21 DEC 96

EDTA.

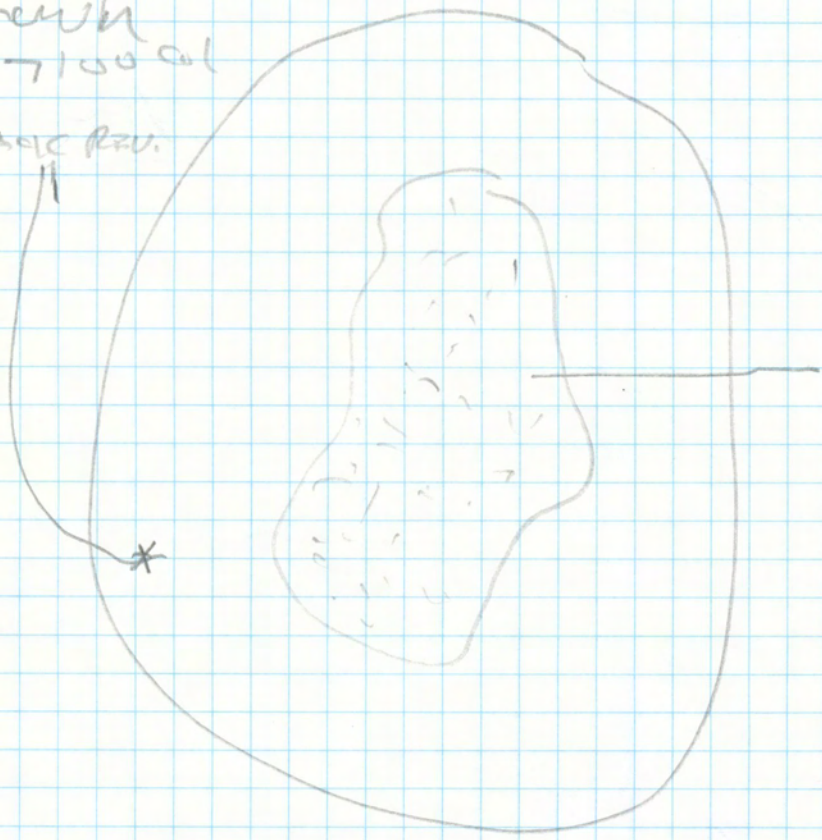
CON - 1 M.
Vol 100 ml
Pg CDC 98 f2 Stock
Freon Filtered wine
Amicon purification filter.
PLATES - BBL CHOC II Agar
Pg 3d 2 Hrs.
Incubated 48/W @ 32° 5% CO_2
H20 4 l sample

Mutant Lot # B-026

Results.

Crush
7100 Col

Basic Rev.



