# Aim

To describe the processing of nasopharyngeal swab specimens (NPS) to determine the presence of *Streptococcus pneumoniae* (pneumococcal) colonisation.

# Principle

*Streptococcus pneumoniae* is a leading bacterial pathogen, especially amongst children in the developing world. Over 90 pneumococcal capsular serotypes have been described: current vaccines can protect against 7-13 (conjugate vaccines, PCV) to 23 (plain polysaccharide vaccines, PPV) serotypes.

Pneumococcal colonisation of the nasopharynx (back of the nose) occurs frequently in infants and young children. Colonisation is an important precursor to infection, both invasive disease and mucosal infections. An individual may be colonised on many occasions with a single or multiple pneumococcal serotypes. Understanding the dynamics, and serotype distribution, of pneumococcal colonisation is important for vaccine planning and implementation (pre- and post-introduction).

Thin wire/plastic swabs (NPS) are used to sample the nasopharynx. These swabs can be stored in skim milk-tryptone-glucose-glycerol (STGG) transport medium at -80°C for prolonged periods after collection to enable batch processing. NPS-STGG specimens are cultured on a selective medium (sheep blood agar supplemented with either gentamicin or colistin-nalidixic acid (CNA)) to maximise the chance of identification of *Streptococcus pneumoniae*. Potential pneumococci are sub-cultured to blood agar and confirmed by conventional means (Gram stain, catalase, optochin disc, bile solubility). Isolates are serotyped by identification of capsular swelling (Quellung reaction) or agglutination (latex reagents) using specific antisera.

The use of the latex antisera on a suspension of organisms from the primary culture plate permits the identification of colonisation by multiple pneumococcal serotypes.

Although the WHO method recommends culture of 10µl STGG (with 50µl and 100µl as options), preliminary work at AHC-COMRU revealed this to be an inadequate volume, hence 100µl selected as standard.

# Method

## Specimen collection

Nasopharyngeal samples should be obtained with a deep nasopharyngeal swab:

* The patient’s head should be tipped slightly backward and the swab passed directly backwards, parallel to the floor of the nasopharynx.
  + The swab should pass without resistance until it reaches the posterior pharynx which is approximately one-half to two-thirds the distance from the nostril to the ear lobe.
  + If resistance is encountered, the swab should be removed, and an attempt should be made to pass the swab through the other nostril.
* Once the swab is in place, rotate the swab 180 degrees or leave it in place for 5 seconds to saturate the tip before removing it slowly.
* Immerse the tip of the swab in a fresh 1ml cryovial of STGG transport medium and cut the shaft with sharp scissors (cleaned in between uses with 70% ethanol).
* Replace the lid on the cryovial, label the specimen, and place immediately into a cool cox or refrigerator.

## Specimen transport and storage

NPS-STGG specimens must be:

* Stored in cool box or refrigerator prior to and during transportation back to the laboratory.
* Frozen at -80°C within 8 hours of collection.

## Specimen processing

### Reception

Enter specimen details into the laboratory database / logbook.

Vortex the NPS-STGG cryovial for 15 seconds.

Using a P1000 pipette and sterile tip, pipette 500µl of STGG into a second sterile cryovial:

* Label both tubes with the specimen number, study code, and date.
* Label the first tube (with swab in situ) “aliquot 1”
* Label the second tube “aliquot 2”

Place both aliquots in the -80°C freezer and note the specimen freezer position (freezer / shelf / rack / box) in the study specimen logbook.

### Culture

Retrieve the required batch of NPS-STGG specimens from the -80°C freezer (use “aliquot 2” (no swab) unless directed otherwise).

Leave on the bench or in the class II biosafety cabinet to fully defrost.

Vortex each cryovial for 15 seconds.

Using a P200 pipette and sterile tip, inoculate a Columbia agar with 5% sheep blood and 5µg/ml gentamicin plate (BA-G) with 100µl STGG and streak the plate in four directions (Figure 1) with a sterile loop.

Incubate the BA-G plates overnight (16 – 20 hours) at 35-37°C in a candle jar (3 – 10% CO2).

Put the NPS-STGG specimen back in the -80°C freezer as soon as possible.

**Figure 1.** Streaking of the BA-G plate

1

2

3

4

# Interpretation

For both plates, record the semi-quantitative growth of all alpha-haemolytic colony types as follows:

1+ Growth in quadrant 1 but <10 colonies in quadrant 2

2+ >10 colonies in quadrant 2 but <10 colonies in quadrant 3

3+ >10 colonies in quadrant 3 but <10 colonies in quadrant 4

4+ >10 colonies in quadrant 4

If no alpha-haemolytic colonises are seen, re-incubate the plates for another 16 – 20 hours before discarding as negative.

## Follow-up using the WHO protocol

### Identification of Streptococcus pneumoniae

Pick one representative colony of each alpha-haemolytic morphologic type seen and sub-culture onto ½ of a plain Columbia agar with 5% sheep blood plate (BA), with an optochin disc placed between the pool and first streak (Figure 2).

Incubate the BA plates overnight (16 – 20 hours) at 35-37°C in a candle jar.

Further follow-up pure isolates as shown in Figure 3.

