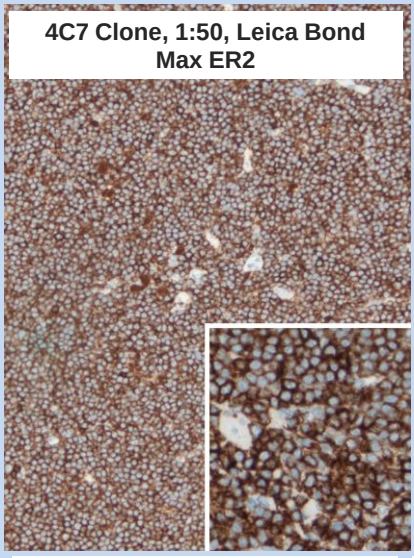
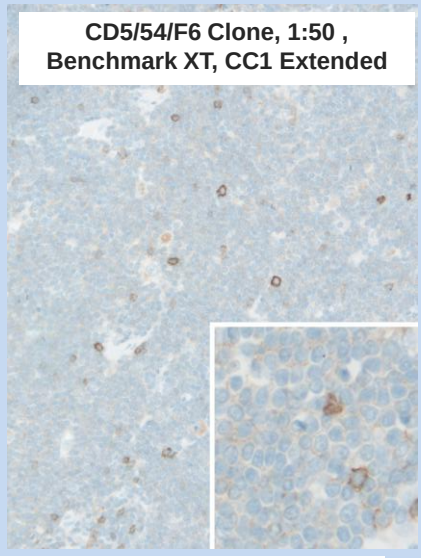
Lymphoma Module: CD5 in Mantle Cell Lymphoma

4C7 Clone, 1:50, Leica Bond Max ER2



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

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



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

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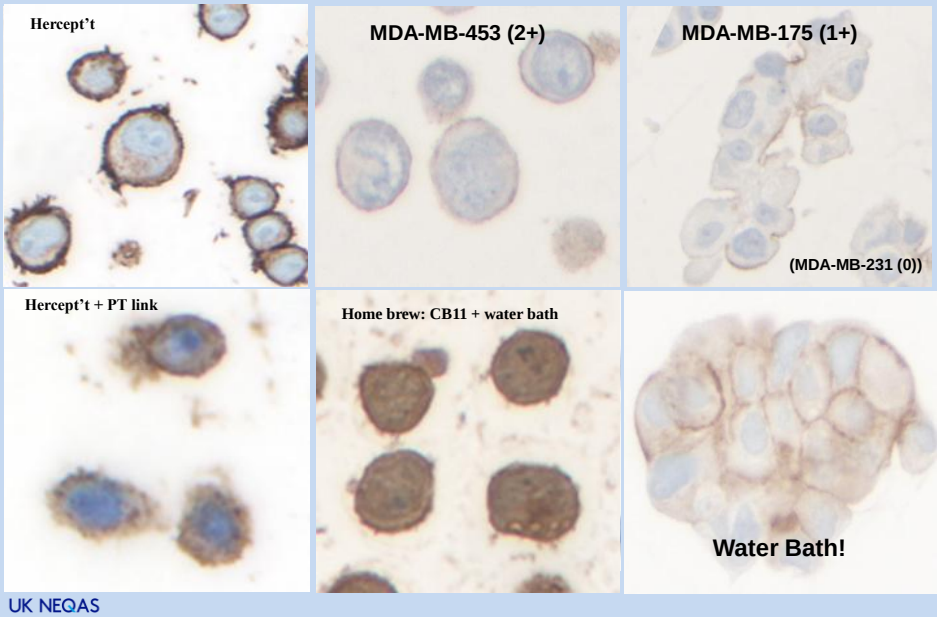
CD5 Summary

- 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’



