

20 Aug 06

Chemistry Forward
Analysis

10

PASS

Chemical: Sodium Trisulfate

Con: 1M In TRIS H₂O Vol = 100 ml.

Target 1g conc 98 extract constant method.

Maxwell Urine.

Agar 2 HR @ +27°C

Mutant lot # B-019

American 100 Filter unit to clean up RNA.

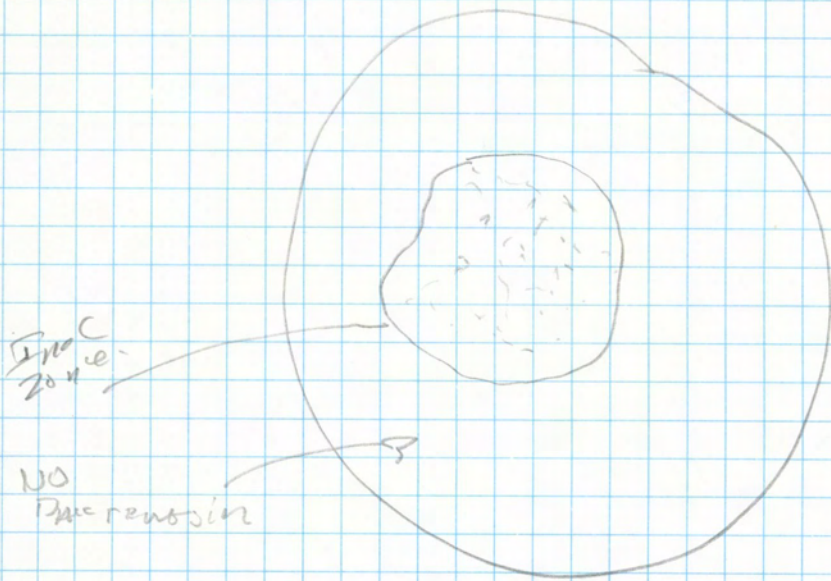
Control

Laurel 4004C Purified RNA on BBL c140 R plate.

Incubated 48 HR Dial - plus. @ 37°C + 5% CO₂

Cult Results

PASS 7 100 colonies
conclusion INTACT RNA



Control 7 100 col.
400 4004C control source
Extract.

Lot. B-018

20 August 06

—

24 Aug 96

Chemistry

Fail

Calcium Acetate.

Con - 1M

Vol 100 ml DLR H₂O

1 ml sample used.

10g conc 98

Age 2 hrs

Urine Fresh filtered.

American Filter for purification.

BBL choc II plates - Control mutant lot # B020

Incubate 48 hrs @ 37°C 5% CO₂

CT Result.

15 colonies

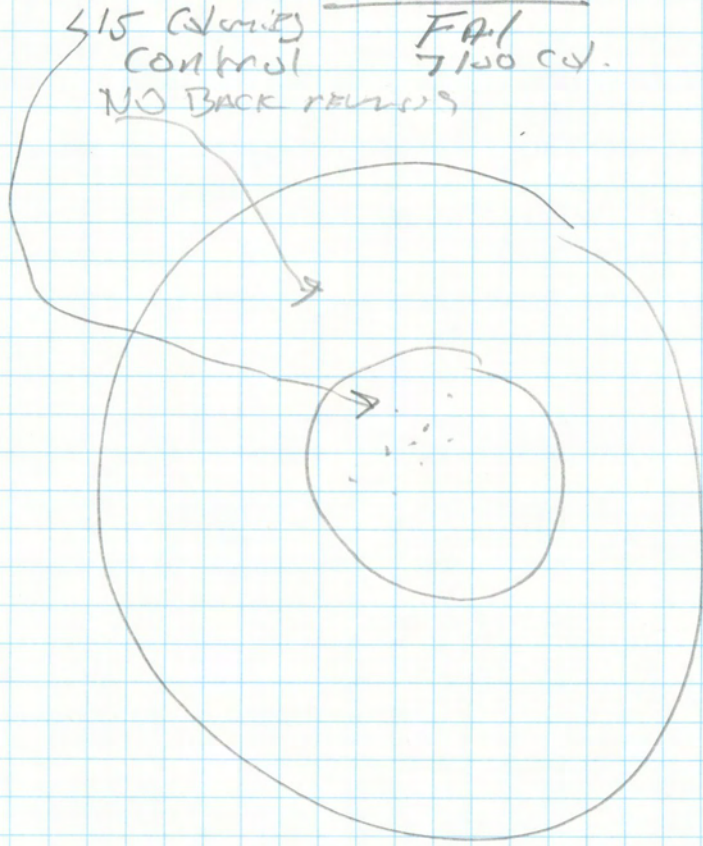
control

NO BACK REACTION

Fail

7100 col.

Fail



Control 7100 col
with 0.1%

TH

10 Sept 96

Chemistry: Caprylic Acid.

FAIL

Con- Jm.

Vol 100 ml

1 pg CDC 93 From stock

1 ml sample

Age 2 hrs @ 25°C

From Filtered urine.

Clean up - Amicon Filter.

1371 CDC II plates.

CREONSTAT Mutant Lot # B-0900

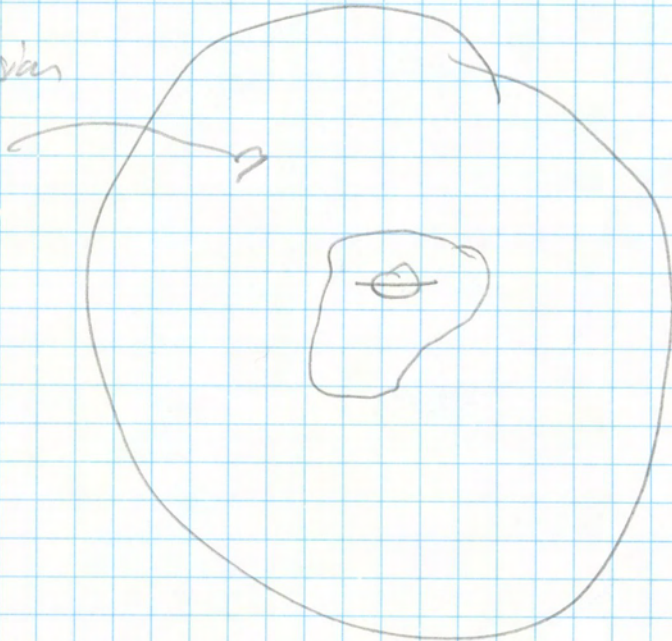
Inoculate 40 plates @ 300 5% CO₂

GTT Results

0 colonies

FAIL

NO
BACKMUTATION



Control = 7/100 col
Lot # 09

—

12 Sept 08

Chemistry LAURYL SARCOSINE

CON 1M

Volume 100ml

1ml sample

1g CDC 98 target from stocks

Age 2 hrs

Urine Fresh Filtered

Amicon purification Filter 24 P100

PLATES BDL class II CANTOSTAT mutant Lot # B-020

Inoculation 400 µl EXTRA

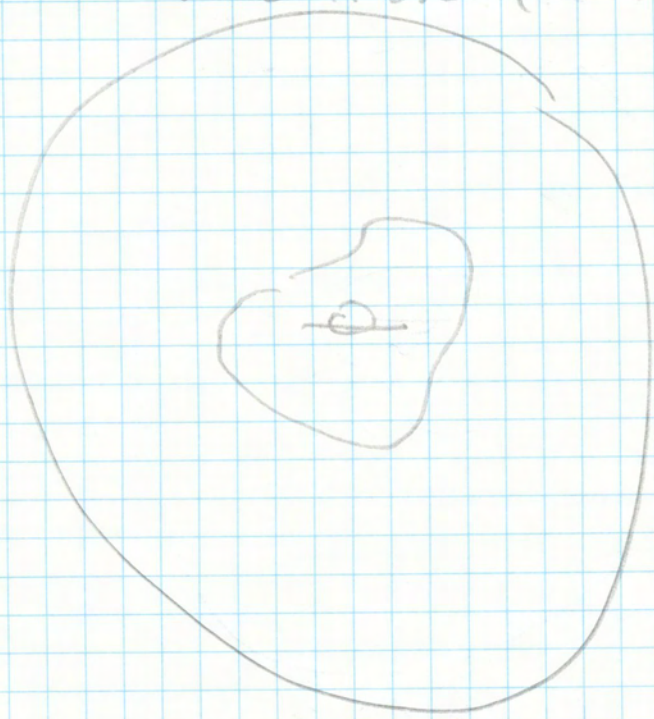
Inoculated 48 (10) C37E 37C

RESULTS.

0 Colonies

NO Backcrossing

Fail



Control 7/0 Col.
lot # G19

~

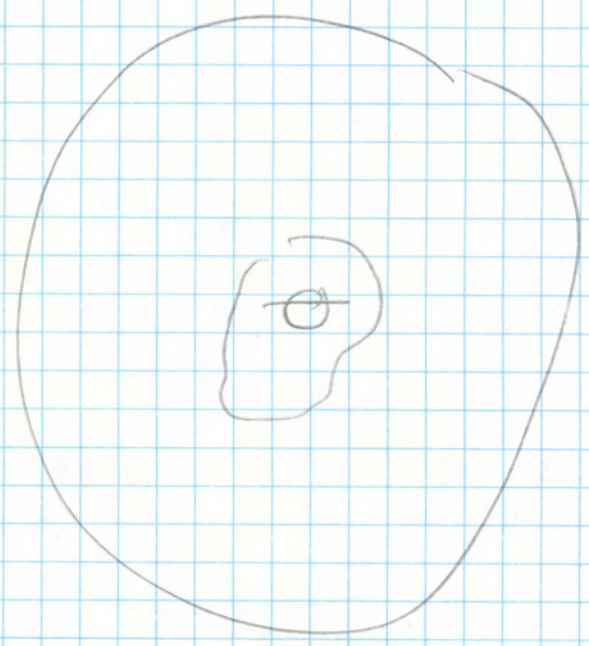
12 sept 96

Chemistry Polyvinyl Chloride

Con 1m.
 Vol 100 ml.
 pp cpc 98 From Fz store. 50
 Age 2 hrs
 Urine Fresh Flin 2p Fluor
 American Filter purification
 BSL class II path. Gonospat m/fmt. with B-GZO
 Incubation 400 ml Extract
 Incubated 48 hrs @ 37°C 5% CO₂

Results

4 Colonies Fail
 NO Bacillus -



Control 7/100 col.
 hold/ag

v6

21 SEPT 06

CHEMISTRY: Sodium m-Periodate

CON - [M.]

