

Immunohistochemical staining methods Round 2, 2023

Specimens

Please find enclosed 5 unstained slides S001 - S005.

Background information

Each participating laboratory receives five unstained slides, one for each staining. Each slide has several sections of paraffin block. The sections of 3-4 µm thickness have been prepared from paraffin embedded tissue fixed in 10% neutral phosphate-buffered formalin (24-48 hours). The sections were cut on slides and have not been dried or otherwise attached to the slides.

Storage and use

The sections should be dried and attached on slides using heat treatment.

Please store the slides at room temperature before and after staining. If all the mentioned antibodies are not included in your selection, you can participate also with selected antibodies according to your order: product 6600 for 1-5 antibodies, product 6600S for 1-3 antibodies. Please note that we will send all the 5 slides in both products. If you have ordered 6600S, please return maximum 3 stained slides.

Please, remember to label each slide clearly with given Labquality account number. If you order multiple sample sets with the same client code, please separate them with roman numerals or the alphabet.

Returning slides and protocol reporting

Please return the stained slides in the same slide mailer box wrapped in bubble wrap at participant's own cost to the address given in the column on the right side of the page unless our local partner has instructed otherwise. An address label is included with your shipment for return.

The slides should be at Labquality's office no later than the date given in the column on the right side of the page. Please note that slides received after that will no longer be included in the evaluation.

For each antibody staining, please fill a separate staining techniques protocol form of the used method via LabScala. We can provide a more comprehensive final report that serves the customer if the method information is given as detailed as possible.

Examination

S001: PR



S004: Ki-67



S002: HER2



S005: HER2 -ISH



S003: ER



2023-09-12

INSTRUCTIONS

Product no. 6600 / 6600S
LQ778323021-025/FI

If the kit is incomplete or contains damaged specimens, please report immediately to info@labquality.fi

The slides should be returned no later than **October 24, 2023.**

Inquiries

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Pia Eloranta
pia.eloranta@labquality.fi

Labquality Oy

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FI-00520 HELSINKI
Finland

Tel. + 358 9 8566 8200

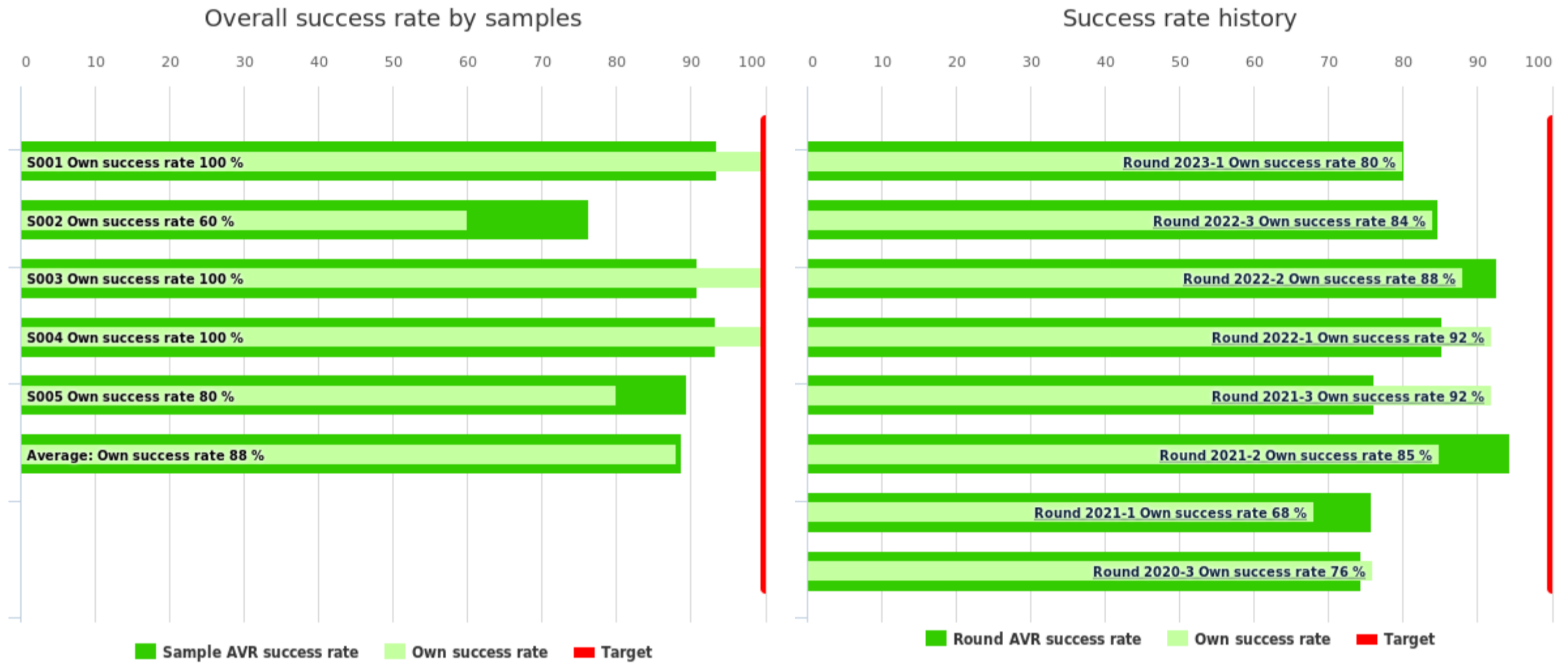
info@labquality.fi
www.labquality.com



Client report

	No of participants	No of responded participants	Response percentage
Immunohistochemical staining methods, limited selection of antibodies, September, 2-2023	34	34	100 %
Immunohistochemical staining methods, September, 2-2023	69	69	100 %

Summary

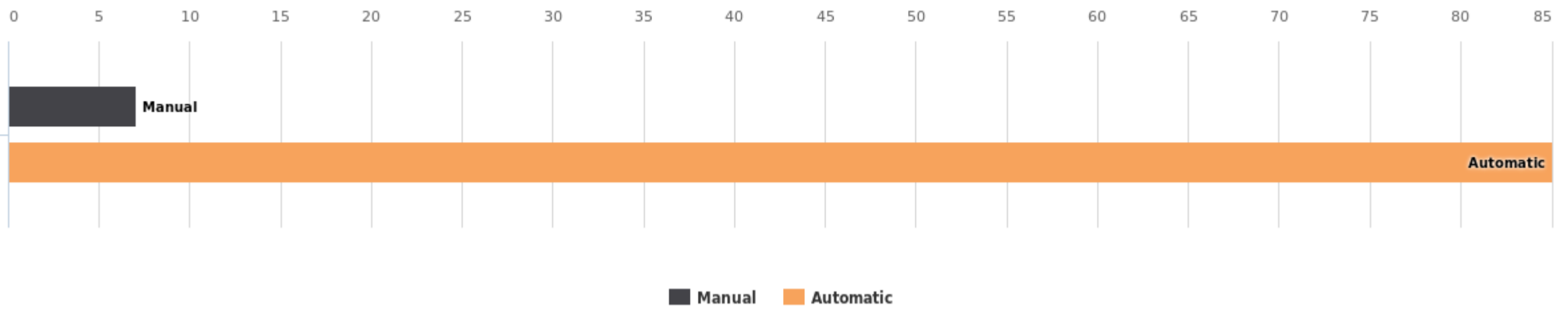


Summary	Own score	Max score	Own success rate	Difference	AVR success rate
S001	5	5	100 %	6.5 %	93.5 %
S002	3	5	60 %	-16.4 %	76.4 %
S003	5	5	100 %	9.2 %	90.8 %
S004	5	5	100 %	6.6 %	93.4 %
S005	4	5	80 %	-9.5 %	89.5 %
Average:			88 %	-0.7 %	88.7 %

History	Test nr.	Own success rate	Difference	AVR success rate
Round 2023-1	1-1	80 %	-0.1 %	80.1 %
Round 2022-3	1-1	84 %	-0.8 %	84.8 %
Round 2022-2	1-1	88 %	-4.7 %	92.7 %
Round 2022-1	1-1	92 %	6.7 %	85.3 %
Round 2021-3	1-1	92 %	15.9 %	76.1 %
Round 2021-2	1-1	85 %	-9.4 %	94.4 %
Round 2021-1	1-1	68 %	-7.8 %	75.8 %
Round 2020-3	1-1	76 %	1.7 %	74.3 %

S001 | Sample S001 PR

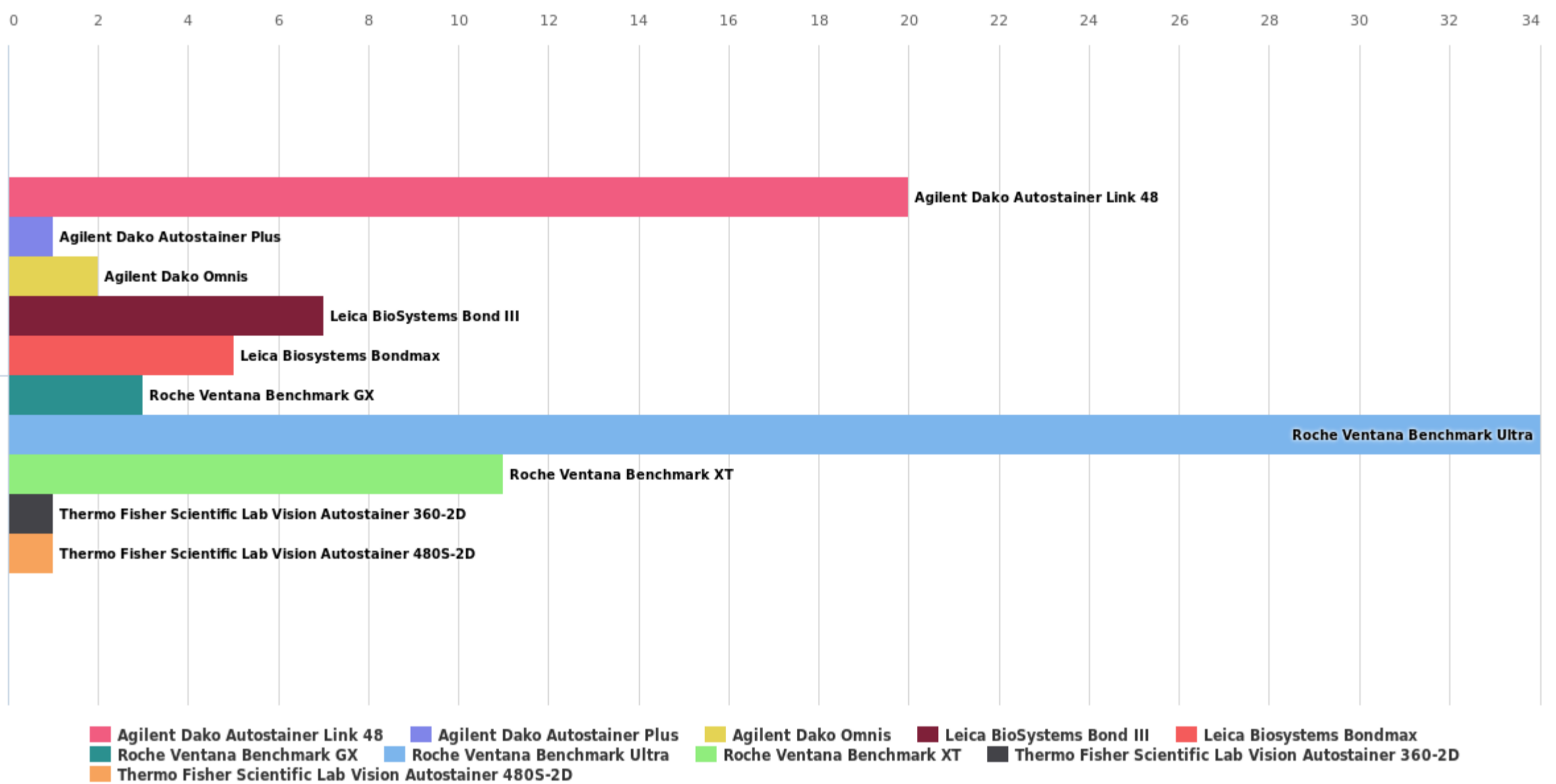
Performance



GENERAL DETAILS OF STAINING

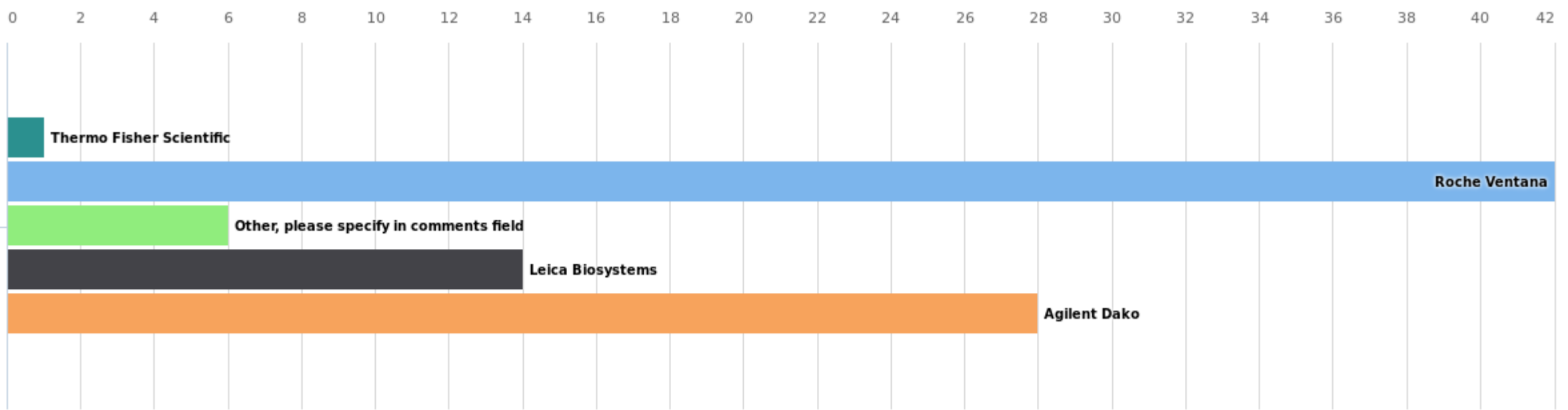
Performance	Performance count
<input checked="" type="radio"/> Automatic	85
Manual	7
Total:	92

Instrument



Instrument	Instrument count
Agilent Dako Autostainer Link 48	20
Agilent Dako Autostainer Plus	1
Agilent Dako Omnis	2
Leica BioSystems Bond III	7
<input checked="" type="radio"/> Leica Biosystems Bondmax	5
Roche Ventana Benchmark GX	3
Roche Ventana Benchmark Ultra	34
Roche Ventana Benchmark XT	11
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Total:	85

Manufacturer

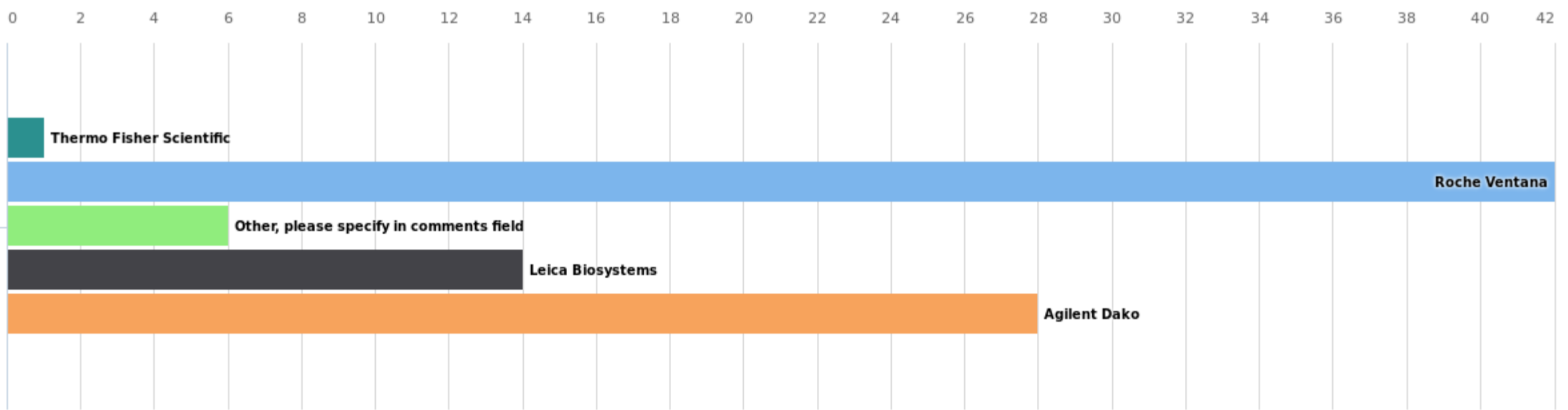


Thermo Fisher Scientific Roche Ventana Other, please specify in comments field Leica Biosystems Agilent Dako

PRIMARY ANTIBODY

Manufacturer	Manufacturer count
<input checked="" type="radio"/> Agilent Dako	28
Leica Biosystems	14
Other, please specify in comments field	6
Roche Ventana	42
Thermo Fisher Scientific	1
Total:	91

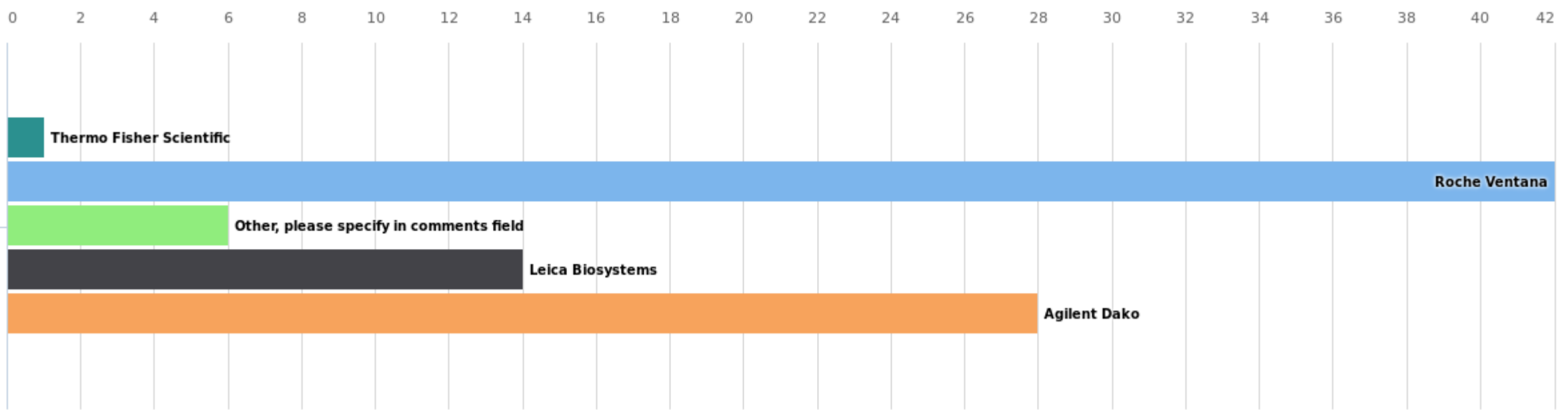
Clonal



Thermo Fisher Scientific Roche Ventana Other, please specify in comments field Leica Biosystems Agilent Dako

Clonal	Clonal count
<input checked="" type="radio"/> Monoclonal	89
Polyclonal	1
Total:	90

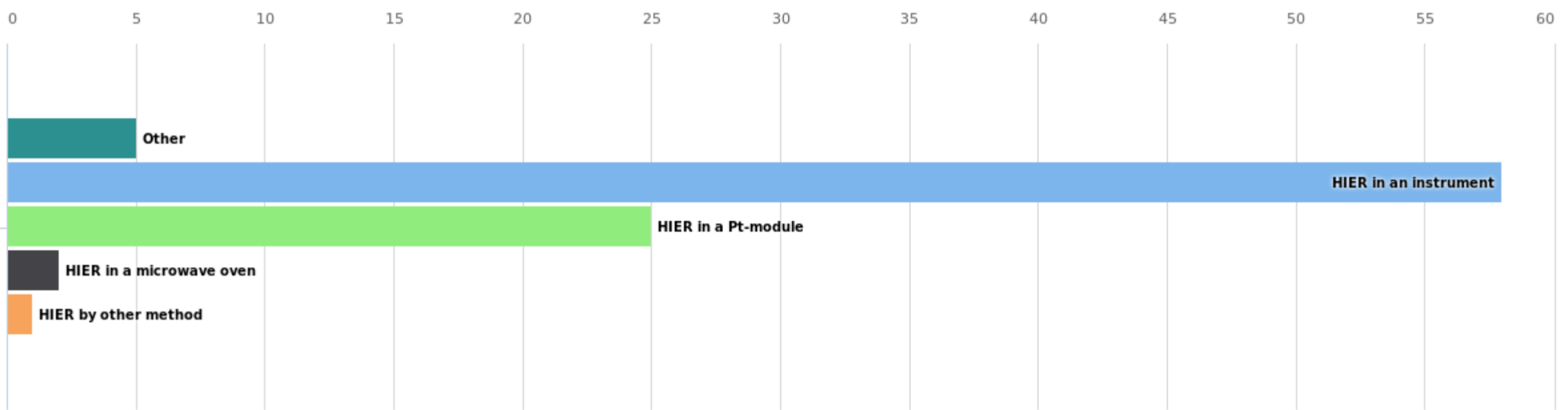
Dilution



Thermo Fisher Scientific Roche Ventana Other, please specify in comments field Leica Biosystems Agilent Dako

Dilution	Dilution count
<input checked="" type="radio"/> Dilution	26
Ready-to-use	65
Total:	91

S001 Pretreatment

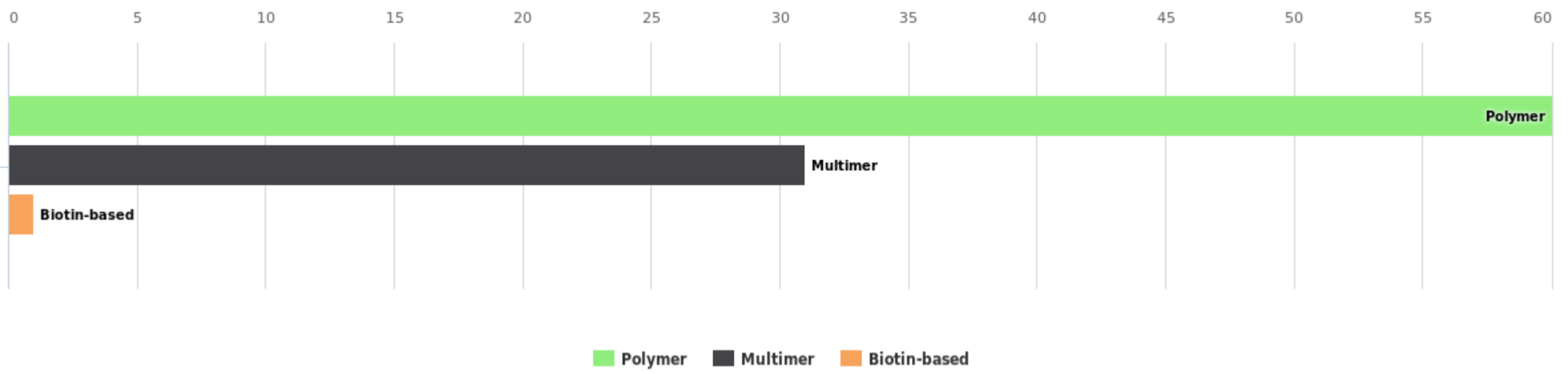


Other HIER in an instrument HIER in a Pt-module HIER in a microwave oven HIER by other method

PRETREATMENT

Pretreatment	Pretreatment count
HIER by other method	1
HIER in a microwave oven	2
HIER in a Pt-module	25
<input checked="" type="radio"/> HIER in an instrument	58
Other	5
Total:	91

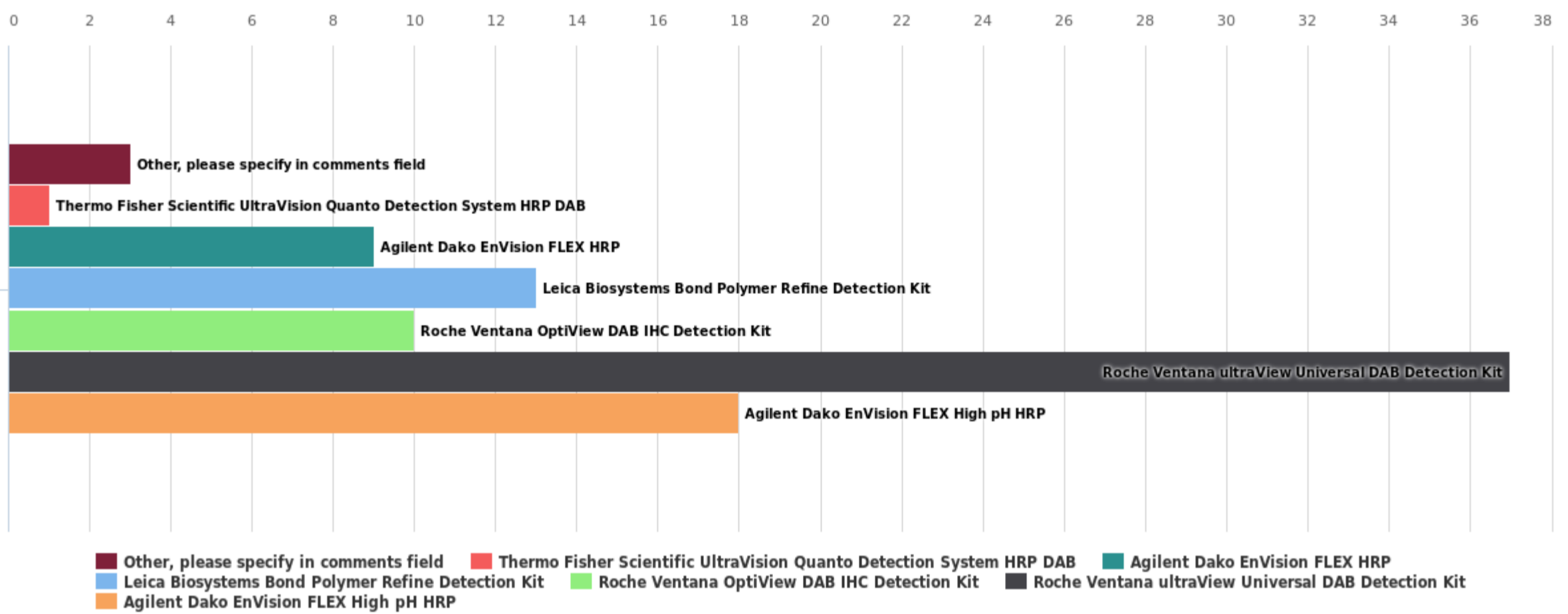
S001 Detection system



DETECTION SYSTEM

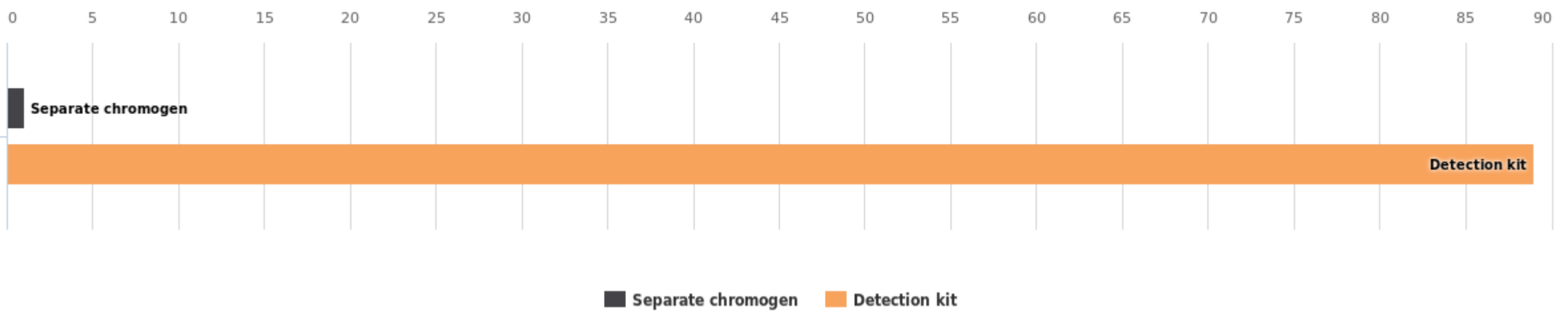
Detection system	Detection system count
Biotin-based	1
Multimer	31
<input checked="" type="radio"/> Polymer	60
Total:	92

Detection system: Instrument



Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	9
Agilent Dako EnVision FLEX High pH HRP	18
<input checked="" type="radio"/> Leica Biosystems Bond Polymer Refine Detection Kit	13
Other, please specify in comments field	3
Roche Ventana OptiView DAB IHC Detection Kit	10
Roche Ventana ultraView Universal DAB Detection Kit	37
Thermo Fisher Scientific UltraVision Quanto Detection System HRP DAB	1
Total:	91

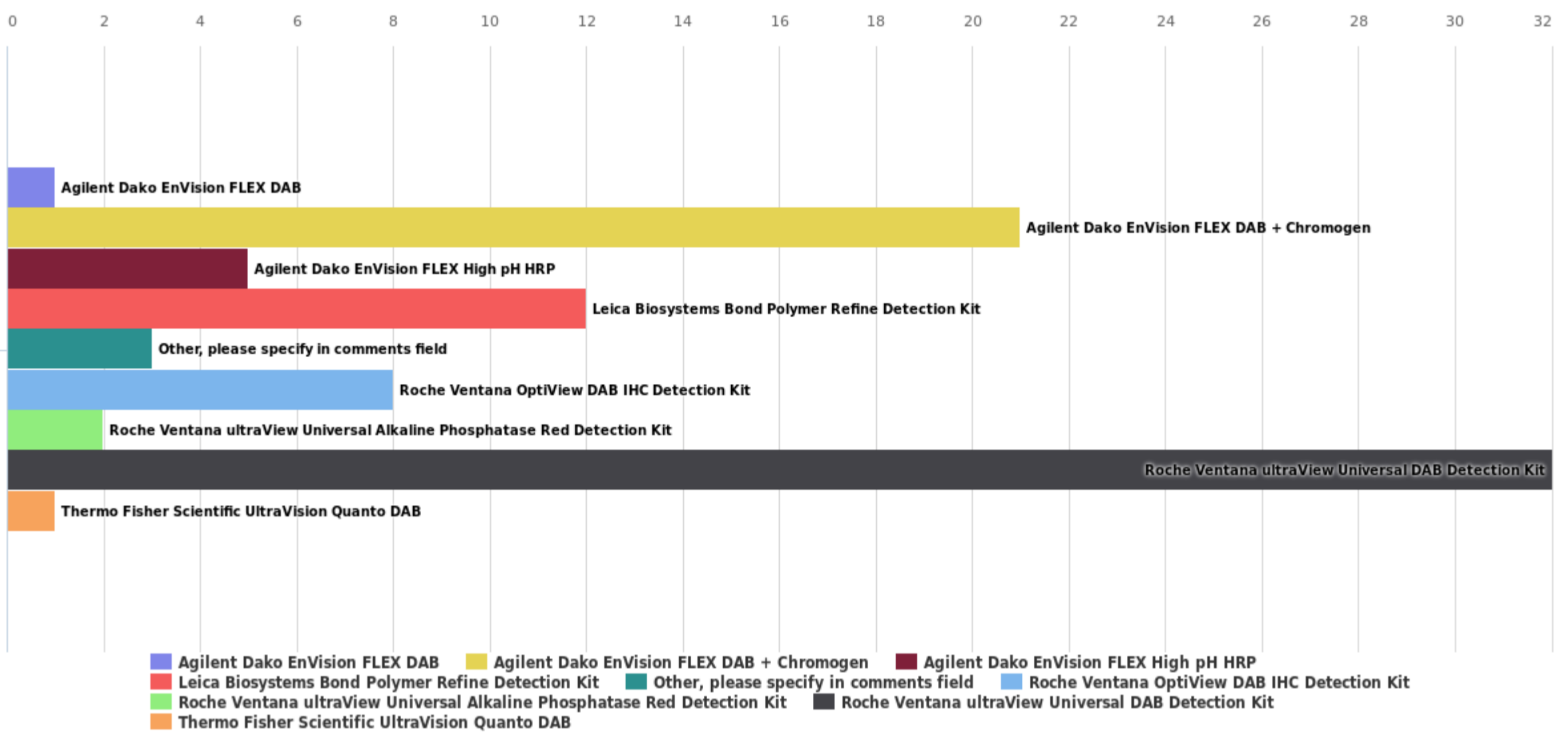
S001 Chromogen



CHROMOGEN

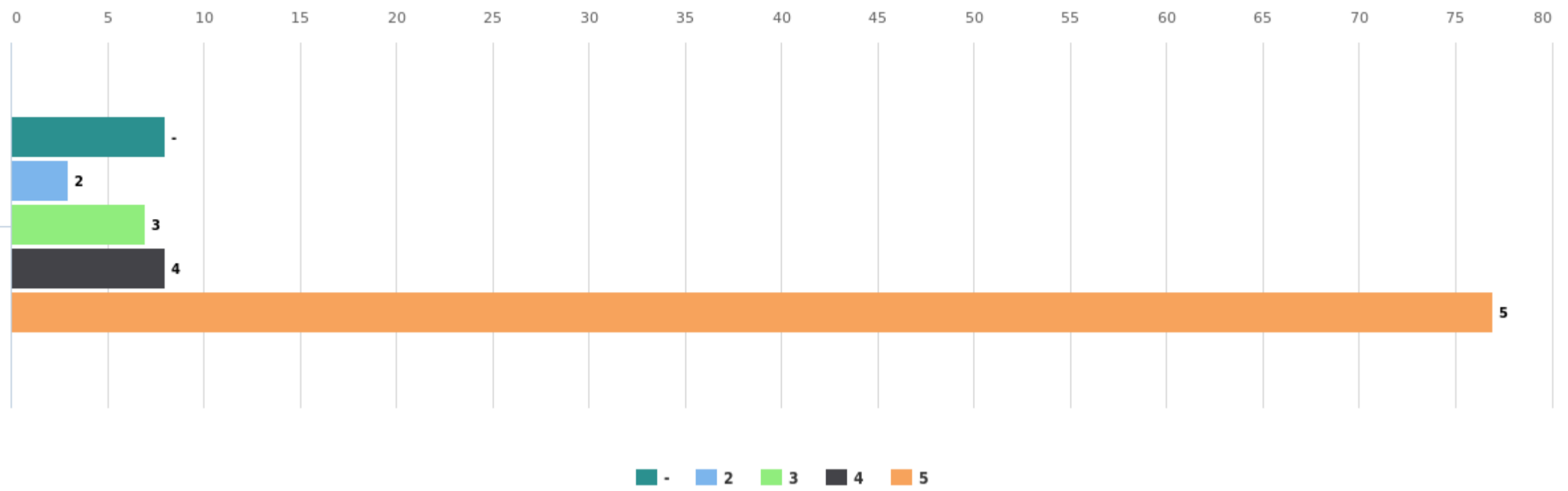
Chromogen	Chromogen count
<input checked="" type="radio"/> Detection kit	89
Separate chromogen	1
Total:	90

Chromogen: Instrument



Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	1
Agilent Dako EnVision FLEX DAB + Chromogen	21
Agilent Dako EnVision FLEX High pH HRP	5
<input checked="" type="radio"/> Leica Biosystems Bond Polymer Refine Detection Kit	12
Other, please specify in comments field	3
Roche Ventana OptiView DAB IHC Detection Kit	8
Roche Ventana ultraView Universal Alkaline Phosphatase Red Detection Kit	2
Roche Ventana ultraView Universal DAB Detection Kit	32
Thermo Fisher Scientific UltraVision Quanto DAB	1
Total:	85

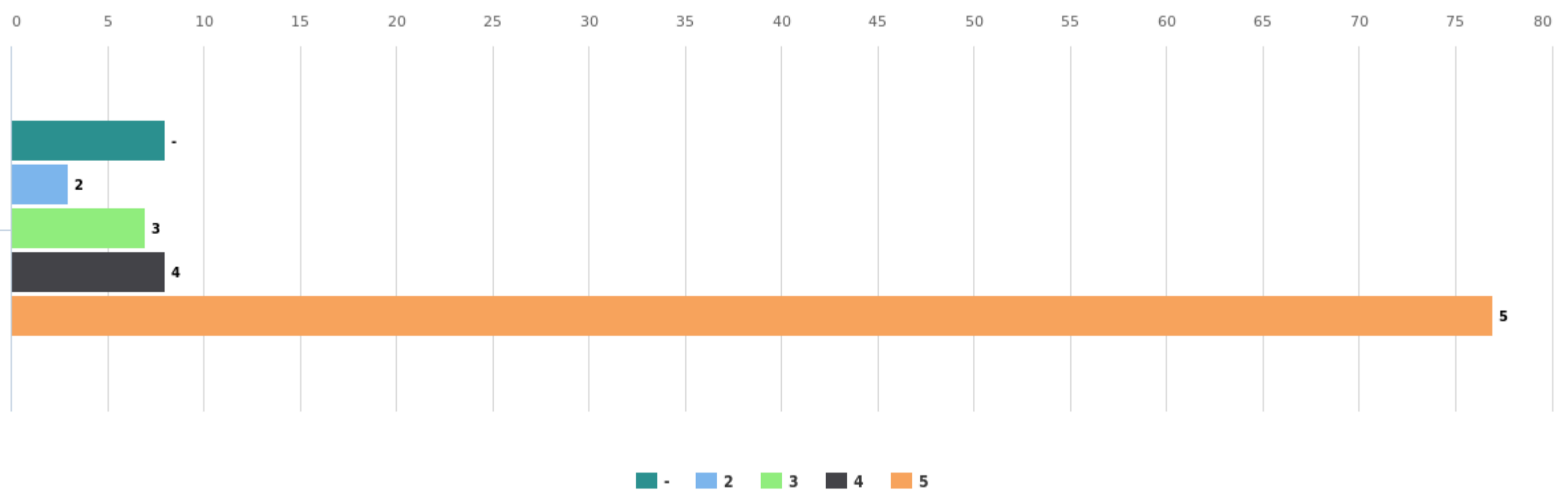
S001 Score



SCORE

Score	Comment
-	Not Available
2	False Negative Staining, Hematoxylin Also Weak, May Endanger Diagnosis False Negative Staining, May Endanger Diagnosis
3	No Hematoxylin, Bubble On Top Of Sample, Incomplete Staining Scraped Samples, Difficult To Evaluate Some False Negative Staining
4	Dark Hematoxylin Hematoxylin Weak Hematoxylin Weak, Staining Intensity A Bit Weak Morphology Impaired Some Background Staining Staining Intensity A Bit Weak
<input checked="" type="radio"/> 5	Embedding Failed <input checked="" type="radio"/> Excellent
Total:	

S001 Score

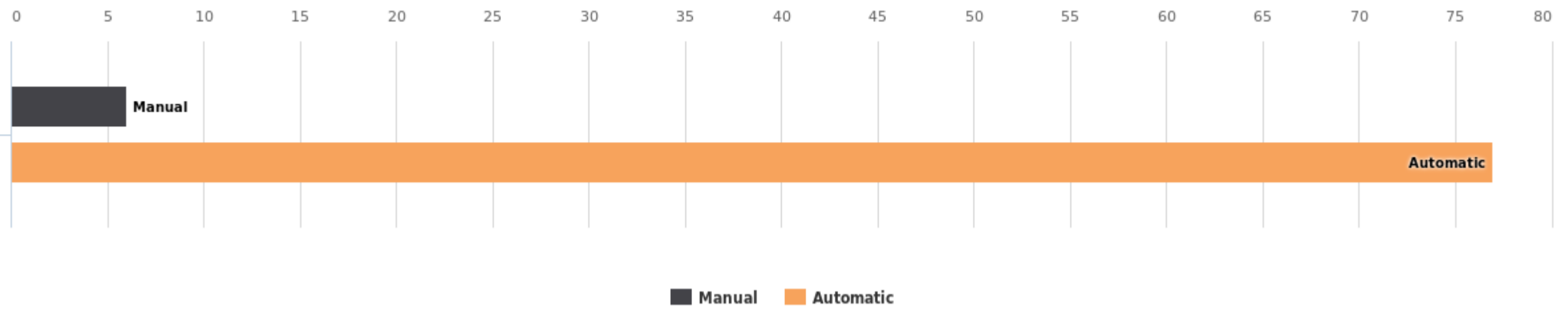


Score	Score count
-	8
2	3

3	7
4	8
<input checked="" type="radio"/> 5	77
Total:	103

S002 | Sample S002 HER2

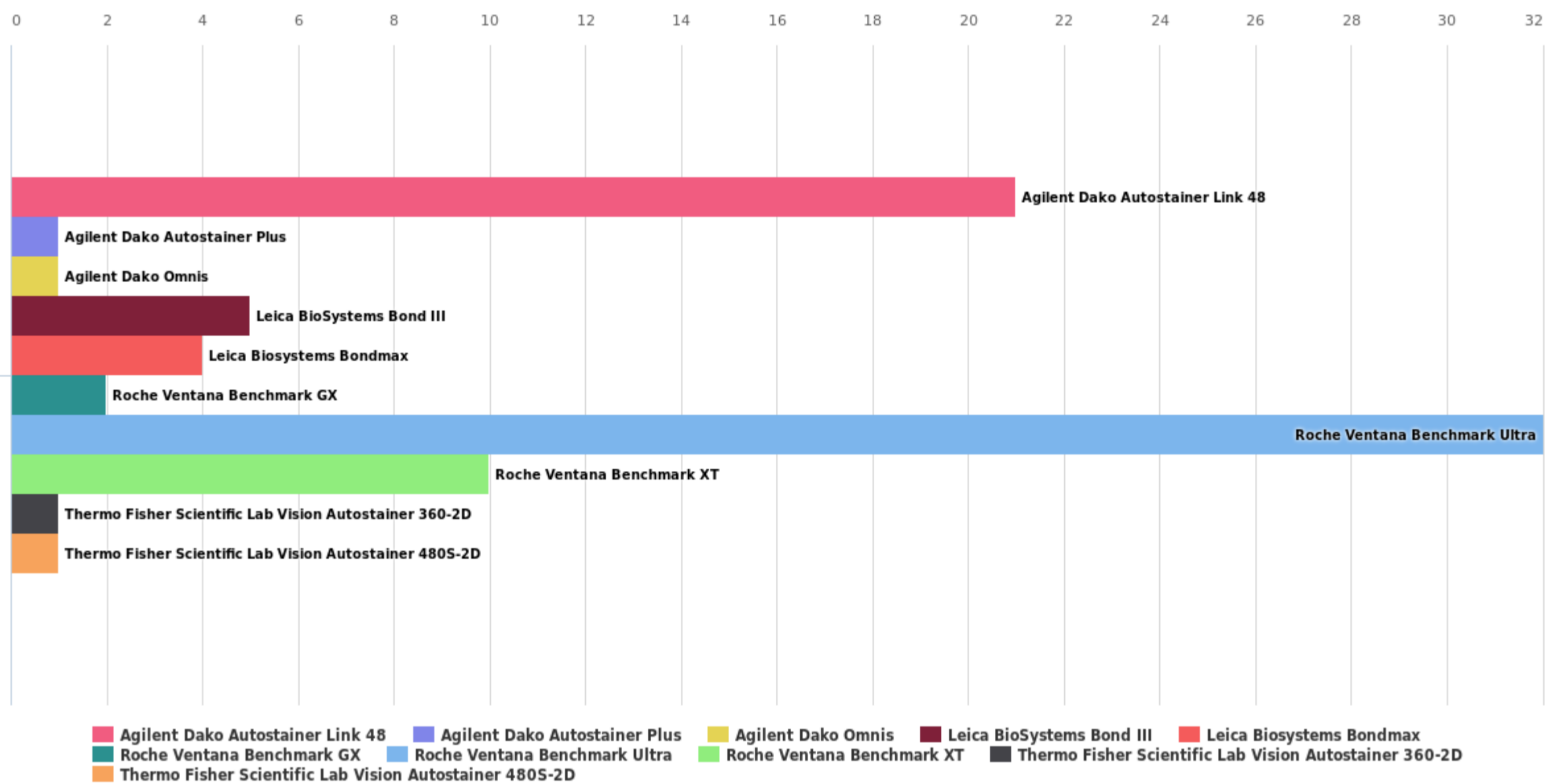
Performance



GENERAL DETAILS OF STAINING

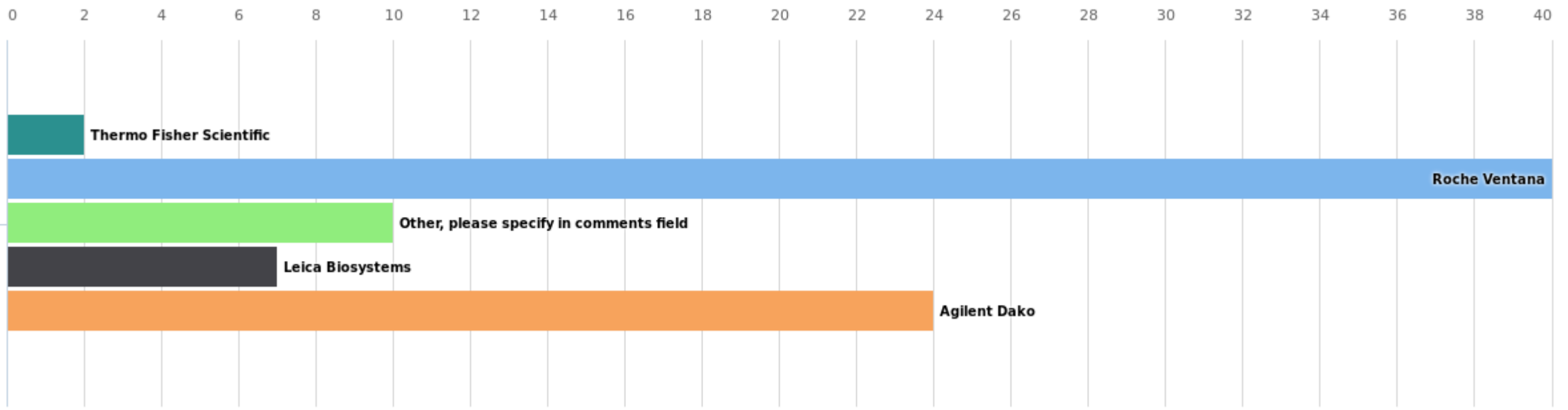
Performance	Performance count
<input checked="" type="radio"/> Automatic	77
Manual	6
Total:	83

