# Aim

To identify bacterial isolates using commercial biochemical test kits (bioMerieux API).

# Principle

API test strips consists of microtubes (cupules) containing dehydrated substrates to detect the enzymatic activity or the assimilation / fermentation of sugars by the inoculated organisms. During incubation, metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents. When the carbohydrates are fermented, the pH within the cupule changes and is shown by an indicator. Assimilation tests are inoculated with a minimal medium (API AUX medium) and the bacteria grow if they are able to utilize the corresponding substrate: a positive result is indicated by growth. Test results are entered into an online database to determine the bacterial identity.

Following presumptive organism identification using Gram’s stain, morphological features and other simple tests, the appropriate API kit should be selected using the table below.

|  |  |  |
| --- | --- | --- |
| **Presumptive Organism ID** | **Which API strip to use** | **Additional notes** |
| Gram negative bacillus* Oxidase positive
* Non-fastidious
* Non-*Enterobacteriaceae*
 | API 20 NE | *Stenotrophomonas* & *Acinetobacter* spp.(oxidase negative) may also be identified using API 20E. |
| Gram negative bacillus* Oxidase negative
* *Enterobacteriaceae* & other non-fastidious GNB
 | API 20 E | *Vibrio* spp. and *Aeromonas* spp. (oxidase positive) may also be identified using API 20NE. |
| Gram positive cocci* Pairs or chains
* Catalase negative
* *Streptococci, Enterococci* & related genera
 | API 20 Strep | *Streptococcus pneumoniae* and groupable beta-haemolytic streptococci do not usually require API testing. |
| Gram negative cocci in pairsPleomorphic nutritionally demanding Gram negative bacilli or coccobacilli*(e.g. Neisseria, Haemophilus, Moraxella)* | API NH | *Moraxella catarrhalis* can be adequately identified using the trybutyrin test if isolated from a non-sterile site.*Haemophilus influenzae* can be adequately identified by XV-factor dependent growth.  |

# Method

The following are summaries from the kit inserts present in each box: refer to these inserts for further details if required.

API strips should only be used to identify pure cultures of an unknown organism. Confirm Gram stain (plus catalase and oxidase if appropriate) before inoculating a test strip. Inoculation of the incorrect strip can result in misidentification.

## General

### Inoculation

*This section applies to all APIs. Differences between each type of strip are described under the appropriate heading.*

1. Label the API carrier tray with specimen number and date.
2. Put 5ml of sterile distilled water into the tray to provide a moist atmosphere which prevent drying of the strip.
3. Lay the strip in the tray.
4. Open an ampoule of suspension medium if required:
	1. Insert the base of the ampoule into the protective plastic guard.
	2. Hold the ampoule vertically in one hand with the white plastic cap uppermost.
	3. Press the cap down as far as possible.
	4. Apply thumb pressure in an outward direction to the flattened part of the cap to snap off the top of the ampoule inside the cap.
	5. Carefully remove the cap and discard it.
5. Make a suspension of the organism and inoculate the wells as described in the section for the type of strip used. The structure of the wells is as follows:

Cupule

Tube

1. Tilt the strip slightly to help the inoculum to run into the tube. Avoid the tendency to trap air, which is most likely if the tubes are filled too rapidly, by carefully allowing the inoculum to run down inside one edge of the tube. The wells are of several types, and must be filled as shown in the diagrams:
	1. Those requiring filling of only the tube, denoted by a plain test name, e.g.: **GLU**
	2. Those requiring filling of both tube and cupule, denoted by a border round the test name, e.g.:

**GEL**

* 1. Those requiring filling of the tube, which is then overlaid with liquid paraffin, denoted by a line under the test name, e.g.: **URE**

Liquid paraffin

1. For strips that are to be incubated overnight or longer, inoculate a purity plate from the organism suspension, using a non-selective medium appropriate for the type of organism.
2. Put the lid on the tray and incubate it for the prescribed time under the appropriate condition

### Profile calculation and interpretation

1. Add any reagents as described for the type of strip used and make any other observations required, e.g.: haemolysis, then construct the profile:
	1. Mark each test as positive or negative on the lid of the tray
	2. The wells are marked off into triplets by black triangles, for which scores are allocated as follows:

1

2

4

* 1. Add up the scores for the positive wells only in each triplet. Supplementary tests, e.g.: oxidase may also be included in the profile. The highest score possible for a triplet is 7 (the sum of 1, 2 and 4) and the lowest is 0, e.g.:

1

+

-

-

2

4

**1**

1

+

-

+

2

4

1+4=**5**

1

-

-

+

2

4

**4**

1

+

+

+

2

4

1+2+4=**7**

1

+

+

-

2

4

1+3=**3**

1

-

-

-

2

4

**0**

1

-

+

4

ox

2

2+4=**6**

+

* 1. The profile for this combination of reactions is therefore **5147306**
1. Identify the organism using api*web*:
	1. Start Internet Explorer or Firefox web browser
	2. Go to: <https://apiweb.biomerieux.com>
		1. Login: pturner
		2. Password: apiweb
		3. Select the correct test (e.g. API 20E).
		4. Enter the numerical profile to obtain the identity.
		5. Record the identity along with comments (% ID and T value) on the results sheet.

