Microgen^{TM} STAPH-ID Identification

Instructions for Use

REF **MID-69 Panel** 20

CE IVD

MICROGEN STAPH ID

Quick Reference

CONFIRMATION	Perform: Gram stain (Gram positive cocci in clusters), Catalase (catalase positive)and Latex Agglutination. Note the presence of colony pigment
INOCULUM	A single colony in suspending medium
INOCULATION	3-4 drops (100µl) per well
OVERLAY WITH OIL	Well 10 and 11 – Urease and Arginine
INCUBATION TIME	18 - 24 hours
TEMPERATURE	35 - 37°C
INITIAL READINGS	Read all tests and record colour changes
ADDITION OF REAGENTS	Well 9: Nitrate - Add 1 drop of Nitrate A and 1 drop of Nitrate B after the β Glucuronide test has been read. Read Nitrate after 30 – 60 seconds Well 12: PYR – Add 1 drop of PYR reagent and read after 10 minutes
FINAL READING (Optional Microgen 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 Staph-ID system employs 12 standardised biochemical substrates in microwells to identify medically important members of the genus *Staphylococcus.* The kit is intended for in vitro diagnostic use only in a professional laboratory.

PRINCIPLE OF THE TEST

The Microgen Staph-ID system comprises a single microwell strip containing 12 standardised biochemical substrates which have been selected on the basis of extensive computer analysis (1) of published databases for the identification of the genus *Staphylococcus* (2, 3, 4). The dehydrated substrates in each well are reconstituted with a suspension of the organism to be identified prepared in the suspending medium provided. If the individual substrates are metabolised by the organism, a colour change occurs during incubation or after addition of specific reagents (see Substrate Reference Table). The permutation of metabolised substrates can be interpreted using the Microgen Identification System Software (MID-60) to identify the test organism.

CONT	KIT PRESENTATION		
BROTH	MID-69b	STAPH-ID Suspending Broth	20 Staph broth 20 x 3ml
TEST STRIPS	MID-69c	STAPH-ID Panel	20 Staph Test strips

Microwell strips containing 12 biochemical substrates for identification of Staphylococcus organisms - see data tables

Staphylococcal Suspending Medium 20 bottles
Holding frame for microwell 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 differentiation. Full definition of these terms is provided with the software Help manual.
   MID-60 software (V 1.1.16.19 onwards) which does not contain the Staphylococcus database may be updated to include the Staph ID data, by visiting the Microgen Bioproducts website www.microgenbioproducts.com)
2) Mineral Oil
3) Nitrate A + B Reagents
4) PYR Reagent
5) Microgen® Staph Latex
6) Sterile pipettes and bacteriological loops
7) Gram stain reagents
8) Hydrogen Peroxide
9) Incubator, not fan-assisted (35-37°C)
10) Bunsen burner.

WARNINGS AND PRECAUTIONS

Safety:

1. The reagents supplied in this kit 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.

Procedural:

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

STORAGE AND SHELF LIFE

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

SPECIMENS

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

PROCEDURE - INOCULATION AND INCUBATION

1. Carry out a Gram stain (Gram positive cocci in clusters), catalase test (catalase positive) and latex agglutination (Microgen® Staph Latex) or slide coagulase test (LAT) on the isolate to confirm that it belongs to the genus *Staphylococcus*.. Note any colony pigment production (CPG).
2. Emulsify a single colony from an 18-24 hour culture in the suspending medium supplied in the kit. Mix thoroughly.
3. Carefully peel back the adhesive strip(s) sealing the microwell strip(s). **Do NOT discard the sealing strip(s) as they will be required later.**
4. Using a sterile pasteur pipette, add 3-4 drops (approximately 100µL) of the bacterial suspension to each well of the strip(s).
5. As a purity check, transfer 1 drop of the bacterial suspension on to a purity plate using a non-selective differential medium. Incubate the plate aerobically at 35-37°C for 18-24 hours.
6. After inoculation, overlay wells 10 and 11 with 3-4 drops of mineral oil. This well is highlighted with a black circle around the well to assist in adding oil to the correct wells.
7. Seal the top of the microwell strip with the adhesive strip removed earlier and incubate at 35-37°C. The microwell strips are read after 18-24 hours incubation.

