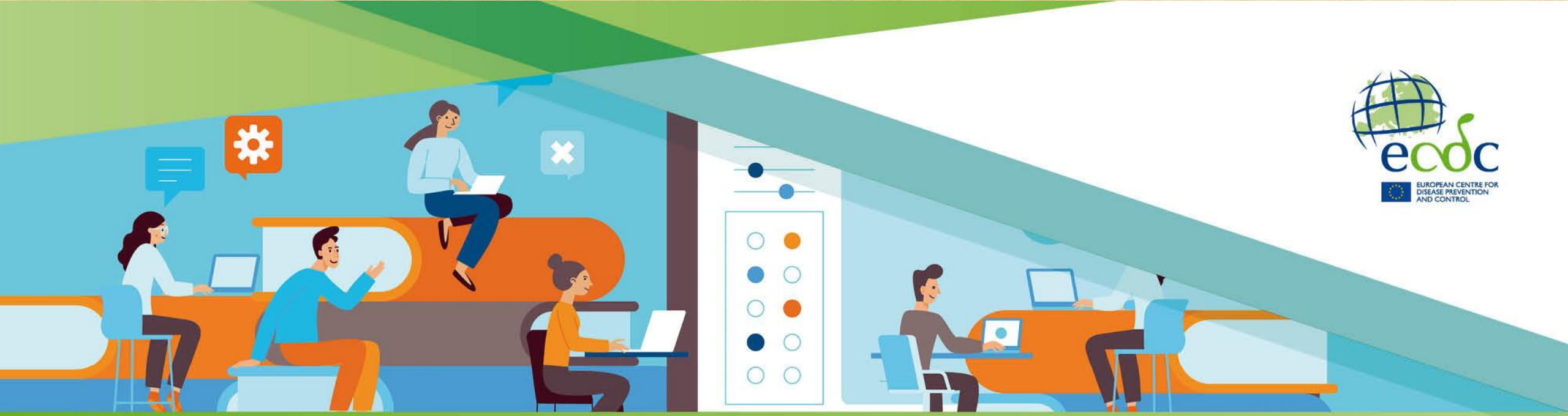

Clinical application of Nanopore-based 16S metagenomic sequencing for diagnostics
GenEpi-BioTrain Virtual Training 27

Clinical application and interpretation

2026-06-05

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Conflict of interest

None to declare

Terminology and abbreviations

16S rRNA gene sequencing = **16S rDNA sequencing** =

16S metagenomics (=amplicon metagenomics) vs shotgun metagenomics

seq: short for sequencing

ONT: Oxford Nanopore Technologies (long-read sequencing)

What's your previous experience with 16S rDNA seq?

Aim and learning objectives

Aim: Acquire a good understanding of the clinical application, interpretation and reporting of 16S rDNA sequencing results

Specific learning objectives: At the end of this session, you will be able to

1. Understand some strengths and weaknesses of 16S rDNA seq
2. Evaluate whether a sample is suitable for 16S rDNA seq or not
3. Know what considerations are important in the clinical reporting of 16S seq
4. Remember that a multiprofessional involvement is encouraged

Outline

This session consists of the following elements

- Culture or 16S rDNA sequencing?
- Sample selection
- ONT report and visualization, what do I consider?
- Clinical cases
- Future perspectives

Culture vs 16S rDNA sequencing

- Culturing enables antimicrobial susceptibility testing, is cheaper, less resource demanding and (used to be) faster

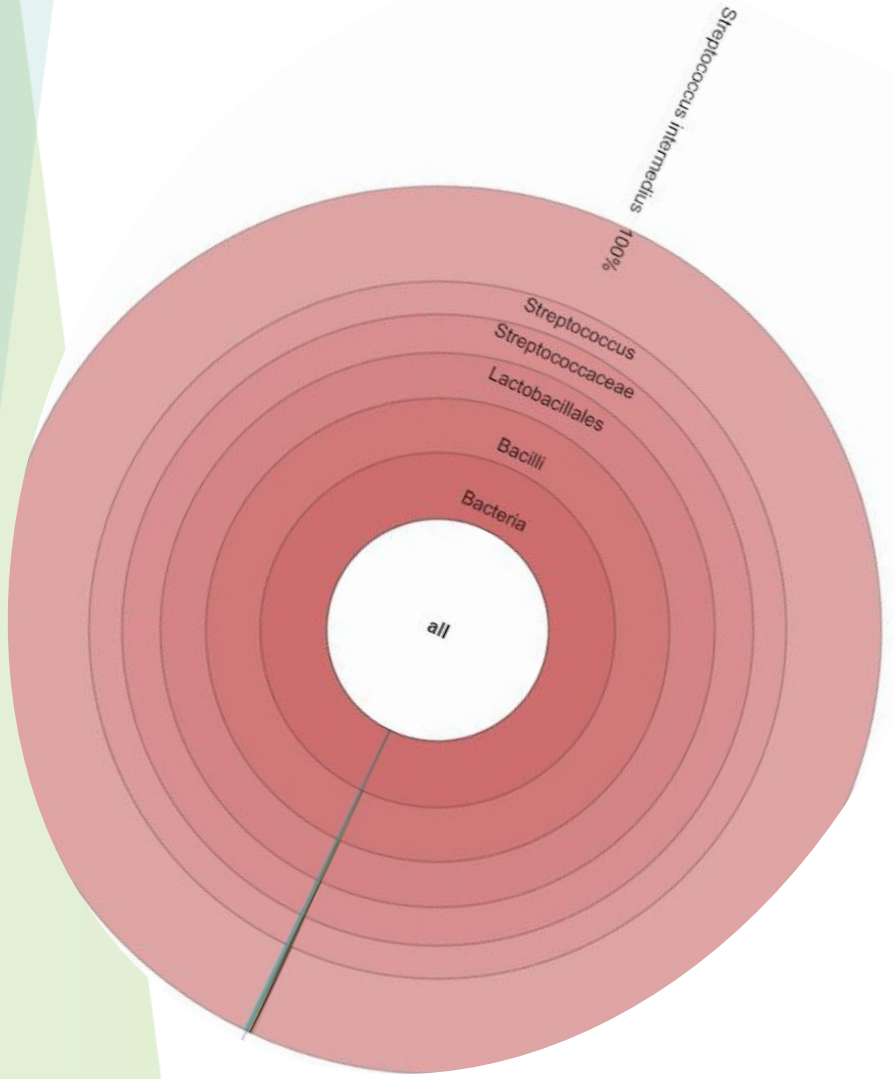
- Normally sterile locations
- Fastidious, anaerobic and non-culturable bacteria (culture-negative suspected infections)
- When antibiotic treatment has been initiated before sampling/treatment failure

ONT 16S rDNA seq: Challenging culture-based methods on being fast

Clinical case 1, pleural effusion

Microbiological diagnostic workup

Pleuravätska	-
Pleuravätska	Ingen växt
Pleuravätska	Ingen växt
Urin	E.coli R*
Nasofarynxsekret	S.aureus
Blod	Ingen växt
Blod	Ingen växt
Nasofarynxsekret	CoV-2 GX: NEG CoV-2 N2/E Ct, 0.0 ej deb IA RNA GX: NEG (Infl A Ct1 GX, 0.0 ej deb) (Infl A Ct2 GX, 0.0 ej deb) IB-RNA GX: NEG (Infl B Ct GX, 0.0 ej deb) RS-RNA GX: NEG (RS Ct GX, 0.0 ej deb)



