Appendix

Appendix 1. Advanced PubMed Search performed on the 20th of June 2024

Study Population	#1	Search: ("Breast Neoplasms"[Mesh]) OR (breast cancer*[tiab] OR breast neoplasm*[tiab] OR breast carcinoma*[tiab] OR breast tumor*[tiab] OR breast tumour*[tiab])	474,590
Outcome	#2	Search: ("Survival"[Mesh] OR "Mortality"[Mesh] OR "mortality" [Subheading]) OR (surviv*[tiab] OR overall survival[tiab] OR OS[tiab] OR PFS[tiab] OR clinical impact[tiab] OR response[tiab] OR objective response rate[tiab] OR ORR[tiab])	4,451,477
Treatment	#3	Search: ("Trastuzumab"[Mesh]) OR (trastuzumab*[tiab] OR Herceptin*[tiab] OR rhuMAb HER2[tiab] OR anti-HER2 monoclonal antibod*[tiab])	16,075
Diagnostic Tests	#4	Search: ("Immunohistochemistry"[Mesh]) OR (immunohistochemistry[tiab] OR immuno histochemistry[tiab] OR immuno-histochemistry[tiab])	767,439
	#5	Search: ("In Situ Hybridization"[Mesh] OR "In Situ Hybridization, Fluorescence"[Mesh]) OR (in situ hybridization[tiab] OR ISH[tiab] OR FISH[tiab] OR CISH[tiab] OR SISH[tiab] OR in situ hybridisation[tiab] OR in-situ hybridization[tiab] OR in-situ hybridisation[tiab])	331,895
	#6	Search: ("Enzyme-Linked Immunosorbent Assay"[Mesh]) OR (enzyme-linked immunosorbent assay[tiab] OR enzyme linked immunosorbent assay[tiab] OR ELISA[tiab] or enzyme-linked immuno sorbent assay[tiab] OR enzyme linked immuno sorbent assay[tiab])	329,116
	#7	Search: ("Blotting, Western"[Mesh]) OR (western blot[tiab] OR western blotting[tiab] OR western blot*[tiab])	419,327
	#8	Search: ("Polymerase Chain Reaction"[Mesh]) OR (polymerase chain reaction[tiab] OR PCR[tiab] OR polymerase chain reaction*[tiab])	996,990
Combination of 2 tests	#9	Search: #4 AND #5	40,029
	#10	Search: #4 AND #6	169,650
	#11	Search: #4 AND #7	102,716
	#12	Search: #4 AND #8	119,105
	#13	Search: #5 AND #6	3,421
	#14	Search: #5 AND #7	9,242
	#15	Search: #5 AND #8	46,065
	#16	Search: #6 AND #7	55,272
	#17	Search: #6 AND #8	66,599
	#18	Search: #7 AND #8	151,628

Either of the test combinations	#19	Search: #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18	526,474
Original Search	#20	Search: #1 AND #2 AND #3 AND #19	500
Final Search with allowed publication type	#21	Search: #1 AND #2 AND #3 AND #19 AND ("adaptive clinical trial"[Publication Type] OR "clinical study"[Publication Type] OR "clinical trial"[Publication Type] OR "clinical trial, phase i"[Publication Type] OR "clinical trial, phase ii"[Publication Type] OR "clinical trial, phase iii"[Publication Type] OR "clinical trial, phase iv"[Publication Type] OR "comparative study"[Publication Type] OR "controlled clinical trial"[Publication Type] OR "evaluation study"[Publication Type] OR "multicenter study"[Publication Type] OR "observational study"[Publication Type] OR "pragmatic clinical trial"[Publication Type] OR "randomized controlled trial"[Publication Type] OR "validation study"[Publication Type])	121

Appendix 2. Advanced CENTRAL Search performed on the 20th of June 2024

Study Population	#1	MeSH descriptor: [Breast Neoplasms] this term only	19911
	#2	(breast NEXT (cancer* or tumor* or tumour* or neoplasm* or carcinoma*)):ti,ab,kw	45895
	#3	#1 OR #2	45895
Outcome	#4	MeSH descriptor: [Survival] this term only	183
	#5	MeSH descriptor: [Mortality] this term only	1031
	#6	(surviv*):ti,ab,kw OR (OS):ti,ab,kw OR (PFS):ti,ab,kw OR ("clinical impact"):ti,ab,kw OR (response):ti,ab,kw	397775
	#7	("objective response rate"):ti,ab,kw OR (ORR):ti,ab,kw	13614
	#8	#4 OR #5 OR #6 OR #7	398606
Treatment	#9	MeSH descriptor: [Trastuzumab] this term only	1198
	#10	(trastuzumab*):ti,ab,kw OR (herceptin*):ti,ab,kw OR ("rhuMAb HER2"):ti,ab,kw OR ("anti-HER2 monoclonal antibody"):ti,ab,kw	3683
	#11	#9 OR #10	3683
Diagnostic Tests	#12	MeSH descriptor: [Immunohistochemistry] this term only	1755
	#13	(immunohistochemistry):ti,ab,kw OR ("immuno histochemistry"):ti,ab,kw OR (immuno-histochemistry):ti,ab,kw	6847
	#14	#12 OR #13	6847
	#15	MeSH descriptor: [In Situ Hybridization] this term only	101
	#16	MeSH descriptor: [In Situ Hybridization, Fluorescence] this term only	298
	#17	("in situ hybridization"):ti,ab,kw OR ("in situ hybridisation"):ti,ab,kw OR ("in-situ hybridization"):ti,ab,kw OR ("in-situ hybridisation"):ti,ab,kw	1487
	#18	(FISH):ti,ab,kw OR (CISH):ti,ab,kw OR (SISH):ti,ab,kw OR (ISH):ti,ab,kw	7973
	#19	#15 OR #16 OR #17 OR #18	8829
	#20	MeSH descriptor: [Enzyme-Linked Immunosorbent Assay] this term only	2937
	#21	("enzyme linked immuno sorbent assay"):ti,ab,kw OR ("enzyme-linked immuno sorbent assay"):ti,ab,kw OR ("enzyme linked immunosorbent assay"):ti,ab,kw OR ("enzyme-linked immunosorbent assay"):ti,ab,kw OR (ELISA):ti,ab,kw	17729
	#22	#20 OR #21	17729