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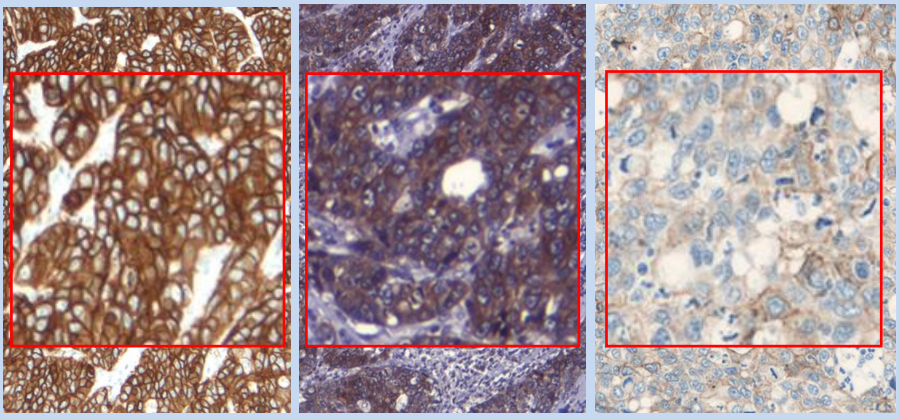
Acceptable and Unacceptable Results

Acceptable 3+
stained using: Ventana 4B5

Unacceptable 3+
stained using: Ventana 4B5

Unacceptable 3+
stained using: SP3 ‘home-brew’



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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-erB-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

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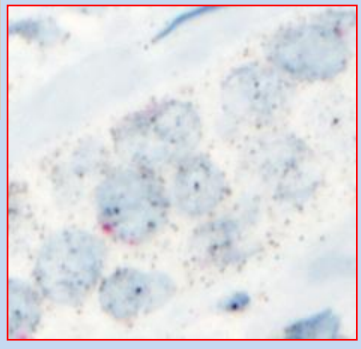
EQA of HER2 ISH

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

Lab interpretation is very Good

	Number of cells counted	Average HER2 Copy Number	Average CEP 17 Number	Average RATIO	Amplified/ Non-Amplified/ Borderline	Score
	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

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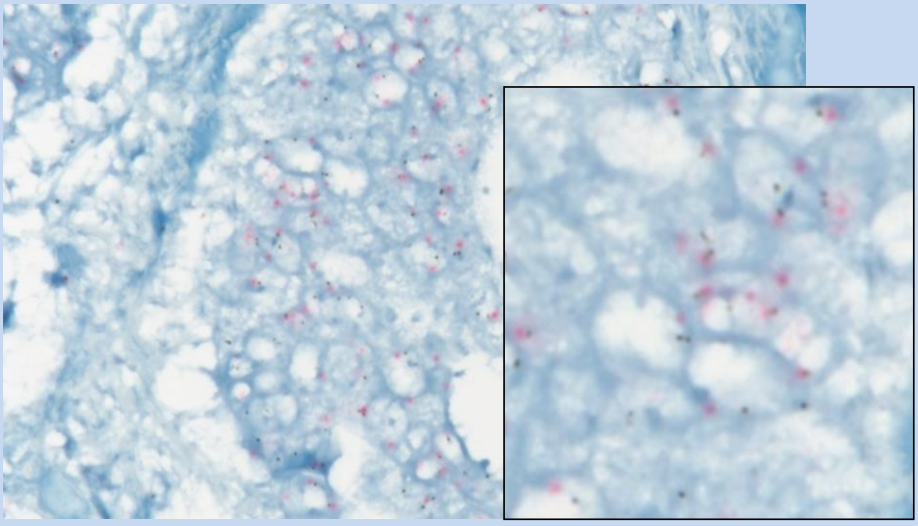
Another Poor Example: Roche/Ventana DDISH On 'Non Amplified' NEQAS Tissue Section



