Results of A National Proficiency Test for 16S rRNA Gene Sequencing and / or MALDI-TOF Testing

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National Molecular Group* [NMG] *Meeting
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[Disclosures: NONE]

Government of Canada / Gouvernement du Canada
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Objectives

- Discuss criteria used to select bacteria for panel
- Review method to contact potential participants
- Review number of respondents
- Review Results
- Next Steps
- Update on GRDI MALDI project

Recap: 2010 - 2013

- 2010: NMG 1st discussed possibility for holding a national proficiency test for 16S
- Test not readily available from commercial proficiency test org, such as CAP, CMPT etc
- NML Special Bact asked to conduct a test
- 2012 PT sent to 13 participants/sites with panel of 2 strains for 16S only
- 2013 PT sent to 29 participant/ 25 sites with 3 strain panel for 16S and/or MALDI

2014 Proficiency Test

- Notified NMG, CMUG & previous participants re: upcoming PT (~ Aug. 1/14)
- 4 bacteria panel sent Oct. 1/14; due date Oct. 15/14
- 3 labs: NO courier acc't no. (no courier; wrong number recd); cost borne by NML
- 34 sites sent results; 32/34 (94%) rec'd by due date
- 4 sites sent >1 result (2-3 people tested)
- Transport media used (2012-13:used SBA)

Selected Four Isolates- varying Complexity

- (1st) strict anaerobe [complexity 'easy']
- Attempt test for awareness of RL3 agents by sending a RL1 'close relative' [nuance/complexity 'moderate']
- Bacterium where lab could not necessarily resolve between 2 species by either method [nuance/ complexity 'moderate']
- Unidentifiable bacterium [complexity 'easy' if referred out; 'difficult' for reference centres]

Participants Submitting 16S Results- asked..

- If all steps done internally (nearly all)
- No. bps obtained (6 sites did full seq (>1400 bps), 14 partial seq (<900 bps), all obtained similar results
- Asked for ID; Scores /degree of identity
- Comments: if would report result definitively; Other Provide comments

Summary: 11 sites did both 16S & MALDI and 9 sites =16S ONLY

Total= 22 16S participants from 20 sites

Participant Submitting MALDI reports

- asked to cite if 'direct' and/or 'extracted' methods used (most [non-NML]used 'direct')
- Provide ID /IDs found by MALDI
- Provide MALDI score;
- Comments: if lab would report definitively
- OTHER Comments

Summary: 11 sites did both 16S and MALDI

- 13 sites= MALDI-TOF only

Total= 29 participants from 24 sites

16 = Bruker; 13 =Vitek MS

NML Methods

- 2 reference and 2 clinical isolates (ID'ed by 16S rRNA gene sequencing (full gene seq))
- Secondary gene target required for 1 strain
- 3 Spec Bac staff did 16S rRNA gene sequencing independently
- 1 staff did MALDI-TOF on Bruker system
- Extraction used for all MALDI work, standard Bruker methods
- Results interpreted after examining 4-24 replicates of same bacterium

External Proficiency Test (EPT)-2014-1

Burkholderia thailandensis (ATCC 700388^{T} (source=soil))

- *B. thailandensis* detected/recovered from soils in SE Asia- also reported as very rare human pathogen (2006 JCM 44:4601-4604)- 'very low' pathogenicity
- Test: a) to see if labs ID'ed B. thailandensis and b) 'nuance' = if labs demonstrated /aware of close relationship to RL3 agents, *Burkholderia pseudomallei & B. mallei* (horse pathogen) by 16S and/or MALDI
- Difficulty: as shipper, required to declare risk level for shipping (influenced some responses???)
- Some clinical labs would elect not to test at all if source had been declared

