Results of the 2015 16S rRNA gene sequencing and/or MALDI-TOF National Proficiency Test

****Sponsored by the NMG and the NML****

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Preparation for the Test

- NMG survey re: 'interest in PT': sent April 2015
- Response 'limited' (sent during AMMI-CACMID?)
- Previous contacts, survey, & 'new' respondents contacted in August 2015 in order to
  - Provide provisional dates for shipment
  - obtain HPTA-required 'Compliance letters'
  - Organize 'Transfer Requests for Pathogens/Toxins'
  - Get a courier no. to pay for shipment of panel

The Panel; Instrumentation at NML

- Panel of 4 bacteria sent Oct. 6, 2015
- 3 fac. anaerobes/aerobes, 1 anaerobe sent on stabs
- Original due date: Monday Oct. 19, 2015
- Amended due date: Friday Oct. 23, 2015
- 35/39 (**90%**) sites reported results by due date; all sites by Oct. 29 2015
- **Instrumentation (at NML)**: Bruker **Microflex**
  - Making NMD entries with this
- 17/18 Bruker Microflex users (1 Autoflex)
- Access only to listings of Vitek IVD 2.0 & RUO 4.10

Summary of Participant Activities

N = 39 sites	16S Only	MALDI (Bruker)	MALDI (Vitek)	Total
MALDI Only	N/A	12 (31%)	7 (18%)	19 (49%)
16S rRNA seq	7 (18%)	6 (16S+Bruker, 15%)	7 (16S+ Vitek, 18%)	20 (51%)
Total	7 (18%)	18 (46%)	14 (36%)	39 (100%)

Each of the 39 sites were counted as 1 except for:
(doing MALDI-TOF only)

- 1 site with 4 participants
- 1 site with 2 participants

Total: **42** participants from **39** sites
(Generally participants at the same site gave rise to Similar or same results)

EPT-1 *Yersinia pseudotuberculosis* (DOD=2)

- First described in 1889, undergone various name changes
- Soil and water-borne, cause of yersiniosis in animals and humans
- *Y. pestis* is a recently (~1,500-20,000y ago) clone of *Y. pseudotuberculosis, '*emerging' shortly before the first pandemics of plague
- Specimen **NML 72-0486**, derived from stool; extensively characterized as surrogate for Yp

Comparison between the Two Species [Safest way to differentiate is in CL3]

Feature	*Y. pseudotuberculosis (Yps)* RL 1 agent	*Y. pestis (Yp)* RL3 agent
Usual symptoms[2]	GI tract, lymphadenitis[2]	Plague: pulmonary , GI, septicemia[2]
Genome size[1]	4.7 million bp	4.6 million bp
13% total genome[1]	Functional	Dysfunctional (genome smaller than Yp)
32 chromosomal genes 2 plasmids[1]	**Absent**	**Found** (genes assoc. with virulence)
16S rRNA gene seq ID CFAs (MIDI system) [3]	99.5-100% to *Yp,* 2 other sp High scores Yps to Yp	99.5-100% to *Yps*, 2 other sp
MALDI-TOF	Bruker[4]: scores of **2.3 – 2.4** to *Y. pestis & Yps*	Vitek MS unknown
Biochemicals[2]- Urease	Positive	Negative
Rham, Melb, Esc, Xyl	+w / + / + / +	- / - / - / -

1. **Achtman M**. et al 1999 PNAS 96:14043-14048; **Chain et al** 2004 PNAS 101 13826-13831
2. **Petersen**, J.M., L.M. **Gladney**, M.E**. Schriefer**. 2015 MCM 11th pg 738-751
3. **NML data**; 4. When tested against both the **Security Relevant Database and Biotyper**.