Clinical case 2, pleural effusion

Background: B-cell lymphoma

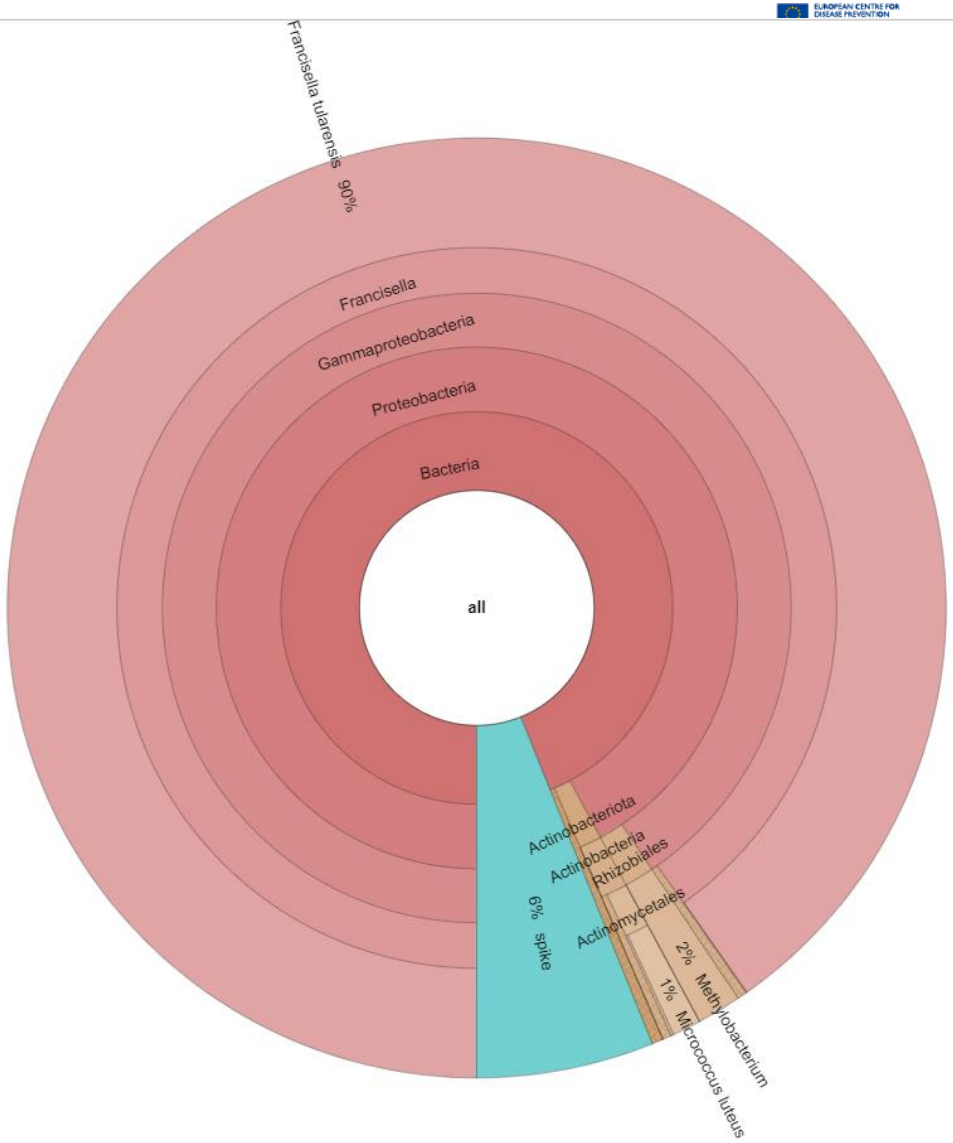
History of present illness: Fever 40 grader, chills and feeling unwell for 2-3 days.

Chest X-ray: Consolidation consistent with pneumonia

→ Benzylpenicillin, but clinical response not satisfactory → Cefotaxime + CT-scan, pleural fluid