Instrument



Instrument	Instrument count
Agilent Dako Autostainer Link 48	21
Agilent Dako Autostainer Plus	1
Agilent Dako Omnis	1
Leica BioSystems Bond III	5
<input checked="" type="radio"/> Leica Biosystems Bondmax	4
Roche Ventana Benchmark GX	2
Roche Ventana Benchmark Ultra	32
Roche Ventana Benchmark XT	10
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Total:	78

Manufacturer

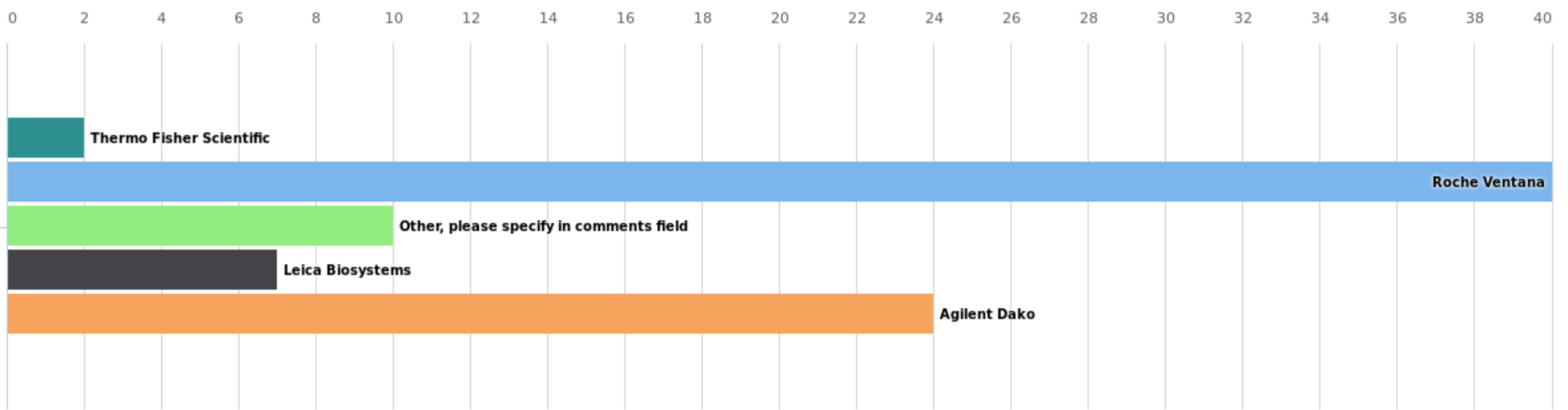


■ Thermo Fisher Scientific
 ■ Roche Ventana
 ■ Other, please specify in comments field
 ■ Leica Biosystems
 ■ Agilent Dako

PRIMARY ANTIBODY

Manufacturer	Manufacturer count
<input checked="" type="radio"/> Agilent Dako	24
Leica Biosystems	7
Other, please specify in comments field	10
Roche Ventana	40
Thermo Fisher Scientific	2
Total:	83

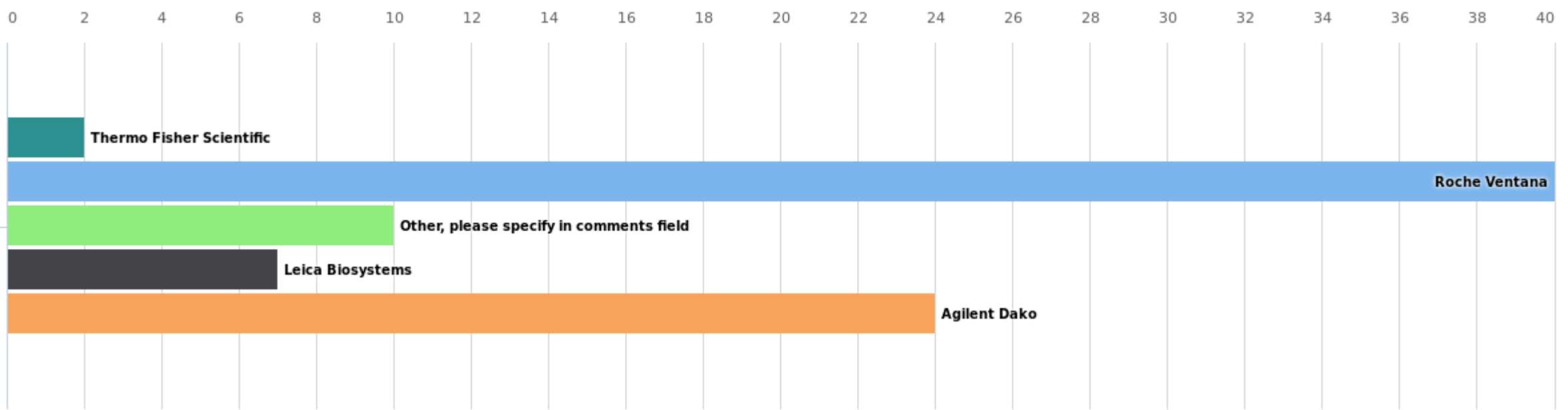
Clonal



■ Thermo Fisher Scientific
 ■ Roche Ventana
 ■ Other, please specify in comments field
 ■ Leica Biosystems
 ■ Agilent Dako

Clonal	Clonal count
Monoclonal	65
<input checked="" type="radio"/> Polyclonal	18
Total:	83

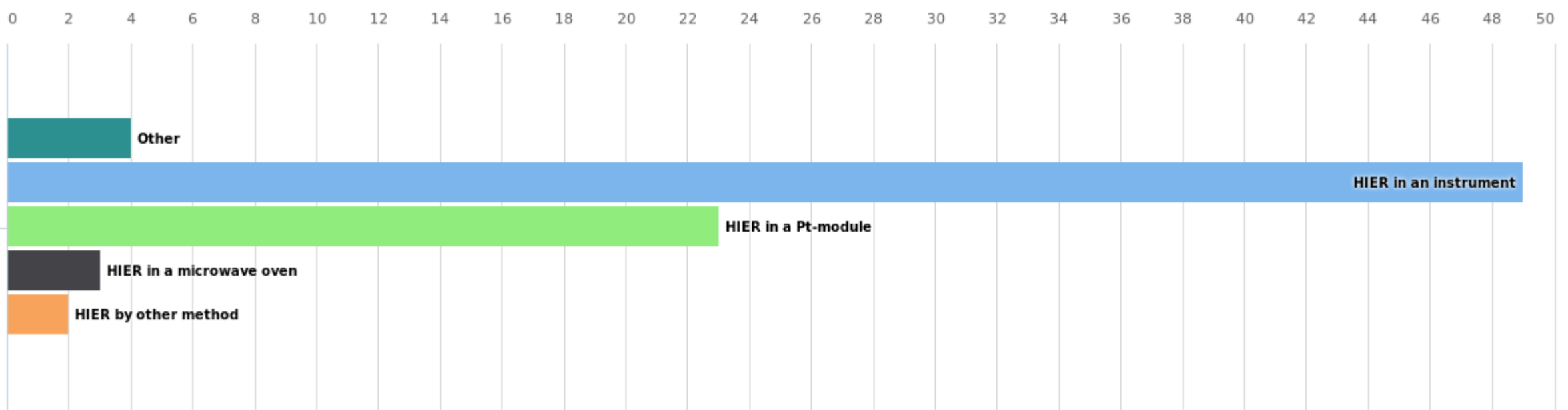
Dilution



Thermo Fisher Scientific Roche Ventana Other, please specify in comments field Leica Biosystems Agilent Dako

Dilution	Dilution count
<input checked="" type="radio"/> Dilution	29
Ready-to-use	54
Total:	83

S002 Pretreatment

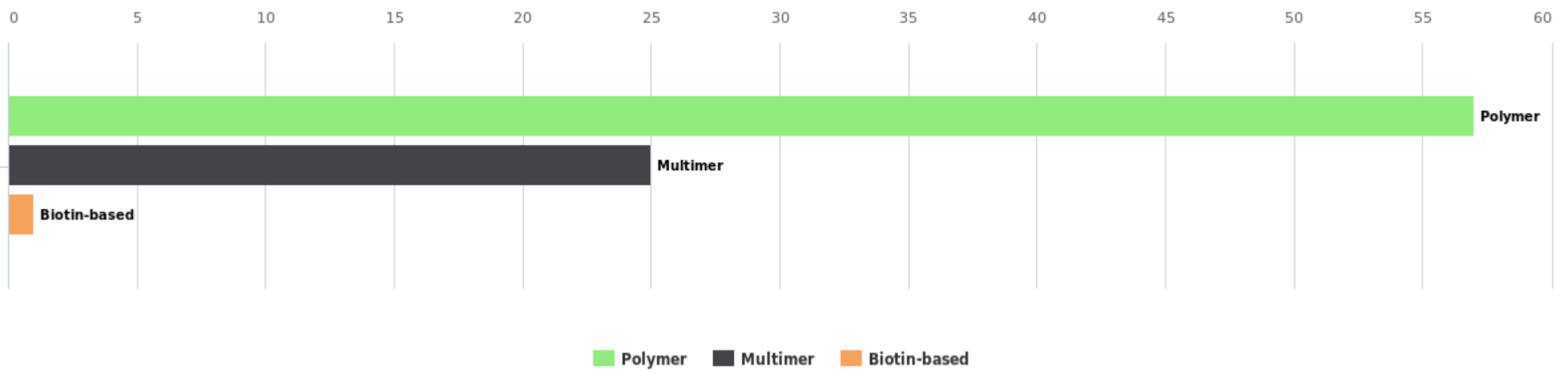


Other HIER in an instrument HIER in a Pt-module HIER in a microwave oven HIER by other method

PRETREATMENT

Pretreatment	Pretreatment count
HIER by other method	2
HIER in a microwave oven	3
HIER in a Pt-module	23
<input checked="" type="radio"/> HIER in an instrument	49
Other	4
Total:	81

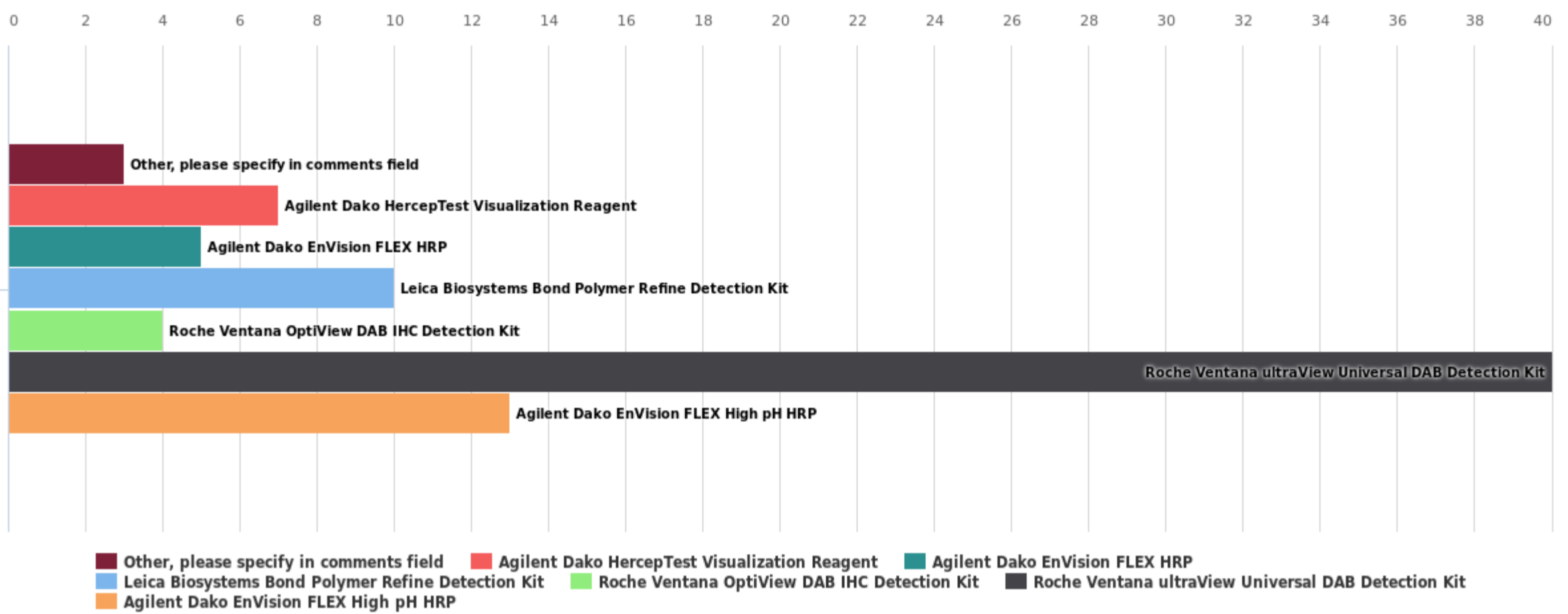
S002 Detection system



DETECTION SYSTEM

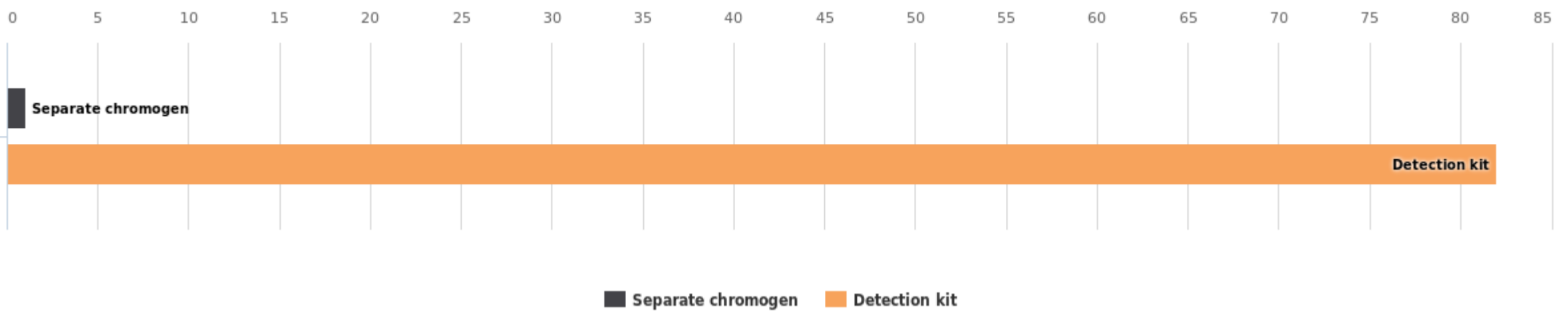
Detection system	Detection system count
Biotin-based	1
Multimer	25
<input checked="" type="radio"/> Polymer	57
Total:	83

Detection system: Instrument



Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	5
Agilent Dako EnVision FLEX High pH HRP	13
Agilent Dako HercepTest Visualization Reagent	7
<input checked="" type="radio"/> Leica Biosystems Bond Polymer Refine Detection Kit	10
Other, please specify in comments field	3
Roche Ventana OptiView DAB IHC Detection Kit	4
Roche Ventana ultraView Universal DAB Detection Kit	40
Total:	82

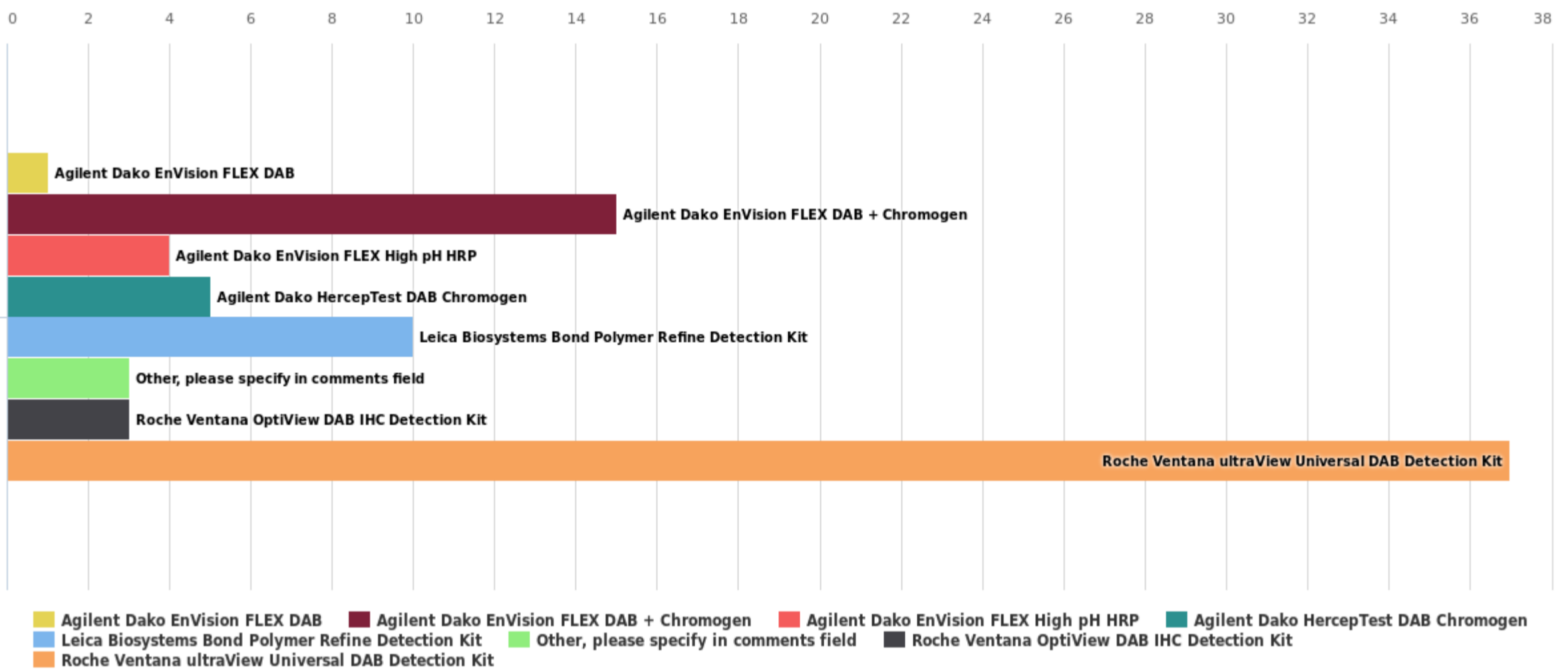
S002 Chromogen



CHROMOGEN

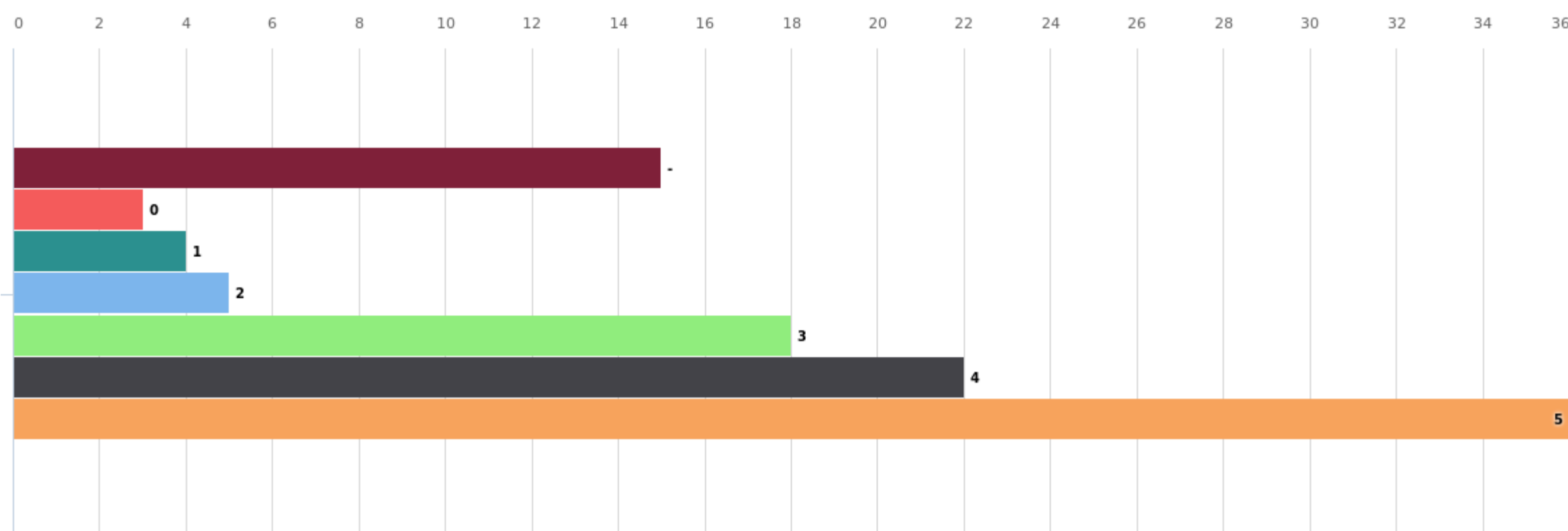
Chromogen	Chromogen count
<input checked="" type="radio"/> Detection kit	82
Separate chromogen	1
Total:	83

Chromogen: Instrument



Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	1
Agilent Dako EnVision FLEX DAB + Chromogen	15
Agilent Dako EnVision FLEX High pH HRP	4
Agilent Dako HercepTest DAB Chromogen	5
<input checked="" type="radio"/> Leica Biosystems Bond Polymer Refine Detection Kit	10
Other, please specify in comments field	3
Roche Ventana OptiView DAB IHC Detection Kit	3
Roche Ventana ultraView Universal DAB Detection Kit	37
Total:	78

S002 Score

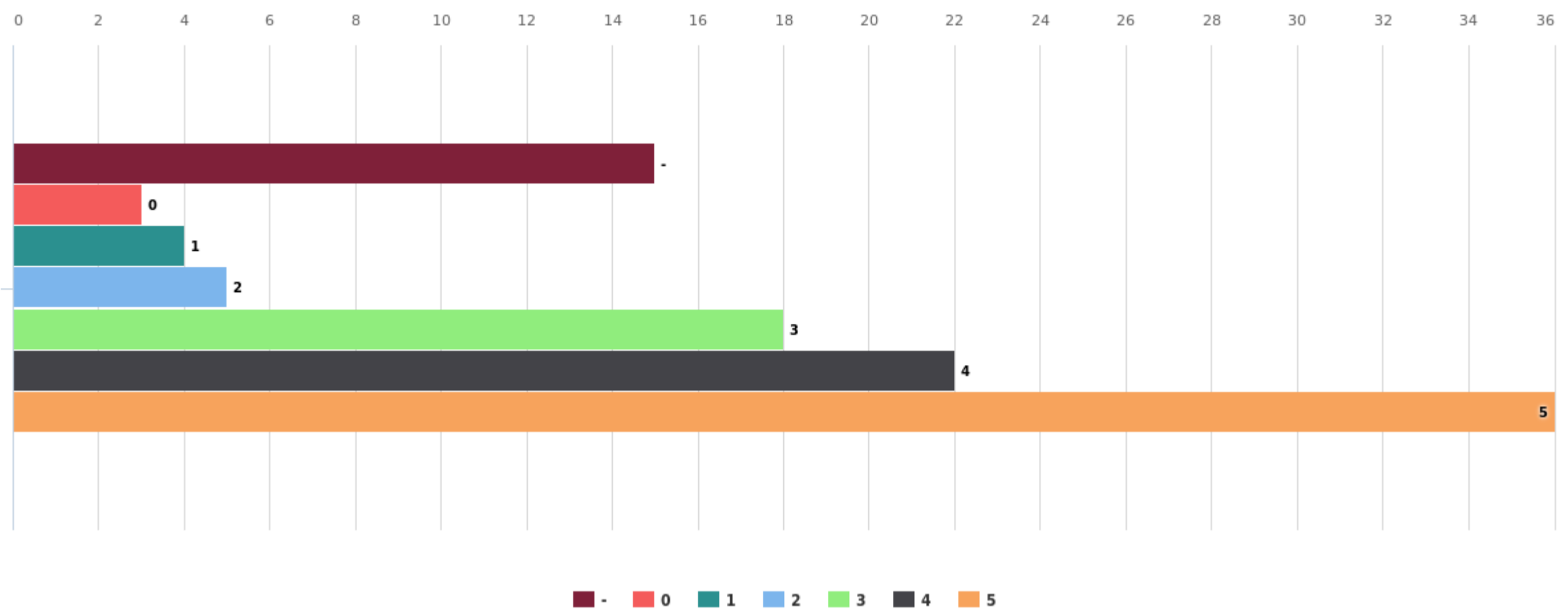


- 0 1 2 3 4 5

SCORE

Score	Comment
-	Not Available
0	Failed, Extreme False Positive Staining, Diagnostically Unacceptable
1	Extreme False Positive Staining, Diagnostically Unacceptable
2	False Positive Staining With Disturbing Strong Background Staining, May Endanger Diagnosis
<input checked="" type="radio"/> 3	False Positive Staining False Positive Staining With Strong Background Staining Strong Background Staining Mainly In The Cytoplasm <input checked="" type="radio"/> Strong Background Staining, Some False Positive Cells Strong Background Staining, Weak Staining Intensity, Hematoxylin Very Weak
4	Mild Background Staining Weak Staining Intensity
5	Excellent
Total:	

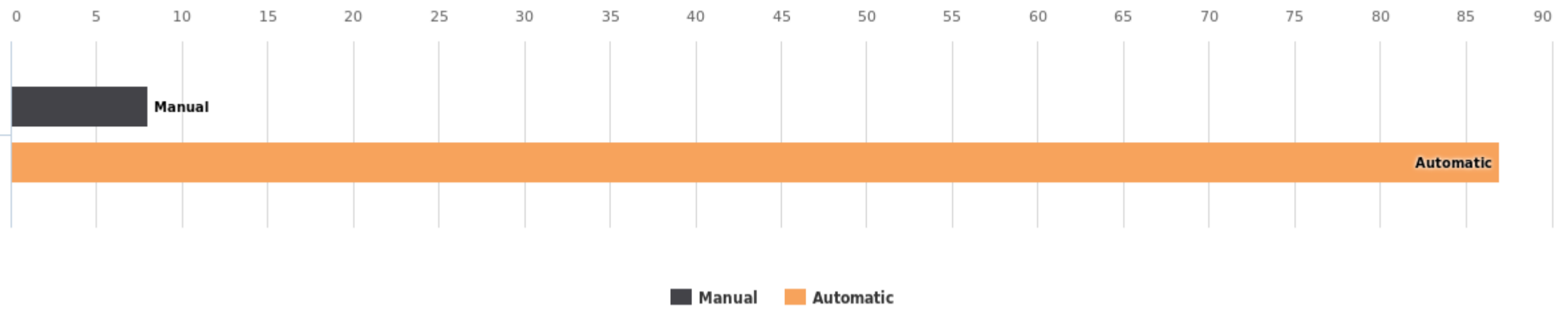
S002 Score



Score	Score count
-	15
0	3
1	4
2	5
<input checked="" type="radio"/> 3	18
4	22
5	36
Total:	103

S003 | Sample S003 ER

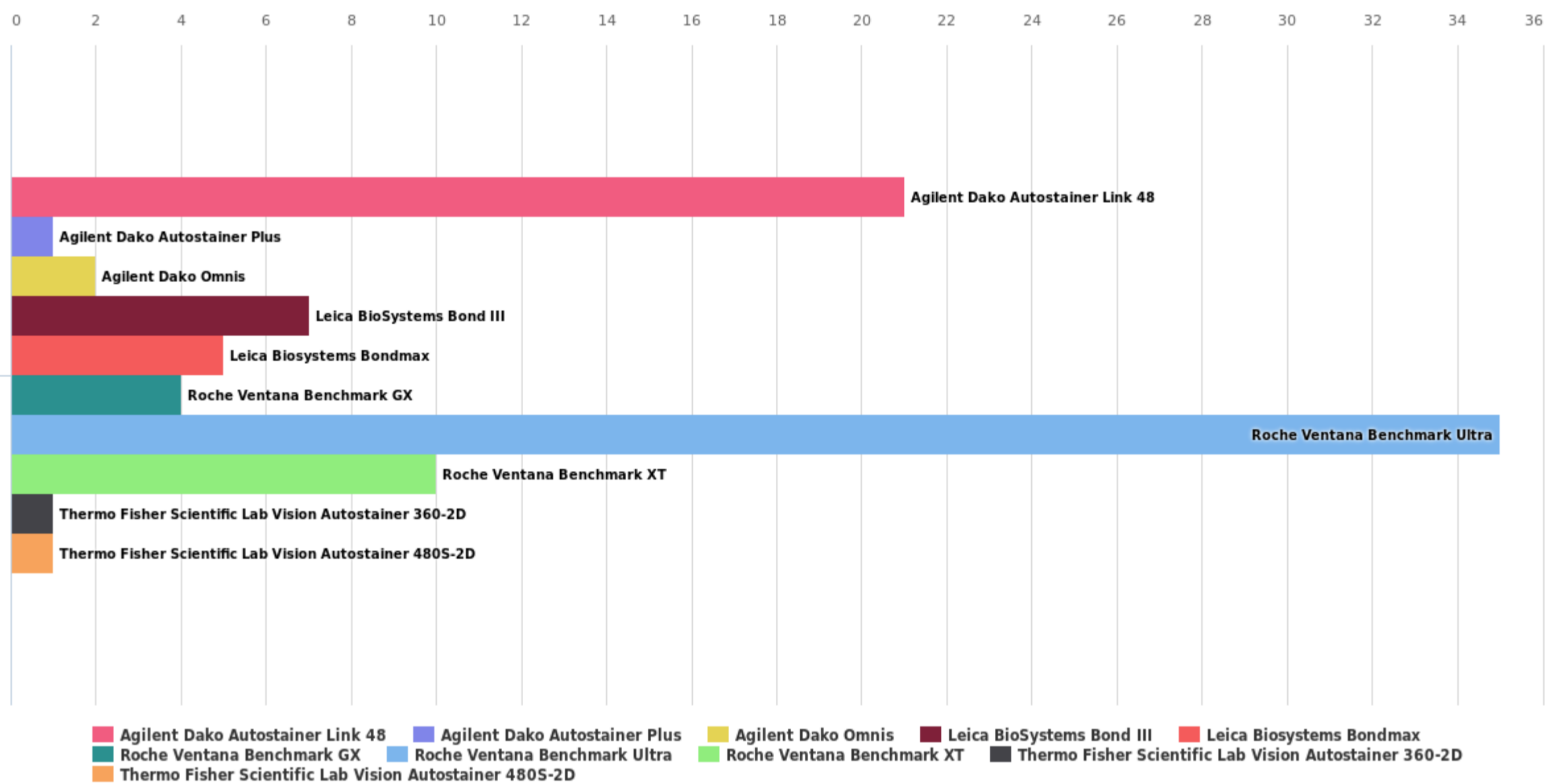
Performance



GENERAL DETAILS OF STAINING

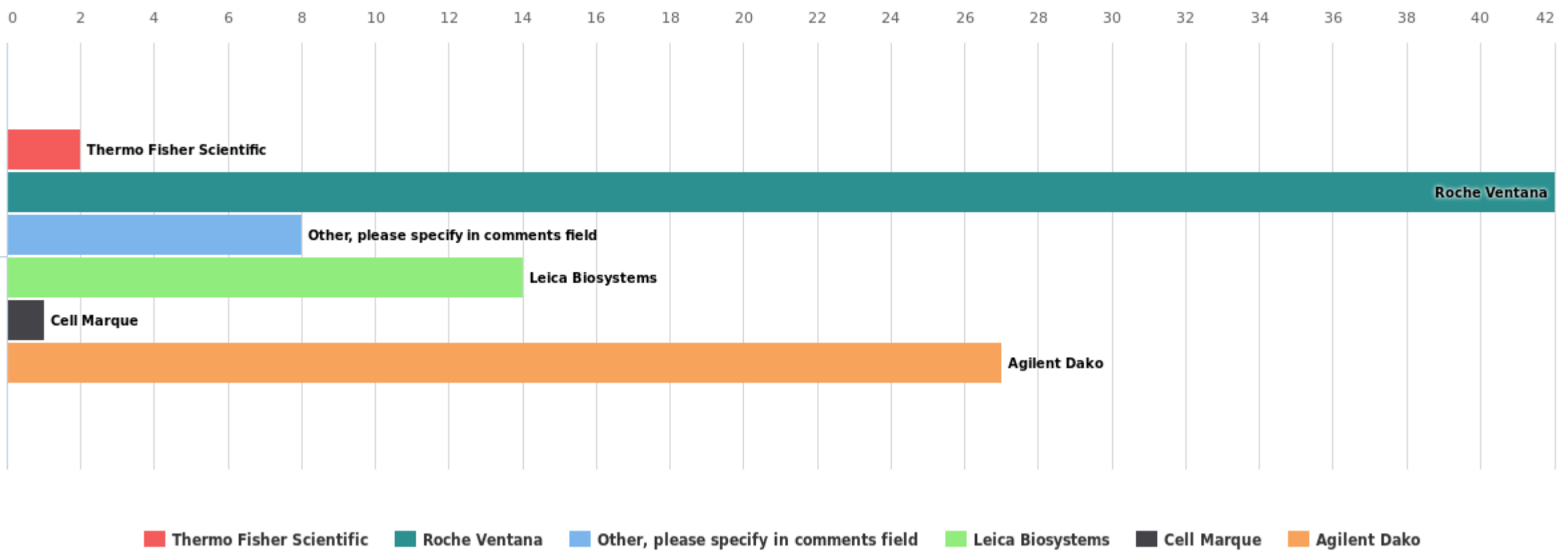
Performance	Performance count
<input checked="" type="radio"/> Automatic	87
Manual	8
Total:	95

Instrument



Instrument	Instrument count
Agilent Dako Autostainer Link 48	21
Agilent Dako Autostainer Plus	1
Agilent Dako Omnis	2
Leica BioSystems Bond III	7
<input checked="" type="radio"/> Leica Biosystems Bondmax	5
Roche Ventana Benchmark GX	4
Roche Ventana Benchmark Ultra	35
Roche Ventana Benchmark XT	10
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Total:	87

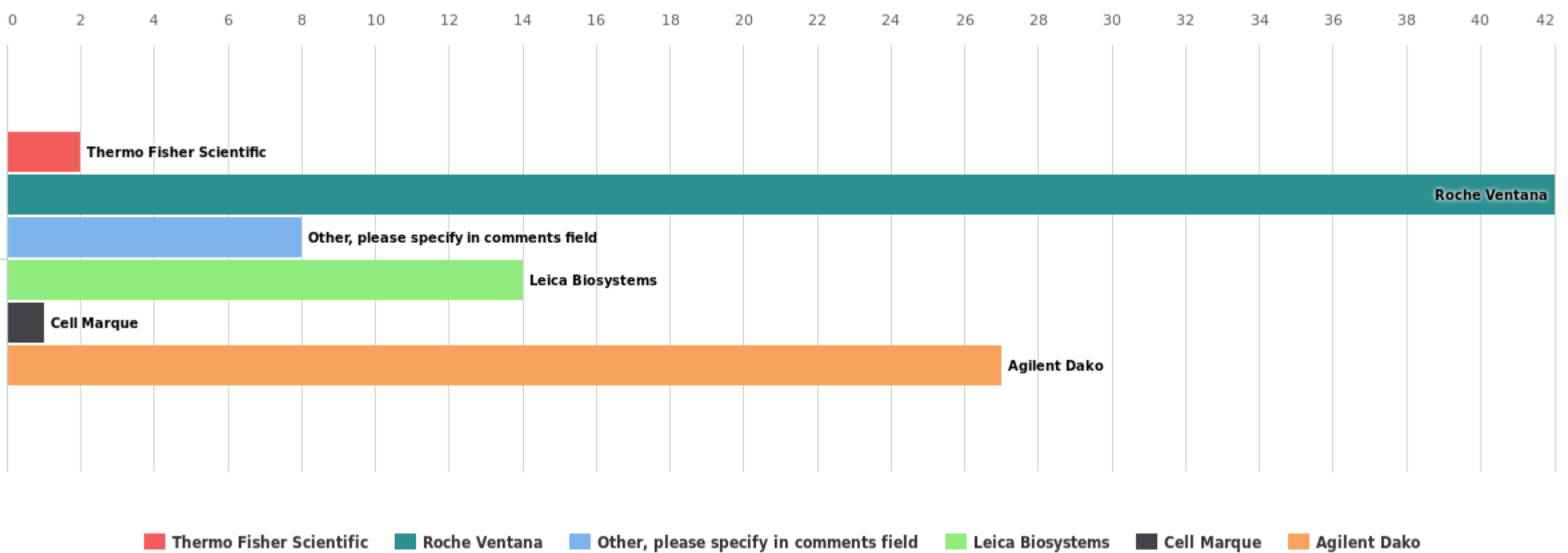
Manufacturer



PRIMARY ANTIBODY

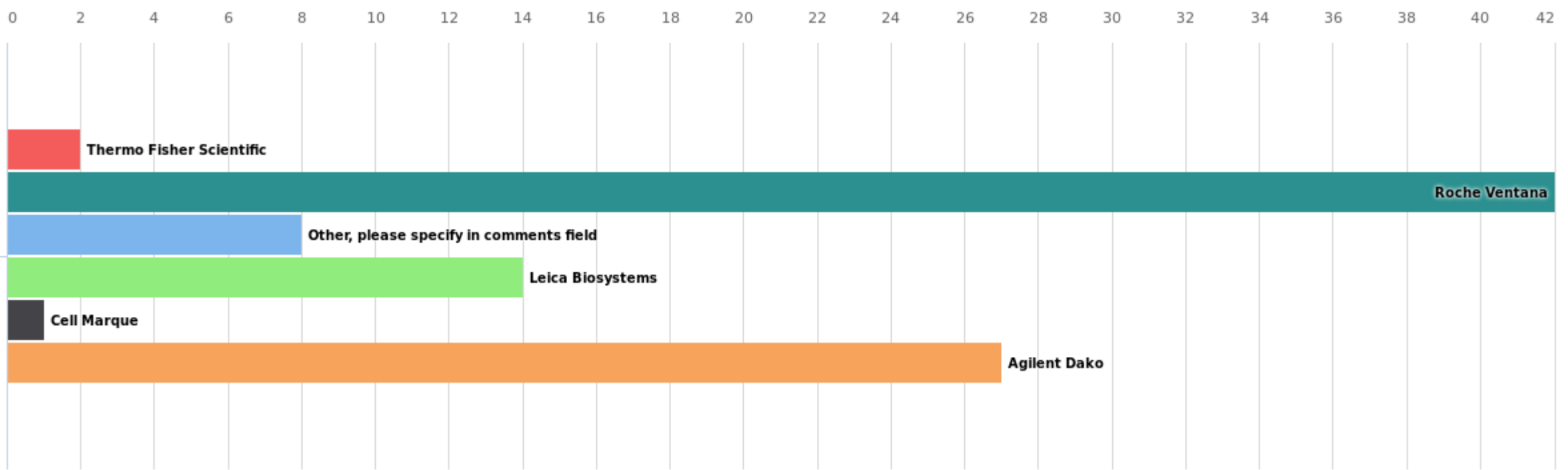
Manufacturer	Manufacturer count
<input checked="" type="radio"/> Agilent Dako	27
Cell Marque	1
Leica Biosystems	14
Other, please specify in comments field	8
Roche Ventana	42
Thermo Fisher Scientific	2
Total:	94

Clonal



Clonal	Clonal count
<input checked="" type="radio"/> Monoclonal	91
Polyclonal	1
Total:	92

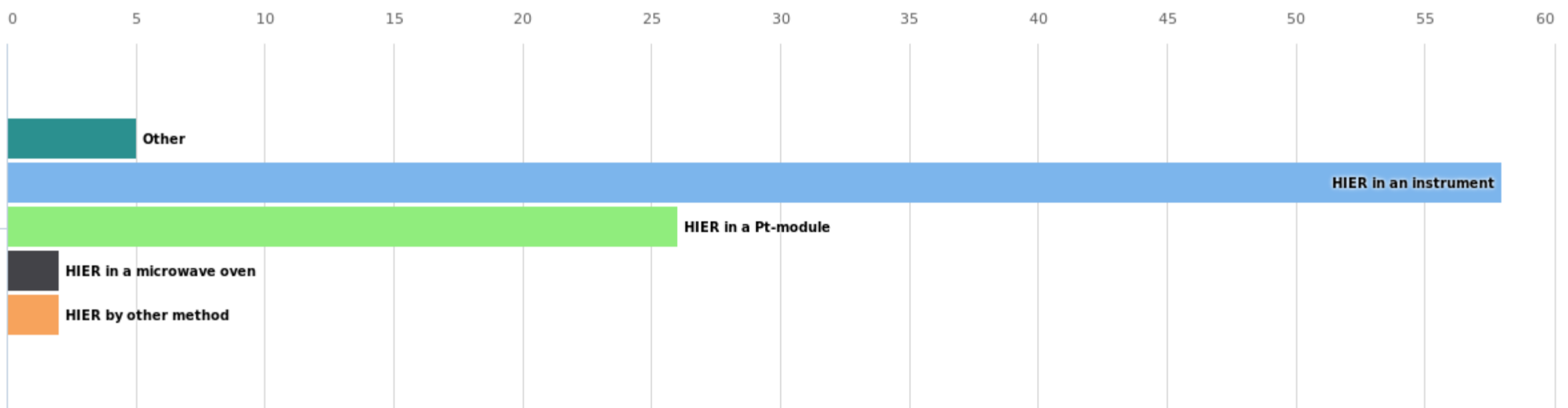
Dilution



Thermo Fisher Scientific Roche Ventana Other, please specify in comments field Leica Biosystems Cell Marque Agilent Dako

Dilution	Dilution count
<input checked="" type="radio"/> Dilution	28
Ready-to-use	66
Total:	94

S003 Pretreatment

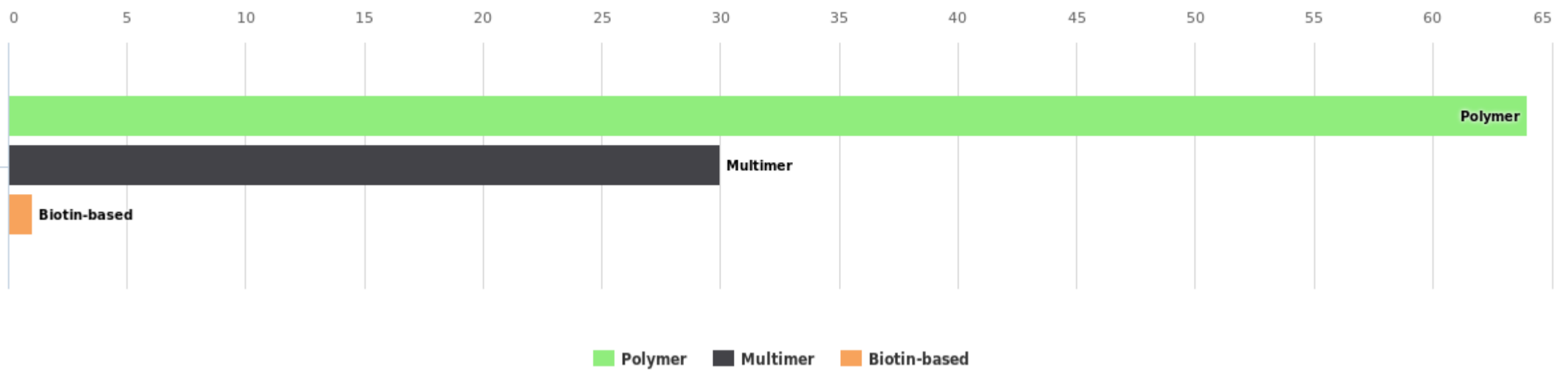


Other HIER in an instrument HIER in a Pt-module HIER in a microwave oven HIER by other method

PRETREATMENT

Pretreatment	Pretreatment count
HIER by other method	2
HIER in a microwave oven	2
HIER in a Pt-module	26
<input checked="" type="radio"/> HIER in an instrument	58
Other	5
Total:	93