Control 7100 Col.
Lot # B-019

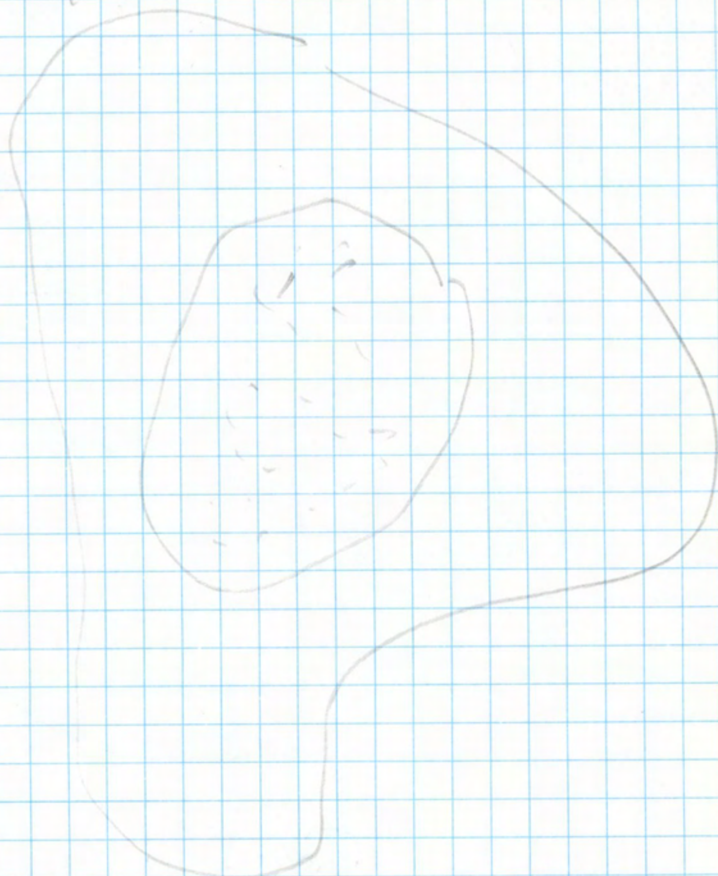
23 DEC 96

EGTA

Con - 1M.
Vol - 100 ml.
1 g c DC 98 f2 stock.
Fresh Filtered urine.
Amicon purification Filters.
PLATES 1 BBL CNA II Agar MUTANT Lot # B-046.
Aged 2 HrS.
Incubated 48 Hrs @ 37°C 5% CO_2
400 µl Sample.

Results

Growth >100 col.
Back grd. 0



Control > 100 col.
Lot # B-019.

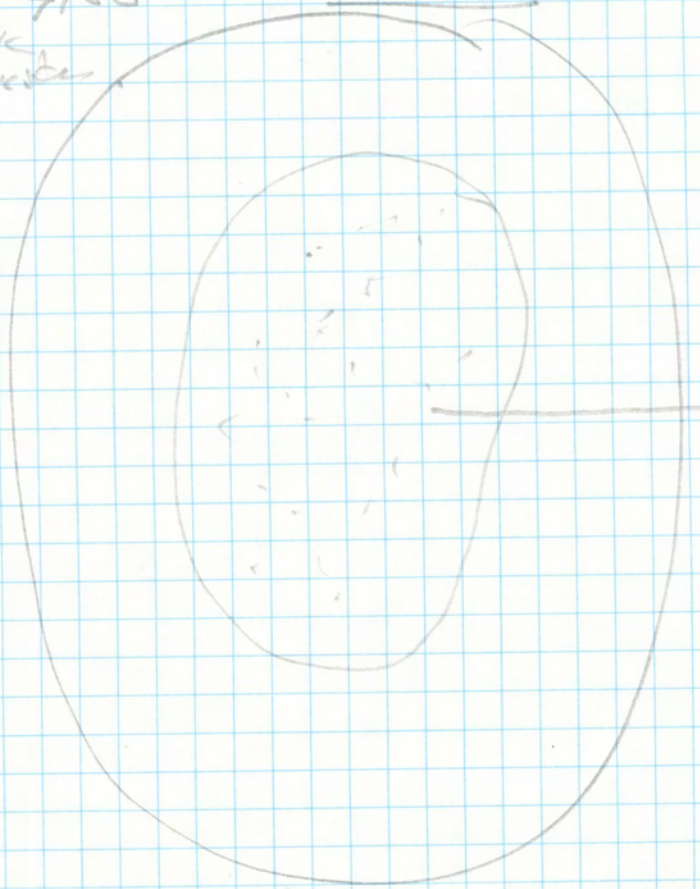
29 DEC 96.

BAPTA

Con 1 m
Vol 100 µl
1 mg cpc 98 f_2 stock
Fresh Filtered Urine
Millipore Purification Filter
PLATES BBL - Chec II Mutant Lot # B-016
100 µl Inoculum
Incubated 48 hrs @ 37°C 5% CO_2
400 µl SAMPLE.

Growth 7/10
NO Back
Round

RESULTS



Control
7/10
Lot # B-019.

FEB 12 97

Enzyme Challenge
DNA modifying Enzymes
Method Homogenous Liquid phase EIA. Direct primary
priming Antibodies conjugated to HRP.

