RCPA - A Guide to Bacterial Identification

1995: L'Année PASTEUR
Musée Institut Pasteur de Lille
Souches originales du BCG (vaccin contre la tuberculose) et microscope d'ALBERT CALMETTE

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Learning objectives

1. Describe the five key steps to identifying bacterial isolates
2. Describe the key microscopic and cultural characteristics of bacteria that can be used in identification
3. Select and describe basic phenotypic tests for the identification of bacteria
4. Interpret preliminary bacterial identification profiles
5. Select methods for definitive identification
6. Identify limitations and pitfalls of both commercial and manual identification methods
7. Apply trouble-shooting principles to facilitate difficult identifications
Preliminary Identification

The Identification Process

The identification process involves collecting information about an unknown organism. This information should include a number of preliminary observations and tests that, when viewed together can be a powerful tool to help determine the identification of an organism either into a broad group or to the genus level.
Steps in the Identification Process

The identification of an isolate should be systematic – don’t try to second guess what the organism is!

Step 1    Record the following:

- Gram stain appearance
- Growth characteristics
- Culture characteristics - colony morphology
- Oxidase reaction
- Catalase reaction
- Metabolism
- Motility
- Haemolysis
- Pigment production

*Observation is the key to success!*
Step 2   Develop a “Profile” of the Organism

These tests together should provide sufficient information for you to place the organism into a broad group or genus which will help you to choose the appropriate identification method for the organism.
Step 3  Choose the identification method

A number of options are available to assist with identification of an isolate: rapid methods have become very popular because they use preformed enzymes harvested from the organism – the test is basically a substrate-enzyme reaction which produces a coloured end product. This method is independent of growth which is an advantage for fastidious or slow-growing organisms.
A number of **semi-automated systems** are available – they vary in cost depending on the number of tests included per identification, how extensive the database is and how often updates occur.

**Automated methods** are expensive but have the advantage of large throughput and quick turn-around time. They too have their limitations as they are best suited to identification of rapidly growing organisms.
Notes, Hints, and trouble-shooting

- It is important to be aware of the limitations of identification systems. They are certainly improving all the time, but they do not always keep pace with current changes in nomenclature, they are not all suited to slow-growing or fastidious organisms and are slow to include newly described species.

- Some rapid identification systems that include preformed enzyme tests can be used to identify organisms not on the database - if the kit provides the tests needed to speciate an organism then use it!
A 4-24hr preformed enzyme method using Rosco tablets (Dutech Pty Ltd)

Remel produce a range of paper disc tests e.g. PYR & LAP discs

API (bioMerieux-Vitek Inc.) produce a range of manual & semi-automated kits

Conventional tube sugars

A number of automated identification systems are now available; Microscan, Vitek, & Phoenix to name a few.
Step 4  Perform identification
Step 5  Check ID Against Description in Text

This is very important!

- Some organisms share similar biochemical profiles but differ in other phenotypic features.
- Refer to a good text – doing this helps to cross-check the identification against other phenotypic features and also broadens your knowledge base.

**Recommended texts include:**
- Koneman, Elmer W., 2006. Colour Atlas and Textbook of Diagnostic Microbiology, Lippincott, Williams & Wilkins, NY
A Few Words on Commercial ID Systems!

- Ideally do not use expired kits or reagents
  - but if you must QC check the kit first
- Do not use reagents that have changed colour
- Always prepare the correct suspension turbidity.
  - this is particularly important with assimilation methods such as the API20NE
- Be aware of the limitations of each method
- Always check manufacturer’s instructions before using a kit for the first time
Now we will examine each of the tests listed in Step 1 in more detail. Then we will put these tests together using some examples to show you how to develop a profile of an unknown organism.
**Gram Stain**

- **Always prepare smears from a young 18-24hr culture**
  There are however, some exceptions - if you are looking for spores or the presence of a rod-coccus cycle, an old culture (2-3 days old) should be examined in parallel with a freshly prepared smear.

- **Examine the Gram stain** and describe what you see in detail and look closely - *I mean really look!*
Listeria monocytogenes – gs x100 - regular looking rods
Corynebacterium spp. – gs x100 regular medium

Gram-positive rods showing palisading
Propionibacterium acnes – gs x100
Rothia dentocariosa – gs x100 note rudimentary branching
Some organisms stain Gram variable even though their cell wall is of the Gram positive type – this may be because the cell wall is thinner for e.g. *Gemella* species.

