

External Quality Assessment Scheme

Immunohistochemical staining methods Round 1, 2020

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 two 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 hrs). The sections were taken on pure slides and attached for one hour in incubator.

Storage and use

Please store the slides at room temperature before and after staining. If all the above-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.

Remember to label slides clearly with given Labquality account number.

Returning slides and protocol reporting

Please return the stained slides so that they are at Labquality's office no later than the date given in the column on the right side of the page.

The slides should be returned in the same slide mailer box wrapped in bubble wrap at participant's own cost. For each antibody staining fill a separate staining techniques protocol form of the used method via LabScala.

S001: LQ778320011 = CD3



S004: LQ778320014 = CD30



S002: LQ778320012 = CD5



S005: LQ778320015 = PAX5



S003: LQ778320013 = CD20



2020-03-17

INSTRUCTIONS

Product no. 6600 / 6600S
LQ778320011-015/FI

Subcontracting: Sample
preparation, Sample pretesting

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

The slides should be
returned no later than
April 24, 2020.

Inquiries

EQA Coordinator
Pia Eloranta
pia.eloranta@labquality.fi

Labquality

Kumpulantie 15
FI-00520 HELSINKI
Finland

Tel. + 358 9 8566 8200
Fax + 358 9 8566 8280

info@labquality.fi
www.labquality.fi

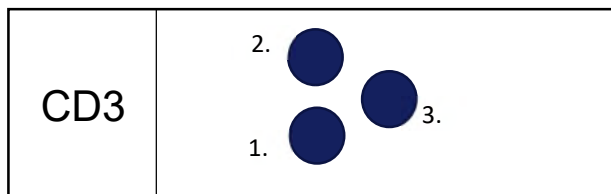


Examinations

The slides are stained as follows:

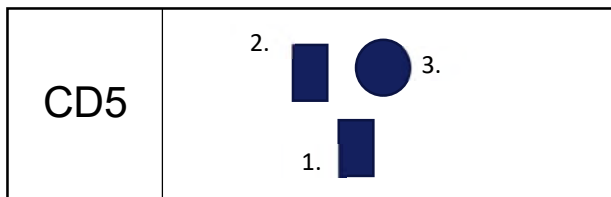
The origin of the tissues:

Sample S001: CD3



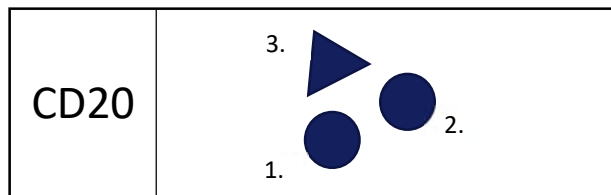
1. Tonsil
2. Peripheral T-cell lymphoma
3. Liver

Sample S002: CD5



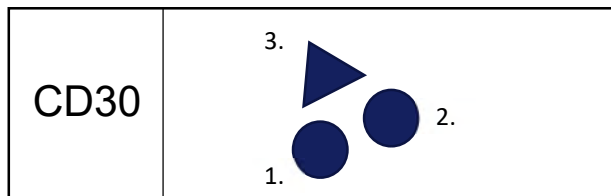
1. Liver
2. Tonsil
3. Mantle cell lymphoma

Sample S003: CD20



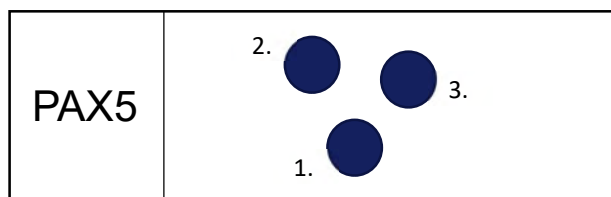
1. Tonsil
2. DLBCL
3. Colon

Sample S004: CD30



1. Embryonal carcinoma
2. Hodgkin's lymphoma
3. Tonsil

Sample S005: PAX5

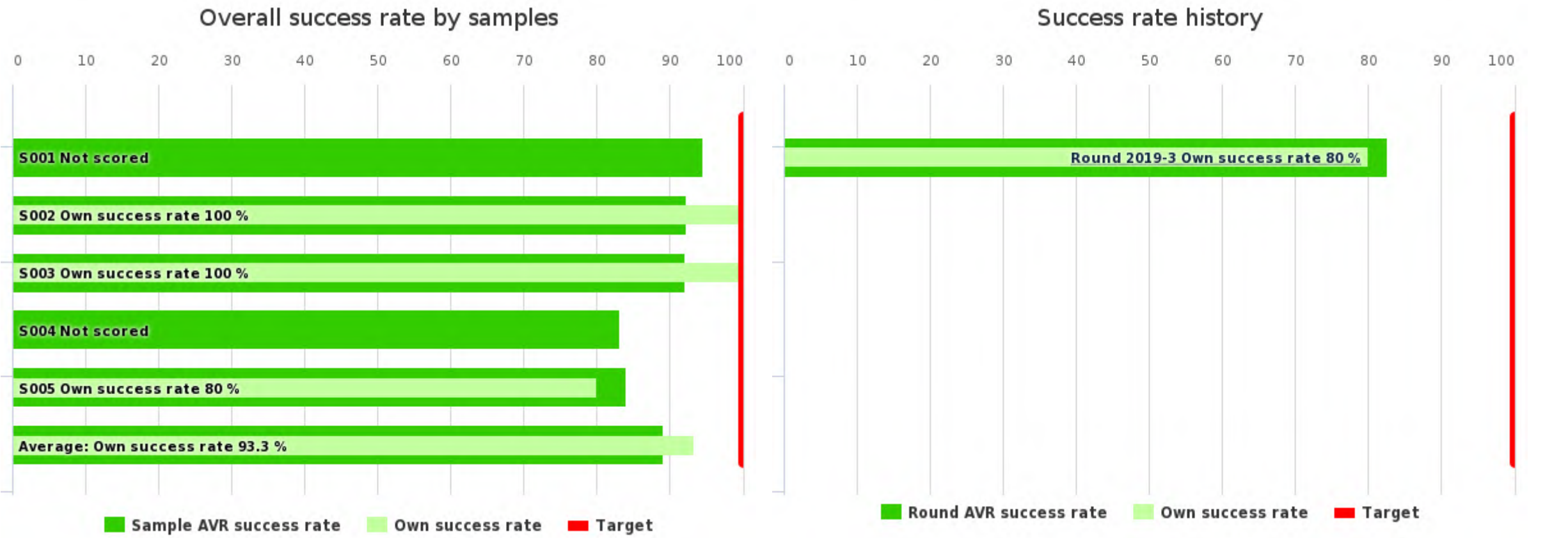


1. DLBCL
2. Hodgkin's lymphoma
3. Tonsil

Client report

	No of participants	No of responded participants	Response percentage
Immunohistochemical staining methods, limited selecton of antibodies, March, 1-2020	17	17	100 %
Immunohistochemical staining methods, March, 1-2020	45	45	100 %

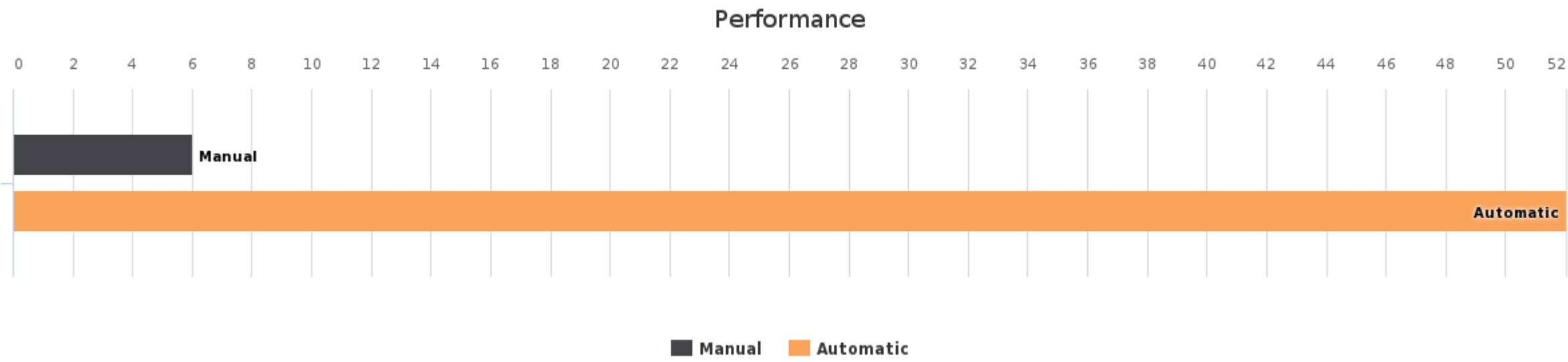
Summary



Summary	Own score	Max score	Own success rate	Difference	AVR success rate
S001	-	-	-	-	94.6 %
S002	5	5	100 %	7.8 %	92.2 %
S003	5	5	100 %	7.9 %	92.1 %
S004	-	-	-	-	83.1 %
S005	4	5	80 %	-4 %	84 %
Average:			93.3 %	4.1 %	89.2 %

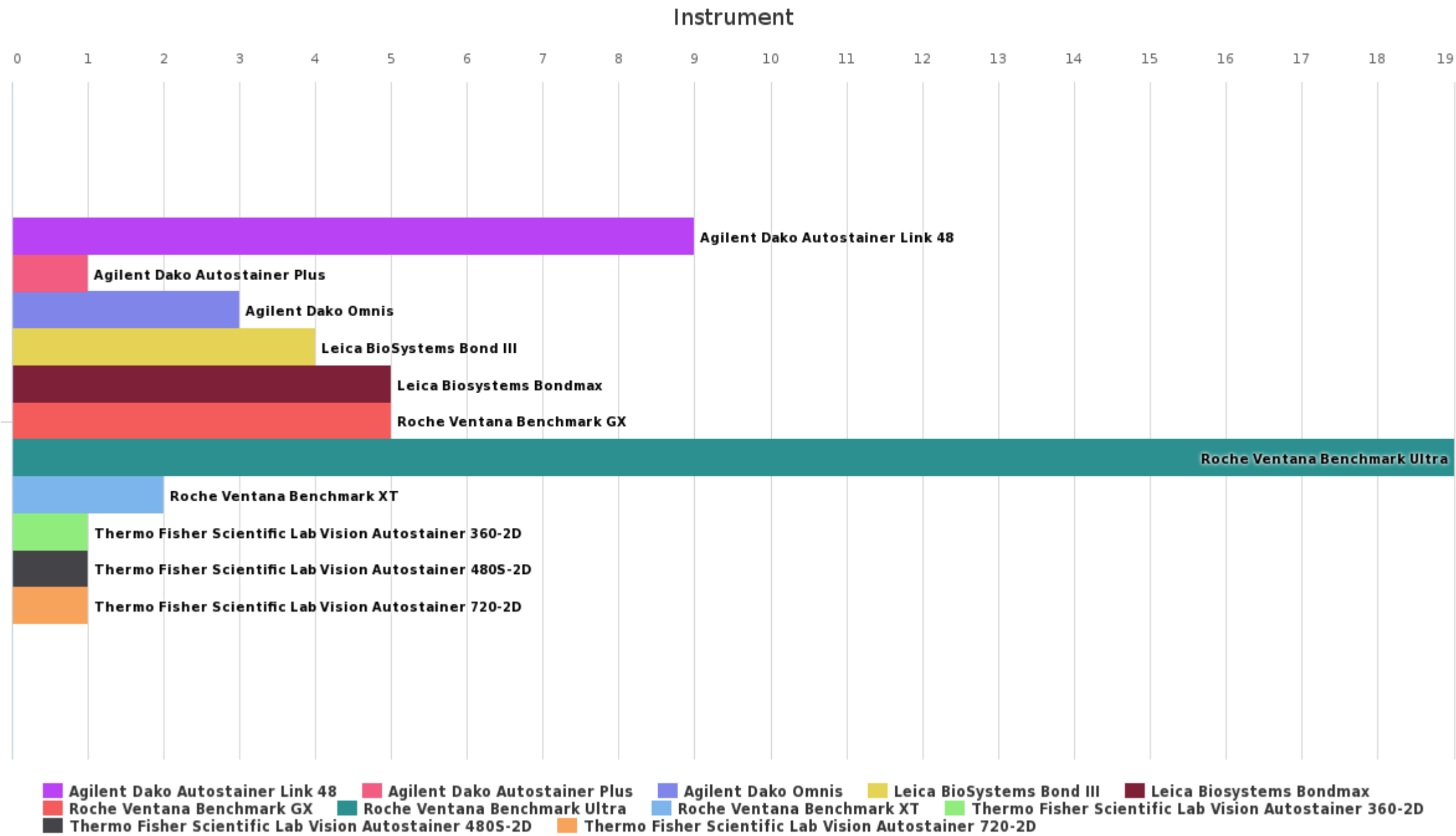
History	Test nr.	Own success rate	Difference	AVR success rate
Round 2019-3	1-1	80 %	-2.7 %	82.7 %

S001 | Sample S001 CD3

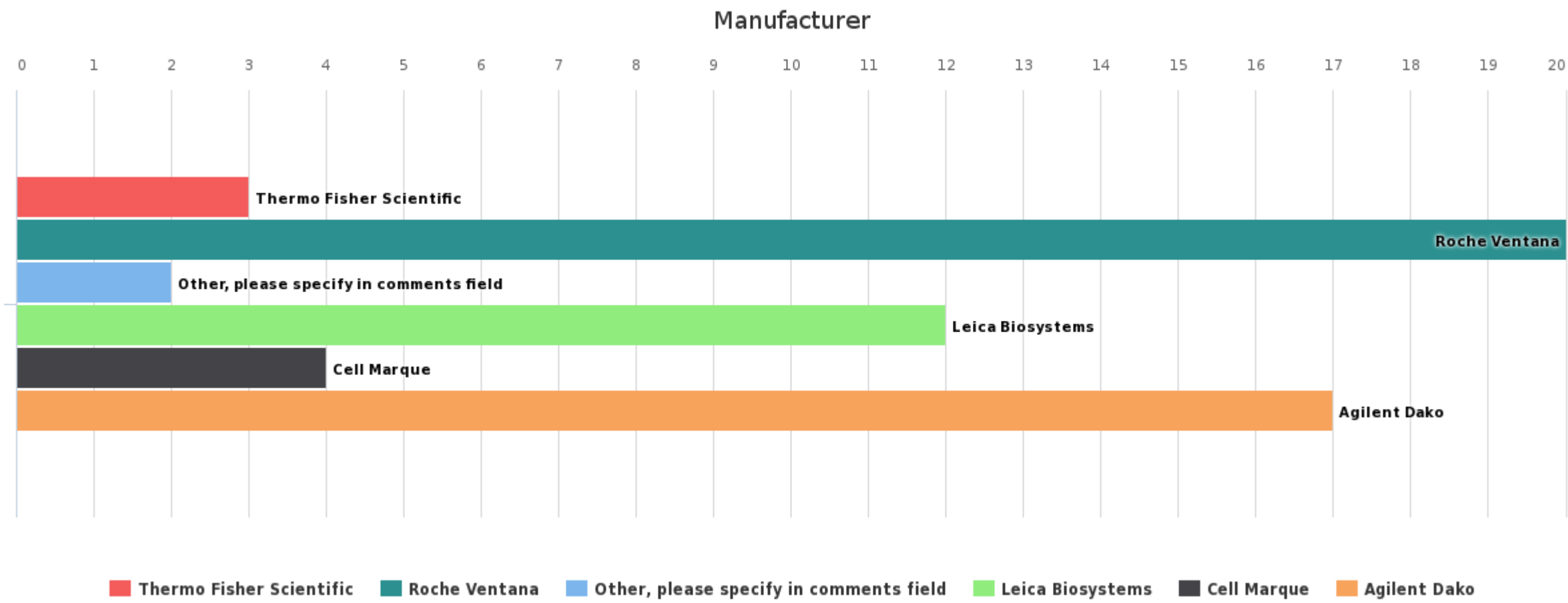


GENERAL DETAILS OF STAINING

Performance	Performance count
Automatic	52
Manual	6
Total:	58

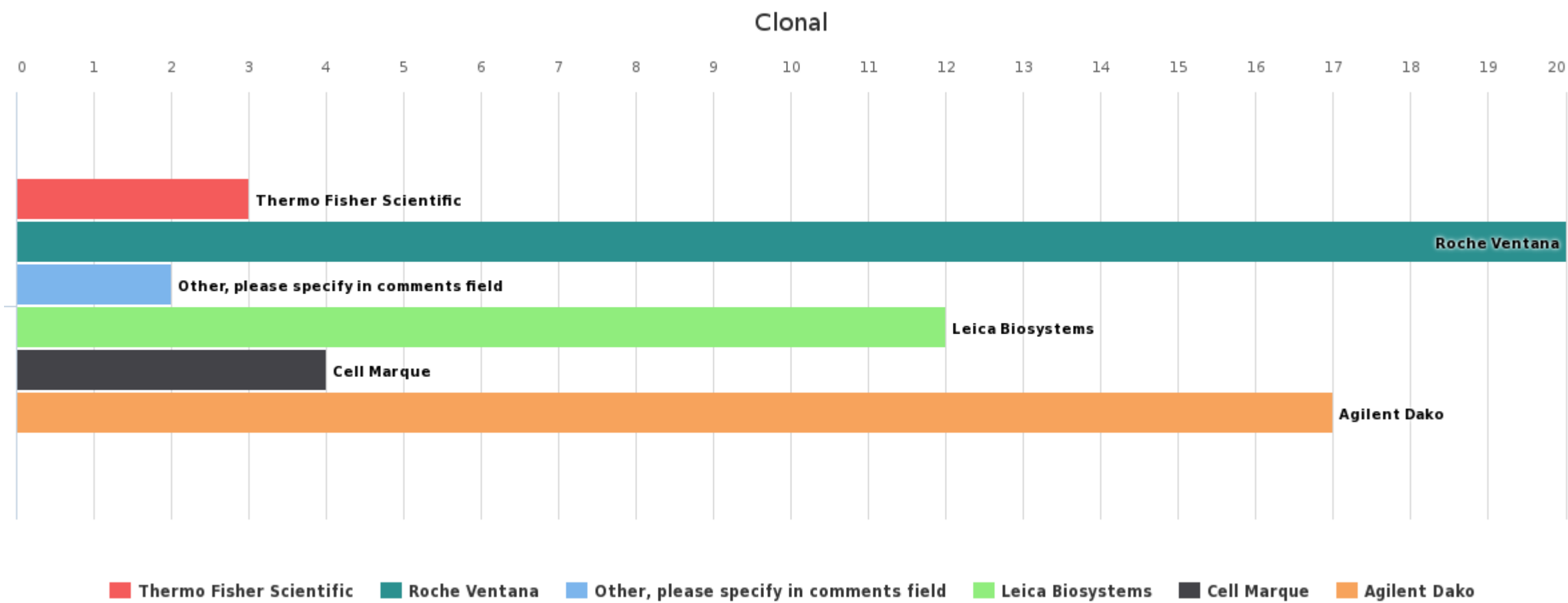


Instrument	Instrument count
Agilent Dako Autostainer Link 48	9
Agilent Dako Autostainer Plus	1
Agilent Dako Omnis	3
Leica BioSystems Bond III	4
Leica Biosystems Bondmax	5
Roche Ventana Benchmark GX	5
Roche Ventana Benchmark Ultra	19
Roche Ventana Benchmark XT	2
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 720-2D	1
Total:	51

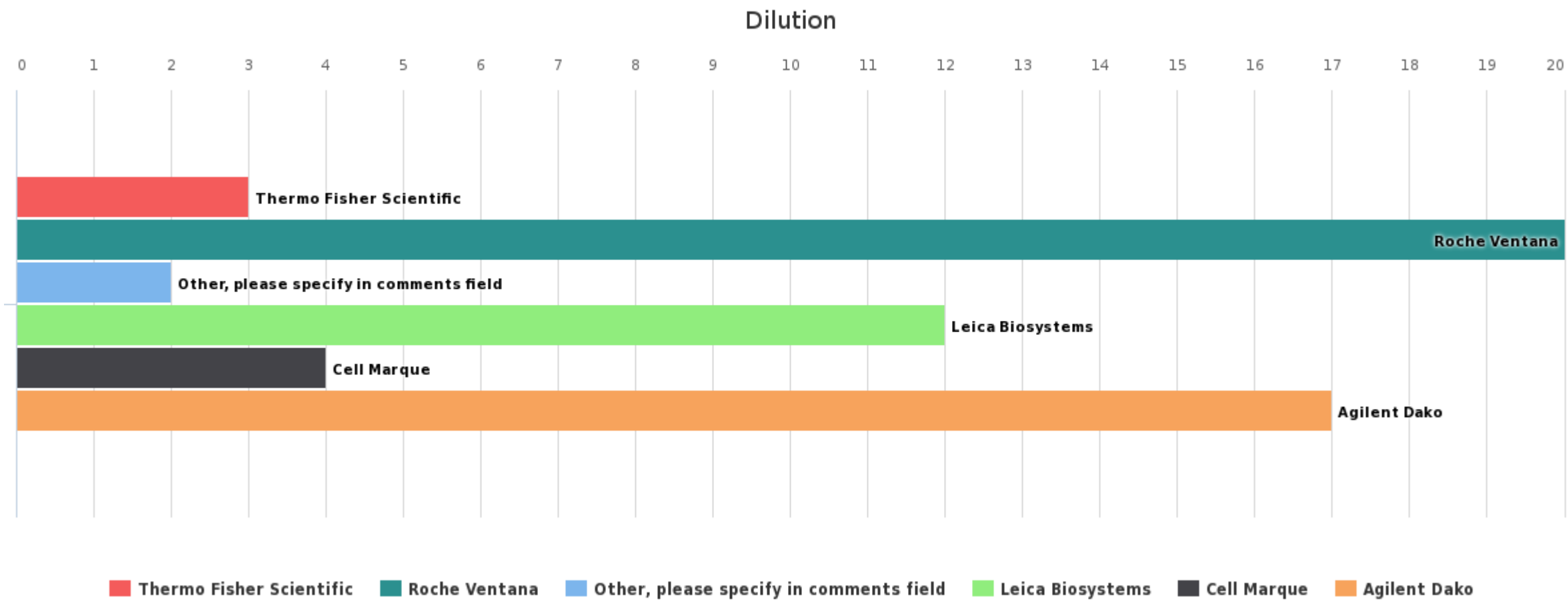


PRIMARY ANTIBODY

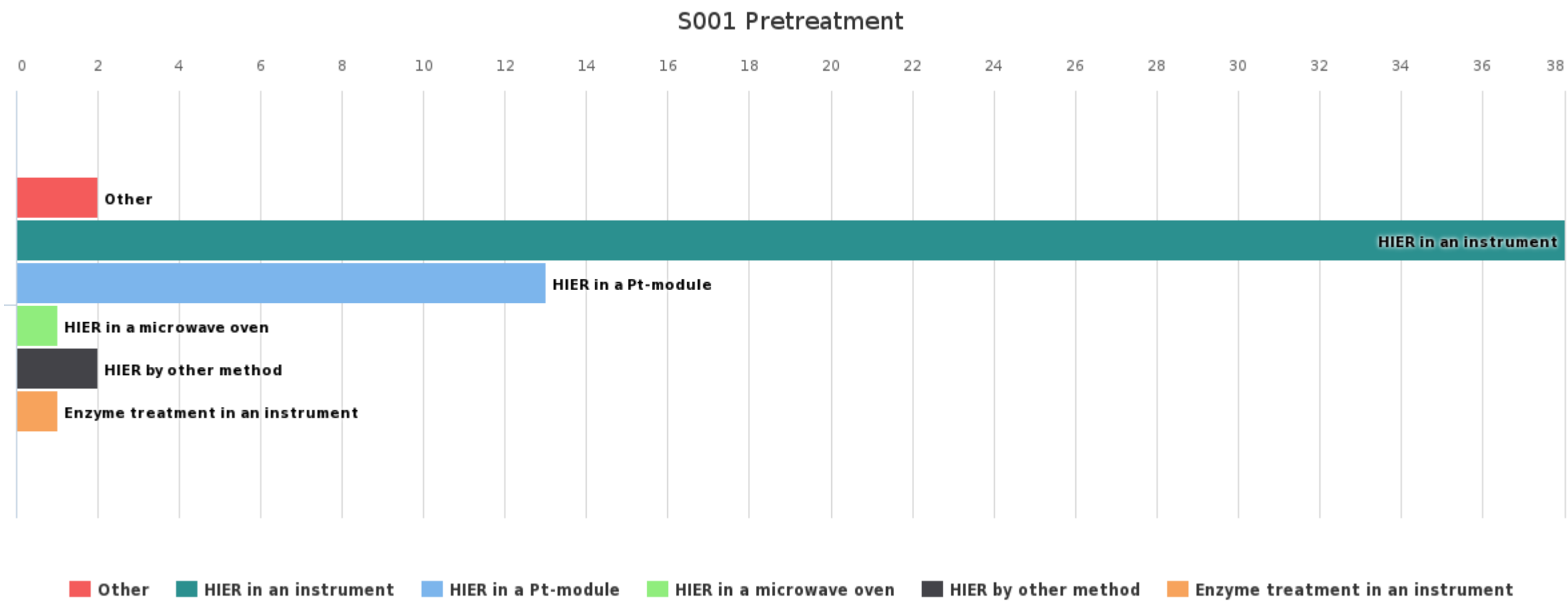
Manufacturer	Manufacturer count
Agilent Dako	17
Cell Marque	4
Leica Biosystems	12
Other, please specify in comments field	2
Roche Ventana	20
Thermo Fisher Scientific	3
Total:	58



Clonal	Clonal count
Monoclonal	40
Polyclonal	17
Total:	57

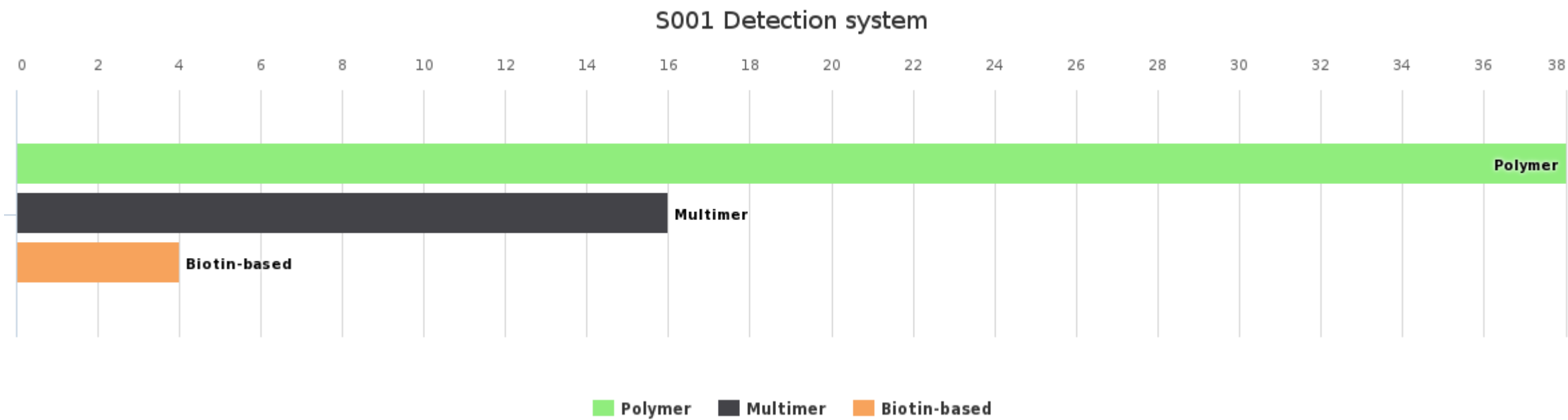


Dilution	Dilution count
Dilution	21
Ready-to-use	36
Total:	57



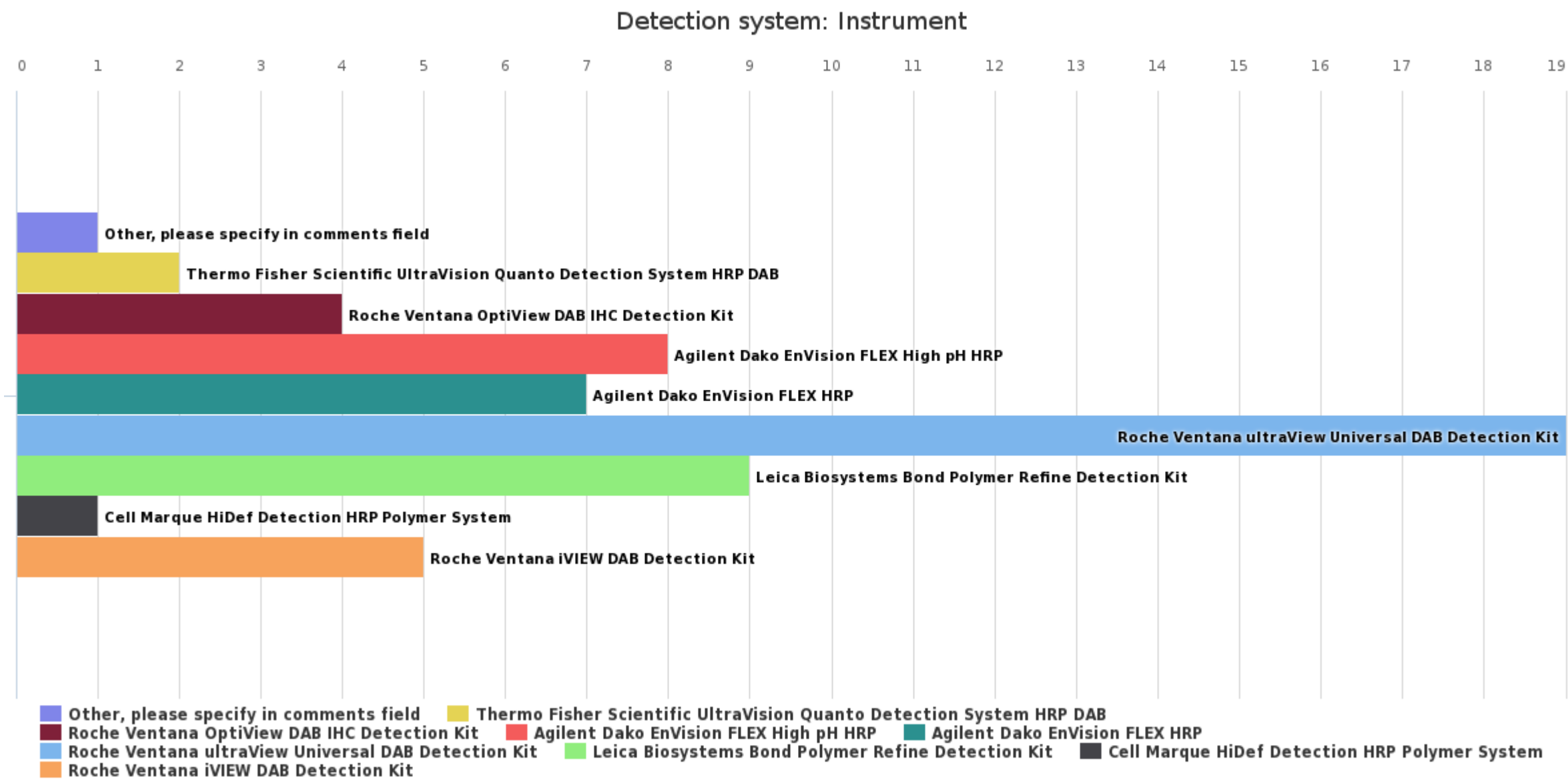
PRETREATMENT

Pretreatment	Pretreatment count
Enzyme treatment in an instrument	1
HIER by other method	2
HIER in a microwave oven	1
HIER in a Pt-module	13
HIER in an instrument	38
Other	2
Total:	57

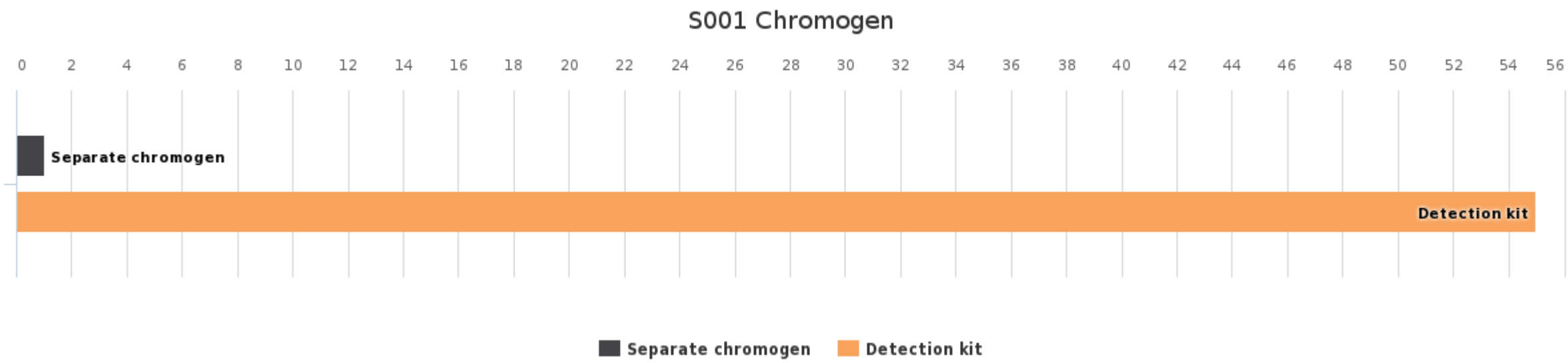


DETECTION SYSTEM

Detection system	Detection system count
Biotin-based	4
Multimer	16
Polymer	38
Total:	58

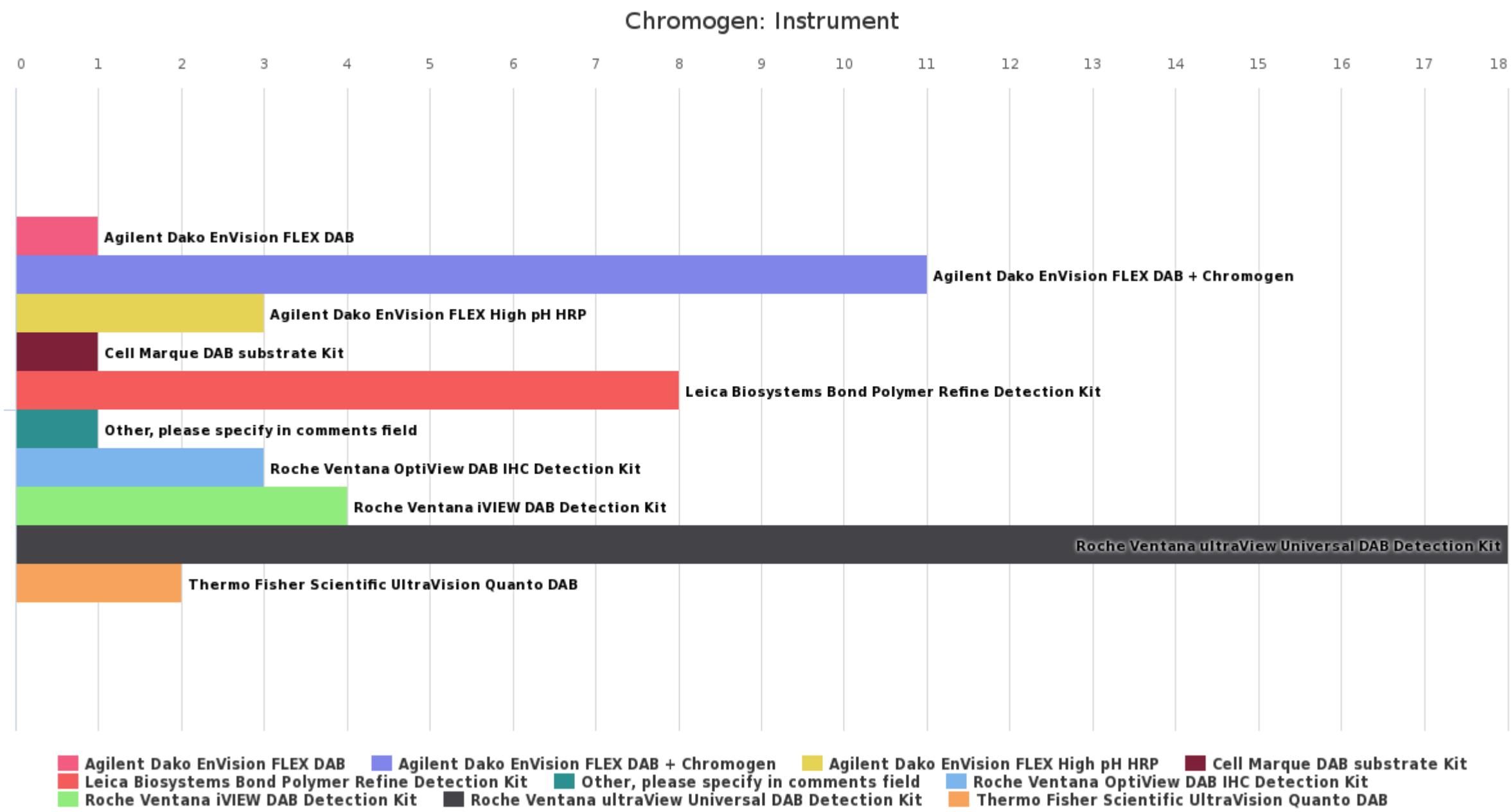


Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	7
Agilent Dako EnVision FLEX High pH HRP	8
Cell Marque HiDef Detection HRP Polymer System	1
Leica Biosystems Bond Polymer Refine Detection Kit	9
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	4
Roche Ventana iVIEW DAB Detection Kit	5
Roche Ventana ultraView Universal DAB Detection Kit	19
Thermo Fisher Scientific UltraVision Quanto Detection System HRP DAB	2
Total:	56

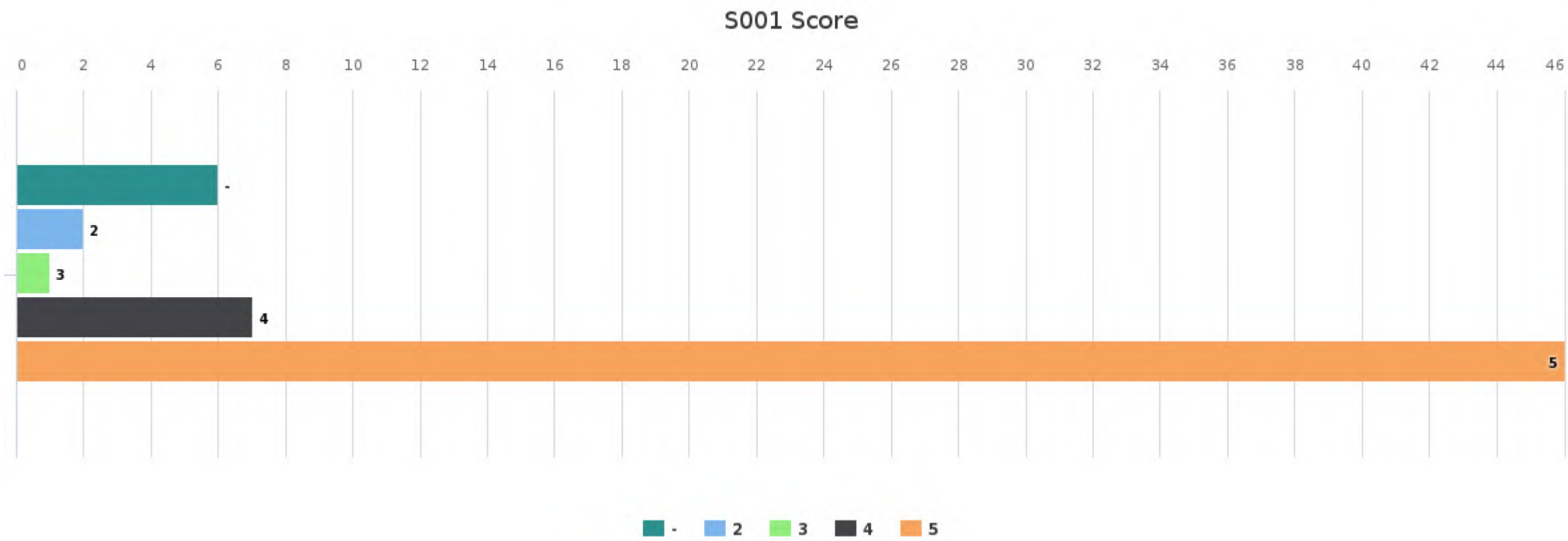


CHROMOGEN

Chromogen	Chromogen count
Detection kit	55
Separate chromogen	1
Total:	56

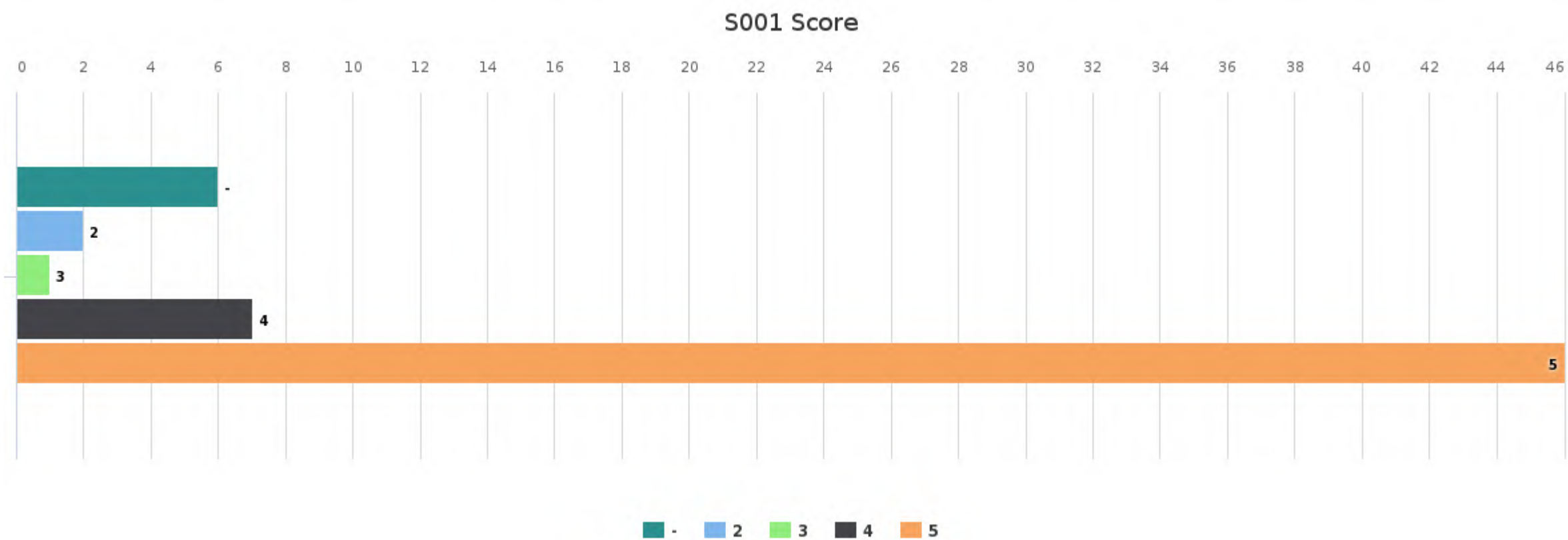


Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	1
Agilent Dako EnVision FLEX DAB + Chromogen	11
Agilent Dako EnVision FLEX High pH HRP	3
Cell Marque DAB substrate Kit	1
Leica Biosystems Bond Polymer Refine Detection Kit	8
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	3
Roche Ventana iVIEW DAB Detection Kit	4
Roche Ventana ultraView Universal DAB Detection Kit	18
Thermo Fisher Scientific UltraVision Quanto DAB	2
Total:	52



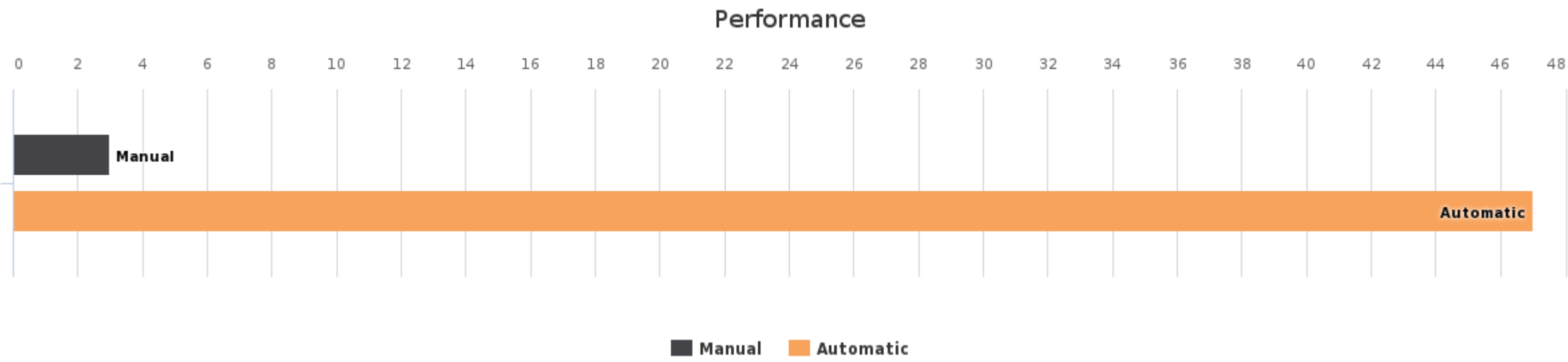
SCORE

Score	Comment
<input checked="" type="radio"/> -	
	<input checked="" type="radio"/> Not available
2	
	Too weak staining in the tonsil and no T-cell staining in the liver.
	Too weak staning, No membrane staining/ not clear membranous staining.
3	
	Too weak staining of the T-cell lymphoma.
4	
	Light background; liver hepatocytes
	Not crisp
	Not crisp; no membranous staining
	Slightly too weak staining.
	Slightly uneaven staining, edge artefacts.
	Slightly weak staining in all tissues.
	Slightly weak. Contrast between hematoxylin and DAB is not optimal.
5	
	Excellent
Total:	



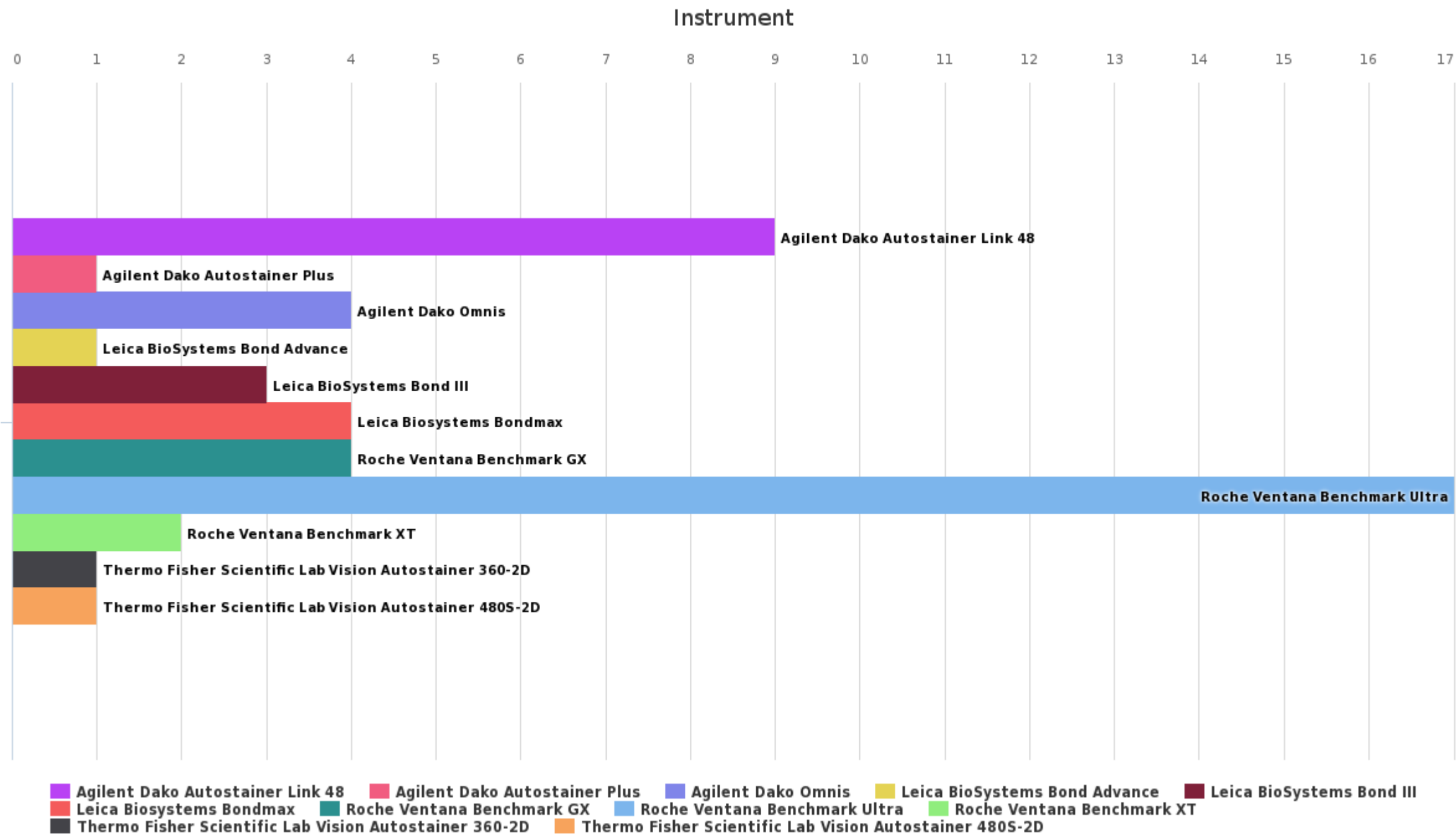
Score	Score count
<input checked="" type="radio"/> -	6
2	2
3	1
4	7
5	46
Total:	62

S002 | Sample S002 CD5

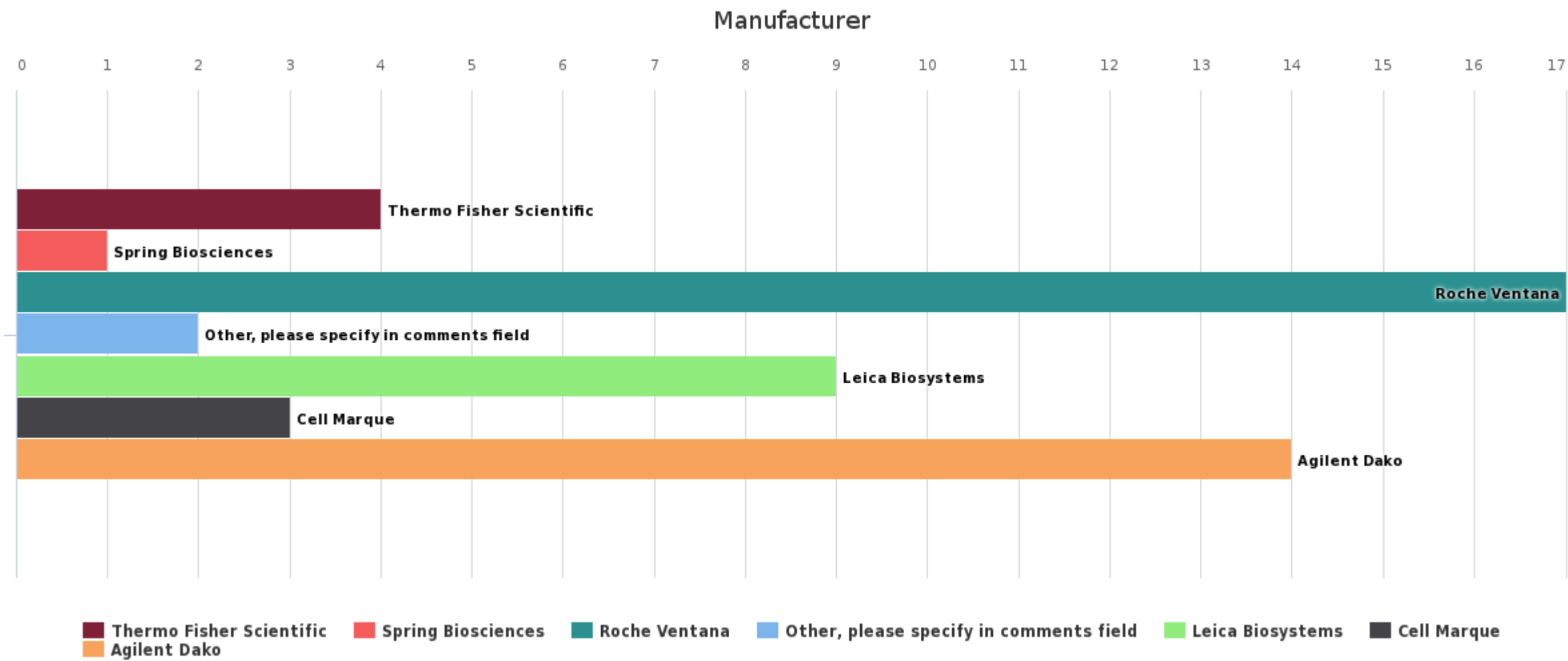


GENERAL DETAILS OF STAINING

Performance	Performance count
<input checked="" type="radio"/> Automatic	47
Manual	3
Total:	50