EPT-2014-1. *Burkholderia thailandensis*- 16S results

Identified as	N=22 participants	Comment
NML *B. thailandensis*	99.9-100% B. thail; >99% *B. pseudomallei & B. mallei*	High % identity and coverage to type strains of species
B. thailandensis	22/22 (100%)	All lab reported species with high scores /% identity
B. thailandensis and would report out as definitive ID	11/22 (50%)	Did not cite detecting RL3 agents (in spite of being in BLAST)
B. thailandensis but also reported *B pseudomallei/mallei*	8/22 (36%)	Cited they would send to reference centre either to r/o RL3 agent or to provide final spp
B. thailandensis but would report out as *Burkholderia* spp	3/22 (14%)	2/3 did not describe pseudo/mallei, only 'Burk spp'; 1/3 mentioned RL3 agents, but would report as 'Burkholderia spp'

EPT-2014-1. *Burkholderia thailandensis*- MALDI-Bruker results

Identified as	**N=16 BRUKER participants**	**Comment**
NML [Bruker] Biotyper (just B. thai) Biotyper +SR* database (all 3 species)	[Bruker type score] *B. thail* 2.05 – 2.25 B. thail as above B. pseudo 2.1-2.1	8x replicates done; compared results to Biotyper alone vs Biotyper + SR* db (*B. mallei* did not come up as significant choice here)
B. thailandensis; also cited as potential IDs *B pseudomallei / mallei*	6/16 (37.5%)	Refer strain to reference centre either to r/o RL3 agent or provide final spp; (possible link to 16S result?)
B. thailandensis; would report out as definitive ID	6/16 (37.5%)	No mention of issues with RL 3 agents
Burkholderia spp or only unacceptable ID found	4/16 (25%)	No mention of RL 3 agents in comments

*'Security Relevant' (SR) – a separate Bruker database which contains RL3 bacteria; these agents are otherwise not found in the Biotyper DB. See 2013 JCM 51:1639-40

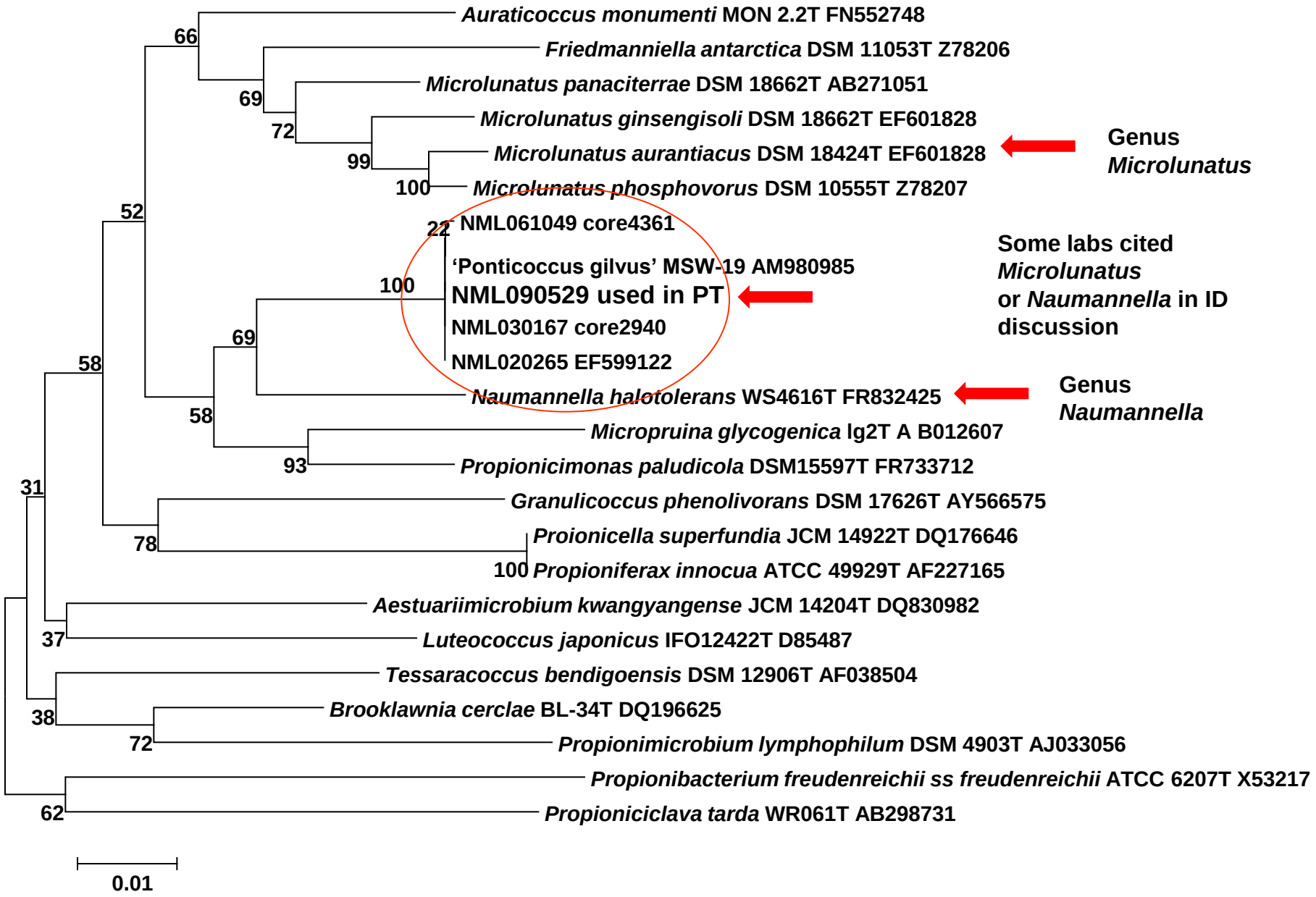
EPT-2014-1. *Burkholderia thailandensis*- MALDI VITEK results

