

SOLUS *LISTERIA* *Biochemical* *Identification Kit*

Biochemical Test System for the Identification of
Listeria species

Insert Number: 54
Issue Number: 0.2
Date of Issue: Mar 24
Product Code(s): LISTID12
Certifying Body: N/A



For the Better

INTRODUCTION

1. INTENDED USE

The Solus *Listeria* identification kit is based on an eight well microstrip system for identifying suspected *Listeria* isolates from selective or non-selective agars. This is achieved through the fermentation of a range of carbohydrate substrates and haemolytic activity.

The assay is designed to identify the following species that are currently included in ISO 11290-1:2017:

- Established *Listeria* spp.: *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. ivanovii*, *L. welshimeri*, *L. grayi*.
- New *Listeria* spp.: *L. marthii*, *L. weihenstephanensis*, *L. fleischmannii* and *L. rocourtiae*.

This identification test is easy to perform; however, it requires microbiological laboratory facilities and must be used by qualified and appropriately trained personnel. Using this kit requires compliance with Good Laboratory Practices (refer to EN ISO 7218).

2. REAGENTS PROVIDED

The Solus *Listeria* Identification Kit contains the following items for performing 24 tests.

Component	Appearance	Comments
2 Assay plates	96-Well microplate with removeable strips.	Wells containing seven dehydrated substrates and Phenol Red indicator
24 Suspension vials	Plastic red-top vials containing a straw-coloured fluid.	Used for the suspension of isolated <i>Listeria</i> colonies.
Haemolysis reagent	Dropper vial containing stabilised red blood cells.	Red blood cells resuspended in a diluent. To be used in well H for the detection of haemolysin producing isolates.
24 Coverslip strips	Clear perforated adhesive sealing strips with white-backed labelling tails.	Used to cover the microwell plate during incubation to minimise evaporative loss, sample labelling, and for safe storage.
Product Instructions	Paper booklet containing instructions for the use of the kit.	Results tables for converting results code into identification result found within.
Solus <i>Listeria</i> ID Report Cards	Card report tables for the record of results of individual identifications	Used to record results and generate a result code for species determination

The kit and its contents should be stored at 2-8°C until the expiry date stated.
Any unused kit components should be returned to storage at 2-8°C immediately after use.

3. Principle of Test.

Each biochemical identification consists of an 8 well microstrip. Each strip contains 7 dehydrated carbohydrate fermentation tests (Rows A – G). An empty well is reserved for the haemolysis test (Row H). The substrates are selected to enable the biochemical identification of all species of *Listeria* described in ISO 11290-1:2017 (E). A sealing perforated coverslip is provided to cover the microstrip. This allows some air exchange in the well but minimises evaporation and prevents any spillage of the well contents.

The test is performed by inoculating 2-3 drops (approximately 100µl) of a suspension of the isolate to be identified into each of the wells. After 18-24 h incubation at 35-37°C the colours of the wells and the haemolysis reaction is recorded on a report card. The results are then interpreted using the reference guide (below) and the code calculated. Using this code an identification can be determined using the Results Tables found within page 9-13 of this insert.

Reaction Type	Substrate Utilisation							Haemolysis Reaction
	Seven dehydrated substrates with phenol red indicator.							
Location	Well 1 (Row A)	Well 2 (Row B)	Well 3 (Row C)	Well 4 (Row D)	Well 5 (Row E)	Well 6 (Row F)	Well 7 (Row G)	Well 8 (Row H)
Substrate	Arabitol	Mannitol	Rhamnose	Xylose	Tagatose	Glucose-1-Phosphate	Methyl-D-Mannose	Add haemolysin reagent
Positive	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Light brown liquid
Negative	Red / Orange	Red / Orange	Red / Orange	Red / Orange	Red / Orange	Red / Orange	Red / Orange	Ring of red cells

4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

The following materials are required for the confirmation that isolates being identified are in fact *Listeria* species.

Sterile bacteriological loops.

Micropipette (100µL) and sterile tips or disposable Pasteur pipettes.

Incubator (35-37°C).

Refrigerator (2-8°C).

Oxidase strips.

Hydrogen peroxide (3%).

Gram stain kit.

Microscope and slides.

Non-selective agar plates.

5. METHOD

Before use of the Solus *Listeria* ID kit the selected isolates should be confirmed as:

- Short Gram-positive rods
- Oxidase negative
- Catalase positive







Motility at 25°C was previously recommended as a distinct feature of *Listeria* spp. but *L. fleischmannii* is non-motile. However, performing a motility test may still provide useful information and help to confirm those species of *Listeria* other than *L. fleischmannii*.

1. Remove the kit from storage at least one hour before use to allow the components to reach ambient temperature (18-25°C).
2. Remove the required number of strips from the pouch, haemolysis reagent, and the required number of suspension fluids for the number of identifications to be performed. Fit the strips into the frame provided. We recommend there is at least one empty space between each test strip.
3. Ensure that the empty haemolysis well is placed within row H of the microwell plate frame.

4. Using a sterile bacterial loop select a well isolated colony from an 18–24-hour agar plate culture. Isolates can be taken from both selective and non-selective media.
5. Emulsify the colony into a single vial of suspension fluid. Mix the suspension in the vial thoroughly.
6. Using a disposable Pasteur pipette dispense 2-3 drops (approximately 100 µl) or using a micropipette with a sterile tip dispense 100µl of the bacterial suspension into all 8 wells of a single test strip.
7. Gently mix the Haemolysis reagent so that all red blood cells are resuspended. DO NOT VORTEX.
8. Place 1 drop of Haemolysis reagent into the final well (row H). Ensure that that dropper does not come in to contact with the inoculated well.
9. Once complete, carefully seal each microwell strip with an adhesive coverslip. Ensure the coverslip has adhered to each well of the microwell strip and is completely covering the circumference of the well.
10. Label each microwell strip using the white-backed labelling tails found on the coverslip.
11. Repeat steps 4-10 for all isolates to be tested until all strips are prepared.
12. Place the assay plate into a 35-37°C incubator and incubate for 18-24 hours.
13. Unused strips, suspension fluid vials and haemolysis reagent should be returned to storage at 2-8°C.