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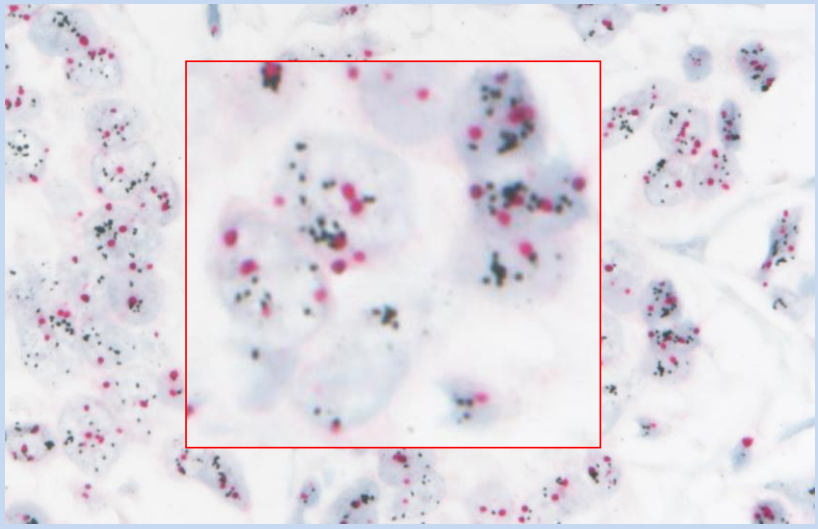
Good Example: Roche DDISH On 'Amplified' In-House Tissue Control



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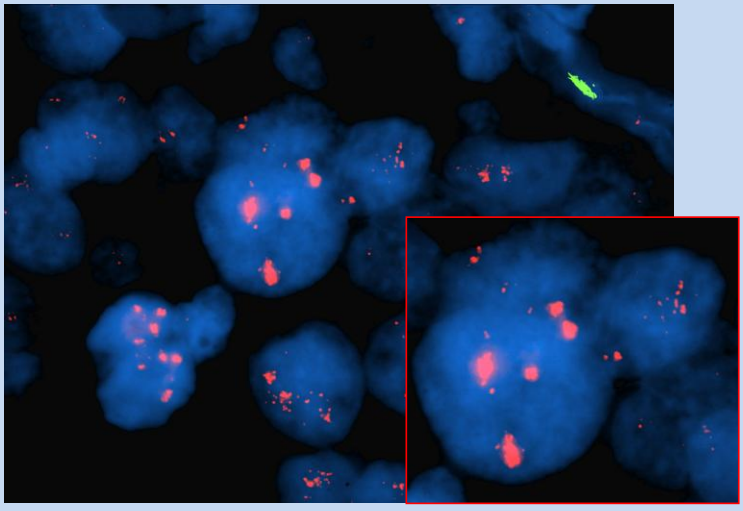
FISH on 'Amplified'
(IHC 2+) NEQAS Cell line



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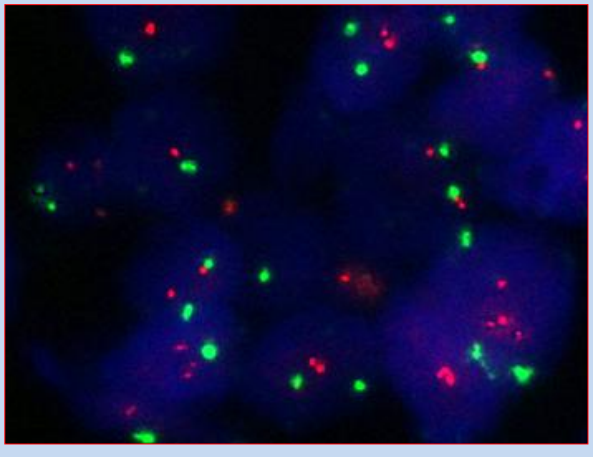
What We Should See!



