MICROGEN GNA-ID
MID-64

GRAM NEGATIVE BACILLUS

TRAINING MANUAL

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INTRODUCTION

The Microgen™ GNA Identification system for Gram Negative Bacilli consists of traditional or conventional biochemical substrates presented in a miniaturised format. When these substrates are inoculated with a suspension of test organism and the organism is able to metabolise the active components, the end products are detected either through a change in colour of a pH indicator or the development of a coloured end product after the addition of a supplementary reagent. The colours are scored as positive or negative and then translated into a digital Reaction Code which when input into the associated software produces an identification.

The Microgen™ GNA Identification system offers a number of features which are able to set it apart from other systems. These are discussed in greater detail in the Features and Benefits Section of this manual. However, when compared to other commercial systems some of the following features are key to the marketing of this product:

- **Convenient 12 substrate identification system for the commonly encountered *Enterobacteriaceae***
- **No assembly required**
- **Compact**
- **Comprehensive analysis tools i.e. Database**
- **Easy-to-read colours**

The Microgen GNA-ID system is suitable for use in the identification of isolates from food or medical samples including urinary pathogens, faecal pathogens and common wound isolates. For wider clinical applications, veterinary, industrial (water testing, pharmaceutical etc) all currently recognized species of *Enterobacteriaceae* and an extensive range of oxidase-positive Gram Negative Bacilli can be identified using the combination of the Microgen GNA + GNB identification panels i.e. 24 substrates. **Note** that the GNB strip of tests cannot be used independently of the GNA strip.

The Microgen-ID software utilises a "DATABASE" which contains the details of the large number of different organisms or species that can be identified. A **KEY FEATURE** that differentiates Microgen™ ID from other systems is not only the detail provided within the database but also the way the database is presented to the customer. The system is **DESIGNED BY MICROBIOLOGISTS FOR MICROBIOLOGISTS**. The databases in the Microgen ID software can be updated free-of-charge by downloading updates from the Microgen web-site. (See the section on the Software)

FEATURES AND BENEFITS OF THE MICROGEN ID GNA SYSTEM (MID-64)

FEATURE	BENEFITS
All tests included are based on conventional methods.	Reactions agree with those found in established reference books and **standard methods.**
Comprises 12 substrates specifically selected to optimize the identification of the most commonly encountered oxidase negative gram negative bacilli including the family *Enterobacteriaceae* and *Acinetobacter* spp.	Uses only those substrates necessary to achieve the desired identification. Other systems such as API 20E and Crystal (BD) employ 20 and 30 substrates respectively to achieve the same levels of identification as can be achieved using the 12 substrates found in the Microgen ID – GNA. **The Microgen ID – GNA is therefore faster to inoculate and read.**
Particular emphasis on important food pathogens i.e. *E.coli* and *Salmonella* spp.	**High levels of confidence** when used in food testing laboratory environments.
Reactions are easy to read with clear positives and negatives.	**No confusion in interpretation leads to more accurate identifications.** The interpretation of difficult tests such as Lysine decarboxylase, is significantly improved. Training of staff simplified.
Compact design – the use of microstrips and a holding trays which holds 4 separate identifications offers a very compact identification package.	Particularly advantageous to laboratories with limited storage and incubator space. In addition, **the holding tray ensures stability of the tests during all stages of use.**
Easy-to-inoculate.	Simply add 3 drops (approximately 100µl) of inoculum to each well. **No awkward cupules to fill**, as is the case with the API system.
Consistency in inoculum	Each well receives the same inoculum unlike the API where some cupules are partially filled whilst others are completely filled. **Gives greater consistency of results**.
Comprehensive systems for interpretation of results. The Identification System Software provides the most comprehensive interpretation with detailed statistical analysis, identification comments etc.	**Provides confidence in use** as helpful comments are provided. Literature references are given and suggested additional tests listed to clarify identifications.

TESTS INCLUDED IN GNA IDENTIFICATION SYSTEM

Well	Reaction	Description	Positive	Negative
1	Lysine	Lysine decarboxylase - Bromothymol blue changes to green/blue indicating the production of the amine cadaverine.	Green / Blue	Yellow
2	Ornithine	Ornithine decarboxylase - Bromothymol blue changes to blue indicating the production of the amine putrescine.	Blue	Yellow / Green
3	H_2S	H_2S production - Thiosulphate is reduced to H_2S that reacts with ferric salts producing a black precipitate.	Black	Straw
4	Glucose	Fermentation - Bromothymol blue changes from blue to yellow as a result of acid produced from the carbohydrate fermentation.	Yellow	Blue / Green
5	Mannitol			
6	Xylose			
7	ONPG	Hydrolysis - ONPG hydrolysis by B-galactosidase results in the production of yellow ortho-nitrophenol.	Yellow	Colourless
8	Indole	Indole is produced from tryptophan and gives a pink/red complex when Kovac's reagent is added.	Pink / Red	Colourless
9	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.	Pink / Red	Straw colour
10	VP	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
11	Citrate	Utilisation of citrate (only carbon source) leading to a pH increase giving a colour change in bromothymol blue from green to blue.	Blue	Green
12	TDA	Indolepyruvic acid is produced from tryptophan by tryptophan deaminase giving a cherry red colour when ferric ions are added. Indole positive isolates may give a brown colour – this is a negative result.	Cherry red	Straw colour

The chemistry of these reactions is described in detail in the extensive list of Technical Bulletins available for download from the Microgen website, www.microgenbioproducts.com.

