

Laboratory validation of the MycAssay™ *Pneumocystis* kit for the detection of *Pneumocystis* *jirovecii* in bronchoalveolar lavage specimens

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Pneumocystis jirovecii

- Severe respiratory infection in immunocompromised individuals
- AIDS patients: 10-20% mortality
- Non-HIV immunosuppressive diseases: 35-55%
- Treatment: high dose trimethoprim-sulfamethoxazole + corticosteroids
 - Side effects: toxicity, severe rash, fever, neutropenia
- 2nd line treatment: clindamycin-primaquine, atovaquone, or pentamidine
 - Side-effects, relapse, recurrence

Diagnosis and Detection

- Symptoms non-specific: fever, cough, dyspnea
- Non-culturable organism
- Traditional: microscopic examination of respiratory samples
 - Calcofluor white
 - Fluorescein-conjugated monoclonal antibodies
 - Grocott Gomori methenamine silver staining
- Various home-brew real-time PCR assays
- Commercial real-time PCR assay:

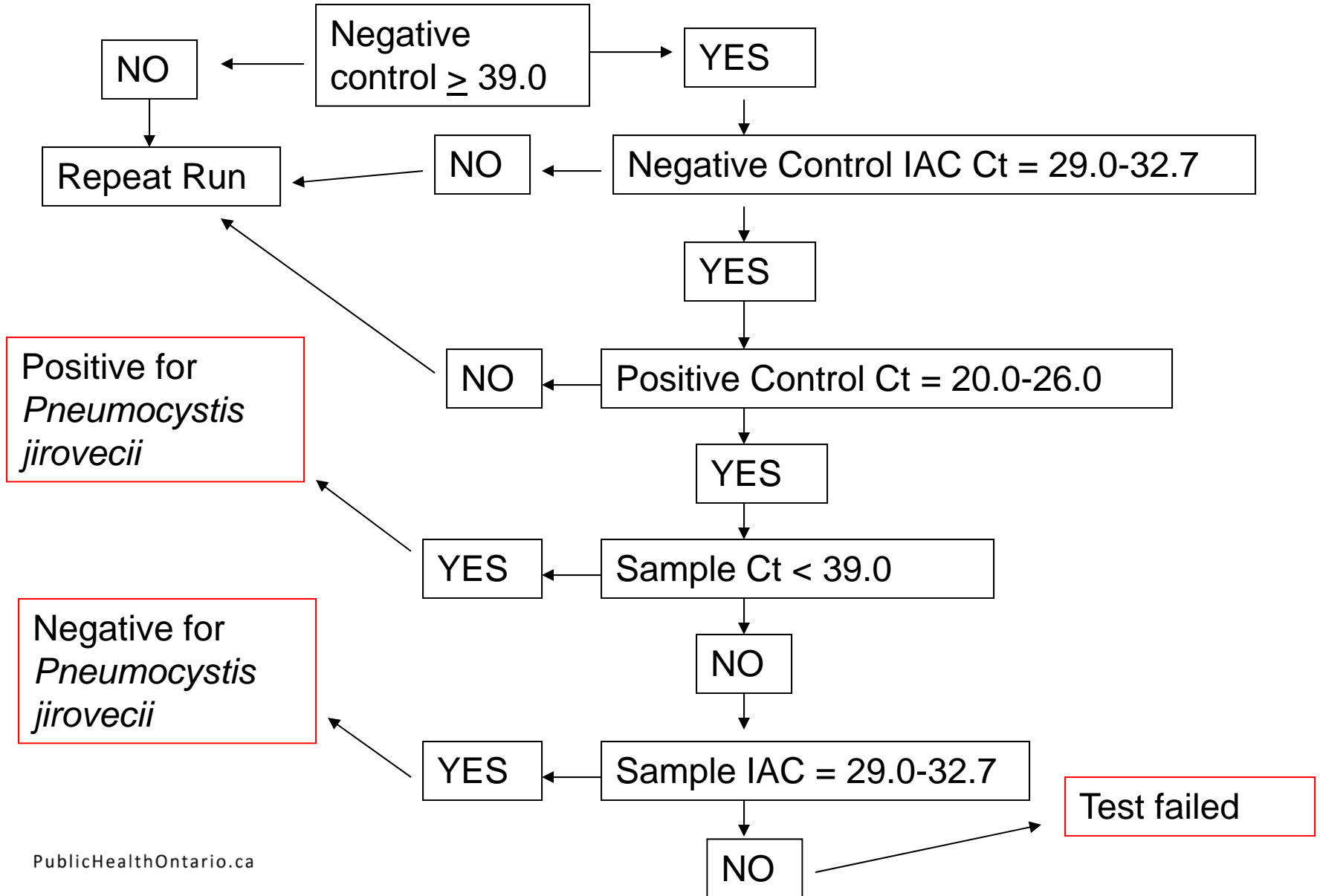
MycAssay™ *Pneumocystis* kit

Myconostica, Ltd. (Manchester, UK) & Alere

MycAssay™ *Pneumocystis* kit

- Real-time PCR assay
- Target: mitochondrial ribosomal large sub-unit (mLSU)
- Molecular Beacon technology
- Applied Biosystems 7500 Real-Time PCR machine + others
- Proprietary reagents + positive and negative controls
- Internal Amplification Control (IAC)
- Specified analysis parameters
- Ct < 39.0 = positive
- Reporting recommendations

MycAssay™ *Pneumocystis* kit



MycAssay™ *Pneumocystis* kit

- Analytical Sensitivity: LoD < 30 copies of mLSU
- Analytical Specificity: Negative results with
 - Fungal Species: *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Blastomyces capitatus*, *Candida albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *Cladosporium spp.*, *Cryptococcus neoformans*, *Doratomyces microsporus*, *Fusarium solani*, *Histoplasma capsulatum*, *Rhizomucor pusillus*, *Rhodotonia rubra*, *Saccharomyces cerevisiae*, *Scedosporium apiospermum*, *S. prolificans*, *Sporothrix schenckii*, *Trichosporon capitatum*
