Pharmaceutical Control and Development Laboratory Co. Ltd.
9. Mexikói Street Budapest, H-1149

Study code: PCDL-0703

FINAL REPORT

Bacterial reverse mutation (Ames) test

of VigRX tablet blend

Initiation of the study: February19, 2007

Experimental period: from February 19 to March 9, 2007

Sponsor:
COFOPEX Ltd.
H-1022 Budapest,
Bimbó út 92.
Contact Person:
István Bara

Study was performed at:
Pharmaceutical Control and
Development Laboratory Co. Ltd.
H-1149 Budapest, Mexikói út 9.
Contact Person:
János Horváth

This report consists of 24 pages plus 5 attachments.

2007

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Bacterial reverse mutation (Ames) test of VigRX tablet blend

Study code: PCDL-0703

SUMMARY

General information:

The plate incorporating method was used in the test with five doses in triplicates on four Salmonella (TA98, TA100, TA1535 and TA1537) and one E. coli [WP2 (uvrA)] tester strains, with and without S9 activation. After cytotoxicity test, a definitive assay and a confirmatory repeat assay were made. Doses of test article were up to 5000 μg/plate. Vehicle (distilled water) and suitable positive controls were used. The plates were counted after 3 days incubation.

Evaluation:

There were no revertants exceeding three times the background average either with or without metabolic activation, and there was no dose-related increase over the range tested, so this study gave negative result to VigRX tablet blend as test article.

The results of the definitive assay showed that the test article had no mutagenic effect to any strain used in this test. The results of the repeat assay confirmed the negative results of the definitive assay.

Bacterial reverse mutation (Ames) test of VigRX tablet blend
Study code: PCDL-0703

Statement of Study Director

I hereby certify that this study report provides a true and complete record of the data generated and that the study was conducted in accordance with the Principles of Good Laboratory Practice as set forth in the following documents:

1. US Food and Drug Administration Title 21, Code of Federal Regulations, Part 58 Good Laboratory Practice Regulations for Nonclinical Laboratory Studies
2. Good Laboratory Practice Regulations (National GLP, Joint Decree 9/2001. (III.30) EüM-FVM)
3. OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98)17 as revised in 1997);

There were no deviations from the aforementioned regulations, which affected the quality or integrity of the study or the interpretation of the results in the report.

Date: April 3, 2007 Signature: János Horváth

János Horváth, M. Sc.
Study Director

Bacterial reverse mutation (Ames) test of VigRX tablet blend

Study code: PCDL-0703

Statement of the Quality Assurance Unit

This study has been inspected and the report audited by the Quality Assurance Unit of PCDL in compliance with the Principles of Good Laboratory Practice. As far as it can be reasonably established, the methods described and the results incorporated in the report accurately reflect the raw data produced during this study.

Inspections concerning adherence to the protocol were performed:

Date of Inspection / Audit	Type or Phase of Inspection	Date of Report to the	
		Study Director	Management
February 15, 2007	Protocol audit	February 15, 2007	February 19, 2007
February 20, 2007	Experimental procedure - cytotoxicity test	February 20, 2007	February 20, 2007
February 23, 2007	Counting procedure - cytotoxicity test	February 23, 2007	February 23, 2007
February 26, 2007	Preparation of inoculums - definitive assay	February 27, 2007	February 27, 2007
February 27, 2007	Experimental procedure - definitive assay	February 27, 2007	February 27, 2007
February 27, 2007	Checking tester strains - definitive assay	February 27, 2007	February 27, 2007
February 28, 2007	Evaluation of checking tester strains - definitive assay	February 28, 2007	February 28, 2007
March 2, 2007	Counting procedure - definitive assay	March 2, 2007	March 2, 2007
March 6, 2007	Experimental procedure - repeat assay	March 6, 2007	March 7, 2007
March 9, 2007	Counting procedure - repeat assay	March 9, 2007	March 10, 2007
April 3, 2007	Draft report audit	April 3, 2007	April 3, 2007

Date: April 4, 2007 Signature: Piroska Molnár

Piroska Molnár, M. Sc.
Biologist
Quality Assurance Unit at PCDL

Staff in Charge

	Signature	Date
Director of the Laboratory:	István Financsek M.D., Ph.D.	04-04-2007
Head of the Toxicological Department	Dr. Tamara Varga Tamara Varga Ph.D., toxicologist	April 4, 2007
Study Director:	János Horváth János Horváth M. Sc. food industry engineer, food safety engineer	April 3, 2007
Deputy Study Director:	Andrea Mózes Andrea Mózes M. Sc. Biologist	April 3, 2007
Quality Assurance Unit:	Piroska Molnár Piroska Molnár M. Sc. Biologist	April 4, 2007
Sponsor:	István Bara István Bara Managing Director COFOPEX Ltd.	April 4, 2007
Monitoring Scientist:	Alexander G. Schauss Ph.D. (AIBMR Life Sciences)	

Staff in Charge

	Signature	Date
Director of the Laboratory:	István Financsek M.D., Ph.D.	04-04-2007
Head of the Toxicological Department	Tamara Varga Ph.D., toxicologist	April 4, 2007
Study Director:	János Horváth M. Sc. food industry engineer, food safety engineer	April 3, 2007
Deputy Study Director:	Andrea Mózes M. Sc. Biologist	April 3, 2007
Quality Assurance Unit:	Piroska Molnár M. Sc. Biologist	April 4, 2007
Sponsor:	István Bara Managing Director COFOPEX Ltd.	April 4, 2007
Monitoring Scientist:	Alexander G. Schauss Ph.D. (AIBMR Life Sciences)	April 11, 2007

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Attachments:

- E-mail from Dr Alex G. Schauss Ph.D. dated March 16, 2007.
- Materials used in the Ames assay PCDL-0703
- Certificate of Analysis of Viga Rx Plus Tablet (Lot: 120657)
- Certificate of Laboratory Analysis of Powder Blend of Vigra RX Tablet (Lot: 120657)
- Certificate of Good Laboratory Practice (GLP) of PCDL Ltd.

