External Quality Assessment Scheme

Mycobacterial stain Round 1, 2023

Specimens

Please find enclosed 2 smears on slides S003 and S004.

Caution

Specimens simulate patient specimens and they should be handled in the same way as corresponding specimens capable of transmitting infectious disease.

Examinations

Mycobacterial stain

Storage and use

After arrival, the samples should be stored at +2...8 °C. Specimens are readyfixed preparations. They are to be stained by the staining method that you routinely use. Note that the identification number is on the foil package and the slide is not marked.

Result reporting

Please enter the results via LabScala (www.labscala.com). Interpretation part will be scored.

S003



S004



2023-02-28

INSTRUCTIONS

Product no. 5240 LQ760923013-014/US UN3373

Subcontracting: Sample testing

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

The results should be reported no later than **April 26, 2023**.

The expected results of the round are published in LabScala in the View Reports section by April 28, 2023.

Inquiries EQA Coordinator

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Labquality Oy

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Mycobacterial staining

Client report

	No of participants	No of responded participants	Response percentage
Mycobacterial culture and stain, March, 1-2023	92	90	97.8 %
Mycobacterial stain, March, 1-2023	37	35	94.6 %

Summary



Summary	Own score	Max score	Own success rate	Difference	AVR success rate
Sample S003	2	2	100 %	3.8 %	96.2 %
Sample S004	0	2	0 %	-93.1 %	93.1 %
Average:			50 %	-44.6 %	94.6 %

History	Test nr.	Own success rate	Difference	AVR success rate
Round 2022-1	1-1	100 %	6.7 %	93.3 %
Round 2021-1	1-1	100 %	15 %	85 %
Round 2019-1	1-1	100 %	1.4 %	98.6 %

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Mycobacterial staining

Sample S003 | Positive / AFB, +++



Sample S003 Mycobacterial staining: Interpretation



Sample S003 Mycobacterial staining: Method



Negative 1-4 AFB/prep. 5-10 AFB/prep. (+) 1-25 AFB/field (++) 1-10 AFB/field (++) > 25 AFB/field (+++) > 10 AFB/field (+++)

MYCOBACTERIAL STAINING: INTERPRETATION

Interpretation	Interpretation count	Own score	Max score	Own success rate	Difference	AVR success rate
 Acid fast bacilli 	125	2	2	100 %	0 %	100 %
No acid fast bacilli = negative	5	-				0 %
Total:	130	2	2	100 %	3.8 %	96.2 %

MYCOBACTERIAL STAINING: METHOD

Method	Negative	1-4 AFB/prep.	5-10 AFB/prep. (+)	1-25 AFB/field (++)	1-10 AFB/field (++)	> 25 AFB/field (+++)	> 10 AFB/field (+++)	Total:
Acridine orange	-	-	-	1	-	2	-	3
Auramine	2	1	1	5	3	14	7	33
Auramine-Rodamine	-	-	-	3	1	4	1	9
Kinyoun	-	-	3	2	3	-	1	9
Tan-Thiam-Hok	-	-	-	-	1	-	-	1
Ziehl-Neelsen	4	1	6	6	22	5	31	75
Total:	6	2	10	17	30	25	40	130

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Mycobacterial staining

Sample S004 | No acid-fast bacilli



Sample S004 Mycobacterial staining: Interpretation



Sample S004 Mycobacterial staining: Method



Negative 1-4 AFB/prep. 5-10 AFB/prep. (+)

MYCOBACTERIAL STAINING: INTERPRETATION

🗾 Acid fast bacilli

🔜 No acid fast bacilli = negative

Interpretation	Interpretation count	Own score	Max score	Own success rate	Difference	AVR success rate
No acid fast bacilli = negative	121	-				100 %
 Acid fast bacilli 	9	0	2	0 %	0 %	0 %
Total:	130	0	2	0 %	-93.1 %	93.1 %

MYCOBACTERIAL STAINING: METHOD

Method	Negative	1-4 AFB/prep.	5-10 AFB/prep. (+)	1-10 AFB/field (++)	> 10 AFB/field (+++)	Total:
Acridine orange	3	-	-	-	-	3
Auramine	37	1	-	-	-	38
Auramine-Rodamine	7	-	-	-	_	7
Kinyoun	8	-	-	1	-	9
Tan-Thiam-Hok	1	-	-	-	_	1
Ziehl-Neelsen	64	3	3	1	1	72
Total:	120	4	3	2	1	130

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Mycobacterial staining

PARTICIPANTS

Altogether 127 laboratories from 14 countries participated in this EQA round.