Vol = 100 ml

1 ml sample

1 pg concn Fe stocks

Age 2 hrs

Urine Fresh Filtered 24/5/11

Amicon purification Filter

Plates Biorad II

Colony count mutant lot H-0.020

Inoculum 100% Extra

Incubated 48 hrs

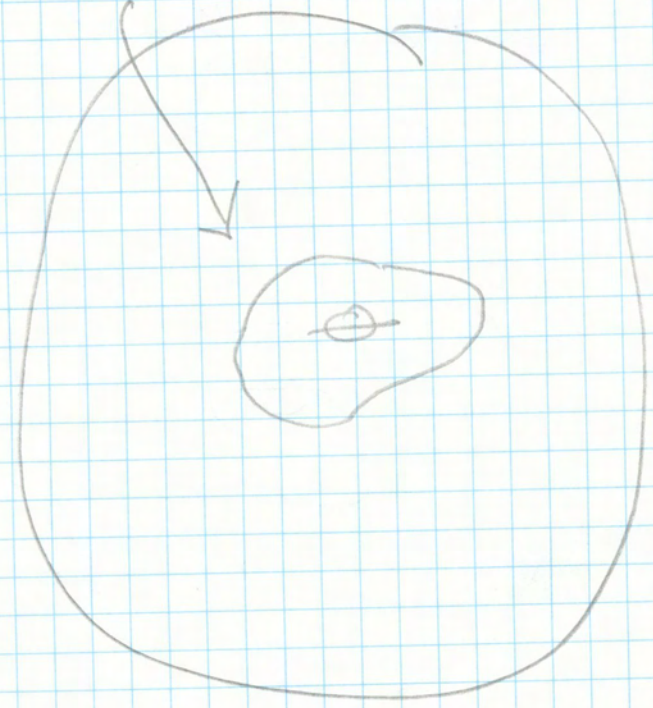
25% CO₂ 37°C

Results

H Colours

Fail

No Backscattering



Control 7/100

lot B-019

[Handwritten signature]

23 Sept 96

CHEMISTRY: TRICHLOROACETIC ACID

Con: 1 M.

Vol = 100 μ l.

1 μ l CDL 98 from F2 stocks.

Time 2 hrs.

Urine FRESH Filtered 2 μ Filter.

Amicon purification Filter.

PLATES BSL check Counted lot # B-020

Inoculum 100 μ l.

1ml Sample

30 μ l.

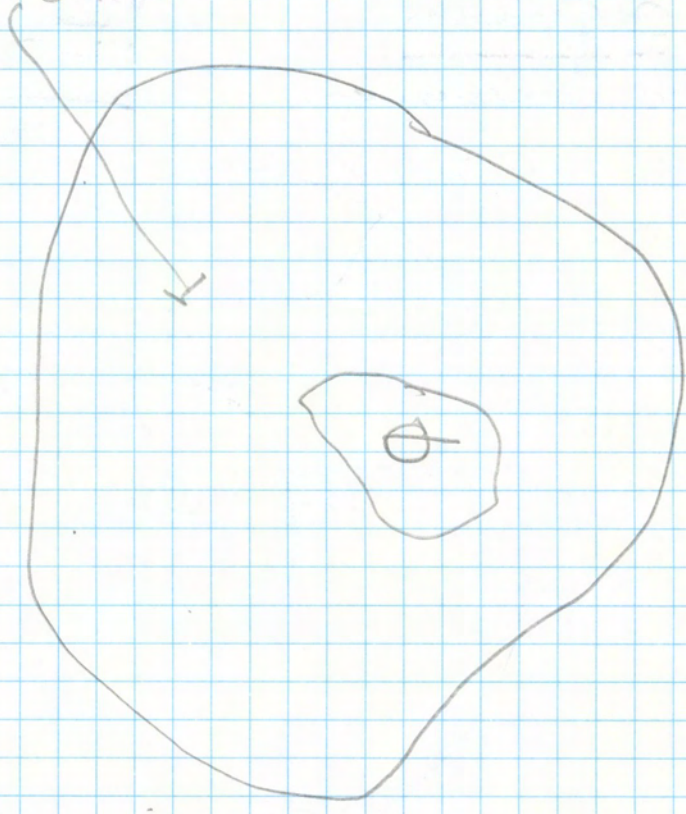
Incubated 48 hr @ 37°C

Results

0 colonies.

FAIL

NO Backreaction



Control 7/100
lot B-019

27 Sept 96

CHEMISTRY: UREA

1 ml sample FAI

Con - 1 ml

Vol 100 ml

100 mg CDC 98 Protein Stock

5041

Agd 2 hrs @ 25°C

Urine From Filtered

Amies purification Filter

DIETES DBL C6C R

Conostat Nutrient Lot B-020

Inoculum 400 µl

Filteral size

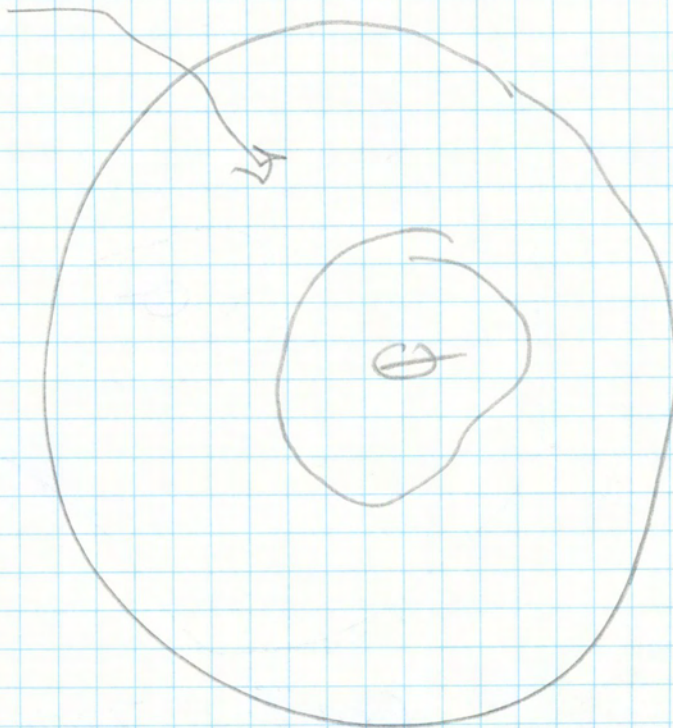
Incubated 48 hrs @ 37°C $\frac{1}{2}$ Ca

RESULTS

0 Growth

No Backreaction

FAI



Control 7/100
Lot #13-09

[Handwritten signature]

02 Oct 96

CHEMISTRY: Inocul.

FAIL

Con 1 M

Vol = 100 ml

Yp, CDC 98 From Extraction Control 2nd 50%
Age - 2 hrs

1ml sample

Urine: Fresh Filtered

2µm can Purification Filter

Plates BBL ChC II 99%

Enoculum 400 µl

Control Lot # B-020

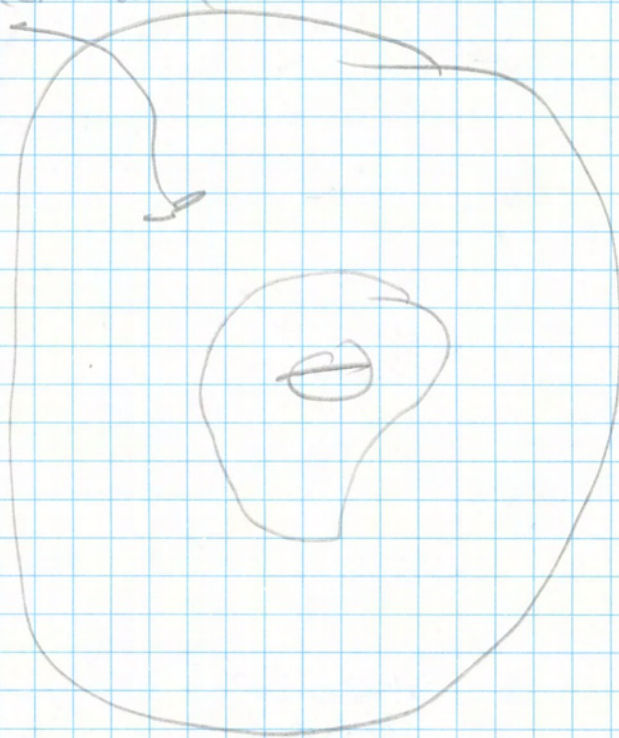
Inoculated 4/3/96 37°C 5% CO₂

RESULTS

7 Colonies

No Bacteremia

FAIL



Control = 7/100

Lot # B-019

as outgo

TRASS

Chemistry Li-chloride.

CON-14.

Vol = 100 ml

1ml sample

1pc - Cocq8 from F₂ stock

50%.

Age 2 hrs. @ 25°C

Amicon purification filter

PLATES BOKKOE II

Constant mutant host # B-020

Incubation 400 yf

Filtered urine.

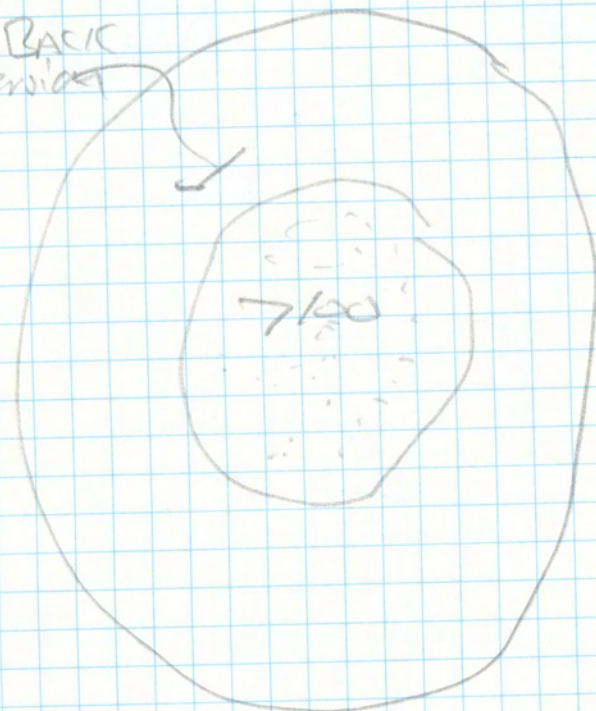
Incubated

48 hrs @ 37°C 5% CO₂

RESULTS

TRASS → 100 col.

NO BASIC
PROPERTIES



Control = 7/100
B-019

TR

08 Oct 96

CHEMISTRY Ammonium Thiocyanate

CON - 1 M.

Vol = 100 ml

1/2 cpc 98 from Fe stock 50%.

Pre 2 hrs @ 25°C

Ammonium purification Filter.

