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METHODS: See Protocol of RNA Modifying Enzymes Data - 12 FEB 97

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Primary Antibodies)

1. E-coli RNA polymerase — monoclonal purified HRP.
2. RNA polymerase T7 — monoclonal purified HRP.
3. T3 RNA polymerase — Monoclonal purified HRP.
4. T-4 RNA Ligase — Monoclonal purified HRP.
5. Poly A-Polymerase — Monoclonal purified HRP.

TREATED

HR	1	2	3	4	5	6	7	
Absorbance	5.00	3.2	2.2	1.0	0.55	0	0	E-coli RNA polymerase
	5.0	1.56	1.0	0.45	0	0	0	RNA polymerase.
	5.0	2.9	1.56	0.55	0	0	0	T3 RNA polymerase.
	5.0	3.45	2.0	1.0	0.98	0	0	T4 RNA Ligase.
	5.0	3.1	1.6	0.5	0	0	0	Poly A polymerase.

UNTREATED

HR	1	2	3	4	5	6	7	
Absorbance	5.0	4.7	3.66	2.54	2.0	2.69	2.45	E-coli RNA polymerase
	5.0	4.9	4.0	3.4	3.0	2.65	2.83	RNA polymerase.
	5.0	4.2	4.0	3.0	3.29	2.48	2.77	T3 RNA polymerase
	5.0	3.85	3.7	3.0	2.65	2.55	2.75	T4 Ligase
	5.0	4.3	3.0	3.22	2.69	2.55	3.10	Poly A polymerase.

RESULTS

THE TREATED RNA degrading Enzymes were significantly Reduced in activity By DNA/RNA protect chemistry in Sodium Thiol & 1 M EDTA

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Enzyme Challenge
DNA Modifying Enzymes

Method Homogenous Liquid phase EIA. Direct primary antibodies conjugated to HRP.

1. E-coli Ligase T-4 monoclonal purified
2. E-coli polymerase T-7 monoclonal purified
3. Exonuclease II monoclonal purified
4. Lambda Exonuclease monoclonal purified
5. Bal 31 Deoxyribonuclease monoclonal
6. S1 Nuclease monoclonal
7. Bap phosphatase monoclonal
8. T4 polynucleotide monoclonal

Each Enzyme (treated) was exposed to a formula of DNA/RNA protect of 1M sodium Thiocyanate and .1 M of EDTA (rapid glass)

Each untreated Enzyme was Incubated into a urine matrix and aged for 8 Hours

Substrate Lan added Incubate 5 min

Measurements were taken every hour which absorbance measurements were made every hour on a spectrophotometer.

Antibody dilutions for each antibody were 1/1000

Wave length was set at 540 nm.

Total volume for each reaction chamber was 1000 ml.

Each enzyme treated and untreated had individual chamber. Chemistry + Enzyme and Enzyme in DI of H_2O were incubated at Ambient room temp. 25°C ± 2°C

DI of H_2O were used to blank each reading.

Total Enzyme activity was measured in absorbance units converted to a scale 0–5 absorbance

Measurements were taken over an 7 Hour period.

Results Treated

Hr	1	2	3	4	5	6	7	
Absorbance	5.50	2.0	1.38	1.00	0.55	0	0	E-coli Ligase
	5.0	2.13	1.22	0.66	0	0	0	E-coli polymerase
	5.0	1.39	1.00	0.55	0	0	0	Exonuclease II
	5.0	2.13	1.25	1.0	0.98	0	0	Lambda Exonuclease
	5.0	1.26	1.11	0.99	0.77	0	0	bal 31 Deoxyribonuclease

Results Untreated

Hr	1	2	3	4	5	6	7	
	5.0	4.5	3.3	3.43	3.52	3.44	3.33	E-coli Ligase Treated
	5.0	4.2	3.5	3.4	3.6	3.50	3.20	E-coli Polymerase
	5.0	4.6	3.7	3.8	3.7	3.50	3.20	Exonuclease II
	5.0	4.0	3.7	3.8	3.5		3.20	Lambda Exonuclease
	5.0	4.3	3.8	3.7	3.2		3.1	bal 31 Deoxyribonuclease

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Effect of Molar Concentration of DNA/RNA protect on Taq Polymerase.

① Controls 1pg/ccc93 fr DNA-
② 1M Sodium Thiocyanate + EDTA 0.1 M.
③ 2M Sodium Thiocyanate + EDTA 0.2M.

TAQs used
1. Taq Gold U. Roche
2. Taq - TAKARA BIO
3. Perkin Elmer
4. Promega
5. Invitrogen
6. Stratagene
7. Qiagen
8. ABI.
9. Imp Biologicals
10. Promega.

Target 1pg CCC93 DNA.

1 unit of each Taq was used.
Sigma master mix protocol.

Target = 1pg CCC93 DNA from stock extracted for constant protocol.
Group 1 = Controls NO CHEMISTRY.
Group 2 1M Sodium Thio + 0.1 M EDTA.
Group 3 2M Sodium Thio + 0.1 M EDTA.