1. General information

1.1. Title of the study

Bacterial reverse mutation (Ames) test of VigRX tablet blend

Initiation of the study: February 19, 2007.

Experimental period was from February 19 to March 9, 2007.

1.2. Objective of the study

The objective of this study was to evaluate the ability of the test article to induce mutagenic response in four strains of Salmonella typhimurium (TA98, TA100, TA1535, and TA1537) and one strain of Escherichia coli [WP2 (uvrA)]. The test article was plated in triplicates, at five concentrations both in presence and absence of S9 metabolic activation. A cytotoxicity assessment was performed prior to the definitive study, and an independent repeat assay was conducted to confirm the negative results.

1.3. Type of the study

Preclinical toxicology study in compliance with the principles of the

- Good Laboratory Practice Regulations for Nonclinical Laboratory Studies of the United States Food and Drug Administration (21 CFR 58) [(2)],
- Good Laboratory Practice Regulations (National GLP, Joint Decree 9/2001. (III.30) EüM-FVM), and
- OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98)17 as revised in 1997).

The study was set up according to the OECD GUIDELINE FOR TESTING OF CHEMICALS (Guideline No.: 471, adopted: 21st July 1997), Bacterial Reverse Mutation Test. [(3)]

1.4. Introduction

The reverse mutation assay (Ames test) is used to evaluate the mutagenic properties of test articles. The test uses histidine-dependent strains of S. typhimurium and tryptophan-dependent strain of E. coli. In the absence of an external histidine or tryptophan source, the cells cannot grow to form colonies. Colony growth is evident if a reversion of mutation occurs, allowing the production of histidine or tryptophan to be resumed. Spontaneous reversions occur with each of the strains; mutagenic compounds cause an increase in the number of revertant colonies relative to the background level.

2. Test article

Name[1]:	VigRX tablet blend, synonyms: VIGRA RX TABLET BLEND (label), Viga Rx Plus Tablet [4], Powder Blend of Vigra Rx Tablet [5]
Specification [4]:	powder of different parts of plants
Manufacturer [4] [5]:	VITA-PURE, Inc. 410 W. 1st Avenue Roselle, NJ 07203 U.S.A.
Lot number [4] [5]:	120657
Identification number at PCDL:	2007/03901
Appearance:	dark brown powder
Package form:	plastic, white bottle, 50g
Storage conditions:	at room temperature
Expiration date [4]:	December, 2009

For ingredients see Certificate of Analysis attached [4].

2.1. Microbiological analysis

There were no data available on the stability of the test article when autoclaved in water, it was not possible to get sterile material. Therefore, a microbial limit test by plate-count method was preformed on the test article according to the United States Pharmacopeia 30. The total plate count was 10 CFU/g.

2.2. Chemical analysis

Certificate of Analysis provided by the Sponsor is attached to this Final Report. Composition of the test article and the analytical control are the Sponsor's responsibility.

2.3. Stability control of the test article

Stability control of the test article is the Sponsor's responsibility. The product was considered to be stable.

3. Test system

3.1. Test system description

All Salmonella strains used are histidine-dependent. Revertants were identified as colonies that grew in low levels of histidine. The E. coli strain used here is tryptophan-dependent. Revertants were identified as colonies that grew in low levels of tryptophan. Frameshift and base-pair substitution defects are represented to identify mutagens of both types. Additional genetic markers enhance sensitivity of the strains to certain types of mutagens. The DNA repair mutations (uvrB and uvrA) eliminate excision repair, a repair pathway for DNA damage from UV light and certain chemical mutagens. The uvrB mutation, present in strains TA98, TA100, TA1535 and TA1537, and the uvrA mutation, present in strain WP2 (uvrA), was indicated by sensitivity to UV light. The rfa mutation changes the properties of the bacterial cell wall, increasing the permeability of cells to certain types of chemicals. The rfa mutation, present in all Salmonella strains, was indicated by sensitivity to crystal violet. The R factor plasmid (pKM101) present in strains TA98 and TA100 makes them more responsive to a variety of mutagens. The plasmid carries an ampicillin resistance gene, therefore, ampicillin resistance indicated that the strains retain the plasmid.

3.2. Test system justification

The five strains of bacteria that were used in this assay were among those recommended by OECD Guideline 471[3] for use in the Ames test. These strains of S. typhimurium and E. coli have been shown to be reliably and reproducibly responsive between laboratories.

3.3. Source and storage of test system

The Salmonella and E. coli strains used in this study were obtained from Xenometrix GmbH (Gewerbestrasse 25, CH-4123 Allschwill, Switzerland), are maintained as frozen stocks at –80°C ± 5°C.

3.4. Identification of test system

Strains were identified by certain characteristics (see Table 1). The strains also yield spontaneous revertant colony plate counts within the frequency ranges of the historical control data.

Table 1. Characteristics of Salmonella and E. coli strains

Strain	Gene Affected	DNA repair	LPS	Biotin requirement	Plasmids	Mutational event
TA98	*hisD*	*uvrB*	*rfa*	*bio-*	*pKM101*	frameshift
TA100	*hisG*	*uvrB*	*rfa*	*bio-*	*pKM101*	base-pair substitution
TA1535	*hisG*	*uvrB*	*rfa*	*bio-*	-	base-pair substitution
TA1537	*hisC*	*uvrB*	*rfa*	*bio-*	-	frameshift
WP2 (uvrA)	*trp*	*uvrA*	-	-	-	base-pair substitution

3.5. Preparation of overnight cell cultures

Frozen stock cultures were grown overnight (for 14-16 hours) at 37 ± 2 °C with shaking in nutrient broth until a cell density of about 10^9 cells/ml was obtained (determined by optical density). Cells were maintained at room temperature until use and during the test.