Plates B12L Choc II

Consistent Mutant Rys

Filtered urine

Inoculum: 400 µl

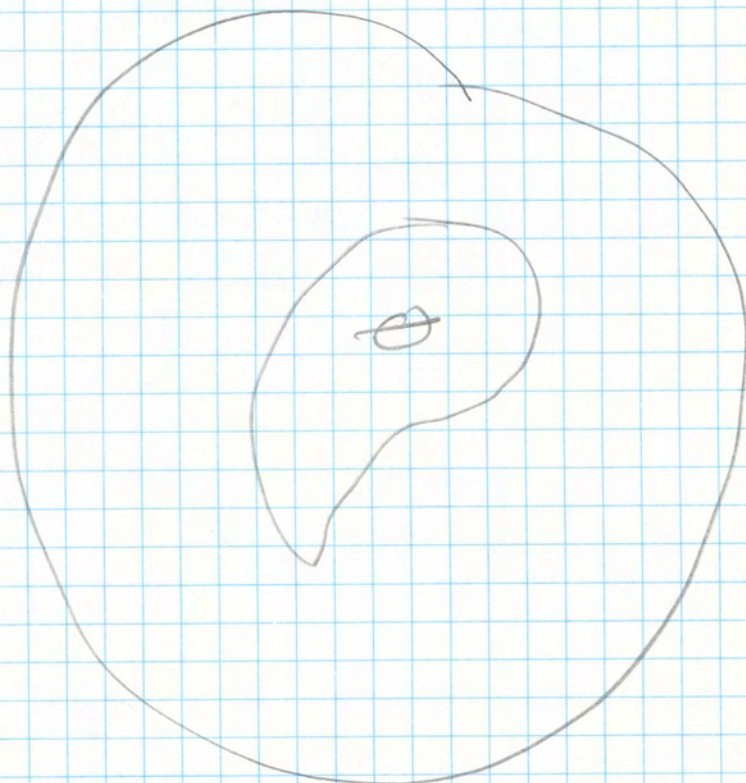
Lot# B-020

Incubated 48 hrs

Results

Fail 0 Colonies
NO BACK REVERSION

Fail



Control 7100 Colonies
Lot# B-019

111 15 Oct 96

CHEMISTRU: LITHIUM HEPARIN

PASS

Toxic to PCR

CON 1-M.

Vol - 100 ml.

1 pg CDC 98 From E2 stock

1 ml sample.

50 yr sample.

AGE 2 hrs @ 25°C

Amicon purification Filter.

PLATE B32 check

Control lot # 13-020

Inoculum - 4/0 ul

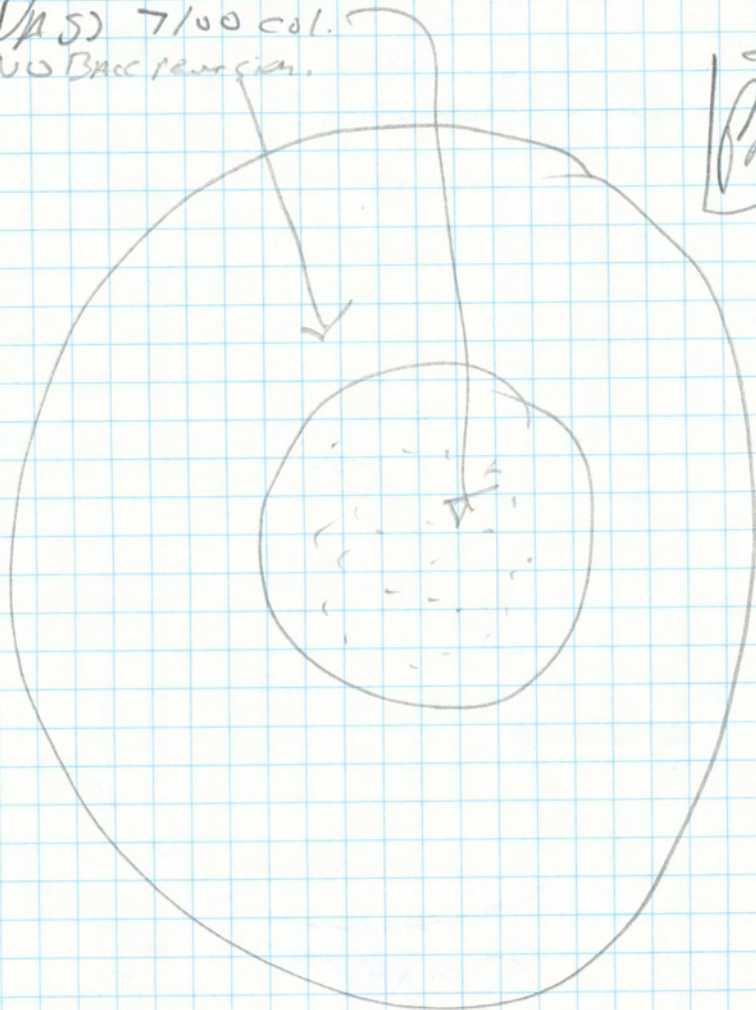
Filtrate urine.

RESULTS

Incubated 40/65 @ 37°C 5/6

PASS 7/100 col.
NO Bacc reaction.

PASS



Control 7/100 col.
13-019

T

21 Oct 96 Chemistry: FERRIC CHLORIDE

FAIL

CON-141

Vol 100 ml 1ml sample.

1pg CDC 98 F₂ sample.

50 yl. extract

ADON CON Purification Filter.

PLATED BGL CHOC II

CONSTAT Lot# B-020

Inoculum - 400 yl.

Filtered urine.

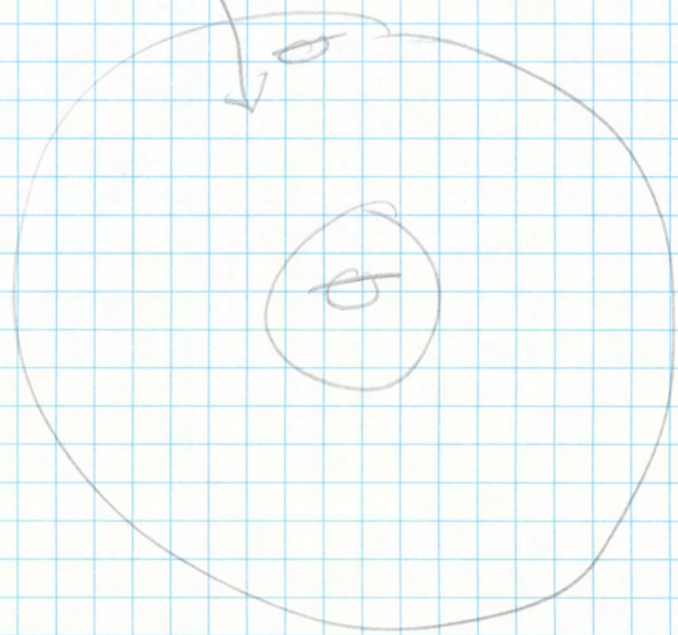
Inoculated 48 hrs. @ 37°C 5% CO₂

Result:

FAIL

NO growth or colonies

NO BACK INVERSION



Controls 1/100 col.

B-019

25 Oct 96 CHEMISTRY: Sulfanilic Acid

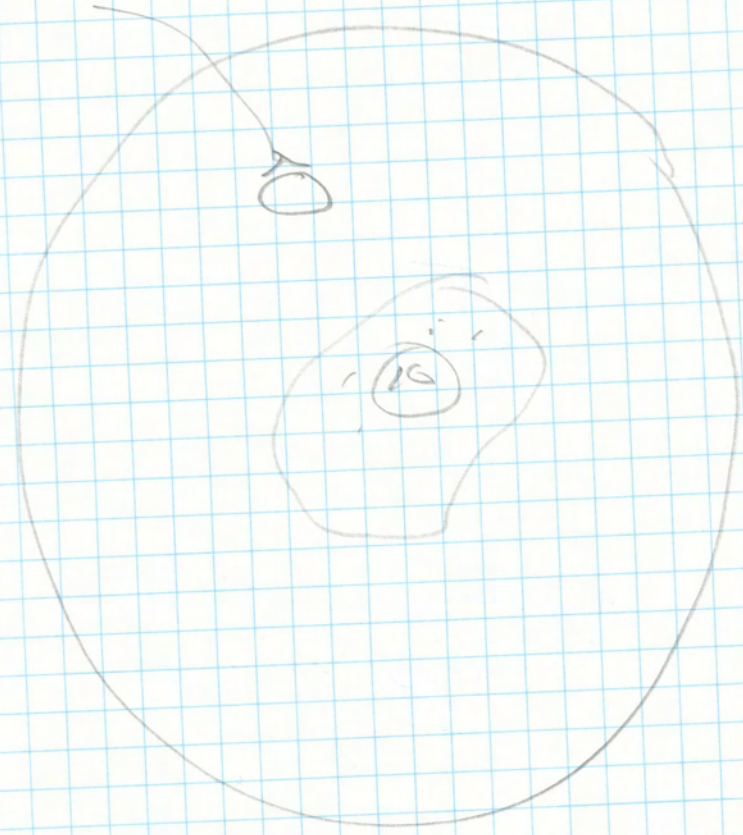
FAIL

CON 1M.
Vol 100 ml
1M CAC 98
1M Pge 2 H₂O
From F₂ stores.
1 ml sample
50 µl sample
Ami Con Filtrus purification
PIATES - BBL choc II
Inoculum 400 µl
Filtered urine.
mutant Lot # B-020
Incubated 48 hrs. 37°C 5% CO₂

RESULT

10 colonies
O Dark purple

FAIL



Control 7/100 col.
lot # B-019

12 Nov 78
Chesley

Ammonium Oxidate

FBI

CON-1M

Vol 100 ml.

1% CAC 98 From F2 stocks

50% sample

Age 2 hrs.

American purification filter:

MATES BBL CAC FF

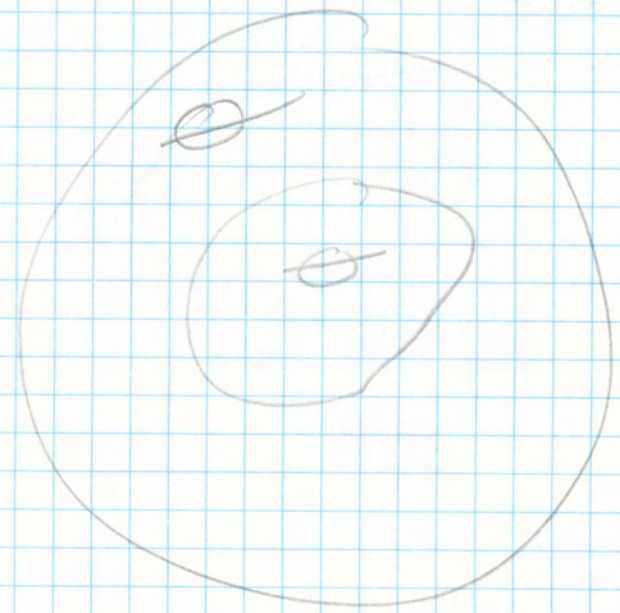
Mutant Lot # B-020

Filtered yrile.