EPT-1: Results 16S rRNA gene sequencing

N= 20 sites/20 participants

Full (>1400 bps) seq, N=4; Partial, N=16; 16S ONLY= 7; Both 16S & MALDI =13

No. phone or emails calls asking if Kathy sent out a strain of PLAGUE: = 2

Response	Comment1: Definitive ID with no comment	Comment 2: Send to ref lab for species specific ID/ ? BT agent/ or ?Yps because source = stool or after biochems	Comment 3: [expect from NML or CLRN labs only] Perform plague r/o NAATs	Found Yps and Yp but provided no comment
Y. pseudo-tuberculosis	1 (5%)	-	-	-
Yersinia spp. (Could not resolve Yps and Yp, other spp)	-	12[1] (60%)	4 (20%)	1 (5%)
Yersinia spp. ['probably Yps OR Yp'; or 'most closely resembling Yps']	-	-	-	2 (10%)

1. 2 labs= refer to Ref centre if motility, urease negative

EPT-1 Results: MALDI-TOF

BRUKER[1] sites = 18 (19 participants); Both 16S & MALDI-Bruker= 6; Bruker only=12

VITEK[2] MS sites=14 (17 participants); Both 16S & MALDI-Vitek = 7; Vitek MS only = 7

Yps, *Yersinia pseudotuberculosis*; Yp *Y. pestis*

Prep Methods: Direct= 88% (Br=15, Vi=13); Extracted only = 12% (Br=3, V=1)

Response	Yps = Definitive ID; no comment OR mentioned Yp but did not elaborate	Yps= Presumptive ID; since Yps not validated for system, would do enterics biochems (to validate)	Yps but report as *Yersinia* species and send to ref lab for Yp r/o	Yps would NOT be reported out; would do enteric w/u or report as 'Not usual enteric pathogen'[4]
32 MALDI sites; 36 partici-pants	Bruker=7[3a] (22%) Vitek=6 (19%)	Bruker=0 (0%) Vitek=3 (9%)	Bruker=10[3b] (31%) Vitek=3 (9%)	Bruker= 1 Vitek=2

1. Bruker scores ~ >2.0 to Yps; same scores if rerun against SR database
2. Vitek scores (usually) ~99.9% to Yps; Yp did not come up
3. 3a lab =definitive Yps (urease positive) 'in containment'; 3b: would test urea & motility
4. 'Not' *Shigella, Salmonella, Campylobacter, E. coli, Y. enterolytica* or send to enterics

Summary, EPT-1 *Y. pseudotuberculosis*

- Shipping: obligation to send bacterium using RL1 or 2 on packaging (important nuance (r/o Yp))
- Source = stool was clue (probable r/o of Yp)
- Responses: all sites got the correct answer (s), regardless of method(s), that is, Yps and/or Yp (by 16S, few other Yersiniae came up)
- *Y. pestis*- 1 entry in Vitek MS IVD and RUO but **did not come up** nor mentioned by any lab (NEG result has not been validated to date in literature…)
- *Y. pestis*- **0** entries in Biotyper; found only in the **Security relevant Database; not discernable** from *Y.ps*
- Suggest **minimal work in CL1/2** if Yps or Yp come up

EPT-2: *Burkholderia cepacia* complex/group (DOD=1-2)

- *B. cepacia complex/*group (21+spp) **CAN NOT BE** readily differentiated by 16S or biochemically
- Differentiated by ***rec*A sequencing** or (as done at NML) **MLST** [seq for 7 alleles sent to European website, generates ST linked to species name]
- Why Do this? Precise species may be required for CF, other patient types
- Test: esp to see if MALDI could provide ID
- Sample: **NML 110041**, *B. cenocepacia* (ST 28) derived from a **sputum**

EPT-2: Results 16S rRNA gene sequencing

N= 20 sites/20 participants

Full (>1400 bps) seq, N=5; Partial, N=15; 16S ONLY= 7; Both 16S & MALDI =13

BCC, *Burkholderia cepacia complex* / group

ID as B. cepacia group / complex. Definitive ID no comment	Definitive ID as BCC but if further ID needed, would do *rec*A or MLST	ID as *Burkholderia* spp OR B. species probably *B. cepacia* or *B. cenocepacia*	ID as *B. cepacia*
9 (45%)	7 (35%)	3 (15%)	1 (5%)

EPT-2 Results: MALDI-TOF

BRUKER[1] sites = 18 (19 participants); Both 16S & MALDI-Bruker= 6; Bruker only=12

VITEK[2] MS sites= 14 (17 participants); Both 16S & MALDI-Vitek = 7; Vitek MS only = 7

32 MALDI sites with 36 participants. BCC, *Burkholderia cepacia* complex

Prep Method: Direct= 88% (Br=14, Vi=12); Extracted only or both= 12%, (Br=2,Vi=2)