3.6. Control of bias

In order to control bias, for each day of test system treatment, all test article doses, as well as controls, were plated against cells from homogenous culture, i.e. from a single flask.

4. Vehicle

Sterile distilled water. To enhance dispersion at least two minutes vortexing was used. The vehicle did not affect the spontaneous mutation level and it was recommended for use in this test.

4.1. Formulation of the test article

The necessary amount of the test article (c.a. 500 mg) was weighted and suspended with vortexing in sterile distilled water not earlier than 30 min prior the start of the experimental procedures. For dilution to final doses, sterile distilled water was used with constant vortexing.

5. Dose levels

5.1. Doses used in the cytotoxicity assessment

A cytotoxicity assessment was performed to determine the appropriate dose range for the definitive assay. Test doses (5, 10, 50, 100, 500, 1000 and 5000 µg/plate), along with negative controls, were plated against strain TA100, in triplicates, both with and without S9 activation, as described in Section 10. Experimental procedures.

Cytotoxicity could have been detected by the absence of a confluent bacterial lawn, the presence of pinpoint colonies, and/or a substantial decrease or lack of revertant colonies, but the test article is not cytotoxic material.

As the total plate count had not disturbed the counting procedure in the cytotoxicity assessment, the doses of test article were not reduced in the definitive and repeat assays.

5.2. Doses used in the definitive assay

Five dose levels of test article were evaluated in the definitive test in triplicates. The concentrations for the test article were 50, 100, 500, 1000 and 5000 µg/plate.

5.3. Doses used in the independent repeat assay

As the definitive assay yielded negative results, an independent repeat assay was performed according to OECD guideline 471[3]. The guideline indicates that study parameters should be modified. There was no other reason for modifying concentration spacing, therefore the S9 content of S9/cofactor mix was increased from 4% to 10%.

6. Metabolic activation [6]

6.1. S9 fraction

Aroclor™ 1254-induced rat liver S9 (frozen-dried) was purchased from Trinova Biochem GmbH, Kerkrader Strasse 10. D-35394, Giessen, Germany.

6.2. S9/cofactor mix

The S9/cofactor mix contained (for 52.5 ml):

	Standard mix	Raised mix
Rat liver S9 (Aroclor-1254 induced)	2.1 ml (4%)	5.25 ml (10%)
Salt solution (1.65 M KCl + 0.4 M $MgCl_2$)	1.05 ml	1.05 ml
1 M glucose-6-phosphate	0.26 ml	0.26 ml
0.1 M NADP solution	2.1 ml	2.1 ml
0.2 M sodium phosphate buffer, pH 7.4	26.25 ml	26.25 ml
Sterile distilled water	20.74 ml	17.6 ml

It was kept on ice during the experiment.

6.3. Buffer

In case S9 mix was not added, sodium phosphate buffer pH 7.4 was used.

7. Tester strain media [6]

7.1 Nutrient broth

The broth used for the overnight cultures consisted of 2.5% Oxoid nutrient broth #2.

7.2. Minimal glucose plates

Ingredient:	Per 0.5 liter	
Agar, granulated	7.5	g
Distilled water	465	ml
50X VB salts	10	ml
40% glucose	25	ml

Plates consisted of 1.5% agar supplemented with 2.0% glucose and 2.0% Vogel-Bonner buffer.

7.3. Top agar

Ingredient:				
	Agar, granulated		6	g
	NaCl, a.r.		5	g
	Distilled water	ad.	1000	ml

The top agar consisted of 0.6% agar and 0.5% NaCl. It was supplemented right before use with (10ml/100ml top agar) 0.5 mM solution of histidine and biotin (for Salmonella) or tryptophan (for E. coli).

7.4. Other materials

All the media and solutions were used in this study were prepared in PCDL Co. Ltd. (see Table 2.). Ingredients of these media and solutions are listed in attachment "Materials used in the Ames assay PCDL-0703".

Table 2: List of media and solutions prepared

Name of media or solution	Lot No.
Oxoid nutrient broth #2	A128
Minimal glucose plate	A146, A150, A153
Top agar	A132
0.5mM Histidine/Biotin	A111
0.5mM Tryptophan	A131
S9/cofactor mix	A149, A152, A156
0.2M sodium phosphate buffer pH 7.4	A136
Sterile distilled water	A137
Sterile DMSO	A144
Histidine/Biotin plate (for control of strains)	A130
Tryptophan plate (for control of strain WP2)	A138
Nutrient agar (for control of strains)	A141
0.1% Crystal violet (for control of strains)	A142
Ampicillin plate (for control of strains)	A145

8. Controls

8.1. Positive controls (Table 3)

Strains were tested with known mutagens to demonstrate that the assay was working effectively and the metabolic activation system was operating. The applied concentrations are listed in Tables 4. and 5.

8.2. Negative controls

All strains were tested for spontaneous revertant counts containing distilled water instead of test article. The characteristic spontaneous revertant counts of the strains are listed in Tables 4. and 5.