REPORT INFO

On the front page you can see a summary of the overall average success rate and sample specific success rates which have been calculated from the scores.

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 button . 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.. If you have not reported results, you will get a note: "You have not responded in time, only global report is available." For information on report interpretation and performance evaluation, please see the "EQAS Interpretation guidelines" in LabScala User instructions. In case you have any questions regarding the

reports, please contact the EQA Coordinator.

SCORING

The results in the "Interpretation" part can be scored 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 reached by reporting the expected correct result 0 points is given for an incorrect/false result

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Report to the clinician

GLOBAL REPORT

	No of participants	No of responded participants	Response percentage
Mycobacterial culture and stain, March, 1-2023	92	87	94.6 %
Mycobacterial nucleic acid detection, March, 1-2023	23	21	91.3 %

Summary





Sample AVR success rate 🛛 💻 Target

Summary	AVR success rate
Sample S001	92 %
Sample S002	98.2 %
Average:	95.1 %

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Report to the clinician

Sample S001 | Mycobacterium intracellulare



Sample S001 results	Responded	AVR success rate	Count
	Report to the clinician	92 %	109
Total:		92 %	109

Sample S001 Mycobacterium intracellulare



Mycobacterium intracellulare	
Mycobacterium avium-intracellul	are complex
Mycobacterium avium complex	Mycobacterium chimaera
Mycobacteria, other than M. tube	erculosis (MOTT)
Negative (M. tuberculosis not de	tected)
Negative (M. tuberculosis comple	x not detected)
📒 Atypical mycobacteria 🛛 🔤 Myco	bacterium sp. 🛛 📕 Acid-fast bacilli
Mycobacterium tuberculosis	Mycobacterium tuberculosis complex
No acid-fast bacilli	

REPORT TO THE CLINICIAN

Finding group	Finding	Finding count	Referred	Not referred	AVR success rate
Mycobacterium intracellulare		109			92 %
	Mycobacterium intracellulare	20	8	12	
	Mycobacterium avium-intracellulare complex	2	1	1	
	Mycobacterium avium complex	1		1	
	Mycobacterium chimaera	2		2	
	Mycobacteria, other than M. tuberculosis (MOTT)	43	26	17	
	Negative (M. tuberculosis not detected)	8	1	7	
	Negative (M. tuberculosis complex not detected)	14	4	10	
	Atypical mycobacteria	4	4		
	Mycobacterium sp.	3	3		
	Acid-fast bacilli	8	5	3	
	Mycobacterium tuberculosis	1	1		
	Mycobacterium tuberculosis complex	1	1		
	No acid-fast bacilli	2		2	
Total:		109			92 %

SCORING SUMMARY

Finding group	Finding	Finding score	Referred	Max score
Mycobacterium intracellulare				4
	Mycobacterium intracellulare	4		4
	Mycobacterium avium-intracellulare complex	4		4

Mycobacteria, March, 1-2023

Report to the clinician

	Mycobacterium avium complex	4		4
	Mycobacterium chimaera	4		4
	Mycobacteria, other than M. tuberculosis (MOTT)	4		4
	Negative (M. tuberculosis not detected)	4		4
	Negative (M. tuberculosis complex not detected)	4		4
	Atypical mycobacteria	4		4
	Mycobacterium sp.	4		4
	Acid-fast bacilli	1	1	4
	Mycobacterium tuberculosis	0		4
	Mycobacterium tuberculosis complex	0		4
	No acid-fast bacilli	0		4
Total:				4

