Microgen® BACILLUS-ID

An identification system for the Mesophilic bacillus species and Genera from Food and Clinical sources

Instructions for Use

REF **MID-66**

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MICROGEN BACILLUS-ID

Quick Reference

CONFIRMATION	Prior to inoculation into the Microgen Bacillus ID, isolates should be checked to ensure they are members of the genus *Bacillus* Gram positive bacillus, Endospore forming Catalase positive Optimal growth temperature between 25 and 45°C i.e. Mesophilic. Isolates growing at < 25°C (Psychrophiles) or isolates growing at >45°C (Thermophiles) are not identified by this product.
INOCULUM	Prepare a 2.0 MacFarland suspension in suspending medium.
INOCULATION	Using a sterile Pasteur pipette transfer 4 drops (100-125µl) of the bacterial suspension to each well of both microwell test strips (BAC1 & BAC2)
OVERLAY WITH OIL	Well 21 – Arginine
INCUBATION TIME	24 & 48 hours
TEMPERATURE	30°C
INITIAL READINGS	Read all tests after 24 hours and record colour changes. Return strips to 30°C for a further 24 hours.
ADDITION OF REAGENTS	After 48 hours incubation add the appropriate reagents to the following microwells in the second microwell test strip: Well 19: Kovacs – Add 2 drops of Kovac's Reagent. Read after 60 seconds. Well 23: VP - Add 1 drop of VPI Reagent followed by 1 drop of VPII Reagent and read after 15 - 30 minutes. Well 20: Nitrate Reduction - Read and record the ONPG result then add 1 drop of Nitrate A and 1 drop of Nitrate B. Read after 60 seconds.
FINAL READING	Record results on report form provided, calculate Octal Code and interpret using MID Software

Note: A black circle around the top of a well indicates a well requiring the addition of mineral oil prior to incubation.

A green circle around the top of a well indicates a well requiring addition of reagents after incubation.

INTENDED USE

The Microgen Bacillus-ID system is intended to be used for the identification of mesophilic *Bacillus spp*. isolated from food or clinical samples. The kit is intended for in vitro diagnostic use only.

The following species can be identified using the Microgen Bacillus-ID system.

***Bacillus* species**	*B. pumilus*
B. cereus group	*B. licheniformis*
B. firmus	*B. megaterium*
B. badius	
B. laevolacticus	***Vergibacillus* species**
B. coagulans	*V. pantothenticus*
B. lentus	
B. amyloliquefaciens	***Paenibacillus* species**
B. subtilis	*P.alvei*
B. circulans	*P. polymyxa*
B. insolitus	*P. macerans*
B. thiaminolyticus	
B. freudenreichii	***Brevibacillus* species**
B. globisporus	*Br. brevis*
B. sphaericus	*Br. laterosporus*

Note: *B.cereus* group consists of *B. cereus, B. thuringiensis* and *B. mycoides* and *B. weihenstephanensis.* On the basis of routinely employed biochemical tests, these species are indistinguishable. (See Limitations of Use)

PRINCIPLE OF THE TEST

The Microgen Bacillus-ID identification system consists of 2 microwell test strips (labelled BAC 1 and BAC 2), each containing 12 dehydrated substrates for the performance of either carbohydrate fermentation tests or other biochemical based tests. The last well in the second microwell test strip is a carbohydrate fermentation control well for use as a reference well in the interpretation of these tests. The selection of the substrates included in the microwell test strip has been determined using computer based analysis of all available substrates for the identification or differentiation of this group of organisms (1).

Identification of isolates is achieved by recording the results visualised by a colour change after 24 and 48 hours incubation at 30°C and the addition of appropriate reagents (Kovac's, Nitrate and VP Reagents) after 48 hours.