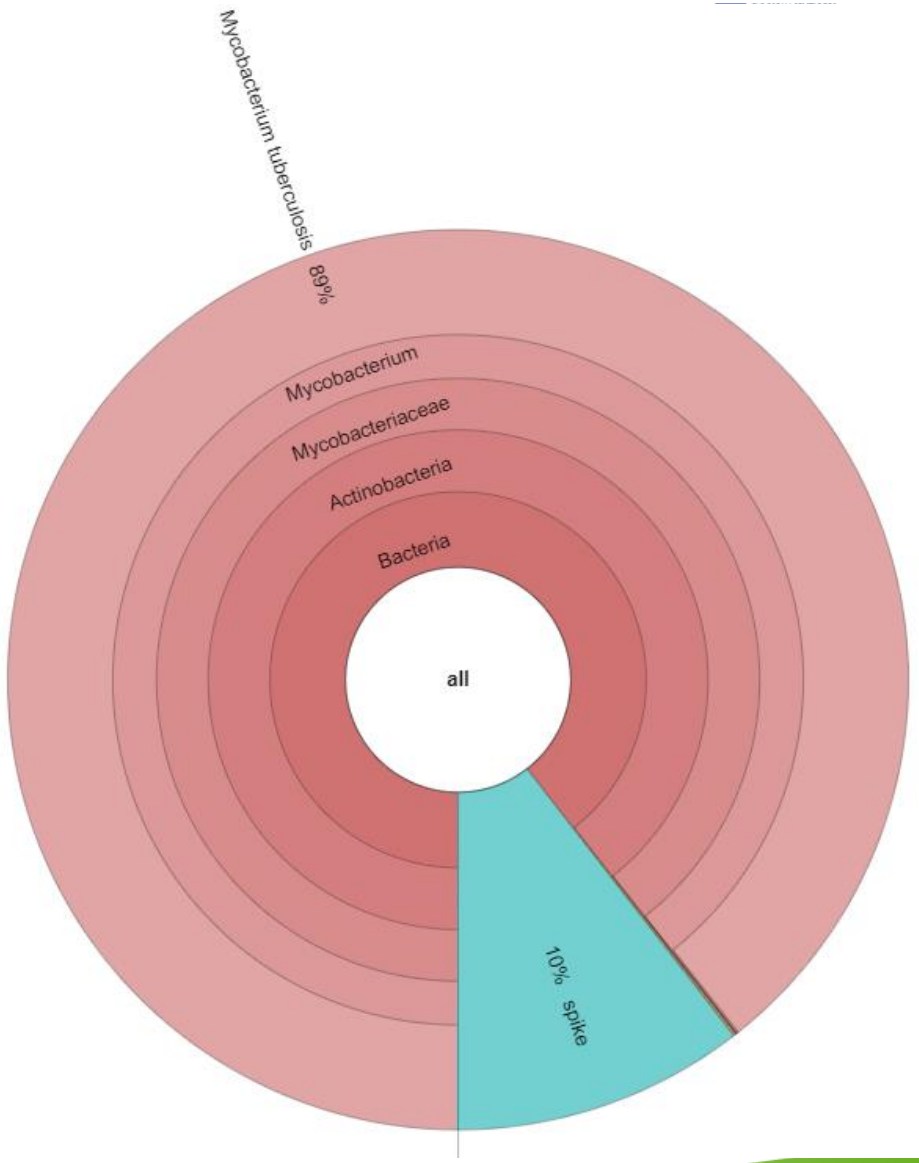
→ Blood culture negative

➢ Pleural tap for 16S rDNA seq



Clinical case 3, hip infection

Provmaterial	Resultat	Provse
Ledvätska	Ingen växt	DC
Urin	Ingen växt	AC
Blod	Ingen växt	AP
Sårsekret	Hudflora	DD
Urin	Uretrafl	AC
Sårsekret	S.epiderm	DD
Blod	Ingen växt	AP
Blod	Ingen växt	AP
Nasofarynxsekret	CoV-2 GX: NEG CoV-2 N2/E Ct, 0.0 ej deb IA RNA GX: NEG (Infl A Ct1 GX, 0.0 ej deb) (Infl A Ct2 GX, 0.0 ej deb) IB-RNA GX: NEG (Infl B Ct GX, 0.0 ej deb) RS-RNA GX: NEG (RS Ct GX, 0.0 ej deb)	CA
Punktat	Ingen växt	DB
Punktat	Ingen växt	DA
Punktat	Ingen växt	DA
Blod	Ingen växt	AP
Blod	Ingen växt	AP
Ledvätska	(Tid ae fl, 101.7 ej deb) Ingen växt	DC
Urin	Ingen växt	AC
Blod	Ingen växt	AP
Blod	Ingen växt	AP



Clinical case 4, abdominal drainage

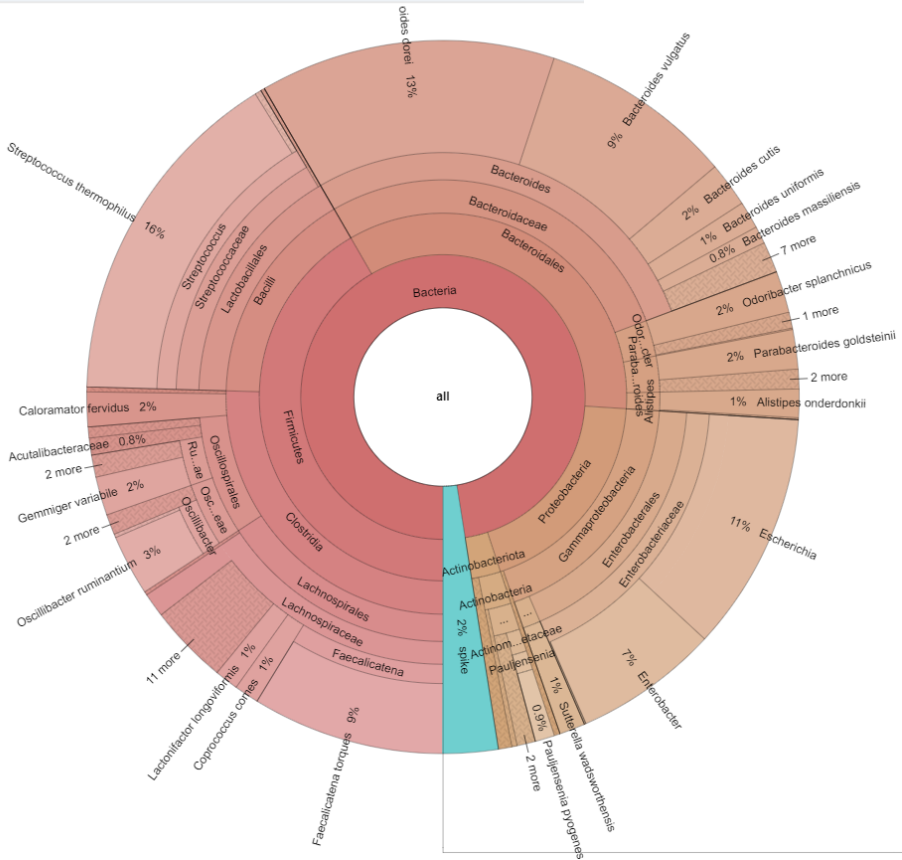
Urin, KAD	Ingen växt	AC
Blod	Ingen växt	AP
Blod	Ingen växt	AP
Blod	Ingen växt	AP
Blod	Ingen växt	AP
Kärlkateterspets	0 bakt<10	DA
Feces	C diff tox B: NEG (C diff bin tox: NEG ej deb) (C diff tcdC del: NEG ej deb) (Tox B Ct, 0.0 ej deb) (Bin tox Ct, 0.0 ej deb) (tcdC del Ct, 0.0 ej deb)	CA
Punktat	0 svamp	DK
Punktat	0 svamp	DK
Punktat	E.coli R* E.cloacae R*	DB
Punktat	Ingen växt	DB
Kärlkateterspets	0 bakt<10	DA
BAL	Ingen växt	DQ
Bronksekret, borste	0<10 borst	DQ
Blod	Calb/trop T2: NEG Cparapsi T2: NEG Ckrus/glab T2: NEG	CC
Serum	Betaglukan: NEG, 39	BS
Blod	Ingen växt	AP
Urin, KAD	Ingen växt	AC
Blod	Ingen växt	AP
Blod	Ingen växt	AP
Urin	Ingen växt	AC

Resultat | SIR | Svar

Odlingsfynd

1. Escherichia coli <BT>Res grå
PTZ: 23:S IMI: 29:S MER: 31:S MES: 31:S ERT: 30:S AMI: 20:S TSI
Preliminärt X->Maldi
2023-08-18 Res.best | Malditof 2.350
2023-08-21 Hylla

2. Enterobacter cloacae <BT>Res vit
PTZ: 21:S IMI: 25:S MER: 30:S MES: 30:S ERT: 30:S AMI: 19:S TSI
I minst 20% av fallen ses resistensutveckling vid behandling med ce
Preliminärt X->Maldi
2023-08-18 Res.best | Malditof 2.380



Which of the following samples would you accept for 16S metagenomics?