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Report to the clinician

Sample S002 | No acid-fast bacilli



Sample S002 results	Responded	AVR success rate	Count
	Report to the clinician	98.2 %	109
Total:		98.2 %	109

Sample S002 No acid-fast bacilli



No acid-fast bacilli Negative (M. tuberculosis not detected) Negative (M. tuberculosis complex not detected) Mycobacterium tuberculosis Mycobacterium tuberculosis complex

REPORT TO THE CLINICIAN

Finding group	Finding	Finding count	Referred	Not referred	AVR success rate
No acid-fast bacilli		109			98.2 %
	No acid-fast bacilli	44	1	43	
	Negative (M. tuberculosis not detected)	21	7	14	
	Negative (M. tuberculosis complex not detected)	42	2	40	
	Mycobacterium tuberculosis	1	1		
	Mycobacterium tuberculosis complex	1	1		
Total:		109			98.2 %

SCORING SUMMARY

Finding group	Finding	Finding score	Max score
No acid-fast bacilli			4
	No acid-fast bacilli	4	4
	Negative (M. tuberculosis not detected)	4	4
	Negative (M. tuberculosis complex not detected)	4	4
	Mycobacterium tuberculosis	0	4
	Mycobacterium tuberculosis complex	0	4
Total:			4

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Mycobacteria, March, 1-2023

Report to the clinician

Report Info

PARTICIPANTS

Altogether 115 laboratories from 14 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 button **()**. 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. If you have not reported results you will get a note: "You have not responded in time, only global report is available". For information on report interpretation and performance evaluation, please see the "EQAS Interpretation guidelines" in LabScala User instructions. In case you have any questions regarding the

reports, please contact the EQA Coordinator.

SCORING

The results in the "Report to the clinician" part can be scored when at least 60% of the participants have reported the correct/expected result and when there are at least three reported results. The report includes a sample specific scoring summary.

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:

4 points is reached by reporting the expected result 1-3 points is given to results that are partly correct/insufficient regarding the expected finding 0 points is given for an incorrect/false result

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Culture media and identification tests

GLOBAL REPORT

	No of participants	No of responded participants	Response percentage
Mycobacterial culture and stain, March, 1-2023	92	86	93.5 %
Mycobacterial nucleic acid detection, March, 1-2023	23	21	91.3 %

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Culture media and identification tests

Sample S001 | Mycobacterium intracellulare

Sample S001 results	Responded	Count
	Culture media	128
	Identification tests: NAT	92
	Identification tests: Other	56
	Identification tests: MALDI-TOF	8
Total:		284



CULTURE MEDIA

Finding group	Method	Growth after 1 week	Growth after 2 weeks	Growth after 3 weeks	Growth after 4 weeks	Growth after 5 weeks	Growth after 8 weeks	No growth	Total:
Mycobacterium intracellulare									128
	BacT/ALERT MP (bioMerieux)	1	-	-	-	-	-	-	1
	Bactec MGIT (BD)	36	1	2	-	-	-	2	41
	Bactec Myco/F Lytic Culture Vials (BD)	-	1	-	-	-	-	-	1
	Coletsos medium	1	3	3	2	1	-	2	12
	Finlayson medium	-	-	1	-	-	-	-	1
	Löwenstein-Jensen	10	28	15	9	2	1	1	66
	Löwenstein-Jensen+pyruvic acid	-	1	-	1	1	-	-	3
	MiddleBrooks	1	-	-	-	-	-	-	1
	Stonebrink medium	-	-	-	-	1	-	-	1
	VersaTREK Myco medium	1	-	-	-	-	-	-	1
	Total:	50	34	21	12	5	1	5	128