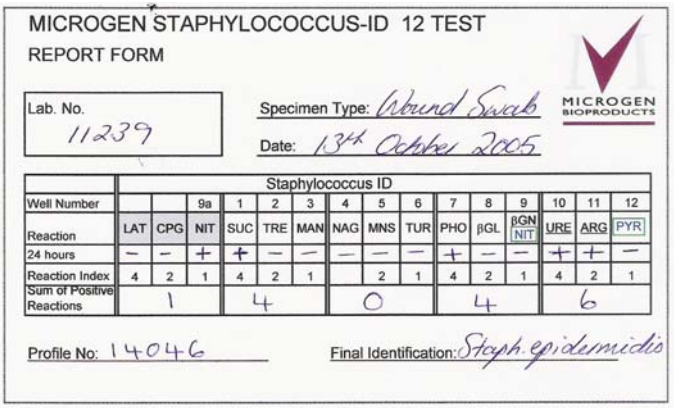
PROCEDURE - READING AND ADDITION OF REAGENTS

1. Remove the adhesive strip and record all positive reactions with the aid of the colour chart (included in this booklet). Record the results on the forms provided.
2. Add the appropriate reagents to the following microwells:
   a) Add 1 drop of PYR reagent to well 12 and read after 10 minutes. Formation of a very deep pink/red colour indicates a positive result.
   b) Perform the nitrate reduction test on well 9 after reading and recording the β Glucuronidase reaction. Add 1 drop of Nitrate A reagent and 1 drop of Nitrate B reagent to the well and read after 60 seconds. The development of a red colour indicates that nitrate has been reduced to nitrite.
3. Record these additional results on the forms provided.

IDENTIFICATION

On the Microgen Staph-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 Profile Number that is used to determine the identity of the isolate. The Profile Number 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 differentiation. 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 STAPHYLOCOCCUS-ID 12 TEST

REPORT FORM

MICROGEN BIOPRODUCTS

Lab. No. 11239

Specimen Type: Wound Swab

Date: 13th October 2005

	Staphylococcus ID														
Well Number			9a	1	2	3	4	5	6	7	8	9	10	11	12
Reaction	LAT	CPG	NIT	SUC	TRE	MAN	NAG	MNS	TUR	PHO	βGL	βGN NIT	URE	ARG	PYR
24 hours	−	−	+	+	−	−	−	−	−	+	−	−	+	+	−
Reaction Index	4	2	1	4	2	1		2	1	4	2	1	4	2	1
Sum of Positive Reactions	1			4			0			4			6		

Profile No: 14046

Final Identification: Staph. epidermidis

Important:

The Microgen Staph-ID microwell strip + external tests will generate a 5 digit Profile Number.

LIMITATIONS OF USE

1. Results should be interpreted by the clinician in the context of all available clinical and laboratory information.
2. The Microgen ID system is intended for identification of those organisms included in the database. It should not be used to identify any other bacteria.
3. Test only pure, single colonies since mixed colonies may give erroneous results.
4. Reactions obtained using Microgen Staph-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. When determining the final identification of an isolate, the source of the isolate, gram staining, colonial morphology, additional tests and tests against the suggested identification should be considered.
8. The development of colony pigmentation (CPG) and the performance of a Staphylococcal Latex Agglutination test (LAT), OR alternatively a slide coagulase test using appropriate plasma e.g. rabbit, must be performed on all isolates prior to inoculation into the Microgen Staph-ID test panel. A 5 digit Profile Number is required to interpret the results using the Microgen Identification System Software.

QUALITY CONTROL

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

Staphylococcus aureus NCTC 8538/ ATCC 12598
Staphylococcus epidermidis NCTC 110472/ ATCC 14990
Staphylococcus saprophyticus NCTC 7292 / ATCC 15305

	LAT	CPG	NIT	SUC	TRE	MAN	NAG	MNS	TUR	PHO	βGL	βGN	URE	ARG	PYR
S. aureus NCTC 8530 / ATCC 12598	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-
S. epidermidis NCTC 11047 / ATCC 14990	-	-	+	+	-	-	-	-	v	+	-	-	+	+	-
S. saprophyticus NCTC 7292 / ATCC 15305	-	-	-	+	+	+	-	-	+	-	-	-	+	-	-

DATABASE

The Microgen Staph-ID systems are based on standard biochemical testing methods. The data provided for interpretation of reaction profiles is based on established literature sources (2,3,4).

PERFORMANCE CHARACTERISTICS

Microgen Staph- ID (MID-69) has been evaluated in comparison with a well-established commercially available product for identification of cultured bacterial isolates. 108 fully characterised strains of *Staphylococcus* were tested with both products.

