For Pathologists

Clinical question 2: What strategies can help ensure optimal performance, interpretation and reporting of established assays in patients with gastroesophageal adenocarcinoma?

1. What are the acceptable methodologies for HER2 IHC (different antibodies) and FISH/ISH/CISH (different probes platforms)?

2. What are the steps/procedures needed to analytically validate a laboratory developed HER2 gastroesophageal adenocarcinoma assay before reporting results on patient samples? (Refer to CAP's IHC Validation Guidelines, CAP molecular guideline, CAP FISH guideline)
   a. Should different validation be performed in gastroesophageal adenocarcinoma and breast specimen?

Table 1: Patient and disease characteristics

Refid	Bibliography	Year	Study Design	Location of study	N of participant	N of specimens	Age			Gender				Specimen Type	WHO/Lauren/ Both	Tumor Stage	Dx Addressed
							Mean/Median	Std dev	Range	N Male	% male	N female	% female				
2	Kinugasa	2015	Prospective cohort	Asia	25	NA/NR	66	NR	29-81	20	80	5	20	Biopsy from primary tumor, serum	Intestinal, Diffuse	Stage III - IV	Primary
74	Kim	2014	Prospective-Restrospective	Asia	89	NA/NR	53	NA/NR	NA/NR	58	65	31	35	NR	Intestinal, Diffuse, Mixed	Stage I - IV	Primary, Recurrent or persistent disease, Metastasis
193	Sheffield	2014	Prospective-Restrospective	Multiple countries	28	28	64.3	NA/NR	42-89	21	79	6	21	Resection	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
228	Kimura	2014	Retrospective cohort	Asia	198	198	NA/NR	NA/NR	NA/NR	128	65	70	35	Resection	Intestinal, Diffuse	Stage I - IV	Primary
257	Tajiri	2014	Prospective cohort	Asia	475	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NR	Papillary adenocarcinoma, Tubular adenocarcinoma, Mucinous adenocarcinoma, Other poorly cohesive carcinoma, Mixed carcinoma	NA/NR	Primary

334	Grin	2013	Prospective-Restrospective	Canada	50	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Mixed	NR	Primary
340	Abrahao-Machado	2013	Prospective-Restrospective	Brazil	199	NA/NR	60.7	NA/NR	27-87	123	62	76	83	NR	Intestinal, Diffuse, Mixed	NR	Primary
356	Pala	2013	Retrospective cohort	Europe	88	NA/NR	61.2	NA/NR	29-81	NA/NR	NA/NR	NA/NR	NA/NR	NR	Papillary adenocarcinoma, Tubular adenocarcinoma, Mucinous adenocarcinoma, Other poorly cohesive carcinoma, Mixed carcinoma	Stage I - IV	Primary
493	Hirschmann	2012	Prospective-Restrospective	Europe	NA/NR	82 breast carcinomas (28 FISH-positive, 2 borderline cases, and 20 cases with difficult previous FISH), 14 samples of normal breast tissue, 25 gastric carcinomas (6 FISH-positi	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	TMA	NA/NR	NR	NA/NR

						ve), and 24 samples of normal gastric mucosa											
498	Kiyose	2012	Prospective-Restrospective	Asia		125 BC and 198 GC	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	TMA	NA/NR	NR	Primary
501	Fassan	2012	Prospective-Restrospective	Europe	NA/NR	275	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Biopsy from primary tumor, Resection	NA/NR	NR	Primary
563	Cho	2012	Prospective cohort	United States	289	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	TMA	NA/NR	NR	NA/NR
565	Fox	2012	Prospective cohort	Australia	NA/NR	100	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Resection	NA/NR	NR	Primary
566	Radu	2012	Prospective-Restrospective	United States	103	NA/NR	Gp1: 65 Gp2: 62	Gp1: 12 Gp2: 10	NA/NR	88	85	15	15	Resection, Tissue from metastatic site	NA/NR	Stage I - IV	Primary, Metastasis
579	Park	2012	Prospective-Restrospective	Asia	1091	NA/NR	55	NA/NR	20-70	738	68	353	32	TMA	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
585	Mrklic	2012	Prospective-Restrospective	Europe	73	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Mixed	NR	Primary
588	Yoon	2012	Prospective-Restrospective	United States	713		63.8	NA/NR	NA/NR	633	89	80	11	Resection	NA/NR	Stage I - IV	Primary
590	Yang	2012	Prospective-Restrospective	Asia	148	265	59	NA/NR	31-89	119	80	29	20	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
623	Tafe	2011	Prospective-Restrospective	United States	135	NA/NR	NA/NR	NA/NR	22-90	103	76	32	24	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Indeterminate, Mixed	NA/NR	Primary, Recurrent or persistent disease, Metastasis
633	Kim	2011	Prospective-Restrospective	Asia	1414 (series A); 615		Gp1: 58 Gp2: 58		Gp1: 25-87 Gp2: 23-89	955 (A); 414 (B)	68 (A); 67 (B)	461 (A); 201 (B)	32 (A); 33 (B)	Resection, TMA	Intestinal, Diffuse	Stage I - IV	Primary, Metastasis

					(series B)												
639	Choritz	2011	Prospective cohort	Multiple countries	NA/NR	42 institutions (10,916 breast results); 15 institutions (982 gastric results)	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
653	Garcia-Garcia	2011	Prospective-Restrospective	Europe	166	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Biopsy from primary tumor, Resection, Tissue from metastatic site	Intestinal, Diffuse, Indeterminate	NR	Primary, Metastasis
660	Langer	2011	Prospective-Restrospective	Europe	142	NA/NR	64	NA/NR	33-83	130	92	12	8	Resection	NA/NR	Stage I - IV	Primary
661	Hu	2011	Prospective-Restrospective	United States	116	NA/NR	65	NA/NR	Gp1: 34-85 Gp2: 43-88	104	90	12	10	Resection	NA/NR	NR	Primary
666	Thompson	2011	Prospective-Restrospective	Australia	89	NA/NR	63.9	NA/NR		74	83	15	17	Resection	NA/NR	Stage I - IV	
675	Im	2011	Prospective-Restrospective	Asia	142	NA/NR	52	NA/NR	15-72	96	68	46	32	Resection	Signet-ring cell carcinoma, Mixed, Intestinal, Diffuse	Stage III - IV	
686	Yan	2011	Prospective-Restrospective	Asia	145	NA/NR	60	NA/NR	NA/NR	95	65	50	35	Resection	Intestinal, Diffuse, Indeterminate	Stage I - IV	
690	Moelans	2011	Prospective-Restrospective	Multiple countries	199	NA/NR	Gp1: 35 Gp2: 68		Gp1: 21-45 Gp2: 47-86	NA/NR	NA/NR	NA/NR	NA/NR	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Mixed	Stage I - IV	Primary

694	Boers	2011	Prospective-Restrospective	Europe	146	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Mixed	NR	Primary
721	Schoppmann	2010	Prospective-Restrospective	Europe	189	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Biopsy from primary tumor, Resection	NA/NR	Stage I - IV	Primary
736	Yan	2010	Prospective-Restrospective	Asia	NA/NR	128	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Resection	Intestinal, Diffuse, Mixed	NR	NA/NR
806	Marx	2009	Prospective-Restrospective	Europe	166	NA/NR	67	NA/NR	28-93	117	70	49	30	Resection, Tissue from metastatic site	Intestinal, Diffuse, Mixed	Stage I - IV	Primary, Metastasis
913	Sekaran	2012	Prospective cohort	Asia	52	NA/NR	55	NA/NR	24-80	34	65	18	35	Resection	Intestinal, Diffuse	Stage I - IV	Primary

Table 2: Test Characteristics

Refid	First Author	Year	Methodology	Neg/0	1+	2+	3+	Amplified	Non-amplified	HER 2 SCORING METHODS	Her2 Result reporting structure	Heterogeneity
2	Kinugasa	2015	IHC/ISH/FISH	17	NR	NR	8	8	17	Ruschoff	NR	NR
74	Kim	2014	ISH/FISH	NR	NR	NR	NR	8	NR		NR	NR
193	Sheffield	2014	IHC/ISH	NR	NR	NR	NR	NR	NR	Toga	NR	NR
228	Kimura	2014	IHC/ISH/FISH	101	48	28	21	12	27	Toga	NR	NR
257	Tajiri	2014	IHC/ISH/FISH	NR	NR	NR	NR	51	424	dako HercepTest recommendations for IHC. FISH scored according to ASCO/CAP guidelines (more than 6 gene copies per nucleus or gene signal/centromere signals >2.2	NR	Intratumoral heterogeneity of ERBB2 amplification, defined as less than 50% of cancer cells positive for ERBB2 amplification, was found in 41% (21/51) of ERBB2-amplified tumors

334	Grin	2013	IHC/DISH/FISH	21	12	10	7	FISH: 6; DFISH: 7	FISH: 44; DFISH: 43	Toga	NR	Tumor heterogeneity was observed in 7 of 9 (78%) HER2-positive cases. The 2 nonheterogenous cases showed diffuse 3+ staining by IHC and were both diffusely highly amplified (HER2:CEP17 ratios >10).
340	Abrahao-Machado	2013	IHC/DISH	varied with antibody; 125, 128, 179	30, 17, 7	20, 34,1	23,18,10 (Table1 in article)			Hofmann		Intratumoral heterogeneity of HER2 expression was observed with all antibodies. # of positive cases was lower in TMA than in whole sections for each antibody tested.
356	Pala	2013	IHC/ISH/FISH/SISH	71	2	3	12	S:18; F:15	S:70; F:72	Hofmann ISH- amplified if HER2/cen17 ratio greater than or equal to 2 within 20 tumor cell nuclei (ToGA)	NR	NR
493	Hirschmann	2012	IHC/ISH/FISH/SISH	NR	NR	NR	NR	NR	NR	ASCO guideline	For IHC, specimens scored as 0 or 1+ were classified as negative and specimens scored as 2+ or 3+ were classified as positive. Gene status was classified as amplified if the SISH or FISH HER2/Chr17 ratio was 2 or more and classified as not amplified if the ratio was less than 2. In some cases, the ISH result was recorded as “cluster” or “focal positivity,” which were both classified as amplified	NR
498	Kiyose	2012	IHC/ISH/FISH/CISH	140	8	13	37	F: 50; C: 52	F: 148; C: 146	HercepTestTM kit guide and Pathvysion HER2 DNA probe kit	a score of 0 or 1+ was considered negative, a score of 2+ was considered weakly positive, and a score of 3+ was considered strongly positive.	