Some *Clostridium* species (*C. ramosum*), and some *Bacillus* species can cause confusion because they stain Gram-negative. **Vancomycin susceptibility** or the KOH string test may help determine the true Gram reaction. Alternatively prepare a smear from an older culture – as the cells prepare for sporulation they often becomes more Gram-positive.

Some organisms are Gram-negative but may stain Gram positive e.g. *Acinetobacter* and *Moraxella* spp.
Gemella haemolysans – gs x100 cocci are often decolourised and may look like Neisseria
Can’t tell if you have a coccobacillus or a coccus?

Subculture isolate with a penicillin disc. Next day prepare a Gram stain smear from edge of zone of inhibition – the differences between cocci and rods will be obvious (see next 3 slides).
LHS – *Bacillus* spp. showing zone (susceptible) to vancomycin 5µg disc and are String test negative (no string formation in 3% KOH)

RHS – *Pseudomonas* spp. showing no zone (resistant) to vancomycin 5µg disc and are String test positive (strong positive string formation in 3% KOH). See next slide for KOH String test.
**String test:** emulsify the organism in a drop of 3% potassium hydroxide (KOH) and observe for formation of string (+ reaction) as above. Positive reaction = Gram-negative organism; Negative reaction = Gram-positive organism
Notes, Hints, and trouble-shooting

Size is important!

Familiarity with the size of an organism on Gram stain can be very helpful.

Examine the next slide to see what I mean.
Note how large these cocci are compared to the image below.

Cocci are much smaller.
Looking for spores?

The best place to find them is in the initial or primary inoculum where the nutrients in the media have been exhausted due to heavy growth in this area.
"Neisseria elongata"
gs x100 from edge of penicillin 10U disc
Coccobacilli elongate to form long filaments

"Neisseria species"
True cocci form “puff balls”
Moraxella osloensis
very coccoid rods
(from plate without penicillin)

*M. osloensis* again - Gram stain from edge of zone to penicillin note that the coccoid rods have become more elongated
Notes, Hints, and trouble-shooting

Some microorganisms do not produce a Gram stain appearance that is characteristic of the genus they belong to.

Examples include:

*Streptococcus mutans*

The Nutritionally Variant Streptococci (NVS)

e.g. *Abiotrophia defectiva* and *Granulicatella adiacens*
Streptococcus mutans – gs x100 from HBA - small Gram-positive coccobacilli that convert to cocci in chains when grown in BHI³ broth
Capnocytophaga ochracea – HBA @ 48hours

Note enhanced growth in CO$_2$ a characteristic of capnophilic organisms
*Abiotrophia defectiva* (NVS)– gs x100 require B6 supplementation to grow ‘normally’. The recommended concentration of pyridoxal hydrochloride (B6) is 0.001%
**Method**

Prepare a wet prep smear using distilled water or saline.

Spores are best observed using x40 phase contrast microscopy. As the spores mature and the spore coat thickens they becomes very refractile.
X40 phase contrast microscopy – *Bacillus* spp. showing refractile oval spores
Growth Characteristics

- **Subculture isolate** to HBA O₂, CO₂, ANO₂, CA (chocolate) and MAC (MacConkey).
- The next day (after 24hrs incubation) quantify growth for each media (score as +, ++, +++). Repeat again at 48 & 72 hours - make sure culture is not mixed!
- **Observe atmospheric requirements** - does the isolate grow better anaerobically, CO₂ or is it a strict aerobe?
- **Optimum temperature for growth** - Generally this is 35-37°C, however some organisms prefer a lower temperature especially for biochemical reactions i.e. 28-30°C for *vibrio* species or higher temperature (42°C) for *Campylobacter jejuni*
- **Examine your culture regularly** - observe for changes in colony morphology as colonies develop.
Propionibacterium acnes – note the increase in growth anaerobically compared to CO₂ and O₂ a characteristic of this organism.
Actinomyces neuii spp. neuii

Note enhanced growth in CO$_2$ and ANO$_2$ compared to O$_2$ a feature of many Actinomyces
Bordetella holmesii – growth O$_2$ and CO$_2$ but no growth anaerobically - typical of oxidative organisms such as Pseudomonas, Burkholderia, Acinetobacter to name a few.
*Alloiococcus otitidis* - HBA @ 48hrs

Note the slow growth rate and again no growth ANO$_2$
The initial inspection of colonies for preliminary identification of bacteria is one of the cornerstones of diagnostic microbiology (Koneman 1997).