6. INTERPRETATION OF RESULTS


1. After incubation, peel back the coverslip and record the results of each test on the Solus *Listeria* ID Report Sheets. Interpretation of results are as follows:

Sugar Metabolism (Rows A-G)	Interpretation	Haemolysis Test (Row H)	Interpretation
<p style="text-align: center;">Red</p> 	Negative	<p style="text-align: center;">Deposited Red Cells (No Haemolysis)</p> 	Negative
<p style="text-align: center;">Orange</p> 	Negative	<p style="text-align: center;">Pellet with partial haemolysis</p> 	Positive
<p style="text-align: center;">Yellow</p> 	Positive	<p style="text-align: center;">Complete Haemolysis</p> 	Positive

2. Interpretation of Sugar Fermentation Reactions (Rows A-G): Red and Orange wells are interpreted as NEGATIVE. Yellow wells are interpreted as POSITIVE.

3. Interpretation of Haemolysis Reaction (Row H): A NEGATIVE reaction will have settled red blood cells in the form of a circle or crescent at the base of the well. A POSITIVE reaction will either appear as total haemolysis of the red blood cells or a partial haemolysis of the red blood cells in the well. A POSITIVE well may look brown or darkened.
4. On the Solus *Listeria* ID Report Sheets record the details of the test including operator, date performed, sample details, and additional biochemical information generated from complementary tests to confirm that the isolate is a *Listeria* species.
5. Interpret the results as described above. Score results as either positive (+ or Tick) or negative (-)
6. To calculate the Identification Code, if a reaction is positive then include the score in the final code.
7. Combine the-scores from reactions 1-3, 4-6, and 7-8 respectively in the "Code" Box below, to form a 3-digit code.
8. Cross reference the code with the Results Table found on pages 9-12 of this insert to determine the species of the sample.

Below is an example of a Solus *Listeria* ID Report Card:



LISTERIA BIOCHEMICAL ASSAY
REPORT CARD

Known Listeria Isolate?		✓	Operator		Tom Wright							
Listeria Confirmation Test/s			Date		08.02.24							
Test	Result	Expected	Sample Type		Sliced Smoked Ham							
Gram Stain	+	+	Code/Designation		AHC-2776-76							
Morphology	Short Rod	Short Rod	Location	ARA	MAN	RHA	XYL	TAG	G1P	MDM	HAEM	
Oxidase	-	-	Score	4	2	1	4	2	1	4	2	
Catalase	+	+	Result (Tick)	✓	-	✓	-	-	-	✓	✓	
Motility	25°C: +	25°C: +	Code	5			0			6		
	37°C: -	37°C: -										
Other: ELISA	2.150	>0.20	Species Determined		L. monocytogenes							

Species Metabolism Percentage Database

	ARA	MAN	RHA	XYL	TAG	G1P	MDM	HAEM
<i>L. monocytogenes</i>	97	0.1	98	0.1	0.1	3	98	99
<i>L. innocua</i>	99	0.1	85	1	0.1	0.1	99.9	0.1
<i>L. marthii</i>	99.9	0.1	0.1	1	0.1	0.1	95	0.1
<i>L. seeligeri</i>	99	0.1	0.1	99.9	0.1	0.1	5	93
<i>L. ivanovii</i>	99.9	0.1	5	95	0.1	91	0.1	90
<i>L. welshimeri</i>	99.9	0.1	85	50	91	0.1	95	0.1
<i>L. grayi</i>	99.9	97	1	0.1	0.1	0.1	94	0.1
<i>L. weihenstephanensis</i>	99	95	95	95	0.1	0.1	0.1	0.1
<i>L. fleischmannii</i>	99.9	95	98	95	0.1	0.1	0.1	0.1
<i>L. rocourtiae</i>	5	95	95	95	1	0.1	95	0.1

The above table contains the percentage likelihood of a strain to metabolise a given substrate, i.e 50% of *L. welshimeri* isolates are found to metabolise Xylose.

7. PRECAUTIONS

1. The Solus *Listeria* ID system is designed for the identification of the *Listeria* species described ISO 11290-1:2017 (E). The kit is not designed for the identification of *Listeria* species not included in this manual or for the identification of any non-*Listeria* species.
2. *Enterococcus*, *Bacillus* and *Staphylococcus* species can grow on *Listeria* selective media and may resemble *Listeria* species. If you are unsure if an isolate is *Listeria* species use the appropriate microbiological tests such as gram-stain, catalase, and oxidase to confirm that the isolate is a *Listeria* spp.
3. *Listeria* species are predominantly Arabitol fermentation positive. Some *Listeria* spp. are Arabitol negative e.g., *L. rocourtiae*. Except for *L. welshimeri*, all *Listeria* species are unable to ferment Tagatose. Therefore, if isolates appear as Arabitol fermentation negative and/ or Tagatose fermentation positive they are potentially *Enterococcus* spp. If isolates present in this way it is recommended that they are confirmed as belonging to *Listeria* spp. using the screening tests described at the start of Section 5.
4. The differentiation between closely related *L. innocua* and *L. marthii* is difficult as it is dependent on Rhamnose fermentation which is variable in *L. innocua*. *L. marthii* has only been isolated from a limited range of geographical locations in the United States (Chapin et. al.), and currently worldwide distribution is unknown. Therefore, all isolates that could be identified as *L. marthii* should be reported as *L. innocua* until further notice.
5. Pick a well isolated colony, preferably from a purity plate to avoid incorrect identifications that may arise due to inoculation with mixed cultures.
6. It is not recommended that the tests are conducted in a CO₂ incubator.
7. The haemolysis reagent contains sheep red blood cells which may deteriorate. Always store at 2 - 8°. Exposure to temperatures below freezing will result in lysis of the cells. Exposure to elevated temperatures i.e., >37°C for prolonged periods may significantly reduce the shelf life of this reagent. Brown colouration of the reagent is indicative of such deterioration.
8. Contamination of the haemolysis reagent will reduce shelf life and give erroneous results. Contact of the dropper with the microwell strip, skin or other surfaces may result in contamination.
9. If the result of the haemolysis test is unclear, the isolate should be inoculated onto a sheep blood agar plate and checked for haemolysis after incubation at 35-37°C for 18 – 24 hours, or alternatively perform a CAMP test (see ISO 11290-1 2017).