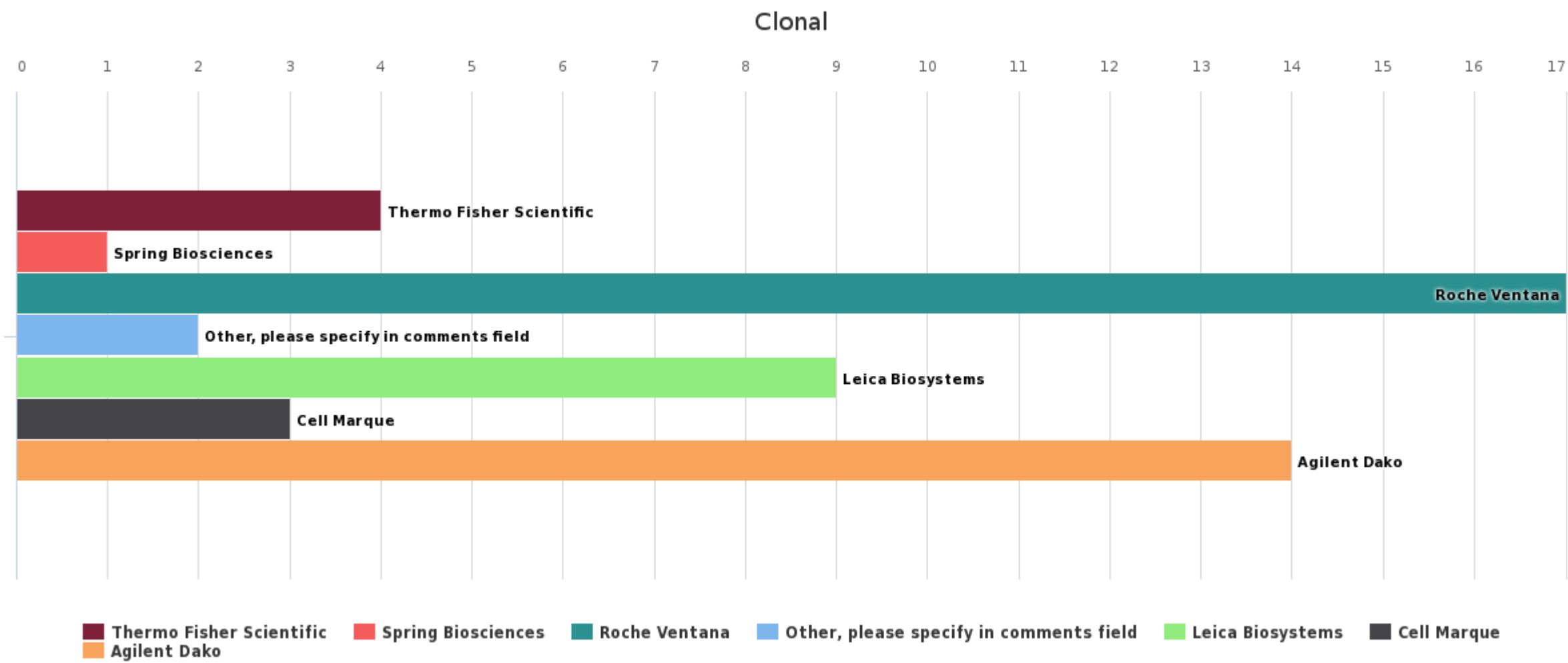


Instrument	Instrument count
<input checked="" type="radio"/> Agilent Dako Autostainer Link 48	9
Agilent Dako Autostainer Plus	1
Agilent Dako Omnis	4
Leica BioSystems Bond Advance	1
Leica BioSystems Bond III	3
Leica Biosystems Bondmax	4
Roche Ventana Benchmark GX	4
Roche Ventana Benchmark Ultra	17
Roche Ventana Benchmark XT	2
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Total:	47

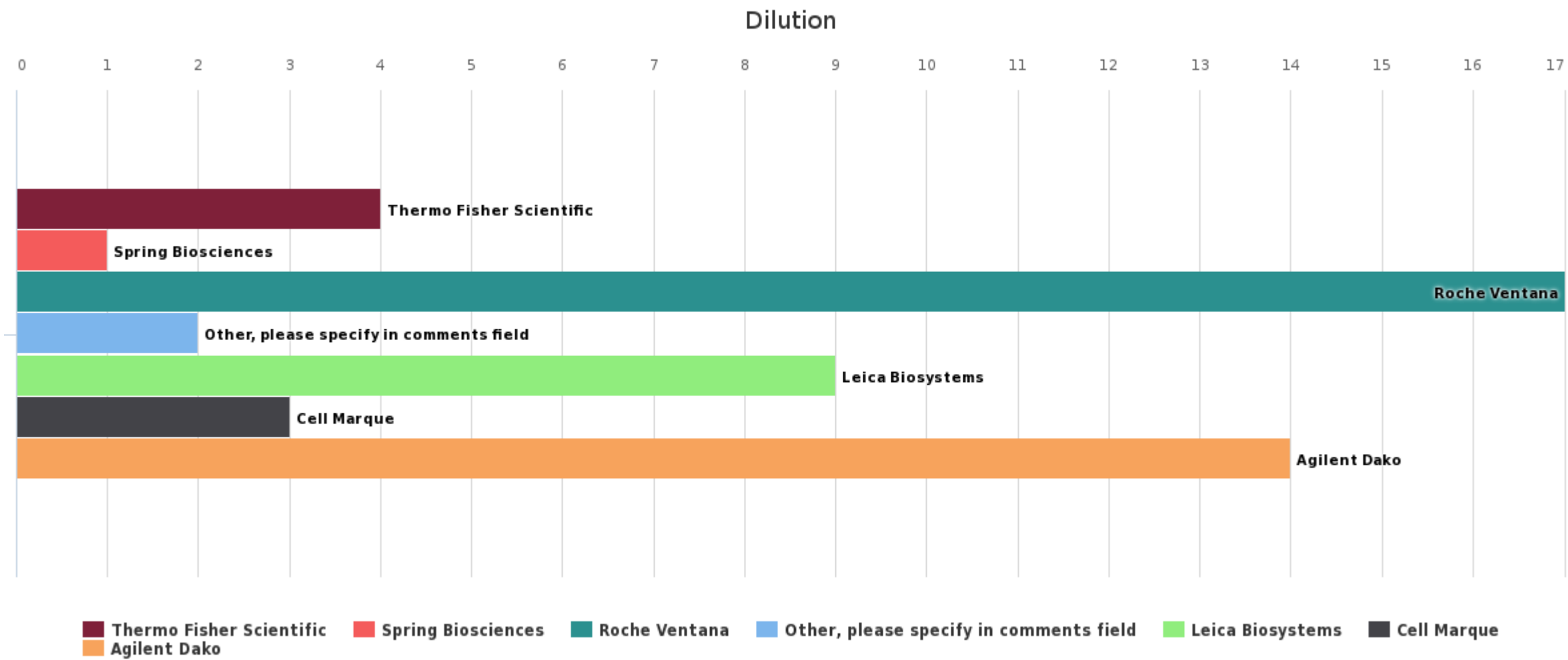


PRIMARY ANTIBODY

Manufacturer	Manufacturer count
<input checked="" type="radio"/> Agilent Dako	14
Cell Marque	3
Leica Biosystems	9
Other, please specify in comments field	2
Roche Ventana	17
Spring Biosciences	1
Thermo Fisher Scientific	4
Total:	50



Clonal	Clonal count
<input checked="" type="radio"/> Monoclonal	49
Polyclonal	1
Total:	50

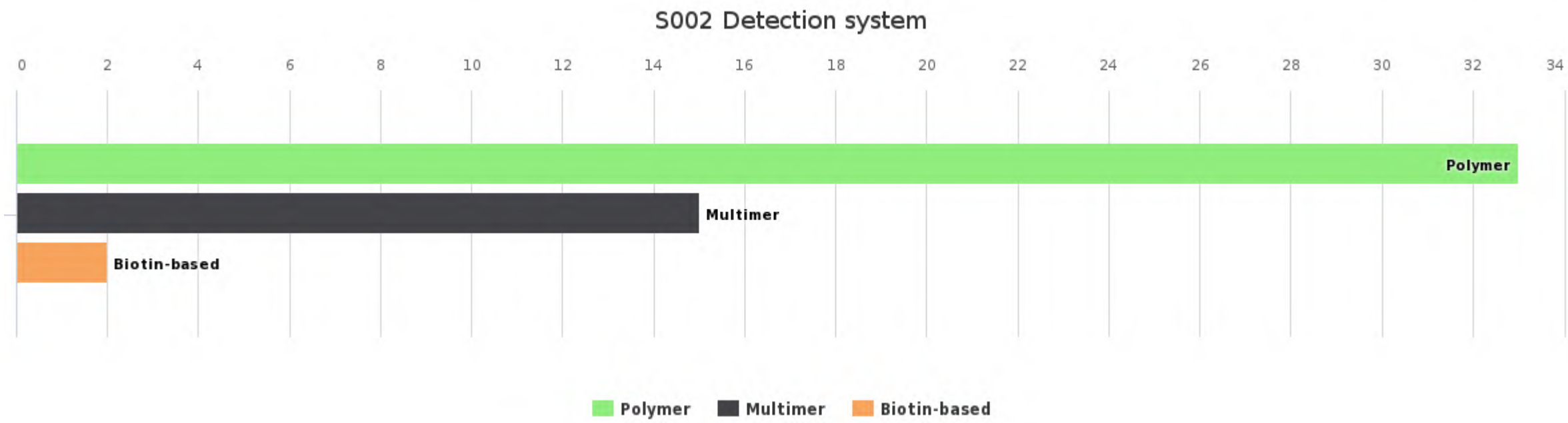


Dilution	Dilution count
Dilution	20
<input checked="" type="radio"/> Ready-to-use	30
Total:	50



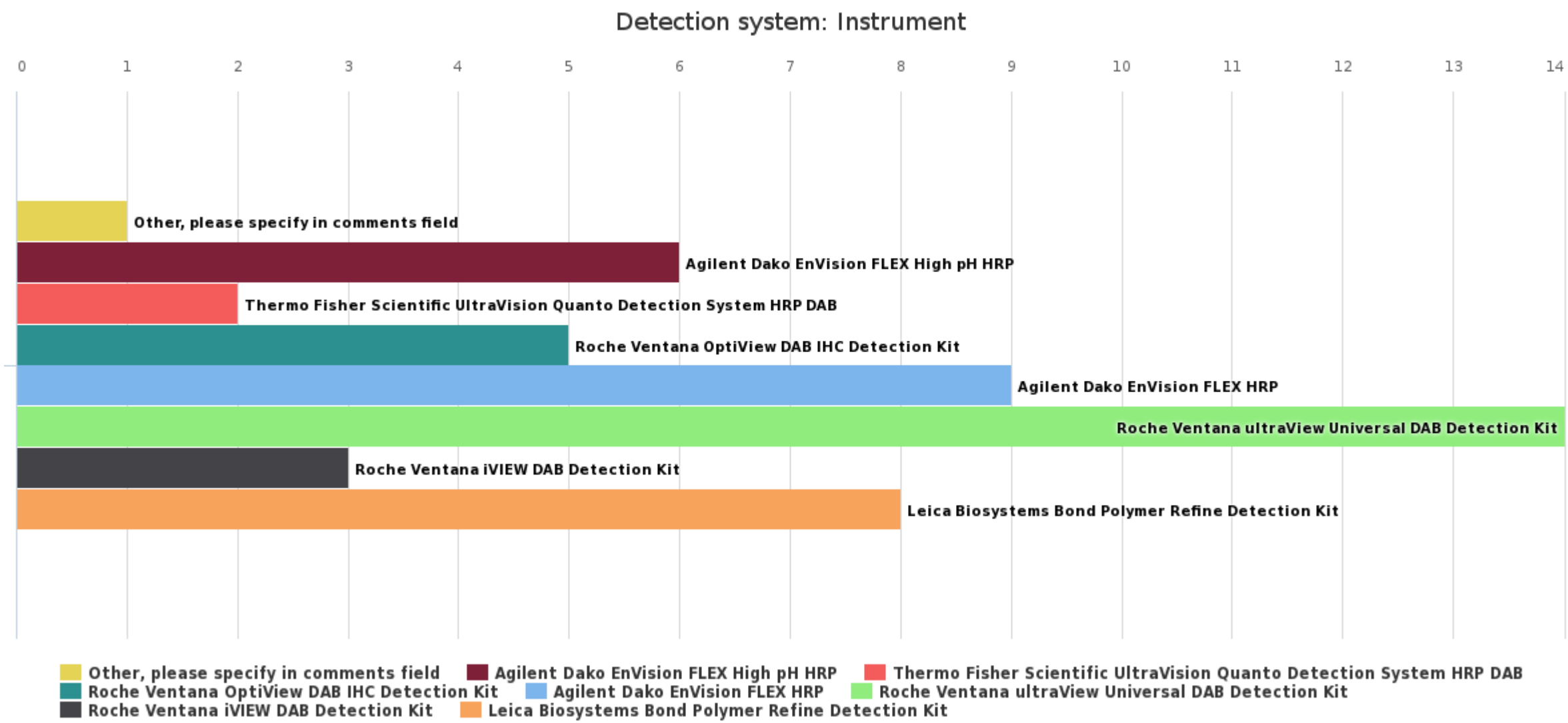
PRETREATMENT

Pretreatment	Pretreatment count
HIER by other method	2
HIER in a Pt-module	12
<input checked="" type="radio"/> HIER in an instrument	32
Other	2
Total:	48

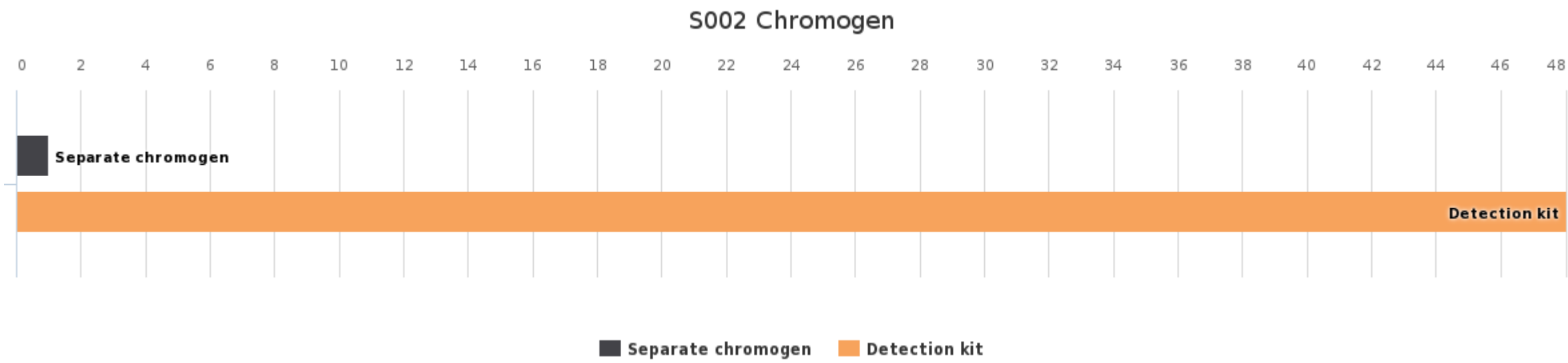


DETECTION SYSTEM

Detection system	Detection system count
Biotin-based	2
Multimer	15
<input checked="" type="radio"/> Polymer	33
Total:	50

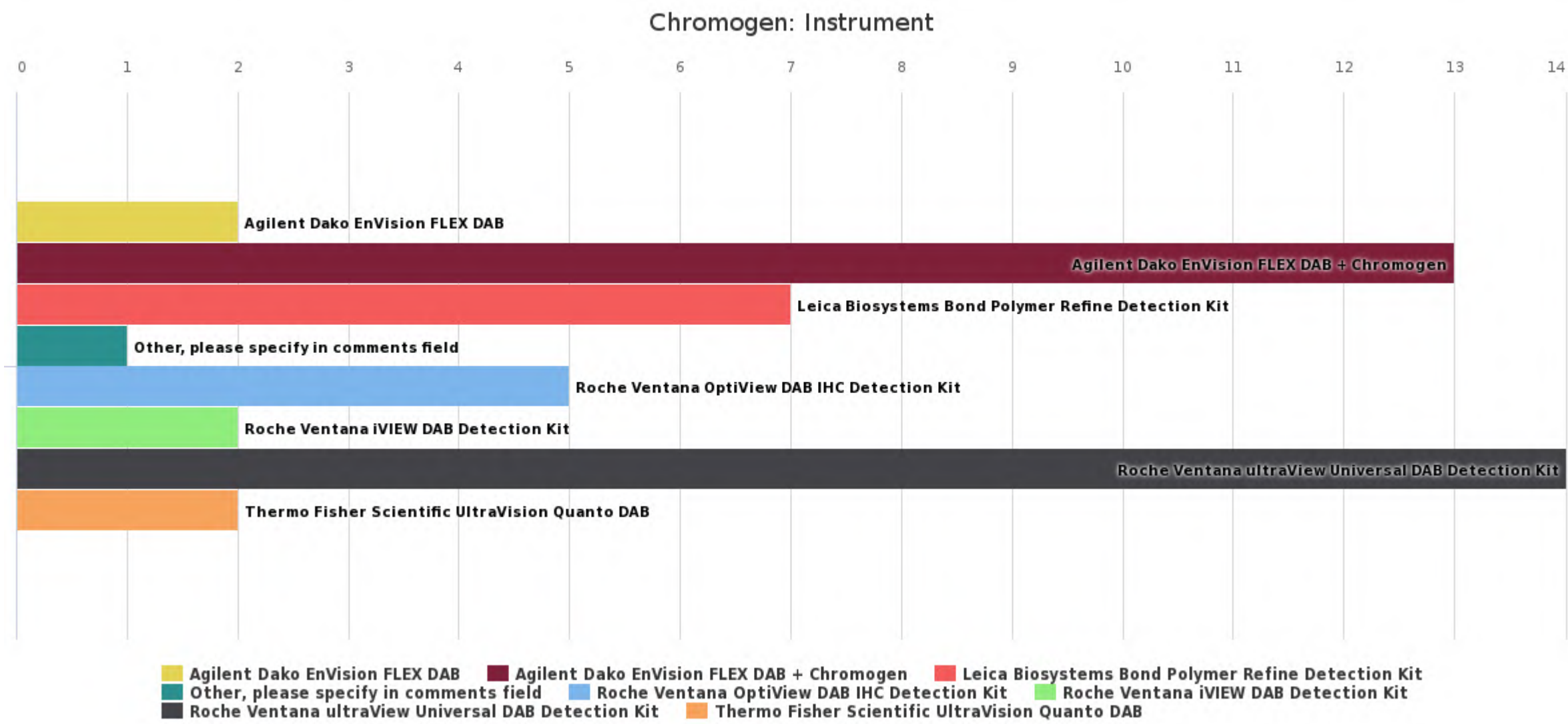


Detection system: Instrument	Detection system: Instrument count
<input checked="" type="radio"/> Agilent Dako EnVision FLEX HRP	9
Agilent Dako EnVision FLEX High pH HRP	6
Leica Biosystems Bond Polymer Refine Detection Kit	8
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	5
Roche Ventana iVIEW DAB Detection Kit	3
Roche Ventana ultraView Universal DAB Detection Kit	14
Thermo Fisher Scientific UltraVision Quanto Detection System HRP DAB	2
Total:	48

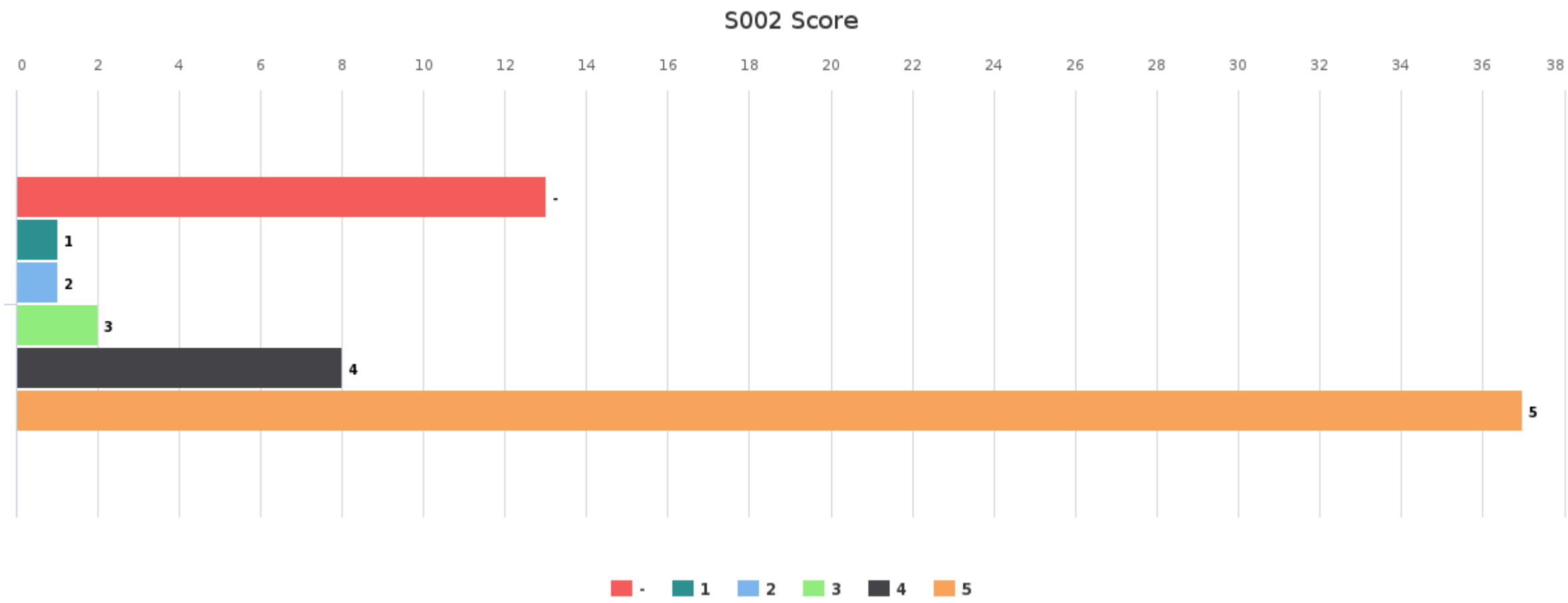


CHROMOGEN

Chromogen	Chromogen count
<input checked="" type="radio"/> Detection kit	48
Separate chromogen	1
Total:	49

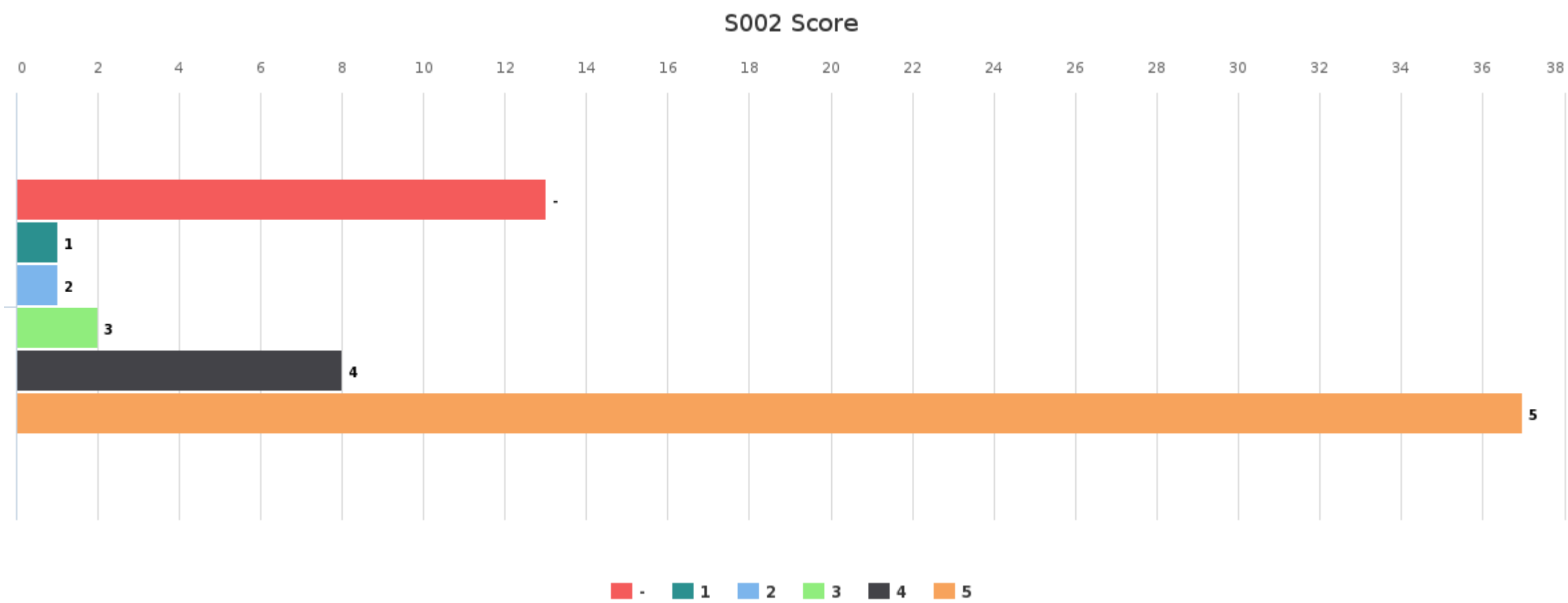


Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	2
<input checked="" type="radio"/> Agilent Dako EnVision FLEX DAB + Chromogen	13
Leica Biosystems Bond Polymer Refine Detection Kit	7
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	5
Roche Ventana iVIEW DAB Detection Kit	2
Roche Ventana ultraView Universal DAB Detection Kit	14
Thermo Fisher Scientific UltraVision Quanto DAB	2
Total:	46



SCORE

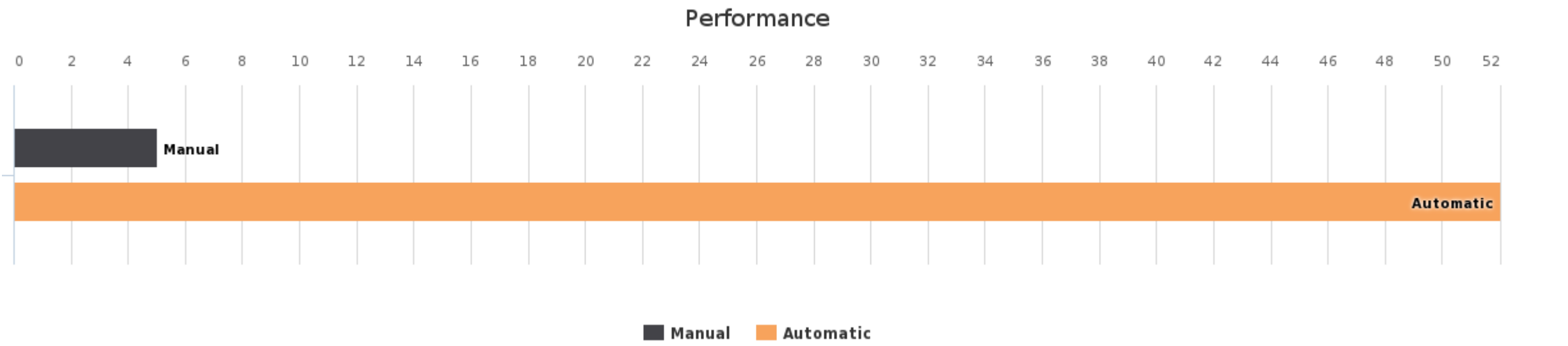
Score	Comment
-	
	Not available
1	
	No specific staining in any tissues.
2	
	False negative neoplastic B-cells in mattle cell lymphoma, weak or no staining of manttle zone B-cells in the tonsil.
3	
	Too strong hematoxylin, stains also cytoplasm. Uneaven staining. Weak staining of the manttle cell lymphoma B-cells and manttle zone B-cells of the tonsil.
	Weak staining, not crisp
4	
	Contrast between hematoxylin and DAB is not optimal.
	Background staining in all tissues.
	Not crisp staining.
	Slight background in all tissues.
	Technical issue; DAB precipitates. Old DAB solution or too few/short washing steps.
	Too strong hematoxylin, not optimal contrast
	Too strong hematoxylin; low contrast.
<input checked="" type="radio"/> 5	
	<input checked="" type="radio"/> Excellent
Total:	



Score	Score count
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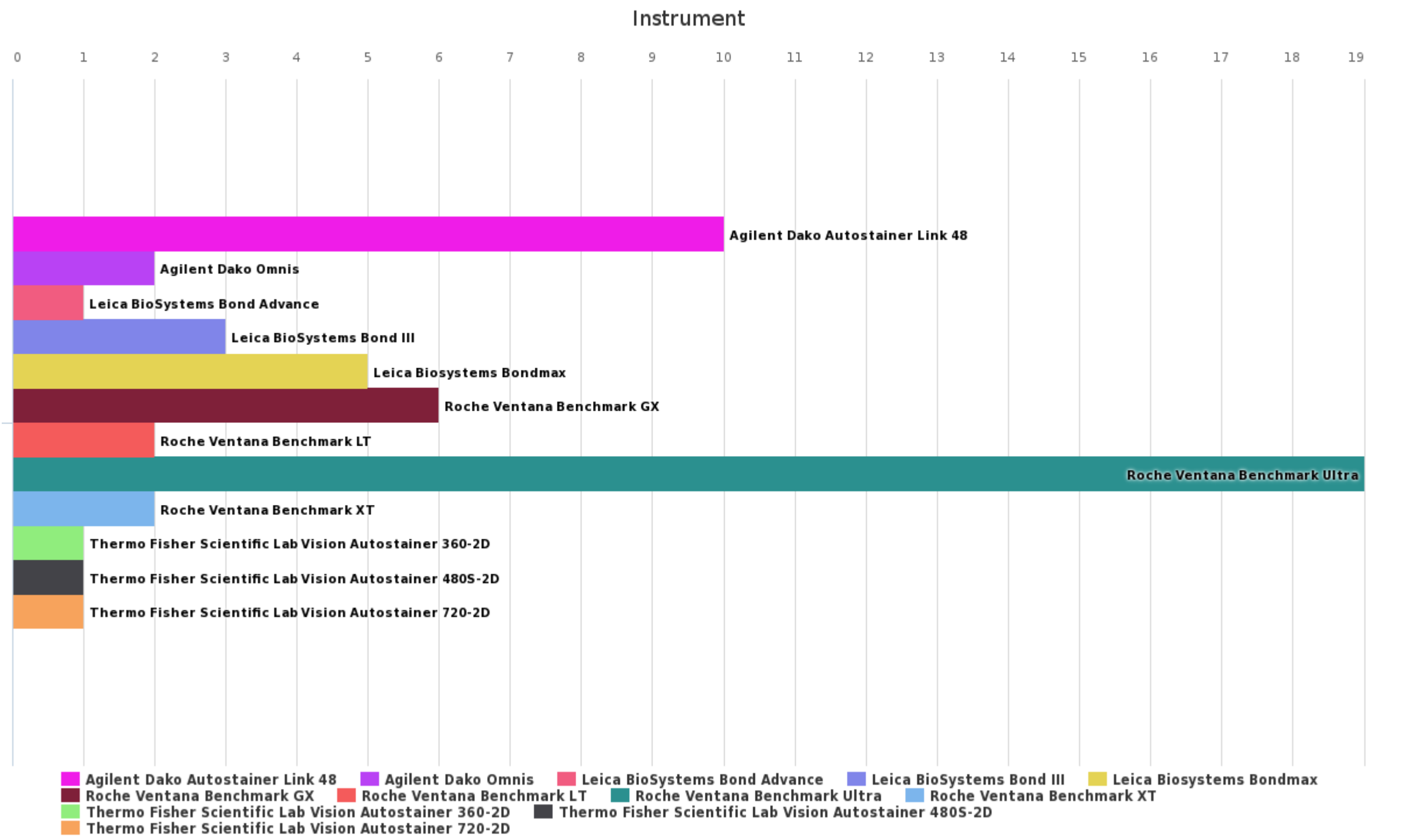
-	13
1	1
2	1
3	2
4	8
<input checked="" type="radio"/> 5	37
Total:	62

S003 | Sample S003 CD20



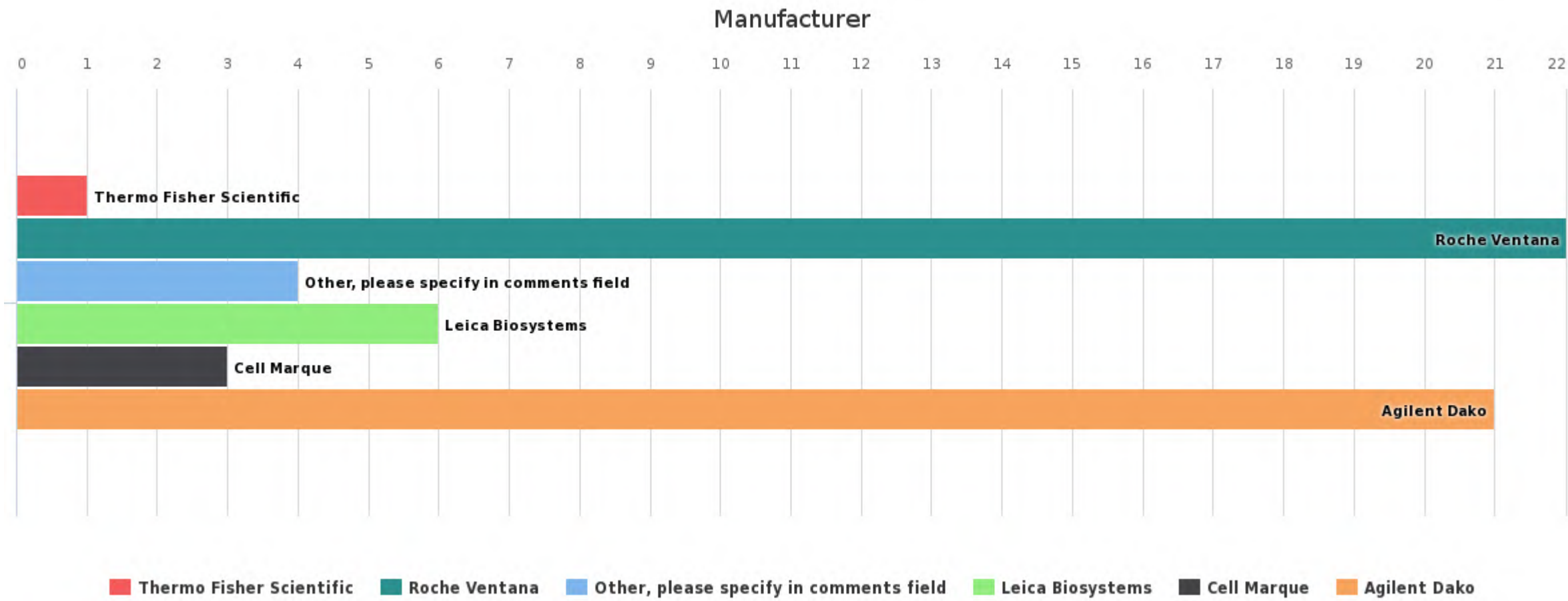
GENERAL DETAILS OF STAINING

Performance	Performance count
<input checked="" type="radio"/> Automatic	52
Manual	5
Total:	57



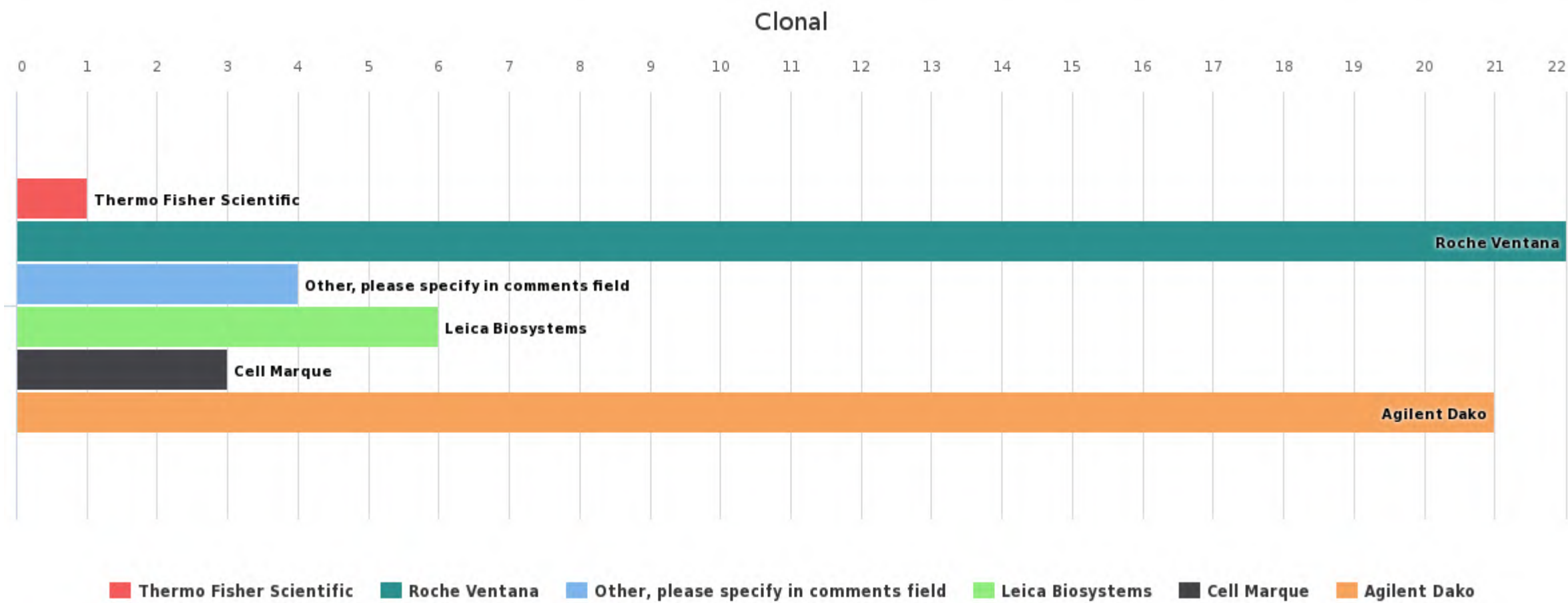
Instrument	Instrument count
<input checked="" type="radio"/> Agilent Dako Autostainer Link 48	10
Agilent Dako Omnis	2
Leica BioSystems Bond Advance	1
Leica BioSystems Bond III	3
Leica Biosystems Bondmax	5
Roche Ventana Benchmark GX	6
Roche Ventana Benchmark LT	2
Roche Ventana Benchmark Ultra	19
Roche Ventana Benchmark XT	2
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1

Thermo Fisher Scientific Lab Vision Autostainer 720-2D	1
Total:	53

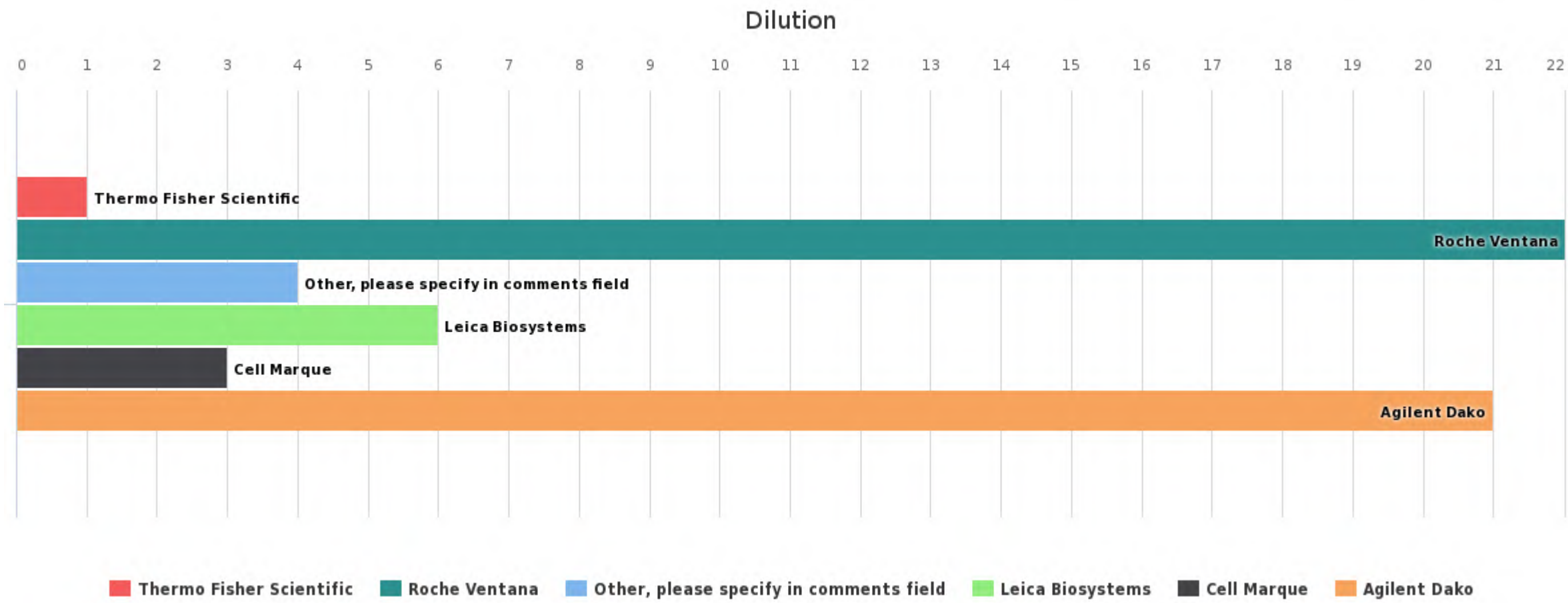


PRIMARY ANTIBODY

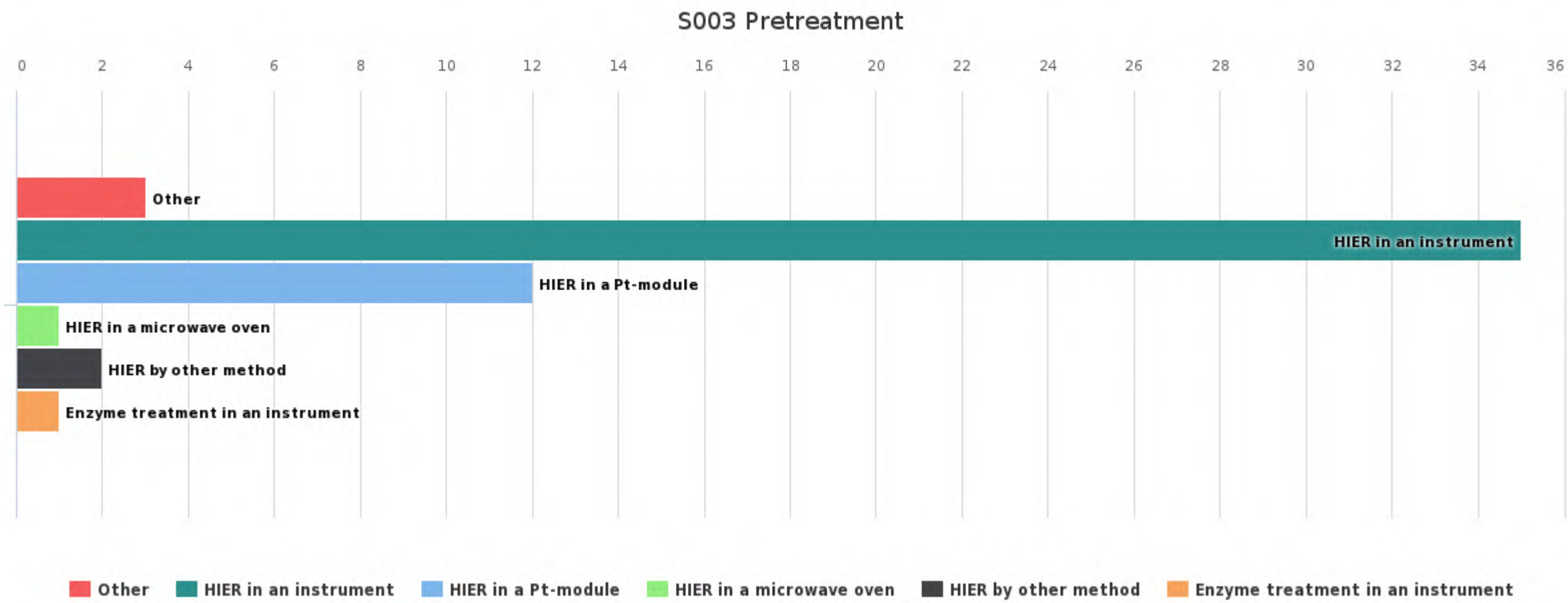
Manufacturer	Manufacturer count
<input checked="" type="radio"/> Agilent Dako	21
Cell Marque	3
Leica Biosystems	6
Other, please specify in comments field	4
Roche Ventana	22
Thermo Fisher Scientific	1
Total:	57



Clonal	Clonal count
<input checked="" type="radio"/> Monoclonal	56
Polyclonal	1
Total:	57

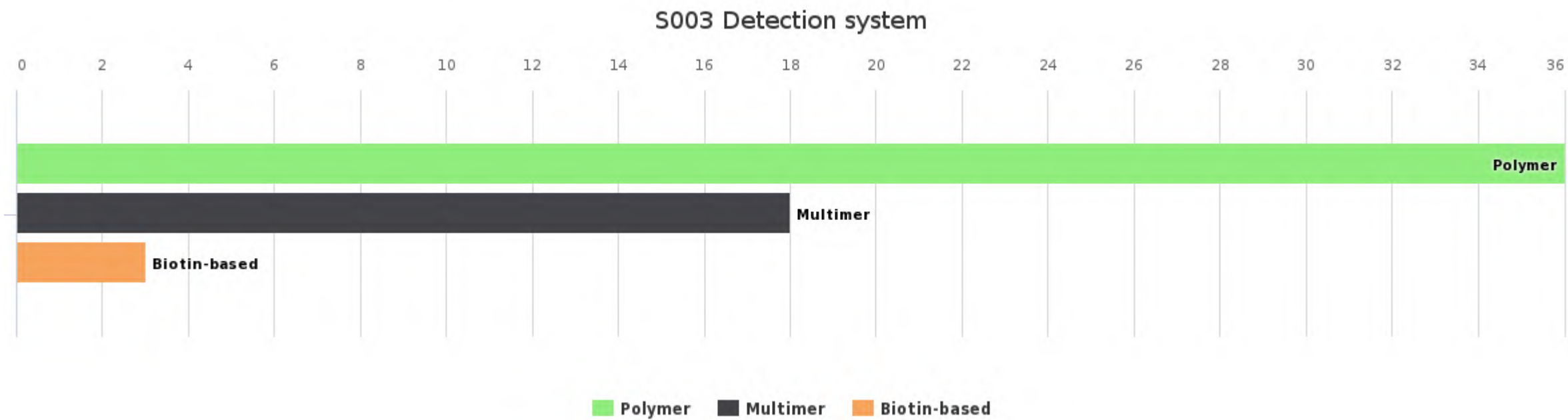


Dilution	Dilution count
Dilution	24
<input checked="" type="radio"/> Ready-to-use	33
Total:	57



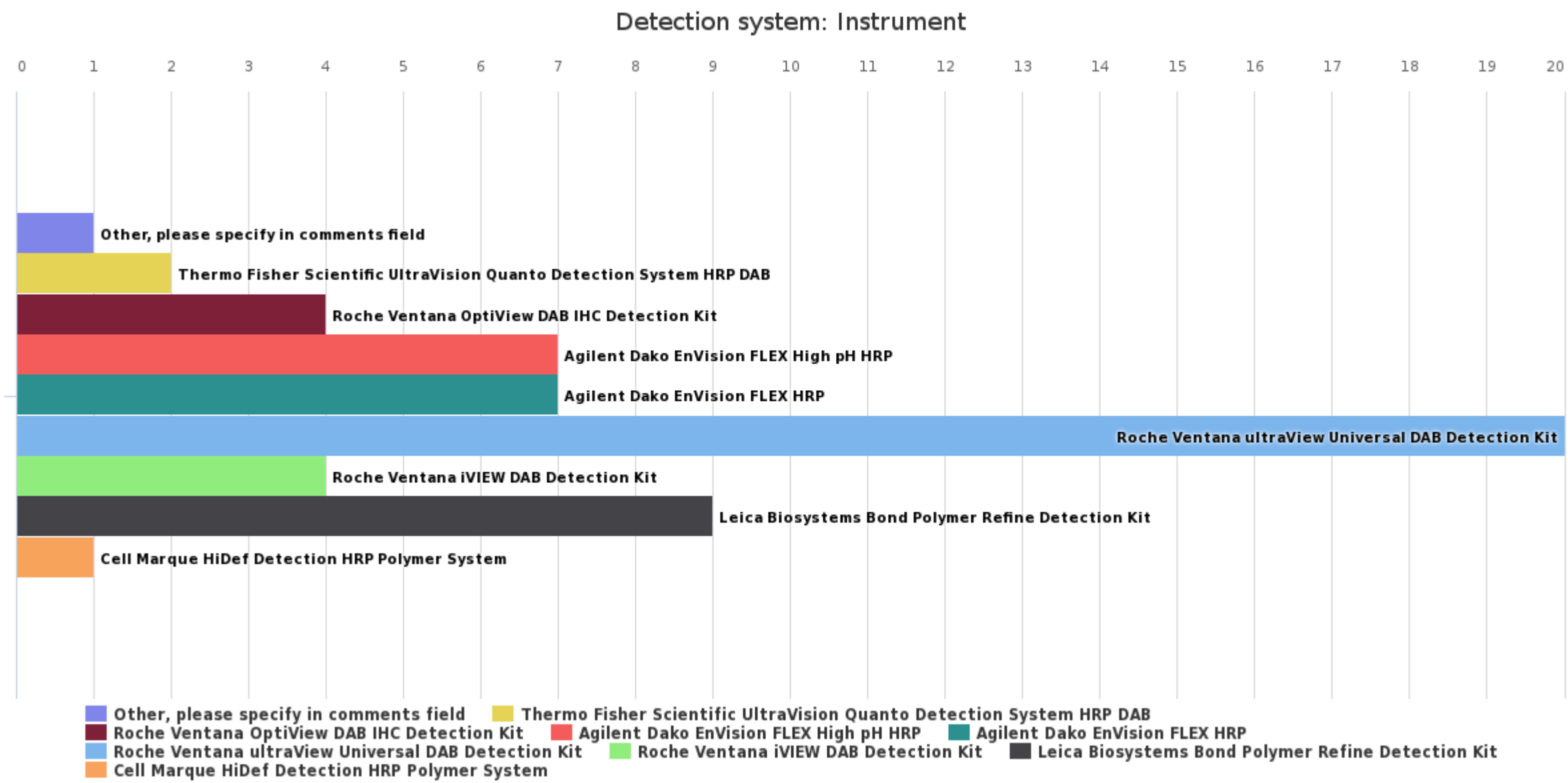
PRETREATMENT

Pretreatment	Pretreatment count
Enzyme treatment in an instrument	1
HIER by other method	2
HIER in a microwave oven	1
HIER in a Pt-module	12
<input checked="" type="radio"/> HIER in an instrument	35
Other	3
Total:	54