1. E-coli Ligase T-4 monoclonal purified
2. E-coli polymerase T7 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.

Treated
Each Enzyme was exposed to a formula of DNA/RNA protect of 1M sodium Thiocyanate. And 0.1 M of EDTA. [illegible]

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

Substrate untreated Incubate 5 m?

Measurements were taken every Hour each Absorbance measurement 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 value converted to a scale 0-6 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	2.44	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

FEB 12 97

Enzyme Challenge II
DNA Modifying Enzymes

RESULTS:

The chemically treated enzymes were modified or destroyed due to the action of Sodium Thiocyanate and EDTA

22 FEB 92.

Methods: See Protocol of RNA Modifying Enzymes Dated - 12 FEB 92

	Primary Antibody(s)	
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	1.10	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	2.35	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 @ 1M Sodium Thiosulfate 0.1 M EDTA

July 19 97

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

① Controls 1pg/cDC93 for DNA-
② 1M Sodium Thiocyanate + EDTA 0.1 M.
③ 2M. Sodium Thiocyanate + EDTA 0.2M.

Taqs used

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

Target 1pg cDC93 DNA.

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

Target = 1pg cDC93 DNA from stock extracted per Qiagen 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	3.7	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	2.36	1.9	1.2	2.75	1.6	2.0	2.6	2.57

Results

1. 1 Molar sodium Thio and 0.1 molar EDTA-Inhibit on Amplif
2. 2 Molar sodium Thio and 0.1 molar EDTA - Inhibition on Taq Pol
3. There was significant variation in performance between Taqs.
4. All Enzymes amplified Template of Low Resolution

Follow up studies

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

october 29 97

Revised Sierra Master mix

DNA - Nucleotide sequence 5' to 3'

Primer set 1 - AGT TAT CTA CAC GAC GG
primer set 2 - GGC GTA CTA TTC ACT CT
primer set 3 - GCG TCA GAC CCC TAT CTA TAA ACT C

Formula

10 mM Tris (ph 8.3)
50 mM KCl
2 mM $MgCl_2$
50 µM Each Deoxyribonucleoside
2.5 units of Taq polymerase (Perkin Elmer)
5% Glycerol
50 pmol of primers / Ampl 2 per 100 µl Reaction

Notes

1. 95 µl of master mix per reaction.
2. Make master mix just prior to Dispensing into reaction tubes.
3. Thermal cycler - 3 cycles @ 94°C + 8 min @ 55°C
4. Amplify DNA by 25 s Denaturation at +94°C and a 25 s Annealing @ 55°C Total of 30 cycles.

June 23, 98 Stability of Gonococcal DNA in various concentrations of chemistry
1 pg gonococcal DNA from extraction was spiked into formulations & DNA/RNA protect. Samples were aged at 37°C for 5 days and run on CHT. 800 µl samples were used.

Formulations

1. 1M Guanidine Thiocyanate + 0.01 M. EDTA.
2. Sodium perchlorate 1M + 0.01 M EDTA.
3. Sodium Thiocyanate 1M + 0.01 M EDTA.
4. Guanidine 1M 0.001 EDTA.

