# Qualitative Real-time PCR Analysis for the Presence of Mycoplasma and Ureaplasma Bacterial Species associated with Urinary Tract Infections

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## Introduction and Objective

Common bacterial infections that cause UTIs are *Escherichia coli*, *Klebsiella sp.*, *Proteus sp.*, and sometimes *Staphylococcus sp.* and *Streptococcus sp.* These infections are identified through culturing techniques using Sheep's Blood Agar (SBA) and MacConkey Agar (MAC) followed by chemistries. Though these bacteria are most commonly identified as the cause of urinary tract infections (UTI), many times a suspected UTI will yield negative culture results and be reported out as negative for growth to the provider. Fastidious microorganisms (such as *Mycoplasma genitalium* and *Ureaplasma urealyticum*) can be considered normal urethral and vaginal flora or a possible source of sexually transmitted infections. Not much is known about the role these bacteria play in common UTIs because they are fastidious and most clinical labs do not include them as a part of routine work up. In order to test for the presence of these fastidious bacteria, urine samples with both positive and negative culture results were included in testing using molecular techniques. Our objective was to determine whether or not patients with complaints of UTI symptoms that culture negative for common bacterial pathogens are testing positive for the more fastidious organisms, *Mycoplasma sp* or *Ureaplasma urealyticum*, via real-time PCR.

## Specimen Collection

Urine samples for suspected urinary tract infections (UTI) from the Student Wellness Center on TTU main campus (an entity of the TTUHSC system) were obtained after physician's orders for each patient had been set up for analysis but prior to sample disposal. Specimens are ones with orders for urine cultures that either did or did not exhibit growth of bacteria under traditional culturing techniques on BSA and MAC agars. Each sample (n=37) was de-identified and the results from this research will have no effect on patient treatment yet. 2 mL of patient urine will be placed in Aptima Urine Collection tubes that contain media for stabilizing the bacteria in the samples until DNA extraction can be completed.

### Interventions

The urine specimens were frozen after collection for up to one year before DNA isolation.

## Methods

Using molecular techniques, we tested for the presence of M. genitalium, and U. urealyticum through a qualitative analysis. The Qiagen DNA isolation kit was used for DNA isolation, and DNA concentration was determined using a nanodrop instrument. DNA concentrations <5µL were confirmed for presence using gel electrophoresis. Species specific Taq-man probes were used in real-time PCR to check for the presence of *Mycoplasma* species or *Ureaplasma urealyticum*. A analytical/ethical sensitivity and specificity analysis was performed along with a chi-square analysis for a comparison of observed vs. expected results.

## Results

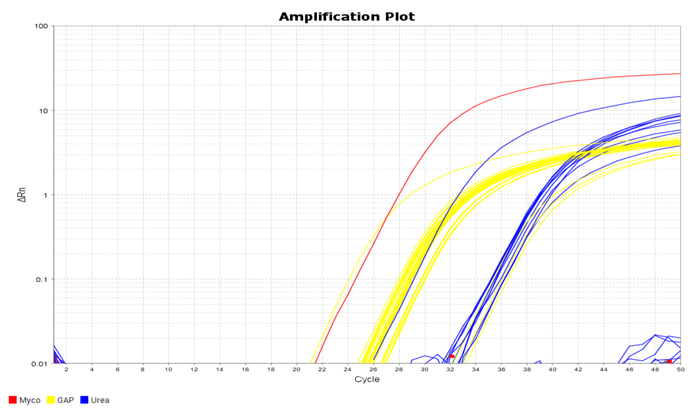


**Figure 1 (above): PCR Amplification Plot for patients 138-158. Patients 147, 151, and 158 tested positive for *Ureaplasma*. All patients tested in triplicate with a GAPDH control.**

**Symmetric Measures**

| | | Value | Approximate Significance |
|---|---|---|---|
| Nominal by Nominal | Phi | .470 | .017 |
| | Cramer's V | .470 | .017 |
| N of Valid Cases | | 37 | |

**Table 2 (above): Symmetric Measures of the data from figure showing that Ureaplasma detection was clinically significant.**

**Ureaplasma urealyticum * Culture results Crosstabulation**

| | | | Culture results | | |
|---|---|---|---|---|---|
| | | | PC | GROWTH | NO GROWTH |
| Ureaplasma urealyticum | N | Count | 9 | 12 | 9 |
| | | Expected Count | 7.3 | 15.4 | 7.3 |
| | | % within Ureaplasma urealyticum | 30.0% | 40.0% | 30.0% |
| | Y | Count | 0 | 7 | 0 |
| | | Expected Count | 1.7 | 3.6 | 1.7 |
| | | % within Ureaplasma urealyticum | 0.0% | 100.0% | 0.0% |
| Total | | Count | 9 | 19 | 9 |
| | | Expected Count | 9.0 | 19.0 | 9.0 |
| | | % within Ureaplasma urealyticum | 24.3% | 51.4% | 24.3% |

**Table 1 (above): Ureaplasma urealyticum Detection in Traditional Cultures. This figure depicts if Ureaplasma was detected using molecular techniques (Y/N) in traditional cultures that were either possibly contaminated, had growth or exhibited no growth.**

**Chi-Square Tests**

| | Value | df | Asymptotic Significance (2-sided) |
|---|---|---|---|
| Pearson Chi-Square | 8.179[a] | 2 | .017 |
| Likelihood Ratio | 10.885 | 2 | .004 |
| Linear-by-Linear Association | .000 | 1 | 1.000 |
| N of Valid Cases | 37 | | |

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 1.70.

**Table 3 (above): Chi-Square analysis showing the significant association between symptomatic patient cultural growth and the presence of *U. urealyticum* with a significance value of 0.05.**

## Discussion

In order to determine if there is an association between symptomatic patients' culture results, a chi-square test of independence was used. Because this analysis requires a minimum of five data points, *Mycoplasma* was excluded. *Ureaplasma* was not found in specimens that produced no growth on traditional cultures or that were probably contaminated. *Ureaplasma* was however detected in seven urine specimens that did produce growth. The results indicate that there is a significant association between symptomatic patients culture results and the presence of U*reaplasma urealyticum*, X^2(2,N=37) = 8.179, p=0.017, Cramer's V = 0.470.

## Conclusion

The results show sufficient evidence that we can reject the null hypothesis for *Ureaplasma urealyticum*. There is an evident association between culture results and the presence of *Ureaplasma urealyticum* using molecular techniques; however, the correlation is not in the culture negative specimens as initially suspected. More specifically, the results show an association between the growth on bacterial plates with the presences of *Ureaplasma*. Results indicate that there is no clear correlation between *Mycoplasma* and UTI specimens. Therefore, we cannot reject the null hypothesis for this group. Further research is needed to explore the possibility of *Ureaplasma urealyticum* being transmitted through sexual routes.

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