Table 3: Positive controls

Strain	**Positive controls (without S9 activation)**	**Positive controls (with S9 activation)**
TA 98	2-nitrofluorene (2NF) CAS# 607-57-8	benzo(a)pyrene (BP) CAS# 50-32-8
TA100	sodium-azide (NaN_3) CAS# 26628-22-8	2-aminoanthracene (2AAn) CAS# 613-13-8
TA1535	sodium-azide (NaN_3) CAS# 26628-22-8	2-aminoanthracene (2AAn) CAS# 613-13-8
TA1537	9-aminoacridine (9AA) CAS# 52417-22-8	benzo(a)pyrene (BP) CAS# 50-32-8
WP2	methyl-methansulfonate (MMS) CAS# 66-27-3	2-aminoanthracene (2AAn) CAS# 613-13-8

Table 4: Controls without S9 mix

Strain	Positive control	Solvent	Dose (µg,µl/plate)	Spontaneous revertant count[6]
TA98	2-NF	DMSO	2.5	10-50
TA100	NaN_3	Water	1.5	60-220
TA1535	NaN_3	Water	1.5	5-50
TA1537	9-AA	DMSO	25	1-25
WP2	MMS	Water	2.5	65-115*

*Stated based on previous studies.

Table 5: Controls with S9 mix

Strain	Positive control	Solvent	Dose (µg/plate)	Spontaneous revertant count[6]
TA98	BP	DMSO	20	10-50
TA100	2-AAn	DMSO	10	60-220
TA1535	2-AAn	DMSO	10	5-50
TA1537	BP	DMSO	20	1-25
WP2	2-AAn	DMSO	10	65-115*

*Stated based on previous studies.

9. Procedures

The experiments were preformed according to the current Standard Operating Procedures of Pharmaceutical Control and Development Laboratory Co. Ltd.

10. Experimental procedures for definitive and repeat assays

The followings were added to each sterile culture tube in triplicates in every dose levels: 0.1 ml of test or control article, 0.5 ml S9/cofactor mix or 0.5 ml phosphate buffer pH 7.4, 0.1 ml of overnight cell culture and 2.0 ml top agar. The tubes were vortexed, poured onto Minimal glucose plates, and evenly distributed. The agar was allowed to harden and the plates were inverted and incubated at 37 °C ± 2 °C for 72 ± 4 hours, then scored.

In the repeat assay was the following modification: the S9 concentration in the S9/cofactor mix was increased to 10%. Concentration spacing was not modified.

11. Counting procedure

All plates for all concentrations were counted by hand.

12. Data provided

Results are presented as the number of revertant colonies per plate. For the assays individual plate counts and the mean number with standard deviations of revertant colonies are provided in tabular form.

13. Evaluation and interpretation of results

13.1. Criteria for a valid assay

The study is considered valid if all of the following criteria are met:

- Tester strains TA98, TA100, TA1535, TA1537, and WP2 (uvrA) exhibit sensitivity to UV light.
- All Salmonella tester strains exhibit sensitivity to crystal violet.
- Tester strains TA98 and TA100 exhibit resistance to ampicillin.
- Tester strains exhibit a characteristic number of spontaneous revertant colonies when plated. The mean should be within the range presented in Tables 4. and 5.
- Tester strains exhibit at least a three-fold increase in mutagen-induced revertant colonies (two-fold for strain TA100) when plated with positive control chemicals.

This study is considered valid, because all criteria were met.

13.2. Statistical analysis of the data

There was no test article related increase in average number of revertant colonies relative to the negative control, dose-related increase has not occurred, so it was not reasonable to make statistical (regression) analysis.

13.3. Evaluation of mutagenicity

A test article considered positive if the assay is valid, and if the following conditions are met, considering biological relevance:

- If the background average is below six colonies, the average number of revertants for any test article dose must exceed twenty colonies/plates.
- One test article dose exceeds three times the background average (two times for strain TA100) either with or without metabolic activation, or there is a dose-related increase over the range tested ($p < 0.025$).

A positive result indicates that the test article induces mutations in Salmonella typhimurium or Escherichia coli cells.

A test article for which the results do not meet the above criteria will be considered non-mutagenic in this test.

Negative results indicate that, under the test conditions, the test article does not produce mutations in test cells.

14. Records maintained

The data obtained in the course of the study are collected in a Study File. The Study Protocol, all data generated during and as a result of the study, the documents and all information in connection with the study, a control sample of the test article, and the Final Report will be stored at least for 15 years in the Archives of the PCDL then offered to the Sponsor.

15. Schedule of the study

Cytotoxicity assessment	February 19 - 23, 2007
Definitive assay	February 26 – March 2, 2007
Repeat assay	March 5 - 9, 2007

16. Results

16.1. Cytotoxicity test

Table 6. Results of cytotoxicity test

Test article	Dose (μg/plate)	S. typhimurium TA 100 Revertant colonies per plate [**Mean** ± S.D.]	
		without activation	with 4% S9 activation
VigRX tablet blend	0 [a)]	171 154 167 [**164** ± 8.9]	141 130 148 [**140** ± 9.1]
	5	157 163 158 [**159** ± 3.2]	136 133 154 [**141** ± 11.4]
	10	170 158 157 [**162** ± 7.2]	126 149 139 [**138** ± 11.5]
	50	171 166 176 [**171** ± 5.0]	153 144 123 [**140** ± 15.4]
	100	177 168 163 [**169** ± 7.1	152 137 141 [**143** ± 7.8]
	500	155 169 158 [**161** ± 7.4]	132 155 140 [**142** ± 11.7]
	1000	166 183 161 [**170** ± 11.5]	131 143 139 [**138** ± 6.1]
	5000	153 163 157 [**158** ± 5.0]	142 133 157 [**144** ± 12.1]
Historical negative [b)]	-	60-220	

[a)] Negative (solvent) control, spontaneous revertant number.

[b)] The historical negative range was formed by reference literature [(7)].

Comments on the cytotoxicity test:

There was normal backround lawn with no significant reduction (more than 50%) in the number of revertant colonies.

There was no cytotoxic effect and no precipitation, so in the definitive and repeat assays the dose range was up to 5000 μg/plate.