501	Fassan	2012	IHC/ISH/SISH	NR	NR	NR	NR	NR	NR	HER2 expression was scored according to the four-tiered Herceptest, as modified for gastric adenocarcinoma as follows:13,18 score 0 (negative), absence of any stain or membrane staining; score 1+ (negative), tumour cell cluster with faint or barely perceptible membrane reactivity irrespective of percentage of tumour cells stained; score 2+ (equivocal), tumour cell cluster with weak to moderate (complete, lateral, or basolateral) reactivity irrespective of percentage of tumour cells stained; score 3+ (positive), tumour cell cluster with moderate to strong (complete, lateral, or basolateral) reactivity irrespective of percentage of tumour cells stained. For scoring purposes, any (nuclear or cytoplasmic) background staining was disregarded. SISH: Only nuclei with a distinct nuclear border were considered; overlapping nuclei were always excluded. The ratio of HER2 to CEP was calculated, and HER2 was considered to be amplified when the ratio of gene-specific HER2 to CEP signals was ‡2.0, or when there was evidence of HER2 signal clusters.	NR	Intratumour heterogeneity was documented in both GC and BAc (using both IHC protocols). The rate of HER2 amplification (using SISH) increased significantly along with IEN dedifferentiation (P <0.001).

563	Cho	2012	IHC/ISH/FISH	Hercep Test: 249; A0485: 243; 4B5: 249; CB11: 262		Hercep Test: 18; A0485: 22; 4B5: 14; CB11: 6	HercepTest: 22; A0485: 24; 4B5: 26; CB11: 21	38	251	Hofmann	A score of 0 or 1+ was considered negative while 2+ and 3+ were considered positive. HER2 gene was considered amplified when HER2/CEP17 was > 2.0	NR
565	Fox	2012	IHC/ISH/FISH/CISH/SISH	NR	NR	NR	NR	NR	NR	Hofmann & Ruschoff	Each IHC comparison was assessed using 2 cutoff points for scoring positivity [IHC3+ = positive, and IHC2+ or IHC3+ (ie, IHC2+/3+) = positive]	NR
566	Radu	2012	IHC/ISH/FISH					30	73	Hofmann & Ruschoff	NR	NR
579	Park	2012	IHC/ISH/FISH/SISH	Hercep Test: 917 ;Pathway: 803	HercepTest: 50 ;Pathway: 137	Hercep Test: 29 ;Pathway: 51	HercepTest: 63 ;Pathway: 68	F: 71; S: 70	F: 517; S: 518	Hofmann	Cases with scores of 2+ or 3+ were considered positive for HER2 overexpression	NR
585	Mrklic	2012	IHC/ISH/CISH	51	9	6	7	10	63	Hofmann	NR	NR
588	Yoon	2012	IHC/ISH/FISH	93	NR	167	84	108	236	Hofmann	A case was considered HER2 positive if it was (i) IHC3+ or (ii) IHC2+ plus gene-amplified (4). Remaining cases (i.e., nonamplified IHC2+ or IHC0-1+) were considered HER2 negative	NR

590	Yang	2012	IHC/ISH/FISH	Biopsy: 125; Resect: 93	NR	Biopsy: 7; Resect: 5	Biopsy: 16; Resect: 19	Biopsy: 18; Resect: 22	Biopsy: 16; Resect: 8	Hofmann	NR	The intratumoral heterogeneity was defined as detection of areas showing different HER2 staining scores in IHC or HER2 gene amplification score in FISH (Fig. 1). Heterogeneous staining was demonstrated in 23 of 29 (79.3%) HER2- positive cases detected by IHC. Further, heterogeneity of HER2 at genetic level was observed in 11/25 (44.0%) FISH positive cases.
623	Tafe	2011	IHC/ISH/FISH	64	44	8	17	20	103	ASCO/CAP breast cancer guideline	Tumors showing 3+ protein expression or gene amplification were considered HER2 positive.	Overall, the rate of heterogeneity in this study was 1.5%.
633	Kim	2011	NR	Sect: 1106; TMA: 350	Sect: 132; TMA: 144	Sect: 66; TMA: 51	Sect: 110; TMA: 50	NR	NR	Hofmann	NR	NR
639	Choritz	2011	IHC/ISH/FISH	NR	NR	NR	16.7±3.2% (breast), 23.2±5.7% (gastric)	17.9±17.0 % (breast), 30.5±12.1 % (gastric)	NR	NR	NR	NR
653	Garcia-Garcia	2011	ISH/FISH/SISH	NR	NR	NR	NR	F:29; S: 35	F: 137; S: 131	NR	NR	NR
660	Langer	2011	IHC/ISH/FISH/bright field ISH	83		13	14	15	81	toga	NR	NR
661	Hu	2011	IHC/ISH/CISH	93	9	9	5	21	95		NR	NR
666	Thompson	2011	IHC/ISH/SISH	63	1	1	11	14	75	Hofmann	NR	NR
675	Im	2011	IHC/ISH/FISH	85	33	10	12	13	127	Hofmann	NR	NR
686	Yan	2011	IHC/ISH/FISH	98	25	12	10	18	127	dako	Scores 0 and 1 were considered negative, while scores 2 and 3 were considered positive	NR

690	Moelans	2011	IHC/ISH/CISH	Early Onset: 106; Late onset: 76		Early Onset: 2; Late onset: 8	Early Onset: 0; Late onset: 7	Early Onset: 5; Late onset: 13	Early Onset: 103; Late onset: 78	Hofmann	NR	CISH showed less heterogeneity than IHC. In 2/199 cases (1%), IHC showed clinically relevant heterogeneity between TMA cores, but all cases with focal IHC 3+ expression were uniformly CISH high level amplified.
694	Boers	2011	IHC/ISH/FISH/SISH	SP3: 125; 4B5: 106	SP3: 4; 4B5: 17	SP3: 6; 4B5: 6	SP3: 11; 4B5: 17	SP3: 22; 4B5: 22 [SISH];;SP3: 22; 4B5: 22 [FISH]	SP3: 124; 4B5: 124 [SISH];;SP3: 20; 4B5: 20 [FISH]	Hofmann	NR	Heterogeneity of HER2-immunoreactivity was the dominant pattern, and areas of HER2 amplification closely matched positive HER2-immunoreactivity. Amplification was heterogeneous in 73% of the adenocarcinomas
721	Schoppmann	2010	IHC/ISH/CISH	143	13	11	22	29	160	Hofmann, Grabsch	All tumors showing either 3+ expression of Her-2 at IHC or 2+ at IHC in combination with amplification of the HER-2 gene at CISH were considered as positive with regard to Her-2 status	NR
736	Yan	2010	IHC/ISH/FISH/CISH	111	4	1	12	15	113	Hofmann	NR	NR
806	Marx	2009	IHC/FISH	134	4	6	22	27	139	Hofmann	NR	NR
913	Sekaran	2012	IHC/ISH/FISH	28	NR	2	22	1	1	Hofmann	HER2 expression was considered positive if IHC 2+ was also positive by FISH and negative if FISH was negative	NR

Table 3: Outcome 1

Refid	First Author	Year	Length of f/u		Number of pts lost to follow-up	Comparisons	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	NND	Reproducibility	Concordance	Obs. variability
			Mean/median	Range										
2	Kinugasa	2015	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
74	Kim	2014	76	5.5-149.3	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

193	Sheffield	2014	NR	NR	NR	IHC	99.1% (95% CI, 98.1%–99.6%)	99.8% (95% CI, 99.6%–100%)	NR	NR	NR	(IHC 3+) cases (k = 0.80 +/- 0.01), negative (IHC 0 or 1+) cases (k = 0.58 +/- 0.01). equivocal (IHC 2+) cases (k= 0.22+/- 0.01).	NR	NR
228	Kimura	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
257	Tajiri	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
334	Grin	2013	NR	NR	NR	FISH/DualISH	NR	NR	NR	NR	NR	NR	98%	NR
340	Abrahao-Machado	2013	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
356	Pala	2013	NR	NR	NR	SISH V FISH	HercepTest: 83.3; A0485: 83.3; HercepTest: 93.3; A0485:93.3	HercepTest: 100; A0485: 95.7; HercepTest: 100; A0485: 95.8	NR	NR	NR	NR	NR	NR
493	Hirschmann	2012	NR	NR	NR	Dual ISH V Gene and protein IHC	NR	NR	NR	NR	NR	97.4%; 100%	NR	NR
498	Kiyose	2012	NR	NR	NR	CISH/FISH	NR	NR	NR	NR	NR	NR	Breast: 98.4%; Gastric: 99%	NR
501	Fassan	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
563	Cho	2012	NR	NR	NR	IHC/FISH			CB11 (85.2%), by HercepTest (75%), 4B5 (72.5%), and A0485 (69.6%)	A0485 (97.5%) HercepTest (96.8%), 4B5 (96.4%), and CB11 (94.3%)	NR	NR	93.1% by A0485 and 4B5, 93.4% by CB11, and 93.8% by HercepTest.	NR
565	Fox	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
566	Radu	2012	NR	NR	NR	IHC/FISH	NR	NR	NR	NR	NR	NR	95%	NR

579	Park	2012	NR	NR	NR	Herceptest/pathway V FISH/dc-SISH	NR	NR	NR	NR	NR	NR	96.1% (k=0.785 (p<0.001)) 98.3% (k=0.927 (p<0.001))	NR
585	Mrklic	2012	NR	NR	NR	Biopsy/Resection among pathologist	NR	NR	NR	NR	NR	NR	94.7% 95%	NR
588	Yoon	2012	12.6yrs	NR	NR	IHC/FISH	NR	NR	NR	NR	NR	NR	IHC0-1+ and IHC3+ groups [k = 0.83 (95% CI: 0.75– 0.91)	NR
590	Yang	2012	NR	NR	NR	IHC V FISH	80% (Biopsy), 96% (resection) 69.6% (biopsy), 95.6% (resection)	NR	NR	NR	NR	NR	93.2% (for biopsy & resection); 93.2% (for biopsy & resection)	NR
623	Tafe	2011	NR	NR	NR	IHC/FISH	NR	NR	NR	NR	NR	NR	s 97% for IHC 0, 93% for IHC 1+, and 100% for IHC 3+.	NR
633	Kim	2011	2.54yrs (A); 3.98ys (B)	3d-3.87ys (A); 18d - 6.5ys (B)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
639	Choritz	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
653	Garcia-Garcia	2011	NR	NR	NR	FISH/dc-SISH	NR	NR	NR	NR	NR	NR	94.4%	NR
660	Langer	2011	NR	NR	NR	IHC, ISH,FISH	NR	NR	NR	NR	NR	NR	There was a highly significant correlation of immunohistochemistry, bright field in situ hybridisation and fluorescent in situ hybridisation (P<0.001 each).	NR

