LABQUALITY

External Quality Assessment Scheme

Gram stain, colonies
Round 1, 2023

Specimens
On the slide there are three air-fixed microbial suspensions S001, S002 and S003.

Caution
The slide is unfixed and unstained and should therefore be handled as infectious materials. Fixing kills the microbes.

Examinations
Gram stain, colonies

Storage and use
The slide is unfixed and unstained. After arrival, the slide should be stored at +2…8 °C. The slide should be fixed, stained and examined by microscope. The microbes included in the samples are common in clinical specimens, even though they do not grow on all kinds of media. Multiple species are not encountered in the samples.

Result reporting
Please enter the results and methods via LabScala (www.labscala.com). Gram staining and Finding parts will be scored. In case of yeast only Finding part will be scored.

S001

S002

S003

2023-01-24

INSTRUCTIONS

Product no. 5040
LQ760523011-013/FI
UN3373

Subcontracting: Sample preparation, sample pretesting

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

The results should be reported no later than **February 21, 2023**.

The expected results of the round are published in LabScala in the View Reports section by February 23, 2023.

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

Quality Management
ISO 9001:2015

LABQUALITY

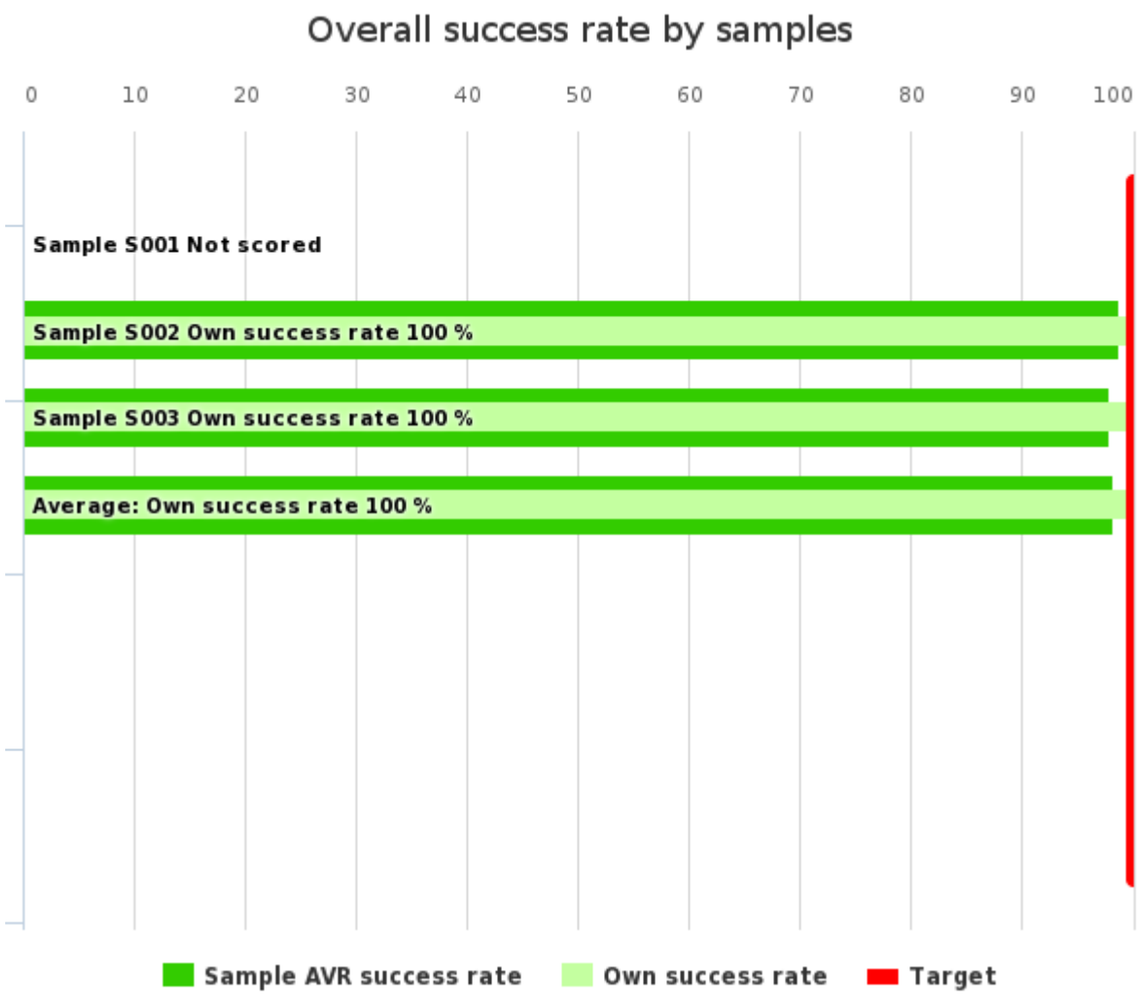
Gram stain, colonies, January, 1-2023

XXXX

Client report

	No of participants	No of responded participants	Response percentage
Gram stain, colonies, January, 1-2023	121	118	97.5 %

Summary

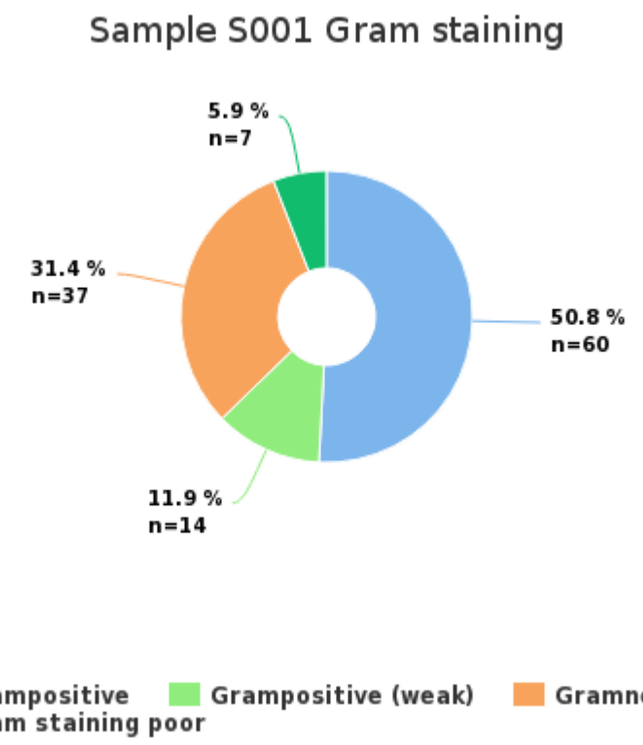


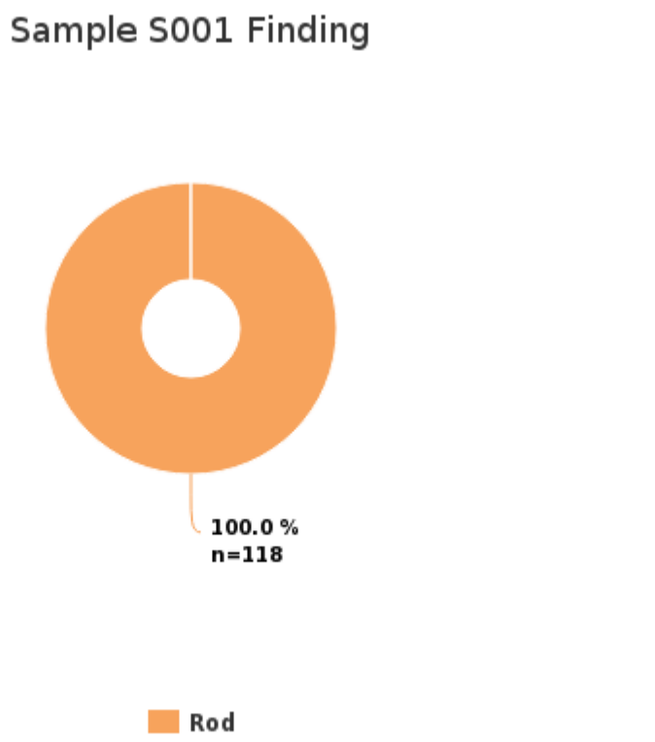
Summary	Own score	Max score	Own success rate	Difference	AVR success rate
Sample S001	-	-	-	-	-
Sample S002	4	4	100 %	1.3 %	98.7 %
Sample S003	4	4	100 %	2.1 %	97.9 %
Average:			100 %	1.7 %	98.3 %

