LABQUALITY

External Quality Assessment Scheme

# Histological staining techniques: HE and Iron (Fe) Round 1, 2023

**Specimens**
Please find enclosed two unstained and unbaked slides.

**Background information**
Each participating laboratory receives two unstained slides. Each slide is labeled according to the staining procedure. The samples have been fixed in 10% neutral buffered formalin.

S001: HE (hematoxylin & eosin) (small intestine, ileum)
S002: Iron (Fe) (lung and kidney)

**Storage and use**
The sections are adhered to the slides for 15 min +60°C.

The slides should be stained with hematoxylin & eosin or iron. The slides can be stored at room temperature before and after staining. If the protocols of your laboratory do not include both stains, you can participate with one slide only. If needed, extra slides can be ordered from Labquality.

**Please remember to label the slides clearly with your Labquality account number**

If you ordered multiple sample sets with the same client code, please separate them with the alphabet.

**Returning slides and protocol reporting**
Please return the stained slides in the same slide mailer box wrapped in bubble wrap at participant's own cost to the address given in the column on the right side of the page unless our local partner has instructed otherwise. The shipment comes with an address label for return, which does not include postage.

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

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

S001: HE

LQ778423011

S002: Iron (Fe)

LQ778423012

**2023-04-25**

**INSTRUCTIONS**

Product no. 6543
LQ778423011-012/FI

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

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The slides should be returned no later than **May 26, 2023.**

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**Inquiries**
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**Labquality Oy**
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www.labquality.com

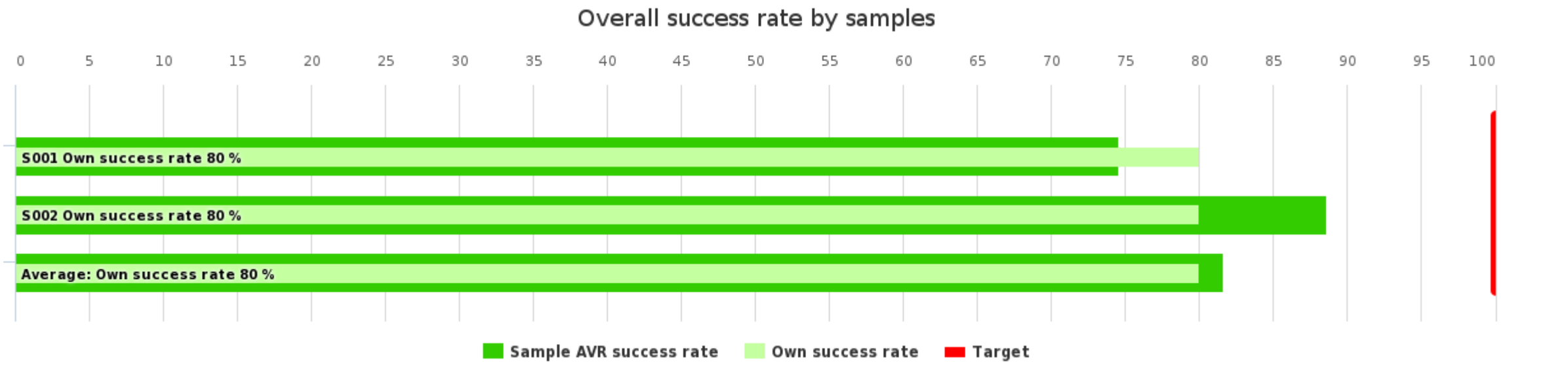
FINAS
Finnish Accreditation Service
PT02 (EN ISO/IEC 17043)

Quality Management
ISO 9001:2015

## Client report

| | No of participants | No of responded participants | Response percentage |
|---|---|---|---|
| Histological staining techniques, April, 1-2023 | 111 | 111 | 100 % |

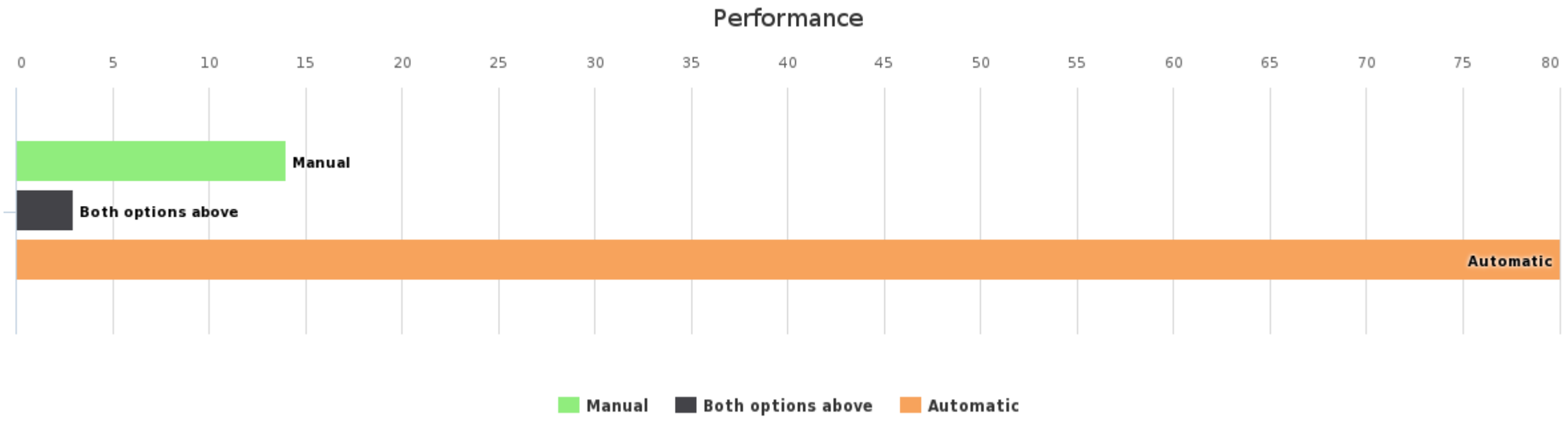
## Summary




| Summary | Own score | Max score | Own success rate | Difference | AVR success rate |
|---|---|---|---|---|---|
| S001 | 4 | 5 | 80 % | 5.4 % | 74.6 % |
| S002 | 4 | 5 | 80 % | -8.6 % | 88.6 % |
| Average: | | | 80 % | -1.6 % | 81.6 % |

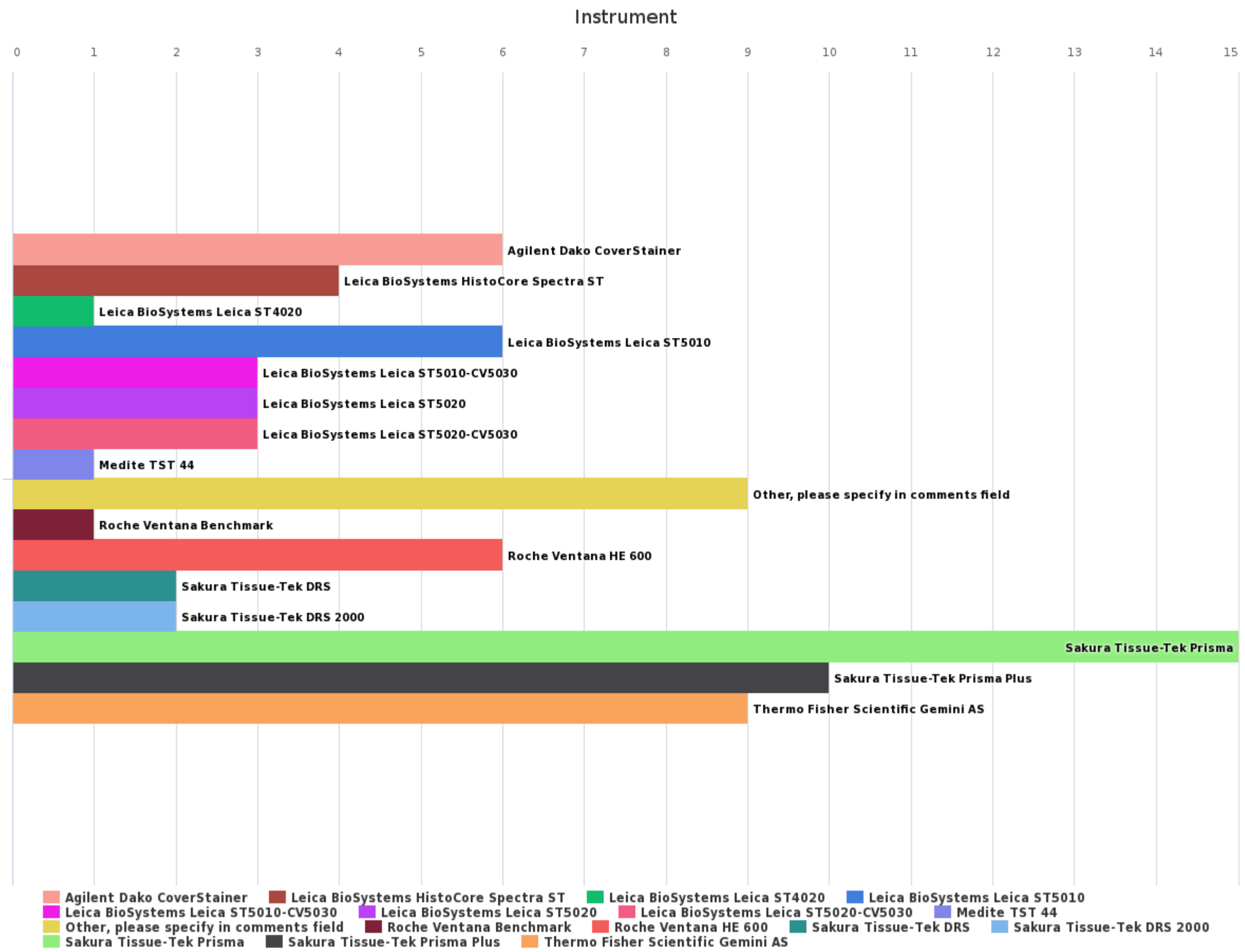
| History | Test nr. | Own success rate | Difference | AVR success rate |
|---|---|---|---|---|
| History not found | | | | |