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Mycobacteria, March, 1-2023

Culture media and identification tests



IDENTIFICATION TESTS: NAT

Finding group	Method	Result	Result count
Mycobacterium intracellulare	BD MAX MDR-TB panel (BD)	Negative	1
		Negative (M. tuberculosis complex not detected)	1
	GenoType MTBC (Hain Lifescience)	Mycobacterium intracellulare	1
	GenoType MTBDRplus 2.0 (Hain Lifescience)	Atypical mycobacteria	1
	GenoType mycobacterium AS (Hain Lifescience)	Mycobacteria, other than M. tuberculosis (MOTT)	1
	GenoType mycobacterium CM (Hain Lifescience)	Atypical mycobacteria	1
		Mycobacterium avium complex	1
		Mycobacterium avium-intracellulare complex	2
		Mycobacterium intracellulare	13
	GenoType NTM-DR (Hain Lifescience)	Mycobacterium intracellulare	6
	In-house hsp65 gene sequencing	Mycobacterium avium-intracellulare complex	1
		Mycobacterium intracellulare	4
	In-house PCR	Mycobacterium intracellulare	1
	MDR/MTB ELITe MGB Kit (ELITechGroup)	Negative (M. tuberculosis complex not detected)	1
	Seegene Anyplex MTB/NTM Real-time Detection	Atypical mycobacteria	1
	Xpert MTB/RIF (Cepheid)	Mycobacteria, other than M. tuberculosis (MOTT)	1
		Negative	1
		Negative (M. tuberculosis complex not detected)	6
		Negative (M. tuberculosis not detected)	3
	Xpert MTB/RIF Ultra (Cepheid)	Mycobacteria, other than M. tuberculosis (MOTT)	1
		Negative	2
		Negative (M. tuberculosis complex not detected)	31
		Negative (M. tuberculosis not detected)	10
		No acid-fast bacilli	1
Total:			92

IDENTIFICATION TESTS: OTHER

Finding group	Method	Result	Result count
Mycobacterium intracellulare	BD MGIT TBc Identification test (BD)	Atypical mycobacteria	1
		Mycobacteria, other than M. tuberculosis (MOTT)	1
		Negative	1
		Negative (M. tuberculosis complex not detected)	5
	In-house Nitrate test	Negative (M. tuberculosis complex not detected)	1
	SD BIOLINE TB Ag MPT64 (SD)	Atypical mycobacteria	1
		Mycobacteria, other than M. tuberculosis (MOTT)	7
		Negative	15
		Negative (M. tuberculosis complex not detected)	13
		Negative (M. tuberculosis not detected)	3
	TB MPT 64 Ag test (ubio Biotechnology Systems)	Mycobacteria, other than M. tuberculosis (MOTT)	2
		Negative	2
		Negative (M. tuberculosis not detected)	2

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Mycobacteria, March, 1-2023

Culture media and identification tests

	TBCheck MPT64 (Hain Lifescience)	Mycobacteria, other than M. tuberculosis (MOTT)	1
		Negative (M. tuberculosis complex not detected)	1
Total:			56



IDENTIFICATION TESTS: MALDI-TOF

Finding group	Method	Result	Score / Probability %	Score / Probability % count
Mycobacterium intracellulare	MALDI Biotyper (Bruker)	Mycobacterium avium-intracellulare complex	≥2	1
		Mycobacterium chimaera	≥2	2
		Mycobacterium intracellulare	≥2	1
			≥1.7<2	1
		Negative	N/A	1
	VITEK MS (bioMérieux)	Mycobacterium intracellulare	99,9 %	1
			99,5 %	1
Total:				8

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Culture media and identification tests

Sample S002 | No acid-fast bacilli

Sample S002 results	Responded	Count
	Culture media	126
	Identification tests: NAT	58
	Identification tests: Other	4
Total:		188







CULTURE MEDIA

Finding group	Method	Growth after 1 week	Growth after 6 weeks	Growth after 7 weeks	Growth after 8 weeks	No growth	Total:
No acid-fast bacilli							126
	BacT/ALERT MP (bioMerieux)	-	-	-	-	1	1
	Bactec MGIT (BD)	1	-	-	-	38	39
	Bactec Myco/F Lytic Culture Vials (BD)	-	-	-	-	1	1
	Coletsos medium	-	-	-	-	11	11
	Finlayson medium	-	-	-	-	1	1
	Löwenstein-Jensen	-	4	1	1	61	67
	Löwenstein-Jensen+pyruvic acid	-	-	-	-	3	3
	MiddleBrooks	-	1	-	-	-	1
	Stonebrink medium	-	-	1	-	-	1
	VersaTREK Myco medium	-	-	-	-	1	1
	Total:	1	5	2	1	117	126