### Disposal

1. After reading the API strip, place the carrier and contents into the autoclave bag in the plastic discard bin.

## API 20E

GEL

GLU

MAN

INO

SOR

RHA

SAC

MEL

AMY

ARA

ONPG

ADH

LDC

ODC

CIT

H2S

URE

TDA

IND

VP

1. Make a suspension of the test organism in 5ml saline.
2. From this suspension inoculate a sheep blood agar / Columbia agar purity plate.
3. Prepare and inoculate the test strip as described above and overlay with sterile liquid paraffin where indicated.
4. Incubate overnight (18-24h) in air at 36C (+/- 2C).
5. Assess strip:
	1. If there are **less than three** **positive** **reactions** (GLU test + or -) after incubation, do not add any reagents. Reincubate for a further 24 hours after checking the purity plate to make sure the organism is growing.
	2. If there are **three or more positive reactions** (GLU test + or -) examine the purity plate to ensure the culture is pure then add the reagents as follows:

|  |  |
| --- | --- |
| **Well** | **Reagent** |
| TDA | One drop of TDA reagent |
| IND | One drop of James reagent |
| VP | One drop of VP1 then one drop of VP2 |
| Also perform an oxidase on the purity plate |

1. Read the results from the following table:

| **TEST** | **REACTION** | **NEGATIVE** | **POSITIVE** |
| --- | --- | --- | --- |
| ONPG | β-galactosidase | Colourless | Yellow (maybe pale) |
| ADH | Arginine dihydrolase | Yellow | Orange or red |
| LDC | Lysine decarboxylase | Yellow | Orange or red |
| ODC | Ornithine decarboxylase | Yellow | Orange or red |
| CIT | Citrate utilisation | Light green | Blue-green or blue |
| H2S | H2S production | Colourless | Black |
| URE | Urea hydrolysis | Yellow | Pink |
| TDA | Tryptophan deamination | Yellow | Dark brown |
| IND | Indole production | Colourless reagent | Pink |
| VP | Acetoin production | Colourless | Pink or red |
| GEL | Gelatin hydrolysis | Colourless | Black diffuse pigment |
| GLU | Glucose fermentation | Blue | Yellow |
| MAN | Mannitol | Blue | Yellow |
| INO | Inositol | Blue | Yellow |
| SOR | Sorbitol | Blue | Yellow |
| RHA | Rhamnose | Blue | Yellow |
| SAC | Sucrose | Blue | Yellow |
| MEL | Melibiose | Blue  | Yellow |
| AMY | Amygdalin | Blue | Yellow |
| ARA | Arabinose | Blue | Yellow |
| Oxidase | Cytochrome oxidase | Colourless | Purple |

1. The tests on the strip plus oxidase are used to determine the first seven digits of the profile number. This is usually sufficient to determine the identity using api*web* software, but supplementary tests can be used to determine a further two digits if required (see kit insert).

## API 20NE

GLU

NO3

TRP

GLU

ADH

URE

ESC

GEL

PNPG

ARA

MNE

MAN

NAG

MAL

GNT

CAP

ADI

MLT

CIT

PAC

1. Make a suspension of the test organism in 2ml sterile saline.
2. Prepare the test strip as described above.
3. Inoculate the NO3 to PNPG (the first 8 cupules) from the saline suspension.
4. Open an API AUX ampoule (provided with the kit) and add 4 drops (200µl) of the saline suspension to it. Use the pipette to mix well without creating bubbles.
5. Use this suspension to inoculate the remainder of the cupules.
6. Overlay wells with liquid paraffin where indicated.
7. Incubate overnight (24h +/- 2h) in air at 29C (+/- 2C).
8. Examine the purity plate to ensure the culture is pure then add the reagents:

|  |  |
| --- | --- |
| **Well** | **Reagent** |
| NIT | One drop of NIT1 and one drop of NIT2 and wait 5 minutes. If there is no reaction (still colourless), add zinc powder, wait 5 minutes and interpret according to the table below |
| TRP | One drop of James reagent |