10. Rare non-haemolytic *L. monocytogenes* strains have been isolated and can be further identified by using the CAMP test or by assessing the phosphatidylinositol phospholipase C activity demonstrated by the presence of a halo around colonies growing on the medium of Ottaviani and Agosti (see ISO 11290-1 2017).
11. Due to the similarities in substrate utilisation and haemolysis in *L. weihenstephanensis* and *L. fleischmannii* isolates, these two species share result codes. If a shared code is recorded, *L. fleischmannii* can be differentiated from *L. weihenstephanensis* through motility testing as *L. fleischmannii* is non-motile and *L. weihenstephanensis* is weakly motile as described in ISO 11290-1:2017.

8. KIT STORAGE AND EXPIRY

The kit and any unused kit components should be stored at 2-8°C. DO NOT FREEZE.

The kit expiry date is displayed on the kit box plus all the kit components within the box.

Any unused microplate strips should be returned to the foil pouch with the desiccant sachet and then sealed completely.
Store at 2-8°C.

9. SAFETY.

Used materials must be disposed of safely by autoclaving, incineration, or immersion into an appropriate disinfectant prior to disposal.

10. REFERENCES

ISO 11290-1:2017 Microbiology of the Food Chain-Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. -Part 1: Detection method.

Bacteriological Analytical Manual Online: <https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam> Bacteriological Analytical Manual Online, Chapter 10 - Detection and Enumeration of *Listeria monocytogenes* in Foods.

Chapin TK, Nightingale KK, Worobo RW, Wiedmann M, Strawn LK. Geographical and meteorological factors associated with isolation of *Listeria* species in New York state produce production and natural environments. *J Food Prot.* 2014;77:1919–1928.

11. MSDS INFORMATION

Material safety data sheets (MSDS) are available for this test on request.

12. WARRANTY

Accurate results depend on the proper use of the kit by following the instructions for use carefully. If the kit fails to perform according to specification, please contact:

Solus Scientific Solutions Ltd
9 Mansfield Networkcentre
Millennium Business Park
Concorde Way Mansfield
Nottinghamshire
NG19 7JZ
Tel - +44 (0)1623 429701
Fax - +44 (0)1623 620977

Copyright Information

This document, including all photographs and illustrations, contains proprietary information that is protected by copyright. No part of this publication may be reproduced in any form whatsoever or translated into any language without the prior, written permission of PerkinElmer. ©2023 PerkinElmer, Inc. All rights reserved.

Trademarks

PerkinElmer is a registered trademark of PerkinElmer, Inc. All other trademarks are the property of their respective owners.

Summary of changes

Change date	Issue Number	Change Summary
Mar2023	0.1	New Document
Mar2024	0.2	Amendment of Result Table and Coverslip Addition

NOTE: Minor changes (e.g. formatting, grammar, correcting typographical errors) are not included in the summary of changes.

For more information visit www.perkinelmer.com

Manufactured at:

Solus Scientific Solutions Ltd.

9 Mansfield Networkcentre,
Millennium Business Park, Concorde
Way, Mansfield, Nottinghamshire
NG19 7JZ
UK
Tel - +44 (0)1623 429701

PerkinElmer US LLC
710 Bridgeport Avenue
Shelton, CT 06484
P: (866) 925-4600
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