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Mycobacteria, March, 1-2023

Culture media and identification tests



IDENTIFICATION TESTS: NAT

Finding group	Method	Result	Result count
No acid-fast bacilli	BD MAX MDR-TB panel (BD)	Negative	1
		Negative (M. tuberculosis complex not detected)	1
	GenoType MTBDRplus 2.0 (Hain Lifescience)	Atypical mycobacteria	1
	GenoType mycobacterium CM (Hain Lifescience)	No acid-fast bacilli	2
	Seegene Anyplex MTB/NTM Real-time Detection	Negative	1
	Xpert MTB/RIF (Cepheid)	Negative	1
		Negative (M. tuberculosis complex not detected)	6
		Negative (M. tuberculosis not detected)	2
	Xpert MTB/RIF Ultra (Cepheid)	Negative	2
		Negative (M. tuberculosis complex not detected)	31
		Negative (M. tuberculosis not detected)	9
		No acid-fast bacilli	1
Total:			58

IDENTIFICATION TESTS: OTHER

Finding group	Method	Result	Result count
No acid-fast bacilli	SD BIOLINE TB Ag MPT64 (SD)	Atypical mycobacteria	1
		Negative	1
		Negative (M. tuberculosis complex not detected)	1
	TB MPT 64 Ag test (ubio Biotechnology Systems)	Negative	1
Total:			4

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Mycobacterial staining

GLOBAL REPORT

	No of participants	No of responded participants	Response percentage
Mycobacterial culture and stain, March, 1-2023	92	90	97.8 %
Mycobacterial stain, March, 1-2023	37	35	94.6 %

Summary



Overall success rate by samples



Summary	AVR success rate
Sample S003	96.2 %
Sample S004	93.1 %
Average:	94.6 %

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Mycobacterial staining

Sample S003 | Positive / AFB, +++



Sample S003 Mycobacterial staining: Interpretation



Sample S003 Mycobacterial staining: Method



Negative 1-4 AFB/prep. 5-10 AFB/prep. (+) 1-25 AFB/field (++) 1-10 AFB/field (++) > 25 AFB/field (+++) > 10 AFB/field (+++)

MYCOBACTERIAL STAINING: INTERPRETATION

Interpretation	Interpretation count	AVR success rate	Interpretation Score
Acid fast bacilli	125	100 %	2
No acid fast bacilli = negative	5	0 %	0
Total:	130	96.2 %	

MYCOBACTERIAL STAINING: METHOD

Method	Negative	1-4 AFB/prep.	5-10 AFB/prep. (+)	1-25 AFB/field (++)	1-10 AFB/field (++)	> 25 AFB/field (+++)	> 10 AFB/field (+++)	Total:
Acridine orange	-	-	-	1	-	2	-	3
Auramine	2	1	1	5	3	14	7	33
Auramine-Rodamine	-	-	-	3	1	4	1	9
Kinyoun	-	-	3	2	3	-	1	9
Tan-Thiam-Hok	-	-	-	-	1	-	-	1
Ziehl-Neelsen	4	1	6	6	22	5	31	75
Total:	6	2	10	17	30	25	40	130

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Mycobacterial staining

Sample S004 | No acid-fast bacilli



Sample S004 Mycobacterial staining: Interpretation



Sample S004 Mycobacterial staining: Method





MYCOBACTERIAL STAINING: INTERPRETATION

🗾 Acid fast bacilli

🔜 No acid fast bacilli = negative

Interpretation	Interpretation count	AVR success rate	Interpretation Score
No acid fast bacilli = negative	121	100 %	2
Acid fast bacilli	9	0 %	0
Total:	130	93.1 %	

