Gram Positive Cocci

Catalase positive

GPC in short chains/pair -> could be Enterococci (produces pseudocatalase).
So API strep.
Do sensitivity.

GPC in cluster

1. Do latex agglutination (e.g. Pastorex).
2. Set up tube coagulase: *3 tubes of 1ml horse serum, inoculate 2-3 colonies of test, positive control, and negative control. Incubate in a hot bath/35 degree – 4 hrs, can keep overnight. Tilt the tube to see the clot.*
3. Set up DNase plate: *pick up few colonies and make a heavy inoculums on DNase plate. Put controls. 35 degreeC/24/air. Next day – flood with 1M HCl, keep for few min, drain excess HCl, look for clearing around the colony (clearing = positive).*
4. Plate selective or chromogenic agar if available. If history suggest MRSA is a possibility consider MRSA chromogenic agar.
5. Put Staphylococcus sensitivity, if suspecting MRSA consider testing antibiotics which can be used.
6. If gram stain shows tetrads – Plate a blood agar with bacitracin (and furazolidone) if available – 35 deg C/24 hr/air.[Bacitracin – Micrococcus S, Staphylococcus R; Furazolidone – Micrococcus R, Staphylococcus S].

8. Ask about possible Staphylococcus aureus sources – skin, soft tissue, bone, joint, line, spine (discitis), endocarditis etc.
9. If suspecting MRSA, consider covering it. – appropriate abx, infection control.

10. Check tube coagulase after 4 hours.

11. Check sensitivity next day – Cefoxitin R – Ask for PBP2 kit/send for mecA PCR/ ask for MRSA selective agar.
12. Check DNase – if you have put it, check and document.
13. If MRSA – mention notification, PIR, infection control etc.
14. Manage St aureus appropriately.
15. If Coagulase negative Staphylococcus – assess if it needs furtehr identification/contaminant – consider API staph, if needs identification.
16. If yellow colony, tetrad in gram stain, suspecting Micrococcus – most likely a contaminant. If need confirmation – you need modified oxidase test – ask for Microdase disc.

©ElaineCloutman-Green2015

Catalase negative

Beta-haemolytic colony, GPC in chains –> Do Lancefield grouping.

Group A
1. Large colony, big zone of beta haemolysis, long chains:
Streptococcus pyogenes.
Optional – PYR + (anginosus group – negative).
Do sensitivity.

Look for skin, soft tissue infection, necrotising fascitis, URTI etc>
Isolate, standard infection control.
Always penicillin sensitive, recommend a suitable course (10 days).
Public health notification (invasive disease).

β-haemolytic or non-halemolytic – See F

Small colony, caramel/sweet smell: Streptococcus anginosus/ milleri group.
Look for abscess.

Group B
Streptococcus agalactiae
Optional – Granada agar/Islam's media – orange colony
Tight zone of beta haemolysis.
Do sensitivity.

Pregnant patient – inform obs-gynae/ midwife
Neonate: Inform ASAP.
From high vaginal swab of non pregnant women – could be normal flora.

Group C/G
Streptococcus dysgalactiae
Cellulitis, diabetic, endocarditis..
Do sens.

Group D
Long chain – Strep gallolyticus/bovis
Short chain – Enterococcus.
It might not be reliable,
Do PYR, if available – Enterococcus positive.
Do API Strep.
Set up sensitivity – put penicillin, Amoxicillin and antibiotics to cover Enterococcus as well.

Enq about bowel/ hepatobiliary malignancy, endocarditis..

Group F
Small colony, caramel smell – anginosus

Non-grouping/ not sure –
Recheck control.

©ElaineCloutman-Green2015

Put API strep.
Do sensitivity.

PYR – Moisten the filter paper disc with deionised water. Do not saturate (will cause false neg). Pick up few colonies and rub on the disc. Let it dry for 2 min and the put 1 drop of PYR reagent. Bright red/pink = positive.

Alpha haemolytic Streptococcus

1. Short chain/pair
Do Pneumococcus latex agglutination.
If negative – do strep group, D only (Enterococcus could be D +).
If BC – ask if the blood in the bottle was haemolysed.
Check blood culture growth graph.
Put blood agar with optochin disc (S>14mm)- Make sure to incubate in 5% CO2.

(S pseudopneumoniae is optochin R in CO2, but sen in ambient atmosphere.)

Plate 2 more blood agar plates.
Put sensitivity for strep/enterococcus.
Do bile solubility, if available.
Put API strep.

Remember E test.
Urine for Pneumococcal antigen, if appropriate.
Public health notification.

If non-pneumo alpha haemolytic Streptococcus (eg – mitis, mutans, oralis etc) – review for endocarditis, ask for repeat blood culture but also consider that it could be a contaminant.
Check oral hygiene.

Non haemolytic colony

API strep.
Sensitivity (put penicillin, Amox and abx for Enterococcus as well)
Do grouping, if possible (D only)

Anginosus could be non haemolytic.

API strep should ID organisms like – Leuconoctoc, Pediococcus, Gemella etc
Leuconostoc and Pediococcus – vancomycin R.

©ElaineCloutman-Green2015

Sensitivity panel

Pneumococcus

Optochin, Oxacillin, Tetracycline, Moxifloxacin, Linezolid,
Amoxicillin (if it turns out to be an Enterococcus, to get an idea of the species).

2nd line
Pen Etest, Ceftriaxone E test, Meropenem E test.

Streptococcus

Penicillin, Erythromycin, Clindamycin, Tetracycline, Vancomycin 5, Linezolid.

(if suspecting Pneumo/ent – put an optochin disc and amoxicillin on a separate blood agar).

Enterococcus

Amox/ampi, Linezolid, Vancomycin, Teicoplanin, Gent 200, Tigecycline.

Staphylococcus

Cefoxitin, Erythromycin, Clindamycin, Gentamicin, Linezolid,
Tetracycline.

Staph day2/extra

Neomycin, Chloramphenicol, Tigecycline, Trimethoprim, Mupirocin,
Rifampicin.

©ElaineCloutman-Green2015