 - Bacterial species: *Bordetella pertussis*, *Corynebacterium diphtheriae*, *Escherichia coli*, *Haemophilus influenzae*, *Lactobacillus plantarum*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *S. pyogenes*, *S. salivarius*
 - Human genomic DNA

MycAssay™ *Pneumocystis* Kit

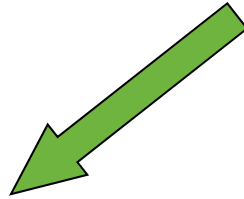
- Seah et al 2011, Med. Mycol. . doi: 10.3109/13693786.2011.598878.
 - Compared to Microscopy
 - Sensitivity: 93.5%
 - Specificity: 95.1%
 - Positive Predictive Value: 70.5%
 - Negative Predictive Value: 99.1%
- Hauser et al 2011, J. Clin. Micro. 49: 1872-1878
 - Compare to Clinical Diagnosis
 - Sensitivity: 93%
 - Specificity: 91%
 - Positive Predictive Value: 59%
 - Negative Predictive Value: 99%

MycAssay™ *Pneumocystis* Kit

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-
- The diagram consists of two boxes on the right side of the slide. The top box has a red border and contains the text 'False negatives?' and 'Low fungal burden/ asymptomatic?'. The bottom box has a green border and contains the text 'Rule out *P. jirovecii*'. Lines connect the circled PPV values to these boxes: a red line connects the 70.5% PPV to the red box, and a green line connects the 99.1% PPV to the green box. Similarly, a red line connects the 59% PPV to the red box, and a green line connects the 99% PPV to the green box.

MycAssay™ Validation

105 BAL specimens



Direct Immunofluorescence Microscopy (IFA)



MycXtra™ Fungal DNA Extraction Kit
(Myconostica, Ltd. & Alere)



Human GAPDH real-time PCR
(Applied Biosystems)



MycAssay™ *Pneumocystis* real-time PCR assay



cdc2 real-time PCR assay (Mayo Clinic)
(Arcenas et al 2006)

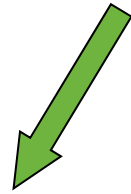


mLSU conventional PCR and sequencing
(Wakefield et al 1990)

Composite standard: If any one of IFA, *cdc2*, or mLSU conventional PCR is positive for *P. jirovecii*, then the sample is considered positive for *P. jirovecii*