History	Test nr.	Own success rate	Difference	AVR success rate
Round 2022-4	1-1	100 %	2.7 %	97.3 %
Round 2022-3	1-1	100 %	3.4 %	96.6 %
Round 2022-2	1-1	100 %	8 %	92 %
Round 2022-1	1-1	100 %	6.5 %	93.5 %
Round 2021-4	1-1	100 %	3.9 %	96.1 %
Round 2021-3	1-1	100 %	6.3 %	93.7 %
Round 2021-2	1-1	100 %	7.3 %	92.7 %
Round 2021-1	1-1	100 %	5.3 %	94.7 %

Sample S001 | Clostridium perfringens, grampositive rod

Sample S001 results	Responded	Own score	Max score	Own success rate	Difference	AVR success rate	Count
	Gram staining	-	-	-	-	-	118
	Finding	-	-	-	-	-	118
	Arrangement / Morphology	-	-	-	-	-	89
Total:		-	-	-	-	-	325





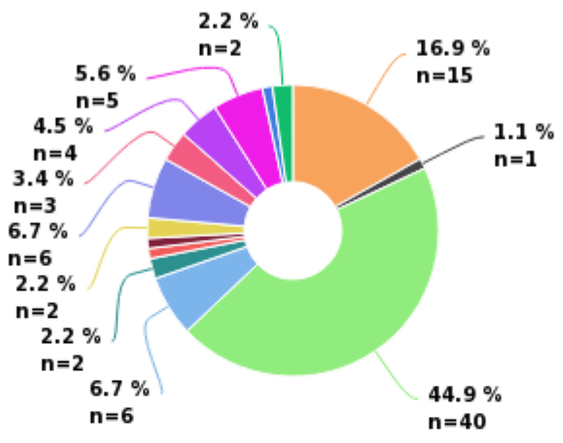
GRAM STAINING

Result	Result count	Own score	Max score	Own success rate	Difference	AVR success rate	Result Score
Grampositive	60	-				-	-
◉ Grampositive (weak)	14	-				-	-
Gramnegative	37	-				-	-
Gram staining poor	7	-				-	-
Total:	118	-	-	-	-		

FINDING

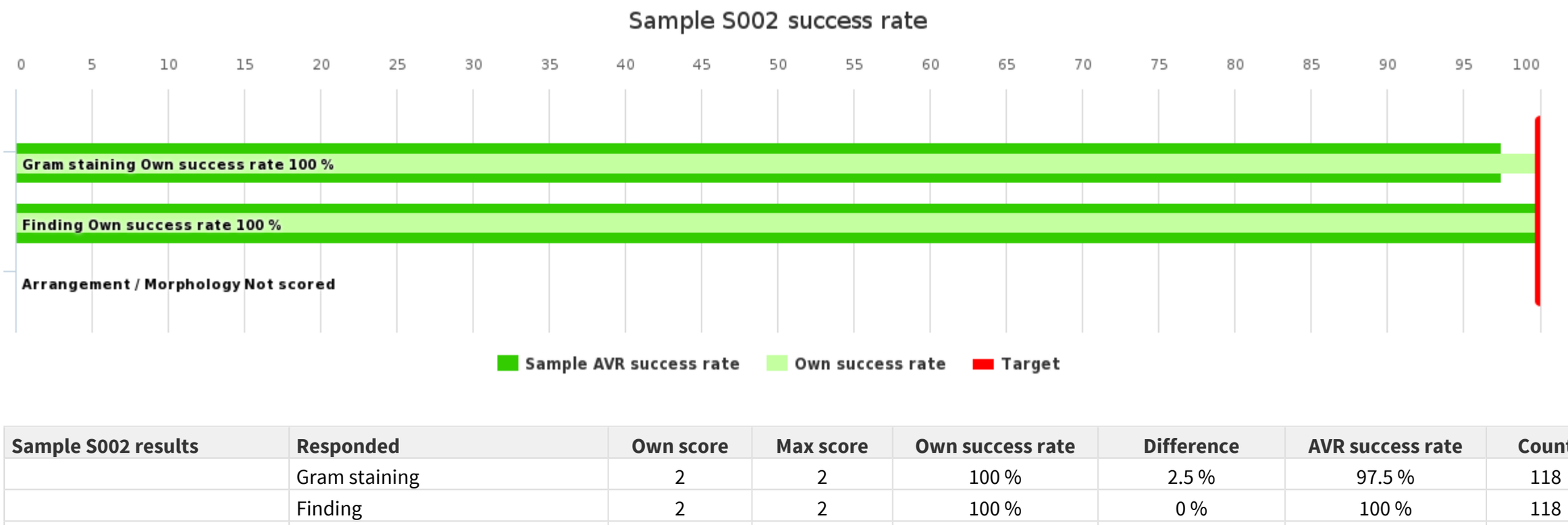
Result	Result count	Own score	Max score	Own success rate	Difference	AVR success rate	Result Score
◉ Rod	118	-				-	-
Total:	118	-	-	-	-		

Sample S001 Arrangement / Morphology

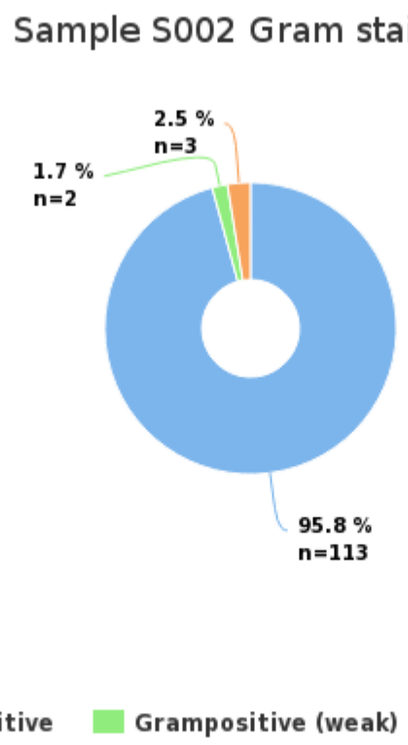


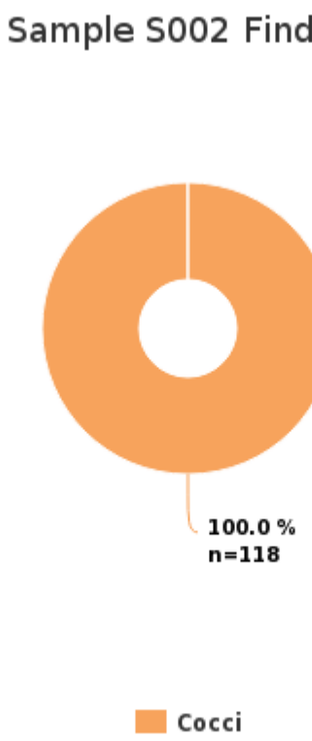
Arrangement / Morphology	Result	Result count
	Blunt rod	15
	Irregular/branching rod	1
	◉ Large rod	40
	Long rod	6
	Other interpretation	2
	Palisade	1
	Rod in chain	1
	Sharp rod	2
	Short/coccoid rod	6
	Thin rod	3
	Variable	4
	Varied rod	5
	Vary in size	1
	With spores	2
	Total:	89