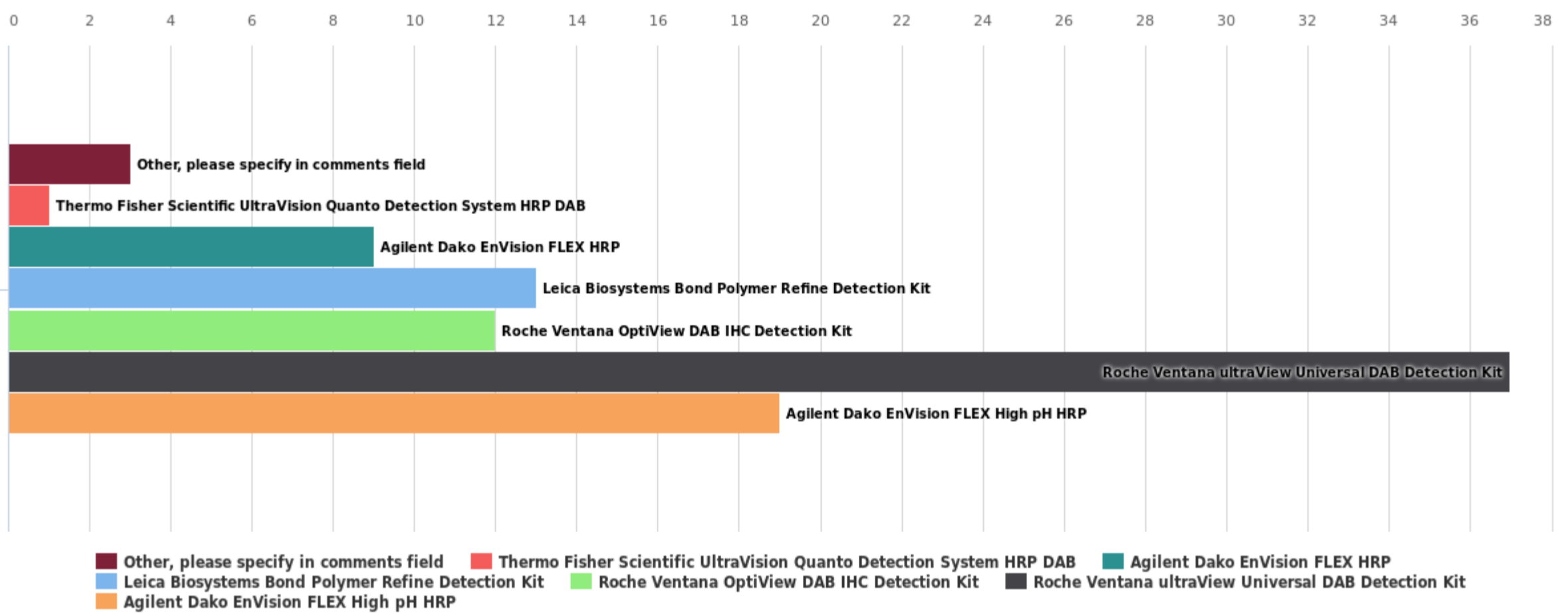
S003 Detection system



DETECTION SYSTEM

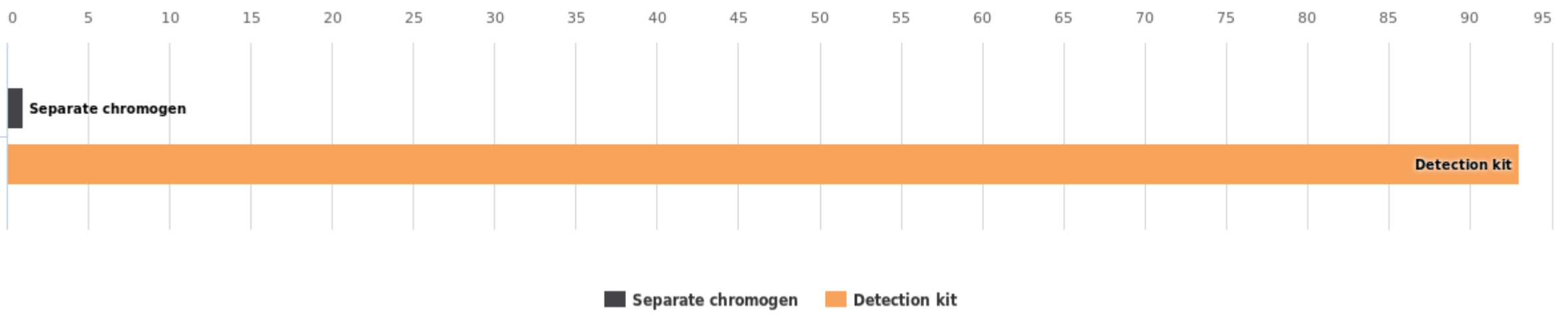
Detection system	Detection system count
Biotin-based	1
Multimer	30
<input checked="" type="radio"/> Polymer	64
Total:	95

Detection system: Instrument



Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	9
Agilent Dako EnVision FLEX High pH HRP	19
<input checked="" type="radio"/> Leica Biosystems Bond Polymer Refine Detection Kit	13
Other, please specify in comments field	3
Roche Ventana OptiView DAB IHC Detection Kit	12
Roche Ventana ultraView Universal DAB Detection Kit	37
Thermo Fisher Scientific UltraVision Quanto Detection System HRP DAB	1
Total:	94

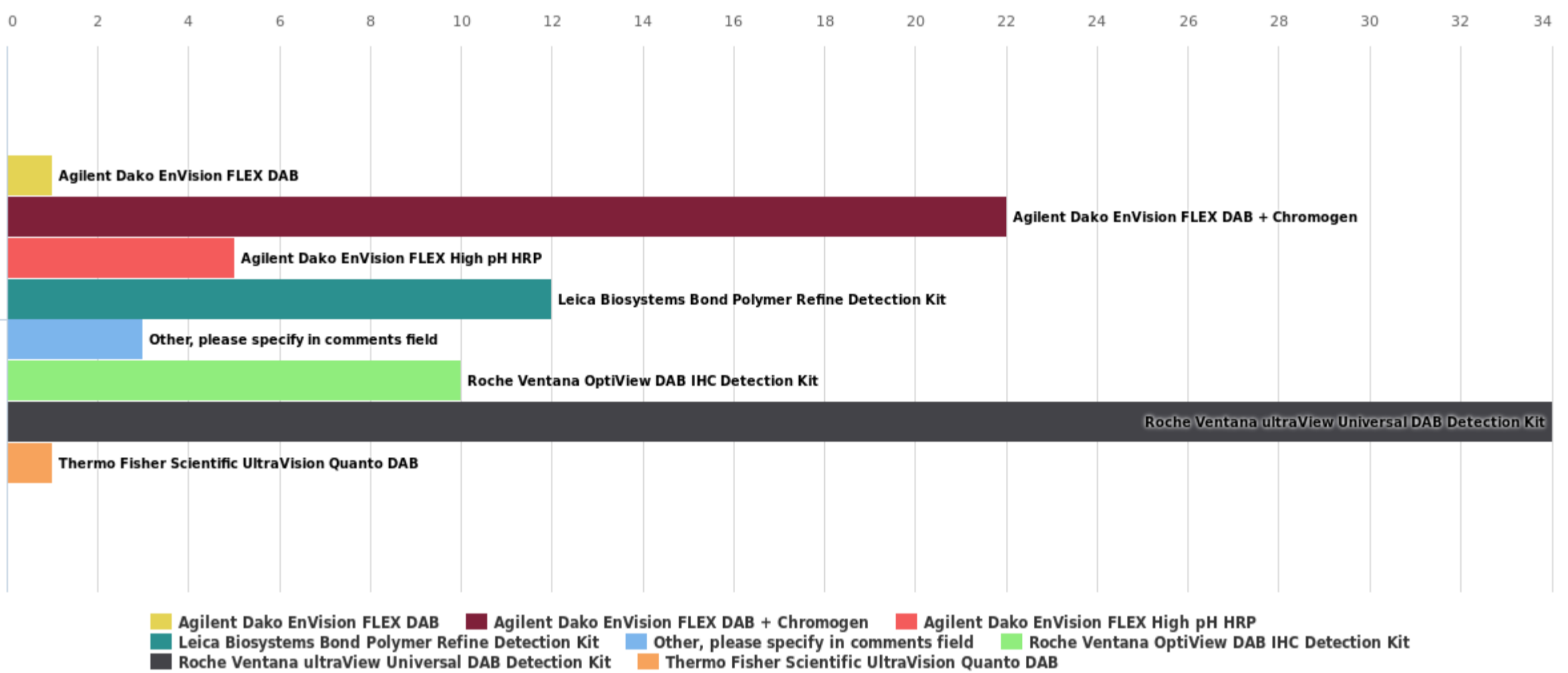
S003 Chromogen



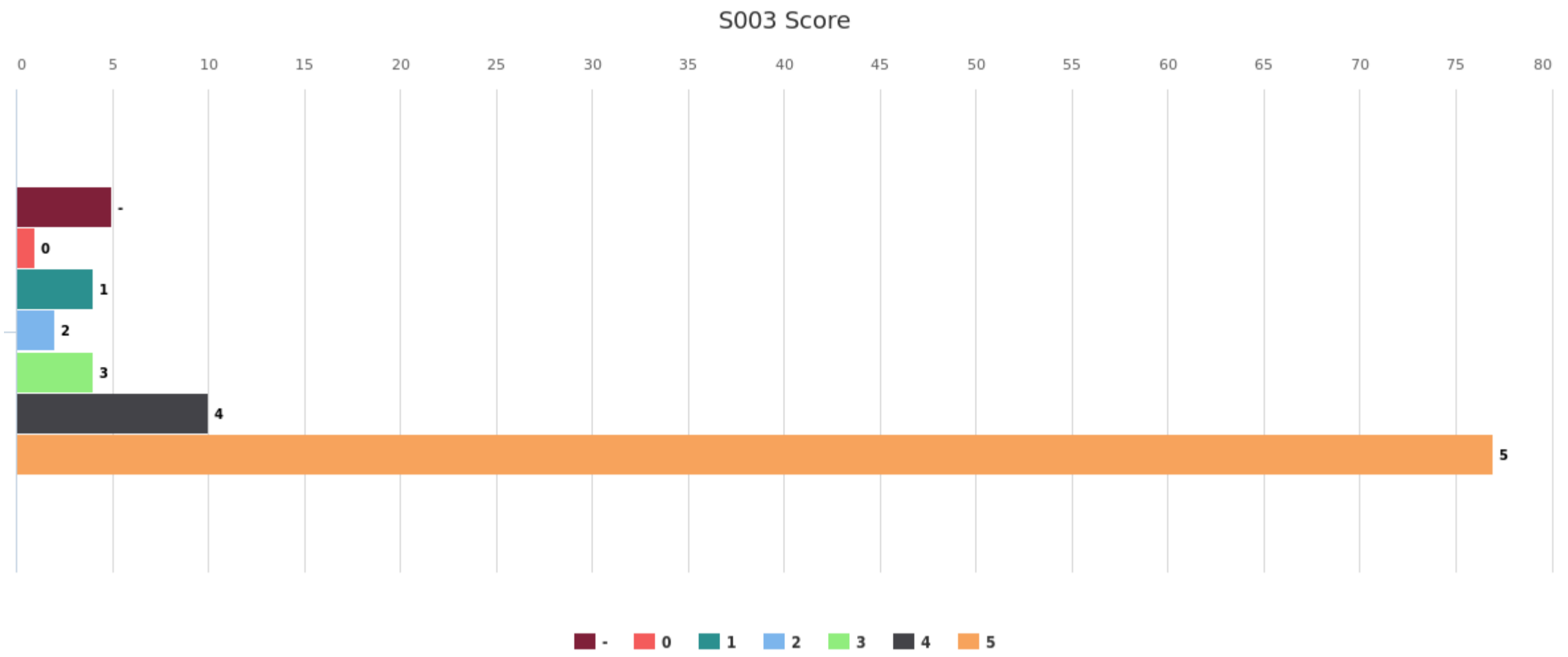
CHROMOGEN

Chromogen	Chromogen count
<input checked="" type="radio"/> Detection kit	93
Separate chromogen	1
Total:	94

Chromogen: Instrument

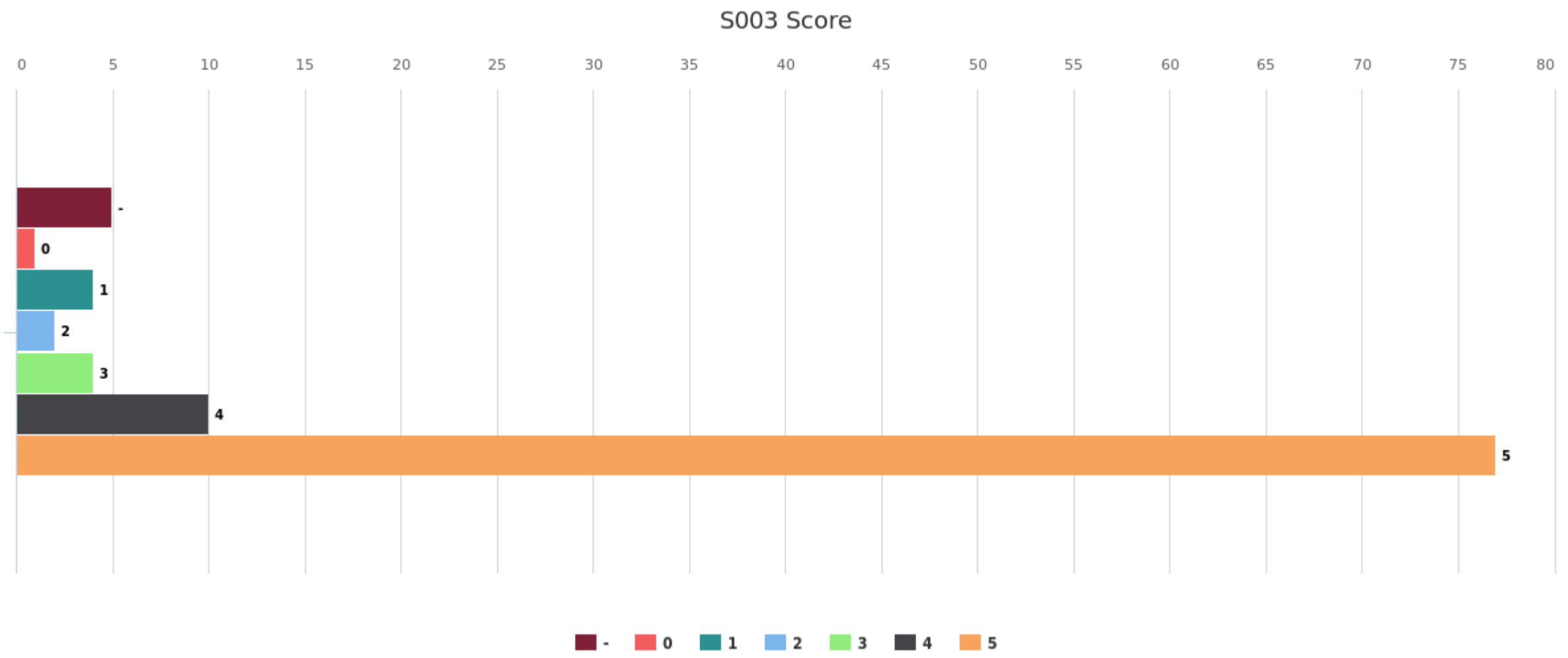


Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	1
Agilent Dako EnVision FLEX DAB + Chromogen	22
Agilent Dako EnVision FLEX High pH HRP	5
<input checked="" type="radio"/> Leica Biosystems Bond Polymer Refine Detection Kit	12
Other, please specify in comments field	3
Roche Ventana OptiView DAB IHC Detection Kit	10
Roche Ventana ultraView Universal DAB Detection Kit	34
Thermo Fisher Scientific UltraVision Quanto DAB	1
Total:	88



SCORE

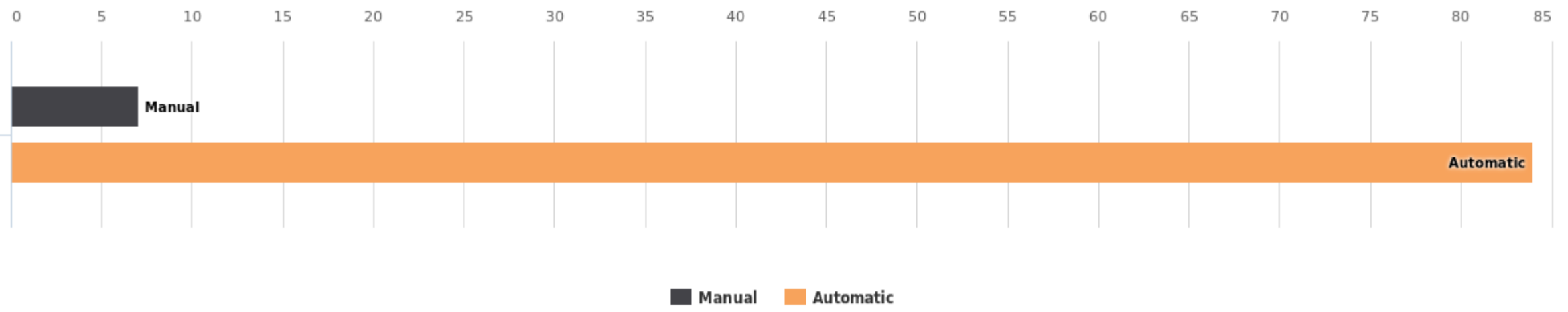
Score	Comment
-	Not Available
0	Poor, No Staining At All, Blanco
1	Unsuccessful, Both False Negative And False Positive Staining, Diagnostically Unacceptable Unsuccessful, False Negative Staining, Diagnostically Unacceptable
2	Diagnosis May Compromise, False Negativity, Staining Intensity Very Weak Diagnosis May Compromise, False Negativity, Staining Intensity Very Weak, Poor Contrast
3	False Negativity, Staining Intensity Weak
4	Staining Intensity A Bit Weak Staining intensity a bit weak
<input checked="" type="radio"/> 5	Contrast Between Hematoxylin And Dab Poor Due To Dark Hematoxylin <input checked="" type="radio"/> Excellent
Total:	



Score	Score count
-	5
0	1
1	4
2	2
3	4
4	10
<input checked="" type="radio"/> 5	77
Total:	103

S004 | Sample S004 Ki-67

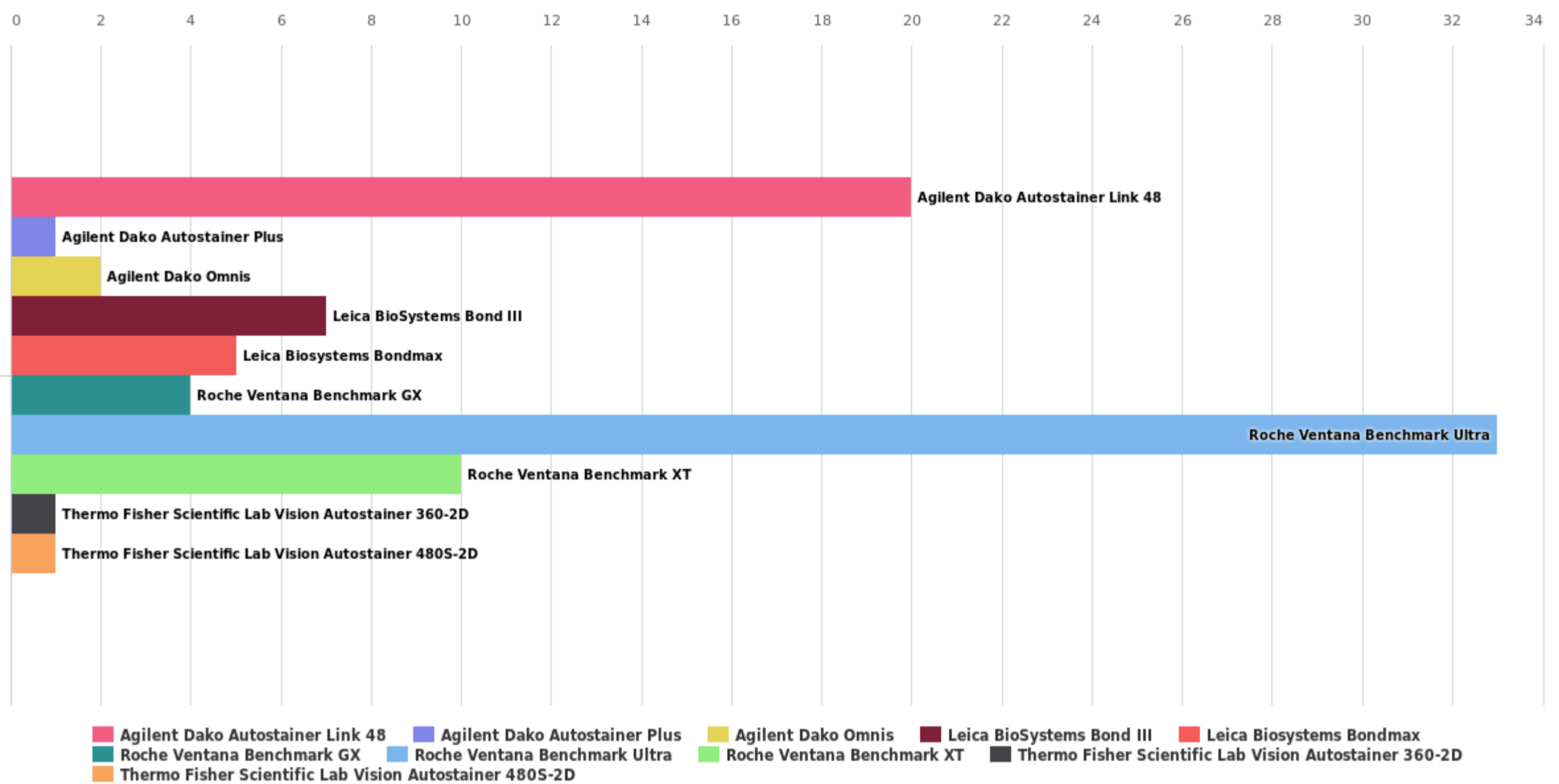
Performance



GENERAL DETAILS OF STAINING

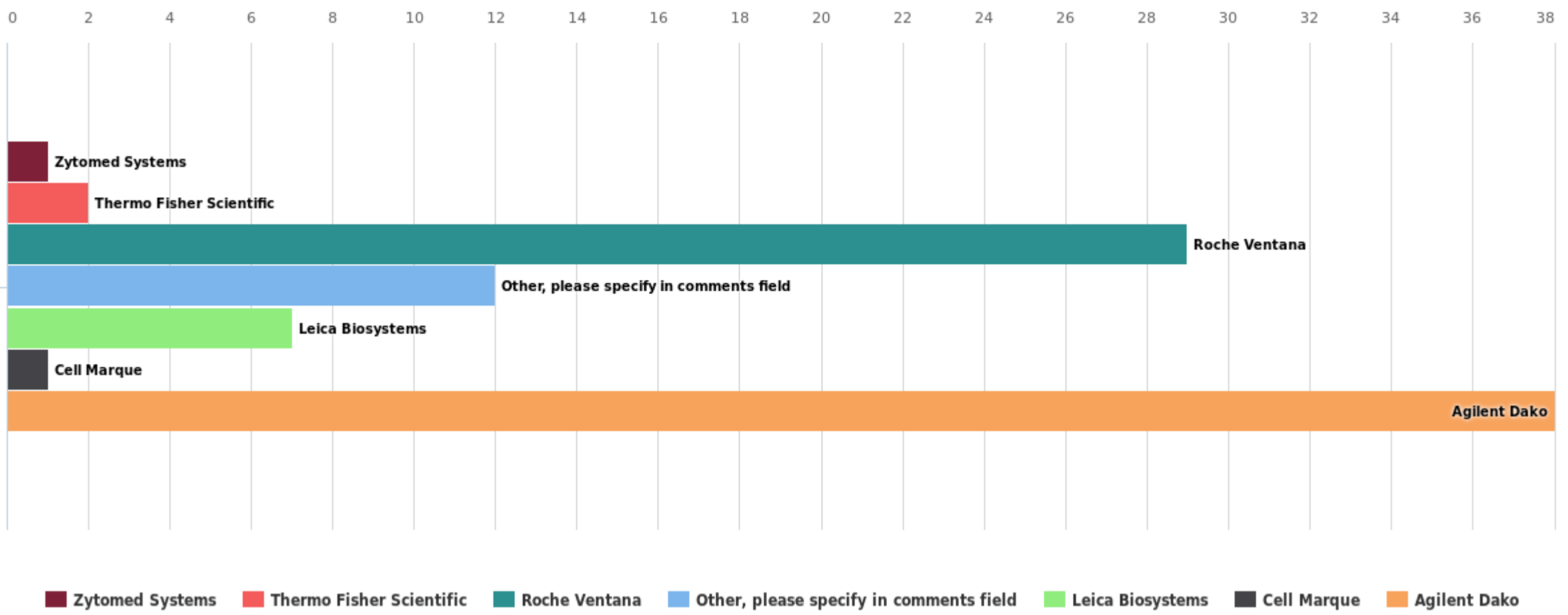
Performance	Performance count
<input checked="" type="radio"/> Automatic	84
Manual	7
Total:	91

Instrument



Instrument	Instrument count
Agilent Dako Autostainer Link 48	20
Agilent Dako Autostainer Plus	1
Agilent Dako Omnis	2
Leica BioSystems Bond III	7
<input checked="" type="radio"/> Leica Biosystems Bondmax	5
Roche Ventana Benchmark GX	4
Roche Ventana Benchmark Ultra	33
Roche Ventana Benchmark XT	10
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Total:	84

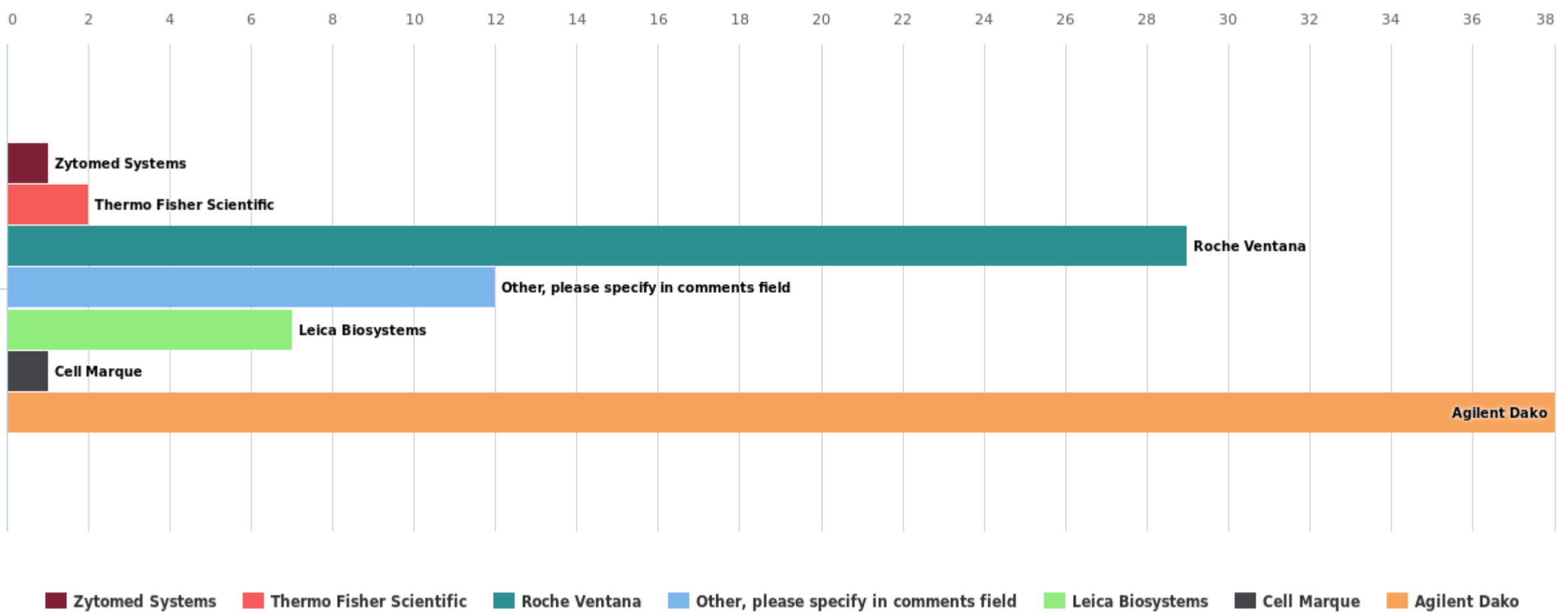
Manufacturer



PRIMARY ANTIBODY

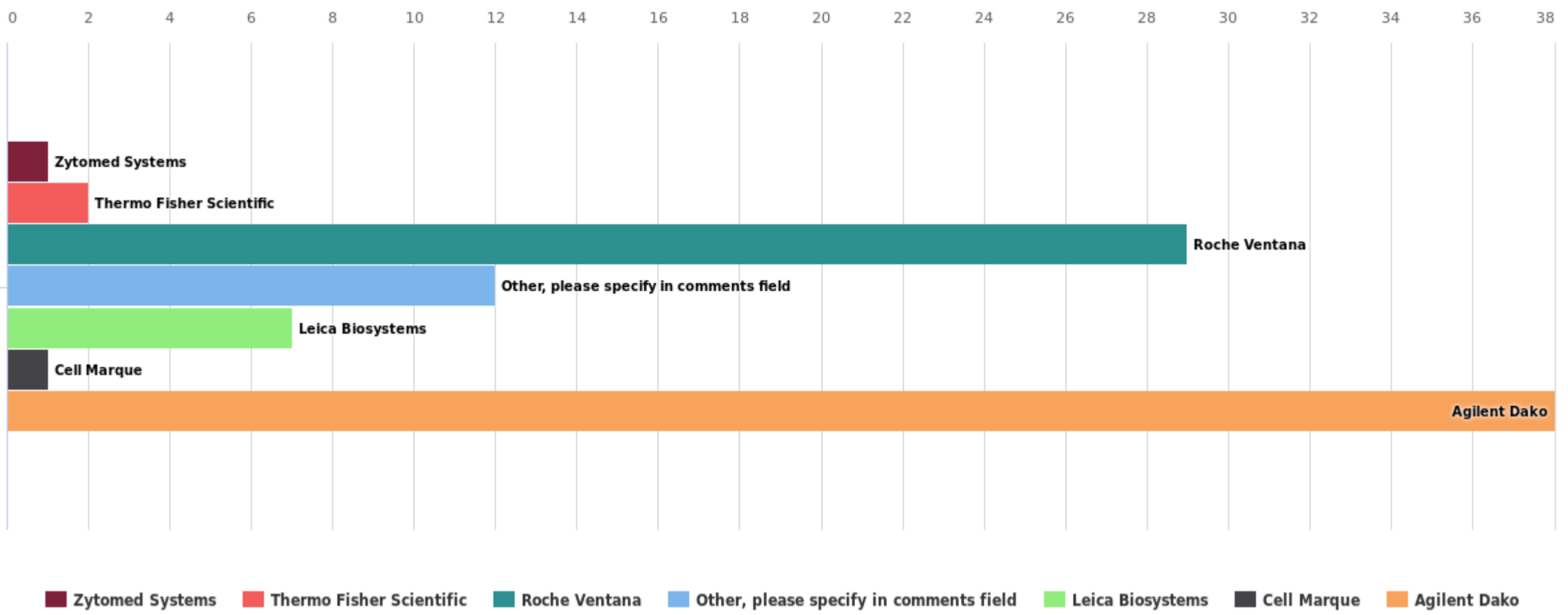
Manufacturer	Manufacturer count
<input checked="" type="radio"/> Agilent Dako	38
Cell Marque	1
Leica Biosystems	7
Other, please specify in comments field	12
Roche Ventana	29
Thermo Fisher Scientific	2
Zytomed Systems	1
Total:	90

Clonal



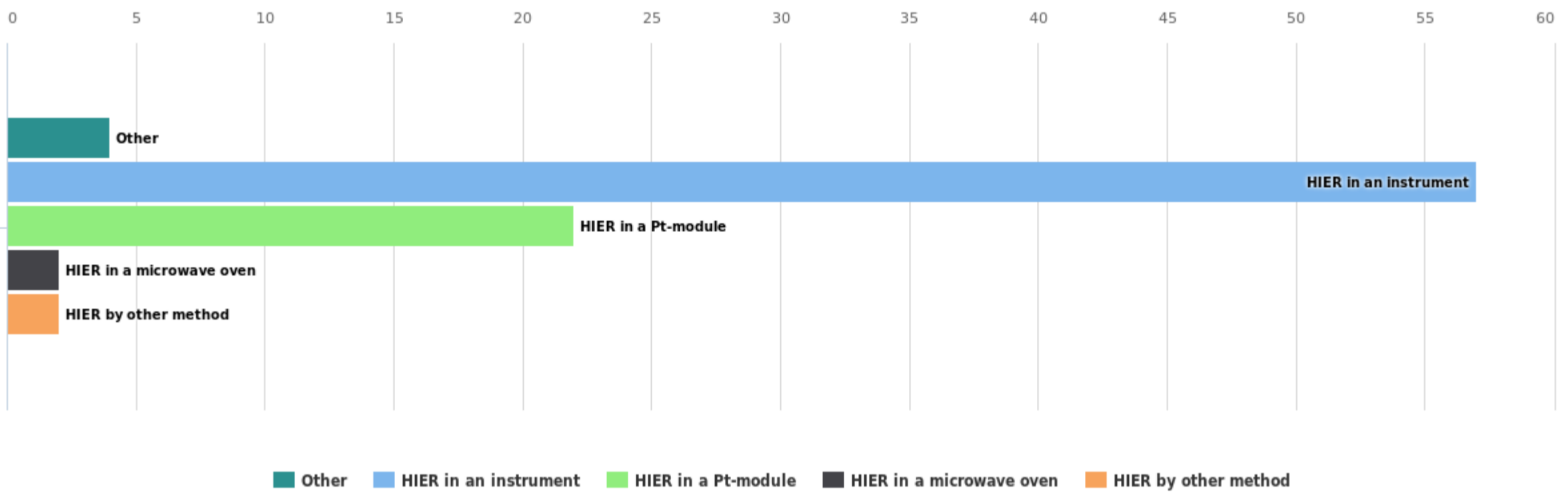
Clonal	Clonal count
<input checked="" type="radio"/> Monoclonal	86
Polyclonal	1
Total:	87

Dilution



Dilution	Dilution count
<input checked="" type="radio"/> Dilution	38
Ready-to-use	51
Total:	89

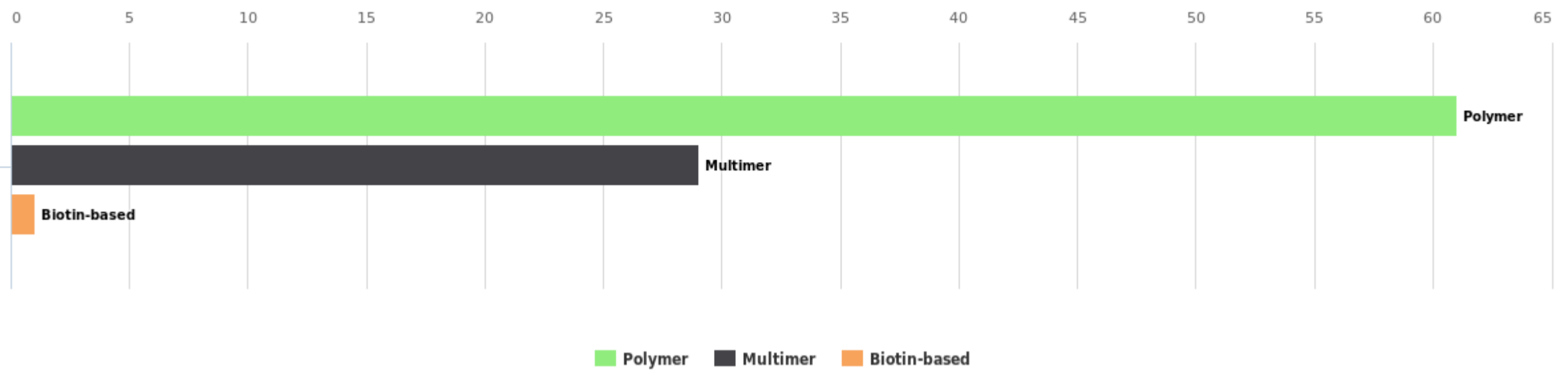
S004 Pretreatment



PRETREATMENT

Pretreatment	Pretreatment count
HIER by other method	2
HIER in a microwave oven	2
HIER in a Pt-module	22
<input checked="" type="radio"/> HIER in an instrument	57
Other	4
Total:	87

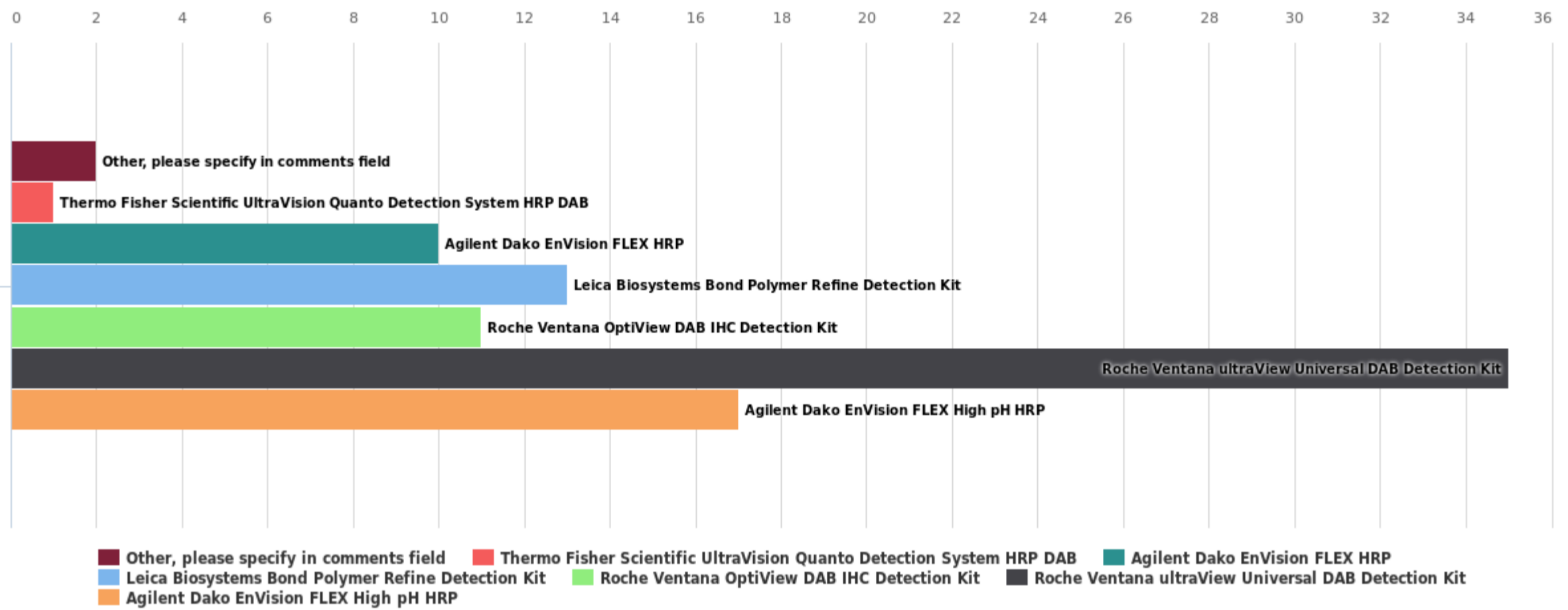
S004 Detection system



DETECTION SYSTEM

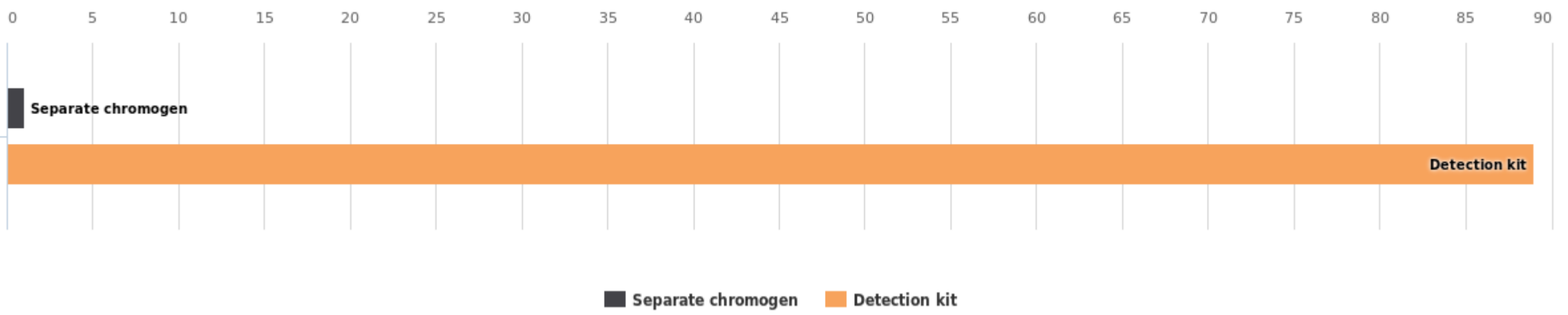
Detection system	Detection system count
Biotin-based	1
Multimer	29
<input checked="" type="radio"/> Polymer	61
Total:	91

Detection system: Instrument



Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	10
Agilent Dako EnVision FLEX High pH HRP	17
<input checked="" type="radio"/> Leica Biosystems Bond Polymer Refine Detection Kit	13
Other, please specify in comments field	2
Roche Ventana OptiView DAB IHC Detection Kit	11
Roche Ventana ultraView Universal DAB Detection Kit	35
Thermo Fisher Scientific UltraVision Quanto Detection System HRP DAB	1
Total:	89

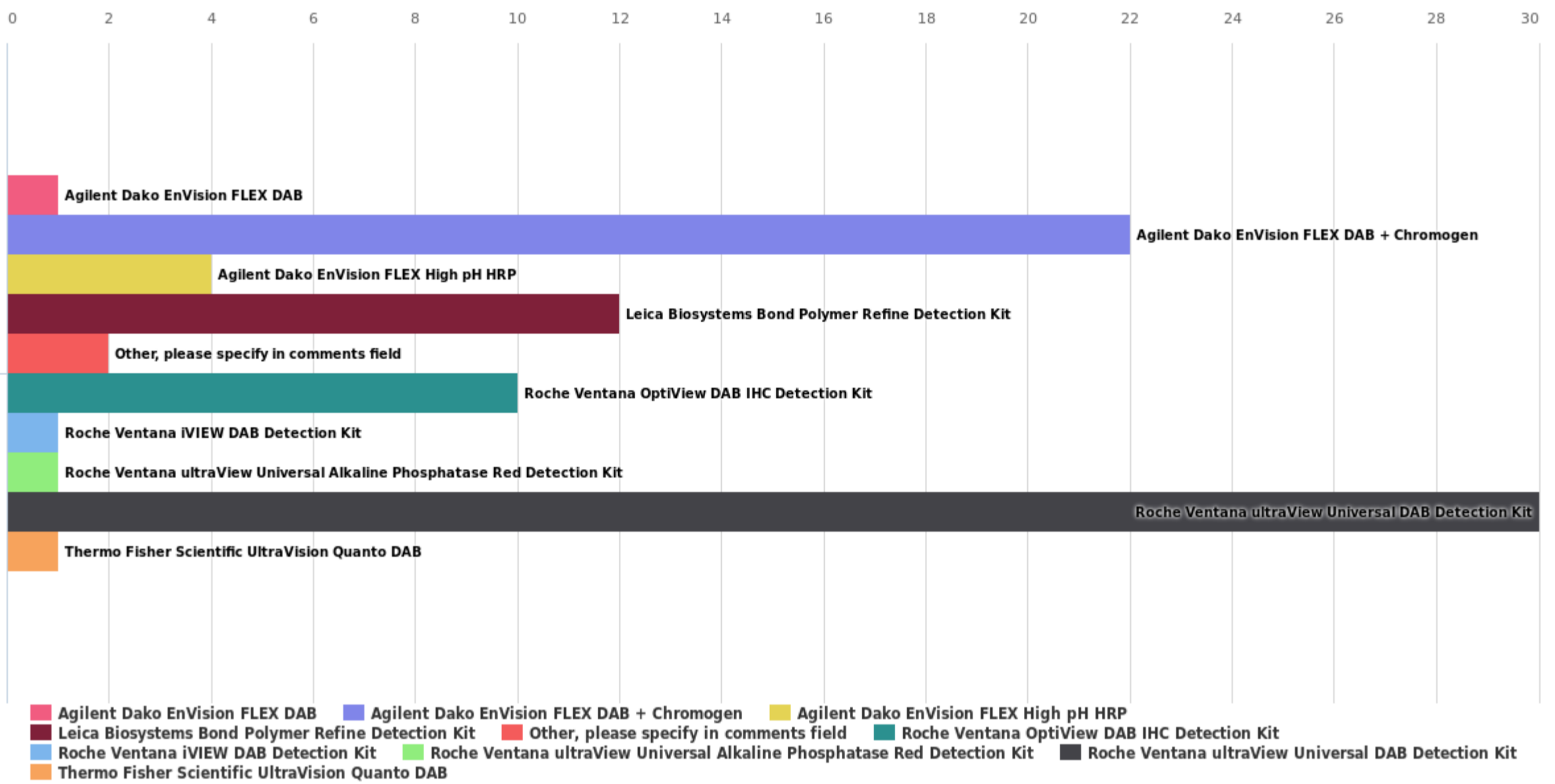
S004 Chromogen



CHROMOGEN

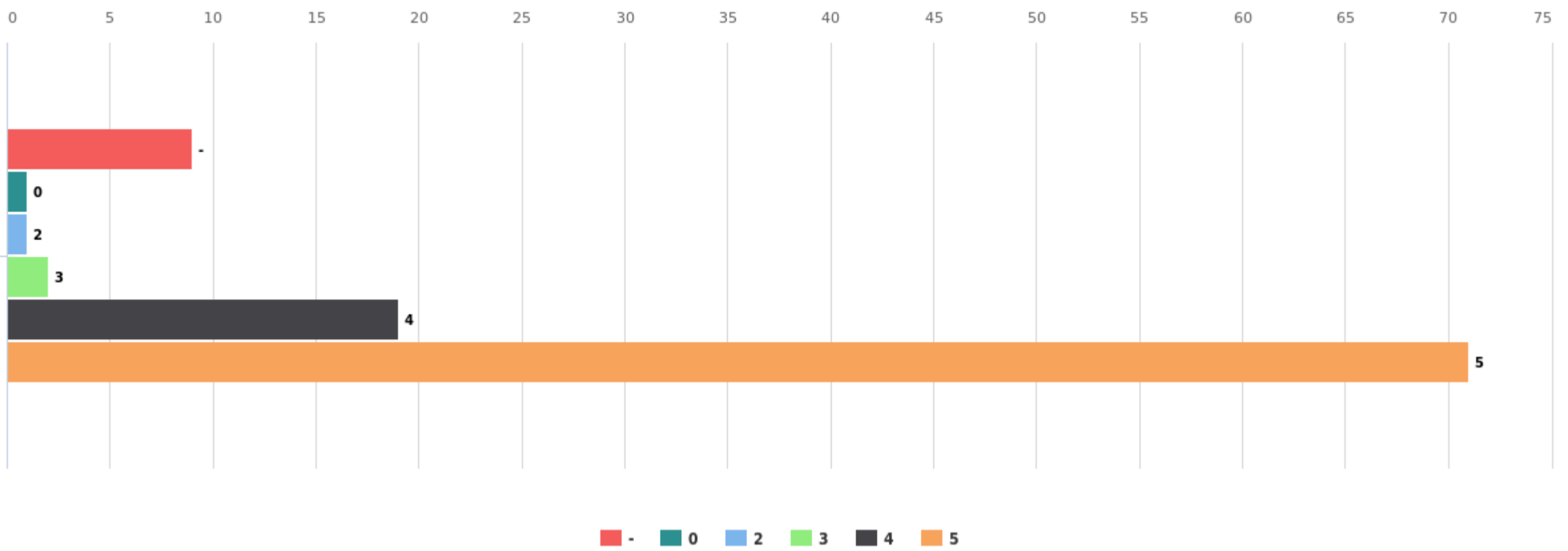
Chromogen	Chromogen count
<input checked="" type="radio"/> Detection kit	89
Separate chromogen	1
Total:	90