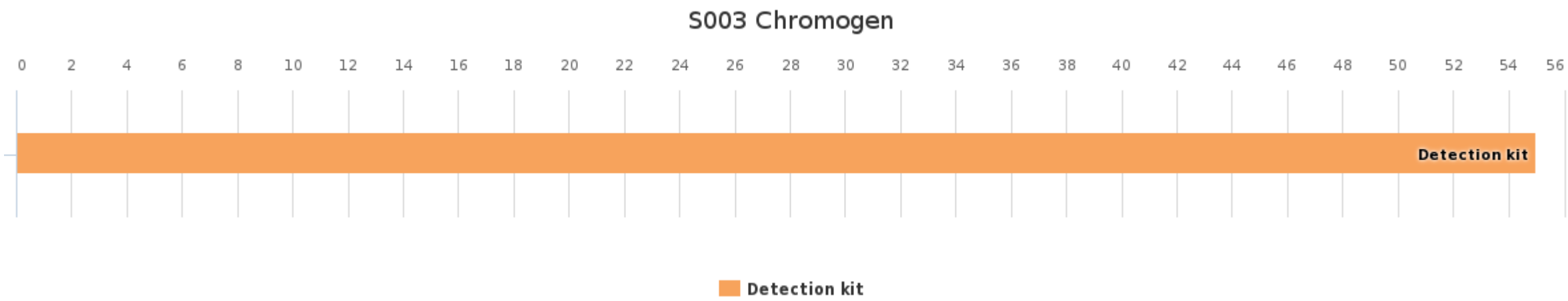


DETECTION SYSTEM

Detection system	Detection system count
Biotin-based	3
Multimer	18
<input checked="" type="radio"/> Polymer	36
Total:	57

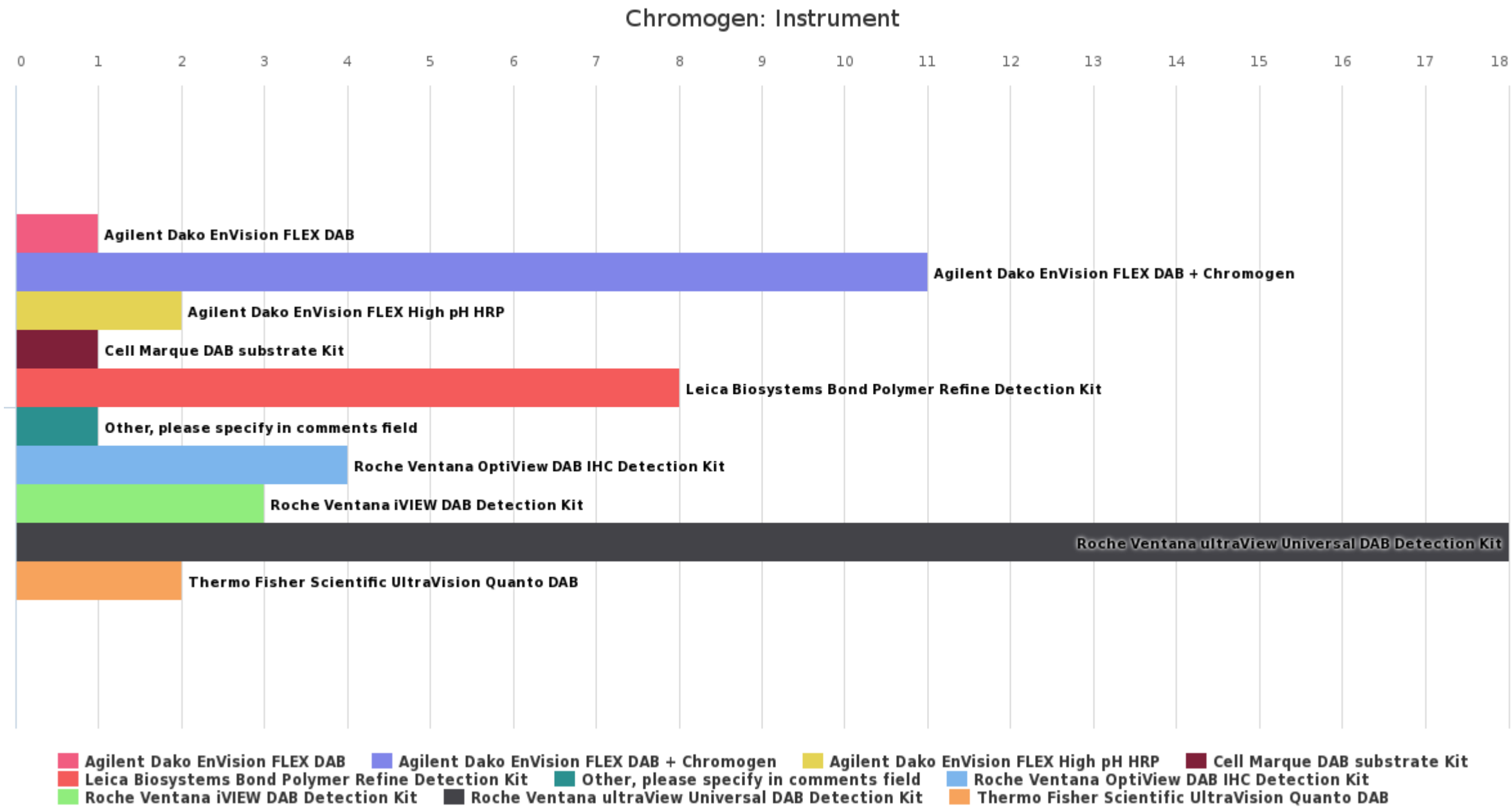


Detection system: Instrument	Detection system: Instrument count
<input checked="" type="radio"/> Agilent Dako EnVision FLEX HRP	7
Agilent Dako EnVision FLEX High pH HRP	7
Cell Marque HiDef Detection HRP Polymer System	1
Leica Biosystems Bond Polymer Refine Detection Kit	9
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	4
Roche Ventana iVIEW DAB Detection Kit	4
Roche Ventana ultraView Universal DAB Detection Kit	20
Thermo Fisher Scientific UltraVision Quanto Detection System HRP DAB	2
Total:	55

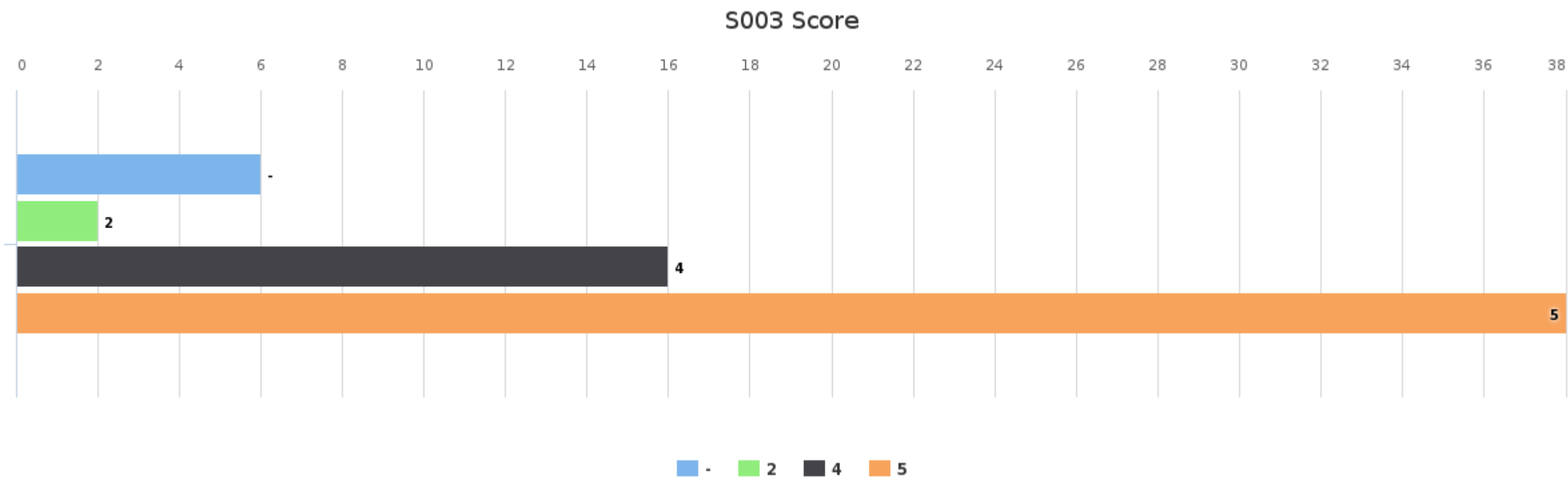


CHROMOGEN

Chromogen	Chromogen count
<input checked="" type="radio"/> Detection kit	55
Total:	55

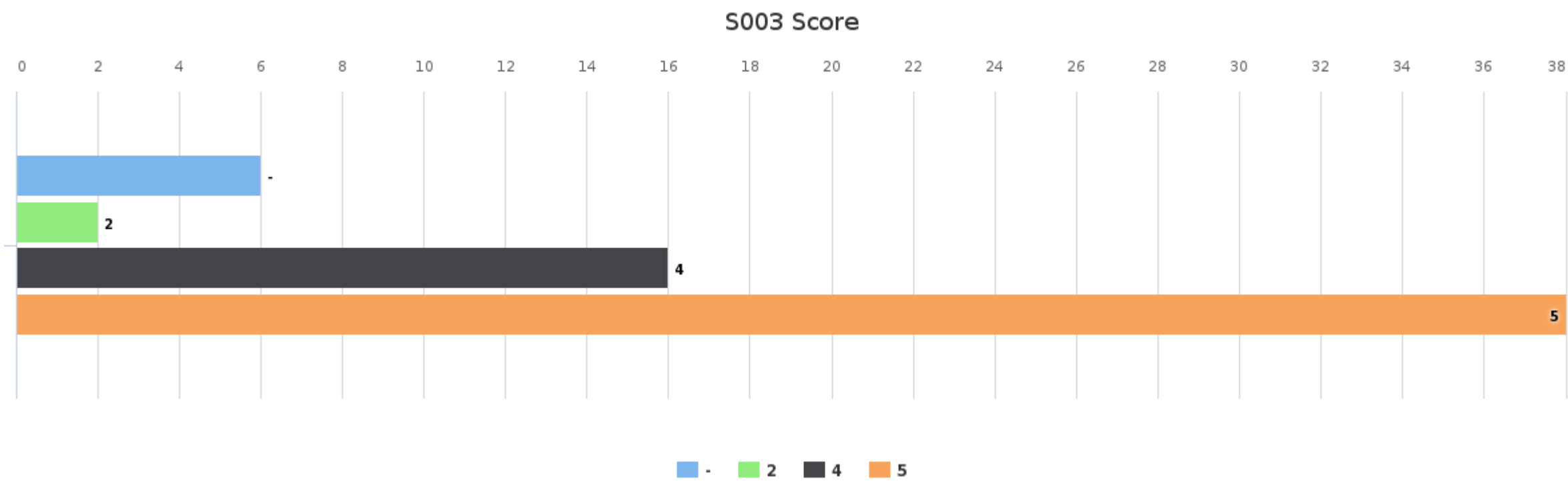


Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	1
<input checked="" type="radio"/> Agilent Dako EnVision FLEX DAB + Chromogen	11
Agilent Dako EnVision FLEX High pH HRP	2
Cell Marque DAB substrate Kit	1
Leica Biosystems Bond Polymer Refine Detection Kit	8
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	4
Roche Ventana iVIEW DAB Detection Kit	3
Roche Ventana ultraView Universal DAB Detection Kit	18
Thermo Fisher Scientific UltraVision Quanto DAB	2
Total:	51



SCORE

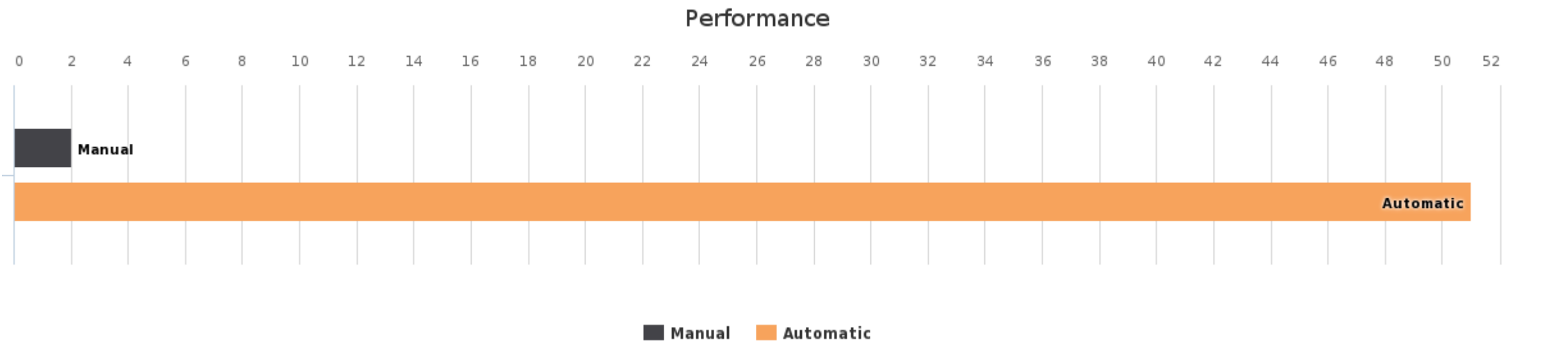
Score	Comment
-	
	Not available
2	
	Too strong hematoxylin, stains also cytoplasm. False negative staining in DLBCL. Uneaven staining.
	Too weak staining in the DLBCL, not optimal contrast between DAB and hematoxylin.
4	
	Contrast between hematoxylin and DAB is not optimal.
	Background staining/ unspecific staining of smooth muscle cells.
	Bakcground staining; smooth muscle cells
	Light background; smooth muscle cells of colon
	Slight background staining; smooth muscle cells.
	Slightly uneven staining
	Slightly weak, not continuous membrane staining, nuclear bubbling (usually due to too strong pretreatment).
	Some background staining of the colon epithelial cells and the smooth muscle cells.
	Staining gradient; technical issue.
	Strong staining, some background staining.
	Technical issue: Precipitates of hematoxylin and DAB
	Technical issue; hematoxylin and DAB precipitate
	Too strong hematoxylin; low contrast.
	Uneven staining
	Uneven staining, not optimal contrast, too strong hematoxylin.
	Unspecific staining of the colon; smooth muscle cells and laminull propria leukocytes, epithelial cells
<input checked="" type="radio"/> 5	
	<input checked="" type="radio"/> Excellent
Total:	



Score	Score count
-	6
2	2
4	16

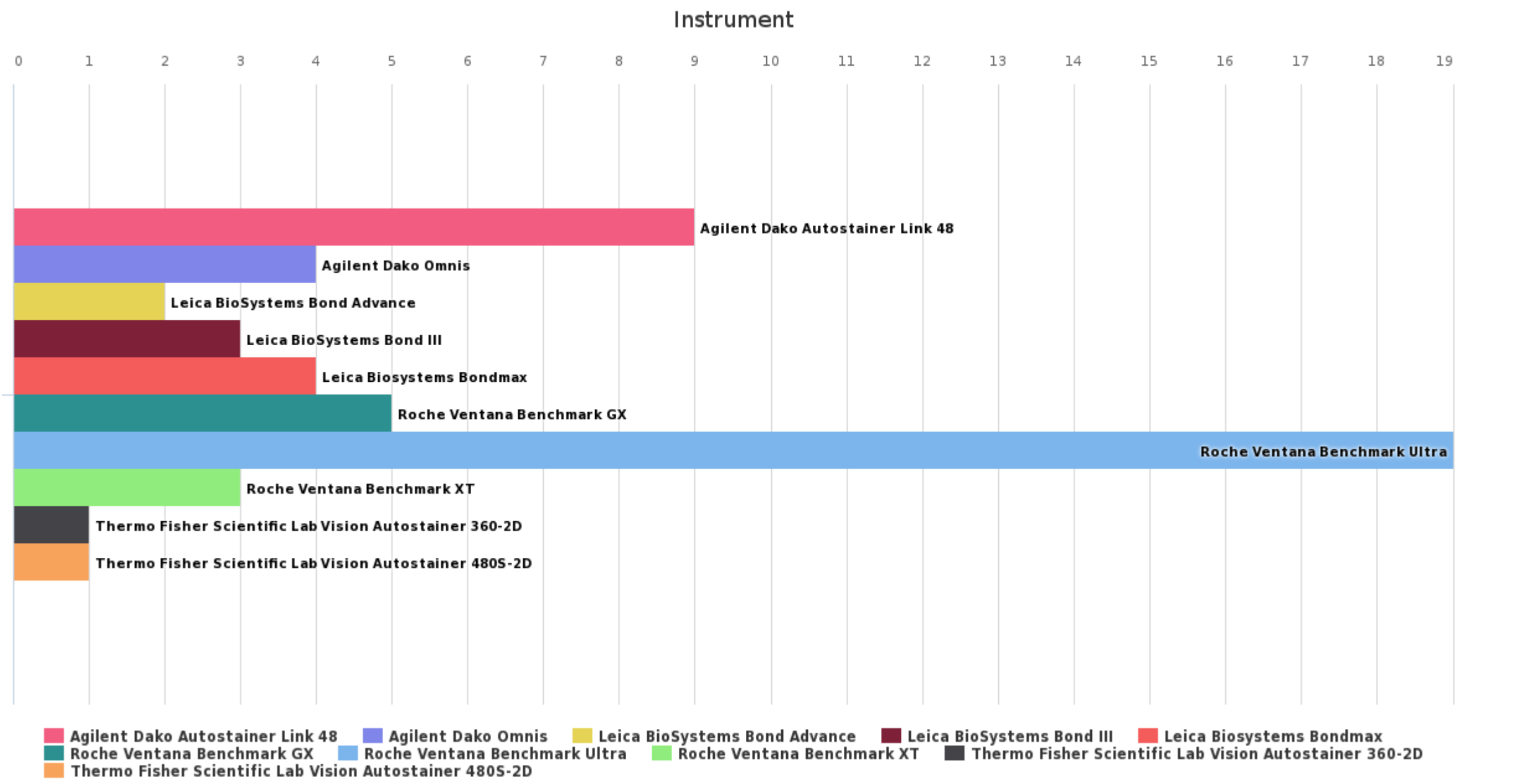
<input checked="" type="radio"/> 5	38
Total:	62

S004 | Sample S004 CD30

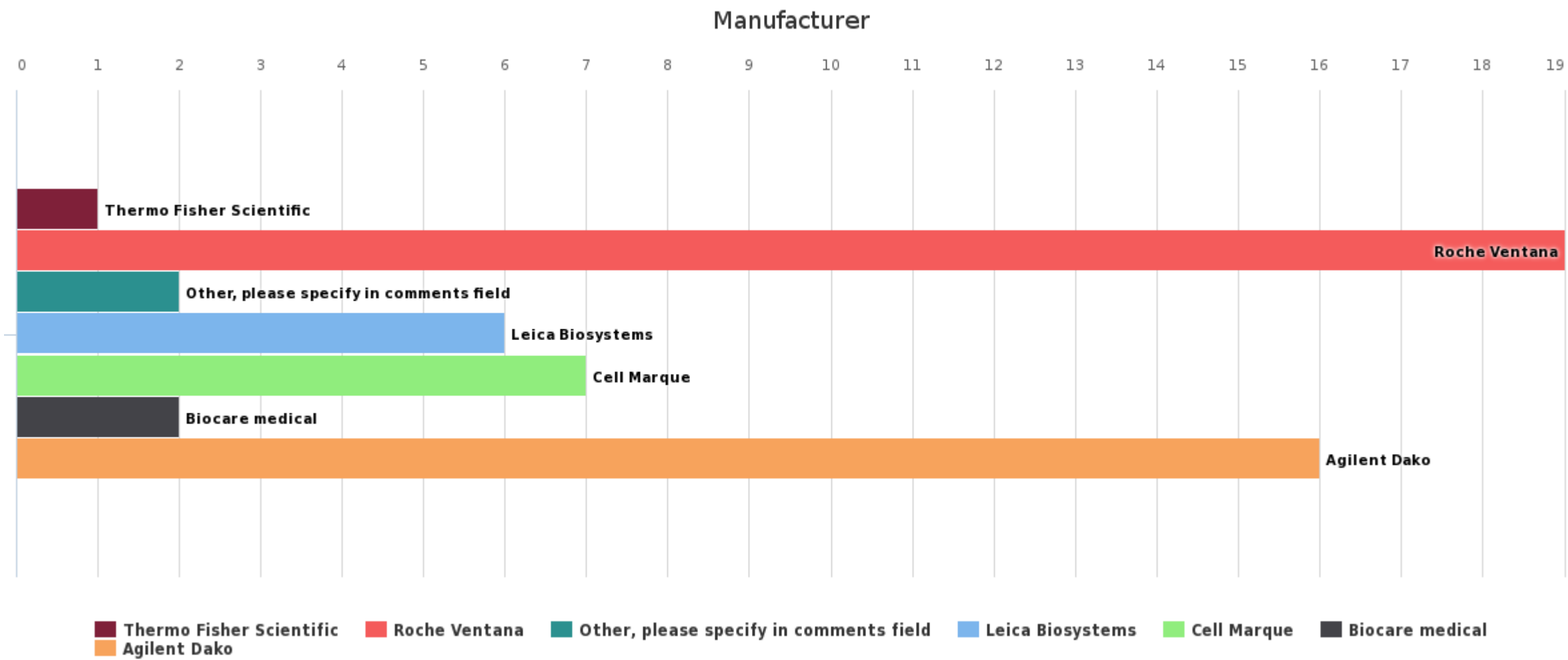


GENERAL DETAILS OF STAINING

Performance	Performance count
Automatic	51
Manual	2
Total:	53

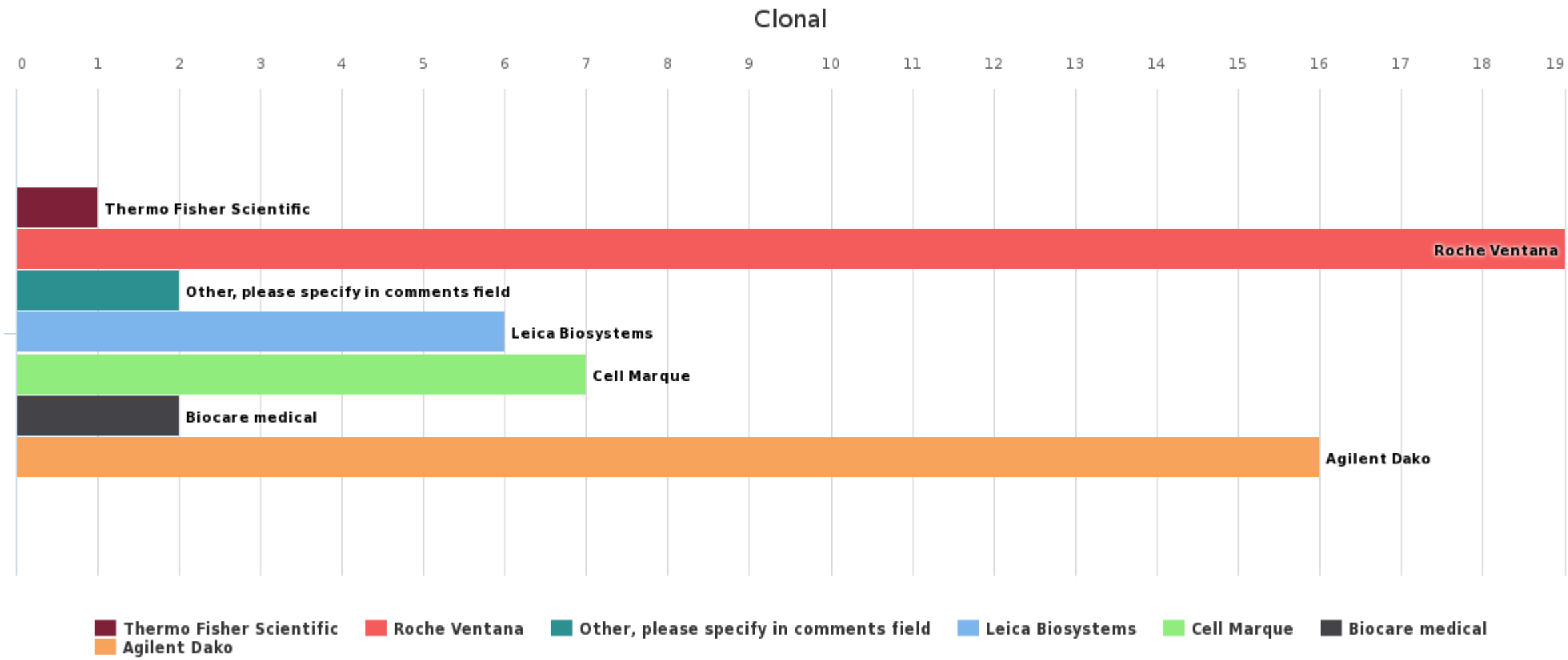


Instrument	Instrument count
Agilent Dako Autostainer Link 48	9
Agilent Dako Omnis	4
Leica BioSystems Bond Advance	2
Leica BioSystems Bond III	3
Leica Biosystems Bondmax	4
Roche Ventana Benchmark GX	5
Roche Ventana Benchmark Ultra	19
Roche Ventana Benchmark XT	3
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Total:	51

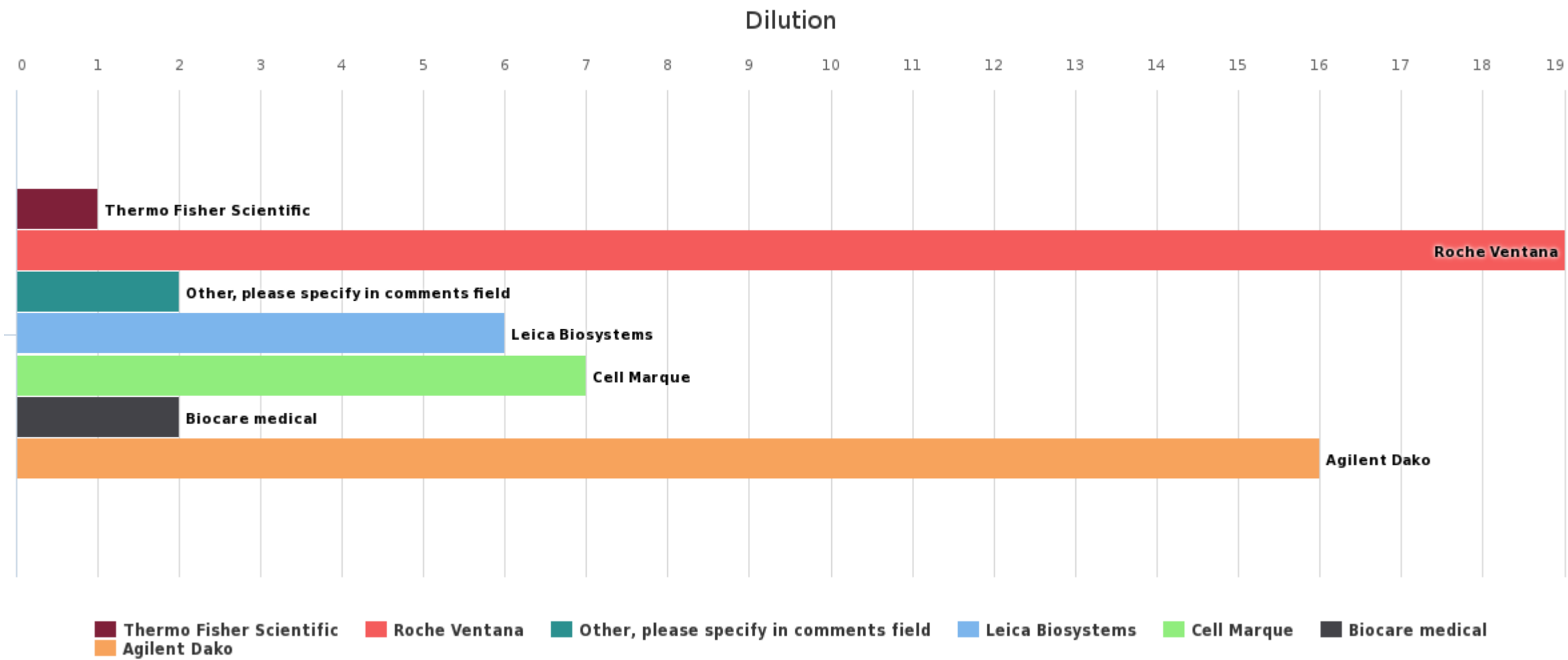


PRIMARY ANTIBODY

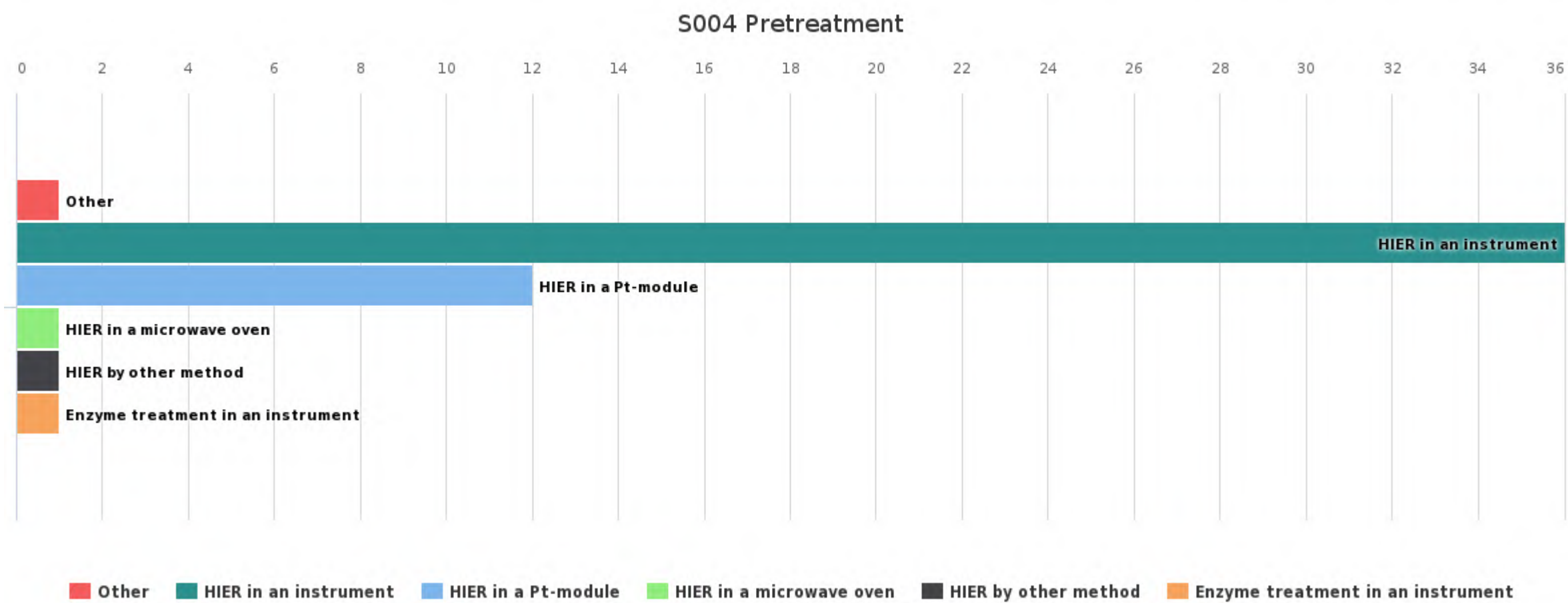
Manufacturer	Manufacturer count
Agilent Dako	16
Biocare medical	2
Cell Marque	7
Leica Biosystems	6
Other, please specify in comments field	2
Roche Ventana	19
Thermo Fisher Scientific	1
Total:	53



Clonal	Clonal count
Monoclonal	53
Total:	53

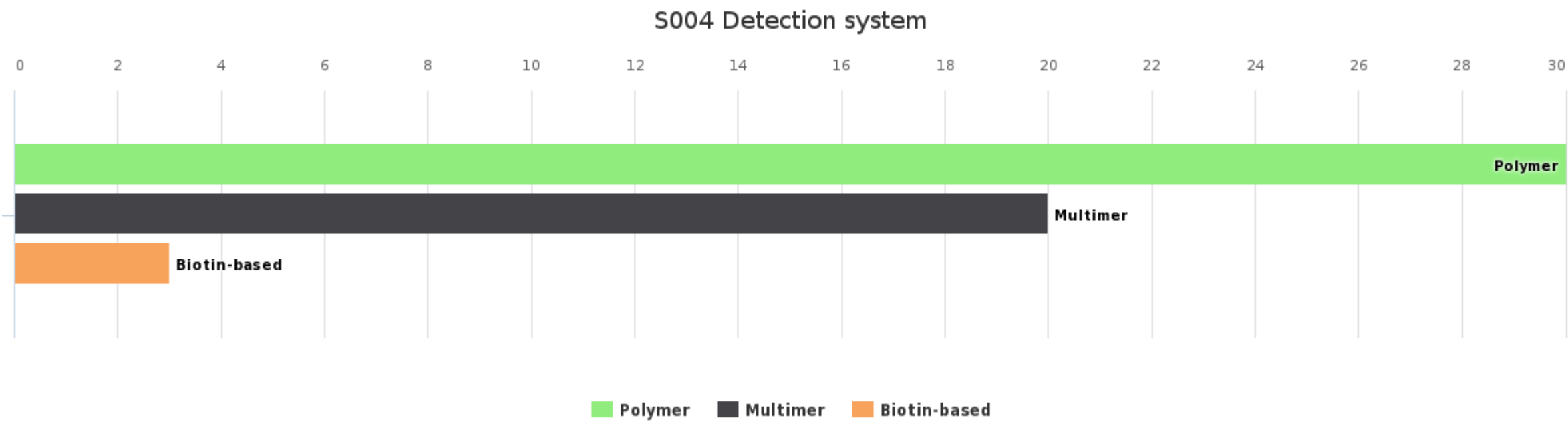


Dilution	Dilution count
Dilution	18
Ready-to-use	35
Total:	53



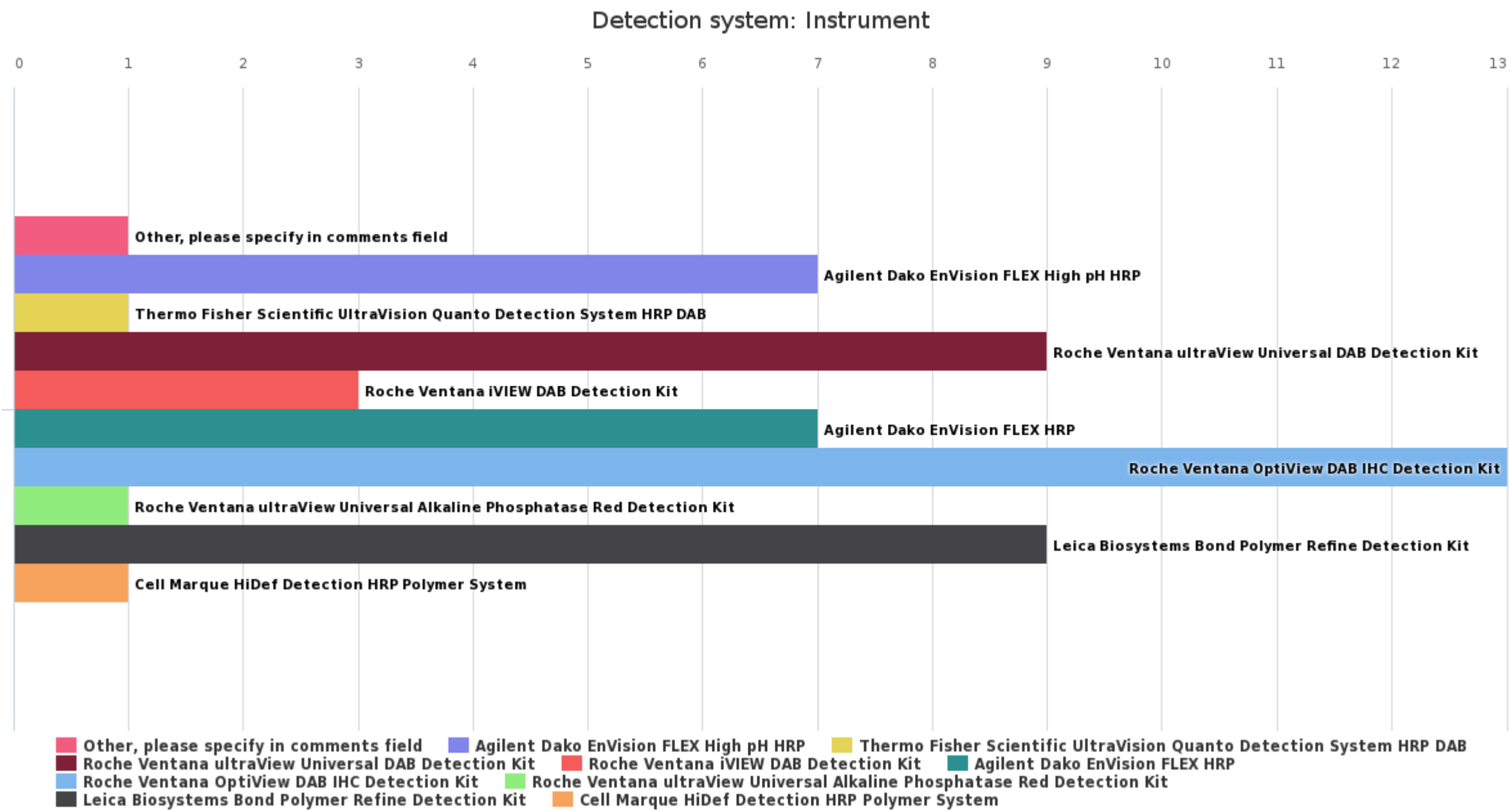
PRETREATMENT

Pretreatment	Pretreatment count
Enzyme treatment in an instrument	1
HIER by other method	1
HIER in a microwave oven	1
HIER in a Pt-module	12
HIER in an instrument	36
Other	1
Total:	52

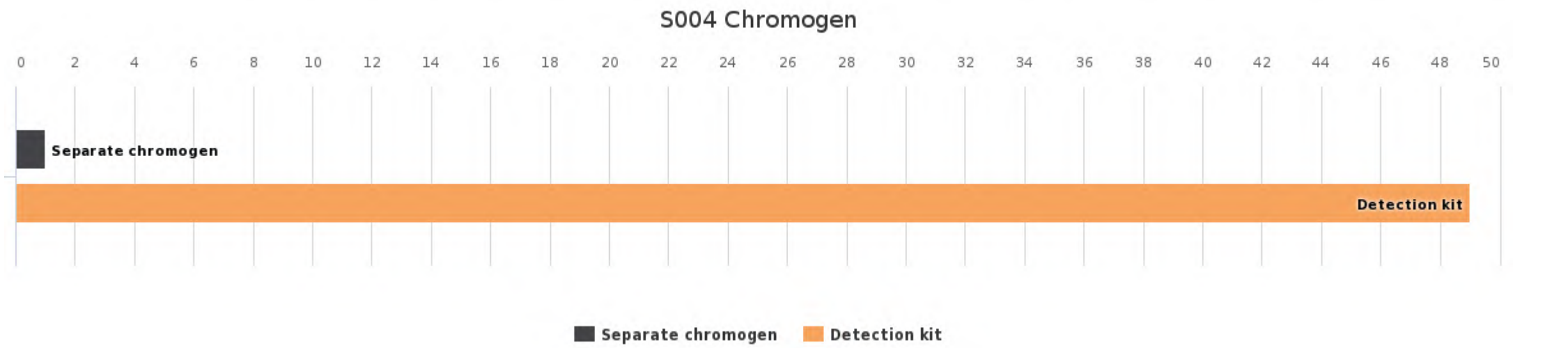


DETECTION SYSTEM

Detection system	Detection system count
Biotin-based	3
Multimer	20
Polymer	30
Total:	53

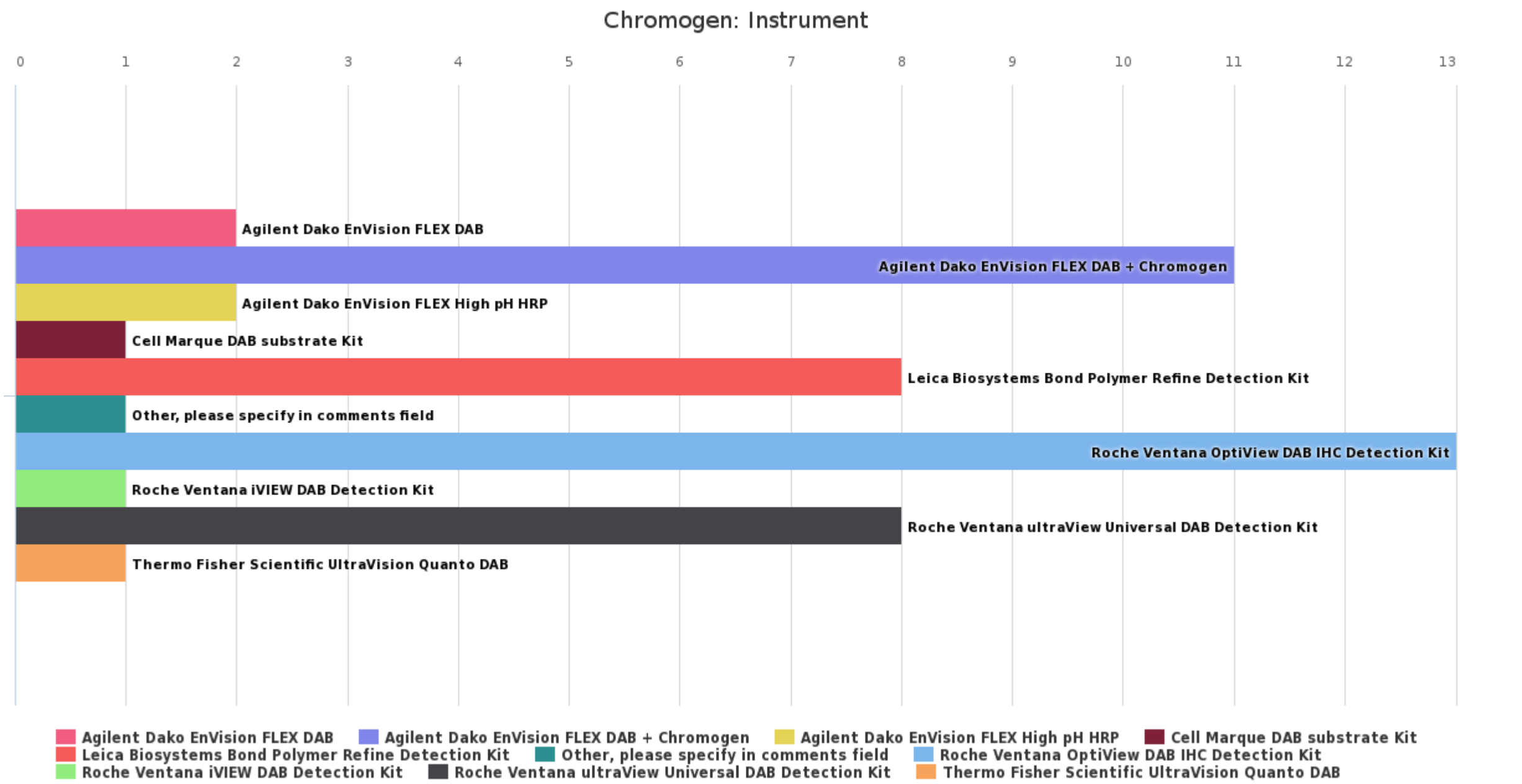


Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	7
Agilent Dako EnVision FLEX High pH HRP	7
Cell Marque HiDef Detection HRP Polymer System	1
Leica Biosystems Bond Polymer Refine Detection Kit	9
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	13
Roche Ventana iVIEW DAB Detection Kit	3
Roche Ventana ultraView Universal Alkaline Phosphatase Red Detection Kit	1
Roche Ventana ultraView Universal DAB Detection Kit	9
Thermo Fisher Scientific UltraVision Quanto Detection System HRP DAB	1
Total:	52

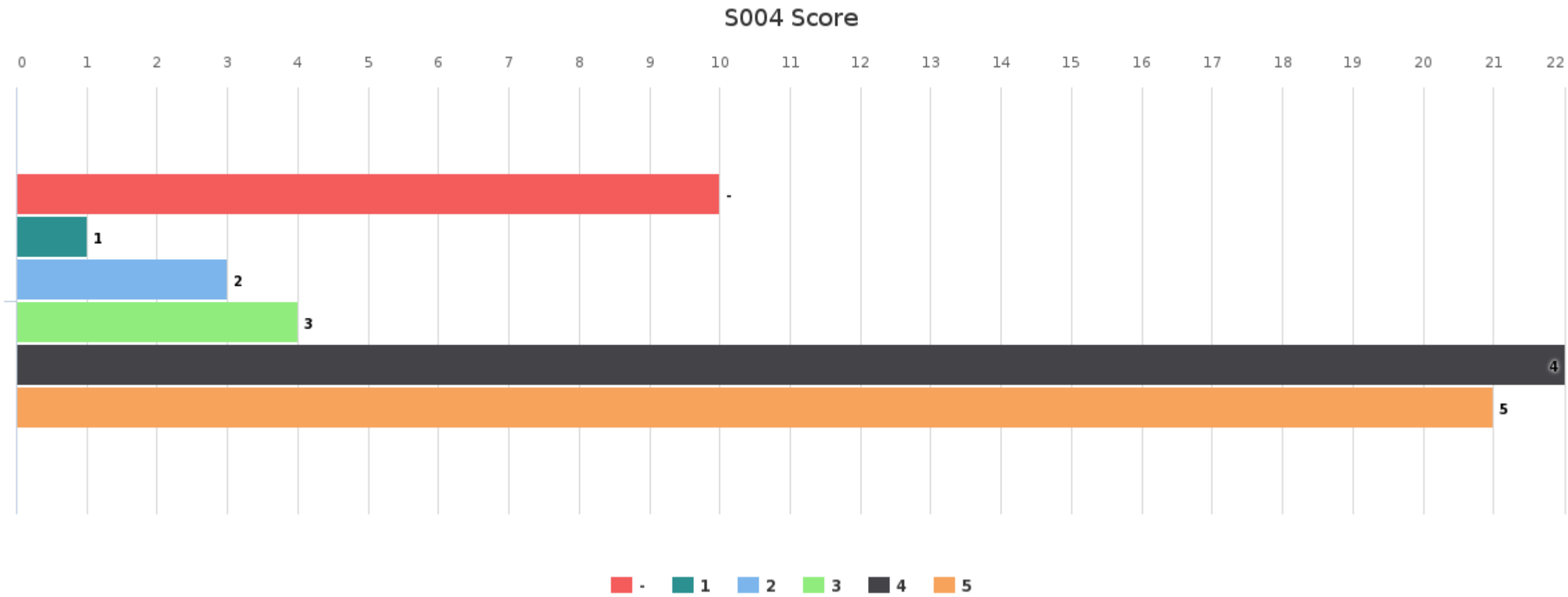


CHROMOGEN

Chromogen	Chromogen count
Detection kit	49
Separate chromogen	1
Total:	50

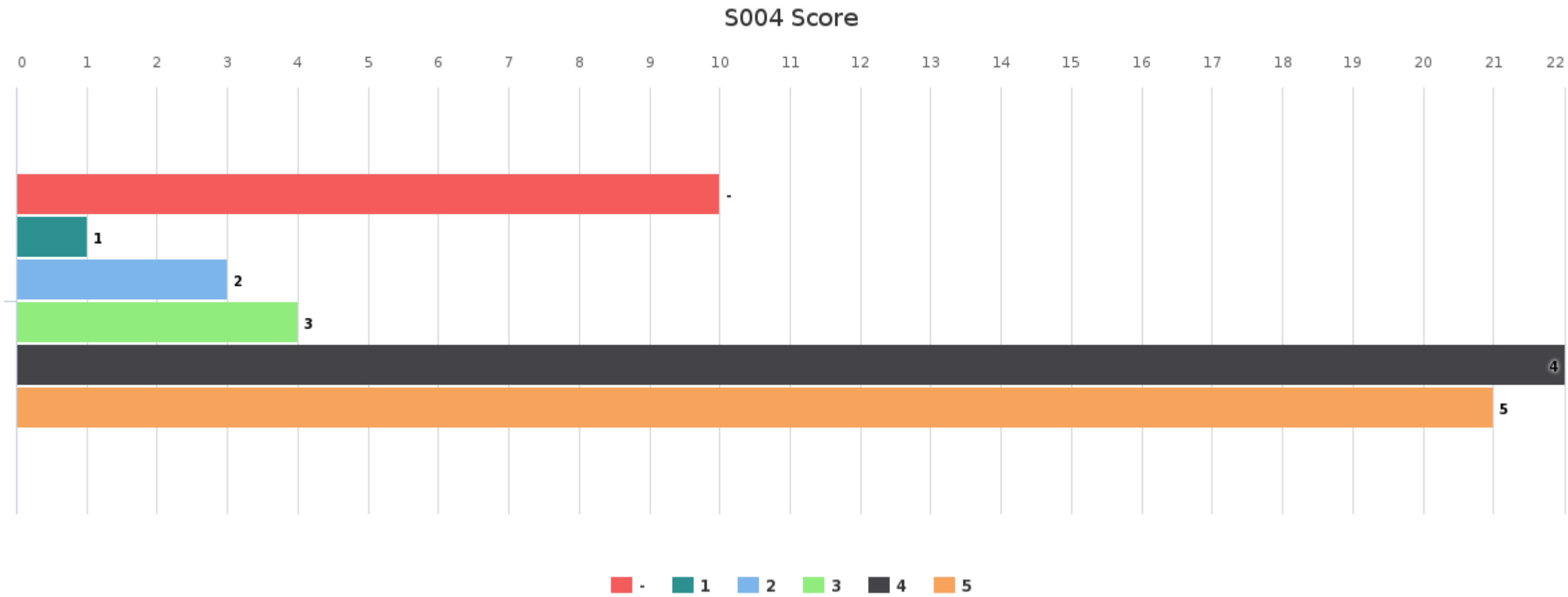


Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	2
Agilent Dako EnVision FLEX DAB + Chromogen	11
Agilent Dako EnVision FLEX High pH HRP	2
Cell Marque DAB substrate Kit	1
Leica Biosystems Bond Polymer Refine Detection Kit	8
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	13
Roche Ventana iVIEW DAB Detection Kit	1
Roche Ventana ultraView Universal DAB Detection Kit	8
Thermo Fisher Scientific UltraVision Quanto DAB	1
Total:	48



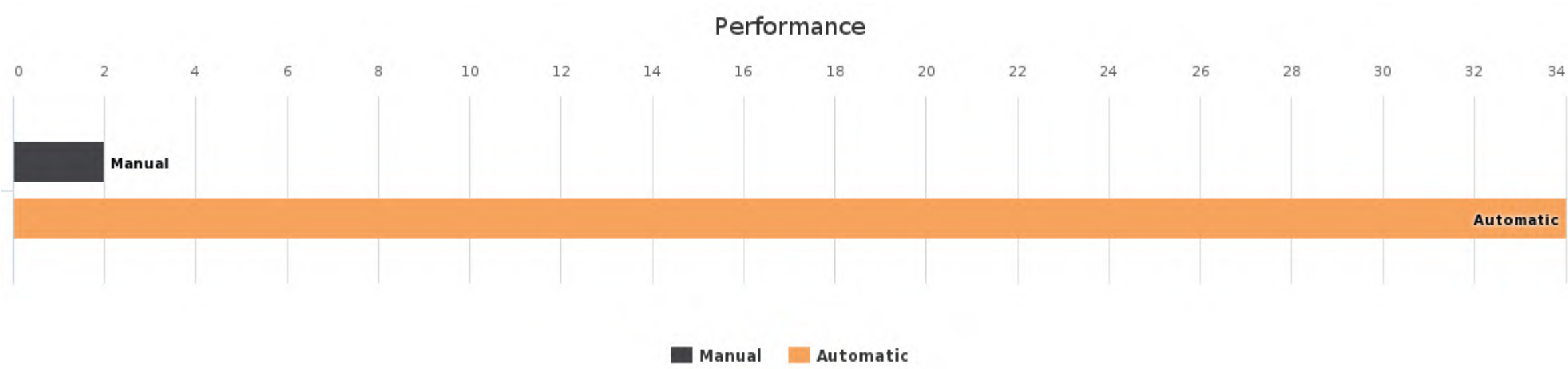
SCORE

Score	Comment
<input checked="" type="radio"/> -	
	<input type="radio"/> Not available
1	
	No activated T- and B-cells are stained in tonsil, hardly any of the RS cells are stained in the Hodgking's lymphoma, very weak staining in theembryonullll ca.
2	
	Too weak in all tissues
	Too weak in all tissues; only scattered RS cells are stained faintly.
	Too weak staining; emryonullll ca
3	
	Background staining, slightly too weakstaining in all tissues
	Strong background staining.
	Too weak staining in all tissues
	Too weak staining of the RS cells, activated B and T cells and embryo carcinoma.
4	
	Background staining in the tonsill; epithelial cells and vessels
	Light background due to RED chromogen
	Light background staining
	Slightly weak staining of activated T- and B-cells in the tonsil.
	Slight background staining
	Slight background staining in the tonsil
	Slight background staining, slightly weak staining in all tissues, not all activated T- and B-cells are stained in the tonsil.
	Slight background staining; Tonsil.
	Slightly too weak staining.
	Slightly weak
	Slightly weak staining in all tissues
	Slightly weak staining especially in the tonsil.
	Slightly weak staining in all tissues.
	Slightly weak staining of activated T- and B-cells in tonsil, nuclear bubbling.
	Slightly weak staining of the activated T- and B-cells in tonsil, nuclear bubbling.
	Slightly weak staining of theembryonullll ca, slight background.
	Slightly weak staining.
	Slightly weak staining in all tissues.
	Technical issue; hematoxylin and DAB precipitate
	Too strong hematoxylin, Slight background.
5	
	Excellent
Total:	