**Figure 2.** Sub-culture of alpha-haemolytic colonies

**Figure 3.** Flow chart for identification of alpha-haemolytic colonies



### Serotyping of pneumococcal isolates

Serotype all isolates of *Streptococcus pneumoniae* using latex agglutination and / or the Quellung reaction as described in SOP MID-005.

### Antimicrobial susceptibility testing of pneumococcal isolates

Antimicrobial susceptibilities will be done later in batches from frozen isolates. Pneumococcal isolates should have antimicrobial susceptibilities determined according to SOP MIC-001. Benzyl penicillin and ceftriaxone Etests should be done on any pneumococci with a zone diameter of <20mm around the oxacillin (OX1) screening disc.

### Storage of pneumococcal isolates

Save pneumococcal isolates (harvest growth from the whole BA plate with a sterile swab) in a labelled fresh vial of STGG medium and store in the -80°C freezer.

Record the isolate freezer position (freezer / shelf / rack / box) in the study isolate logbook.

## Follow-up using the latex sweep protocol

Using a sterile loop or swab, make a heavy suspension (2-3 MacFarland) of the colonies on the primary BA-G plate in 0.5 – 2.0ml of 0.85% saline (volume required will depend on colony density) in a sterile 5ml polystyrene bijou or test tube.

Label the tube with the specimen number.

Perform serotyping using latex reagents as described in SOP MID-005:

* Follow-up all positive pool reactions with the appropriate group, type, and factor antisera to identify all pneumococcal serotypes cultured from the specimen (the specimen may contain more than one).

It is possible to determine the presence of non-typeable pneumococci in a culture: a weak reaction with Pool B and Group 19 latexes, but no reaction with the Group 19 factor latexes, is observed. Report this result as “?NT”.

**Note:** SSI Diagnostica report known cross-reactions for some antisera. These should be resolved by working through the additional factor antisera results for the isolate (and referring to Table 1).

**Table 1.** SSI known antisera cross-reactions

|  |  |
| --- | --- |
| **Antiserum** | **Cross-reacting types** |
| Group 25 | 38 |
| Type 29 | 35B |
| Group 35 | 42, 47F |
| Type 42 | 20, 31, 33A, 35A, 35B, 35C |

## Reporting

Research results on NPS specimens will not be reported to the clinical team, since colonisation by pneumococci is a normal event and does not represent infection requiring treatment.

Report results as specified on the appropriate study laboratory case record form. If not specified, the following should be recorded:

* Quantity (1+ to 4+)
* Colony morphology
* Optochin zone diameter (mm) +/- bile solubility result
* Serotype
* Antimicrobial susceptibilities
* Latex sweep serotyping result

# Quality assurance

Media and identification tests should be quality controlled according to the relevant SOP. Serotyping should only be performed on young / healthy cultures (i.e. overnight growth).

# Limitations

Prior antimicrobial use may result in negative NPS cultures. Pneumococcal serotypes present at relatively low abundance <10%, may not be detected by the latex sweep serotyping method unless a larger volume of NPS-STGG specimen is cultured or an appropriately dense suspension of colonies is tested.

# References

O'Brien KL, Nohynek H. Report from a WHO Working Group: standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*. Pediatr Infect Dis J. 2003;22(2):e1-11.

* This document is currently under revision (Satzke C, et al. 2013). Changes arising from a meeting of experts at WHO (Geneva, March 2012) have been incorporated into this SOP pending publication of the revised method paper.

Turner P, Hinds J, Turner C, Jankhot A, Gould K, Bentley SD, et al. Improved detection of nasopharyngeal co-colonisation by multiple pneumococcal serotypes by use of latex agglutination or molecular serotyping by microarray. J Clin Microbiol. 2011;49(5):1784-9.

Turner P, Turner C, Jankhot A, Phakaudom K, Nosten F, Goldblatt D. Field Evaluation of Culture plus Latex Sweep Serotyping for Detection of Multiple Pneumococcal Serotype Colonisation in Infants and Young Children. PLoS ONE. 2013;8(7):e67933.

# Synopsis / Bench aid



# Risk assessment

|  |  |
| --- | --- |
| **COSHH risk assessment - University of Oxford COSHH Assessment Form** | |
| **Description of procedure**  Culture of nasopharyngeal swab specimens to identify *Streptococcus pneumoniae* colonisation | **Substances used**  STGG transport medium  Sheep blood agar  Optochin discs  10% sodium deoxycholate (bile) solution   * harmful if swallowed; irritant   Pneumococcal antisera (plain and latex-bound; prepared in rabbits)   * potential allergen |
| **Quantities of chemicals used**  Small | **Frequency of SOP use**  Daily |
| **Hazards identified**  1. Autoclaved liquid  2. Potentially infectious material in sample  3. Potentially pathogenic bacteria | **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) | |
| **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 Adviser  2. Use eyewash for splashes  3. Clean up spills using 1% Virkon or chemical spill kit | **Waste disposal procedures**:  1. Sharps discarded into appropriate rigid containers for incineration  2. Infectious waste discarded into autoclave bags or 1% Virkon solution prior to autoclaving and subsequent incineration  3. Chemical waste disposed of according to manufacturer’s instructions |