Identified as	**N=13 VITEK MS Participants**	**Comment**
NML [Bruker] Biotyper (just B. thai) Biotyper +SR database (all 3 species)	[Bruker type score] *B. thail* 2.05 – 2.25 B. thail as above B. pseudo 2.1-2.1	8x reps done; compared results to Biotyper alone vs Biotyper + SR db (*B. mallei* did not come up as significant choice); NML only have access to Vitek MS ver 4.10 sp list
B. thailandensis also noted *B pseudomallei / mallei*	0/13 (0%)	None [note: IVD lacks B thail entry; RUO: has *B pseudomallei* SS; 20+ Burk species, including ref S for *B thailandensis*
Burkholderia spp due to low discrimination among several spp	4/13 (31%)	Stated would send to reference centre to identify species or confirm by different method
Burkholderia species; would report out as definitive ID or 'spp'	9/13 (69%)	MALDI= low or high score to *B. cepacia* group species (6)*, *B. gladioli* (2) or *Burkholderia* spp (1)

***B. cepacia* group species cited: *B cepacia, B. multivorans, B. vietnamiensis*, other**

EPT-2014-2 Unidentifiable, fits Family *Propionibacteriaceae*

- 4 referrals in ~12 years, 2 from CSF, 1 from lymph node, NML 090529 used here, from lung; 2 from BC, 2 from QC;
- Catalase +, oxidase v, non-motile, aerobe
- Pleomorphic Gram positive coccobacillus
- Poorly, slowly reactive biochemically
- NML 02-0265=GB access.no. EF599122
- EF599122 & 3 other strains: ~99.9% identity with 'Ponticoccus gilvus' [Lee & Lee 2008 J. Microbiol. 46:508-512-never 'validated']



Bootstrap 1000 replicates; Alignment by Clustal W and Neighbour-Joining software found in MEGA 6.02. Contains all Genera in family Propionibacteriaceae and NML strains closest to 'Ponticoccus gilvus'

EPT-2014-2 What Does "Not Validated" mean?