Sample S002 | Finegoldia magna, grampositive cocci



Sample S002 results	Responded	Own score	Max score	Own success rate	Difference	AVR success rate	Count
	Gram staining	2	2	100 %	2.5 %	97.5 %	118
	Finding	2	2	100 %	0 %	100 %	118
	Arrangement / Morphology	-	-	-	-	-	108
Total:		4	4	100 %	1.3 %	98.7 %	344





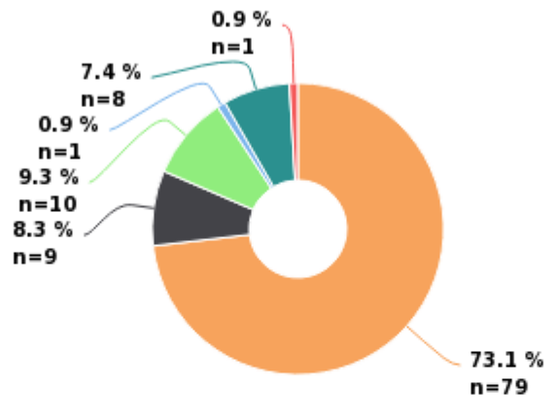
GRAM STAINING

Result	Result count	Own score	Max score	Own success rate	Difference	AVR success rate	Result Score
◉ Grampositive	113	2	2	100 %	0 %	100 %	2
Grampositive (weak)	2	-				100 %	2
Gramnegative	3	-				0 %	0
Total:	118	2	2	100 %	2.5 %	97.5 %	

FINDING

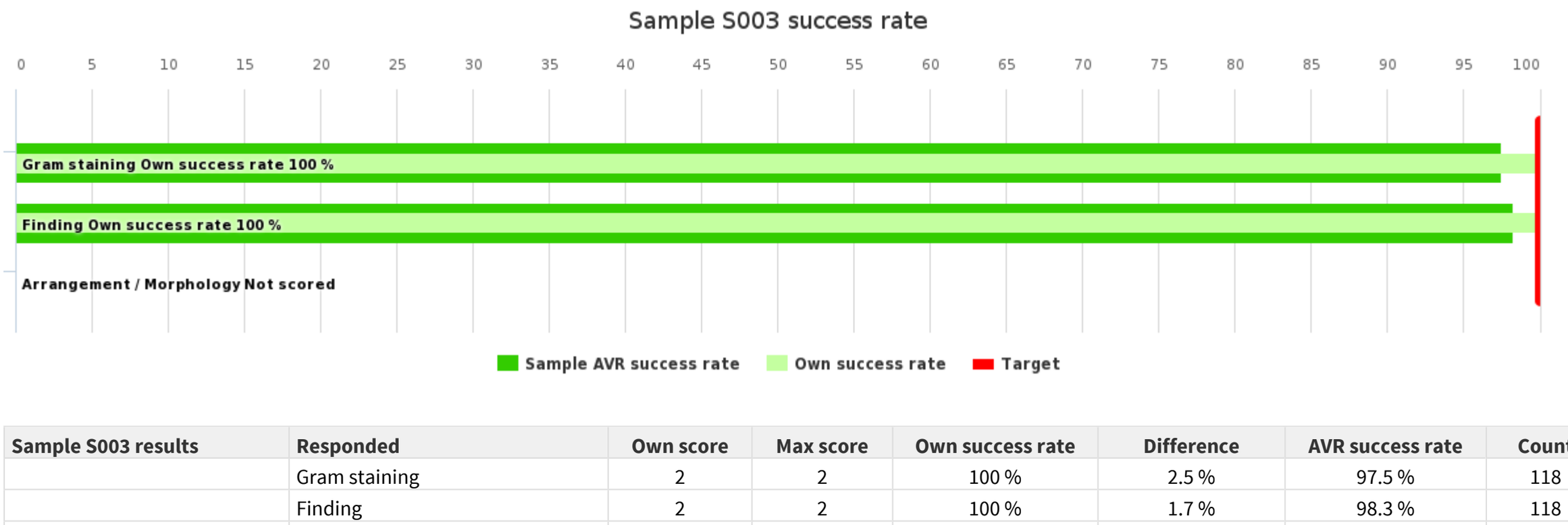
Result	Result count	Own score	Max score	Own success rate	Difference	AVR success rate	Result Score
◉ Cocci	118	2	2	100 %	0 %	100 %	2
Total:	118	2	2	100 %	0 %	100 %	

Sample S002 Arrangement / Morphology

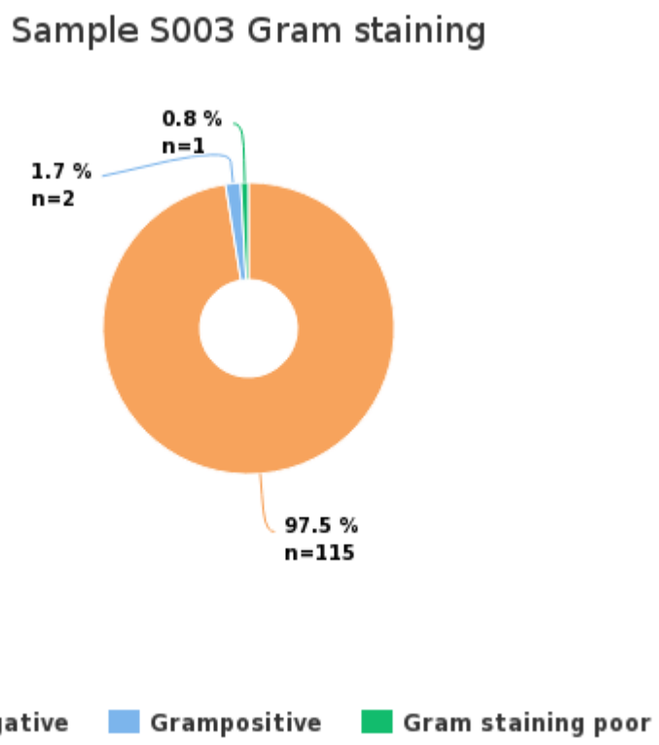


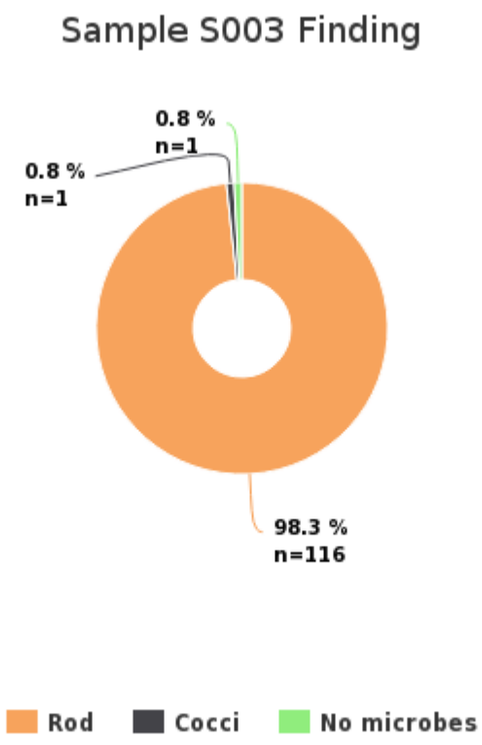
Arrangement / Morphology	Result	Result count
	◉ Cocci by group	79
	Diplococcus	9
	Large coccus	10
	Small coccus	1
	Tetrads	8
	Vary in size	1
	Total:	108