661	Hu	2011	39mo	0.03-142mo	NR	IHC/CISH	NR	NR	NR	NR	NR	NR	76%	NR
666	Thompson	2011	20.6mo	NR	NR	IHC/SISH	NR	NR	NR	NR	NR	NR	k=0.636 (p<0.0001	NR
675	Im	2011	NR	NR	NR	IHC/FISH	100	92.9	59	100	NR	NR	k=0.638, p=0.01	NR
686	Yan	2011	NR	NR	NR		NR	NR	NR	NR	NR	NR		NR
690	Moelans	2011	NR	NR	NR	IHC/CISH	NR	NR	NR	NR	NR	NR	92%	NR
694	Boers	2011	NR	NR	NR	IHC V IHC	SP3: 77.3; 4B5: 95.5	SP3: 100; 4B5: 98.4	SP3: 100; 4B5: 91.3	SP3: 96.1; 4B5: 99.2	NR	NR	NR	NR
721	Schoppmann	2010	NR	NR	NR		NR	NR	NR	NR	NR	NR	NR	NR
736	Yan	2010	NR	NR	NR	FISH/CISH	NR	NR	NR	NR	NR	NR	100%	NR
806	Marx	2009	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
913	Sekaran	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Table 4: Outcome 2

Refid	First Author	Year	Comparisons	HR (CI)	Median/ % OS (Her2+)	Median/ % OS (Her2-)	p value for OS	HR for DFR	Median/ % DFS (Her2+)	Median/ % OS (Her2-)	p value for DFS	Quality	Algorithm	xtra info
2	Kinugasa	2015	ddPCR on FFPE or IHC/FISH	NR	124 days	321 days	0.01	NR	NR	NR	NR	NR	NR	The median HER2 ratio of the tissue samples was 0.25 (range: 0.18–0.53), whereas the median HER2 ratio of the serum samples was 1.05 (range: 0.51–1.14)The concordance rate of HER2 amplification examined in FFPE samples with ddPCR and IHC/FISH was 92% (23 out of 25). The concordance rate of FFPE with ctDNA was not high (62.5%); however, patients who were HER2-positive by ctDNA had significantly shorter survival compared with HER2-negative patients. Age, sex, tumour stages, and tumour histology were not significantly different between the patients who were HER2-positive or HER2-negative based on ctDNA analysis

74	Kim	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	This study tested the Ion AmpliSeq Cancer Hotspot Panel v2 and nCounter Copy Number Variation Assay in 89 formalin-fixed paraffin-embedded gastric cancer samples to determine whether they are applicable in archival clinical samples for personalized targeted therapies. Results were validated with Sanger sequencing, real-time quantitative PCR, FISH, and IHC. Frequently detected somatic mutations included TP53 (28.17%), APC (10.1%), PIK3CA (5.6%), KRAS (4.5%), SMO (3.4%), STK11 (3.4%), CDKN2A (3.4%) and SMAD4 (3.4%). Amplifications of HER2, CCNE1, MYC, KRAS and EGFR genes were observed in 8 (8.9%), 4 (4.5%), 2 (2.2%), 1 (1.1%) and 1 (1.1%) cases, respectively. In the cases with amplification, FISH for HER2 verified gene amplification and IHC for HER2, EGFR and CCNE1 verified the overexpression of proteins in tumor cells.

193	Sheffield	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	Unstained TMA slides were distributed to laboratories participating in a quality control exercise on a voluntary basis. Stained slides were returned for review and analysis by 2 pathologists with expertise in IHC quality assurance and gastrointestinal pathology , who achieved a consensus interpretation of these cases.	NR	interlaboratory agreement study using 5 Her2 antibodies in 37 participating laboratories
228	Kimura	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	HER2 expression differed according to the IHC method and antibodies used. HER2 IHC3+ tumors were identified in 21 (10%) and 7 (3.5%) cases by hand-operated and automated IHC, respectively
257	Tajiri	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	The fraction of amplification-positive cells in each tumor ranged from less than 10% to almost 100%.The combined analysis of MLPA and fluorescence in situ hybridization revealed that ERBB2 was coamplified with EGFR in 7 tumors, FGFR2 in 1 tumor, and FGFR2 and MET in 1 tumor; however, the respective genes were amplified in mutually exclusive cells. Coamplified ERBB2 and MYC coexisted within single nuclei in 4 tumors, and one of these cases had suspected coamplification in the same amplicon of ERBB2 with MYC

334	Grin	2013	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	One discrepant case was nonamplified by FISH but showed focal amplification by Dual ISH. Discrepancy was attributed to tumor heterogeneity, which was a frequent finding (78% of HER2-positive cases). There was excellent correlation between Dual ISH and FISH for assessment of HER2 amplification (0.97; p<0.001)
340	Abrahao-Machado	2013	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	HER2-positive expression (3+) in the whole-tissue sections was observed in 23 cases (11.6%) using the 4B5 antibody, in 18 cases (9.1%) using the SP3 antibody and in 10 cases (5.1%) using the HercepTest antibody. In the TMAs, 11 positive cases (5.6%) were identified using SP3 antibody, 9 (4.6%) using the 4B5 antibody and 6 (3%) using the HercepTest antibody. The sensitivity using whole-tissue sections and TMA, respectively, was 95.2% and 42.9% with 4B5, 90.5% and 66.7% with SP3 and 47.6% and 42.9% with HercepTest. The accuracy, calculated from the area under the ROC curve, using whole-tissue sections and TMA, respectively, was 0.91 and 0.79 by 4B5, 0.86 and 0.80 by SP3 and 0.73 and 0.71 by HercepTest. The concordance of the results obtained using whole tissue sections and TMA was 97.4% (Kappa 0.75) using HercepTest, 85.6% (Kappa 0.56) using SP3 and 84.1% (Kappa 0.38) using 4B5
356	Pala	2013	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Of the 18 cases, 4 showed focal heterogeneous low level amplification by SISH. Focal amplification was noted in only 2 cases by FISH.The concordance between HercepTestTM/A0485 IHC and ISH is perfect in (3+) cases.

493	Hirschmann	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	HER2 gene detection results using the gene and protein detection platform (Dual ISH) agreed with conventional FISH results in 76 (96.2%) of 79 (95% CI = 89.4-98.7]) and 41 (95.4%) of 43 (95% CI = 84.5-98.7]) breast and gastric carcinomas, respectively. HER2 protein detection results using the gene and protein detection platform IHC (PATHWAY HER2 [4B5]) agreed with the single-staining IHC results (clone CB11 antibody) in 58 (76.3%) of 76 (95% CI = 65.6-84.5) and 36 (85.7%) of 42 (95% CI = 72.2-93.3) breast and gastric carcinomas, respectively.
498	Kiyose	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	The polysomy of chromosome 17 was defined as the presence of three or more CEP17 signals in at least 10% of the tumor cells. In the 50 BC cases and 54 GC cases displaying chromosome 17 polysomy, the concordance between FISH and CISH was 98.0% and 98.1%, respectively.
501	Fassan	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	In both oesophageal and gastric samples, the rate of HER2 overexpression rose significantly from low-grade to high-grade IEN to adenocarcinoma (P < 0.001), with the two IHC protocols showing consistent staining (consistency 95%; k = 0.78; P < 0.001). Neither native nor metaplastic mucosa samples (obtained from either stomach or oesophagus) ever showed HER2 amplification. There was excellent agreement between HER2 amplification and protein overexpression (both IHC protocols: SISH／4B5 consistency 97.8%, k = 0.89, P < 0.001; SISH／CB11— consistency 97.8%, k = 0.91, P < 0.001).

563	Cho	2012												Discordant IHC results were seen in 23 cases (8.0%) with the four antibodies. All HER2 3+ cases (n=22) by HercepTest were positive with A0485 and 4B5, while one was negative with CB11. CB11 was found to be negative in 10 HercepTest 2+ and FISH+ cases.
565	Fox	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Interlaboratory agreement on IHC3+ scoring was good (k = 0.76), and there was good/very good agreement between IHC (positivity defined as IHC3+) and ISH when HER2 copy number was used (k = 0.72 to 0.87). Agreement on CISH/SISH scoring was good/very good when HER2 copy number was used (k = 0.68 to 0.86), and agreement between CISH/SISH and FISH using HER2 copy number was very good (k = 0.88 to 0.91). Agreement was reduced when HER2:chr17 ratio was used. The good agreement for HER2 copy number determined by bright-field ISH suggests that this is the optimal method for testing in GC/GJC cases.
566	Radu	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	as a screening test for FISH amplification, the Ventana Medical Systems (Tucson, AZ) 4B5 antibody demonstrated superior sensitivity (87%) compared with the DAKO (Carpinteria, CA) A0485 (70%). Of the cases, 28 were IHC 3+ or IHC 2+/FISH-amplified with the 4B5 assay compared with only 22 cases with the A0485 assay, representing a large potential difference in patient eligibility for anti-HER2 therapy. Cases with low-level FISH amplification (HER2/CEP17, 2.2-4.0) express lower levels of HER2 protein compared with cases with high-level amplification (HER2/CEP17, ≥4.0), raising the possibility of a differential response to anti-HER2 therapy.