TAQ	1	2	3	4	5	6	7	8	9	10
Group 1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1
Group 2	2.99	3.1	3.1	2.3	2.7	2.99	1.9	2.3	3.0	2.5
Group 3	3.1	2.3	2.86	1.9	1.2	2.75	1.6	2.0	2.6	2.57

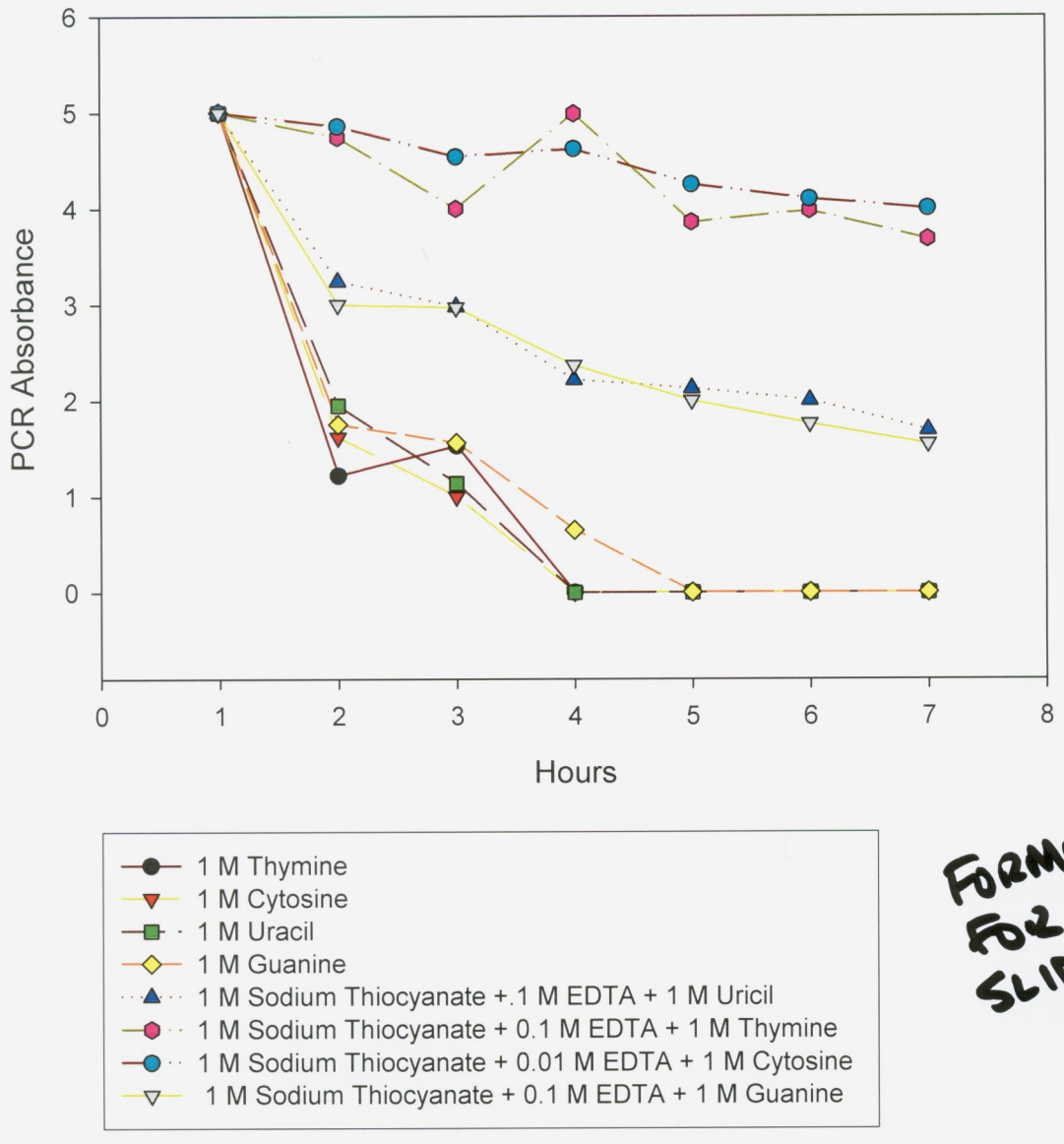
Results

1. 1 Molar Sodium Thio and 0.1 molar used to Inhibit on Amplification
2. 2 Molar Sodium Thio and 0.1 molar used to Inhibit on Amplification
3. There was significant variation in performance between Taqs.
4. All Enzymes Amplified Target of Low Replication.

Follow up studies

1. Increase units of Taq to 2 units.
2. Optimize performance by Dilution and Buffer Experiments.

Comparison of single purines and pyrimidines to combination chemistries containing Thymine, Uracil, Cytosine and Guanine, in the preservation and stabilization of 1 pg of gonoccocal DNA in fresh human urine.