(Percentages shown based on instrument type)

B. cenocepacia but report as BCC; some would refer out; (*B. cenocepacia* scores 2.25-2.4)	*B. cenocepacia*, definitive ID (*B. cenocepacia* scores 2.2- 2.27)	*Burkholderia* spp (refer out) OR (VITEK) *B. viet/cepacia* 50/50 [presump ID]	*B. cepacia* (Refer out) OR sp. not validated- (Refer out) OR = Bc, Definitive ID OR = report as BCC OR = *B. cepacia / B cenocepacia*
Bruker=11 (61%) Vitek= 0 (0%)	Bruker=4 (22%) Vitek=0 (0%)	Bruker= 3 (17%) Vitek= 2 (14%)	Bruker= 1 (5%) Vitek= 12 (86%)

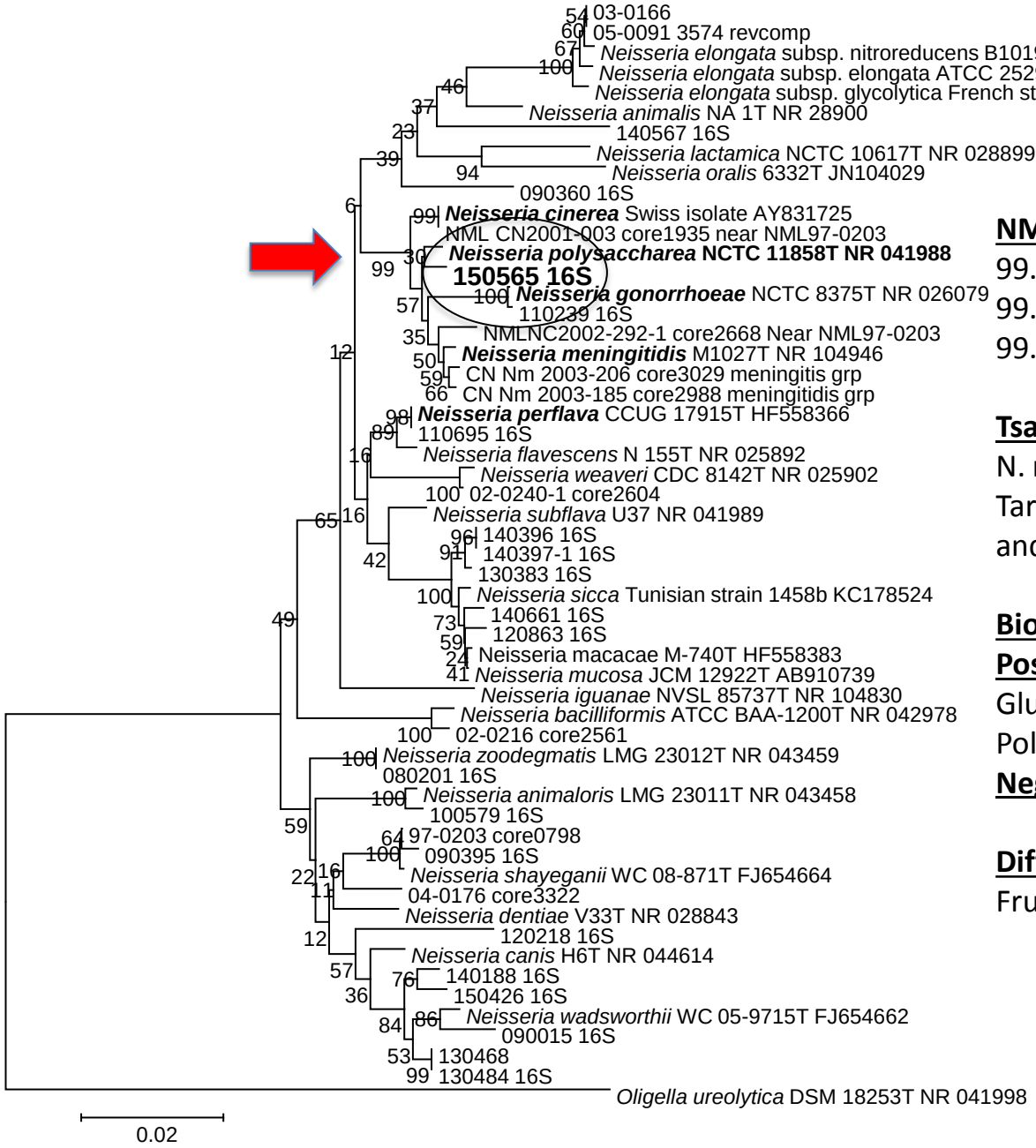
Summary, EPT-2: *B. cepacia* complex / *B. cenocepacia*

