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CDC Shigella Study Culture, RT PCR with DNA/RNA Protect Sponge Swab Device

A Novel Device to Prevent Inhibition of Amplification in Cervical and Urethral Swab Specimens for N. Gonorrhea and Chlamydia Trachomatis

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Methodology

The DNA/RNA device consists of a sterile polyurethane sponge impregnated with 700µl of the Protect chemistry in a sterile tube.(Starplex)The collection swab was a sterile rayon swab.The control device was a standard rayon swab in a sterile tube(Copan Diagnostics (#155C -160C)

All specimens were collected in duplicate. All samples were transported and processed within 8 hours of collection. Current assay reagents and direction inserts were used at each site.A second amplified assay was utilized to challenge all positives to verify true positives. LCx was refereed by PCR, and SDA,TMA, PCR were all refereed by LCx. All positive extracts

Four amplification assays were included in this study. LCx® Abbott Diagnostics, The Probe-Tec® Becton Dickinson, TMA™ GenProbe, and PCR®Roche Diagnostics. Four separate sites were utilized one for each assay platform. Specimens were collected at STD clinics using existing collection sop’s. 50 specimens were collected at each site.

Inhibition Of Amplification is a significant problem with STD specimens from both Cervical and urethral sites. Estimates of inhibition range from (2-20%) This study compared a novel collection device containing **DNA/RNA Protect™** chemistry to a standard swab collection device.

Study Site Analysis

- Site 1 Cervical Chlamydia (asymptomatic)
- Site 2 Urethral Gonorrhea(symptomatic)
- Site 3 Cervical Chlamydia(asymptomatic)
- Site 4 Urethral Gonorrhea(symptomatic)
- Prevalence: All sites had a prevalence of > 15% for both gonorrhea and chlamydia

Results

1. DNA/RNA Protect™ swabs yielded a statistically significant increase in amplification at all sites compared to standard unprotected swab
2. There was no statistically significant difference between gonorrhea and chlamydia specimens for inhibition.
3. There was a statistically significant

presence of target inhibitors in both unprotected gonorrhea and chlamydia specimens. Lactoferrin, Hydrogen peroxide,
Methemoglobin, Gamma interferon, Lactic acid, Leukocyte esterase were all associated with inhibited specimens.

That were unprotected were subjected to GC/MS analysis to confirm the presence of substances known to cause inhibition in amplified assay systems. Target substances were leukocyte esterase, Methemoglobin, lactoferrin, hydrogen peroxide, Lactic Acid. Immunoassays were preformed to detect the presence of the following, Gamma Interferon, and Mucosal IgA .

Immunoassay Data for Unprotected Inhibited Specimens

- IgA Cervical Correlation
- Gamma Interferon Urethral and cervical correlation
- Protein Oxidation (hydroxy-nonenal) Activity Urethral correlation only

GC/MS Urethral data for Unprotected Inhibited Specimens

- Neutrophil Esterase >15μl(achieved Peaks)
- Hydrogen Peroxide(no quantitation done)
- Zinc 110μg/dl
- All had Statistically Significant Correlation with inhibited specimens

GC/MS Summary Cervical Data Unprotected inhibited specimens

- Lactoferrin >175g/mg
- Methemoglobin >8mg/dl
- Leukocyte esterase>15/μL
- Lactic Acid (no quantitation done)
- All had Statistically significant correlation with inhibited specimens

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Introduction

Inhibition of amplification is a significant problem with STD specimens from both cervical and urethral sites. Based on a review of the literature, estimates of inhibition range from 2-20% for specimens collected with a swab. This study compares a novel swab collection device containing **DNA/RNA PROTECT™** chemistry to a standard dry swab collection device and demonstrates that Protect chemistry can be utilized to significantly minimize the effects of inhibition, thereby reducing the incidence of false negative results.

Methodology

The novel swab device consists of a sterile polyurethane sponge impregnated with 700µl of Protect chemistry, which is housed in the bottom of an empty sterile tube. The specimen is collected on a separate sterile rayon swab and inserted into the above tube (Starplex). Once the swab has been inserted in the tube, the swab comes into contact with the sponge and absorbs the Protect chemistry, which preserves the specimen and removes inhibitors by covalently linking them to the Protect chemistry. The control device used for comparison was a standard dry rayon swab in a sterile tube (Copan Diagnostics #155C -160C)

Four well-known amplification assays were included in this study: LCx® (Abbott Diagnostics), Probe-Tec® (BD Diagnostic Systems), TMA™ (Gen-Probe), and PCR® (Roche Diagnostics). Four separate laboratories were utilized to conduct the experiment, one for each assay platform.