Results Table 1

Grey Codes: Most Probable Identification Choices, Probability <1/100

Code	First ID Choice Species	Probability (%)	Second ID Choice Species	Probability (%)	Comments
776	<i>L. rocourtiae</i>	46.0	<i>L. welshimeri</i>	45.9	TAG +ve – possible <i>Enterococcus</i> check Catalase
774	<i>L. rocourtiae</i>	49.9	<i>L. welshimeri</i>	49.6	TAG +ve – possible <i>Enterococcus</i> check Catalase
772	<i>L. ivanovii</i>	97.6			TAG +ve – possible <i>Enterococcus</i> check Catalase
770	<i>L. fleischmannii/ weihenstephanensis</i>	94.0			TAG +ve – possible <i>Enterococcus</i> check Catalase
766	<i>L. rocourtiae</i>	49.9	<i>L. welshimeri</i>	49.8	TAG +ve – possible <i>Enterococcus</i> check Catalase
764	<i>L. rocourtiae</i>	49.9	<i>L. welshimeri</i>	49.8	TAG +ve – possible <i>Enterococcus</i> check Catalase
762	<i>L. fleischmannii/ weihenstephanensis</i>	93.9			TAG +ve – possible <i>Enterococcus</i> check Catalase
760	<i>L. fleischmannii/ weihenstephanensis</i>	94.4			TAG +ve – possible <i>Enterococcus</i> check Catalase
756	<i>L. ivanovii</i>	37.4	<i>L. rocourtiae</i>	36.3	
754	<i>L. rocourtiae</i>	97.7			
752	<i>L. ivanovii</i>	97.7			
750	<i>L. fleischmannii/ weihenstephanensis</i>	98.0			Refer to motility for species outcome
746	<i>L. rocourtiae</i>	95.4			
744	<i>L. rocourtiae</i>	97.6			
742	<i>L. fleischmannii/ weihenstephanensis</i>	98.1			Refer to motility for species outcome
740	<i>L. fleischmannii/ weihenstephanensis</i>	98.7			Refer to motility for species outcome
736	<i>L. monocytogenes</i>	98.6			TAG +ve – possible <i>Enterococcus</i> check Catalase
734	<i>L. welshimeri</i>	94.5			
732	<i>L. ivanovii</i>	77.5	<i>L. monocytogenes</i>	30.5	TAG +ve – possible <i>Enterococcus</i> check Catalase
730	<i>L. fleischmannii/ weihenstephanensis</i>	66.6	<i>L. welshimeri</i>	30.9	TAG +ve – possible <i>Enterococcus</i> check Catalase
726	<i>L. monocytogenes</i>	67.8	<i>L. welshimeri</i>	28.4	TAG +ve – possible <i>Enterococcus</i> check Catalase
724	<i>L. welshimeri</i>	94.6			
722	<i>L. fleischmannii/ weihenstephanensis</i>	52.6	<i>L. welshimeri</i>	21.9	TAG +ve – possible <i>Enterococcus</i> check Catalase
720	<i>L. fleischmannii/ weihenstephanensis</i>	66.6	<i>L. welshimeri</i>	27.7	TAG +ve – possible <i>Enterococcus</i> check Catalase
716	<i>L. monocytogenes</i>	99.9			
714	<i>L. rocourtiae</i>	51.7	<i>L. grayi</i>	22.2	
712	<i>L. ivanovii</i>	78.1	<i>L. monocytogenes</i>	21.2	
710	<i>L. fleischmannii/ weihenstephanensis</i>	98.5			Refer to motility for species outcome
706	<i>L. monocytogenes</i>	99.6			
704	<i>L. rocourtiae</i>	55.4	<i>L. grayi</i>	23.7	
702	<i>L. fleischmannii/ weihenstephanensis</i>	70.4	<i>L. monocytogenes</i>	28.2	Refer to motility for First ID species outcome
700	<i>L. fleischmannii /weihenstephanensis</i>	98.6			Refer to motility for species outcome
676	<i>L. ivanovii</i>	89.8	<i>L. seeligeri</i>	5.1	TAG +ve – possible <i>Enterococcus</i> check Catalase
674	<i>L. rocourtiae</i>	48.9	<i>L. welshimerii</i>	48.8	TAG +ve – possible <i>Enterococcus</i> check Catalase
672	<i>L. ivanovii</i>	99.9			TAG +ve – possible <i>Enterococcus</i> check Catalase
670	<i>L. fleischmannii/ weihenstephanensis</i>	48.3	<i>L. ivanovii</i>	43.9	TAG +ve – possible <i>Enterococcus</i> check Catalase
666	<i>L. seeligeri</i>	50.9	<i>L. rocourtiae</i>	24.0	TAG +ve – possible <i>Enterococcus</i> check Catalase
664	<i>L. rocourtiae</i>	50.0	<i>L. welshimerii</i>	49.0	TAG +ve – possible <i>Enterococcus</i> check Catalase
662	<i>L. seeligeri</i>	89.9			TAG +ve – possible <i>Enterococcus</i> check Catalase
660	<i>L. fleischmannii/ weihenstephanensis</i>	86.0			TAG +ve – possible <i>Enterococcus</i> check Catalase
656	<i>L. ivanovii</i>	94.0			
654	<i>L. rocourtiae</i>	67.4	<i>L. grayi</i>	29.5	
652	<i>L. ivanovii</i>	99.9			
650	<i>L. fleischmannii /weihenstephanensis</i>	51.0	<i>L. ivanovii</i>	46.0	
646	<i>L. seeligeri</i>	93.6			
644	<i>L. rocourtiae</i>	69.3	<i>L. fleischmannii</i>	29.4	
642	<i>L. seeligeri</i>	90.3			
640	<i>L. fleischmannii/ weihenstephanensis</i>	96.0			Refer to motility for species outcome
636	<i>L. grayi</i>	57.3	<i>L. monocytogenes</i>	35.9	TAG +ve – possible <i>Enterococcus</i> check Catalase
634	<i>L. grayi</i>	97.0			TAG +ve – possible <i>Enterococcus</i> check Catalase
632	<i>L. ivanovii</i>	99.8			TAG +ve – possible <i>Enterococcus</i> check Catalase
630	<i>L. grayi</i>	94.0			TAG +ve – possible <i>Enterococcus</i> check Catalase
626	<i>L. grayi</i>	95.5			TAG +ve – possible <i>Enterococcus</i> check Catalase
624	<i>L. grayi</i>	93.1			TAG +ve – possible <i>Enterococcus</i> check Catalase
622	<i>L. grayi</i>	88.5	<i>L. ivanovii</i>	6.3	TAG +ve – possible <i>Enterococcus</i> check Catalase
620	<i>L. grayi</i>	96.0			TAG +ve – possible <i>Enterococcus</i> check Catalase
616	<i>L. grayi</i>	59.9	<i>L. monocytogenes</i>	37.5	
614	<i>L. grayi</i>	99.9			
612	<i>L. ivanovii</i>	99.8			
610	<i>L. grayi</i>	96.8			
606	<i>L. grayi</i>	97.9			
604	<i>L. grayi</i>	99.9			
602	<i>L. grayi</i>	90.2			
600	<i>L. grayi</i>	98.1			