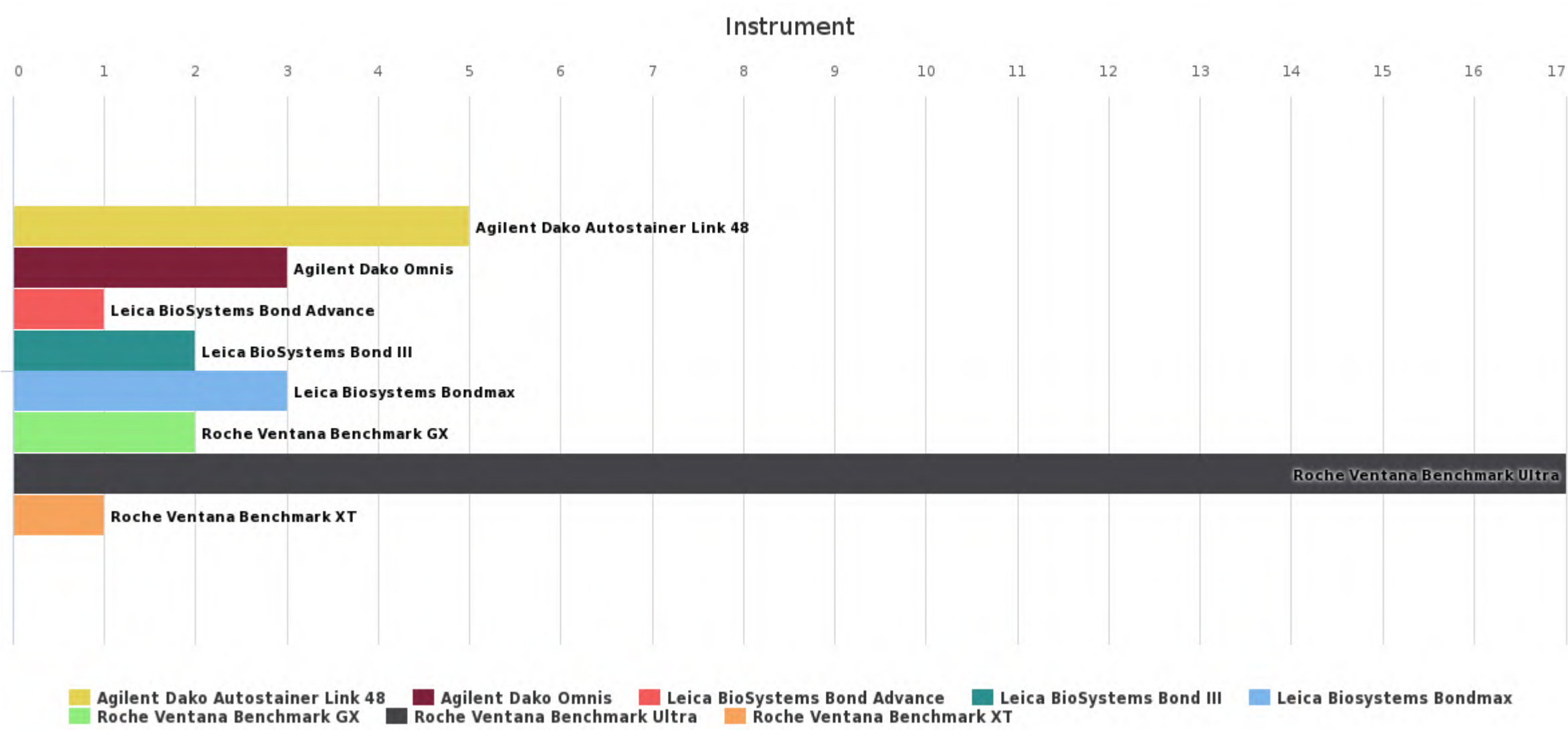
Score	Score count
<input checked="" type="radio"/> -	10
<input type="radio"/> 1	1
<input type="radio"/> 2	3
<input type="radio"/> 3	4
<input type="radio"/> 4	22
<input type="radio"/> 5	21
Total:	61

S005 | Sample S005 PAX5

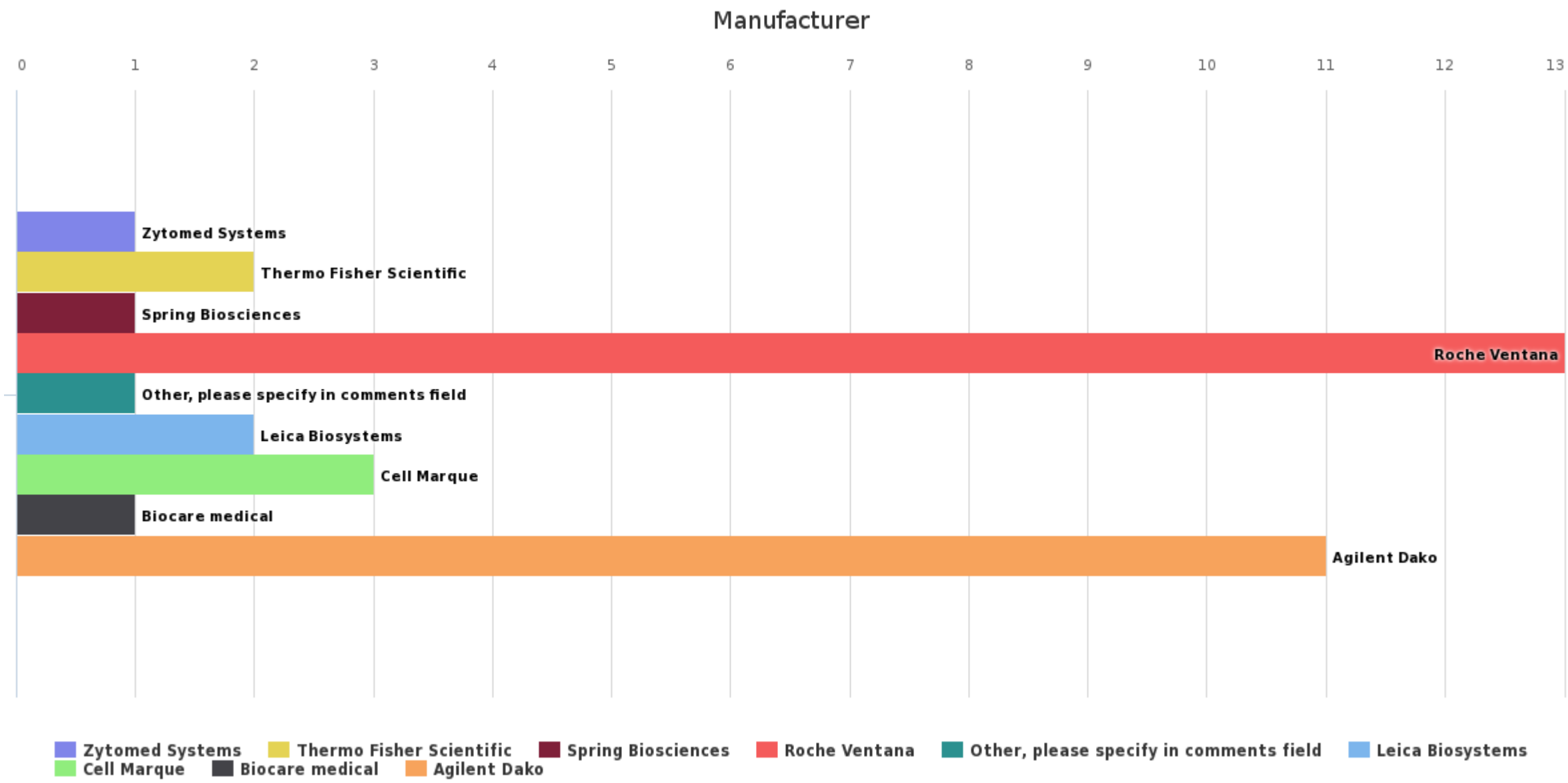


GENERAL DETAILS OF STAINING

Performance	Performance count
<input checked="" type="radio"/> Automatic	34
Manual	2
Total:	36

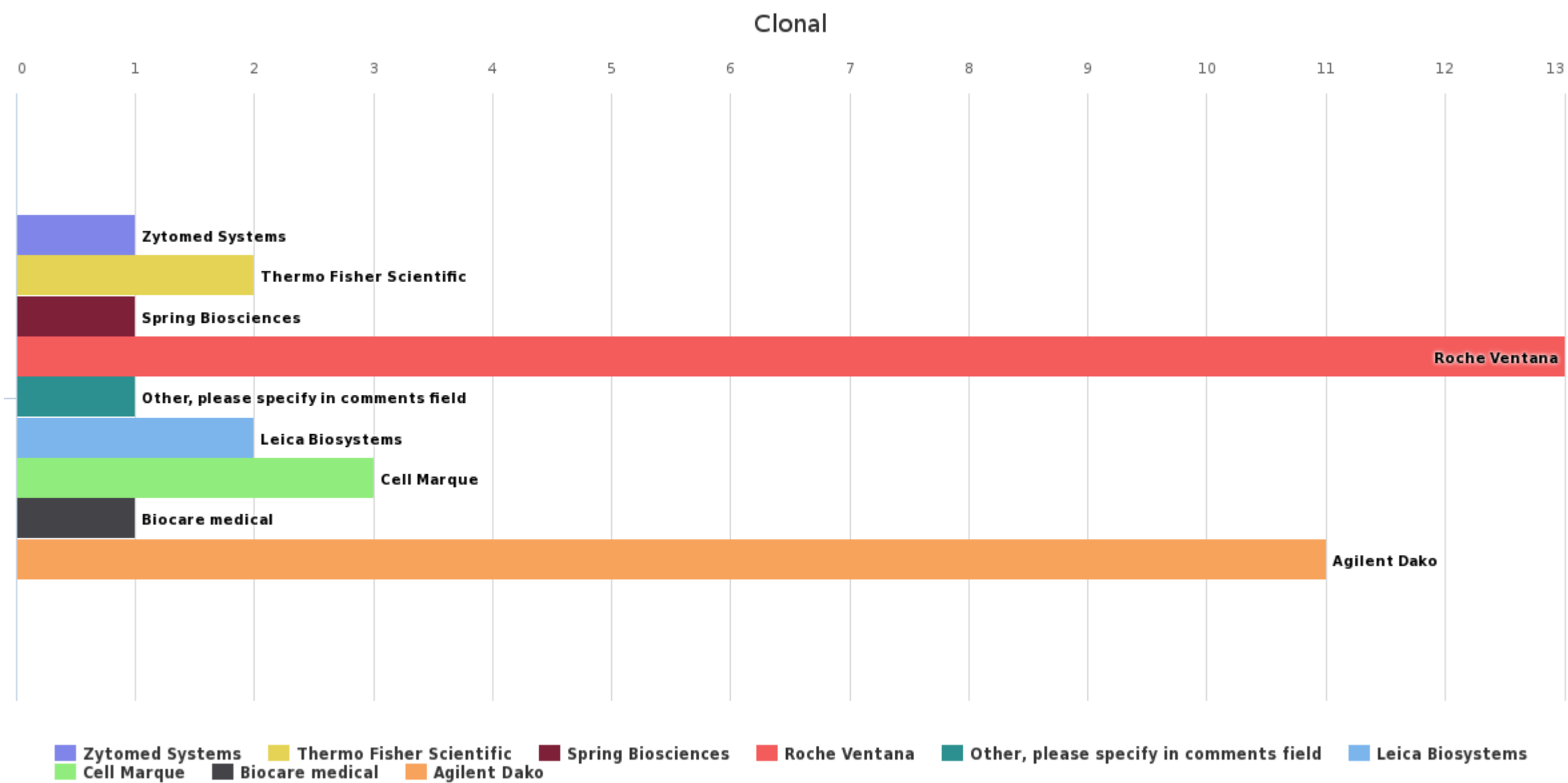


Instrument	Instrument count
<input checked="" type="radio"/> Agilent Dako Autostainer Link 48	5
Agilent Dako Omnis	3
Leica BioSystems Bond Advance	1
Leica BioSystems Bond III	2
Leica Biosystems Bondmax	3
Roche Ventana Benchmark GX	2
Roche Ventana Benchmark Ultra	17
Roche Ventana Benchmark XT	1
Total:	34

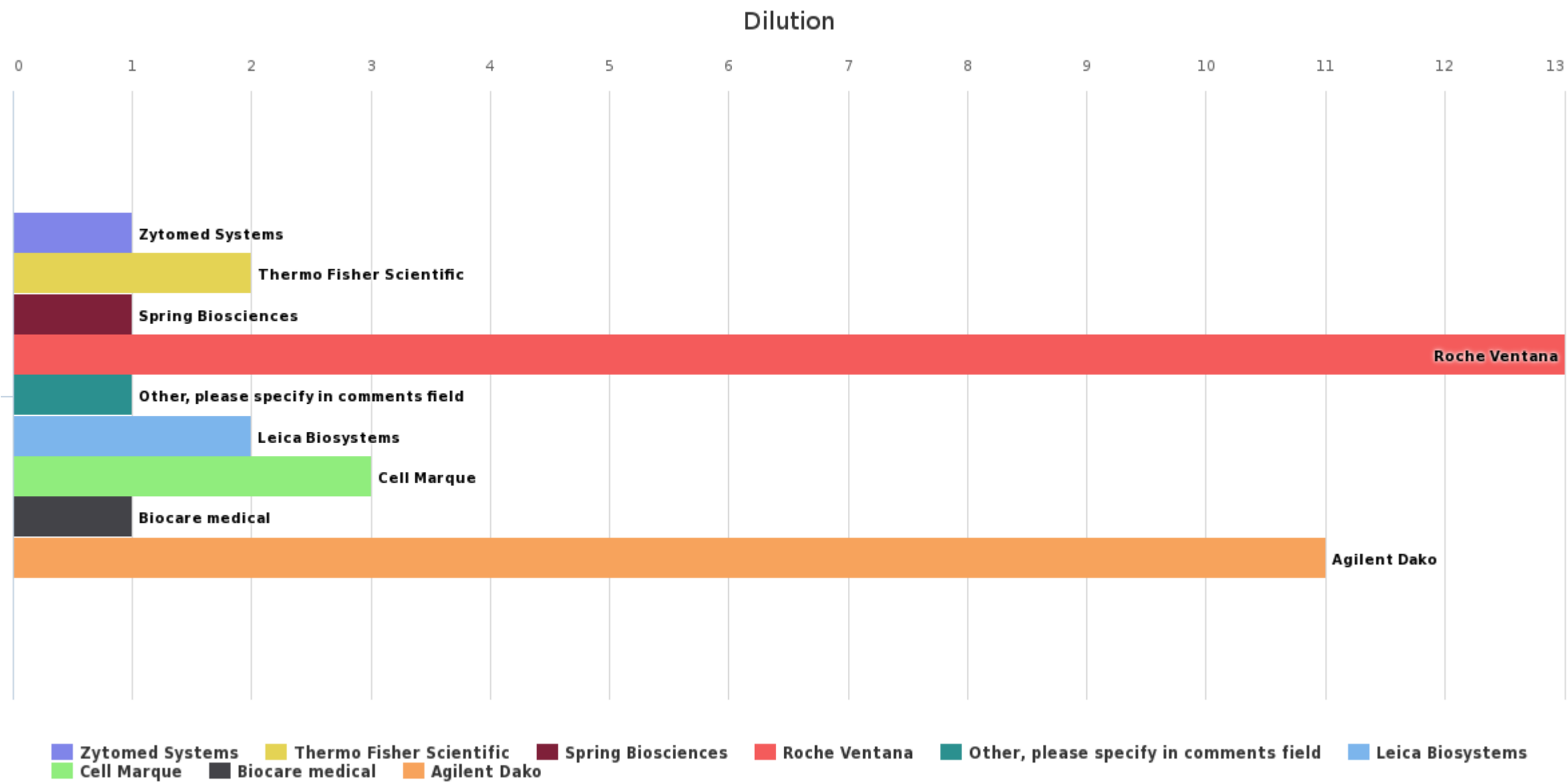


PRIMARY ANTIBODY

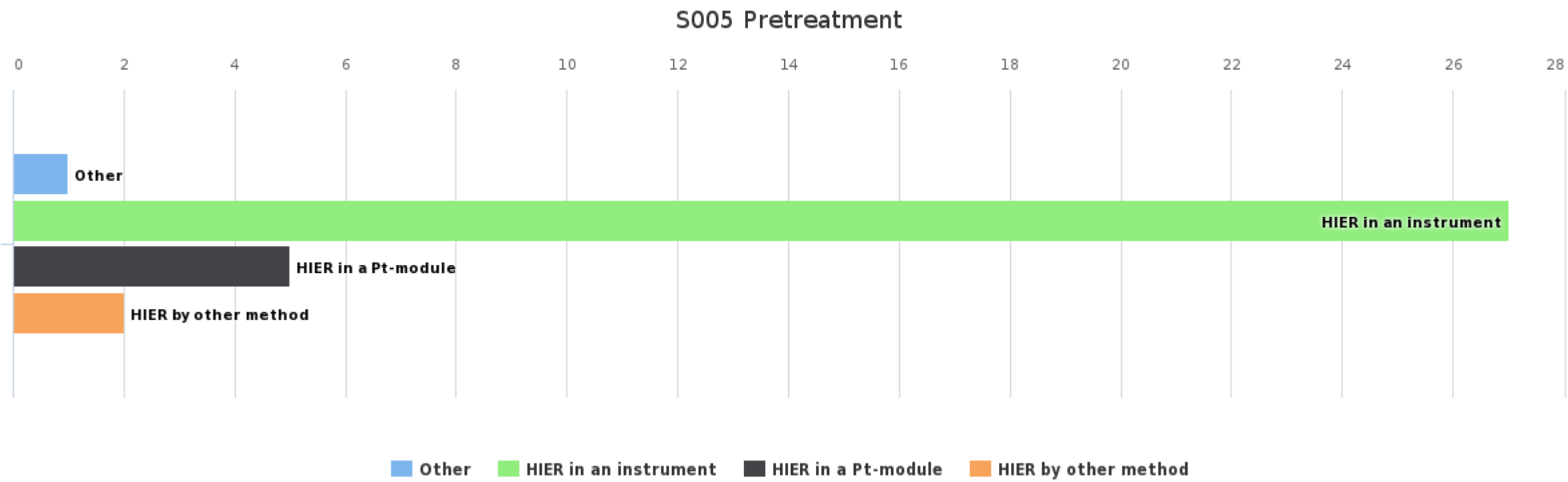
Manufacturer	Manufacturer count
<input checked="" type="radio"/> Agilent Dako	11
Biocare medical	1
Cell Marque	3
Leica Biosystems	2
Other, please specify in comments field	1
Roche Ventana	13
Spring Biosciences	1
Thermo Fisher Scientific	2
Zytomed Systems	1
Total:	35



Clonal	Clonal count
<input checked="" type="radio"/> Monoclonal	32
Polyclonal	3
Total:	35

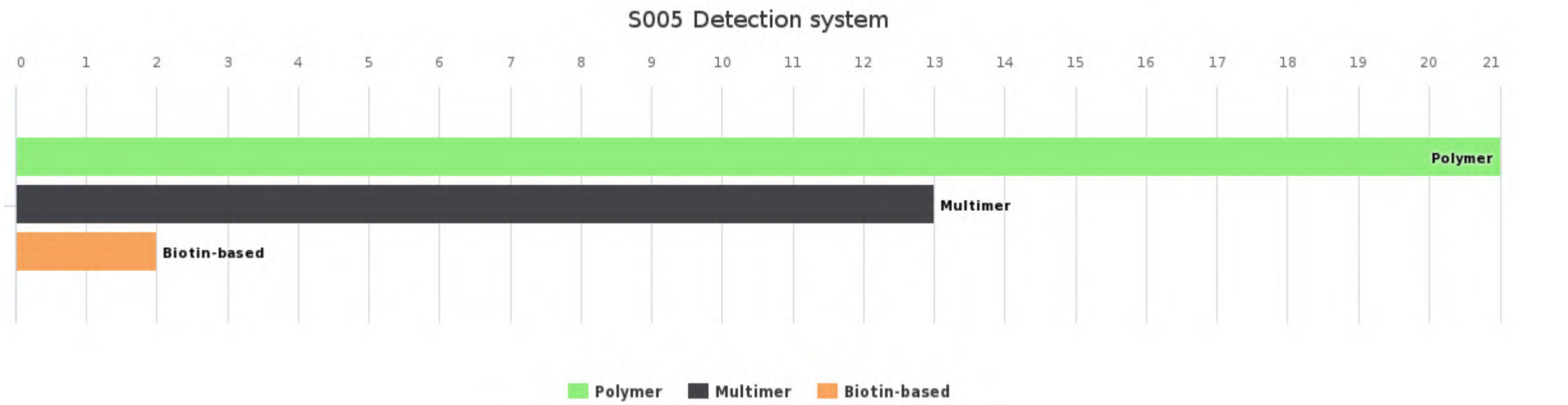


Dilution	Dilution count
Dilution	11
<input checked="" type="radio"/> Ready-to-use	24
Total:	35



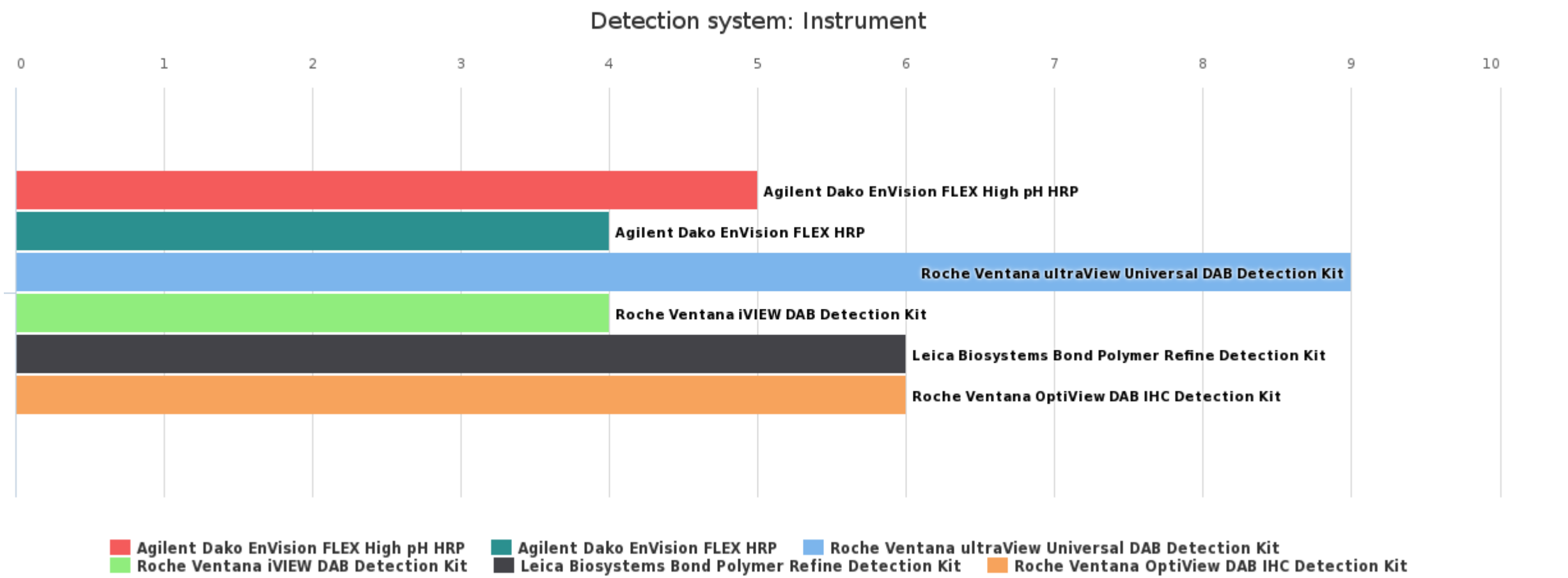
PRETREATMENT

Pretreatment	Pretreatment count
HIER by other method	2
HIER in a Pt-module	5
<input checked="" type="radio"/> HIER in an instrument	27
Other	1
Total:	35

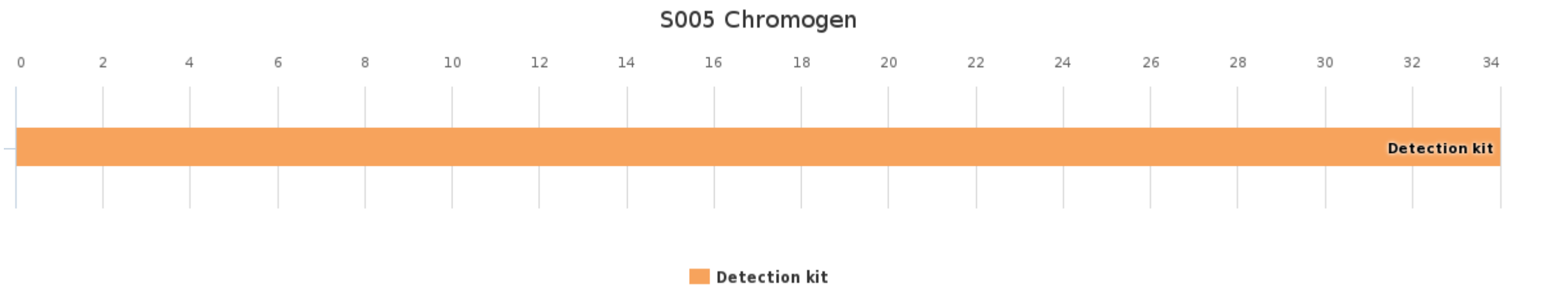


DETECTION SYSTEM

Detection system	Detection system count
Biotin-based	2
Multimer	13
<input checked="" type="radio"/> Polymer	21
Total:	36



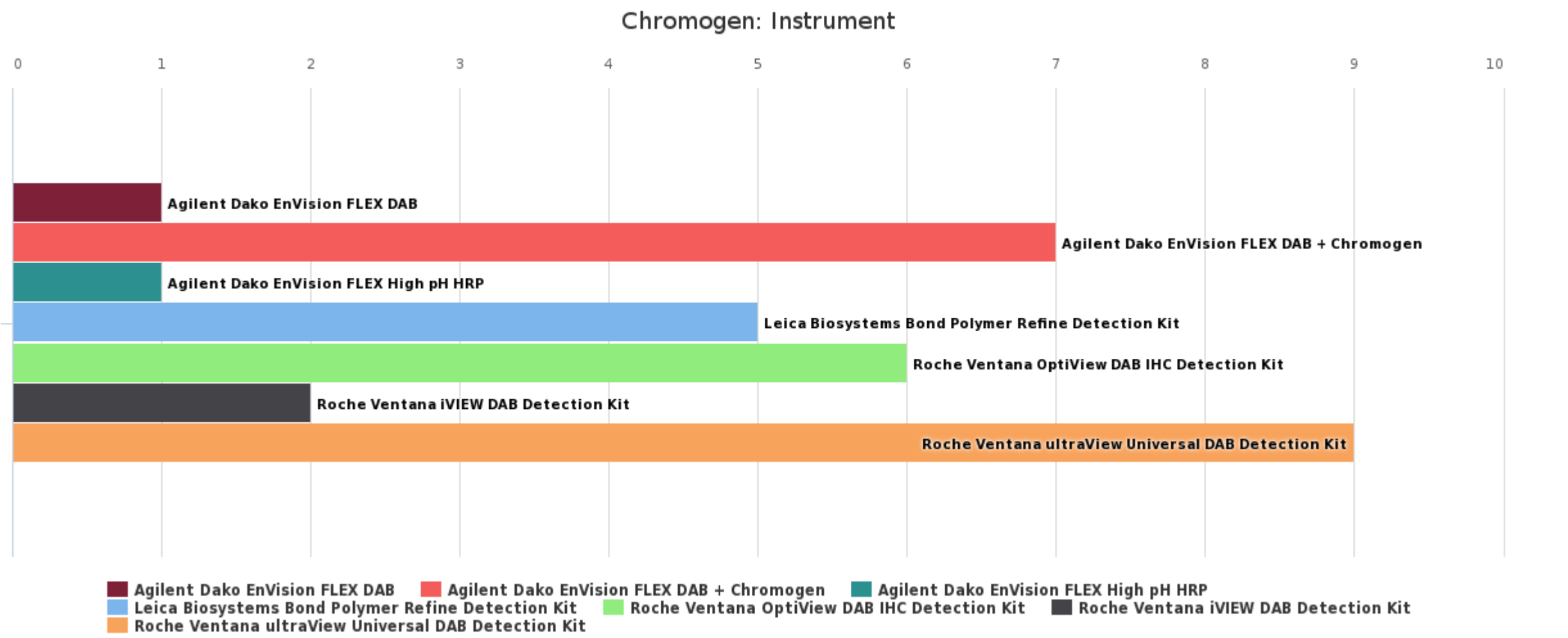
Detection system: Instrument	Detection system: Instrument count
<input checked="" type="radio"/> Agilent Dako EnVision FLEX HRP	4
Agilent Dako EnVision FLEX High pH HRP	5
Leica Biosystems Bond Polymer Refine Detection Kit	6
Roche Ventana OptiView DAB IHC Detection Kit	6
Roche Ventana iVIEW DAB Detection Kit	4
Roche Ventana ultraView Universal DAB Detection Kit	9
Total:	34



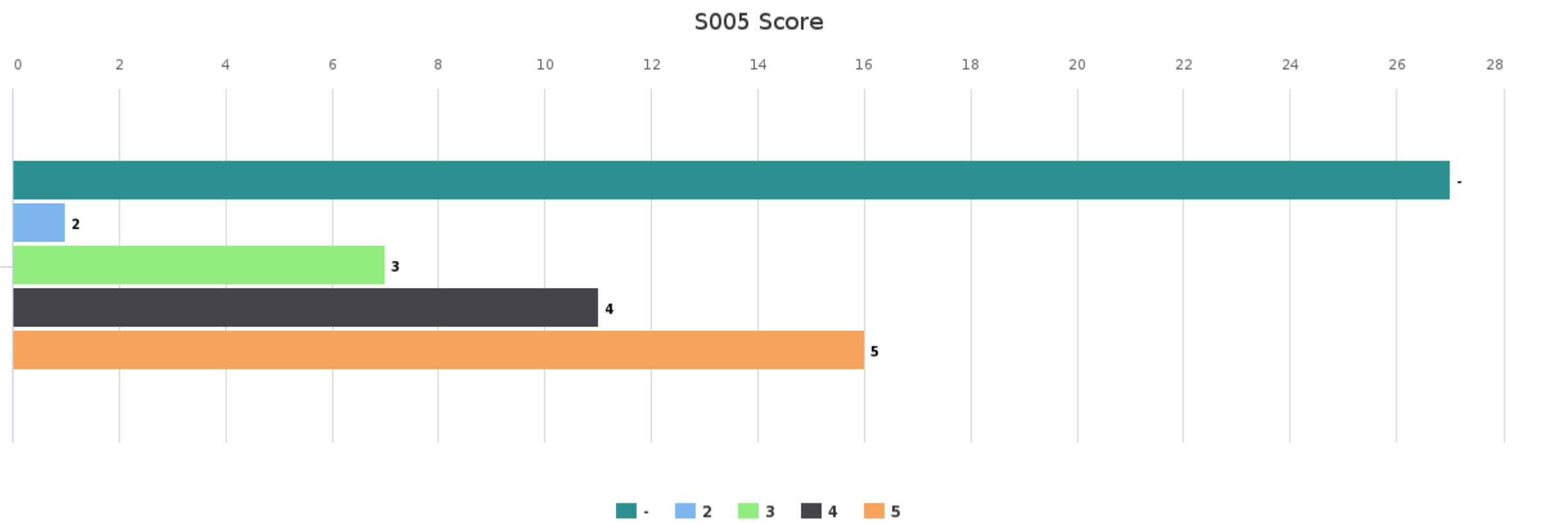
CHROMOGEN

Chromogen	Chromogen count
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<input checked="" type="radio"/> Detection kit	34
Total:	34



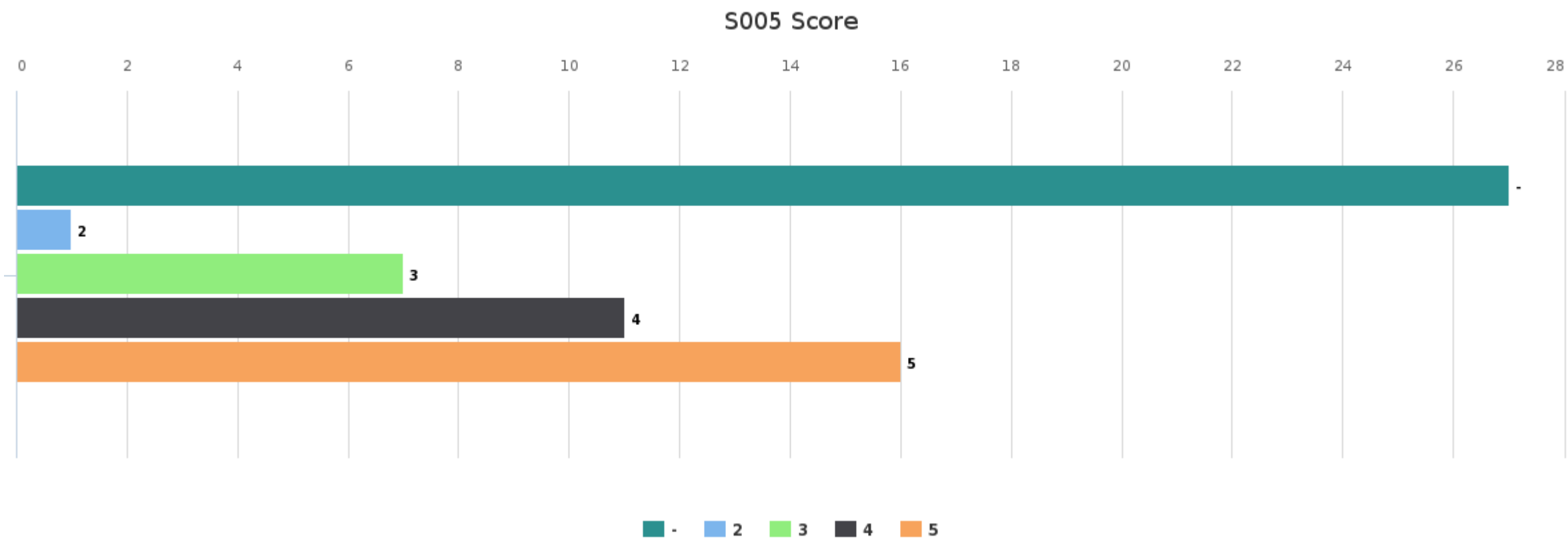
Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	1
<input checked="" type="radio"/> Agilent Dako EnVision FLEX DAB + Chromogen	7
Agilent Dako EnVision FLEX High pH HRP	1
Leica Biosystems Bond Polymer Refine Detection Kit	5
Roche Ventana OptiView DAB IHC Detection Kit	6
Roche Ventana iVIEW DAB Detection Kit	2
Roche Ventana ultraView Universal DAB Detection Kit	9
Total:	31



SCORE

Score	Comment
-	Not available
2	No staining in the RS cells of Hodgking's lymphoma. Weak staining in the tonsil. Background staining on all tissues.
3	Good staining, but nuclear morphology has suffered (nuclear bubbling and shrinking) Too weak staining of the RS-cells Too weakly stained RS-cells. Unspecific cytoplasmic staining; endothelial cells and macrophage positivity

	Unspecific cytoplasmic staining; endothelial positivity
	Unspecific staining of endothelial cells and macrophages. Weak staining of RS cells in Hodgkins lymphoma.
	Weak HTX staining. Slightly weak staining of the RS cells. Unspecific cytoplasmic staining and staining of endothelial cells. Nuclear shrinking due to pretreatment.
<input checked="" type="radio"/> 4	
	Background staining; unspecific staining of macrophages and endothelial cells.
	<input checked="" type="radio"/> Nuclear bubbling. Ususally due to too strong pretreatment.
	Nuclear shrinking (drying after pretreatment or too strong pretreatment).
	Slight background staining; endothelial cells and macrophages
	Slightly weak
	Slightly weak staining in RS-cells
	Slightly weak staining of the RS cells, unspecific staining of macrophages
	Slightly weak staining of the RS cells.
	Slightly weak staining of the RS-cells and unspecific cytoplasmic staining of endothelial cells.
	Slightly weak staining.
	Too weak stainig of the RS cells, unspecific background staining in the endotheal cells and macrophages
5	
	Excellent
Total:	

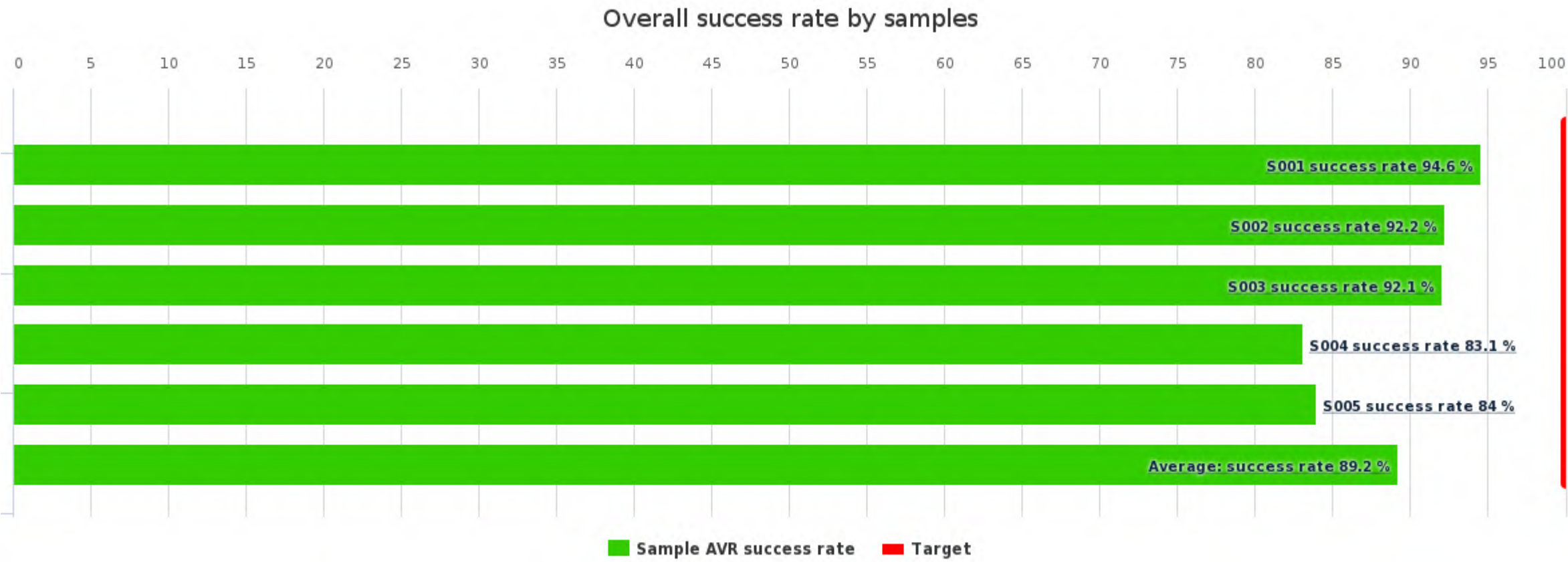


Score	Score count
-	27
2	1
3	7
<input checked="" type="radio"/> 4	11
5	16
Total:	62

GLOBAL REPORT

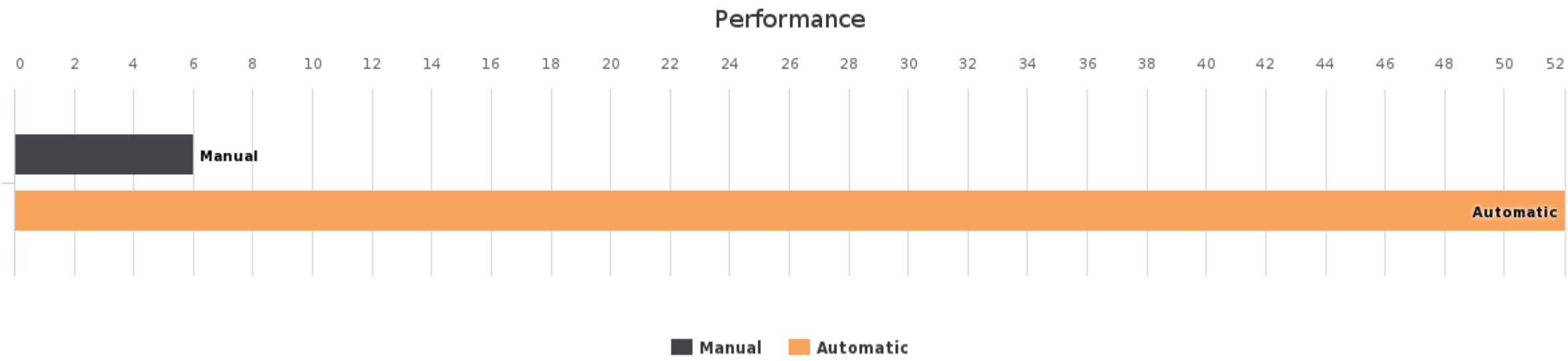
	No of participants	No of responded participants	Response percentage
Immunohistochemical staining methods, limited selecton of antibodies, March, 1-2020	17	17	100 %
Immunohistochemical staining methods, March, 1-2020	45	45	100 %

Summary



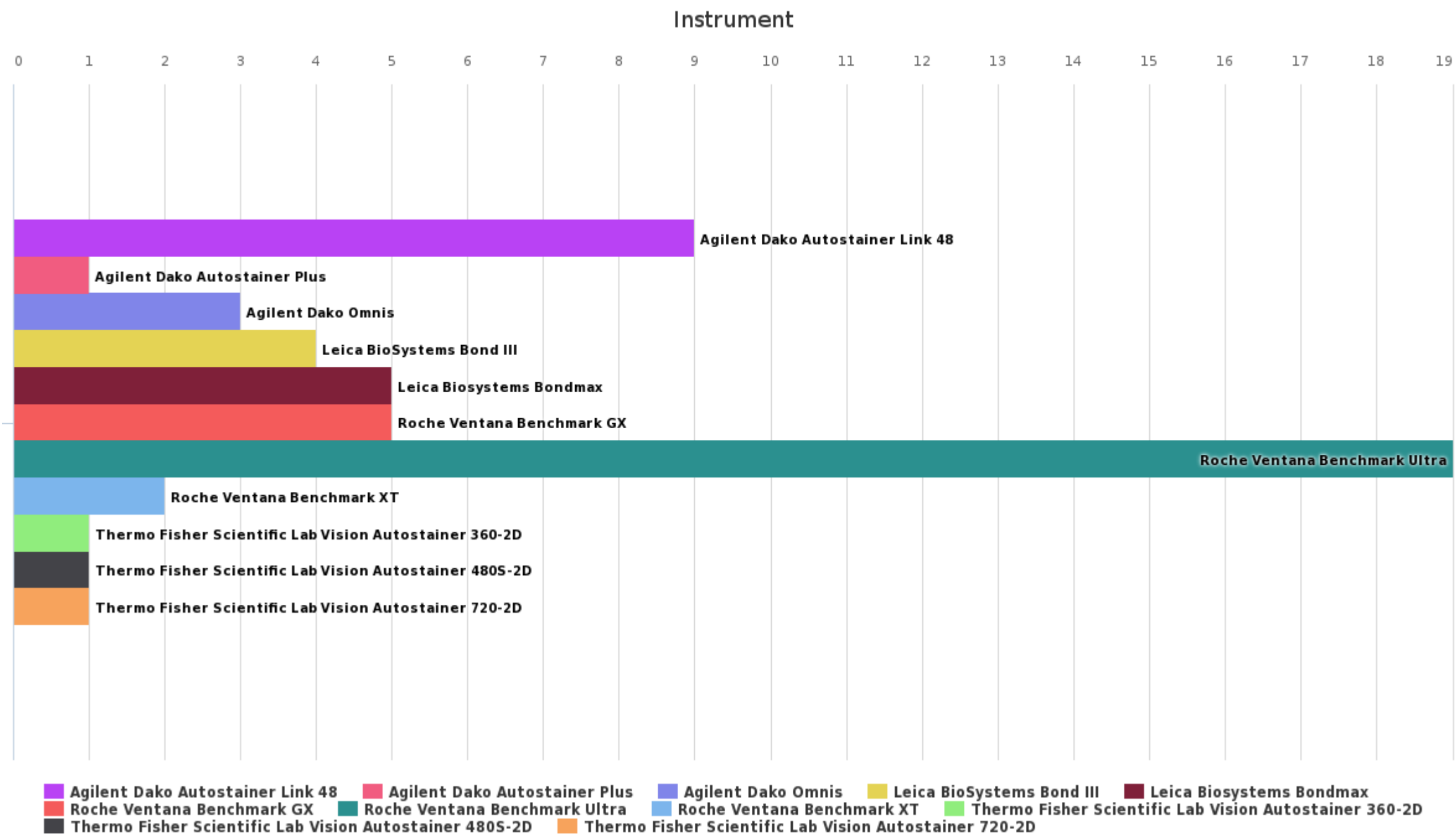
Summary	AVR success rate
S001	94.6 %
S002	92.2 %
S003	92.1 %
S004	83.1 %
S005	84 %
Average:	89.2 %

S001 | Sample S001 CD3

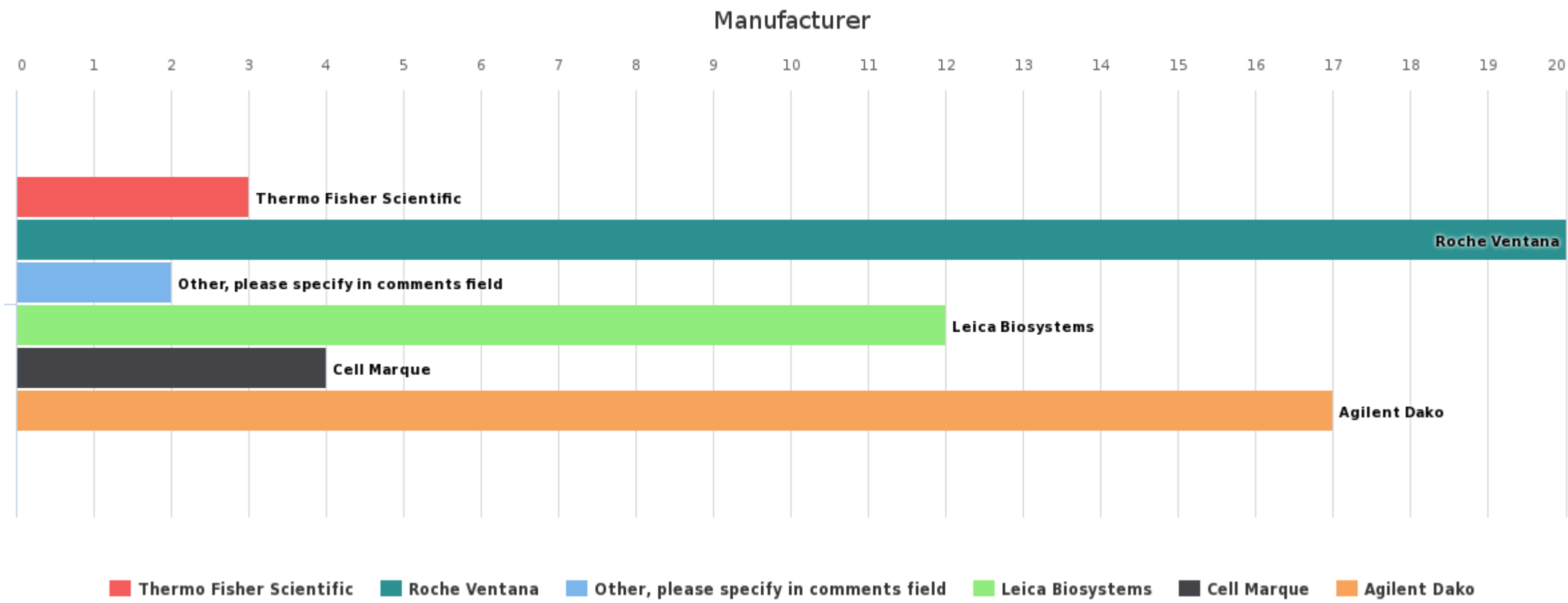


GENERAL DETAILS OF STAINING

Performance	Performance count
Automatic	52
Manual	6
Total:	58

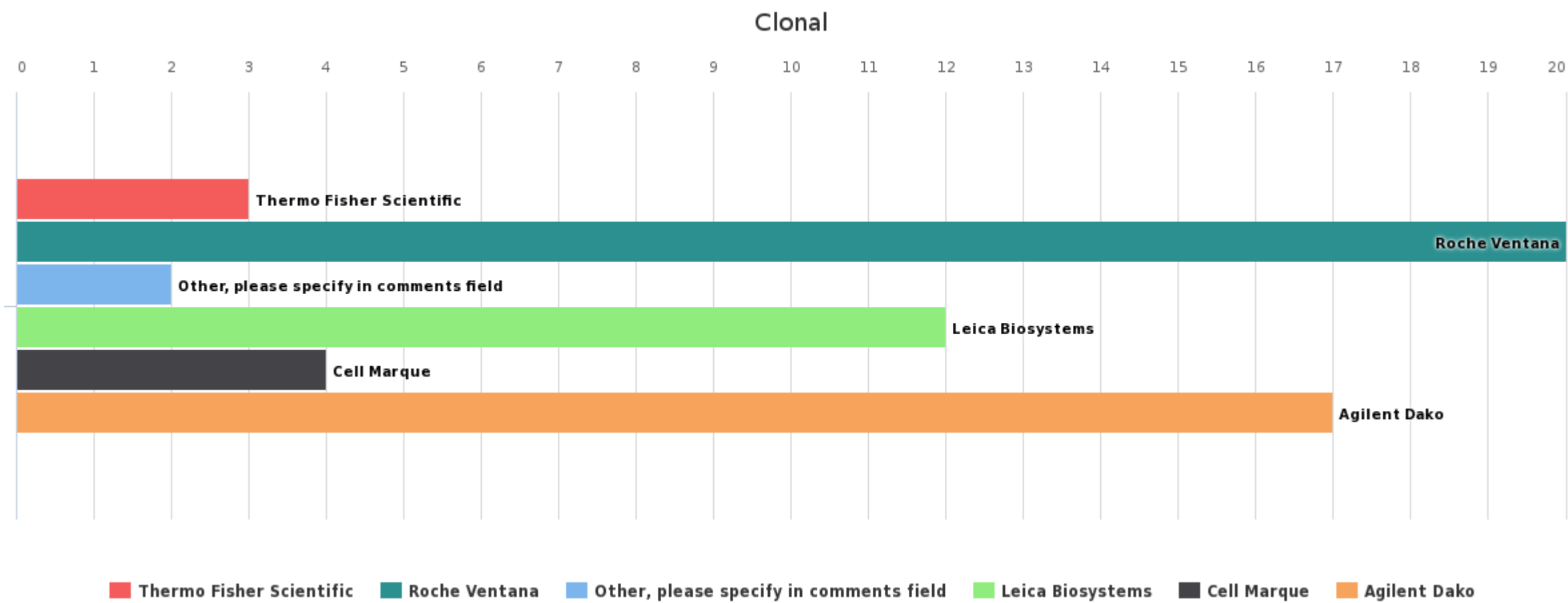


Instrument	Instrument count
Agilent Dako Autostainer Link 48	9
Agilent Dako Autostainer Plus	1
Agilent Dako Omnis	3
Leica BioSystems Bond III	4
Leica Biosystems Bondmax	5
Roche Ventana Benchmark GX	5
Roche Ventana Benchmark Ultra	19
Roche Ventana Benchmark XT	2
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 720-2D	1
Total:	51