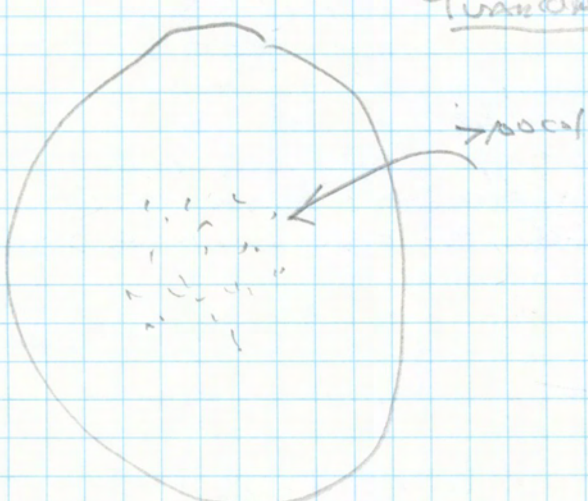
Results

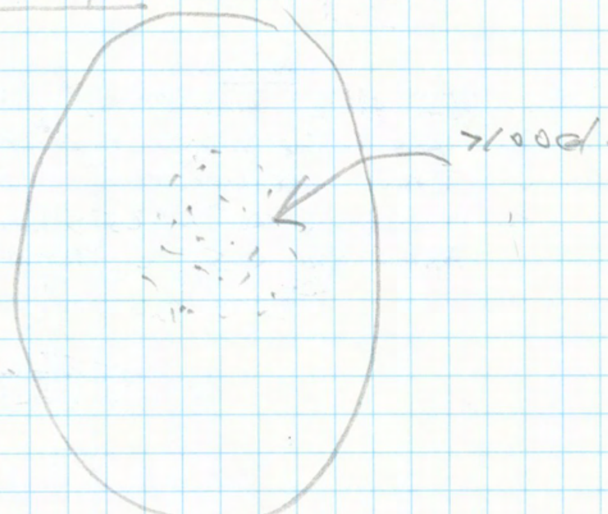
CHT Results

Time 0

7 Days

Guanidine Thiocyanate + 0.01 M EDTA

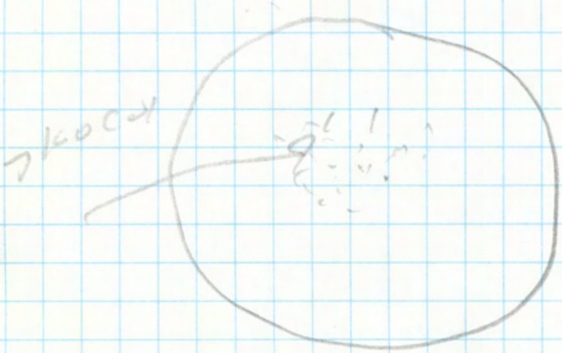


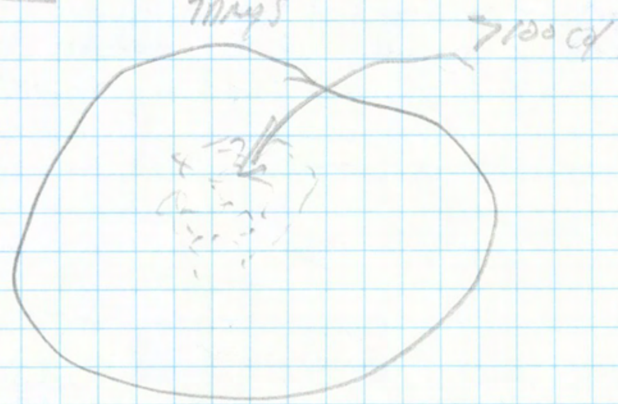


Sodium perchlorate + 0.01 M EDTA.

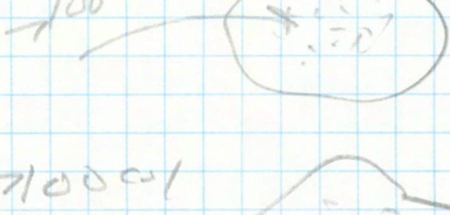
Time 0

7 Days





Sodium Thiocyanate + 0.01 EDTA



Guanidine 1M + 0.001 EDTA.

July 12 99.

Survival of 1pg Gonococcal DNA in Room Temp. In unprotected Urines.

Method 10 Urine were obtained from [illegible] and were Brought to Room Temp 26°C. Volume used was 2ml

Urines - Normal male Normal Dipstick Clean.

Normal Female

Diabetic Urine (Non Insulin Dependent)

Diabetic Urine (Insulin Dependent)

Acid Urine

Basic Urine

High Nitrates

E coli UTI > [illegible]

Mixed UTI (Spinal Cord Inj.).

Urine was Inoculated with 1pg Gonococcal DNA and Incubated for 6 Hours. The G-II was used as the marker for Viable DNA. Colonies were counted on BSL Choc II plates.