	Total Tested	MID-69	Competitor
S. aureus	45	45	45
S. epidermidis	19	19	19
S. haemolyticus	12	11	12
S. simulans	8	8	8
S. capitis subsp. *capitis*	3	3	3
S. capitis subsp. *urealyticus*	2	2	2
S. warneri	5	5	5
S. saprophyticus	2	2	2
S. ludergensis	2	2	2
S. chromogenes	2	2	2
S. hominis	3	3	3
S. cohni subsp. *cohni*	1	1	1
S. lentus	1	1	1
S. xylosus	1	1	0
S. caprae	1	1	1
S. auricularis	1	1	1
Total	**108**	**107**	**107**

REPRODUCIBILITY

Intra-batch: A panel of bacterial cultures was tested using three batches of Microgen Staph-ID. Each batch of product was used on three occasions using a different operator on each occasion. Test results obtained by the three operators correlated very closely giving an overall intra-assay reproducibility of >99%.

Inter-batch: Three batches of Microgen Staph-ID were tested using a panel of five bacterial cultures. This gave an overall inter-batch reproducibility of >99%.

REFERENCES

1. Lapage S.P, Bascombe S, Willcox W.R and Curtis M.A. (1973) Identification of Bacteria by Computer: General Aspects and Perspectives J.Gen. Microbiol. **77**: 273 -290
2. Murray, Baron, Pfaller, Tenover, Yolken Manual of Clinical Microbiology, 6th Edition
3. Murray P.R. (Ed) (1999) Manual of Clinical Microbiology 7th Edition. American Society for Microbiology, Washington, DC
4. Murray P.R. (Ed) (2003) Manual of Clinical Microbiology 7th Edition. American Society for Microbiology, Washington, DC

SUBSTRATE REFERENCE TABLE

Well	Reaction	Description	Positive	Negative
1	Sucrose	Fermentation – Phenol red changes from red to yellow as a result of acid produced from the carbohydrate fermentation.	Yellow – Yellow/ Orange	Red
2	Trehalose			
3	Mannitol			
4	N-Acetyl Glucosamine			
5	Mannose			
6	Turanose			
7	Alkaline Phosphatase	Hydrolysis of p-nitrophenyl phosphate by alkaline phosphatase results in the production of yellow p-nitrophenol.	Yellow	Colourless
8	Glucosidase	Hydrolysis of p-nitrophenyl β D glucopyranoside by β Glucosidase results in the production of yellow o-nitrophenol	Yellow	Colourless
9	Glucuronidase	Hydrolysis of o-nitrophenyl β D glucuronide by β Glucuronidase results in the production of yellow p-nitrophenol	Yellow	Colourless
9	Nitrate	Nitrate is reduced to nitrite which forms a deep red complex after the addition of α-Naphthylamine and Sulphanilic Acid	Red	Yellow
10	Urease	Hydrolysis of urea results in the formation of ammonia leading to an increase in pH which turns phenol red from yellow to pink / red.	V. Deep Pink	Straw to pale salmon pink colour
11	Arginine	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
12	PYR	Hydrolysis of L-pyrrolidonyl-α-naphthylamide by the enzyme pyrrolidonyl arylamidase.	Red/ Deep Pink	Colourless/ Very pale Pink
	LAT	Latex Agglutination test for Coagulase and Protein A, or a slide or tube coagulase test		
	CPG	The development of visible colony pigmentation (cream to golden in colour)		

Species identified using Microgen Staph ID

Staphylococcus spp.

S. aureus subsp. *aureus*
S. aureus subsp. *anaerobius*
S. auricularis
S. caprae
S. capitis subsp. *capitis*
S. capitis subsp. *urealyticus*
S. carnosus
S. chromogenes
S. cohnii subsp. *conhii*
S. cohnii subsp. *urealyticum*
S. epidermidis
S. haemolyticus
S. hominis subsp. *hominis*
S. hominis subsp. *novobiosepticus*
S. hyicus
S. intermedius
S. lentus
S. lugdenensis
S. saccharolyticus
S. saprophyticus
S. schleiferi subsp. *schleiferi*
S. schleiferi subsp. *coagulans*
S. sciuri
S. simulans
S. warneri
S. xylosus

Kocuria spp.

K. kristinae
K. rosea
K. carniphila

Kytococcus spp.

Ky. sedentarius

Micrococcus spp.