- After 2008, authors should have sent 'P. gilvus' to IJSEM for 'validation', as 1st paper published in journal not IJSEM [ND or (more likely) would fail review]
- 'P. gilvus' <78% identity with TS of validly-named gen. nov. sp nov. *Ponticoccus litoralis,* a Gram neg bacillus, assigned to the *Rhodobacteraceae* [Hwang & Cho, 2008, IJSEM 58:1332-38]
- 'P gilvus' NOT consistent with 2008 *Ponticoccus gen nov* description
- Lee & Lee description suggestive of gen nov sp nov in *Propionibacteriaceae*, or sp nov in existing genus (Someone needs to formally propose change)

EPT-2014-2
Unidentifiable Family Propionibacteriaceae -16S results

Identified as	**N=22 participants**	**Comment**
NML: UNID, "P. gilvus" GB access AM980985 NML 020265 (EF 599122)	99.9% identity 99.9% identity	NML report read: UNID, Family *Propionibacteriaceae*, close to (non-valid) 'P. gilvus', <96% to *Microlunatus, Naumannella*
UNID, 'P. gilvus' (not valid), family level ID cited, other such wording	14/22 (64%)	Most said would refer to reference lab if required
Ponticoccus gilvus (definitive ID)	6/22 (27%)	Some would refer out if required
Other	2/22 (9%)	1: Microlunatus spp 99% (would refer) 2: No product amplified

EPT-2014-2 UNIDENTIFIED Family Propionibacteriaceae- MALDI

Identified as	**No. with result**	**Comment:**
NML: Unidentified, near 'Ponticoccus gilvus' (AM980985)/ NML 020265 (EF599122) *Microlunatus /Numannella,* other	Bruker, numerous replicates all **No reliable ID**	'No reliable ID' interpreted as per Bruker instructions where low scores (<1.699) and low consistency values (C, (-))
Bruker N= 16 No reliable ID	16/16 (100%)	Most stated would send to ref centre if ID required; many cited GPCB type Gram stain
Vitek MS N=13 NO ID	8/13 (62%)	Most stated would send to ref centre if ID required; many cited GPCB type Gram
Vitek MS N=13 Other responses	5/13 (38%)	2: NO ID and *Lactobacillus* species (*L. salivarius* (98-99%)); 1=Gram not like Lactobacillus 1: *Lactobacillus salivarius* (99.9%) 1: *Weissella viridescens* (99.9% 3x) 1: low scores to 4 taxa

EPT-2014-3. *Corynebacterium propinquum*

- GPR, recovered from eye (NML 110512); rare pathogen, increasingly recovered as sole pneumonia agent/other sites
- Resembles *C. pseudodiphtheriticum* by several methods
- Historically differed by urease rx, [C. pseudodip= urease pos; C propin= urease neg]
- >99.4% identity to each other by 16S
- By MALDI, literature = IDs species correctly but 2nd species can come up with very similar & high score
- Species emended in 2013 IJSEM 63:2146-2154 to include urease positive *C. propinquum*, discerned from *C. pseudodiphtheriticum* by *rpo*B sequencing
- Selected for PT: would participants detect/cite close relationship of these species by either method?
- clinically, treatment similar, whichever ID comes up

EPT-2014-3. *Corynebacterium propinquum* -16S results

Identified as	**N=22 participants**	**Comment**
NML (3 people): *C. propinquum* *C pseudodiphtheriticum*	% identity 99.7-99.8% 99.4%	Species can not be resolved by 16S alone, would do *rpo*B seq
C. propinquum & *C. pseudo-diphtheriticum cited,* various wordings	15/22 (68%)	Several would discern 2 species by biochemical / urease testing; some would refer to ref centre
C. propinquum Definitive ID	4/22 (18%)	
Corynebacterium spp	3/22 (14%)	? Conjecture…may be routine practise not to ID 'diphtheroids' to genus and species by 16S, depending on site = call *Corynebacterium* spp.