- One *B. cenocepacia* entry (LMG 12614 HAM, not the TS) in BIOTYPER provided good (2.25-2.40) scores for many Bruker labs; has 1 other cenocepacia entry, assoc. with poorer scores to test strain
- *B. cenocepacia* did **not come up** with Vitek MS; note **IVD** has: cepacia, multivorans, stabilis, vietnamensis; **RUO** has: many more BCC species, including a SS for *B. cenocepacia*
- Vitek users ID'ed as *B cepacia* or BCC or ID not validated; unique 'split' feature cited by 1 lab.
- Suggested ID here = ***B. cepacia complex / group;*** refer out /or perform *rec*A/MLST if need definitive ID

EPT-3: *Neisseria species,* closest to *N. polysaccharea* (DOD=4)

Described as a 'difficult' identification since:

- NML 16S did not provide an unambiguous ID
- NML biochemical testing- ID ambiguous
- NML Bruker gave ID as *N. meningitidis,* which was incorrect with respect to genetic testing
- Isolate less easy to recover from stab (2 labs got **NG,** 1= Vitek & 16S; 1 Bruker only user]
- Number sites forEPT-3**= 37**
- Sample: **NML 150565**, source described as respiratory



NML [SB] 16S

99.5% N. poly NCTC 11858^{T} (NR_041988)

99.3% *N. cinerea* ATCC 14685^{T} (NR_121687)

99.0% N. mening M1027T (NR_104946)

Tsang lab: Genetically **NEGATIVE FOR:**

N. mening. capsule transport gene (*ctr*A),

Targets for all known serogroup, serotypes and subserotypes

Biochemically, most like *N. perflava*:

Positive (CTA sugars):

Glucose, maltose, sucrose, fructose, GGT, Polysaccharide production

Negative for: sucrose, tributyrin;

Differed from *N. polysaccharea* by:

Fructose positive

EPT-3: Results 16S rRNA gene sequencing

N= 19 sites/19 participants, 1 NG

Full (>1400 bps) seq, N=5; Partial, N=14; 16S ONLY= 7; Both 16S & MALDI =12

Np, *Neisseria polysaccharea*; Nc *Neisseria cinerea*, Nm *Neisseria meningitidis;*

p, partial sequence, F, nearly full sequence

ID = *N. meningitidis* Definitive ID -No Comment OR -Refer out OR -'Respiratory, so considered as normal flora' [P=5, F=1]	ID = *Neisseria* sp (possible novel sp.) OR Np/Nm OR Np/Nc OR most like Nc -Would refer out to R/O Nm OR do molecular testing to R/O NM (N=6) -Would also do Biochems (N=4) - Would treat as RL3[1] (N=1); [P=9, F=4]
6 (32%)	13 (68%)

1. NM is assigned to SCHEDULE 2*(Subsections 3(1), 9(2) and (3) and 10(1))*RISK GROUP 2 HUMAN PATHOGENS in the HPTA. See also description for Nm in Pathogen Safety Data Sheet found at http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/neisseria-men-eng.php

EPT-3 Results: MALDI-TOF

BRUKER sites= 17 (18 participants); Both 16S & MALDI-Bruker=5; Bruker only=12, NG =1

VITEK MS sites= 13(16 participants); Both 16S & MALDI-Vitek=7; Vitek MS only=6; NG=1

30 MALDI sites with 34 participants. Nm, *Neisseria meningitis*; N. sp, *Neisseria* spp.,

Prep Method: Direct= 77% (Br=14, Vi=9); Extracted only or both= 23%, (Br=3,Vi=4)

No. calls/emails to Kathy as to why we 'sent *N. meningitidis* to labs which be handled by those who are not immunized and may have to be put on prophylactics': 2