Sample S003 | Fusobacterium nucleatum, gramnegative rod



Sample S003 results	Responded	Own score	Max score	Own success rate	Difference	AVR success rate	Count
	Gram staining	2	2	100 %	2.5 %	97.5 %	118
	Finding	2	2	100 %	1.7 %	98.3 %	118
	Arrangement / Morphology	-	-	-	-	-	156
Total:		4	4	100 %	2.1 %	97.9 %	392





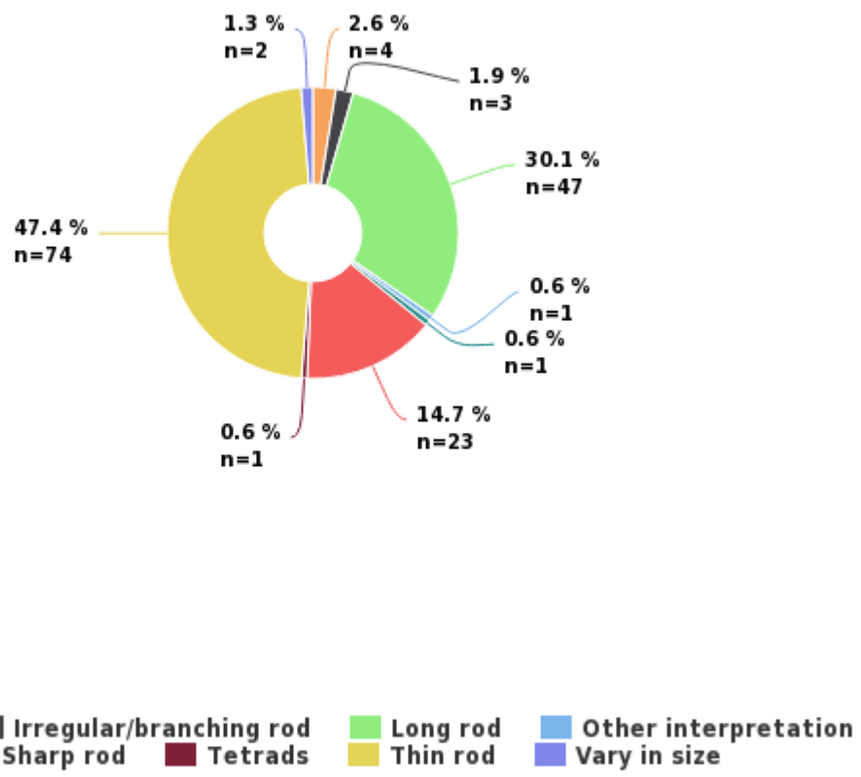
GRAM STAINING

Result	Result count	Own score	Max score	Own success rate	Difference	AVR success rate	Result Score
◉ Gramnegative	115	2	2	100 %	0 %	100 %	2
Grampositive	2	-				0 %	0
Gram staining poor	1	-				0 %	0
Total:	118	2	2	100 %	2.5 %	97.5 %	

FINDING

Result	Result count	Own score	Max score	Own success rate	Difference	AVR success rate	Result Score
◉ Rod	116	2	2	100 %	0 %	100 %	2
Cocci	1	-				0 %	0
No microbes	1	-				0 %	0
Total:	118	2	2	100 %	1.7 %	98.3 %	

Sample S003 Arrangement / Morphology



Arrangement / Morphology	Result	Result count
	Blunt rod	4
	Irregular/branching rod	3
	◉ Long rod	47
	Other interpretation	1
	Palisade	1
	◉ Sharp rod	23
	Tetrads	1
	◉ Thin rod	74
	Vary in size	2
	Total:	156

Report Info

PARTICIPANTS

Altogether 121 laboratories from 16 countries participated in this EQA round.

REPORT INFO

On the front page you can see summaries of overall success rate and sample specific success rates which have been calculated from the scores. The reported results and the scores are presented in the same report but in separate tables.
In general, the expected results are marked with green color. Accepted results may also be indicated with yellow color. Laboratory's own results are indicated with a black radio ◉. In the participant specific report there is also a laboratory specific scoring table for each sample, where you can find your own result and the scores given.
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SCORING

Results reported for the Gram staining and Finding parts are scored. In case of yeast only Finding part is scored. Scoring is implemented when at least 60% of the participants have reported the correct/expected result and when there are at least three reported results. Laboratory's scores have been converted to percentage (own success rate, % from maximum scores) with a target at 100%. Own success rate is compared with the success rate of all results.

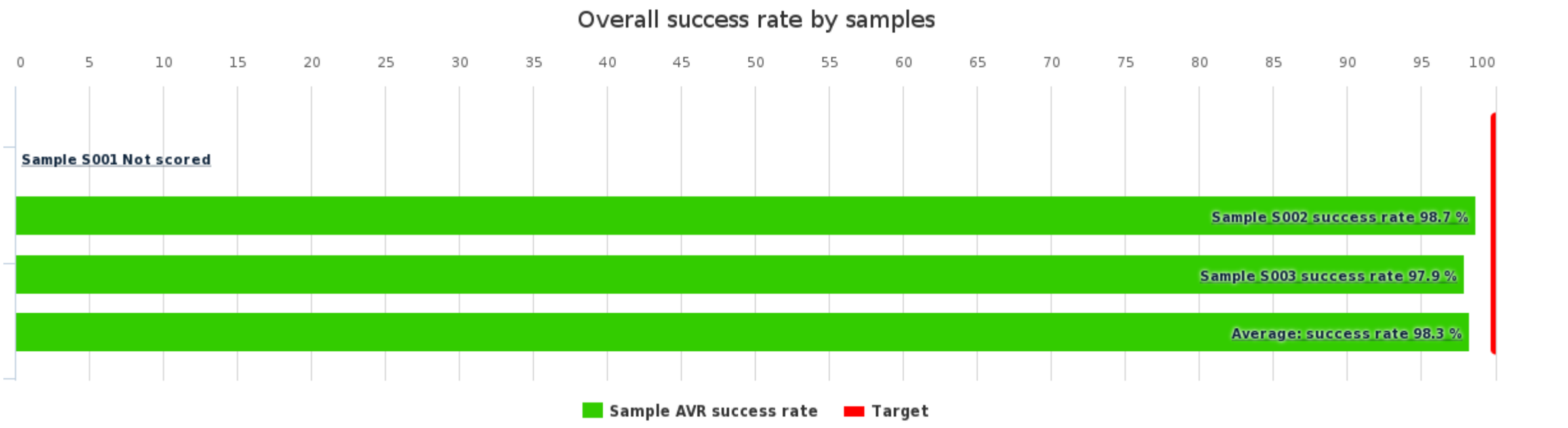
The following general rules are applied:
2 points is given to results that are correct/accepted regarding the expected result
1 point can be given to results that are partly correct/insufficient regarding the expected result
0 point is given to results that are incorrect/false regarding the expected result