16.2. Definitive assay

Table 7. Results of definitive assay, without metabolic activation

Test article	Dose (µg/plate)	Revertant colonies per plate [Mean ± S.D.]				
		TA98	TA100	TA1535	TA1537	WP2 (uvrA)
VigRX tablet blend	0[a)]	45 51 40 [**45** ± 5.5]	193 174 168 [**178** ± 13.1]	41 39 47 [**42** ± 4.2]	14 13 10 [**12** ± 2.1]	104 78 92 [**91** ± 13.0]
	50	40 39 44 [**41** ± 2.6]	169 171 190 [**177** ± 11.6]	47 38 39 [**41** ± 4.9]	16 15 10 [**14** ± 3.2]	86 75 94 [**85** ± 9.5]
	100	45 41 47 [**44** ± 3.1]	182 171 168 [**174** ± 7.4]	38 36 50 [**41** ± 7.6]	13 15 12 [**13** ± 1.5]	92 102 96 [**97** ± 5.0]
	500	45 39 42 [**42** ± 3.0]	161 187 197 [**182** ± 18.6]	47 49 41 [**46** ± 4.2]	16 9 10 [**12** ± 3.8]	107 93 88 [**96** ± 9.8]
	1000	40 49 50 [**46** ± 5.5]	184 178 195 [**186** ± 8.6]	48 42 41 [**44** ± 3.8]	17 12 9 [**13** ± 4.0]	87 82 110 [**93** ± 14.9]
	5000	48 41 46 [**45** ± 3.6]	166 175 200 [**180** ± 17.6]	47 44 38 [**43** ± 4.6]	11 15 10 [**12** ± 2.6]	88 102 91 [**94** ± 7.4]
Positive control	[b)]	498 517 461 [**492** ± 28.5]	884 964 1052 [**967** ± 84.0]	586 610 696 [**631** ± 57.8]	81 111 75 [**89** ± 19.3]	1524 1638 1488 [**1550** ± 78.3]
Historical negative [c)]	-	10-50	60-220	5-50	1-25	65-115

[a)] Negative (solvent) control, spontaneous revertant number.

[b)] Dose of the suitable mutagen material, see Table 4.

[c)] The historical negative ranges were formed by our lab experiences and reference literature [(7)].

Table 8. Results of definitive assay, with 4% S9 activation

Test article	Dose (μg/plate)	Revertant colonies per plate [**Mean** ± S.D.]				
		TA98	TA100	TA1535	TA1537	WP2 (uvrA)
VigRX tablet blend	0 [a)]	41 39 45 [**42** ± 3.1]	189 156 171 [**172** ± 16.5]	40 37 42 [**40** ± 2.5]	12 8 16 [**12** ± 4.0]	102 91 108 [**100** ± 8.6]
	50	48 44 40 [**44** ± 4.0]	170 161 188 [**173** ± 13.7]	34 36 42 [**37** ± 4.2]	13 10 13 [**12** ± 1.7]	88 91 100 [**93** ± 6.2]
	100	41 45 39 [**42** ± 3.1]	184 160 170 [**171** ± 12.1]	37 42 45 [**41** ± 4.0]	10 17 11 [**13** ± 3.8]	81 131 106 [**106** ± 25.0]
	500	43 37 41 [**40** ± 3.1]	159 190 174 [**174** ± 15.5]	40 40 36 [**39** ± 2.3]	17 12 11 [**13** ± 3.2]	93 108 106 [**102** ± 8.1]
	1000	44 48 35 [**42** ± 6.7]	197 164 181 [**181** ± 16.5]	41 39 33 [**38** ± 4.2]	14 12 10 [**12** ± 2.0]	86 96 92 [**91** ± 5.0]
	5000	43 31 43 [**39** ± 6.9]	157 166 188 [**170** ± 15.9]	34 41 36 [**37** ± 3.6]	10 16 14 [**13** ± 3.1]	111 94 99 [**101** ± 8.7]
Positive control	[b)]	234 217 264 [**238** ± 23.8]	692 709 803 [**735** ± 59.8]	714 684 756 [**718** ± 36.2]	59 49 57 [**55** ± 5.3]	350 306 386 [**347** ± 40.1]
Historical negative [c)]	-	10-50	60-220	5-50	1-25	65-115

[a)] Negative (solvent) control, spontaneous revertant number.

[b)] Dose of the suitable mutagen material, see Table 5.

[c)] The historical negative ranges were formed by our lab experiences and reference literature [(7)].

Comments on the definitive assay:

The backround lawn was normal at every dose. The positive and negative (solvent) control values were appropriate for the respective strains.

16.3. Repeat assay

Table 9. Results of repeat assay, without metabolic activation

Test article	Dose (μg/plate)	Revertant colonies per plate [**Mean** ± S.D.]				
		TA98	TA100	TA1535	TA1537	WP2 (uvrA)
VigRX tablet blend	0 [a)]	37 30 38 [**35** ± 4.4]	169 177 184 [**177** ± 7.5]	27 37 29 [**31** ± 5.3]	13 7 7 [**9** ± 3.5]	63 70 82 [**72** ± 9.6]
	50	34 32 38 [**35** ± 3.1]	182 178 165 [**175** ± 8.9]	29 29 33 [**30** ± 2.3]	12 6 12 [**10** ± 3.5]	79 61 77 [**72** ± 9.9]
	100	34 36 40 [**37** ± 3.1]	170 177 174 [**174** ± 3.5]	31 30 36 [**32** ± 3.2]	9 6 16 [**10** ± 5.1]	63 79 67 [**70** ± 8.3]
	500	31 31 42 [**35** ± 6.4]	181 169 179 [**176** ± 6.4]	20 38 35 [**31** ± 9.6]	8 10 10 [**9** ± 1.2]	61 65 78 [**68** ± 8.9]
	1000	36 34 32 [**34** ± 2.0]	172 182 177 [**177** ± 5.0]	36 29 28 [**31** ± 4.4]	7 9 11 [**9** ± 2.0]	74 67 64 [**68** ± 5.1]
	5000	34 34 36 [**35** ± 1.2]	177 172 181 [**177** ± 4.5]	27 32 39 [**33** ± 6.0]	5 13 10 [**9** ± 4.0]	71 76 60 [**69** ± 8.2]
Positive control	[b)]	462 388 558 [**469** ± 85.2]	772 827 889 [**829** ± 58.5]	460 484 590 [**511** ± 69.2]	63 51 41 [**52** ± 11.0]	1340 1488 1124 [**1317** ± 183.1]
Historical negative [c)]	-	10-50	60-220	5-50	1-25	65-115

[a)] Negative (solvent) control, spontaneous revertant number.