579	Park	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	gastric cancer scoring system (GCSS) and the breast cancer scoring system (BCSS)	GCSS was significantly more sensitive for detecting SISH positivity than was BCSS in both antibodies (polyclonal, P = .003; monoclonal, P < .001), but specificity was higher in BCSS than GCSS (polyclonal, P = .004; monoclonal, P< .001).
585	Mrklic	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	her2/neu overexpression was more common in intestinal type gastric cancers (22.5%) than diffuse type (3.7%). Mixed type tumors showed no overexpression.
588	Yoon	2012	NR	0.76 (0.59-0.96)	NR	NR	0.024	0.79	NR	NR	0.066	NR	NR	HER2 positivity was significantly associated with lower tumor grade, less invasiveness, fewer malignant nodes, and the presence of adjacent Barrett's esophagus (BE). EACs with BE had higher odds of HER2 positivity than EACs without BE, independent of pathologic features [OR =1.8 (95% CI: 1.1–2.8), P = 0.014]. Among all cases, HER2 positivity was significantly associated with disease-specific survival (DSS) in a manner that differed by the presence or absence of BE (Pinteraction = 0.0047). In EACs with BE, HER2 positivity was significantly associated with improved DSS [HR = 0.54 (95% CI: 0.35–0.84), P =0.0065] and overall survival (P = 0.0022) independent of pathologic features, but was not prognostic among EACs without BE
590	Yang	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

623	Tafe	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Human epidermal growth factor receptor 2 positivity was strongly associated with tumor grade (moderately differentiated , poorly differentiated, P <.001) and histologic subtype (intestinal , diffuse, P = .007). Array comparative genomic hybridization analysis was successful in 31 tumors (14 FISH+ and 17 FISH-). Fluorescence in situ hybridization and array comparative genomic hybridization results were highly concordant in both HER2-positive and HER2-negative groups (93% and 100% concordance, respectively).
633	Kim	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Among samples scored 3+, 90.1% stained >50% of the tumor area, but only 40.9% in score 2+ cases stained >50% of the tumor area. In whole-tissue sections, HER2-positivity was correlated with age (P = 0.002), histological type (differentiated or intestinal, P<0.001), lymphovascular invasion (P = 0.005), and lymph node metastasis (P = 0.009). In TMAs, HER2- positivity was correlated only with age (P = 0.003) and histological type (P<0.001). Multivariate analyses of the differentiated GC subgroup revealed that HER2-positivity was an independent poor prognostic factor (P = 0.042). The cases with HER2-positive in >50% of the tumor area showed worse prognosis than those of<50% (P = 0.021).

639	Choritz	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	Pathologists regularly determined the number of HER2+ positive cases (HER2 3+, HER2 2+/amplified or amplified) in their laboratory, and figures were continuously entered into a central website. The overall positivity rate of each participant was calculated and compared with the average rates of all other institutes (n=42).	NR	A total of 10,916 test results on breast cancer and 982 on gastric cancer were entered into the system. Positivity rates for HER2 in breast cancer ranged from 7.6% to 31.6%. Statistically, the results from six institutions qualified as outliers (p<0.000005). From the remaining institutions encompassing 10,916 assessments, the mean proportion of positive cases was 16.7±3.2% (99% confidence interval 16.6–16.8). The results from six institutions were in between the 95% and 99.5% confidence intervals. For gastric cancer, there was one outlier and the mean positivity rate was 23.2±5.7%.
653	Garcia-Garcia	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Heterogeneity was identified in up to 52% of cases. All six discordant cases were positive by SISH and negative by FISH. On review of the FISH slides, all contradictory cases were polysomic and were confirmed to be negative for amplification by real-time PCR. Interestingly, all ratios in this latter group were between 2.06 and 2.50, so setting the cut-off for amplification at >3 resulted in perfect concordance
660	Langer	2011	IHC/ISH	NR	25 months (95% CI; 7–41 months)	73 months (95% CI; 26–120 months)	0.002	NR	18 months (95% CI; 6–30 months)	60 months (95% CI; 22–97 months)	0.004	NR	NR	ErbB2 positivity was observed more frequently in tumours with lower differentiation grades (P =0.029).

661	Hu	2011	High density microarrays CISH	NR	21mo 25mo	25mo 23mo	0.27 0.19	NR	NR	NR	0.709	NR	NR	HER2 amplification does not associate with poor prognosis in total 232 esophageal adenocarcinoma patients by CISH and high-density microarrays. further analysis confirm similar frequency of HER2 amplification by CISH (18%; 21 out of 116) and SNP 6.0 microarrays (16%, 19 out of 116) in esophageal adenocarcinoma. HER2 protein overexpression was observed in 12% (14 out of 116) of esophageal adenocarcinoma and 7% (1 out of 15) of high-grade dysplasia. No HER2 amplification or overexpression was identified in Barrett's esophagus or low-grade dysplasia. All HER2 protein overexpression cases showed HER2 gene amplification. Gene amplification was found to be more frequent by CISH than protein overexpression in esophageal adenocarcinoma (18 vs 12%).
666	Thompson	2011	IHC/SISH	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	HER2 amplification was seen more commonly in pT1 (25%) and pT4 tumors (27%) versus pT2 (9%) and pT3 (11%) tumors, but this difference was not significant (P = .25). The presence of low or high HER2 amplification did not influence any other patient or tumor characteristic

675	Im	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	HER2 IHC 3+ cases were more common in the intestinal-type tumors compared with diffuse-type tumors (16.7% vs. 5.1%, respectively; P = 0.049), and a nonsignificant trend was observed using fluorescence in situ hybridization (14.3% vs. 9.2%, respectively; P= 0.399). HER2 gene amplification was more frequent in stage IV (M0) than stage III disease (15.4% vs. 4.0%, respectively; P = 0.037). Interestingly, HER2-amplified disease was more common than nonamplified disease in patients with nodal stage 3 tumors (76.9% vs. 38.6%, respectively; P = 0.009); a similar pattern was observed using IHC. HER2 overexpression correlated with nodal stage, and a lymph node ratio greater than 0.5 was more common in HER2-amplified tumors than HER2-nonamplified tumors (69.2% vs. 43.3%, respectively; P= 0.086).
686	Yan	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	HER-2 status was not correlated with the sex and age of patients, and tumor size, location or differentiation, but with the depth of invasion, TNM stage, lymph node and distant metastasis as well as histopathological classification of gastric cancer (P < 0.05).

690	Moelans	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Proximal GC had more HER2 amplification (9% versus 3%) and overexpression (7% versus 2%) than distal tumours although this difference was not significant (p=0.181 and p=0.182 respectively). HER2 CISH showed more high level amplification in the intestinal type (7%, 16% if low-level included) compared to the mixed (5%, 5% if low-level included) and diffuse type (3%, 4% if low-level included) GCs (p=0.029). A similar association was seen for HER2 IHC and histologic type (p=0.008). Logistic regression indicated a significant association between HER2 expression and age, which remained significant when adjusted for both location and histological type.
694	Boers	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Results of FISH performed in 42 cases were identical to SISH. 24% of the oesophago-gastric carcinomas and 7% of distal stomach tumours were amplified. Assessment of polysomy – often a striking finding in tumours in our study – did not contribute to the prediction of amplification.
721	Schoppmann	2010	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Positive Her-2-status was more common in dysplastic Barrett mucosas compared with nondysplastic ones (P= 0.04). In 26% of the patients with ACs who had received neoadjuvant chemotherapy (n = 39), the Her-2 status of pretherapeutic biopsies was different compared with subsequent surgical specimens. There was no statistically significant correlation between Her-2 status and patients' survival.

736	Yan	2010												In the analyses of various clinicopathological parameters with HER2 status, a significant inverse correlation between HER2 protein overexpression (3+) status and overall survival in intestinal-type gastric cancers was found ($p<0.05$).
806	Marx	2009	FISH	NR	NR	NR	0.48	NR	NR	NR	NR	NR	NR	Amplification was associated with intestinal tumor phenotype but unrelated to survival, grading, pT, pN, or pM. Identical HER-2 status was found in primary tumor and their matched lymph node metastases. HER-2 and Topoisomerase IIα coamplification analysis of 3 to 16 large sections from 8 Her-2–positive gastric cancers did not reveal any heterogeneity of the amplicon site.
913	Sekaran	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	There was no difference in HER2 overexpression (positivity) or negativity in relation to age, gender, tumor site, histological subtype, tumor differentiation, serosal involvement or lymph nodal status. HER2 overexpression rates were similar for intestinal type as compared to diffuse histological type (OR 1.84), as also for proximal as compared to distal gastric cancers (OR 0.81)

For Pathologists

Clinical question 2: What strategies can help ensure optimal performance, interpretation and reporting of established assays in patients with gastroesophageal adenocarcinoma?

1. What is the optimal testing algorithm for the assessment of HER2 status?
    a. Which testing modality or algorithm is most cost effective?
    b. When and how should reflex (FISH/ISH) testing be done?

Table 1: Patient and disease characteristics

Refid	Bibliography	Year	Study Design	Location of study	N of participant	N of specimens	Age			Gender				Specimen Type	WHO/Lauren/ Both	Tumor Stage	Dx Addressed
							Mean/Medain	Std dev	Range	N Male	% male	N female	% female				
181	Koopman	2015	Prospective-Restrospective	Europe	323	NA/NR	NA/NR	NA/NR	NA/NR	221	68.4	102	31.6	Biopsy from primary tumor, Resection, Tissue from metastatic site	Intestinal, Diffuse, Indeterminate, Mixed	NR	Primary, Metastasis
228	Kimura	2014	Retrospective cohort	Asia	198	198	NA/NR	NA/NR	NA/NR	128	65	70	35	Resection	Intestinal, Diffuse	Stage I - IV	Primary
565	Fox	2012	Prospective cohort	Australia	NA/NR	100	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Resection	NA/NR	NR	Primary
579	Park	2012	Prospective-Restrospective	Asia	1091	NA/NR	55	NA/NR	20-70	738	68	353	32	TMA	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
590	Yang	2012	Prospective-Restrospective	Asia	148	265	59	NA/NR	31-89	119	80	29	20	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
623	Tafe	2011	Prospective-Restrospective	United States	135	NA/NR	NA/NR	NA/NR	22-90	103	76	32	24	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Indeterminate, Mixed	NA/NR	Primary, Recurrent or persistent disease, Metastasis
633	Kim	2011	Prospective-Restrospective	Asia	1414 (series A); 615 (series B)		Gp1: 58 Gp2: 58		Gp1: 25-87 Gp2: 23-89	955 (A); 414 (B)	68 (A); 67 (B)	461 (A); 201 (B)	32 (A); 33 (B)	Resection, TMA	Intestinal, Diffuse	Stage I - IV	Primary, Metastasis