Gram stain, colonies, January, 1-2023

GLOBAL REPORT

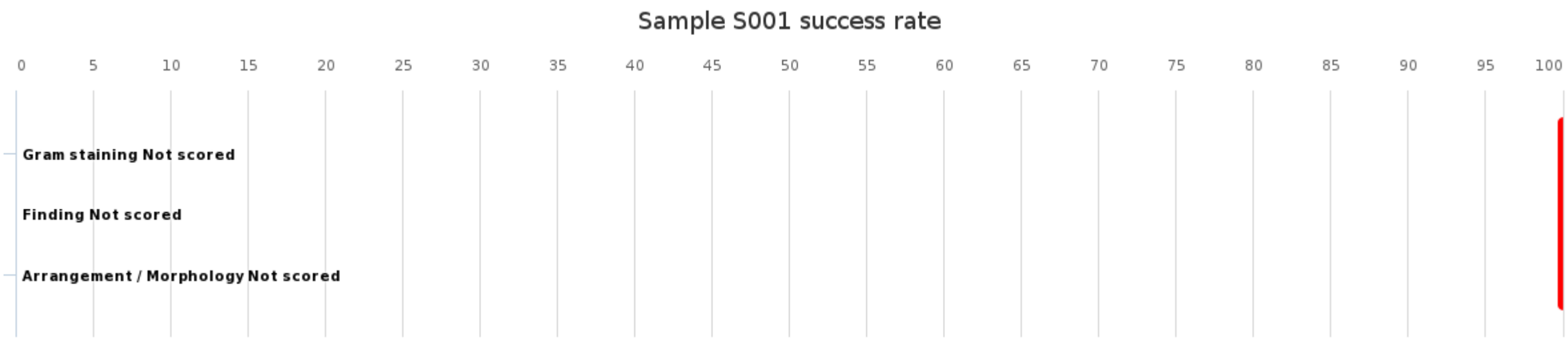
	No of participants	No of responded participants	Response percentage
Gram stain, colonies, January, 1-2023	121	118	97.5 %

Summary

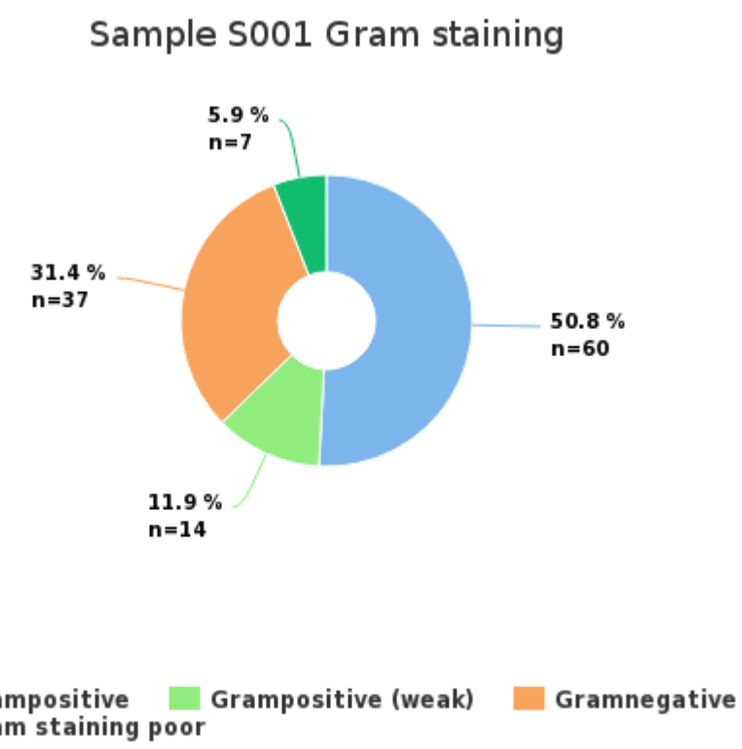


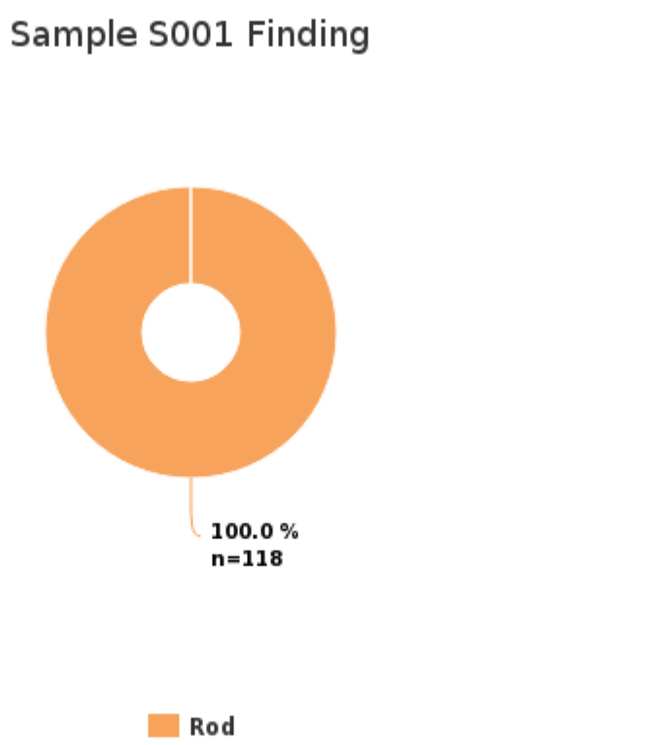
Summary	AVR success rate
Sample S001	-
Sample S002	98.7 %
Sample S003	97.9 %
Average:	98.3 %

Sample S001 | Clostridium perfringens, grampositive rod



Sample S001 results	Responded	AVR success rate	Count
	Gram staining	-	118
	Finding	-	118
	Arrangement / Morphology	-	89
Total:		-	325





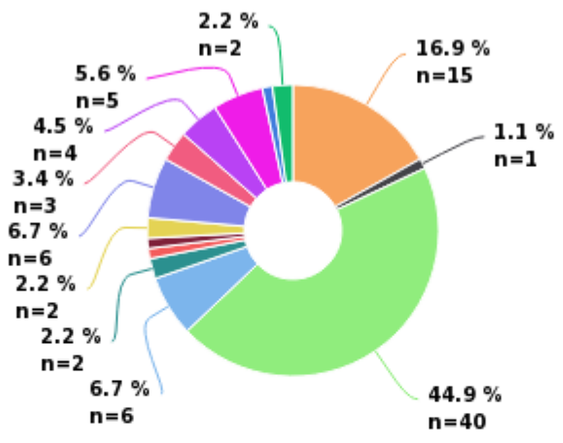
GRAM STAINING

Result	Result count	AVR success rate	Result Score
Grampositive	60	-	-
Grampositive (weak)	14	-	-
Gramnegative	37	-	-
Gram staining poor	7	-	-
Total:	118		