These results are then analysed using the Microgen Identification System Software (MID-60)

Each Microgen Bacillus-ID test consists of the following 24 biochemical reactions:

Well	Substrate	Reaction	Positive	Negative
1	Arabinose	Fermentation of specific sugars producing acid end products changes the Phenol Red indicator from red to yellow	Yellow	Red
2	Cellobiose			
3	Inositol			
4	Mannitol			
5	Mannose		Yellow	Red

6	Raffinose	Fermentation of specific sugars producing acid end products changes the Phenol Red indicator from red to yellow		
7	Rhamnose			
8	Salicin			
9	Sorbitol			
10	Sucrose			
11	Trehalose			
12	Xylose	Fermentation of specific sugars producing acid end products changes the Phenol Red indicator from red to yellow	Yellow	Red
13	Adonitol			
14	Galactose			
15	Methyl-D-Mannoside			
16	Methyl-D-Glucoside			
17	Inulin			
18	Melezitose			
19	Indole	Indole is produced from tryptophan and gives a pink/red complex when Kovac's reagent is added.	Pink / Red	Colourless / Yellow
20	ONPG	Hydrolysis - ONPG hydrolysis by B-galactosidase results in the production of yellow ortho-nitrophenol.	Yellow	Colourless
20 Plus reagent	Nitrate	Nitrate is reduced to nitrite which forms a deep red complex after the addition of α-Naphthylamine and Sulphanilic Acid	Red	Colourless/ yellow
21	Arginine Dihydrolase	Arginine is converted to ornithine, ammonia and CO_2 by arginine dihydrolase resulting in an increase in pH and a change in colour of the bromothymol blue from green to blue. At 48 hours green reactions are negative.	Green/ Blue Blue	Yellow Yellow / Green
22	Citrate Utilisation	Utilisation of citrate (only carbon source) leading to a pH increase giving a colour change in bromothymol blue from yellowy green to blue.	Blue	Yellow / Light Green
23	Voges Proskauer	Acetoin production from glucose is detected by the formation of a pink / red complex after the addition of alpha naphthol and creatine in the presence of KOH.	Pink / Red	Straw colour
24	Control	Carbohydrate Control	Red	Red

CONT	KIT PRESENTATION		
BROTH	MID66b	BACILLUS-ID Suspending Broth	20 x 3ml
TEST STRIP	MID66c	BACILLUS-ID Microwell Test Strip	20 Test strips

20 pairs of microwell test strips (BAC1 and BAC2) in foil pouches

Holding frame for microwell test strips
Result forms
Instructions for Use

Additional Requirements:

1) Microgen Identification System Software (MID-60) - Provides identification based on probability, % probability and likelihood with an analysis of the quality of separation. Full definition of these terms is provided with the software Help manual. MID-60 software should be registered at the Microgen Bioproducts website so that database updates are notified and can be downloaded (www.microgenbioproducts.com).
2) Mineral Oil
3) VP I + VP II Reagents
4) Nitrate A & B Reagents
5) Kovac's Reagent
6) Blood agar / Nutrient agar plates
7) Sterile pipettes, swabs and bacteriological loops
8) Incubator (30°C), not fan assisted
9) Refrigerator (2 - 8°C)
10) Gram stain reagents
11) Catalase test reagents
12) Microscope + slides
13) Vortex mix

Items 1-5 above can be purchased from Microgen Bioproducts Ltd.

WARNINGS AND PRECAUTIONS

Safety:

1. The Microgen Bacillus-ID system is intended for use by qualified laboratory personnel using aseptic techniques, appropriate microbiological precautions and after reading these Instructions for Use. The reagents supplied are for *in vitro* diagnostic use only
2. Appropriate precautions should be taken when handling or disposing of potential pathogens. After use, dispose of all contaminated materials by autoclaving, incineration or immersion in an appropriate disinfectant e.g. sodium hypochlorite at a final concentration of 3% for 30 minutes. Liquid waste containing acid must be neutralised before treatment.
3. Care should be taken when handling additional reagents as they may contain corrosive or irritant materials. Refer to the individual reagent bottles for further information.
4. Microgen Bacillus-ID System is intended to be used to identify the mesophilic bacillus species listed in the database which are involved in food poisoning and spoilage. However, it is possible that other bacilli, categorised as ACDP3, if tested in Microgen Bacillus-ID will produce Octal Codes which can be misinterpreted as organisms in the database. To minimise the likelihood of this occurrence, users are advised to follow the culture conditions and pre-test methods strictly.

Procedural:

1. The Microgen Bacillus-ID system should be used according to the kit instructions.
2. The microwell test strips must **not** be incubated in a CO_2 or fan-forced incubator
3. Incorrect incubation, inadequate filling of wells, or inadequate inoculum density may give false

results.