KIT CONTENTS

MID-64 60 Test A Panel
60 x A microwell test strips
Frame for microwell strips
Result forms
Instructions for Use

ADDITIONAL MATERIALS REQUIRED (NOT SUPPLIED IN THE KIT)

Microgen GN-ID Reagents including:

MID-61a	Nitrate A Reagent
MID-61b	Nitrate B Reagent
MID-61c	VPI Reagent
MID-61d	VPII Reagents
MID-61e	TDA Reagent
MID-61f	Indole (Kovac's) Reagent
MID-61g	Oxidase strips
MID-61h	Mineral Oil

MID-60 Microgen Identification System Software

Colour Chart for reading results (available from your distributor on request)

Sterile physiological saline (0.85%) 3 – 5ml
Sterile pipettes and bacteriological loops
Incubator (35 - 37°C), not fan assisted
Motility medium
Bunsen Burner
Sterile horse serum (if *Actinobacillus* spp**.** or *Pasteurella* spp**.** are suspected)

STORAGE

The microwell strips are stable in the unopened foil pouches at 2 - 8°C until the expiry date on the label. Once the foil pouch has been opened, unused microwell strips should be replaced into the pouch and the pouch resealed. Microwell strips in resealed pouches should be used within 14 days.

DATABASE

The following organisms are included in the GNA database within the Microgen Identification System Software.

Acinetobacter baumannii
Acinetobacter lwoffii
Acinetobacter haemolyticus
Citrobacter freundii
Citrobacter diversus
Edwardsiella tarda
Enterobacter aerogenes
Enterobacter cloacae
Enterobacter agglomerans
Enterobacter gergoviae
Enterobacter sakazakii
Escherichia coli
Escherichia coli - inactive
Shigella dysenteriae (Group A)
Shigella flexneri (Group B)
Shigella boydii (Group C)
Shigella sonnei (Group D)
Hafnia alvei
Klebsiella pneumoniae
Klebsiella oxytoca
Klebsiella ozaenae
Klebsiella rhinoscleromatis
Morganella morganii
Proteus mirabilis
Proteus vulgaris
Providencia rettgeri
Providencia stuartii
Providencia alcalifaciens
Salmonella Group I
Salmonella typhi
Salmonella cholerae-suis
Salmonella paratyphi A
Salmonella gallinarum
Salmonella pullorum
Salmonella Group II
Salmonella Group IIIa
Salmonella Group IIIb
Salmonella Group IV
Salmonella Group V
Salmonella Group VI
Serratia marcescens
Serratia liquefaciens
Serratia rubidaea
Yersinia enterocolitica

All of currently recognized species of Enterobacteriaceae and an extensive range of Oxidase Positive Gram Negative Bacilli can be identified using the combination of the Microgen GNA + GNB identification panels i.e. 24 substrates.

Refer to product instructions for full details.

PROCEDURE

STEP 1

Select a single colony of the isolate to be identified

STEP 2

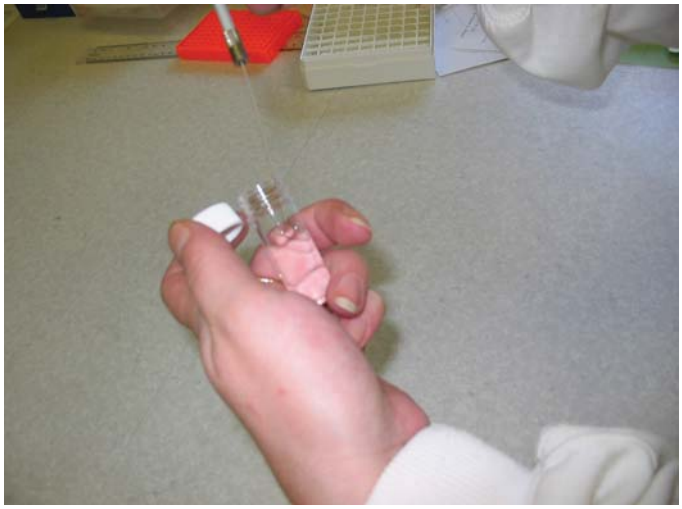
Emulsify colony in 3ml saline (A) or 5mls saline (A+B)