FINDING

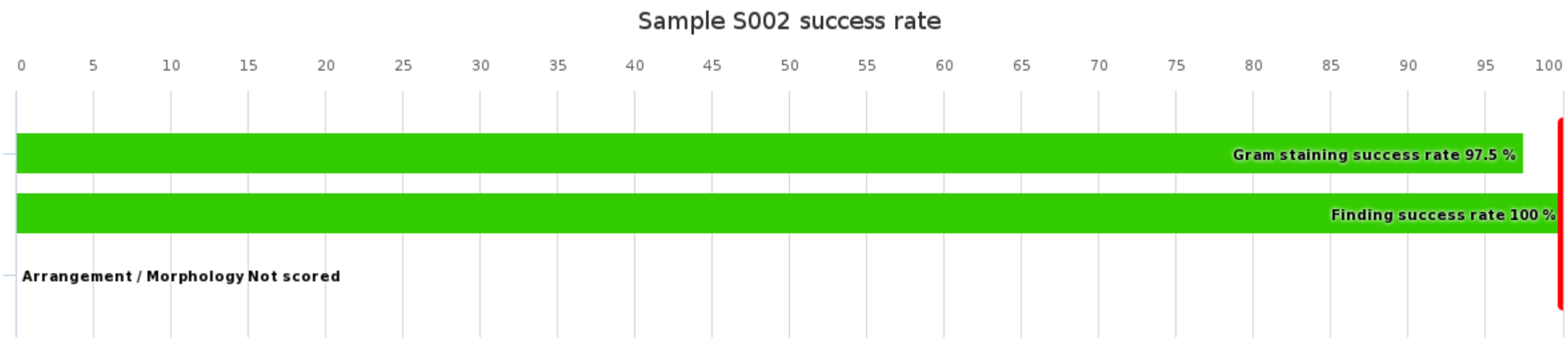
Result	Result count	AVR success rate	Result Score
Rod	118	-	-
Total:	118		

Sample S001 Arrangement / Morphology

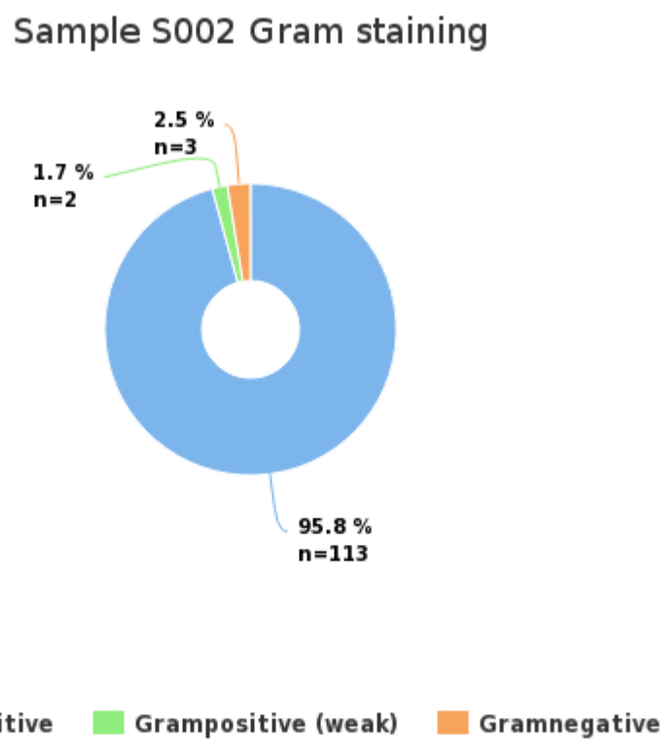


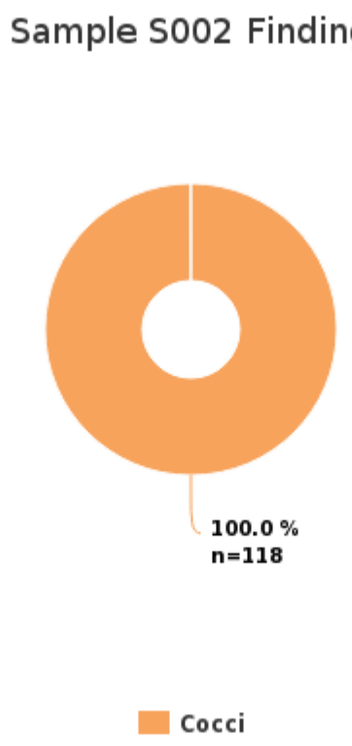
Arrangement / Morphology	Result	Result count
	Blunt rod	15
	Irregular/branching rod	1
	Large rod	40
	Long rod	6
	Other interpretation	2
	Palisade	1
	Rod in chain	1
	Sharp rod	2
	Short/coccoid rod	6
	Thin rod	3
	Variable	4
	Varied rod	5
	Vary in size	1
	With spores	2
	Total:	89

Sample S002 | Finegoldia magna, grampositive cocci



Sample S002 results	Responded	AVR success rate	Count
	Gram staining	97.5 %	118
	Finding	100 %	118
	Arrangement / Morphology	-	108
Total:		98.7 %	344





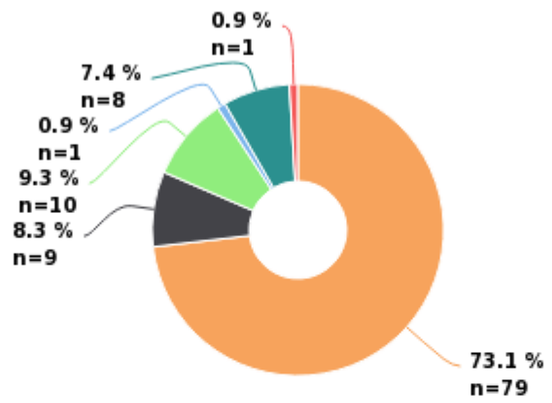
GRAM STAINING

Result	Result count	AVR success rate	Result Score
Grampositive	113	100 %	2
Grampositive (weak)	2	100 %	2
Gramnegative	3	0 %	0
Total:	118	97.5 %	

FINDING

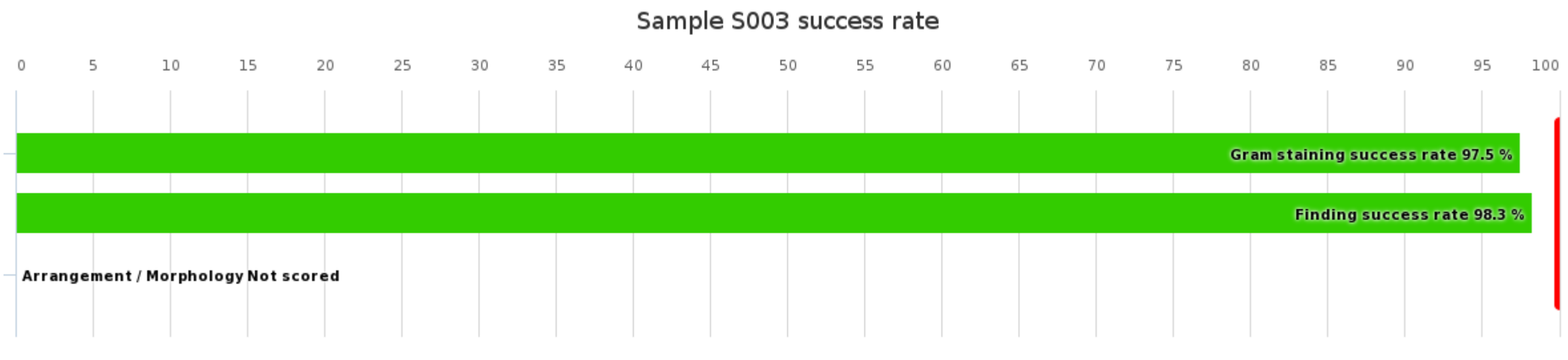
Result	Result count	AVR success rate	Result Score
Cocci	118	100 %	2
Total:	118	100 %	