4. Always read carbohydrate fermentation tests with reference to the Control microwell (well 24, strip 2).
5. Carbohydrate fermentation tests should be read after both 24 and 48 hours incubation. If a test is positive after 24 hours incubation but is negative after 48 hours incubation, the positive result should be recorded.

STORAGE AND SHELF LIFE

Microgen Bacillus-ID microwell test strips are stable in unopened foil pouches at 2 - 8°C until the expiry date on the label. Opened pouches of microwell test strips can be stored for up to 14 days at 2 - 8°C provided that the pouch is resealed and contains the desiccant sachets. The Bacillus suspending broth should be stored at 2 – 8°C.

SPECIMENS

A pure 18 - 24 hour culture of the bacterial isolate to be identified must always be used.

PROCEDURE – SELECTION OF COLONIES, INOCULUM PREPARATION, INOCULATION AND INCUBATION

1. Selection of colonies for identification

1.1. Isolates must be tested from a pure culture on non selective media eg Blood Agar. Subculturing from a primary plate will be required.

1.2 Prior to inoculation into the Microgen Bacillus ID, isolates should be checked to ensure they are members of the genus Bacillus

1.2.1. Gram positive bacillus,
1.2.2. Endospore forming
1.2.3. Catalase positive
1.2.4. Optimal growth temperature between 25 and 45°C i.e. Mesophilic. Isolates growing at < 25°C (Psychrophiles) or isolates growing at >45°C (Thermophiles) are not identified by this product.

2. Inoculum preparation

2.1 Bring the suspending broth and microwell test strips to room temperature before inoculation.

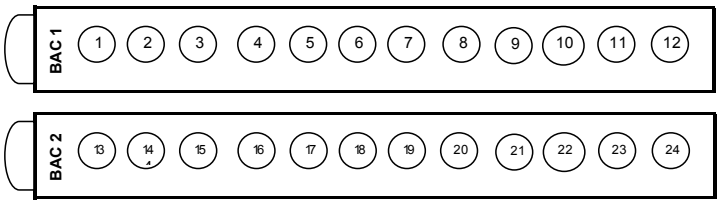
2.2 Remove colonies from an 18-24 hour pure culture using a sterile loop or swab and emulsify it in a vial of Bacillus Suspending medium. Several sweeps with the swab may be required.

2.3 Mix thoroughly eg using a vortex mixer suspension equivalent to a MacFarland 2.0 standard and allow particulates to settle prior to inoculating the microwell test strips. More than one plate of pure culture may be required to achieve this.

2.4 Inoculate the microwell test strips within 10 minutes of preparing and mixing the suspension.

3. Inoculation and Incubation

3.1 Remove the microwell test strips from the foil pouch and place in the holding tray.



3.2 Carefully peel back the adhesive tape sealing the microwells. **Do NOT discard the sealing strips as they will be required later.**

3.3 Using a sterile Pasteur pipette transfer 4 drops (100-125µl) of the bacterial suspension to each well of both microwell test strips.

3.4 After inoculation, overlay well 21 (arginine) with 3-4 drops of mineral oil.

3.5 Seal the top of the microwells with the adhesive tape peeled back earlier and incubate at 30°C for 24 hours and 48 hours. **Ensure that the punctures in the adhesive tape are positioned above the citrate and ONPG microwells, on the BAC 2 strip, and that a good seal is achieved.**

3.6 As a purity check, transfer 1 drop of the organism suspension onto an appropriate non-selective agar plate. Incubate the plate aerobically at 30°C for 18 - 24 hours

PROCEDURE - READING AND ADDITION OF REAGENTS

1. After 24 hours peel back the adhesive tape and record all positive results for wells 1 to 18 (carbohydrates) with reference to the control well. Anything more orange or yellow in colour compared to the control well should be scored as positive. The arginine, ONPG and citrate results should be read against the colour chart (included in the booklet) and recorded. Record the results on the forms provided, carefully reseal the adhesive tape making sure the punctures are correctly aligned with the wells, and return the strips to 30°C for a further 24 hours.

2. After 48 hours incubation, add the appropriate reagents to the following microwells in the second microwell test strip:

2.1 Add 2 drops of Kovac's reagent to well 19. Read and record the results after 60 seconds. Formation of a pink/red colour indicates a positive result.