N. meningitidis, Definitive ID - May do ox, Gram - Do prep in a BSC (Percentages shown based on instrument type)	*Neisseria species* OR Nm/Np with comment such as: - sp. not validated on MALDI (eg. API NM, Vitek 2 NH) - Confirm with additional Biochems eg. API NH or 16S - Did biochems (results odd): API, growth on selective TM, colistin sens, atmosphere, optimal temp - Refer to other lab for species level ID
Bruker = 11 (65%) Vitek = 9 (69%)	Bruker = 6 (35%) Vitek= 4 (31%)

Summary, EPT-3: *Neisseria species, closest to N. polysaccharea*

- 16S rRNA gene seq results more likely to suggest that isolate **could not be** unambiguously identified to spp.
- Biochemicals slightly off for several described species
- Both MALDI-TOF systems suggested *N. meningitidis* as ID with **high degree of confidence.** Consider:
  - 31-35% of MALDI users still questioned or wanted to substantiate ID/ validation for Nm / other
  - Findings eg by Cunningham et al (**2014 JCM 52:2270-2271**) describe that even with in-house constructed MALDI entries, **can not** unambiguously resolve N poly. from *N. meningitidis*
  - This error **MAY INVOKE** unnecessary PH efforts, changes in patient management as well as imposition of unnecessary prophylactics or rush immunization to Nm for staff

EPT-4: *Eggerthella lenta* (DOD = 1)

- First described by Eggerth in 1935
- For many years called *Eubacterium lentum;* reassigned as *Eggerthella lenta i*n 1999 by Wade
- Biochemically, mostly inert
- Gram positive rod, strict anaerobe
- Literature with respect to MALDI scant (eg Schmitt et al 2013 JCM 51:782-786, expected to ID correctly)
- Sample: **NML 140177**, source described as from a blood culture

EPT-4: Results for 16S rRNA gene sequencing and MALDI-TOF

16S rRNA Sequencing

Full (>1300 bps) seq, N=5; Partial, N=15; 16S ONLY= 7; Both 16S & MALDI =13

p, partial sequence, F, nearly full sequence p=15; F= 5

ID = *E. lenta* Definitive ID with one of: No Comment (16) OR Would refer out to confirm (N=3) OR 'most closely resembling E. *lenta* (N=1)
20 (100%)

MALDI-TOF: Direct N=16 (V=8, Br=8); Extracted or both N=16 (V=6, Br=10)

ID = *E. lenta* Definitive ID with: - No Comment (N=16) OR - Presumptive; confirm with bios (N=1) OR - Would confirm with 16S/refer out (N=2) OR - Call it 'Eggerthella sp' or 'most closely resembling E. lenta' (N=2) - Note: BC not normally tested at their lab (N=1)	No ID obtained, after both direct and extraction, on Vitek MS
31 (97%)	**1 (3%)**

Summary, EPT-4: *Eggerthella lenta*

- All labs successfully grew this, a strict anaerobe
- 1 lab did not get good ID in spite of trying both direct and extraction preps
- Generally both 16S and MALDI-TOF did excellent jobs of unequivocably identifying isolate
- 16S **unambiguous**, whether partial or full sequence
- MALDI generally unambiguous, whether direct prep or extracted (1 exception) and on either platform
- MALDI-Bruker, scores though unambiguous, were slightly lower than expected (1.89-2.2)

Update on other PT available for 16S,MALDI

- In 2016, panel for **16S testing** will become available for 1st time (as trial) from **QCMD**
- Provides QC panels for many infectious agents
- Have provided MALDI-TOF panel for several yrs
- Headquartered in Glasgow Scotland
- NML enrolled in 4 of their panels for 2016; each costs **£300** (including 16S but not MALDI)
- Website: **www.qcmd.org**
- **CAP users**: 1 US lab given citation as did not have disclaimer on report where ID=MALDI (Nov 12/15 listserv entry)

UPDATES on NMD and CMUG

- Starting process to hire new manager, as CH won a new job elsewhere at NML (congrats, Chris!)
- CH current has ~300 well curated entries in NMD; targeting 1000 before end of pilot project
  - Currently **~20% of NMD entries** are new genera and/or species
- Tried unsuccessfully to get access to a Vitek MS as loaner

CMUG:

- plan to meet at AMMI-CACMID either Thursday or Friday around the lunch hour for 1.5h