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Bacterial 16S rRNA gene detection and sequencing

The bacterial 16S rRNA gene encodes for the ribosomal RNA small subunit. It contains DNA sequences that are common to all bacteria and some that are unique to each species. It can be used in the pathology laboratory as a tool to identify bacteria in culture negative infections, to speed up the identification of slow-growing bacteria or to identify difficult or rare bacteria in subculture.

We aim to provide a positive identification of an infectious agent within 1-2 days.

We use a specific manual extraction process (including protease, lysozyme and lysostaphin) to lyse bacteria and minimise the effects of inhibitors and contaminating DNA. We then use an in-house assay to amplify a short, conserved region of the 16S rRNA gene. The amplification reaction includes negatives to control for possible contamination of i) the extraction procedure and ii) the assay reagents.

Any product amplified from the unknown sample is sequenced and compared to known sequences available in Genbank (Benson et al, Nucleic Acids Research, 2008;36:D25-3), usually using the BLAST tool (Altschul et al, J. Mol. Biol. 1990;215:403-410).

Suitable samples:

- This test is only useful for samples from normally sterile sites (e.g. joint aspirates, CSF). Samples from areas that have a normal flora (e.g. the oropharynx or genitourinary tract) will yield mixed sequences that cannot be distinguished from one another. Samples must be handled in an aseptic manner prior to dispatch to our laboratory to minimise the risk of contamination by other bacteria or their DNA.
- We can attempt to extract DNA from any body fluids, tissues or implants (such as artificial heart valves).
- We will accept formalin fixed, wax-embedded tissues, but fresh tissue will provide the best chance of detecting DNA.
- The assay detects DNA from living and dead bacteria.
- Slow-growing bacteria – a dry swab can be used to sample tiny colonies in subculture. This will provide more than enough DNA for our purposes.

- Empyema samples – we have found that it is normally possible to detect *Streptococus pneumoniae* DNA in these samples. We will therefore first test for this organism specifically, and if negative, go on to test for the 16S rRNA gene. This does not normally incur any extra cost and may even result in a saving.

Sensitivity and specificity:

- The test will detect the equivalent of about 1,000 colony-forming units per mL. This intentionally low sensitivity increases the confidence that any sequences amplified are derived from the sample rather than accidental contaminants.
- In theory, the test can identify any type of eubacteria, however our *in silico* investigations have shown that the primers we use are less likely to detect some Chlamydiae and Spirochaetes.
- The degree of certainty of species identification relies on the quality and quantity of the sequence data available in Genbank. This has been a problem in the past but sequences available in Genbank increase at an exponential rate and there a number of other databases available that collate good quality sequence data specifically for the purposes of bacterial 16S rRNA gene identification.
- Sometimes the bacterial 16S rRNA gene fragment we amplify is not sufficiently discriminative to allow us to identify to species level. In these instances, we will name the identified genus and provide the names of the species to which the sequence is most similar.
- The test does not provide antibiotic sensitivities.
- **We have a range of species-specific tests available (see our website for more information). These are more sensitive and rapid than the 16S rRNA assay. Please provide sufficient clinical information to allow us to select appropriate tests and/or to aid in interpretation of the results.**

The following are examples of some bacteria identified in our laboratory using this test and the site from which they were isolated; more examples are listed in the appendix:

Bartonella quintana	from an aortic valve.
Brucella genus	pus from a man with a 10-year history of knee abscess, culture negative.
Campylobacter sp.	from a liver aspirate.
Capnocytophaga sp.	vitreous aspirate from a woman bitten by a dog.
Cardiobacterium hominis	from an aortic valve.
Citrobacter koseri	CSF from a 1 month old baby, culture negative, treated with antibiotics for 48h prior to sample.
Coxiella burnetii	aortic valve vegetation, culture negative.

Enterobacter sp.	from the lung tissue of a child with haemoptysis.
Fusobacterium sp.	pus from a liver abscess.
Granulicatella adiacens	slow-growing organism from the blood culture of patient with AML.
Kingella kingae	pus from a hip joint, culture negative.
Kluyvera intermedia	CSF from a 2 month old baby causing sudden infant death syndrome.
Legionella sp.	sputum, urine antigen positive.
Leuconostoc spp.	extradural pus sample from a sudden death victim.
Prevotella sp.	pus from a brain abscess, culture negative.
Streptobacillus monilliformis	isolate from blood culture of rat bite victim.

Please contact Dr John Thomas or Dr Jennifer Morris-Cottell to discuss any aspect of the bacterial 16S RNA service.

Appendix:

Abiotropha defectiva
Achromobacter sp
Acidovorax sp.
Acinetobacter sp.
Actinomyces sp.
Aerococcus sp.
Aggregatibacter sp.
Amycolatopsis species.
Arcanobacterium haemolyticum
Bacillus sp.
Bacteroides fragilis
Bartonella henselae
Bartonella quintana
Bilophila wadsworthia
Bordetella sp.
Brucella sp.
Burkholderia cepacia complex
Campylobacter spp.
Capnocytophaga canimorsus
Carnobacterium divergens
Citrobacter spp.
Clostridium sp.
Corynebacterium sp.
Coxiella burnetii
Dermabacter hominis
Dermacoccus sp.
Desulfovibrio fairfieldensis
Dialister micraerophilus

Dialister pneumosintes
Dietzia cinnamea
Dolosigranulum pigrum
Escherichia coli
Eggerthella lenta
Eikenella corrodens
Elizabethkingia spp
Enhydrobacter sp.
Enterobacter sp.
Enterococcus sp.
Facklamia sp.
Finegoldia magna
Fusobacteria necrophorum
Fusobacterium nucleatum
Fusobacterium sp.
Gardnerella vaginalis
Gemella morbillorum
Gordonia polyisoprenivorans
Gordonia sp
Gordonia sputi
Granulicatella adiacens
Granulicatella spp.
Haematobacter sp.
Haemophilus influenzae
Haemophilus parainfluenzae
Haemophilus sp.
Herbaspirillum sp.
Janibacter sp.
Klebsiella pneumoniae
Moraxella catarrhalis
Moraxella lacunata
Moraxella nonliquefaciens
Negativicoccus succinicivorans
Neisseria elongata
Neisseria gonorrhoeae
Neisseria meningitidis
Neisseria weaveri
Nocardia farcinica
Nocardia spp.
Ochrobactrum spp.
Oscillibacter sp.
Paenibacillus sp.
Pandoraea sp
Paracoccus sp.
Parvimonas micra

Pasteurella multocida
Peptoniphilus spp.
Ponticoccus gilvus
Porphyromonas endodontalis
Porphyromonas gingivalis
Prevotella sp.
Propionibacterium sp.
Pseudomonas spp.
Rothia sp.
Ruminococcus gnavus
Salmonella enterica
Salmonella sp.
Serratia sp.
Sphingomonas sp
Staphylococcal aureus
Staphylococcus epidermidis
Staphylococcus lugdunensis
Staphylococcus saccharolyticus
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus bovis group
Streptococcus bovis/gallolytics
Streptococcus dysgalactiae
Streptococcus mitis
Streptococcus mutans
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus salivarius
Streptococcus sanguinis
Trichococcus sp.
Tsukamurella sp.
Ureaplasma sp.
Vagococcus sp.
Veillonella montpellierensis
Veillonella sp.
Wautersiella falsenii