**S001 | Sample S001 HE**

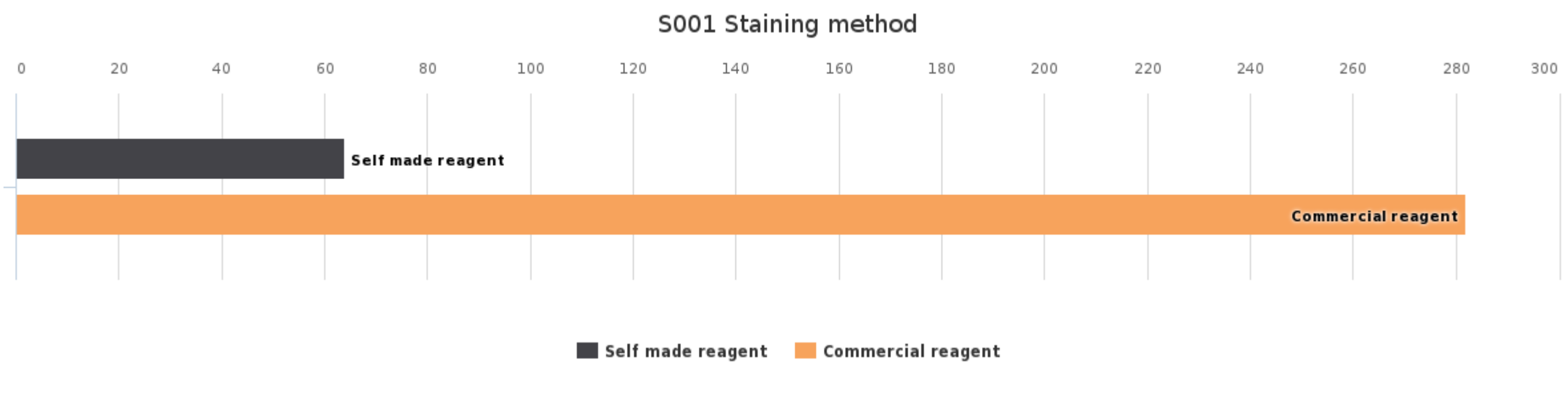



## GENERAL DETAILS OF STAINING

| Performance | Performance count |
|---|---|
| Automatic | 80 |
| Both options above | 3 |
| ◉ Manual | 14 |
| Total: | 97 |

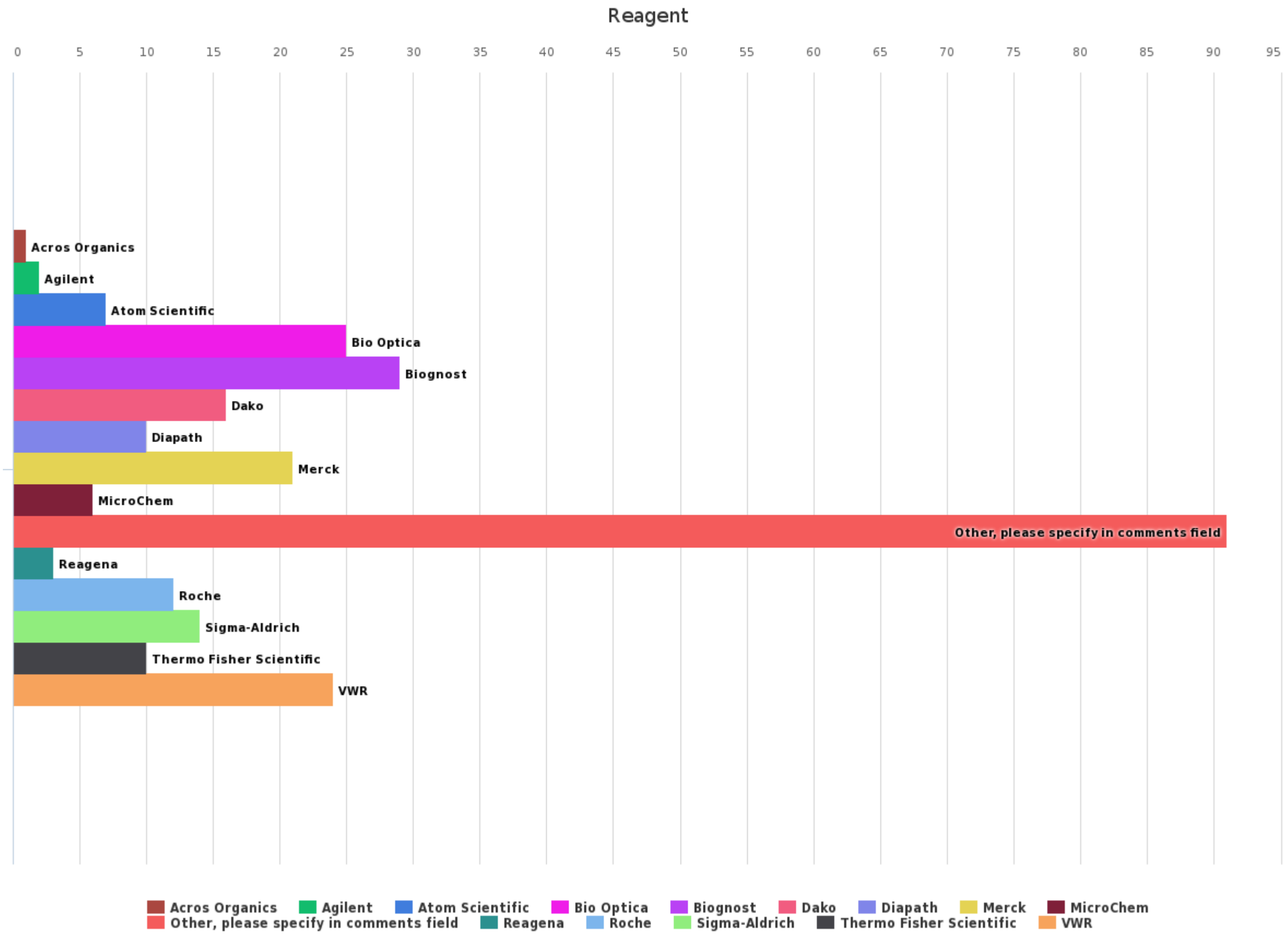


| Instrument | Instrument count |
|---|---|
| Agilent Dako CoverStainer | 6 |
| Leica BioSystems HistoCore Spectra ST | 4 |
| Leica BioSystems Leica ST4020 | 1 |
| Leica BioSystems Leica ST5010 | 6 |
| Leica BioSystems Leica ST5010-CV5030 | 3 |
| Leica BioSystems Leica ST5020 | 3 |
| Leica BioSystems Leica ST5020-CV5030 | 3 |
| Medite TST 44 | 1 |
| Other, please specify in comments field | 9 |
| Roche Ventana Benchmark | 1 |
| Roche Ventana HE 600 | 6 |
| Sakura Tissue-Tek DRS 2000 | 2 |
| Sakura Tissue-Tek Prisma | 15 |
| Sakura Tissue-Tek Prisma Plus | 10 |
| Thermo Fisher Scientific Gemini AS | 9 |
| Total: | 81 |

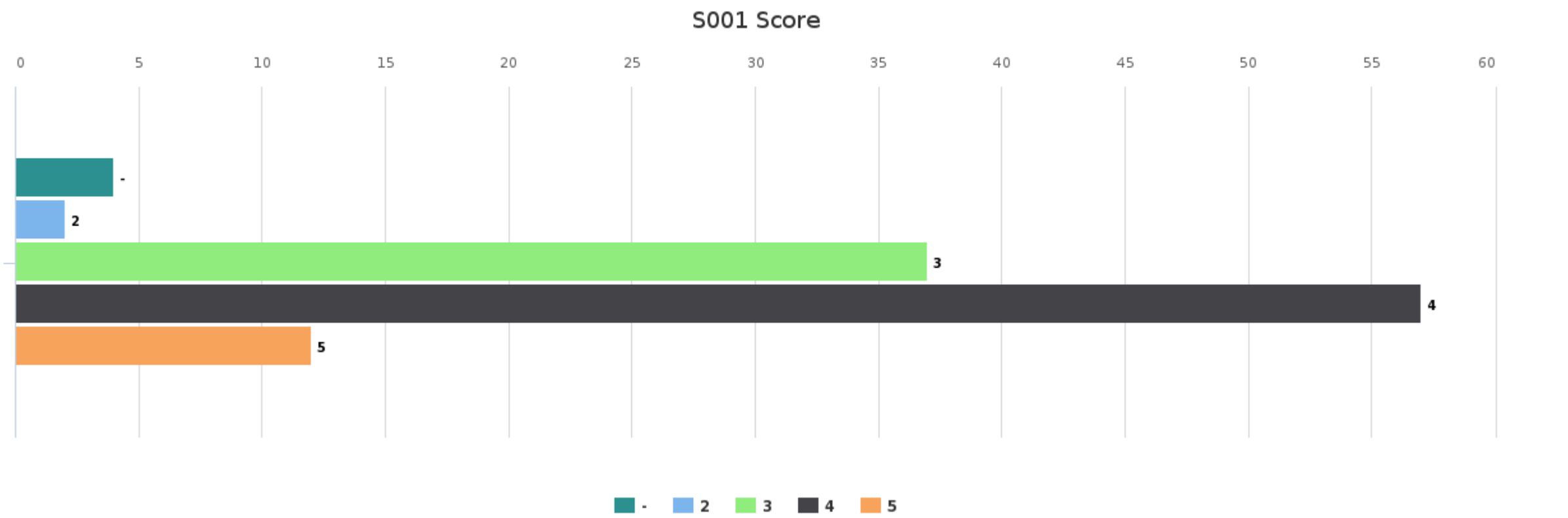



**STAINING METHOD**

| Reagent | Reagent count |
|---|---|
| ◉ Commercial reagent | 282 |
| Self made reagent | 64 |
| Total: | 346 |




| Manufacturer | Manufacturer count |
|---|---|
| Acros Organics | 1 |
| Agilent | 2 |
| Atom Scientific | 7 |
| Bio Optica | 25 |
| Biognost | 29 |
| ◉ Dako | 16 |
| Diapath | 10 |
| Merck | 21 |
| MicroChem | 6 |
| Other, please specify in comments field | 91 |
| Reagena | 3 |
| Roche | 12 |
| Sigma-Aldrich | 14 |
| Thermo Fisher Scientific | 10 |
| VWR | 24 |
| Total: | 271 |

