



PHLN

Public Health Laboratory Network

Invasive Group A Streptococcal disease (iGAS) | Streptococcus pyogenes

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions to inform the diagnosis of key diseases in Australia. This document contains the laboratory case definition (LCD) for invasive streptococcus pyogenes, Group A streptococcus or (iGAS).

Version	Status	Authorisation	Consensus Date
1.0	Initial PHLN Laboratory Case Definition	PHLN	22 November 2023

PHLN summary laboratory definition

1.1 Condition:

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- Invasive Group A Streptococcal Disease (iGAS). Forms of invasive GAS disease include necrotising soft tissue infection, pregnancy-associated infection, and bacteraemia.

1.1.1 Definitive Criteria

- Isolation of *Streptococcus pyogenes* from a normally sterile site (e.g., blood, cerebrospinal fluid, joint fluid, peritoneal fluid, bone, internal organ tissue).

OR

- Detection of nucleic acid of *S. pyogenes* from a normally sterile site.

1.1.2 Suggestive Criteria

- Detection (Isolation or nucleic acid detection) of *Streptococcus pyogenes* from a non-sterile site (e.g., pus, wound swab, placenta, etc) in a patient with clinically compatible severe illness (e.g., necrotising fasciitis, puerperal sepsis, streptococcal toxic shock syndrome (STSS), or postpartum endometritis).

There are a number of iGAS syndromes that require clinical as well as laboratory input to diagnose. They are important syndromes to include but having clinical criteria in a laboratory definition is problematic. However, it is more important to have accurate case numbers than semantic correctness. These syndromes are from a laboratory definition listed as possible/probable and infection severity is an important clinical factor:

Isolation of GAS from a non-sterile site (i.e., throat, sputum, wound, skin abscess, placenta, cervix, etc), no other microbial aetiology likely; and a clinically compatible severe illness (e.g., puerperal sepsis, necrotising fasciitis, streptococcal toxic shock syndrome (STSS), postpartum endometritis). Illness severity is an important determining factor (e.g., the presence of hypotension and multi-organ involvement).

1.1.3 Special Considerations / Guide for Use

- iGAS manifestations can present as several clinical syndromes where bacteria are isolated (bacteraemia, deep soft tissue infection, meningitis, or infection of other

usually sterile body sites); and/or the release of toxins causing STSS, a syndrome of hypotension with multi organ failure. Invasive GAS infections are defined as bacteraemia, pneumonia, osteomyelitis, septic arthritis, or any other infection associated with the isolation of GAS from a normally sterile body site. Invasive infections also include necrotising fasciitis and spontaneous gangrenous myositis.

- STSS is a clinical syndrome which may or may not have supportive laboratory evidence.
- A normally sterile site contains no microorganisms in healthy individuals.
- Illness severity (hypotension and multi-organ failure) must be assessed as part of iGAS assignment.

Introduction

2 Group A Streptococci (GAS) are human specific bacteria, also known as *Streptococcus pyogenes* and Streptococcal A (Strep. A). There are many different strains or types (classified in various ways such as emm types) and some types are more likely to be associated with disease than others. Non-S. pyogenes Group A streptococci (e.g., *Streptococcus dysgalactiae* subspecies *equisimilis*) can be excluded from the surveillance.

Colonisation/Carriage: GAS commonly resides on people's skin, upper respiratory, gastrointestinal, and anogenital tracts. When it resides on a person without causing disease, the person is said to be 'colonised' or have GAS 'carriage' and the person is not 'infected'.

However, GAS can cause infection and a wide variety of clinical disease in humans – including for people who are usually healthy. The balance of GAS 'colonisation' versus 'infection' depends on:

- a person's comorbidities, demographics, immune status, living conditions, and the particular strain of GAS involved and its ability to cause harm.

GAS can cause a wide spectrum of clinical infections that range from non-invasive to invasive, and from non-severe to severe. Complications of GAS infection include:

- Acute GAS infection, non-invasive: mild skin or throat infections, scarlet fever (toxin-mediated).

- Invasive GAS disease, or iGAS: bacteraemia, necrotising fasciitis, post-partum sepsis, severe pneumonia, and meningitis.
- STSS: a complication of invasive GAS disease characterised by shock and multi-organ failure. The pathology is associated with capillary leakage and tissue damage due to release of inflammatory cytokines induced by streptococcal toxins.
- Immune-mediated complications following an episode of GAS infection (of varying severity):
 - Acute post-streptococcal glomerulonephritis, which can lead to chronic renal failure
 - Acute rheumatic fever, which can lead to rheumatic heart disease.