Chromogen: Instrument



Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	1
Agilent Dako EnVision FLEX DAB + Chromogen	22
Agilent Dako EnVision FLEX High pH HRP	4
<input checked="" type="radio"/> Leica Biosystems Bond Polymer Refine Detection Kit	12
Other, please specify in comments field	2
Roche Ventana OptiView DAB IHC Detection Kit	10
Roche Ventana iVIEW DAB Detection Kit	1
Roche Ventana ultraView Universal Alkaline Phosphatase Red Detection Kit	1
Roche Ventana ultraView Universal DAB Detection Kit	30
Thermo Fisher Scientific UltraVision Quanto DAB	1
Total:	84

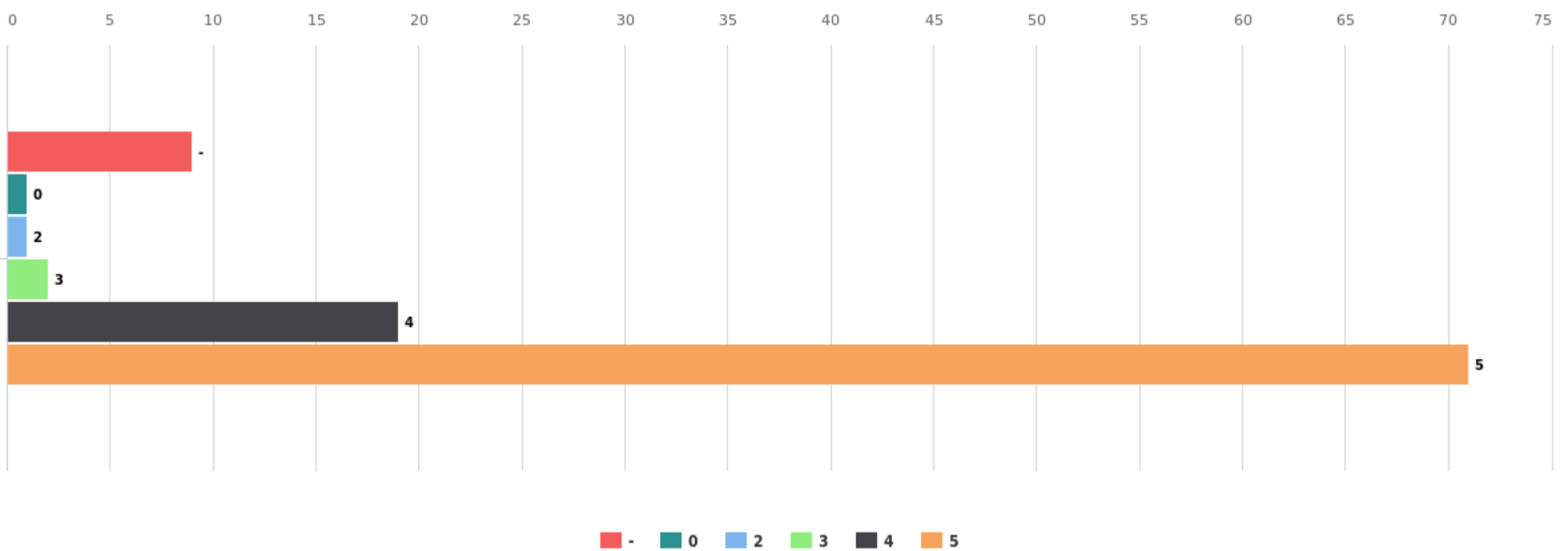
S004 Score



SCORE

Score	Comment
-	Not Available
0	No Staining At All, Blanco, Not Acceptable For Daignosis,Failed
2	Diagnosis May Be Compromised, False Negativity, Staining Intensity Weak
3	False Negativity, Staining Intensity Weak
4	Embedding Failed, Difficult To Evaluate Hematoxylin Very Dark Hematoxylin Very Weak Membranes Of Adipocytes Stained Staining Intensity A Bit Weak
<input checked="" type="radio"/> 5	<input checked="" type="radio"/> Excellent
Total:	

S004 Score

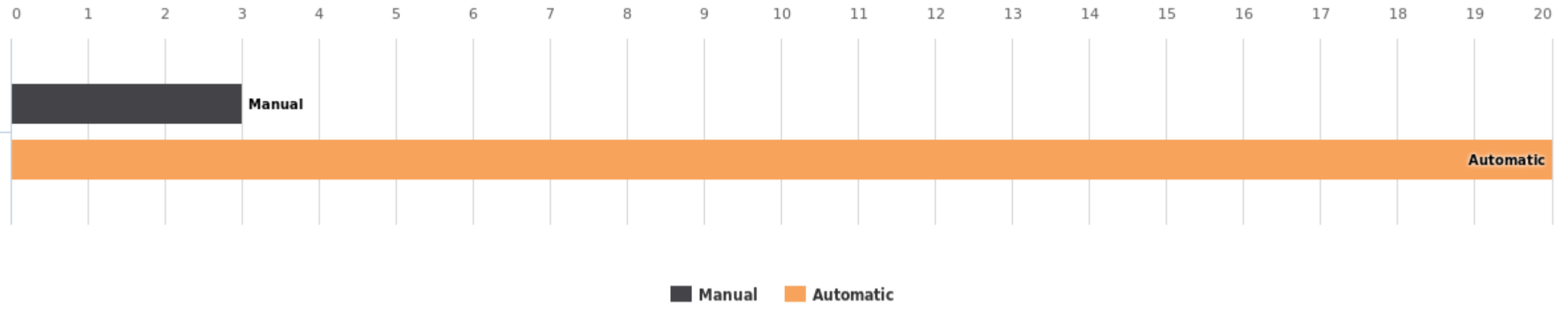


Score	Score count
-	9

0	1
2	1
3	2
4	19
<input checked="" type="radio"/> 5	71
Total:	103

S005 | Sample S005 HER2 -ISH

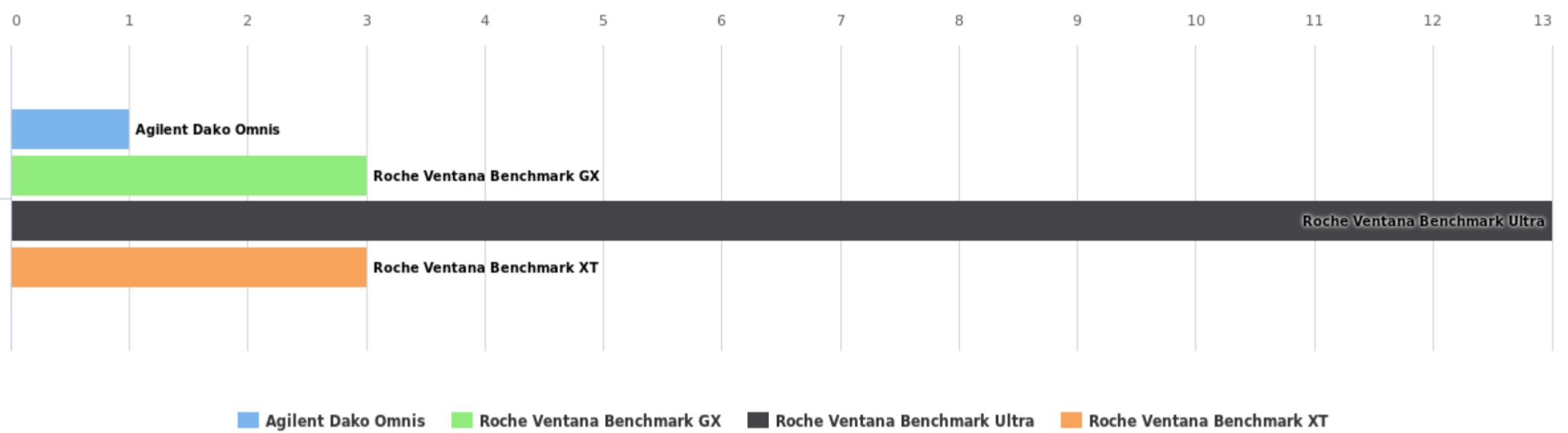
Performance



GENERAL DETAILS OF STAINING

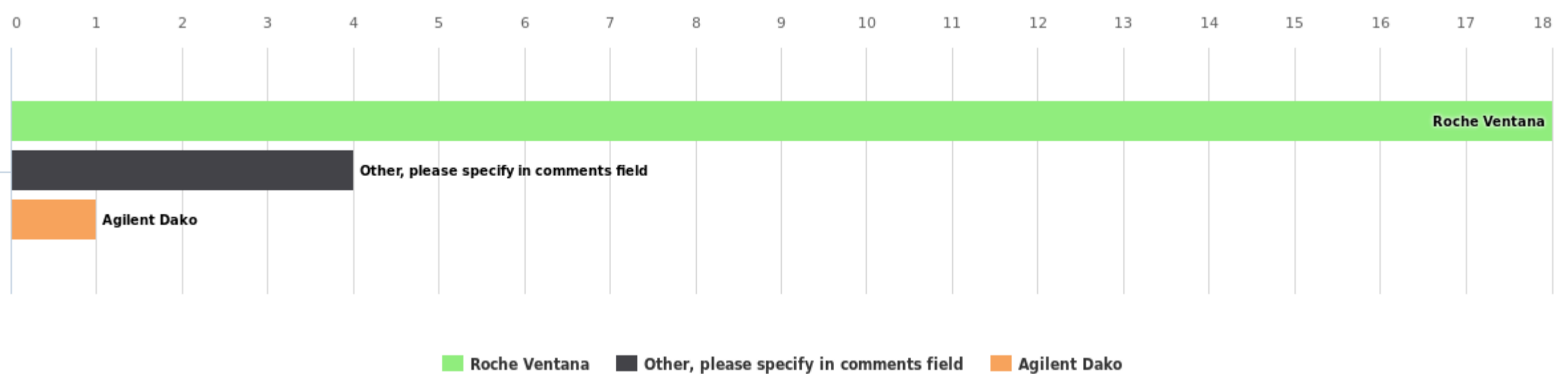
Performance	Performance count
Automatic	20
<input checked="" type="radio"/> Manual	3
Total:	23

Instrument



Instrument	Instrument count
Agilent Dako Omnis	1
Roche Ventana Benchmark GX	3
Roche Ventana Benchmark Ultra	13
Roche Ventana Benchmark XT	3
Total:	20

Manufacturer

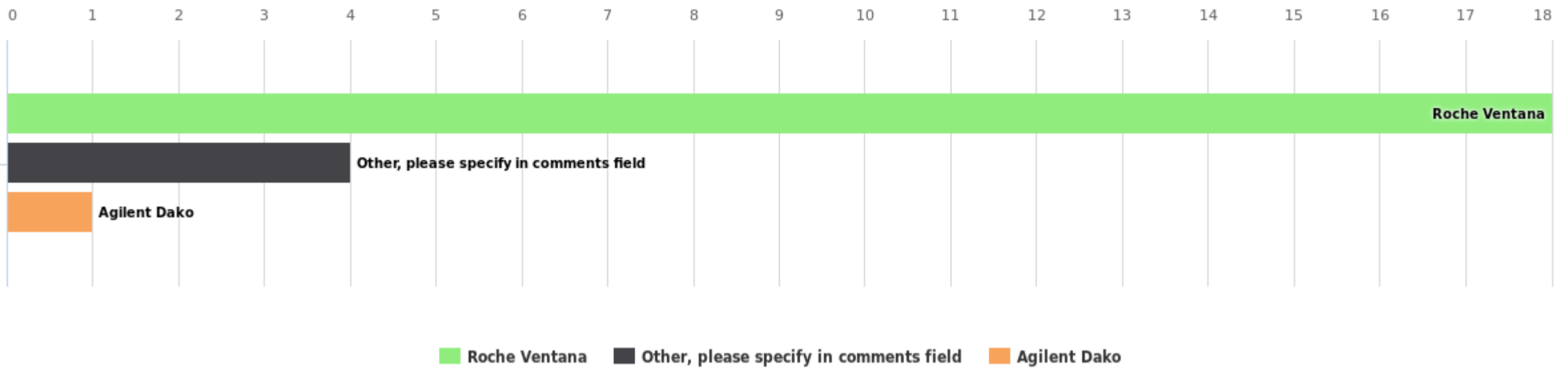


PRIMARY ANTIBODY

Manufacturer	Manufacturer count
--------------	--------------------

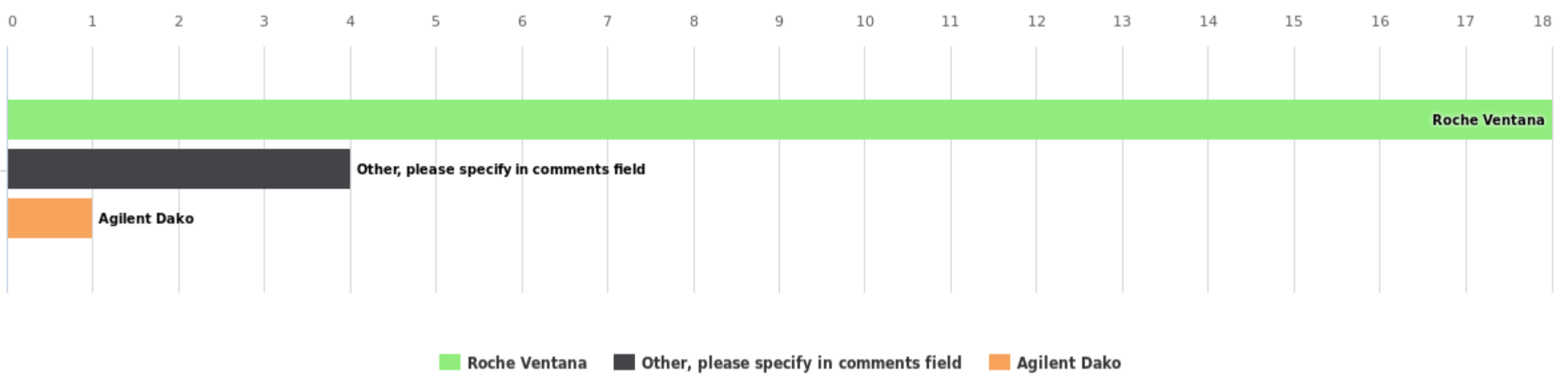
Agilent Dako	1
<input checked="" type="radio"/> Other, please specify in comments field	4
Roche Ventana	18
Total:	23

Clonal



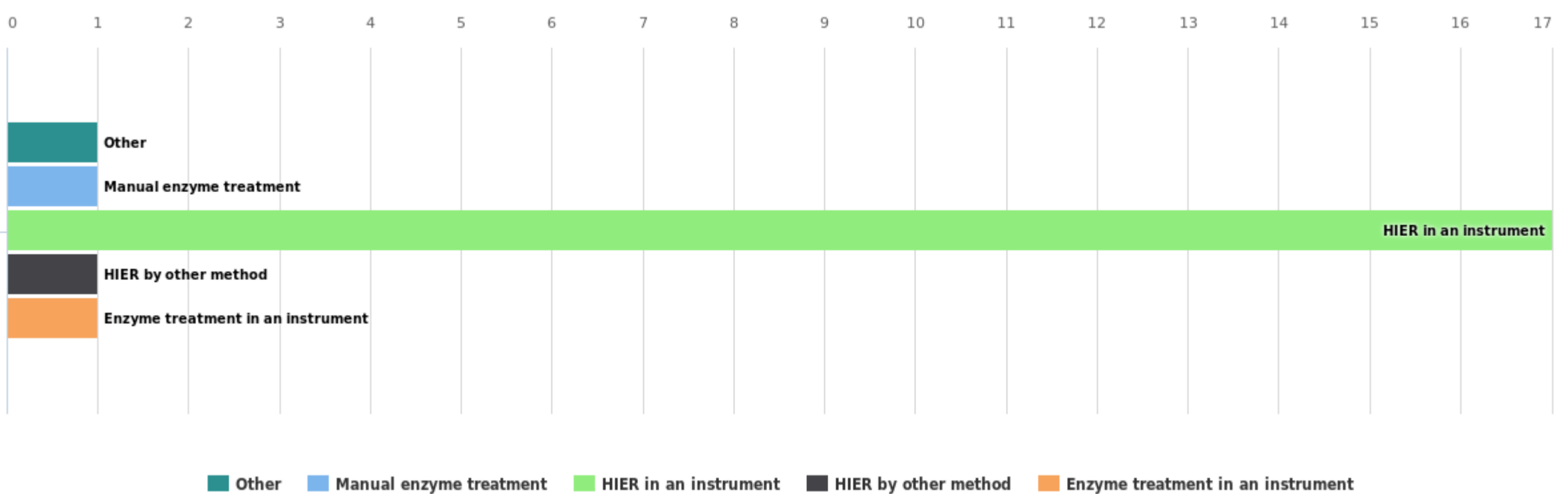
Clonal	Clonal count
Monoclonal	7
Polyclonal	3
Total:	10

Dilution



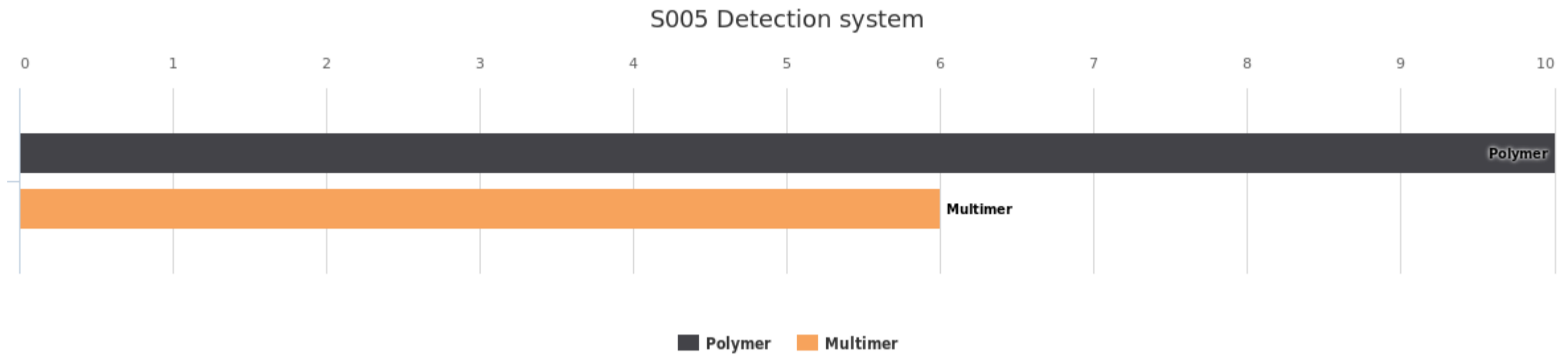
Dilution	Dilution count
<input checked="" type="radio"/> Ready-to-use	21
Total:	21

S005 Pretreatment



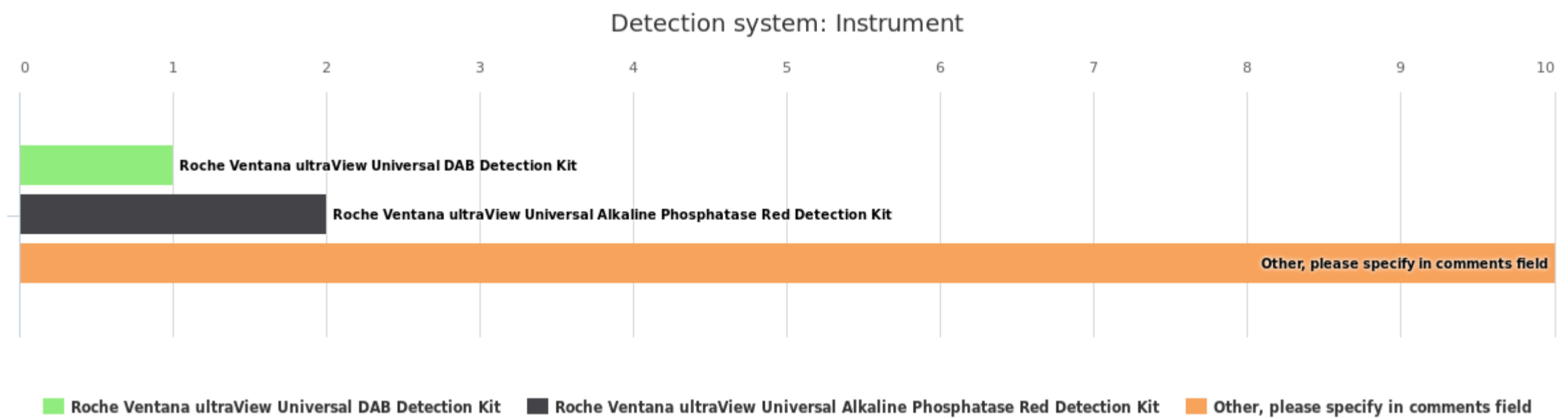
PRETREATMENT

Pretreatment	Pretreatment count
Enzyme treatment in an instrument	1
<input checked="" type="radio"/> HIER by other method	1
HIER in an instrument	17
Manual enzyme treatment	1
Other	1
Total:	21

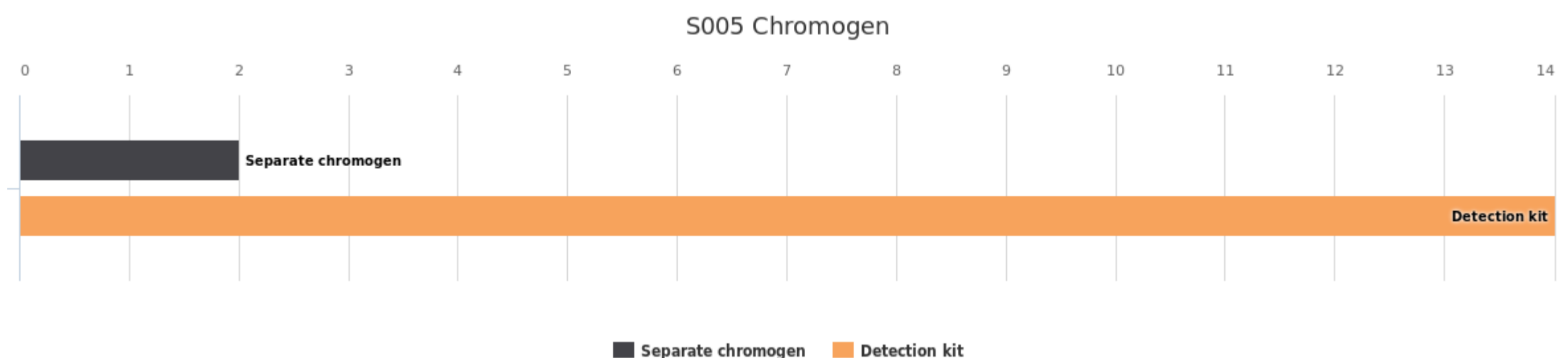


DETECTION SYSTEM

Detection system	Detection system count
Multimer	6
<input checked="" type="radio"/> Polymer	10
Total:	16



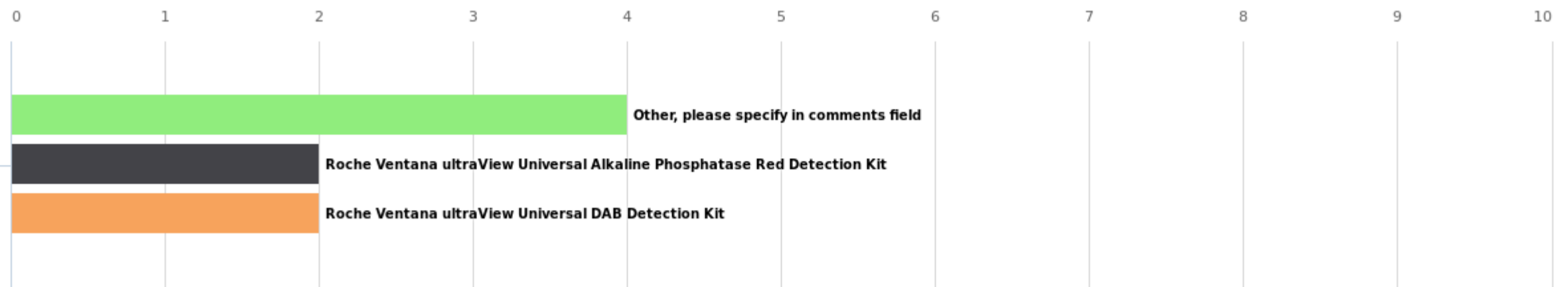
Detection system: Instrument	Detection system: Instrument count
<input checked="" type="radio"/> Other, please specify in comments field	10
Roche Ventana ultraView Universal Alkaline Phosphatase Red Detection Kit	2
Roche Ventana ultraView Universal DAB Detection Kit	1
Total:	13



CHROMOGEN

Chromogen	Chromogen count
<input checked="" type="radio"/> Detection kit	14
Separate chromogen	2
Total:	16

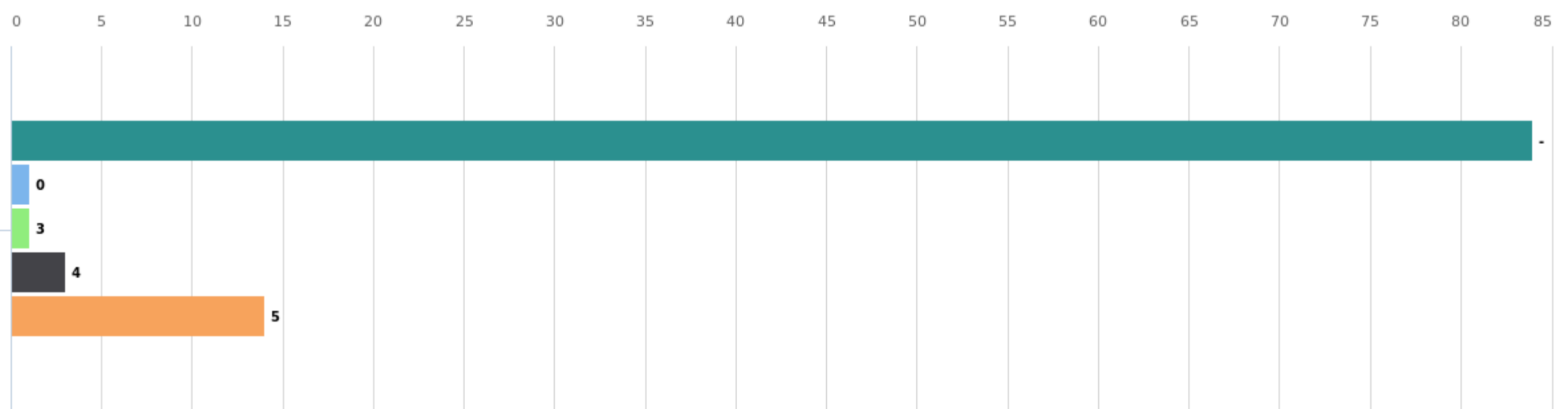
Chromogen: Instrument



■ Other, please specify in comments field
 ■ Roche Ventana ultraView Universal Alkaline Phosphatase Red Detection Kit
 ■ Roche Ventana ultraView Universal DAB Detection Kit

Chromogen: Instrument	Chromogen: Instrument count
<input checked="" type="radio"/> Other, please specify in comments field	4
Roche Ventana ultraView Universal Alkaline Phosphatase Red Detection Kit	2
Roche Ventana ultraView Universal DAB Detection Kit	2
Total:	8

S005 Score



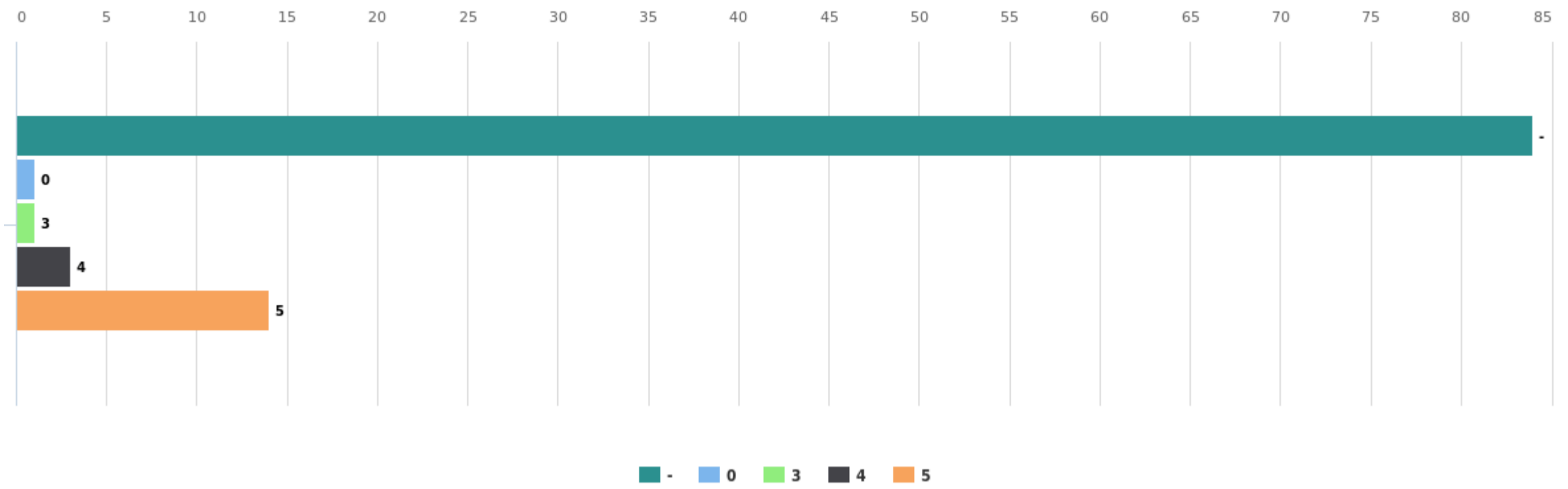
■ -
 ■ 0
 ■ 3
 ■ 4
 ■ 5

SCORE

Score	Comment
-	FISH, Not Evaluated Not Available
0	No Sample On The Slide
3	One of the sample blanco, one of the samples scraped, two optimal
<input checked="" type="radio"/> 4	<input checked="" type="radio"/> A Bit Smudgy, Cytoplasmic Background Staining May Block The View Of Ish Signals CHR17 Signals Should Be Enhanced One Of The Samples "Grilled"
5	Excellent IHC+ISH

Total:

S005 Score



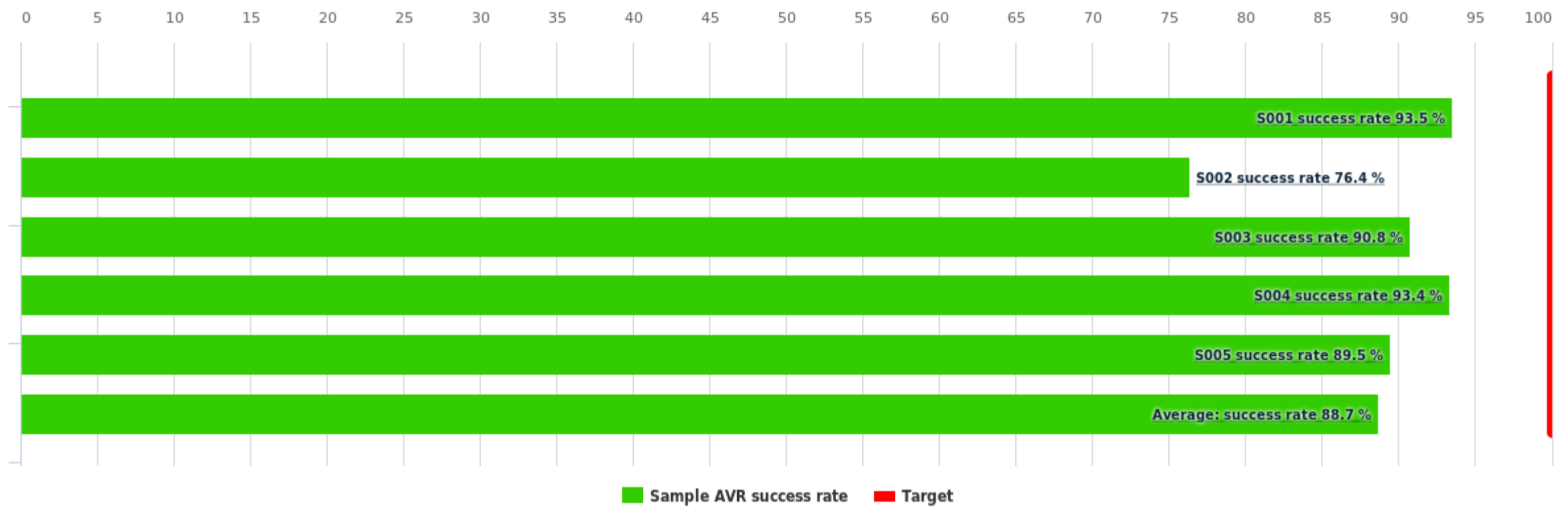
Score	Score count
-	84
0	1
3	1
<input checked="" type="radio"/> 4	3
5	14
Total:	103

GLOBAL REPORT

	No of participants	No of responded participants	Response percentage
Immunohistochemical staining methods, limited selection of antibodies, September, 2-2023	34	34	100 %
Immunohistochemical staining methods, September, 2-2023	69	69	100 %

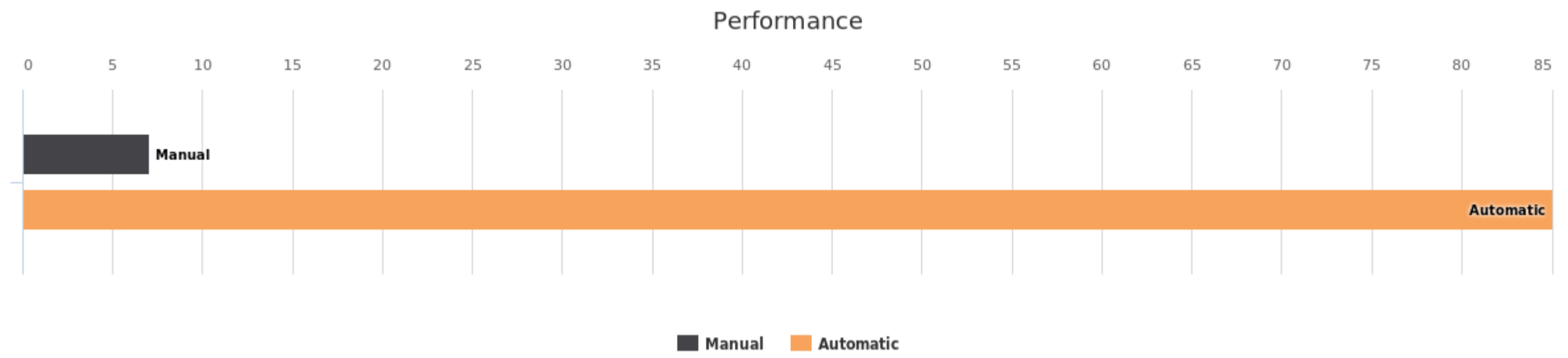
Summary

Overall success rate by samples



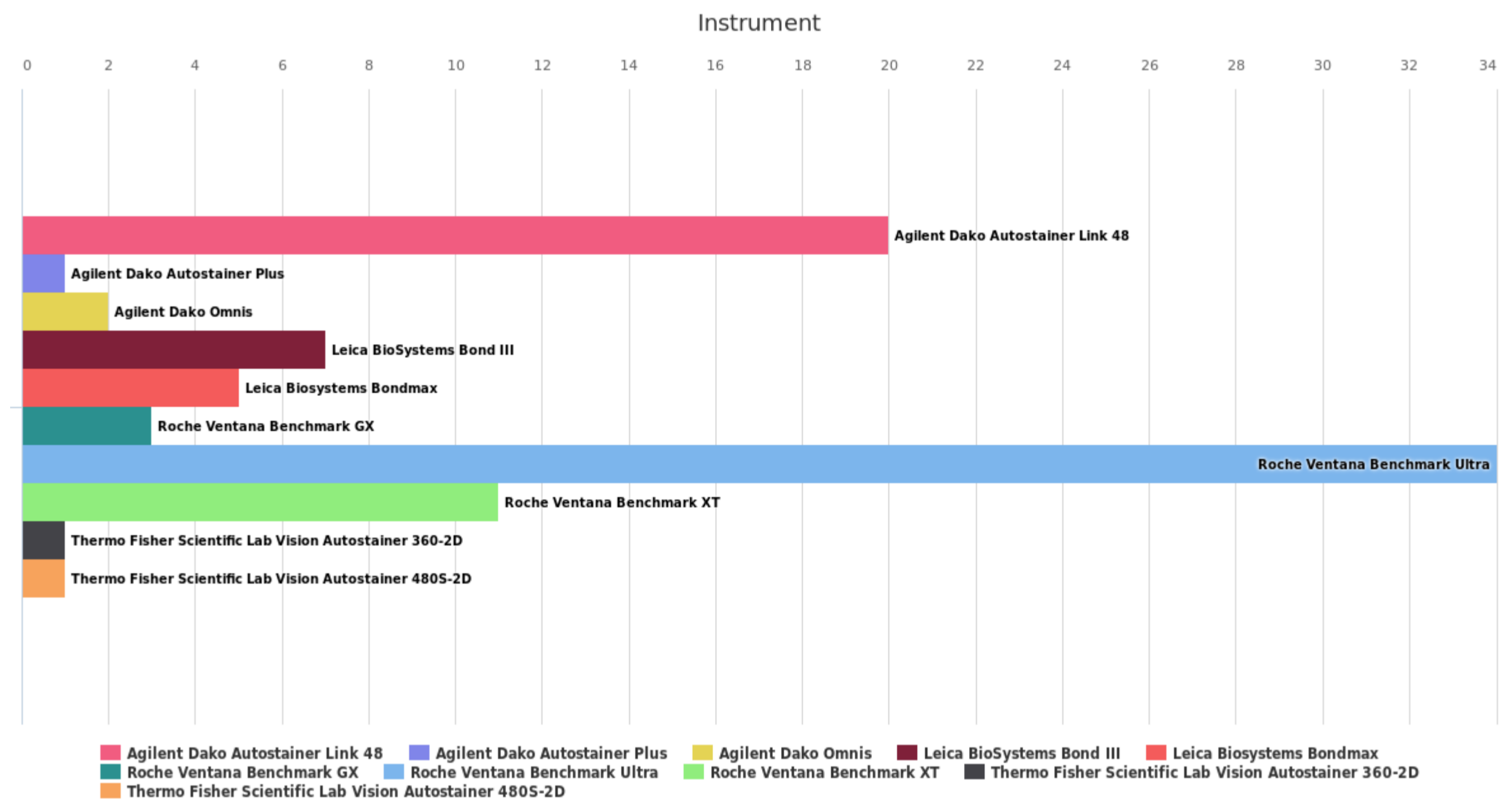
Summary	AVR success rate
S001	93.5 %
S002	76.4 %
S003	90.8 %
S004	93.4 %
S005	89.5 %
Average:	88.7 %

S001 | Sample S001 PR



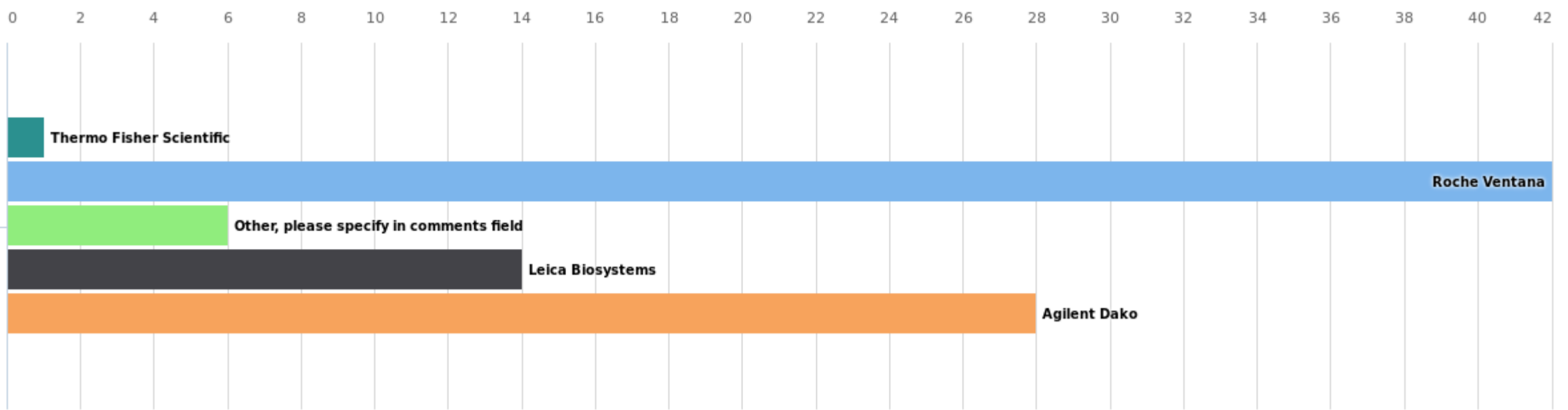
GENERAL DETAILS OF STAINING

Performance	Performance count
Automatic	85
Manual	7
Total:	92



Instrument	Instrument count
Agilent Dako Autostainer Link 48	20
Agilent Dako Autostainer Plus	1
Agilent Dako Omnis	2
Leica BioSystems Bond III	7
Leica Biosystems Bondmax	5
Roche Ventana Benchmark GX	3
Roche Ventana Benchmark Ultra	34
Roche Ventana Benchmark XT	11
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Total:	85

Manufacturer

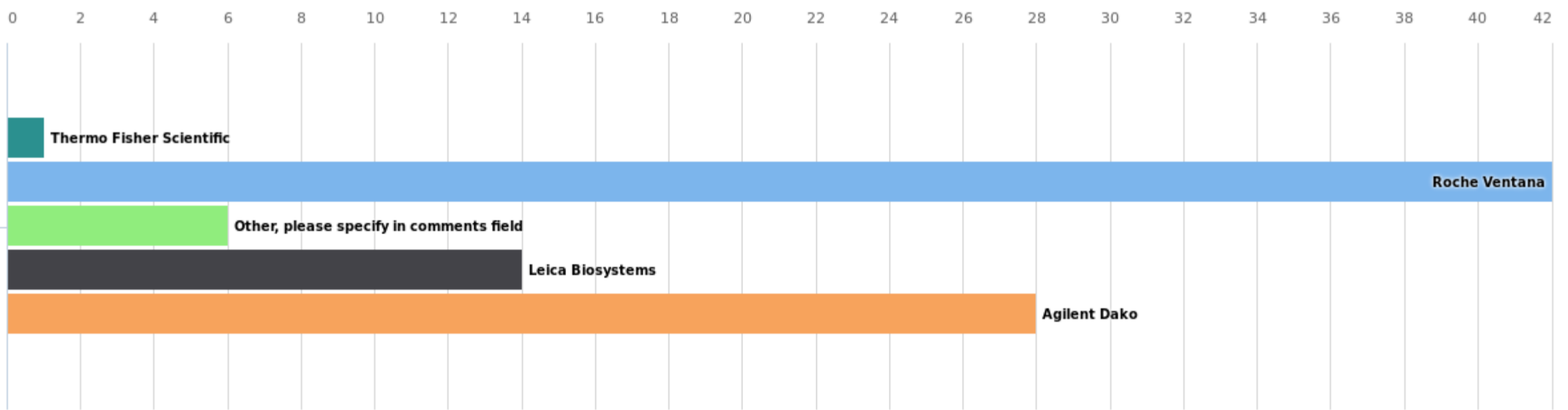


Thermo Fisher Scientific Roche Ventana Other, please specify in comments field Leica Biosystems Agilent Dako