639	Choritz	2011	Prospective cohort	Multiple countries	NA/NR	42 institutions (10,916 breast results); 15 institutions (982 gastric results)	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR
694	Boers	2011	Prospective-Restrospective	Europe	146	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Mixed	NR	Primary
736	Yan	2010	Prospective-Restrospective	Asia	NA/NR	128	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Resection	Intestinal, Diffuse, Mixed	NR	NA/NR
806	Marx	2009	Prospective-Restrospective	Europe	166	NA/NR	67	NA/NR	28-93	117	70	49	30	Resection, Tissue from metastatic site	Intestinal, Diffuse, Mixed	Stage I - IV	Primary, Metastasis
814	Barros-Silva	2009	Prospective-Restrospective	Portugal	463	NA/NR	67	NA/NR	26-91	145 of 256	56.6	101 of 256	43.4	Resection	Intestinal, Diffuse, Indeterminate	Stage I - IV	Primary

Table 2: Test Characteristics

Refid	First Author	Year	Methodology	Neg/0	1+	2+	3+	Amplified	Non-amplified	HER 2 SCORING METHODS	Her2 Result reporting structure	Heterogeneity
181	Koopman	2015	IHC/ISH/CISH	182		99	42	47	89	Hofmann & Ruschoff	NR	NR
228	Kimura	2014	IHC/ISH/FISH	101	48	28	21	12	27	Toga	NR	NR
565	Fox	2012	IHC/ISH/FISH/CISH/SISH	NR	NR	NR	NR	NR	NR	Hofmann & Ruschoff	Each IHC comparison was assessed using 2 cutoff points for scoring positivity [IHC3+ = positive, and IHC2+ or IHC3+ (ie, IHC2+/3+) = positive]	NR

566	Radu	2012	IHC/ISH/FISH					30	73	Hofmann & Ruschoff	NR	NR
579	Park	2012	IHC/ISH/FISH/SISH	Hercep Test: 917 ;Pathway: 803	HercepTest: 50 ;Pathway: 137	Hercep Test: 29 ;Pathway: 51	HercepTest: 63 ;Pathway: 68	F: 71; S: 70	F: 517; S: 518	Hofmann	Cases with scores of 2+ or 3+ were considered positive for HER2 overexpression	NR
590	Yang	2012	IHC/ISH/FISH	Biopsy: 125; Resect: 93	NR	Biopsy: 7; Resect: 5	Biopsy: 16; Resect: 19	Biopsy: 18; Resect: 22	Biopsy: 16; Resect: 8	Hofmann	NR	The intratumoral heterogeneity was defined as detection of areas showing different HER2 staining scores in IHC or HER2 gene amplification score in FISH (Fig. 1). Heterogeneous staining was demonstrated in 23 of 29 (79.3%) HER2- positive cases detected by IHC. Further, heterogeneity of HER2 at genetic level was observed in 11/25 (44.0%) FISH positive cases.
623	Tafe	2011	IHC/ISH/FISH	64	44	8	17	20	103	ASCO/CAP breast cancer guideline	Tumors showing 3+ protein expression or gene amplification were considered HER2 positive.	Overall, the rate of heterogeneity in this study was 1.5%.
633	Kim	2011	NR	Sect: 1106; TMA: 350	Sect: 132; TMA: 144	Sect: 66; TMA: 51	Sect: 110; TMA: 50	NR	NR	Hofmann	NR	NR
639	Choritz	2011	IHC/ISH/FISH	NR	NR	NR	16.7±3.2% (breast), 23.2±5.7% (gastric)	17.9±17.0 % (breast), 30.5±12.1 % (gastric)	NR	NR	NR	NR
694	Boers	2011	IHC/ISH/FISH/SISH	SP3: 125; 4B5: 106	SP3: 4; 4B5: 17	SP3: 6; 4B5: 6	SP3: 11; 4B5: 17	SP3: 22; 4B5: 22 [SISH];;SP3: 22; 4B5: 22 [FISH]	SP3: 124; 4B5: 124 [SISH];;SP3: 20; 4B5: 20 [FISH]	Hofmann	NR	Heterogeneity of HER2-immunoreactivity was the dominant pattern, and areas of HER2 amplification closely matched positive HER2-immunoreactivity. Amplification was heterogeneous in 73% of the adenocarcinomas
736	Yan	2010	IHC/ISH/FISH/CISH	111	4	1	12	15	113	Hofmann	NR	NR
806	Marx	2009	IHC/FISH	134	4	6	22	27	139	Hofmann	NR	NR
814	Barros-Silva	2009	IHC/FISH	414	6	18	25	38	218	NR	NR	NR

Table 3: Outcome 1

Refid	First Author	Year	Length of f/u		Number of pts lost to follow-up	Comparisons	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	NND	Reproducibility	Concordance	Obs. variability
			Mean/median	Range										
181	Koopman	2015	NR	NR	NR	IHC	NR	NR	NR	NR	NR	NR	NR	0.78
228	Kimura	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
565	Fox	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
579	Park	2012	NR	NR	NR	Herceptest/pathway V FISH/dc-SISH	NR	NR	NR	NR	NR	NR	96.1% (k=0.785 (p<0.001)) 98.3% (k=0.927 (p<0.001))	NR
590	Yang	2012	NR	NR	NR	IHC V FISH	80% (Biopsy), 96% (resection) 69.6% (biopsy), 95.6% (resection)	NR	NR	NR	NR	NR	93.2% (for biopsy & resection); 93.2% (for biopsy & resection)	NR
623	Tafe	2011	NR	NR	NR	IHC/FISH	NR	NR	NR	NR	NR	NR	s 97% for IHC 0, 93% for IHC 1+, and 100% for IHC 3+.	NR
633	Kim	2011	2.54yrs (A); 3.98ys (B)	3d-3.87ys (A); 18d - 6.5ys (B)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
639	Choritz	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
694	Boers	2011	NR	NR	NR	IHC V IHC	SP3: 77.3; 4B5: 95.5	SP3: 100; 4B5: 98.4	SP3: 100; 4B5: 91.3	SP3: 96.1; 4B5: 99.2	NR	NR	NR	NR
736	Yan	2010	NR	NR	NR	FISH/CISH	NR	NR	NR	NR	NR	NR	100%	NR
806	Marx	2009	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
814	Barros-Silva	2009	52.8mo	1-133mo		FISH/CISH	NR	NR	NR	NR	NR	NR	100%	NR

Table 4: Outcome 2

Refid	First Author	Year	Comparisons	HR (CI)	Median/ % OS (Her2+)	Median/ % OS (Her2-)	p value for OS	HR for DFR	Median/ % DFS (Her2+)	Median/ % OS (Her2-)	p value for DFS	Quality	Algorithm	xtra info
181	Koopman	2015	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Interobserver variability on IHC scoring using the currently standard modified HER2 scoring system was determined among three clinical pathologists. Most disagreement was found in diffuse or mixed tumor types and in weak to moderate stained samples (IHC 2+). The HER2 IHC scoring system is sensitive in differentiating HER2 status before ISH
228	Kimura	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	HER2 expression differed according to the IHC method and antibodies used. HER2 IHC3+ tumors were identified in 21 (10%) and 7 (3.5%) cases by hand-operated and automated IHC,respectively
565	Fox	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Interlaboratory agreement on IHC3+ scoring was good (k = 0.76), and there was good/very good agreement between IHC (positivity defined as IHC3+) and ISH when HER2 copy number was used (k = 0.72 to 0.87). Agreement on CISH/SISH scoring was good/very good when HER2 copy number was used (k = 0.68 to 0.86), and agreement between CISH/SISH and FISH using HER2 copy number was very good (k = 0.88 to 0.91). Agreement was reduced when HER2:chr17 ratio was used. The good agreement for HER2 copy number determined by bright-field ISH suggests that this is the optimal method for testing in GC/GJC cases.

579	Park	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	gastric cancer scoring system (GCSS) and the breast cancer scoring system (BCSS)	GCSS was significantly more sensitive for detecting SISH positivity than was BCSS in both antibodies (polyclonal, P = .003; monoclonal, P < .001), but specificity was higher in BCSS than GCSS (polyclonal, P = .004; monoclonal, P< .001).
590	Yang	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
623	Tafe	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Human epidermal growth factor receptor 2 positivity was strongly associated with tumor grade (moderately differentiated , poorly differentiated, P <.001) and histologic subtype (intestinal , diffuse, P = .007). Array comparative genomic hybridization analysis was successful in 31 tumors (14 FISH+ and 17 FISH-). Fluorescence in situ hybridization and array comparative genomic hybridization results were highly concordant in both HER2-positive and HER2-negative groups (93% and 100% concordance, respectively).

633	Kim	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Among samples scored 3+, 90.1% stained >50% of the tumor area, but only 40.9% in score 2+ cases stained >50% of the tumor area. In whole-tissue sections, HER2-positivity was correlated with age (P = 0.002), histological type (differentiated or intestinal, P<0.001), lymphovascular invasion (P = 0.005), and lymph node metastasis (P = 0.009). In TMAs, HER2- positivity was correlated only with age (P = 0.003) and histological type (P<0.001). Multivariate analyses of the differentiated GC subgroup revealed that HER2-positivity was an independent poor prognostic factor (P = 0.042). The cases with HER2-positive in >50% of the tumor area showed worse prognosis than those of<50% (P = 0.021).
639	Choritz	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	Pathologists regularly determined the number of HER2+ positive cases (HER2 3+, HER2 2+/amplified or amplified) in their laboratory, and figures were continuously entered into a central website. The overall positivity rate of each participant was calculated and compared with the average rates of all other institutes (n=42).	NR	A total of 10,916 test results on breast cancer and 982 on gastric cancer were entered into the system. Positivity rates for HER2 in breast cancer ranged from 7.6% to 31.6%. Statistically, the results from six institutions qualified as outliers (p<0.000005). From the remaining institutions encompassing 10,916 assessments, the mean proportion of positive cases was 16.7±3.2% (99% confidence interval 16.6–16.8). The results from six institutions were in between the 95% and 99.5% confidence intervals. For gastric cancer, there was one outlier and the mean positivity rate was 23.2±5.7%.