Spectrum of disease caused by Group A Streptococci

GAS can be acquired by direct skin contact or by respiratory contact with infectious secretions from an infected person's nose or mouth, or spread by large droplets when they cough or sneeze, or from contact with skin sores or wounds. GAS can also be transmitted by direct contact with contaminated environment such as clothes and linen.

The incidence of iGAS is highest among the very young and the elderly; and persons with chronic medical conditions, immune-suppression and Aboriginal and Torres Strait Islander populations. Pregnancy associated infections are also well recognised causes of infant and maternal mortality. Prognosis varies depending on the type of infection, but a case fatality of 10-20% is reported particularly in the very young and persons with necrotising fasciitis or STSS.

- 3 Invasive GAS infection often occurs sporadically; however, clusters and outbreaks of
3.1. invasive GAS infection are documented.

Laboratory diagnosis

Test method

Bacterial culture is the current gold standard; however Nucleic Acid Amplification Test (NAAT) assays are becoming more widely available to analyse blood, sterile tissues, and fluids.

Traditional blood agar plate culture methods (combined with Gram stain which is suggestive but non-specific) rely on incubation of microbiological processed samples in a CO₂ supplemented atmosphere to enhance growth.

Identification relies on a combination of methods (no single method is 100% sensitive) and includes Gram stain morphology, presence of beta-haemolysis on blood agar, Latex agglutination (Lancefield) grouping, bacitracin sensitivity, and PYR (pyrrolidonyl aminopeptidase) testing. PYR is used for the detection of pyrrolidonyl arylamidase enzyme activity in GAS. MALDI-TOF (Matrix-assisted laser desorption/ionisation-time of flight) mass spectrometry is also a validated method of identification, as are commercial biochemical panels.

NAAT testing can include commercial molecular panels or in-house detections. One molecular target amplified is the highly conserved *sdaB* gene, which encodes for Dnase B, an extracellular antigen of GAS, and the basis for the anti-Dnase B antibody test used to help identify immune related post streptococcal infection syndromes.

An alternative target is *speB*, also highly conserved, and it encodes for streptococcal pyrogenic exotoxin B or SPEB, a cysteine protease, major virulence factor, and superantigen whose expression mediates toxic shock seen with various syndromes of severe acute pyrogenic infections due to GAS.

Both of these target genes are specific for GAS, and both are single gene copies in the *Streptococcus pyogenes* chromosome.

Suitable specimen types

Blood culture (sterile procedure), CSF, body fluid (such as fluid aspirate(s)), surgical biopsy material (i.e., fasciitis), pus and swabs (with transport media) from sterile sites. Throat and ano-genital swabs from non-sterile sites in patients presenting with possible STSS.

Specimen collection and handling

Keep specimens moist and collect into sterile containers, use sterile saline wrapped gauze, if necessary to avoid surgical specimens drying out. Transport and process promptly.

Test sensitivity

Nucleic acid testing offers an alternative way to improve speed and accuracy in GAS diagnosis. With respect to pharyngitis, NAAT has been shown to have superior sensitivity and specificity compared to conventional throat cultures and clinical diagnosis.

However, similar studies have not been performed for iGAS, and as culture positivity rates can be influenced by specimen collection, transport, and time to processing, NAAT remains an option if clinical circumstances are suggestive of iGAS syndromes.

Test specificity

NAAT assays in Group A Streptococcal pharyngitis are reported as having lower specificity than culture due likely due to the NAAT Strep A assay having higher sensitivity as compared to throat culture. Large comparator studies have not been performed for sterile site iGAS yet.

Strain differentiation

Various typing systems are available for epidemiological purposes. GAS has been classically subdivided based upon serotyping of surface-expressed M and major pilus subunit protein T typing. M-typing using specific antisera has been largely replaced by *emm*-typing, using DNA sequencing of the variable region of the *emm* gene. While useful it may not be specific enough when common *emm* types occur geographically, hence multi-locus sequence typing (MLST) and the even more specific whole genome sequencing (WGS) are also used. *Emm* type of GAS strains can also be determined from the WGS results.

4 Antimicrobial susceptibility

GAS remains universally penicillin sensitive despite 85 years of antibiotic selection pressure. Alternative agents are also tested due to the need to treat patients with allergies and include vancomycin, macrolides, trimethoprim/sulfamethoxazole, and clindamycin. Though usually susceptible, antimicrobials can develop resistance, such as clindamycin which may develop constitutional or inducible reduced susceptibility.