[b)] Dose of the suitable mutagen material, see Table 4.

[c)] The historical negative ranges were formed by our lab experiences and reference literature [(7)].

Table 10. Results of repeat assay, with 10% S9 activation

Test article	Dose (μg/plate)	Revertant colonies per plate [**Mean** ± S.D.]				
		TA98	TA100	TA1535	TA1537	WP2 (uvrA)
VigRX tablet blend	0 [a)]	46 40 41 [**42** ± 3.2]	179 166 171 [**172** ± 6.6]	23 24 31 [**26** ± 4.4]	15 11 16 [**14** ± 2.6]	72 67 81 [**73** ± 7.1]
	50	45 46 39 [**43** ± 3.8]	165 183 177 [**175** ± 9.2]	30 24 26 [**27** ± 3.1]	12 9 14 [**12** ± 2.5]	60 79 64 [**68** ± 10.0]
	100	41 38 45 [**41** ± 3.5]	169 176 169 [**171** ± 4.0]	25 22 30 [**26** ± 4.0]	14 11 12 [**12** ± 1.5]	71 75 65 [**70** ± 5.0]
	500	36 38 49 [**41** ± 7.0]	168 179 173 [**173** ± 5.5]	28 25 25 [**26** ± 1.7]	15 13 17 [**15** ± 2.0]	62 65 79 [**69** ± 9.1]
	1000	39 45 44 [**43** ± 3.2]	167 176 170 [**171** ± 4.6]	21 27 28 [**25** ± 3.8]	15 12 9 [**12** ± 3.0]	65 77 66 [**69** ± 6.7]
	5000	39 48 38 [**42** ± 5.5]	165 167 178 [**170** ± 7.0]	23 24* 28 [**25** ± 2.6]	16 13 15 [**15** ± 1.5]	74 66 70 [**70** ± 4.0]
Positive control	[b)]	488 415 468 [**457** ± 37.7]	1356 1472 1196 [**1341** ± 138.6]	680 600 602 [**627** ± 45.6]	115 116 111 [**114** ± 2.6]	382 321 356 [**353** ± 30.6]
Historical negative [c)]	-	10-50	60-220	5-50	1-25	65-115

[a)] Negative (solvent) control, spontaneous revertant number.

[b)] Dose of the suitable mutagen material, see Table 5.

[c)] The historical negative ranges were formed by our lab experiences and reference literature [(7)].

* One colony of mould formed on the plate.

Comments on the repeat assay:

The backround lawn was normal at every dose. The positive and negative (solvent) control values were appropriate for the respective strains. The test shows similar results to those of the definitive assay.

17. Conclusion

There were no revertants exceeding three times the background average either with or without metabolic activation, and there was no dose-related increase over the range tested, so this study gave negative result to VigRX tablet blend as test article.
The results of definitive assay showed that the test article had no mutagenic effect to any strain used in this test. The results of the repeat assay confirmed the negative results of the definitive assay.

April 3, 2007
János Horváth, M. Sc.
Study Director

18. References

(1) E-mail from Dr Alex G. Schauss Ph.D. dated March 16, 2007 (see attached) and Amendment to the Protocol PCDL-0703 dated March 21, 2007.
(2) Good Laboratory Practices (GLP) Regulations for non-clinical laboratory studies by the U.S. Food and Drug Administration (21 CFR 58), current versions as of April 2003.
(3) Organization for Economic Cooperation and Development (OECD) Section 4 of the OECD Guidelines for the Testing of Chemicals: Bacterial Reverse Mutation Test, Guideline 471, adopted 21st July 1997.
(4) Certificate of Analysis of Viga Rx Plus Tablet, Lot 120657, issued by VITA-PURE, Inc., dated 12/2006 (see attached).
(5) Certificate of Laboratory Analysis of Powder Blend of Vigra Rx Tablet Lot 120657 issued by Sani-Pure Food Laboratories, dated 1/22/2007 (see attached).
(6) Maroon, D. and Ames B. (1983) Mutation Res. 113, p.173-215.
(7) Invittox on line http://embryo.ib.amwaw.edu.pl/invittox/prot/30.htm

Financsek Istvan

From: "Alex Schauss" <alex@aibmr.com>
To: "Istvan Bara" <barai@mail.datanet.hu>; "Istvan Financsek, MD, PhD" <financse@t-online.hu>
Sent: 2007. március 16. 5:20
Attach: 0702-EP.DOC
Subject: From Alex Schauss FW: Acute tox VIGRA Rx

Dear Dr. Financsek:

I have a dilemma just discovered. We just noticed that the C of A for the VigRX tablet blend incorrectly reported the name of the product as "Vigra RX". This is incorrect. The name is VigRX. The manufacturer's analytical lab just realized this error and now our client is concerned that the report of the acute toxicity study you are doing will have the wrong name for the product and they can not use the findings because of the error. Can this be resolved before issuing the draft final report? I realize this is difficult because it is a GLP study and you rely on the C of A (from Sani-Pure) to determine the product's name.