Incubated 48 hrs @ 37°C 5% CO₂

RESULTS

- Colours
- BACTERIAL



FBI

Controls > 100%
13-019

—

ISNOX 96
BARIUM CHLORIDE

FAIL

CON-1M

Vol 100 ml

1 ps CPC 9g

Age 2 Hour

Amicon Purification Filter

PLATES DBL CHOC II

INOCULUM 400 ul

Filtered urine

Incubated 48 hrs @ 27°C 5% CO₂

1 ml sample
Fresh Extract Pro 2 CA scrub

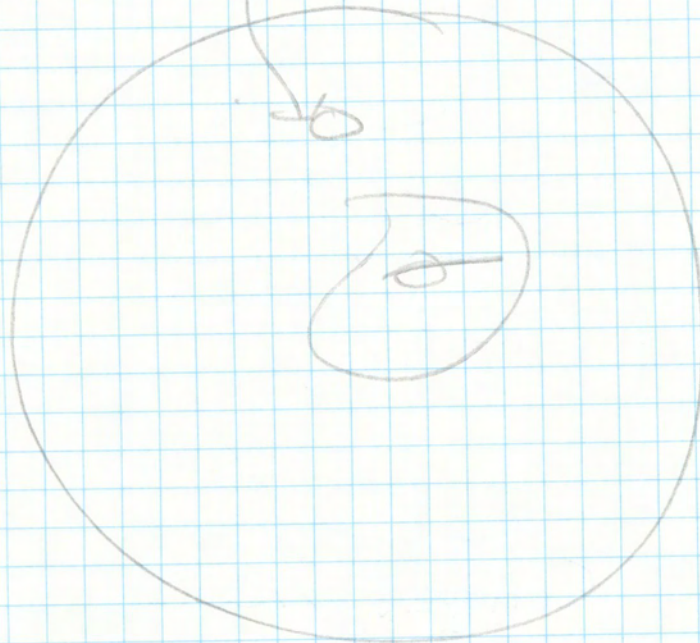
CRONOSAT nutrient

Lot # 13-020

RESULTS

FAIL

- Growth
- BACIGRAMS



Control > 100 col

13-019

10 Nov 96

Cesium Chloride

Fail

CON 1-M.

Vol - 100 ml.

1 kg Sample

1 g concn Fresh Extract From QA Samples

AGE 2 hrs.

Amicon Purification Filter

Two columns 400 ml.

PLATES BBH CHOCTHAPAL

Lot # B-030

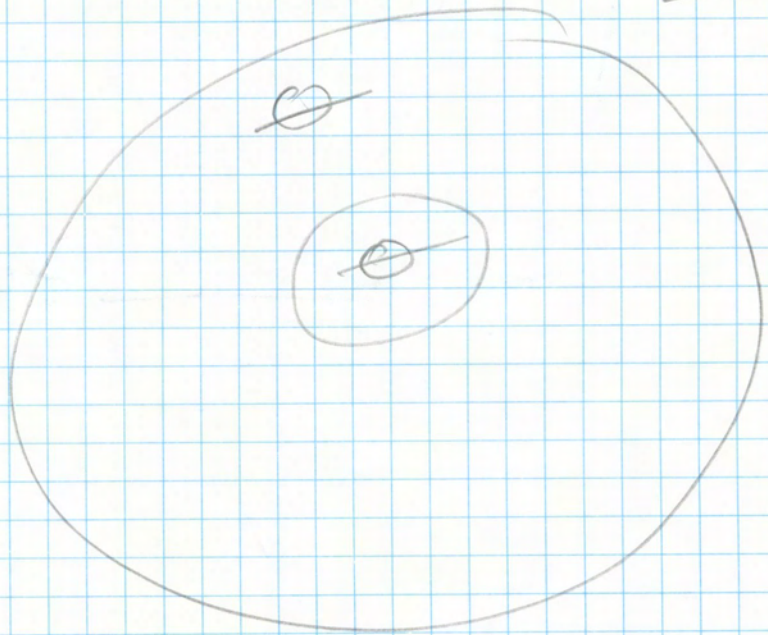
Filtered urine.

Inoculated 4 vials 37°C (5), Co2

RESULTS

- Growth
- BACK REVERSION

Fail



Controls 7/100 Col.
Lot # B-030

22 NOV 98

GUANIDINE.

CAN-1M

VOL 100 ml

1g CD egg E stocks

Agar 2/15

Yeast BCL choc #

American purification filters

Incubation 50°C

Filtered urine 2g

Incubated 48 hrs 5% CO₂

RESULTS

7100 colonies

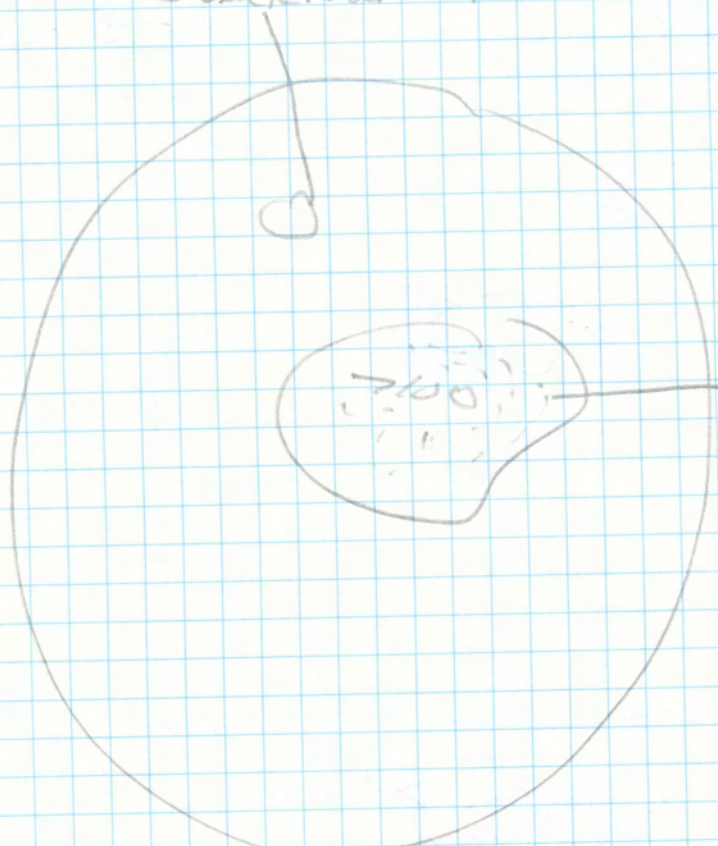
0 Back reaction

PASS

1 ml sample
50 µl

GONOSTAT method
Lot# B-020

PASS



Control Cell morphology

Controls: 7100 col
Lot# B-019

TR

26 Nov 96 MAGNESIUM ACETATE

FAIL

CON-1M:

Vol. 100 ml

1 ml sample.

Filtered vial. 12

18 cpc 18 F₂ stocks.

Agar 2 Hours.

Antibiotic BBL chc II

Control mutant

Amicon Purification Filter

Lot # B-020

Inoculum 50%

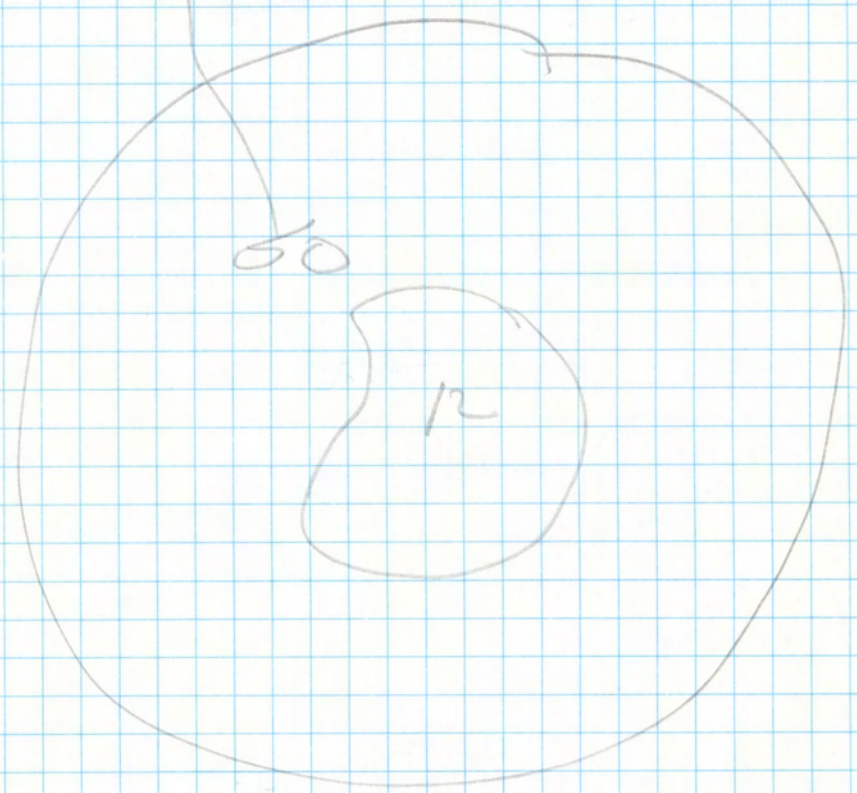
Incubated 40 hrs @ 37°C 5% CO₂

RESULTS

FAIL

12 colonies

↳ BACKBROTH



Controls > 100 cpc.