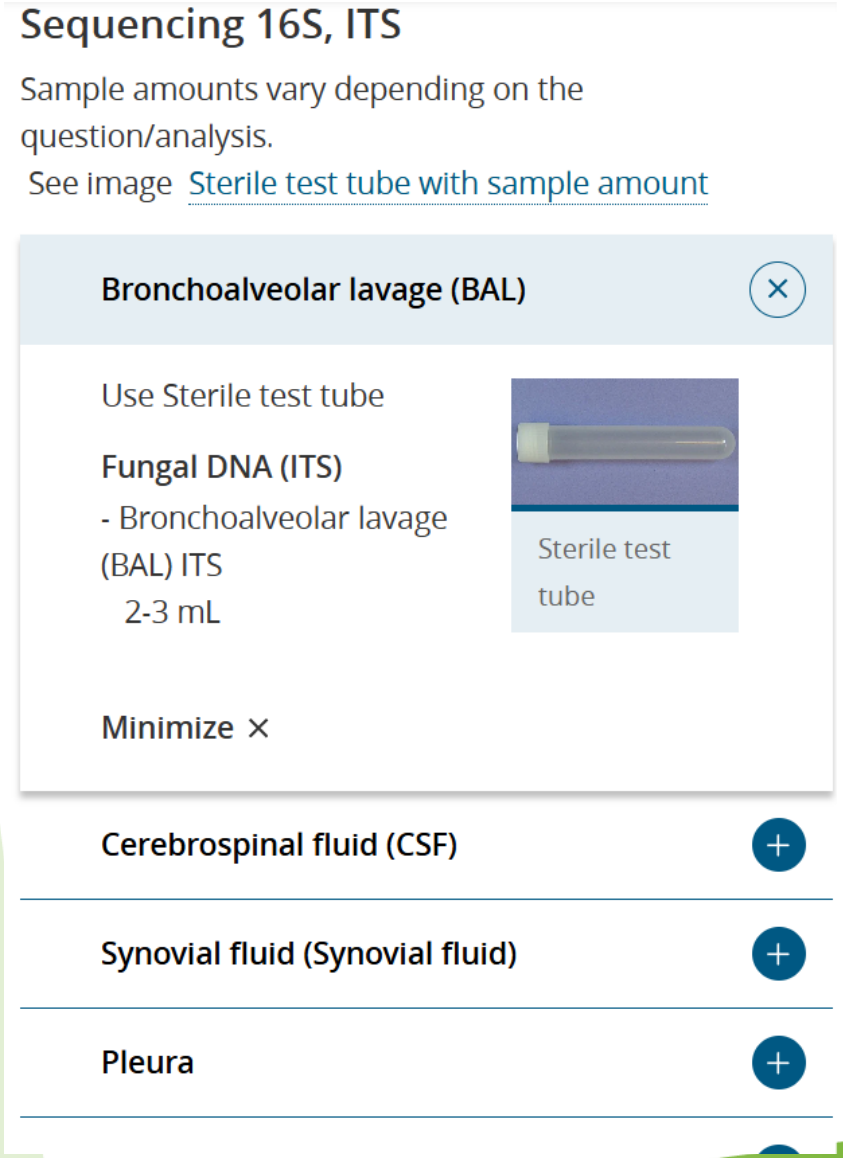
In summary

- Culture-based methods are still often the primary diagnostic method for bacterial infections
- 16S is rarely required, hard to pin-point when it is, but potentially essential for the outcome in a minority of cases

So how can clinical laboratories help guide clinicians to identify right situations/samples for 16S rDNA sequencing?

Communicate indications and limitations

- Sampling instructions on web
- Guidance in requisition form
- Directed diagnostic stewardship: ID-, orthopaedic-, surgical- and ENT-units
- Follow-up systematic errors of referral



Sample accessioning (potential rejection) and processing

Yes: Sterile sample material (cerebrospinal fluid, synovial fluid, pleural/pericardial effusion, deep tissue samples)

Selection: Orthopaedic tissue samplings: 5 to culture, 1-5 to 16S rDNA seq: we analyse 2, rest if requested

No: Material from drain, non-sterile (chronic wound, pressure wound, amputation wound…), airways

Grey zone:

- Abscesses: Puncture yes, drain no. Superficial usually no, deep usually yes
- Deep tissue samples from "small, non-sterile body parts" i.e. feet, hands
- Ear samplings (from middle ear, not ear canal)

Ear samples kept rising, how to handle?

- Surprisingly, rarely a rich microbiota although we receive swabs taken through ear canal. Compiled data 2024-2025:
  - 30% of samples: only normal microbiota
  - 45% of samples: finding that was already found in culture
  - **25% of samples: primary pathogen, not found in culture**

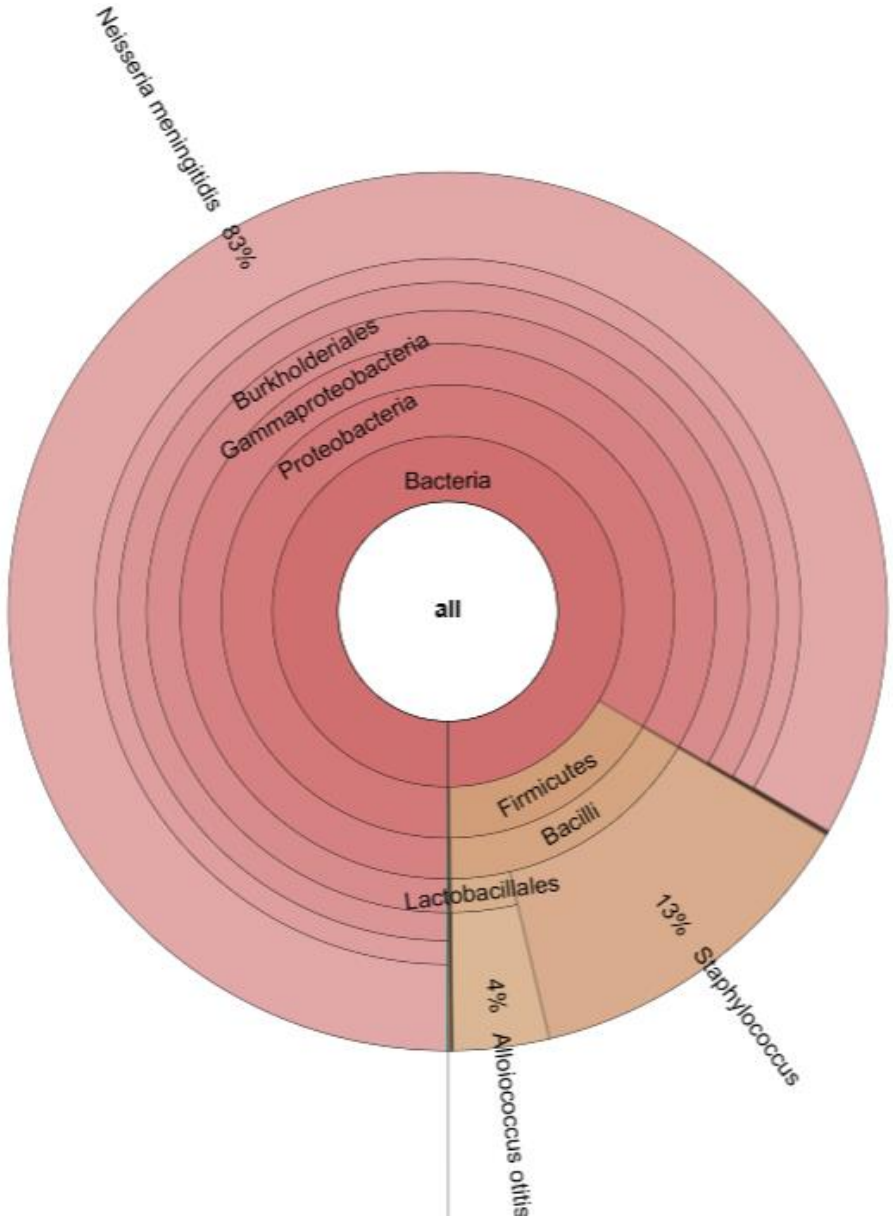
- Strategy: accept ear samples provided that
  - Sampling from ENT ward
  - Paracentesis (ear drum has been punctured for sampling from middle ear)
  - Do not report other species if a primary pathogen is present
  - Comment findings normally present in ear canal ”Belongs to the normal flora, clinically relevant?”