Any assistance you to provide or instructions on how this can be solved now would be greatly appreciated. The draft report is scheduled to be released on March 29th.

Regards,

Alex Schauss

------ Forwarded Message
From: Financsek Istvan <financse@t-online.hu>
Organization: GYEL Kft
Date: Tue, 20 Feb 2007 16:13:20 +0100
To: Alex Schauss <alex@aibmr.com>
Cc: Bara Istvan <barai@mail.datanet.hu>
Subject: Acute tox VIGRA Rx

Dear Dr Schauss,

Attached please find the Study Protocol of the VIGRA Rx acute tox study for comments and approval.

Best regards,

I.Financsek

------ End of Forwarded Message

2007.03.19.

Materials used in the Ames assay PCDL-0703

Name of material	Manufacturer	Quality	Lot/Batch No.	Expiry date
2-Aminoanthracene	SIGMA Trinova Biochem	for Ames test	39H0945	February, 2008
2-Nitrofluorene	SIGMA-ALDRICH	98%	S08447-244	-
9-Aminoacridine	MERCK	for synthesis	S03761	July, 2011
Agar-agar, granulated	MERCK	purified and free from inhibitors for microbiology	VM603814	January, 2010
Ampicillin trihydrate	SIGMA-ALDRICH	≥96%	035K0521	April, 2009
Benzo(a)Pyrene	SIGMA Trinova Biochem	for Ames test	084K1371	May, 2008
Cristal violet, C.I.42555	REANAL	for microscopy	828489	June, 2008
D(+)-Biotin	MERCK	for biochemistry	D472614	November, 2007
Dimethyl sulfoxide dried	MERCK	max. 0,05% H_2O	K35155131	September, 2008
Glucose-6-phosphate disodium salt dihydrate	AppliChem	98.8%	4T11080	April, 2008
L-Histidine	MERCK	for biochemistry	K34168851	December, 2009
L-Tryptophane	MERCK	for biochemistry	K31536974	November, 2007
Lyoph. SD Rat liver S9, Aroclor 1254 induced	Trinova Biochem	for Ames test	2057	September, 2008
Methyl methanesulfonate	FLUKA	purum, ≥98% (GC)	1098804 24704051	-
NADP-Na_2	AppliChem	97.2%	6V002232	January, 2009
Nutrient Broth No. 2.	OXOID	-	378206	January, 2010
Sodium azide	MERCK	for synthesis	S4230635	November, 2009
Sterile distilled water	PCDL	sterile	A137	April, 2007

VITA-PURE, INC.
410 W. 1ST AVENUE
ROSELLE, NJ 07203
TEL: (908) 245-1212 FAX: (908) 245-1999

CERTIFICATE OF ANALYSIS

SF 2226

Our Invoice #
Quantity:
Product: Viga Rx Plus Tablet

Date: 12/06
Lot:# 120657
Exp:# 12/09

PHYSICAL CHARACTERISTICS

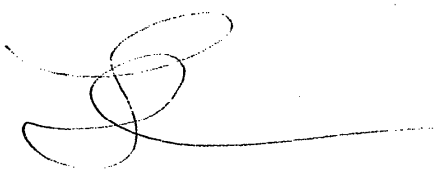
Size: 750"
Average Weight: 1200 mg
Disintergration: Within 60 minutes
Hardness: 5KG/CM2
Thickness: 0.320"
Description: Redish Orange Tablet

Shape:Oval

Method-USP
Complies: x
Complies: x

Assay: Each Tablet Contains:	Label Claim mg	Results mg
Ingredients:		
Korean Red Ginseng (root)	100 mg	100 mg*
Saw Palmetto (berry)	100 mg	100 mg*
Hawthorne (berry)	100 mg	100 mg*
Ginkgo Biloba (leaf)	100 mg	100 mg*
Damiana (Leaf)	100 mg	100 mg*
Tribulus Terrestris (vine)	75 mg	75 mg*
Catuaba 4:1 extract (bark)	50 mg	50 mg*
Muira Puama 4:1 extract (bark)	50 mg	50 mg*
Cuscuta 4:1 extract (seed)	25 mg	25 mg*
Epimedium 4:1 extract (leaf)	15 mg	15 mg*
Bioperine	5 mg	5 mg*

*BASED ON INPUT



MEMBER ACIL

Sani-Pure Food Laboratories

CHEMISTS TO THE FOOD INDUSTRIES

178-182 Saddle River Road • Saddle Brook, New Jersey 07663-4619

VOICE (201) 843-2525 • FAX (201) 843-4934 • E-MAIL sanipure.labs@verizon.net

Certificate of Laboratory Analysis

FOR INFORMATIONAL PURPOSES ONLY

Vita-Pure, Inc.
410 W. 1st Avenue
Roselle, New Jersey 07203

Date Received:	1/15/2007
Date Reported:	1/22/2007
Lab Number:	07,015,c,130
Purchase Order No.	17455
Page:	1 of 1

Product Identification:	Powder Blend of Vigra Rx Tablet
Label	n/a
Net Contents	Plastic Bottle
Lot Number	120657
Exp Date	n/a

TEST REQUIRED	*RESULT*	LIMIT	*REFERENCE METHOD*
Total Plate Count	40 CFU per g	5,000 CFU per g	USP 29 <61>
Salmonella	ABSENT	ABSENT	USP 29 <61>
Total Coliforms	ABSENT	ABSENT	USP 29 <61>
E. coli	ABSENT	ABSENT	USP 29 <61>
Yeasts	< 10 CFU per g	< 500 CFU per g	USP 29 <61>
Molds	< 10 CFU per g	< 500 CFU per g	USP 29 <61>

CFU: COLONY FORMING UNITS

Respectfully submitted,

Ronald A. Schnitzer
Director

ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET
NATIONAL INSTITUTE OF PHARMACY
GENERAL DIRECTOR
H-1051 Budapest V., Zrínyi u. 3.
✉: 1372 POB. 450.
☎/fax: 317-4044, 317-1462
Email: tpaal@ogyi.hu

Budapest, 3rd July, 2006
No.: 239/48/2006
Our ref.: Szilvia Karsai
Annex: -
Subject: GLP Certificate

GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

Based on the Inspection report and the discussion of follow up activities it is hereby certified that the test facility

Pharmaceutical Control and Development Laboratory Ltd.
Toxicological Department, Microbiological Assay Group
(H-1149 Budapest, Mexikói út 9., Hungary)

is able to carry out **toxicity, mutagenicity studies** in compliance with the Principles of GLP (Good Laboratory Practice).