Results Table 2

Grey Codes: Most Probable Identification Choices, Probability <1/100

Code	First ID Choice Species	Probability (%)	Second ID Choice Species	Probability (%)	Comments
576	<i>L. welshimeri</i>	84.6			TAG +ve – possible <i>Enterococcus</i> check Catalase
574	<i>L. welshimeri</i>	99.9			TAG +ve – possible <i>Enterococcus</i> check Catalase
572	<i>L. ivanovii</i>	99.9			TAG +ve – possible <i>Enterococcus</i> check Catalase
570	<i>L. fleischmannii/ weihenstephanensis</i>	92.2			TAG +ve – possible <i>Enterococcus</i> check Catalase
566	<i>L. welshimeri</i>	99.7			TAG +ve – possible <i>Enterococcus</i> check Catalase
564	<i>L. welshimeri</i>	99.9			
562	<i>L. welshimeri</i>	77.4	<i>L. ivanovii</i>	15.0	TAG +ve – possible <i>Enterococcus</i> check Catalase
560	<i>L. welshimeri</i>	95.6			
556	<i>L. ivanovii</i>	59.0	<i>L. monocytogenes</i>	40.0	
554	<i>L. welshimeri</i>	78.0	<i>L. innocua</i>	14.0	
552	<i>L. ivanovii</i>	99.9			
550	<i>L. fleischmannii/ weihenstephanensis</i>	49.4	<i>L. ivanovii</i>	47.8	Refer to motility for First ID species outcome
546	<i>L. monocytogenes</i>	90.2			
544	<i>L. welshimeri</i>	79.0	<i>L. innocua</i>	14.0	
542	<i>L. ivanovii</i>	69.0	<i>L. fleischmannii/ weihenstephanensis</i>	15.0	Refer to motility for Second ID species outcome
540	<i>L. fleischmannii/ weihenstephanensis</i>	94.8			Refer to motility for First ID species outcome
536	<i>L. monocytogenes</i>	98.7			TAG +ve – possible <i>Enterococcus</i> check Catalase
534	<i>L. welshimeri</i>	99.7			
532	<i>L. ivanovii</i>	77.8	<i>L. monocytogenes</i>	21.5	TAG +ve – possible <i>Enterococcus</i> check Catalase
530	<i>L. welshimeri</i>	99.5			TAG +ve – possible <i>Enterococcus</i> check Catalase
526	<i>L. monocytogenes</i>	68.0	<i>L. welshimeri</i>	31.0	TAG +ve – possible <i>Enterococcus</i> check Catalase
524	<i>L. welshimeri</i>	99.8			
522	<i>L. welshimeri</i>	53.0	<i>L. monocytogenes</i>	45.0	TAG +ve – possible <i>Enterococcus</i> check Catalase
520	<i>L. welshimeri</i>	99.8			TAG +ve – possible <i>Enterococcus</i> check Catalase
516	<i>L. monocytogenes</i>	99.9			
514	<i>L. innocua</i>	67.0	<i>L. monocytogenes</i>	27.0	
512	<i>L. ivanovii</i>	78.4	<i>L. monocytogenes</i>	20.0	
510	<i>L. fleischmannii/ weihenstephanensis</i>	45.0	<i>L. ivanovii</i>	44.0	
506	<i>L. monocytogenes</i>	99.9			
504	<i>L. innocua</i>	92.0			
502	<i>L. monocytogenes</i>	98.6			
500	<i>L. fleischmannii/ weihenstephanensis</i>	88.7			Refer to motility for species outcome
476	<i>L. ivanovii</i>	92.0			TAG +ve – possible <i>Enterococcus</i> check Catalase
474	<i>L. welshimeri</i>	99.8			TAG +ve – possible <i>Enterococcus</i> check Catalase
472	<i>L. ivanovii</i>	99.9			TAG +ve – possible <i>Enterococcus</i> check Catalase
470	<i>L. ivanovii</i>	92.0			TAG +ve – possible <i>Enterococcus</i> check Catalase
466	<i>L. seeligeri</i>	68.0	<i>L. welshimeri</i>	32.0	TAG +ve – possible <i>Enterococcus</i> check Catalase
464	<i>L. welshimeri</i>	99.9			
462	<i>L. seeligeri</i>	91.9			TAG +ve – possible <i>Enterococcus</i> check Catalase
460	<i>L. welshimeri</i>	90.0			TAG +ve – possible <i>Enterococcus</i> check Catalase
456	<i>L. ivanovii</i>	94.1			
454	<i>L. ivanovii</i>	47.4	<i>L. marthii</i>	27.0	Report all <i>L. marthii</i> as <i>L. innocua</i> until further notice
452	<i>L. ivanovii</i>	99.9			
450	<i>L. ivanovii</i>	99.3			
446	<i>L. seeligeri</i>	99.8			
444	<i>L. marthii</i>	51.6	<i>L. seeligeri</i>	19.0	Report all <i>L. marthii</i> as <i>L. innocua</i> until further notice
442	<i>L. seeligeri</i>	92.3			
440	<i>L. seeligeri</i>	56.0	<i>L. fleischmannii/ weihenstephanensis</i>	40.5	Refer to motility for Second ID species outcome
436	<i>L. monocytogenes</i>	89.0			TAG +ve – possible <i>Enterococcus</i> check Catalase
434	<i>L. welshimeri</i>	94.0			TAG +ve – possible <i>Enterococcus</i> check Catalase
432	<i>L. ivanovii</i>	99.9			TAG +ve – possible <i>Enterococcus</i> check Catalase
430	<i>L. welshimeri</i>	55.5	<i>L. ivanovii</i>	43.8	TAG +ve – possible <i>Enterococcus</i> check Catalase
426	<i>L. welshimeri</i>	52.0	<i>L. monocytogenes</i>	45.0	TAG +ve – possible <i>Enterococcus</i> check Catalase
424	<i>L. welshimeri</i>	94.0			
422	<i>L. ivanovii</i>	63.0	<i>L. welshimeri</i>	17.0	TAG +ve – possible <i>Enterococcus</i> check Catalase
420	<i>L. welshimeri</i>	95.4			TAG +ve – possible <i>Enterococcus</i> check Catalase
416	<i>L. monocytogenes</i>	93.4			
414	<i>L. marthii</i>	73.0	<i>L. innocua</i>	23.0	Report all <i>L. marthii</i> as <i>L. innocua</i> until further notice
412	<i>L. ivanovii</i>	99.7			
410	<i>L. ivanovii</i>	94.0			
406	<i>L. monocytogenes</i>	94.0			
404	<i>L. marthii</i>	73.0	<i>L. innocua</i>	24.0	Report all <i>L. marthii</i> as <i>L. innocua</i> until further notice
402	<i>L. ivanovii</i>	74.7	<i>L. seeligeri</i>	16.0	
400	<i>L. marthii</i>	90.5			Report all <i>L. marthii</i> as <i>L. innocua</i> until further notice

