



# STUDY

## SUMMARY OF STUDIES OF MULTI-GYN ACTIGEL

DR. ANNELIZE GOEDBLOED



## SUMMARY OF STUDIES OF MULTI-GYN ACTIGEL

### Content

1. In vivo studies of Multi-Gyn® ActiGel.....	3
2. In vitro studies.....	6
3. Toxicity .....	8
4. Irritation and sensitization.....	9

## 1. In vivo studies of Multi-Gyn® ActiGel

### 1.1 Exploratory study with Multi-Gyn® ActiGel in women with vaginal discomforts

**Nr. of participants:** 50

**Study design:** evidence based

**Duration of application:** 5 days

**Results:** ActiGel improved the main symptoms, 43% improvement of itching, 60% improvement with vaginal discharge and 7% improvement on malodour. This efficacy on BV-related complaints was also supported by the vaginal pH data obtained from 9 patients. The mean vaginal pH values in 9 patients decreased from 6.2 to 4.7. In consideration of this pH change and the above-mentioned changes in the symptoms, the improvement of vaginal discomforts was considered to have been presented by re-balancing of vaginal flora and recovery of vaginal self-purification effects.

**Side effects:** none

**Investigators and Study site:**

Kunio Kitamura, MD, Japan Family Planning Association

Yukihiro Shibui, MD, Cimema Art Clinic

Kanako Hanaoka, MD, Hanaoka Ladies' Clinic

Sayoko Makabe, MD, Kanda Daini Clinic

Harumi Kubo, MD, NPO Japan Reproductive Health Association

**Publication/Report:** Prepared by Kunio Kitamura, MD and others, January 2013, Tokyo, Japan

### 1.2 First Line Treatment and Relief of Bacterial Vaginosis-related Vaginal Complaints with Metronidazole and Multi-Gyn® ActiGel

**Nr. of participants:** 47

**Study design:** Open label study comparing a 2QR-based product with Metronidazol

**Duration of application:** 1 week treatment results and after 3 months follow up

**Results:** One week post-treatment all the women in the metronidazole group and 84% in the ActiGel group presented with less than three of the Amsel criteria, and no confirmed BV. After three months, 83% in the metronidazole group and 87% of the women in the ActiGel group were free from BV. The findings suggest that physicians may encourage the use of this vaginal gel for self-care in order to treat and relieve BV related symptoms and thus reduce the prescription of antibiotics and the emergence of resistance.

**Side effects:** none

**Investigators and Study site:** Tatjana Bojovic, Djordje Bojovic, François-Xavier Boyer de La Tour and Babette Lamers.

Dr Bojovic Clinique for Fertility and Gynecology, Belgrade, Serbia. 2011.

**Publication/Report:** Published: October 2012: European Obstetrics & Gynecology, 2012:7(2):103–6

### 1.3 Observational pharmacy survey on the efficacy of Multi-Gyn ActiGel in the treatment of uncomplicated vaginal conditions

**Nr. of participants:** 390

**Study design:** Survey

**Duration of application:** 5 days treatment results

**Results:** The survey was conducted in Italy by Multi-Gyn partner Corman in autumn 2012 with the collaboration of 102 gynecologists and 98 pharmacies that collected the treatment cards of 390 patients aged between 15 and 65. The gynecologists worked with a VVS (Visual View Scale) and the feedback of the patients was filled out in a VAS (Visual Analysing Scale) and sent back to the pharmacists. The survey consisted of a five day treatment, morning and evening, with Multi-Gyn ActiGel and a daily score kept score of the VAS by the patient. Multi-Gyn ActiGel is proven to be effective in all target age groups as perceived by patients.

**Side effects:** none

**Investigators and Study site:** Conducted by Corman Spa. Multi-Gyn Distributor Italy

**Publication/Report:** Study Report, prepared by Corman Spa. Multi-Gyn Distributor Italy

### 1.4 A pilot study to evaluate the efficacy of Multi-Gyn® ActiGel in the treatment of nonspecific vulvitis and vaginitis.

