NMG and NML *Bordetella pertussis/parapertussis* proficiency panel

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Bordetella spp.

- *B. pertussis*
  - "whooping cough"
  - Severe in young children
- *B. parapertussis*
  - Can mimic whooping cough by *B. pertussis*
  - Generally milder disease
  - Thought be a small percentage (10-20%) of mild pertussis infections
- *B. holmesii*
  - Uncommon cause of sepsis
  - Similar respiratory infection to *B. pertussis*, milder
- Respiratory colonization
  - *B. bronchiseptica*

- Waning immunity
- Lack of immunization

History

- A number of laboratories across Canada are performing PCR testing for *Bordetella pertussis* and/or *B. parapertussis and/or B. holmesii*
- Most assays used are in-house (conventional block, real time) with few comparisons having been done between assays
- A European external quality assessment of 6 laboratories using 7 different assays showed significant differences in sensitivity: 4 to 30,000 cfu/mL LOD (Muyldermas et al., 2005)
- Proficiency testing is limited mostly to CAP specimens (may not challenge the assays adequately)

NML Pertussis Workshop

- Representatives from across Canada met in March 2006 to discuss pertussis testing
- Recommendations:
  - "PCR testing for diagnosis should be encouraged and made widely available"
  - "NML will study feasibility of providing a PCR proficiency "
- Following an initial panel, NML was not able to continue providing proficiency material

Objectives of the Panel

- NMG will work with NML to set up a panel of specimens to allow participating laboratories to assess the sensitivity and specificity of their existing assays
- Focus on the assays (leave out extraction at this point)
- Results will be shared with all participants

Composition of the Panel

- *Isolates received from NML (R. Tsang)*
  - *B. pertussis*
    - 4 ten-fold dilutions of purified nucleic acid including those at or near the detection limit
  - *B. parapertussis*
    - 4 ten-fold dilutions of purified nucleic acid at or near the detection limit
  - *B. holmesii*
    - single dilution purified nucleic acid
  - B. avium
    - single dilution purified nucleic acid
  - *B. bronchiseptica*
    - single dilution purified nucleic acid
- Negatives (buffer)

Time line

- Invitation to participate March 2013 through NMG email list
- Complete production of panel and pre-test to ensure it performs as expected (March-April 2013)
- Develop questionnaire to capture methods used
- Send out panels April 2013 (n=16)
  - One site performed and reported results for two different assays
  - One panel shipped to company that was developing assay
- Results by May 30, 2013
- Analyze results and send out cumulative anonymous results with code for each participating site
- Present at NMG October 2013

Panel Composition

Specimen #	
BPP1	*B. parapertussis* 10
BPP2	Buffer
BPP3	*B. avium*
BPP4	*B. pertussis* 1
BPP5	*B. pertussis* 100
BPP6	*B. parapertussis* 1000
BPP7	*B. parapertussis* 1
BPP8	Buffer
BPP9	*B. pertussis* 1000
BPP10	Buffer
BPP11	Buffer
BPP12	*B. bronchiseptica*
BPP13	*B. parapertussis* 100
BPP14	*B. pertussis* 10
BPP15	*B. holmesii*
TOTAL BP	4
TOTAL BPP	4
BH	Yes

Assays Used

- In-house
  - Conventional
  - Real-time
  - Real-time Multiplexes
- Commercial assays
  - Diagenode Bordetella (pertussis/parapertussis) Real Time Assay
  - Seegene Anyplex II RB5
  - Cepheid ASR
  - Focus Diagnostics
  - RIDA Gene Bordetella Assay

Gene Targets

- B. pertussis
  - IS481
    - Except for one commercial assay (Anyplex; BP485)
    - Known cross-reactivity with B. homesii
- B. parapertussis
  - IS1001
    - Used by all, when specified
- B. holmesii
  - hIS1001
  - recA
- One site confirmed BP and BPP by second target IS1002
  - BP and BPP positive
  - BH negative

Results

- All 17 assays used detected *B. pertussis*
  - Some noted cross-reactivity with *B. holmesii* and *B. paratuberculosis*
  - Some reported a cut-off of Negative at Ct>40 and IND of Ct >=36 and <=40
- 5 had assays that discriminated *B. holmesii*
- 12 had assays that detected *B. parapertussis* (either as a multiplex or uniplex)

Results - *B. pertussis* (n=17)

Specimen	% Positivity
1000	100% (17/17)
100	100% (17/17)
10	64.7% (11/17) + 3 IND
1	35.3% (6/17) + 1 IND

IND Ct >36 but <40

Results - *B. parapertussis* (n=12)

Specimen	% Positivity
1000	100% (12/12)
100	100% (12/12)
10	75.0% (9/12) + 1 IND
1	41.5% (5/12) + 2 IND

IND Ct >36 but <40

In-House

- B. pertussis
  - Ranged from 2/4 to 4/4
  - Multiplex vs. uniplex (no real difference)
  - Conventional 2/4
- B. parapertussis
  - Ranged from 2/4 to 4/4
- B. holmesii
  - All detected
  - Those that tried to discriminate were able to do so

Commercial Assays*

Assay	BP	BPP	BH
Cepheid ASR kit	3 of 4	2 of 4	√ (as BP)
Diagenode	4 of 4	4 of 4	√ (as BP)
Focus Diagnostics	4 of 4	4 of 4	√ (as BP)
Rida GENE	4 of 4	3 of 4	√ (as BH)
Anyplex II Seegene	2 of 4	4 of 4	X

* Used extracted material. Not necessarily as described by package insert

Specificity

- No false positives detected in negative specimens (buffer)
- No positives detected with B. avium or B. bronchiseptica
- No cross-reactivity seen between B. pertussis and B. parapertussis

Issues

- One panel arrived thawed with no dry ice remaining (sent a second-arrived OK)
- One site reported a tube was received with nothing in it (didn't want a second panel sent)
  - Turned out to be a negative
- One site reported a tube labeled as "" on top of tube and "" on side of tube
- One site asked us whether we would be grading the results as "pass" or "fail"
  - We are not a licensed proficiency testing provider
  - Educational only
- Sending this out-even just to 16 sites is no small feat

Where do we go from here?

- Do we do this again?
- Quantitate load of organism to determine limit of detection
- Send mock samples
  - Assess extraction as well as amplification
  - Assays performed directly on specimens (no extraction)
- Determine if we need this on a more permanent basis.

Thanks

- NML-Dr. Raymond Tsang
  - Sent us isolates
- Waufa Naqvi MLT
  - Extracted, made dilutions, determined end-point, made aliquots, packaged and sent out panels
- All the participants
  - Thanks for your patience

Questions?

Whooping Crane (Grus americana)