Results

HR	N Male	N Female	Diabetic ND	Diabetic ID	Acid Urine	Basic Urine	E-coli UTI	Mixed Spinal Injury	Preg F [illegible]
1	200	200	200	200	200	200	200	200	200
2	103	115	110	102	101	106	100	103	105
3	65	56	55	48	51	53	51	56	58
4	46	39	43	38	37	41	43	47	49
5	12	14	13	11	14	9	10	10	9
6	0	0	0	0	0	0	0	0	0

Result - All urines Degraded Gonococcal DNA At About 5 Hours in Urine > [illegible]°C.

Notes - Data analyzed at 4 Hours and 6 Hours. G-II Controls were >100 Choc II plates were not [illegible].
No Background [illegible] on plate Results were [illegible] Control.

July 22 99

Eight Day survival study 1µg Gonococcal DNA Biologically Active in Fresh Urine.

10 ml fresh urine was inoculated with 1µg DNA from GC 98 DNA extracted via shown method - Hood(?) samples. GT1 frozen controls used. Temp 37°C

Results	# Transformants	Controls	Background
Day 0	>100	>100	2
Day 2	0	>100	1
Day 3	0	>100	0
Day 4	0	>100	1
Day 5	0	>100	2
Day 6	0	>100	0
Day 7	0	>100	3
Day 8.	0	>100	1

Summary

Biologically Active Gonococcal DNA Did Not survive past 1 Day.

[illegible signature]

August 1998

Survival study 1 µg gonococcal DNA in normal and disease state serum.

Pt disease was spiked with 1 µg of gonococcal DNA and incubated for 10 hrs. 800 µl samples were taken every hour for 10 hrs. Controls were Pt GX controls.

Results # transformants.

HR	N-serum M	N-serum F	Abnormal ser M	AB-serum F	Controls	Pt
1	110	100	32	61	>100	1
2	87	91	38	31	>100	1
3	42	55	29	22	>100	0
4	25	32	10	0	>100	2
5	17	20	0	0	>100	1
6	14	10	0	0	>100	0
7	0	10	0	0	>100	0
8	0	0	0	0	>100	1
9	0	0	0	0	>100	2
10	0	0	0	0	>100	0

Results: Gonococcal DNA is destroyed in fresh Pt serum. Results seem to parallel what is seen in urine - no analysis of enzymes present in serum.

— [initials] —

August 22 98

Eight Hour Survival Study of DNA/RNA protect for Fresh Urine.

Method

100 ml fresh urine was collected.
10 ml urine was spiked with 1 pg GPC 98 DNA. Temp 37°C
Protected urine was Sodium Thiocyanate 1M + EDTA 0.01 M
PCR was Run using standard screen primers. Antibodies were Tagged with HRP.
Samples of Amplification Reaction mix were Sampled Every Hr.

Results

	DNA/RNA protect	Unprotected
Hr. 0	3.7	3.2
1	3.7	3.4
2	3.1	2.6
3	3.0	2.0
4	3.0	1.7
5	3.0	1.0
6	3.1	0.6
7	2.9	0

Results

Sodium Thiocyanate + 0.1 M EDTA is protective of Gonococcal DNA at 37°C for 7 Hours.

[signature]

Sept 19, 98

Single Agent Protection of 1pg Gonococcal DNA

Method

CTT used for detection
Fresh Unil @ 37°C
1pg gonococcal DNA CDC 98 Sierra Extraction 0.5 pg. method.
2 BDC/GC 2 Agar.
3 Target Agent 24hrs @ 37°C.
Time Sampled 0, 7, 16, 24 hrs.

Results

Chloroform 1 Sarca 1 M.