This step together with the other steps described here provide the microbiologist with the necessary tools to select the appropriate tests to complete the identification of most bacteria either to the species level or at the very least, to place the organism into a broad group.
- How would you describe the size, shape and texture of the colonies?
- Does your isolate swarm?
- Does it have an odour?
- Does it “pit” the agar?
- Does the growth have a sheen?
- Are colonies adherent, mucoid or tenacious?
- Does the isolate prefer to grow on chocolate agar?

The following slides show examples of characteristic colony features that may assist with early recognition of a particular organism.
Eikenella corrodens
spreading & pitting

Pseudomonas species
Metallic sheen
Capnocytophaga ochracea spreading growth

Mucoid, rose pink colonies
*Roseomonas* spp.
**Pseudomonas stutzeri**
Wrinkled colonies

**Pseudomonas oryzihabitans**
Dry, adherent, yellow colonies
Stenotrophomonas maltophilia
Lilac sheen on HBA

Myroides odoratus
Spreading growth & sickly sweet odour
Colonies resemble a Bacillus spp.
Notes, Hints, and trouble-shooting

Some microorganisms e.g. *Capnocytophaga* spp. lose their characteristic culture appearance due to the effects of SPS (sodium polyanethol sulphonate) included in most blood culture media. These organisms require multiple subcultures before they return to their ‘normal’ appearance.

It is not uncommon for SPS-affected organisms to become inert in biochemical tests. The same remedy applies!
Notes, Hints, and trouble-shooting

Observe growth characteristics over several days. Some organisms grow very slowly e.g. *Actinomyces israelii*, others grow quickly but still require 2 or more days for characteristic cultural features to appear e.g. *Rothia dentocariosa*. Reincubate plates every day for up to 5 days if necessary.
Rothia dentocariosa

HBA @ 5 days

Note ‘pinwheel’ colony forms
**Oxidase**

**Principle of the test**

To determine the presence of intracellular oxidase enzymes.

All aerobic bacteria obtain their energy by respiration, a process responsible for oxidation of various substrates.

Oxidases catalyse many of the oxidation/reduction reactions involved in this process.
Oxidase test – positive reaction

- Reagent must be fresh - addition of 0.5-1% ascorbic acid will prevent oxidation of reagent. Discard reagent if it is deep blue
- Positive reactions generally occur within 10 seconds
- False negative reactions can occur if testing is performed on MacConkey agar or glucose containing media.
Catalase

Principle of the test

Catalase is an enzyme that breaks down hydrogen peroxide into water and oxygen. If it is not broken down but allowed to accumulate, it is lethal to bacterial cells.

- Always use fresh reagent and QC regularly
- Never perform catalase test on HBA plate
- False positives do occur e.g. *Lactobacillus* & *Enterococcus*
Catalase test – positive reaction

Note – placing a cover slip on the slide after mixing the reagent with the organism will enhance weak catalase reactions.
Metabolism

Principle of the test

Saccharolytic organisms break down glucose (and other sugars) either fermentatively or oxidatively.

*The metabolic processes of microorganisms determines the tests and procedures used to identify them.*
Oxidative organisms can only degrade glucose in the presence of oxygen and are therefore strict aerobes. The acids formed from aerobic oxidation are very weak, hence reactions are slower – this is why oxidative organisms tend not to perform as well in fully automated systems.

Fermentative organisms can use both the aerobic and anaerobic or fermentative pathway (EMP) and are generally facultative anaerobes. The acids formed via this pathway are relatively strong mixed acids. Organisms using the fermentative pathway grow more quickly and are very well suited to rapid automated identification systems.
Kligler’s Iron Agar – How it works

KIA contains 2 carbohydrates: 1% lactose and 0.1% glucose. Some organisms ferment both carbohydrates others ferment only glucose (yellow colour); still others do not ferment either lactose or glucose but utilise the peptones in the media resulting in an alkaline reaction (red colour). Carbohydrate fermentation may occur with or without formation of gas. Fermentation occurs both aerobically (on the slant) and anaerobically (in the butt).