Sample S002 Arrangement / Morphology

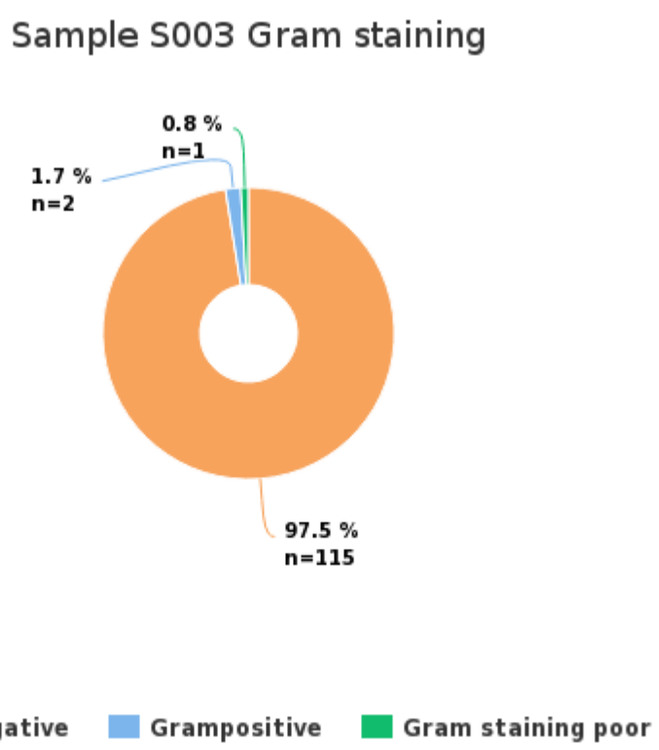


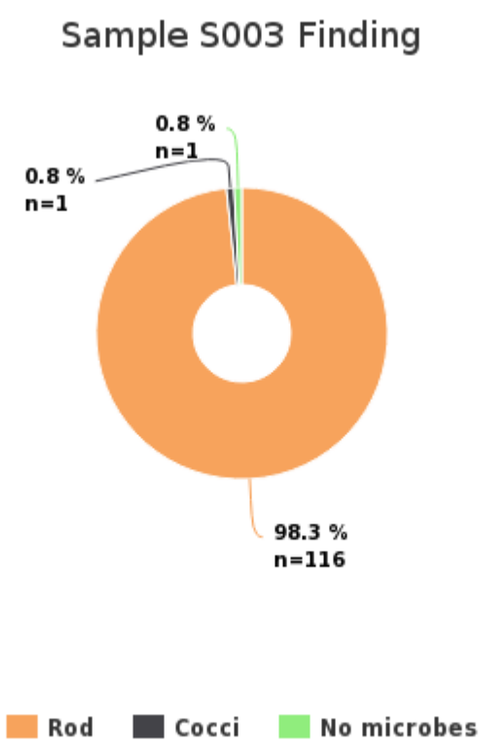
Arrangement / Morphology	Result	Result count
	Cocci by group	79
	Diplococcus	9
	Large coccus	10
	Small coccus	1
	Tetrads	8
	Vary in size	1
	Total:	108

Sample S003 | Fusobacterium nucleatum, gramnegative rod



Sample S003 results	Responded	AVR success rate	Count
	Gram staining	97.5 %	118
	Finding	98.3 %	118
	Arrangement / Morphology	-	156
Total:		97.9 %	392





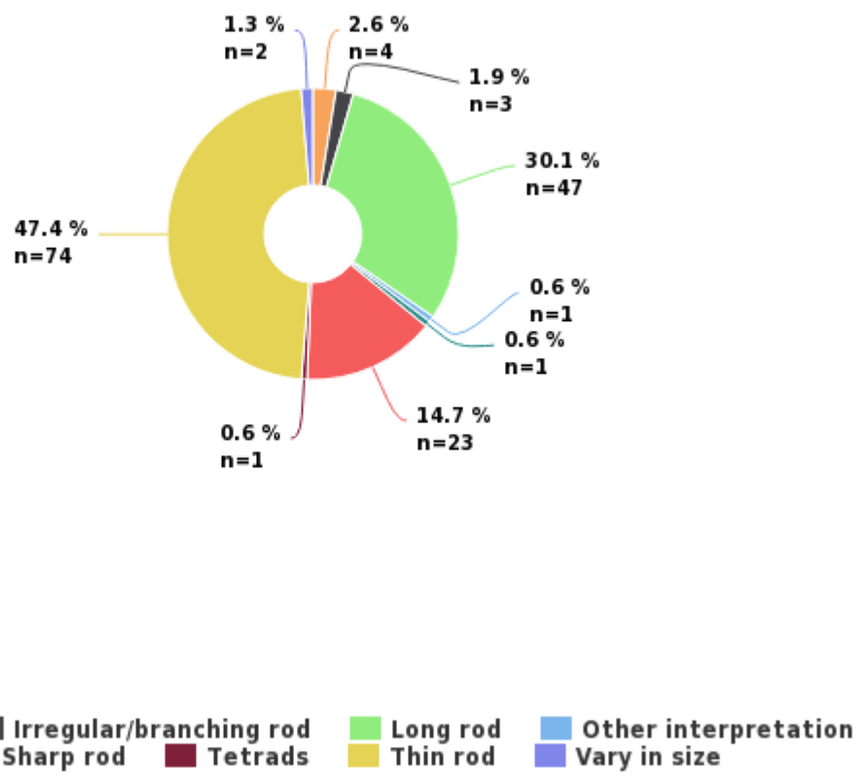
GRAM STAINING

Result	Result count	AVR success rate	Result Score
Gramnegative	115	100 %	2
Grampositive	2	0 %	0
Gram staining poor	1	0 %	0
Total:	118	97.5 %	

FINDING

Result	Result count	AVR success rate	Result Score
Rod	116	100 %	2
Cocci	1	0 %	0
No microbes	1	0 %	0
Total:	118	98.3 %	

Sample S003 Arrangement / Morphology



Arrangement / Morphology	Result	Result count
	Blunt rod	4
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	Other interpretation	1
	Palisade	1
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LABQUALITY

External Quality Assessment Scheme

Gram stain, colonies
Round 1, 2023

Specimens
The samples were three air-fixed microbial suspensions on a slide. The sample lots were tested in an accredited Finnish reference laboratory. Based on the quality controls conducted by the sample material manufacturer, pre-testing and the results obtained in the round, the sample lots are to be considered as homogeneous, stable and suitable for external quality assessment. The materials were sent without temperature control packaging.

The content of the samples was as follows:

Sample S001 (LQ760523011)
Clostridium perfringens, grampositive rod

Sample S002 (LQ760523012)
Finegoldia magna, grampositive cocci

Sample S003 (LQ760523013)
Fusobacterium nucleatum, gramnegative rod

Report info
Please see the description of the data analysis on the last page of the laboratory-specific reports and global reports. It is important to read the Final report first, because it contains important information of the samples and results in each round.

Comments − Expert
In this round, a grampositive rod and coccus, and a gramnegative rod were included. The average success rate was 98.3%. The sample S001 was quite challenging and thus this sample was not scored. I hope that this training sample will give new insights about interpreting the gram staining.