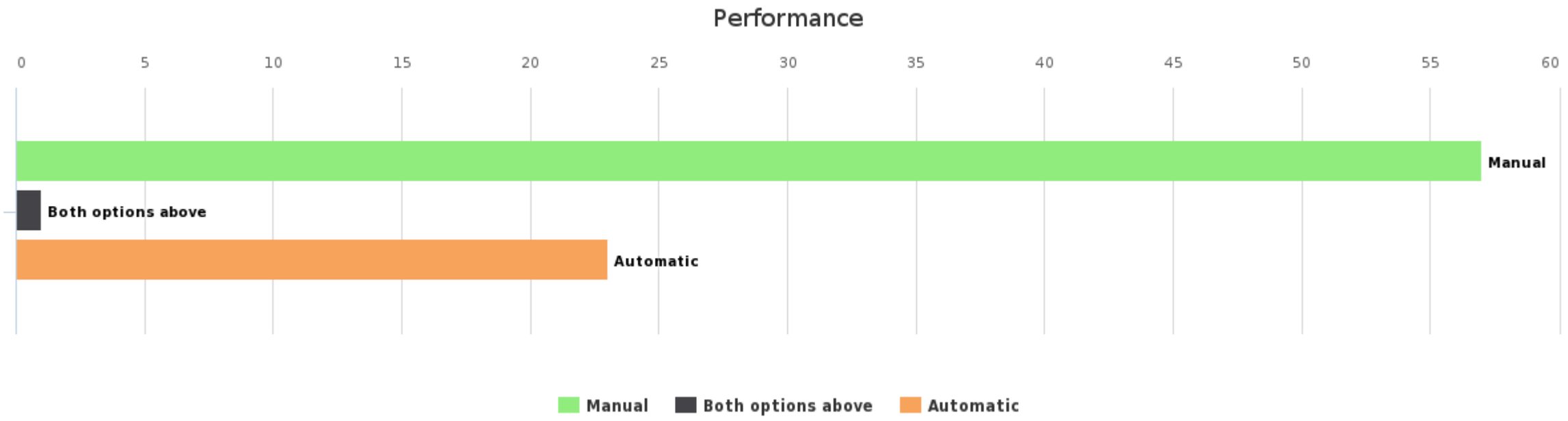



**SCORE**

| Score | Comment |
|---|---|
| 4 | |
| | weak contrast |

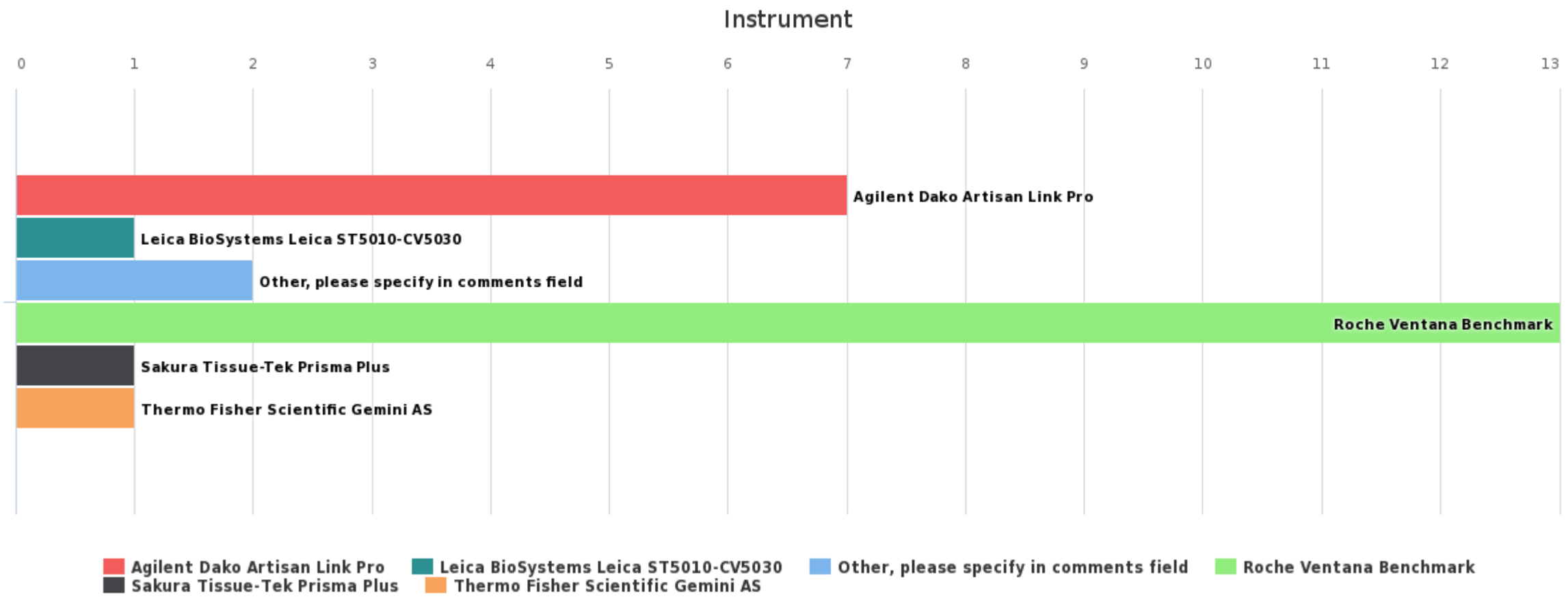
| Score | Score count |
|---|---|
| - | 4 |
| 2 | 2 |
| 3 | 37 |
| ◉ 4 | 57 |
| 5 | 12 |
| Total: | 112 |

**S002 | Sample S002 Iron**

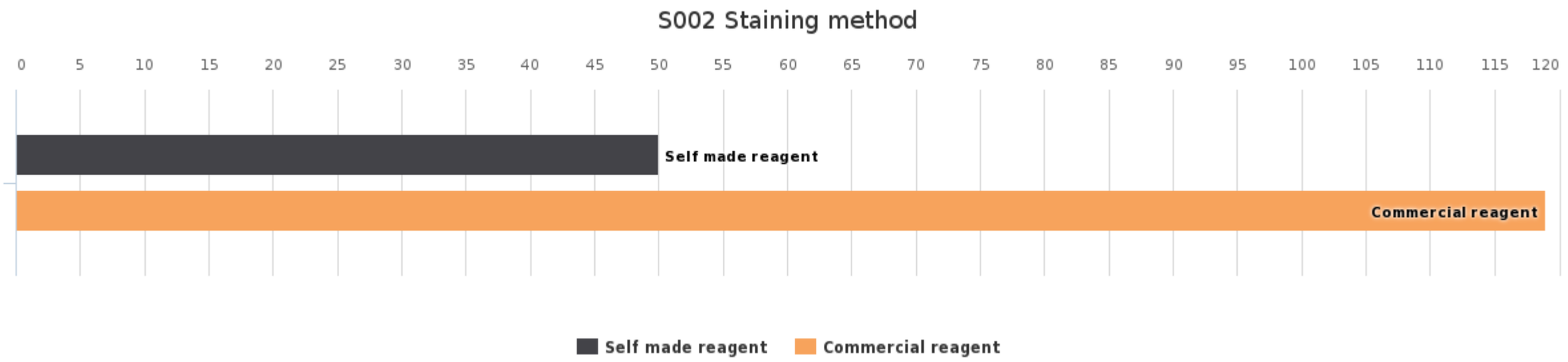



## GENERAL DETAILS OF STAINING

| Performance | Performance count |
|---|---|
| Automatic | 23 |
| Both options above | 1 |
| ◉ Manual | 57 |
| Total: | 81 |