EPT-2014-3. *Corynebacterium propinquum* –MALDI results

Identified as	**MALDI**	**Comment. Most used direct; some both direct & extracted; NML used Extracted**
NML 12 reps, 1st choice: *C. propinquum* (11/12) *C pseudodiphtheriticum* (1/12)	Bruker Score 2.0-2.3 2.0	2013 IJSEM, *C. propinquum* usual 1st choice, but not always
[Bruker] *C. propinquum* definitive	10/16 (63%)	1 comment: urease not consistent with ID
[Bruker] *C. prop & pseudodiph*	2/16 (12%)	
[Bruker] *Corynebacterium spp* or other	4/16 (25%)	3 got *C. propinquum* but would report as *Corynebacterium* spp; 1= poor data
[VITEK MS] *Corynebacterium* spp, as response "split" between C. prop & *C pseudodiph.*	8/13 (61%)	Some cited urease test to discern between species; splits usually ~ 50:50 or 60:40
[VITEK MS] *Corynebacterium pseudodiphtheriticum* definitive	4/13 (31%)	1: confirmed by positive urease
[VITEK MS] *Corynebacterium propinquum* definitive	1/13 (8%)	

EPT-2014-4. *Bacteroides fragilis*

- One of the most common anaerobes recovered from clinical materials
- ATCC 25285T used, recovered from appendix abscess
- Based on NML results and literature values, anticpated that all participants should be able to ID this, regardless of method used.

EPT-2014-4. *Bacteroides fragilis* results

Identified as	**participants**	**Comment**
NML 16S *B. fragilis* (NR_074784) Bruker Score	% identity 100%; 2.3-2.4	Inambiguous identification by either approach
[16S] *B. fragilis, or B. fragilis* but report as *Bacteroides spp*	22/22 (100%)	98%-100% identities reported; 20 as definitive, 2 as *Bacteroides* sp ml *B fragilis*
[MALDI, Bruker] *B. fragilis, or B. fragilis* but report as *Bacteroides spp*	16/16 (100%)	1: also recovered 2 other taxa (facultative anaerobes, GPC) - scores 2.2-nearly 2.6 - All would report as definitive
[MALDI, VITEK MS] *B. fragilis*	13/13 (100%)	1: score= 98.7%; 12/13 score= 99.9% -all would report as definitive ID

Conclusions

- 9 (26%) additional respondents in 2014, primarily more MALDI systems in use (compared with 2013)
- Use of a RL1 surrogate for a RL3 agent demonstrated potential for unsafe exposure of bench technologists / MALDI instruments requiring decom, partly due to:
  - Bruker opting to separate RL1 and 2 agents (in Biotyper DB) from RL3 bacteria (in SR database); lab may not own the SR database
  - VITEK MS users testing bacteria against IVD which lacks RL3 bacteria; Saramis did not seem to perform as well as expected
- EPT-2 most found organism 'unidentifiable' by either method
- EPT -3, -4 were identified reasonably well by both methods by participants
  - VITEK MS provided 'split' result feature; clue that 2 species (C. propinquum/ C. pseudodiphtheriticum) were very close by MALDI (Bruker did not)

Next Steps / Other Comments

- 'Proof of participation' available from NML;
- Within NML, results for participants used in ISO accreditation documentation for several labs
- Expect similar sort of test/ panel for fall 2015
- Some labs were missed due to method / time available to analyze notification emails from August -eg key contacts changed jobs, changed email address etc/ other
- Feedback regarding degree of complexity etc welcome
- Next year, will add 'source' to specimen description (mentioned by several participants)

GRDI Brief Update

- GRDI funding rec'd June 2014
- Hired FT staff (Chris Huynh) in July 2014 for 2.5y, to perform technical part of project; instrumental in mounting this EPT
- Chris trained in MALDI, will start making Bruker MSPs after purchase of extra Bruker software
- Biomerieux approached re: Vitek MS loaner; response= not free, may have to negotiate a 'lease to own' type deal (not started yet)
- In contact with international project managers undertaking similar initiatives, try to establish 'data exchange' if possible