**Nr. of participants:** 50

**Study design:** evidence based

**Duration of application:** 1 week treatment results

**Results:** 64 % of participants reported positive results after one week of treatment. Disappearance of vaginal complaints in 16 participants

**Side effects:** none

**Investigators and Study site:** Dr. M.E. Boon, Dr. M. C. Dersjant, Leiden Cytology and Pathology Laboratory, Leiden, the Netherlands

*The practices of the GP investigators: Dr. W. F. Beelaarts van Blokland (Hengelo), Dr. R. M. Gaaymans (Breda)*

*Gynecologists: Dr. G. Beyer (Rode Kruis Ziekenhuis, The Hague, Department Gynecology and Obstetrics) and Dr. M. Csanky (Academic Hospital LUMC, Leiden)*

**Publication/Report:** Study Report, prepared by Dr. Annelize Goedbloed, 1996  
Approved by the investigators

### 1.5 Multi-Gyn® ActiGel in the Prevention and Treatment of Bacterial Vaginosis: an evidence-based cytological evaluation.

**Nr. of participants:** 128

**Study design:** evidence based

**Duration of application:** 1 week treatment results

**Results:** After treatment 74 % lactobacilli, 26 % mixed flora, 78 % relief of complaints.

**Side effects:** none

**Investigators and Study site:** Dr. M.E. Boon, Leiden Cytology and Pathology Laboratory, Leiden, the Netherlands

**Publication/Report:** Study Report, prepared by Dr. Annelize Goedbloed and Dr. M.E. Boon, 1999

### 1.6 Relief and prevention of Vaginal Complaints in (Post) Menopausal Women with Multi-Gyn® ActiGel

**Nr. of participants:** 68

**Study design:** placebo controlled

**Duration of application:** one week treatment results, 6 months maintenance results

**Results:** decrease of all complaints, improvement of pH and flora

**Side effects:** none

**Investigators and Study site:** Gynecologist: Dr. Boris Divic, Dr. Marta Savic, Dr. Zoran Sretenovic, Dr. Marjan Huljic, Sanja Djurovi and Gordana Obradovic, Belgrade, Serbia and Leiden Cytology and Pathology Laboratory (LCPL) Leiden, The Netherlands, October 2005.

**Publication/Report:** Study Report, prepared by Dr. Annelize Goedbloed, Dr. M.E. Boon and Dr. Matthijs Boon (statistician), 2005  
Approved by the investigators

### 1.7 Influence of Multi-Gyn® ActiGel on the vaginal pH.

**Nr. of participants:** 26

**Study design:** evidence based

**Duration of application:** pH measurement before application and after 4 hours

**Results:** 6 women with starting pH of 4-4.5: no change. 20 women with pH > 5: direct decrease of pH

**Side effects:** none

**Investigators and Study site:** In house study by BioClin B.V, Delft, 2003.

**Publication/Report:** Study Report, prepared by Dr. Annelize Goedbloed, 2003

## 2. In vitro studies

### 2.1 An In Vitro Study of the Effects of Self Care Gels on Desirable and Undesirable Vaginal Microbiota

Vaginal self care gels are used to relieve and treat vaginal discomforts and to maintain and restore the healthy vaginal flora and pH. The effects of four vaginal self care gels on desirable and undesirable vaginal microbiota were evaluated in a challenge study. Results: Exposure to RepHresh, Gynofit and Balance Activ resulted in a decrease in lactobacilli. Multi-Gyn ActiGel does not substantially affect lactobacilli. Candida was decreased by exposure to Gynofit, Balance Activ and Multi-Gyn ActiGel. Exposure to RepHresh resulted in an increase.

- **Conclusions:** Based on the data of this in vitro study, it appears to be unlikely that Balance Activ, Gynofit and RepHresh will have the desired effect of restoration and maintenance of healthy vaginal microbiota. It is proposed that in vitro testing on microbicidal properties is advisable for all vaginal gels that claim to correct and maintain the pH.
- **Authors:** Joke AM Dols<sup>1,2</sup> and Mathilde E Boon<sup>2</sup>  
 1. Resident, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands and Leiden Cytology and Pathology Laboratory (LCPL), Leiden, the Netherlands, 2. Former Senior Pathologist and Director, Leiden Cytology and Pathology Laboratory (LCPL), Leiden, the Netherlands.
- **Publication/Report:** Published: October 2012: *European Obstetrics & Gynecology*, 2012;7(2):111-114

### 2.2 A charged poly-mannose-containing fraction of Aloe vera gel inhibits binding of Helicobacter pylori to salivary mucin.