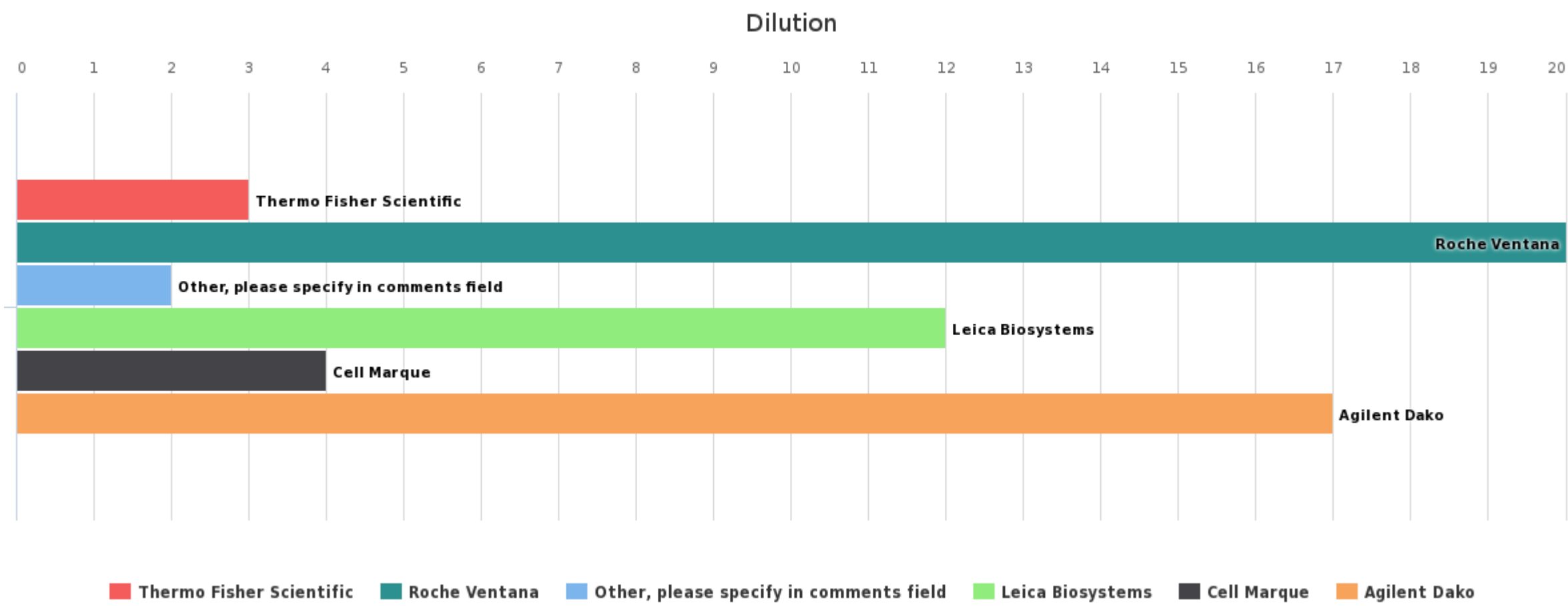


PRIMARY ANTIBODY

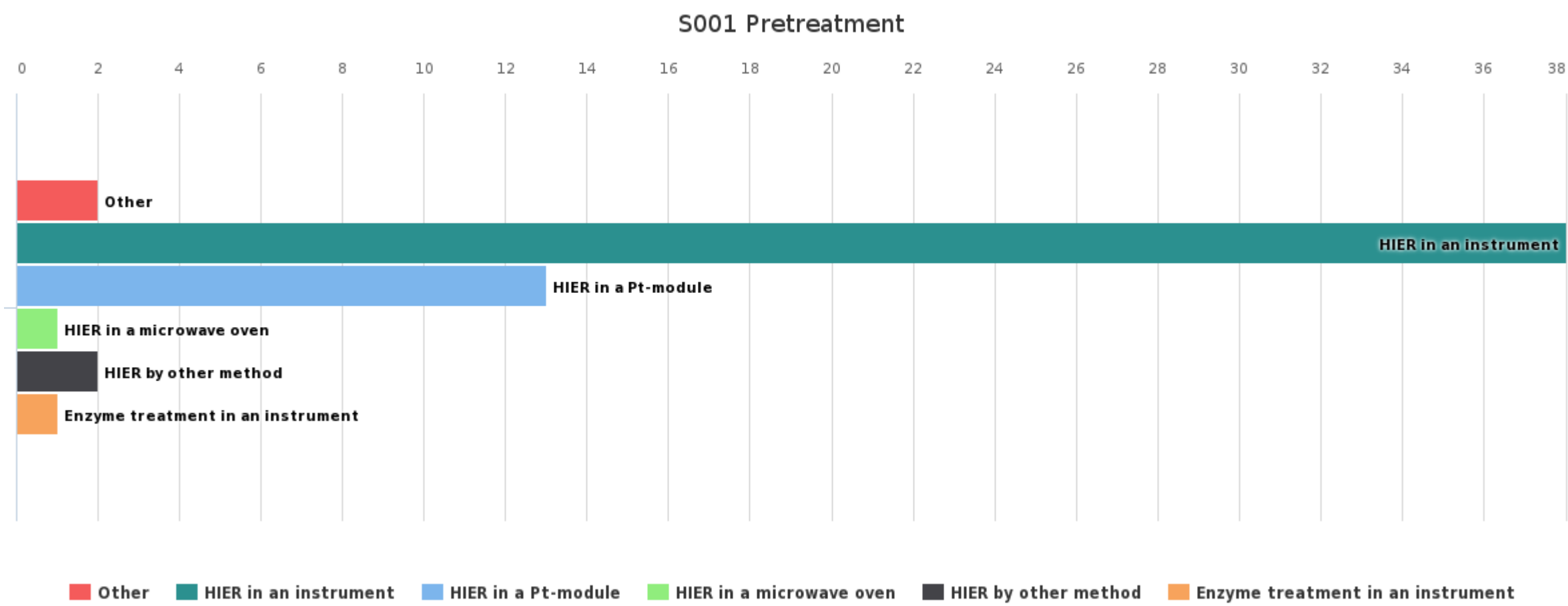
Manufacturer	Manufacturer count
Agilent Dako	17
Cell Marque	4
Leica Biosystems	12
Other, please specify in comments field	2
Roche Ventana	20
Thermo Fisher Scientific	3
Total:	58



Clonal	Clonal count
Monoclonal	40
Polyclonal	17
Total:	57

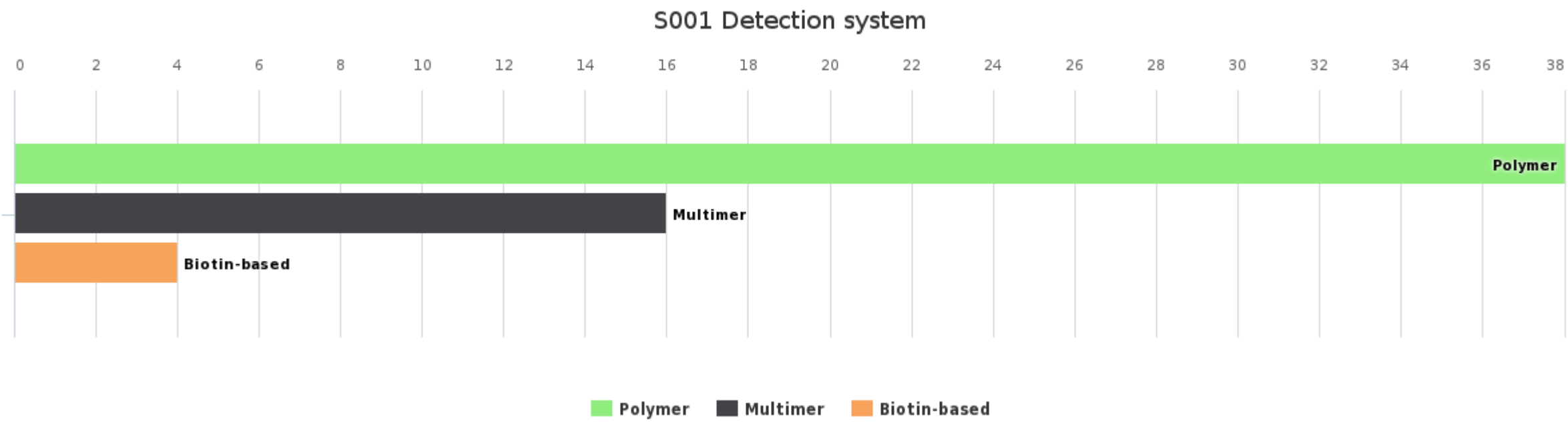


Dilution	Dilution count
Dilution	21
Ready-to-use	36
Total:	57



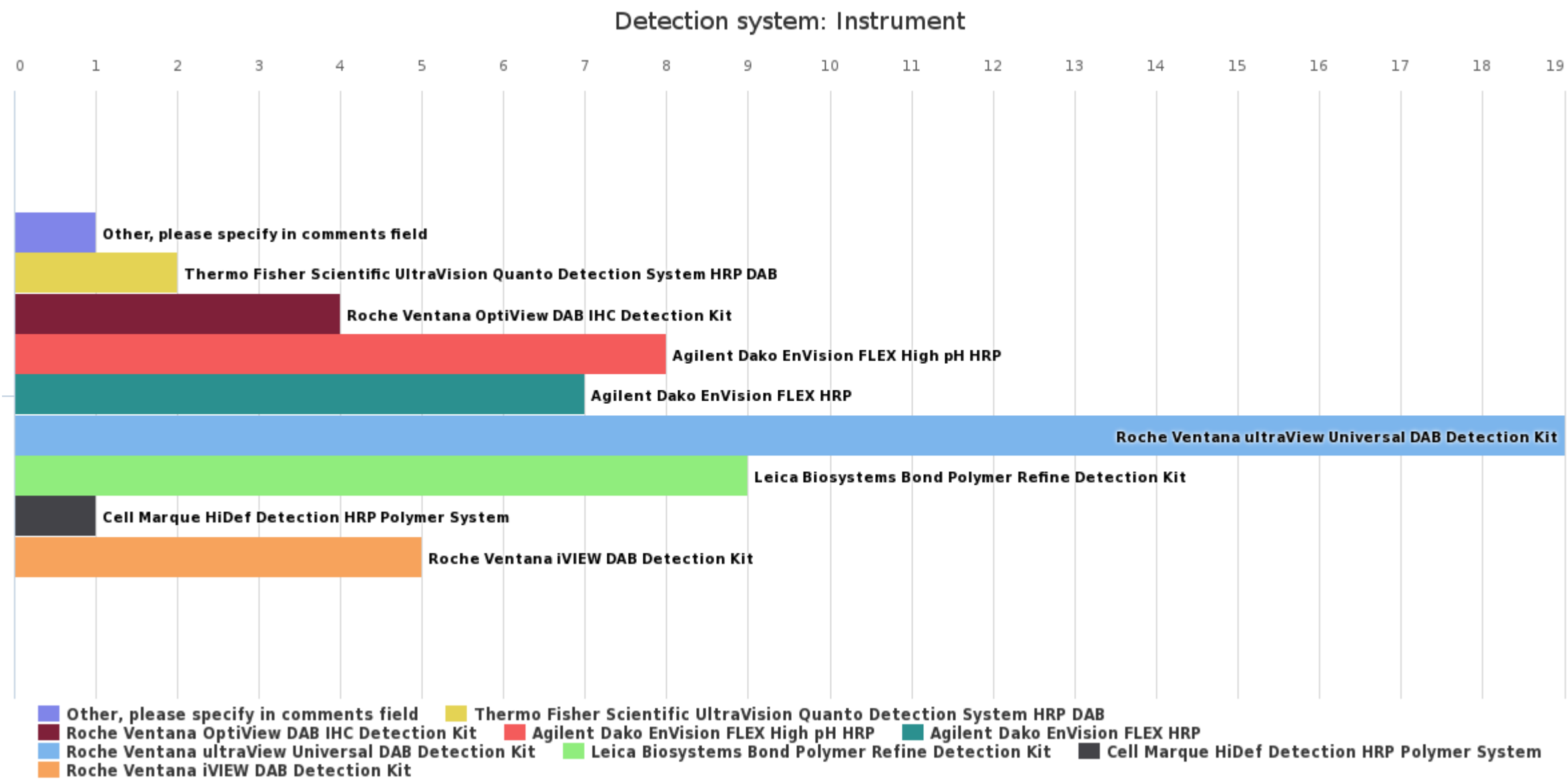
PRETREATMENT

Pretreatment	Pretreatment count
Enzyme treatment in an instrument	1
HIER by other method	2
HIER in a microwave oven	1
HIER in a Pt-module	13
HIER in an instrument	38
Other	2
Total:	57

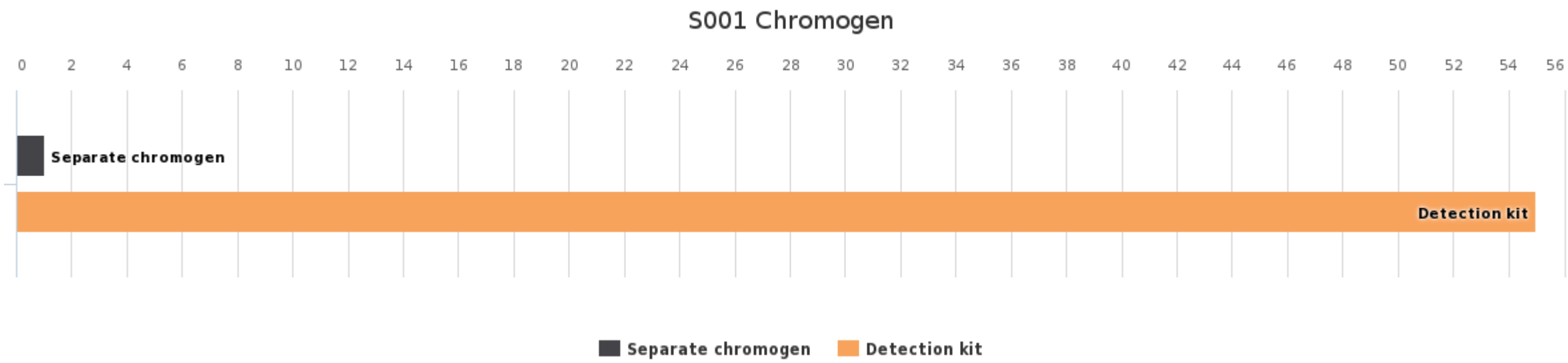


DETECTION SYSTEM

Detection system	Detection system count
Biotin-based	4
Multimer	16
Polymer	38
Total:	58

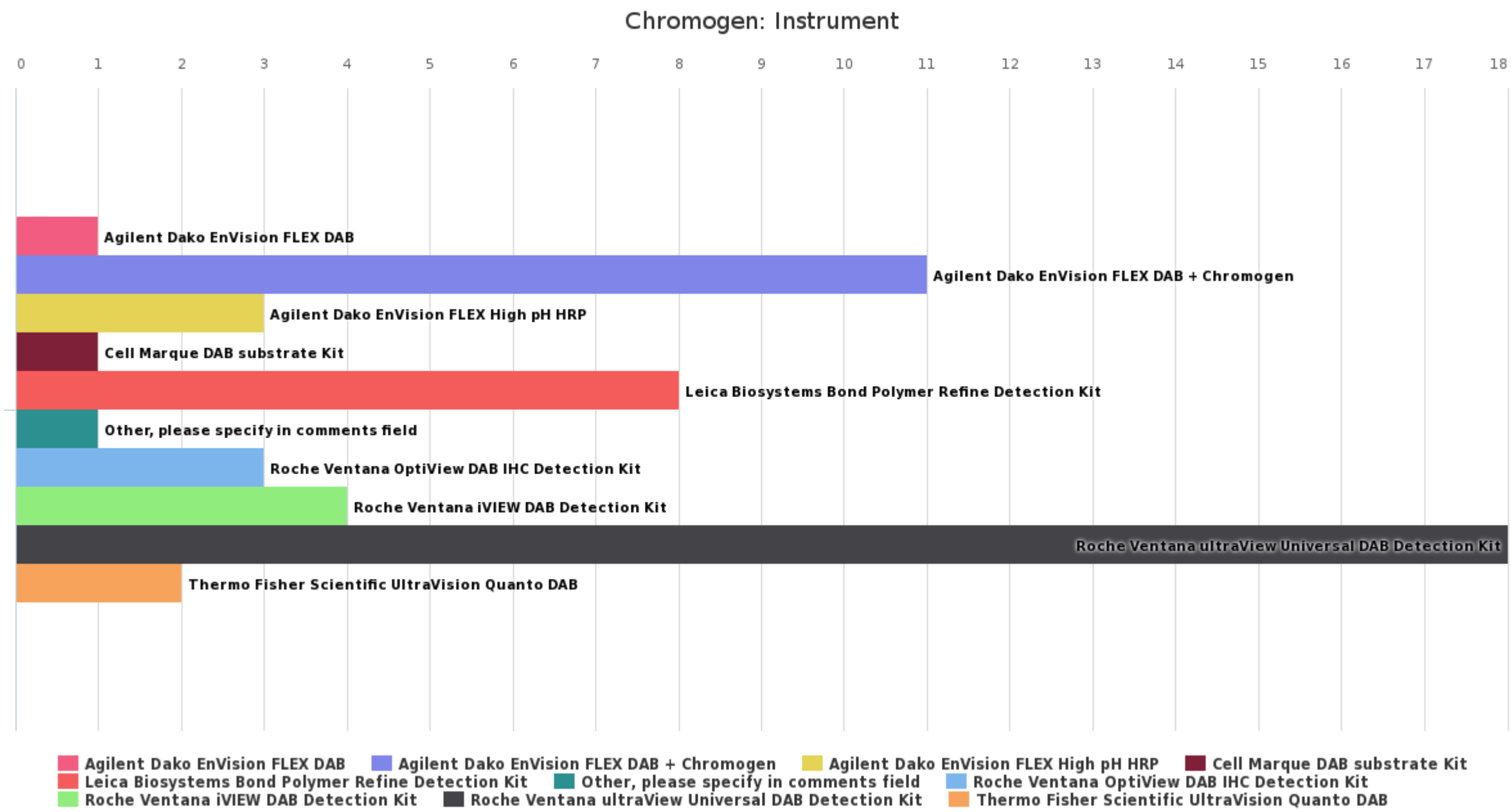


Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	7
Agilent Dako EnVision FLEX High pH HRP	8
Cell Marque HiDef Detection HRP Polymer System	1
Leica Biosystems Bond Polymer Refine Detection Kit	9
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	4
Roche Ventana iVIEW DAB Detection Kit	5
Roche Ventana ultraView Universal DAB Detection Kit	19
Thermo Fisher Scientific UltraVision Quanto Detection System HRP DAB	2
Total:	56

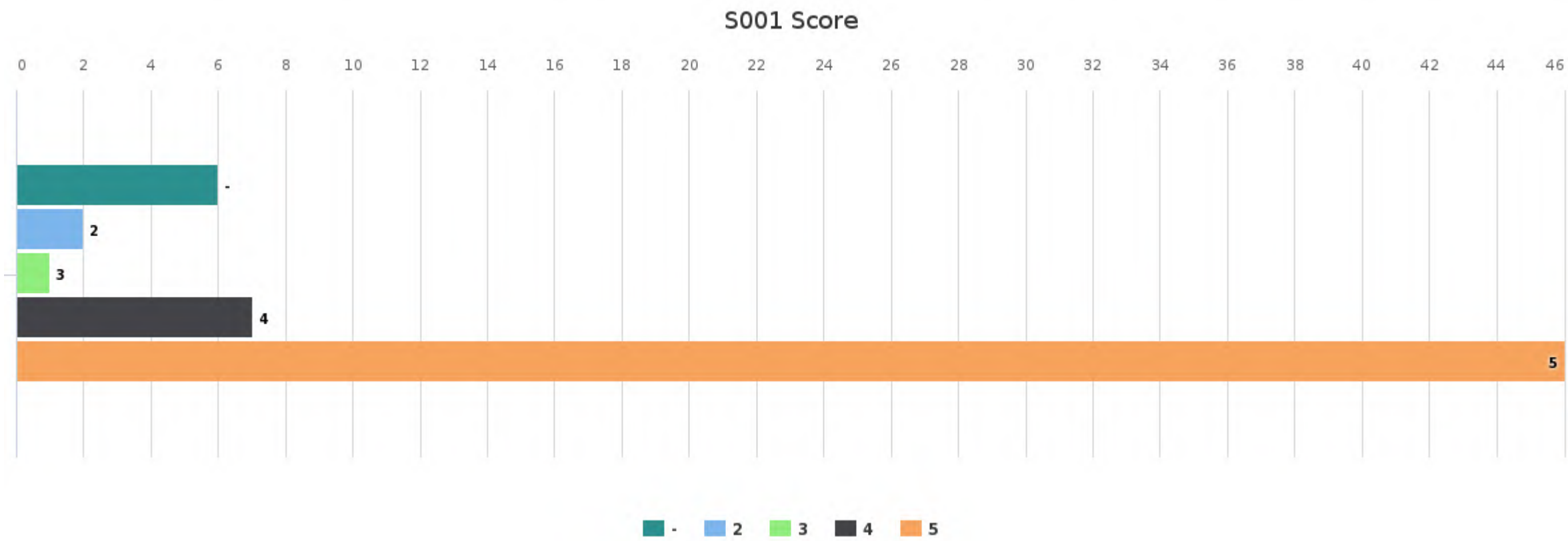


CHROMOGEN

Chromogen	Chromogen count
Detection kit	55
Separate chromogen	1
Total:	56

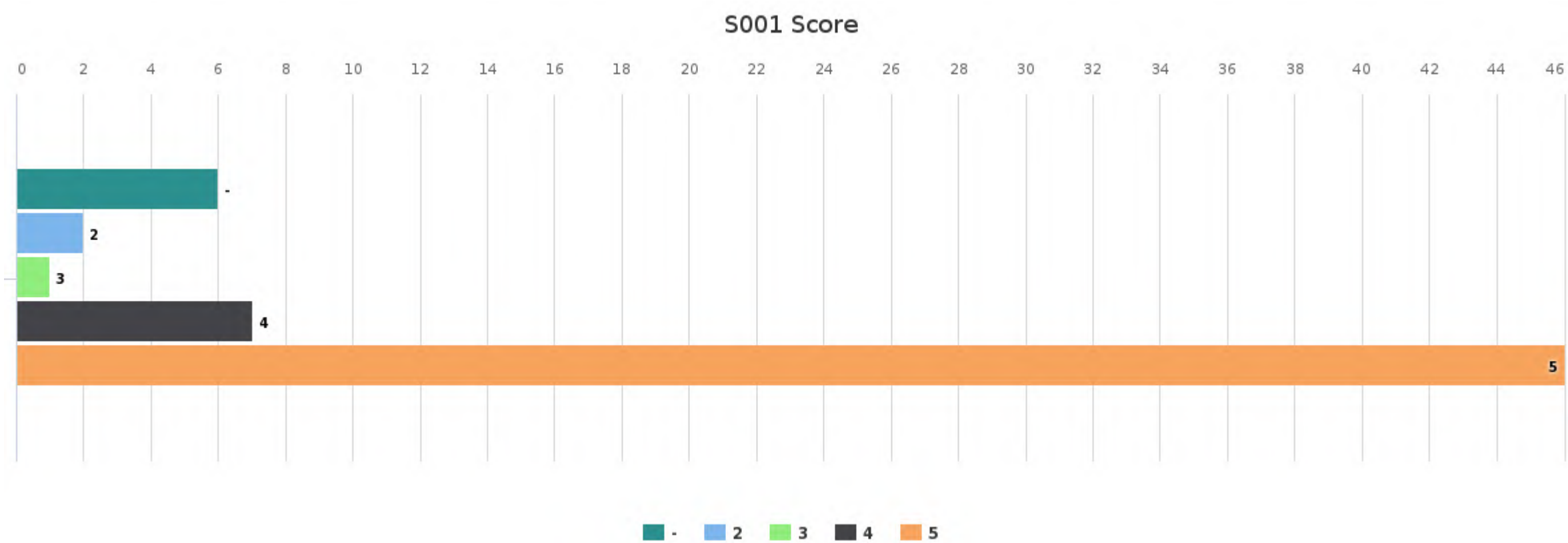


Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	1
Agilent Dako EnVision FLEX DAB + Chromogen	11
Agilent Dako EnVision FLEX High pH HRP	3
Cell Marque DAB substrate Kit	1
Leica Biosystems Bond Polymer Refine Detection Kit	8
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	3
Roche Ventana iVIEW DAB Detection Kit	4
Roche Ventana ultraView Universal DAB Detection Kit	18
Thermo Fisher Scientific UltraVision Quanto DAB	2
Total:	52



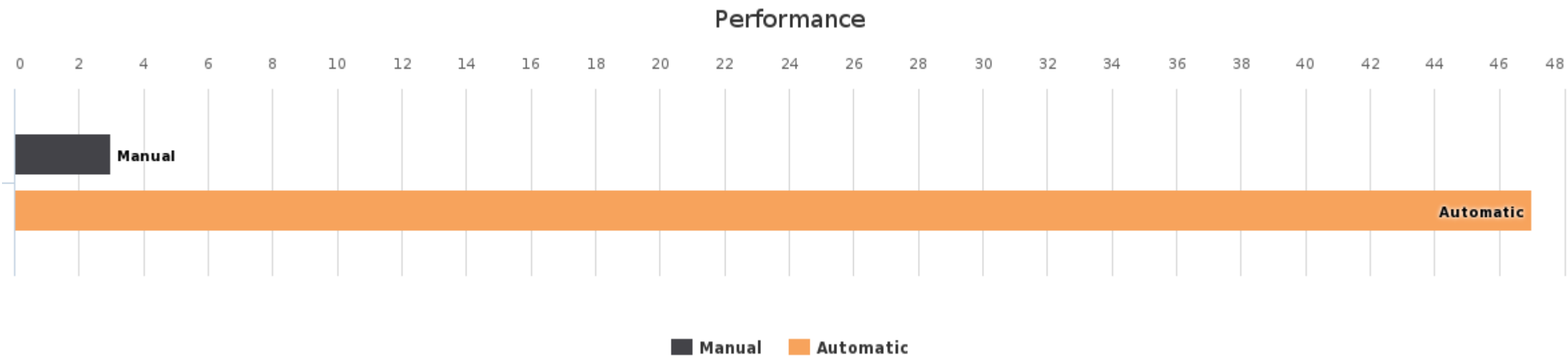
SCORE

Score	Comment
-	
	Not available
2	
	Too weak staining in the tonsil and no T-cell staining in the liver.
	Too weak staning, No membrane staining/ not clear membranous staining.
3	
	Too weak staining of the T-cell lymphoma.
4	
	Light background; liver hepatocytes
	Not crisp
	Not crisp; no membranous staining
	Slightly too weak staining.
	Slightly uneven staining, edge artefacts.
	Slightly weak staining in all tissues.
	Slightly weak. Contrast between hematoxylin and DAB is not optimal.
5	
	Excellent
Total:	



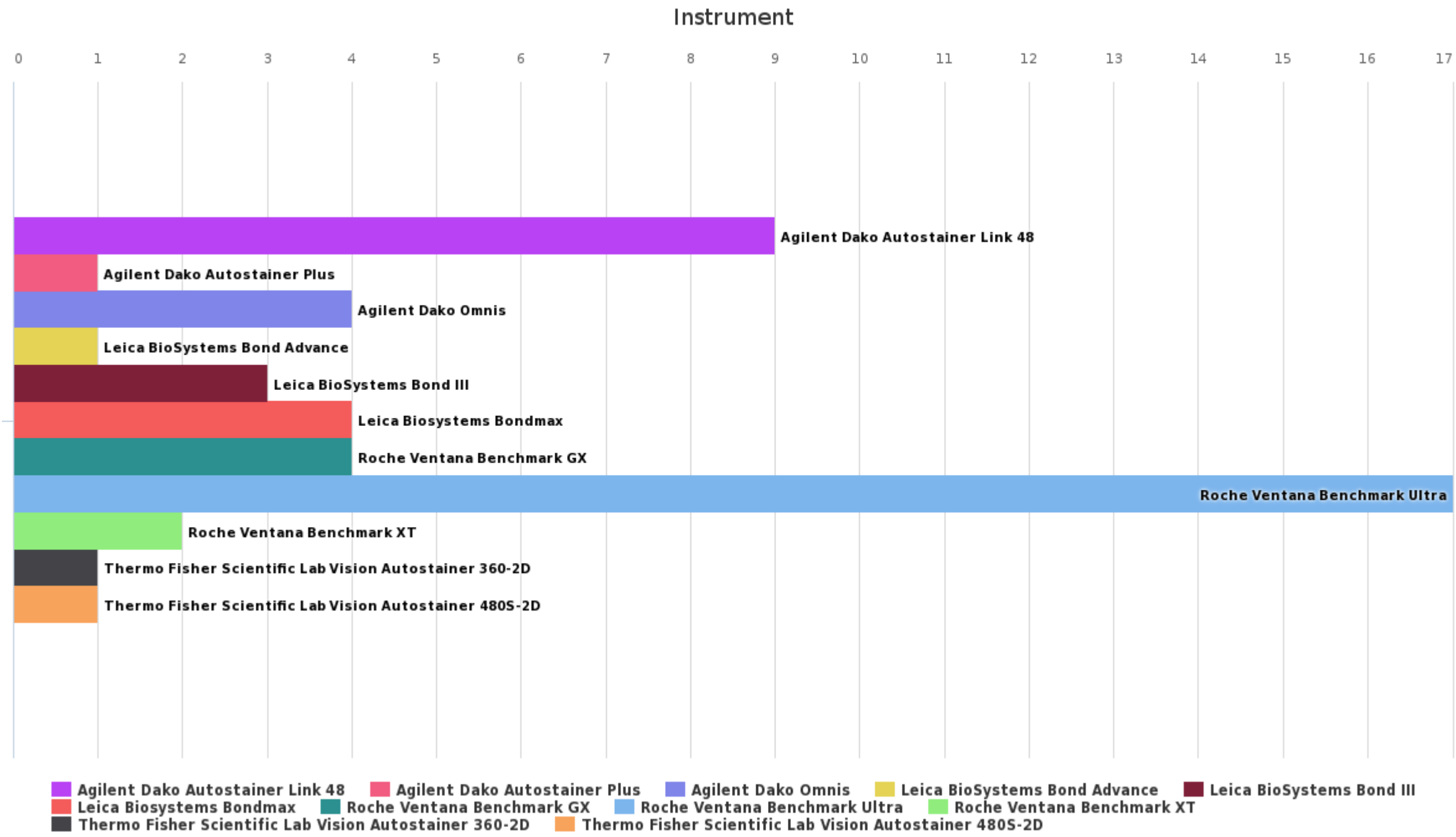
Score	Score count
-	6
2	2
3	1
4	7
5	46
Total:	62

S002 | Sample S002 CD5

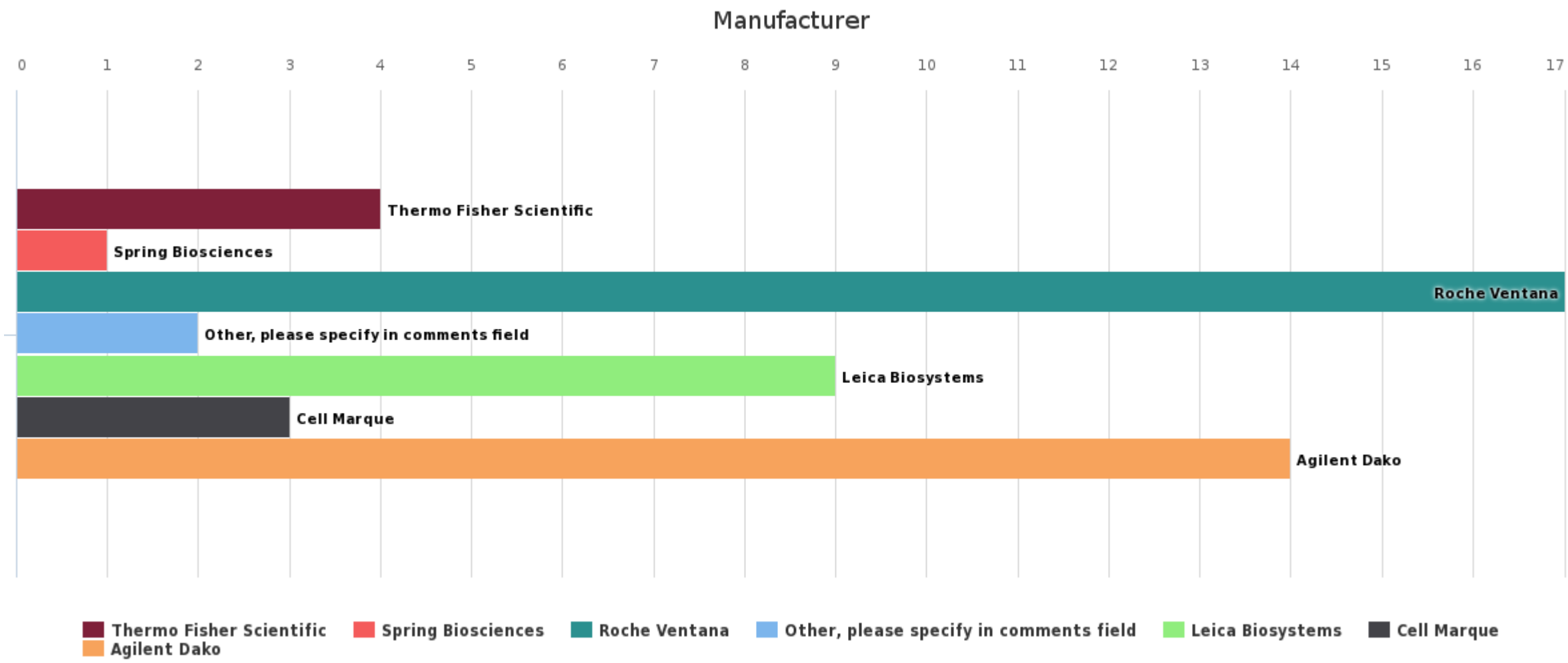


GENERAL DETAILS OF STAINING

Performance	Performance count
Automatic	47
Manual	3
Total:	50

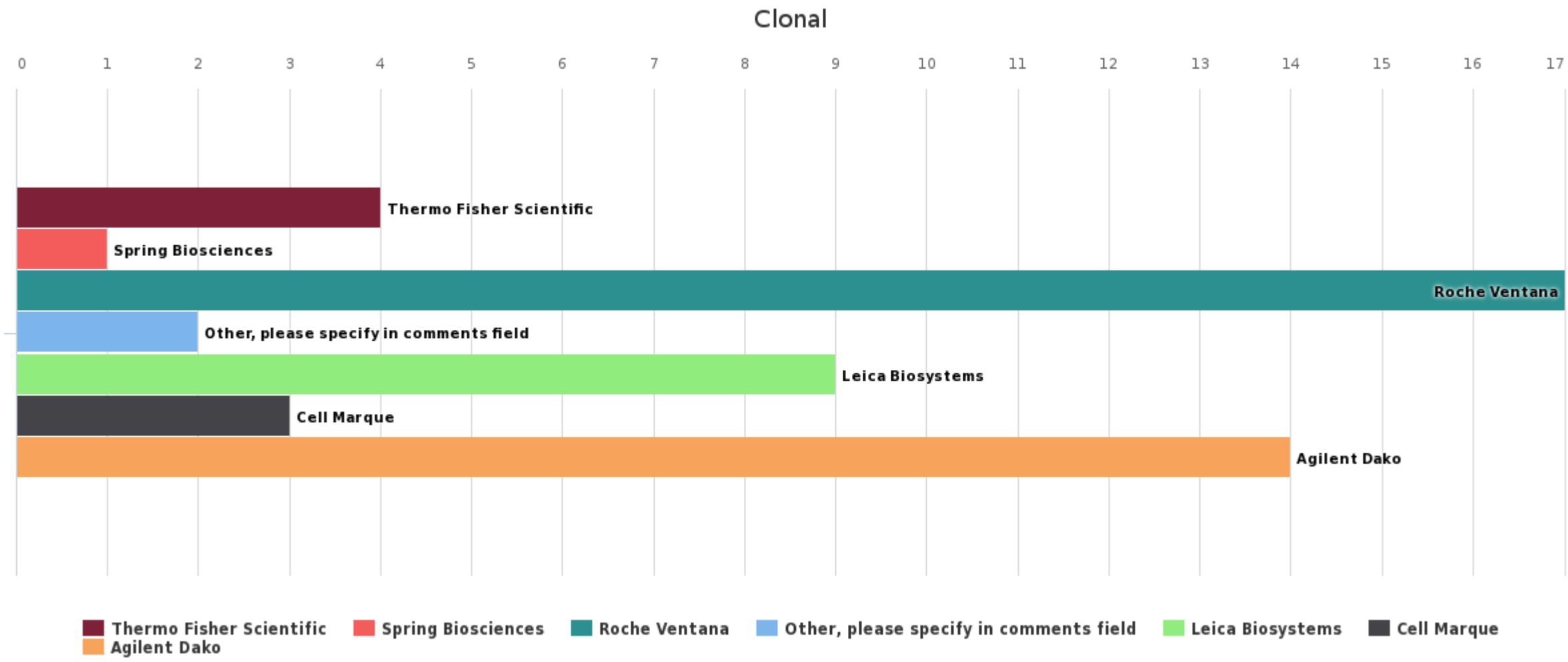


Instrument	Instrument count
Agilent Dako Autostainer Link 48	9
Agilent Dako Autostainer Plus	1
Agilent Dako Omnis	4
Leica BioSystems Bond Advance	1
Leica BioSystems Bond III	3
Leica Biosystems Bondmax	4
Roche Ventana Benchmark GX	4
Roche Ventana Benchmark Ultra	17
Roche Ventana Benchmark XT	2
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Total:	47

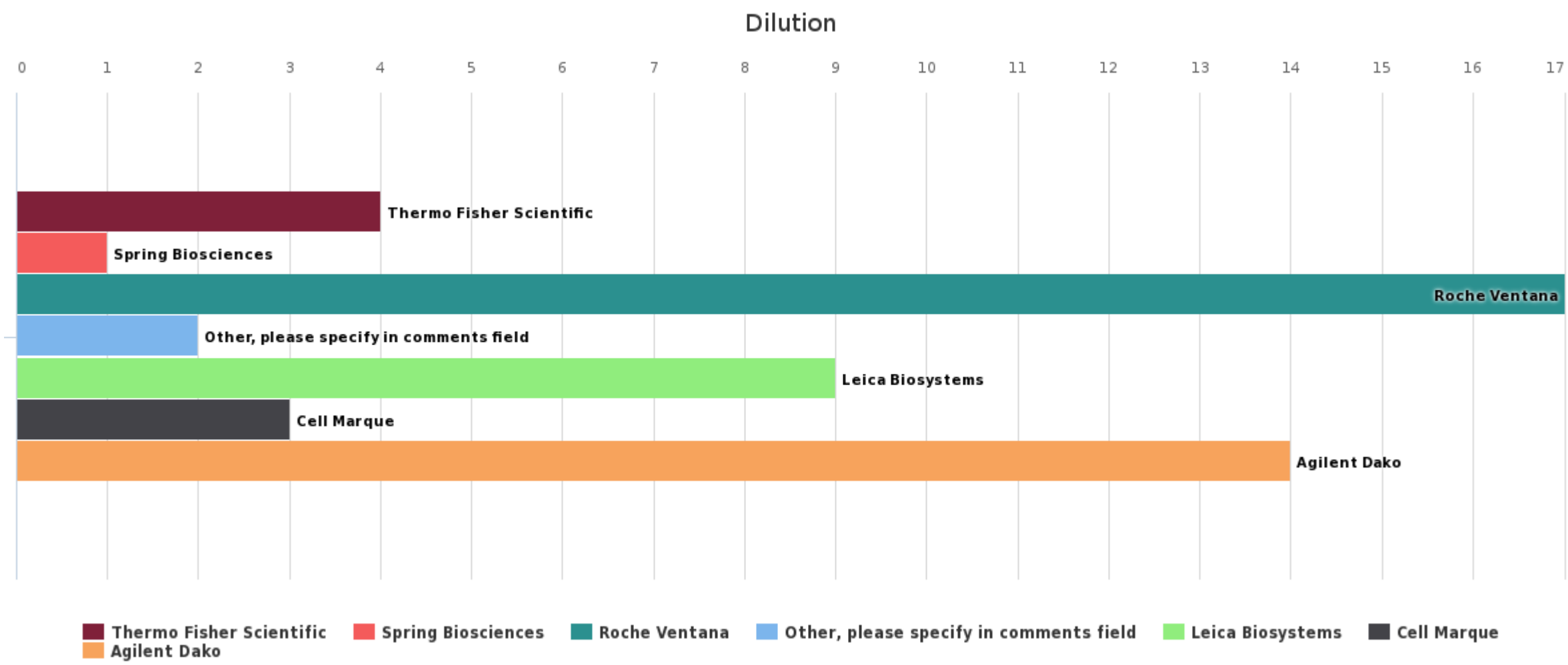


PRIMARY ANTIBODY

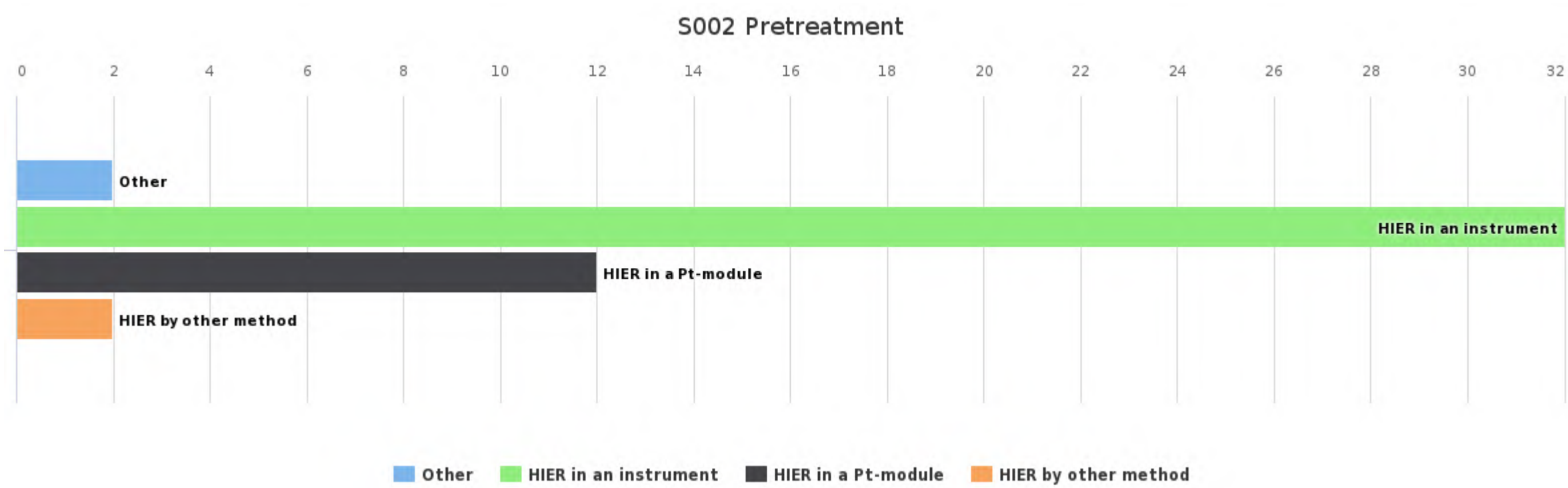
Manufacturer	Manufacturer count
Agilent Dako	14
Cell Marque	3
Leica Biosystems	9
Other, please specify in comments field	2
Roche Ventana	17
Spring Biosciences	1
Thermo Fisher Scientific	4
Total:	50



Clonal	Clonal count
Monoclonal	49
Polyclonal	1
Total:	50

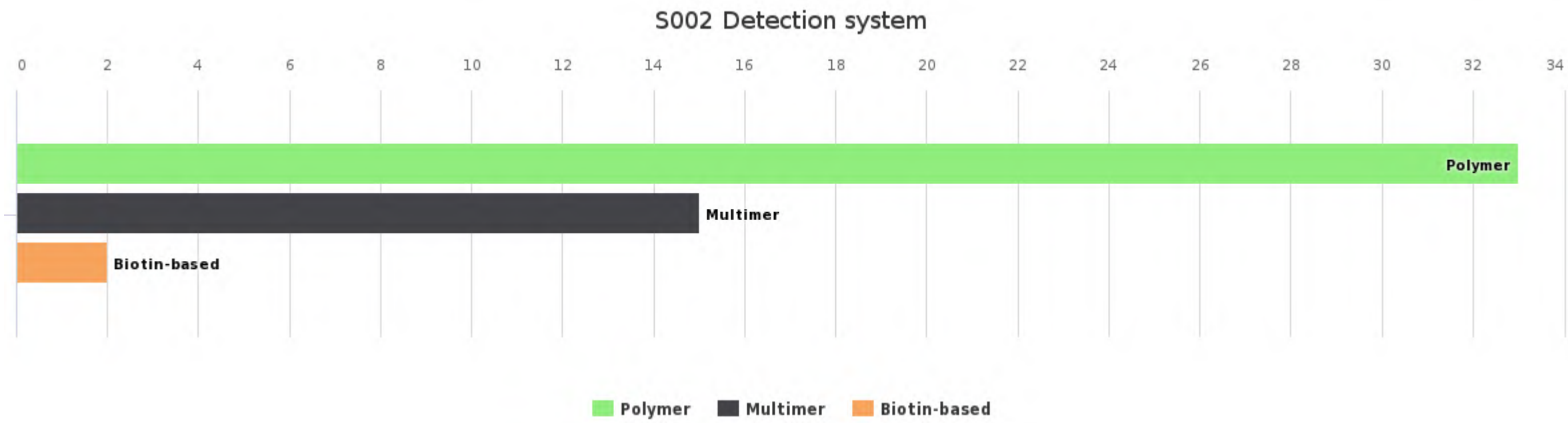


Dilution	Dilution count
Dilution	20
Ready-to-use	30
Total:	50



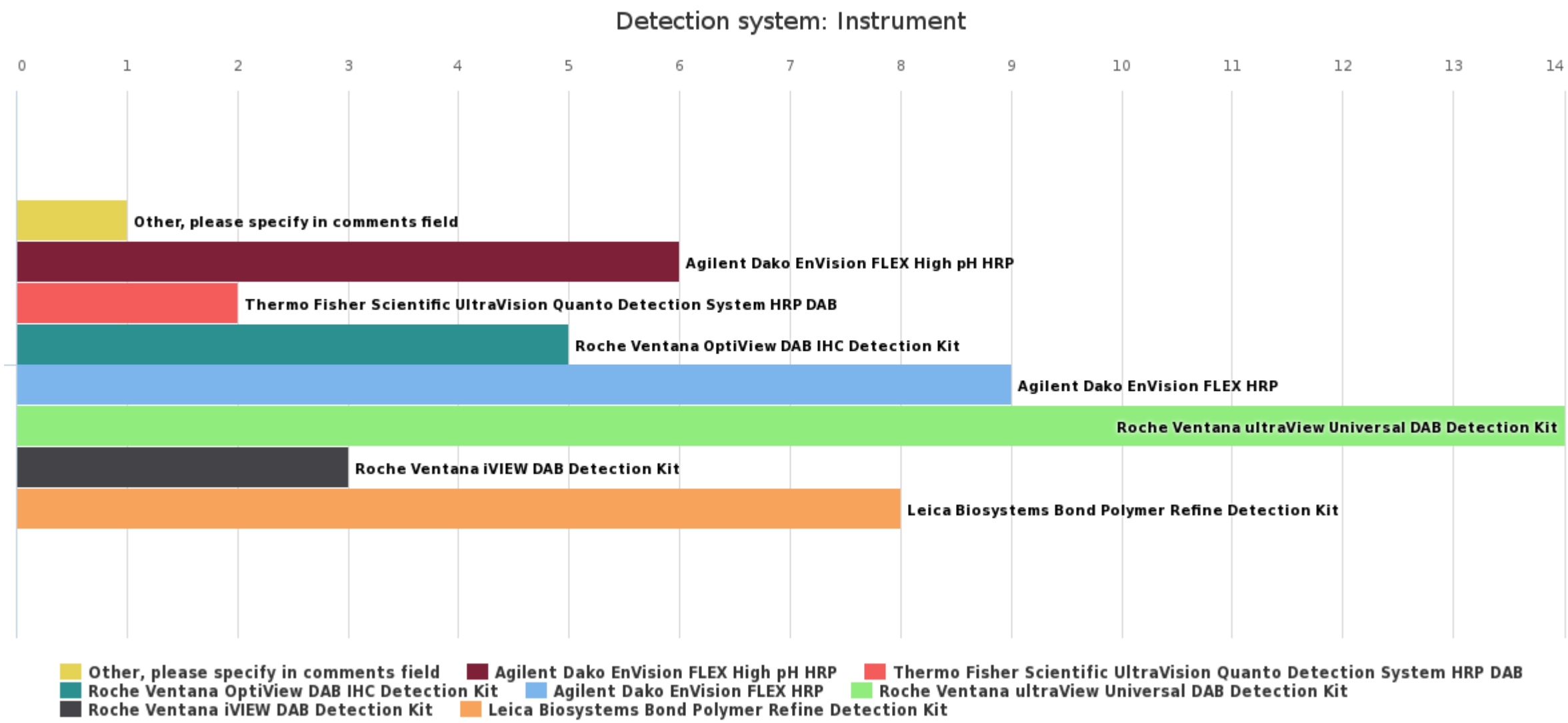
PRETREATMENT

Pretreatment	Pretreatment count
HIER by other method	2
HIER in a Pt-module	12
HIER in an instrument	32
Other	2
Total:	48

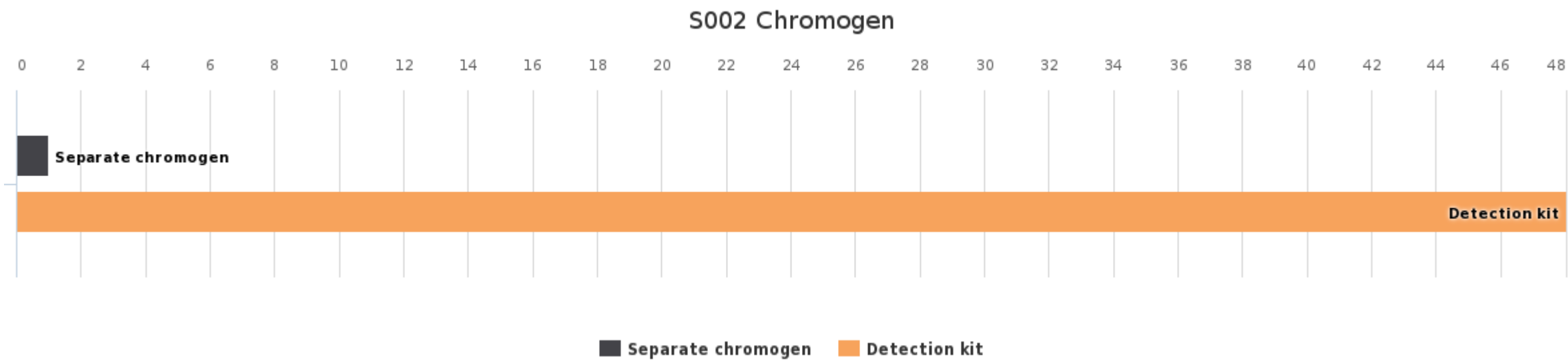


DETECTION SYSTEM

Detection system	Detection system count
Biotin-based	2
Multimer	15
Polymer	33
Total:	50