694	Boers	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Results of FISH performed in 42 cases were identical to SISH. 24% of the oesophago-gastric carcinomas and 7% of distal stomach tumours were amplified. Assessment of polysomy – often a striking finding in tumours in our study – did not contribute to the prediction of amplification.
736	Yan	2010												In the analyses of various clinicopathological parameters with HER2 status, a significant inverse correlation between HER2 protein overexpression (3+) status and overall survival in intestinal-type gastric cancers was found ($p<0.05$).
806	Marx	2009	FISH	NR	NR	NR	0.48	NR	NR	NR	NR	NR	NR	Amplification was associated with intestinal tumor phenotype but unrelated to survival, grading, pT, pN, or pM. Identical HER-2 status was found in primary tumor and their matched lymph node metastases. HER-2 and Topoisomerase IIα coamplification analysis of 3 to 16 large sections from 8 Her-2–positive gastric cancers did not reveal any heterogeneity of the amplicon site.
814	Barros-Silva	2009	FISH	NR	35.3%	43.2%	0.222	NR	NR	NR	NR	NR	NR	ERBB2 amplification was associated with gastric carcinomas of intestinal type (P = 0.007) and with an expansive growth pattern (P =0.021). ERBB2 amplification was detected in both histological components of two mixed carcinomas, indicating a common clonal origin. A statistically significant association was found between ERBB2 amplification and worse survival in patients with expansive gastric carcinomas (P = 0.011).

For Pathologists

Clinical question 2: What strategies can help ensure optimal performance, interpretation and reporting of established assays in patients with gastroesophageal adenocarcinoma?

1. What is the best scoring method for IHC and ISH in gastroesophageal adenocarcinoma specimens?
    - **a.** Can HER2 copy numbers be used to define HER2 status in addition to HER2:CEP17 ratios (i.e. in cases with apparent polysomy) in ISH testing as a positive result?
    - b. Should the scoring criteria be the same for biopsy specimen vs resection specimen?
    - c. How should Her2 heterogeneity be interpreted/reported?
    - d. When should a specimen be reported as indeterminate?

Table 1: Patient and disease characteristics

Refid	Bibliography	Year	Study Design	Location of study	N of participant	N of specimens	Age			Gender				Specimen Type	WHO/Lauren/ Both	Tumor Stage	Dx Addressed
							Mean/Medain	Std dev	Range	N Male	% male	N female	% female				
355	Gasljevic	2013	Prospective-Restrospective	Europe	302	NA/NR	67	12	33-87	199	66	103	34	NR	Papillary adenocarcinoma, Tubular adenocarcinoma, Mucinous adenocarcinoma, Signet-ring cell carcinoma, Other poorly cohesive carcinoma, Mixed, Intestinal, Diffuse, Indeterminate	Stage I - IV	Primary
356	Pala	2013	Retrospective cohort	Europe	88	NA/NR	61.2	NA/NR	29-81	NA/NR	NA/NR	NA/NR	NA/NR	NR	Papillary adenocarcinoma, Tubular adenocarcinoma, Mucinous adenocarcinoma, Other poorly cohesive carcinoma, Mixed carcinoma	Stage I - IV	Primary

358	Ormenisan	2013	Retrospective cohort	United States	68	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Biopsy from primary tumor, Resection, Fine needle aspiration (FNA) or cytology sample,	Tubular adenocarcinoma, Other poorly cohesive carcinoma	NR	Primary, Metastasis
403	Cruz-Reyes	2013	Prospective-Restrospective	Mexico	269	NA/NR	61	NA/NR	24-93	142	52.8	127	48.2	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Mixed	Stage III - IV	Primary
451	Warneke	2013	Prospective-Restrospective	Europe	454	NA/NR	67.3	11.1	NA/NR	283	62.3	171	37.7	Resection, TMA	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
565	Fox	2012	Prospective cohort	Australia	NA/NR	100	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Resection	NA/NR	NR	Primary
579	Park	2012	Prospective-Restrospective	Asia	1091	NA/NR	55	NA/NR	20-70	738	68	353	32	TMA	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
585	Mrklic	2012	Prospective-Restrospective	Europe	73	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Mixed	NR	Primary
590	Yang	2012	Prospective-Restrospective	Asia	148	265	59	NA/NR	31-89	119	80	29	20	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Mixed	Stage I - IV	Primary
623	Tafe	2011	Prospective-Restrospective	United States	135	NA/NR	NA/NR	NA/NR	22-90	103	76	32	24	Biopsy from primary tumor, Resection	Intestinal, Diffuse, Indeterminate, Mixed	NA/NR	Primary, Recurrent or persistent disease, Metastasis
653	Garcia-Garcia	2011	Prospective-Restrospective	Europe	166	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Biopsy from primary tumor, Resection, Tissue from metastatic site	Intestinal, Diffuse, Indeterminate	NR	Primary, Metastasis

Table 2: Test Characteristics

Refid	First Author	Year	Methodology	Neg/0	1+	2+	3+	Amplified	Non-amplified	HER 2 SCORING METHODS	Her2 Result reporting structure	Heterogeneity
355	Gasljevic	2013	IHC/FISH	190	38	57	20	69	430	Ruschoff	NR	NR
356	Pala	2013	IHC/ISH/FISH/SISH	71	2	3	12	S:18; F:15	S:70; F:72	Hofmann ISH- amplified if HER2/cen17 ratio greater than or equal to 2 within 20 tumor cell nuclei (ToGA)	NR	NR
358	Ormenisan	2013	IHC/ISH/FISH	51 (0 & 1+)		3	14	14	51	Hofmann FISH- used ASCO/CAP breast guidelines, not ToGA (1.8 to 2.2 equivocal; > 2.2, amplified)	NR	NR
403	Cruz-Reyes	2013	IHC/FISH/CISH	255	3	6	5	10	172	Hofmann	NR	NR
451	Warneke	2013	IHC/ISH/SISH	417	NR	NR	37	37	417	Ruschoff	NR	NR
565	Fox	2012	IHC/ISH/FISH/CISH/SISH	NR	NR	NR	NR	NR	NR	Hofmann & Ruschoff	Each IHC comparison was assessed using 2 cutoff points for scoring positivity [IHC3+ = positive, and IHC2+ or IHC3+ (ie, IHC2+/3+) = positive]	NR
579	Park	2012	IHC/ISH/FISH/SISH	Hercep Test: 917 ;Pathway: 803	HercepTest: 50 ;Pathway: 137	Hercep Test: 29 ;Pathway: 51	HercepTest: 63 ;Pathway: 68	F: 71; S: 70	F: 517; S: 518	Hofmann	Cases with scores of 2+ or 3+ were considered positive for HER2 overexpression	NR
585	Mrklic	2012	IHC/ISH/CISH	51	9	6	7	10	63	Hofmann	NR	NR

590	Yang	2012	IHC/ISH/FISH	Biopsy: 125; Resect: 93	NR	Biopsy: 7; Resect: 5	Biopsy: 16; Resect: 19	Biopsy: 18; Resect: 22	Biopsy: 16; Resect: 8	Hofmann	NR	The intratumoral heterogeneity was defined as detection of areas showing different HER2 staining scores in IHC or HER2 gene amplification score in FISH (Fig. 1). Heterogeneous staining was demonstrated in 23 of 29 (79.3%) HER2- positive cases detected by IHC. Further, heterogeneity of HER2 at genetic level was observed in 11/25 (44.0%) FISH positive cases.
623	Tafe	2011	IHC/ISH/FISH	64	44	8	17	20	103	ASCO/CAP breast cancer guideline	Tumors showing 3+ protein expression or gene amplification were considered HER2 positive.	Overall, the rate of heterogeneity in this study was 1.5%.
653	Garcia-Garcia	2011	ISH/FISH/SISH	NR	NR	NR	NR	F:29; S: 35	F: 137; S: 131	NR	NR	NR

Table 3: Outcome 1

Refid	First Author	Year	Length of f/u		Number of pts lost to follow-up	Comparisons	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	NND	Reproducibility	Concordance	Obs. variability
			Mean/median	Range										
355	Gasljevic	2013	2.28yrs	0.04-10.27yrs	12	1st core Vs. 2nd core	69.5 % (58.4–79.2 %) 67.4 % (57.0–76.6)	91.0 % (95 % CI: 86.2–94.6 %). 95.8 % (91.9–98.1)	76; 88.9	88; 85.4	NR	NR	84.8 %(κ=0.62, 95 % CI: 0.51–0.72) 86.3 % (κ=0.67, 0.58–0.76)	NR
356	Pala	2013	NR	NR	NR	SISH V FISH	HercepTest: 83.3; A0485: 83.3; HercepTest: 93.3; A0485:93.3	HercepTest: 100; A0485: 95.7; HercepTest: 100; A0485: 95.8	NR	NR	NR	NR	NR	NR
358	Ormenisan	2013	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
403	Cruz-Reyes	2013	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
451	Warneke	2013	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

565	Fox	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
579	Park	2012	NR	NR	NR	Herceptest/pathway V FISH/dc-SISH	NR	NR	NR	NR	NR	NR	96.1% (k=0.785 (p<0.001)) 98.3% (k=0.927 (p<0.001))	NR
585	Mrklic	2012	NR	NR	NR	Biopsy/Resection among pathologist	NR	NR	NR	NR	NR	NR	94.7% 95%	NR
590	Yang	2012	NR	NR	NR	IHC V FISH	80% (Biopsy), 96% (resection) 69.6% (biopsy), 95.6% (resection)	NR	NR	NR	NR	NR	93.2% (for biopsy & resection); 93.2% (for biopsy & resection)	NR
623	Tafe	2011	NR	NR	NR	IHC/FISH	NR	NR	NR	NR	NR	NR	s 97% for IHC 0, 93% for IHC 1+, and 100% for IHC 3+.	NR
653	Garcia-Garcia	2011	NR	NR	NR	FISH/dc-SISH	NR	NR	NR	NR	NR	NR	94.4%	NR

Table 4: Outcome 2

Refid	First Author	Year	Comparisons	HR (CI)	Median/ % OS (Her2+)	Median/ % OS (Her2-)	p value for OS	HR for DFR	Median/ % DFS (Her2+)	Median/ % OS (Her2-)	p value for DFS	Quality	Algorithm	xtra info

355	Gasljevic	2013	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	The overall concordance of IHC and FISH on cores was 75.7 %. The level of amplification correlated with the IHC score. Relationship between the intestinal and papillary types and tumour grade was observed for tumours with over-expression and amplification, whereas tumour location was related only to over-expression. There was a statistically significant difference in the overall survival of the patients, which was related to HER2 amplification
356	Pala	2013	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Of the 18 cases, 4 showed focal heterogeneous low level amplification by SISH. Focal amplification was noted in only 2 cases by FISH.The concordance between HercepTestTM/A0485 IHC and ISH is perfect in (3+) cases.
358	Ormenisan	2013	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Image cytometric algorithm used in breast cancer	Of the 14 visually HER2 IHC positive, 13 were positive by image cytometry (93% concordance), all 13 were amplified by HER2 FISH (100% concordance). Of the 3 cases equivocal both visually and by image cytometry, only 1 was FISH amplified. Fifty-one were negative by IHC visually and 52 by image cytometry (98% concordance). None of the 5 HER2 IHC negative were amplified by FISH
403	Cruz-Reyes	2013	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Amplified tumors were intestinal adenocarcinomas located throughout the different regions of the stomach. Heterogeneity was documented in 4 widely sampled tumors. HER2 amplification was restricted to the intestinal phenotype.