(TSI – triple Sugar Iron agar may be substituted for KIA but it differs in also containing sucrose).
Reactions: (K=alkaline/red; A=acid/yellow; NC=no change)

K/NC or K/K
Oxidative fermentation of glucose occurs on the slant but this reaction is masked by alkaline reversion due to breakdown of peptones in media by oxidative Gram-negative rods e.g. *Pseudomonas* spp.

K/A with or without gas glucose fermenter e.g. *E. coli*

A/A with or without gas glucose and lactose fermenter e.g. *Enterobacter* spp.

A/NC
fastidious fermentative organism e.g. *Pasteurella* or *Bacillus* spp.
O/F Glucose
Fermentative organisms utilise glucose in both tubes and grow to the bottom of both tubes i.e. colour change from orange to yellow

Oxidation fermentation medium is an alternative method of determining the metabolic pathway of an organism

O/F Glucose – oxidative organism
Oxidative organisms only utilise glucose in the open tube (without oil overlay). Note visible line of growth in aerobic portion of tube only
Vitek printout – the GLU and OFG reactions can be interpreted in the same way as the O/F glucose tubes. **OFG** = open tube    **GLU** = closed tube
Motility Test

Principle of test

To determine if an organism is motile or non-motile.

Bacteria are motile by means of flagella. Number and position of flagella varies with bacterial species and culture conditions. The optimum temperature for flagella development is 25°C for a number of species. Occasionally motile bacteria produce non-motile variants.
- Use a good quality liquid medium eg **BHI broth**

- Perform test at **25°C or RT**

- Examine wet mount using x40 phase contrast at 0hrs, 4hrs and 24hrs (floating coverslip method)

- Do not perform the Motility test from differential or selective media i.e. MAC

- Motility differs from Brownian motion because bacteria change direction and do not just bop up & down on the spot!

- Some bacteria e.g **Sphingomonas paucimobilis** may only have a small proportion of the population that are motile, hence the name.
Notes, Hints, and trouble-shooting

Gliding motility as described for *Capnocytophaga* spp. is not true motility. They do not have flagella and will therefore not be motile in broth. They are tactile, requiring a moist surface to glide over.

Koneman, Elmer W., 2002
Semi-solid motility media may not be sensitive enough to detect the motility of some genera e.g. *Campylobacter*. In this case the preferred method is to prepare a direct smear in Brain Heart Infusion broth (BHI) from a freshly grown culture of the organism.
Bacteria generally produce their most characteristic morphology in broth media e.g. streptococci form chains, staphylococci form clusters, *Actinomyces* spp. form ‘microcolonies’.

Brain Heart Infusion broth (BHI) can be used to examine bacterial morphology as well as motility.

Organisms are grown in BHI broth for 4-24hrs at 37°C and then examined using x40 phase contrast microscopy. The next 2 slides highlight the difference in morphology when organisms are grown in a broth medium.
Streptococcus spp. (from HBA) GPC’S appear in clusters of large cocci

The same organism grown overnight in BHI broth
Gram-positive cocci in clusters (from HBA) – x100
*Streptococcus* spp. grown overnight in BHI broth form long chains of cocci. *Lactobacilli* also form long chains of rods in BHI broth which is helpful to differentiate them from *Actinomyces* spp.
Haemolysis

- Correct interpretation of haemolysis is important because the choice of subsequent tests is predicted on this initial evaluation (Koneman 2005)
- There are 3 types of haemolysis on blood agar. They are recorded as:
  - alpha (partial haemolysis of erythrocytes resulting in greenish discolouration in the media)
  - beta (complete lysis)
  - non-haemolytic.
*Kingella kingae* – HBA plate showing ‘soft’ beta haemolysis
Streptococcus pyogenes HBA plate showing β-haemolysis and a zone around Bacitracin disc

Plate held up to the light – the best way to observe haemolysis!
“Viridans” *streptococcus* – HBA showing alpha haemolysis
This is partial haemolysis of erythrocytes results in greenish
discolouration in the media
Notes, Hints, and trouble-shooting

- Haemolysis is best observed by examining colonies on the anaerobic HBA plate.

- Haemolysis is best observed at both 24 & 48hrs because weak β-haemolysis may be missed at 24hrs. This is particularly important with organisms such as Arcanobacterium haemolyticum as colonies develop slowly and haemolysis may be overlooked.
Pigment Production

- The swab technique can be very helpful because the pigment of some organisms is not obvious until you observe the underside of the growth i.e. by passing the swab through the growth.