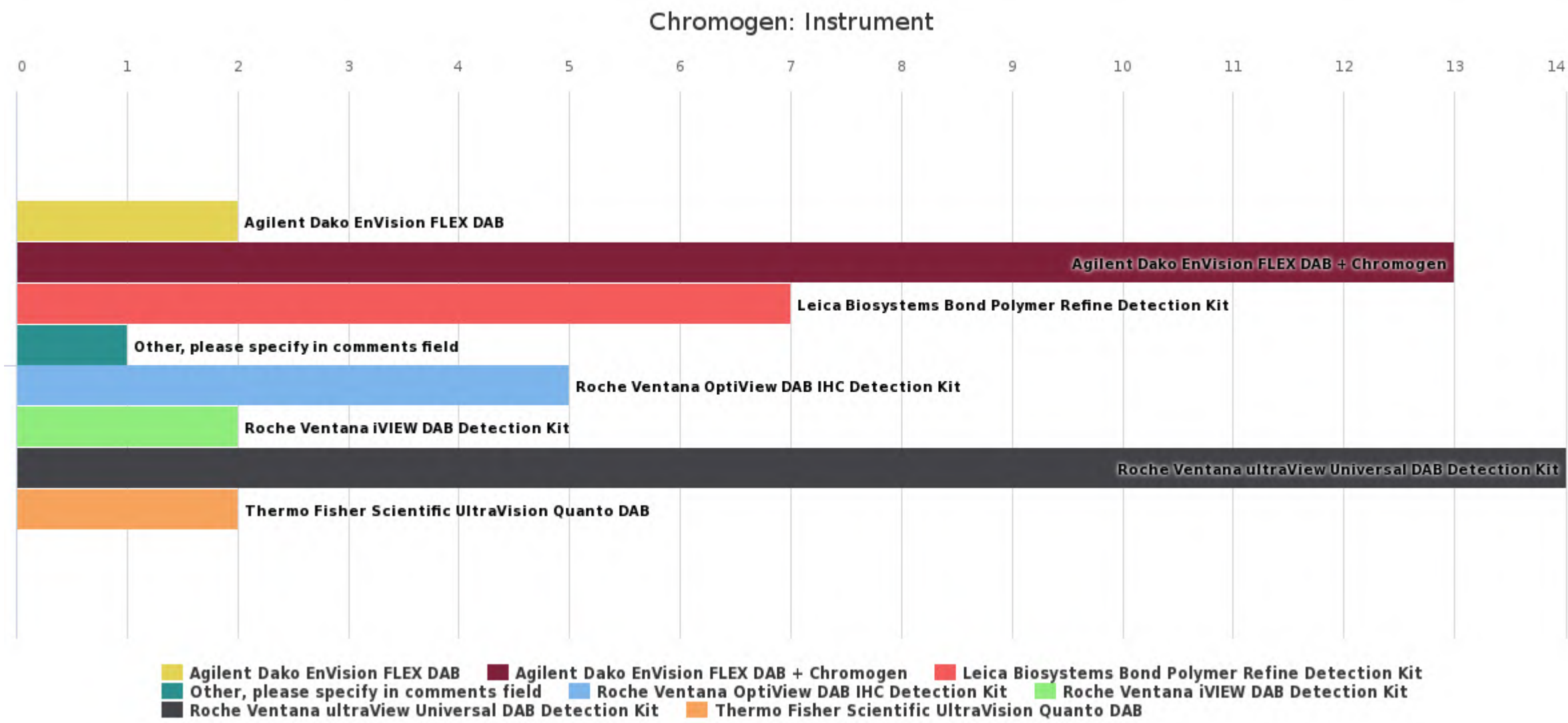


Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	9
Agilent Dako EnVision FLEX High pH HRP	6
Leica Biosystems Bond Polymer Refine Detection Kit	8
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	5
Roche Ventana iVIEW DAB Detection Kit	3
Roche Ventana ultraView Universal DAB Detection Kit	14
Thermo Fisher Scientific UltraVision Quanto Detection System HRP DAB	2
Total:	48

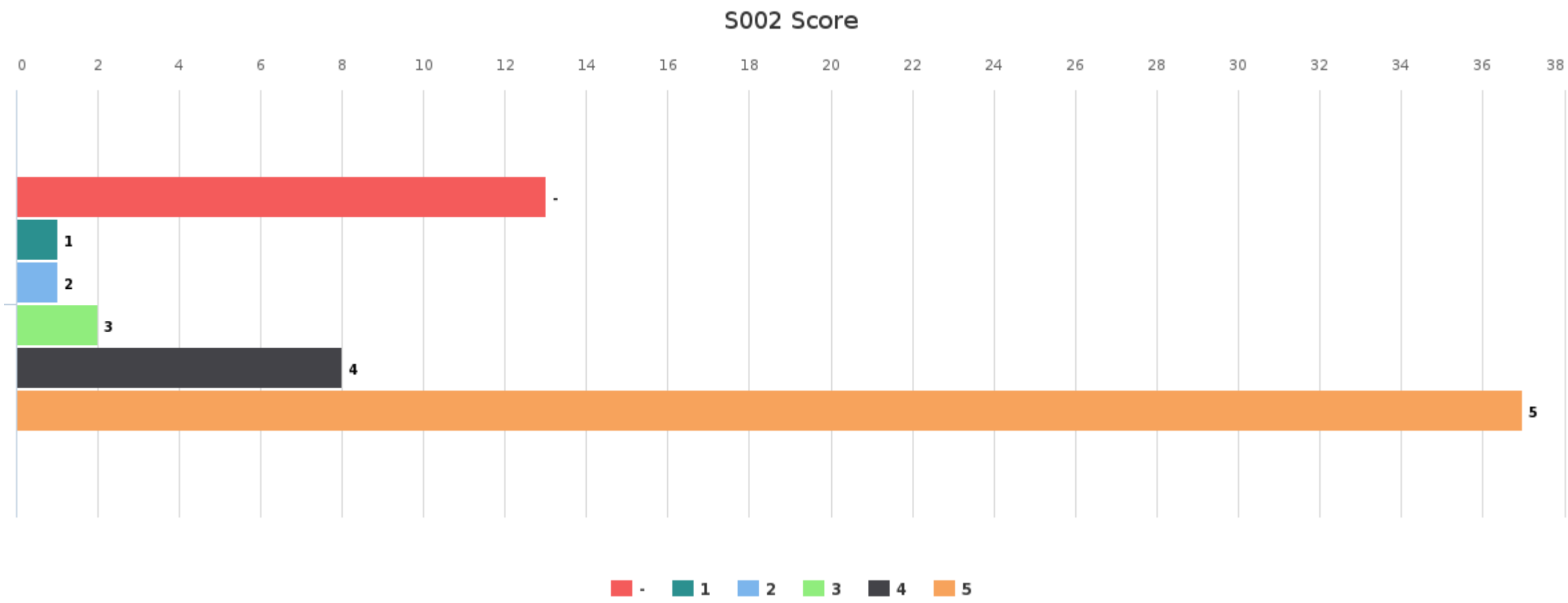


CHROMOGEN

Chromogen	Chromogen count
Detection kit	48
Separate chromogen	1
Total:	49

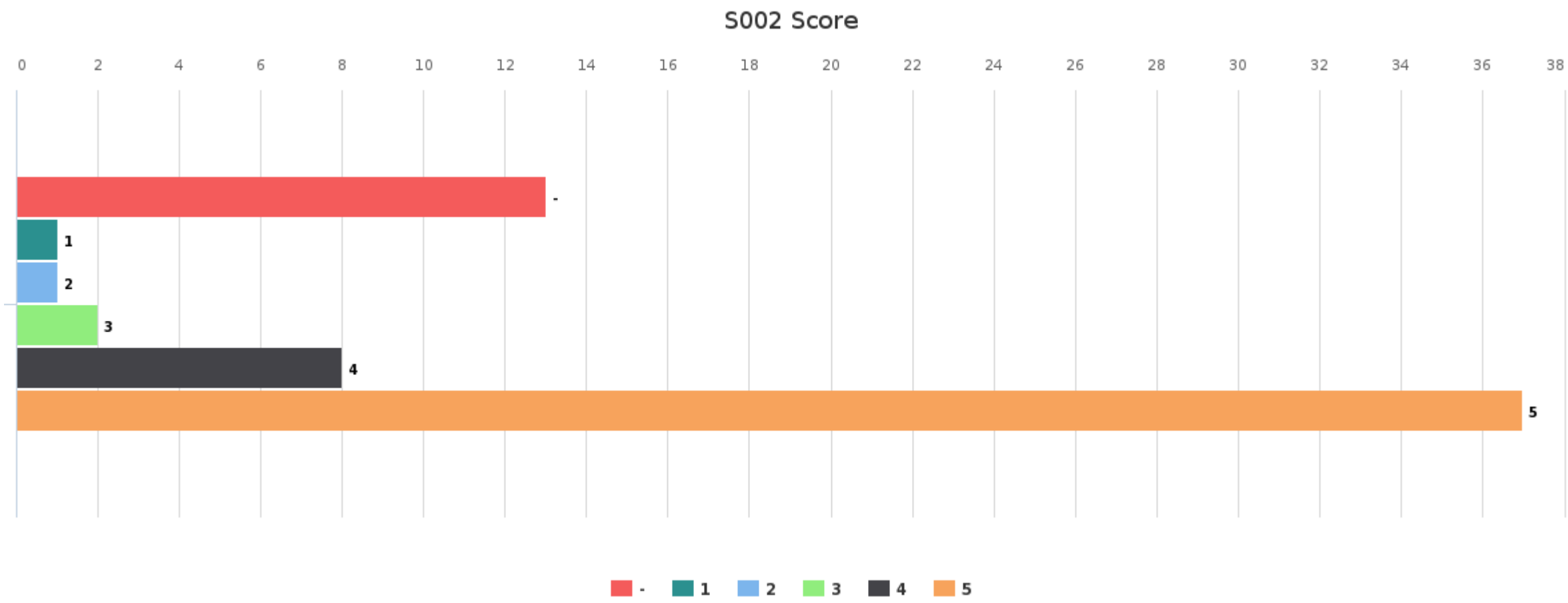


Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	2
Agilent Dako EnVision FLEX DAB + Chromogen	13
Leica Biosystems Bond Polymer Refine Detection Kit	7
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	5
Roche Ventana iVIEW DAB Detection Kit	2
Roche Ventana ultraView Universal DAB Detection Kit	14
Thermo Fisher Scientific UltraVision Quanto DAB	2
Total:	46



SCORE

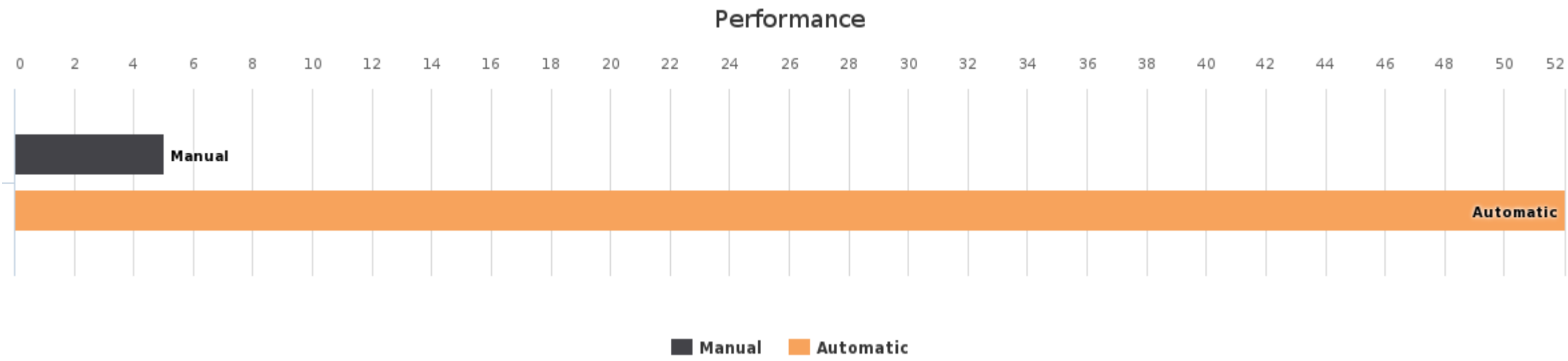
Score	Comment
-	
	Not available
1	
	No specific staining in any tissues.
2	
	False negative neoplastic B-cells in mattle cell lymphoma, weak or no staining of manttle zone B-cells in the tonsil.
3	
	Too strong hematoxylin, stains also cytoplasm. Uneaven staining. Weak staining of the manttle cell lymphoma B-cells and manttle zone B-cells of the tonsil.
	Weak staining, not crisp
4	
	Contrast between hematoxylin and DAB is not optimal.
	Background staining in all tissues.
	Not crisp staining.
	Slight background in all tissues.
	Technical issue; DAB precipitates. Old DAB solution or too few/short washing steps.
	Too strong hematoxylin, not optimal contrast
	Too strong hematoxylin; low contrast.
5	
	Excellent
Total:	



Score	Score count
-------	-------------

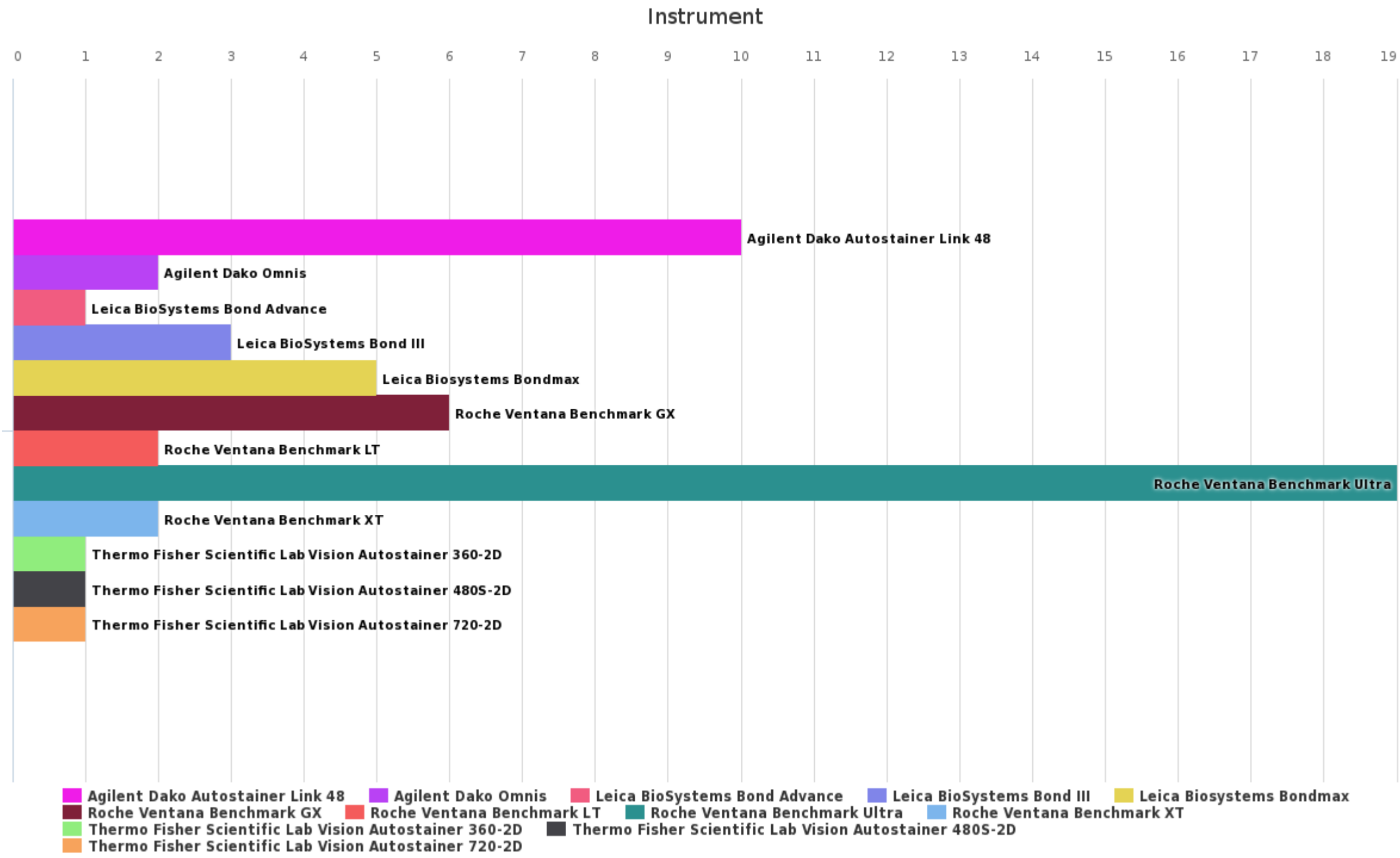
-	13
1	1
2	1
3	2
4	8
5	37
Total:	62

S003 | Sample S003 CD20



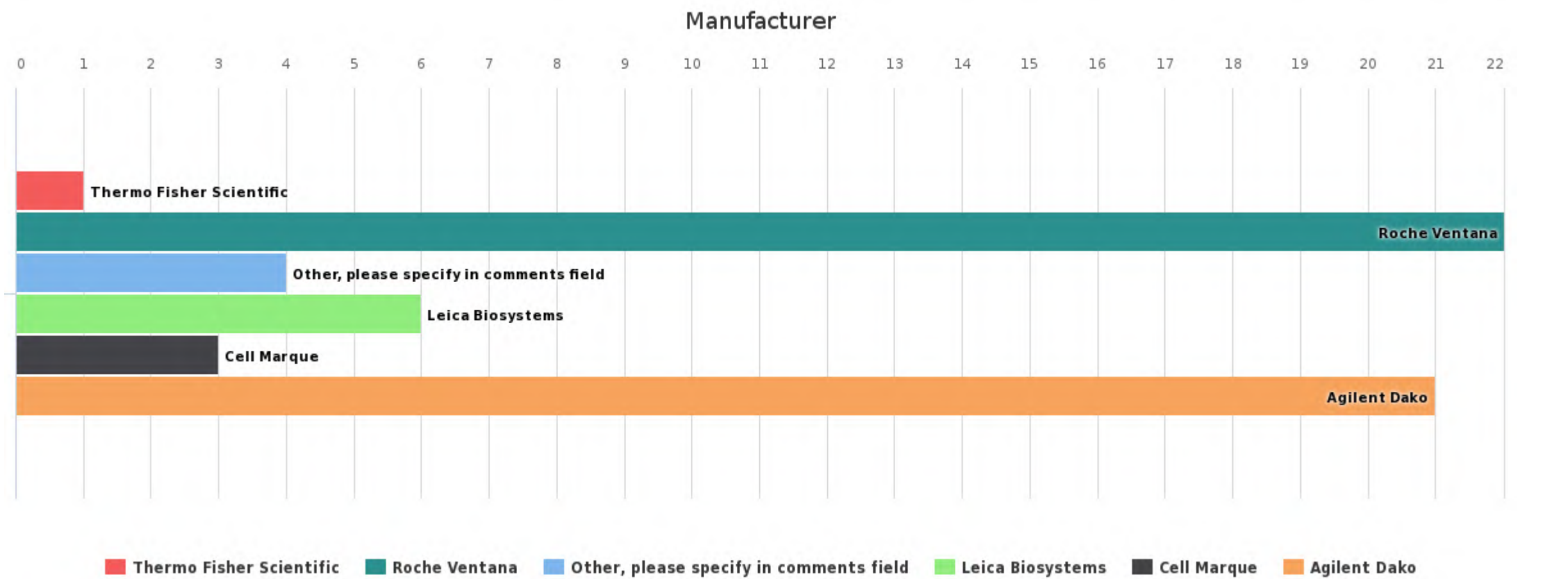
GENERAL DETAILS OF STAINING

Performance	Performance count
Automatic	52
Manual	5
Total:	57



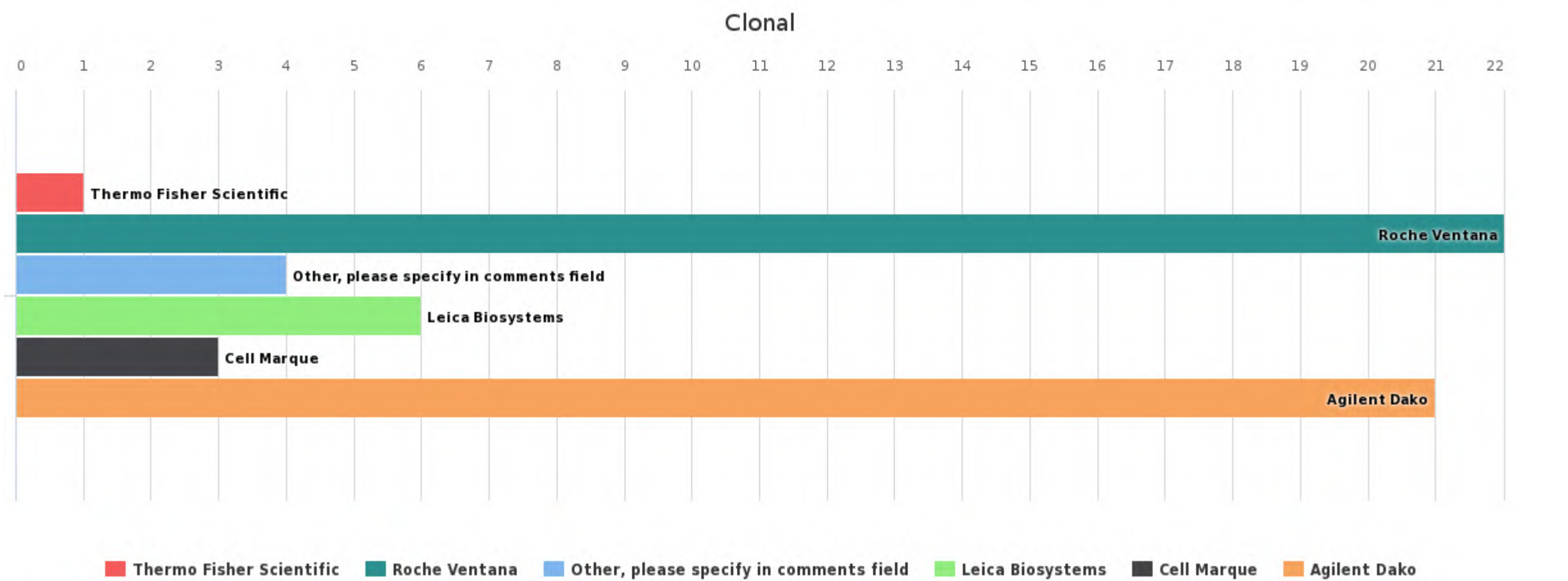
Instrument	Instrument count
Agilent Dako Autostainer Link 48	10
Agilent Dako Omnis	2
Leica BioSystems Bond Advance	1
Leica BioSystems Bond III	3
Leica Biosystems Bondmax	5
Roche Ventana Benchmark GX	6
Roche Ventana Benchmark LT	2
Roche Ventana Benchmark Ultra	19
Roche Ventana Benchmark XT	2
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 720-2D	1

Total:	53
--------	----

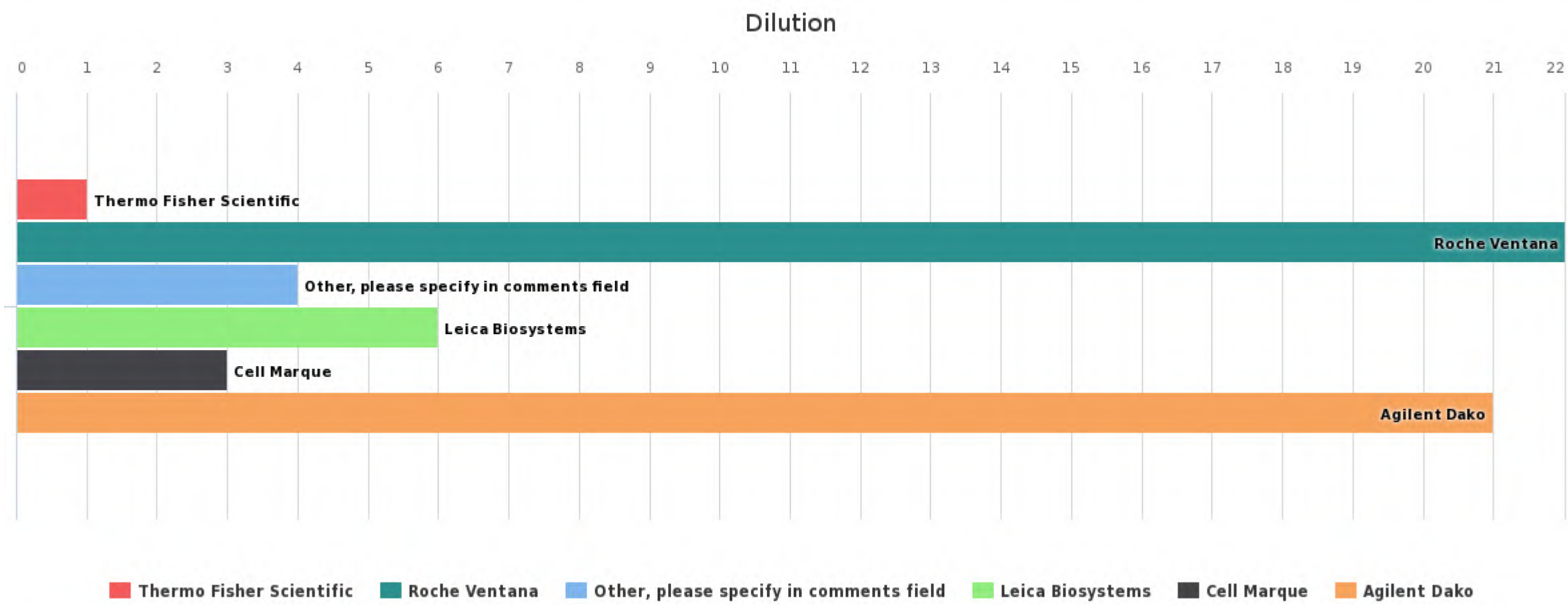


PRIMARY ANTIBODY

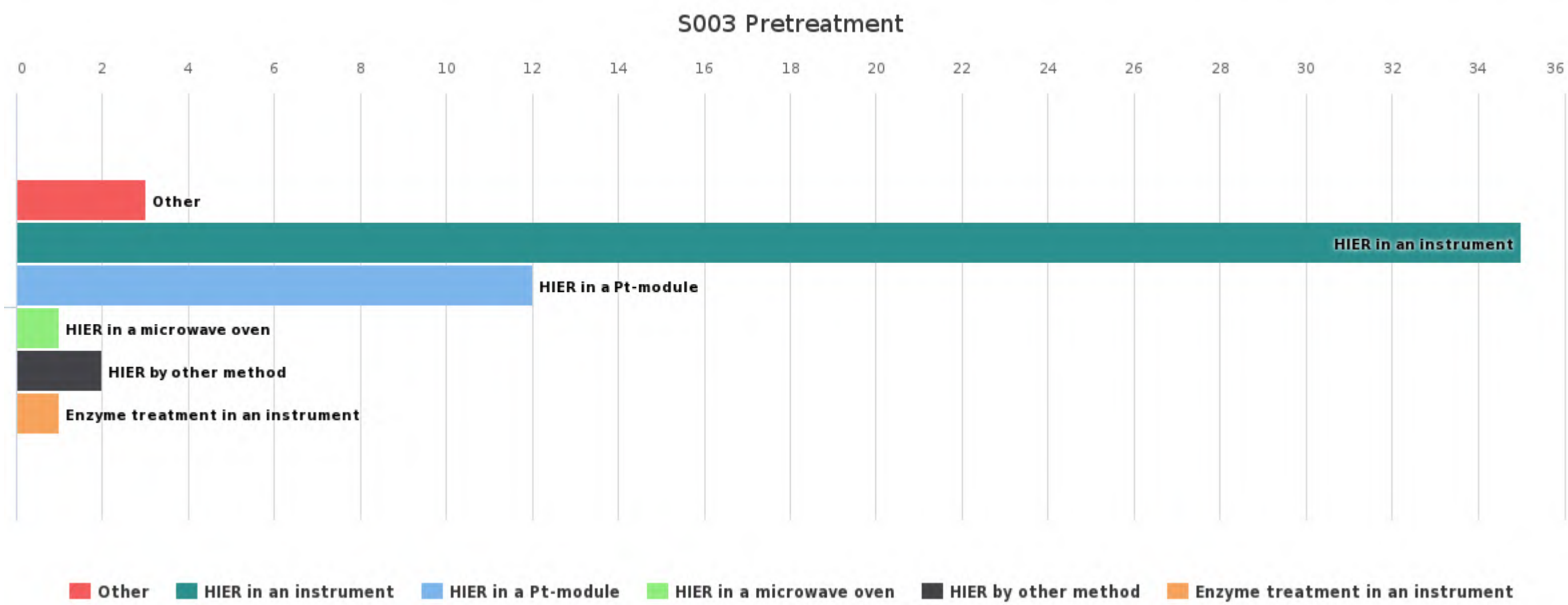
Manufacturer	Manufacturer count
Agilent Dako	21
Cell Marque	3
Leica Biosystems	6
Other, please specify in comments field	4
Roche Ventana	22
Thermo Fisher Scientific	1
Total:	57



Clonal	Clonal count
Monoclonal	56
Polyclonal	1
Total:	57

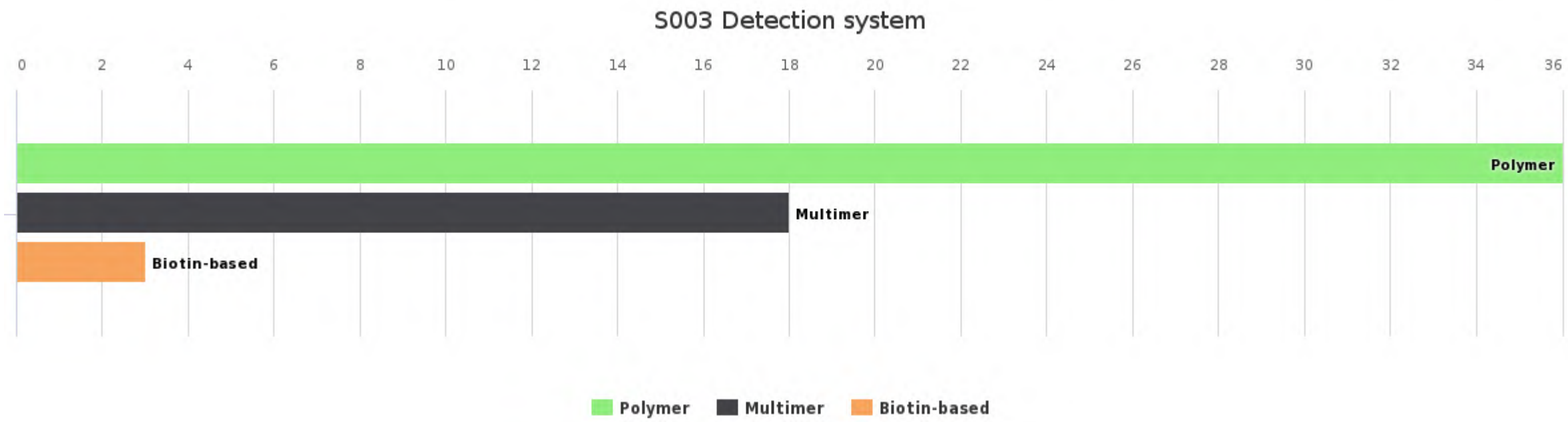


Dilution	Dilution count
Dilution	24
Ready-to-use	33
Total:	57



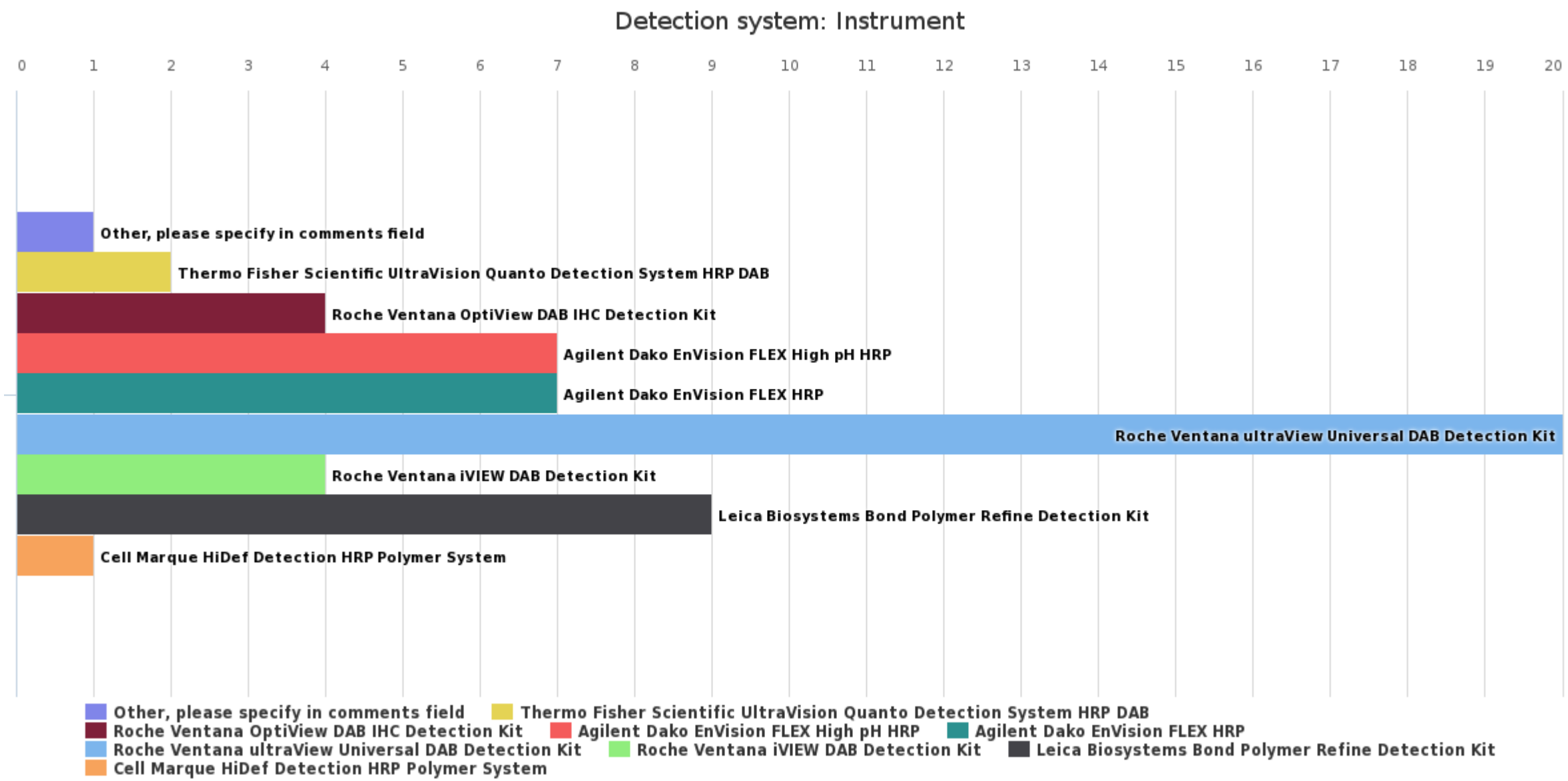
PRETREATMENT

Pretreatment	Pretreatment count
Enzyme treatment in an instrument	1
HIER by other method	2
HIER in a microwave oven	1
HIER in a Pt-module	12
HIER in an instrument	35
Other	3
Total:	54

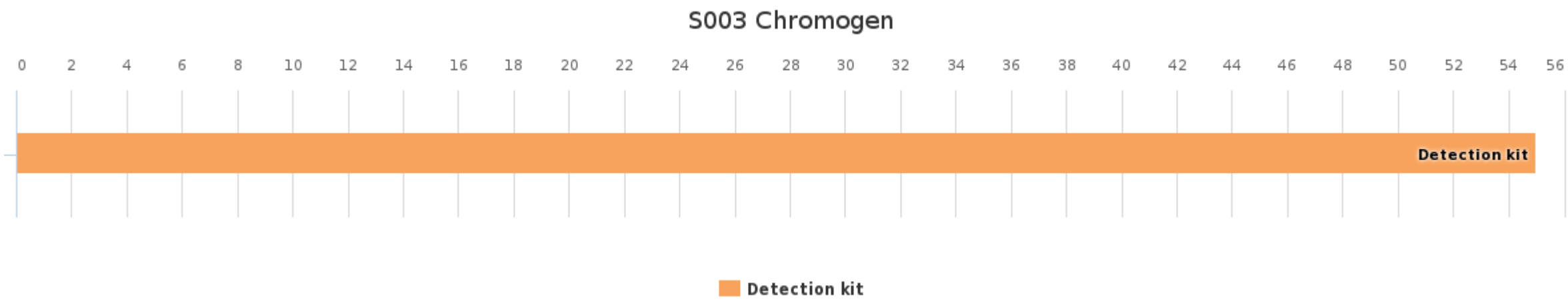


DETECTION SYSTEM

Detection system	Detection system count
Biotin-based	3
Multimer	18
Polymer	36
Total:	57

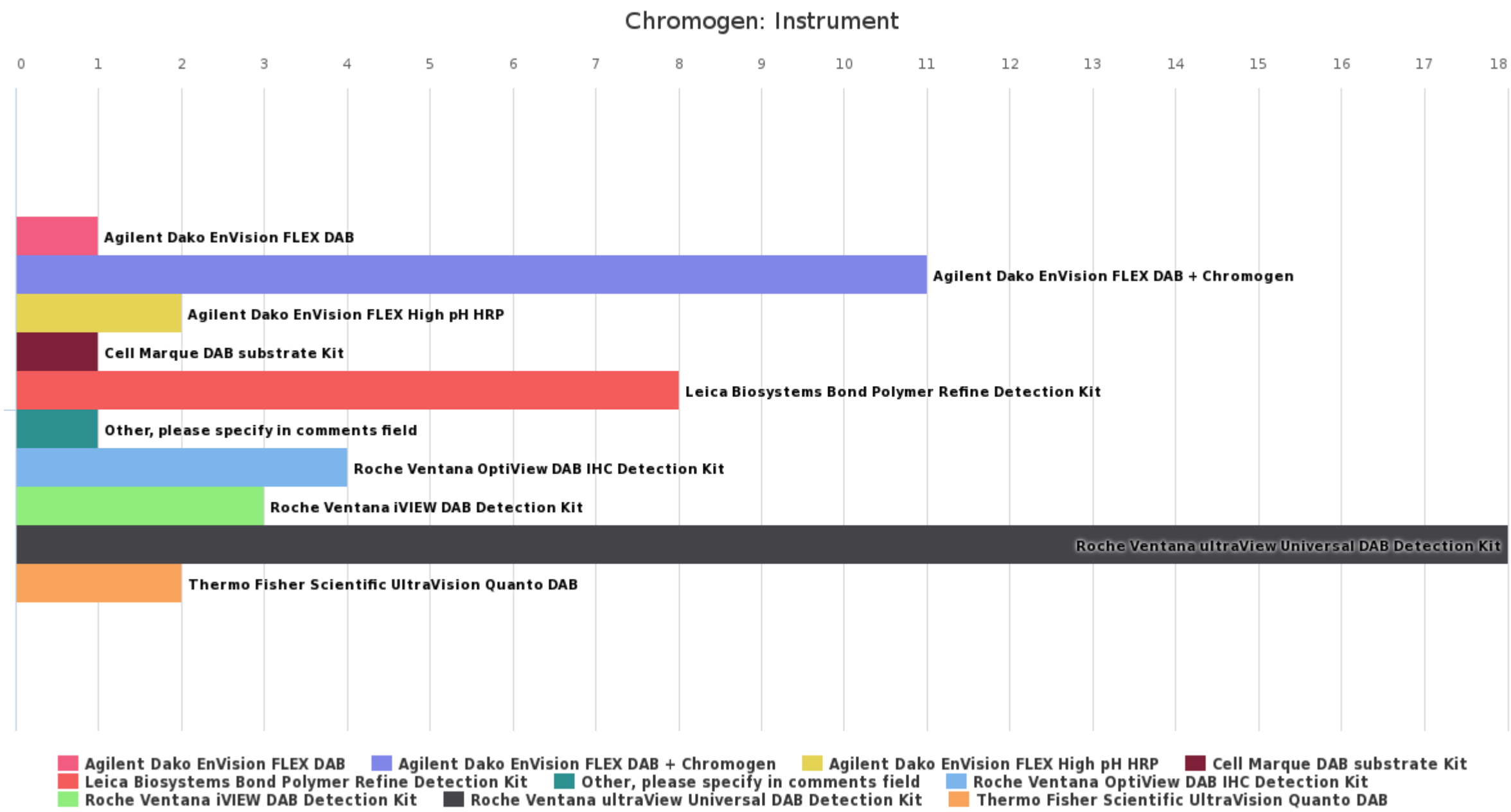


Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	7
Agilent Dako EnVision FLEX High pH HRP	7
Cell Marque HiDef Detection HRP Polymer System	1
Leica Biosystems Bond Polymer Refine Detection Kit	9
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	4
Roche Ventana iVIEW DAB Detection Kit	4
Roche Ventana ultraView Universal DAB Detection Kit	20
Thermo Fisher Scientific UltraVision Quanto Detection System HRP DAB	2
Total:	55

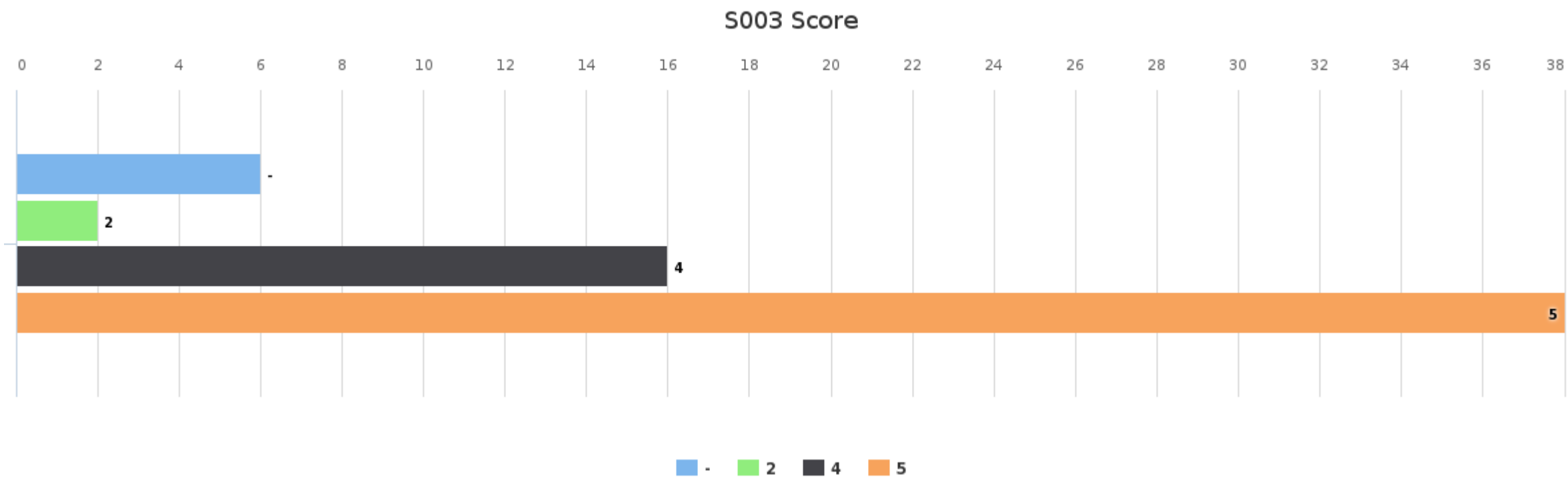


CHROMOGEN

Chromogen	Chromogen count
Detection kit	55
Total:	55

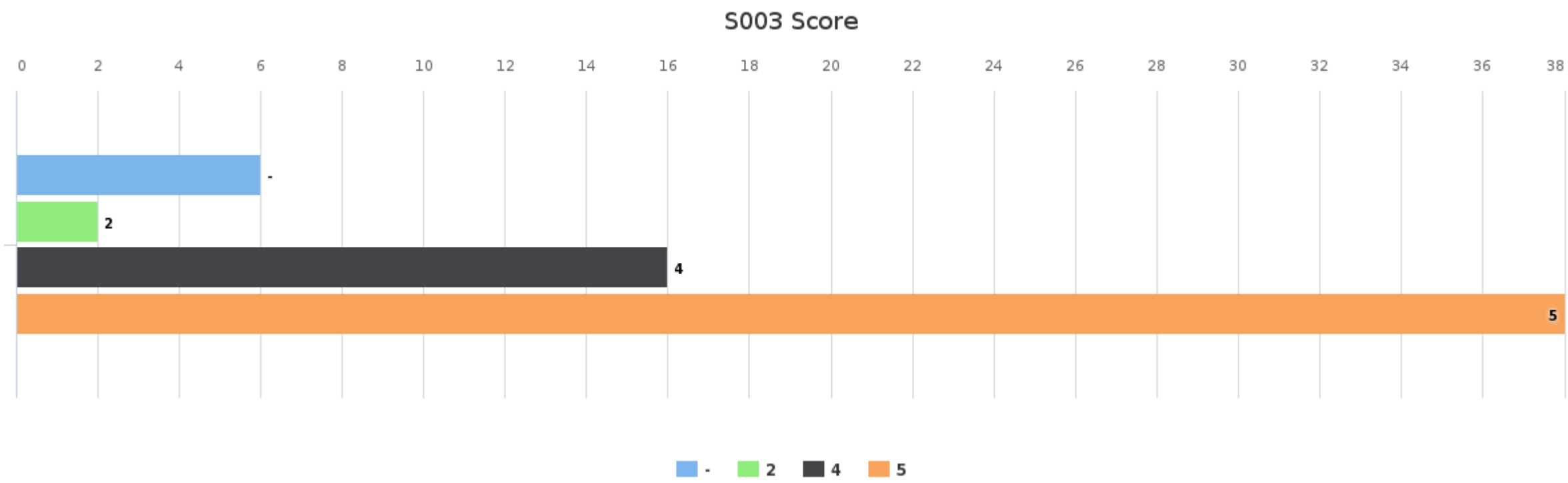


Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	1
Agilent Dako EnVision FLEX DAB + Chromogen	11
Agilent Dako EnVision FLEX High pH HRP	2
Cell Marque DAB substrate Kit	1
Leica Biosystems Bond Polymer Refine Detection Kit	8
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	4
Roche Ventana iVIEW DAB Detection Kit	3
Roche Ventana ultraView Universal DAB Detection Kit	18
Thermo Fisher Scientific UltraVision Quanto DAB	2
Total:	51



SCORE

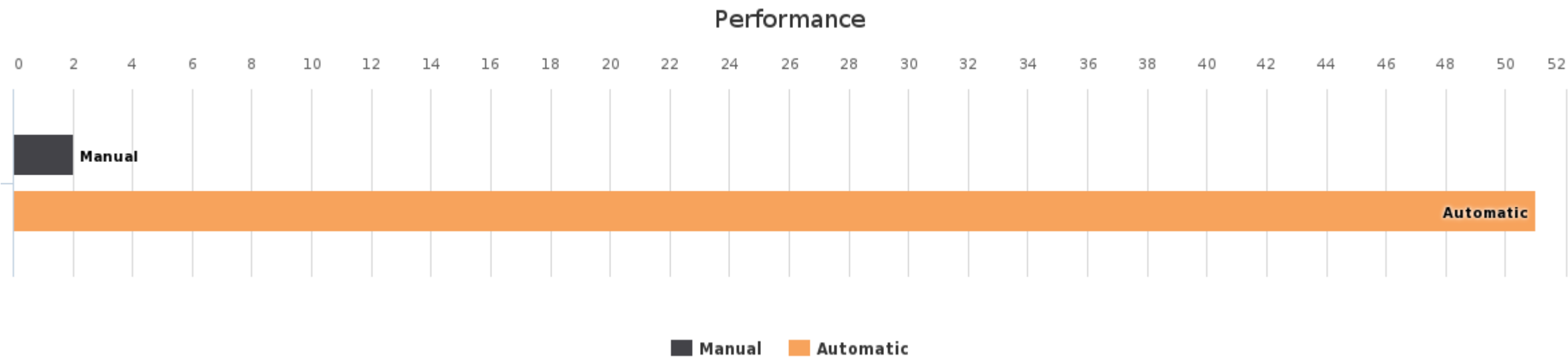
Score	Comment
-	
	Not available
2	
	Too strong hematoxylin, stains also cytoplasm. False negative staining in DLBCL. Uneaven staining.
	Too weak staining in the DLBCL, not optimal contrast between DAB and hematoxylin.
4	
	Contrast between hematoxylin and DAB is not optimal.
	Background staining/ unspecific staining of smooth muscle cells.
	Bakcground staining; smooth muscle cells
	Light background; smooth muscle cells of colon
	Slight background staining; smooth muscle cells.
	Slightly uneven staining
	Slightly weak, not continuous membrane staining, nuclear bubbling (usually due to too strong pretreatment).
	Some background staining of the colon epithelial cells and the smooth muscle cells.
	Staining gradient; technical issue.
	Strong staining, some background staining.
	Technical issue: Precipitates of hematoxylin and DAB
	Technical issue; hematoxylin and DAB precipitate
	Too strong hematoxylin; low contrast.
	Uneven staining
	Uneven staining, not optimal contrast, too strong hematoxylin.
	Unspecific staining of the colon; smooth muscle cells and laminull propria leukocytes, epithelial cells
5	
	Excellent
Total:	