Sample S001: *Clostridium perfringens*
Clostridium perfringens is a grampositive rod. The cells are large (0.6–2.4 µm x 1.3–19 µm), straight and they have blunted ends. Some laboratories commented about the large size and the blunt ends of the cells. *C. perfringens* is a spore-forming bacterium, but the spores are rarely seen on *in vitro* conditions. The spores are large, oval, central or subterminal. A few laboratories reported that they had seen endospores in the slide. Gram staining is variable which is typical for clostridia. This caused a challenge to many laboratories in this round, and this is why I am going to focus on this problem in these comments.

In the final interpretation of gram staining, it is good to consider other characteristics of the bacterium than just what it looks like at first glance. You can also pay attention to different properties when evaluating the staining. For example, gramnegative bacterium does not produce endospores, thus bacterium, which seems to produce endospores is most probably grampositive bacterium than gramnegative. *C. perfringens* rods are quite large and in clinical samples there are only few if any of these large gramnegative rods known. You can use these extra hints to get you on the correct path. It is also good to pay attention if there are even some grampositive cells on the slide or the colour is very intense. These should remind you about the possibility of grampositive rather than gramnegative bacterium. You can even ask what bacterium this might be. Could this be clostridium which has lost its colour?

2023-03-15

FINAL REPORT

Product no. 5040

Subcontracting: Sample preparation, Sample pretesting

Samples sent	2023-01-24
Round closed	2023-02-21
Expected results	2023-02-23
Final report	2023-03-15

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
Jaana Paakkanen
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Expert
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Päivi Mähönen,
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Quality Management
ISO 9001:2015

In addition to notifying the different properties, you can use the microscope to help you. In microscope, true grampositive bacteria disappear quickly when the sharpness is changing (don't be too fast). In contrast, gramnegative bacteria get pale before the cells finally disappear. You can train your eyes for example with *C. perfringens* and *E. coli*. Considering the other properties of the bacteria and also using this disappearance technique, it is possible to interpret a completely gramnegatively stained bacterium correctly as grampositive. It is important to learn to interpret the gram staining more comprehensively as this quick method can help the clinicians to choose the correct antibiotics to the patient more quickly.

The average success rate was 81.4% for this sample. Only 62.7% of the participating laboratories reported gram staining correctly as grampositive (success in previous rounds had varied between 69-88%) and all reported the cells correctly as rod (previously 97-100%). This sample was not scored. Let's see in coming rounds how these tips have been put to testing and use.

Sample S002: *Finegoldia magna*

Finegoldia magna is a grampositive coccus. The size of the cells is variable (ca. 0.8 to 1.9 µm in diameter). Thus, the cell can be small, mid-size or large. In this round, the cells seem to be quite small. The cells are mostly arranged in groups and occasionally in pairs and short chains. Many laboratories commented the arrangement in groups. Gram staining may be variable but, in this round, it was not a problem at all. 97.5% of the participating laboratories reported gram staining correctly as grampositive and all reported the cells correctly as cocci. An average success rate of this specimen (including report of the gram staining and the finding) was 98.7%. This was the easiest sample in this round.

Sample S003: *Fusobacterium nucleatum*

Fusobacterium nucleatum is a gramnegative rod. The cells are thin and long although the length of the cells can vary a lot (from 3 µm to 10 µm). However, usually the cells appear in uniform length in actively growing cells. Tapered to pointed ends of the rods are characteristic to *F. nucleatum* cells. It is good to notice that gram staining of *F. nucleatum* is pale that is typical to many anaerobic gramnegative bacteria. 97.5% of the participating laboratories reported gramstaining correctly as gramnegative (success in previous rounds has varied between 88–96%) and 99% reported the cells correctly as rod (previously 93–96%). About half of the participants commented the thinness and lengthiness of the cells. In addition, many of the laboratories commented the sharpness of the rods.

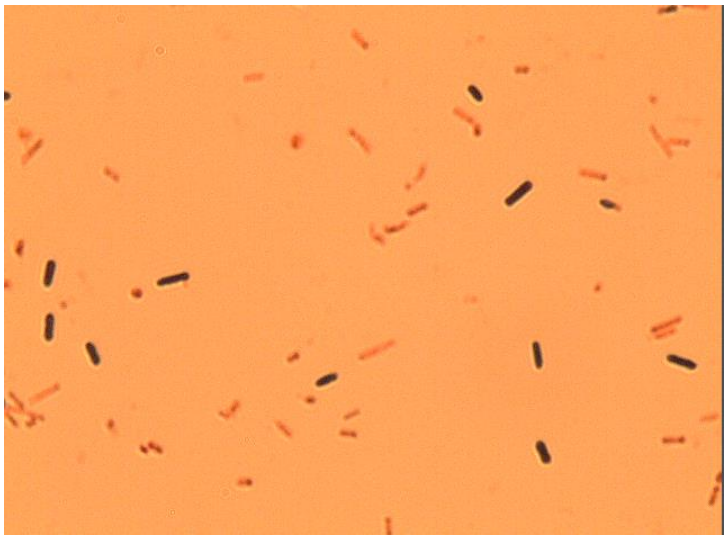
Exceptions in scoring

Sample S001 was not scored as the interpretation of gram staining was too challenging.

End of report

LABQUALITY

5040 Gram stain, colonies 1, 2023
5040 Gramvärjäys, pesäkevärjäys 1, 2023



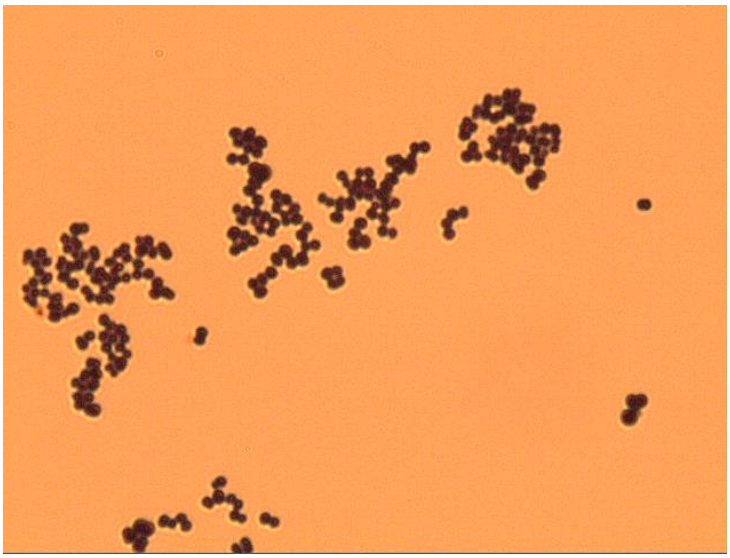
S001 *Clostridium perfringens*

grampositive rod

Characteristics: a large rod with blunt-ends, variable gram staining.

grampositiivinen sauva

Tunnusomaista: iso, tylppäpäinen sauva, värjäytyvyys vaihteleva.



S002 *Finegoldia magna*

grampositive cocci

Characteristics: a small or quite large coccus, arrangement in groups or in short chains.

grampositiivinen kokki

Tunnusomaista: pieni tai melko iso kokki, asettuminen ryhmiin tai lyhyiksi ketjuiksi.

S003 *Fusobacterium nucleatum*

gramnegative rod

Characteristics: a thin and long rod with tapered ends, pale gram staining colour.

gramnegatiivinen sauva

Tunnusomaista: ohut ja pitkä teräväpäinen sauva, hailakka värjäytyvyys.

Pictures taken with 100 x lens. Kuvattu 100 x objektiivilla.

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