- Keep a plate at 25-30°C for several days to check for pigment development.

- Media containing amino acids can be used to enhance pigment production eg MHA (Mueller Hinton agar) or tyrosine agar.
Pigment detection using the swab technique

*Brevundimonas vesicularis*
Carotenoid pigment at 48hrs
Some Words of Wisdom

- Don’t second guess the identification
- Be systematic - perform all preliminary tests
- Examine Gram stain closely
- Remember – all ID methods have limitations including molecular techniques! (Refer to JCM 2006, 44:3469-3470)
- Dichotomous keys – use only as a guide
  - they indicate probabilities not certainties
- Check identification against description in text
- If you cannot identify an organism – remember It might just might be novel!
Some Examples

Following are a few examples that may help to explain how to profile an organism based on the tests just outlined.

*Note: The growth characteristics are very important.*

*Observe the direction of optimum growth as indicated by the green arrow*  i.e. *is the growth of your isolate best in O₂ and CO₂ or is it growing better in the opposite direction.*
PRELIMINARY IDENTIFICATION

Accession No:      External/Internal: bench
Name:  ISOLATE 1
Date Received: 

Gram Stain:  Medium GNR

Catalase:  +

Oxidase:  +

* KIA:  K/NC  H₂S:  -  GAS:  -
* OF Glucose:  oxidative / Fermentative

Motility: (BHI broth)  RT  +  (0 hr)
RT  (4 hr)
RT  (24 hr)

Haemolysis:  α,  β,  γ,

Pigment:  Pale yellow  (check pigment production @ 24 & 48hrs)

Optimum Temperature for Growth:  37°C

Colonial Morphology:  @ 24hrs  1-1.5mm grey, shiny, convex, entire
@ 48hrs  2-3.0mm grey, shiny, convex, entire

Burkholderia pseudomallei – 24hrs HBA & MAC

Note: Some oxidative GNR’S are lactose fermenter on MacConkey agar

Isolate 1 - Pseudomonas aeruginosa NLF on MacConkey agar
Notes, Hints, and trouble-shooting

- Most pseudomonads fit the description on the previous slide, however a few species do not grow on MAC e.g. _Sphingomonas_ (Pseudomonas) _paucimobilis_.
- A few are oxidase negative e.g. _Ps. oryzihabitans_ and _Ps. Luteola_
- Some _Burkholderia_ spp. are lactose fermenters on MAC
- KIA reaction is always K/NC or NC/NC for all oxidative Gram-negative rods (GNR’S) irrespective of oxidase reaction.
- _Sphingobacterium, Chryseobacterium_ and unnamed _Flavobacterium_ spp. share the same profile, but all are non-motile, many are yellow pigmented, they do not grow on MAC and may be polymyxin B resistant.
Preliminary Identification

Accession No: External/Internal: bench
Name: ISOLATE 2
Date Received:

Gram Stain: Plump GNCB and rods

<table>
<thead>
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<th>Growth Characteristics @ 35°C</th>
<th>24hrs</th>
<th>48hrs</th>
<th>72hrs</th>
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<tbody>
<tr>
<td>BA</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAC</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BA</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHOC</td>
<td>++</td>
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<td></td>
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</table>

* KIA: K/NC
H₂S: -
GAS: -
* OF Glucose: oxidative / fermentative

Motility: (BHI broth)
RT - (0 hr)
RT - (4 hr)
RT - (24 hr)

Haemolysis: α, β, γ

Pigment: None (check pigment production @ 24 & 48hrs)

Optimum Temperature for Growth: 37°C

Colonial Morphology:
@ 24hrs 1.5mm white-grey, shiny, convex
@ 48hrs 2.5mm white-grey, shiny, convex

Acinetobacter and Moraxella spp. are characteristically “plump” Gram-negative rods, that tend to resist decolourising unlike the medium to slender rods of Pseudomonas spp. and hence may appear Gram-positive.