Do not use PBS

Do not use Distilled Water

Optimum density to MacFarland 0.5

Final suspension should be just visibly turbid/ cloudy



STEP 3

Add 3 - 4 drops (100µL) of the suspension to each well of the strip(s)

Well should be approximately 30 - 40% full

Do not overfill - reagent addition may not be possible

STEP 4

Overlay appropriate wells with mineral oil

Wells indicated by black highlight

Use 3 drops of mineral oil

STEP 5

Seal the inoculated strip with the adhesive tape.
Incubate 18 - 24hours at 35 - 37°C

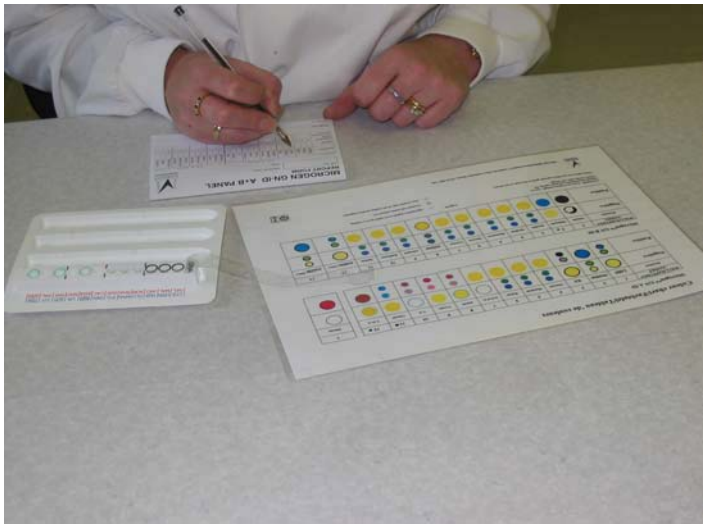
STEP 6

Add reagents:

- Indole – read within a few seconds
- VP1 and VP 11
    - Reagents must be added in order
    - Colour development of positive reactions should begin within 5 minutes and the intensity should increase over the next 15 – 20 minutes
- TDA – read within a few seconds

STEP 7

Interpret results using colour chart for guidance



STEP 8

Record results on report form

Complete the octal code and determine the Profile Number

MICROGEN GN-ID A+B PANEL

REPORT FORM

Lab. No. 3341

Specimen Type: CHEESE SANDWICH

Date: 28TH JANUARY 2002

MICROGEN BIOPRODUCTS

Well Number				GN A wells									GN B wells											
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Reaction	Oxidase	Motility	Nitrate	Lysine	Ornithine	H2S	Glucose	Mannitol	Xylose	ONPG	Indole	Urease	V.P.	Citrate	TDA	Gelatine	Malonate	Inositol	Sorbitol	Rhamnose	Sucrose	Lactose	Arabinose	Adonitol	Raffinose	Salicin	Arginine
Result				+	+	−	+	+	+	+	+	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−
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	4	2	1
Sum of Positive Reactions				6			7			6			0			0			7			6			0		