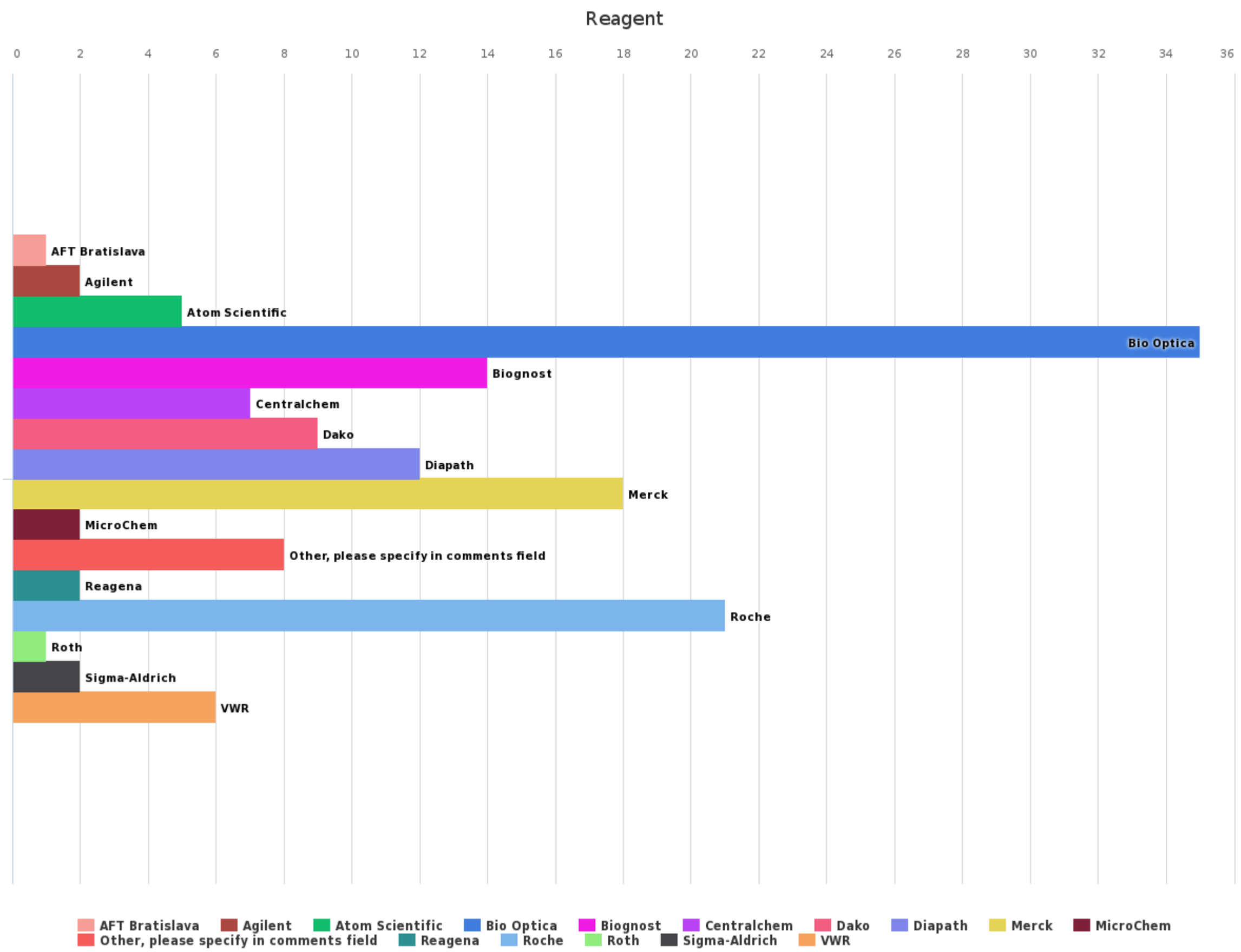



| Instrument | Instrument count |
|---|---|
| Agilent Dako Artisan Link Pro | 7 |
| Leica BioSystems Leica ST5010-CV5030 | 1 |
| Other, please specify in comments field | 2 |
| Roche Ventana Benchmark | 13 |
| Sakura Tissue-Tek Prisma Plus | 1 |
| Thermo Fisher Scientific Gemini AS | 1 |
| Total: | 25 |



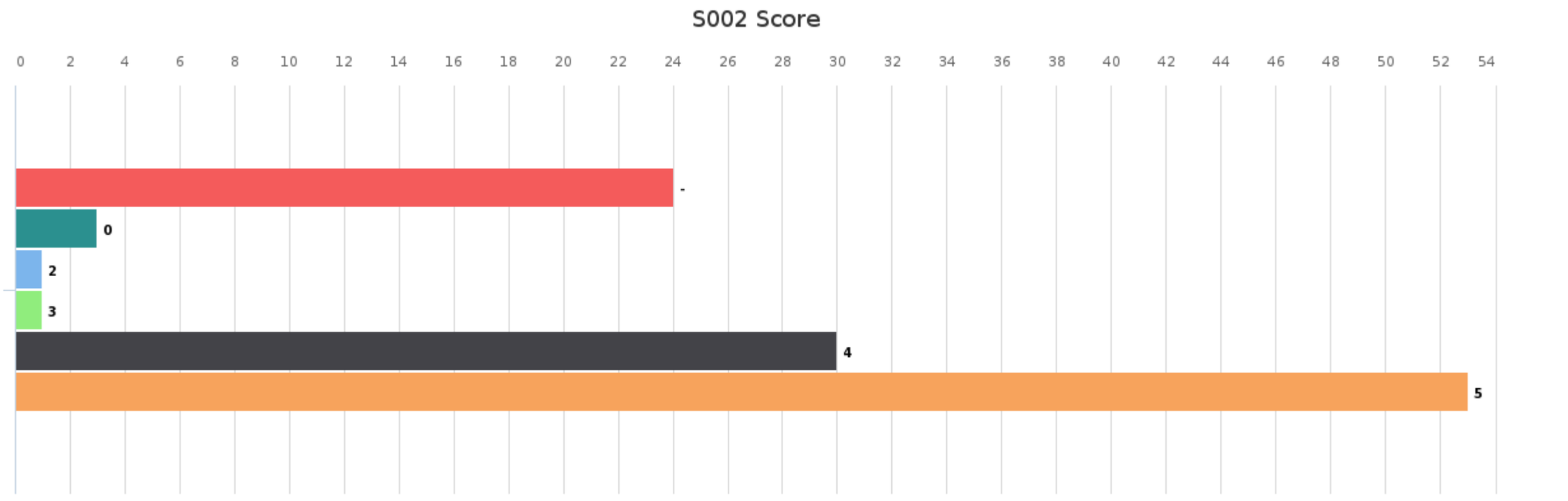

**STAINING METHOD**

| Reagent | Reagent count |
|---|---|
| ◉ Commercial reagent | 119 |
| Self made reagent | 50 |
| Total: | 169 |




| Manufacturer | Manufacturer count |
|---|---|
| AFT Bratislava | 1 |
| Agilent | 2 |
| ◉ Atom Scientific | 5 |
| Bio Optica | 35 |

| | |
|---|---|
| Biognost | 14 |
| Centralchem | 7 |
| Dako | 9 |
| Diapath | 12 |
| Merck | 18 |
| MicroChem | 2 |
| Other, please specify in comments field | 8 |
| Reagena | 2 |
| Roche | 21 |
| Roth | 1 |
| Sigma-Aldrich | 2 |
| VWR | 6 |
| Total: | 145 |




**SCORE**

| Score | Comment |
|---|---|
| 4 | |
| | background |

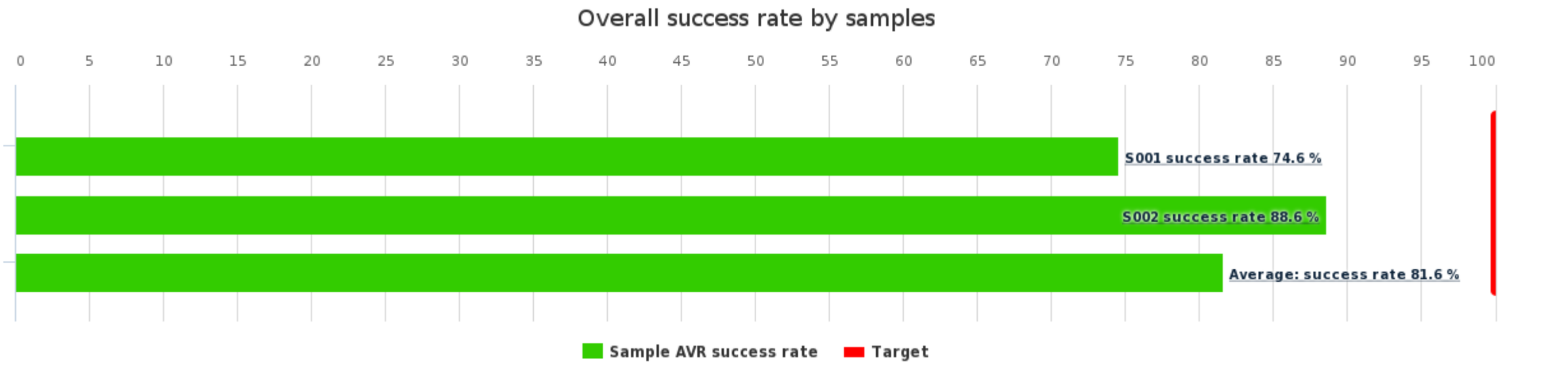
| Score | Score count |
|---|---|
| - | 24 |
| 0 | 3 |
| 2 | 1 |
| 3 | 1 |
| ◉ 4 | 30 |
| 5 | 53 |
| Total: | 112 |

# Histological staining techniques, April, 1-2023

## GLOBAL REPORT

| | No of participants | No of responded participants | Response percentage |
|---|---|---|---|
| Histological staining techniques, April, 1-2023 | 111 | 111 | 100 % |

## Summary




| Summary | AVR success rate |
|---|---|
| S001 | 74.6 % |
| S002 | 88.6 % |
| Average: | 81.6 % |

**S001 | Sample S001 HE**




## GENERAL DETAILS OF STAINING

| Performance | Performance count |
|---|---|
| Automatic | 80 |
| Both options above | 3 |
| Manual | 14 |
| Total: | 97 |