2.2 Add 1 drop each of the VP I and VP II reagents to well 23 and read after 15-30 minutes. Formation of a pink/red colour indicates a positive result.

2.3 Perform the nitrate reduction test on well 20 after reading and recording the ONPG result. Add 1 drop each of Nitrate A and Nitrate B reagents to the well and read after 60 seconds.

3. Record these additional results on the forms provided:

IDENTIFICATION

On the Microgen Bacillus-ID Report Form, the substrates have been organised into triplets (sets of 3 reactions) with each substrate assigned a numerical value (1, 2 or 4). The sum of the positive reactions for each triplet forms a single digit of the Octal Code that is used to determine the identity of the isolate. The Octal Code is entered into the Microgen Identification System Software (MID-60), which generates a report of the five most likely organisms in the selected database. The software provides an identification based on probability, % probability and likelihood with an analysis of the quality of separation. Full definitions of these terms and an explanation of their usefulness in interpretation are provided with the software Help manual.

Example of Report Form:

MICROGEN BACILLUS-ID 24 TEST

REPORT FORM

Lab. No. F4560

Specimen Type Cooked Rice

Date 12-10-04

MICROGEN BIOPRODUCTS

	Bacillus Strip 1 (BAC 1)											Bacillus Strip 2 (BAC 2)											
Well Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	20	24
Reaction	ARA	CEL	INO	MAN	MNS	RAF	RHA	SAL	SOR	SUC	TRE	XYL	ADO	GAL	MDM	MDG	INU	MLZ	IND	ONPG	ARG	CIT	VP	NIT	NEG
24 hours	+	−	−	−	−	−	−	+	−	+	+	−	−	−	−	−	−	−		−	+	−			
48 hours	−	−	−	−	−	−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	+	−	+	+	
Reaction Index	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	
Sum of Positive Reactions	0			0			2			6			0			0			1			3			

Profile No: 00260013 Final Identification: Bacillus cereus group

Important: The Microgen Bacillus-ID microwell test strips will generate an 8 digit Octal Code.

LIMITATIONS OF USE

1. Results should be interpreted by the clinician in the context of all available clinical and laboratory information.
2. The Microgen Bacillus-ID identification system is designed to identify bacteria belonging to the genus Bacillus. It cannot be used to identify organisms belonging to other genera.
3. Test only pure, single colonies since mixed colonies may give erroneous results.
4. Reactions obtained using Microgen Bacillus-ID may differ from published data obtained using alternative substrate formulations or reagents.
5. Some bacterial strains may have atypical biochemical reactions and may be difficult to identify.
6. Computer generated identification results should be interpreted by suitably trained personnel.
7. On the basis of routinely employed biochemical tests, B. *cereus* group consists of B. *cereus*, B. *thuringiensis* and B. *mycoides* and B. *weihenstephanensis.* These species are indistinguishable. The following information may assist further in achieving satisfactory differentiation.

Organism	Motility
B. *cereus*	+
B. *thuringiensis*	+
B. *mycoides*	-
B. *weihenstephanensis*	?

8. B. *subtilis*, B. *amyloliquefaciens*, B. *licheniformis* and B. *pumilus* belong to the B. *subtilis* group. As these species are closely related the performance of some additional tests may be required to achieve satisfactory differentiation.
9. Inoculation of a purity plate from the suspending broth used is recommended as it will confirm that a single species was inoculated into the microwell test strips.
10. The Microgen Bacillus-ID identification system will only identify organisms with an optimal growth temperature between 25 and 45°C i.e. Mesophilic. Isolates growing at <25°C (Psychrophiles) or isolates growing at >45°C (Thermophiles) are not identified by this product.