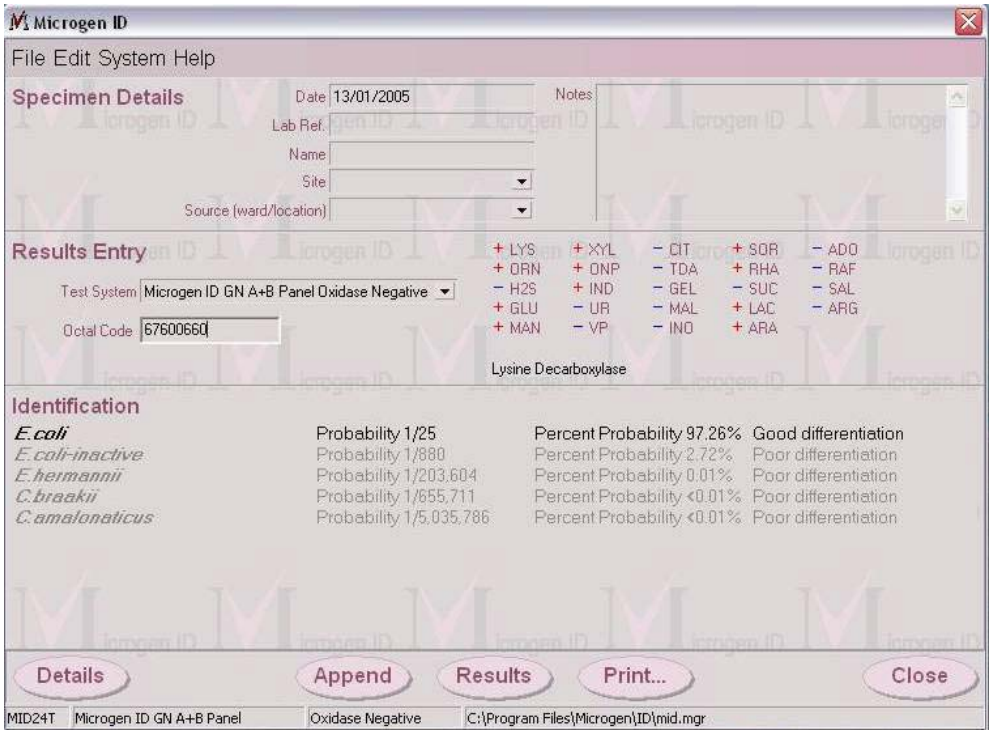
Profile No: 67600760

Final Identification: E.coli

WF6125/01/12

STEP 9

Analyse using Microgen Identification System Software or Code Book.



FEATURES/BENEFITS OF THE MICROGEN ID SYSTEM SOFTWARE MID-60

FEATURE	BENEFIT
Easy navigation by drop-down box to required ID system	Convenient. Easy to find the required ID system
Automatic prompt for Oxidase status	Confidence that the right system is in use
User configurable fields for specimen details	Convenient. Easily adaptable for individual circumstances
Space available for freeform notes	Convenient. Any required notes can be kept and retained with identification
Results entry by Reaction Code or Substrate result	Convenient. Results can be entered as preferred
Results can be individually changed to assess data	Confidence. If an individual result is suspect it can be investigated.
Details page contains all required data including probability, likelihood and tests against.	Confidence. Full data availability provides ability to fully assess the data.
Specifies additional tests to aid identification	Confidence. No need to consult other data sources.
Provides comments on taxonomy and other aids to identification e. g. serology	Confidence. System based on latest taxonomy.
Desktop software	Not dependent on internet to use
Updates regularly available from web site and easily downloaded and installed.	Convenient. Databases are always current an new systems can be added and updated quickly.
Results can be stored and sorted and edited for future reference.	Convenient. Results are easily available in the chosen format.
Results can be exported to other data packages.	Convenient.

KEY IDENTIFICATION DEFINITIONS IN MID SOFTWARE

These definitions must be understood for the correct interpretation of the results obtained using the MID System Software. It is **very important** that you and your customers have these clear in your mind.

1. **Probability**

   The chance or probability that the identification provided is correct when compared to all the organisms in the database. The system displays the 5 most probable choices.

2. **Percent Probability**

   The percentage chance that the ID given is correct when compared to the top 5 choices shown. This provides a measure of the extent to which the 5 choices provided are separated or differentiated from each other.

Percentage Probability		Interpretation
First Choice	**Second Choice**	
99.9	<0.01	The first choice is 10,000 times more likely than the second choice i.e. the two choices are very well separated
99	<1	The first choice is 100 times more likely than the second choice i.e. the two choices are well separated
70	25	The first choice is 3 times more likely than the second choice i.e. the two choices are poorly separated

3. **Likelihood**

   The closeness that the unknown strain matches the typical strain in the database. A likelihood of 100% indicates that the unknown organism has given a pattern of reactions absolutely typical for an organism of that type. The larger the number of tests against (Details Page of MID Software) the more atypical an isolate will be and therefore the lower the likelihood.

   For every organism in the database there is a "typical strain" in that e.g. in 95% of cases the typical strain may metabolise e.g. ARG. If an isolate does not, it could mean it is an atypical strain of that organism or that it is a different organism. The other tests will help this decision

4. Differentiation

The degree to which the identification choice is different from one or more of the other choices. In the event of the words “poor differentiation**” i**t means that one or more choices are poorly separated or "differentiated" and as such a clear choice cannot be made on the basis of the tests provided and that factors such as colonial morphology, site of isolation or additional tests should be taken into consideration.

As an example, the typical pattern for Salmonella 7702 will return 5 choices, each of Salmonella group something. On the basis of the biochemical tests, these cannot be better separated because biochemically they are indistinguishable. The groupings of Salmonella are based on "O" and "H" serology. In this case all choices have similar percentage positive results (20 - 25%) and probabilities 1/1 or 1/2.

5. Identification comments

These comments i.e. Excellent, Very Good, Good, Acceptable and Poor Differentiation refer to both the Percentage Probability and the Likelihood. Basically it provides a comment on the confidence an operator can have in a result based on these two parameters. A high Likelihood (100%) and a high Percentage Probability will provide a comment of Excellent i.e. typical strain which is well separated from the other choices. A low Likelihood (70%) due to one reaction being atypical for a strain of that type and a low Percentage Probability (70%) indicating that the first choice is only 3 – 4 times more likely than the second will return a comment of Poor Diferentiation. In such a situation additional tests may be useful (Refer to Details Page of Program).