# Histological staining techniques, April, 1-2023

| Instrument | Instrument count |
|---|---|
| Agilent Dako CoverStainer | 6 |
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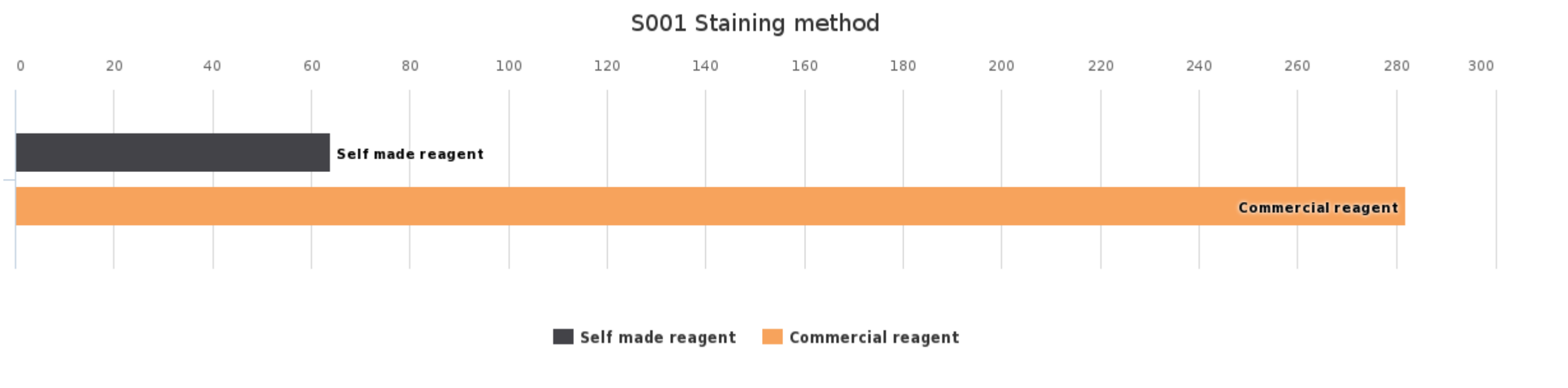
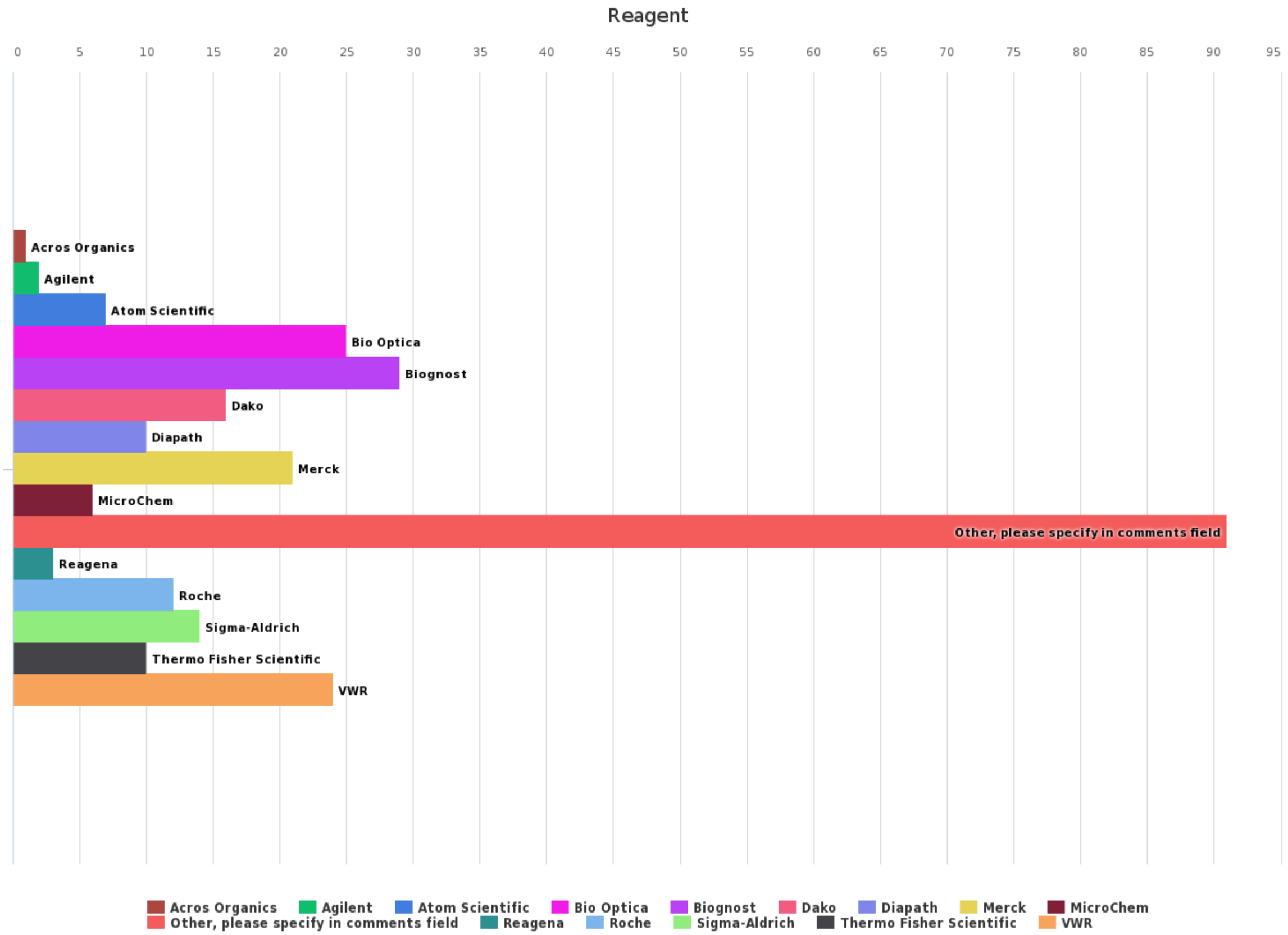
| Leica BioSystems Leica ST5020 | 3 |
| Leica BioSystems Leica ST5020-CV5030 | 3 |
| Medite TST 44 | 1 |
| Other, please specify in comments field | 9 |
| Roche Ventana Benchmark | 1 |
| Roche Ventana HE 600 | 6 |
| Sakura Tissue-Tek DRS 2000 | 2 |
| Sakura Tissue-Tek Prisma | 15 |
| Sakura Tissue-Tek Prisma Plus | 10 |
| Thermo Fisher Scientific Gemini AS | 9 |
| Total: | 81 |




**STAINING METHOD**

| Reagent | Reagent count |
|---|---|
| Commercial reagent | 282 |
| Self made reagent | 64 |
| Total: | 346 |

# Histological staining techniques, April, 1-2023




| Manufacturer | Manufacturer count |
|---|---|
| Acros Organics | 1 |
| Agilent | 2 |
| Atom Scientific | 7 |
| Bio Optica | 25 |
| Biognost | 29 |
| Dako | 16 |
| Diapath | 10 |
| Merck | 21 |
| MicroChem | 6 |
| Other, please specify in comments field | 91 |
| Reagena | 3 |
| Roche | 12 |
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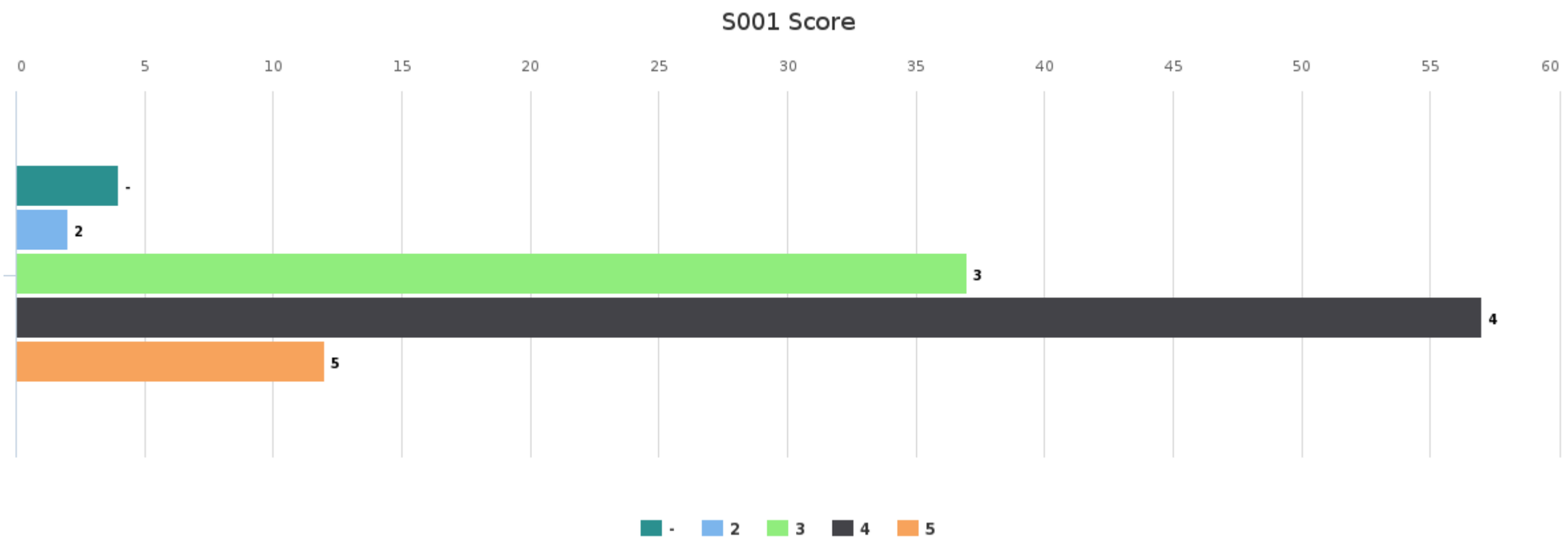
| VWR | 24 |
| Total: | 271 |