Score	Score count
-	6
2	2
4	16

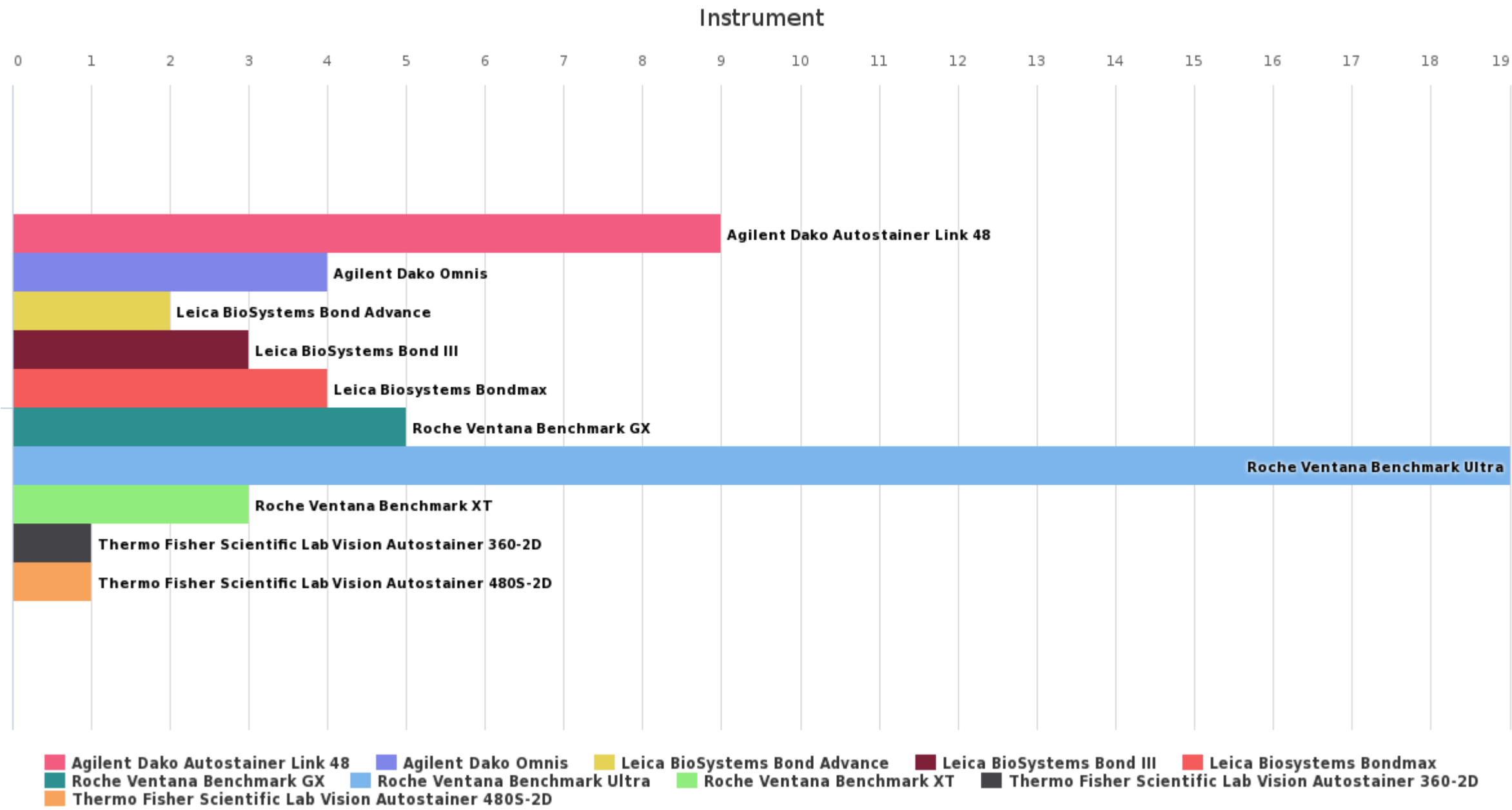
5	38
Total:	62

S004 | Sample S004 CD30

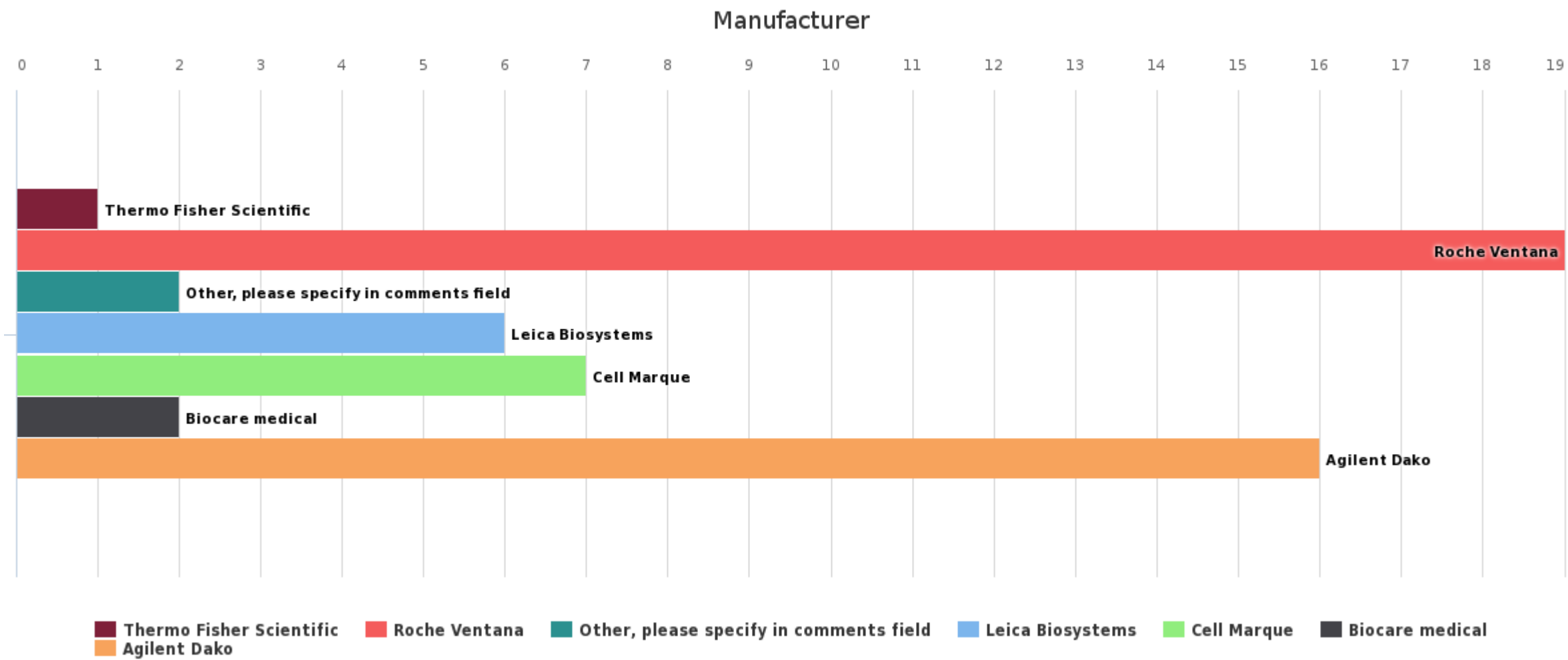


GENERAL DETAILS OF STAINING

Performance	Performance count
Automatic	51
Manual	2
Total:	53

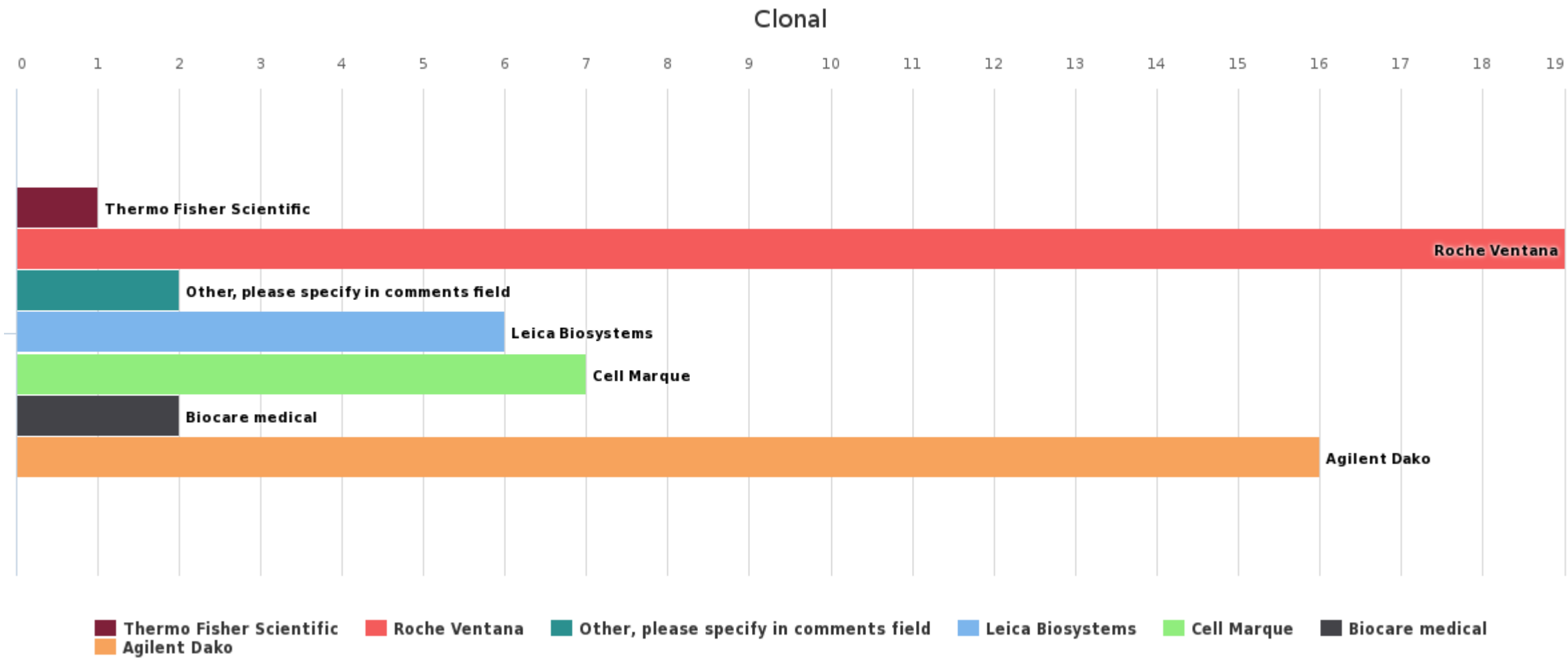


Instrument	Instrument count
Agilent Dako Autostainer Link 48	9
Agilent Dako Omnis	4
Leica BioSystems Bond Advance	2
Leica BioSystems Bond III	3
Leica Biosystems Bondmax	4
Roche Ventana Benchmark GX	5
Roche Ventana Benchmark Ultra	19
Roche Ventana Benchmark XT	3
Thermo Fisher Scientific Lab Vision Autostainer 360-2D	1
Thermo Fisher Scientific Lab Vision Autostainer 480S-2D	1
Total:	51

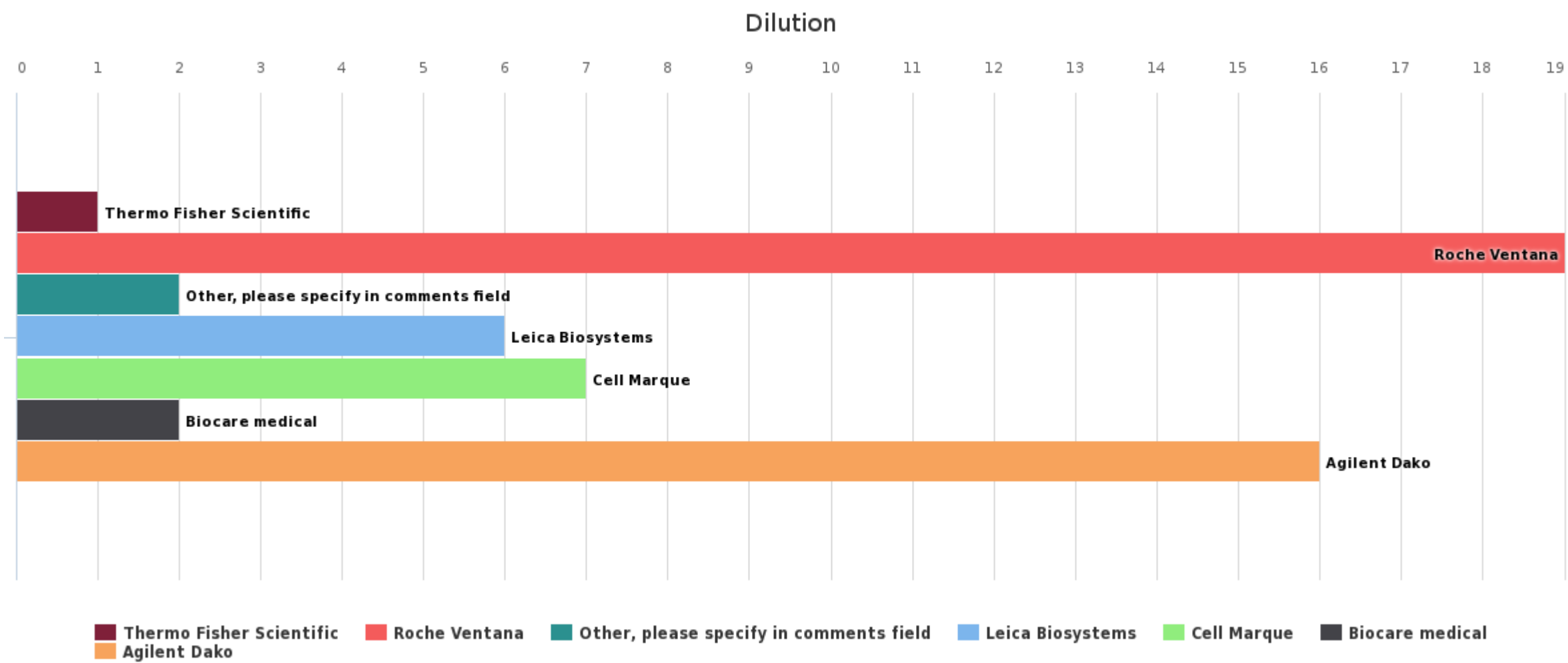


PRIMARY ANTIBODY

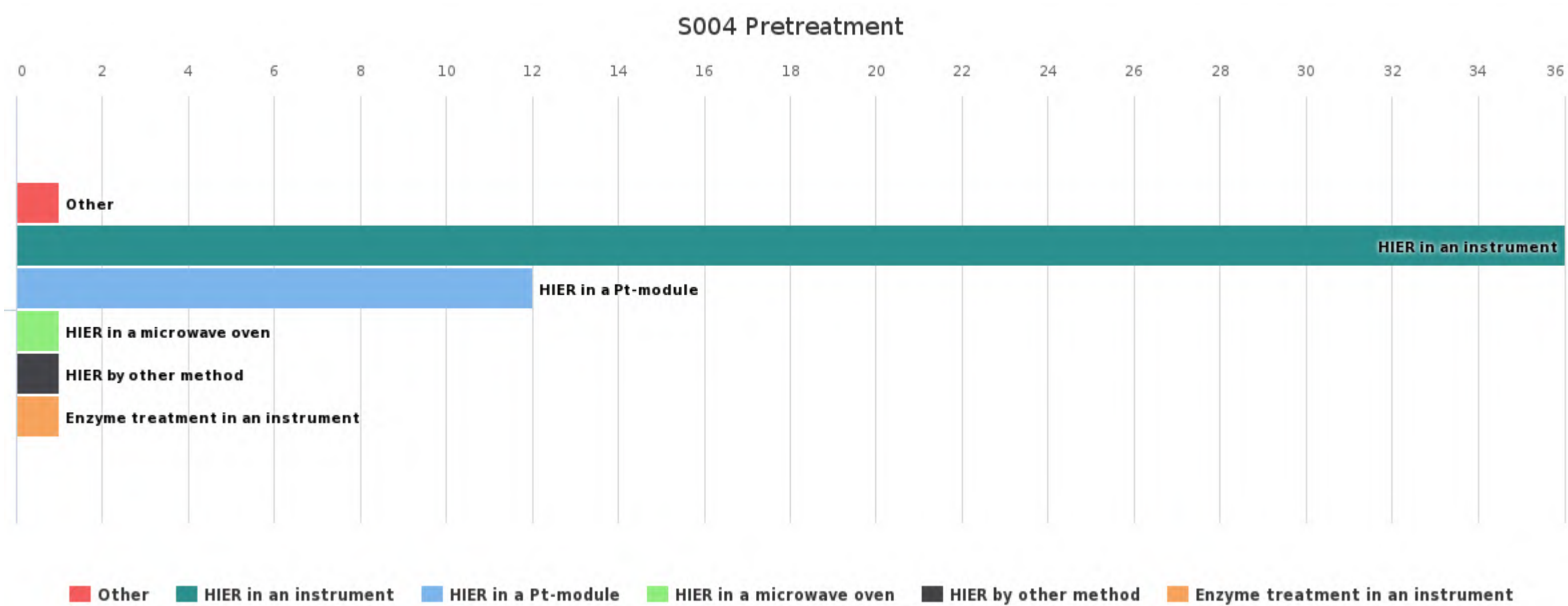
Manufacturer	Manufacturer count
Agilent Dako	16
Biocare medical	2
Cell Marque	7
Leica Biosystems	6
Other, please specify in comments field	2
Roche Ventana	19
Thermo Fisher Scientific	1
Total:	53



Clonal	Clonal count
Monoclonal	53
Total:	53

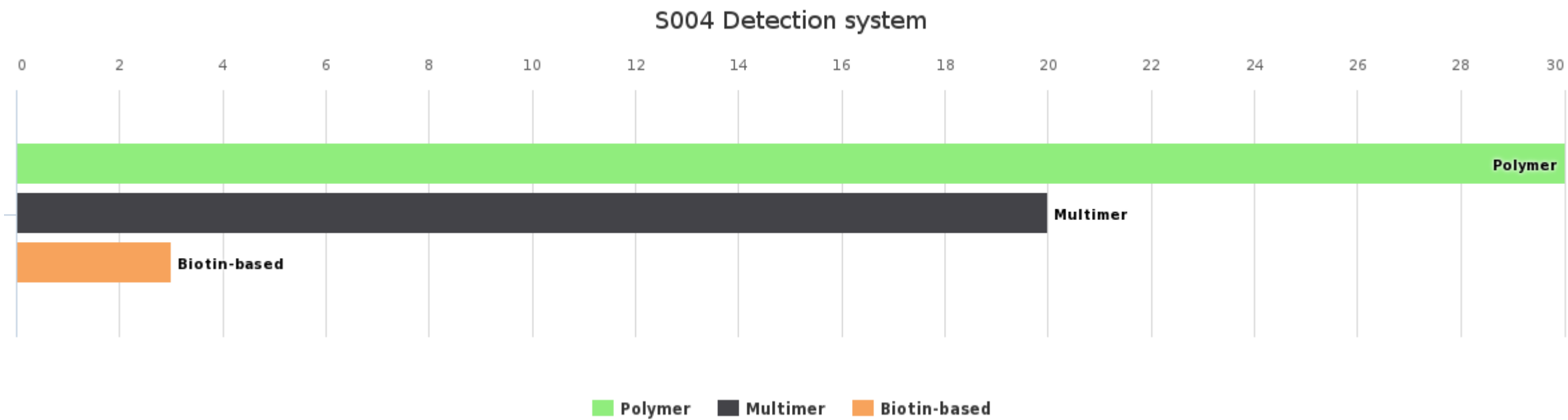


Dilution	Dilution count
Dilution	18
Ready-to-use	35
Total:	53



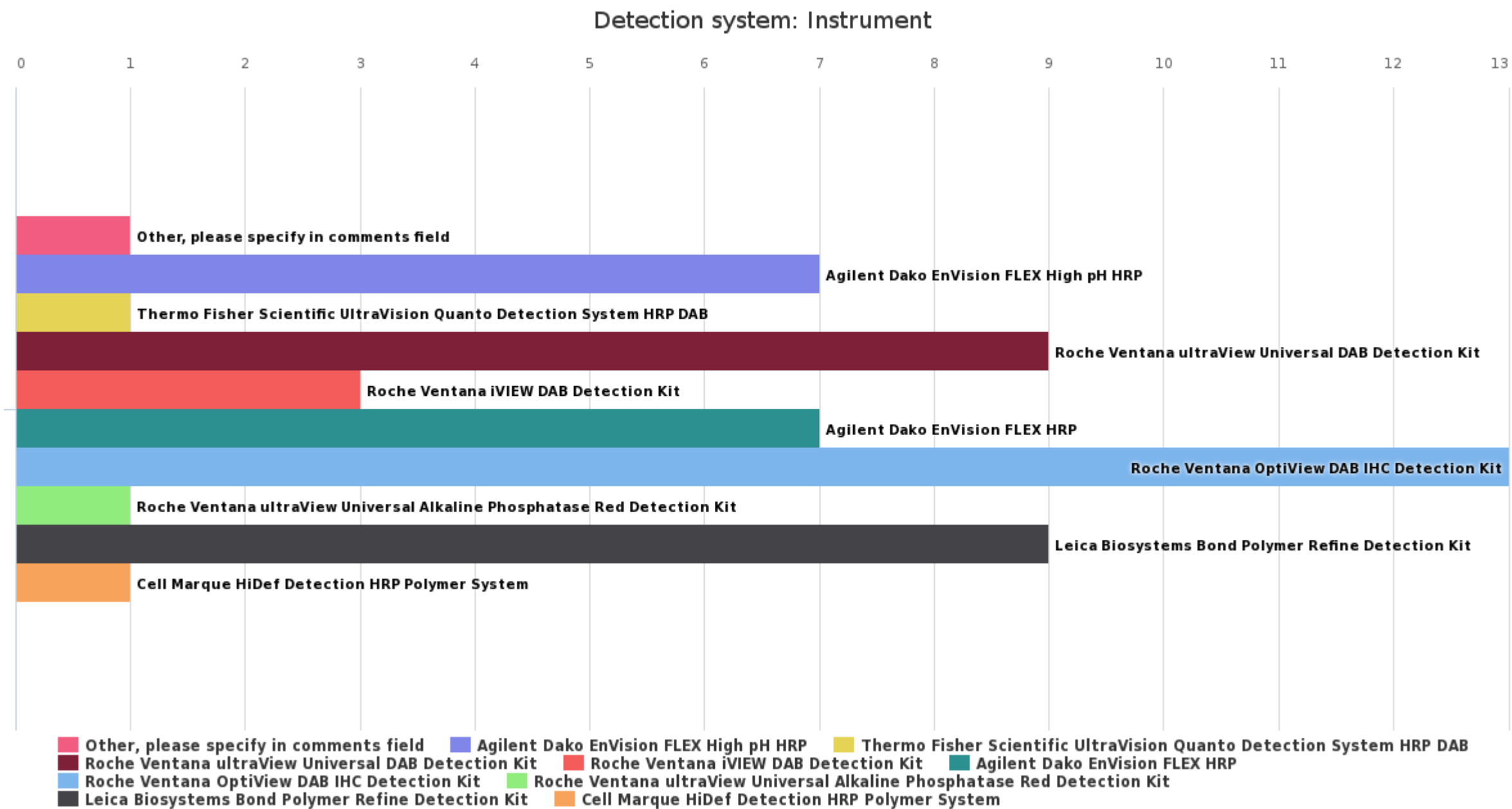
PRETREATMENT

Pretreatment	Pretreatment count
Enzyme treatment in an instrument	1
HIER by other method	1
HIER in a microwave oven	1
HIER in a Pt-module	12
HIER in an instrument	36
Other	1
Total:	52

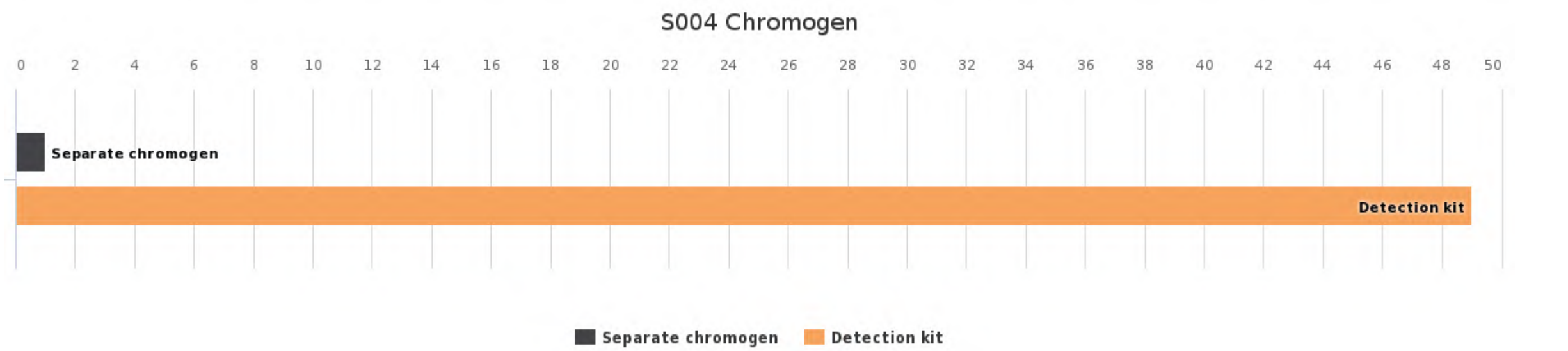


DETECTION SYSTEM

Detection system	Detection system count
Biotin-based	3
Multimer	20
Polymer	30
Total:	53

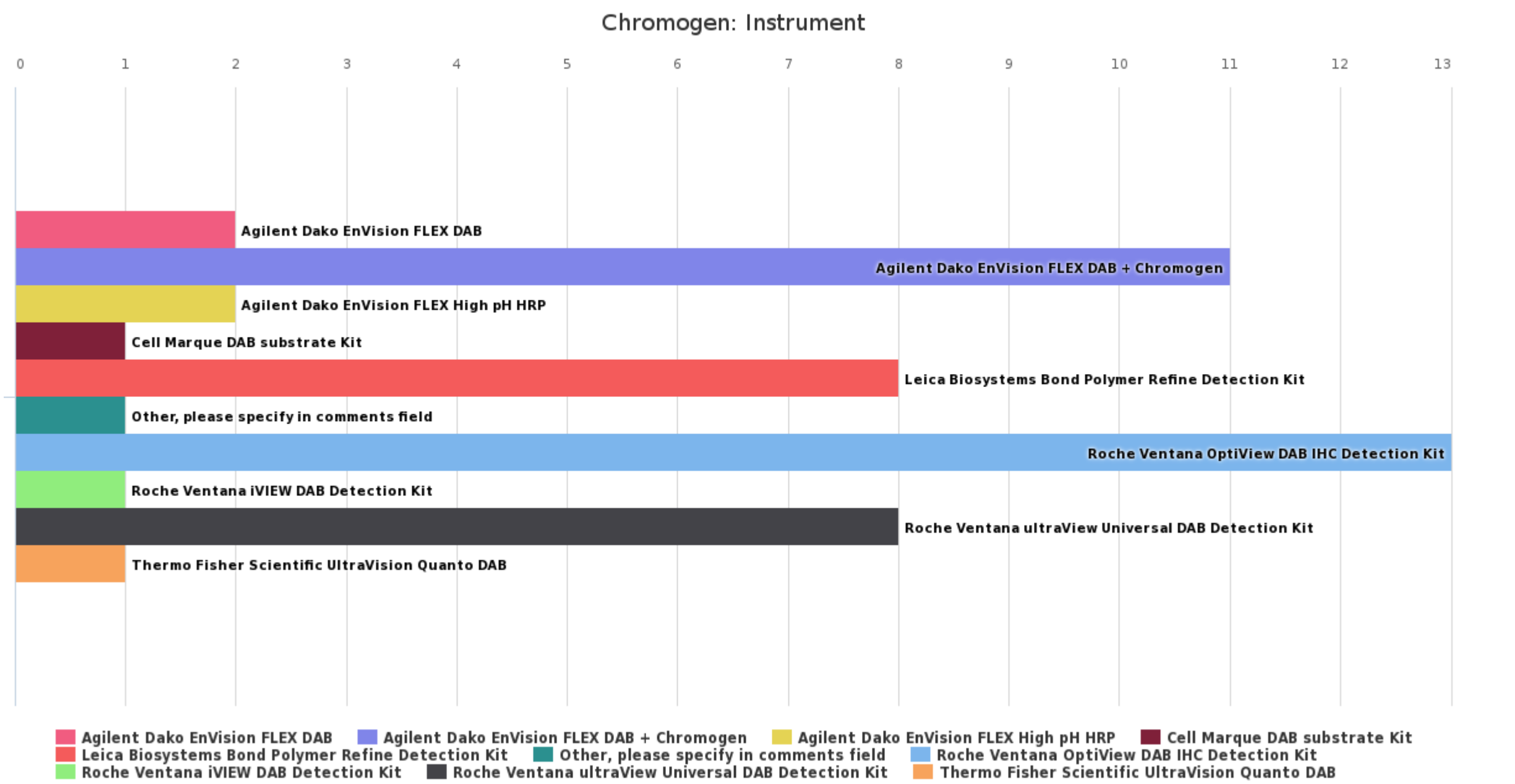


Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	7
Agilent Dako EnVision FLEX High pH HRP	7
Cell Marque HiDef Detection HRP Polymer System	1
Leica Biosystems Bond Polymer Refine Detection Kit	9
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	13
Roche Ventana iVIEW DAB Detection Kit	3
Roche Ventana ultraView Universal Alkaline Phosphatase Red Detection Kit	1
Roche Ventana ultraView Universal DAB Detection Kit	9
Thermo Fisher Scientific UltraVision Quanto Detection System HRP DAB	1
Total:	52

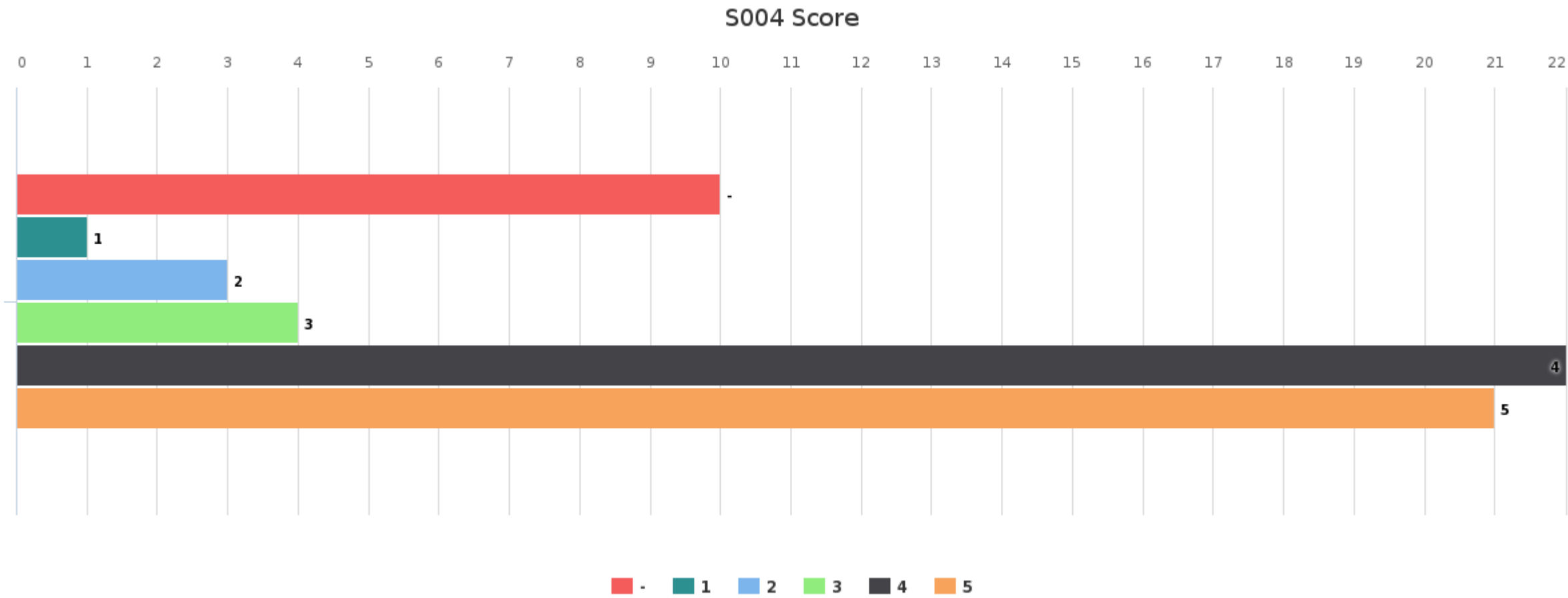


CHROMOGEN

Chromogen	Chromogen count
Detection kit	49
Separate chromogen	1
Total:	50

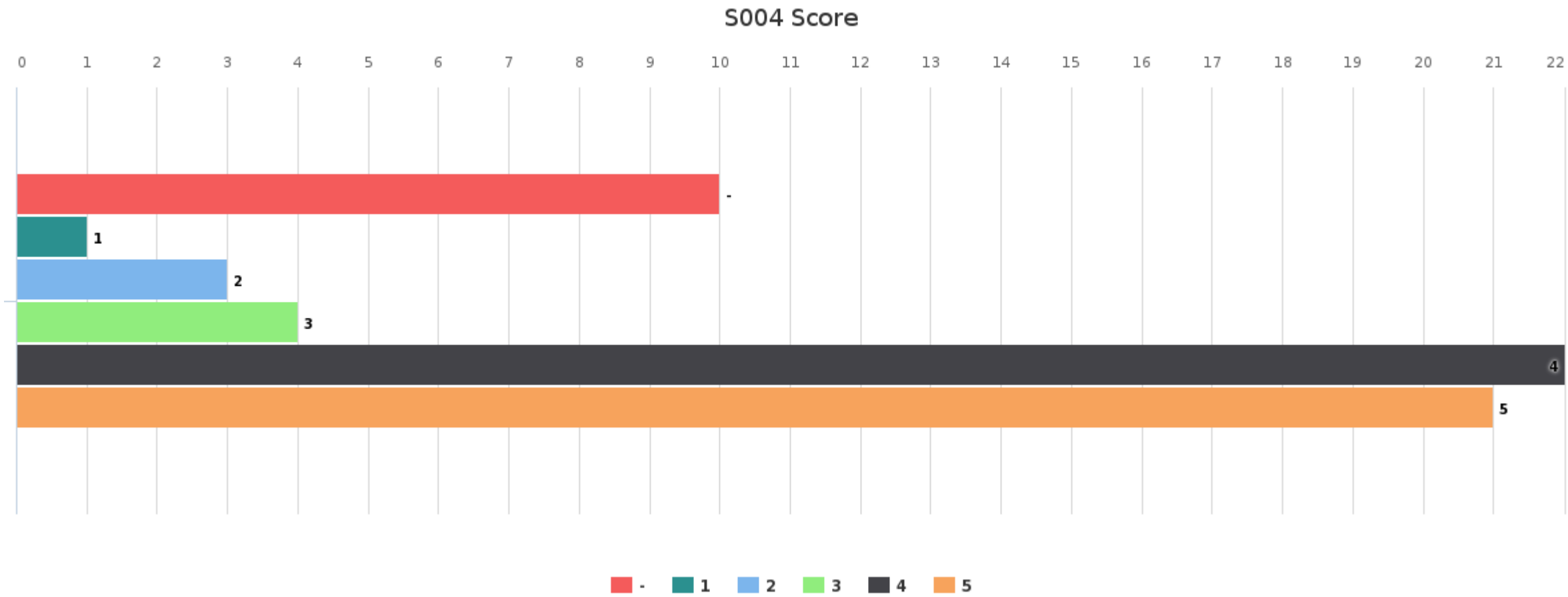


Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	2
Agilent Dako EnVision FLEX DAB + Chromogen	11
Agilent Dako EnVision FLEX High pH HRP	2
Cell Marque DAB substrate Kit	1
Leica Biosystems Bond Polymer Refine Detection Kit	8
Other, please specify in comments field	1
Roche Ventana OptiView DAB IHC Detection Kit	13
Roche Ventana iVIEW DAB Detection Kit	1
Roche Ventana ultraView Universal DAB Detection Kit	8
Thermo Fisher Scientific UltraVision Quanto DAB	1
Total:	48



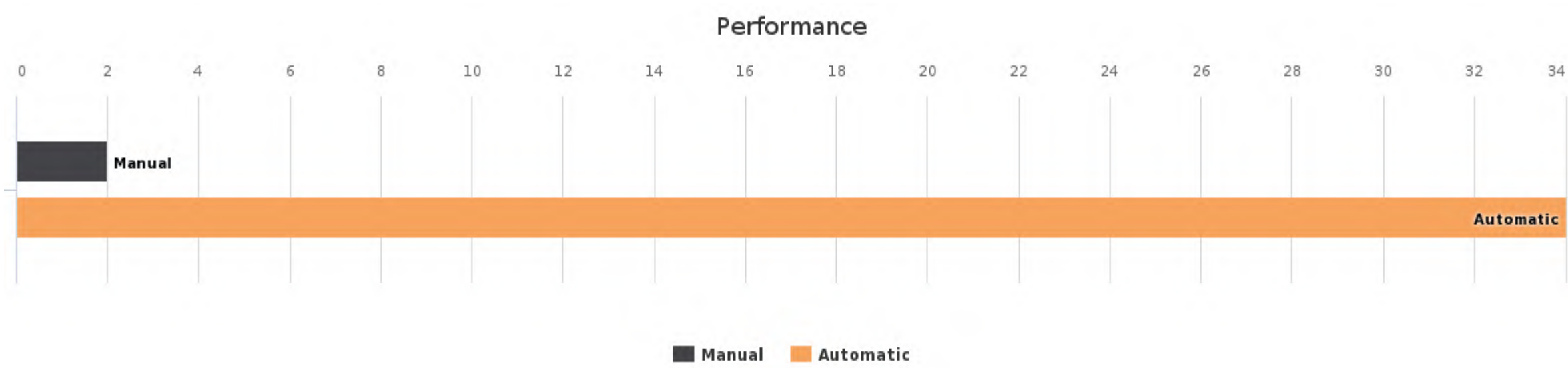
SCORE

Score	Comment
-	
	Not available
1	
	No activated T- and B-cells are stained in tonsil, hardly any of the RS cells are stained in the Hodgking's lymphoma, very weak staining in theembryonulll ca.
2	
	Too weak in all tissues
	Too weak in all tissues; only scattered RS cells are stained faintly.
	Too weak staining; emryonulll ca
3	
	Background staining, slightly too weakstaining in all tissues
	Strong background staining.
	Too weak staining in all tissues
	Too weak staining of the RS cells, activated B and T cells and embryo carcinoma.
4	
	Background staining in the tonsill; epithelial cells and vessels
	Light background due to RED chromogen
	Light background staining
	Slightly weak staining of activated T- and B-cells in the tonsil.
	Slight background staining
	Slight background staining in the tonsil
	Slight background staining, slightly weak staining in all tissues, not all activated T- and B-cells are stained in the tonsil.
	Slight background staining; Tonsil.
	Slightly too weak staining.
	Slightly weak
	Slightly weak staining in all tissues
	Slightly weak staining especially in the tonsil.
	Slightly weak staining in all tissues.
	Slightly weak staining of activated T- and B-cells in tonsil, nuclear bubbling.
	Slightly weak staining of the activated T- and B-cells in tonsil, nuclear bubbling.
	Slightly weak staining of theembryonulll ca, slight background.
	Slightly weak staining.
	Slightly weak staining in all tissues.
	Technical issue; hematoxylin and DAB precipitate
	Too strong hematoxylin, Slight background.
5	
	Excellent
Total:	



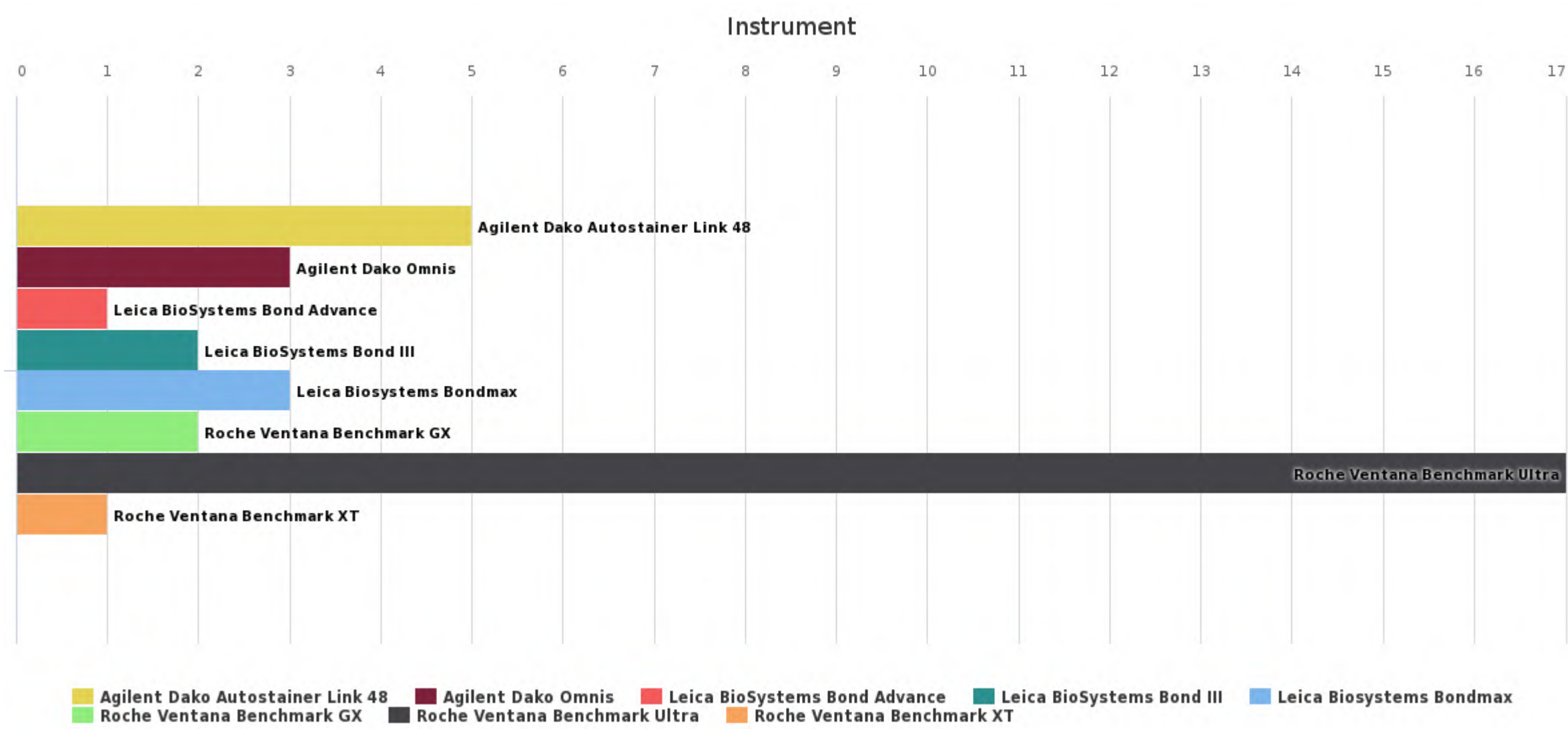
Score	Score count
-	10
1	1
2	3
3	4
4	22
5	21
Total:	61

S005 | Sample S005 PAX5

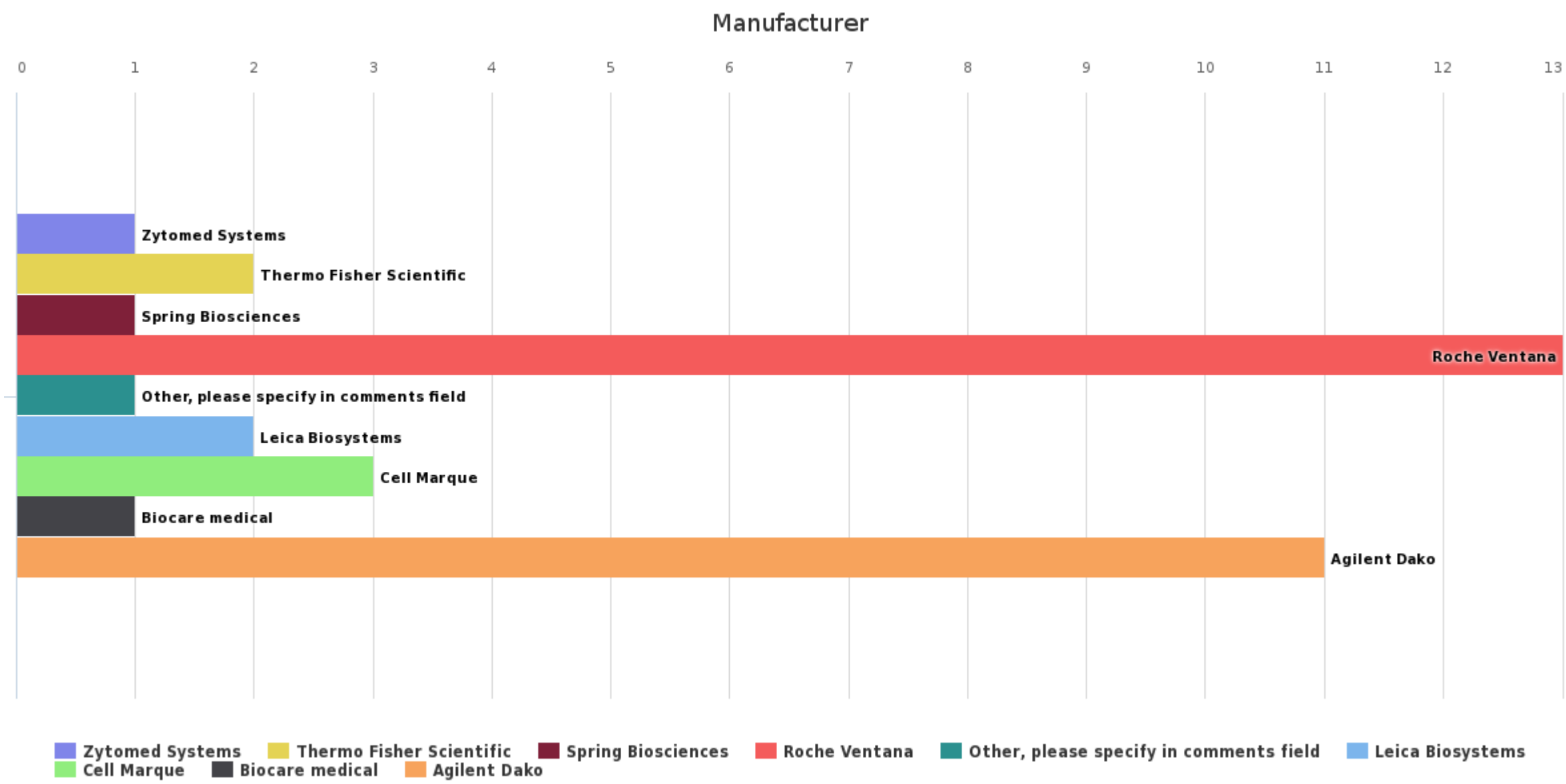


GENERAL DETAILS OF STAINING

Performance	Performance count
Automatic	34
Manual	2
Total:	36

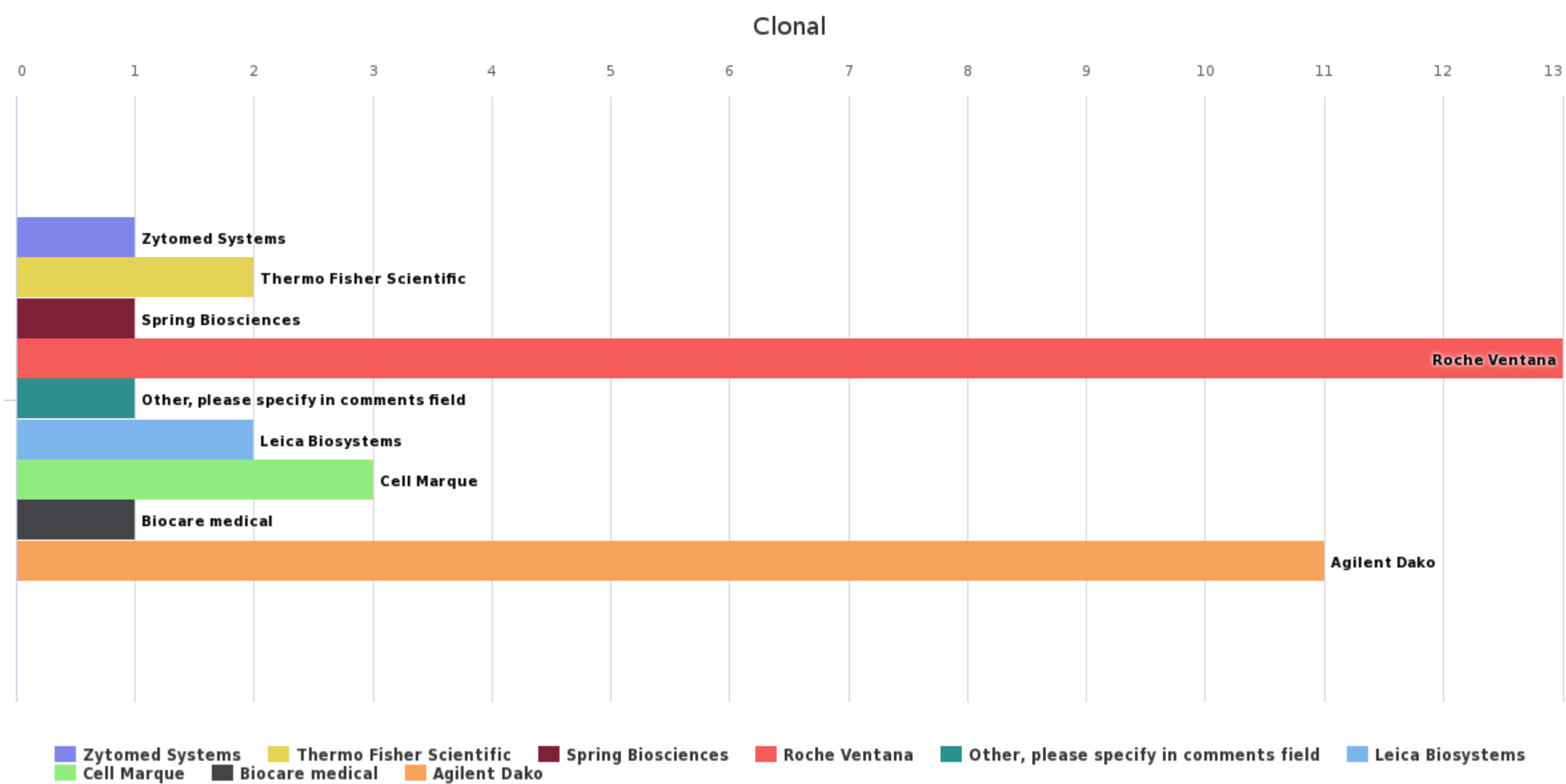


Instrument	Instrument count
Agilent Dako Autostainer Link 48	5
Agilent Dako Omnis	3
Leica BioSystems Bond Advance	1
Leica BioSystems Bond III	2
Leica Biosystems Bondmax	3
Roche Ventana Benchmark GX	2
Roche Ventana Benchmark Ultra	17
Roche Ventana Benchmark XT	1
Total:	34



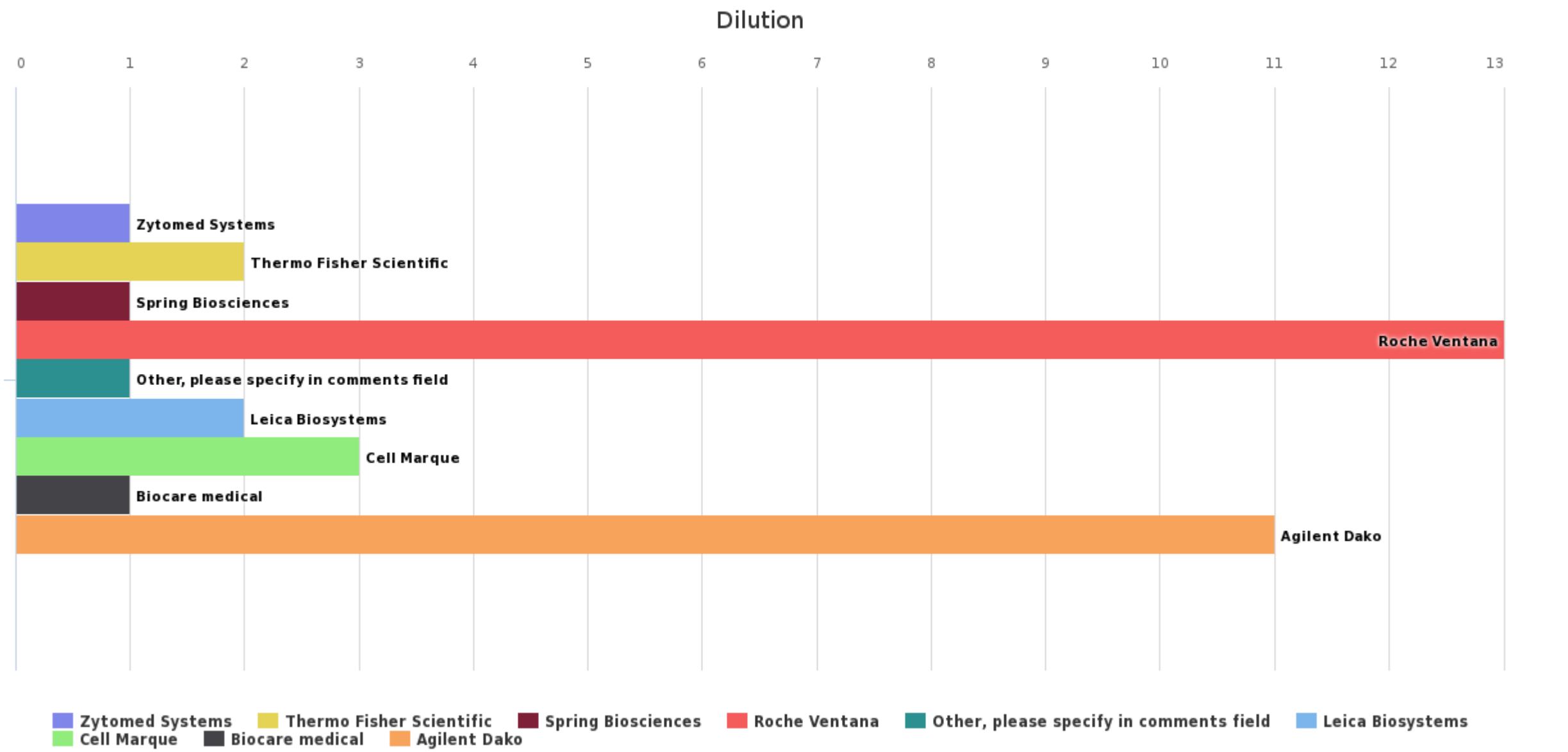
PRIMARY ANTIBODY

Manufacturer	Manufacturer count
Agilent Dako	11
Biocare medical	1
Cell Marque	3
Leica Biosystems	2
Other, please specify in comments field	1
Roche Ventana	13
Spring Biosciences	1
Thermo Fisher Scientific	2
Zytomed Systems	1
Total:	35