Lot # B-019

7/15/96

SODIUM SALICYLATE

PASS

CON - 1M
 VOL = 100 ML
 1.6 CDE 98 F₂ STOCKS
 AGED 2 HRS
 PLATES DBL CHITR
 FRESH FILTERED URINE
 100 μ l Urine
 Total tested 43 Hrs @ 37°C 5% CO₂

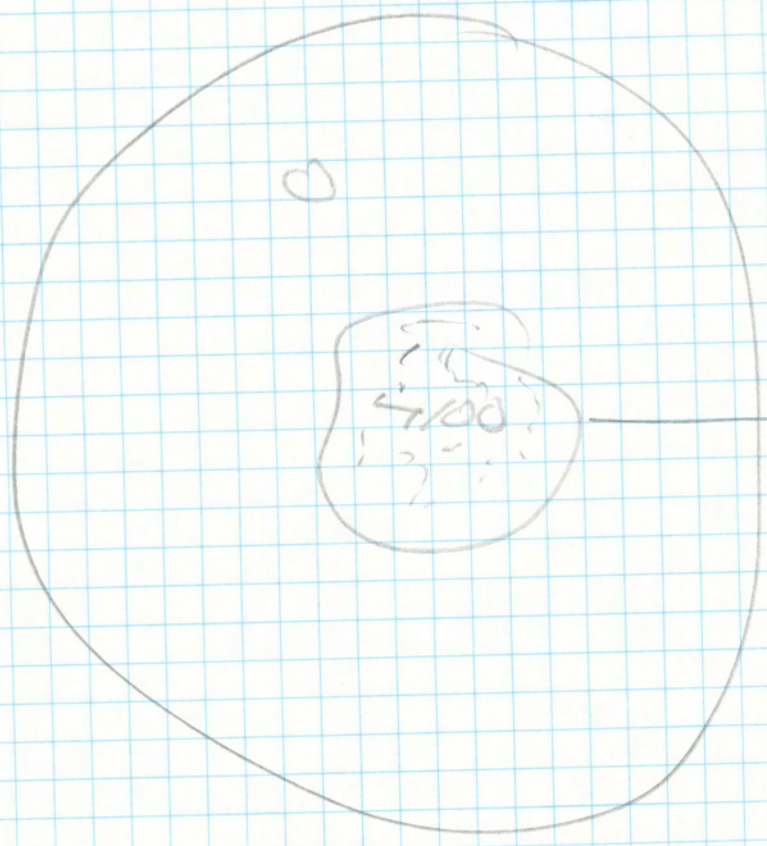
1 ml Sample
 50 μ l

CROCODAR Lot # B-020
 "

RESULTS

7100 Colonies
 O BACITRACIN

PASS



Control
 7100

Control 7100
 B-019

4/1/22 C96 POTASSIUM ACETATE

FAIL

Con - 1M.
Vol - 100 ml.
COC98 From FL Stock
Aged 2 hours.
PLATES BBL chct#
Fresh Filtered urine.

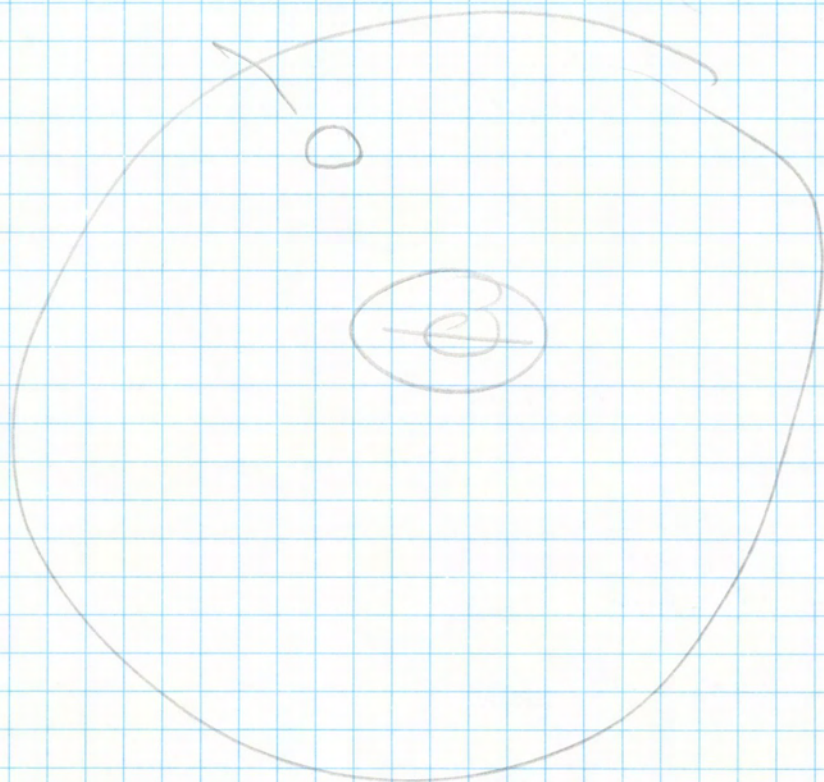
CONSTAT Lot# R220

400 ul sample.
Incubated 48 hrs @ 37°C

RESULTS

FAIL

- o colonies
- o BACK formation



Controls 7/00
B-019

05 DEC 96

MESO-TETRA (N-Methyl-4PYRIDYL) PYRPHYZIN

CON - 1 M
Vol - 100 ml
AGED 2 HRS
FRESH Filtered Urine
PLATES BBL CHOCTI AGAR
Ami con purification FILTER
400 ul sample
1 pk conc 98 E ster.

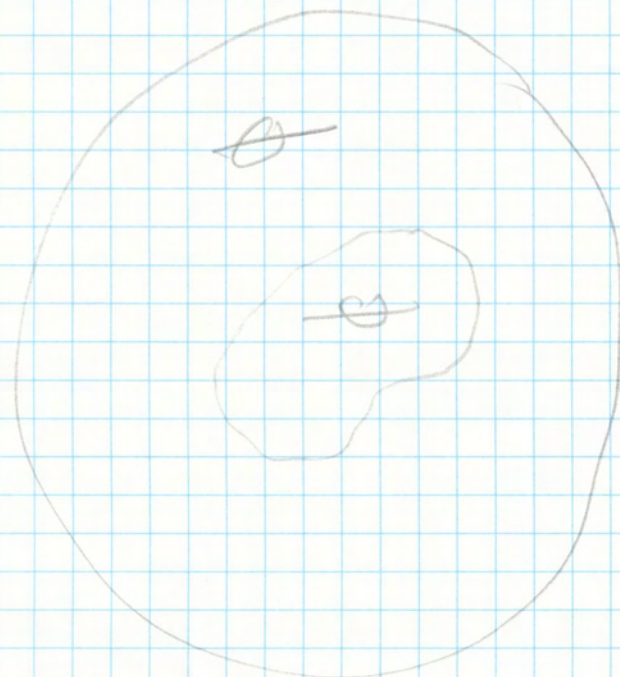
Fail

INSTANT Lot E13-020

Incubated 48 HRS @ 37°C
5/1/02

RESULTS

- Growth
- Back-titration



Fail

Control 7/00
B-019.

TRIFLAMETHYL AMMONIUM CHLORIDE

10 DEC 96
CON - 1M
VOL = 100 ml
AGED 2 HRS -
FRESH URINE
PLATES BBL 200 BBL
AMICON Purification Filter
400 ml sample
CDE 98 From fe stores -
Inoculated
48 HRS

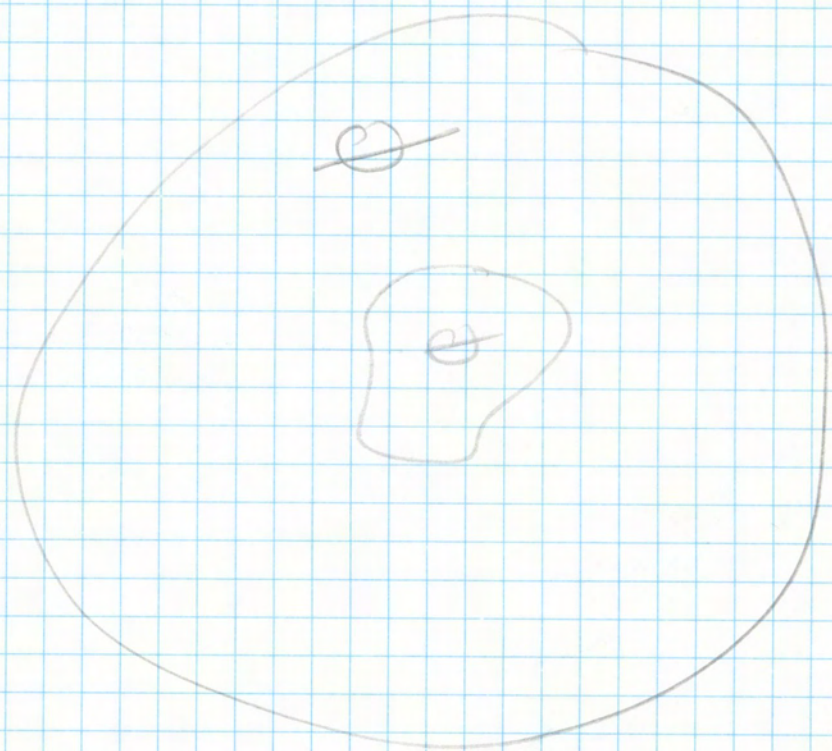
1 ml Sample (Fail)

CONSTANT MUTANT
Lot # B-046

RESULTS

Fail

- Growth
- Back-reversion



CONTROL 5 = 7/100 GI.
Lot # B-019

—

12 DEC 96

SODIUM PERYCHLORATE

PASS

CON - 1 M.

Vol - 100 ml

1 ml Sample.
50 pl.

1 pg CDC 980 f₂ stock.

AS 30 2 hrs

FRESH Filtered urine.

PLATES BBA CHECKS

American purification filter

100 pl sample.

Goodwater lab
Mutant # B-076

Incubated 48 hrs @ 37°C
5/100

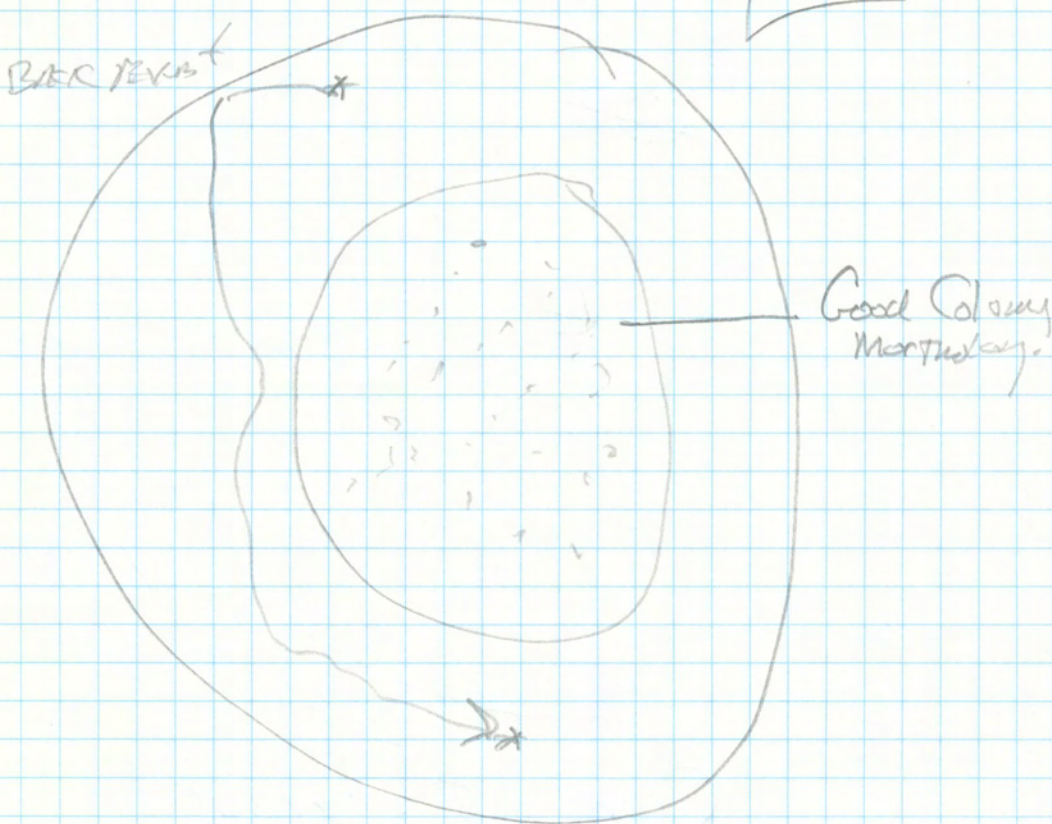
400 pl sample.