In an experimental study by **Van Dijk et al**, VU Medical center, Amsterdam, The Netherlands, it was seen that a negatively charged polymannose containing fraction of Aloe vera gel inhibits binding of Helicobacter pylori to salivary mucin. This was studied using an ELISA assay in which micro titer wells coated with an adhesion containing extract of H.Pylori. Aloe vera extracts inhibited the interactions with mucins in a dose dependent way when they were co inhibited with a fixed concentration of mucin. These results indicate that the inhibiting components of A.vera bind to adhesins in the H.Pylori layer extract. The studies suggest that negatively charged polymannose containing high molecular (>10kDa) fraction of Aloe vera gel extract can be applied to prevent infection.

The discovery of this new compound lead to the patent: Free patent on line: EP20010205253  
 Publication Date:07/02/2003 Filing Date:12/27/2001.

- **Authors:** Willem van Dijk, Ben Bruyneel, Bert Van het Hof, Maarten Nietfeld, José de Jong, Patricia Celi, Iris Nijrolder, Clyde Viegas, Hans Niessen and Ferry Namavar.  
  
*Glycoimmunology Group, Dept. Molecular Cell Biology & Immunology, Dept. of Pathology, and Dept. Medical Microbiology and Parasitology, VU Medical Center, Amsterdam, The Netherlands, 2004.*

### 2.3 Anti-adhesive effect of an acidic polysaccharide from Aloe vera L. var. chinensis (Haw.) Berger on the binding of Helicobacter pylori to the MKN-45 cell line.

The results of the study conducted by **Van Dijk et al** were recently confirmed in a publication by **Xu C.** of the Nanjing University, Nanjing, China, on the Anti-adhesive effect of an acidic polysaccharide from Aloe vera L. var. chinensis (Haw.) Berger on the binding of Helicobacter pylori to the MKN-45 cell line. In their search for alternative therapies for the treatment of antibiotic-resistant Helicobacter pylori strains it was shown that the adherence of H. pylori was effectively inhibited in vitro by a fraction that contained significant amounts of significant amounts of galacturonic acid, galactose and arabinose. This fraction was also shown to have a potent anti-adhesive effect against Escherichia coli. This acetylated polymannose fraction appears to be identical to the one discovered by van Dijk that was patented as 2QR-complex (Galactoarabinan Polyglucuronic Acid Crosspolymer).

- **Authors and journal:** Xu C, Ruan XM, Li HS, Guo BX, Ren XD, Shuang JL, Zhang Z. *J Pharm Pharmacol.* 2010 Dec;62(12):1753-9. doi: 10.1111/j.2042-7158.2010.01181.x. Epub 2010 Oct 5.

### 2.4 Dynamic Challenge Test of 2QR-complex against vaginal flora.

It is proven with clinical data that Multi-Gyn ActiGel improves the vaginal flora by neutralizing the harmful bacteria and by supporting the desired Lactobacillus flora within 5 days. The effectiveness of Multi-Gyn ActiGel is proven with in vivo and in vitro tests.

In a dynamic challenge test performed by the microbiological laboratory Bactimm Lab. report Nr. 05.005-01 it is shown that the beneficial bacteria Lactobacillus acidophilus are not affected by 2QR. The test model in this research project for the analysis of the restoration of the balance of the vaginal flora through 2QR shows the direct neutralizing effect on pathogenic microorganisms such as Gram- anaerobic, E. Coli and P. mirabilis. In vivo the microbial ecosystem is always subject to moving boundaries and the results in vitro cannot be translated equally in vivo because of different conditions. However, the selective neutralizing effect on pathogens in vitro can be translated into an effect in vivo.