PRIMARY ANTIBODY

Manufacturer	Manufacturer count
Agilent Dako	28
Leica Biosystems	14
Other, please specify in comments field	6
Roche Ventana	42
Thermo Fisher Scientific	1
Total:	91

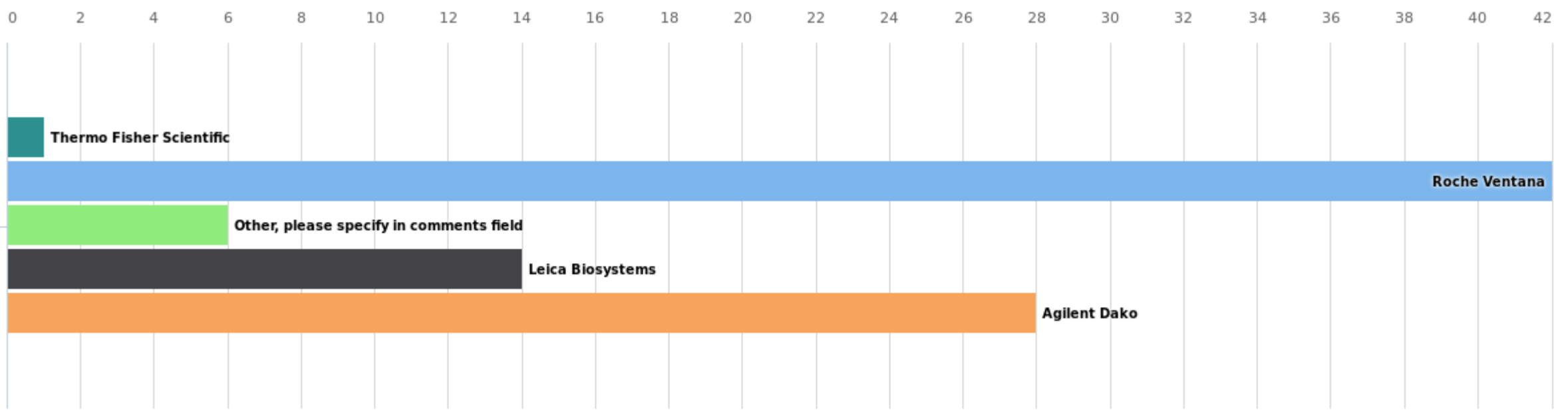
Clonal



Thermo Fisher Scientific Roche Ventana Other, please specify in comments field Leica Biosystems Agilent Dako

Clonal	Clonal count
Monoclonal	89
Polyclonal	1
Total:	90

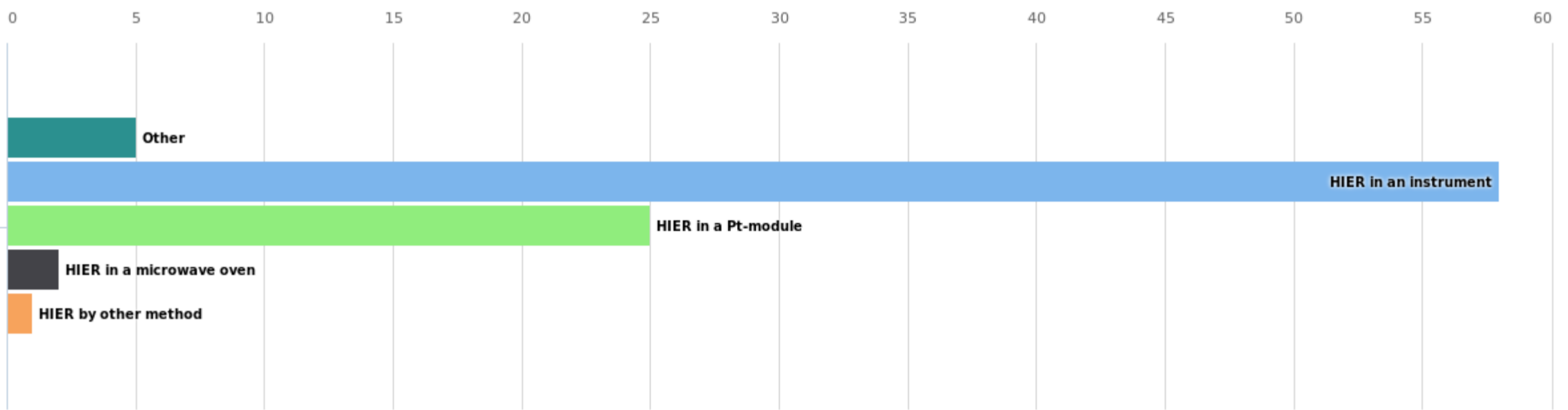
Dilution



Thermo Fisher Scientific Roche Ventana Other, please specify in comments field Leica Biosystems Agilent Dako

Dilution	Dilution count
Dilution	26
Ready-to-use	65
Total:	91

S001 Pretreatment

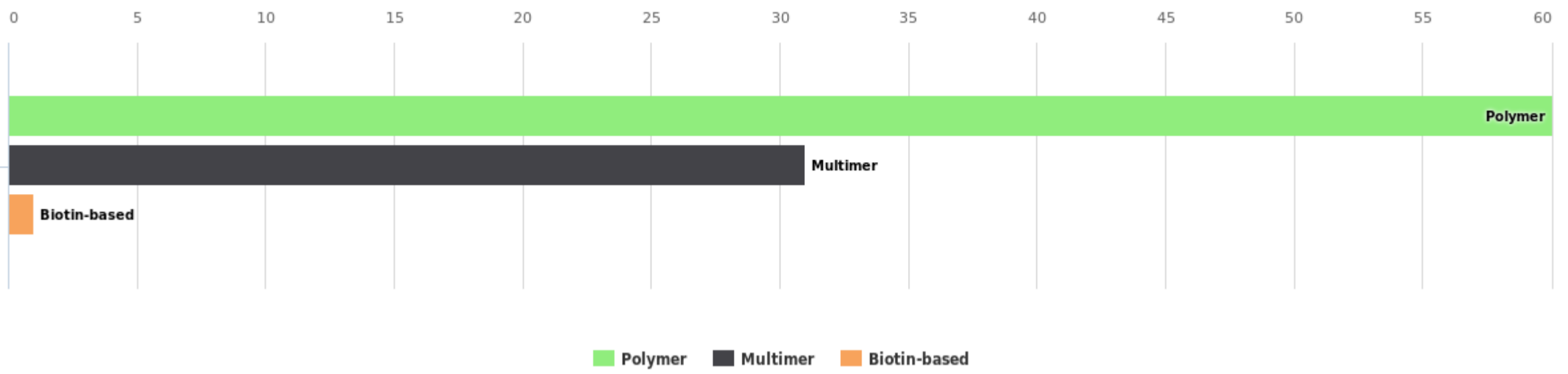


Other HIER in an instrument HIER in a Pt-module HIER in a microwave oven HIER by other method

PRETREATMENT

Pretreatment	Pretreatment count
HIER by other method	1
HIER in a microwave oven	2
HIER in a Pt-module	25
HIER in an instrument	58
Other	5
Total:	91

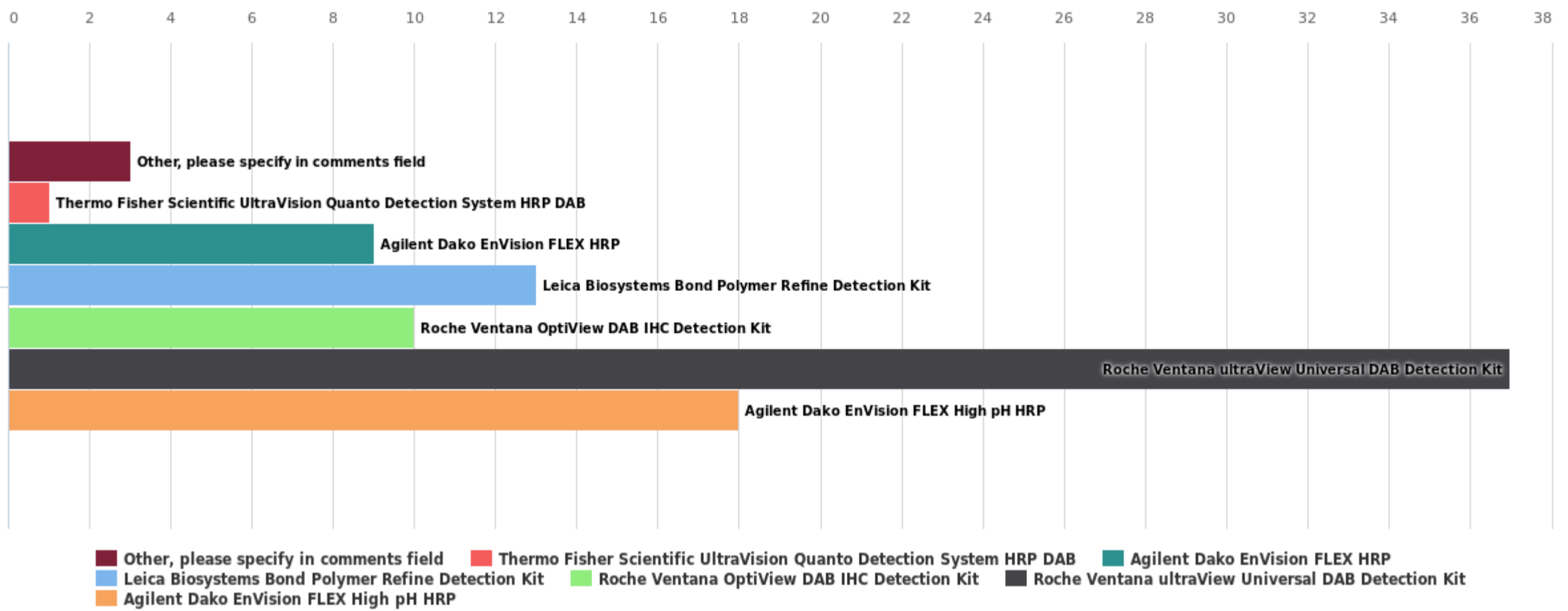
S001 Detection system



DETECTION SYSTEM

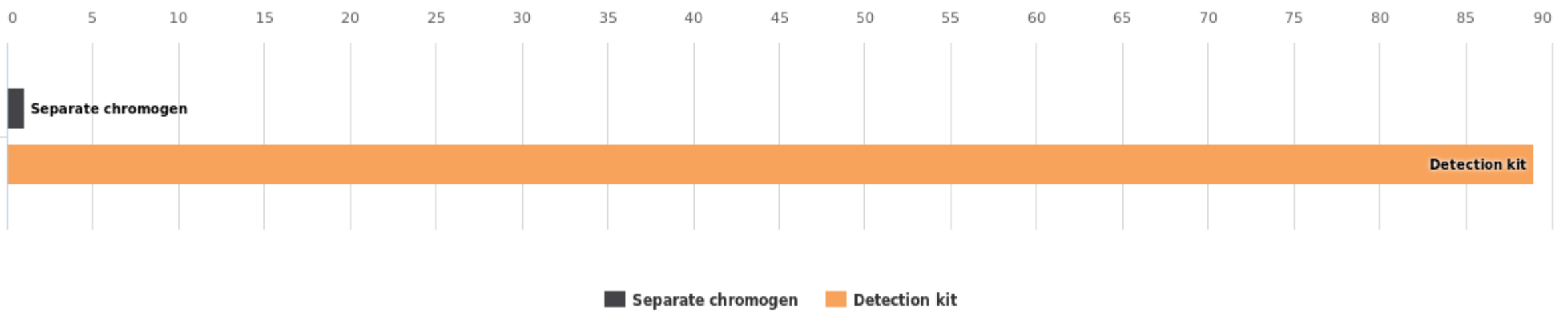
Detection system	Detection system count
Biotin-based	1
Multimer	31
Polymer	60
Total:	92

Detection system: Instrument



Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	9
Agilent Dako EnVision FLEX High pH HRP	18
Leica Biosystems Bond Polymer Refine Detection Kit	13
Other, please specify in comments field	3
Roche Ventana OptiView DAB IHC Detection Kit	10
Roche Ventana ultraView Universal DAB Detection Kit	37
Thermo Fisher Scientific UltraVision Quanto Detection System HRP DAB	1
Total:	91

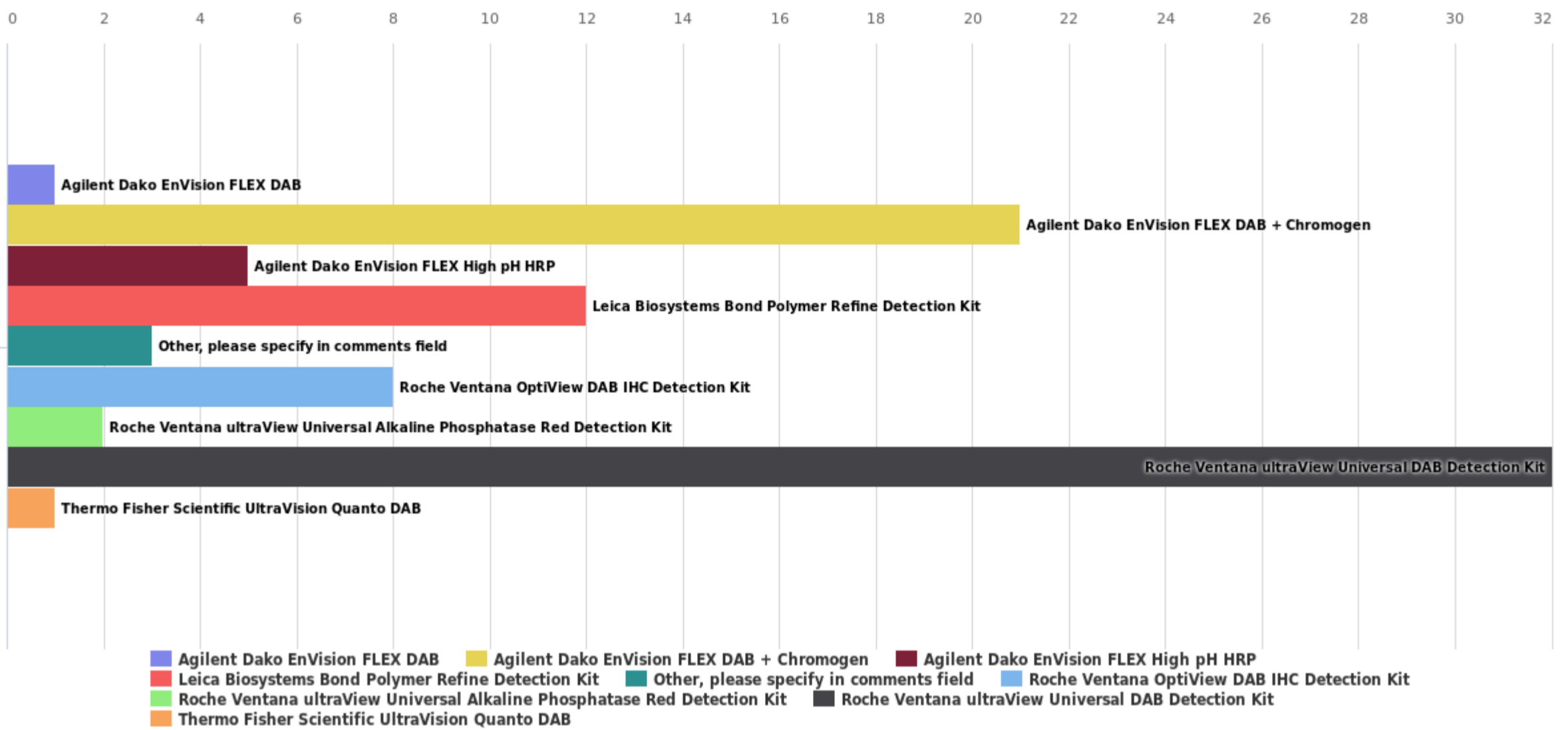
S001 Chromogen



CHROMOGEN

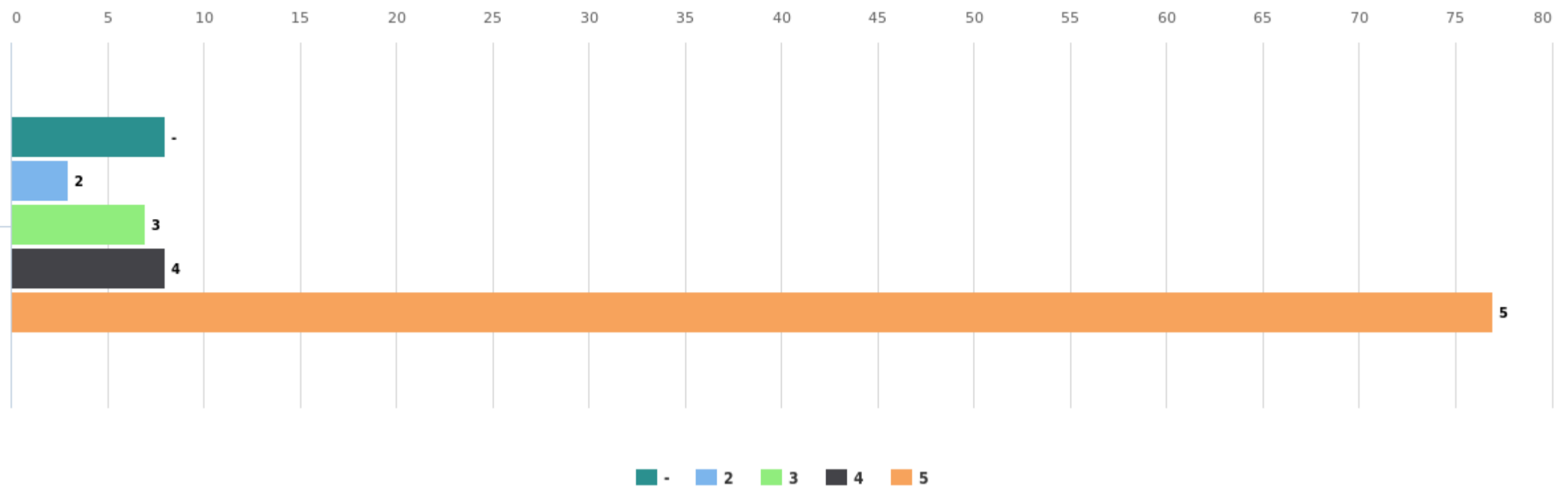
Chromogen	Chromogen count
Detection kit	89
Separate chromogen	1
Total:	90

Chromogen: Instrument



Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	1
Agilent Dako EnVision FLEX DAB + Chromogen	21
Agilent Dako EnVision FLEX High pH HRP	5
Leica Biosystems Bond Polymer Refine Detection Kit	12
Other, please specify in comments field	3
Roche Ventana OptiView DAB IHC Detection Kit	8
Roche Ventana ultraView Universal Alkaline Phosphatase Red Detection Kit	2
Roche Ventana ultraView Universal DAB Detection Kit	32
Thermo Fisher Scientific UltraVision Quanto DAB	1
Total:	85

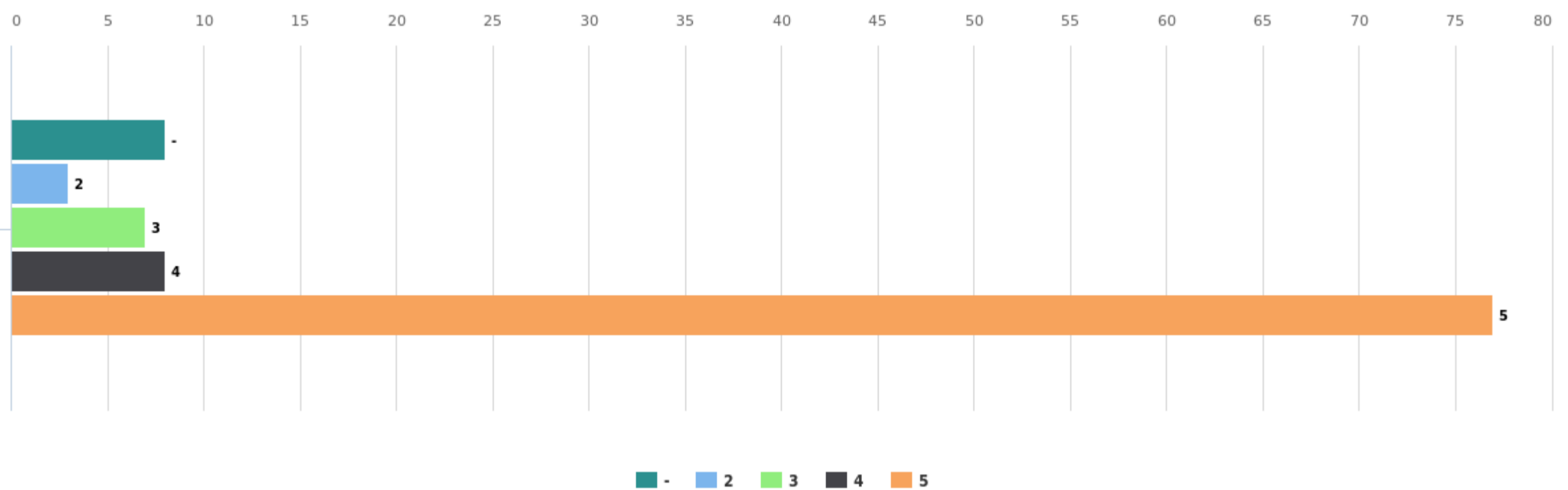
S001 Score



SCORE

Score	Comment
-	Not Available
2	False Negative Staining, Hematoxylin Also Weak, May Endanger Diagnosis False Negative Staining, May Endanger Diagnosis
3	No Hematoxylin, Bubble On Top Of Sample, Incomplete Staining Scraped Samples, Difficult To Evaluate Some False Negative Staining
4	Dark Hematoxylin Hematoxylin Weak Hematoxylin Weak, Staining Intensity A Bit Weak Morphology Impaired Some Background Staining Staining Intensity A Bit Weak
5	Embedding Failed Excellent
Total:	

S001 Score

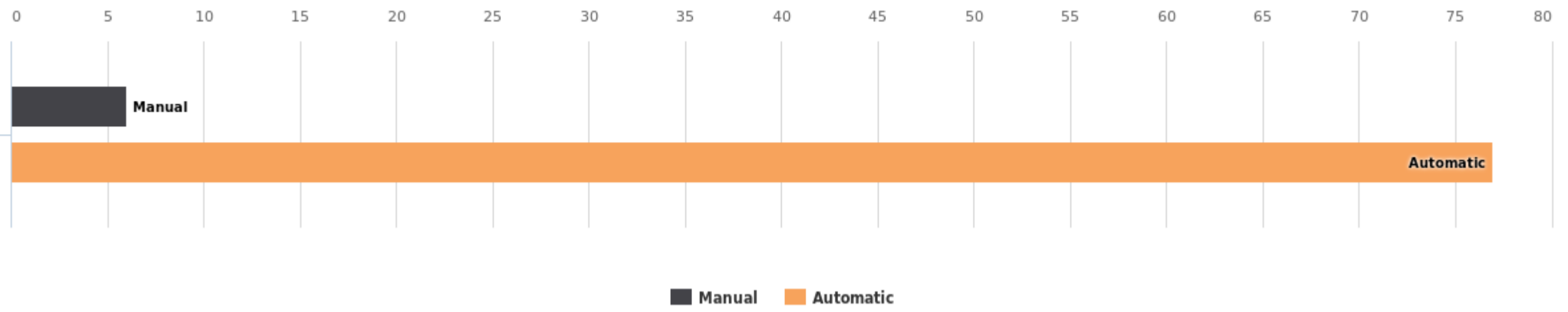


Score	Score count
-	8
2	3

3	7
4	8
5	77
Total:	103

S002 | Sample S002 HER2

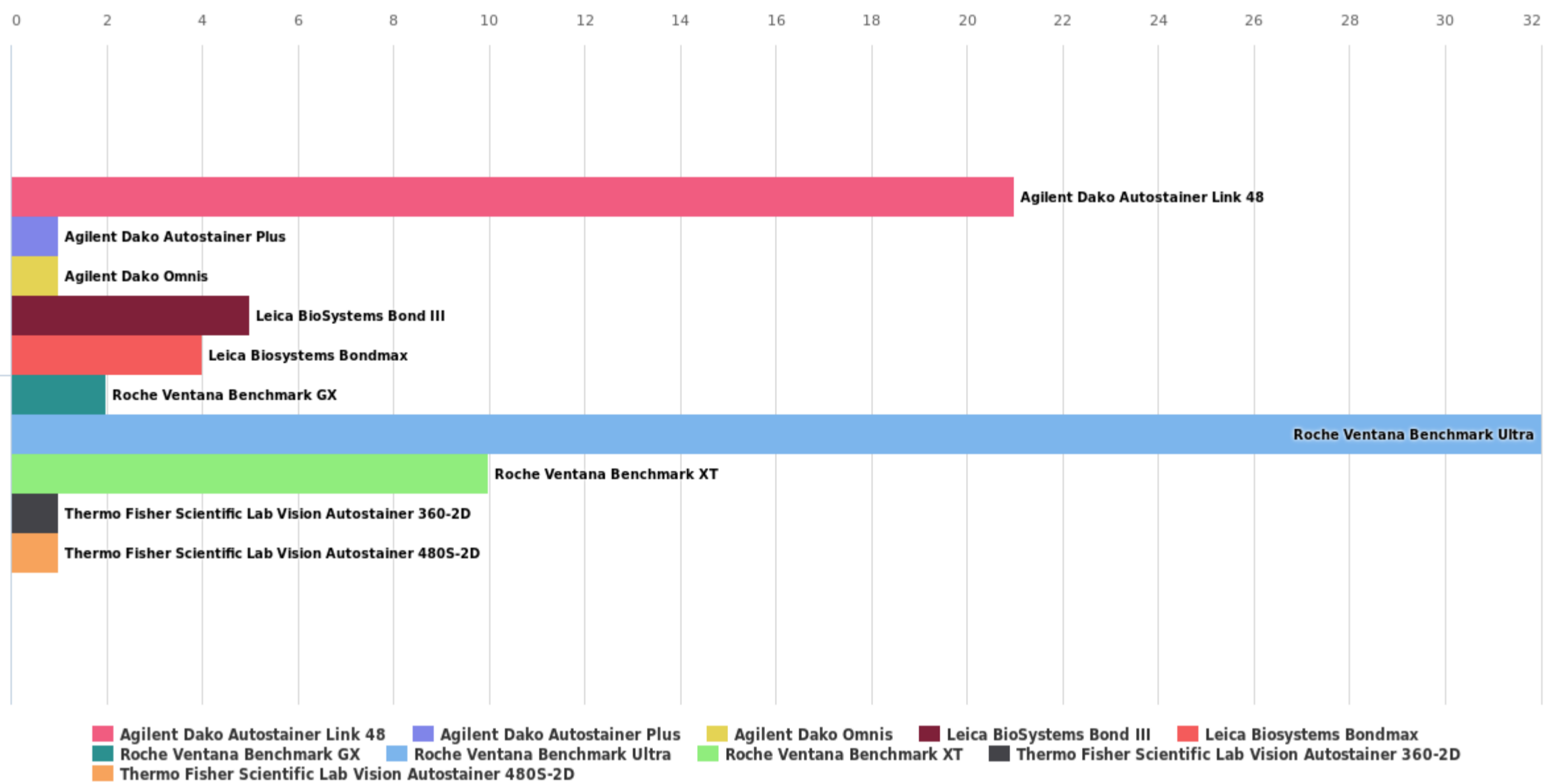
Performance



GENERAL DETAILS OF STAINING

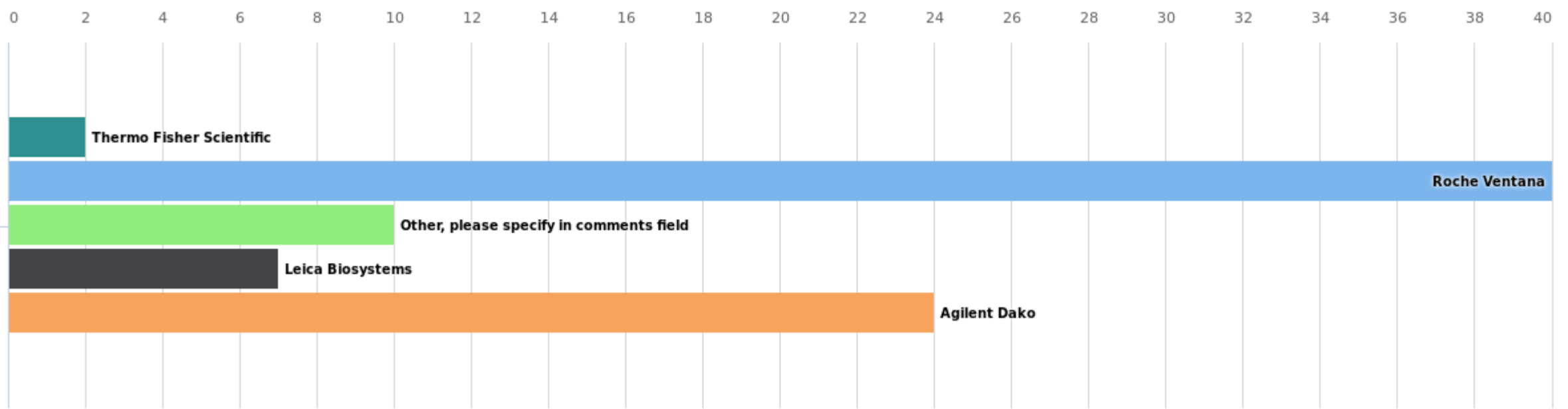
Performance	Performance count
Automatic	77
Manual	6
Total:	83

Instrument



Instrument	Instrument count
Agilent Dako Autostainer Link 48	21
Agilent Dako Autostainer Plus	1
Agilent Dako Omnis	1
Leica BioSystems Bond III	5
Leica Biosystems Bondmax	4
Roche Ventana Benchmark GX	2
Roche Ventana Benchmark Ultra	32
Roche Ventana Benchmark XT	10
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Total:	78

Manufacturer

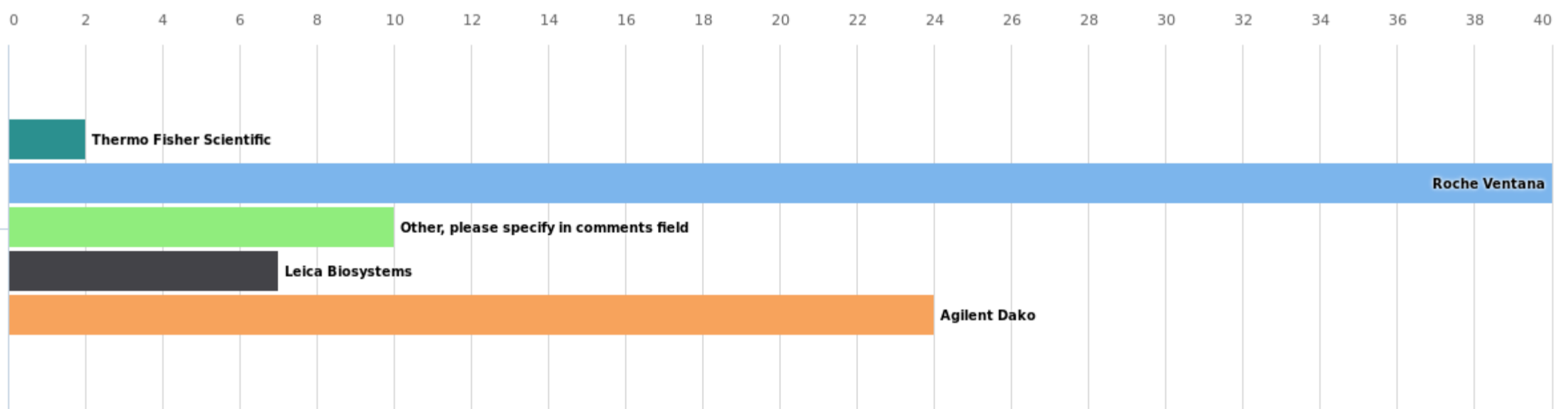


■ Thermo Fisher Scientific
 ■ Roche Ventana
 ■ Other, please specify in comments field
 ■ Leica Biosystems
 ■ Agilent Dako

PRIMARY ANTIBODY

Manufacturer	Manufacturer count
Agilent Dako	24
Leica Biosystems	7
Other, please specify in comments field	10
Roche Ventana	40
Thermo Fisher Scientific	2
Total:	83

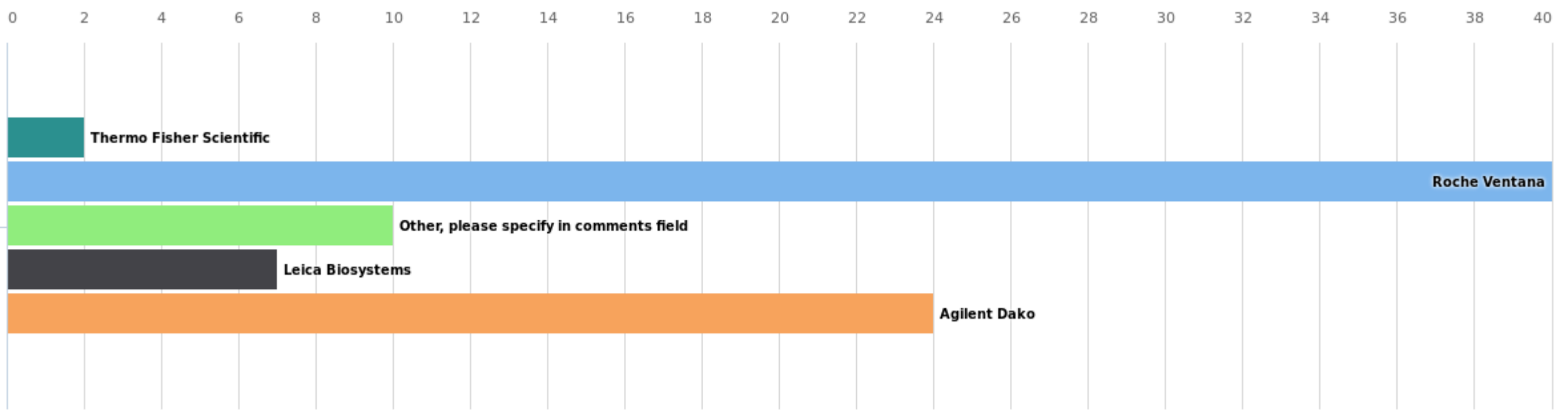
Clonal



■ Thermo Fisher Scientific
 ■ Roche Ventana
 ■ Other, please specify in comments field
 ■ Leica Biosystems
 ■ Agilent Dako

Clonal	Clonal count
Monoclonal	65
Polyclonal	18
Total:	83

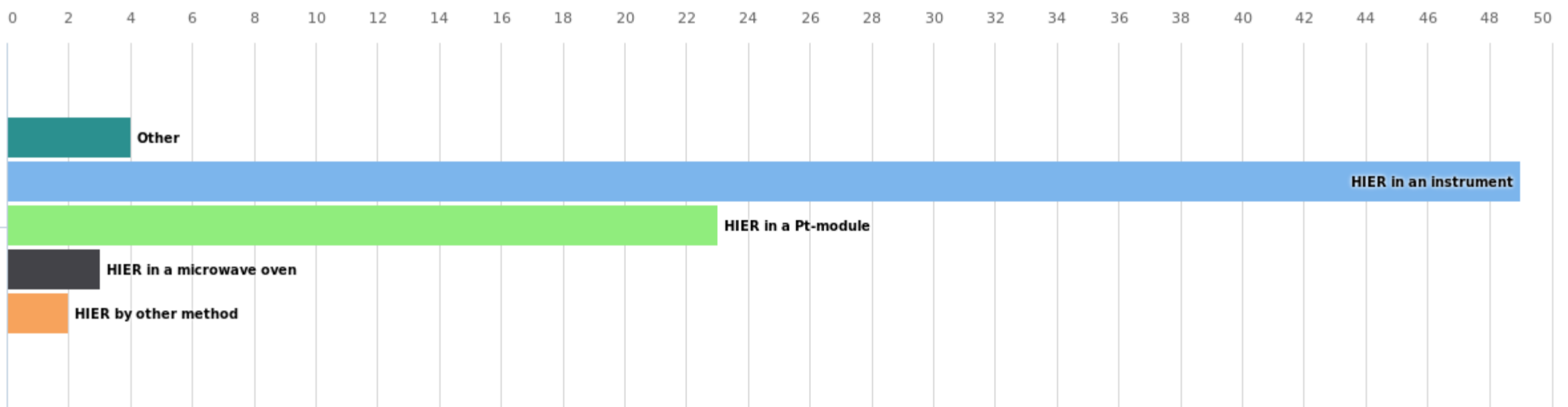
Dilution



■ Thermo Fisher Scientific ■ Roche Ventana ■ Other, please specify in comments field ■ Leica Biosystems ■ Agilent Dako

Dilution	Dilution count
Dilution	29
Ready-to-use	54
Total:	83

S002 Pretreatment

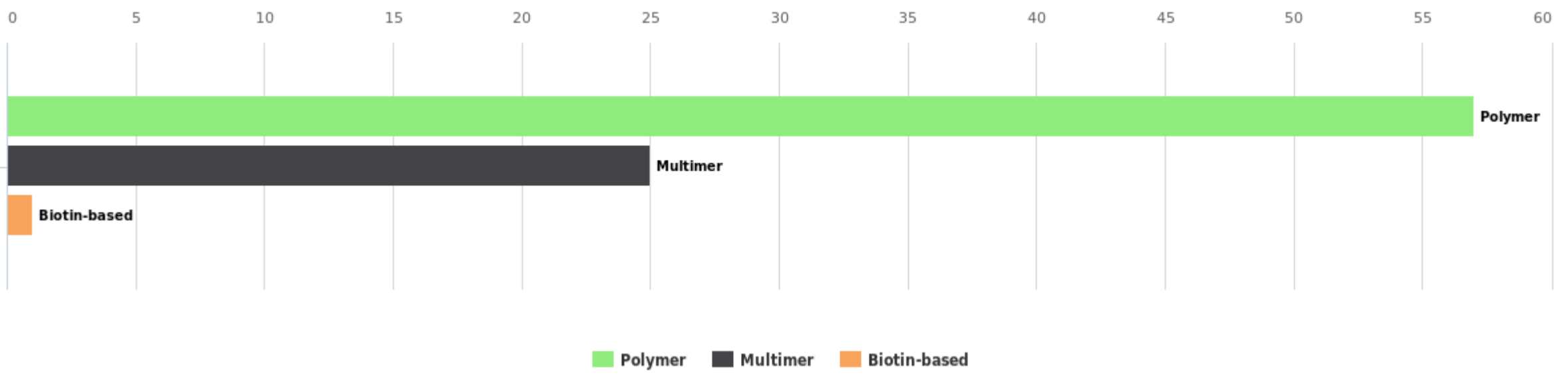


■ Other ■ HIER in an instrument ■ HIER in a Pt-module ■ HIER in a microwave oven ■ HIER by other method

PRETREATMENT

Pretreatment	Pretreatment count
HIER by other method	2
HIER in a microwave oven	3
HIER in a Pt-module	23
HIER in an instrument	49
Other	4
Total:	81

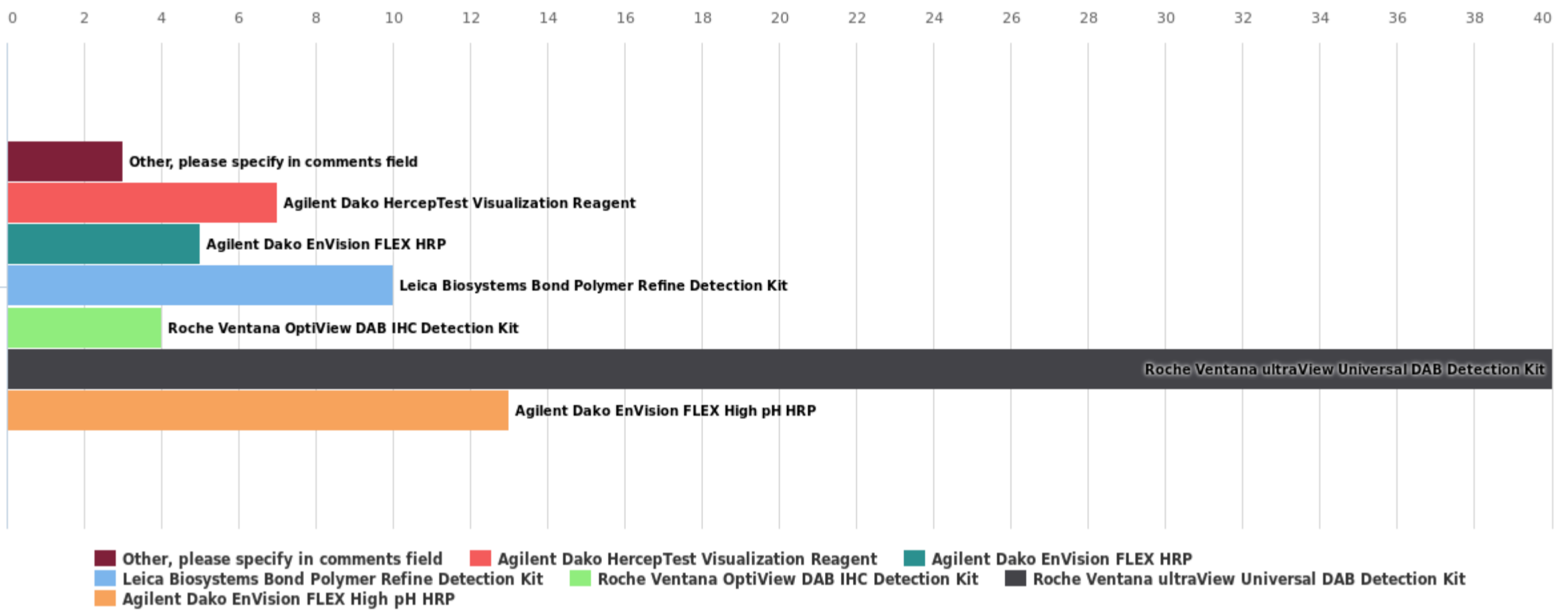
S002 Detection system



DETECTION SYSTEM

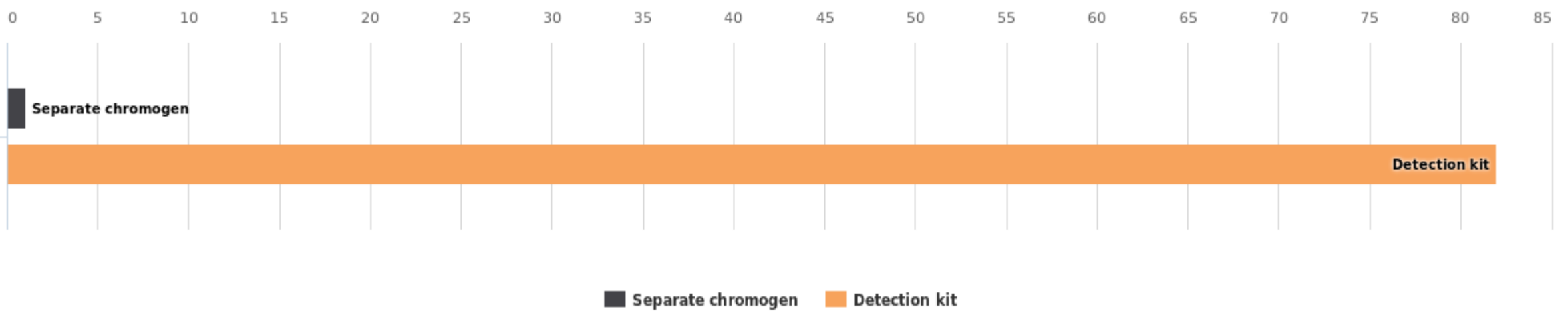
Detection system	Detection system count
Biotin-based	1
Multimer	25
Polymer	57
Total:	83

Detection system: Instrument



Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	5
Agilent Dako EnVision FLEX High pH HRP	13
Agilent Dako HercepTest Visualization Reagent	7
Leica Biosystems Bond Polymer Refine Detection Kit	10
Other, please specify in comments field	3
Roche Ventana OptiView DAB IHC Detection Kit	4
Roche Ventana ultraView Universal DAB Detection Kit	40
Total:	82