1. Examine the assimilation tests for bacterial growth. An OPAQUE cupule indicates a POSITIVE REACTION. Occasionally, a cupule may show weak growth. In this case the results should be noted as +/- or -/+ by comparison to other tests on the strip. Once these readings have been made, identification should be possible.
2. Read the results from the following table:

| **TEST** | **REACTION** | **NEGATIVE** | **POSITIVE** |
| --- | --- | --- | --- |
| NO3→NO2NO2→N2 | Reduction of potassium nitrate | ColourlessRed/pink | Red (NIT1+NIT2)Colourless (Zn) |
| TRP | Indole production from tryptophan | Yellow | Pink |
| GLU | Glucose fermentation | Blue/green | Yellow |
| ADH | Arginine hydrolysis | Yellow | Orange/pink/red |
| URE | Urea hydrolysis | Yellow | Orange/pink/red |
| ESC | Aesculin hydrolysis | Yellow | Grey/brown/black |
| GEL | Gelatin hydrolysis | No pigment diffusion | Diffusion of black pigment |
| PNPG | p-nitrophenyl-βD-galactopyranoside hydrolysis | Colourless | Yellow |
| GLU | Glucose assimilation | Transparent | Opaque |
| ARA | Arabinose assimilation | Transparent | Opaque |
| MNE | Mannose assimilation | Transparent | Opaque |
| MAN | Mannitol assimilation | Transparent | Opaque |
| NAG | N-acetyl-glucosamine assimilation | Transparent | Opaque |
| MAL | Maltose assimilation | Transparent | Opaque |
| GNT | Gluconate assimilation | Transparent | Opaque |
| CAP | Caprate assimilation | Transparent | Opaque |
| ADI | Adipate assimilation | Transparent | Opaque |
| MLT | Malate assimilation | Transparent | Opaque |
| CIT | Citrate assimilation | Transparent | Opaque |
| PAC | Phenyl-acetate assimilation | Transparent | Opaque |
| Oxidase | Cytochrome oxidase | Colourless | Purple |

1. Construct the numerical profile and look up the number using api*web*
	1. In the following cases, the strip must be reincubated:
		1. Low discrimation, unacceptable or doubtful profile on api*web.*
		2. If the following note is added to profile obtained from api*web*: **IDENTIFICATION NOT VALID BEFORE 48 HOURS INCUBATION.**
	2. In these events, immediately remove the contents of the NO3 and TRP wells using a pipette and fill with mineral oil so that a convex meniscus is formed, to prevent escape of acidic vapour. Reincubate the strip at 29C (+/- 2C) for a further 18-24 hours and read all the tests once more excepting NO3, TRP and GLU which must be read once only, at 18-24 hours.
	3. Reread the tests and construct a new profile.

## API NH

LIP
ProA

PAL
GGT

βGAL
IND

PEN

GLU

FRU

MAL

SAC

ODC

URE

Unlike API 20E/20NE/Strep, API NH relies on detection of preformed enzymes (not growth).

***Important:*** *suspensions of suspected N. meningitidis must be prepared in the BSLII safety cabinet*

1. Using a swab make a heavy suspension (McFarland 4) of the organism in 2ml of 0.85% sterile saline provided with the kit.
2. Into wells PEN to URE dispense about 50 l of this suspension. Fill the tube and cupule of the last 3 tubes (LIP/ProA, PAL/GGT, BGAL/IND).
3. Cover the first seven tests with mineral oil.
4. Incubate the strip in air at 36C (+/- 2C) for 2 hours.
5. **Before adding any reagents** record the primary results using the table below.
6. Add the reagents as follows:

**Note:** ZYM B **is very light sensitive** and loses activity within a few days of opening. Check that the date the ampoule was opened is within the last two weeks. If you start a new ampoule, write the date on the bottle.

|  |  |
| --- | --- |
| **Well** | **Reagent** |
| Wells 8 & 9(LIP/ProA & PAL/GGT) | One drop of ZYM B |
| Well 10(BGAL/IND) | One drop of James reagent |