- Acinetobacter spp. can be very coccoid
- Acinetobacter and Moraxella differ in their oxidase reaction
  
  Acinetobacter (-), Moraxella (+)

- Acinetobacter and Moraxella are non-pigmented but occasional strains of Acinetobacter baumannii produce a reddish-brown pigment on MAC and Mueller Hinton agar.
Notes, Hints, and trouble-shooting

- β-lactamase producing strains of *Moraxella* spp. have been reported
- Tributyrin positive strains of *M. osloensis* have also been reported which may result in the organism being misidentified as *M. catarrhalis* (GNC) - correct Gram stain morphology is critical (it is wise to check morphology using penicillin challenge)
PRELIMINARY IDENTIFICATION

Accession No: ISOLATE 3
Date Received: 

Gram Stain: Tiny GN CB and cocci

1. Growth in O2  
   BA +/ -  +/-  
   MAC No growth

2. Growth in CO2  
   BA +/ -  ++

3. Growth in ANO2  
   BA +/ -  +

Catalase: +
Oxidase: -
KIA: NC/NC or A/NC  
H2S: -  
GAS: -

* OF Glucose: oxidative / Fermentative

Motility: (BHI broth)  
   RT - (0 hr)
   RT - (4 hr)
   RT - (24 hr)

Haemolysis: α, β, γ

Pigment: Pale yellow  
(check pigment production @ 24 & 48hrs)

Optimum Temperature for Growth: 37°C

Colonial Morphology:  
   @ 24hrs 0.1mm translucent to whitish
   @ 48hrs 1.0mm dry growth, white-grey, adherent

Preliminary identification: Presumptive HACEK organism - ? Actinobacillus actinomycetemcomitans
The “HACEK*” Group

What defines this group of organisms?

- Fastidious Gram-negative rods
- Do not grow on MAC
- Capnophilic
- Non-motile
- Slow–growth - require 48hrs for visible colonies to form
- Fermentative (exception *E. corrodens*)

*Haemophilus; Actinobacillus; Cardiobacterium; Eikenella; Kingella*
Each member of the HACEK group has a characteristic that sets it apart from the others i.e. the Gram stain, colony appearance, haemolysis or distinctive biochemical reaction.

The Gram stain morphology of HACEK organisms is very characteristic. Those that look similar i.e. *Haemophilus (Aggregatibacter) aphrophilus* and *A. actinomycetemcomitans* differ in their catalase reaction and sugar reactions.

Examine the following Gram stain smears of each of the HACEK organisms.
Cardiobacterium hominis, gs x100
*Kingella kingae*, gs x100

Plumpish rods that line up in parallel rows of pairs & short chains
*Eikenella corrodens* - gs x100

rods are slender and regular with some variation in length
Haemophilus aphrophilus, gs x100
small pleomorphic Gram-negative coccobacilli
Actinobacillus actinomycetemcomitans
gs x100
Tiny Gram-negative cocci and coccobacilli

Brucella melitensis
gs x100
Tiny Gram-negative cocci and coccobacilli
# PRELIMINARY IDENTIFICATION

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**Accession No:**

<table>
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<tr>
<th>Gram Stain: GPR, coryneform, palisades</th>
<th>Growth Characteristics @ 35°C</th>
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<tbody>
<tr>
<td>Catalase: +</td>
<td>1. Growth in O₂</td>
</tr>
<tr>
<td></td>
<td>24hrs</td>
</tr>
<tr>
<td>Oxidase: -</td>
<td>BA</td>
</tr>
<tr>
<td></td>
<td>MAC</td>
</tr>
<tr>
<td>* KIA:</td>
<td>H₂S:</td>
</tr>
<tr>
<td>* OF Glucose: F</td>
<td>oxidative / Fermentative</td>
</tr>
<tr>
<td>Motility: (BHI broth)</td>
<td>RT -</td>
</tr>
<tr>
<td></td>
<td>RT -</td>
</tr>
<tr>
<td></td>
<td>RT -</td>
</tr>
<tr>
<td>Haemolysis: α, β, γ,</td>
<td></td>
</tr>
<tr>
<td>Pigment:: None</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(check pigment production @ 24 &amp; 48hrs)</td>
</tr>
</tbody>
</table>

**Optimum Temperature for Growth:** 37°C

**Colonial Morphology:**

- @ 24hrs 1.0mm shiny, convex, entire
- @ 48hrs 1.5mm shiny, convex, entire

**Preliminary identification:** *Corynebacterium* spp.
Notes, Hints, and trouble-shooting

- *Corynebacterium* spp. are regular Gram-positive rods, they may be curved, barred and pallisading but they NEVER have the rudimentary elements demonstrated by the *Actinomyces* and other irregular Gram-positive rods.