451	Warneke	2013	IHC/SISH	12.4-17.0 (Her2-); 5.5-25.2 (her2+)	15.4±5.0	14.7±1.2	0.452	NR	NR	NR	NR	observers were blinded with regard to the clinicopathological patient characteristics. After independent evaluation of the whole tissue sections and the TMAs by both observers, a final consensus evaluation was carried out with three observers	NR	In whole tissue sections, 37 (8.1%; observer 1) and 38 (8.4%; observer 2) of the GCs, and in the corresponding TMAs, 28 (6.3%; observer 1) and 28 (6.3%; observer 2) of the GCs were classified as Her2/neu-positive (kappa value 98.5% and 96.2%; P < 0001). Comparison of whole tissue sections with corresponding TMAs showed a false-negative rate of 24% and a false-positive rate of 3% for TMAs
565	Fox	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Interlaboratory agreement on IHC3+ scoring was good (k = 0.76), and there was good/very good agreement between IHC (positivity defined as IHC3+) and ISH when HER2 copy number was used (k = 0.72 to 0.87). Agreement on CISH/SISH scoring was good/very good when HER2 copy number was used (k = 0.68 to 0.86), and agreement between CISH/SISH and FISH using HER2 copy number was very good (k = 0.88 to 0.91). Agreement was reduced when HER2:chr17 ratio was used. The good agreement for HER2 copy number determined by bright-field ISH suggests that this is the optimal method for testing in GC/GJC cases.
579	Park	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	gastric cancer scoring system (GCSS) and the breast cancer scoring system (BCSS)	GCSS was significantly more sensitive for detecting SISH positivity than was BCSS in both antibodies (polyclonal, P = .003; monoclonal, P < .001), but specificity was higher in BCSS than GCSS (polyclonal, P = .004; monoclonal, P< .001).

585	Mrklic	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	her2/neu overexpression was more common in intestinal type gastric cancers (22.5%) than diffuse type (3.7%). Mixed type tumors showed no overexpression.
590	Yang	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
623	Tafe	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Human epidermal growth factor receptor 2 positivity was strongly associated with tumor grade (moderately differentiated , poorly differentiated, P <.001) and histologic subtype (intestinal , diffuse, P = .007). Array comparative genomic hybridization analysis was successful in 31 tumors (14 FISH+ and 17 FISH-). Fluorescence in situ hybridization and array comparative genomic hybridization results were highly concordant in both HER2-positive and HER2-negative groups (93% and 100% concordance, respectively).
653	Garcia-Garcia	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Heterogeneity was identified in up to 52% of cases. All six discordant cases were positive by SISH and negative by FISH. On review of the FISH slides, all contradictory cases were polysomic and were confirmed to be negative for amplification by real-time PCR. Interestingly, all ratios in this latter group were between 2.06 and 2.50, so setting the cut-off for amplification at >3 resulted in perfect concordance

For Pathologists

Clinical question 2: What strategies can help ensure optimal performance, interpretation and reporting of established assays in patients with gastroesophageal adenocarcinoma?

1. How should HER2 results be reported? Use CAP biomarkers template? Hoffman method? Package insert?

Table 1: Patient and disease characteristics

Refid	Bibliography	Year	Study Design	Location of study	N of participant	N of specimens	Age			Gender				Specimen Type	WHO/Lauren/ Both	Tumor Stage	Dx Addressed
							Mean/Medain	Std dev	Range	N Male	% male	N female	% female				
247	Kushima	2014	Prospective cohort	Asia		50	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Resection	NA/NR	NR	Primary
498	Kiyose	2012	Prospective-Restrospective	Asia		125 BC and 198 GC	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	TMA	NA/NR	NR	Primary

Table 2: Test Characteristics

Refid	First Author	Year	Methodology	Neg/0	1+	2+	3+	Amplified	Non-amplified	HER 2 SCORING METHODS	Her2 Result reporting structure	Heterogeneity
247	Kushima	2014	IHC	15	14	8	13	NR	NR	NR	NR	NR
498	Kiyose	2012	IHC/ISH/FISH/CISH	140	8	13	37	F: 50; C: 52	F: 148; C: 146	HercepTestTM kit guide and Pathvysion HER2 DNA probe kit	a score of 0 or 1+ was considered negative, a score of 2+ was considered weakly positive, and a score of 3+ was considered strongly positive.	NR

Table 3: Outcome 1

Refid	First Author	Year	Length of f/u		Number of pts lost to follow-up	Comparisons	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	NND	Reproducibility	Concordance	Obs. variability
			Mean/median	Range										
247	Kushima	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
498	Kiyose	2012	NR	NR	NR	CISH/FISH	NR	NR	NR	NR	NR	NR	Breast: 98.4%; Gastric: 99%	NR

Table 4: Outcome 2

Refi d	First Author	Year	Comparisons	HR (CI)	Median/ % OS (Her2+)	Median/ % OS (Her2-)	p value for OS	HR for DFR	Median/ % DFS (Her2+)	Median/ % OS (Her2-)	p value for DFS	Quality	Algorithm	xtra info
247	Kushima	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	The educational QA/QC program comprised three parts: comments and explanation of pre-interpretation cases, lecture, and presentation of typical and special cases for discussion. To confirm the effectiveness of the educational program, pathologist scores before and after the educational program were compared and the increase in the rate of concordance was determined	NR	The JGC ring study demonstrated good agreement in the interpretation of HER2-immunohistochemistry. Kappa coefficients among the five observers were 0.73 (substantial) and 0.84 (almost perfect) in 4×4 and 3×3 cross tests, respectively. In the second study, the concordance rate and kappa coefficients improved from preeducational program levels of 78.6 % and 0.68, respectively, to post-educational program levels of 87.1 % and 0.79, respectively.
498	Kiyose	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	The polysomy of chromosome 17 was defined as the presence of three or more CEP17 signals in at least 10% of the tumor cells. In the 50 BC cases and 54 GC cases displaying chromosome 17 polysomy, the concordance between FISH and CISH was 98.0% and 98.1%, respectively.

For Pathologists

Clinical question 2: What strategies can help ensure optimal performance, interpretation and reporting of established assays in patients with gastroesophageal adenocarcinoma?

1. What is adequate specimen handling for gastroesophageal adenocarcinoma testing? (the second part does not add anything and the term 'indeterminate' for a specimen is not intuitively clear)
    a. Ischemic time
    b. Fixation time, fixative
    c. Tissue processing
    d. Decalcification
    e. Tissue degeneration

Table 1: Patient and disease characteristics

Refid	Bibliography	Year	Study Design	Location of study	N of participant	N of specimens	Age			Gender				Specimen Type	WHO/Lauren/ Both	Tumor Stage	Dx Addressed
							Mean/Medain	Std dev	Range	N Male	% male	N female	% female				
951	Gullo	2015	Prospective-retrospective	Europe	103	504	69	NR	37-90	75	73	28	27	Biopsy from primary tumor, resection	Intestinal, Diffuse	NR	Primary

Table 2: Test Characteristics

Refid	First Author	Year	Methodology	Neg/0	1+	2+	3+	Amplified	Non-amplified	HER 2 SCORING METHODS	Her2 Result reporting structure	Heterogeneity
951	Gullo	2015	IHC/ISH/FISH	67	NR	16	23	20	64	Ruschoff	NR	NR

Table 3: Outcome 1

Refid	First Author	Year	Length of f/u		Number of pts lost to follow-up	Comparisons	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	NND	Reproducibility	Concordance	Obs. variability
			Mean/median	Range										
951	Gullo	2015	NR	NR	NR	Surgical samples Virtual biopsies	 91.9	 97	NR	NR	NR	NR	IHC/FISH: 97.1	NR

Table 4: Outcome 2

Refid	First Author	Year	Comparisons	HR (CI)	Median/ % OS (Her2+)	Median/ % OS (Her2-)	p value for OS	HR for DFR	Median/ % DFS (Her2+)	Median/ % OS (Her2-)	p value for DFS	Quality	Algorithm	xtra info
951	Gullo	2015	Surgical samples Virtual biopsies	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

For Pathologists

Clinical question 2: What strategies can help ensure optimal performance, interpretation and reporting of established assays in patients with gastroesophageal adenocarcinoma?

1. What is the appropriate morphologic correlation for interpretation of ISH?

Table 1: Patient and disease characteristics

Refid	Bibliography	Year	Study Design	Location of study	N of participant	N of specimens	Age			Gender				Specimen Type	WHO/Lauren/ Both	Tumor Stage	Dx Addressed
							Mean/Medain	Std dev	Range	N Male	% male	N female	% female				
257	Tajiri	2014	Prospective cohort	Asia	475	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NR	Papillary adenocarcinoma, Tubular adenocarcinoma, Mucinous adenocarcinoma, Other poorly cohesive carcinoma, Mixed carcinoma	NA/NR	Primary

Table 2: Test Characteristics

Refid	First Author	Year	Methodology	Neg/0	1+	2+	3+	Amplified	Non-amplified	HER 2 SCORING METHODS	Her2 Result reporting structure	Heterogeneity
257	Tajiri	2014	IHC/ISH/FISH	NR	NR	NR	NR	51	424	dako HercepTest recommendations for IHC. FISH scored according to ASCO/CAP guidelines (more than 6 gene copies per nucleus or gene signal/centromere signals >2.2	NR	Intratumoral heterogeneity of ERBB2 amplification, defined as less than 50% of cancer cells positive for ERBB2 amplification, was found in 41% (21/51) of ERBB2-amplified tumors

Table 3: Outcome 1

Refid	First Author	Year	Length of f/u	Number of	Comparisons	Sensitivit	Specificity	PPV (%)	NPV (%)	NND	Reproducib	Concordance	Obs.