Results Table 3

Grey Codes: Most Probable Identification Choices, Probability <1/100

Code	First ID Choice Species	Probability (%)	Second ID Choice Species	Probability (%)	Comments
376	<i>L. rocourtae</i>	99.9			ARA -ve & TAG +ve - possible Enterococcus check Catalase
374	<i>L. rocourtae</i>	99.9			ARA -ve & TAG +ve - possible Enterococcus check Catalase
372	<i>L. ivanovii</i>	50.4	<i>L. rocourtae</i>	48.5	ARA -ve & TAG +ve - possible Enterococcus check Catalase
370	<i>L. rocourtae</i>	99.7			ARA -ve & TAG +ve - possible Enterococcus check Catalase
366	<i>L. rocourtae</i>	99.9			ARA -ve & TAG +ve - possible Enterococcus check Catalase
364	<i>L. rocourtae</i>	99.9			ARA -ve & TAG +ve - possible Enterococcus check Catalase
362	<i>L. rocourtae</i>	97.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
360	<i>L. rocourtae</i>	97.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
356	<i>L. rocourtae</i>	99.9			ARA -ve - possible non-listeria check Confirmation Tests
354	<i>L. rocourtae</i>	99.9			ARA -ve - possible non-listeria check Confirmation Tests
352	<i>L. ivanovii</i>	89.0			ARA -ve - possible non-listeria check Confirmation Tests
350	<i>L. rocourtae</i>	82.0			ARA -ve - possible non-listeria check Confirmation Tests
346	<i>L. rocourtae</i>	99.9			ARA -ve - possible non-listeria check Confirmation Tests
344	<i>L. rocourtae</i>	99.9			
342	<i>L. rocourtae</i>	81.0			ARA -ve - possible non-listeria check Confirmation Tests
340	<i>L. rocourtae</i>	82.0			
336	<i>L. monocytogenes</i>	58.8	<i>L. rocourtae</i>	41.0	ARA -ve & TAG +ve - possible Enterococcus check Catalase
334	<i>L. rocourtae</i>	99.9			ARA -ve & TAG +ve - possible Enterococcus check Catalase
332	<i>L. ivanovii</i>	39.7	<i>L. rocourtae</i>	38.3	ARA -ve & TAG +ve - possible Enterococcus check Catalase
330	<i>L. rocourtae</i>	99.6			ARA -ve & TAG +ve - possible Enterococcus check Catalase
326	<i>L. rocourtae</i>	93.6			ARA -ve & TAG +ve - possible Enterococcus check Catalase
324	<i>L. rocourtae</i>	99.9			ARA -ve & TAG +ve - possible Enterococcus check Catalase
322	<i>L. rocourtae</i>	97.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
320	<i>L. rocourtae</i>	97.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
316	<i>L. monocytogenes</i>	95.5			ARA -ve - possible non-listeria check Confirmation Tests
314	<i>L. rocourtae</i>	99.9			ARA -ve - possible non-listeria check Confirmation Tests
312	<i>L. ivanovii</i>	61.0	<i>L. monocytogenes</i>	32.0	ARA -ve - possible non-listeria check Confirmation Tests
310	<i>L. rocourtae</i>	82.0			ARA -ve - possible non-listeria check Confirmation Tests
306	<i>L. rocourtae</i>	69.0	<i>L. monocytogenes</i>	31.0	ARA -ve - possible non-listeria check Confirmation Tests
304	<i>L. rocourtae</i>	99.9			
302	<i>L. rocourtae</i>	71.0	<i>L. fleischmannii/ weihenstephanensis</i>	16.0	ARA -ve - possible non-listeria check Confirmation Tests
300	<i>L. rocourtae</i>	82.0			ARA -ve - possible non-listeria check Confirmation Tests
276	<i>L. rocourtae</i>	98.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
274	<i>L. rocourtae</i>	99.9			ARA -ve & TAG +ve - possible Enterococcus check Catalase
272	<i>L. ivanovii</i>	99.5			ARA -ve & TAG +ve - possible Enterococcus check Catalase
270	<i>L. rocourtae</i>	99.4			ARA -ve & TAG +ve - possible Enterococcus check Catalase
266	<i>L. rocourtae</i>	99.9			ARA -ve & TAG +ve - possible Enterococcus check Catalase
264	<i>L. rocourtae</i>	99.9			ARA -ve & TAG +ve - possible Enterococcus check Catalase
262	<i>L. rocourtae</i>	53.0	<i>L. seeligeri</i>	44.0	ARA -ve & TAG +ve - possible Enterococcus check Catalase
260	<i>L. rocourtae</i>	99.8			ARA -ve & TAG +ve - possible Enterococcus check Catalase
256	<i>L. rocourtae</i>	82.0			ARA -ve - possible non-listeria check Confirmation Tests
254	<i>L. rocourtae</i>	99.9			ARA -ve - possible non-listeria check Confirmation Tests
252	<i>L. ivanovii</i>	98.5			ARA -ve - possible non-listeria check Confirmation Tests
250	<i>L. rocourtae</i>	85.0			ARA -ve - possible non-listeria check Confirmation Tests
246	<i>L. rocourtae</i>	98.9			ARA -ve - possible non-listeria check Confirmation Tests
244	<i>L. rocourtae</i>	99.9			ARA -ve - possible non-listeria check Confirmation Tests
242	<i>L. seeligeri</i>	85.0			ARA -ve - possible non-listeria check Confirmation Tests
240	<i>L. rocourtae</i>	92.0			ARA -ve - possible non-listeria check Confirmation Tests
236	<i>L. rocourtae</i>	53.7	<i>L. monocytogenes</i>	33.6	ARA -ve & TAG +ve - possible Enterococcus check Catalase
234	<i>L. rocourtae</i>	95.7			ARA -ve & TAG +ve - possible Enterococcus check Catalase
232	<i>L. ivanovii</i>	99.6			ARA -ve & TAG +ve - possible Enterococcus check Catalase
230	<i>L. rocourtae</i>	94.4			ARA -ve & TAG +ve - possible Enterococcus check Catalase
226	<i>L. rocourtae</i>	93.3			ARA -ve & TAG +ve - possible Enterococcus check Catalase
224	<i>L. rocourtae</i>	95.7			ARA -ve & TAG +ve - possible Enterococcus check Catalase
222	<i>L. rocourtae</i>	92.8			ARA -ve & TAG +ve - possible Enterococcus check Catalase
220	<i>L. rocourtae</i>	93.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
216	<i>L. monocytogenes</i>	77.0	<i>L. rocourtae</i>	14.0	ARA -ve - possible non-listeria check Confirmation Tests
214	<i>L. rocourtae</i>	70.1	<i>L. grayi</i>	29.8	ARA -ve - possible non-listeria check Confirmation Tests
212	<i>L. ivanovii</i>	98.6			ARA -ve - possible non-listeria check Confirmation Tests
210	<i>L. rocourtae</i>	59.0	<i>L. grayi</i>	31.0	ARA -ve - possible non-listeria check Confirmation Tests
206	<i>L. rocourtae</i>	62.0	<i>L. grayi</i>	26.0	ARA -ve - possible non-listeria check Confirmation Tests
204	<i>L. rocourtae</i>	70.1	<i>L. grayi</i>	23.4	ARA -ve - possible non-listeria check Confirmation Tests
202	<i>L. rocourtae</i>	45.0	<i>L. grayi</i>	29.4	ARA -ve - possible non-listeria check Confirmation Tests
200	<i>L. rocourtae</i>	65.1	<i>L. grayi</i>	33.5	ARA -ve - possible non-listeria check Confirmation Tests