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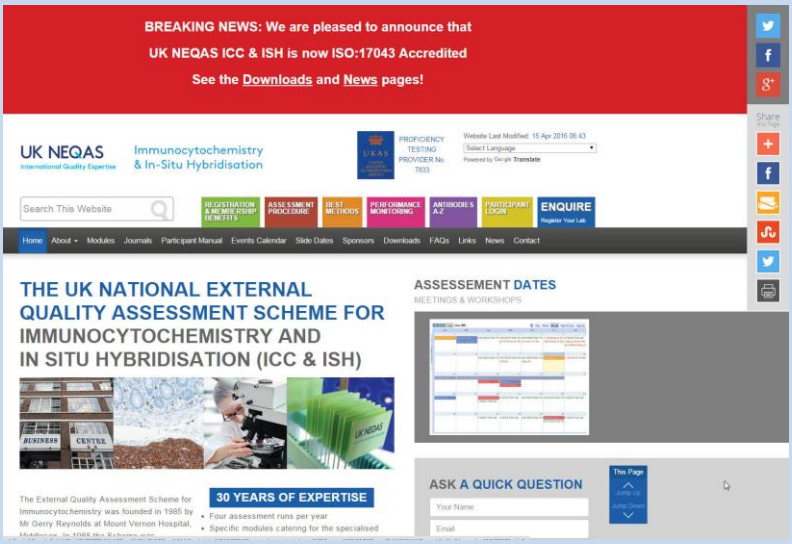
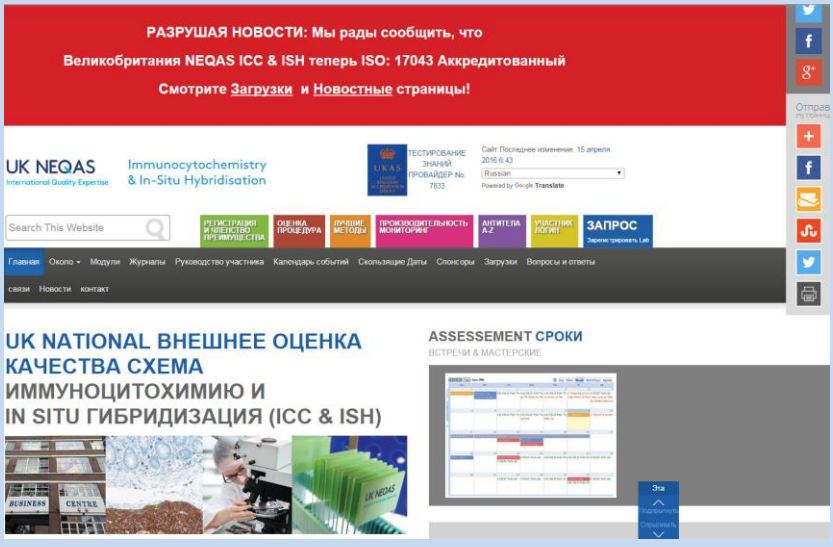
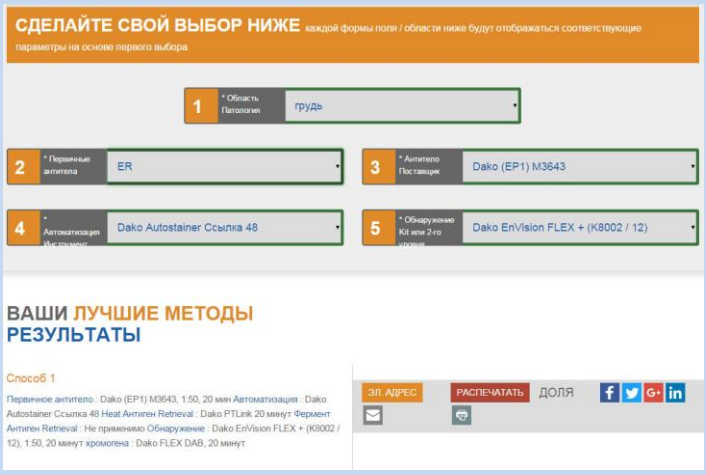
UK NEQAS ICC & ISH Website



UK NEQAS ICC & ISH Website



Searchable 'Best Methods' Database



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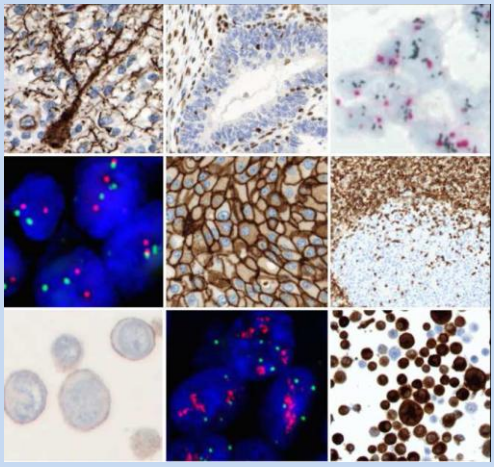
UK NEQAS Workshop Meetings

Bangkok

Thank You



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