Testing is classically done by phenotypic disc-based methods, but MIC (minimum inhibitory concentration) testing and broth micro-dilution are also available. Breakpoints and standardised methods are protocol based on either EUCAST (European Committee on Antimicrobial Susceptibility Testing) or CLSI (Clinical and Laboratory Standards Institute). Mutations in GAS penicillin binding protein genes have been noted in many countries but have not yet caused penicillin resistance, but do confer some reduced susceptibility to other beta lactam antibiotics.

Cephalosporins, clindamycin, and macrolides are alternatives for patients who are allergic to penicillin, however resistance is increasing.

Clindamycin is often added together with a beta-lactam antibiotic for treatment of STSS; the beta-lactam antibiotic is continued in case the infection is caused by an isolate that is resistant to clindamycin. Other potential advantages of clindamycin for treatment of iGAS include: (i) clindamycin suppresses bacterial toxin production, and (ii) clindamycin efficacy, if susceptible, is not affected by inoculum size.

Adjunctive therapies with varying evidence efficacy for treatment of iGAS infection include intravenous immune globulin (IVIG), and hyperbaric oxygen.

SNOMED CT terms

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SNOMED CT code	Term name	Description
406614006	Invasive Group A beta-haemolytic streptococcal disease	<i>Disorder</i>
80166006	Streptococcus pyogenes	<i>Organism</i>

References

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Glossary

Ag/Ab – Antigen/Antibody

AMR – Antimicrobial resistance

7 **ARTG** – Australian Register of Therapeutic Goods

BA – Blood agar

Biotype – Strain distinguished from other microorganisms of the same species by its physiological properties or a group of organisms with the same genotype

CCNA – Cell cytotoxicity neutralisation assay

(US) CDC – Centers for Disease Control and Prevention

CDNA – Communicable Diseases Network Australia

CDS – Calibrated dichotomous susceptibility

CIA – Chemiluminescent immunoassay

Clade – Group of organisms composed of a common ancestor and all its lineal descendants

CLSI – Clinical and Laboratory Standards Institute

CSF – Cerebrospinal fluid

Ct – Cycle threshold

DFA – Direct fluorescent antibody

DNA – Deoxyribonucleic acid

EDTA – Ethylenediaminetetraacetic acid

EIA – Enzyme immunoassay

ELISA – Enzyme linked immunosorbent assay

EUCAST – European Committee on Antimicrobial Susceptibility Testing

HI – Haemagglutination inhibition

ICT – Immunochromatographic test

IFA – Immunofluorescent antibody

IgA – Immunoglobulin A

IgG – Immunoglobulin G

IgM – Immunoglobulin M

IVD (device) – In vitro diagnostic medical device

In vitro – performed in a test tube, culture dish, or elsewhere outside a living organism

In vivo – performed or taking place in a living organism

ITS – Inter-genic spacer region

LAMP – Loop-mediated isothermal amplification

LPS – Lipopolysaccharide

MALDI-TOF – Matrix-assisted laser desorption ionisation-time of flight

MAT – Microscopic agglutination test

MDST – Molecular drug susceptibility testing

MDR – Multidrug resistant

MIA – Microsphere immunoassay

MLST – Multilocus sequence typing

NAAT – Nucleic acid amplification test/ing

NATA - National Association of Testing Authorities, Australia

NGS – Next generation sequencing

NPAAC – National Pathology Accreditation Advisory Council

NRL – National Serology Reference Laboratory

PCR – Polymerase chain reaction

PC2 laboratory – Physical containment level 2 laboratory

PC3 laboratory – Physical containment level 3 laboratory

PC4 laboratory – Physical containment level 4 laboratory

PFGE – Pulsed field gel electrophoresis

POC – Point-of-care

QAP – Quality assurance program

QC – Quality control

RAPD – Random amplified polymorphic DNA

RCPA – Royal College of Pathologists of Australasia

RFLP – Restriction fragment length polymorphism

RNA – Ribonucleic acid

RT – Reverse transcriptase

RT-PCR – Reverse transcription polymerase chain reaction

SBT – Sequence based typing

Serotype – Pathogens of the same species that are antigenically different

SNT – Serum neutralisation

SSBA – Security sensitive biological agent

STI – Sexually transmitted infection

Strain – Variant that possesses unique and stable phenotypic characteristics

SQAP – Serology quality assurance program

Test sensitivity – Ability of a test to correctly identify patients with a disease

Test specificity – Ability of a test to correctly identify people without the disease

TGA – Therapeutic Goods Administration

UTM – Universal transport medium

VTM – Viral transport media

WGS – Whole genome sequencing

WHO – World Health Organization

WHO CC – WHO Collaborating Centre

XDR – Extensively drug resistant