1. Wait three minutes and read the results from the following table:

|  |  |  |  |
| --- | --- | --- | --- |
| **TEST** | **REACTION** | **NEGATIVE** | **POSITIVE** |
| PEN  | Penicillinase production | Blue | Yellow |
| GLU  | Glucose | Red | Yellow/orange |
| FRU  | Fructose | Red | Yellow/orange |
| MAL  | Maltose | Red | Yellow/orange |
| SAC  | Saccharose | Red | Yellow |
| ODC  | Ornithine decarboxylase | Yellow | Blue |
| URE  | Urease | Yellow | Pink/violet |
| LIP  | Lipase | Colourless | Blue (+ precipitate) |
| PAL  | Alkaline phosphatase | Colourless  | Yellow |
| βGAL  | β Galactosidase | Colourless  | Yellow |
| **AFTER ADDITION OF REAGENTS (ZYMB / JAMES)** |
| ProA | Proline arylamidase | Yellow | Orange |
| GGT | γ Glutamyl transferase | Yellow | Orange |
| IND | Indole | Colourless | Pink |

1. Construct a four digit profile from the results as described above, **ignoring the PEN result** and starting with the GLU, FRU, MAL triplet. The third digit is derived from the upper three tests in the bifunctional wells and the fourth from the lower three.
2. Determine the organism identity using api*web.*

## API Strep

LAP

VP

HIP

ESC

PYRA

αGAL

βGURUR

βGAL

PAL

ADH

RIB

ARA

MAN

SOR

LAC

TRE

INU

RAF

AMD

GLYG

The heavy density of the inoculum allows detection of preformed enzymes in some tests, allowing identification within 4 hours.

1. Subculture a single colony of the organism to be tested onto sheep blood agar and incubate for 24 or 48 hours until sufficient growth is obtained. Note the type of haemolysis.
2. Make a heavy (McFarland 4) suspension of the test organism in 2ml sterile distilled water.
3. Prepare the test strip as described above.
4. Using this suspension, inoculate VP to LAP with 100l suspension, and fill the tube portion only of the ADH.
5. Open an API Strep Medium ampoule (provided with the kit) and transfer about 0.5 ml of the suspension to it. Use the pipette to mix well without creating bubbles.
6. Distribute this into the remaining tests (Rib to GLYG).
7. Overlay wells with liquid paraffin where indicated.
8. Incubate in air at 36C (+/- 2C) for 4 hours to obtain the initial profile.
9. Add the reagents as follows:

**Note:** ZYM B **is very light sensitive** and loses activity within a few days of opening. Check that the date the ampoule was opened is within the last two weeks. If you start a new ampoule, write the date on the bottle.

|  |  |
| --- | --- |
| **Well** | **Reagent** |
| VP | One drop of VP1 and one drop of VP2 and wait 10 minutes.  |
| HIP | One drop of NIN and read after 10 minutes |
| PYRA toLAP | One drop of ZYM A followed by one drop of ZYM B and read after ten minutes. If necessary decolourise with intense light. |

1. Read the results from the following table.

| **TEST** | **REACTION** | **NEGATIVE** | **POSITIVE** |
| --- | --- | --- | --- |
| VP | Acetoin production | Colourless | Pink / red |
| HIP | Hippurate | Colourless | Dark blue / violet |
| ESC | Aesculin hydrolysis | 4hrs - colourless24hrs - pale grey | 4hrs - grey / black24hrs - black |
| PYRA | Pyrrolidonylaryl-amidase | Colourless / pale orange | Orange |
| αGAL | α-galactosidase | Colourless | Violet |
| βGUR | β-glucuronidase | Colourless | Blue |
| βGAL | β-galactosidase | Colourless / pale violet | Violet |
| PAL | Alkaline phosphatase | Colourless / pale violet | Violet |
| LAP | Leucine arylamidase | Colourless | Orange |
| ADH | Arginine dihydrolase | Yellow | Red |
| RIB | Ribose fermentation | 4hrs - Red24hrs - Red / orange | 4hrs - Orange / yellow24hrs - Yellow |
| ARA | Arabinose fermentation | 4hrs - Red24hrs - Red / orange | 4hrs - Orange / yellow24hrs - Yellow |
| MAN | Mannitol fermentation | 4hrs - Red24hrs - Red / orange | 4hrs - Orange / yellow24hrs - Yellow |
| SOR | Sorbitol fermentation | 4hrs - Red24hrs - Red / orange | 4hrs - Orange / yellow24hrs - Yellow |
| LAC | Lactose fermentation | 4hrs - Red24hrs - Red / orange | 4hrs - Orange / yellow24hrs - Yellow |
| TRE | Trehalose fermentation | 4hrs - Red24hrs - Red / orange | 4hrs - Orange / yellow24hrs - Yellow |
| INU | Inulin fermentation | 4hrs - Red24hrs - Red / orange | 4hrs - Orange / yellow24hrs - Yellow |
| RAF | Raffinose fermentation | 4hrs - Red24hrs - Red / orange | 4hrs - Orange / yellow24hrs - Yellow |
| AMD | Starch fermentation | 4hrs - Red24hrs - Red / orange | 4hrs - Orange / yellow24hrs - Yellow |
| GLYG | Glycogen fermentation | 4hrs - Red24hrs - Red / orange | 4hrs - Orange / yellow24hrs - Yellow |
| βHAEM | Haemolysis | No haemolysis | β haemolysis present |