			Mean/median	Range	pts lost to follow-up		y (%)	(%)				ility		variability
257	Tajiri	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Table 4: Outcome 2

Refid	First Author	Year	Comparisons	HR (CI)	Median/ % OS (Her2+)	Median/ % OS (Her2-)	p value for OS	HR for DFR	Median/ % DFS (Her2+)	Median/ % OS (Her2-)	p value for DFS	Quality	Algorithm	xtra info
257	Tajiri	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	The fraction of amplification-positive cells in each tumor ranged from less than 10% to almost 100%.The combined analysis of MLPA and fluorescence in situ hybridization revealed that ERBB2 was coamplified with EGFR in 7 tumors, FGFR2 in 1 tumor, and FGFR2 and MET in 1 tumor; however, the respective genes were amplified in mutually exclusive cells. Coamplified ERBB2 and MYC coexisted within single nuclei in 4 tumors, and one of these cases had suspected coamplification in the same amplicon of ERBB2 with MYC

For Pathologists

Clinical question 2: What strategies can help ensure optimal performance, interpretation and reporting of established assays in patients with gastroesophageal adenocarcinoma?

1. What are the optimal quality assurance/quality control standards that labs should adhere to?
    a. Proficiency testing
    b. Lab volume
    c. Ongoing personnel training
    d. Appropriate control (breast, gastric cell lines)

Table 1: Patient and disease characteristics

Refid	Bibliography	Year	Study Design	Location of study	N of participant	N of specimens	Age			Gender				Specimen Type	WHO/Lauren/ Both	Tumor Stage	Dx Addressed
							Mean/Medain	Std dev	Range	N Male	% male	N female	% female				
247	Kushima	2014	Prospective cohort	Asia		50	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Resection	NA/NR	NR	Primary
565	Fox	2012	Prospective cohort	Australia	NA/NR	100	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Resection	NA/NR	NR	Primary
639	Choritz	2011	Prospective cohort	Multiple countries	NA/NR	42 institutions (10,916 breast results); 15 institutions (982 gastric results)	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR

Table 2: Test Characteristics

Refid	First Author	Year	Methodology	Neg/0	1+	2+	3+	Amplified	Non-amplified	HER 2 SCORING METHODS	Her2 Result reporting structure	Heterogeneity
247	Kushima	2014	IHC	15	14	8	13	NR	NR	NR	NR	NR

565	Fox	2012	IHC/ISH/FISH/CISH/SISH	NR	NR	NR	NR	NR	NR	Hofmann & Ruschoff	Each IHC comparison was assessed using 2 cutoff points for scoring positivity [IHC3+ = positive, and IHC2+ or IHC3+ (ie, IHC2+/3+) = positive]	NR
639	Choritz	2011	IHC/ISH/FISH	NR	NR	NR	16.7±3.2% (breast), 23.2±5.7% (gastric)	17.9±17.0 % (breast), 30.5±12.1 % (gastric)	NR	NR	NR	NR

Table 3: Outcome 1

Refid	First Author	Year	Length of f/u		Number of pts lost to follow-up	Comparisons	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	NND	Reproducibility	Concordance	Obs. variability
			Mean/median	Range										
247	Kushima	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
565	Fox	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
639	Choritz	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Table 4: Outcome 2

Refid	First Author	Year	Comparisons	HR (CI)	Median/ % OS (Her2+)	Median/ % OS (Her2-)	p value for OS	HR for DFR	Median/ % DFS (Her2+)	Median/ % OS (Her2-)	p value for DFS	Quality	Algorithm	xtra info

247	Kushima	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	The educational QA/QC program comprised three parts: comments and explanation of pre-interpretation cases, lecture, and presentation of typical and special cases for discussion. To confirm the effectiveness of the educational program, pathologist scores before and after the educational program were compared and the increase in the rate of concordance was determined	NR	The JGC ring study demonstrated good agreement in the interpretation of HER2-immunohistochemistry. Kappa coefficients among the five observers were 0.73 (substantial) and 0.84 (almost perfect) in 4×4 and 3×3 cross tests, respectively. In the second study, the concordance rate and kappa coefficients improved from preeducational program levels of 78.6 % and 0.68, respectively, to post-educational program levels of 87.1 % and 0.79, respectively.

565	Fox	2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	Interlaboratory agreement on IHC3+ scoring was good (k = 0.76), and there was good/very good agreement between IHC (positivity defined as IHC3+) and ISH when HER2 copy number was used (k = 0.72 to 0.87). Agreement on CISH/SISH scoring was good/very good when HER2 copy number was used (k = 0.68 to 0.86), and agreement between CISH/SISH and FISH using HER2 copy number was very good (k = 0.88 to 0.91). Agreement was reduced when HER2:chr17 ratio was used. The good agreement for HER2 copy number determined by bright-field ISH suggests that this is the optimal method for testing in GC/GJC cases.
639	Choritz	2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	Pathologists regularly determined the number of HER2+ positive cases (HER2 3+, HER2 2+/amplified or amplified) in their laboratory, and figures were continuously entered into a central website. The overall positivity rate of each participant was calculated and compared with the average rates of all other institutes (n=42).	NR	A total of 10,916 test results on breast cancer and 982 on gastric cancer were entered into the system. Positivity rates for HER2 in breast cancer ranged from 7.6% to 31.6%. Statistically, the results from six institutions qualified as outliers ($p<0.000005$). From the remaining institutions encompassing 10,916 assessments, the mean proportion of positive cases was 16.7±3.2% (99% confidence interval 16.6–16.8). The results from six institutions were in between the 95% and 99.5% confidence intervals. For gastric cancer, there was one outlier and the mean positivity rate was 23.2±5.7%.

For Pathologists

Clinical question 2: What strategies can help ensure optimal performance, interpretation and reporting of established assays in patients with gastroesophageal adenocarcinoma?

1. Is there a role for HER2 genomic testing?

Table 1: Patient and disease characteristics

Refid	Bibliography	Year	Study Design	Location of study	N of participant	N of specimens	Age			Gender				Specimen Type	WHO/Lauren/ Both	Tumor Stage	Dx Addressed
							Mean/Medain	Std dev	Range	N Male	% male	N female	% female				
2	Kinugasa	2015	Prospective cohort	Asia	25	NA/NR	66	NR	29-81	20	80	5	20	Biopsy from primary tumor, serum	Intestinal, Diffuse	Stage III - IV	Primary
14	Schmitt	2015	Retrospective cohort	Europe	NA/NR	79	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	45 frozen HER2+++ tumors and 34 FFPE HER2 +++ tumors	NA/NR	NR	NR
257	Tajiri	2014	Prospective cohort	Asia	475	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	NR	Papillary adenocarcinoma, Tubular adenocarcinoma, Mucinous adenocarcinoma, Other poorly cohesive carcinoma, Mixed carcinoma	NA/NR	Primary

Table 2: Test Characteristics

Refid	First Author	Year	Methodology	Neg/0	1+	2+	3+	Amplified	Non-amplified	HER 2 SCORING METHODS	Her2 Result reporting structure	Heterogeneity
2	Kinugasa	2015	IHC/ISH/FISH	17	NR	NR	8	8	17	Ruschoff	NR	NR
14	Schmitt	2015	NR	NR	NR	NR	79	NR	NR	NR	NR	NR

257	Tajiri	2014	IHC/ISH/FISH	NR	NR	NR	NR	51	424	dako HercepTest recommendations for IHC. FISH scored according to ASCO/CAP guidelines (more than 6 gene copies per nucleus or gene signal/centromere signals >2.2	NR	Intratumoral heterogeneity of ERBB2 amplification, defined as less than 50% of cancer cells positive for ERBB2 amplification, was found in 41% (21/51) of ERBB2-amplified tumors

Table 3: Outcome 1

Refid	First Author	Year	Length of f/u		Number of pts lost to follow-up	Comparisons	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	NND	Reproducibility	Concordance	Obs. variability
			Mean/median	Range										
2	Kinugasa	2015	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
14	Schmitt	2015	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
257	Tajiri	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

Table 4: Outcome 2

Refid	First Author	Year	Comparisons	HR (CI)	Median/ % OS (Her2+)	Median/ % OS (Her2-)	p value for OS	HR for DFR	Median/ % DFS (Her2+)	Median/ % OS (Her2-)	p value for DFS	Quality	Algorithm	xtra info
2	Kinugasa	2015	ddPCR on FFPE or IHC/FISH	NR	124 days	321 days	0.01	NR	NR	NR	NR	NR	NR	The median HER2 ratio of the tissue samples was 0.25 (range: 0.18–0.53), whereas the median HER2 ratio of the serum samples was 1.05 (range: 0.51–1.14)The concordance rate of HER2 amplification examined in FFPE samples with ddPCR and IHC/FISH was 92% (23 out of 25). The concordance rate of FFPE with ctDNA was not high (62.5%); however, patients who were HER2-positive by ctDNA had significantly shorter survival compared with HER2-negative patients. Age, sex, tumour stages, and tumour histology were not significantly different between the patients who were HER2-positive or HER2-negative based on ctDNA analysis

14	Schmitt	2015	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	The sensitivity of BT474, HCC2218, UACC-812, HCC1419, HCC1954, and HCC1569 cell lines was analyzed with increasing doses of trastuzumab (from 0 to 500 µg/ml). Among the 6 cell lines, -A- BT474, UACC-812, and HCC2218 were sensitive to trastuzumab (IC50 = 1µg/ml, 5µg/ml, and 8µg/ml, respectively) and -B- the 3 others were resistant (IC50 > 500µg/ml). 8-gene-expression combination was identified that predicted the response to treatment with an accuracy of 76%. Based on public microarray data, study also showed that the expression profile was specific to first-line trastuzumab + docetaxel-based treatment with an accuracy of 85%.
257	Tajiri	2014	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	The fraction of amplification-positive cells in each tumor ranged from less than 10% to almost 100%.The combined analysis of MLPA and fluorescence in situ hybridization revealed that ERBB2 was coamplified with EGFR in 7 tumors, FGFR2 in 1 tumor, and FGFR2 and MET in 1 tumor; however, the respective genes were amplified in mutually exclusive cells. Coamplified ERBB2 and MYC coexisted within single nuclei in 4 tumors, and one of these cases had suspected coamplification in the same amplicon of ERBB2 with MYC