QUALITY CONTROL

The performance of the Microgen Bacillus-ID system should be monitored using appropriate control strains. The following are recommended for independent laboratory assessment:

Well Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	20	24
Reaction	ARA	CEL	INO	MAN	MNS	RAF	RHA	SAL	SOR	SUC	TRE	XYL	ADO	GAL	MDM	MDG	INU	MLZ	IND	ONP	ARG	CIT	VP	NIT	NEG
B. licheniformis ATCC 14580, NCTC 10341	-	-	-	+	+	-	-	+	+/-	-	+	+/-	-	-	-	+/-	-	-		+	+	-			
	-	-	+	+	+	+/-	+/-	+	+	+	+	+	-	+/-	-	+	-	-	-	+	+	+	+	+	
B. cereus ATCC 11778, NCTC 10320	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-		-	+	-			
	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	
P. macerans ATCC 8244, NCTC 6355	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+/-	+		+	-	-			
	+	+	+	+	+	+	+	+	+/-	+	+	+	-	+	+	+	+	+	-	+	-	-	+	+	
P. alvei ATCC 6344, NCTC 6352	-	+/-	+/-	-	-	+	-	+	-	+	+	-	+	-	-	-	-	-		+	-	-			
	-	+/-	+/-	-	-	+	-	+	-	+	+	-	+	+/-	-	+	-	-	+	+	-	-	-	-	

DATABASE

Microgen Bacillus-ID is based on standard biochemical testing methods. The data provided for interpretation of reaction profiles is based on established literature sources.

PERFORMANCE CHARACTERISTICS

Microgen Bacillus-ID (MID-66) has been evaluated in comparison with an established commercially-available product for identification of cultured bacterial isolates.

	Total	Microgen-ID Bacillus (MID-66)	Competitor Product
B. firmius	2	2	2
B. cereus	11	11	11
B. licheniformis	11	11	11
P. macerans	1	1	1
B. megaterium	1	1	1
B. pumilis	4	4	4
B. sphaericus	3	3	3
B. subtilis[(1)]	11	11	11
B. thuringiensis[(2)]	2	2	0
B. alvei	1	1	1
B. circulans	2	2	2
Agreement		100%	95.9%

(1) Two isolates identified as *B. amyloliquefaciens*, a member of the *B. subtilis* Group.

(2) Two *B. thuringiensis* not included in the competitor database. Isolates identified as *B. cereus. B. thuringiensis* is a member of the *B. subtilis* Group. If the identification of *B. cereus* is considered correct, then agreement of the competitor is 100%

REPRODUCIBILITY

Intra-batch: A panel of six bacterial cultures was tested using one batch of Microgen Bacillus-ID, on three occasions using a different operator on each occasion. Test results obtained by the three operators correlated very closely giving an overall intra-batch reproducibility of 98%.

Inter-batch: Three batches of Microgen Bacillus-ID were tested using a panel of six bacterial cultures. This gave an overall inter-batch reproducibility of 98%.