	#23	MeSH descriptor: [Blotting, Western] this term only	392
	#24	(western NEXT (blot*)):ti,ab,kw	1906
	#25	#23 OR #24	2117
	#26	MeSH descriptor: [Polymerase Chain Reaction] this term only	1675
	#27	("polymerase chain reaction"):ti,ab,kw OR (PCR):ti,ab,kw	21720
	#28	#26 OR #27	21720
Combination of 2 tests	#29	#14 AND #19	801
	#30	#14 AND #22	294
	#31	#14 AND #25	318
	#32	#14 AND #28	950
	#33	#19 AND #22	182
	#34	#19 AND #25	28
	#35	#19 AND #28	557
	#36	#22 AND #25	501
	#37	#22 AND #28	1739
	#38	#25 AND #28	802
Either of the test combinations	#39	#29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38	4942
Publication Types to be Excluded	#40	(("Trial registry record" OR conference* OR "Clinical trial protocol" OR Retraction* OR Preprint* OR Erratum* OR "Dissertation thesis" OR Editorial* OR Letter* OR Comment* OR Note* OR "Multimedia" OR "Expression of concern")):pt (Word variations have been searched)	760691
Final Search	#41	#3 AND #8 AND #11 AND #39 NOT #40	69

Appendix 3. Adapted ROBINS-I tool

Domain 1: Confounding

- **Question 1.1:** If present, did the study control for confounding variables that could affect the outcome within the cohort?
- **Response Options**: Yes/Probably Yes/No Information/Probably No/No
- **Bias**: Low/Moderate/High/Unclear

Domain 2: Classification of Interventions

- **Question 2.1**: Was/were the intervention(s) clearly defined?
- **Question 2.2**: Was the information used to define intervention groups recorded at the start of the intervention?
- **Question 2.3**: Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?
- **Response Options**: Yes/Probably Yes/No Information/Probably No/No
- **Bias**: Low/Moderate/High/Unclear

Domain 3: Deviations from Intended Interventions

- **Question 3.1**: Were there deviations from the intended intervention beyond what would be expected in usual practice?
- **Question 3.2**: If Y/PY to 3.1: were deviations from the intended intervention unlikely to have affected the outcome or was this corrected for in the analysis?
- **Response Options**: Yes/Probably Yes/No Information/Probably No/No
- **Bias**: Low/Moderate/High/Unclear

Domain 4: Missing Data

- **Question 4.1**: Was the data complete within the cohort?
- **Question 4.2**: If N/PN to 4.1: Were adjustment techniques used in the analysis that are likely to correct for the presence bias due to missing data or was the missing data not likely to cause bias?
- **Response Options**: Yes/Probably Yes/No Information/Probably No/No
- **Bias**: Low/Moderate/High/Unclear

Domain 5: Measurement of Outcomes

- **Question 5.1**: Were the outcome assessors blinded to the intervention status within the cohort?
- **Question 5.2**: If N/PN to 5.1: Could the outcome measure have been influenced by knowledge of the intervention received?
- **Response Options**: Yes/Probably Yes/No Information/Probably No/No
- **Bias**: Low/Moderate/High/Unclear

Domain 6: Selection of the Reported Result

- **Question 6.1**: Was the reported result selected based on the pre-specified analysis plan within the cohort?
- **Response Options**: Yes/Probably Yes/No Information/Probably No/No
- **Bias**: Low/Moderate/High/Unclear

Appendix 4. Adapted QUADAS-C tool

1. Patient Selection

- **Question 1.1**: Was the selection of patients consecutive or random?
- **Question 1.2**: Was a case-control design avoided?
- **Question 1.3**: Did the study avoid inappropriate exclusions?
- **Question 1.4**: Was the patient spectrum representative of the patients who will receive the tests in practice?
- **Question 1.5**: Was a fully paired or randomized design used?
- **Question 1.6**: If a randomized design used: Was the allocation sequence random and was the allocation sequence concealed until patients were enrolled and assigned to tests?

Response Options: Yes/No/Unclear
Bias: Low/Moderate/High/Unclear

2. Tests

- **Question 2.1**: Were the tests clearly described?
- **Question 2.2**: Were the tests conducted as per the protocol?
- **Question 2.3**: Was the interpretation of the tests conducted without knowledge of other test results?
- **Question 2.4**: Is undergoing one test unlikely to affect the performance of the other test(s)?
- **Question 2.5**: Were the tests conducted and interpreted without advantaging one of the tests?

Response Options: Yes/No/Unclear
Bias: Low/Moderate/High/Unclear

3. Flow and Timing

- **Question 3.1**: Was there an appropriate interval between the tests and follow-up?
- **Question 3.2**: Did all patients receive the same tests regardless of the results of the index tests/ did all patients receive both (or all) tests?
- **Question 3.3**: Are the proportions and reasons for missing data similar across the tests? If yes, was this appropriately handled or unlikely to affect the result?

Response Options: Yes/No/Unclear
Bias: Low/Moderate/High/Unclear