	HR	Transformants	Control		BK
	0	200	7200	(TNTC)	2
	7	0	7200	(TNTC)	0
Sept 19	16	0	7200	TNTC)	1
	24	0	7200	TNTC	2

EDTA 1M

	HR				
	0	200	7200	TNTC	0
	7	0	7200	TNTC	0
Sept 20	16	0	7200	TNTC	1
	24	0	7200	TNTC	1

EGTA 1M

	HR				
	0	200	7200	TNTC	0
	7	0	7200	TNTC	1
Sept 21	16	0	7200	TNTC	0
	24	0	7200	TNTC	0

Sodium salicylate

	HR				
	0	200	7200	TNTC	1
	7	6	7200	TNTC	0
Sept 22	16	0	7200	TNTC	0
	24	0	7200	TNTC	2

Guanidine HCl 1M

	HR			
	0	200	7100	1
	7	0	7100	1
Sept 23	16	0	7100	0
	24	0	7100	1

Sodium Thiocyanate 1M

	HR			
	0	200	7200	0
	7	10	7200	1
Sept 24	16	0	7200	0
	24	0	7200	0

Sodium perchlorate 1M.

	0	200	7200	1
Sept 25	7	0	7200	2
	16	0	7200	0
	24	0	7200	0

SEPT 19 <u>cont.</u> Conclusion Single agent [illegible] not [illegible]
at concentration of TH1 at 7 days.
[illegible]

Nov 12 99.

Single agent vs combination agents for protection of gonococcal DNA in [illegible]

Methods

The experiment extended over a period of 5 weeks.

10 ml of urine was inoculated with 10⁶ gonococcal [illegible] and incubated for a total of 60 hours. PCR was done [illegible] + [illegible] added to reaction vial [illegible] @ 540, 0.01 M of chelators + 1 M of enhancers (chaotropes).

DNA was extracted using [illegible] extraction procedure.

Each [illegible] 60 [illegible].

Results

HR	EGTA 0.01 M + Guanidine 1M	EGTA 0.1 M + Sodium Thio (1M)	EGTA 0.1M + Sodium Perchlorate (1M)	EDTA + Sodium Thiocyanate 1M	EDTA 0.1 M + Sodium Perchlorate	PBS Buffer No prot.	Guanidine
0	75.0	75.0	75.0	75.0	75.0	75.0	75.0
10	4.0	3.9	4.1	4.2	4.0	0.12	0
15	3.8	3.6	3.7	3.8	3.45	0	0
20	3.4	3.2	3.56	3.8	3.22	0	0
25	3.22	3.0	3.12	3.43	3.0	0	0
30	3.0	2.70	2.86	3.2	2.39	0	0
35	2.8	2.65	2.40	3.0	3.10	0	0
40	2.7	2.55	2.63?	2.74	2.99	0	0
50	2.5	2.76	2.54	2.66	2.72	0	0
60	2.4	2.57	2.62	2.52	2.62	0	0

HR	EDTA 0.01M	EGTA 0.01M	BAPTA 0.01M	Sodium Perchlorate 1M	Sodium Thiocyanate	LiCl 1M
0	75	75	75	75	75	75
10	0.25	0	0	0	0.30	0
15	0	0	0	0	0	0
20	0	0	0	0	0	0
25	0	0	0	0	0	0
30	0	0	0	0	0	0
35	0	0	0	0	0	0
40	0	0	0	0	0	0
50	0	0	0	0	0	0
60	0	0	0	0	0	0

[illegible signature]

FEB 22 99. spiked concentrations

Effects of Methemoglobin on amplification of Gonococcal DNA in Serum. Serum Treated at 37°C and All aliquoted into [illegible] vessels.

Method: 1 µg of Gonococcal DNA Extracted from CDC [illegible]. PCR was run against increasing concentrations of Methemoglobin. Concentrations were 14 gm/dL ——→ 0 gm/dL. Urine was spun, collected and filtered to sterility.
400 µl samples were processed at [illegible] and [illegible]
5%. Samples incubated at 37°C for 10 min prior to processing.

Results

Methemoglobin g/dL	Absorbance
14	0
13	0
12	0
11	0
10	1
9	1.2
8	1.8
7	1.9
6	2.2
5	2.5
4	3.0
3	3.5
2	3.7
1	4.2
0	

Results:
Methemoglobin is an inhibitor of PCR at concentrations of > 10 mg/dL of inhibitor.
Note: Need clinical follow-up to confirm. Clinical samples may have more than one inhibitor. Need to evaluate another molecular platform.