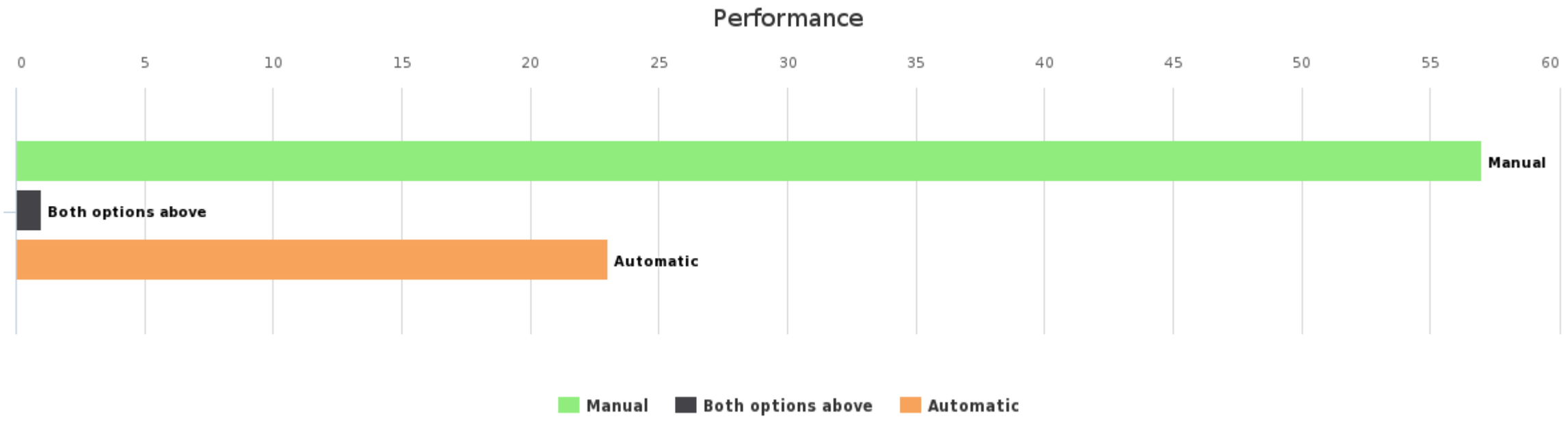

**SCORE**

| Score | Comment |
|---|---|
| - | |
| | Not available |
| 2 | |
| | clearly ovarstained, mucus |
| | light, Panetth cells and eosinophils poorly discernible |
| 3 | |
| | dirty |
| | hematoxylin overstained |
| | hematoxylin overstained, mucus |
| | hematoxylin overstained, strong mucus |
| | light |
| | overstained |
| | overstained, mucus |
| | poor contrast |
| | strong hematoxylin |
| | strong hematoxylin, weak contrast |
| | strong hematoxylin, weak Paneth cells |
| | too strong eosin |
| | too strong hematoxylin |
| | unclear contrast, mucus |
| | weak |
| | weak contrast |
| | weak contrast, mucus too strong |
| | weak contrast, weak eosinophils |
| | weak eosin, Paneth cells not discernible |
| | weak eosinophils |
| | weak eosinophils, mucus, dirty |
| | weak eosinophils, poorly discernible |
| | weak Paneth cells and eosinophils |
| 4 | |
| | eosin too strong |
| | eosinophils poorly discernible |
| | hematoxylin strong |
| | light |
| | light, weak Paneth cells |
| | overstained |
| | slightly weak |
| | slightly weak contrast |
| | some overstained |
| | strong eosin |
| | strong hematoxylin |
| | strong mucus, very blue |
| | too strong eosin |

| | |
|---|---|
| | weak |
| | weak a little |
| | weak contrast |
| | weak contrast, too strong hematoxylin |
| | weak eosin |
| | weak eosinohils |
| | weak eosinophils |
| | weak eosinophils, mucus |
| | weak Paneth cells |
| | weak Paneth cells and eosinophils |
| 5 | |
| | Excellent |
| | mucus |

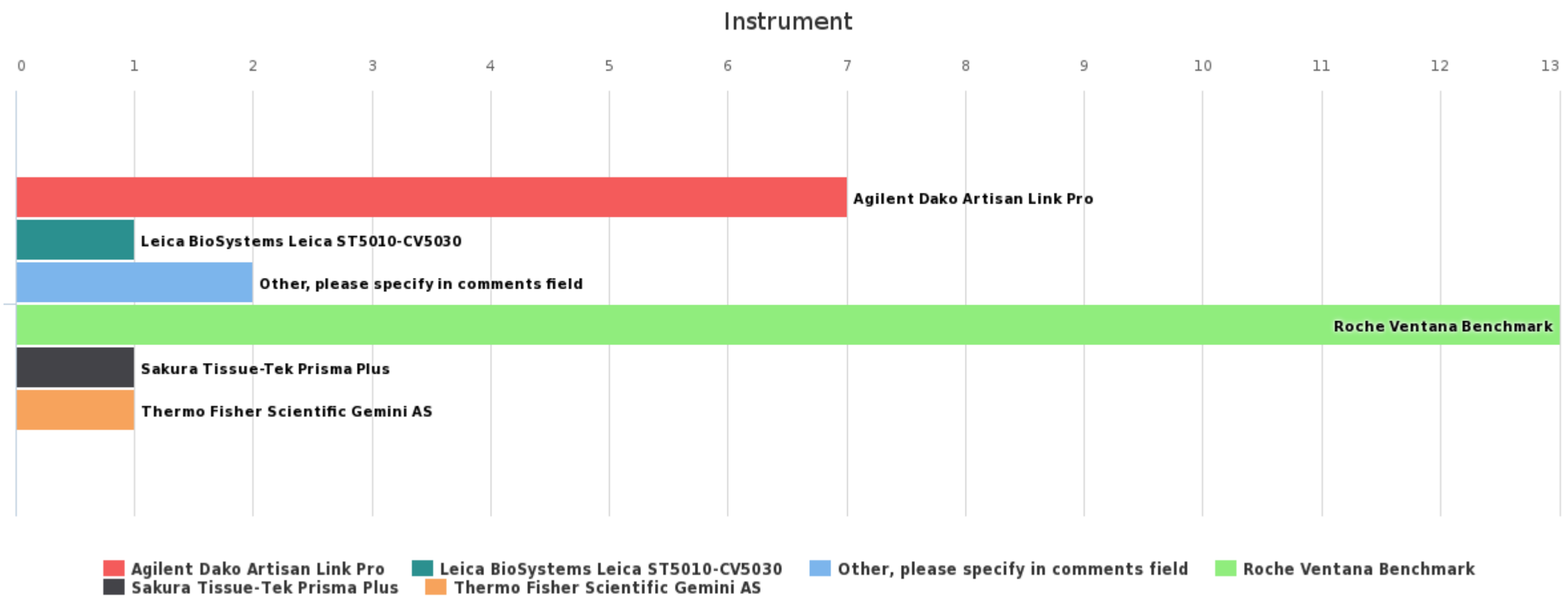
| Score | Score count |
|---|---|
| - | 4 |
| 2 | 2 |
| 3 | 37 |
| 4 | 57 |
| 5 | 12 |
| Total: | 112 |

**S002 | Sample S002 Iron**

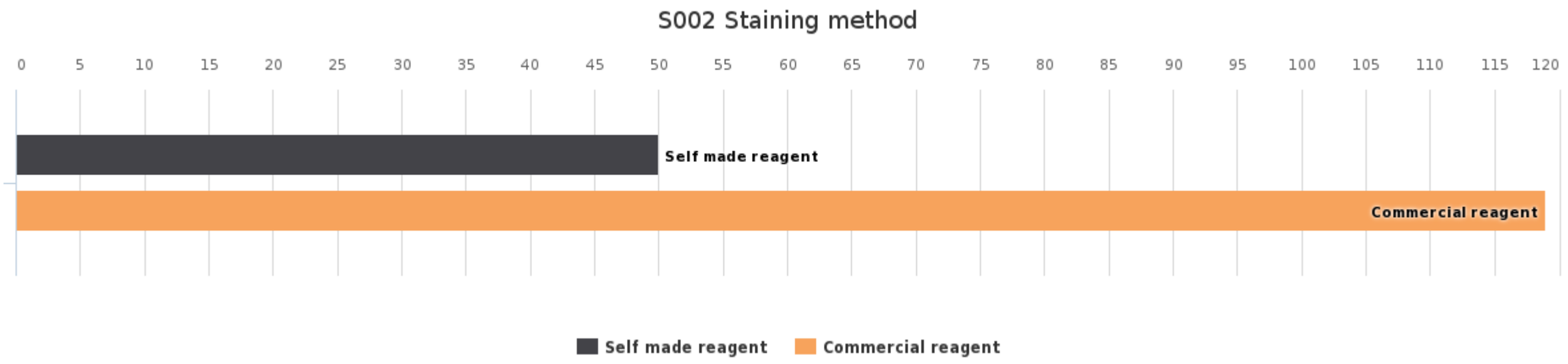



## GENERAL DETAILS OF STAINING

| Performance | Performance count |
|---|---|
| Automatic | 23 |
| Both options above | 1 |
| Manual | 57 |
| Total: | 81 |