Based on the above, BioClin has established the recommended use of ActiGel once a day for 5 days. In this way the product has enough time to neutralize the harmful bacteria, to reduce the counts of candida albicans and to support the growth of the desired flora. This conform the objective of the product to restore the balance in the vaginal flora by selectively neutralizing pathogens whilst supporting the desired and useful microbial flora.

Dynamic antimicrobial efficacy of 2QR/N (0,8 mg/g) 100% against vaginal flora, Bactimm B.V. Boven-Leeuwen 19-09-06.

## Lab. Report Nr. 05.005-01 (DYNAMIC CHALLENGE TEST REPORT, DCT) ESTIMATED COUNTS DURING TEST

test strains: wild mix	t=0 13-09- 06	t=15min 13-09- 06	t=30min 13-09- 06	t=1h 13-09- 06	t=3h 13-09- 06	t=6h 13-09- 06	t=24h 14-09- 06
	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g	cfu/g
Lactobacillus	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>
Coryneb. + Strep.B	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>
Gram- anaerobic	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>4</sup>	0
E.coli + P.mirabilis	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>4</sup>	0
Candida	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>2</sup>

### 3. Toxicity

To support its expected non-toxicity BioClin had Multi-Gyn Gel tested on human as well as sheep red blood cells in the very elegant and very sensitive Red Blood Cell Lysis Test. (Laboratory Report "The effect of Multi-Gyn Gel on the osmotic fragility of erythrocytes") This test showed no lysis. Therefore it can conclude that the product is noncytotoxic. With this test method BioClin deferred from the test method that is offered for consideration by ISO 10993-5:1999. \*

The RBC test system is specifically useful to provide extra information in the research and development of products that are in contact with mucous tissues. The RBC test system is applied as an alternative to the testing on animals. Moreover it also contains its own natural sensitive indicator for both cell and protein damage. The measurement of protein denaturation is used as an alternative test method for ocular irritancy and can be correlated to the results of the Rabbit Eye Test (Draize). It allows a good differentiation and can be brought into accordance with the usually used ranks such as: non irritant, irritant etc.

The procedure used for the RBC test on Multi-Gyn Gel is described in John D. Bauer 'Red blood cell pathology' in Gradwohl's Clinical laboratory methods and diagnosis (Ed. S. Frankel, S. Reitman, A.C. Sonnenwirth), C.V. Mosby Company, 1970 and also in Dacie (Dacie J.V. The hemolytic anemia's, congenital and acquired, New York, 1962, Grune & Stratton, Inc.)

Red blood cells are perhaps the most sensitive cells in the human body. From the results of the RBC lysis test as conducted, it may be concluded that Multi-Gyn Gel does not cause harm to other body cells if applied topically.



## 4. Irritation and sensitization

In view of the available information there is no rationale for animal testing as offered for consideration by ISO 10993-10:1999(E).<sup>\*</sup> Aloe extracts have been researched for the prevention of allergic reactions of the skin. For example Aloe showed prevention of ultraviolet radiation induced suppression of contact and delayed hypersensitivity (J Invest Dermatol: VOL 102, ISS 2, 1994, P197-204. Strickland FM: Pelley RP: Kripke ML) and radiation (Phase III double-blind evaluation of an aloe vera gel as a prophylactic agent for radiation-induced skin toxicity. Williams-MS: Burk-M: Loprinzi-CL: Hill-M: Schomberg-PJ: Nearhood-K: O'Fallon-JR: Laurie-JA: Shanahan-TG: Moore-RL: Urias-RE: Kuske-RR: Engel-RE: Eggleston-WD. Int-J-Radiat-Oncol-Biol-Phys. 1996 Sep 1; 36(2): 345-9 ) and dry skin and contact dermatitis (Evaluation of aloe vera gel gloves in the treatment of dry skin associated with occupational exposure. West DP, Zhu YF. Am J Infect Control. 2003 Feb;31(1):40-2)

To support its expected non-irritability BioClin had Multi-Gyn Gel tested on 50 human volunteers by the department of Dermatology of the AMC.