Results Table 4

Grey Codes: Most Probable Identification Choices, Probability <1/100

Code	First ID Choice Species	Probability (%)	Second ID Choice Species	Probability (%)	Comments
176	<i>L. welshimeri</i>	74.0	<i>L. monocytogenes</i>	10.0	ARA -ve & TAG +ve - possible Enterococcus check Catalase
174	<i>L. welshimeri</i>	91.0	<i>L. welshimeri</i>	47.4	ARA -ve & TAG +ve - possible Enterococcus check Catalase
172	<i>L. ivanovii</i>	99.8			ARA -ve & TAG +ve - possible Enterococcus check Catalase
170	<i>L. welshimeri</i>	84.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
166	<i>L. welshimeri</i>	90.5			ARA -ve & TAG +ve - possible Enterococcus check Catalase
164	<i>L. welshimeri</i>	90.9			ARA -ve & TAG +ve - possible Enterococcus check Catalase
162	<i>L. welshimeri</i>	70.4	<i>L. ivanovii</i>	13.0	ARA -ve & TAG +ve - possible Enterococcus check Catalase
160	<i>L. welshimeri</i>	87.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
156	<i>L. monocytogenes</i>	55.0	<i>L. ivanovii</i>	41.0	ARA -ve - possible non-listeria check Confirmation Tests
154	<i>L. rocourtiae</i>	99.6			ARA -ve - possible non-listeria check Confirmation Tests
152	<i>L. ivanovii</i>	99.9			ARA -ve - possible non-listeria check Confirmation Tests
150	<i>L. fleischmannii/ weihenstephanensis</i>	44.0	<i>L. ivanovii</i>	42.0	ARA -ve - possible non-listeria check Confirmation Tests
146	<i>L. rocourtiae</i>	67.0	<i>L. monocytogenes</i>	31.0	ARA -ve - possible non-listeria check Confirmation Tests
144	<i>L. rocourtiae</i>	99.7			
142	<i>L. ivanovii</i>	57.0	<i>L. seeligeri</i>	25.0	ARA -ve - possible non-listeria check Confirmation Tests
140	<i>L. fleischmannii/ weihenstephanensis</i>	77.0	<i>L. rocourtiae</i>	18.0	ARA -ve - possible non-listeria check Confirmation Tests
136	<i>L. monocytogenes</i>	99.2			ARA -ve & TAG +ve - possible Enterococcus check Catalase
134	<i>L. welshimeri</i>	99.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
132	<i>L. ivanovii</i>	65.0	<i>L. monocytogenes</i>	34.0	ARA -ve & TAG +ve - possible Enterococcus check Catalase
130	<i>L. welshimeri</i>	99.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
126	<i>L. monocytogenes</i>	82.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
124	<i>L. welshimeri</i>	99.5			ARA -ve & TAG +ve - possible Enterococcus check Catalase
122	<i>L. monocytogenes</i>	63.0	<i>L. welshimeri</i>	36.0	ARA -ve & TAG +ve - possible Enterococcus check Catalase
120	<i>L. welshimeri</i>	99.2			ARA -ve & TAG +ve - possible Enterococcus check Catalase
116	<i>L. monocytogenes</i>	99.9			ARA -ve - possible non-listeria check Confirmation Tests
114	<i>L. monocytogenes</i>	63.0	<i>L. rocourtiae</i>	23.0	ARA -ve - possible non-listeria check Confirmation Tests
112	<i>L. ivanovii</i>	65.0	<i>L. monocytogenes</i>	34.0	ARA -ve - possible non-listeria check Confirmation Tests
110	<i>L. fleischmannii/ weihenstephanensis</i>	38.0	<i>L. ivanovii</i>	37.0	ARA -ve - possible non-listeria check Confirmation Tests
106	<i>L. monocytogenes</i>	99.9			
104	<i>L. rocourtiae</i>	60.0	<i>L. innocua</i>	20.0	ARA -ve - possible non-listeria check Confirmation Tests