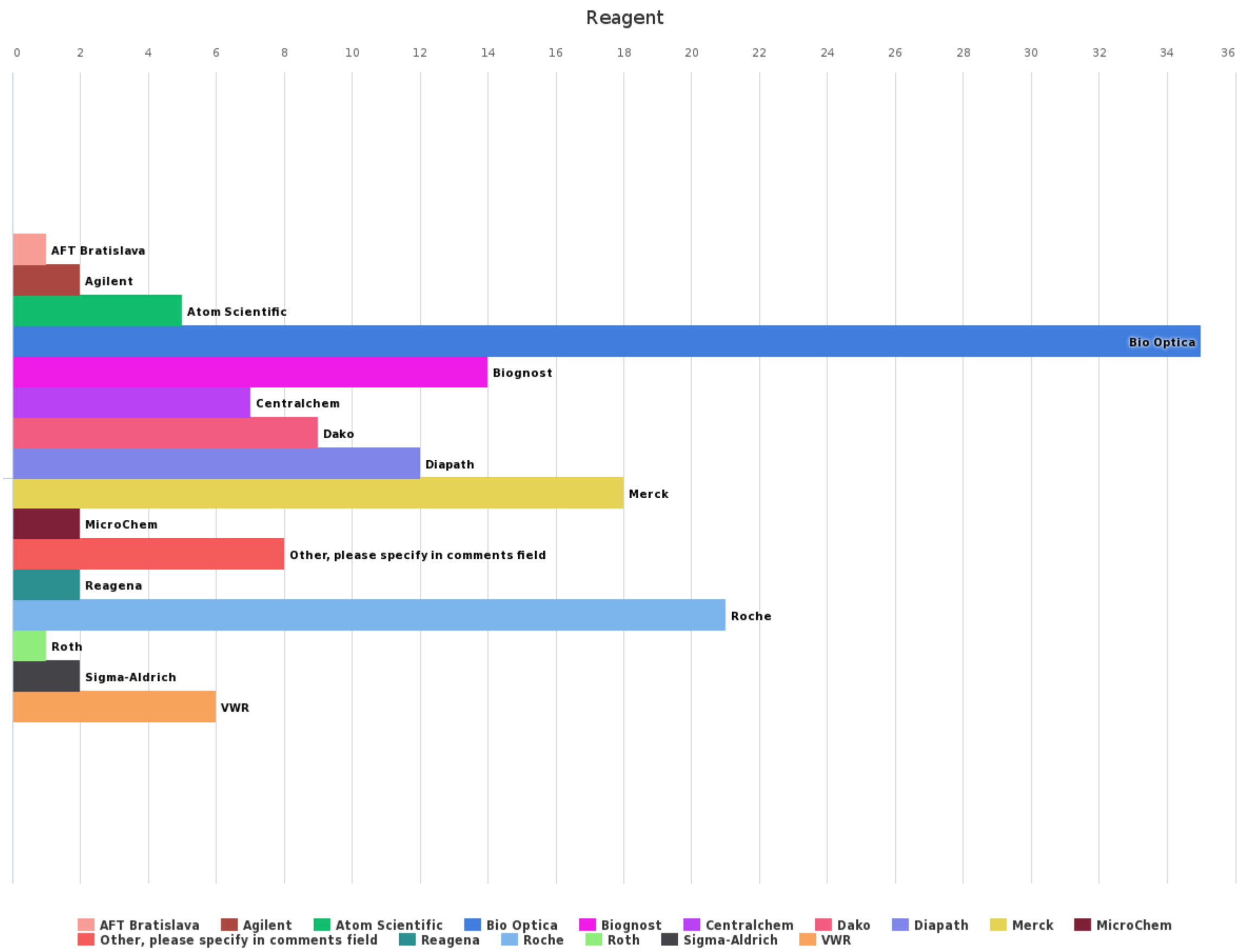



| Instrument | Instrument count |
|---|---|
| Agilent Dako Artisan Link Pro | 7 |
| Leica BioSystems Leica ST5010-CV5030 | 1 |
| Other, please specify in comments field | 2 |
| Roche Ventana Benchmark | 13 |
| Sakura Tissue-Tek Prisma Plus | 1 |
| Thermo Fisher Scientific Gemini AS | 1 |
| Total: | 25 |



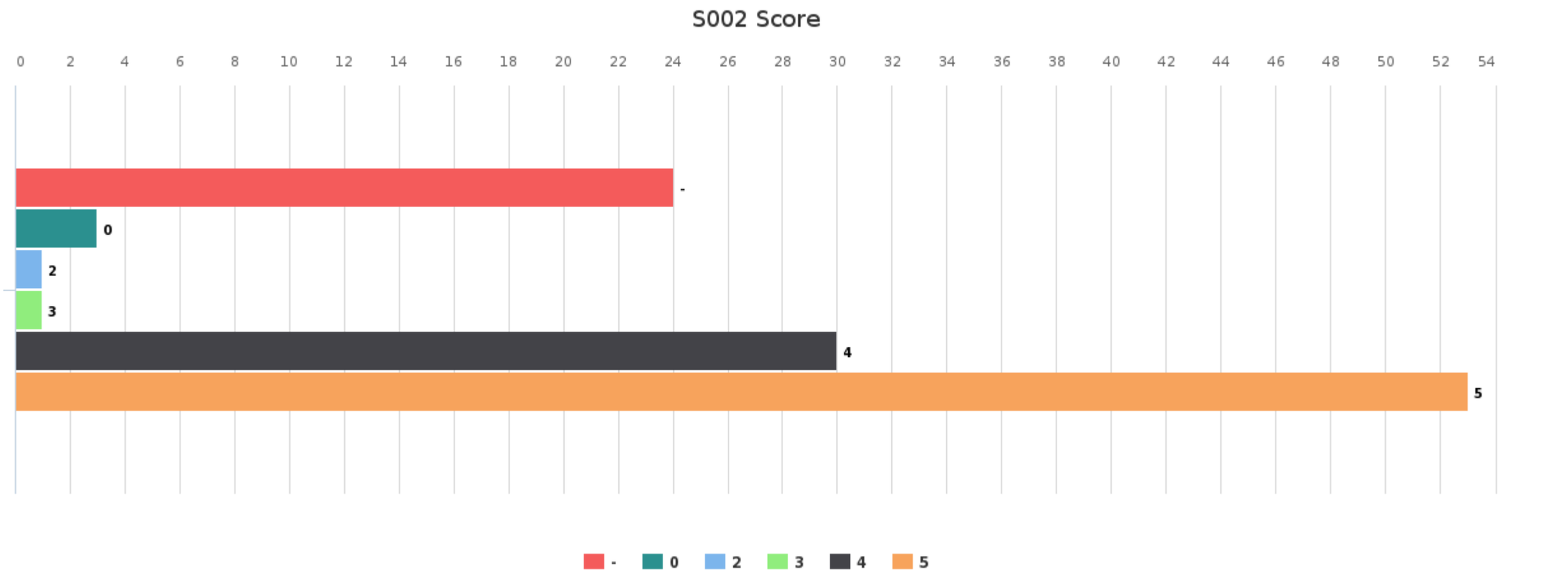

**STAINING METHOD**

| Reagent | Reagent count |
|---|---|
| Commercial reagent | 119 |
| Self made reagent | 50 |
| Total: | 169 |




| Manufacturer | Manufacturer count |
|---|---|
| AFT Bratislava | 1 |
| Agilent | 2 |
| Atom Scientific | 5 |
| Bio Optica | 35 |

| | |
|---|---|
| Biognost | 14 |
| Centralchem | 7 |
| Dako | 9 |
| Diapath | 12 |
| Merck | 18 |
| MicroChem | 2 |
| Other, please specify in comments field | 8 |
| Reagena | 2 |
| Roche | 21 |
| Roth | 1 |
| Sigma-Aldrich | 2 |
| VWR | 6 |
| Total: | 145 |




**SCORE**

| Score | Comment |
|---|---|
| - | |
| | Not available |
| 0 | |
| | HE stain done |
| | iron not stained, probably HE done |
| | negative, iron not stained |
| 2 | |
| | clearly ovarstained, background |
| 3 | |
| | weak, uneven |
| 4 | |
| | almost optimal |
| | background |
| | overstained |
| | some background |
| | weak |
| 5 | |
| | Excellent |

| Score | Score count |
|---|---|
| - | 24 |
| 0 | 3 |
| 2 | 1 |
| 3 | 1 |
| 4 | 30 |

| | |
|---|---|
| 5 | 53 |
| Total: | 112 |

LABQUALITY

External Quality Assessment Scheme

# Histological staining techniques: HE and Iron (Fe) Round 1, 2023

**Specimens**
Sample S001-S002 (LQ778423011-LQ778423012) were two slides, each with an unstained paraffin section.

S001: HE (hematoxylin & eosin) (small intestine, ileum)
S002: Iron (Fe) (lung and kidney)

**Report info**
The final report contains distribution of results and used methods. Laboratory's own result and method is marked with a black radio button (⊙). 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.

**Evaluation scale: 0-5**
3-5 points indicate good enough staining for diagnosis.
0-2 points mean that the staining is insufficient for diagnosis or failed.

| | |
|---|---|
| **5 points** | optimal, excellent |
| **4 points** | almost optimal, practically faultless, slight over/understaining, slightly uneven or patchy staining |
| **3 points** | acceptable for diagnosis, but distinct over/understaining, uneven or patchy staining, stain deposits etc. |
| **2 points** | borderline, weak staining, uncertain for diagnosis |
| **1 point** | poor, failed, some scanty but inadequate staining observed/ notifiable overstaining. |
| **0 points** | negative staining or fully false positive, failed. |

| Points | HE (n) | Iron (n) |
|---|---|---|
| **5** | **12** | **53** |
| **4** | **57** | **30** |
| **3** | **37** | **1** |
| **2** | **2** | **1** |
| **1** | | |
| **0** | | **3** |
| **Mean** | **3.73** | **4.43** |

**Comments – Expert**
**S001: Criteria for optimal HE –staining**
Blue hematoxylin and red eosin should clearly differentiate tissue components. The intensity of staining was to be adequate, and overstaining was not allowed. Staining of mucus should not disturb interpretation. Cell membranes and chromatin were to be distinct. The Paneth cells and granules of eosinophils must be clearly discernible.

**Results**
In the survey of HE -stain 61/108 (56.5 %) of the participants got 4 or 5 points which means excellent performance. Only 2/108 (1.9 %) showed insufficient performance (2 points). As a whole, mean of the performance was 3.73 points.

