Development of Synthetic Multiplexed External Controls for Monitoring the Performance of Qualitative Laboratory Nucleic Acid Testing Panels Used for Identification of Lower Respiratory Pathogens

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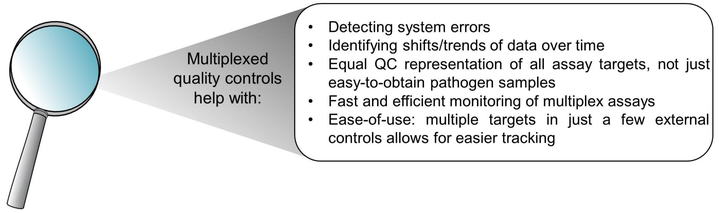
Background

Pneumonia is an infection of the lungs that results in hospitalization of over a million Americans per year in the United States. Classical microbiological methods take days to obtain results. For patients hospitalized with pneumonia, a fast diagnosis is critical to prevent mortality. Panel-based molecular diagnostic assays that can identify multiple pathogens and antibiotic resistance are newly available to aid in the diagnosis of lower respiratory infections and significantly shorten the time to effective targeted therapy. Performance of all clinical assays must be closely monitored to identify shifts, trends, and random errors in order to ensure accurate results. A multiplex control panel has been designed to monitor all analytes detected by the FDA-cleared Curetis Unyvero Lower Respiratory Tract (LRT) Application.



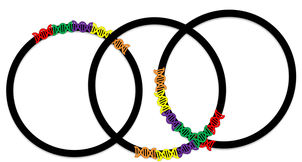
Materials and Methods

The synthetic, multiplex molecular controls contain genome segments of all 19 bacterial pathogens and their corresponding 10 resistance markers detected by the FDA-cleared Curetis Unyvero LRT Application. Pathogen segments were designed in silico, dispersed among several large pieces of synthetic DNA, ligated into engineered vectors, and transformed to create stable frozen clone stocks. DNA plasmids were purified, quantified by 260/280 UV spec, and formulated in a proprietary matrix which carries the control DNA first through a rigorous lysis process and ending in a sensitive array detection system (Figure 1).

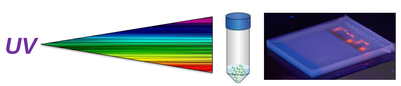
Figure 1

1) *In silico* DNA synthesis of genomic segments from pathogens detected by the Unyvero LRT Application

2) Sub-cloning onto MMQCI engineered vectors



3) Plasmid Purification/Quantification



4) MMQCI Formulation (Matrix and stabilizers)

Table 1: Unyvero LRT Application pathogens and resistance assays.

Unyvero LRT/Pneumonia POS A (M405)	Unyvero Microorganism Classification
Enterobacter cloacae complex	*Enterobacteriaceae* (Gram-negative)
Escherichia coli	*Enterobacteriaceae* (Gram-negative)
Klebsiella oxytoca	*Enterobacteriaceae* (Gram-negative)
Klebsiella pneumoniae	*Enterobacteriaceae* (Gram-negative)
Klebsiella variicola	*Enterobacteriaceae* (Gram-negative)
Serratia marcescens	*Enterobacteriaceae* (Gram-negative)
Staphylococcus aureus	Gram-positive
Pseudomonas aeruginosa	Non-fermenting
Mycoplasma pneumoniae	Other
ctx-M	Antibiotic resistance marker (3rd Gen. Cephalosporins)
kpc	Antibiotic resistance marker (Carbapenems)
ndm	Antibiotic resistance marker (Carbapenems)
oxa-48	Antibiotic resistance marker (Carbapenems)
vim	Antibiotic resistance marker (Carbapenems)

Unyvero LRT/Pneumonia POS B (M406)	Unyvero Microorganism Classification
Citrobacter freundii	*Enterobacteriaceae* (Gram-negative)
Morganella morganii	*Enterobacteriaceae* (Gram-negative)
Proteus spp.	*Enterobacteriaceae* (Gram-negative)
Staphylococcus aureus	Gram-positive
Streptococcus pneumoniae	Gram-positive
Acinetobacter spp.	Non-fermenting
Stenotrophomonas maltophilia	Non-fermenting
Chlamydia pneumoniae	Other
Haemophilus influenzae	Other
Legionella pneumophila	Other
Moraxella catarrhalis	Other
mecA	Antibiotic resistance marker (Oxacillin/Cefoxitin)
oxa-24	Antibiotic resistance marker (Carbapenems)
oxa-23	Antibiotic resistance marker (Carbapenems)
oxa-58	Antibiotic resistance marker (Carbapenems)
tem	Antibiotic resistance marker (Penicillins)

Unyvero LRT/Pneumonia NEG (M407)	Unyvero Microorganism Classification
No targets present	*N/A*