- *Corynebacterium* spp. always grow best either aerobically or are facultative anaerobes – that is, they ALWAYS grow better in $O_2$ and $CO_2$ with reduced growth anaerobically.

- Some *Corynebacterium* spp. have an oxidative metabolism others are fermentative.
Coryneform morphology – V-shapes, pallisades, “chinese” letters
Colony size difference between non-lipophilic and lipophilic *Corynebacterium* spp.

Lipophilic *Corynebacterium* spp. require the addition of lipids in the form of sterile bovine or foetal calf serum to fermentation tests. Alternatively prepare a very heavy inoculum to enhance reactions. Tween 80 also provides a readily available source of lipids and can be added at a concentration of 0.5-1.0% to liquid or solid media to improve growth.
**Tween 80 hydrolysis test**

C=control tube (no added tween); T=test (tween added)

Note increased turbidity in the test compared to the control tube for the lipophilic species, but equal growth in the test and control tubes for the non-lipophilic species.
Lipophilic *Corynebacterium* species before and after addition of Tween 80 to HBA
PRELIMINARY IDENTIFICATION

Accession No: External/Internal: bench
Name: ISOLATE 5
Date Received:

<table>
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<th>Gram Stain:</th>
<th>Growth Characteristics @ 35°C</th>
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<tr>
<td>GPR, curved, some cells Gram</td>
<td>1. Growth in O₂</td>
</tr>
<tr>
<td>Variable “elbows”</td>
<td>24hrs 48hrs 72hrs</td>
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<tr>
<td>Catalase:</td>
<td>BA +</td>
</tr>
<tr>
<td>+</td>
<td>MAC No growth</td>
</tr>
<tr>
<td>Oxidase:</td>
<td>-</td>
</tr>
<tr>
<td>KIA:</td>
<td>2. Growth in CO₂</td>
</tr>
<tr>
<td>H₂S:</td>
<td>BA +++</td>
</tr>
<tr>
<td>GAS:</td>
<td>CA ++</td>
</tr>
<tr>
<td>*</td>
<td>3. Growth in ANO₂</td>
</tr>
<tr>
<td>* OF Glucose:</td>
<td>BA ++</td>
</tr>
<tr>
<td>oxidative / Fermentative</td>
<td>BHV</td>
</tr>
<tr>
<td>Motility: (BHI broth)</td>
<td>4. Other:</td>
</tr>
<tr>
<td>RT - (0 hr)</td>
<td></td>
</tr>
<tr>
<td>RT - (4 hr)</td>
<td></td>
</tr>
<tr>
<td>RT - (24 hr)</td>
<td></td>
</tr>
</tbody>
</table>

Haemolysis: α, β, γ,
Pigment: None (check pigment production @ 24 & 48hrs)

Optimum Temperature for Growth: 37°C

Colonial Morphology: @ 24hrs 0.1mm grey, dry, entire colonies
@ 48hrs 0.5mm grey, dry, entire colonies

Preliminary identification: Actinomyces spp. or Propionibacterium spp. (check spot indole)
Notes, Hints, and trouble-shooting

- Some of the newer *Actinomyces* spp. show very little branching and may appear almost coryneform – look closely for rudimentary elements as seen in earlier gram stain slide.

- Colony appearance also varies from classic slow-growing molar tooth colonies of *A. israelii*, tiny α-haemolytic colonies of *A. turicensis* to the large, shiny white colonies of *A. neuii* spp. *neuii*

- Colonies vary from weakly to strongly α-haemolytic to occasional β-haemolytic species.
Hints that an isolate may be an *Actinomyces* are:

- **Biochemical reactions** - Fermentation of xylose or lactose, or aesculin hydrolysis on the API Coryne strip
- **Growth conditions** - *Actinomyces* spp. always grow better in CO₂ and anaerobic conditions or they grow equally in all atmospheres BUT they never grow better in an aerobic atmosphere, hence their growth is opposite to that of *Corynebacterium* spp.
- **API Coryne ID**: *Microbacterium/Cellulomonas* or *Gardnerella vaginalis* may be a *Actinomyces* species
Actinomyces israelii – gs x100 microcolony grown in BHI broth

Actinomyces neuii spp. neuii gs x100 coryneform morphology
Thank you for your participation

We would really appreciate your feedback. Please click on the link to complete a 2 minute online survey