Human GAPDH

105 BAL specimens



14 negative

91 positive

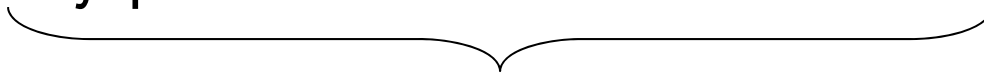


10 MycAssay™
P.j. negative

4 MycAssay™
P.j. positive



Excluded as
indeterminate

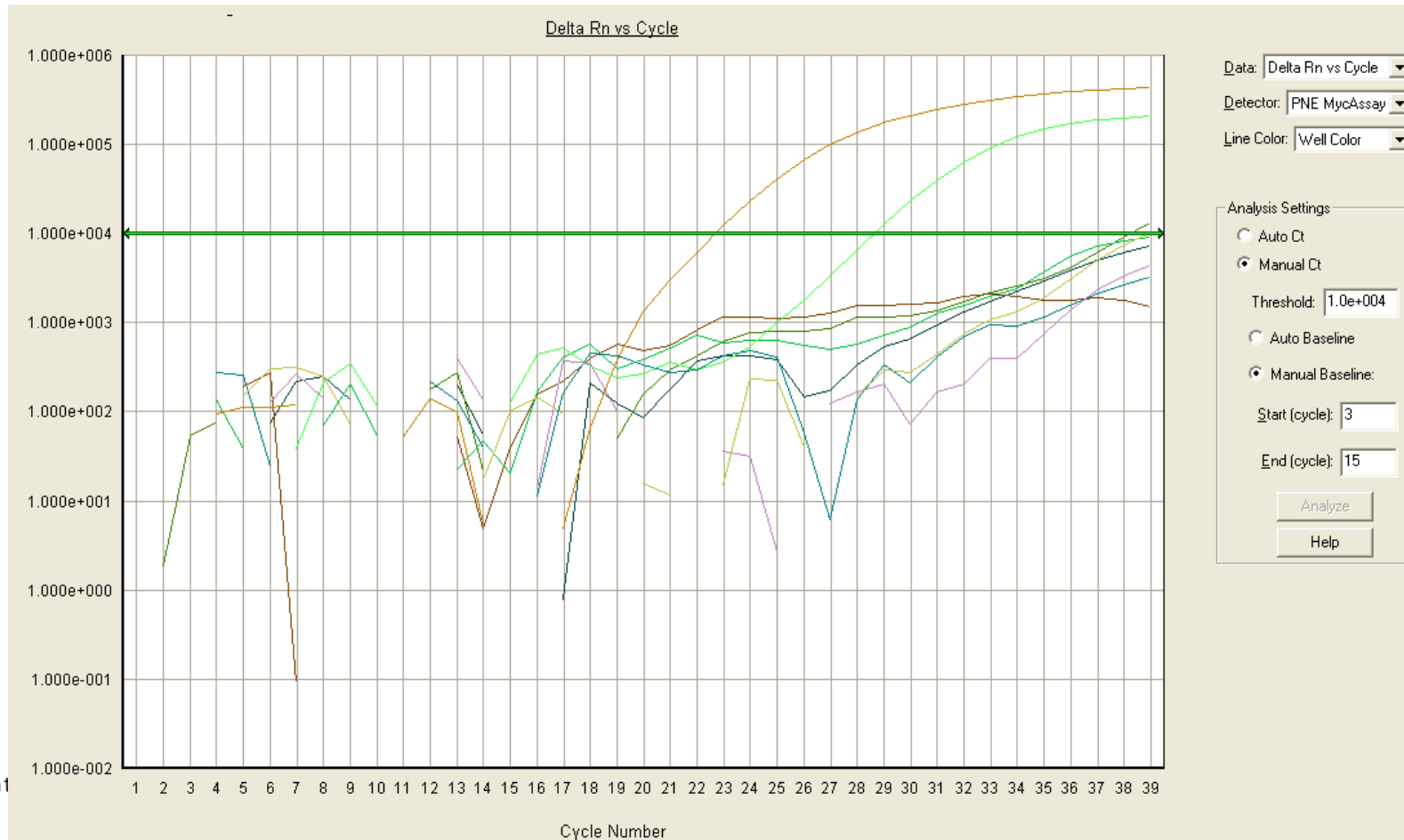


Further analysis

MycAssay™ *Pneumocystis* Results

- 95 samples: 27 positive, 68 negative
- 2 samples had Ct < 39.0 but no exponential amplification pattern → negative

Threshold Setting:
10,000



MycAssay™ vs. IFA Microscopy

IFA

MycAssay™
Pneumocystis
real-time PCR

	Pos.	Neg.	Total
Pos.	9	18	27
Neg.	0	68	68
Total	9	86	95

- Sensitivity: 100%
- Specificity: 79%
- Suggested that MycAssay™ *Pneumocystis* real-time PCR more sensitive than IFA
- Positive Predictive Value: 33%
- Negative Predictive Value: 100%

MycAssay™ vs. *cdc2* real-time PCR

cdc2 real-time PCR

MycAssay™
Pneumocystis
real-time PCR

	Pos.	Neg.	Total
Pos.	16	11	27
Neg.	0	68	68
Total	16	79	95

- Sensitivity: 100%
- Specificity: 86%
- *cdc2* is a single-copy target while MycAssay™ *Pneumocystis* real-time PCR targets mLSU, a multiple-copy target
- Positive Predictive Value: 59%
- Negative Predictive Value: 100%