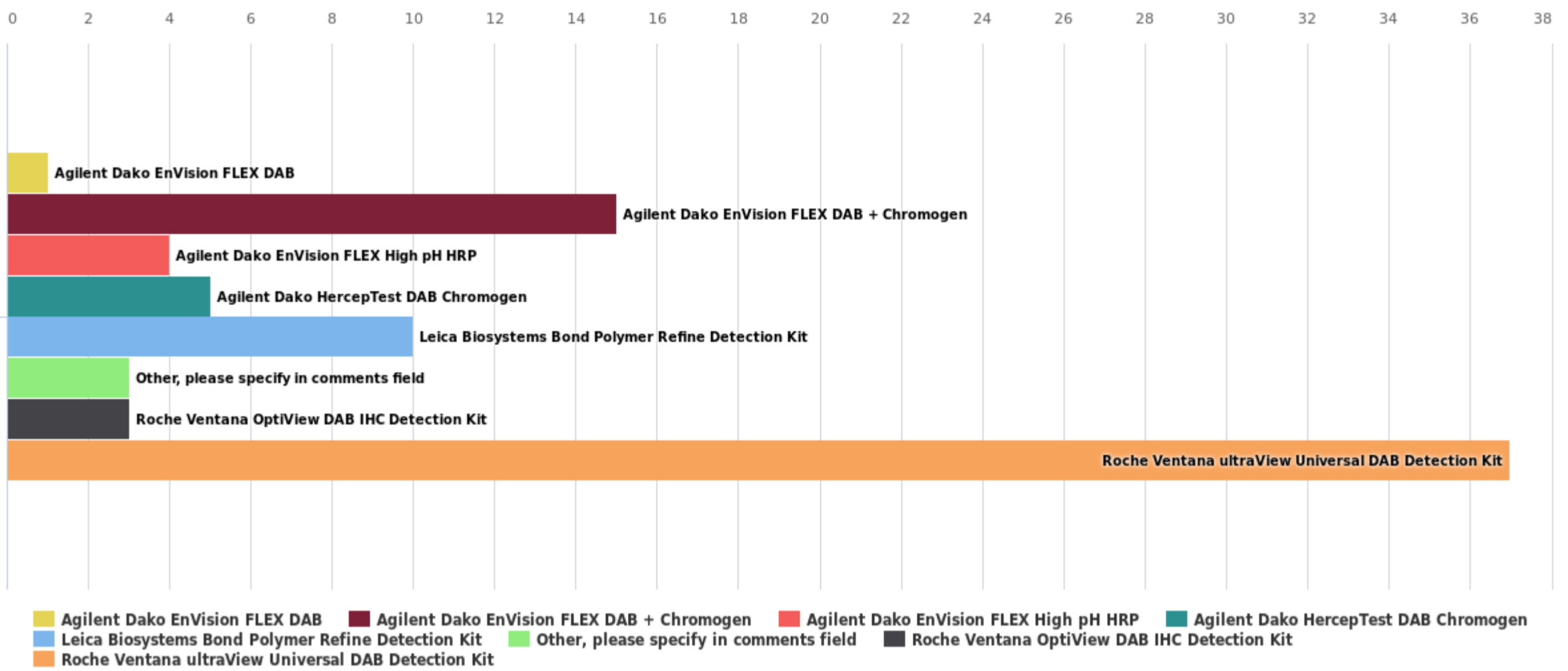
S002 Chromogen



CHROMOGEN

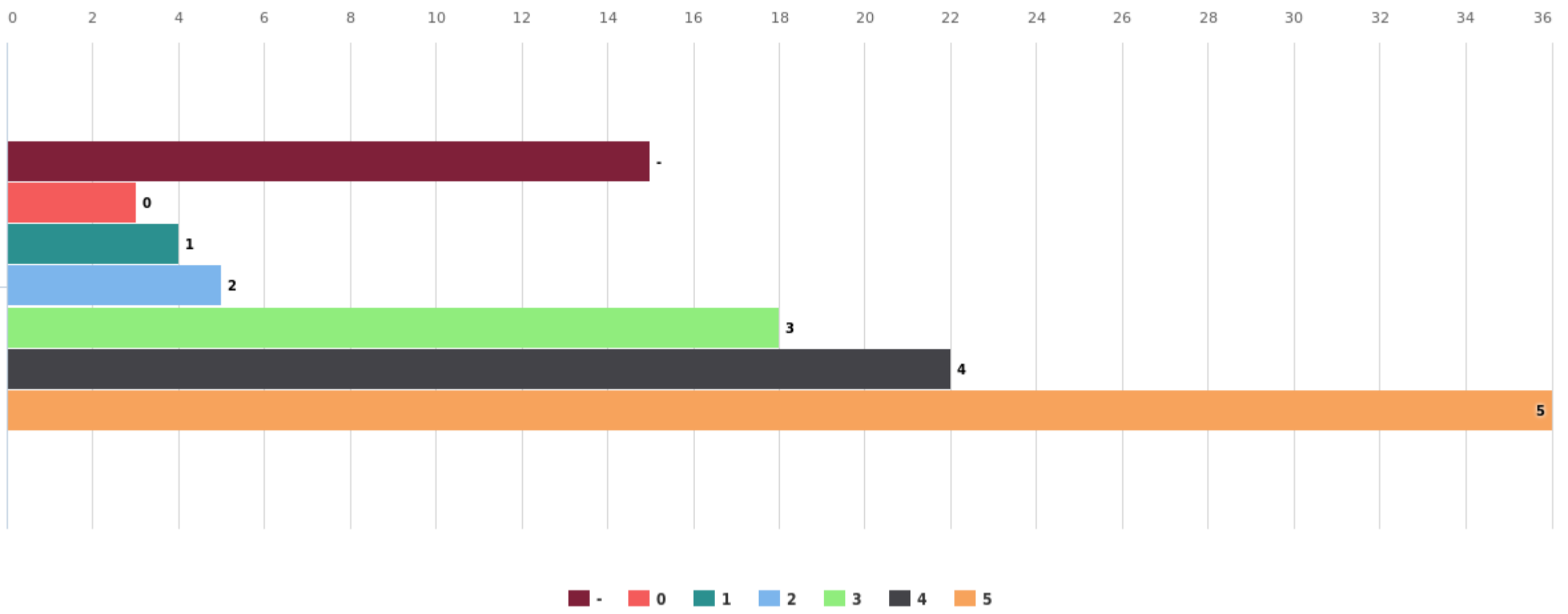
Chromogen	Chromogen count
Detection kit	82
Separate chromogen	1
Total:	83

Chromogen: Instrument



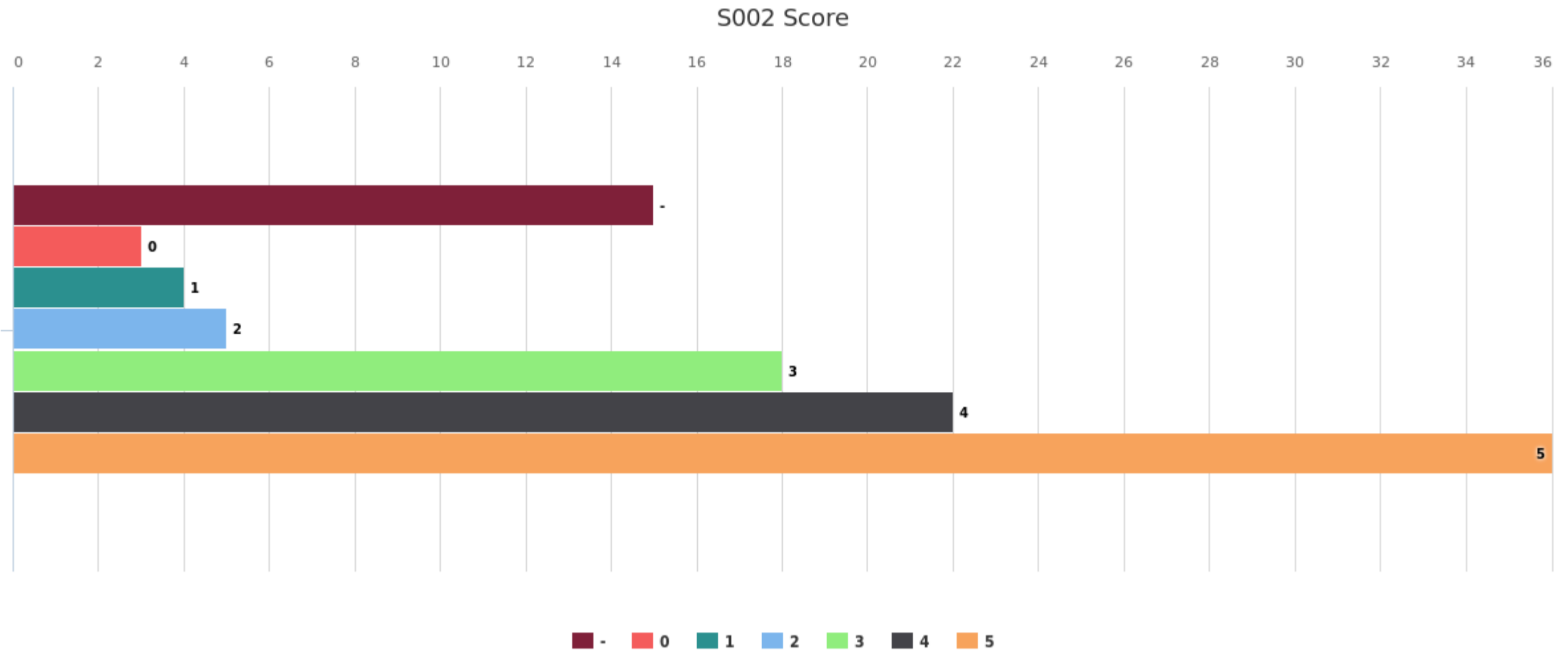
Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	1
Agilent Dako EnVision FLEX DAB + Chromogen	15
Agilent Dako EnVision FLEX High pH HRP	4
Agilent Dako HercepTest DAB Chromogen	5
Leica Biosystems Bond Polymer Refine Detection Kit	10
Other, please specify in comments field	3
Roche Ventana OptiView DAB IHC Detection Kit	3
Roche Ventana ultraView Universal DAB Detection Kit	37
Total:	78

S002 Score



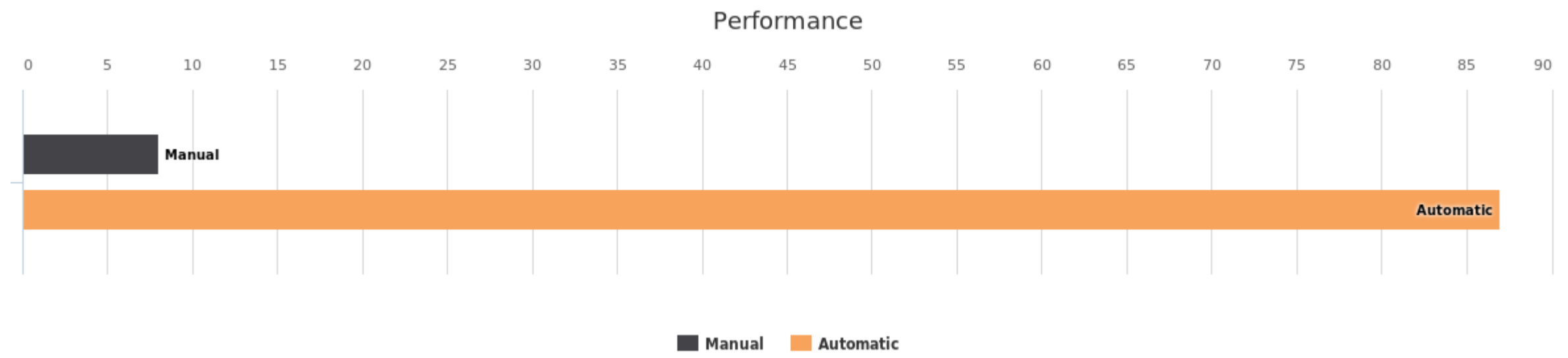
SCORE

Score	Comment
-	Not Available
0	Failed, Extreme False Positive Staining, Diagnostically Unacceptable
1	Extreme False Positive Staining, Diagnostically Unacceptable
2	False Positive Staining With Disturbing Strong Background Staining, May Endanger Diagnosis
3	False Positive Staining False Positive Staining With Strong Background Staining Strong Background Staining Mainly In The Cytoplasm Strong Background Staining, Some False Positive Cells Strong Background Staining, Weak Staining Intensity, Hematoxylin Very Weak
4	Mild Background Staining Weak Staining Intensity
5	Excellent
Total:	



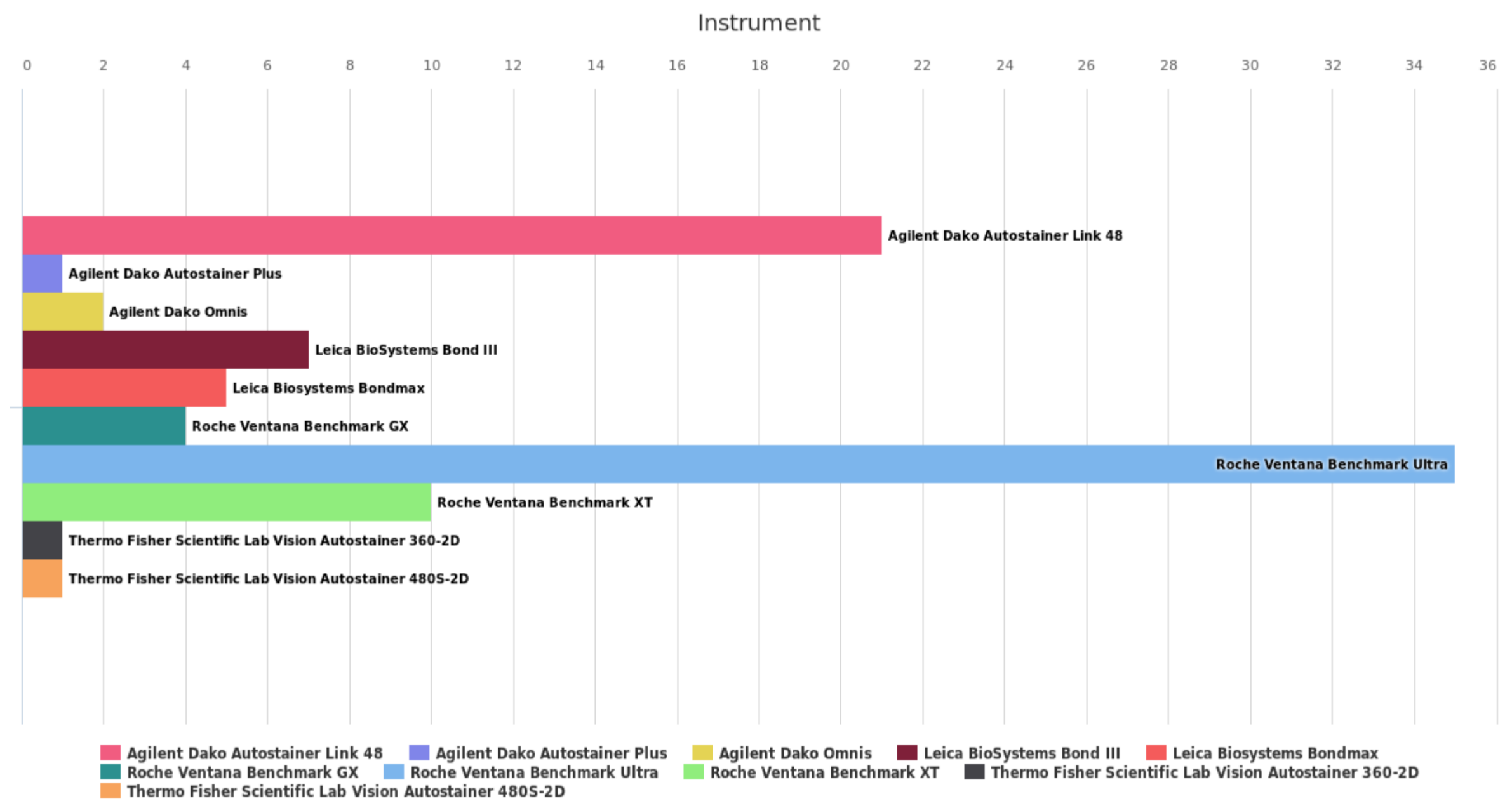
Score	Score count
-	15
0	3
1	4
2	5
3	18
4	22
5	36
Total:	103

S003 | Sample S003 ER



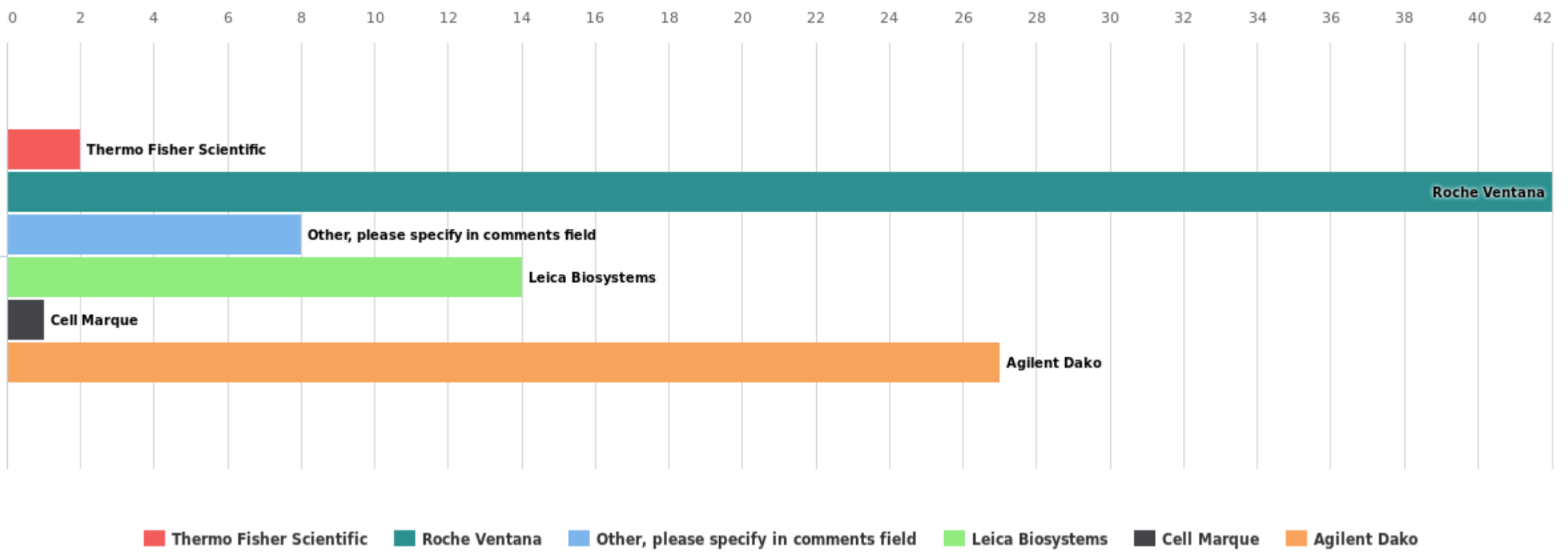
GENERAL DETAILS OF STAINING

Performance	Performance count
Automatic	87
Manual	8
Total:	95



Instrument	Instrument count
Agilent Dako Autostainer Link 48	21
Agilent Dako Autostainer Plus	1
Agilent Dako Omnis	2
Leica BioSystems Bond III	7
Leica Biosystems Bondmax	5
Roche Ventana Benchmark GX	4
Roche Ventana Benchmark Ultra	35
Roche Ventana Benchmark XT	10
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Total:	87

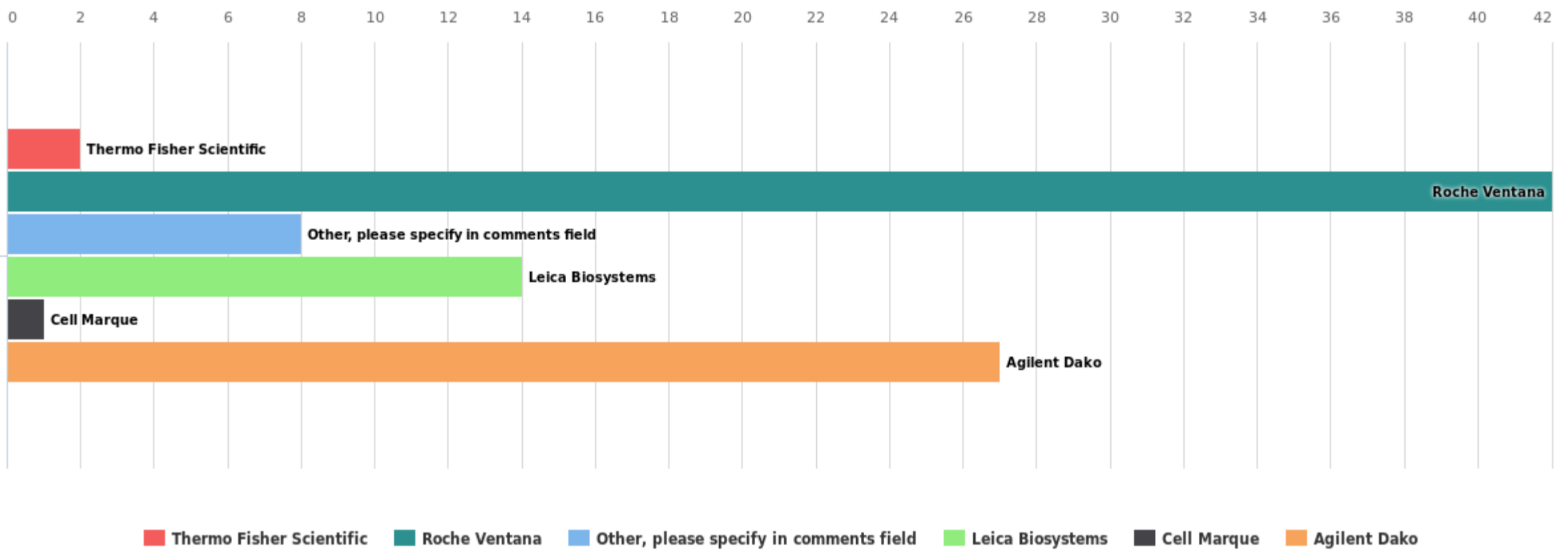
Manufacturer



PRIMARY ANTIBODY

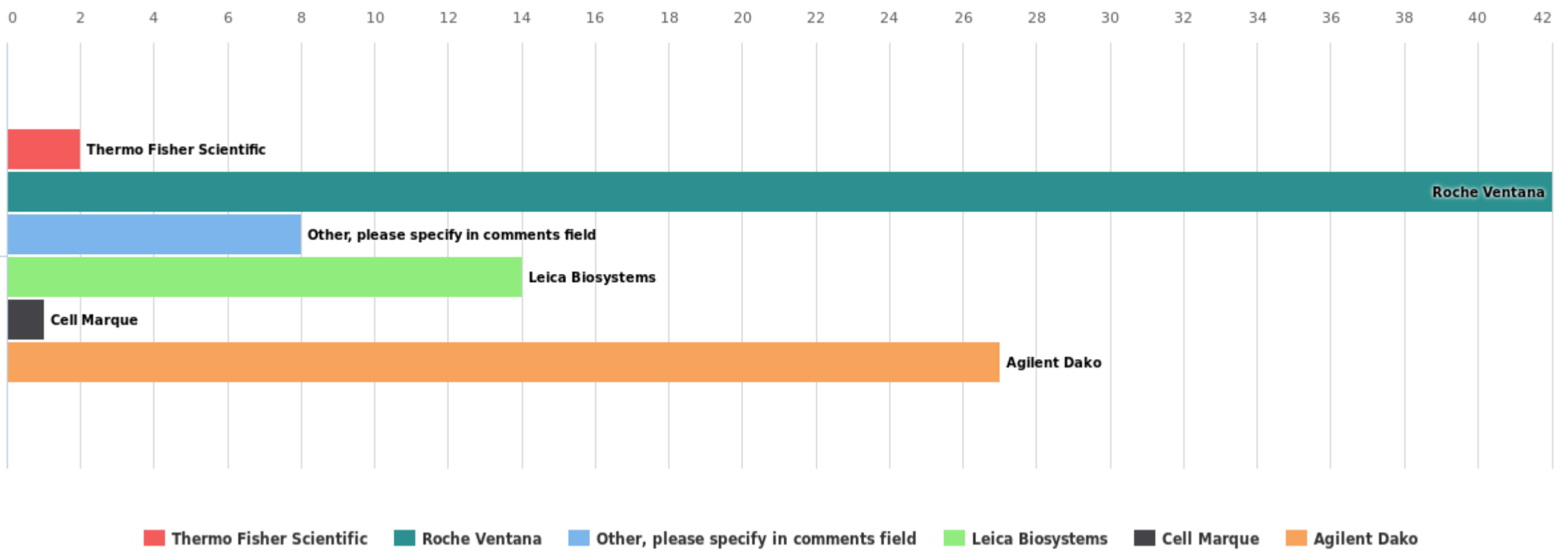
Manufacturer	Manufacturer count
Agilent Dako	27
Cell Marque	1
Leica Biosystems	14
Other, please specify in comments field	8
Roche Ventana	42
Thermo Fisher Scientific	2
Total:	94

Clonal



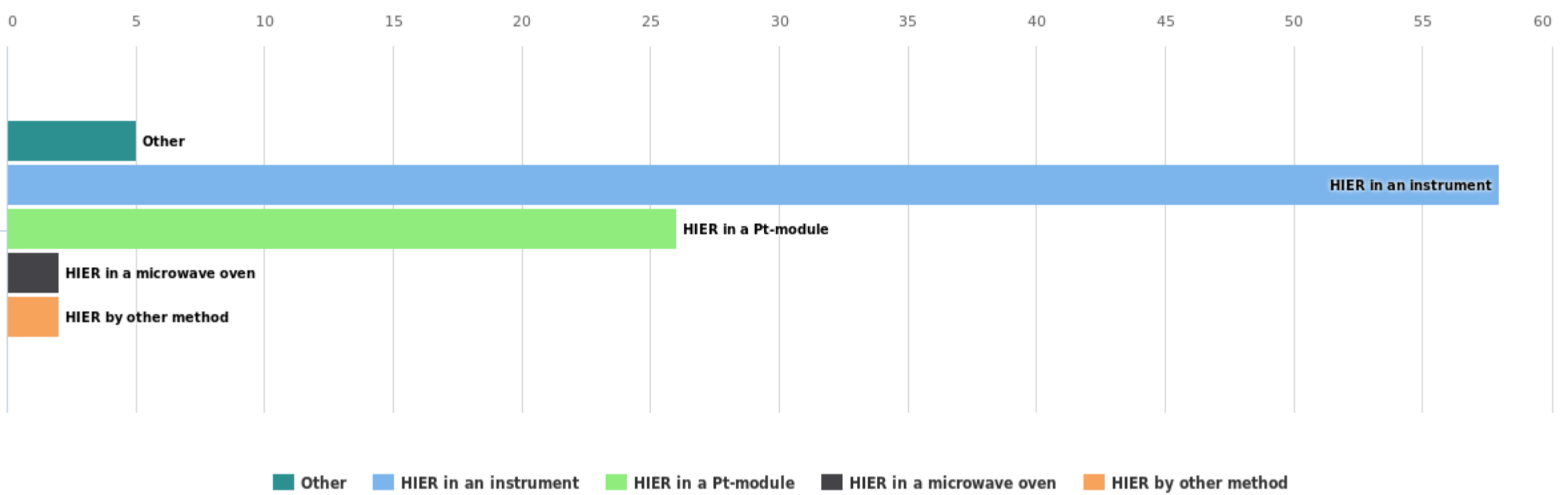
Clonal	Clonal count
Monoclonal	91
Polyclonal	1
Total:	92

Dilution



Dilution	Dilution count
Dilution	28
Ready-to-use	66
Total:	94

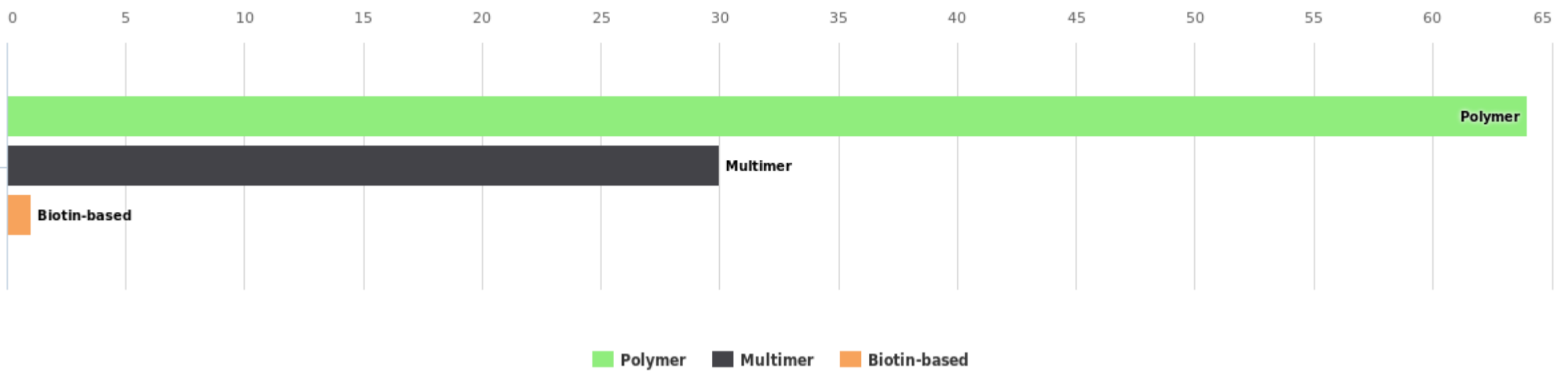
S003 Pretreatment



PRETREATMENT

Pretreatment	Pretreatment count
HIER by other method	2
HIER in a microwave oven	2
HIER in a Pt-module	26
HIER in an instrument	58
Other	5
Total:	93

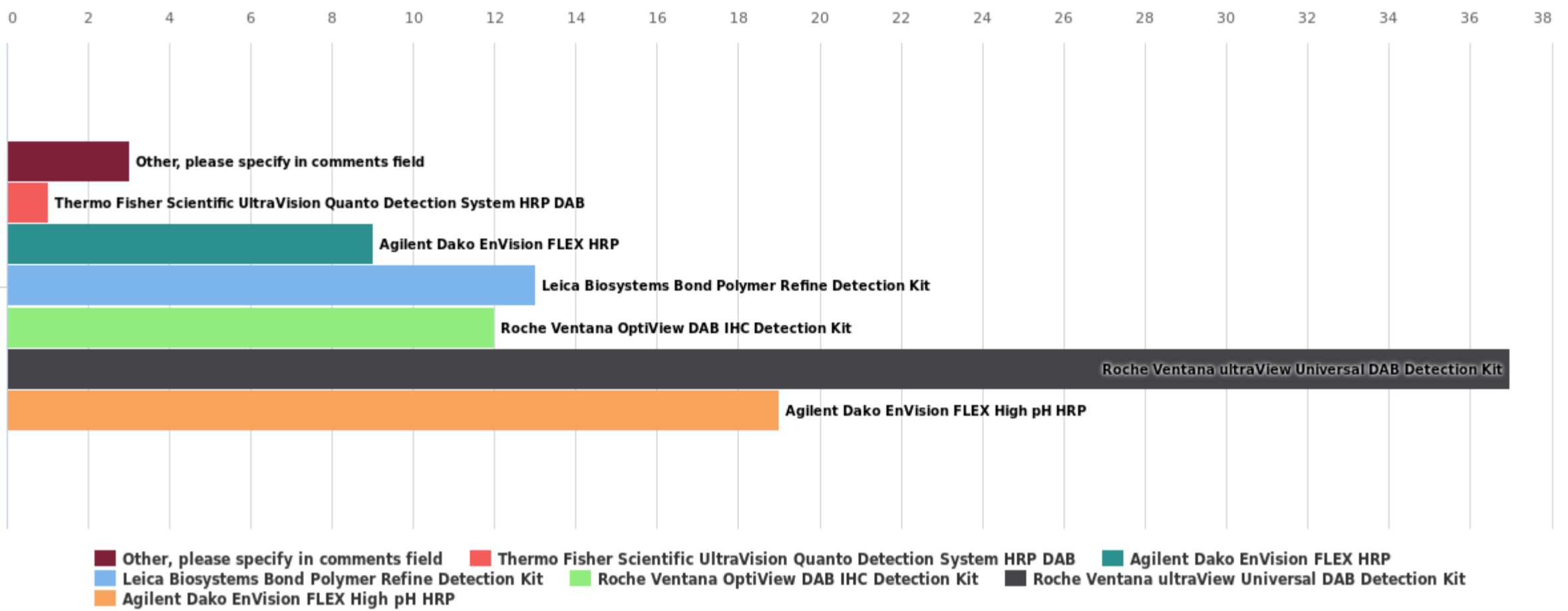
S003 Detection system



DETECTION SYSTEM

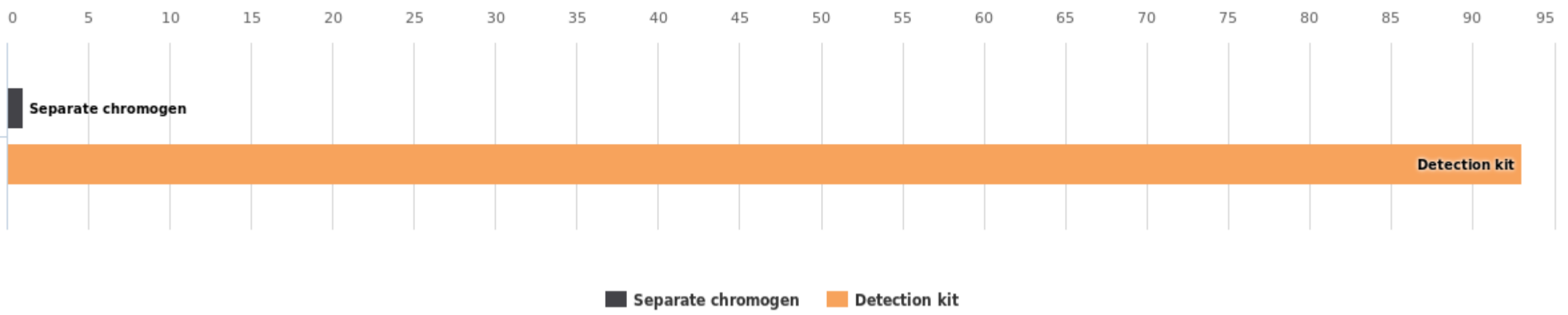
Detection system	Detection system count
Biotin-based	1
Multimer	30
Polymer	64
Total:	95

Detection system: Instrument



Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	9
Agilent Dako EnVision FLEX High pH HRP	19
Leica Biosystems Bond Polymer Refine Detection Kit	13
Other, please specify in comments field	3
Roche Ventana OptiView DAB IHC Detection Kit	12
Roche Ventana ultraView Universal DAB Detection Kit	37
Thermo Fisher Scientific UltraVision Quanto Detection System HRP DAB	1
Total:	94

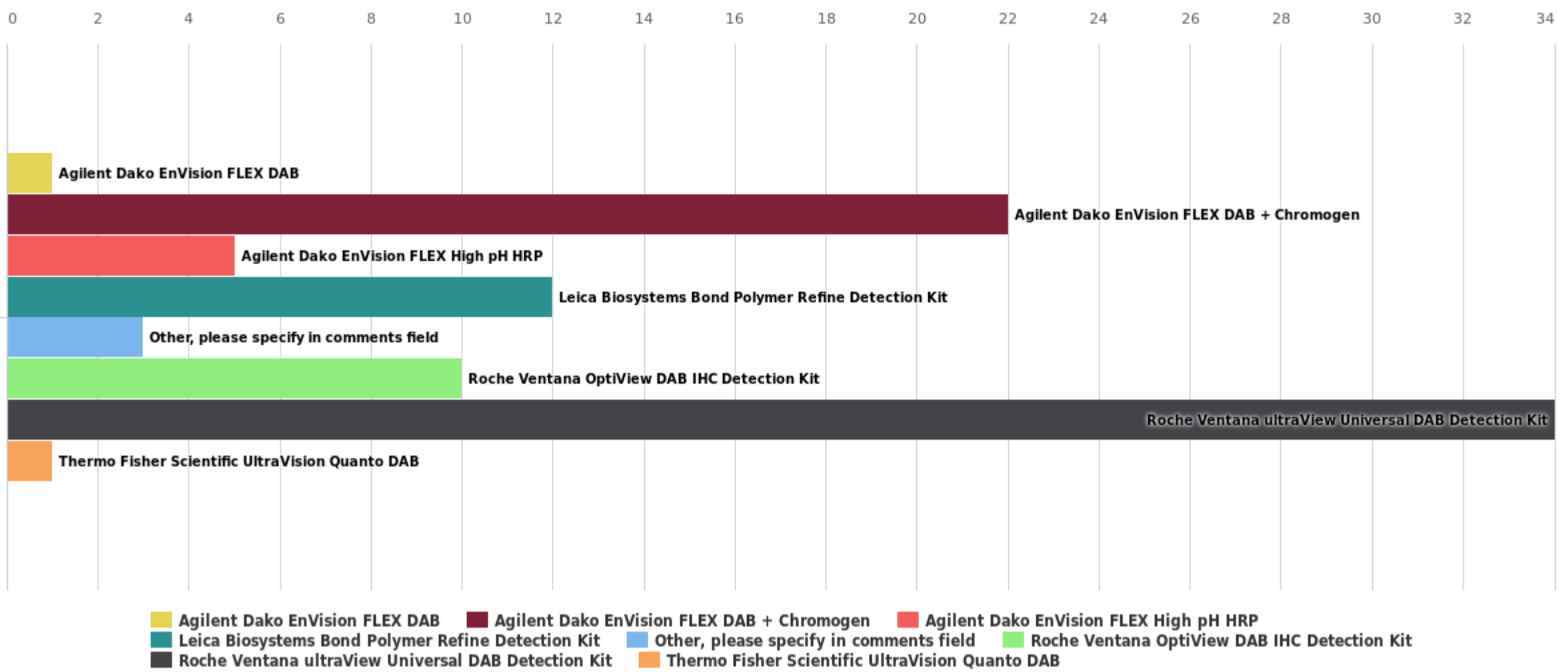
S003 Chromogen



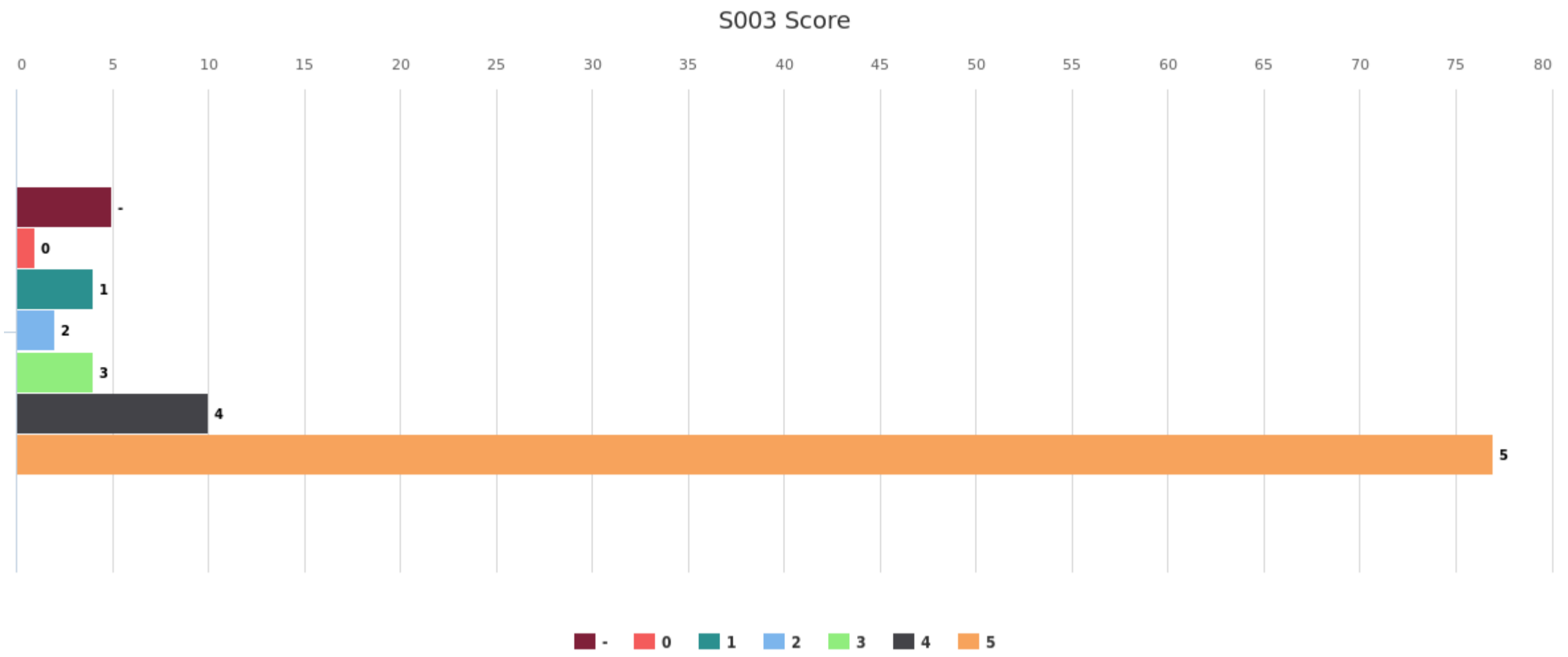
CHROMOGEN

Chromogen	Chromogen count
Detection kit	93
Separate chromogen	1
Total:	94

Chromogen: Instrument

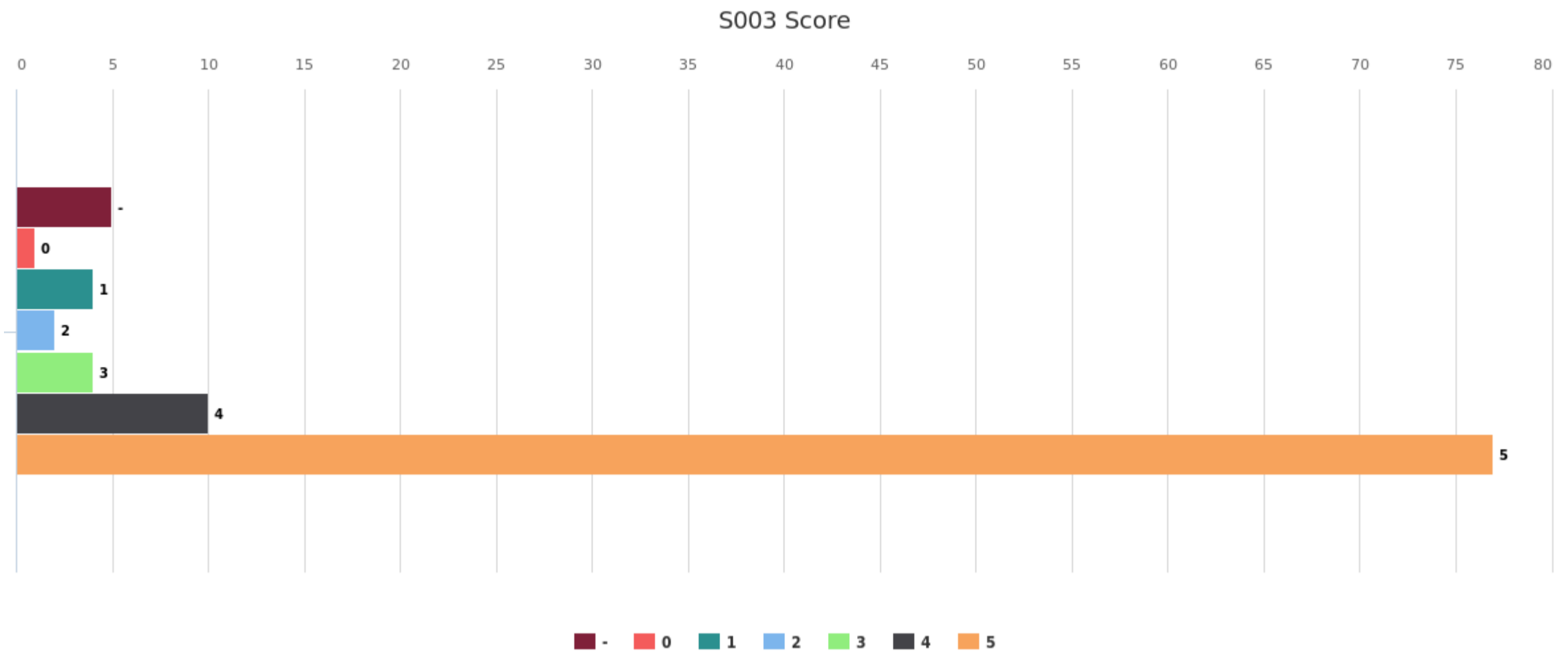


Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	1
Agilent Dako EnVision FLEX DAB + Chromogen	22
Agilent Dako EnVision FLEX High pH HRP	5
Leica Biosystems Bond Polymer Refine Detection Kit	12
Other, please specify in comments field	3
Roche Ventana OptiView DAB IHC Detection Kit	10
Roche Ventana ultraView Universal DAB Detection Kit	34
Thermo Fisher Scientific UltraVision Quanto DAB	1
Total:	88