MYCOBACTERIAL STAINING: METHOD

Method	Negative	1-4 AFB/prep.	5-10 AFB/prep. (+)	1-10 AFB/field (++)	> 10 AFB/field (+++)	Total:
Acridine orange	3	-	-	-	-	3
Auramine	37	1	-	-	-	38
Auramine-Rodamine	7	-	-	-	-	7
Kinyoun	8	-	-	1	-	9
Tan-Thiam-Hok	1	-	-	-	-	1
Ziehl-Neelsen	64	3	3	1	1	72
Total:	120	4	3	2	1	130

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Mycobacteria, March, 1-2023

Mycobacterial staining

Report Info

PARTICIPANTS

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The following general rules are applied: 2 points is reached by reporting the expected correct result 0 points is given for an incorrect/false result

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External Quality Assessment Scheme

Mycobacterial culture and stain (5220) Mycobacterial nucleic acid detection (5221) Mycobacterial stain (5240) Rounds 1, 2023

Specimens

The round included two lyophilized samples and/or two smears on slides. The sample lots were tested in an accredited Finnish reference laboratory and the results were consistent with the certificates provided by the sample manufacturer. Based on the quality controls conducted by the sample material manufacturer, 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 use of samples only for external quality assessment. The consent of Labquality must be requested for the use of the microbial strains contained in the samples for other purposes.

The content of the samples was as follows:

Sample S001 (LQ760923011) *Mycobacterium intracellulare* N000062 Background information: simulated sputum

Sample S002 (LQ760923012) No acid-fast bacilli Background information: simulated sputum

Sample S003 (LQ760923013) Positive / AFB, +++

Sample S004 (LQ760923014) No acid-fast bacilli

Test method: Reference laboratory stained the slides with the direct auramin method.

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

This round consisted of *Mycobacterium intracellulare* in sample S001 and a negative sample S002. Slide S003 was strong positive and slide S004 was negative.

Staining

The average success rate for the positive slide S003 was 96.2% with 5/130 false negative results, and for the negative slide S004 the success rate was 93.1% with 9/130 false positive results, of which 4 were weak positives (1-4 cells/slide). Three laboratories had possibly mixed up the samples and reported crosswise correct results. Additionally, two laboratories had discrepancies between parallel initial results and/or final reports.

2023-05-09

FINAL REPORT

Product no. 5220, 5221, 5240

Subcontracting: Sample testing

2023-02-28
2023-04-26
2023-04-28
2023-05-09

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 jaana.paakkanen@labquality.fi

Expert

Microbiologist, Ph.D, Docent Silja Mentula, National Institute for Health and Welfare, Finland

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Culture and identification

Sample S001

The average success rate for sample S001 was 92% with 25/109 (23%) species level identifications, 50 NTM identifications, TB was ruled out by 22 participants, 8 reported acid-fast bacilli, 2 false TB findings and 2 false negative results. All species in the *avium-intracellulare* complex were accepted as correct result (*M. avium, M. intracellulare, M. chimaera*). Species level identification was performed by Hain Genotype in 22, by In-house PCR / sequencing in 6 and by MALDI-TOF in 8 laboratories. Some of the laboratories used multiple methods. "No growth" was reported by 5 laboratories. There were altogether 92 nucleic acid amplification test results, 55 immunologic test results, 8 MALDI-TOF results and 1 nitrate result. Culture was performed by 82 laboratories.

One laboratory reported "no acid-fast bacilli" after a negative result from TB detection test, hence, the correct reporting option would have been "negative (*M. tuberculosis* not detected)". There were also two reporting errors with correct initial TB negative result but false final TB positive report.

Sample S002

The average success rate for the negative sample S002 was 98.2% with TB ruled out by 63 participants, 44 reported no acid-fast bacilli and 2/109 reported false TB findings. Growth was reported by 5 laboratories.

Conclusions

The round went well though the cell counts in staining scattered somewhat and there were some reporting errors.

Exceptions in scoring No exceptions.

End of report

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