RESULTS

Overnight 7/100 col.

2 BACK row mutants

PASS



Controls

7/100 col.
13-019

1 BACK row.

UREA

Failed

Con 1M.
Vol - 100 ml
1M CPC 98 f2 FROZEN

FRESH Filtrated urine .27 F.

PLATES BBL abc II

Amicon purification filter.
1/100 y1 sample.

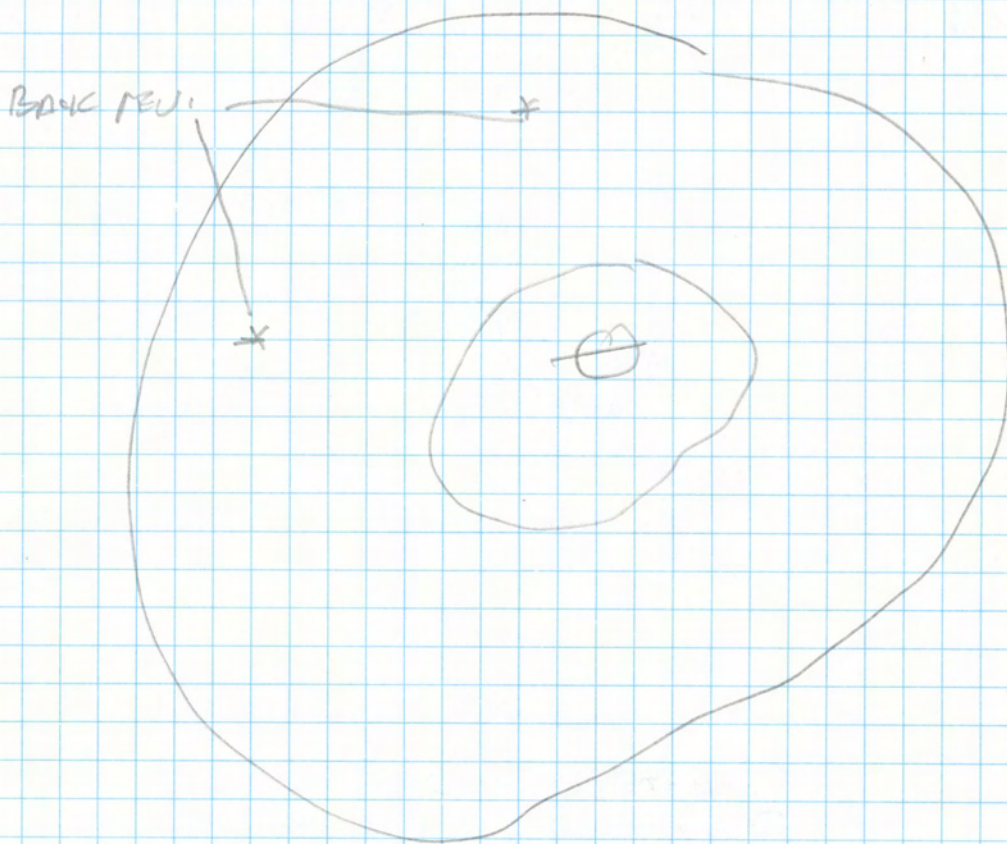
Failed

Amicon purification filter.

Encountered 484P E37E 5/102 LOT# B-09

PREV 15

grown in
BASIC MEDIA 2



Controls
7/100
B-019

2 BASIC MEDIA

100 mg c 98

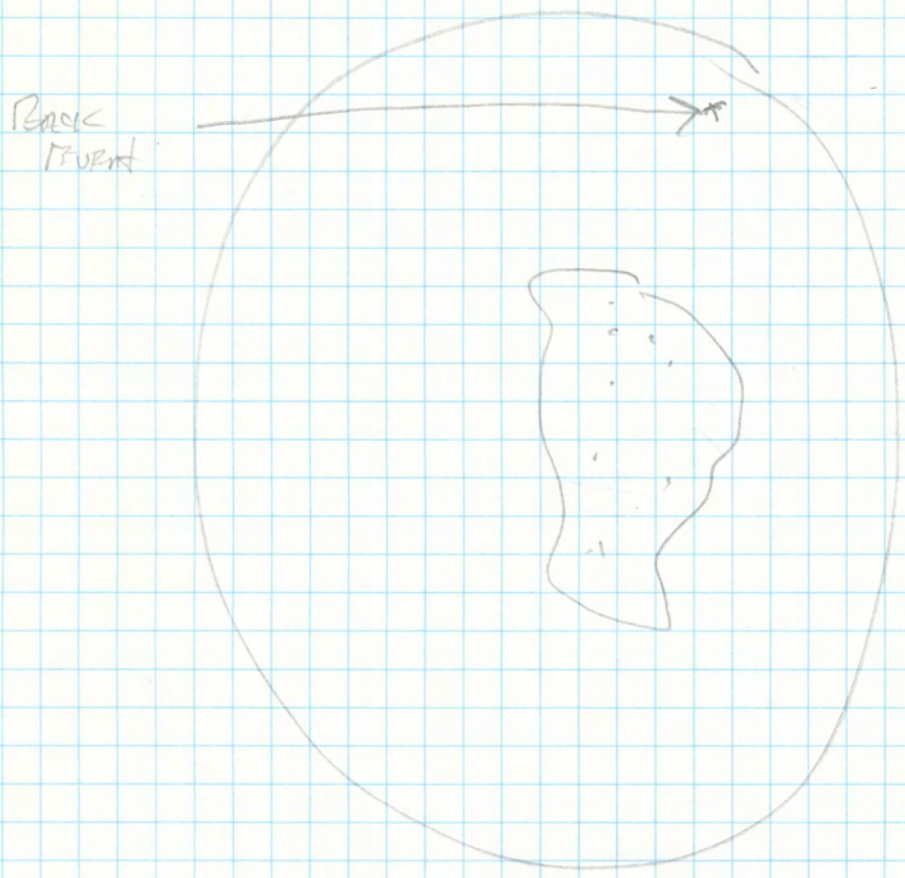
ZINC CHLORIDE

CON-1M
 Vol - 100 ml
 1% concn for stock
 Fresh Filtered urine 2 ml
 Amicon purification filter.
 PLATES BBI/ Ciba II
 Aged 2 hrs.
 Incubated @ 48 hrs. @ 37°C 5% CO₂
 100 µl sample.

Mutnant
Lot # B046

Control 21 col.
1 Background

RESULTS



Control 7/00
 Background 1
 Lot # B-049.

Handwritten signature

19 DEC 96 SODIUM TUNGSTATE PASS

CON - 14.
Vol - 100 ml
MIC 98 f stock
FRESH Filtered urine.
AMMON purification FILTER
PLATES DRY CHECK
Inoculated 400 μ l
Inoculated 48 hrs @ 37°C 5% CO₂

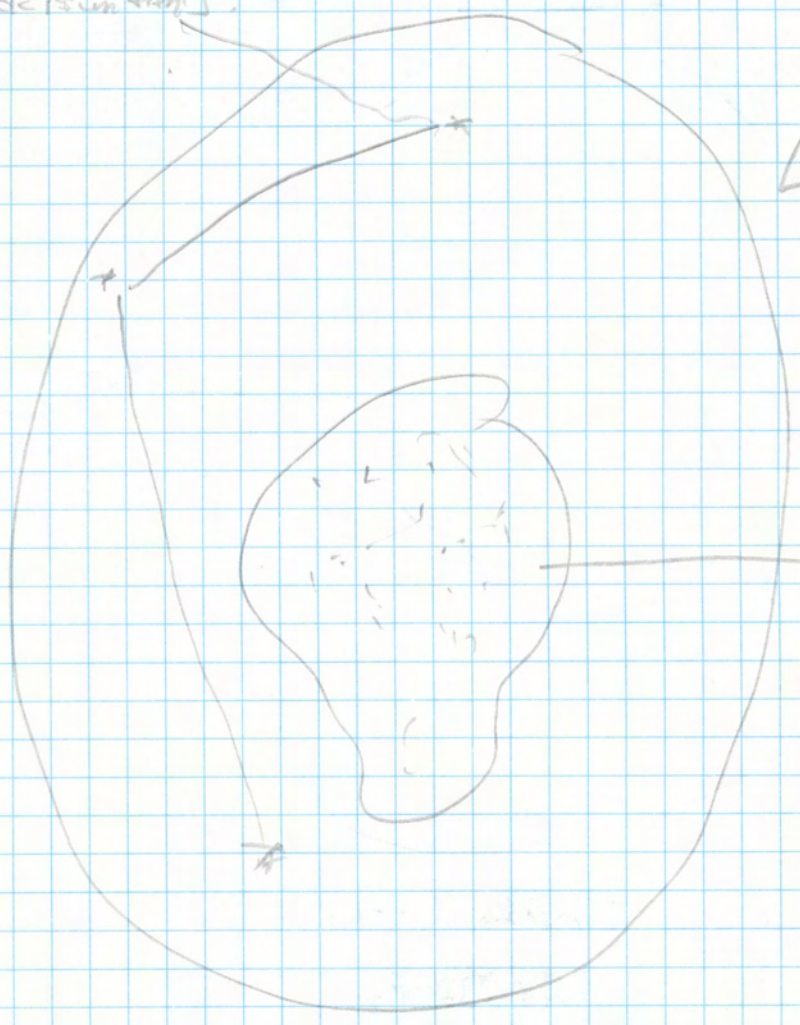
1m Sample

mutant
not

RESULTS

Growth > 100
3 back countants

PASS



Excellent
Colony
morphology

REPEAT TEST

21 DEC 96

EDTA.

CON - 1 M.

Vol 100 ml

1 μ CDC 98 f2 StovC

Fresh filtered wine

Amicon purification filter.

PLATES - BBL CHOC II AGAR

Agar 2 hrs.