FORMULATE FOR SLIDE 60

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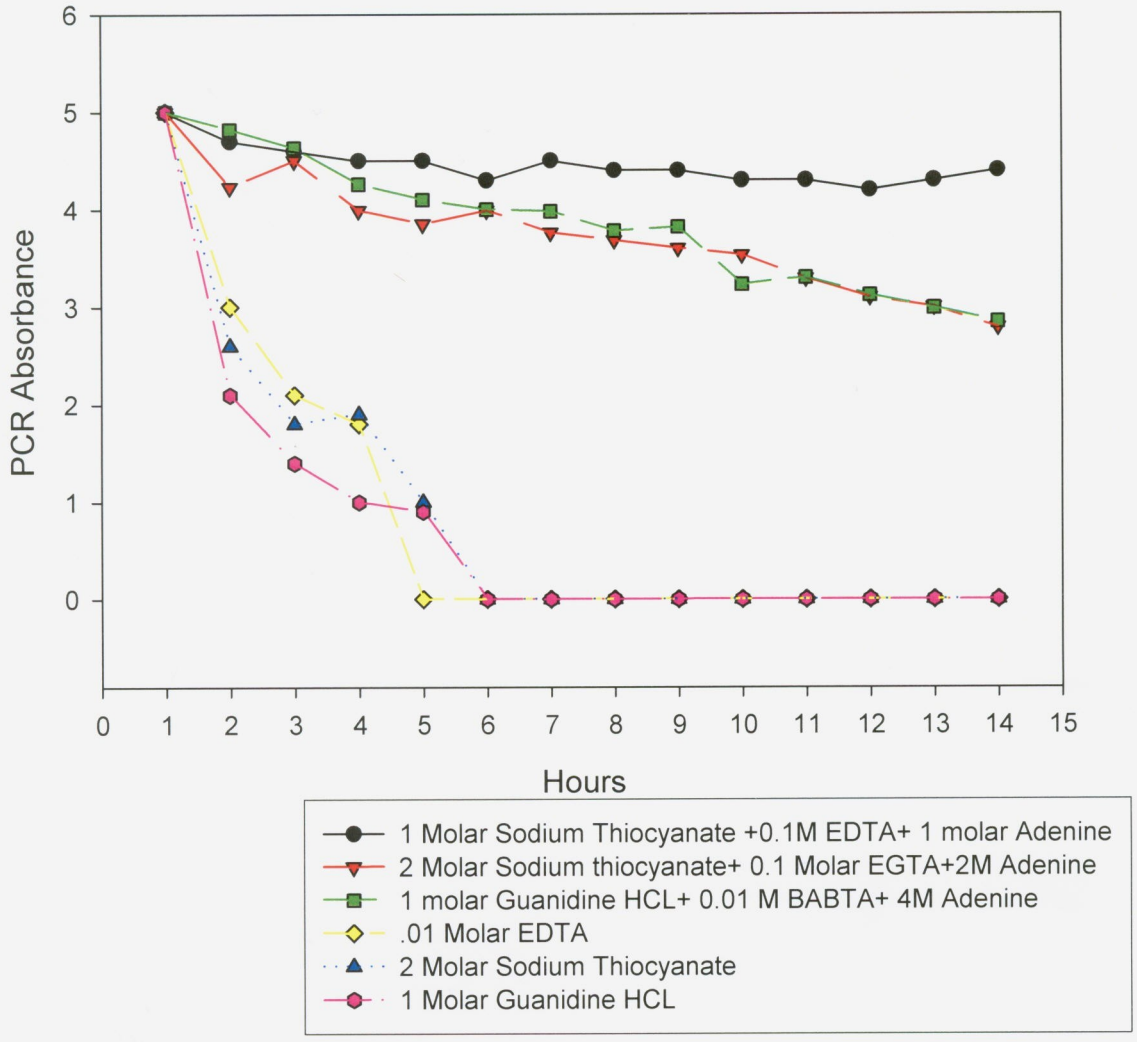
Comparison of single agents to combination agents
in protecting 1 pg of gonococcal DNA in human urine



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	1	2	3	4	5	6
1	5.0000	5.0000	5.0000	5.0000	5.0000	5.0000
2	4.7000	4.2300	4.8200	3.0000	2.6000	2.1000
3	4.6000	4.5000	4.6300	2.1000	1.8000	1.4000
4	4.5000	3.9900	4.2600	1.8000	1.9000	1.0000
5	4.5000	3.8500	4.1000	0.0000	1.0000	0.9000
6	4.3000	3.9900	4.0000	0.0000	0.0000	0.0000
7	4.5000	3.7600	3.9800	0.0000	0.0000	0.0000
8	4.4000	3.6800	3.7800	0.0000	0.0000	0.0000
9	4.4000	3.6000	3.8200	0.0000	0.0000	0.0000
10	4.3000	3.5300	3.2300	0.0000	0.0000	0.0000
11	4.3000	3.2900	3.3000	0.0000	0.0000	0.0000
12	4.2000	3.1000	3.1200	0.0000	0.0000	0.0000
13	4.3000	3.0000	2.9900	0.0000	0.0000	0.0000
14	4.4000	2.7900	2.8500	0.0000	0.0000	0.0000
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