M. luteus
M. lylae

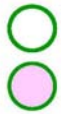
Staphylococcus Data Table

	LAT	CPG	NIT	SUC	TRE	MAN	NAG	MNS	TUR	PHO	BGL	BGN	URE	ARG	PYR
S. aureus subsp. aureus	99.9	85	85	98	97	98	90	93	95	99.9	99.9	0.1	82	90	0.1
S. aureus subsp. anaerobius	0.1	0.1	0.1	99.9	0.1	0.1	0.1	0.1	0.1	99	0.1	0.1	0.1	60	0.1
S. auricularis	0.1	0.1	75	50	99.9	5	0.1	45	0.1	0.1	0.1	0.1	0.1	90	99.9
S. caprae	0.1	0.1	99.9	0.1	87	25	5	80	0.1	99.9	0.1	0.1	65	99.9	50
S. capitis subsp. capitis	0.1	0.1	80	45	0.1	50	0.1	75	0.1	20	0.1	0.1	30	80	0.1
S. capitis subsp. urealyticus	0.1	30	99.9	99.9	0.1	90	0.1	96	0.1	0.1	0.1	0.1	95	80	0.1
S. carnosus	0.1	0.1	99.9	0.1	90	99.9	90	99.9	0.1	85	0.1	0.1	0.1	99.9	99.9
S. chromogenes	0.1	75	99.9	99.9	99.9	23	35	90	50	96	40	0.1	95	89	65
S. cohnii subsp. conhii	0.1	0.1	20	50	93	89	0.1	60	0.1	98	0.1	0.1	0.1	2	0.1
S. cohnii subsp. urealyticum	0.1	68	0.1	0.1	99.9	96	90	95	0.1	95	0.1	99.9	94	0.1	0.1
S. epidermidis	0.1	0.1	90	97	0.1	0.1	0.12	70	50	82	50	0.1	85	73	0.1
S. haemolyticus	0.1	45	82	99.9	95	66	85	7	50	4	78	0.1	4	85	50
S. hominis subsp. hominis	0.1	55	80	95	85	25	45	2	99.9	24	0.1	0.1	85	40	0.1
S. hominis subsp. novobiosepticus	0.1	0.1	90	99	0.1	0.1	0.1	30	99.9	0.1	10	0.1	99	0.1	0.1
S. hyicus	50	0.1	90	89	90	7	92	90	0.1	93	50	99.9	66	99.9	0.1
S. intermedius	50	0.1	99.9	99.9	99.9	99.9	99.9	75	4	99.9	50	0.1	50	80	0.1
S. lentus	0.1	0.1	99.9	99.9	99.9	99.9	90	92	99.9	20.1	99.9	0.1	0.1	0.1	0.1
S. lugdenensis	0.1	75	95	99.9	95	0.1	99.9	85	50	5	99.9	0.1	50	0.1	99.9
S. saccharolyticus	0.1	0.1	95	0.1	60	0.1	0.1	10	70	0.1	0.1	0.1	0.1	95	0.1
S. saprophyticus	0.1	80	25	99.9	95	89	70	0.1	99.9	0.1	50	0.1	84	30	0.1
S. schleiferi subsp. schleiferi	90	0.1	99.9	0.1	70	0.1	99.9	99.9	0.1	95	0.1	0.1	0.1	99.9	0.1
S. schleiferi subsp. coagulans	0.1	0.1	99.9	24	0.1	48	80	99.9	49	99	0.1	0.1	99.9	99.9	20
S. sciuri	0.1	49	99.9	99.9	99.9	99.9	83	99.9	60	98	99.9	0.1	0.1	0.1	99.9
S. simulans	0.1	0.1	90	95	95	85	90	50	0.1	25	5	90	85	95	99.9
S. warneri	0.1	62	30	99.9	94	80	8	50	50	0.12	99.9	60	92	72	0.1
S. xylosus	0.1	47	80	90	95	90	85	90	50	65	99.9	99.9	85	5	99.9
K. kristinae	0.1	19	10	99.9	98	5	0.1	99.9	0.1	0.1	99.9	0.1	15	0.1	95
K. rosea	0.1	0.1	90	12	0.1	10	0.1	8	0.1	0.1	99.9	35	0.1	0.1	0.1
K. carniphila	0.1	85	83	15	10	0.1	0.1	10	0.1	0.1	0.1	0.1	85	0.1	15
Ky. sedentarius	0.1	24	5	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	70	0.1
M. luteus	0.1	80	4	15	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	32	0.1	85
M. lylae	0.1	0.1	10	5	0.1	0.1	7	5	0.1	0.1	0.1	0.1	0.1	0.1	50

Colour chart/Farbtafel/Tableau 'de couleurs

Microgen™ Staphylococcus ID MID- 69

Read strips at 18 to 24 hours

WELL/NAPFCHEN /GODET	1 to 6	7	8	9	9a	10	11	12
Reaction	Carbohydrate Fermentation	PHS	βGL	βGN	(βGN) Nitrate	Urease	Arginine	PYR
Negative								
Positive					 	 	 	 

CAUTION: Keep out of direct sunlight. Due to laminate discolouration and paper ageing, the colours on this chart will change.

These colours are provided as general guide to the range of test colours.

Legend:

- Appropriate reagents to be added **at 18 to 24 hours**, prior to reading.
- Overlaid with sterile mineral oil.

MICROGEN BIOPRODUCTS

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