1. Construct the numerical profile and look up the number using api*web:*
	1. In the following cases, the strip must be reincubated overnight:
		1. If the profile cannot be found in api*web.*
		2. If the following note is printed for the profile obtained: **IDENTIFICATION NOT VALID BEFORE 24 HOURS INCUBATION.**
	2. After 24hrs incubation, reread ESC, ADH and RIB to GLYG then construct a new profile.

# Quality assurance

No specific procedures required, aside from using the kits as described above and in the manufacturer’s manuals (kit inserts).

# Limitations

API kits should only be used to identify organisms as specified in this SOP or the relevant kit insert. Inoculation of an organism with inappropriate presumptive identification test results may result in an incorrect result.

If an unexpected / no result is obtained, it is important to recheck basic tests such as Gram result, catalase, oxidase, and ensure that the purity plate is satisfactory. Repeat the test with a pure culture if the purity indicates a mixture.

# References

1. bioMerieux API kit inserts (20 100 (API 20E); 20 050 (API 20NE); 10 400 (API NH); 20 600 (API 20 Strep)).
2. Procedure for the use of API Strips. Whittington Hospital SOP MB/040.04 (2005).
3. Which API to use. LOMWRU SOP BIP 005-01 (2012).

# Synopsis / Bench aid

|  |
| --- |
| **API 20E** |
| **Negative** | API_20_E_NEG |
| **Positive** | API_20_E_POS |
| **API 20NE** |
| **Negative** | API_20_NE_NEG |
| **Positive** | API_20_NE_POS |
|  |  |
| **API NH** |
|  | **Before addition of reagents** | **After addition of reagents** |
| **Negative** | API_NH_NEG | API_NH_NEG2 |
| **Positive** | API_NH_POS1 | API_NH_POS2 |
| **API 20 Strep** |
| **Negative** |  |
| **Positive** |  |

# Risk assessment

|  |
| --- |
| **COSHH risk assessment - University of Oxford COSHH Assessment Form** |
| **Description of procedure**Biochemical identification of bacteria | **Substances used**1. Pathogenic bacteria
2. API test strips and associated reagents
 |
| **Quantities of chemicals used**Small | **Frequency of SOP use**Daily |
| **Hazards identified**JAMES (HCl) is an irritant to the eyes, skin or other mucous membranesNIN (Dimethylsulfoxide (DMSO) and methanol) is a severe irritant and causes chemical burns if in contact with the eyes, skin, ingested or inhaled. Methyl alcohol is very flammable.NIT1&2 contain acetic acid, which is a severe irritant and causes chemical burns if in contact with the eyes, skin, ingested or inhaled.TDA (Ferric chloride)VP1 (potassium hydroxide) is a severe irritant and causes chemical burns if in contact with the eyes, skin, ingested or inhaled.VP2 (alpha naphthol and ethyl alcohol) is a severe irritant and causes chemical burns if in contact with the eyes, skin, ingested or inhaled. Also very flammable.Zinc powder is highly flammable.ZYM A & B is flammable and toxic. There is a danger of severe irreversible effects through inhalation, in contact with skin and if swallowed.Potential risk of infection from bacterial suspensions. | **Could a less hazardous substance be used instead?** No |
| **What measures have you taken to control risk?** 1. Training in good laboratory practices (GLP)2. Appropriate PPE (lab coat, gloves, eye protection)3. Use of biosafety cabinet for reading of plates / follow-up of BSL-3 organisms (e.g. *B. pseudomallei*) |
| **Checks on control measures**Observation and supervision by senior staff |
| **Is health surveillance required?**No | **Training requirements:**GLP |
| **Emergency procedures**:1. Report all incidents to Safety Adviser2. Clean up spills using 1% Virkon or chemical spill kit3. In the event of any solution going on the eyes, flush with eye was for 15 minutes. Wash hands if any solution gets on your hands. If swallowed do not induce vomiting, if conscious drink copious amounts of water. | **Waste disposal procedures**:1. Sharps discarded into appropriate rigid containers for incineration2. Infectious waste (incl. API strips) discarded into autoclave bags or 1% Virkon solution prior to autoclaving and subsequent incineration3. Chemical waste disposed of according to manufacturer’s instructions |