Incubated 48 hrs @ 32 $^{\circ}$ 5% CO $_2$

400 μ l sample.

mutant Lot # B-016

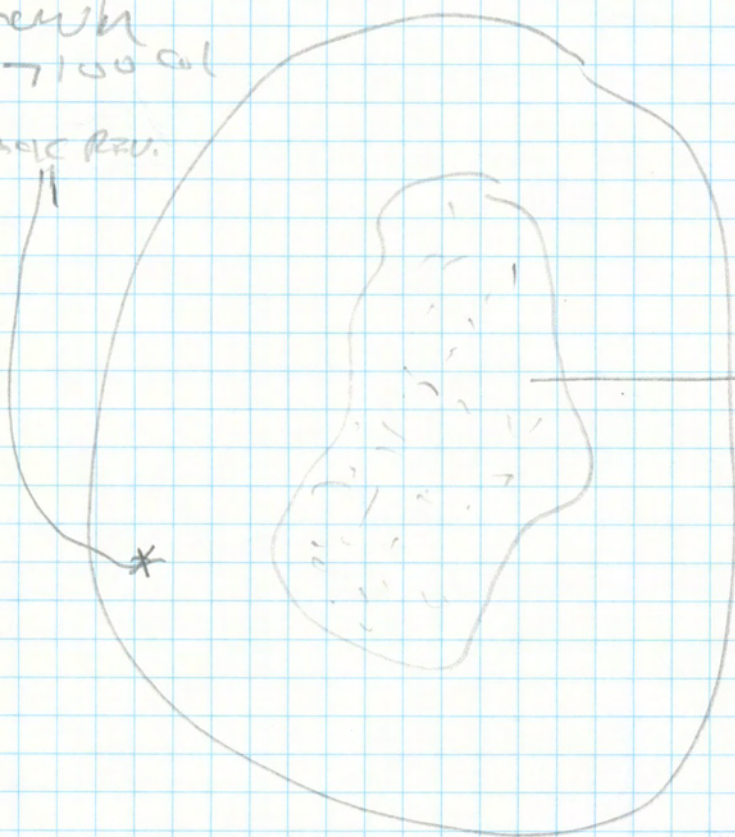
Results.

Growth
7100 col

BAC REV.

||

*



Excitization of
colony
manifestly.

Control 7100 col.

lot # B-019

23 DEC 96

EGTA

CON - 1M.

Vol - 100 ml.

1M egg fz stock.

FRESH Filtered URINE.

American Purification Filter.

PLATES - BBL CHOICE AGAR

Need 2 H₂S.

Incubated 48 hrs @ 37°C 5% CO₂

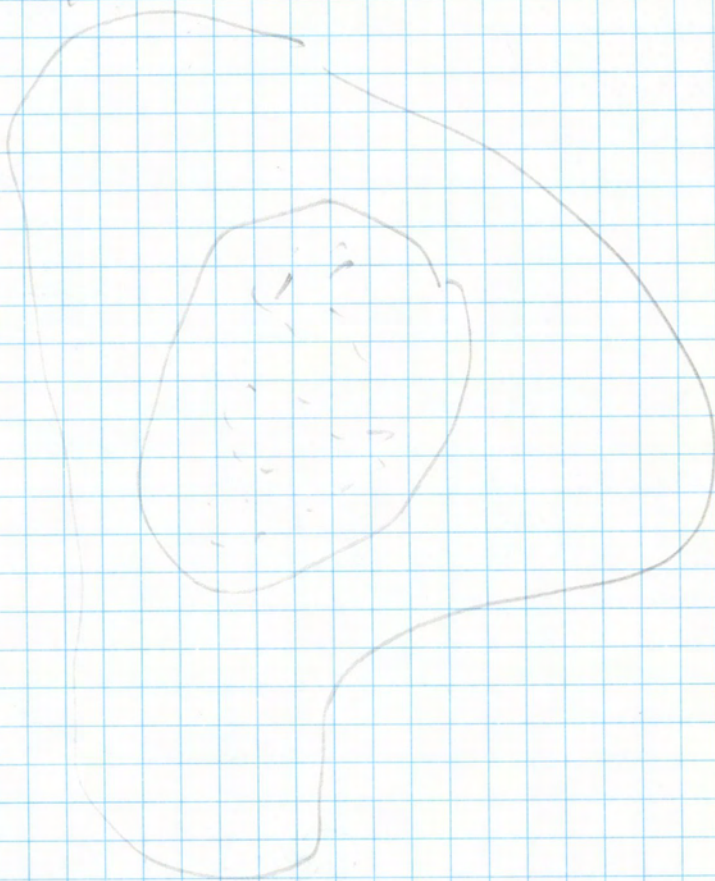
400 μ l sample

Mutant lot #
B-046.

RESULTS

Control 7/100 col.

Back row. 0



Control 7/100 col.

lot # B-019.

29 DEC 96.

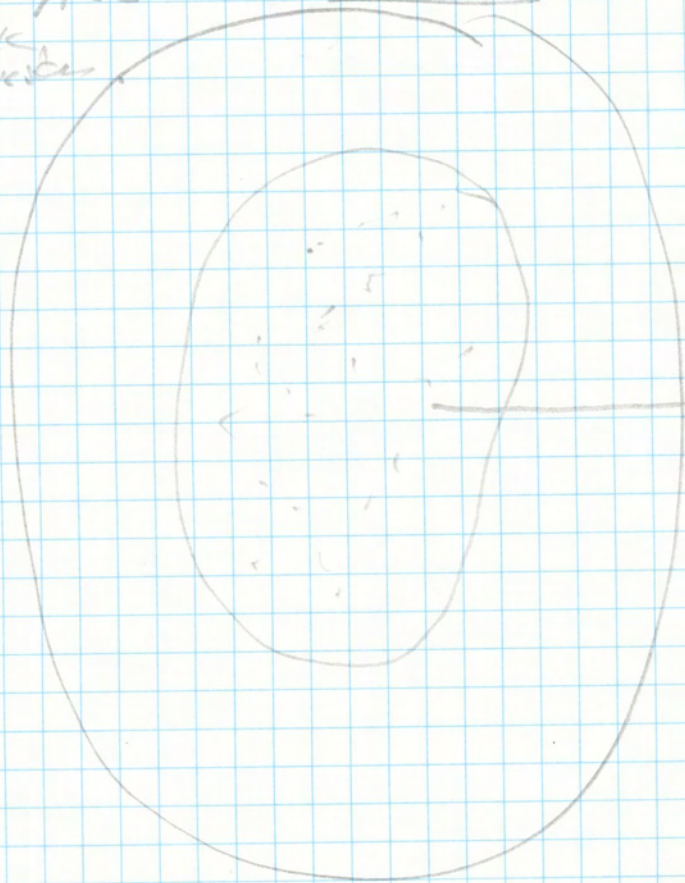
BAPTA

Con 1m
 Vol 100 μ l
 1m cpe98 f2 stock
 Fresh Filtered Urine
 Amicon Purification Filter
 PLATES BBL - CHOC II
 400 μ l Inoculum
 Incubated 48hrs @ 27°C 5% CO₂
 400 μ l Sample.

Mutant lot # B-045

RESULTS

Growth 7/100
 NO BACK
 PLATES



Excellent Colony morphology

Control
 7/100
 Lot # B-019.

Enzyme Challenge

DNA modifying Enzymes
 Method: Homogeneous Liquid phase EIA. Direct detection
 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 Deoxyribose Nuclease monoclonal
6. S1 Nuclease monoclonal
7. Bsp phosphatase monoclonal
8. T4 polynucleotide monoclonal

Each Enzyme was exposed to a formula of DNA/RNA
 product of 1M sodium thiocyanate. And 0.1 M of EDTA

Each individual Enzyme was incubated into a urine
 matrix Aug April for 8 hours

Substrate
 was added
 through
 SWZ

Measurements were taken every hour (each absorbance measurement)
 were made every hour on a spectrophotometer @

Antibody dilutions for each antibody were 1/1000
 urine height was set at 540 nm.

Total Volume for each reaction chamber was 1000 ml.

Each enzyme treated and untreated had individual chambers.

Chemistry + Enzyme

And Enzyme in DEUT H₂O were incubated at
 Ambient room temp. 25°C ± 2°C

DEUT H₂O were used to blank each reading.

TOTAL Enzyme activity was measured in Absorbance
 values 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	3.9	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 91

Enzyme Challenge II DNA Modifying Enzymes.

RESULTS:

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

[Handwritten signature]

22 FEB 07

METHODS: See Protocol of RNA modification
Enzymes used - 12 FEB 07

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. T4 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	2.5	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 @ 1M SODIUM TRIS @ 1M EDTA

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July 19 97

Effect of Molar Concentration of DNA/RNA protect on Tag Polymerase

① Controls 1pg / coc 98 for DUA-

② 1M Sodium Thiocyanate + EDTA 0.1M

③ 2M Sodium Thiocyanate + EDTA 0.2M

Tags used

1. Tag Gold (Roche)
2. Tag - TAKARA BIO
3. Rockin' Primer
4. Promega
5. Invitrogen
6. STRATAGENE
7. Qiagen
8. ABI
9. MP Biomedicals
10. Promega

Target 1pg COC98 DNA

1 unit of each Tag was used.
Sigma master mix used.

Target = 1pg COC98 DNA from stock extracted per constant protocol.

Group 1 = Controls no chemistry

Group 2 1M Sodium Thio + 0.1M EDTA

Group 3 2M Sodium Thio + 0.1M EDTA

Tag 1 2 3 4 5 6 7 8 9 10

Group 1 3.1 3.3 3.1 3.1 3.1 3.1 3.1 3.1 3.1 3.1

Group 2 2.97 3.1 3.1 2.3 2.7 2.99 1.9 2.3 3.0 2.5

Group 3 3.1 2.8 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 EDTA - Impact on Amplification
2. 2 Molar sodium Thio and 0.1 molar EDTA - Impact on Amplification
3. There was significant variation in performance between Tags.
4. All enzymes amplified despite of LOP restriction

Follow up studies

1. Increase units of Tag 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 CCL TAT CTA TAA ACTC

Formula

- 10 mM Tris (pH 8.3)
- 50 mM KCl
- 2 mM MgCl₂
- 50 μM Each deoxyribonucleoside
- 2.5 units of Taq polymerase (Pierce & Warriner)
- 5% Glycerol
- 50 pmol of primers 1 and 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 cycle - 3 cycles @ 94°C + 2 min @ 55°C
4. Amplify DNA by 25 S Denaturation at 94°C and a 25 S Annealing @ 55°C Total of 30 cycles.

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June 28, 98 Stability of Conjugated DNA

In various concentrations of elements

1 μ g Conjugated DNA solution Extraction was spiked into 6 Formulations
& 100 μ g protected samples were aged at 37°C for
5 days and then an 800 μ l sample was used.