Clinical case 4, labyrinthitis

- Inner ear infection. Rare complication to acute media otitis
- Bacterial aetiology: Pneumococci, *H. influenzae,* Group A Streptococci*, M. catarrhalis,* meningococci
- Child, 3 days of pain in ear, develops dizziness despite PcV. ER → cefotaxime, sampling:

1. Adenovirus-DNA, Qiastat		Negativ
2. Adenovirus-DNA, Qiastat, Ct	0	
3. Bordetella pertussis-DNA, Qiastat		Negativ
4. Bordetella pertussis-DNA, Qiastat, Ct	0	
5. Bocavirus-DNA, Qiastat		Negativ
6. Bocavirus-DNA, Qiastat, Ct	0	
7. Chlamydophila pneumoniae-DNA, Qiastat		**POSITIV**
8. Chlamydophila pneumoniae-DNA, Qiastat, Ct	29.94	
9. Coronavirus 229E-RNA, Qiastat		Negativ
10. Coronavirus 229E-RNA, Qiastat, Ct	0	
11. Coronavirus HKU1-RNA, Qiastat		Negativ

Öronsekret	Ingen växt
Nasofarynxsekret	Pneumokock R*



The immunocompromised patient

- Increased susceptibility of infections in general
- More prone to acquire rare infections with fastidious or unculturable bacteria
- Might not produce antibodies – compromises serological diagnostics

Clinical case 5

Young, healthy person with peripheral facial palsy

Provmaterial	Resultat	Provserie
Serum	TBE G Virotech: NEG, 1.2 TBE M Virotech: NEG, 2.0	BA
Cerebrospinalvätska	Borr G csv: POS, 198.4 Borr M csv: POS, 79.1 Index G Index M	BA
Serum	Borr G: POS, 139 Borr M: NEG, 10	BA
Cerebrospinalvätska	Neg Bakt	CC
Cerebrospinalvätska	Cryptoc Q: NEG (Cryptoc Q Ct, 0 ej deb) Entero Q: NEG (Entero Q Ct, 0 ej deb) E.coli K1 Q: NEG (E.coli K1 Q Ct, 0 ej deb) H.infl Q: NEG (H.infl Q Ct, 0 ej deb) HSV1 Q: NEG (HSV1 Q Ct, 0 ej deb) HSV2 Q: NEG (HSV2 Q Ct, 0 ej deb) HHV6 Q: NEG (HHV6 Q Ct, 0 ej deb) L.mono Q: NEG (L.mono Q Ct, 0 ej deb) M.pn Q: NEG (M.pn Q Ct, 0 ej deb) N.menin Q: NEG (N.menin Q Ct, 0 ej deb) Parecho Q: NEG (Parecho Q Ct, 0 ej deb) S.agalact Q: NEG (S.agalact Q Ct, 0 ej deb) S.pneumo Q: NEG (S.pneumo Q Ct, 0 ej deb) S.pyogen Q: NEG (S.pyogen Q Ct, 0 ej deb) VZV Q: NEG (VZV Q Ct, 0 ej deb)	CA
Cerebrospinalvätska	Ingen växt	AR

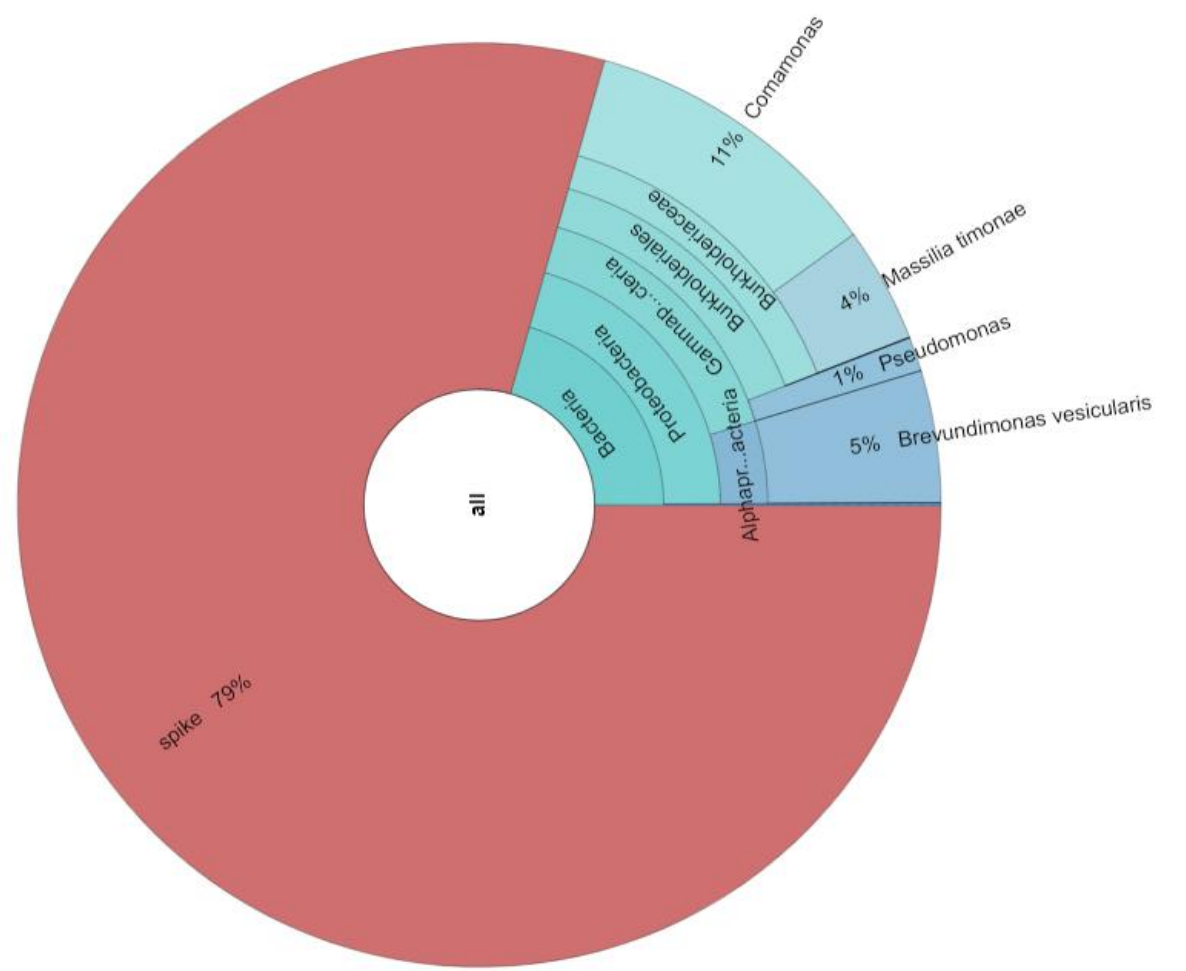
Clinical case 6

Elderly patient with leukaemia, headache (immunocompromised)