Specimens were collected at four separate STD clinics using best-practice collection methods. At each collection site, 50 patients provided duplicate specimens for an aggregate of 200 Protected samples and 200 unprotected samples. All samples were transported to the laboratory at room temperature and processed within 8 hours of collection.

Current assay reagents and direction inserts were used to perform the amplification assay. A second amplified assay was utilized to challenge all positives to confirm that they were really true positives. LCx was refereed by PCR, and SDA, TMA, and PCR were all refereed by LCx. Additionally, all positive extracts that were unprotected (dry) were subjected to GC/MS analysis to confirm the presence of substances known to cause inhibition in amplified assay systems. Target substances were leukocyte esterase, methemoglobin, lactoferrin, hydrogen peroxide, and lactic acid. Furthermore, immunoassays were preformed to detect the presence of the following inhibitors:

- Gamma interferon
- Mucosal IgA
- Non-target bacterial DNA

Data:

1) Comparison between True Positives using DNA/RNA Protect™ and an unprotected control

Number of collection sites: 4

- Collection site 1: Cervical Chlamydia (asymptomatic)
- Collection site 2: Urethral Gonorrhea (symptomatic)
- Collection site 3: Cervical Chlamydia(asymptomatic)
- Collection site 4: Urethral Gonorrhea (symptomatic)

Number of Samples that were Protected: 200 (50 from each collection site)

Number of Samples that were unprotected: 200 (50 from each collection site)

Test Site #/ Assay	Number of Samples	Positives- **DNA/RNA Protected**	Prevalence	Number of Samples	Positives- **Unprotected control**	Prevalence
1 - LCx	50	8	16%	50	6	12%
2 - Probe-Tec	50	7	14%	50	4	8%
3 - TMA	50	5	10%	50	3	6%
4 - PCR	50	6	12%	50	3	6%
Totals :	200	**26**	**13%**	200	**16**	**8%**

2) GC/MS Cervical Data for Unprotected Inhibited Specimens:

Lactoferrin >175g/mg

Methemoglobin >8mg/dl

Leukocyte esterase >15/µL

Lactic Acid: present, but not quantified

*All had statistically significant correlation with inhibited specimens

3) GC/MS Urethral data for Unprotected Inhibited Specimens:

Neutrophil Esterase >15µl (achieved peaks)

Hydrogen peroxide: present, but not quantified

Zinc 110µg/dl

*All had statistically significant correlation with inhibited specimens

4) Immunoassay Data for Unprotected Inhibited Specimens:

IgA cervical correlation

Gamma Interferon urethral and cervical correlation

Protein oxidation (hydroxy-nonenal) activity urethral correlation only

Results

1) **DNA/RNA Protect™** swabs yielded a statistically significant increase in amplification at all sites compared to a standard unprotected swab.
2) There was no statistically significant difference between gonorrhea and chlamydia specimens with regard to their inhibition characteristics.
3) There was a statistically significant presence of target inhibitors in both unprotected gonorrhea and chlamydia specimens.
4) Lactoferrin, hydrogen peroxide, methemoglobin, gamma interferon, lactic acid, leukocyte esterase were all associated with inhibited specimens.

End.

Paired t-test:

Normality Test: Passed (P > 0.200)

Treatment Name	N	Missing	Mean	Std Dev	SEM
Col 1 Protected	4	0	8.750	2.062	1.031
Col 2 Control	4	0	6.250	1.708	0.854
	4	0	2.500	0.577	0.289

Difference 2.500 0.577 0.289

t = 8.660 with 3 degrees of freedom. (P = 0.003)

95 percent confidence interval for difference of means: 1.581 to 3.419

The change that occurred with the treatment is greater than would be expected by chance; there is a statistically significant change (P = 0.003)

Power of performed test with alpha = 0.050: 0.999

Descriptive Statistics:

Column	Size	Missing	Mean	Std Dev	Std. Error	C.I. of Mean
Col 1	4	0	**8.750**	**2.062**	**1.031**	**3.280**
Col 2	4	0	**6.250**	**1.708**	**0.854**	**2.718**

Column	Range	Max	Min	Median	25%	75%
Col 1	5.000	11.000	6.000	9.000	7.500	10.000
Col 2	4.000	8.000	4.000	6.500	5.000	7.500

Column	Skewness	Kurtosis	K-S Dist.	K-S Prob.	Sum	Sum of Squares
Col 1	-0.713	1.785	0.298	0.232	35.000	319.000
Col 2	-0.753	0.343	0.192	0.657	25.000	165.000