SCORE

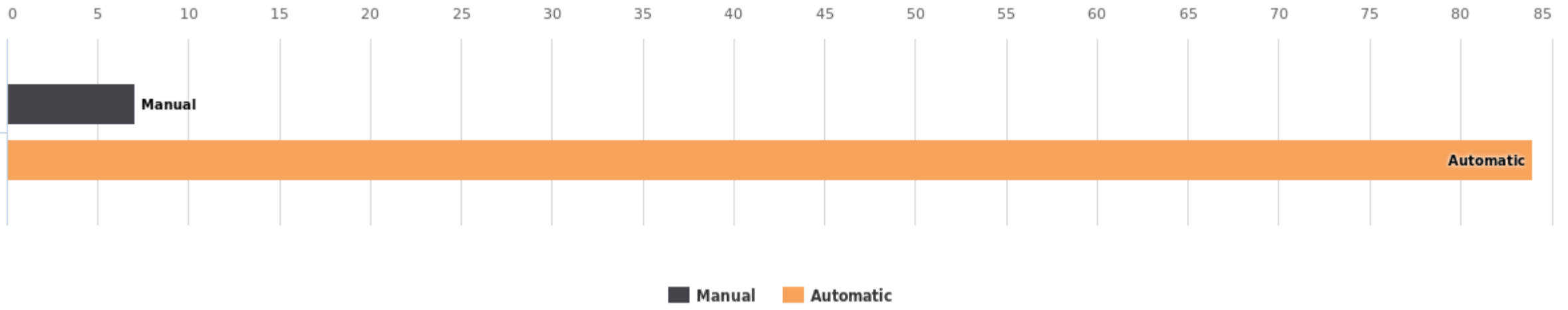
Score	Comment
-	Not Available
0	Poor, No Staining At All, Blanco
1	Unsuccessful, Both False Negative And False Positive Staining, Diagnostically Unacceptable Unsuccessful, False Negative Staining, Diagnostically Unacceptable
2	Diagnosis May Compromise, False Negativity, Staining Intensity Very Weak Diagnosis May Compromise, False Negativity, Staining Intensity Very Weak, Poor Contrast
3	False Negativity, Staining Intensity Weak
4	Staining Intensity A Bit Weak Staining intensity a bit weak
5	Contrast Between Hematoxylin And Dab Poor Due To Dark Hematoxylin Excellent
Total:	



Score	Score count
-	5
0	1
1	4
2	2
3	4
4	10
5	77
Total:	103

S004 | Sample S004 Ki-67

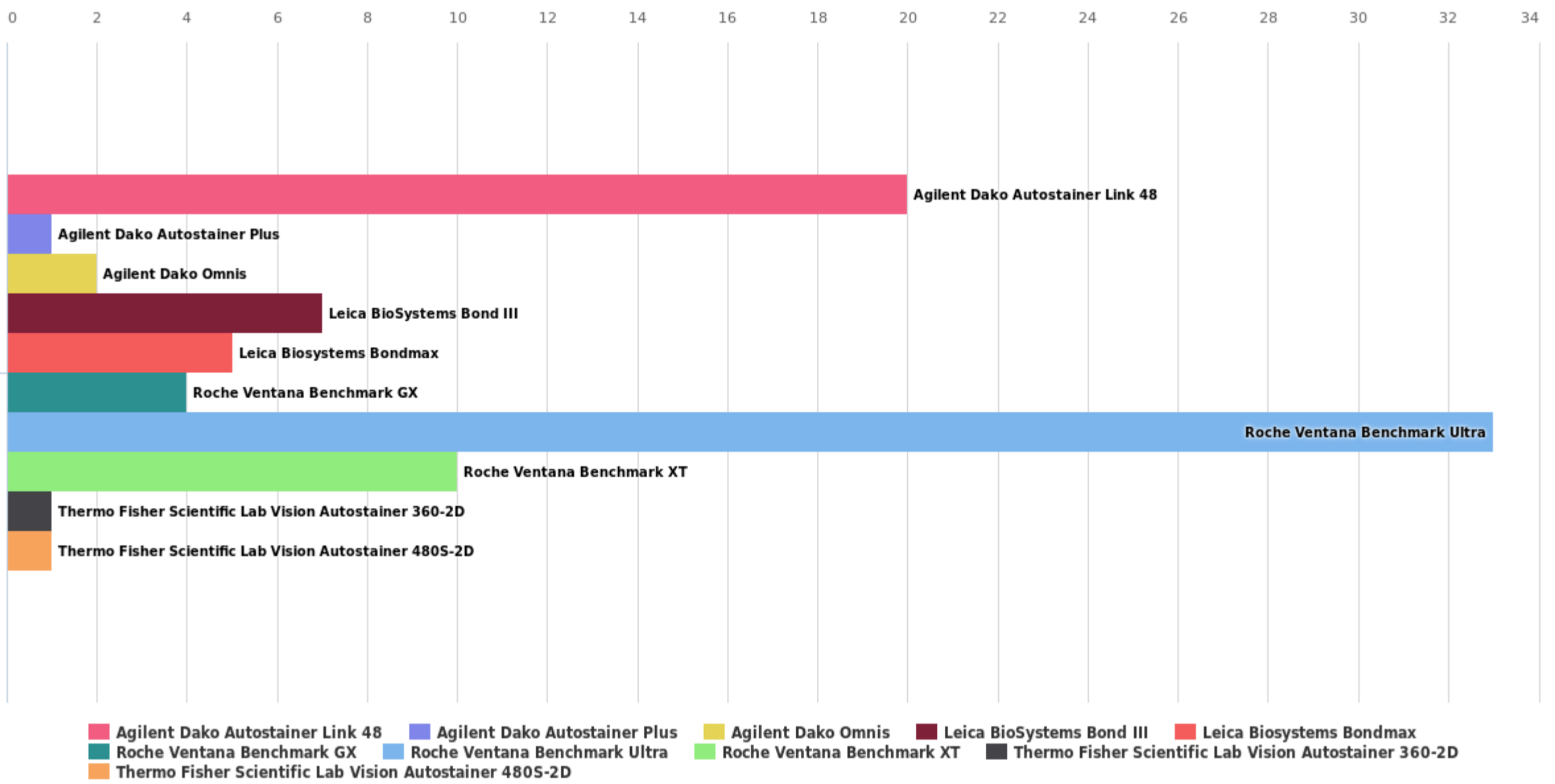
Performance



GENERAL DETAILS OF STAINING

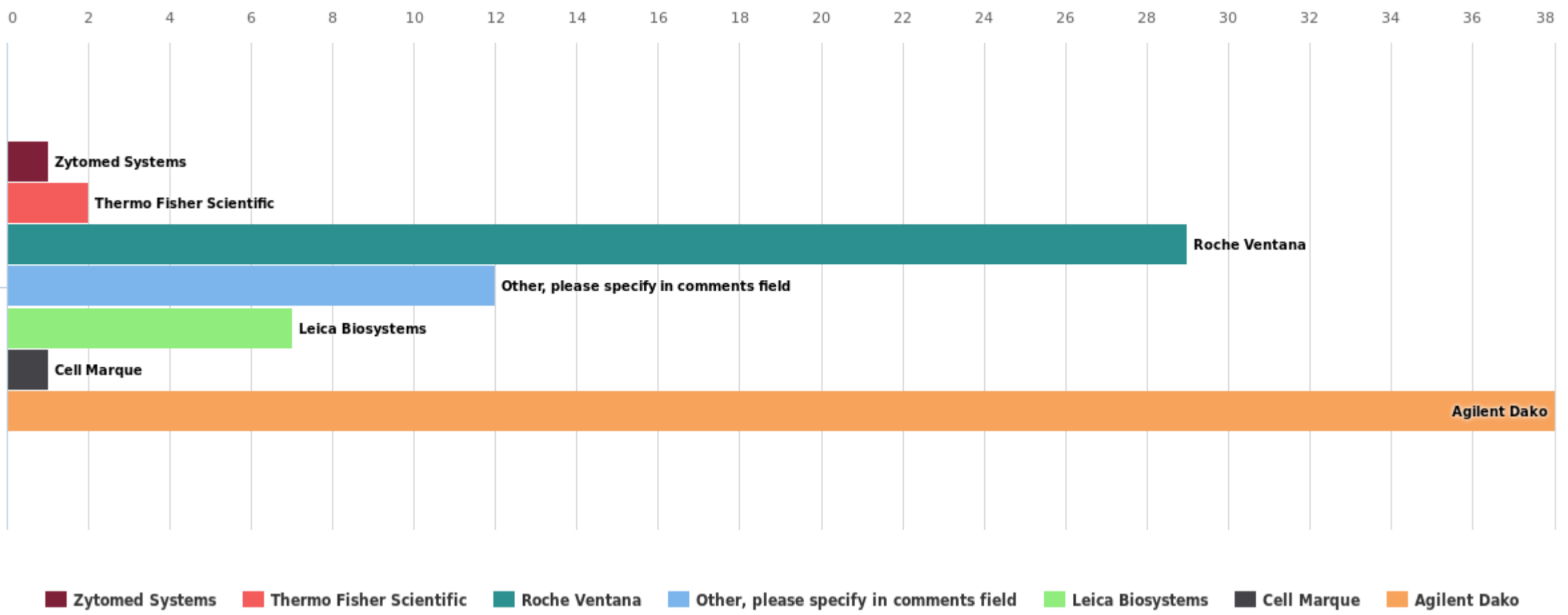
Performance	Performance count
Automatic	84
Manual	7
Total:	91

Instrument



Instrument	Instrument count
Agilent Dako Autostainer Link 48	20
Agilent Dako Autostainer Plus	1
Agilent Dako Omnis	2
Leica BioSystems Bond III	7
Leica Biosystems Bondmax	5
Roche Ventana Benchmark GX	4
Roche Ventana Benchmark Ultra	33
Roche Ventana Benchmark XT	10
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Total:	84

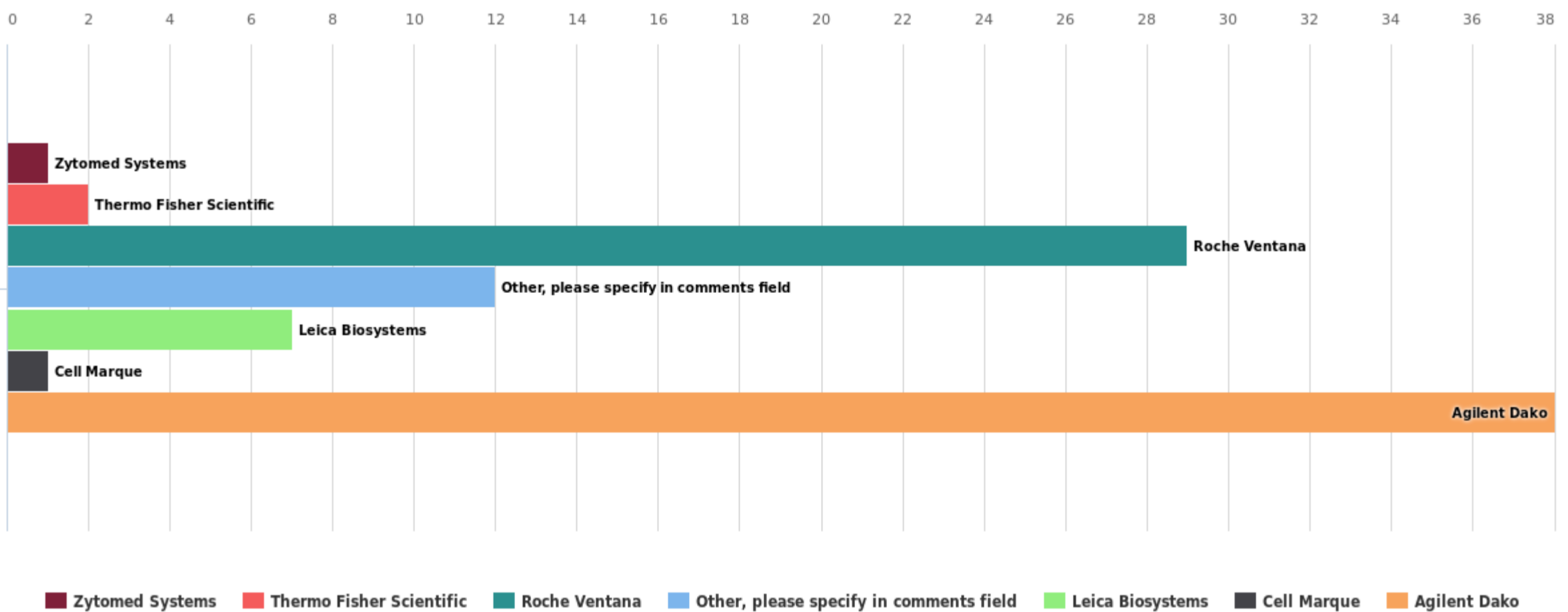
Manufacturer



PRIMARY ANTIBODY

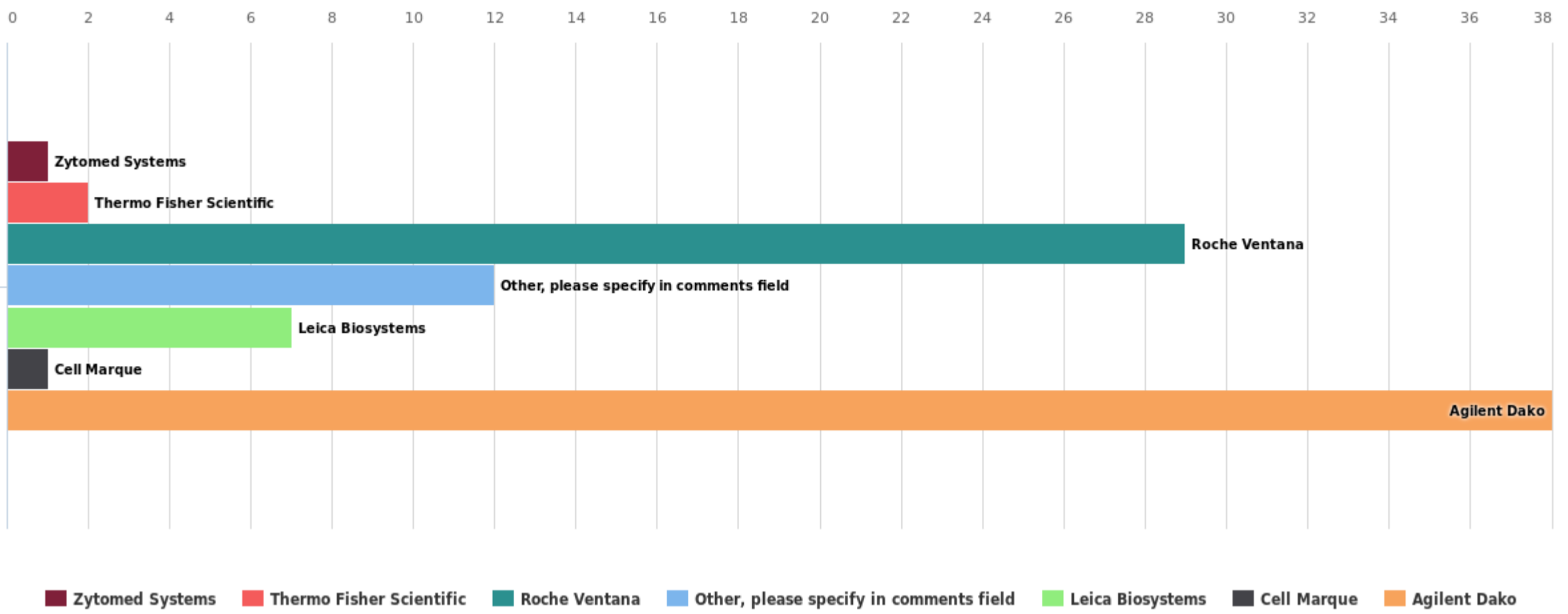
Manufacturer	Manufacturer count
Agilent Dako	38
Cell Marque	1
Leica Biosystems	7
Other, please specify in comments field	12
Roche Ventana	29
Thermo Fisher Scientific	2
Zytomed Systems	1
Total:	90

Clonal



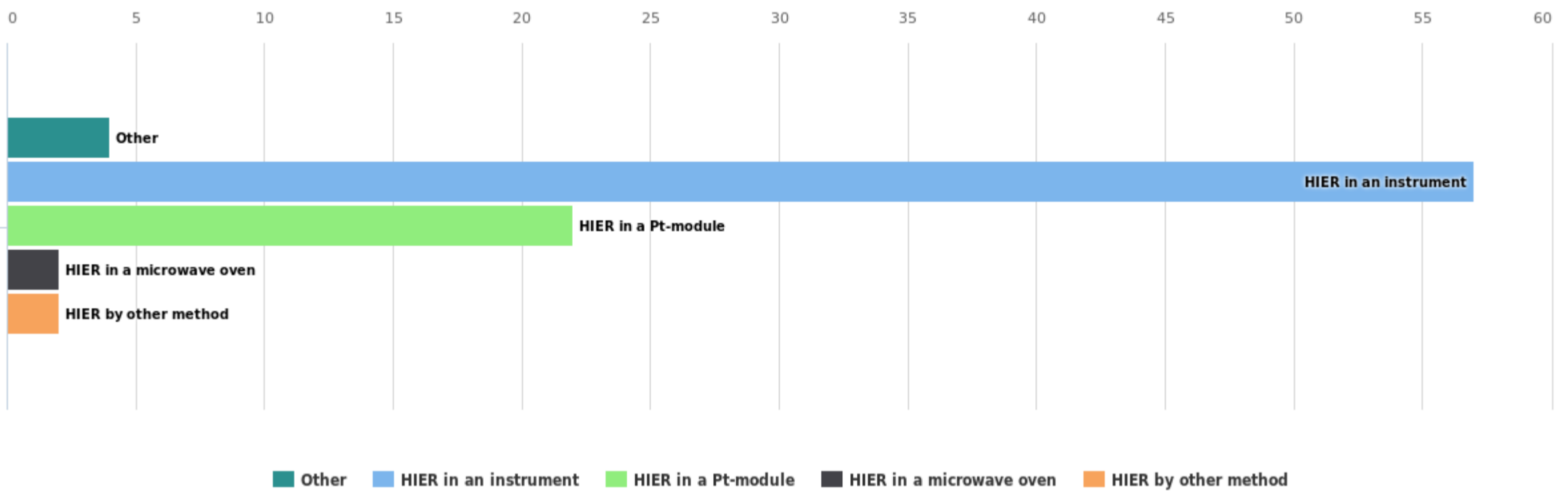
Clonal	Clonal count
Monoclonal	86
Polyclonal	1
Total:	87

Dilution



Dilution	Dilution count
Dilution	38
Ready-to-use	51
Total:	89

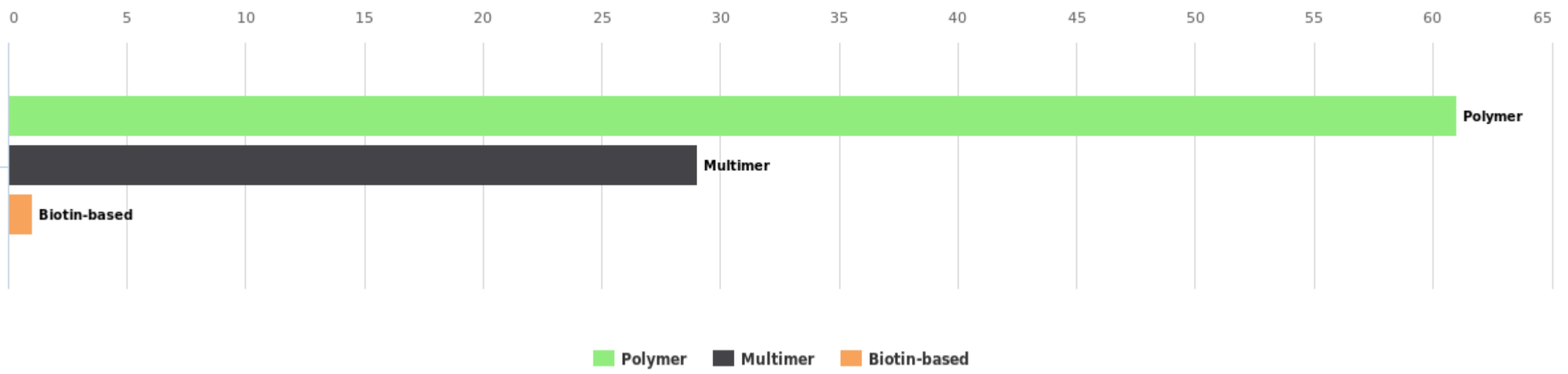
S004 Pretreatment



PRETREATMENT

Pretreatment	Pretreatment count
HIER by other method	2
HIER in a microwave oven	2
HIER in a Pt-module	22
HIER in an instrument	57
Other	4
Total:	87

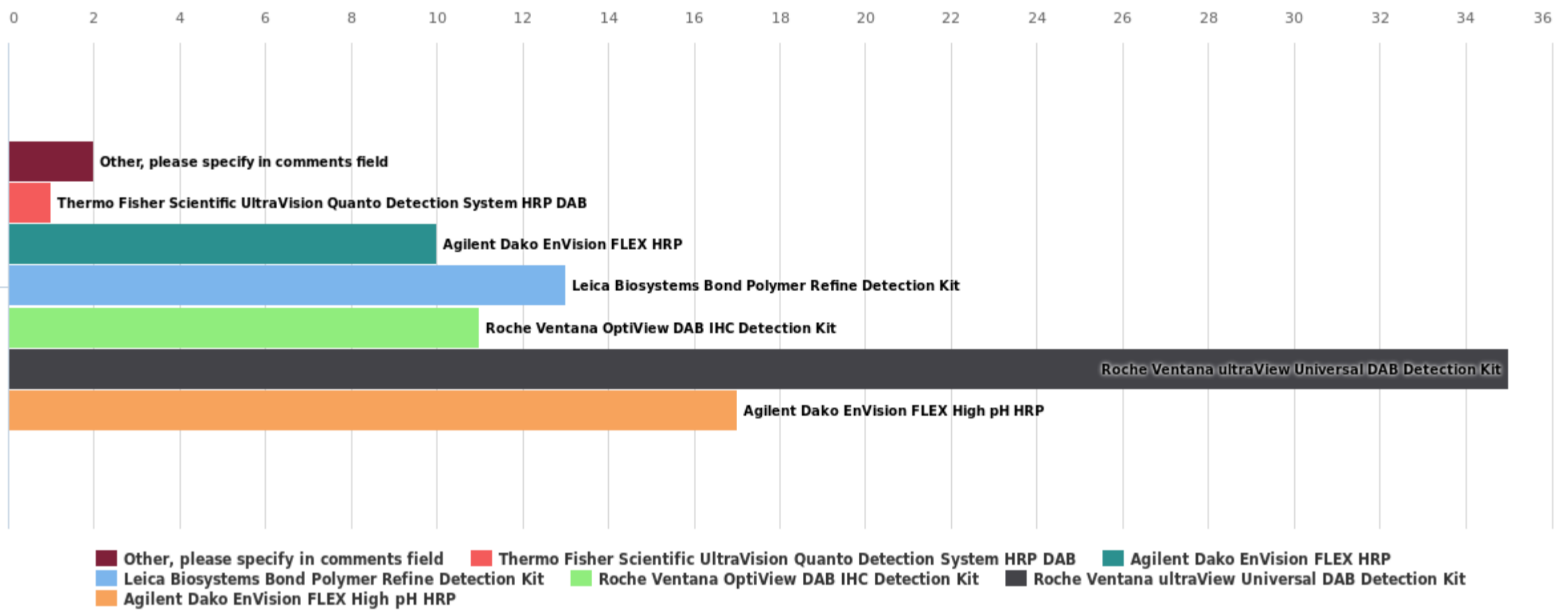
S004 Detection system



DETECTION SYSTEM

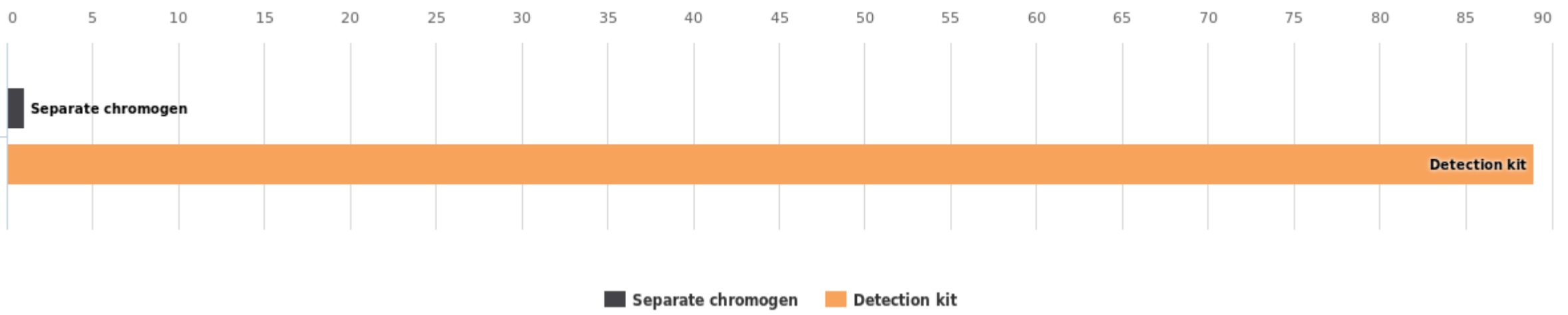
Detection system	Detection system count
Biotin-based	1
Multimer	29
Polymer	61
Total:	91

Detection system: Instrument



Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	10
Agilent Dako EnVision FLEX High pH HRP	17
Leica Biosystems Bond Polymer Refine Detection Kit	13
Other, please specify in comments field	2
Roche Ventana OptiView DAB IHC Detection Kit	11
Roche Ventana ultraView Universal DAB Detection Kit	35
Thermo Fisher Scientific UltraVision Quanto Detection System HRP DAB	1
Total:	89

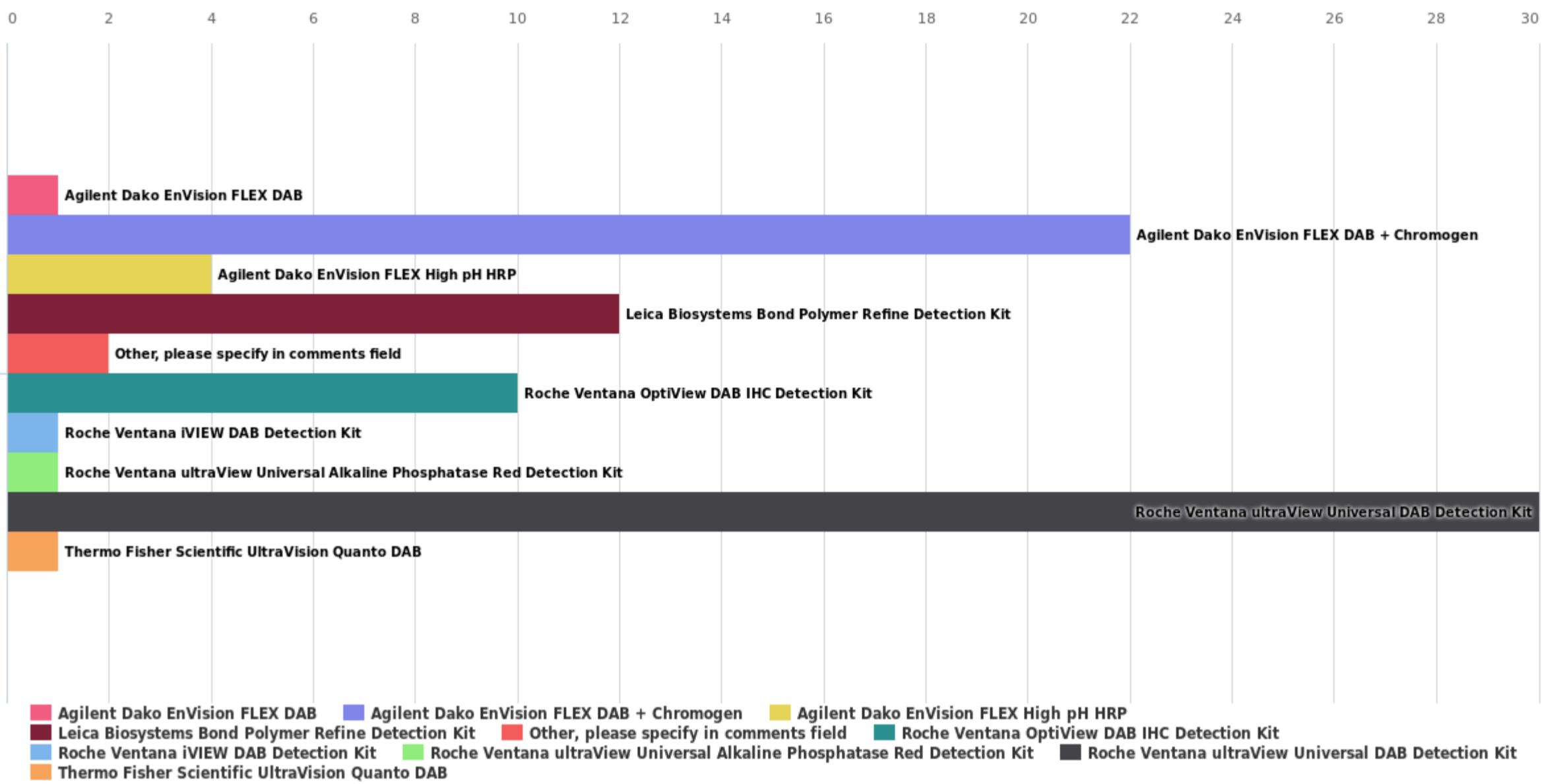
S004 Chromogen



CHROMOGEN

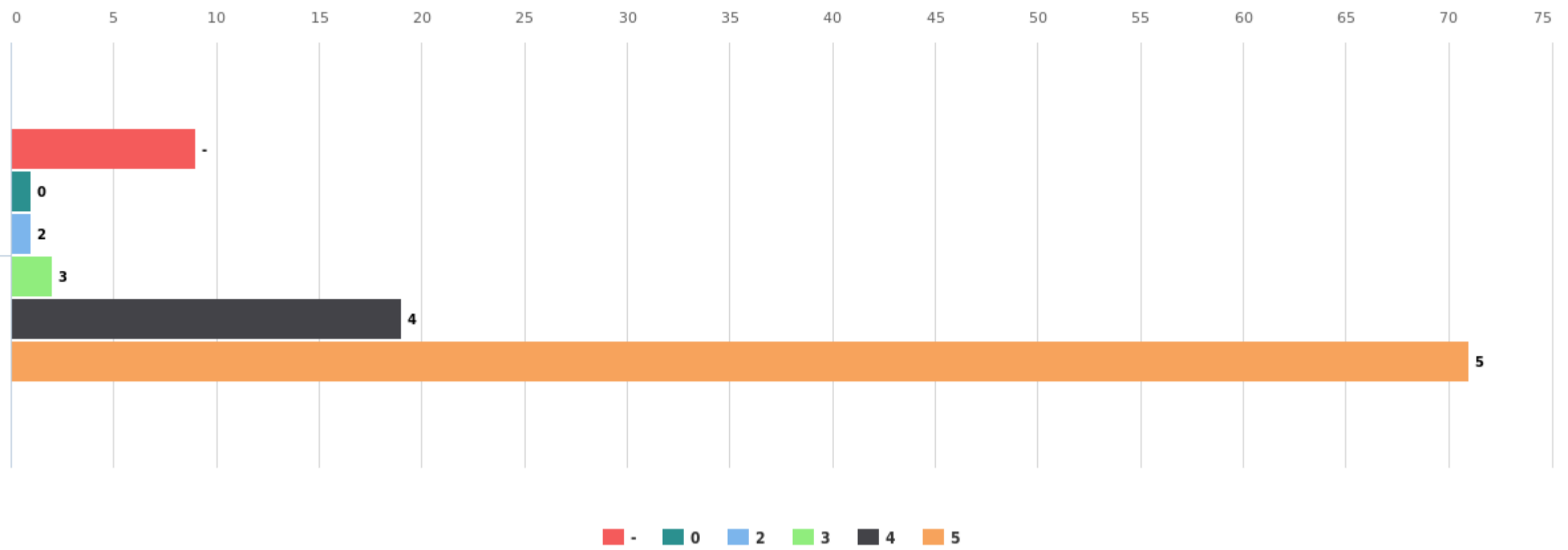
Chromogen	Chromogen count
Detection kit	89
Separate chromogen	1
Total:	90

Chromogen: Instrument



Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	1
Agilent Dako EnVision FLEX DAB + Chromogen	22
Agilent Dako EnVision FLEX High pH HRP	4
Leica Biosystems Bond Polymer Refine Detection Kit	12
Other, please specify in comments field	2
Roche Ventana OptiView DAB IHC Detection Kit	10
Roche Ventana iVIEW DAB Detection Kit	1
Roche Ventana ultraView Universal Alkaline Phosphatase Red Detection Kit	1
Roche Ventana ultraView Universal DAB Detection Kit	30
Thermo Fisher Scientific UltraVision Quanto DAB	1
Total:	84

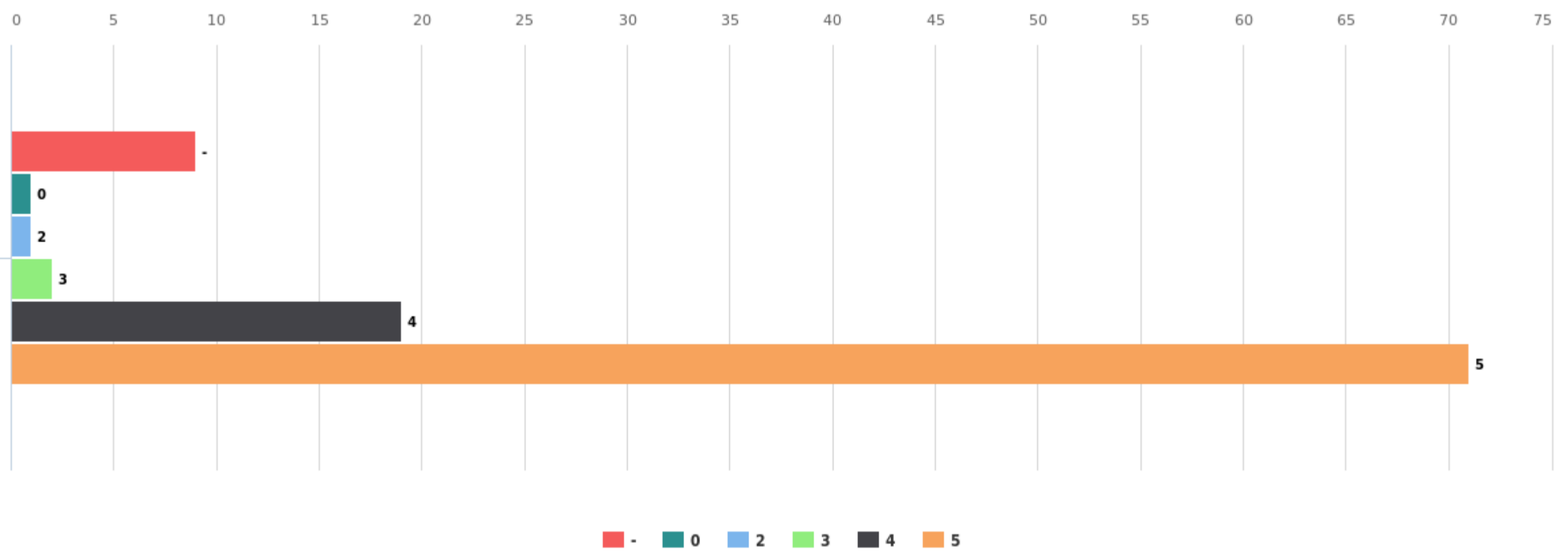
S004 Score



SCORE

Score	Comment
-	Not Available
0	No Staining At All, Blanco, Not Acceptable For Daignosis,Failed
2	Diagnosis May Be Compromised, False Negativity, Staining Intensity Weak
3	False Negativity, Staining Intensity Weak
4	Embedding Failed, Difficult To Evaluate Hematoxylin Very Dark Hematoxylin Very Weak Membranes Of Adipocytes Stained Staining Intensity A Bit Weak
5	Excellent
Total:	

S004 Score

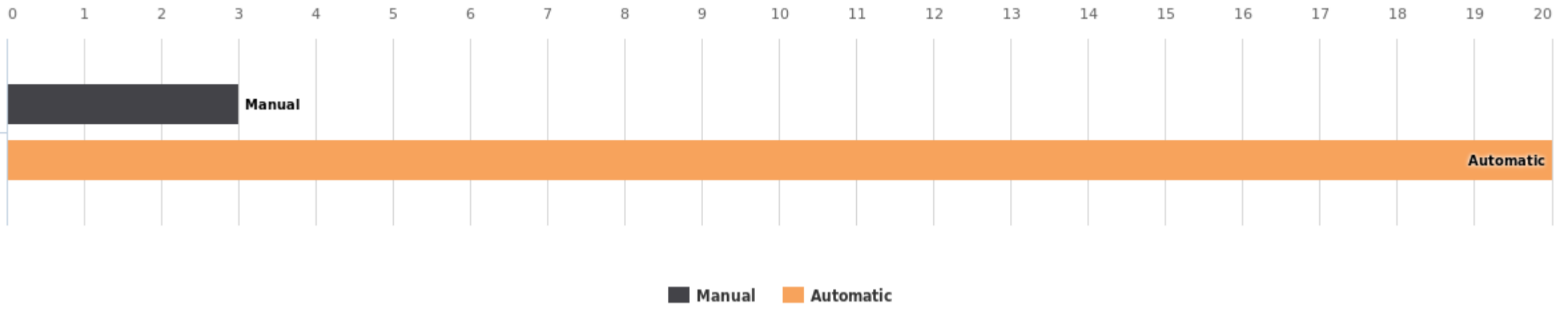


Score	Score count
-	9

0	1
2	1
3	2
4	19
5	71
Total:	103

S005 | Sample S005 HER2 -ISH

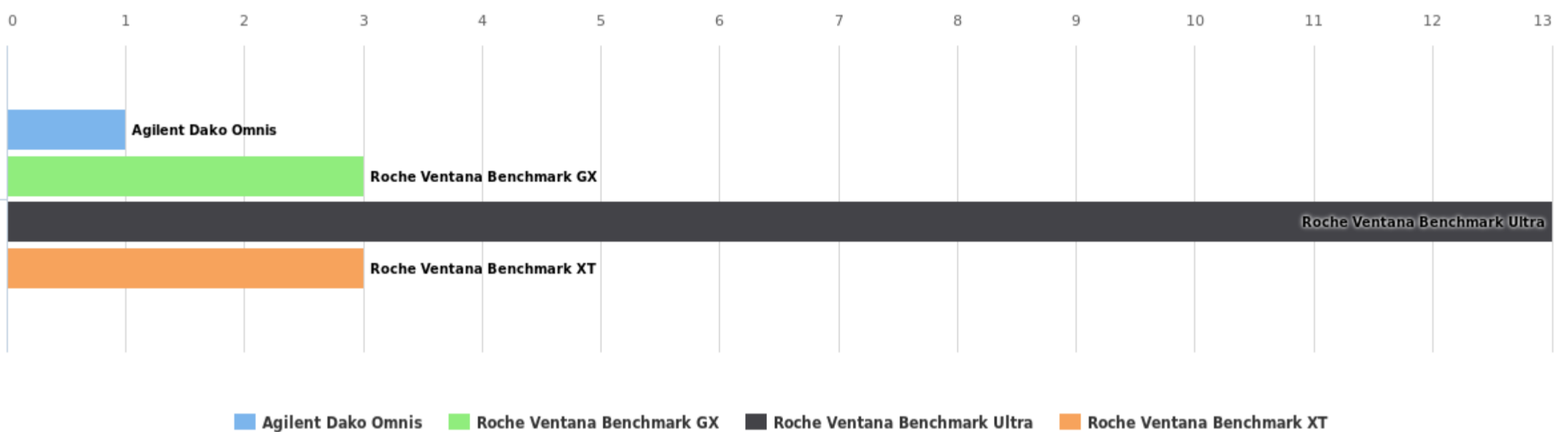
Performance



GENERAL DETAILS OF STAINING

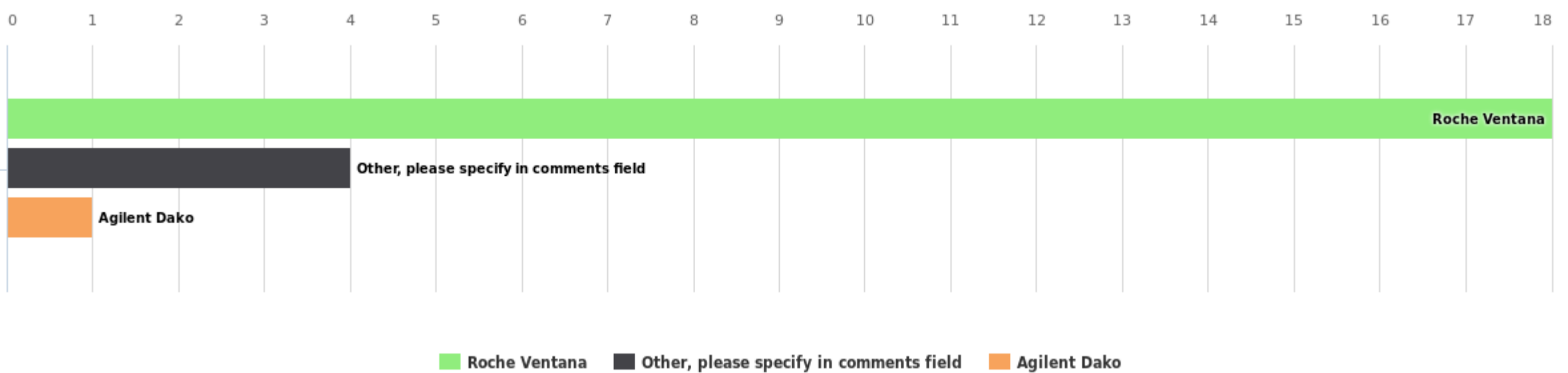
Performance	Performance count
Automatic	20
Manual	3
Total:	23

Instrument



Instrument	Instrument count
Agilent Dako Omnis	1
Roche Ventana Benchmark GX	3
Roche Ventana Benchmark Ultra	13
Roche Ventana Benchmark XT	3
Total:	20

Manufacturer

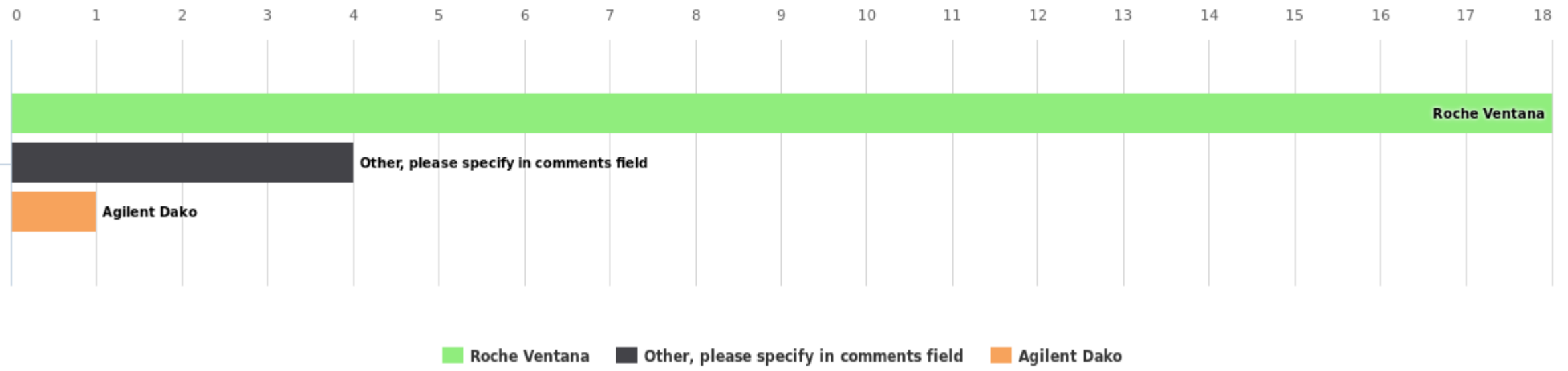


PRIMARY ANTIBODY

Manufacturer	Manufacturer count
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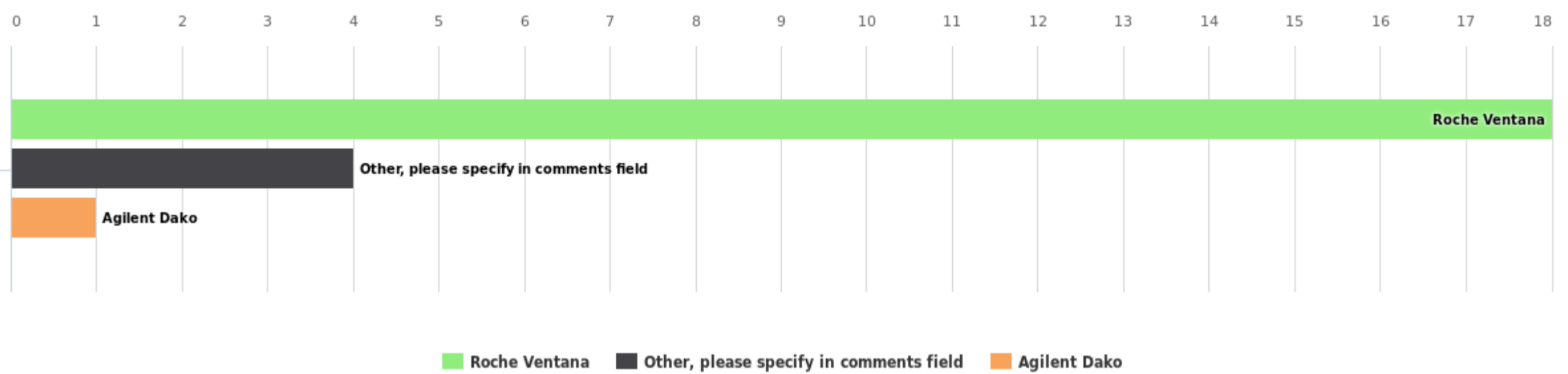
Agilent Dako	1
Other, please specify in comments field	4
Roche Ventana	18
Total:	23