Serum	Borr G: NEG, <5 Borr M: NEG, <2
Serum	Syf TPPA: NEG, <80
Cerebrospinalvätska	Borr G csv: NEG, <0.2 Borr M csv: NEG, <0.0 S TPPA csv: NEG, <8
Cerebrospinalvätska	Ingen växt
Blod	Tox DNA: NEG, 0.00
Cerebrospinalvätska	Ent prb: NEG, 0.00 E.coli-DNA: NEG H.infl-DNA: NEG HSV1-DNA AKUT: NEG, 0.00 HSV2-DNA AKUT: NEG, 0.00 Listeria-DNA: NEG Meningo-DNA: NEG S.agalact-DNA: NEG S.pneum-DNA: NEG VZ DNA: NEG, 0.00
Blod	Ingen växt
Blod	Ingen växt
Nasofarynxsekret	CoV-2 Neu: NEG IA-RNA Neu: NEG IB-RNA Neu: NEG RS-RNA Neu: NEG
Serum	EBV DNA kv: NEG, 0.00 Parvo kvant: NEG, 0.00
Plasma	CMVDNA kv: NEG, 0.00
Serum	TBE G Virotech: NEG, 8.4 TBE M Virotech: NEG, 0.0

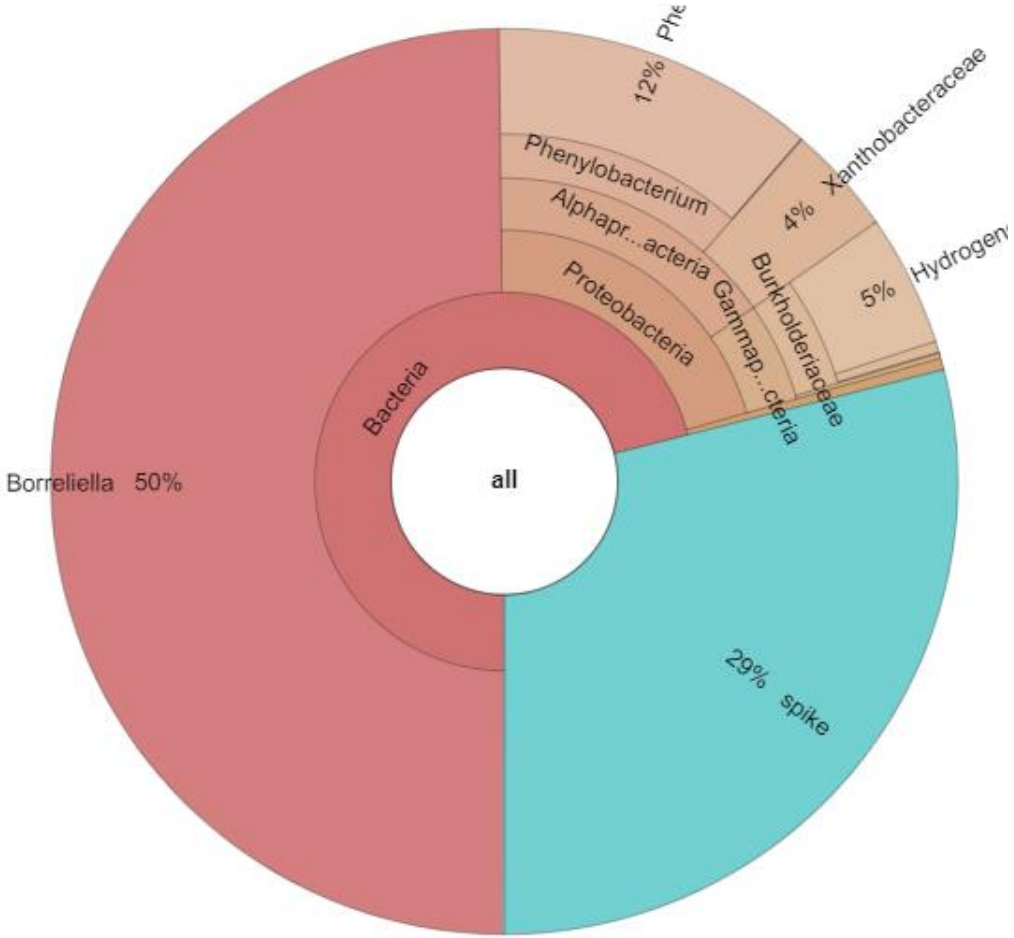
Clinical case 5

Young, healthy person with peripheral facial palsy



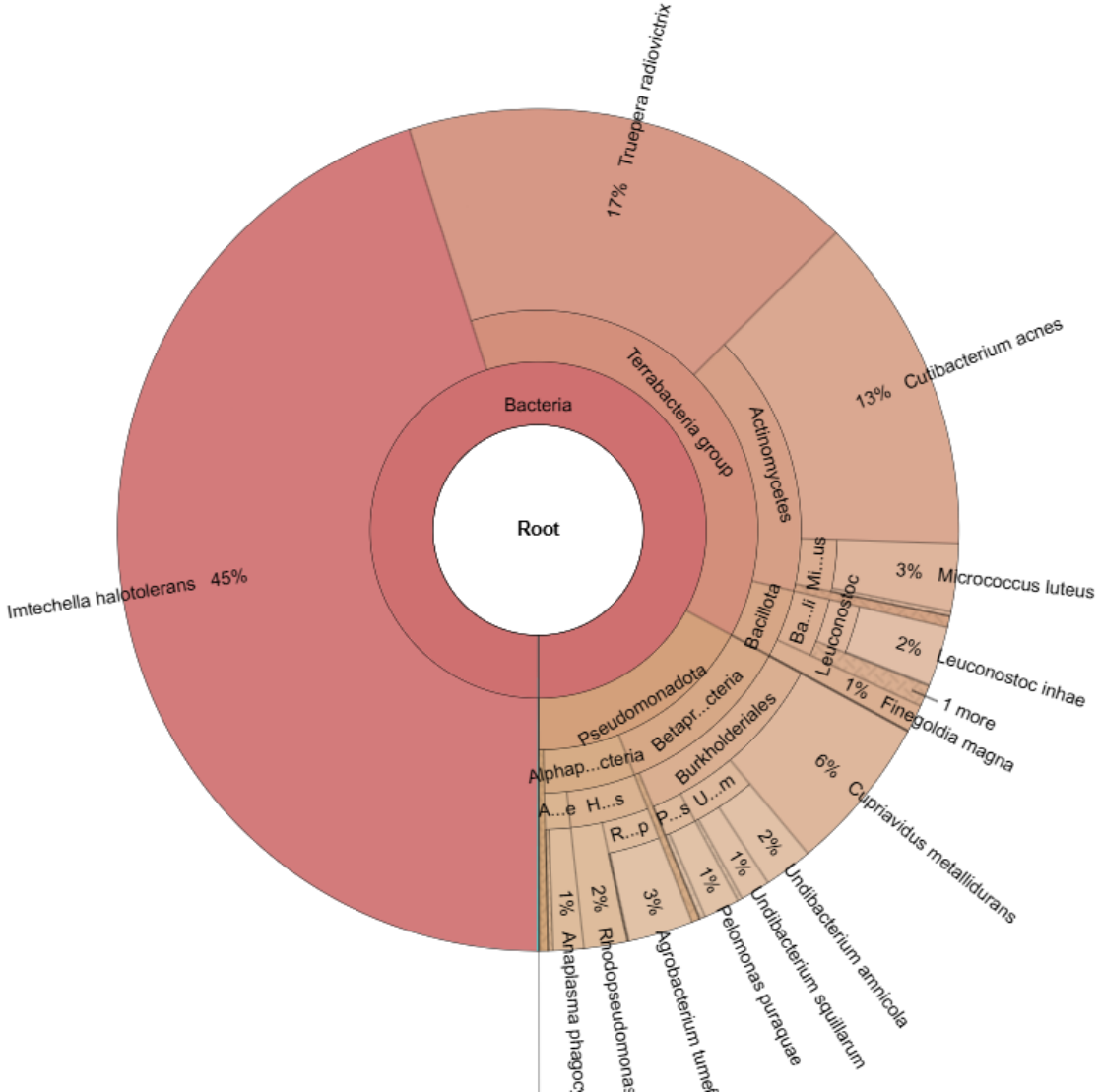
Clinical case 6

Elderly patient with leukaemia, headache (immunocompromised)



ONT 16S output and visualization

Negative control



16S TRANA Report

Sample Name: 16SNEG260504

Run: 16s_ont_260505_anma-20260506-075011

Date: 2026-05-06

Pipeline version: TRANA v0.6.0

Summary Statistics

Number of reads before downsampling	85788
Mean Read Length	1545.4 bp
Mean Read Quality	17.2
Median Read Length	1551.0 bp
Median Read Quality	17.5
Read Length N50	1551.0 bp
STDEV Read Length	43.6 bp
Total Bases	132.6 Mbp
Number of mapped reads	30000

Phred Quality Score (Q)

Q10: 10% error rate
Q20: 1% error rate
Q30: 0.1% error rate
Q40: 0.01% error rate

< Q15 Not acceptable	> Q15 Good	> Q20 Excellent

Abundance