**Automation and reagents**
The mean of those who used automates was 3.76 points which is almost the same as 3.64 points achieved by manual process. The mean was 3.67 points when the protocols were not reported. The participants mentioned 14 different

**2023-06-28**

**FINAL REPORT**

Product no. 6543

Subcontracting: Sample preparation, sample pretesting

| | |
|---|---|
| Samples sent | 2023-04-25 |
| Round closed | 2023-05-30 |
| Final report | 2023-06-28 |

**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**
Chief of department Heikki Aho, MD, PhD
Turku university hospital, pathology

**Evaluation of the slides with experts**
Chief of department Mirva Söderström, MD, PhD
Cell biologist Iina Tuominen, PhD
Turku university hospital, pathology

**Labquality Oy**
Kumpulantie 15
FI-00520 HELSINKI
Finland

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info@labquality.fi
www.labquality.com

FINAS Finnish Accreditation Service PT02 (EN ISO/IEC 17043)

Quality Management ISO 9001:2015

automates. Four automates achieved 5 points and 12 got 4 or 5 points which means that there were no essential differences in performance between the automates. The same automate model may result to 3, 4 or 5 points. There were 10 automates and 2 manual processes in the group of 5 points. The reagents varied from variable commercial products of different sources to home-made ones.

**Deficiencies in the HE -staining**
Usual deficiencies were overstaining, deficient staining and disturbance caused by staining of mucus. Also deficient staining of the Paneth cells and eosinophils reduced the points. Only seldom both Paneth cells and eosinophils were clearly distinct. The chromatin was mostly well stained.

**Protocols that produced optimal staining**
The type of automate used and the reagents, usually commercial, varied a lot and it is very difficult to give any preferable protocols. Is the final result good or less good is also often a matter of taste. The type of hematoxylin, however, influence on the staining of mucus.

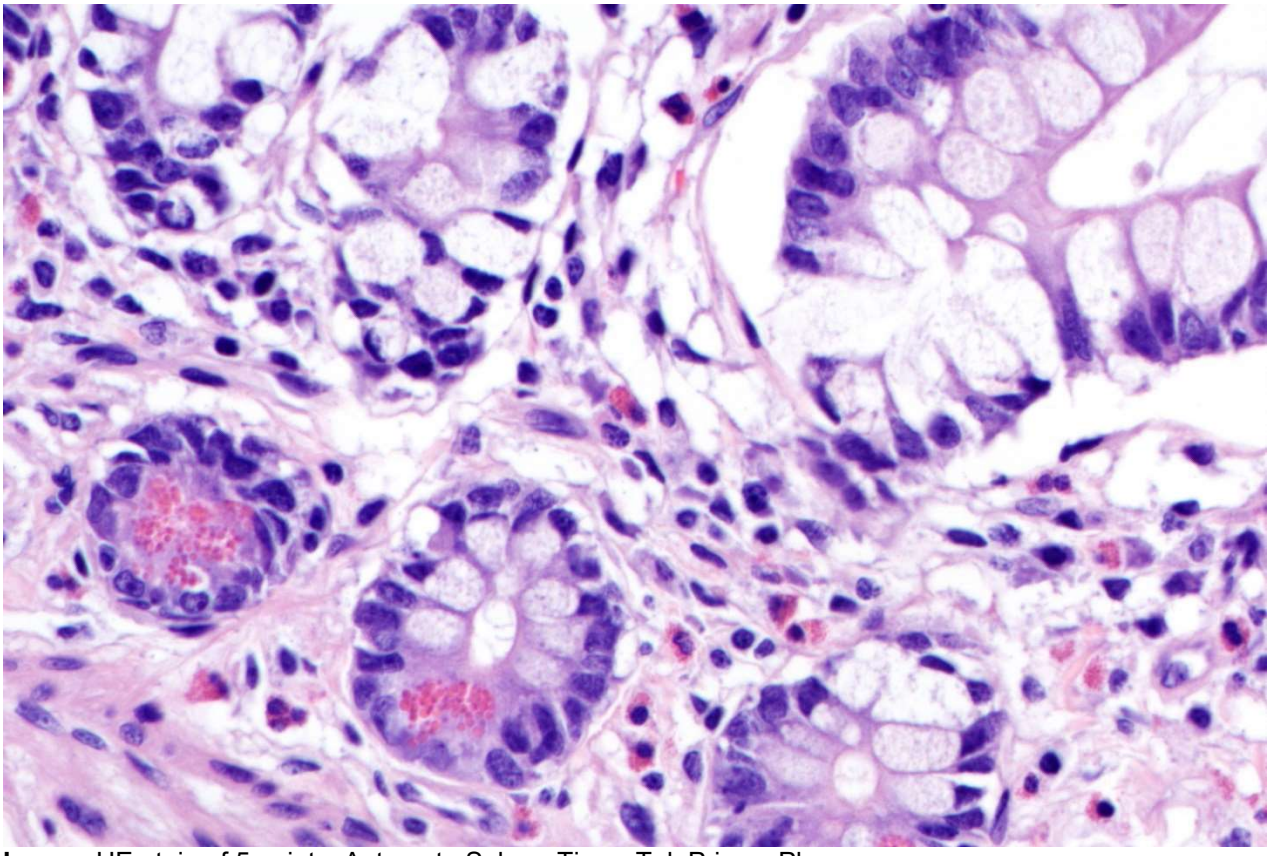

**Image:** HE-stain of 5 points. Automate Sakura TissueTek Prisma Plus.

**S002: Criteria for optimal Iron –staining**
A lot of hemosiderin, mostly in macrophages, was present in this specimen from the lung. The iron should be clearly discernible in granules of macrophages without diffuse spread to surroundings.

**Results**
Staining of iron was successful, 4 or 5 points were achieved by 83/88 (94.3 %) of the participants. Only 4 (4.5 %) were unsuccessful (0-2 points).

**Automation and reagents**
Automate was used in 23 laboratories, mean 4.22 points. Manual process was used in 54 laboratories, mean 4.59 points. Manual process seemed to be a little more reliable. Six automates were named and all achieved 4 or 5 points. When protocol information was not given, the performance was 4,18 points. Those who got the best 5 points result used usually commercial reagents but also with home-made reagents it was possible to get the best performance.

**Deficiencies in the Iron staining**
One slide was heavily overstained and 3 were totally negative (only HE-stain done). Spread to surroundings was considered as a minor deficiency.

**Protocols that produced optimal staining**

Optimal result was possible both with automate and by manual process. The manual process, however, was a little reliable. Incubation periods varied considerably between 20 sec to 80 min and the reagents were variable, so it is difficult to give any recommendable protocol when almost all laboratories succeeded excellently.

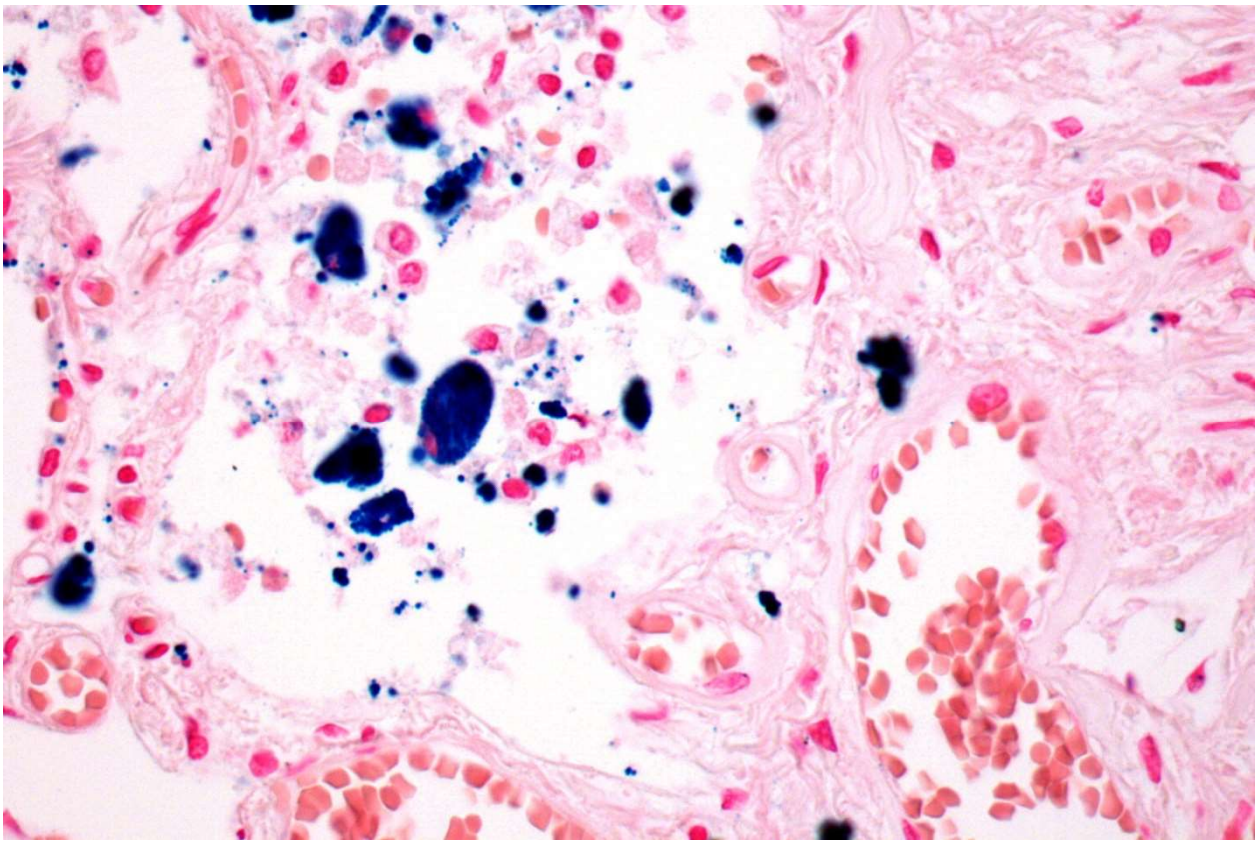

**Image: Iron stain of 5 points.** Manual process. Perls Diapath.

**End of the report**