Date of inspection: **20-22 February, 2006.**

This GLP Certificate is valid for 2 years.

ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET

Prof. Tamás L. Paál
Director-General

V. C

AMENDMENT TO THE PROTOCOL

Study code: PCDL-0703

copy N°5

Title of the study:

Bacterial reverse mutation (Ames) test of Vigra RX Tablet Blend

Number of Amendment: 1.

Type: modification

Reason: In the attached e-mail Dr Alex G. Schauss Ph.D. (monitoring scientist on behalf of the Sponsor) indicated that the name of the test article, Vigra RX Tablet Blend as used in Study Protocol and on all documents generated during and in connection with the study is incorrect. The manufacturer would like to have issued the Report of the study using a name VigRX tablet blend for the test article.

As the lot number 120657 of the test article seems to identify the test article wearing different names on different documents (Ref. 1, 2, 3, 4), we decided to amend the Study Protocol and issue the Report using the suggested name in the Report however listing all synonymous names.

Therefore, the Study Protocol is amended as follows: (Original text: *italics*, altered text: vertical letters)

1.1 Title of the study

Bacterial reverse mutation (Ames) test of Vigra RX Tablet Blend.

1.1 Title of the study

Bacterial reverse mutation (Ames) test of VigRX tablet blend.

2. Test article

Name: *Vigra RX Tablet Blend (as on the label)*

2. Test article

Name: VigRX tablet blend,
synonyms: VIGRA RX TABLET BLEND (label),
Viga Rx Plus Tablet [3],
Powder Blend of Vigra Rx Tablet [4]

References to the present Amendment:

(1) E-mail from Dr Alex G. Schauss Ph.D. dated March 16, 2007 (see attached)

(2) Label of the plastic container delivering the test article (copy attached).

(3) Certificate of Analysis of Viga Rx Plus Tablet, Lot 120657, issued by VITA-PURE, Inc., dated 12/2006 (see attached).

(4) Certificate of Laboratory Analysis of Powder Blend of Vigra Rx Tablet Lot 120657 issued by Sani-Pure Food Laboratories, dated 1/22/2007 (see attached).

Justified by ..... March 21, 2007
János Horváth, M.Sc. Date
(Study Director)

Informed about the Modification	Signature	Date
Management:	.....	21-03-2007
Head of the Toxicological Dept.:	.....	March 21, 2007
QAU:	.....	March 21, 2007
Sponsor:	.....	28.03.2007.
Monitoring Scientist:	.....	March 28, 2007

Financsek Istvan

From: "Alex Schauss" <alex@aibmr.com>
To: "Istvan Bara" <barai@mail.datanet.hu>; "Istvan Financsek, MD, PhD" <financse@t-online.hu>
Sent: 2007. március 16. 5:20
Attach: 0702-EP.DOC
Subject: From Alex Schauss FW: Acute tox VIGRA Rx

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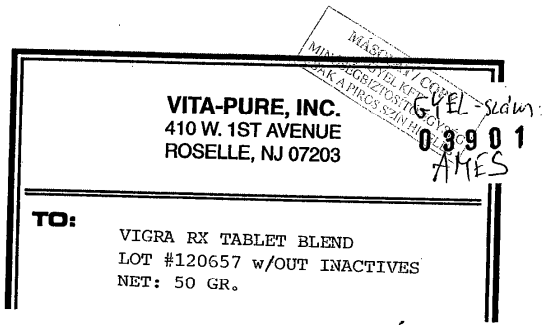
------ End of Forwarded Message



VITA-PURE, INC.
410 W. 1ST AVENUE
ROSELLE, NJ 07203

GYEL - Sildm
03901
AMES

TO:

VIGRA RX TABLET BLEND
LOT #120657 w/OUT INACTIVES
NET: 50 GR.

VITA-PURE, INC.
410 W. 1ST AVENUE
ROSELLE, NJ 07203
TEL: (908) 245-1212 FAX: (908) 245-1999

CERTIFICATE OF ANALYSIS

SF 2226

Our Invoice #
Quantity:
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Exp:# 12/09

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Method-USP
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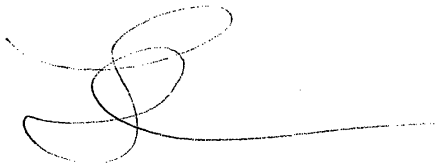

178-182 Saddle River Road • Saddle Brook, New Jersey 07663-4619
VOICE (201) 843-2525 • FAX (201) 843-4934 • E-MAIL sanipure.labs@verizon.net

Certificate of Laboratory Analysis

FOR INFORMATIONAL PURPOSES ONLY

Vita-Pure, Inc.		Date Received:	1/15/2007
410 W. 1st Avenue		Date Reported:	1/22/2007
Roselle, New Jersey 07203		Lab Number:	07,015,c,130
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		Page:	1 of 1

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Molds	< 10 CFU per g	< 500 CFU per g	USP 29 <61>

CFU: COLONY FORMING UNITS

Respectfully submitted,

Ronald A. Schnitzer
Director