Species with low abundance (<0.5%) are shown in light grey.

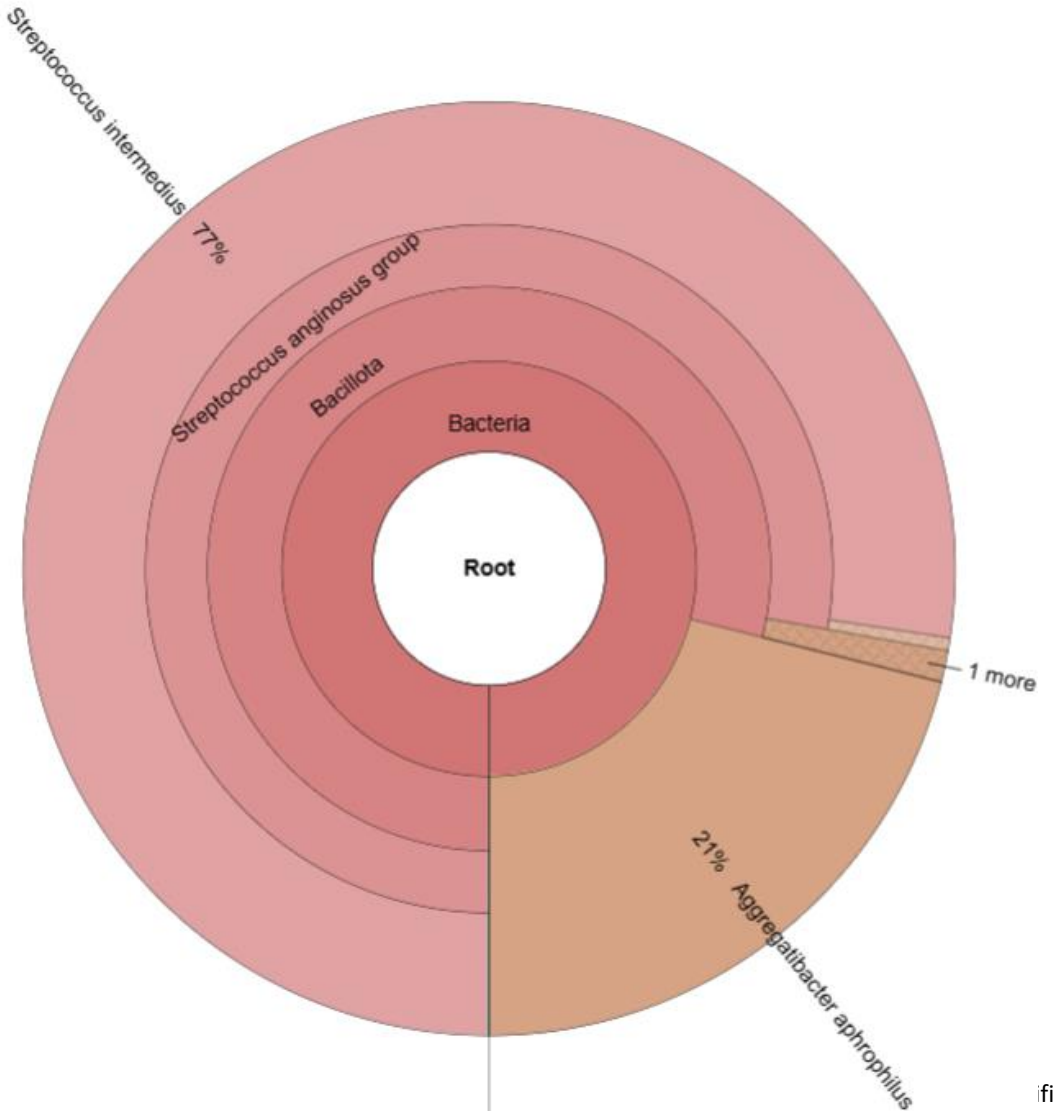
Negative control

row	abundance	species	genus	family	tax id	estimated read counts	median aligned identity	median aligned coverage
1	45.05%	Imtechella halotolerans	Imtechella	Flavobacteriaceae	1165090	13512	99.23%	100.00%
2	17.49%	Truepera radiovictrix	Truepera	Trueperaceae	332249	5244	99.38%	96.04%
3	12.98%	Cutibacterium acnes	Cutibacterium	Propionibacteriaceae	1747	3893	99.39%	95.77%
4	6.02%	Cupriavidus metallidurans	Cupriavidus	Burkholderiaceae	119219	1804	99.39%	96.10%
5	2.60%	Micrococcus luteus	Micrococcus	Micrococcaceae	1270	780	99.25%	96.01%
6	2.50%	Agrobacterium tumefaciens	Agrobacterium	Rhizobiaceae	358	750	99.43%	96.04%
7	2.29%	Leuconostoc inhae	Leuconostoc	Leuconostocaceae	178001	687	99.52%	99.93%
8	1.87%	Undibacterium amnicola	Undibacterium	Oxalobacteraceae	1834038	562	98.08%	97.21%
		Rhodopseudomonas						

Purple rows indicate spike species

ONT 16S output and visualization

Positive clinical sample: Cerebrospinal fluid



Summary Statistics

Number of reads before downsampling	280103
Mean Read Length	1569.5 bp
Mean Read Quality	17.5
Median Read Length	1587.0 bp
Median Read Quality	17.9
Read Length N50	1587.0 bp
STDEV Read Length	68.6 bp
Total Bases	439.6 Mbp
Number of mapped reads	30000

Phred Quality Score (Q)

Q10: 10% error rate
Q20: 1% error rate
Q30: 0.1% error rate
Q40: 0.01% error rate

< Q15 Not acceptable	> Q15 Good	> Q20 Excellent

Abundance

Species with low abundance (<0.5%) are shown in light grey.