Results

Table 2: Verification of Unyvero LRT/Pneumonia Control Panel M404

Unyvero LRT/Pneumonia POS A (M405)	No. samples tested	No. valid tests	No. samples detected	Percent samples detected
Enterobacter cloacae complex	37	31	31	100%
Escherichia coli	37	31	31	100%
Klebsiella oxytoca	37	31	30	96.8%
Klebsiella pneumoniae	37	31	31	100%
Klebsiella variicola	37	31	31	100%
Serratia marcescens	37	31	31	100%
Staphylococcus aureus	37	31	30	96.8%
Pseudomonas aeruginosa	37	31	31	100%
Mycoplasma pneumoniae	37	31	31	100%
ctx-M	37	31	31	100%
kpc	37	31	31	100%
ndm	37	31	31	100%
oxa-48	37	31	31	100%
vim	37	31	31	100%
Unyvero LRT/Pneumonia POS B (M406)	**No. samples tested**	**No. valid tests**	**No. samples detected**	**Percent samples detected**
Citrobacter freundii	44	44	44	100%
Morganella morganii	44	44	44	100%
Proteus spp.	44	44	44	100%
Staphylococcus aureus	44	44	44	100%
Streptococcus pneumoniae	44	44	43	97.7%
Acinetobacter spp.	44	44	44	100%
Stenotrophomonas maltophilia	44	44	44	100%
Chlamydia pneumoniae	44	44	44	100%
Haemophilus influenzae	44	44	44	100%
Legionella pneumophila	44	44	44	100%
Moraxella catarrhalis	44	44	44	100%
mecA	44	44	44	100%
oxa-24	44	44	44	100%
oxa-23	44	44	44	100%
oxa-58	44	44	44	100%
tem	44	44	44	100%

Table2. M405 POS A: Overall, 30/31 runs showed 100% concordance on the Unyvero A50 System. Six runs were removed from analysis due to a manufacturing defect in 1 particular cartridge lot. One run showed false negatives for PCR pool 6 assays *K. oxytoca* and *S. aureus* due to chamber 6 internal control failure. **M406 POS B:** Overall, 43/44 runs showed 100% concordance. One run showed a false negative for PCR pool 6 assay *S. pneumoniae* due to chamber 6 internal control failure.

Unyvero LRT/Pneumonia NEG (M407)	No. samples tested	No. valid tests	No. samples not detected	Percent samples not detected
Enterobacter cloacae complex	38	38	38	100%
Escherichia coli	38	38	38	100%
Klebsiella oxytoca	38	38	38	100%
Klebsiella pneumoniae	38	38	38	100%
Klebsiella variicola	38	38	38	100%
Serratia marcescens	38	38	38	100%
Staphylococcus aureus	38	38	38	100%
Pseudomonas aeruginosa	38	38	38	100%
Mycoplasma pneumoniae	38	38	38	100%
ctx-M	38	38	38	100%
kpc	38	38	38	100%
ndm	38	38	38	100%
oxa-48	38	38	38	100%
vim	38	38	38	100%
Citrobacter freundii	38	38	38	100%
Morganella morganii	38	38	38	100%
Proteus spp.	38	38	38	100%
Staphylococcus aureus	38	38	38	100%
Streptococcus pneumoniae	38	38	38	100%
Acinetobacter spp.	38	38	38	100%
Stenotrophomonas maltophilia	38	38	38	100%
Chlamydia pneumoniae	38	38	38	100%
Haemophilus influenzae	38	38	38	100%
Legionella pneumophila	38	38	38	100%
Moraxella catarrhalis	38	38	38	100%
mecA	38	38	38	100%
oxa-24	38	38	38	100%
oxa-23	38	38	38	100%
oxa-58	38	38	38	100%
tem	38	38	38	100%

Table 2 continued. M407 NEG: No targets were detected in 38/38 negative control runs on the Unyvero A50 System (100% concordant).

Figure 2a

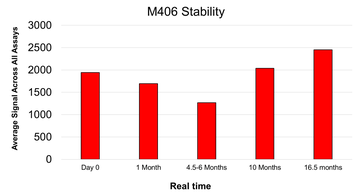


Figure 2b

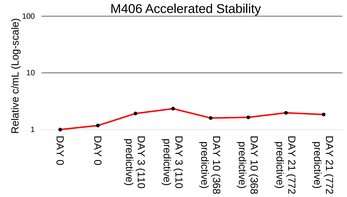


Figure 2: Representative Real-time Stability and Accelerated stability of Unyvero LRT/Pneumonia POS B (M406) a) One control lot of M406 was tested on the Unyvero A50 system over the course of 16.5 months. Signal across all LRT assays were averaged together for the specific time point. Replicates: Day 0 (n=10), 1 month (n=6), 4.5-6 months (n=10), 10 months (n=5), 16.5 months (n=4). **b)** One control lot was placed at 56°C for 3-21 days to assess accelerated stability. Samples were extracted using the QIAmp DNA Blood kit. An in-house MMQCI qPCR assay was run on the Roche Light Cycler to determine c/mL. Relative c/mL from day 0 was graphed on a log-scale. Using the Arrhenius temperature coefficient equation with a Q10 value of 2, this would be predictive of a ~2.1 years stability when stored at 2-8°C.

Conclusions

- MMQCI's synthetic, multiplex controls are designed to be part of an essential clinical laboratory quality control program. The multiplex nature of the controls streamlines the laboratory's Individualized Quality Control Plan (IQCP) without decreasing stringency.
- MMQCI's proprietary matrix and stabilization buffers allow for stable, reliable controls that can be carried through the Unyvero mechanical/chemical lysis, cartridge purification, PCR, and array detection.
- Controls performed robustly with ≥96% accuracy across all targets on the Unyvero LRT application.
- MMQCI's Curetis Unyvero controls are ready-to-use, non-infectious and well-characterized quality control panels for use in the clinical laboratory.
- Current accelerated stability data indicates stability of 24-months. On-going real-time stability demonstrates stability of over 12 months

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