MycAssay™ vs. mLSU conventional PCR + sequencing

mLSU conventional PCR + sequencing

MycAssay™
Pneumocystis
real-time PCR

	Pos.	Neg.	Total
Pos.	26	1	27
Neg.	0	68	68
Total	27	69	95

- Sensitivity: 100%
- Specificity: 98.5%
- Single discrepant result attributed to different polymerases and reaction mixtures.
- Positive Predictive Value: 96.3%
- Negative Predictive Value: 100%

MycAssay™ vs. Composite Standard

Composite Standard

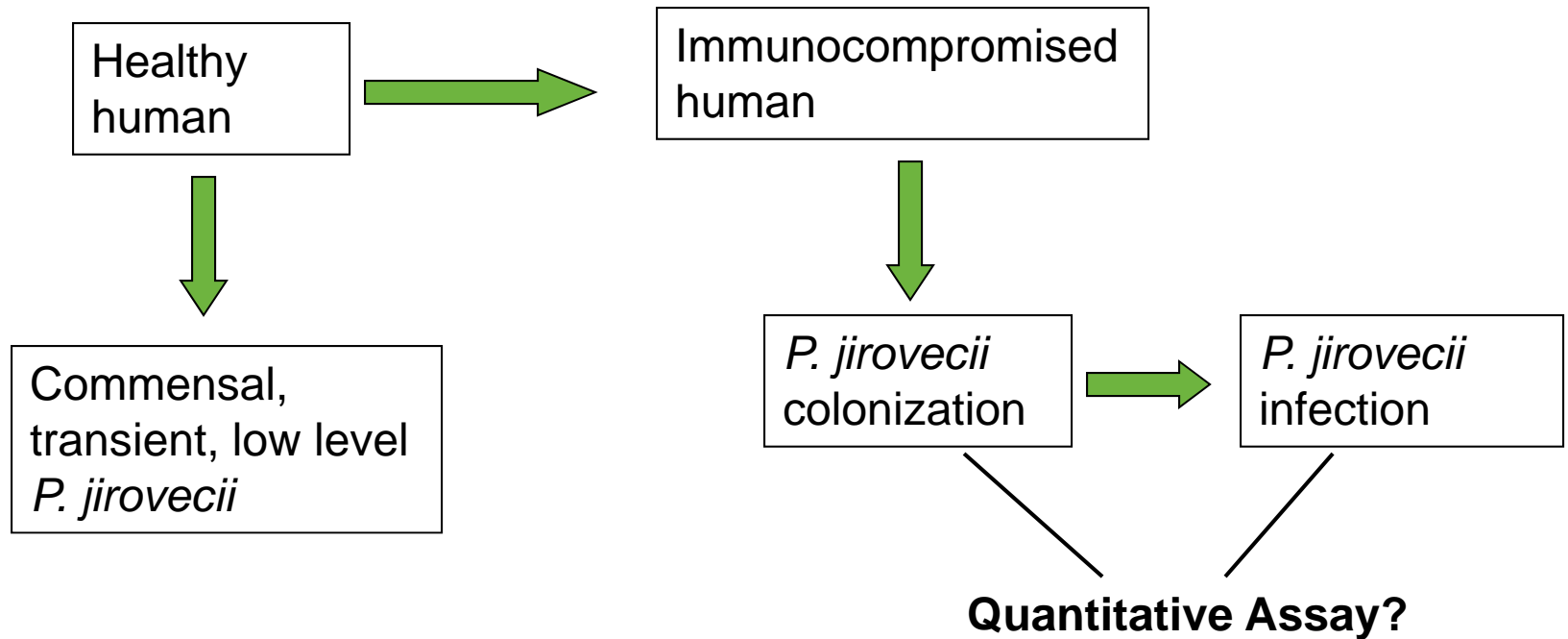
MycAssay™
Pneumocystis
real-time PCR

	Pos.	Neg.	Total
Pos.	27	0	27
Neg.	0	68	68
Total	27	86	95

- Sensitivity: 100%
- Specificity: 100%
- Positive Predictive Value: 100%
- Negative Predictive Value: 100%

Infection vs. Colonization

- Detection of *P. jirovecii* \neq disease



- Assay results interpreted in context of clinical symptoms

Conclusion

- MycAssay™ *Pneumocystis* kit provides a sensitive real-time PCR assay for the detection of *P. jirovecii* in respiratory specimens.
- Validated at PHO-CPHL using a composite standard of IFA, *cdc2* real-time PCR, and mLSU conventional PCR and sequencing.
- Results must be interpreted in the context of clinical symptoms.

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- Dr. Nancy Wengenack

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 - Filip Ralevski
 - Dylan Pillai



References

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