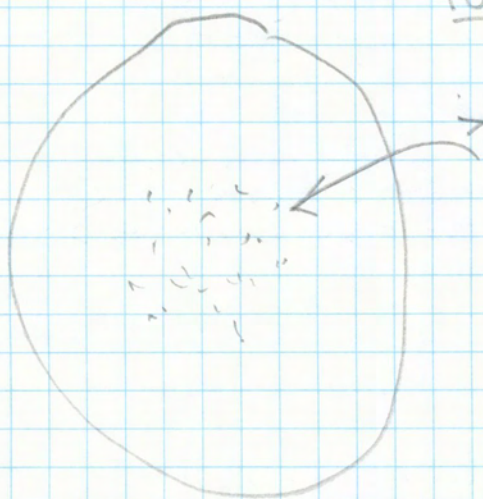
Formulations

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

Results

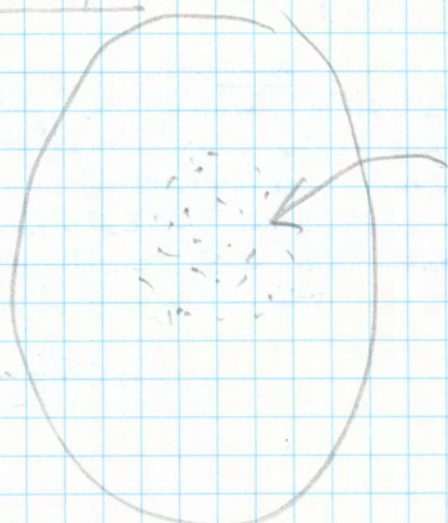
Cell Results

Time 0

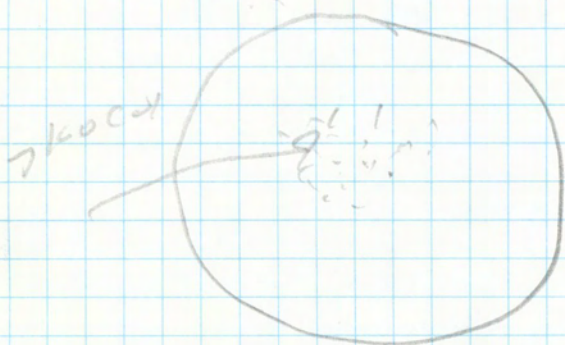


7 Days

Guanidine Thiocyanate + 0.01M EDTA



Time 0



Sodium perchlorate + 0.01M EDTA

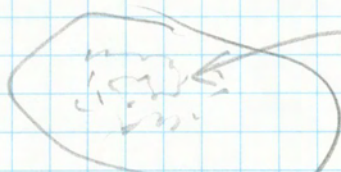
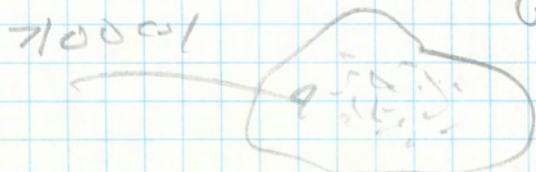
7 Days



Sodium Thiocyanate + 0.01M EDTA



Guanidine 1M + 0.001 EDTA



July-12 99.

Survival of 1st Generation DNA in Room Temp. In unprotected urine.

- Method: 10 urine used obtained from unsexed and urine brought to room temp 26°C. Volume used was 2 ml.
- Male - Normal urine Normal Dip stick test.
 - Normal Female
 - Diabetic urine (Ward Insulin dependent)
 - Diabetic urine (Ward insulin dependent)
 - Acid urine
 - Basic urine
 - High urea
 - E.coli uti 7 Macfarlane's
 - Mixed uti (Special Care Dept).

Urine was inoculated with 1st generation DNA and incubated for 6 hours. The G-IT was used as the marker for viable DNA. Colonies were counted on BSLC (see II page).

RESULTS	N MALE	N FEMALE	Diabetic UD	Diabetic PD	Acid urine	Basic urine	E.coli uti	Mixed special care	Pres. R. 2hr
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 1st generation DNA at about 5 hours in urine > 26°C.

Notes - Data analyzed at 4 hours and 6 hours. G-IT controls were 700 c.f.u. II plates were not exposed. No background counts on plate results were in control.

[Handwritten signature]

JULY 22 98

Eight Day survival study - 199 C. elegans. Biol. Calyces
FA Fresh urine.

10 ml FRESH urine was inoculated w/ln. 19 DNA
from cyc 98 DNA extracted w/ a standard method. H₂O₂ samples
GTT FROZEN CONTROLS used. Temp 37°C

RESULTS	# Transformed	Control	Back row.
DAY 0	>100	7/100	2
DAY 2	0	7/100	1
DAY 3	0	7/100	0
DAY 4	0	7/100	1
DAY 5	0	7/100	2
DAY 6	0	7/100	0
DAY 7	0	7/100	3
DAY 8	0	700	1

Summary

Biological Active C. elegans did not
survive past 1 day.

[Signature]

August 1898

Subcutaneous study 1 pg Gonococcal DNA in normal
milk disease state serum.

F₂ Disease was spread with 1 pg of Gonococcal DNA. Milk
Encapsulated for 10 hrs. 200 µl samples were taken
every hour for 10 hrs. Controls were F₂ OX Controls.

RESULTS HR	# Transformants				AB-Units	Controls	Rx
	N-Serum M	U-Serum F ₂	Abnormal Serum				
1	110	100	32	61	7100	1	
2	87	91	38	31	7100	1	
3	42	55	29	22	7100	0	
4	25	32	10	0	7100	2	
5	17	20	0	0	7100	1	
6	14	100	0	0	7100	0	
7	0	100	0	0	7100	0	
8	0	0	0	0	7100	1	
9	0	0	0	0	7100	2	
10	0	0	0	0	7100	0	

Result: Gonococcal DNA is destroyed in fresh
F₂ serum. Results seem to parallel what is seen
in urine - no analysis of enzymes present in serum.

—

August 22 98

Eight Hour Survival Study of DNA/RNA protected to Fresh Urine.

Method

Two ml Fresh urine was collected.

10ml urine was spiked with 1 μ g CPC 98 RNA. Temp 37°C
Protected urine was Sodium Trisacrylate + EDTA 0.01 M
PKU's Run using standard sigma phos. Antibodies were
those with Hsp.

Amounts of Purification Reaction were Sampled Every
1 hr.

RESULTS

	DNA/RNA protected	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 Trisacrylate + 0.1 M EDTA
is protective of genomic RNA at 37°C + Hsp?
Hsp.

Sept 19, 98

Single Agent Protection of 1/4 Gaseous

DNA
Method

Cell used for detection

Pres. Unit @ 35°

1/4 gaseous DNA CDC 98 Sierra Extraction 0.5 Ag.
Method.

2 BAC 2 Agar.

2 Target Agent 2 plus @ 35°.

Time Sweep 0, 7, 16, 24 hrs.

RESULTS

Chemistry 1/3 Ag. 1/4.

	HR	Transferrin S.	CONTR (TUTC)	13K
Sept 19	0	200	7200 (TUTC)	2
	7	0	7200 (TUTC)	0
	16	0	7200 (TUTC)	1
	24	0	7200 TUTC	2

	HR	EDTA 1u1	7200 TUTC	
Sept 20	0	200	7200 TUTC	0
	7	0	7200 TUTC	0
	16	0	7200 TUTC	1
	24	0	7200 TUTC	1

	HR	EGTA 1u1	7200 TUTC	
Sept 21	0	200	7200 TUTC	0
	7	0	7200 TUTC	1
	16	0	7200 TUTC	0
	24	0	7200 TUTC	0

	HR	Sodium Salicylate	7200 TUTC	
Sept 22	0	200	7200 TUTC	1
	7	0	7200 TUTC	0
	16	0	7200 TUTC	0
	24	0	7200 TUTC	2

	HR	Guanidine 1u1	7100	
Sept 23	0	200	7100	1
	7	0	7100	1
	16	0	7100	0
	24	0	7100	1

	HR	Sodium Thiocyanate 1u1	7200	
Sept 24	0	200	7200	0
	7	10	7200	1
	16	0	7200	0
	24	0	7200	0

	HR	Sodium perchlorate 1u1	7200	
Sept 25	0	200	7200	1
	7	0	7200	2
	16	0	7200	0
	24	0	7200	0

SEPT 19 cont. Conducted Single Agent and not protected
at concentration of 100 mg/kg.
P.B.M.D.J.

A

Single repeat US (Confirmation Agents for protection of 1st GONOCOCCAL RUN IN FRESHWATER.

The Equipment Extended over a period of 5 weeks.

Each run 60 hrs.

10 ml of urine was inoculated with 100 Gonococcal cells and incubated for a total of 60 hours. A 1 ml was

Done and 1 ml of the suspension + 100 µl was added to reaction vial and read @ 540. 0.01M of CHEMICALS + 1 mg of inhibitors (CHASTROF).

DNA was extracted using silica extraction procedure.

Results

HR.	EDTA 0.1M + Guanidine M.	EDTA 0.1M + Sodium Thiocyanate	EDTA 0.1M + Sodium Perchlorate	EDTA + Sodium Thiocyanate	EDTA 0.1M + Sodium Perchlorate	DBS Buffer No. per	Guanidine
0	5.0	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	0.00	0
20	3.4	3.2	3.56	3.8	3.22	0.00	0
25	3.22	3.0	3.12	3.43	3.0	0.00	0
30	3.0	2.70	2.86	3.2	2.39	0.00	0
35	2.8	2.65	2.40	3.0	3.10	0.00	0
40	2.7	2.55	2.63	2.74	2.99	0.00	0
50	2.5	2.76	2.54	2.66	2.72	0.00	0
60	2.4	2.57	2.62	2.52	2.62	0.00	0

HR.	EDTA 0.1M	EDTA 0.01M	BAPTA 0.01M	Sodium Perchlorate 1M	Sodium Thiocyanate	Lic. 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

12

FEB 22 99.

SPYKOR CHROMATOGRAPHS

EFFECTS of METHENEMOPHON on amplification of
 Genococci DNA in R. S. Serum. Serum ^{incubated at 37°C and} diluted into 2 volumes.
 method: 1 µg of Genococci DNA extracted from R. S. Serum.
 PCR was run against increasing concentrations of
 Methenemophon. Concentrations were 11.9 µg/L → 0 µg/L.
 Uptake was shown on a gel and filtered to sterilize.
 400 µl samples were processed at 400 µg/ml and 400 µg/ml.
 500 µg samples were incubated at 37°C for 10 min prior to processing.

RESULTS

METHENEMOPHON 9/2L	ABSTINENCE
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	2.7
1	4.2
0	

RESULTS:

METHENEMOPHON is an inhibitor of PCR at concentrations
 of 7.10 µg/L of DNA.

Note: Need clinical follow up to confirm. Clinical samples may
 have more than one mixture. NEED to establish an order
 molecular platform.