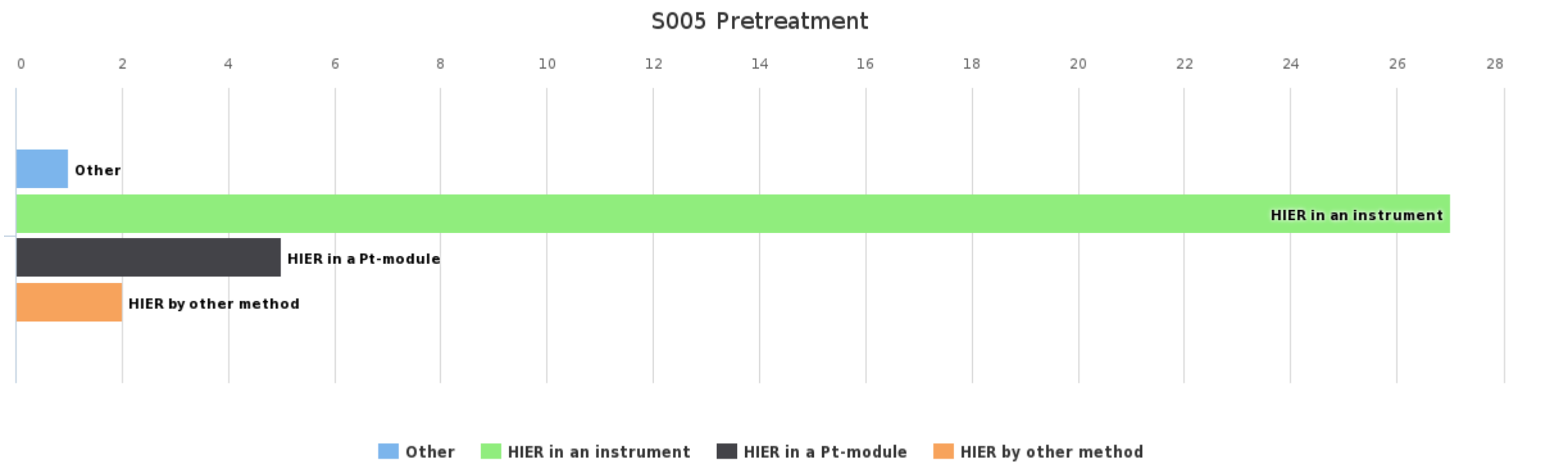


Clonal	Clonal count
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Monoclonal	32
Polyclonal	3
Total:	35

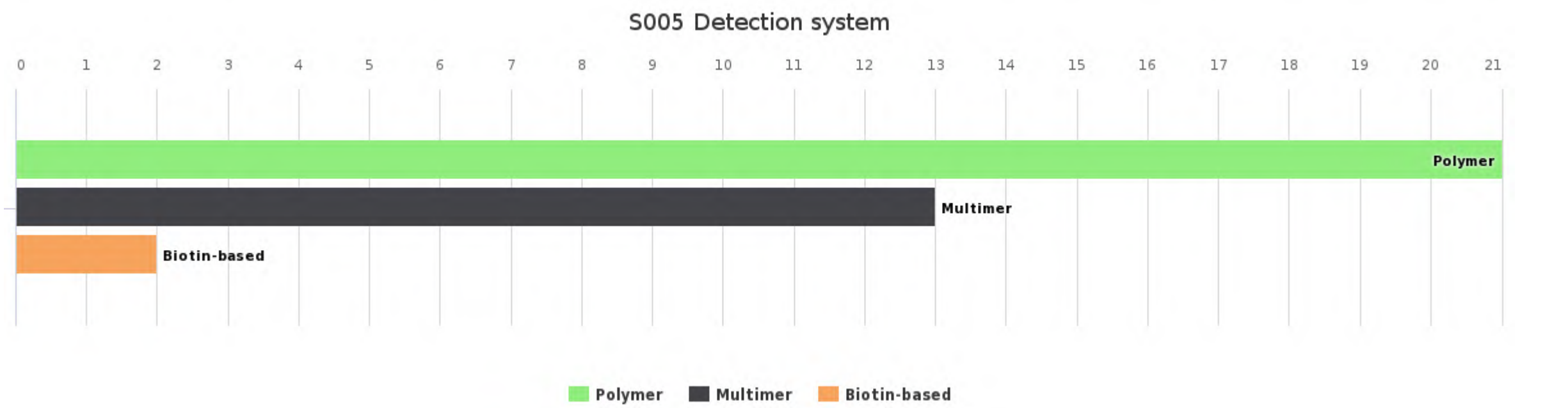


Dilution	Dilution count
Dilution	11
Ready-to-use	24
Total:	35



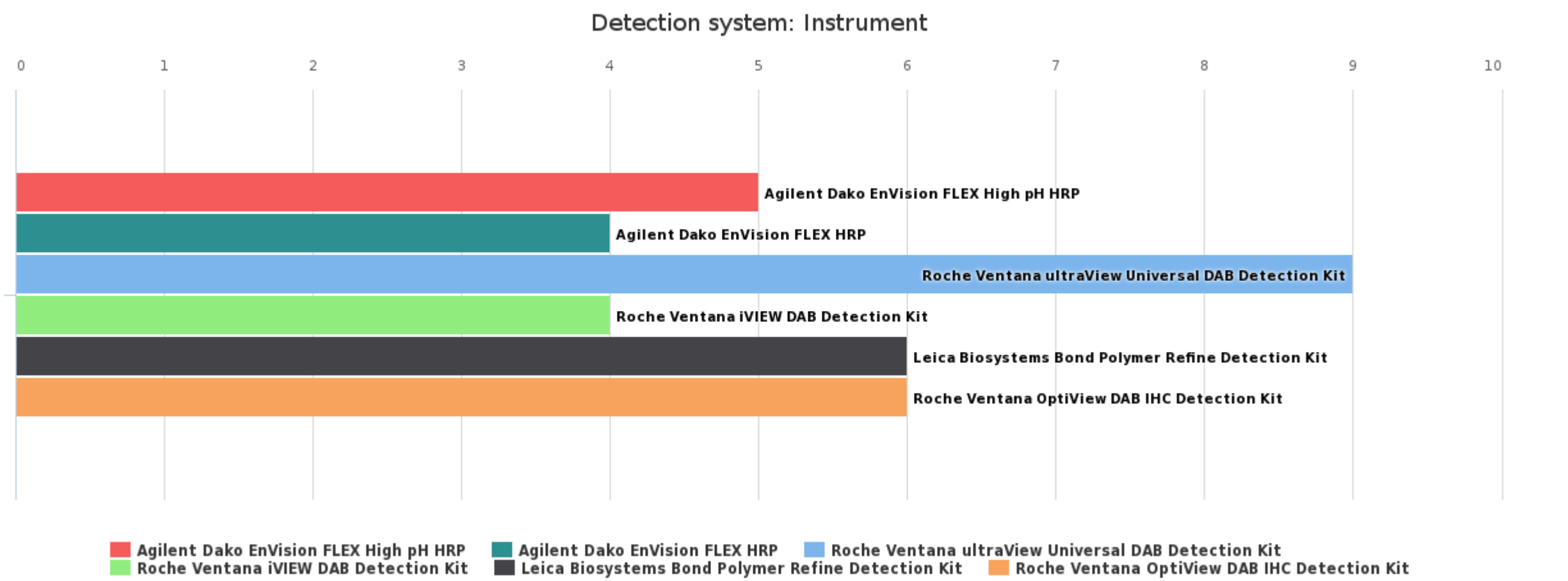
PRETREATMENT

Pretreatment	Pretreatment count
HIER by other method	2
HIER in a Pt-module	5
HIER in an instrument	27
Other	1
Total:	35

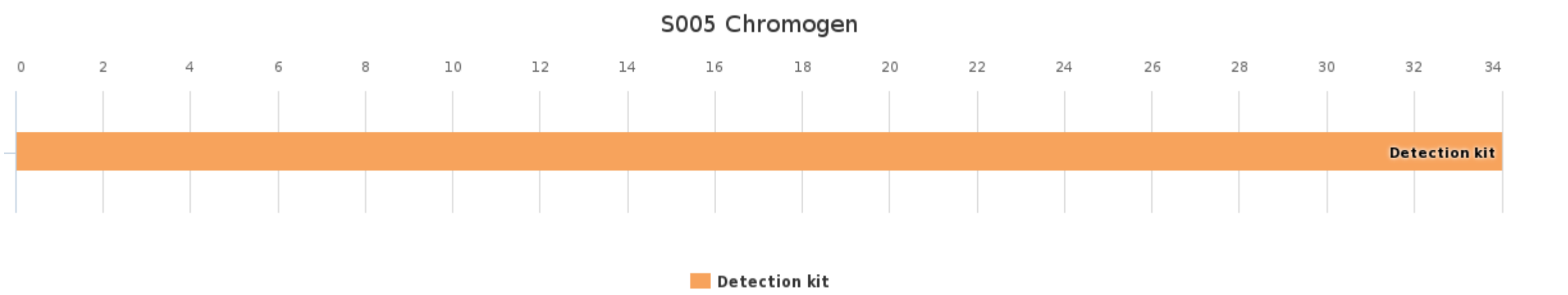


DETECTION SYSTEM

Detection system	Detection system count
Biotin-based	2
Multimer	13
Polymer	21
Total:	36



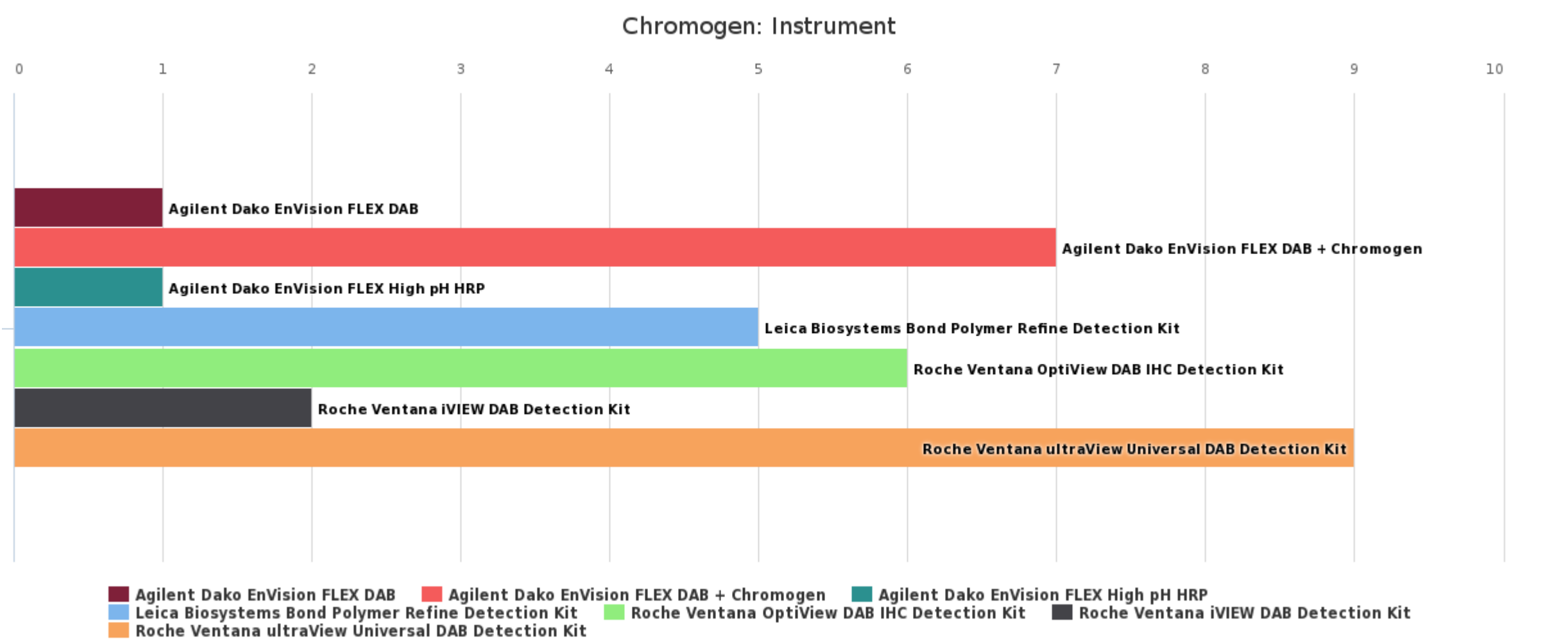
Detection system: Instrument	Detection system: Instrument count
Agilent Dako EnVision FLEX HRP	4
Agilent Dako EnVision FLEX High pH HRP	5
Leica Biosystems Bond Polymer Refine Detection Kit	6
Roche Ventana OptiView DAB IHC Detection Kit	6
Roche Ventana iVIEW DAB Detection Kit	4
Roche Ventana ultraView Universal DAB Detection Kit	9
Total:	34



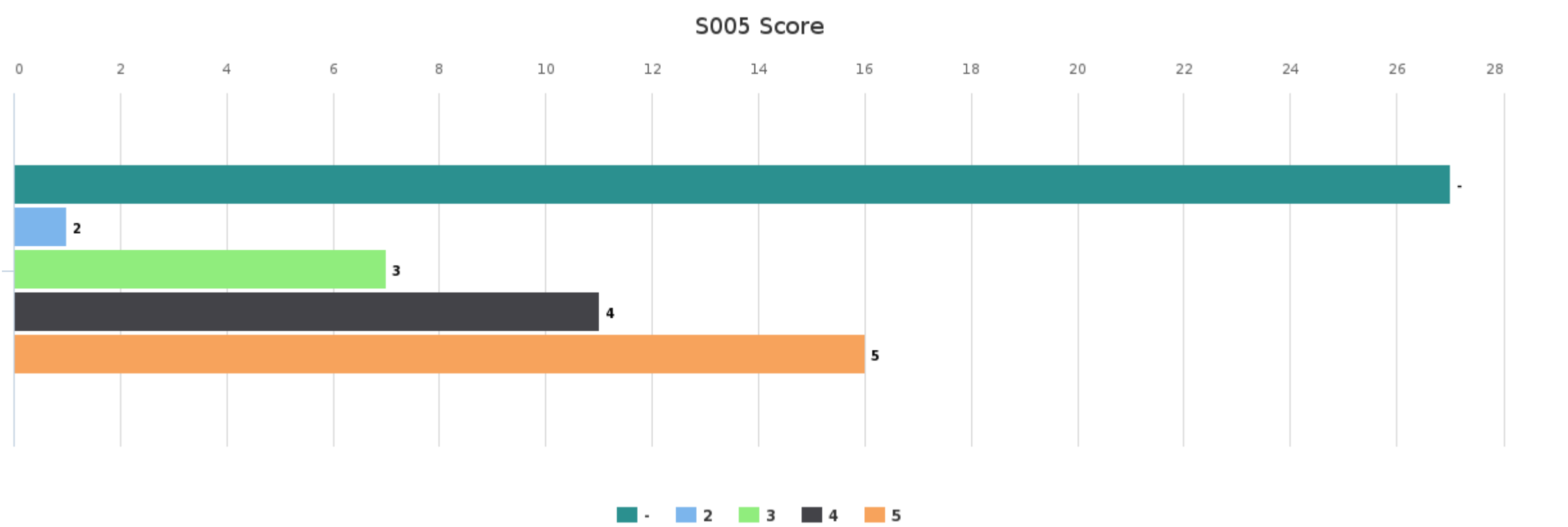
CHROMOGEN

Chromogen	Chromogen count
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Detection kit	34
Total:	34



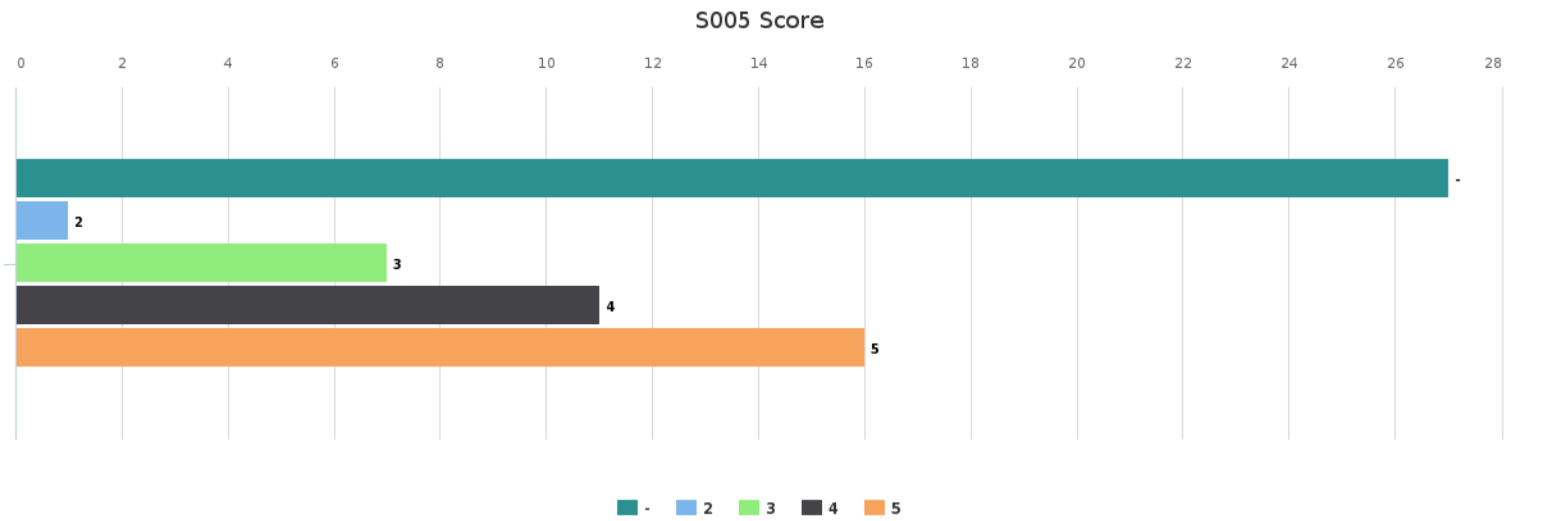
Chromogen: Instrument	Chromogen: Instrument count
Agilent Dako EnVision FLEX DAB	1
Agilent Dako EnVision FLEX DAB + Chromogen	7
Agilent Dako EnVision FLEX High pH HRP	1
Leica Biosystems Bond Polymer Refine Detection Kit	5
Roche Ventana OptiView DAB IHC Detection Kit	6
Roche Ventana iVIEW DAB Detection Kit	2
Roche Ventana ultraView Universal DAB Detection Kit	9
Total:	31



SCORE

Score	Comment
-	
	Not available
2	No staining in the RS cells of Hodgking's lymphoma. Weak staining in the tonsil. Background staining on all tissues.
3	Good staining, but nuclear morphology has suffered (nuclear bubbling and shrinking) Too weak staining of the RS-cells Too weakly stained RS-cells. Unspecific cytoplasmic staining; endothelial cells and macrophage positivity

	Unspecific cytoplasmic staining; endothelial positivity
	Unspecific staining of endothelial cells and macrophages. Weak staining of RS cells in Hodgkins lymphoma.
	Weak HTX staining. Slightly weak staining of the RS cells. Unspecific cytoplasmic staining and staining of endothelial cells. Nuclear shrinking due to pretreatment.
4	
	Background staining; unspecific staining of macrophages and endothelial cells.
	Nuclear bubbling. Ususally due to too strong pretreatment.
	Nuclear shrinking (drying after pretreatment or too strong pretreatment).
	Slight background staining; endothelial cells and macrophages
	Slightly weak
	Slightly weak staining in RS-cells
	Slightly weak staining of the RS cells, unspecific staining of macrophages
	Slightly weak staining of the RS cells.
	Slightly weak staining of the RS-cells and unspecific cytoplasmic staining of endothelial cells.
	Slightly weak staining.
	Too weak stainig of the RS cells, unspecific background staining in the endotheal cells and macrophages
5	
	Excellent
Total:	



Score	Score count
-	27
2	1
3	7
4	11
5	16
Total:	62

External Quality Assessment Scheme

Immunohistochemical staining methods Round 1, 2020

Specimens

Sample S001 (LQ778320011), S002 (LQ778320012), S003 (LQ778320013), S004 (LQ778320014) and S005 (LQ778320015) 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

Criteria for staining

CD3

1. Tonsil

Interfollicular T-cells, dispersed mantle zone T-cells and germinal center T-cells show a moderate to strong distinct membranous staining reaction. No staining shown in B-cells or other cell types. (Image 1)

2. Peripheral T-cell lymphoma

An at least weak to moderate and distinct membranous staining reaction in the neoplastic T-cells.

3. Liver

A strong and distinct membranous staining of dispersed normal T-cells. No staining in other cells.

2020-08-21

FINAL REPORT

Product no. 6600 /6600S

Subcontracting: Sample preparation, sample pretesting

Samples sent	2020-03-17
Round closed	2020-05-22
Final report	2020-08-21

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
pia.eloranta@labquality.fi

Expert

Tiia-Maria Kukkonen, M.D., pathologist
Sanna Kirjavainen, Ph.D., Medical cell biologist
Satu Remes, Fil. Lic., Medical cell biologist

Labquality Oy

Kumpulantie 15
FI-00520 HELSINKI
Finland

Tel. + 358 9 8566 8200
Fax + 358 9 8566 8280

info@labquality.fi
www.labquality.fi



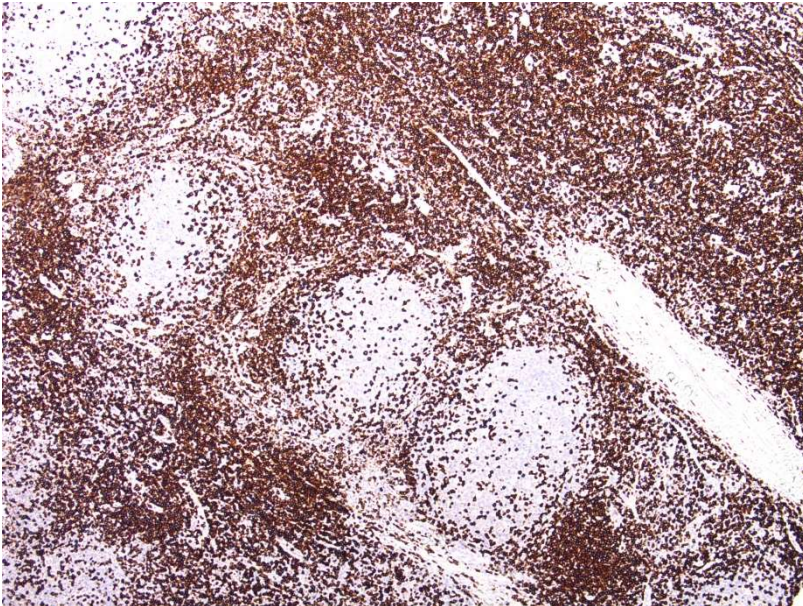


Image1. CD3: Optimal staining in tonsil.

CD5

1. Liver

A strong and distinct membranous staining of dispersed normal T-cells.
No staining in other cells.

2. Tonsil

A moderate to strong and distinct membranous staining reaction of the T-cells in both T-zones and in germinal centers. A weak to moderate distinct membranous staining reaction in dispersed B-cells in the mantle zone. No staining in germinal center B-cells.

3. Mantle cell lymphoma

A moderate to strong distinct membranous staining reaction of all neoplastic cells and T-cells between neoplastic cells. Image 2.

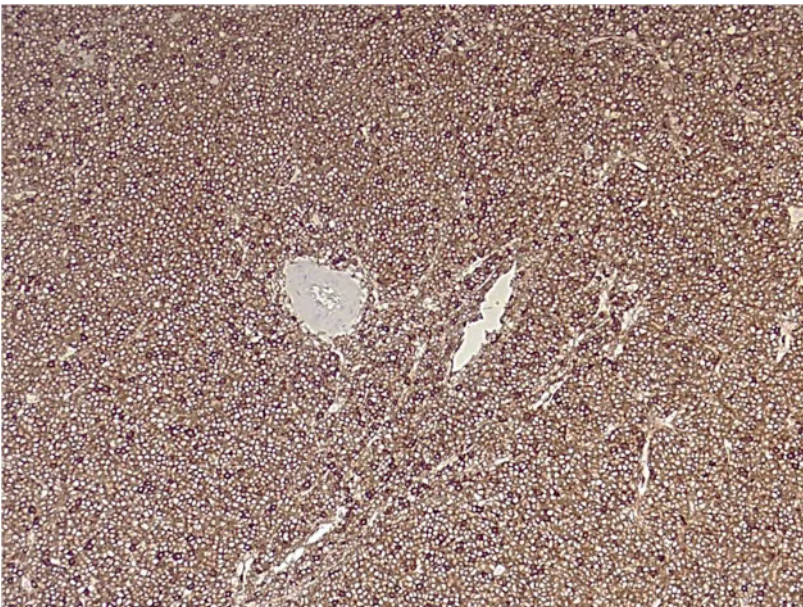


Image 2. CD5: Optimal staining in mantle cell lymphoma

CD20

1. Tonsil

A strong to very strong membranous staining of the mantle zone B-cells, the germinal center B-cells and the inter-follicular B-cells in the tonsil. Image 1.

2. DLBCL

A strong membranous staining of vast majority of the neoplastic cells.

3. Colon

A strong membranous staining of the B-cells in the submucosal lymph follicles (image 3A) in a lamina propria. No staining in other cells (image 3B).

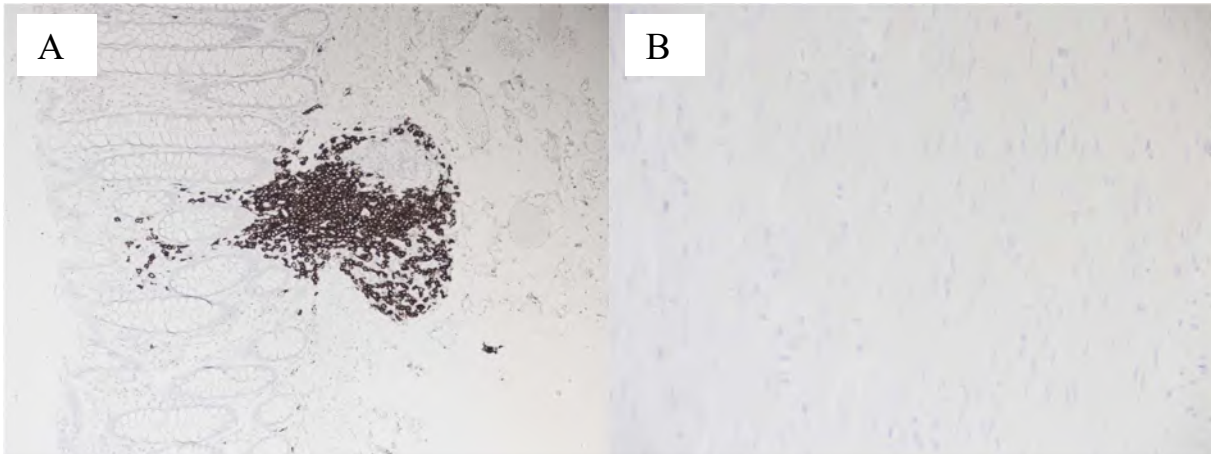


Image 3 CD20: Optimal staining in colon.

A: Submucosal lymph follicle,
B: Smooth muscle cells of muscularis externa

CD30

1. Embryonal carcinoma

A moderate to strong, predominantly membranous staining reaction of the neoplastic cells in the embryonal carcinoma.

2. Hodgkin's lymphoma

An at least moderate predominantly membranous staining reaction of all the neoplastic cells in the Hodgkin lymphoma. A moderate to strong predominantly membranous and a Golgi zone (cytoplasmic) staining reaction of the at least majority of Hodgkin and Reed-Sternberg cells. Image 4.

3. Tonsil

An at least weak distinct membranous staining reaction of the activated T-cells and a subpopulation of the activated B-cells primarily located in the rim of the germinal centers. Image 5.

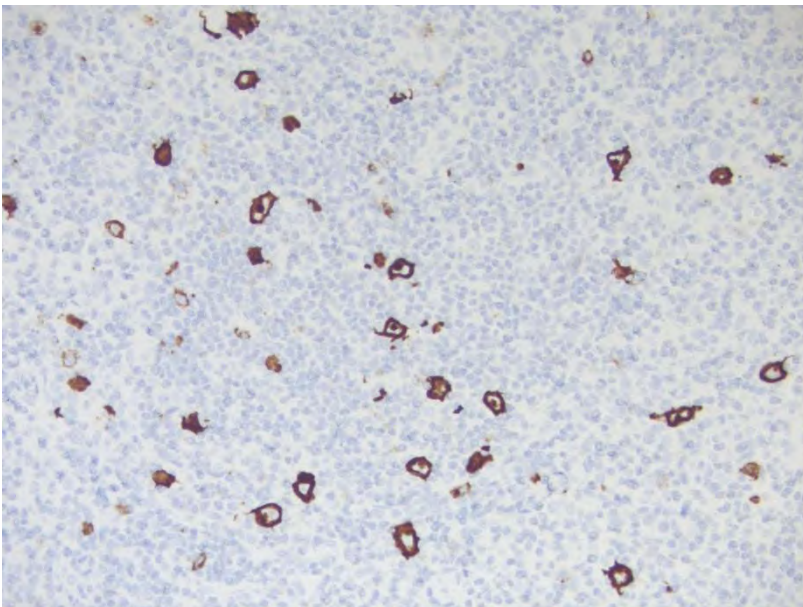


Image 4. CD30: Optimal staining in Hodgkin's Lymphoma

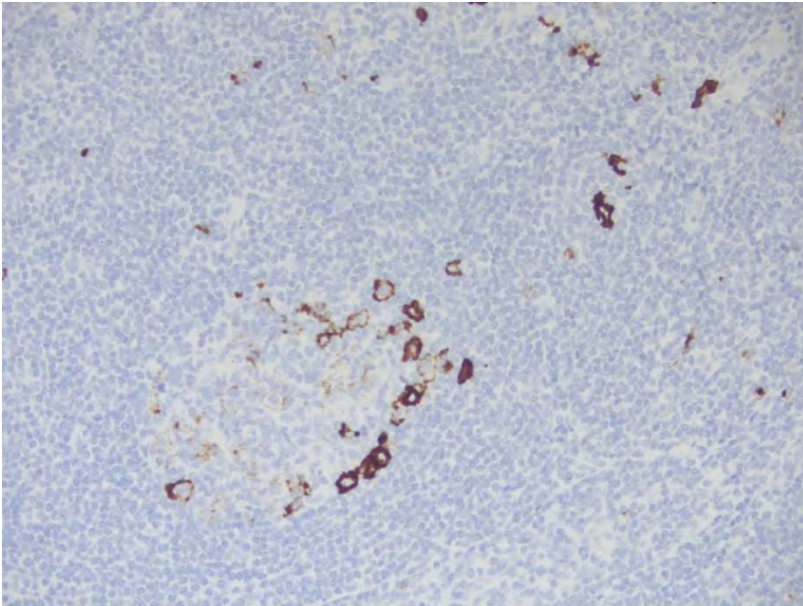


Image 5. CD30: Optimal staining in tonsil.

PAX5

1. DLBCL

A strong distinct nuclear staining reaction of the neoplastic cells.

2. Hodgkin's lymphoma

A weak to moderate nuclear staining reaction of a majority of the Hodgkin and the Reed-Sternberg cells. Image 6.

3. Tonsil

A distinct and strong nuclear staining reaction of the mantle zone B-cells, the germinal center B-cells and the inter-follicular B-cells. A weak cytoplasmic staining reaction in the B-cells is accepted. Image 7.

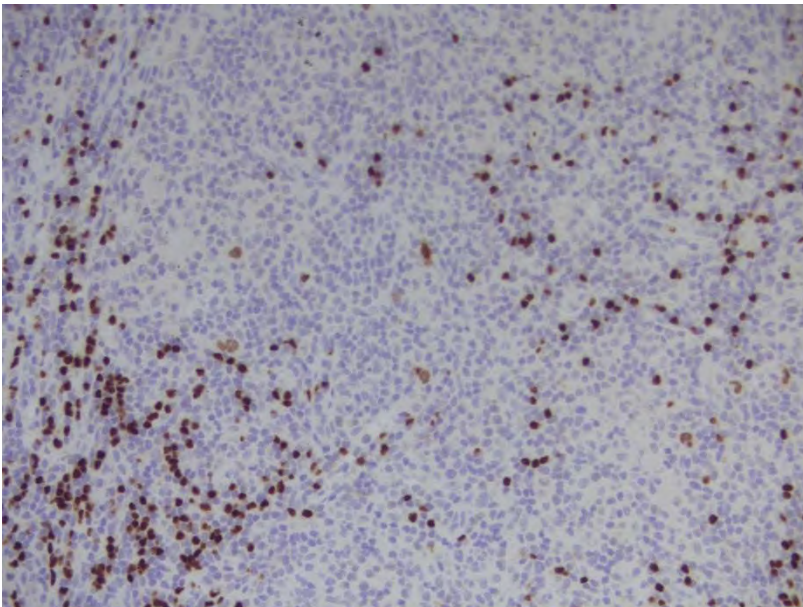


Image 6. PAX5: Optimal staining in Hodgkin's lymphoma.

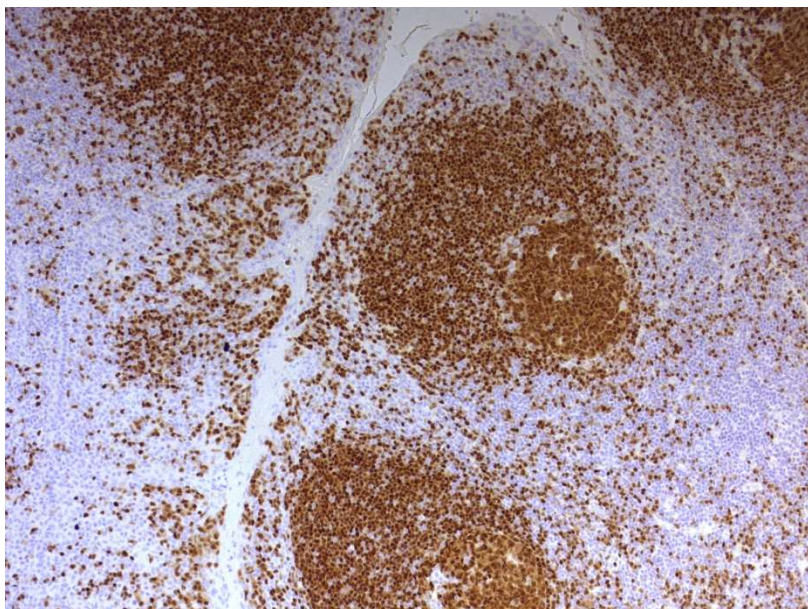


Image 7. PAX5: Optimal staining in tonsil.

General requirements

- No or only very weak background staining.
- No or minimal amount of DAB or hematoxylin precipitates, air bubbles.
- An even and distinct staining reaction throughout the sample.
- A good contrast between hematoxylin and DAB colors.

Results and conclusions

S001 CD3

96% of the laboratories obtained a score 4-5 and 82% of laboratories scored optimal 5.

A RTU antibodies gave optimal (score 5) result in the 94% of the evaluated slides. Whereas only 58% of the slides stained with the concentrated antibodies achieved the optimal results.

The most common cause for not optimal staining result was due to too weak staining or not crisp membranous staining.

S001 CD3 Concentrated antibodies and protocols with optimal results

An optimal result was obtained with the polyclonal antibody and monoclonal antibodies with the clones of rabbit monoclonal antibody (rMab) 2GV6, mouse monoclonal antibody (mMab) LN10, mMab PS1 and rMab SP7.

The polyclonal antibody manufactured by Agilent/Dako was diluted in the range of 1:80-1:400 and stained on Auto-stainer Link, Omnis, Leica's BondMAX or on Ventana BenchMark Ultra strainers. Most of the laboratories used high pH HIER pretreatment of 20 to 64 minutes. The primary antibody was incubated for 30 to 32 minutes in RT or 37°C. The optimal results were obtained with Dako Envision FLEX-, Ventana's UltraView- and Leica's Refine detection.

Leica's polyclonal antibody was diluted 1:40 and stained on Dako Autostainer link 48 instrument. A pretreatment was performed with the High pH buffer and Dako's Envision FLEX was used for detection.

Leica's L10 clone was used in the dilution of 1:25-1:100. It was stained on Dako Autostainer Link48, Leica's BondIII and on Ventana's BM XT instruments using High pH HIER target retrieval for 20 to 60 minutes. Antibody incubation time was 30-56 minutes either in RT or in 37°C. Dako's Envision FLEX, Leica's Refine and Ventana's UltraView kits were used for the optimal detection.

Leica's PS1 clone was diluted in 1:400. It was stained on Leica's Bond III instrument. Low pH target retrieval buffer ER I was used for HIER for 10 minutes. The antibody was incubated for 15 minutes in RT. Leica's Refine was used for detection.

Thermo Fisher Scientific SP7 was diluted 1:200 and incubated for 30 minutes in RT with Dako's target retrieval of High pH buffer for 20 minutes. Dako's Envision FLEX kit was used for the detection. Staining was performed on Dako Autostainer Link 48.

CD3 Concentrated antibodies

Clone	Vendor	n	5	4	3	2	1	Optimal results
LN10	Leica biosystems	7	4	2	1			57 %
PS1	Leica biosystems	1	1					(100 %)
F7.2.38	Agilent/Dako	2	2					100 %
Polyclonal	Agilent/Dako	3	1	2				33 %
	Agilent/Dako	1	1					(100%)
SP7	Thermo Fisher Scientific	3	1	1		1		33 %
SP7	Cell Marque	3	1	1		1		33 %
All concentrated CD3 antibodies								58 %

S001 CD3 Ready-To-Use antibodies and protocols with optimal results

An optimal result was obtained with polyclonal antibody and monoclonal antibodies with the clones of mAb LN10 and rMab 2GV6.

Agilent/Dako polyclonal antibody was stained either on Autostainer Link48 or Plus strainers using high pH HIER pretreatment of 20 minutes. Agilent/Dako Omnis users used Low pH target retrieval buffer. The primary antibody was incubated for 20 minutes in RT. Omnis users incubated the primary antibody for 30 minutes and used rabbit linker for the signal enhancement. Envision FLEX kit was used for detection on Agilent/Dako platforms.

Leica Biosystem's clone LN10 was used in Leica BondMAX or Bond III instruments with Epitope retrieval 2 (High pH) solution 20-30 minutes and primary antibody incubation of 10 to 30 minutes in RT combined to Refine polymer detection.

Ventana 2GV6 clone was used on Ventana Benchmark Ultra-, XT and GX instruments. High pH target retrieval buffer CC1 was used for HIER for 30-64 minutes. Primary antibody was incubated for 12 to 32 minutes in RT or 36-38°C. Most of the laboratories used Ventana's UltraView for the detection but also Optiview and iView kits produced optimal results.

CD3 RTU antibodies

Clone	Vendor	n	5	4	3	2	1	Optimal results
2GV6	Ventana	19	19					100 %
LN10	Leica biosystems	5	5					100 %
Polyclonal	Agilent/Dako	10	8	2				80 %
All RTU CD3 antibodies								94 %

S002 CD5

90% of the laboratories obtained sufficient or optimal staining results (score 4-5) and 73% obtained optimal score RTU antibodies gave optimal (score 5) result in 82% of the evaluated slides and concentrated antibodies in 63% of slides.

Most common cause for not optimal staining result was too weak staining or not distinct membranous staining. In some cases contrast between strong hematoxylin staining and IHC was too low.

S002 CD5 Concentrated antibodies and protocols with optimal results

An optimal staining result was obtained by using rMab SP19- and mAb 4C7- clones.

Leica's 4C7 clone was used with Ventana's Benchmark ultra, Dako's Omnis and Leica's Bond III, -Advance instruments. Pretreatment was performed with the High pH buffer for 20 to 88 minutes. The antibody was diluted in the range of 1:100 to 1:400 and incubated for 30 minutes in RT or 60 min in 37°C. Optiview detection was used with Ventana instruments and Refine detection kit with Leica's instruments.

Agilent/Dako 4C7 clone was used with Dako's autostainer Link48 and Leica's BondMAX instruments. Both High and Low pH epitope retrieval buffers were used. The primary antibody was diluted in the range of 1:50-1:100 and incubated for 20-30 minutes in RT. Envision FLEX and Leica's Refine kits were used for the optimal detection.

SP19 clone from Cell Marque produced optimal result by dilution of 1:10 with Ventana's BM Ultra instrument using high pH CC1 buffer for pretreatment of 48 minutes and primary antibody incubation of 36 minutes in RT. Optiview was used for detection.

Spring Bioscience's polyclonal antibody gained optimal result when stained in Dako's Omnis with the dilution of 1:40, antibody incubation of 30 minutes in 32°C. Dako's Envision FLEX was used for detection.

Thermo Fisher Scientific SP19 clone was used on Ventana Benchmark GX and Dako autostainer Link 48 instruments. The high pH epitope retrieval buffer was used in both instruments. Antibody was diluted 1:80-1:100 and incubated for 30-32 minutes either in RT or 37°C. Ventana's iView and Dako's Envision FLEX kits were used for the detection.

CD5 Concentrated antibodies

Clone	Vendor	n	5	4	3	2	1	Optimal results
4C7	Agilent/Dako	4	2	1	1			50%
EP77	Cell Marque	2	1				1	50%
4C7	Leica biosystems	6	5	1				83%
Polyclonal	Spring Biosciences	1	1					(100%)
SP19	Thermo Fisher Scientific	2	2					100%
4C7	Thermo Fisher Scientific	2	1	1				(50%)
EP77	Zeta Corp	1				1		-
No information		1	1					
All concentrated CD5 antibodies								63%

S002 CD5 Ready-To-Use antibodies and protocols with optimal results

An optimal staining result was obtained by using rMab SP19 and mAb 4C7 clones.

Agilent/Dako clone 4C7 was used on Dako Omnis, Autostainer Link48 and Autostainer plus instruments. Pretreatment of 20-30 minutes was performed in High pH target retrieval buffer or in wash buffer. Antibody incubation of 20-30 minutes in RT or in 32°C. Envision FLEX kit was used for detection.

Leica's prediluted 4C7 clone was used on Bond III and BondMAX instruments. The high pH epitope retrieval buffer 2 was used for HIER of 10 to 30 minutes. Primary antibody incubation was 10-30 minutes in RT. Refine kit was used for detection.

Ventana's SP19 clone was used on Ventana Ultra and XT instruments. The pretreatment was performed in the high pH buffer CC1 for 30 to 64 minutes. The primary antibody was incubated for 16 to 80 minutes in RT or 36-37°C. Both Optiview and Ultraview detection kits were used to achieve optimal staining results.

CD5 RTU antibodies

Clone	Vendor	n	5	4	3	2	1	Optimal results
SP19	Ventana	15	15					100%
4C7	Agilent/Dako	10	5	5				50%
4C7	Leica biosystems	3	3					100%
All RTU CD5 antibodies								82%

S003 CD20

96% of the laboratories obtained sufficient or optimal staining results (score 4-5) and 70% obtained an optimal score. RTU antibodies gave optimal (score 5) result in 81% of the evaluated slides and concentrated antibodies in 57% of slides.

The most common cause for not optimal staining result was the background staining especially in the smooth muscle cells of a colon or too weak staining of the DLBCL. In some cases the contrast between strong the hematoxylin staining and IHC-detection was too low.

S003 CD20 Concentrated antibodies and protocols with optimal results

All the laboratories with the optimal results used mAb L26 clone from different vendors.

Agilent/Dako clone L26 was diluted in the range of 1:50-1:1000. Dilution of 1:200 being the most common giving the optimal staining. Autostainer Link 48 and different generations of Ventana BenchMark instruments as well as Leica BondMAX instruments were used for optimal staining. The high pH HIER pretreatment of 30 to 52 minutes was the most common, except for BondMAX with which low pH ER1 buffer was used for the HIER. The primary antibody was incubated for 20 to 44 minutes in RT, 36°C or 37°C. The optimal results were obtained with Agilent/Dako Envision FLEX - and Leica's Refine detection kit as well as with Ventana's iView and UltraView detection.

Cell Marque clone L26 gave optimal results when stained on Dako's Autostainer Link 48 or on Ventana's Benchmark GX instruments. The target retrieval was performed with the high pH buffer for 20 minutes. The antibody dilution was between 1:100 and 1:200. Primary antibody incubation was 30-37 minutes either in RT or in 37°C. Ventana's iView and Dako's Envision FLEX were used for detection. Optimal result was also obtained by manual staining with Cell Marque's high pH target retrieval solution (5+5minutes) and HiDef HPR-detection kit.

Leica's L26 was stained on Leica BondIII with the dilution of 1:1000, the primary antibody incubation of 30 min, RT using the low pH epitope retrieval buffer 1. Leica's Refine detection kit was used for detection. On Ventana's Benchmark LT instrument the antibody was used in the dilution of 1:300 using incubation time of 40 min in 37°C and the high pH epitope retrieval of 64 min in buffer CC1. Ventana's Ultraview was used for detection. Thermo fisher Scientific clone L26 was used on BondMAX instrument diluted to 1:175 and incubated for 20 min in RT. The target retrieval for 20 min was performed with the low pH epitope retrieval 1 buffer and Leica's Refine kit was used for the detection.

CD20 Concentrated antibodies								
Clone	Vendor	n	5	4	3	2	1	Optimal results
L26	Agilent/Dako	13	7	4		2		54%
L26	Cell Marque	3	3					100%
L26	Leica biosystems	3	2	1				67%
L26	Thermo Fisher Scientific	1	1					(100%)
L26	Diagnostic BioSystems	2		2				-
L26	Biogenex	1		1				-
All concentrated antibodies								57%

S003 CD20 Ready-To-Use antibodies and protocols with optimal results

All the laboratories with optimal results used mAb L26 clone from Agilent/Dako, Ventana or Leica Biosystems. Agilent/Dako RTU antibody was used on Omnis, Autostainer Link48 and Ventana Benchmark Ultra instruments. High pH HIER was used for pretreatment of 20 to 36 minutes. Primary antibody incubation was 12,5-28 minutes in RT or 37°C. Dako Envision FLEX kit was used for detection in Agilent/Dako platforms. Short antibody incubation was compensated with 3-layer detection with mouse linker. UltraView detection kit was used on Ventana instrument.

Ventana's L26 was used on Ventana Benchmark Ultra, LT and GX instruments. Pretreatment of 8-48 minutes was performed with high pH buffer CC1. Antibody incubation was 10-40 minutes in 36-37°C. Most of the laboratories used Optiview kit for the detection but also UltraView and iView detection kits were able to produce optimal result. Leica's L26 was used on BondMAX instruments with low pH epitope retrieval and Refine detection (not all information available).

CD20 RTU antibodies								
Clone	Vendor	n	5	4	3	2	1	Optimal results
L26	Ventana	21	17	4				81%
L26	Agilent/Dako	8	6	2				75%
L26	Leica biosystems	2	1	1				(100%)
All CD20 RTU antibodies								77%

S004 CD30

84% of the laboratories obtained sufficient or optimal staining results (score 4-5). Optimal score 5 was given to 67% of the evaluated slides.

RTU antibodies gave optimal (score 5) result in 44% of the evaluated slides and concentrated antibodies in 63% of slides.

The most common cause for not optimal staining result was too weak staining of the activated T- and B-cells in tonsil or due to background staining.

S004 CD30 Concentrated antibodies and protocols with optimal results

An optimal results were obtained with mAb BER-H2 clone.

Agilent/Dako clone BER-H2 was diluted 1:50 and stained by using Autostainer Link48, Omnis or Ventana BenchMark Ultra strainers using the high pH HIER pretreatment of 20 to 64 minutes. The primary antibody was incubated for 20 to 72 minutes in RT, 32°C, 36°C or 37°C. The optimal result was obtained with Agilent/Dako Envision FLEX detection as well as with Ventana's Optiview, iView and UltraView detection kits. Amplification (3 layer detection) was used for Ultraview detection system.

Cell Marque clone BER-H2 gave the optimal results when stained on Ventana Benchmark Ultra instrument with the dilution of 1:50, using pretreatment of Ventana's CC1 for 88 minutes and primary antibody incubation of 60 minutes in 37°C with Optiview detection.

Thermo Fisher Scientific BER-H2 antibody got the optimal results using Leica BondMAX instrument. Antibody was used in the dilution of 1:50 using Epitope retrieval solution 2 (20 minutes) and primary antibody incubation of 20 minutes in RT. Leica's Refine was used for the detection.

CD30 Concentrated antibodies

Clone	Vendor	n	5	4	3	2	1	Optimal results
BER-H2	Agilent/Dako	8	4	4				50%
BER-H2	Cell Marque	7	1	4		2		14%
BER-H2	Thermo Fisher Scientific	1	1					(100%)
BER-H2	BioSB	1		1				-
BER-H2	Menarini	1			1			-
No information		1	1					-
All concentrated CD30 antibodies								37%

S004 CD30 Ready-To-Use antibodies and protocols with optimal results

An optimal result was obtained with mAb JCM182 clone and with the most commonly used mAb BER-H2 clone. Agilent/Dako clone BER-H2 was stained either on Autostainer Link48 or on Omnis strainers using Low pH HIER pretreatment of 20 minutes (info from 1 laboratory). Primary antibody was incubated for 20 minutes in RT or 25 °C. Envision FLEX was used for detection.

Leica Biosystems clone JCM182 was used on Leica BondMAX or on Bond III instruments with Epitope retrieval 1 (Low pH) solution for 20 minutes and the primary antibody incubation of 30 minutes combined to Refine polymer detection.

Ventana BER-H2 produced optimal staining results with CC1 HIER pretreatment of 30-88 minutes. Primary antibody incubation was 16-60 minutes in RT or 36°C. Optimal result was obtained with Optiview and UltraView-detection or Optiview detection with amplification.

Biocare BER-H2 produced optimal staining results with CC1 HIER pretreatment of 64 minutes. Primary antibody incubation was 40 minutes in 37°C. Optimal result was obtained with the UltraView-detection.

CD30 RTU antibodies

Clone	Vendor	n	5	4	3	2	1	Optimal results
BER-H2	Ventana	18	8	4	2	1	1	44%
BER-H2	Agilent/Dako	8	3	4	1			38%
JCM182	Leica biosystems	4	2	2				50%
BERH2+CON6D/B5	Biocare	1		1				-
BER-H2	Biocare	1	1					(100%)
All CD30 RTU antibodies								44%

S005 PAX5

76% of the laboratories obtained sufficient or optimal staining results (score 4-5). Optimal score 5 was given to 44% of the evaluated slides.

RTU antibodies gave the optimal (score 5) result in 43% of the evaluated slides and concentrated antibodies in 45% of slides.

The most common cause for not optimal staining result was nonspecific cytoplasmic staining of endothelia and macrophage positivity or too weak or not existing staining of the RS-cells.

S005 PAX5 Concentrated antibodies and protocols with optimal results

The optimal result was obtained with mAb DAK-PAX5- and mAb IHC005-clones and with polyclonal PAX5-antibody.

Agilent/Dako clone DAK-PAX5 was diluted in the range of 1:20-1:200. Autostainer Link48, Omnis and Leica Bond III instruments were used for staining with the high pH HIER pretreatment of 20 to 30 minutes. Primary antibody was incubated for 20 to 30 minutes in RT or 32°C. Agilent/Dako Envision FLEX detection kit was used both with and without mouse linker.

GenomeMe's IHC005 antibody was used in the dilution of 1:100 on Leica BondMAX instrument. Epitope retrieval was performed using ER2 solution. The primary antibody was incubated for 20 minutes in RT. Leica's Refine was used for detection.

Thermo Fisher Scientific's polyclonal antibody provided optimal results on Ventana Benchmark GX instrument with CC1 pretreatment and primary antibody incubation of 20 minutes in RT with dilution of 1:100. Ventana's iView was used for the detection.

PAX5 Concentrated antibodies

Clone	Vendor	n	5	4	3	2	1	Optimal results
DAK-PAX5	Agilent/Dako	4	3		1			75%
SP34	Cell Marque	2		1	1			-
IHC005	GenomeMe	1	1					(100%)
Polyclonal	Thermo Fisher Scientific	2	1	1				50%
SP34	Spring Biosciences	1			1			-
Polyclonal	Zytomed	1			1			-
All concentrated PAX5 antibodies								45%

S005 PAX5 Ready-To-Use antibodies and protocols with optimal results

An optimal result was obtained with mAb DAK-PAX5-, mAb 1EW- and rAb SP34 clones.

Agilent/Dako clone DAK-PAX5 was stained either on Autostainer Link48 or on Omnis strainers using High pH HIER pretreatment of 20 to 30 minutes. The primary antibody was incubated for 20 minutes in 22-37°C. Most of the laboratories used mouse linker to enhance the signal.

Leica Biosystem's clone 1EW was used on Leica BondMAX or on Bond III instruments with epitope retrieval 2 solution 20-30 minutes and primary antibody incubation of 20-30 minutes combined to Refine polymer detection.

Ventana SP34 produced optimal staining results with CC1 (high pH) HIER pretreatment of 64-76 minutes. The primary antibody incubation was 28-32 minutes in RT or 36°C. The optimal result was obtained by using Optiview or iView-detection kits.

PAX5 RTU antibodies

Clone	Vendor	n	5	4	3	2	1	Optimal results
SP34	Ventana	12	3	6	3			25%
DAK-PAX5	Agilent/Dako	7	4	2	1			57%
1EW	Leica biosystems	2	2					100%
24	Cell Marque	1				1		-
24	Biocare Medical	1	1					(100%)
All PAX5 RTU antibodies								43%

End of report

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