102	<i>L. monocytogenes</i>	99.9			ARA -ve - possible non-listeria check Confirmation Tests
100	<i>L. fleischmannii/ weihenstephanensis</i>	73.0	<i>L. rocourtiae</i>	17.0	ARA -ve - possible non-listeria check Confirmation Tests
076	<i>L. ivanovii</i>	87.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
074	<i>L. welshimeri</i>	91.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
072	<i>L. ivanovii</i>	99.7			ARA -ve & TAG +ve - possible Enterococcus check Catalase
070	<i>L. ivanovii</i>	92.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
066	<i>L. seeligeri</i>	79.0	<i>L. welshimeri</i>	18.0	ARA -ve & TAG +ve - possible Enterococcus check Catalase
064	<i>L. welshimeri</i>	90.9			ARA -ve & TAG +ve - possible Enterococcus check Catalase
062	<i>L. seeligeri</i>	95.6			ARA -ve & TAG +ve - possible Enterococcus check Catalase
060	<i>L. welshimeri</i>	79.0	<i>L. seeligeri</i>	9.0	ARA -ve & TAG +ve - possible Enterococcus check Catalase
056	<i>L. ivanovii</i>	89.0			ARA -ve - possible non-listeria check Confirmation Tests
054	<i>L. rocourtiae</i>	86.0			ARA -ve - possible non-listeria check Confirmation Tests
052	<i>L. ivanovii</i>	99.7			ARA -ve - possible non-listeria check Confirmation Tests
050	<i>L. ivanovii</i>	98.5			ARA -ve - possible non-listeria check Confirmation Tests
046	<i>L. seeligeri</i>	99.4			ARA -ve - possible non-listeria check Confirmation Tests
044	<i>L. rocourtiae</i>	91.8			ARA -ve - possible non-listeria check Confirmation Tests
042	<i>L. seeligeri</i>	95.6			
040	<i>L. seeligeri</i>	68.0	<i>L. fleischmannii/ weihenstephanensis</i>	24.0	ARA -ve - possible non-listeria check Confirmation Tests
036	<i>L. monocytogenes</i>	94.6			ARA -ve & TAG +ve - possible Enterococcus check Catalase
034	<i>L. welshimeri</i>	94.5			ARA -ve & TAG +ve - possible Enterococcus check Catalase
032	<i>L. ivanovii</i>	99.1			ARA -ve & TAG +ve - possible Enterococcus check Catalase
030	<i>L. welshimeri</i>	55.0	<i>L. ivanovii</i>	42.0	ARA -ve & TAG +ve - possible Enterococcus check Catalase
026	<i>L. monocytogenes</i>	62.3	<i>L. welshimeri</i>	36.0	ARA -ve & TAG +ve - possible Enterococcus check Catalase
024	<i>L. welshimeri</i>	94.8			ARA -ve & TAG +ve - possible Enterococcus check Catalase
022	<i>L. ivanovii</i>	41.5	<i>L. seeligeri</i>	31.8	ARA -ve & TAG +ve - possible Enterococcus check Catalase
020	<i>L. welshimeri</i>	95.0			ARA -ve & TAG +ve - possible Enterococcus check Catalase
016	<i>L. monocytogenes</i>	99.8			ARA -ve - possible non-listeria check Confirmation Tests
014	<i>L. marthii</i>	53.9	<i>L. seeligeri</i>	34.2	ARA -ve - possible non-listeria check Confirmation Tests
012	<i>L. ivanovii</i>	53.0		22.0	ARA -ve - possible non-listeria check Confirmation Tests
010	<i>L. ivanovii</i>	95.0			ARA -ve - possible non-listeria check Confirmation Tests
006	<i>L. monocytogenes</i>	97.0			ARA -ve - possible non-listeria check Confirmation Tests
004	<i>L. marthii</i>	95.0			ARA -ve - possible non-listeria check Confirmation Tests
002	<i>L. ivanovii</i>	61.0	<i>L. seeligeri</i>	26.0	ARA -ve - possible non-listeria check Confirmation Tests
000	<i>L. marthii</i>	93.0			ARA -ve - possible non-listeria check Confirmation Tests