Sample

row	abundance	species	genus	family	tax id	estimated read counts	median aligned identity	median aligned coverage
1	77.36%	Streptococcus intermedius	Streptococcus	Streptococcaceae	1338	23209	99.53%	96.22%
2	21.10%	Aggregatibacter aphrophilus	Aggregatibacter	Pasteurellaceae	732	6330	99.37%	96.11%
3	1.09%	Parvimonas micra	Parvimonas	Peptoniphilaceae	33033	326	96.91%	96.01%
4	0.26%	Streptococcus constellatus	Streptococcus	Streptococcaceae	76860	79	92.75%	91.21%
5	0.19%	Streptococcus anginosus	Streptococcus	Streptococcaceae	1328	56	92.82%	91.21%
6	0.00%	nan	nan	nan	unmapped	0	nan%	nan%
7	0.00%	nan	nan	nan	mapped_unclassified	0	nan%	nan%

Purple rows indicate spike species
Green rows indicate species not found in negative control or with a normalised abundance ratio ≥ 25.

Negative control

row	abundance	species	genus	family	tax id	estimated read counts	median aligned identity	median aligned coverage
1	45.05%	Imtechella halotolerans	Imtechella	Flavobacteriaceae	1165090	13512	99.23%	100.00%
2	17.49%	Truepera radiovictrix	Truepera	Trueperaceae	332249	5244	99.38%	96.04%
3	12.98%	Cutibacterium acnes	Cutibacterium	Propionibacteriaceae	1747	3893	99.39%	95.77%
4	6.02%	Cupriavidus metallidurans	Cupriavidus	Burkholderiaceae	119219	1804	99.39%	96.10%
5	2.60%	Micrococcus luteus	Micrococcus	Micrococcaceae	1270	780	99.25%	96.01%
6	2.50%	Agrobacterium tumefaciens	Agrobacterium	Rhizobiaceae	358	750	99.43%	96.04%
7	2.29%	Leuconostoc inhae	Leuconostoc	Leuconostocaceae	178001	687	99.52%	99.93%

ONT 16S output and visualization

Negative clinical sample: Cerebrospinal fluid

Summary Statistics

Number of reads before downsampling	45075
Mean Read Length	1539.4 bp
Mean Read Quality	17.7
Median Read Length	1551.0 bp
Median Read Quality	18.0
Read Length N50	1552.0 bp
STDEV Read Length	63.1 bp
Total Bases	69.4 Mbp
Number of mapped reads	30000

Phred Quality Score (Q)

Q10: 10% error rate
Q20: 1% error rate
Q30: 0.1% error rate
Q40: 0.01% error rate

< Q15 Not acceptable	> Q15 Good	> Q20 Excellent

Abundance

Species with low abundance (<0.5%) are shown in light grey.

Sample

row	abundance	species	genus	family	tax id	estimated read counts	median aligned identity	median aligned coverage
1	34.40%	Imtechella halotolerans	Imtechella	Flavobacteriaceae	1165090	9518	99.31%	100.00%
2	13.81%	Truepera radiovictrix	Truepera	Trueperaceae	332249	3822	99.45%	96.17%
3	11.50%	Cutibacterium acnes	Cutibacterium	Propionibacteriaceae	1747	3182	99.46%	95.77%
4	7.84%	Cupriavidus metallidurans	Cupriavidus	Burkholderiaceae	119219	2169	99.52%	96.79%
5	7.75%	Agrobacterium tumefaciens	Agrobacterium	Rhizobiaceae	358	2143	99.51%	96.11%
6	7.22%	Allobacillus halotolerans	Allobacillus	Bacillaceae	570278	1999	98.95%	100.00%
7	3.84%	Aquabacterium parvum	Aquabacterium	nan	70584	1062	99.15%	100.00%
8	2.30%	[Propionibacterium]	Cutibacterium	Propionibacteriaceae	1574624	636	99.37%	99.65%

Purple rows indicate spike species
Green rows indicate species not found in negative control or with a normalised abundance ratio ≥ 25.

Negative control

row	abundance	species	genus	family	tax id	estimated read counts	median aligned identity	median aligned coverage
1	24.75%	Imtechella halotolerans	Imtechella	Flavobacteriaceae	1165090	7426	99.31%	100.00%
2	23.50%	Agrobacterium tumefaciens	Agrobacterium	Rhizobiaceae	358	7049	99.51%	96.04%
3	9.96%	Truepera radiovictrix	Truepera	Trueperaceae	332249	2988	99.45%	96.30%
4	8.73%	Cutibacterium acnes	Cutibacterium	Propionibacteriaceae	1747	2619	99.46%	95.77%
5	5.86%	Cupriavidus metallidurans	Cupriavidus	Burkholderiaceae	119219	1757	99.52%	96.59%
6	3.99%	Staphylococcus equorum	Staphylococcus	Staphylococcaceae	246432	1196	99.67%	96.21%
7	2.83%	Paucibacter sp. KCTC 42545	Paucibacter	nan	1768242	850	97.97%	96.02%

Purple rows indicate spike species

as ECDC

Summary, considerations when reporting

- What is in the negative control?
- What are we used to see in "our" background?
- What is recurring in the background, in this run?

- Identity
- Coverage
- Abundance
- "No hits" in database

- What is clinically relevant?
- Do I need to take any urgent actions? (Risk Group 3 pathogens, severely ill patient on inefficient antibiotics)
- Do I need to take any important, less urgent actions? (specific methods (TB), notifiable communicable diseases, verify, send to reference laboratory, typing etc)

Present vs future perspectives

- Present workflow (TAT 3-8 days)
  - Steadily increasing sample load
  - 16S often requested simultaneously as culture

- ONT workflow (TAT <1 day)
  - 16S will become even more attractive and impactful
  - Easier to refrain from 16S before culture?

- Collaborate! Multiprofessional teams highly encouraged!

Break till 11.30

Don't leave this meeting
Just turn off the camera

Reusing this content

Acknowledgements

The creation of this training material was commissioned by ECDC to Karolinska University Hospital with the direct involvement of Anna Norén, Christian Giske, Elin Loo, Lili Andersson-Li, Lina Guerra Blomqvist, Martin Vondracek, Samuel Lampa, Sofia Stamouli