Clonal



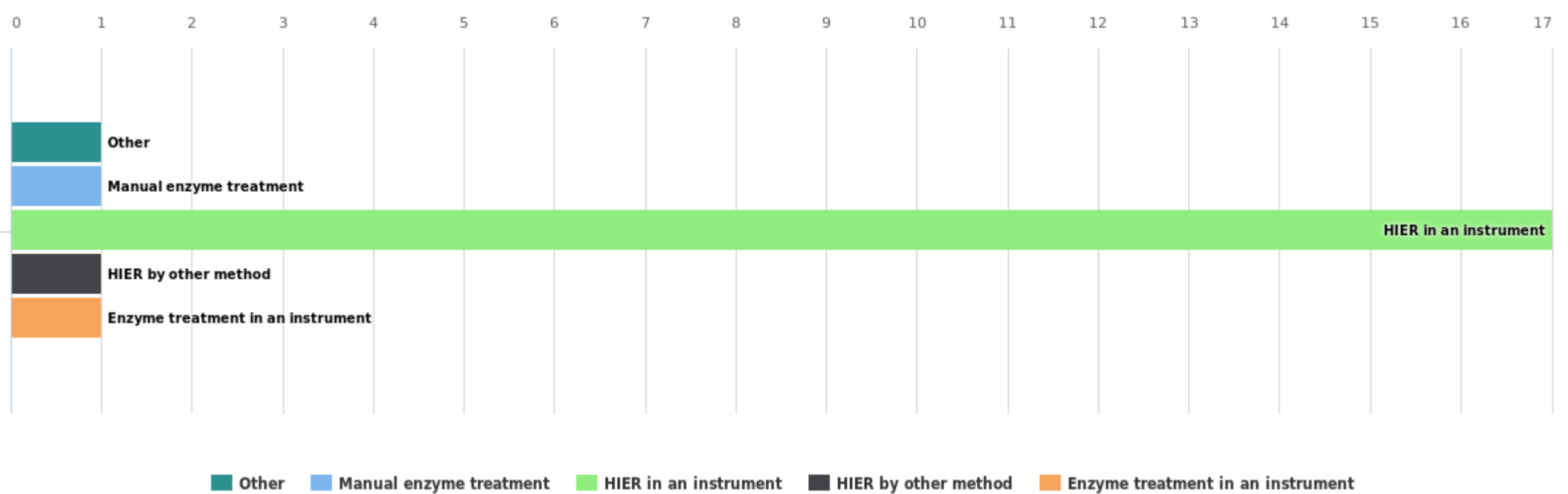
Clonal	Clonal count
Monoclonal	7
Polyclonal	3
Total:	10

Dilution



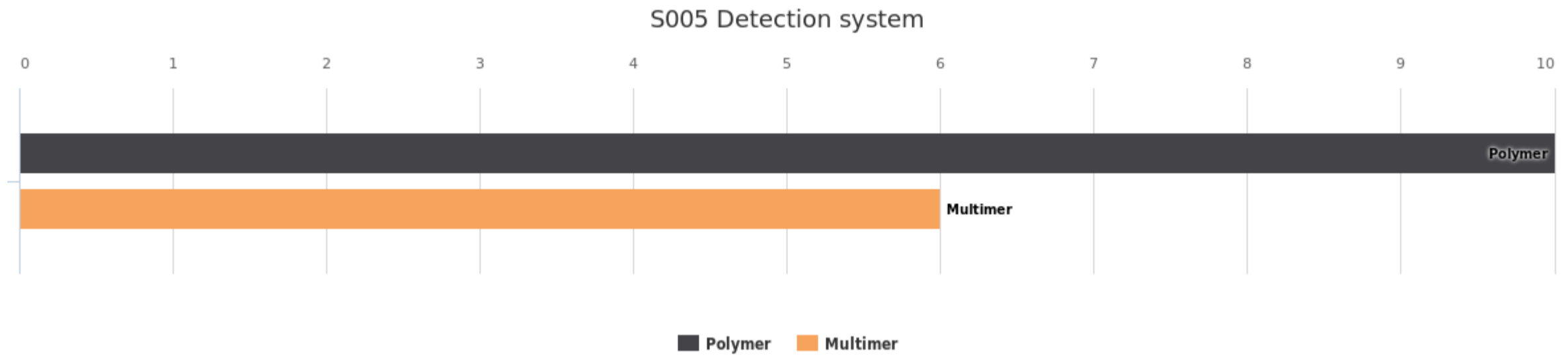
Dilution	Dilution count
Ready-to-use	21
Total:	21

S005 Pretreatment



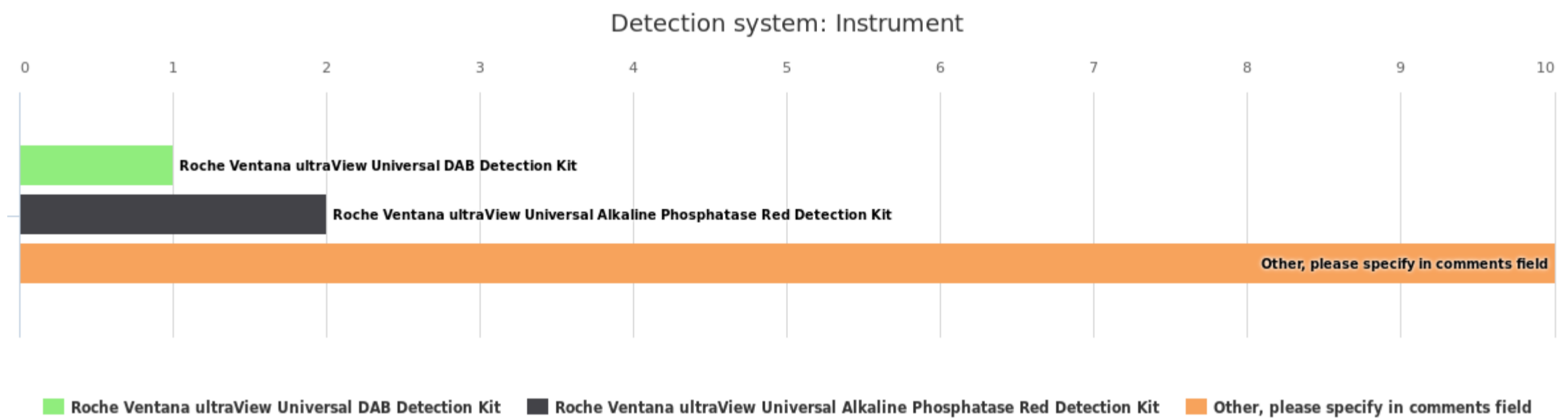
PRETREATMENT

Pretreatment	Pretreatment count
Enzyme treatment in an instrument	1
HIER by other method	1
HIER in an instrument	17
Manual enzyme treatment	1
Other	1
Total:	21

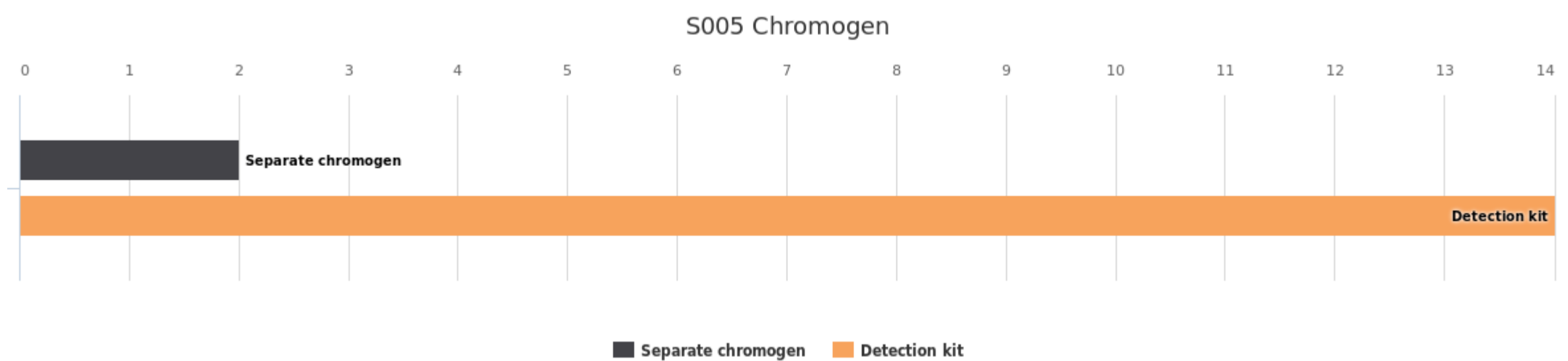


DETECTION SYSTEM

Detection system	Detection system count
Multimer	6
Polymer	10
Total:	16



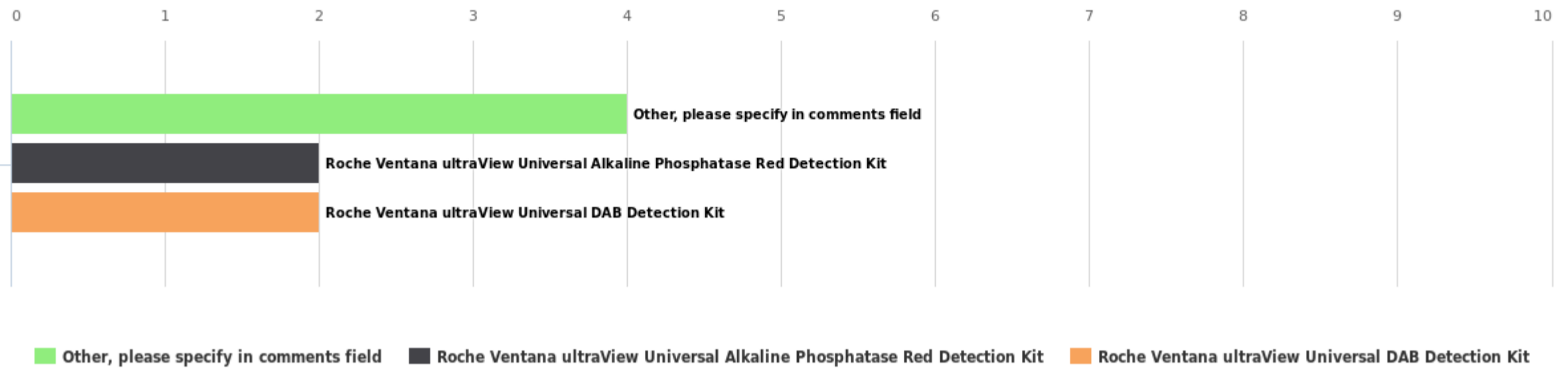
Detection system: Instrument	Detection system: Instrument count
Other, please specify in comments field	10
Roche Ventana ultraView Universal Alkaline Phosphatase Red Detection Kit	2
Roche Ventana ultraView Universal DAB Detection Kit	1
Total:	13



CHROMOGEN

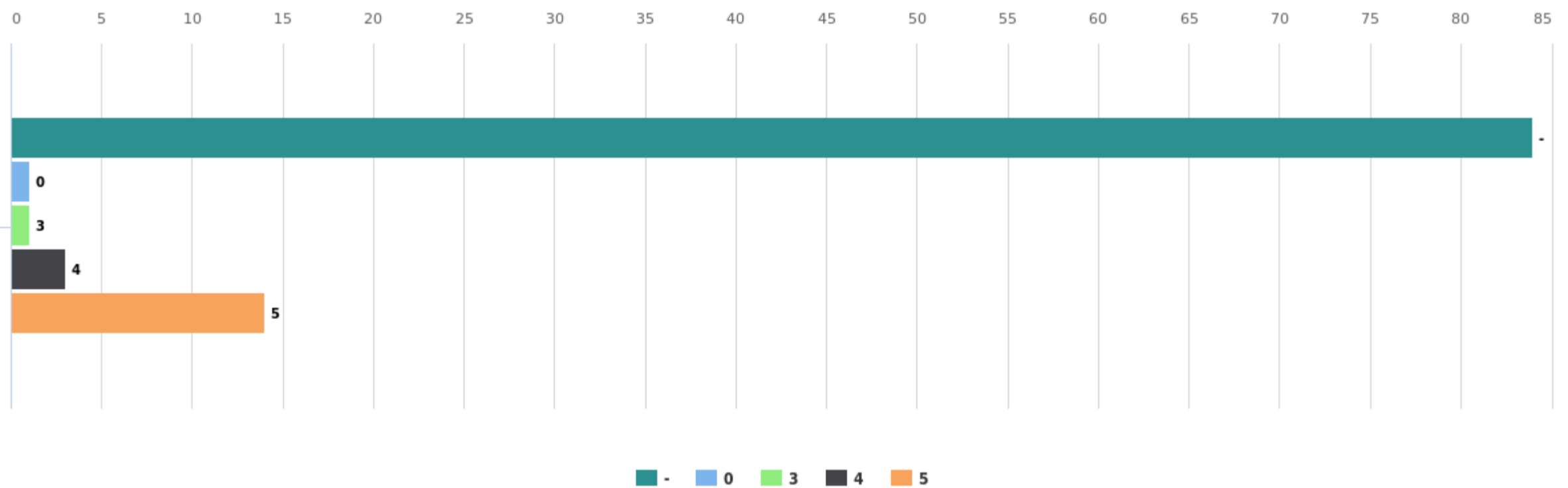
Chromogen	Chromogen count
Detection kit	14
Separate chromogen	2
Total:	16

Chromogen: Instrument



Chromogen: Instrument	Chromogen: Instrument count
Other, please specify in comments field	4
Roche Ventana ultraView Universal Alkaline Phosphatase Red Detection Kit	2
Roche Ventana ultraView Universal DAB Detection Kit	2
Total:	8

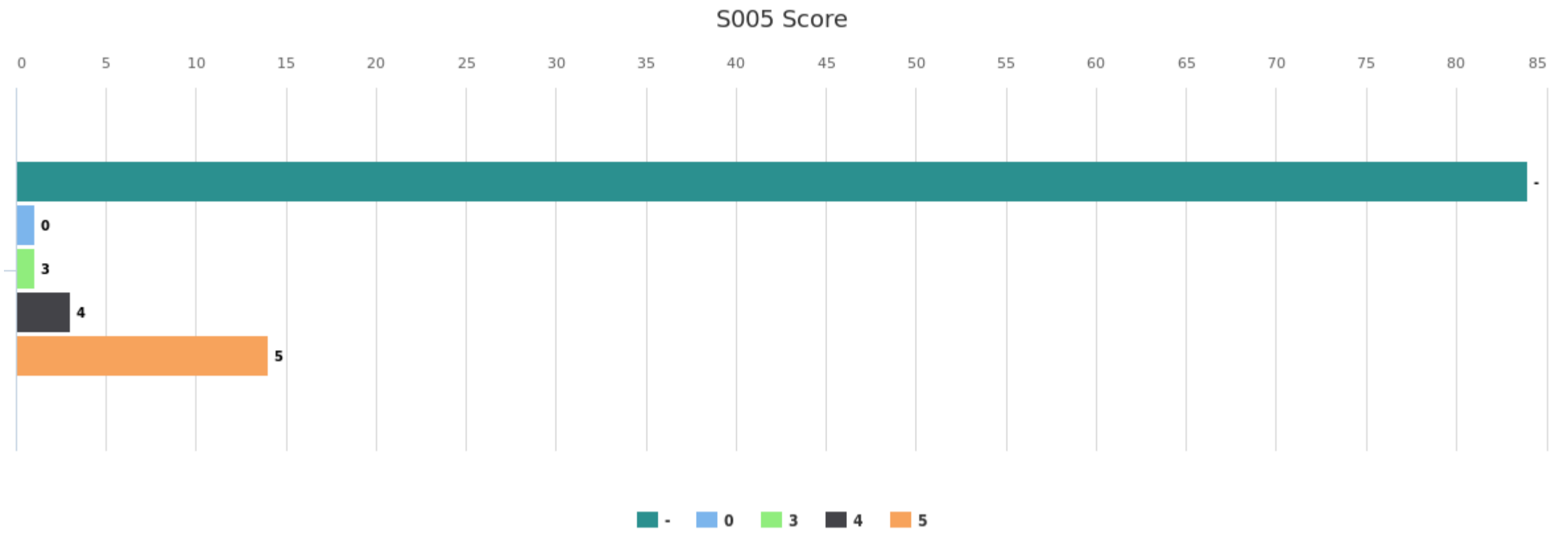
S005 Score



SCORE

Score	Comment
-	FISH, Not Evaluated Not Available
0	No Sample On The Slide
3	One of the sample blanco, one of the samples scraped, two optimal
4	A Bit Smudgy, Cytoplasmic Background Staining May Block The View Of Ish Signals CHR17 Signals Should Be Enhanced One Of The Samples "Grilled"
5	Excellent IHC+ISH

Total:



Score	Score count
-	84
0	1
3	1
4	3
5	14
Total:	103

External Quality Assessment Scheme

Immunohistochemical staining methods Round 2, 2023

Specimens

Sample S001-S005 (LQ778323021-LQ778323025) were slides with unstained paraffin sections. The materials were sent without temperature control packaging.

Report info

A numerical score given in a six-step scale 0–5 is based on consensus. The results of all participants are presented in a table form. A laboratory specific score report is enclosed as well. Scoring reports help your laboratory in following the development of your own stainings. Guidelines how to interpret the reports can be found under "LabScala user instructions" in LabScala. The scoring principles are presented below.

Scoring

Evaluation scale: 0–5

3-5 points indicate good enough staining for diagnosis. 0-2 points mean that the staining is uncertain for diagnosis or failed.

5 points =	excellent = optimal
4 points =	practically faultless, slight over/understaining, slightly uneven or patchy staining
3 points =	good enough for diagnosis, but distinct over/understaining, uneven or patchy staining, stain deposits etc.
2 points =	weak staining, uncertain for diagnosis
1 point =	poor = failed, some scanty but inadequate staining observed/ notifiable overstaining leading to uncertain diagnosis,
	too much background, false positive or false negative
0 point =	negative or false positive - failed

Summary of the results

On this round of immunohistochemical stainings the participants were provided with glass slides with four tissue samples. All the slides have the same four breast cancer samples with known different characteristics. The expected staining patterns for each antibody or in situ hybridization staining are provided later in the report.

Table 1. Summary of the results, number of the participants / score

Score	PR	HER2	ER	Ki-67	HER2-ISH
5	77	36	77	71	14
4	8	22	10	19	3
3	7	18	4	2	1
2	3	5	2	1	0
1	0	4	4	0	0
0	0	3	1	1	1
Average	4.7	3.8	4.5	4.7	4.5

2023-12-27

FINAL REPORT

Product no. 6600/6600S

Subcontracting: Sample preparation, sample pretesting

Samples sent	2023-09-12
Round closed	2023-10-27
Final report	2023-12-27

Request for correction

Typing errors in laboratory's result forms are on laboratory's responsibility. Labquality accepts responsibility only for result processing. Requests must be notified by writing within three weeks from the date of this letter.

Authorized by

EQA Coordinator
Pia Eloranta
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Experts

Joanna Ilvesaro-Maijala, PhD.,
Medical cell biologist
Marita Laurila, MD., Pathologist

Labquality Oy

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FI-00520 HELSINKI
Finland

Tel. + 358 9 8566 8200

info@labquality.fi
www.labquality.com



Table 2. Participants obtaining sufficient staining score (4-5)

Staining	Percentage
PR	89.5%
HER2	65.9%
ER	88.8%
Ki-67	95.7%
HER2-ISH	89.5%

S001: PR

PR staining was evaluated from samples of known Progesterone Receptor status. Progesterone receptor staining should only be seen in the nuclei of the cells. Staining should be intensive, strong and clearly restricted to nuclei. One of the samples should have strong positive nuclear staining with no cytoplasmic staining whereas the other should be totally negative. The intensity of the staining, background staining and possible false positivity were evaluated in each protocol.

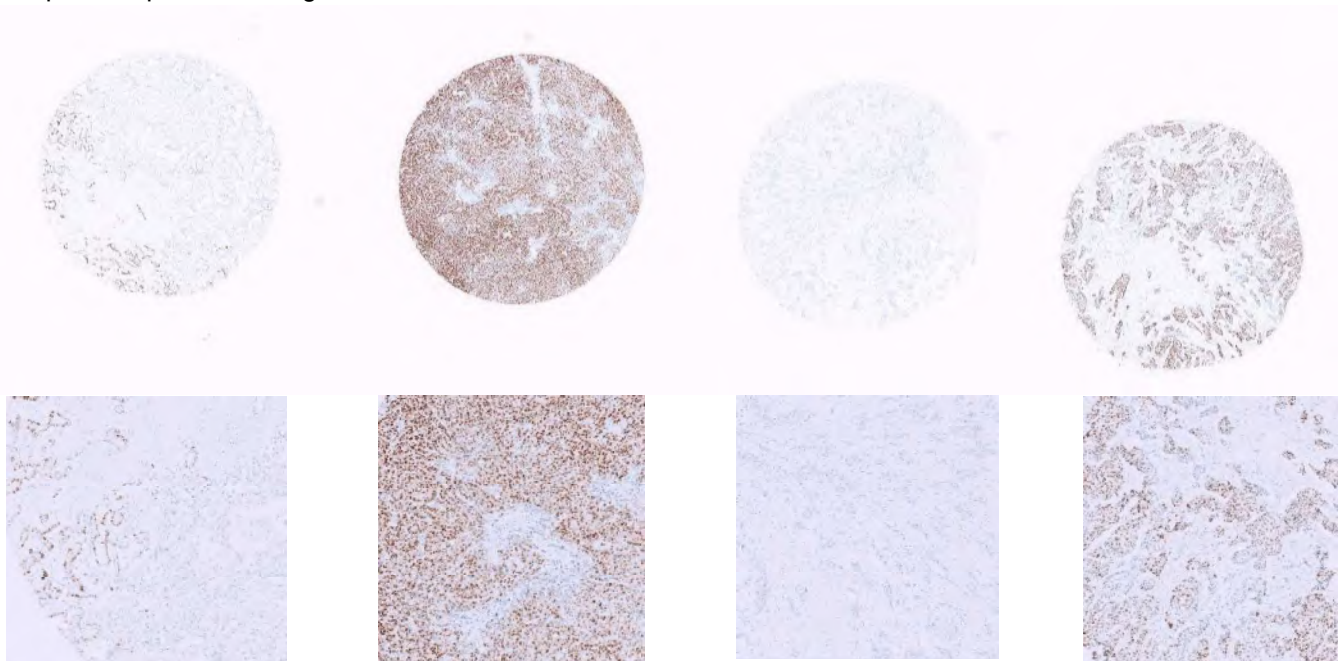
Optimal results and protocols

The average value of the PR stainings was 4.67 points. 95 laboratories took part on this evaluation and 77 laboratories were given optimal results. Only three laboratories received 2 points (Uncertain Diagnosis). Points were deducted due to false negative staining of the slides.

Many different clones were used successfully, also polyclonal antibodies were reported on the round. Both automated and manually stained samples were evaluated on this round, both receiving optimal results.

Since most laboratories used an automated staining instrument, pretreatments were performed according to the instrument, or the staining kit used. Markedly different times and temperatures were used in both the pretreatments and antibody incubation times and dilutions even when using the same staining instruments or antibodies.

Examples of optimal staining results



The expected PR positive percentages for each breast cancer sample are as follows:

- Sample 1: 10-20%
- Sample 2: close to 100%
- Sample 3: 0%
- Sample 4: 70-80%

Staining platforms and protocol steps of the slides with optimal score

Staining platforms: Roche Ventana Ultra , GX and XT, Dako Autostainer and Omnis, Leica Bond III and Bondmax, Thermo Fischer Scientific Lab Vision Autostainer immunoautomates
Pretreatment: pH 9 10-64 min, also pH6
Antibody type: RTU and concentrates

Clones: 1E2, PgR 636, Clone 16, SP2, PgR1294, RBT22, P21-S and SP3

Primary antibody incubation: 10-90 min, 20-37°C

Detection system: Roche Ventana UltraView Universal Detection kit and Optiview kit, Agilent Dako Envision FLEX HRP, Leica Biosystems Bond Polymer Refine Detection kit, Thermo Fischer Scientific UltraVision Quanto Detection System HRP DAB

S002: HER2

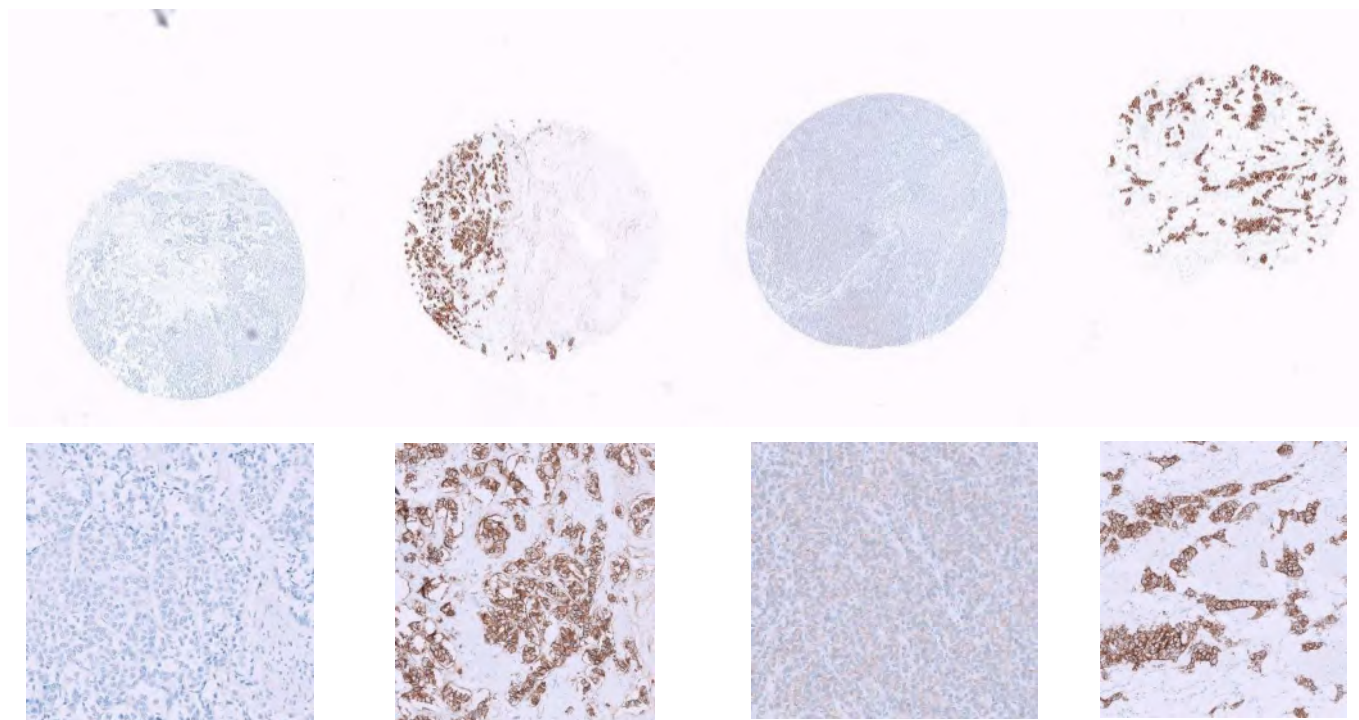
Her2 staining criteria are as follows: the positive staining should be clearly distinguishable and mostly seen in the cell membranes. Cytoplasmic staining is allowed as long as the membranous staining is clearly visible. The scores were lowered if there was lots of background staining or the staining was very weak. The intensity of the staining, background staining and possible false positivity were evaluated in each protocol.

The average value of the HER2 stainings was 3.82 points. 88 laboratories took part on this evaluation and 36 laboratories were given optimal results. 12 laboratories received 0-2 points (Failed - Uncertain Diagnosis). Points were deducted mainly due to false positive staining of the slides.

Many different clones were used successfully, also polyclonal antibodies were reported on the round. Both automated and manually stained samples were evaluated on this round, both receiving optimal results.

Since most laboratories used an automated staining instrument, pretreatments were performed according to the instrument or the staining kit used. Markedly different times and temperatures were used in both the pretreatments and antibody incubation times and dilutions even when using the same staining instruments or antibodies.

Examples of optimal staining results



The expected Her2 positive score for each breast cancer sample are as follows:

Sample 1: 0
Sample 2: 3+
Sample 3: 1-2+
Sample 4: 3+

Staining platforms and protocol steps of the slides with optimal score

Staining platforms: Roche Ventana Ultra, GX and XT, Dako Autostainer and Omnis, Leica Bond III and Bondmax, Thermo Fischer Scientific Lab Vision Autostainer immunoautomates

Pretreatment: pH 9 8-60 min, also pH6

Antibody type: RTU and concentrates

Clones: 4B5, SP3, CB11, Herceptest, Her2/neu, EP3, c-ERB

Primary antibody incubation: 10-60 min, 20-37°C

Detection system: Roche Ventana UltraView Universal Detection kit and Optiview kit, Agilent Dako Herceptest Visualisation Reagent, Agilent Dako Envision FLEX HRP, Leica Biosystems Bond Polymer Refine Detection kit

S003:ER

In the positive tissue the positivity should only be seen in the nuclei of the cells. The staining should be strong, distinct and clearly restricted to the nuclei. Any cytoplasmic staining decreased the points given. Also, if any false positivity was seen in the nuclei, evaluation points were decreased. The intensity of the staining, background staining and possible false positivity were evaluated in each protocol.

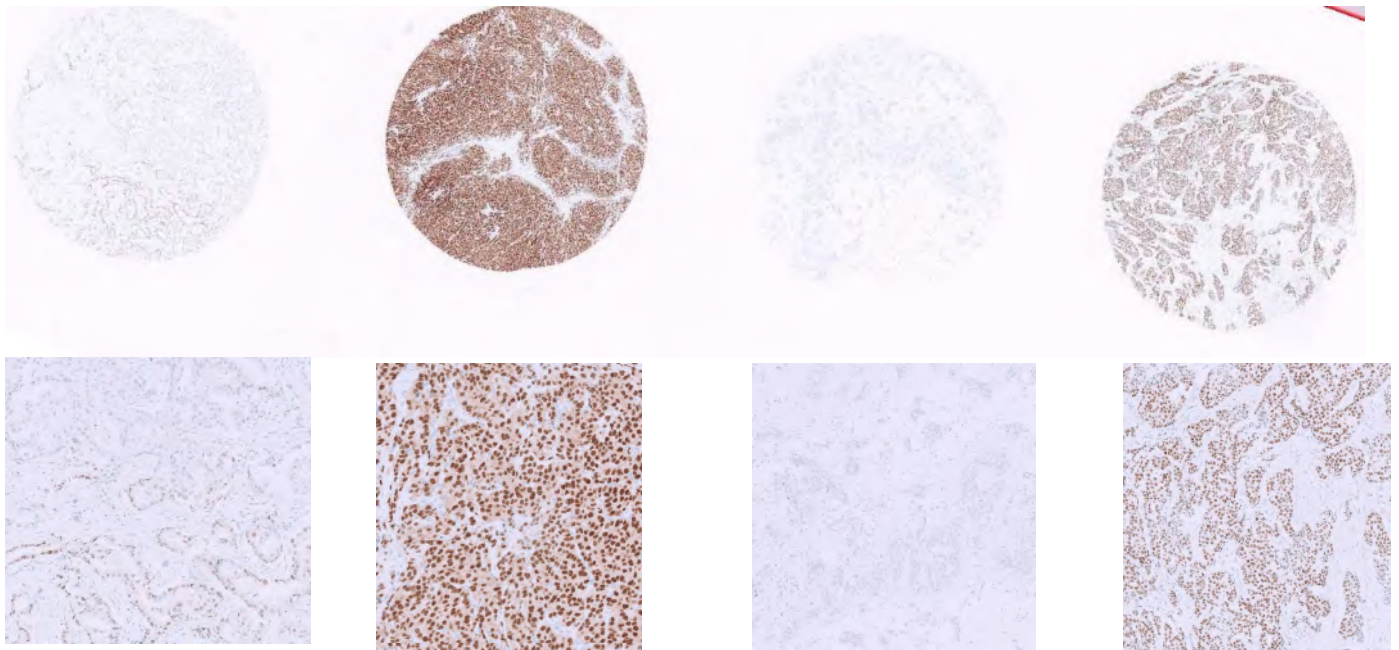
Optimal results and protocols

The average value of the ER stainings was 4.54 points. 98 laboratories took part on this evaluation and 77 laboratories were given Optimal results. 7 laboratories received 0-2 points (Failed - Uncertain Diagnosis). Points were deducted mainly due to false negative staining of the slides.

Many different clones were used successfully, also polyclonal antibodies were reported on the round. Both automated and manually stained samples were evaluated on this round, both receiving optimal results.

Since most laboratories used an automated staining instrument, pretreatments were performed according to the instrument or the staining kit used. Markedly different times and temperatures were used in both the pretreatments and antibody incubation times and dilutions even when using the same staining instruments or antibodies.

Examples of optimal staining results



The expected ER positive percentages for each breast cancer sample are as follows:

- Sample 1: app. 10%
- Sample 2: close to 100%
- Sample 3: 0%
- Sample 4: 80-90%

Staining platforms and protocol steps of the slides with optimal score

Staining platforms: Roche Ventana Ultra, GX and XT, Dako Autostainer and Omnis, Leica Bond III and Bondmax, Thermo Fischer Scientific Lab Vision Autostainer immunoautomates

Pretreatment: pH 9 4-64 min, also pH6

Antibody type: RTU and concentrates

Clones: SP1, 6F11, EP1, BSB-1, S21-V ja ZCL223

Primary antibody incubation: 10-90 min, 20-37°C

Detection system: Roche Ventana UltraView Universal Detection kit and Optiview kit, Agilent Dako Envision FLEX HRP, Leica Biosystems Bond Polymer Refine Detection kit, Thermo Fischer Scientific UltraVision Quanto Detection System HRP DAB

S004: Ki-67

Ki-67 should be seen in the nuclei of cells. Staining should be intensive and clearly distinguishable from background. No cytoplasmic staining is accepted. The intensity of the staining, background staining, possible false positivity were evaluated in each protocol. Ki-67 staining was evaluated from two samples of known Ki-67 status.

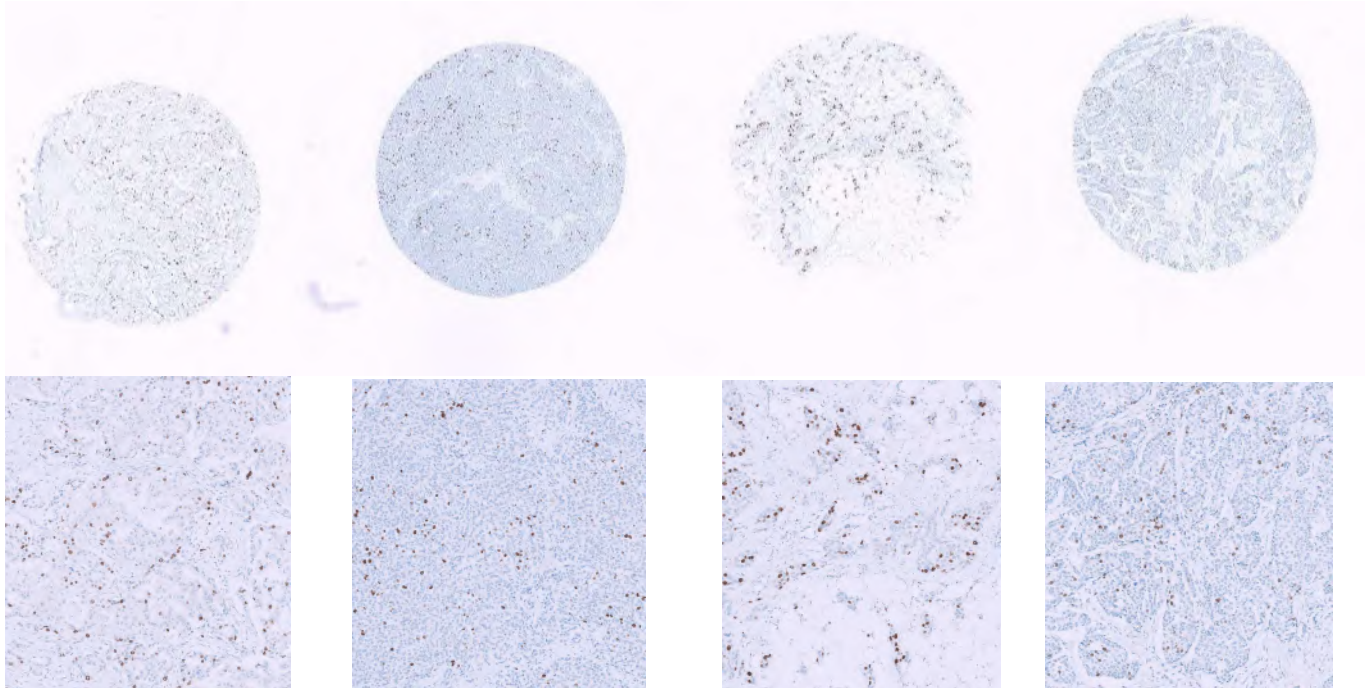
Optimal results and protocols

The average value of the Ki-67 stainings was 4.67 points. 94 laboratories took part on this evaluation and 71 laboratories were given optimal results. Only two laboratories received 0-2 points (Failed - Uncertain Diagnosis). Points were deducted mainly due to false negative staining of the slides.

Many different clones were used successfully, also polyclonal antibodies were reported on the round. Both automated and manually stained samples were evaluated on this round, both receiving optimal results.

Since most laboratories used an automated staining instrument, pretreatments were performed according to the instrument or the staining kit used. Markedly different times and temperatures were used in both the pretreatments and antibody incubation times and dilutions even when using the same staining instruments or antibodies.

Examples of optimal staining results



The expected Ki67 positive percentages for each breast cancer sample are as follows:

Sample 1:	app. 10%
Sample 2:	app. 20%
Sample 3:	app. 20%
Sample 4:	app. 15%

Staining platforms and protocol steps of the slides with optimal score

Staining platforms: Roche Ventana Ultra, GX and XT, Dako Autostainer and Omnis, Leica Bond III and Bondmax, Thermo Fischer Scientific Lab Vision Autostainer immunoautomates

Pretreatment: pH 9 4-64 min, also pH6

Antibody type: RTU and concentrates

Clones: 30-9, MIB-1, SP6, k2, MM1, RM360, M17-1, K2

Primary antibody incubation: 10-90 min, 20-37°C

Detection system: Roche Ventana UltraView Universal Detection kit and Optiview kit, Agilent Dako Envision FLEX HRP, Leica Biosystems Bond Polymer Refine Detection kit, Thermo Fischer Scientific UltraVision Quanto Detection System HRP DAB

S005: HER2-ISH

Only chromogenic HER2 - Chr17 in situ hybridization stainings were evaluated on this Round. Her2 and Chr 17 signals should be clearly separated intensive with no or minimal smudge. No staining outside nuclei is allowed. Hematoxylin should not be too weak or too dark.

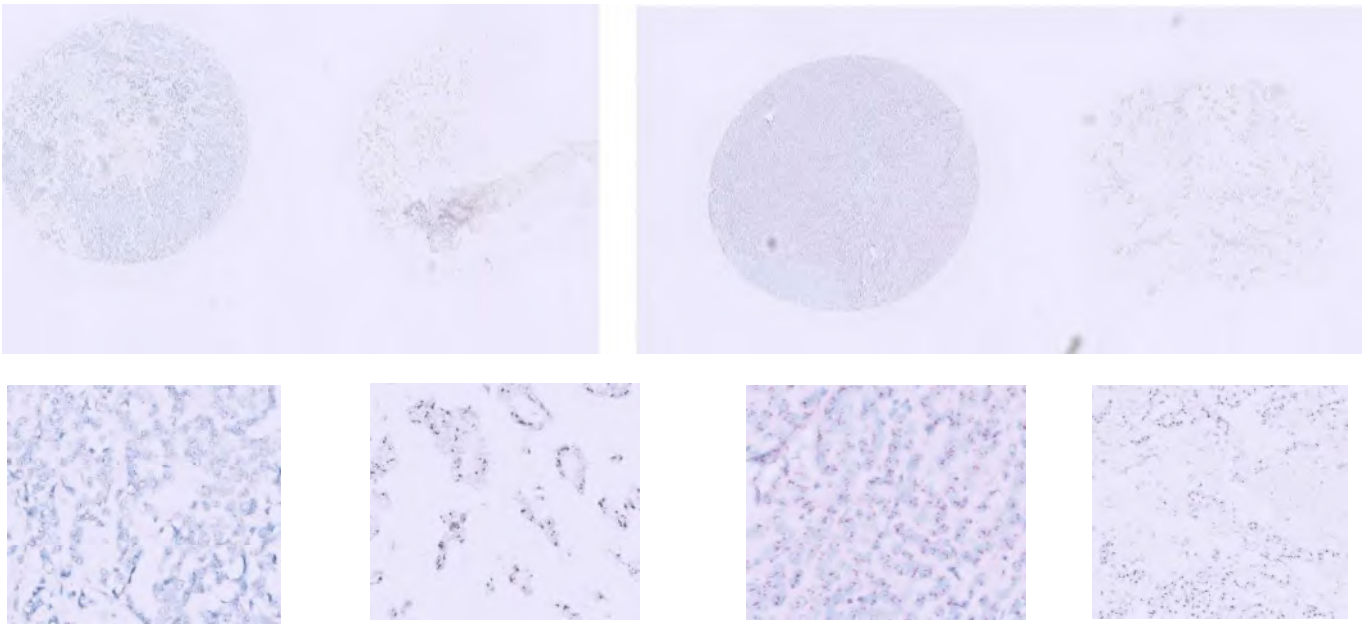
Optimal results and protocols

The average value of the HISH stainings was 4.47 points. 19 laboratories took part on this evaluation and 14 laboratories were given Optimal results. Only one laboratory received 0 points (Failed) with no signal whatsoever thus being false negative.

Only two automated platforms were used on this staining, still manual stainings were also performed, both receiving optimal results. Number of different probes was also significantly less than with the antibody staining rounds.

The variations between different protocols were significantly fewer than with the different antibody stainings. This is due to “closed” protocols, which enable only a few parameters that can be modified by the user.

Examples of optimal staining results



The expected HISH scores for each breast cancer sample are as follows:

- Sample 1: negative
- Sample 2: amplified, cluster positive
- Sample 3: negative
- Sample 4: amplified, cluster positive

Staining platforms and protocol steps of the slides with optimal score

Staining platforms: Roche Ventana Ultra, GX and XT and Dako Omnis

Probes: Ventana HER2 Dual ISH DNA Probe Cocktail

Detection system: Roche Ventana Silver ISH DNP, Ventana Red ISH DIG, Zytovision ZytoDot2C

End of report

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Immunohistochemical staining methods Round 2, 2023

Specimens

Please find enclosed 5 unstained slides S001 - S005.

Background information

Each participating laboratory receives five unstained slides, one for each staining. Each slide has several sections of paraffin block. The sections of 3-4 µm thickness have been prepared from paraffin embedded tissue fixed in 10% neutral phosphate-buffered formalin (24-48 hours). The sections were cut on slides and have not been dried or otherwise attached to the slides.

Storage and use

The sections should be dried and attached on slides using heat treatment.

Please store the slides at room temperature before and after staining. If all the mentioned antibodies are not included in your selection, you can participate also with selected antibodies according to your order: product 6600 for 1-5 antibodies, product 6600S for 1-3 antibodies. Please note that we will send all the 5 slides in both products. If you have ordered 6600S, please return maximum 3 stained slides.

Please, remember to label each slide clearly with given Labquality account number. If you order multiple sample sets with the same client code, please separate them with roman numerals or the alphabet.

Returning slides and protocol reporting

Please return the stained slides in the same slide mailer box wrapped in bubble wrap at participant's own cost to the address given in the column on the right side of the page unless our local partner has instructed otherwise. An address label is included with your shipment for return.

The slides should be at Labquality's office no later than the date given in the column on the right side of the page. Please note that slides received after that will no longer be included in the evaluation.

For each antibody staining, please fill a separate staining techniques protocol form of the used method via LabScala. We can provide a more comprehensive final report that serves the customer if the method information is given as detailed as possible.

Examination

S001: PR



S004: Ki-67



S002: HER2



S005: HER2 -ISH



S003: ER



2023-09-12

INSTRUCTIONS

Product no. 6600 / 6600S
LQ778323021-025/FI

If the kit is incomplete or contains damaged specimens, please report immediately to info@labquality.fi

The slides should be returned no later than **October 24, 2023.**

Inquiries

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