Bacillus Data Table

	TESTS																							
	ARA	CEL	INO	MAN	MNS	RAF	RHA	SAL	SOR	SUC	TRE	XYL	ADO	GAL	MDM	MDG	INU	MLZ	IND	ONP	ARG	CIT	VP	NIT
P. alvei (2)	0.1	50	52	0.1	35	45	44	21	20	52	43	0.1	98	43	0.1	59	0.1	9	99	65	0.1	0.1	94	1
B. amyloliquefaciens (4)	86	99.9	45	55	79	67	0.1	99	94	99	90	66	0.1	23	0.1	96	40	1	0.1	68	0.1	56	94	74
B. badius	0.1	0.1	1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	99.9	0.1	0.1
B. cereus Group (1)	0.1	67	2	0.1	8	0.1	0.1	73	0.1	54	87	1	0.1	5	0.1	1	0.1	1	0.1	1	62	50	47	64
B. circulans	89	98	36	89	98	96	48	99.9	63	99	99	96	0.1	66	14	51	38	30	0.1	63	1	2	39	17
B. coagulans	52	60	26	34	91	62	39	68	24	86	93	60	6	96	10	57	4	3	0.1	81	0.1	0.1	93	25
B. firmus	6	12	1	74	20	4	0.1	7	7	92	70	3	0.1	6	1	2	4	1	4	20	5	26	75	63
B. freudenreichii	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	33	0.1	99.9
B. globisporus	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	99.9	0.1	0.1	0.1	0.1
B. insolitus	99.9	99.9	50	99.9	0.1	50	0.1	99.9	50	99.9	50	99.9	0.1	50	50	99.9	50	50	0.1	99.9	0.1	99.9	99.9	50
B. laevolacticus	0.1	99.9	0.1	99.9	99.9	99.9	60	99.9	99.9	99.9	99.9	0.1	0.1	99.9	80	99.9	99.9	99.9	0.1	60	20	0.1	99.9	20
B. lentus	57	74	11	65	98	67	50	72	26	89	84	37	2	35	50	26	22	26	17	45	0.1	18	55	8
B. licheniformis (4)	50	50	50	95	98	65	59	98	93	98	98	99	43	50	37	50	83	30	1	90	95	72	92	86
B. megaterium	66	63	57	74	12	75	1	45	51	88	79	63	0.1	63	2	34	50	43	0.1	70	0.1	31	53	19
V. pantothenticus (5)	0.1	94	28	50	99.9	0.1	99.9	99.9	78	89	99.9	0.1	0.1	99.9	0.1	99.9	0.1	0.1	0.1	78	0.1	22	50	50
B. pumilus (4)	93	99	20	99	98	70	10	99	9	99	99	99	30	50	68	44	12	1	1	50	1	60	92	1
B. sphaericus	0.1	0.1	1	1	1	1	0.1	0.1	0.1	1	2	0.1	0.1	4	0.1	0.1	0.1	0.1	0.1	6	1	47	11	14
B. subtilis (4)	95	98	79	96	79	47	51	91	94	99	99	74	1	17	1	93	82	1	0.1	84	0.1	60	99	65
B. thiaminolyticus	0.1	91	91	0.1	99.9	99.9	0.1	99.9	0.1	99.9	99.9	0.1	9	99.9	91	99.9	0.1	99.9	27	99.9	4	99.9	27	18
P. polymyxa (2)	50	50	0.1	50	50	50	17	50	0.1	50	99	95	0.1	95	24	99	74	30	0.1	95	0.1	10	92	72
P. macerans (2)	99	99	25	97	99	97	77	96	45	99.9	99.9	99	2	99.9	54	85	85	78	0.1	95	1	1	64	36
Br. brevis (3)	1	7	7	16	1	1	1	1	1	6	8	2	0.1	5	0.1	1	1	0.1	1	24	6	31	50	53
Br. laterosporus (3)	3	77	3	82	82	0.1	3	88	3	6	99	15	0.1	1	0.1	3	1	0.1	13	3	0.1	31	79	70

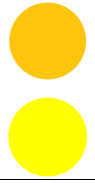
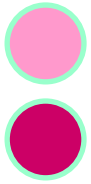
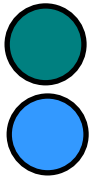
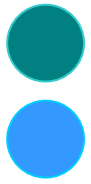
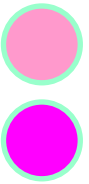
Notes:

1. *B.cereus* Group includes *B. cereus*, *B. thuringiensis, B. mycoides and B. weihenstephanensis*
2. *B. alvei*, *B. polymyxa* and *B. macerans* now *Paenibacillus* spp.
3. *B.brevis* and *B.laterosporus* now *Brevibacillus* spp.
4. *B. subtilis, B. amyloliquefaciens, B. licheniformis* and *B.pumilus* belong to the *B.subtilis* group
5. *B. pantothenicus now Vergibacillus pantothenicus*

Figures denote percentage positive strains

Colour chart/Farbtafel/Tableau 'de couleurs **Microgen™ Bacillus ID MID-66**

Read strips at 24 and 48 hours

WELL/ NAPFCHEN /GODET	24	1 to 18	1 to 18	19	20	21	22	23	(20) + Reagents
Reaction	Carbohydrate Negative Control 24/48 hours	Carbohydrate Fermentation 24 hours	Carbohydrate Fermentation 48 hours (*)	Indole 48 hours	O.N.P.G 24/48 hours	Arginine 24/48 hours	Citrate 24/48 hours	VP 48 hours	Nitrate 48 hours
Negative (Well 24 (*))									
Positive									

These colours are provided as general guide to the range of test colours.
CAUTION: Keep out of direct sunlight. Due to paper ageing and discolouration, the colours on this chart will change

Legend: ○ Appropriate reagents to be added at 48 hours, prior to reading. ◯ Overlaid with sterile mineral oil.

(*) With some Bacillus the negative colour may shift from red to orange over time. When scoring a result it must be with reference to the negative control